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Diversity and Distribution of Forest Insects

Edited by

Dariusz J. Gwiazdowicz

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Editorial

Diversity and Distribution of Forest Insects

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When we ponder or discuss present-day challenges faced by entomology, we focus first of all on climate change, since the warming climate contributes to a dramatic extension in the ranges of many invertebrate species. This leads to predictions concerning imminent health hazards because certain insect species are vectors of life-threatening diseases. For example, female mosquitoes from the genus *Anopheles* transmit protozoa from the genus *Plasmodium*, which cause malaria. These are the problems investigated by specialists in medical entomology. Another important field of research is connected with economic entomology, which is a subdiscipline of entomology and focuses on the effects of insects on the human economy, primarily with regard to agriculture or forestry. Food production is a key issue for the survival of human populations; however, large-area crop cultures are exposed to a considerable risk of infestation by numerous pests. The control of their populations while preventing any potential deterioration in food quality is an extremely important and complex task, particularly considering consumer expectations to be provided healthy, quality food while meeting the demand of the global food market.

However, when analysing the challenges faced by present-day forest entomology, it may be concluded that this problem is far more complex that the examples given above might indicate. On the one hand, we observe outbreaks (mass-scale emergence) of many insect species threatening not only single trees but also large forest complexes. The resulting losses reported by foresters may be substantial and amount to millions of cubic metres of timber annually. A dramatic example we have seen in recent years is connected with the dynamic population growth of the European spruce bark beetle *Ips typographus*, which is leading to the die-back of spruce forests in Europe. This forces foresters to implement measures inhibiting the bark beetle outbreaks and thus minimising economic losses. At the same time such measures are sometimes considered controversial. Many conservationist groups and NGOs oppose foresters' interference, arguing that these are natural processes and, as such, they should be accepted. It is true that many insect species, including the European spruce bark beetle, create specific conditions since their feeding galleries are microhabitats colonised, e.g., by several dozen fungal species and over 100 mite species. For this reason, the role of insect species commonly considered to be pests while being negative, at the same time, may also be considered positive in other respects.

Another aspect of forest entomology is connected with studying the richness of nature. The forest environments, particularly tropical forests, are habitats for many thousands of species, among which insects are the most numerous group. Unfortunately, even today we do not know the exact number of all species living on our planet; instead, we may only talk of rough estimates of that number. Assuming that, at present, we know approx. 2 million species, it may be stated that most species are still unknown to science. In this vast extent of our ignorance, an important part relates to invertebrates, particularly insects. For this reason, it is urgent to identify, describe and name species new to science. However, this is a long-term, tedious process, and while present-day taxonomy uses new research methods, such as, e.g., molecular techniques, there are justified concerns that some species will become extinct before we have a chance to identify them.

Apart from the numerous, abundant species posing a threat to economic prosperity, there are other species, which are rare and endangered. We are responsible for their protec-

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tion, and in order to ensure their survival, we need extensive knowledge on their biology and ecology, which may be provided by advanced entomological research conducted also in forested areas. Knowing their localities, ranges of occurrence and habitat preferences we may protect these tiny, sometimes hardly visible animals more effectively. Sometimes to ensure such protection for invertebrate assemblages we identify "umbrella species", which are the species selected as the basis for conservation-related decisions, because protecting these species indirectly protects the many other species that make up the ecological community of their habitat (the umbrella effect). In order to analyse environmental changes—both of natural origin and those caused by anthropogenic factors—bioindicator species are frequently used, such as many invertebrate species, also including insects.

This relatively superficial overview of problems investigated by forest entomology clearly shows its complexity and multidimensionality. If we additionally consider the outstanding richness of species colonising the forest environment in different geographical regions, as well as correlations between a multitude of fungal, plant and animal species, it becomes obvious that we face a long-term cognitive process, a practically never-ending quest for scientific knowledge. This is what the Special Issue of the *Forests* journal, entitled "Diversity and Distribution of Forest Insects" and comprising 10 papers, aims to do.

The publication by Gwiazdowicz et al. [1] concerning invertebrates found on a relict tree species *Zelkova abelicea* (Lam.) Boiss on the Greek island of Crete is fully in line with the trend focusing on nature conservation. Relict tree species (the term stemming from Latin *relictum*, "that which is left/forsaken/abandoned/left untouched", the participle form *relictus*—"abandoned, having been abandoned") were widely distributed on Earth thousands and even millions of years ago. As a result of changing climatic and environmental conditions, at present, they are found only sporadically and solely in those locations with conditions conducive to their survival. There is a general consensus among the scientific community that relict trees play a significant role, since they constitute specific microhabitats and, as such, promote biodiversity.

In the analysed material coming from eight experimental sites, representatives of Collembola (10,285 individuals) were most numerous. Moreover, representatives of 11 orders belonging to the class Insecta were also reported, among which Psocoptera (422), Hymenoptera (245) and Thysanoptera (163) were the most abundant. In turn, the class Arachnida was most numerously represented by individuals from the orders Acari (2237) and Araneae (212), while representatives of Pseudoscorpiones (20) were the least abundant. A total of 33 Collembola species were reported, among which *Xenylla maritima* was most abundant (3844 individuals). Moreover, 11 species new to science were identified, for which descriptions will be presented in a separate publication.

Among other things, based on the PCoA analysis (the centroid in the central part of the graph), it was stated that the most stable assemblage is found at the Gerakari locality, where old, magnificent trees are found, the trunks and branches of which are covered by abundant lichens and mosses. In the other localities, the trees were less showy, occasionally forming low thickets composed of shrubs browsed on by goats. This has obviously determined the richness of microhabitats and, as a consequence, also the species richness of micro-arthropods and the unique character of the assemblage.

Present-day forestry focuses on satisfying social needs, both tangible or material (e.g., providing timber) and non-tangible or spiritual (such as the forest being a source of creative inspiration), while simultaneously preserving natural richness (e.g., biodiversity). In this way, the multi-faceted role of the forest environment is being underlined, particularly stressing the fact that the forest is a habitat ensuring survival for endangered species. Effective protection of invertebrates frequently consists of the preservation of specific sites and microhabitats, including, e.g., rotting wood colonised by rare insect species.

Resources of dying and dead trees, decaying fragments of stems, stumps and branches, i.e., coarse woody debris (CWD), are an important structural element of biocenoses and are drivers of biodiversity. The aim of a study published by Mazur et al. [2] was to describe assemblages of saproxylic beetles in pine stands of western Poland in view of rotting wood resources. The authors present faunistic (species identity) and quantitative (species and individual counts) data from two types of stands:

- Unmanaged pine stands, in which no trees have been extracted for over 30 years, with processes connected with tree dying and self-thinning of stands being undisturbed;
- Managed pine stands, in which routine tending operations extracting trees are performed in accordance with forest management plans, and naturally dying trees are removed in the course of tending and sanitary logging.

The authors reported 2006 individuals, which were classified to 216 insect species, and they showed the impact of the adopted forest management method on biodiversity. Managed stands with the implemented sanitation cutting regime, in which trees that are naturally dying are removed, are characterised by lower species diversity indices. Moreover, the authors noted that unmanaged stands were characterised by a high share of zoophagous, mycetophagous and saproxylic species. In contrast, managed stands showed a high share of xylophagous beetles [2].

In turn, ambrosia beetles (which live in a nutritional symbiosis with ambrosia fungi) were investigated by Holuša et al. [3], who focused on oak stands. Those authors presented a hypothesis that a limited amount of sunlight and a greater moisture content in those stands promote the growth of fungi, which determines an increased abundance of ambrosia beetles. In addition, the biodiversity of phloexylophagous insects is greater in old-growth oak stands than in many other types of forest stands because old-growth oak stands have more rotting wood, including dry branches in treetops. The authors tested the hypotheses that:

- Ambrosia beetle occurrence will depend on the degree of canopy closure, the abundance of oak trees, the abundance of rotting wood and the abundance of dead oak branches;
- Ambrosia beetle occurrence is greater in unmanaged oak forests than in commercial forests.

Those authors captured beetles into traps in 10 control plots in Czechia, where they caught over four thousand individuals of ambrosia beetles classified to six species. The two most abundant species, *A. dispar* and *X. saxesenii*, represented 98% of the trapped beetles. Both of these ambrosia beetle species were more abundant in oak-dominated forests with a high canopy closure rate, indicative of a stable and humid environment suitable for the growth of ambrosia fungi, compared to oak forests with a low canopy closure level. Furthermore, a greater abundance of dead oak branches in the canopy was found to be an important factor promoting the occurrence of *A. dispar*. Although the abundance of some species was slightly higher in unmanaged forests, no statistically significant differences in ambrosia beetle abundance was found in managed vs. unmanaged forests [3].

An important trend in forestry research comprises studies investigating the biology and ecology of potential pest species. An example in this respect may be provided by the red-haired pine bark beetle *Hylurgus ligniperda*, which is a well-known forest insect that colonizes the phloem of pine species and which can attack stumps, freshly cut logs and stored timber. Usually, *H. ligniperda* does not kill trees and is considered a secondary pest. Previously, when researchers recorded its original distribution, it included Europe, Russia, the Mediterranean and the nearby Atlantic islands. It was also reported as introduced to South Africa, Japan, South Korea, Sri Lanka, Australia, New Zealand, the USA (NY and CA), Brazil, Uruguay and Chile. It should be noted that the distribution of *H. ligniperda* in East Asia is ambiguous in the literature. For this reason, Lin et al. [4] wanted to clarify its status, asking whether it is a native or invasive species in China or Korea. To answer this question, those authors not only collected the material for analyses in the forest habitats, but they also examined specimens deposited in museum collections.

The first reports on *H. ligniperda* in this geographical region come from the 1930s in Japan. Currently, the distribution of this beetle covers the entire Honshu Island. Those authors' investigations and analyses showed that the previous record of the occurrence of *H. ligniperda* in China was likely misplaced, whereas new observations suggest it has recently invaded and successfully colonized parts of the Shandong Province, China. In

South Korea it has spread rapidly in recent years, and its current distribution effectively covers a major part of this country [4].

In turn, the spongy moth (formerly known as a gypsy moth) *Lymantria dispar* is a polyphagous pest that defoliates various species of trees in the genera *Populus*, *Salix*, *Quercus*, *Acer* and *Pinus*. This species has been introduced to several continents and is now found in Europe, Africa, Asia, North America and South America. Its polyphagous larvae live on a variety of deciduous and coniferous trees and can cause severe damage in years of mass reproduction. Due to these features, *L. dispar* is listed among the world's 100 worst invasive alien species.

Every year *L. dispar asiatica* destroys hundreds of hectares of forests, resulting in thousands of dollars in losses. To conserve forests from spongy moth invasions, Akram et al. [5] studied the features of this pest through morphological, molecular and flight analyses to gain insight into its spread rate. The authors hypothesized that the age of moths and locality, by which morphological features differ, influence the flight ability of female *L. dispar asiatica* from five distinct localities in China.

An experiment showed that the female Asian spongy moth from the Xifeng region in China has a significant potential for quick dispersal because of its flying range. The authors also found that 1-day-old females travel faster and have more potential to fly strong than 2- and 3-day-old adult females. They concluded that age is the most important factor affecting an insect's flight, predominantly for Asian spongy moths [5].

As a result of climate warming, the environmental conditions change and become conducive to the occurrence of alien species, frequently being invasive species. Alien species from the Scolytinae subfamily naturalized in Europe represent over 12% of all European Scolytinae, of which most are ambrosia beetles. Ambrosia beetles (Coleoptera, Scolytinae and Platypodinae) are a polyphyletic group covering numerous species from the Scolytinae subfamily, mainly Xyleborini and Xyloterini tribes, and from the Platypodinae subfamily, which differ from bark beetles in the foraging type: bark beetles feed on the phloem, while ambrosia beetles feed on fungi growing in tunnels or galleries created within a host plant. These species usually infect weakened or dead trees and rarely kill healthy plants. However, fungi carried in their mycangia might be pathogenic to particular host species, leading to economic losses.

Gnathotrichus materiarius is one of the alien ambrosia beetles spreading across Europe since 1930. This is a technical pest of coniferous wood in the USA and Europe and due to excavation galleries in the lower part of the trunk it is of economic importance as a pest, decreasing the technical quality and economic value of affected timber. Witkowski et al. [6] developed a theoretical model for the distribution of this species in Europe considering forecasted climate change by the years 2050 and 2070.

The authors indicate that the species has not yet already reached its maximum range within the climatically suitable area and can reach more sites because a low level of niche saturation is typical of the beginning of the second stage of invasion—the 'log phase', which comes after the 'lag phase'. The prediction developed for current climate conditions pointed out the most likely areas where the species can spread. The Balkans and the Baltic countries are highly suitable regions situated a short distance from the known locations of *G. materiarius*, and there are no natural barriers that would prevent the colonisation of this area. Results of these investigations may be used by phytosanitary service agencies to limit the spread of this species to new areas, e.g., together with sold timber [6].

In temperate forests within Europe, early-flushing (EF) deciduous trees are often heavily infested by early spring leaf-eating Lepidoptera, while late-flushing (LF) trees are phenologically better protected against such heavy infestations, as spring moth larvae begin to appear before their buds burst. Sarvašová et al. [7] studied whether the infestation of LF trees by spring Lepidoptera can be affected by EF ones if they grow in their immediate vicinity. The authors compared spring assemblages of leaf-eating larvae of Lepidoptera on LF *Quercus cerris* with those on EF *Q. pubescens* in several microhabitats in Slovakia.

Those authors found that the species composition of larval assemblages on the two oak species was similar. In contrast, on small groups and on lone trees, the lepidopteran larvae were significantly less abundant on LF trees than EF ones. In the case of young trees, the abundance of larvae and the composition of their assemblages on both oaks were comparable in the forest. In the open habitat, LF trees were less infested by larvae than EF ones, and the assemblages of moth larvae differed between the two. The authors revealed the effect (associational susceptibility) of EF trees on LF ones when growing in a close vicinity. This means that the phenological protection of LF trees may not be sufficient if they grow close to or are surrounded by EF ones [7].

The European spruce bark beetle *Ips typographus* is a dangerous pest of spruce stands, sometimes leading to huge economic losses. The activity of this species is stimulated, e.g., by climate warming, local droughts or air pollution, which weakens local spruce populations and makes them more susceptible to infestation by this beetle. For this reason, an important role is played by methods applied to control its population and measures aimed at increasing the effectiveness of currently used traps. Heber et al. [8] conducted an experiment, in which three main components of current monitoring techniques were tested in terms of their potential for mass trapping of *I. typographus*. These are attractant composition, application rate and trap type.

The conclusions of this article state that during early spring, pheromone traps are highly attractive to individuals in search of breeding sites, meaning that it is the best time for mass trapping. A temporary increase in the application rate for this first swarming period could contribute to a more marked reduction in population sizes, while trap types with a high selectivity would spare antagonists. This would increase the chances to sustainably reduce the population of *I. typographus* to a level not harmful for standing trees or at the least mitigate the peak of a mass outbreak. However, the significant increase in total trap catches achieved by the three-directional approach of this study does not seem sufficient to effectively reduce the number of beetles in an outbreak situation. Thus, the replacement of salvage logging and sanitation felling as the most effective treatments in bark beetle management by mass trapping is not yet an option [8].

The Special Issue contains information concerning not only insects, but also microarthropods closely related with these insects, such as mites. A study by Błoszyk et al. [9] analysed the character of assemblages of mites from the order Uropodina colonising a nature reserve in Poland. Mites from the suborder Uropodina are tiny arthropods, typically of max. 1 mm in body size. They inhabit diverse forest microhabitats such as, e.g., leaf litter, rotting wood, subcortical feeding galleries of insects, ant nests but also bird nests, in which they feed on fungi growing there. They typically spread through a specific zoochory, called phoresis, in which one organism attaches itself to an insect’s body and uses it for transportation.

No economic activity is conducted in the Jakubowo nature reserve and the natural environment is affected generally only by natural factors. In that area, monitoring of environmental changes has been carried out for over 40 years, and the primary aim of this work was to assess stability of uropodid mite assemblages depending on environmental changes such as, e.g., secondary plant succession or increases in the mass of decaying wood.

It was observed that within the 40-year monitoring period, the species composition of uropodid mites has changed. Certain species recorded in 1982 retreated, such as, e.g., *Cilliba rafalskii* and *Trachytes lamda*. In turn, the appearance of species previously not reported, i.e., *Oodinychus obscurasimilis* and *Neodiscopoma splendida*, in the opinion of the authors, is a result of climate warming and increased ranges of these species. Moreover, the natural succession of plant cover observed in each research plot undoubtedly has a bearing on the changes in the communities of the discussed group of mites in terms of both their species diversity and abundance. The observed trend showing an increase in the number of *Olodiscus minima* and *Trachytes pauperior*, i.e., species with higher moisture requirements, in the reserve is most likely caused by the formation of an undergrowth layer, leading to additional shading and helping to maintain greater soil moisture [9].

Bursaphelenchus xylophilus, known as the pine wood nematode or pine wilt nematode (PWN), is a nematode feeding in resin canals of pines thus leading to their clogging and resulting in tree death. This species originally was found in North America; however, in the 20th century, it was also recorded in Asia, e.g., China, Japan and Korea, as well as Europe, in Portugal and Spain, among others. The pine wilt nematode is spread by a number of bark beetles and wood borers, and it is most often associated with beetles from the genus *Monochamus*, the pine sawyers. In view of serious concerns related to the spread of this pest, new studies have been conducted on insects being vectors of PWN. An example in line with this research may be provided by a publication of Chu et al. [10], who investigated the colonization sites (phloem and xylem) and colonization sequences of xylophagous beetles on pine trees and analysed the species richness and spatial distribution of these beetles along PWN-infected tree boles during different stages of the pine wilt disease.

The research was conducted on selected trees infected with PWN in a *Pinus massoniana* forest of the Fujian province and a *Pinus thunbergii* forest of the Shandong Province in China. According to molecular identification of the COI gene and comprehensive identification of the morphological characteristics, those authors identified 20 insect species, and *B. xylophilus* was isolated only from *Monochamus alternatus* in both *P. massoniana* and *P. thunbergia*. No PWN was detected from other xylophagous beetles and other insects in this study. This important information may facilitate a limitation of the spread of this dangerous nematode [10].

Summing up the subject matter presented in the Special Issue of the *Forests* journal entitled "Diversity and Distribution of Forest Insects", it is evident that it is in line with the main trends in forest entomology research. It covers the two primary research trends related to the protection of rare species colonising the forest environment and methods of effective biodiversity preservation, as well as ecology of species causing damage in the forest environment and potential for a more effective limitation of such damage. All these aspects indicate that in the nearest decades, these two main research trends will continue to be of importance within the broadly understood forestry sciences.

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Article

Microarthropods Living on the Endemic Tree *Zelkova abelicea* (Ulmaceae) with Particular Attention to Collembola Diversity

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Abstract: *Zelkova abelicea* is an endemic tree species growing in several localities in the mountainous regions of Crete, Greece. To date, the microarthropod species associated with this tree species have not been identified. Since *Z. abelicea* populations are isolated and fragmented, it was hypothesized that the characteristics of microarthropod assemblages, particularly in the case of springtails (Collembola), would vary and differ among localities. Moreover, rare microarthropod species that colonize microhabitats not included in previous studies on *Zelkova* trees were expected to be recorded. Samples were collected from the bark and twigs of *Z. abelicea* at eight localities in all main mountain ranges. Among the collected material, Collembola were the most numerous (10,285), followed by Acari (2237) and representatives of Psocoptera (422). The obtained material and statistical analyses showed that the arthropod assemblages differed considerably at each experimental site, with the most distinct assemblage characteristics observed at the Gerakari site on Mt. Kedros in central Crete. The most numerous specimens were species of Collembola: *Xenylla maritima* (3844), *Xenylla* sp. 2 (*maritima* complex) (3364) and *Xenylla* sp. 1 (*maritima* complex) (2631). A total of 33 Collembola species were recorded, of which 19 had not been previously reported in Crete. Among them, 11 species were likely new to science and will be the subject of separate taxonomic studies.

Keywords: Collembola; Arachnida; Insecta; biodiversity; ecology of arthropods; zoogeography

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1. Introduction

Relict tree species were originally widely distributed on Earth thousands or even millions of years ago. As a result of changing climatic and environmental conditions, they are presently found only sporadically in places where they encounter appropriate conditions for their survival [1]. Examples of such relict trees include species belonging to the genera *Aesculus*, *Laurus*, *Liquidambar*, *Juglans*, *Parrotia*, *Pterocarya*, *Rhododendron* and *Zelkova* [2,3].

Relict trees play a tremendous role from a scientific perspective and for effective biodiversity preservation [4,5]. Many relict tree species are relatively rare, and as such, they are under legal protection in some countries. In turn, a considerable number of old that constitute unique microhabitats remain, thus promoting conducive conditions for the preservation of biodiversity [6]. These microhabitats, sometimes referred to in the literature as "tree-related microhabitats" [7,8], shelter a wide range of organisms, from fungi to bryophytes, invertebrates, birds and mammals.

The genus *Zelkova* (Ulmaceae) is a relict genus from the so-called Arcto-Tertiary geoflora [9], whose members were important components of forests in the Northern Hemisphere during the Paleogene. The six extant species are distributed throughout western and eastern Asia (Caucasus: *Z. carpinifolia* (Pall.) Koch), East Asia (*Z. serrata* (Thunb.) Makino) and China (*Z. schneideriana* Hand.-Mazz and *Z. sinica* Schneid.), although two found are on Mediterranean islands (Sicily, Italy: *Z. sicula* Di Pasq., Garfì & Quézel, and Crete, Greece: *Z. abelicea* (Lam.) Boiss.). Habitat loss, logging, increased drought periods and limited reproduction represent major threats for these species. Both Mediterranean species have been assigned a high threat level according to the IUCN Red List of threatened species [10,11].

Some *Zelkova* species have been the subject of entomological studies, with the most spectacular results presented by Barbagallo [12], who described *Zelkovaphis trinacriae*, which is a new Eriosomatine aphid genus and species that lives on *Z. sicula* on Sicily. In turn, Mazzeo et al. [13] presented a list of 23 insect species of Hemiptera, while Campo et al. [14] summed up the knowledge on insect and fungal species associated with this tree species from Sicily. Hsin-Ting et al. [15] inspected insects at monthly intervals and recorded insects that fed on or utilized *Z. serrata* in a 100-hectare investigation plot in Pingtung County (Southern Taiwan). A total of 91 insect species were recorded, including Coleoptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Orthoptera and Psocoptera. With regard to feeding guilds, 32 species were recognized as defoliators, 12 species were recognized as sap suckers, 3 species were recognized as stem borers, 31 species were recognized as dead wood feeders, and 13 species utilized this tree species in ways other than the above categories. Ohsawa [16] conducted investigations to elucidate the life cycle and ecological characteristics of the beetle *Trachys yanoi*, an important pest of *Z. serrata* in Japan. Two new species of eriophyoid mites (*Tegolophus zelkofoliae* and *Rectalox dorsoenodis*) were found on *Z. carpinifolia* in Golestan Province, Iran. Both new species were vagrants on the leaf underside, and no damage was observed on the infested plants [17].

Zelkova abelicea (Lam.) Boiss is an endemic species growing in several localities in all mountainous regions of Crete above 900 m a.s.l. [18]. A majority of specimens show stunted growth and a dwarfed, bushy plant habit primarily due to browsing by goats. Tree specimens, which are much less common, reach 15–20 m in height [19]. They frequently grow in the vicinity of abandoned shepherd shelters and have historically been pollarded to use the leaves for summer forage [20,21].

To date, specific research has not been conducted on invertebrates living on *Z. abelicea*. Only two Phytoseiidae mites (Acari) have been recorded [22], and one Hymenoptera species has been recorded [23]. This research gap encouraged the authors to initiate a series of studies focused on assemblages of invertebrates colonizing this endemic tree species.

Populations of *Z. abelicea* on Crete are situated in mountainous regions. In Crete, the five main mountain ranges are isolated from each other by lowland areas. Thus, trees in one locality have little to no contact with those growing in other sites, which is supported by the limited genetic exchange among *Z. abelicea* trees between mountain ranges and, in some situations, between populations within a mountain [24]. Thus, our research hypothesis was that the characteristics of microarthropod assemblages, especially springtails (Collembola) on *Z. abelicea* trees, will vary and differ from locality to locality. The aim of this study was to determine the assemblage characteristics in each locality. Then, based on these results, we conducted a statistical analysis to identify the diversity of species of each arthropod group for every locality. Considering that some arthropod species are known to be closely associated with specific tree species, we expected to record very rare arthropod species or to potentially find species new to science.

2. Methods

2.1. Field Studies

The material was collected at eight experimental sites distributed over the entire range of *Z. abelicea* on Crete (Figure 1).

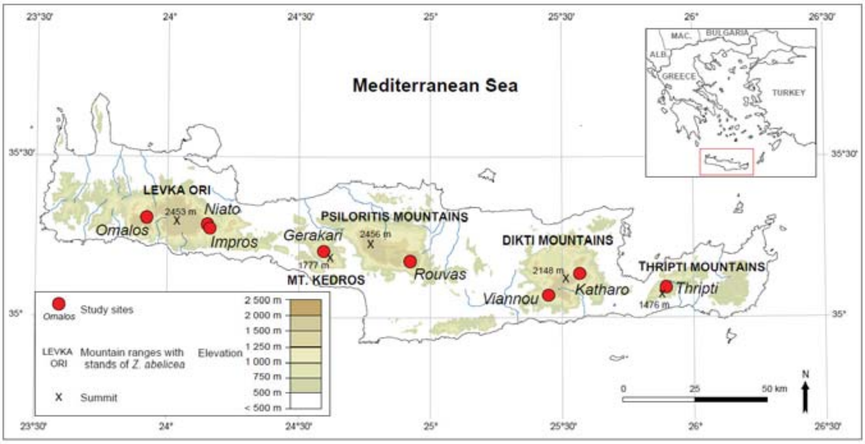

Figure 1. Sampled localities (red dots) on Crete (Greece) with *Zelkova abelicea* trees.

1. Omalos, Levka Ori (Latitude 35, 31901; Longitude 23, 91871), Altitude—1160 m a.s.l., topology: Slope, microhabitat: Bark of arborescent trees, date—21 May 2019, Coll. D. Ghosn;
2. Niato, Levka Ori (35, 287527; 24, 145503), 1215 m a.s.l., doline, branches of dwarfed individuals, 21 May 2019, Coll. D. Ghosn;
3. Impros, Levka Ori (35, 270546; 24, 15315), 1175 m a.s.l., slope, bark of arborescent trees, 21 May 2019, Coll. D. Ghosn;
4. Gerakari, Mt. Kedros (35, 194829; 24, 606713), 1255 m a.s.l., slope, bark of arborescent trees, 11 October 2018, Coll. D.J. Gwiazdowicz;
5. Rouvas, Psiloritis Mountains, (35, 164333; 24, 922794), 1320 m a.s.l., slope, bark of arborescent trees, 10 October 2018, Coll. D.J. Gwiazdowicz;
6. Viannou, Dikti Mountains, (35, 064291; 25, 469778), 1320 m a.s.l., slope, bark of arborescent trees, 9 October 2018, Coll. D.J. Gwiazdowicz;
7. Katharo, Dikti Mountains, (35, 148004; 25, 567558), 1160 m a.s.l., slope, bark of arborescent trees, 9 October 2018, Coll. D.J. Gwiazdowicz;
8. Thripti, Thripti Mountains, (35, 080588; 25, 887408), 1150 m a.s.l., doline, branches of dwarfed individuals, 14 May 2019, Coll. D. Ghosn.

At each sampling site, samples were collected from five trees (one tree—one sample) growing at a distance of a few to tens of meters apart. A sample of the outer trunk bark layer was cut off with a knife from well-developed arborescent trees. In the case of dwarfed specimens, branches were cut off with pruning shears. It was due to the fact that the bark on the trunk of young or dwarf trees is smooth and thin, while on old and large trees it is thick, cracked and frequently colonized by mosses and lichens (Figure 2). The collected material was placed in paper bags. The weight of each sample ranged from 200 to 250 g.

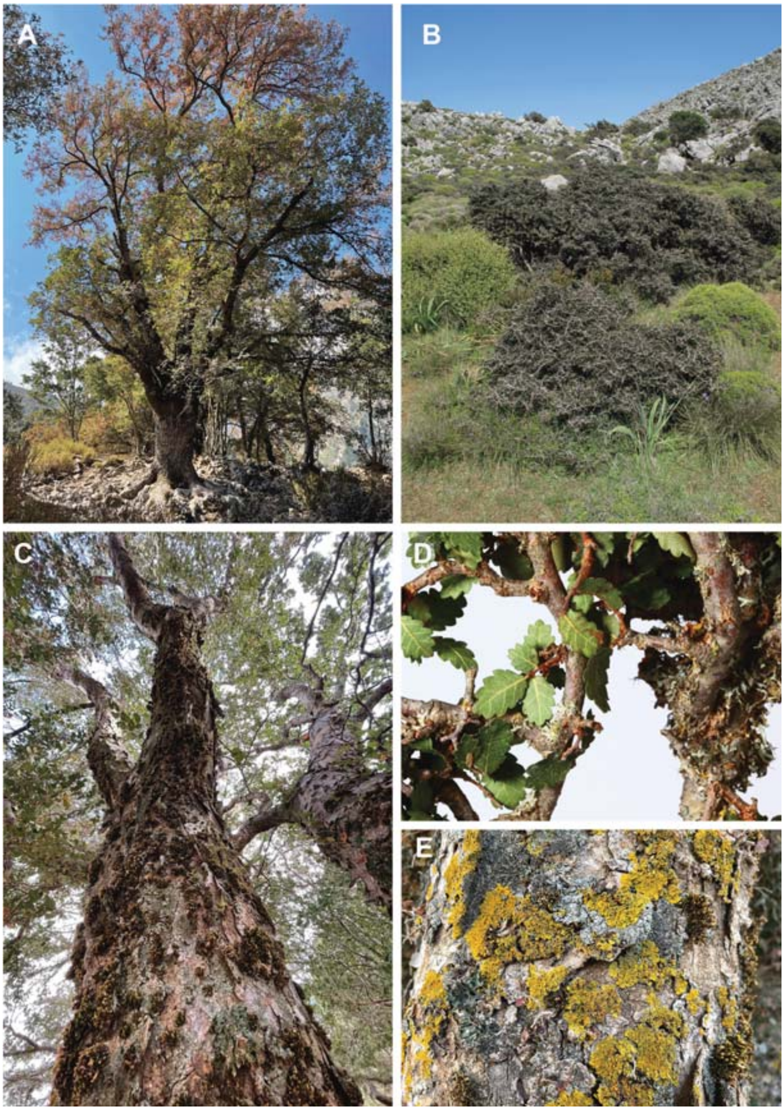

Figure 2. *Zelkova abelicea* trees with microhabitats for invertebrates. (**A**) Large trees (Omalos). (**B**) Dwarfed individuals heavily browsed by goats (Thripti). (**C**) Bark of large trees (Gerakari). (**D**) Bark of browsed individuals with lichens (Thripti). (**E**) Bark of large tree covered by several species of lichens form the genera *Xanthoria*, *Pleurostica* and *Physconia* (Gerakari) (Photos: G. Kozlowski—(**A**–**C**,**E**); Hans-Rüdiger Siegel—(**D**)).

2.2. Laboratory Procedures

The collected samples were placed into Tullgren funnels for 72 h and extracted in 96% ethanol. The extracted arthropods were classified into several groups of arthropods, e.g., spiders, mites, springtails and insects. For this purpose, a Zeiss Stemi 2000 stereoscopic microscope was used.

At this stage of the study, species determinations were limited to the most numerous group, which were springtails (Collembola). A Nikon Eclipse E600 phase contrast microscope was used to identify the Collembola. The extracted specimens of springtails were cleared in Nesbitt's fluid and slide-mounted in a Hoyer medium to prepare semi-permanent microscopic slides necessary for taxonomic analysis. The taxonomic identification of Collembola was carried out based on the following papers: Gisin [25], Stach [26,27], Massoud [28], Ellis [29], Kaprus' & Weiner [30], Jordana et al. [31], Pomorski & Skarżyński [32], Simon Benito & Deharveng [33], Fjellberg [34,35], Pomorski [36], Bretfeld [37], Carapelli et al. [38], Potapov [39], Thibaud et al. [40], Gioia Cipola et al. [41], Skarżyński et al. [42] and Lafooraki et al. [43]. For each site, the number of specimens found for each taxonomic group was counted. In the case of Collembola, the number of recorded species was also provided.

Among the insect material, Acari, Pseudoscorpionida, Myriapoda, and Insecta specimens are stored in the collection of Poznan University of Life Sciences at the Department of Forest Entomology and Pathology, Collembola specimens are stored in the collection of the University of Wrocław at the Department of Invertebrate Biology, Evolution and Conservation, and Araneae are stored in the collection of Adam Mickiewicz University at the Faculty of Biology, Poznań, Poland.

2.3. Statistical Analyses

A cluster analysis [44,45] performed to detect groups of similar sites was run on a Bray-Curtis distances matrix. This method analyzed the Hellinger-transformed the number of specimens using the Manhattan distance matrix and the Ward method. The Hellinger distance is widely used in ecological studies [46]. Cluster analysis is a numerical method that does not consider trends found in community data. Therefore, to compare the species diversity among experimental sites, a principal coordinate analysis (PCoA) [47] based on Bray-Cutris distances was applied. Since the focus of the study was to compare localities in terms of Collembola species, only the ordination method was used to analyze variation throughout arthropod assemblages. Collembola species that preferred a given habitat were identified using multilevel pattern analysis [48]. To describe the Collembola communities, Simpson's diversity index [49], Pielou's evenness index [50] and the dominance index [51] were estimated for each location. To verify whether the number and population size of a species were dependent on the geographical location or altitude of the sampling site, a Mantel test [45] was applied to compare the distance matrix established for geographical coordinates and altitude for the collected samples with the community dissimilarity matrix. All calculations were performed in the R 3.6.1 environment [52] using the vegan [53], indicator species [48], and stats packages.

3. Results

3.1. Diversity of Microarthropod Assemblages in Separate Zelkova abelicea Localities

The class Collembola had by far the highest number of specimens (10,285) in the samples (Table 1). Representatives of 11 orders belonged to the class Insecta, among which the most numerous were Psocoptera (422), Hymenoptera (245) and Thysanoptera (163). Within the class Arachnida, the most numerously represented orders were Acari (2237) and Araneae (212), while the least numerous order was Pseudoscorpiones (20). The highest mean number of specimens per sampling site was reported for Collembola (258), while the lowest number was reported for Lepidoptera (0.05) and Rhaphidioptera (0.03). Representatives of these two orders of insects were detected only sporadically, with a maximum of one specimen per sampling site (Table 1).

Table 1. Total number of specimens in each arthropod group for every sampling site in each of the five mountain ranges. The average number of specimens per tree with the standard error as well as the minimum and maximum are shown.

Systematics/Mountains		**Levka Ori**			**Kedros**	**Psiloritis**	**Dikti**		**Thripti**	
Group of Arthropods/ Localities		**Omalos**	**Niato**	**Impros**	**Gerakari**	**Rouvas**	**Viannou**	**Katharo**	**Thripti**	**Mean ± SE (min, max) Number of Specimens per Tree**
Arachnida	Araneae	109	16	10	15	12	40	2	8	5.3 ± 1.6 (0, 52)
	Pseudoscorpiones	2	0	4	5	0	0	0	9	0.5 ± 0.2 (0, 6)
	Acari	593	44	55	1248	45	61	48	143	55.9 ± 16.9 (0, 427)
Myriapoda		7	0	0	4	0	0	0	0	0.3 ± 0.2 (0, 5)
Collembola		1521	84	951	5325	40	283	355	1726	258.0 ± 76.1 (0, 2279)
Insecta	Coleoptera	2	0	0	22	1	8	1	17	1.3 ± 0.5 (0, 15)
	Dermaptera	0	0	5	0	0	0	0	0	0.1 ± 0.1 (0, 3)
	Diptera	0	0	0	8	0	0	5	1	0.4 ± 0.2 (0, 8)
	Entognatha	12	0	20	25	0	0	3	15	1.9 ± 0.8 (0, 20)
	Hemiptera	4	0	0	4	2	1	1	7	0.5 ± 0.2 (0, 3)
	Heteroptera	0	0	0	3	1	0	1	9	0.4 ± 0.2 (0, 9)
	Hymenoptera	190	0	25	27	0	0	2	1	6.1 ± 3.3 (0, 102)
	Lepidoptera	0	0	0	1	0	0	1	0	0.05 ± 0.03 (0, 1)
	Psocoptera	122	13	18	53	65	37	36	78	10.6 ± 2.1 (0, 51)
	Rhaphidioptera	0	1	0	0	0	0	0	0	0.03 ± 0.03 (0, 1)
	Thysanoptera	13	15	16	20	12	13	54	20	4.1 ± 1.0 (0, 30)
TOTAL		2575	173	1104	6760	178	443	509	2034	345.3 ± 86.8 (0, 2279)
Mean ± SE (min, max) number of specimens per tree		515.0 ± 120.9 (167, 844)	34.6 ± 12.35 (0, 64)	220.8 ± 38.0 (99, 294)	1352.0 ± 402.3 (435, 2501)	35.6 ± 14.7 (0, 64)	88.6 ± 38.1 (0, 189)	107.8 ± 24.3 (47, 183)	408.0 ± 250.4 (29, 1396)	

The variation in the number of arthropod specimens per sampled tree is represented in Figure 3 for every sampling locality. The highest number of specimens was observed at the Gerakari site (per tree average: 1352), where the scatter in the number of specimens in the sampled trees was also the greatest (min: 435, max: 2501). The three sites in the Levka Ori showed strong variations in terms of the numbers of specimens. Indeed, Omalos had the highest number of specimens per sampled tree (average: 515.) and a heterogeneous distribution in the number of specimens per sampled tree (min: 167, max: 844). Impros had an intermediate value (average: 221, min: 99, max: 294), while Niato had the lowest number of specimens (average: 35, min: 0 max: 64). The two sites from Dikti were similar in terms of sample sizes per tree. However, in Katharo, fewer samples predominated (the graph is wider at the bottom), whereas in Viannou, samples with a higher number of specimens predominated. Rouvas was similar to Niato and had small samples. In Thripti, the scatter in the number of specimens in the sampled trees was large because one sample included 1396 specimens while the other four samples had between 29 and 290 specimens. Moreover, certain samples from Niato, Rouvas and Viannou had no microarthropods at all (Table 1).

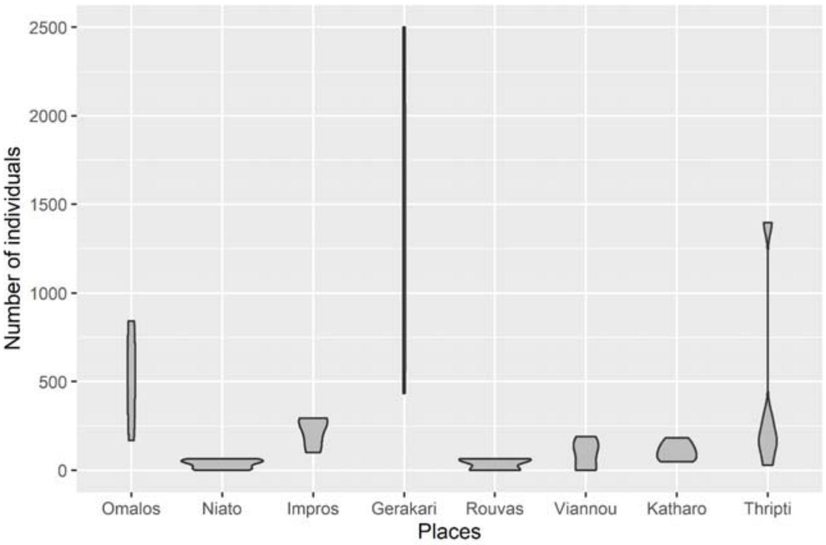

Figure 3. Number of microarthropod specimens per study site. The width of the violin plots is representative of the frequency of data points.

To more accurately illustrate the observed trends in terms of similarity of the microarthropod community, an ordination method based on PCoA distances was applied, with the Hellinger method used to transform data (Figure 4). The centroids and overlapping areas form four groups of sites that are similar in terms of their microarthropod communities: (1) Impros, Niato and Viannou, (2) Thripti and Katharo, (3) Omalos and Rouvas, and (4) Gerakari, which presented a centroid separate from the others (relation to the first axis), although the site had certain commonalities with several other sites. The highest number of specimens by far as well as the highest number of microarthropod groups (14 out of 16) were recorded in Gerakari. Samples from Gerakari were quite close together on the PCoA graph, especially along the first axis, which shows their great similarity. Thripti accounted for a very large area, which is consistent with the results presented in Table 1, with as many as 12 out of 16 microarthropod groups found at this site, although the samples were spaced apart in relation to both axes (they are not very similar). Rouvas accounted for the smallest area and was contained completely within Thripti and Omalos. Thus, all of the microarthropod orders found at Rouvas were also found at the two other sites. At the two sites in the Dikti Mountains, the total number of microarthropod groups was 12 in Katharo and 7 in Viannou. All the orders found in Viannou were also recorded in Katharo. The number of specimens in most microarthropod groups was greater in Viannou than in Katharo except for Collembola and Thysanoptera. For the three sites in the Levka Ori, the lowest number of specimens was recorded in Niato, which also presented the lowest number of microarthropod groups (6), whereas 11 and 8 arthropod groups were found in Omalos and Impros, respectively, and these groups also presented a much higher number of specimens. Samples from Niato and Omalos were spaced apart with regard to the first axis but spaced quite close together with regard to the second axis. Samples from Impros were similar (close to each other) along the first axis and dissimilar (far apart) along the second axis.

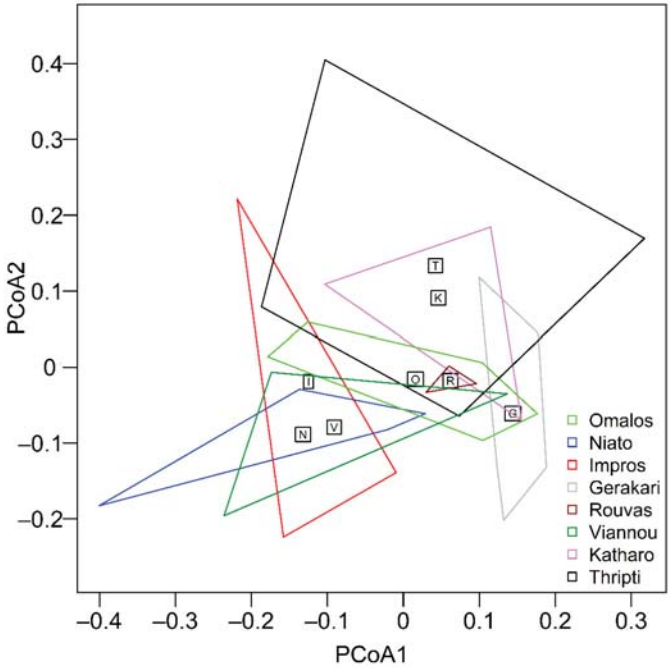

Figure 4. Centroids determined from the PCoA analysis showing the numerical diversity among the samples for each study site (% of total variability: PCoA1—32%, PCoA2—25%). The center of each centroid is indicated by a lettered square representing the study sites (i.e., O: Omalos, N: Niato, I: Impros, G: Gerakari, R: Rouvas, V: Viannou, K: Katharo, T: Thripti).

3.2. Diversity of Collembola Communities on *Zelkova abelicea* Trees

In the collected material, Collembola was by far the most numerous group of microarthropods. Therefore, this class was analyzed in more detail. A total of 33 species were recorded, among which 19 have not been previously reported from Crete [29,32,43,54–59]. Among these 19 species, 11 are likely new to science and will be the subject of separate taxonomic studies (Table 2). Three species of the genus *Xenylla* were represented in the greatest number. *Xenylla maritima* (3844) was dominant, followed by *Xenylla* sp. 2 (*maritima* complex) (3364) and *Xenylla* sp. 1 (*maritima* complex) (2631) (Table 2). Most species were represented by single specimens. Due to the small population size and the presence of juvenile forms, some specimens were only identified at higher taxonomic units, e.g., Anurophorinae 1 and Anurophorinae 2.

Table 2. List of Collembola species in systematic order with the number of recorded specimens per site. Additionally, the average number of species per tree (±standard error, minimum and maximum), the average number of specimens per tree (±standard error, minimum and maximum), the Simpson index (measure of diversity), the Pielou Index (measure of evenness) and the dominance index are shown. * Species new to the fauna of Crete, ** species likely new to science.

	Mountain		**Levka Ori**		**Kedros**	**Psiloritis**	**Dikti**		**Thripti**
	Site	**Omalos**	**Niato**	**Impros**	**Gerakari**	**Rouvas**	**Viannou**	**Katharo**	**Thripti**
1.	*Hypogastrura* cf. *gisini* **				2				
2.	*Xenylla* sp. 1 (*maritima* complex) **	1436	40	933		25	139		58
3.	*Xenylla* sp. 2 (*maritima* complex) **	38	24	8	3233		6		55
4.	*Xenylla maritima*	1	15		1759		132	354	1583
5.	*Protanura* sp. **				1				
6.	*Deutonura* sp. **				2				
7.	*Endonura* sp. **				1				
8.	*Friesea* cf. *cassagnaui* **				1				
9.	*Friesea* sp. **				4				
10.	*Pseudachorutella* sp. **				2				
11.	*Protaphorura aurantiaca*				4				
12.	*Thalassaphorura franzi* *	2							1
13.	*Metaphorura affinis*				1				
14.	Anurophorinae 1 *			2				1	
15.	Anurophorinae 2 *			1					
16.	*Folsomia ksenemani*				2		1		
17.	*Folsomia quadrioculata* *				36				
18.	*Hemisotoma pontica*				6				
19.	*Isotoma* sp.				2				
20.	*Isotomurus fucicolus* *				3				
21.	*Uzelia* cf. *kuehnelti* **	8	4	7	5	7	1		10
22.	*Vertagopus arboreus* *				6				
23.	*Vertagopus* cf. *persicus* **				72				
24.	*Entomobrya handschini*	25							
25.	*Entomobrya multifasciata*	4	1		77	3			5
26.	*Lepidocyrtus lanuginosus*				1				
27.	*Lepidocyrtus* cf. *lignorum*				3				
28.	*Lepidocyrtus* sp. 1				2				
29.	*Lepidocyrtus* sp. 2				2				
30.	*Orchesella taurica* *	1			96				
31.	*Pseudosinella octopunctata*	6			1				
32.	*Seira ferrarii* *					5	4		14
33.	*Sminthurinus alpinus bisetosus*				1				

Table 2. *Cont.*

Mountain		**Levka Ori**		**Kedros**	**Psiloritis**	**Dikti**		**Thripti**
Site	**Omalos**	**Niato**	**Impros**	**Gerakari**	**Rouvas**	**Viannou**	**Katharo**	**Thripti**
TOTAL SPECIES	9	5	5	27	4	6	2	7
TOTAL SPECIMENS	1521	84	951	5325	40	283	355	1726
Average number of species	4.00 ± 0.84 (2, 6)	1.80 ± 0.58 (0, 3)	2.00 ± 0.45 (1, 3)	9.60 ± 3.11 (4, 21)	2.00 ± 0.84 (0, 4)	1.4 ± 0.6 (0, 3)	1.20 ± 0.20 (1, 2)	3.60 ± 0.68 (2, 6)
Average number of specimens	304.2 ± 117.4 (24, 661)	16.8 ± 6.04 (0, 33)	190.2 ± 41.38 (57, 273)	1065 ± 392.4 (105, 2279)	8 ± 3.51 (0, 18)	56.6 ± 31.34 (0, 133)	77 ± 24.06 (32, 165)	346.4 ± 256.2 (16, 1359)
Simpson Index	0.18 ± 0.11 (0.01, 0.61)	0.37 ± 0.13 (0.0, 0.53)	0.05 ± 0.02 (0.0, 0.11)	0.47 ± 0.12 (0.07, 0.8)	0.52 ± 0.05 (0.43, 0.57)	0.23 ± 0.21) (0.01, 0.65)	0.0 ± 0.002 (0.0, 0.2)	0.32 ± 0.12 (0.04, 0.77)
Pielou's Index	0.25 ± 0.13 (0.03, 0.72)	0.82 ± 0.05 (0.75, 0.92)	0.19 ± 0.04 (0.11, 0.23)	0.44 ± 0.09 (0.13, 0.7)	0.77 ± 0.04 (0.69, 0.83)	0.37 ± 0.30 (0.06, 0.98)	0.09 ± NA (0.09, 0.09)	0.48 ± 0.14 (0.11, 0.88)
Dominance Index	0.82 ± 0,11 (0.39, 0.99)	0.63 ± 0,13 (0.47, 1.00))	0.95 ± 0.02 (0.89, 1.00)	0.53 ± 0.12 (0.2, 0.93)	0.48 ± 0.05 (0.43, 0.57)	0.77 ± 0.21 (0.35, 0.99)	1.0 ± 0.004 (0.98, 1.00)	0.68 ± 0.12 (0.23, 0.96)

The highest number of Collembola species (27) and the greatest number of specimens by far (5325) were recorded in Gerakari, followed by Omalos (9 spp., 1521) and Thripti (7 spp., 1726) (Table 2). Katharo had the lowest number of species (2 spp.), while Rouvas had the lowest number of specimens (40). Within the Levka Ori, Omalos stands out as having a higher number of Collembola species compared to the two other sites (9 spp. and 5 spp.). Niato and Impros not only had lower biodiversity than Omalos but also had a lower number of specimens (Table 2, Figure 5). All three aforementioned sites shared three common species, although this species differed significantly in terms of its population size among the sites. The two sites in Dikti had a similar mean number of species per sample but showed differences in the total number of species, with six in Viannou and only two in Katharo. Only one species was shared between these two sites. None of the species was shared among all sampling sites; however, *Uzelia* cf. *kuehnelti* was found at all sites except for Katharo and all three *Xynella* species were found at all sites except two (Table 2). Twenty-two species occurred only once, among which all (except two) were restricted to the trees at Gerakari.

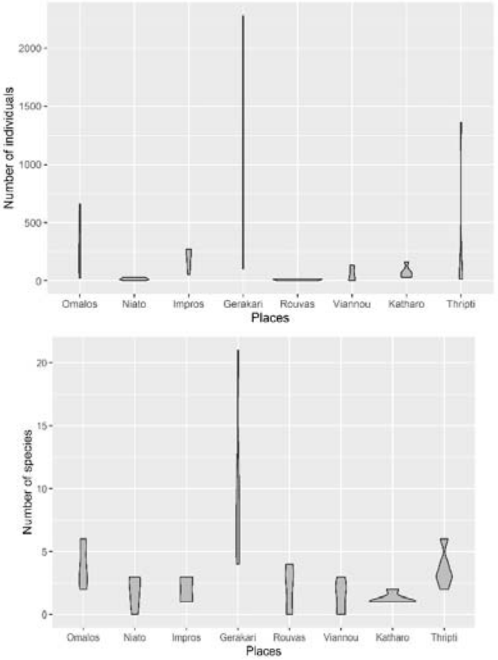

Figure 5. Number of specimens and number of species of Collembola per study site. The width of the violin plots is representative of the frequency of data points.

The cluster analysis of similarities showed that Collembola assemblages in Gerakari differed markedly from all the other assemblages. The sites in Omalos and Impros, which are located in the Levka Ori Mountains, showed considerable similarity, whereas the Niato site, which is geographically very close to Impros, was completely different and positioned closer to Thripti. It may be related to the character of the microhabitat from which the samples were collected. In both Niato and Thripti, twigs were harvested, while bark was harvested from trunks in other sites. In turn, the Collembola assemblages at the two sites located in the Dikti Mountains (Katharo and Viannou) differed slightly (Figure 6).

The PCoA analysis for Collembola showed that Gerakari stands out compared to all other sites due to the presence of many species that are not found elsewhere. In addition, Katharo did not share a common area with any of the other sites because only two species were recorded there, making the site difficult to position. Most of the other sites overlapped. Thripti and Impros were almost fully encompassed in Niato. Impros, Niato and Omalos had a large common area (Figure 7). Moreover, these areas overlapped with those distinguished by samples from Rouvas and Viannou. For these areas, one common indicator species was identified: *Xenylla* sp. 1 (*maritima* complex) (Table 3). Similar to the cluster analysis, samples from Katharo were located closest to the Viannou site, which confirmed their considerable similarity.

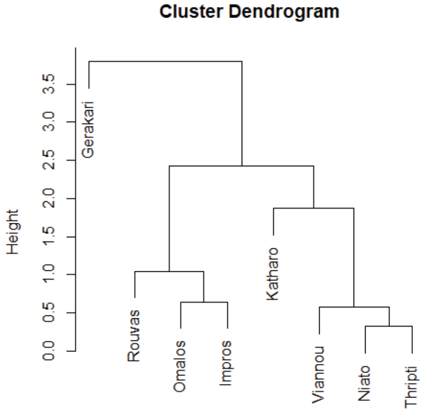

Figure 6. Cluster analysis showing the similarity of localities depending on the Collembola communities.

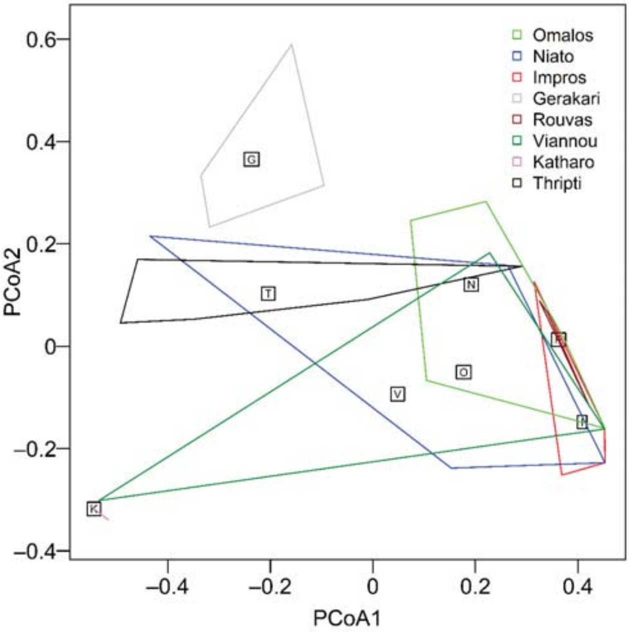

Figure 7. Centroids obtained from the PCoA analysis showing the numerical diversity of Collembola in the study sites (% of total variability: PCoA1—56%, PCoA2—21%). The center of each centroid is indicated by a lettered square representing each study site (i.e., O: Omalos, N: Niato, I: Impros, G: Gerakari, R: Rouvas, V: Viannou, K: Katharo, T: Thripti).

Table 3. Indicator species for localities based on a multilevel pattern analysis.

	Stat	*p* Value
Group Gerakari		
Orchesella taurica Stach, 1960	0.809	0.0020
Lepidocyrtus cf. *lignorum* (Fabricius, 1793)	0.742	0.0120
Vertagopus cf. *persicus* Potapov, Yoosefi & Shayanmehr, 2020	0.717	0.0143
Group Gerakari + Rouvas		
Entomobrya multifasciata (Tullberg, 1871)	0.632	0.0150
Group Katharo + Thripti		
Xenylla maritima Tullberg, 1869	0.682	0.0037
Group Gerakari + Niato + Thripti		
Xenylla sp. 2 (*maritima* complex)	0.621	0.0160
Group Impros + Niato + Omalos + Rouvas + Viannou		
Xenylla sp. 1 (*maritima* complex)	0.743	0.0003

The multilevel pattern analysis identified the indicator species shared by the highest number of sampling sites. These indicator species are the most numerously represented species found in many localities and in many samples (Table 3).

A Mantel test was conducted to compare the distance matrix established for the geographical coordinates and altitude where the samples were collected with the community dissimilarity matrix. The test (r = 0.2329, $p = 0.116$) showed no dependence between the community dissimilarity matrix and distances between locations matrix (calculated from the longitude and latitude as well as the elevation of a given site). Thus, the occurrence of Collembola species was not found to be dependent on the location or altitude of a given site.

4. Discussion

Arthropods are known to contribute to a very important fraction of global biodiversity [60]. However, limited research has focused on this group of organisms. Moreover, even fewer studies have investigated microarthropod communities, especially Collembola communities living on trees or associated with tree microhabitats in the Mediterranean, let alone Crete [61,62]. Our study provides a small but significant addition to this field of study, as corroborated by the 19 recorded Collembola species that had never been reported for Crete, which included 11 species, i.e., almost one-third of the recorded species) that are likely new to science.

Several studies have shown that the presence, range or continuity and connection of some species, groups or even populations of organisms distributed on Crete are highly influenced by the strong topographical and/or geological history and structures present on the island [24,63–70]. The character of arthropod assemblages is also influenced by natural conditions, e.g., the host plant, which creates specific microhabitats [71]. The influence of such factors was also observed in our study, at least for Collembola, with each *Z. abelicea* locality presenting a specific assemblage. Indeed, longitudinal, latitudinal or altitudinal trends were not observed for the Collembola species, which suggests that the distribution and diversity of Collembola assemblages growing on *Z. abelicea* trees are not influenced by macroenvironmental conditions (e.g., precipitation, temperature, and drought) or by between-site epiphytic lichen and bryophyte community differences. In fact, the diversity and distribution of epiphytic lichens and bryophytes growing on *Z. abelicea* were found to differ along a longitudinal gradient, which was likely related to differences in regional climatic patterns [72–74].

In addition, the cluster analysis (Figure 6) revealed that sites situated within the same mountain range showed greater similarity than sites situated in other mountain ranges. A striking exception to this statement was observed for Niato, which was dissimilar to all Levka Ori sites despite being geographically very close to Impros but similar to the easternmost site Thripti. A possible explanation is that both Niato and Thripti were the only sites where samples were collected from dwarfed *Z. abelicea* communities and the only sites situated in flat dolines, whereas samples from all other sites were gathered from arborescent trees situated on sloped areas. Therefore, we can conclude that at least in the case of arborescent trees or trees situated on slopes, the absence of a continuous *Z. abelicea* population between mountains associated with the complex topography of Crete seems to be a determining factor for Collembola assemblages. Further and more in-depth analyses should be undertaken to investigate this matter.

Based on the PCoA and cluster analysis, the most different character of assemblages was recorded at the Gerakari site on Mt. Kedros, where the highest number of species (27) and specimens of Collembola (5325) were recorded. The multilevel pattern analysis identified the indicator species for this site, including the xeroresistant species *Orchesella taurica*, which lives in forests and open sites in SE Europe [26,75,76]; the eurytopic species *Lepidocyrtus* cf. *lignorum*, which is widely distributed in the Holarctic [35]; and the species *Vertagopus* cf. *persicus*, which is likely new to science. Indeed, the Gerakari site on Mt. Kedros proved to be the richest in terms of the number of specimens collected as well as the number of arthropod groups and Collembola species found, and it showed striking differences in its arthropod population compared to all other sites. Indeed, from the 27 Collembola species recorded at Gerakari, twenty were found nowhere else, which indicates the uniqueness of the site and its dissimilarity with the other sampling sites. These results can be assessed based on the study by Fazan et al. [77], who found a richer diversity of epiphytic bryophytes on *Z. abelicea* growing at the same study site on Mt. Kedros. Although the reasons behind these differences exceed the scope of the present article, some hypotheses can be proposed. Indeed, arthropod communities, including Collembola, are known to be very sensitive to a multitude of different factors, including microhabitat conditions [78–80], vegetation type and plant richness [81–83], landscape heterogeneity [84] and land-use practices, such as grazing [84–92], which are known to impact arthropod communities. All of these factors, and many more, could explain to some extent the variations found between study sites in our experiment.

Based on the analyses, such as the PCoA, we can state that the most stable assemblage occurred at the Omalos site, which presented large, monumental trees with trunks and branches that were abundantly covered with lichens and bryophytes. At the other sites, the trees were smaller and occasionally grew as low shrubs that were nibbled upon by goats. This obviously determined the richness of the microhabitats and, as a consequence, the species richness of microarthropods and the different characteristics of the assemblage. Moreover, the three other xeroresistant species should be highlighted: *Entomobrya multifasciata*, which was characteristic of the Gerakari and Rouvas sites and is common in forests and open sites in Palearctic [35,76]; *Xenylla maritima*, which distinguished the Katharo and Tripti sites and has been recorded from mosses, lichens and bark in Europe and New Zealand [42]; and finally, *Xenylla* sp. 1 (*maritima* complex), which distinguished Impros, Niato, Omalos, Rouvas and Viannou sites and is probably new to science.

The discovery of nineteen species of Collembola new to the fauna of Crete, including eleven that are likely new to science, showed how much new information can be provided by the study of specific microhabitats, which was the tree *Z. abelicea* in our study case. The research conducted thus far in Crete, although extensive, has focused mainly on soil and litter assemblages [29,55–58]. The results of the current taxonomic research on the abovementioned species that are likely new to science will be published in separate works. These species may provide a basis for a deeper consideration of the specificity of the Collembola assemblages associated with *Z. abelicea*.

5. Conclusions

At each site where *Z. abelicea* trees were found, a different characteristic of microarthropod assemblages was recorded. Among the collected material, the most numerous groups of organisms were Collembola (10,285), Acari (2237) and representatives of Psocoptera (422). The analyses focused on Collembola showed that the site at Gerakari (Mt. Kedros) stood out in terms of the species assemblage. Moreover, relatively rare species, as well as eleven species likely new to science, were recorded, highlighting the specific character and value of Collembola assemblages that colonize endemic *Z. abelicea* trees. The observation of 19 previously unrecorded species on Crete species and 11 species likely new to science and the results of these pilot studies justify the need for further research on the microarthropods colonizing this unique endemic tree.

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The Structure of Saproxylic Beetle Assemblages in View of Coarse Woody Debris Resources in Pine Stands of Western Poland

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Abstract: Background: Resources of dying and dead trees, decaying fragments of stems, stumps and branches, i.e., coarse woody debris (CWD), are an important structural element of biocenoses and are drivers of biodiversity. The aim of this study was to describe assemblages of saproxylic beetles in pine stands of western Poland in view of dead wood resources. We present faunistic (species identity) and quantitative (species and individual counts) data from two types of stands: 1. unmanaged pine stands, in which no trees have been extracted for over 30 years, with processes connected with tree dying and self-thinning of stands being undisturbed, 2. managed pine stands, in which routine tending operations extracting trees are performed in accordance with forest management plans and naturally dying trees are removed in the course of tending and sanitary logging; Methods: Beetles were captured in the years 2013–2014 using window flight traps. Assemblages of saproxylic beetles were assessed based on the indices of dominance, diversity (the Shannon–Weiner index), and species richness (Margalef's index) as well as the estimated habitat fidelity index, feeding habits, and zoogeographical distribution. Similarity between the assemblages was evaluated applying cluster analysis. Dependence between dead wood resources and the diversity and species richness indices were analysed; Results: A total of 2006 individuals classified to 216 species were captured. Assemblages show considerable similarity on the local scale. Higher values of species diversity indicators were observed in unmanaged stands, in which no sanitation cuttings are performed; Conclusions: The decision to refrain from sanitation logging in pine monocultures results in increased CWD resources, which nevertheless does not lead to a marked increase in the values of biodiversity indicators. Unmanaged stands were characterised by a high share of zoophagous, mycetophagous, and saproxylic species. In contrast, managed stands were characterised by a high share of xylophagous beetles.

Keywords: deadwood; biodiversity; *Pinus sylvestris*; Coleoptera

1. Introduction

The present-day European forest management system is considered to be multifunctional (serving social, economic, and ecological functions) and sustainable [1]. A major objective of such a management is to preserve biodiversity. Forest habitats in Central Europe are characterised by high species diversity (with approx. 65% plant and animal species being forest species) [2,3]. Factors threatening species diversity include increased timber harvesting combined with reduced rotation, promotion of fast-growing species regardless of habitat conditions, devastating management methods (e.g., large-area clear-cuts, deep ploughing, restrictive snag removal, cuttings performed regardless of local conditions, increased fertilisation), application of pesticides, selective breeding of forest trees, introduction of alien tree species, and afforestation excluding any open spaces [4].

Beetles (Coleoptera), particularly saproxylic species, i.e., those biologically associated with dying trees, are a diverse group both in terms of their ecology and taxonomy [5,6]. Some of them, including several species legally protected in Europe, constitute excellent indicators of biodiversity and the impact of forest management on forest ecosystems [7–15].

Protection of natural processes of tree dieback and reduced harvesting of snag as a biotope of many specific saproxylic species [16,17] seem necessary to preserve high species diversity. This need poses questions concerning the volume and quality of coarse woody debris left to decompose naturally, particularly in commercial forests [18]. The saproxylic fauna is modified not only by the obvious, significant role of CWD resources [19], but it is also influenced by the tree or wood species [20–23], humidity, insolation and type of the microhabitat [13,24,25], the geographical region and the size of the forest complex [26], as well as natural disturbances in bark beetle gradations [27], windthrows [28], and fires [29,30].

The problem of threshold dead wood resources in European forests has been discussed in detail [18,19]. Some sources of data indicate that a significant increase in species richness of saproxylic beetles is observed only at a very high accumulation of coarse woody debris on a regional scale [31–34].

In 2020 in Polish forests the mean CWD resources amounted to approx. 8.2 m^3/ha [35], whereas as recently as in the years 2005–2009 it was on average 5.7 m^3/ha [36,37]. Dead wood resources vary depending on the region. Thus, CWD resources are below the mean level in central Poland, where pine stands are dominant, while they are much greater in mixed broadleaf forests of southern Poland (where they range from 14.8 to 20.1 m^3/ha) [36].

A compromise needed to combine the sanitary regime in pine stands (as the most common type of commercial stands in the central, lowland part of Europe) with prescribed allocation of a portion of wood to be left in the forest to decompose naturally [34] may be attained only when considering a variety of aspects. A considerable role is played by the geographical location and the related climate, as well as the size of populations of harmful insects and the intensity of forest management in those forests [38]. The date of logging is also of importance, since it stimulates the population size in the case of bark beetles [39].

A considerable body of data on subcortical and saproxylic beetles of pine coniferous forests comes from Scandinavia. Among other things it was found that forest management significantly determines the composition and quantitative ratios of subcorticular beetles [40]. The volume of dead wood resources does not always affect the species diversity or numbers of subcorticular beetles [41], while the absence of dead trees of large diameters may result in the disappearance of species obligatorily associated with old trees and stands [42]. Seminatural pine forests are distinguished first of all by much greater CWD resources, which are also maintained in the first stages of succession of the young tree generation and such forest habitats are biotopes for threatened saproxylic species [43]. In order to protect saproxylic fauna it is important not always to increase the total volume of dead wood, but also its continuity and diversity in commercial forests, because each type of coarse woody debris (stumps, snag, branches, twigs) produces unique microhabitats [44–48].

Species diversity of saproxylic beetles in pine coniferous forests of Poland have been investigated depending on the method of forest management and the degree of ecosystem transformation [10].

Boggy pine forests as a refuge for saproxylic beetles were investigated in eastern Poland [49]. Obtained results indicate a local character of their assemblages and a significant role of commercial pine stands for the preservation of the natural habitat value and its high biodiversity.

Processes modifying the saproxylic fauna in hurricane-damaged pine coniferous forests were observed in the Puszcza Piska Forest [50]. Analyses showed no statistically significant differences in saproxylic beetle assemblages between hurricane-damaged stands cleared after the hurricane and unaffected managed stands. The rapid accumulation of dead wood resources resulted in an increase in the number of individuals and the concentration of some species on the local scale, although it did not affect the number of species.

Pine coniferous forests were also investigated in areas of high nature value with the aim of finding model patterns. Examples in this respect include the Kampinos Forest [51] and the Białowieża Forest, in which the structure of subcorticular beetle assemblages was analysed [11,52] as a tool in the assessment of anthropogenic transformations of primeval forest habitats. In pine coniferous forests of the Białowieża Forest, observations were also made on the impact of fire on alfa and gamma diversity of beetles [53]. In the initial period of habitat regeneration, the diversity indices were higher for the fire-affected stand; however, in the successive years of observations the differences between the stand destroyed by the fire and the control stand were disappearing.

The aim of this study was to identify dependencies between CWD resources and characteristics of assemblages of saproxylic and subcorticular beetles in lowland pine stands differing in the management system (unmanaged vs. managed), determining dead wood resources.

2. Materials and Methods

Investigations were conducted in the years 2013–2014 in central-western Poland in terms of its landscape representing the North European Plain (Figure 1).

The objects of the study included stands of similar valuation parameters, such as age and species composition, but differing in intensity of snag removal, based on which it was divided into two categories:

A. unmanaged stands, originally commercial forests, with established protection functions, such as, e.g., landscape, water, or soil protection), in which no logging operations have been conducted for over 30 years and in which tree dieback and self-thinning processes have been undisturbed. They are permanent experimental sites of the Department of Silviculture, PULS, in the Torzym and Gubin Forest Districts [54,55] as well as a stand in the Drawieński National Park subjected to the same principles.

B. managed stands, in which routine tending operations (logging) are performed in accordance with the forest management plan, and naturally dying trees are removed in the course of tending operations and sanitation cuttings.

In these experimental sites, CWD resources were measured, stand dynamics trends were assessed, beetles and saproxylic fungi were inventoried [57,58], and losses of benefits due to the allocation of wood to decompose naturally were estimated [59] (Table 1).

Mean volume in unmanaged stands was 356.7 m^3/ha, at the mean volume of dead wood (CWD) of 12.99 m^3/ha, the percentage share of dead wood in relation to growing stock was 3.63%. In managed stands the mean volume was higher and amounted to 389 m^3/ha, while values of the other parameters were approx. 3-fold lower; the mean volume of dead wood was 4.04 m^3/ha and the share of dead wood in relation to growing stock was 1.05%.

Identification of species diversity in Coleoptera. Insects were captured with window flight traps commonly used in inventorying saproxylic beetles in Poland and considered to be highly effective [10,50,60–62]. Two traps of 3800 cm^2 were hung in each of the experimental sites using for this purpose dying trees, windsnaps as well as broken branches and parts of the crown lying on the ground. Beetle capture operations were conducted from 23 April 2013 to 15 November 2014. Window flight traps were operating for a total of 1692 trap-days (the number of days of trap operation × the number of traps).

The beetles for determination were prepared using techniques recommended for individual families (e.g., with separation of mating apparats). The available keys and guides for family and species identification as well as taxonomic revisions of selected groups of beetles were used for the determinations. It was used for this, among others: series of keys for determination of beetles in Poland for example [63–65] and Central

Europe [66,67], taxonomic monographs of beetle families [68–70]. The markings were verified using illustrated keys and beetle iconography [71,72]. The specimens are deposited in the authors' collections at the Department of Forest Entomology and Pathology, Poznan University of Life Sciences, Poland.

Figure 1. Locations of experimental sites. Source: Google Earth Pro 7.3.4.8248 (64-bit) Data SIO, NOAA, U.S. Navy, NGA, GEBCO. Image Landasat / Copernicus (2021) [56].

Results of capture operations from individual stands were totalled, distinguishing beetle assemblages, which were assessed based on such parameters as the number of species (S), the number of individuals (n), Margalef's index (d—species richness) [73], Shannon's index (H'—species diversity) [74,75] and the percentage share of trophic forms (zoophagous, mycetophagous, xylophagous, saproxylophagous). Identified species in terms of their trophic levels were divided into five groups: xylophagous, mycetophagous, zoophagous, saproxylic species, and the other trophic groups. Next species richness and the number of individuals in each of the groups were determined. Among the captured insects, also the percentage shares of species were calculated. The species, the share of which exceeded 5%, were considered dominant, while codominant species were those whose share ranged from 2% to 5%.

Similarity between the *Coleoptera* assemblages was assessed using cluster analysis. The analysis used the species dominance index (where the number of individuals n = 2006, the number of taxa S = 244) in the studied stands. The Euclidean distance was taken as the measure of clustering distances. The analyses were performed using the Statistica13.3 software by StatSoft®. The volume of dead wood, the number of species in an assemblage as well as percentage shares of individual trophic groups were vectors of observations. The Euclidean distance was applied as the metric.

Table 1. Characteristics of experimental sites—unmanaged and managed pine stands in western Poland.

Location and Description/Symbol of the Site	**Geographical Coordinates WGS**	**Area (ha)**	**Stand Volume Excluding Dead Wood (m^3/ha)**	**Volume of Dead Wood (CWD) (m^3/ha)**	**Age**	**Share of Dead Wood/Growing Stock (%)**
Unmanaged pine stands						
Gubin Forest District compartment 73j—uneven-aged stand/UG1	N: 51.9817 E: 14.8611	4.03	248 170	8.03	195 62	1.92
Gubin Forest District compartment 57a/UG2	N: 51.9737 E: 14.8195	20.18	317	16.51	92	5.45
Torzym Forest District compartment 264a/UT3	N: 52.2603 E: 15.1254	11.42	481	23.43	85	5.85
Drawieński National Park, compartment 288h/UD4	N: 53.0839 E: 15.9347	6.08	270 35	4.0	145 105	1.31
Managed pine stands						
Drawieński National Park, compartment 15b/MD5	N: 53.1889 E: 16.0235	7.33	310	3.51	125	1.13
Gubin Forest District compartment 56a/MG6	N: 51.9758 E: 14.8229	20.14	303	3.7	113	1.17
Torzym Forest District compartment 263a, c/MT7	N: 52.2625 E: 15.1288	8.04	401	7.52	91	1.56
Jarocin Forest District compartment 200a/MC8	N: 52.1109 E: 17.4994	8.23	449	1.43	63	0.32

Assessment of specificity of Coleoptera assemblages. Characteristics of species and their relationships with the habitat were based on the estimated fidelity classes proposed by A. Szujecki [76,77] and their later modifications taking into consideration the character of the study and the specific nature of beetle groups [78–80] as well as the Catalogue of Polish Fauna and the National Biodiversity Information System website [81].

The following modified estimated fidelity classes were adopted:

F4: obligate characteristic species—strictly (obligately) related, i.e., in terms of their trophic levels and biology, with processes of dieback in coniferous trees, cambio-, xylo-, and endophytophagous (the first stages in the succession chains in the subcorticular habitat).

F3: selective characteristic species—found numerously in a given habitat, although also present in other habitats; included predatory, parasitic, and ambrosia beetles as well as mycetophagous species, the occurrence of which is dependent on the presence of host organisms.

F2: auxiliary (accompanying) species—found in the subcortical habitat less numerously than in other habitats or showing no preference to any habitat type; included species facultatively (periodically) appearing in the subcortical habitat, searching for shelter or food.

F1: species alien to a given habitat.

F0: ubiquitous, cosmopolitan species.

R: relic species, of special faunistic value, reported in single localities, related with primeval forests.

Data on the occurrence of clown beetles (Histeridae) was published in a study by Mazur et al. [61].

Saproxylic beetles (understood as species in terms of their ecology associated with dying, dead, and decaying trees, in which the utilisation of wood has led to morphological, anatomical, and metabolic adaptations to the habitat) [82,83] were classified in further analyses to species from fidelity classes R, F4, F3, and F2.

3. Results

A total of 2006 beetles belonging to 244 taxa were captured in the period of the study. Of that number, 1820 individuals were identified to species, which accounts for 90.7% all captured insects. They were classified to 216 species, whereas the other beetles (9.3%) were identified to the level of genus or family (Supplementary Materials). Among them 194 (89.8%) are species permanently or periodically associated with wood, they were represented by 1702 (93.7%) individuals. Characteristics of assemblages in the investigated stands are given in Table 2.

Table 2. Characteristics of Coleoptera assemblages in managed and unmanaged pine stands in western Poland.

Characteristics of Assemblages	Unmanaged					Managed				
	UG1	UG2	UT3	UD4	Mean (±SD)	MD5	MG6	MT7	MC8	Mean (±SD)
Number of individuals (*n*)	267	137	126	323	213.25 (±84.2)	531	108	46	282	241.75 (±188.08)
Number of species (S)	64	45	39	84	58 (±17.62)	75	25	18	62	45 (±24.07)
Xylophagous species (%)	14.1	13.3	15.4	22.6	16.3 (±3.7)	28.0	16.0	16.7	22.6	20.8 (±4.9)
Mecytophagous species (%)	15.6	22.2	23.1	14.3	18.8 (±3.9)	16.0	10.7	16.7	27.4	17.7 (±6.1)
Zoophagous species (%)	45.3	51.1	33.3	41.7	42.9 (±6.4)	40.0	46.7	44.4	29.0	40.0 (±6.8)
Saproxylophagous species (%)	9.4	8.9	7.7	6.0	8.0 (±1.3)	8.0	6.7	5.6	9.7	7.5 (±1.5)
Species from other trophic groups (%)	15.6	4.4	20.5	15.5	14.0 (±5.9)	8.0	20.0	16.7	11.3	14.0 (±4.6)
Margalef's index (d)	11.28	8.94	7.86	14.37	10.61 (±2.5)	11.79	5.13	4.44	10.99	8.09 (±3.32)
Shannon's index (H')	3.49	3.32	2.80	3.92	3.38 (±0.4)	2.56	2.77	2.50	3.35	2.8 (±0.33)

In unmanaged stands, a total of 167 beetle species were reported, which were represented by 936 individuals, whereas in managed stands 153 species were captured, represented by 1067 individuals. On average, in each of the unmanaged stands 55.5 species (SD = 14.8) were identified, while in managed stands it was 46.6 species (SD = 22.6).

Species captured in unmanaged stands, in the number of minimum 10 specimens, which were not shown in managed stands, included *Phloeonomus pusillus* (Staphylinidae), *Dryophthorus corticalis*, *Orthotomicus longicollis*, *Hylurgops palliatus*, *Hylastes brunneus* (Curculionidae), *Platydema violacea* (Tenebrionidae), and *Latridius hirtus* (Latridiidae). In contrast, *Cardiophorus ruficollis* (Elateridae) and *Xylosandrus germanus* (Curculionidae) were species recorded in managed stands.

Analyses of individual stand showed the highest number of beetle species in the oldest stands (UG1, MD5, UD4), aged over 140 years, which was reflected in the high values of species richness (d) and species diversity (H'). An exception in this respect was found for the commercial (managed) pine stand established on former farmland (MC8), in which biodiversity indices were relatively high (Table 2). The lowest qualitative and quantitative richness of beetles was recorded in commercial stands in the Gubin and Torzym Forest Districts (MG6, MT7) (Table 2), as it was by 21.2% and 63.5% fewer species compared to unmanaged stands located in their vicinity (UG2, UT3).

Among all the captured beetles *Ampedus balteatus* (Elateridae) was the dominant species (D = 13.9%), while co-dominant species (2% < D ≤ 5%) included *Enicmus rugo-*

sus (Latridiidae), *Tomicus piniperda* (Curculionidae), *Phloeostiba lapponica* (Staphylinidae), *Spondylis buprestoides* (Cerambycidae), *Melanotus villosus* (Elateridae), and *Nicrophorus vespilloides* (Silphidae) (Supplementary Materials). Groups of dominant species in individual stands differed (Table 3). Only *Enicmus rugosus* reached the dominance index above 5% in five stands. Species, which were dominant (D > 5%) in at least three stands included *Phloeostiba lapponica* reported with a very high dominance index in the protected stand in the Torzym Forest District (UT3) and in two managed stands (MT7, MG6); *Spondylis buprestoides* dominant in managed stands as well as *Nicrophorus vespilloides* recorded as dominant only in managed stands. Although *Ampedus balteatus* was the most numerously represented among the captured insects, it was dominant only in one of the analysed stands (MD5).

Table 3. Dominant beetle species (D > 5%) captured in individual stands.

Species	Dominance Index in the Stand							
	UG1	**UG2**	**UT3**	**UD4**	**MD5**	**MG6**	**MT7**	**MC8**
Enicmus rugosus	16.5	16.1	7.1	-	-	5.6	6.5	-
Dryophthorus corticalis	9.0	-	-	-	-	-	-	-
Placusa tachyporoides	7.5	-	-	-	-	5.6	-	-
Orthotomicus longicollis	5.2	-	-	-	-	-	-	-
Cerylon ferrugineum	-	10.2	-	-	-	-	-	-
Platydema violacea	-	7.3	-	-	-	-	-	-
Plegaderus caesus	-	5.1	-	-	-	-	-	-
Spondylis buprestoides	-	5.1	-	-	6.4	10.2	-	-
Phloeostiba lapponica	-	-	34.9	-	-	12.1	10.9	-
Paromalus parallelepipedus	-	-	5.5	-	-	-	-	-
Placus atrata	-	-	5.5	-	-	-	-	-
Phloeonomus pusillus	-	-	-	8.4	-	-	-	-
Rhizophagus depressus	-	-	-	7.4	-	-	-	-
Tomicus piniperda	-	-	-	6.8	-	-	-	8.5
Ampedus balteatus	-	-	-	-	49.3	-	-	-
Nicrophorus vespilloides	-	-	-	-	-	13.9	28.2	9.6
Crypturgus hispidulus	-	-	-	-	-	12.9	-	-
Epuraea thoracica	-	-	-	-	-	7.4	8.7	-
Abraeus perpusillus	-	-	-	-	-	-	6.5	-
Salpingus ruficollis	-	-	-	-	-	-	6.5	-
Melanotus villosus	-	-	-	-	-	-	-	17.7
Cerylon impressum	-	-	-	-	-	-	-	5.3

Results of the analyses of Coleoptera assemblages indicate considerable similarity between the stands located the closest geographically (Figure 2). The assemblages form three basic agglomerations: 1. beetles found in unmanaged stands in the Gubin Forest District (UG1, UG2), 2. beetle assemblages in managed stands in the Gubin and Torzym Forest Districts together with the protected stand in the Torzym Forest District (MT7, UT3, MG6), and 3. beetle assemblages in the managed stand in the Jarocin Forest District and assemblages found in the Drawieński National Park (MC8, MD5, UD4). The assemblage of beetles found in stand MD5 is the most distant from all analysed.

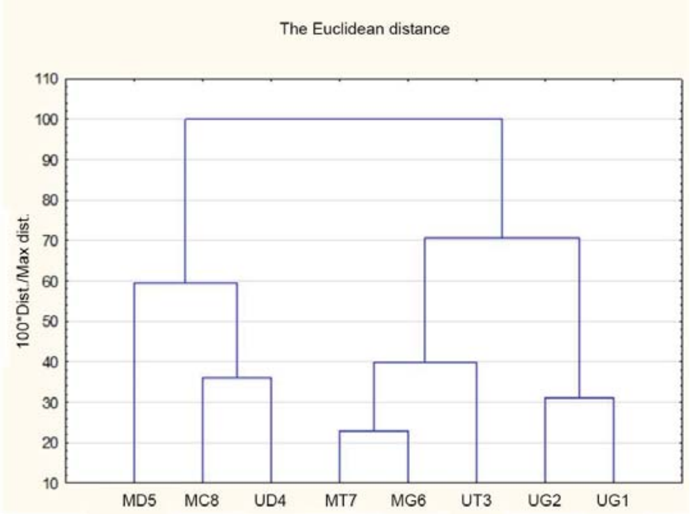


Figure 2. Dendrogram of similarity of species dominance in Coleoptera assemblages in managed (MD5, MG6, MT7, MC8) and unmanaged (UG1, UG2, UT3, UD4) pine stands in western Poland.

The percentage shares of individual trophic groups in the beetle assemblages differ in the analysed stands (Table 4).

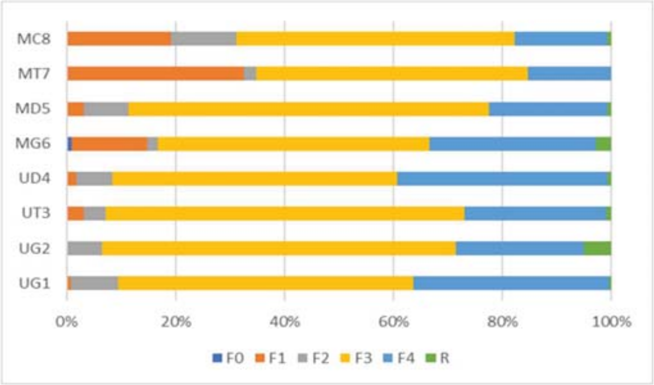
Table 4. Mean percentage shares of beetle species and individuals captured in managed and unmanaged pine stands in western Poland in terms of trophic groups.

Trophic Group	Unmanaged Stands		Managed Stands	
	Mean Share of Species in the Trophic Group (%)	**Mean Frequency of Individuals in the Trophic Group (%)**	**Mean Share of Species in the Trophic Group (%)**	**Mean Frequency of Individuals in the Trophic Group (%)**
Saproxylophagous	8.0 (±1.3)	5.7 (±4.1)	7.5 (±1.5)	15.5 (±20.0)
Xylophagous	16.3 (±3.7)	13.0 (±5.0)	20.8 (±4.9)	21.6 (±8.6)
Mycetophagous	18.8 (±3.9)	22.2 (±7.9)	17.7 (±6.1)	11.4 (±3.5)
Zoophagous	42.9 (±6.4)	52.5 (±6.2)	40.0 (±6.8)	31.6 (±9.8)
other	14.0 (±6.6)	6.6 (±3.2)	14.0 (±4.6)	20.0 (±11.5)

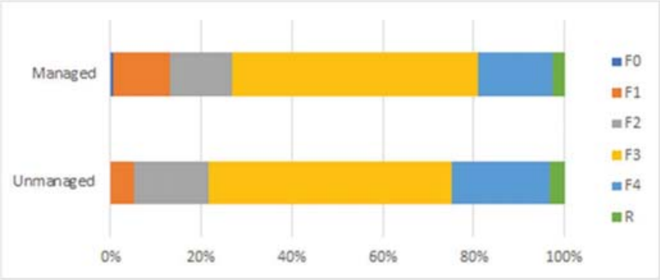
When comparing mean shares of species in the trophic groups in both stand management types, most identified species were zoophages. Species richness of xylophagous and mycetophagous beetles is similar. The lowest share was recorded for saproxylophagous species. In managed stands, a greater share of xylophagous species was reported, while unmanaged stands are generally characterised by a greater share of all trophic groups except for xylophages. The shares of species belonging to the other trophic groups are practically identical.

In unmanaged stands, the highest frequency was recorded for specimens of zoophagous species. The shares of xylophagous and mycetophagous beetles exceeded 10%, while the lowest values were found for saproxylophages and the other trophic groups. In managed stands, the highest number of individuals was also reported in the group of zoophagous specimens; however, their share was much lower than in unmanaged stands. The other trophic groups account for approx. 1/5 of all the individuals, while the frequency of xylophagous and mycetophagous beetles was high, considerably exceeding that in unmanaged stands.

The shares of individuals classified to the adopted fidelity classes varied in the investigated stands (Figure 3). This pertains particularly to the shares of individuals representing alien species in managed stands, which may even exceed 30% (the managed stand in the Torzym Forest District, MT7). Shares of individuals representing obligate characteristic species ranged from 15% to 39%. The highest share of these species was observed in unmanaged stands in the Gubin Forest District (UG1) and the Drawieński National Park (UD4) (Figure 3a).



(**a**)



(**b**)

Figure 3. Percentage shares of species in terms of fidelity classes in analysed managed and unmanaged pine stands in western Poland (**a**); percentage shares of species depending on fidelity classes in managed and unmanaged pine stands in western Poland (**b**).

The total number of species belonging to the four fidelity classes most strongly associated with the subcorticular habitat (R, F4, F3, F2) is higher in unmanaged stands compared to managed stands (Figure 3b). Occurrence of single relic species (R) was observed in almost all the stands, both unmanaged and managed, with the exception of MT7, where no species from that fidelity class were recorded. Selective characteristic species (F3) and alien species (F1) were more numerous in managed stands, whereas ubiquitous species (F0) had only a slight share in the assemblage (Figure 4).

Indices of species richness (Margalef's index) and species diversity (Shannon's index) in the investigated stands reached higher values in the group of unmanaged stands (Table 2).

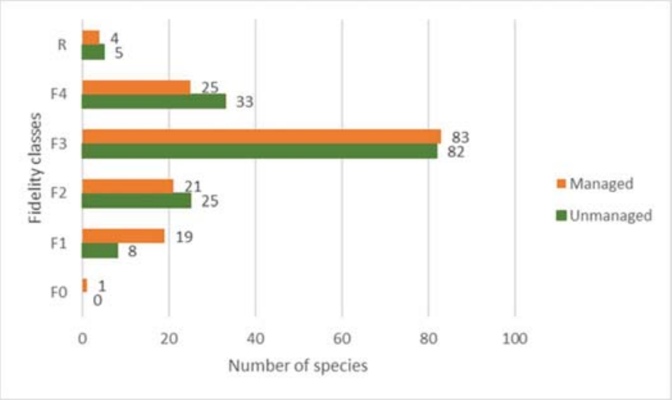


Figure 4. Numbers of species depending on fidelity classes in Coleoptera assemblages in managed and unmanaged pine stands in western Poland.

4. Discussion

Information on saproxylic beetles of pine coniferous forests in view of the area covered by these forests and their role in the forest management system in central Europe is not proportional. Data comes mainly from northern and central Europe [40,42,43]. In turn, studies in Poland were conducted primarily in the eastern part of the m which differs significantly in terms of climate from western Poland, which results in different habitats and plant communities [84]. Western Poland is under the influence of the Atlantic climate, and the pine forests form communities in the type of sub-Atlantic fresh forest—*Leucobryo-Pinetum*. Northeast Poland, on the other hand, is under the influence of a subcontinental climate, it is an area of pine forests of the *Peucedano-Pinetum* type [85]. This affects the length of the growing season, which may determine the distribution of some beetle species (including saproxylic beetles) [86].

The use of screen traps as the only trapping method was dictated by utilitarian goals. In managed stands, standing dead trees and broken tree crowns were the most common microbiotopes, where saproxylic species may occur. One trapping method, although assessed as very effective, narrows the scale of captured species and does not allow direct comparison of the results. The use of various types of traps (e.g., emergence, screen traps in different colours, etc.), dedicated to specific ecological groups of beetles, results in an increase in the number of species caught [61].

Species richness of saproxylic beetles in the investigated pine stands of western Poland differs from that observed in central and eastern parts of the country [50,52,53,80]. Studies conducted to date were frequently based on many methods of insect capture and observation, which considerably hinders comparability of the obtained results. Nevertheless, in relation to forest ecosystems considered natural, which often are treated as a model, to which research results on species diversity are referred, the number of recorded species was lower. In the Białowieża Forest, a total of 630 species were reported [53], while in mixed coniferous forests of the Kampinos Forest it was 433 species [51]. However, they are one of richest and best preserved forest ecosystems in this part of Europe. On the other hand, studies on the fauna of pine stumps showed 178 species [87], whereas assemblages of saproxylic beetles in boggy pine coniferous forests it was as few as 121 species [50].

As it was shown by the studies conducted in stands affected by hurricane and wild fires, a rapid increase in CWD resources does not cause an equally dynamic increase in species diversity [88,89]. Already in the 5th year after stand damage the diversity indices were more favourable for undamaged unmanaged stands with dead wood resources of 7.8–15 m^3/ha. For damaged stands, this index reached a similar value, with a slight advantage of unclear stands (mean CWD resources of 225.5 m^3/ha) over cleared stands

(94.5 m^3/ha) [50]. These observations show that for the maintenance of species diversity continuous access to dead wood is more important than its abundance. Natural self-thinning processes, which are less dynamic, facilitate full utilisation of microhabitats formed over the years. A broad spectrum of trees at various stages of dieback and wood decomposition distributed as a mosaic in the ecosystem promotes survival of individual species more effectively than a large amount of substrate of similar parameters. This was confirmed by extensive research conducted in France [24,90], showing a significant effect of not only CWD resources, but also the presence of den trees, diversity of arboreal fungi and openness of the habitat providing available light and an advantageous microclimate. Moreover, for saproxylic species developing in dying pine trees a significant role is played by the diameter of the stem or branches, the presence of bark, as well as height above the ground [47].

Presented results show the course of transformation of managed forests into forests with the dominant protection function focused on saproxylic insects. The reported observations confirm that the decision to cease removing dying and dead trees has an advantageous effect on species diversity. Analysis of Coleoptera assemblages showed that sites located closest geographically are most similar (Figure 2), while higher values of diversity and species richness indices within agglomerations were recorded for unmanaged forests (Table 2).

In unmanaged forests, the shares and numbers of obligate characteristic and characteristic species were greater. It is also of interest that the shares of species being strictly saproxylic in both stand categories were comparable. Thus, how can the relatively high share of this trophic group be explained in managed stands, where CWD resources are much smaller?

Such a situation may be explained by the specific character of individual forest ecosystems, determined by their history and intensity of management operations affecting the continuity of natural processes of tree dieback and accumulation of dead wood with all its specific microhabitats. Thus, utilisation of the current abundance of dead wood as an indicator feature of biodiversity may lead to a bias. This parameter does not take into consideration the size and degree of decomposition of dead wood, which determine the occurrence of several specific/stenotopic beetle species [91]. It is obvious that in the case of the accumulation of large CWD amounts a wider spectrum of microhabitats is more probable, although some cases suggest that it is not always the rule [28].

Observed high values of diversity and species richness indices at limited CWD resources in managed stands may be explained as a certain island effect [92]. Dying trees constitute a biotope for several species, while in sterile managed forest they may function as hotspots in a microscale.

Probably a reduced number of dying and dead trees of a given species within a certain range may cause the accumulation of specific fauna, and thus increased species richness on individual trees.

In stands not covered by sanitation regime, in which tree dieback is a common process taking place over larger areas, with an increase in CWD resources the concentration of fauna is observed, which in turn may result in a decrease in the values of diversities and species richness indices.

Posing questions on the volume of dead wood resources as the only factor determining insect assemblages associated with this habitat is an oversimplification of the complex biocenotic associations taking place during tree dieback and wood decomposition between several organisms, among which beetles constitute a considerable group. Assessment and strategies to preserve biodiversity need to take into consideration not only α-diversity, but also β- and γ-diversity [93].

A characteristic differentiating the investigated pine stands in western Poland is connected with the share of species dominant on the local scale. *Ampedus balteatus* (Elateridae) is a superdominant, found at a very high frequency in stand MD5. *Ampedus balteatus* is a zoophagous species developing in pine stumps [80,94], an obligatory saproxylic species [95].

It was captured in traps with retusol and sulcatol in southern Finland [96] and in fermental traps [40], while in studies on subcorticular beetles in natural and managed forests with a share of pine and spruce it was recorded only in managed forests [41]. In hurricane-affected stands in the Piska Forest it was co-dominant and found commonly, although its population size did not increase under the influence of the accumulation of hurricane-damaged timber [50]. Its presence in the investigated stands needs thus to be associated with the intensive process of tree dieback and increased presence of organisms constituting its food source.

A list of species dominant in the analysed stands indicates that most identified assemblages have different dominant species. Only *Enicmus rugosus* (Latridiidae) and *Phloeostiba lapponica* (Staphylinidae) were found with the dominant share in three or more assemblages (Table 3). The minute brown scavenger beetle *E. rugosus* is a mycetophagous species, with an extensive distribution in Europe, obligatorily associated with hardwood CWD as well as softwood CWD of larger diameters [95,97]. It does not show a close association to a specific type of forest habitat [95,98], colonising wood dominated by slime mould Eumycetozoa [99]. In many forest habitats it is found as a dominant [79,87,100], colonising tall stumps [87,101], tree hollows [102], and lying boles (trunks) [103] as well as old, solitary oak trees [104].

Another species recorded with high frequency was *Phloeostiba lapponica*, a holarctic species [105]. It is a small, predatory subcorticular beetle staying under tree bark, typically in bark beetle galleries on coniferous trees. In eastern Finland it was reported with high frequency on burnt trees, in habitats subjected to controlled burns to renaturalise managed boreal forests [106].

It also needs to be stressed here that groups of dominant species, which are unique rather than common indicate high specificity of each of the assemblages. A comparison of the dominant species systems between the group of managed vs. unmanaged stands identifies dominant species for each category of stands. In unmanaged stands the most numerous were *Enicmus rugosus* and *Phloeostiba lapponica*, while in managed stands it was *Ampedus balteatus*, *Nicrophorus vespilloides*, *Melanotus villosus,* and *Tomicus piniperda.* The presence of large subcorticular predators (Ampedus, Melanotus) accompanying cambiophages (Tomicus) may indicate the initiated processes of tree dieback, in which the first succession stages are taken by cambiophages of large body size (part of Scolytinae, Cerambycidae, Buprestridae) [107,108].

When analysing the frequency of families (Supplementary Materials), it is noticed that representatives of families Bothrideridae, Dryophthoridae, Eucnemidae, Trogossitidae, and Zopheridae occur only in unmanaged stands. However, in these stands with a greater frequency than in managed stands, species from the following families occur: Ciidae, Melandryidae, Monotomidae, and Scydmaenidae. They are representatives of typically saproxylic families [6]. Only species of Silvanidae have been found in the managed stands, and bark beetles (Curculionidae) are more numerous than in unmanaged stands.

Research results indicate that the leaving naturally dying trees in pine stands results in a slow accumulation of CWD resources and influences directions of succession for assemblages of subcorticular and saproxylic beetles.

This study may illustrate an example of the transition of a stand from the managed type to the unmanaged type as well as changes in fauna with an increase in CWD resources and directions of succession for beetle assemblages associated with processes of dieback and dead wood accumulation.

5. Conclusions

These analyses showed differences between assemblages of saproxylic beetles found in managed and unmanaged stands. These differences are smaller in terms of the number of species, while they are higher in terms of the frequency of individuals in the identified ecological and trophic groups of beetles. However, the number of saproxylic species greater by eight percentage points recorded in unmanaged stands shows that the management method affects species diversity in this group of insects.

Clustering being the result of analysis of similarity between the analysed stands indicates a significant role of the geographical location of these sites. The closer they were located to one another, the greater the similarity despite their different management methods.

Based on the identified objects, no relationship was confirmed between the species diversity indices and growing CWD resources; however, a trend may be observed for the organisation of *Coleoptera* assemblages towards an increase in the share of zoophagous, saproxylic, mycetobiontic, and relic species. Managed stands with the implemented sanitation cutting regime, in which trees naturally dying are removed, are characterised by lower species diversity indices and a greater share of xylophages.

Simulated dependencies between dead wood resources and species richness and diversity indices in pine coniferous forest with different management regimes may indicate that at a comparable level of biodiversity in managed stands trends are evident towards a grouping (concentration) of fauna dependent on dead trees, while in unmanaged stands with an increase in CWD resources saproxylic beetles are scattered.

Pine coniferous forests of central Europe may serve a considerable role in the system of protection for saproxylic beetles. These forests are habitats transformed by traditional forestry towards monocultures of limited diversity and low dead wood resources, particularly large-sized wood. Nevertheless, this habitat is colonised by numerous saproxylic species, in which numbers of individuals and concentration are directly dependent on the presence of coarse woody debris. Thus, active protection of the forest habitat needs to be focused on increasing this parameter through protection of natural and spontaneous phenomena of tree dieback.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12111558/s1. The alphabetical list of beetle species caught on dead trees in protected and commercial pine stands of western Poland in 2013–2014.

Author Contributions: Concept of the study, methodological assumptions: A.M., A.Ł., R.K.; field inventories and desk studies (identification of experimental sites, collection and segregation of beetles): A.M., A.Ł., R.K., R.W.; identification of beetles: A.M., R.W.; elaboration of results: A.M., R.W., R.K.; editing of manuscript: A.M., R.W.; measurement of dead wood resources: R.J., M.T.; identification of fungal assemblages, members of research team: H.K., P.Ł., description of dynamic trends in pine stands: J.S., analysis of economic values, member of research team: K.A., project manager: A.Ł. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data is included in the text and in the Supplementary Materials. Entomological collections are available at the Department of Forest Entomology and Phytopathology, Poznan University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznań, Poland.

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Article

Ambrosia Beetles Prefer Closed Canopies: A Case Study in Oak Forests in Central Europe

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Abstract: Research Highlights: The percentage of canopy closure was found to be the main factor associated with ambrosia beetle abundance and species richness. The latter two variables increased as canopy closure increased, probably because a high percentage of canopy closure provides a stable and humid environment suitable for the growth of ambrosia fungi. Objectives: Oak is a common host tree for ambrosia beetles (Coleoptera: Curculionidae: Scolytinae), which have independently evolved a nutritional mutualism with fungi. We suspected that ambrosia beetles might have specific habitat preferences that are different from those of other saproxylic beetles and that reflect the specific habitat preferences of their food, i.e., ambrosia fungi. Methods: We assessed ambrosia beetle abundance with ethanol-lured traps in five old-growth oak dominated forests and five managed oak dominated forests (one trap per forest) during the vegetation period in 2020. We determined whether ambrosia beetle abundance and species richness depend on forest type (managed vs. unmanaged), degree of canopy closure, abundance of oak trees, abundance of coarse deadwood, and abundance of dead oak branches. Results: In total, 4137 individuals of six species of ambrosia beetles associated with oaks were captured. The native ambrosia beetle *Anisandrus dispar* represented the majority of trapped ambrosia bark beetles. *A. dispar* along with another ambrosia beetle, *Xyleborinus saxesenii*, represented 99% of all captured beetles. Conclusions: In addition to canopy closure, the abundance of oak trees and the abundance of dead oak branches were significantly associated with ambrosia beetle abundance and species richness. The abundance of *A. dispar* was mainly correlated with dead oak branch abundance and the degree of canopy closure, whereas the abundances of *X. saxesenii* and of the invasive species *Xyleborinus attenuatus* and *Cyclorhipidion bodoanum* were mainly correlated with the net area occupied by oak trees.

Keywords: *Anisandrus dispar*; *Cyclorhipidion bodoanum*; deadwood; invasive species; *Xyleborus saxesenii*; *Xyleborinus attenuatus*; *Xylosandrus germanus*; Scolytinae; Quercus

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1. Introduction

European temperate oak woodlands have a rich and unique biodiversity, which can be mainly attributed to the life history traits and structural characteristics of the oak trees *Quercus robur* and *Q. petraea* [1–3]. In Central Europe, oak trees are components of temperate broadleaf and mixed forests. Oak is a common host tree for ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) [4,5].

The ambrosia beetles have independently evolved a nutritional mutualism with fungi [6]. Most species of ambrosia beetles depend on recently dead or stressed woody plants in which the beetles bore their tunnel systems ("galleries"). In the galleries, ambrosia beetles actively farm one or several fungal mutualists, which serve as their essential food source [6]. Some species of ambrosia beetles are among the most damaging forest pests, and species of quarantine significance are frequently moved intercontinentally [7,8].

Because oak trees attacked by other bark beetles provide suitable breeding substrates and other resources for ambrosia beetles, the volume of oaks and the area occupied by oaks under attack by other bark beetles are likely to affect the abundance of ambrosia beetles in a region [9]. Unlike other bark beetles that can fly tens of kilometers [10], ambrosia beetles can fly only up to about a hundred meters to a few kilometers [11,12]. The short dispersal distance of ambrosia beetles also suggests that the abundance of ambrosia beetles in an oak forest should be affected by the area that is occupied by oak.

Many studies in boreal and temperate forests have indicated that insect diversity increases as stands become more open because of higher temperatures and other changes in the microclimate [13–16]. Sun exposure was found to be the most important factor affecting the composition of buprestids and cerambycids in oak forests [17]. There has been sparse research on the influence of the canopy closure on the occurrence of scolytids, but some research suggests that the effects of canopy closure may differ among scolytid species. For example, *Scolytus intricatus* Ratzeburg, 1837 prefers oaks with a high canopy closure [18], but *Scolytus mali* Bechstein, 1805 prefers orchards with open canopies [19]. Similarly, the position of trap in the forest (edge vs. interior) also generally does not affect the occurrence of bark beetles [20–22], but it does affect the occurrence of some species. The scolytids *Hylurgops palliatus* Gyllenhal, 1813 and *H. glabratus* Zetterstedt, 1828, for example, require the shaded environment of the forest interior for feeding and are found in higher numbers in the forest interior than at the forest edge [23]. Two ambrosia beetles showed opposite patterns patterns: *Xylosandrus crassiusculus* Motschulsky, 1866 is more common at the forest edge, while *Xyleborinus andrewesi* Blandford, 1896 is more common in the forest interior [24]. Similarly, the bark beetle *Hylesinus taranio* Danthoine, 1788 prefers canopy closure at the forest edge [25]. We do not know of research focusing on the effect of canopy closure on ambrosia beetles.

Based on our unpublished observations, however, we suspect that ambrosia beetles are not primarily dependent on the sun exposure provided by an open canopy. On the contrary, as wet and warm conditions are important for the growth of their symbiotic fungi [26], ambrosia beetles are likely to be more abundant in wetter and warmer localities [27,28]. Localities are likely to be wetter and to have a more stable microclimate if the canopy is substantially closed rather than open [29].

The biodiversity of phloexylophagous insects is greater in old-growth oak stands than in many other kinds of forest stands, because old-growth oak stands have more deadwood, including dry branches in treetops [30,31]. For ambrosia species, this dependence was confirmed only in beech stands in that the abundance of ambrosia beetles was found to be higher in unmanaged than in managed stands [32].

In the current study, we tested the hypotheses that ambrosia beetle occurrence will depend on the degree of canopy closure, the abundance of oak trees, the abundance of deadwood, and abundance of dead oak branches. We also tested the hypothesis that ambrosia beetle occurrence is greater in unmanaged oak forests than in commercial forests [31,33,34].

2. Materials and Methods

2.1. Study Plots

The study was conducted in the biogeographically isolated area (the Chebsko-sokolovský bioregion) of "Western European broadleaf forests" in the western Czech Republic (Figure 1) (https://en.wikipedia.org/wiki/Western_European_broadleaf_forests, accessed on 12 August 2021). At lower altitudes in this area, "Western European broadleaf forest" is the main forest type. The natural vegetation of the bioregion consists mainly of acidophilic oaks (*Quercion roboris* Malcuit, 1929), but only 6% of the region is currently occupied by oaks. These are mostly in commercial oak forests, and old-growth oak forests have survived in only a few localities [35].

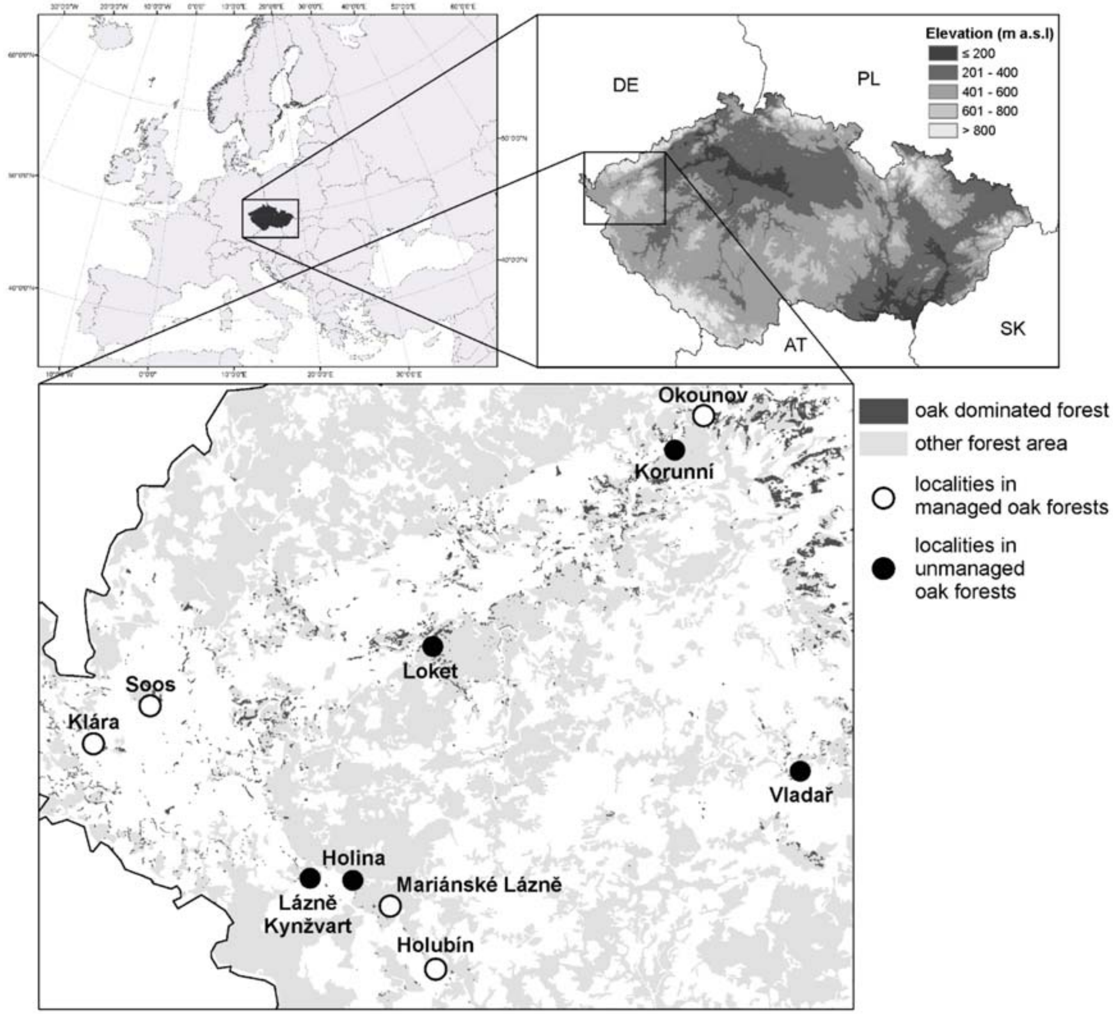

Figure 1. Locations of unmanaged oak dominated forests and managed oak dominated forests in western Bohemia where ambrosia beetle abundance and species richness were determined.

For assessing ambrosia beetle abundance by trapping, we selected oak dominated forests in which oaks represented > 60% of the trees (Only in study plot Soos the oak representation is 40%, and the rest of forest is cover by birch *Betullus* sp.) and that were >80 years old and ≥1 ha. The forests were at altitudes between 400–700 m a.s.l. (Table 1, Figure 1). The study plot is located in the mesophytic zone, which is characterized by an average annual rainfall of around 550–700 mm and an average annual temperature of 7.5 °C. Orographically, the study plots are located on flat land, with only the Korunní and Loket plots (northwest orientation) and the Vladař plot (south orientation) being on a steep slope.

Table 1. Characteristics of the studied oak dominated forests.

Study Plot	Longitude; Latitude	Altitude (m a.s.l.)	Age	Forest Type	DBH (cm)	Net Area Occupied by Oaks (ha)	Volume of Oak Wood (m^3/ha)	Volume of of Coarse Deadwood (m^3/10 m^2)	Abundance of Dead Oak Branches (No. per Tree)	Canopy Closure (%)	Distance from Stand Boundary (m)
Vladař	50°4′31′′ N, 13°12′33′′ E	605	130	Unmanaged	40	5.2	264	30	3	66	100
Mariánské Lázně	49°58′50′′ N, 12°41′40′′ E	700	185	Managed	58	0.5	369	0	0.1	48	20
Soos	50°8′51′′ N, 12°24′19′′ E	440	80	Managed	30	0.3	131	20	0.2	31	20
Okounov	50°21′45′′ N, 13°6′28′′ E	440	80	Managed	40	0.5	58	5	0	35	10
Korunní	50°20′9′′ N, 13°4′11′′ E	500	150	Unmanaged	50	1.5	262	20	1	66	50
Klára	50°7′7′′ N, 12°19′59′′ E	440	90	Managed	40	0.3	215	20	0.2	45	20
Holubín	49°55′44′′ N, 12°44′53′′ E	615	90	Managed	35	1.8	255	0	0.33	63	20
Loket	50°11′13′′ N, 12°45′33′′ E	410	110	Unmanaged	20	2.5	123	15	1	64	40
Holina	50°0′8′′ N, 12°38′58′′ E	700	180	Unmanaged	50	0.5	361	50	5	68	30
Lázně Kynžvart	50°0′19′′ N, 12°35′48′′ E	565	200	Unmanaged	120	0.7	245	5	1	66	10

Quercus robur was dominant in all localities except Vladař and Loket (see Figure 2), where both *Q. petraea* and *Q. robur* grow but *Q. petraea* dominates.

Figure 2. Views of managed 80-year-old oak dominated forests at Holubín (**a**) and unmanaged oak forests at Kynžvart (**b**) and Vladař (**c**).

2.2. Traps and Lures

To estimate the abundance and species richness of ambrosia beetles [4,36], one trap baited with ethanol was placed in the center of each study plot. Ethanol was released from a plastic-vial dispenser (ca. 250 mg·day^{-1}). These dispensers were made of polyethylene with foam and were 5 cm in diameter and 5 cm high. Each dispenser was placed in a Theysohn® trap that was located ca. 1.3 m above the ground and that faced the main wind direction. The traps were emptied, and the ethanol was replaced every 2 weeks from the beginning of April to the end of August in 2020. All trapped insects were preserved in 70% ethanol.

The insects were identified by the second author, who used Pfeffer's key [37]. Dr. Miloš Knížek (Prague) confirmed the identification of *C. bodoanum*.

2.3. Environmental Variables

Forest type. We recognized two types of forests according to management. Mature-managed forests were oak dominated forests between 80 and 120 years old. All trees in each managed forest were the same age and were very homogenous; cut stumps were abundant. Forests in this category had reached maturity (i.e., had attained their maximum annual increase in volume) and represented typical state-owned forests. The volume of deadwood and dead branches was low (Table 1). These forests were last managed ten years ago.

Most of the oldest trees in unmanaged forests were >120 years old, and the forests had not been managed for the last 70 years. The forests in this category represented the closest-to-natural forests that remain in western Bohemia. The only signs of human interference were a few scattered stumps from past selective cuttings. Unlike the managed forests, the unmanaged forests included trees of all ages including small areas with young trees. The volume of deadwood and dead branches was high (Table 1). These forests were last managed more than fifty years ago. The unmanaged forest at Kynžvart (Figure 2b) had been modified into a park with grasslands, but more than 60% of the area was covered with trees, which grew in large unbroken patches. Because the structure of this stand was otherwise similar to old-growth stands, we included this stand in the unmanaged forest category (Figure 2b). In contrast, the forest at Soos, although located in a protected area, was classified as a managed forest because it was a homogeneous stand that had been planted in a meadow.

Net area occupied by oaks: The net area was calculated as the total area of the stand multiplied by the tree density and the percentage represented by oak. Data were obtained from the regional inventory of forests.

Volume of oak wood: Data for the volume of oak trees (m^3/ha) in the oak dominated forests were obtained from the forest management plan, which contained detailed data for all forest stands.

Volume of coarse deadwood: Deadwood volume was quantified in five areas of 10 m^2/area. The diameters and lengths of the dead trees and dead branches were measured manually.

Abundance of dead oak branches: Numbers of large dead branches were determined on 10 oak trees along a transect running through each study area; the values were subsequently expressed as the mean number of dead branches per tree. The transects were located in the central part of each study area (one transect per study area) and were about 50-m long. Dead oak branches included all standing and lying dead wood with a diameter greater than 7 cm and with a hard consistency based on resistance to finger pressure.

Canopy closure: Canopy closure at each study area was assessed by photographing the sky from the ground straight up. The sky was photographed on ten places with distances of 20 m. The photographed surface was ca 200 m^2. The photographs were analysed for the percentages of white (sky) and black (canopy) using ImageJ software (v.1.47). The percentage of the area of the sky that was black in the photographs was considered equivalent to the percentage of canopy closure.

Distance from stand boundary: The distance of each trap from the nearest boundary of the studied oak dominated forest stand (not the forest edge, stand means a homogeneous unit of the spatial distribution of the forest) was measured to the nearest meter.

Values of all variables are presented in Table 1.

2.4. Statistical Analysis

The importance of forest stand characteristics for ambrosia beetle abundance and species richness was evaluated by implementing a random forest algorithm using conditional inference trees as base learners provided in the party package (function cforest, 10,000 trees generated) in R 4.0.2 software (The R Foundation, Vienna, Austria). This method was used because it is a highly effective for evaluating the importance of explanatory variables, it can manage different types of variables, and is robust with respect to the multicollinearity of variables [38]. Because certain tested explanatory variables exhibited multicollinearity in our dataset, a conditional computation of the importance was performed (option conditional = TRUE). In addition, an unbiased random forest model was constructed (option control = cforest_unbiased), because the tested predictors were both quantitative and categorical variables. The response variable in the models was represented by the number of ambrosia beetle individuals and species in specific samples (sample =

beetles captured in one trap during per 2-week-period deployment as described earlier in the Methods). The statistical significance ($\alpha = 0.05$) of each explanatory variable was evaluated using the permutation-based attribute selection algorithm provided in the Boruta package. Finally, the marginal effect of the selected significant variables on the number of trapped ambrosia beetle individuals and species was visualised with a partial dependence plot using the package pdp (functions partial followed by plotPartial).

Ordination analyses of the relationship between ambrosia beetle abundance (i.e., species composition of their assemblages) and forest stand characteristics were performed in Canoco 5 (Wageningen University & Research, Wageningen, Netherlands). Based on preliminary analysis of the data (gradient length of response data was 2.2 SD units), redundancy analysis (RDA) was used. Data on the abundance of species were log-transformed and centered by species. After a global Monte-Carlo permutation test (10,000 permutations) of a full model (including all of the available explanatory variables) confirmed the overall significance of the relationship between response and explanatory variables (pseudo-$F = 4.2$, $p = 0.001$), a forward selection of explanatory variables was performed to identify the forest stand characteristics most closely associated with ambrosia beetle abundance and species richness.

3. Results

A total of 4179 individuals and 15 species of scolytid beetles were captured in the traps that had been deployed in 10 localities with oak trees in the western Czech Republic. Among the 15 species, six (represented by 4137 individuals) were ambrosia beetle species related to oak (Appendix A, Figure 3). *Anisandrus dispar* was the most abundant beetle trapped with an average of 40.0 individuals per sample. The 3520 specimens of *A. dispar* represented 84% of the trapped beetles (Appendix B). *Xyleborinus saxesenii* Ratzeburg, 1837 was the second most abundant species with an average of 6.5 individuals per sample. The 576 specimens of *X. saxesenii* represented 14% of the trapped beetles. The other four species were represented by fewer than 20 trapped specimens, i.e., they represented less than 1% of all trapped specimens (Appendix A, Figure 3).

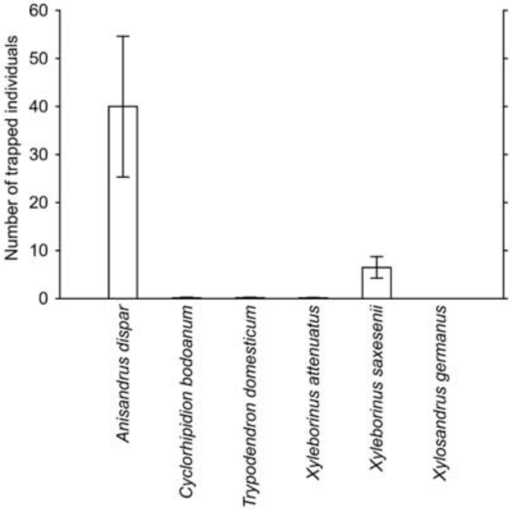

Figure 3. Number (mean ± SE) of individuals of ambrosia beetle species captured per sample. Each sample represented the beetles captured in one trap during 2-week period.

The number of ambrosia beetle individuals caught in the traps was significantly related to date of sampling, the percentage of canopy closure, and the abundance of dead oak branches per tree (Figure 4a). The number of ambrosia beetles trapped increased with the percentage of canopy closure, but the increase was considerable only when canopy closure exceeded 45% (Figure 4b). The number of beetles caught also increased with the number of dead oak branches per tree, but the relationship plateaued with four dead oak branches per tree (Figure 4c).

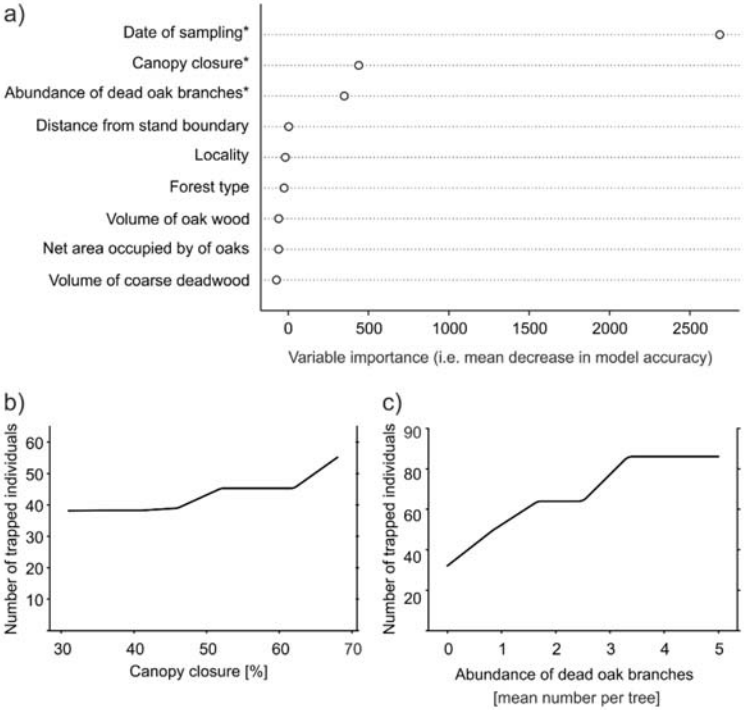

Figure 4. Results of random forest regression of number of ambrosia beetle individuals caught in traps as the response variable: (**a**) variable importance plot (based on the decrease of mean model accuracy with omission of the variable) showing the importance of particular variables for the number of ambrosia beetle individuals caught in the traps. Variables with a significant effect ($p < 0.05$) are denoted with an asterisk (*); (**b**,**c**) partial dependence plots showing the marginal effect of selected significant explanatory variables on the mean number of ambrosia beetle individuals trapped during the eight 2-week periods from April to August.

The number of ambrosia beetle species trapped was most strongly associated with the date of sampling (see Appendix B) and the percentage of canopy closure. Volume of oak wood and abundancy of dead oak branches were also associated with the number of ambrosia beetle species, whereas the associations with study plot and net area occupied by oaks were weak (even if statistically significant) (Figure 5a). The numbers of ambrosia beetle species trapped increased slightly with percentage of canopy closure, volume of oak wood, abundance of dead oak branches, and net area occupied by oaks (Figure 5b–e). The resulting curves were more or less sigmoidal, with most of the increase in the number of trapped species restricted to a narrow interval of explanatory variable values. This interval was between 40% and 50% for canopy closure; 200 and 250 m^3 for volume of oak wood; 2–3 for number of dead oak branches per tree; and 1.0–2.5 ha for the net area occupied by oaks.

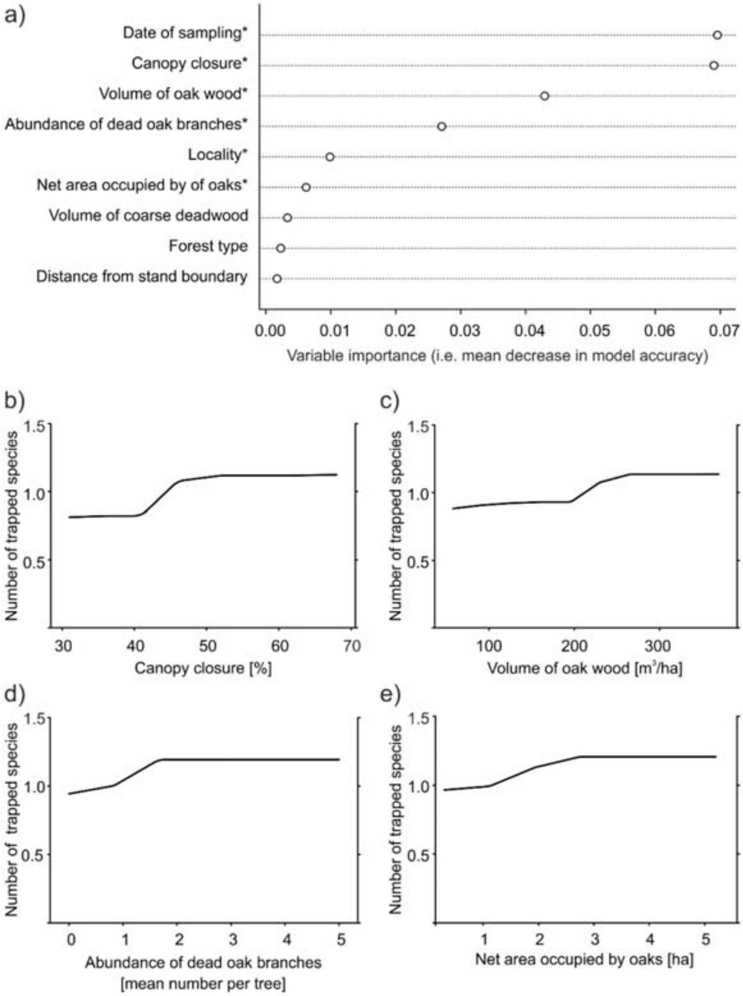

Figure 5. Results of random forest regression of the mean number of ambrosia beetle species captured per trap per 2-week period from April to August as the response variable: (**a**) plot of variable importance (based on the decrease of mean model accuracy with omission of the variable). Variables with a significant effect ($p < 0.05$) are denoted with asterisk (*); (**b**–**e**) partial dependence plots showing the marginal effect of the indicated explanatory variables on the mean number of ambrosia beetle individuals trapped during the eight 2-week periods from April to August.

The partial RDA analysis with the date of sampling treated as a covariable confirmed the significant associations between the studied explanatory variables and the occurrence and the numbers of ambrosia beetle individuals and species. Forward selection of explanatory variables indicated that two explanatory variables had significant effects: canopy closure and net area occupied by oaks (Table 2). Canopy closure had by far the highest explanatory power followed by net area occupied by oaks and the abundance of dead oak branches. The model including these three explanatory variables explained 24.4% of the variability in species occurrence and the numbers of ambrosia beetle individuals and species (Table 2).

Table 2. Results of the partial redundancy analysis forward selection of the percentage of variability in ambrosia species occurrence explained by the indicated forest stand variables. Conditional effects of the explanatory variables are shown. Effect of the date of trap deployment was removed by considering it as a covariable. The upper three explanatory variables highlighted in bold were included in the model based on the results of the forward selection process. Although it was not statistically significant, the abundance of dead oak branches was included in the model because it helped explain the variability in the occurrence of the species.

Forest Stand Variable	Explained Variability in Species Occurrence (%)	Pseudo-*F*	*p*
Canopy closure	**16.5**	**15.6**	**0.002**
Net are occupied by oaks	**6.6**	**6.7**	**0.002**
Abundance of dead oak branches	**1.3**	**1.3**	**0.234**
Volume of coarse deadwood	1.0	1.0	0.360
Distance from forest stand boundary	0.8	1.0	0.368
Forest type	0.4	0.5	0.652
Volume of oak wood	0.2	0.2	0.834

The RDA analysis revealed several associations between the abundance of ambrosia beetles and measured variables. The abundance of *A. dispar* was positively correlated with canopy closure and the abundance of dead oak branches. The abundances of *X. saxesenii* and the invasive species *X. attenuatus* and *C. bodoanum* were positively correlated with the net area occupied by oaks. The abundances of *T. domesticum* and *X. germanus* were positively but weakly correlated with the abundance of dead oak branches (Figure 6).

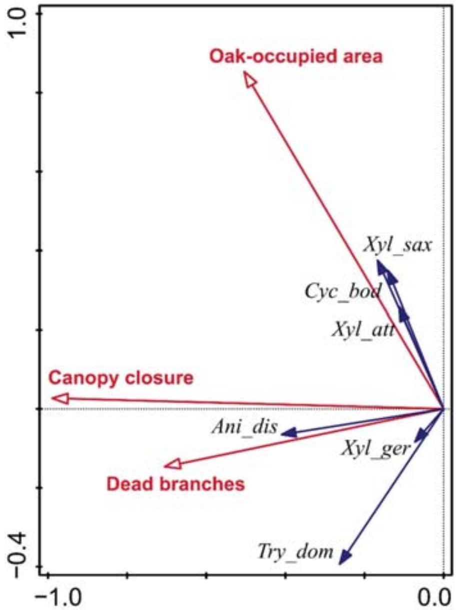

Figure 6. Results of redundancy analysis (RDA) of the relationship between presence of the ambrosia beetle species (blue arrows) and the most important characteristics of forest stand (red arrows). The species and the forest stand characteristics have been abbreviated to simplify the plot. The shown projection of the 1st and the 2nd axes represents 23.8% of the variability in species occurrence. Effect of the date of trap deployment was removed by considering it a covariable (*Ani_dis—Anisandrus dispar*, *Cyc_bod—Cyclorhipidion bodoanum*, *Try_dom—Trypodendron domesticum*, *Xyl_att—Xyleborinus attenuatus*, *Xyl_ger—Xylosandrus germanus*, *Xyl_sax—Xyleborinus saxesenii*).

4. Discussion

Anisandrus dispar and *X. saxesenii* were the most abundant ambrosia beetles detected in this study in western Bohemia (the Czech Republic) and are also very abundant in many other countries [9,39–42]. *Anisandrus dispar* is recognized as a serious pest of fruit and hazelnut trees [43]. It tends to infest trees that have been weakened by biotic and/or abiotic factors [44]. *X. saxesenii* is rarely considered to be a pest [43].

Anisandrus dispar is probably considered to be a pest more often than *X. saxesenii* because it can develop on thinner branches [44]. It is therefore able to attack hazelnut and fruit trees [43,44], which have thinner branches than forest trees. Given the high abundance of *A. dispar* in the current study, one would suppose that this species could cause substantial damage in the region, but such damage occurs only rarely and only on trees with thin stems [45]. Even in regions where *A. dispar* damages fruit trees, the damage it causes to oaks and beeches is insignificant [46], although it has been associated with oak damage [47].

Unlike *A. dispar* females, *X. saxesenii* females directly bore into tree trunks and form a radial entry tunnel [48]. *Xyleborinus saxesenii* females therefore require relatively thick branches and find relatively few resources in forests.

Trypodendron domesticum was the third most abundant species, but it was much less abundant than *A. dispar* and *X. saxesenii*. *Trypodendron* spp. attack the surfaces of tree trunks and thicker branches [37]. A high availability of suitable breeding substrate (e.g., wind-damaged or highly stressed trees) at the forest-stand scale seems to enhance *T. domesticum* population densities and attack rates, e.g., [49] break of bark beetles on spruce has resulted in the increased harvesting of spruce and the suspension of deciduous forest harvesting. As a result, suitable host trees are scarce in oak forests, and the abundance of *T. domesticum* has been low see also [50].

We found only a few individuals of the three species of invasive ambrosia beetles, i.e., *C. bodoanum*, *X. germanus*, and *X. attenuatus*. *Cyclorhipidion bodoanum* and *X. germanus* have recently spread from the west into the Czech Republic, and their abundance remains low [51,52]. In places where both *X. germanus* and *C. bodoanum* have established, however, they are the most abundant Scolytinae species [9,39,40,53].

The introduced ambrosia beetles are considered pests in Europe [54,55]. They can detect stress-induced ethanol emissions from weakened oak trees and can rapidly colonize those trees [56]. Once a forest begins to decline, trees lose vigor, which increases their susceptibility to secondary pests and pathogens [57–61]. If the abundances of invasive species increase overtime, which is likely, these invasive species are likely to contribute to the mortality of trees in weakened oak forests.

The number of ambrosia beetle individuals as well as ambrosia beetle species trapped in the traps was significantly related to date of sampling because of phenology beetles in our study (Figures 4 and 5). The time distribution of the two most abundant species (Appendix B) is in accordance with known seasonal flight activity of these two species [62–64]. The lower numbers of specimens caught in the second half of May are related to the rainy weather.

In this study, we found that the abundance of ambrosia beetles was significantly associated with the percentage of canopy closure and the abundance of dead oak branches (Figure 4). Rather than reflecting the preferences of all species of ambrosia beetles, these associations might mainly reflect the preferences of *A. dispar*, the dominant species in our study (Figure 3). On the other hand, increases in these two variables also increased the number of ambrosia beetle species trapped (Figure 5), suggesting that the percentage of canopy closure and the abundance of dead oak branches may affect species in addition to *A. dispar*. In support of that possibility, the abundance of *T. domesticum* was also positively correlated with the abundance of dead oak branches (there is a positive relationship also in *X. germanus*, but we have trapped only one specimen) (Figure 6). A high percentage of canopy closure is an indication of a stable and humid environment that is suitable for the growth of ambrosia fungi [65,66]. Because ambrosia bark beetles require these fungi as a food source for development, both the fungi and the beetles are more frequent in wetter and warmer localities than in drier and colder localities [26–28]. In addition, ambrosia beetles generally prefer to inhabit the lower parts of tree canopies, such that most bark beetles are caught in traps at a height of 35–200 cm [36,67–71].

The volume of oak wood and the net area occupied by oaks represent the quantity and distribution of ambrosia beetle hosts. Ambrosia and other bark beetles are dependent on ephemeral and generally scattered breeding substrates [72,73], and it is therefore reasonable that the abundance of ambrosia beetle individuals and species at a site will increase with the concentration of host trees (Figures 5 and 6). The abundance of scolytids increases with resource availability [9,74]. In addition, the abundances of the invasive ambrosia beetles *X. attenuatus* and *C. bodoanum* were previously found to be positively correlated with the net area occupied by oaks, because both of these species live in oaks and other deciduous trees [40,49,50,75].

The number of species of ambrosia beetles trapped was significantly affected by study plot (Appendix A). On the other hand, the distance from the stand boundary had no effect on either the species spectrum or the number of captured beetles, which shows that a single trap was sufficient for monitoring the abundance of ambrosia beetle species and individuals at a study plot. This is reasonable because the beetles are lured to the traps by the bait, which was ethanol in the current study. Although not well studied, the population dynamics of ambrosia beetles are probably similar to those of other scolytids. Most individuals that hatched at a given site will probably tend to develop at the same site, but some individuals will disperse to search for new sites with suitable resources [73,76,77].

Although the number of ambrosia individuals trapped was not significantly associated with oak forest type (managed vs. unmanaged; Figure 4a), the abundance of many species was higher in the unmanaged forests than in the managed forests (Appendix A). We therefore cannot draw clear conclusions from these results. The unmanaged forests in the current study were abandoned coppicing forests in three cases (Vladař, Korunní, and Loket), a remnant of an old-growth forest that had been converted into a park in one case (Mariánské Lázně), and a reserve that resembled a virgin forest in only one case (Korunní) (Figure 1). We also captured many beetles at one managed site (Holubín) (Appendix A), which may help explain why the number of beetles captured was not significantly lower in the managed than in the unmanaged sites. Undisturbed, old-growth primary forests are generally considered to support high species richness [78], but species richness for some arthropod assemblages did not differ between primary forests and secondary or degraded (logged) forests in earlier studies, e.g., [79–82]. In a recent study, anthribid species richness did not significantly differ between primary and secondary forests [83], and anthribid species richness was greatly affected by the presence of suitable dead or dying fungus-infested wood, e.g., [84–86].

5. Conclusions

Six species of ambrosia beetles were recorded during the present study. The two most abundant species, *A. dispar* and *X. saxesenii*, represented 98% of the trapped beetles. Both of these ambrosia beetle species were more abundant in oak dominated forests with a high percentage of canopy closure, indicative of a stable and humid environment suitable for the growth of ambrosia fungi, compared to oak forests with a low percentage of canopy closure. Further, a higher abundance of dead oak branches in the canopy was found to be an important factor promoting the occurrence of *A. dispar*. Although the abundance of some species was slightly higher in unmanaged forests, no statistically significant differences in ambrosia beetle abundance in managed vs. unmanaged forests was found. We also have no evidence that particularly high abundances of *A. dispar* in the several studied unmanaged forests would lead to substantial damage to the surrounding forests. In the study area, the abundance of the recorded invasive ambrosia beetles (*C. bodoanum*, *X. attenuated* and *X. germanus*) was low but will probably increase over time. Once the availability of weakened trees increases locally, invasive ambrosia beetles could contribute to oak decline.

Author Contributions: Data curation, T.F.; formal analysis, J.H. and J.F.; methodology, J.H. and T.F.; writing—original draft, J.H., T.F. and J.F.; writing—review and editing, J.H., T.F. and J.F. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in Table 1 and Appendix A.

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

Appendix A

Table A1. Total numbers of bark beetles that were trapped at the 10 studied localities in the western Czech Republic (ambrosia beetle species that use oak trees as hosts are in bold).

	Study Plot										
Species	**Vladař**	**Mariánské Lázně**	**Soos**	**Okounov**	**Korunní**	**Klára**	**Holubín**	**Loket**	**Holina**	**Lázně Kynžvart**	**Total**
Anisandrus dispar **Fabricius, 1792**	**122**	**70**	**2**	**1**	**296**	**2**	**226**	**810**	**1558**	**433**	**3520**
Xyleborinus saxesenii **Ratzeburg, 1837**	**154**	**4**	**49**		**15**	**5**	**57**	**194**	**3**	**95**	**576**
Trypodendron domesticum **Linnaeus, 1758**		**1**							**15**		**16**
Cyclorhipidion bodoanum **Reitter, 1913**	**13**										**13**
Trypodendron lineatum Olivier, 1795		8							1	2	11
Dryocoetes autographus Ratzeburg, 1837		5					1		5		11
Xyleborinus attenuatus **Blandford, 1894**	**9**							**1**			**10**
Hylurgops palliatus Gyllenhal, 1813		6									6
Hylesinus varius Fabricius, 1775		4								1	5
Pityogenes chalcographus Linnaeus, 1761		1			1					1	3
Polygraphus grandiclava C.G. Thomson, 1886					1		1				2
Polygraphus poligraphus Linnaeus, 1758	1						1				2
Hylastes cunicularius Erichson, 1836									1		1
Scolytus intricatus Ratzeburg, 1837								1			1
Xylosandrus germanus **Blandford, 1894**										**1**	**1**
Ips typographus Linnaeus, 1758		1									1
Total	299	100	51	1	313	7	286	1006	1583	533	4179

Appendix B

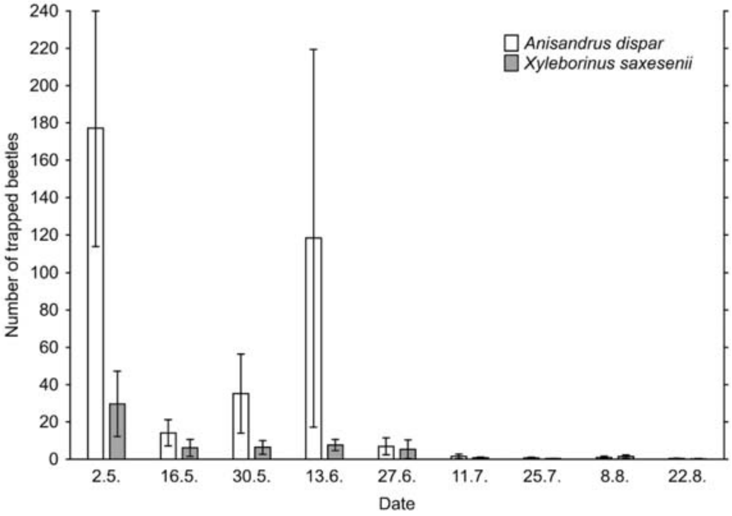

Figure A1. Number (mean ± SE) of individuals of the two most abundant ambrosia beetle species captured per sample in particular sampling periods from April to August 2020. Each sample represented the beetles captured in one trap during 2-week period.

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forests

Article

Native or Invasive? The Red-Haired Pine Bark Beetle *Hylurgus ligniperda* (Fabricius) (Curculionidae: Scolytinae) in East Asia

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Abstract: The red-haired pine bark beetle, *Hylurgus ligniperda* (Fabricius), is one of the most rapidly spreading invasive forest insects. Originally from Eurasia, it has subsequently been introduced to Oceania, North, and South America. Yet, the status of *H. ligniperda* in East Asia is ambiguous. Here, investigation and analysis were conducted on the beetle in China, South Korea, and Japan. New occurrences in China and South Korea were recorded by field surveys and the expansion of *H. ligniperda* spreading in East Asia was analyzed. The results show that *H. ligniperda* is likely an invasive species in East Asia, initially invading Japan, then South Korea. Now it has invaded and successfully colonized Shandong province, China. Furthermore, the species has spread rapidly and it is now widely distributed in South Korea and Japan.

Keywords: pine; *Pinus*; invasion track; new distribution; alien; trap

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1. Introduction

The red-haired pine bark beetle, *Hylurgus ligniperda* (Fabricius), is a well-known forest insect that colonizes the phloem of pine species [1,2]. It can attack stumps, freshly cut logs, and stored timber [1]. In some areas, *H. ligniperda* has become a predominantly root-dwelling species that usually bores into the host through the soil. Overwintering adults have been observed feeding on the root collars of 1–2-year-old seedlings [3,4].

Usually *H. ligniperda* does not kill trees and is considered a secondary pest. Part of the damage is mechanical and direct through brood galleries and feeding tunnels formed under the bark. Decay or sap stain fungi can also be introduced indirectly, which can reduce the quality and value of logs [5,6]. Blue stain fungi, *Leptographium* spp. (Ascomycota: Ophiostomatales), were found transmitted from beetles' galleries into the wood [3,7,8]. In New Zealand, the main damage by *H. ligniperda* in plantation forests is from wood-staining and decay fungal associations that enter with the adults into the brood galleries, and as a quarantine pest that may necessitate treatment of export logs and timber, which greatly increases the cost of exports [5].

Previously, when researchers recorded the original distribution, it included Europe, Russia, the Mediterranean area, and the nearby Atlantic Ocean islands. It was also reported as introduced to South Africa, Japan, South Korea, Sri Lanka, Australia, New Zealand, USA

(NY and CA), Brazil, Uruguay, and Chile [1,9–12]. It should be noted that the distribution of *H. ligniperda* in East Asia is ambiguous in literature. Wood and Bright [9] recorded the distribution of *H. ligniperda* as "Asia ('Manchuria' in China/ Japan)". Hoebeke [13] wrote "It is native to Eurasia and north Africa" and "introduced to Japan". Kim [8] only stated "It is native to Europe" without referencing Asia. CABI [12] listed that "native to Asia (China/Turkey)" and "Japan is invasive", which is reference to a misquotation of "Wood and Bright (1992)". So, whether *H. ligniperda* is native to Asia is still unclear.

In this paper, we aim to clarify the history of recorded collections and known records of *H. ligniperda* in East Asia (Japan, South Korea, and China) using new survey results. Our aim is to determine the status of the insect in East Asia based on the limited collection data. We tested whether the museum specimens and the new records conform to the scenario of native but rarely reported insect (old samples, widely distributed), or whether they conform to the scenario of recent introduction and rapid spread.

We present new records in China and South Korea. The updated distribution in East Asia will contribute to status as an introduced or native species that directly relates to its control and management.

2. Materials and Methods

The new occurrences in China and South Korea were recorded by field surveys from 2013 to 2020. In China, traps were set initially to monitor pine pests in several provinces (Table 1). The lures include pine resin, α-pinene, monochamol, ipsenol, or ipsdienol. After *H. ligniperda* was first found in Shandong, two wood borer pest surveys that focused on damage of pine wood nematode *Bursaphelenchus xylophilus* were processed in Yantai and Weihai. Coincidently, more *H. ligniperda* were found. All those records were included. In Korea, nine sites were surveyed with a lure using a funnel trap. The lures were either alcohol + α-pinene or ipsenol + monochamol.

Table 1. Survey of *Hylurgus ligniperda* in China and Korea in this study.

Country	Location	Periods of Time	Methods [1]	Number of Traps	Number of *H. ligniperda*
China	Fu'an, Fujian prov.	September–October 2018; June–October 2019	trap and search	10–12	0
China	Fuzhou, Fujian prov.	May–August 2018; June–September 2019	trap and search	5	0
China	Zhuhai, Guangdong prov.	2018–2020	trap and search	4	0
China	Shenzhen, Guangdong prov.	2016–2017	trap and search	4	0
China	Kunming, Yunnan prov.	May–August 2019	trap and search	6	0
China	Xishuangbanna, Yunnan prov.	June–August 2014	trap and search	5–8	0
China	Taian, Shandong prov.	July–August 2019	trap and search	9	4
China	Yantai, Shandong prov.	October 2020	search	N/A	10
China	Weihai, Shandong prov.	October 2020–June 2021	search	N/A	20
Korea	Heuksan Island, Jeollanam-do	N/A	trap	N/A	0
Korea	Wando-gun, Jeollanam-do	N/A	trap	N/A	0
Korea	Sacheon-si, Gyeongsangnam-do	N/A	trap	N/A	0
Korea	Gwangju-si, Gyeonggi-do	N/A	trap	N/A	0
Korea	Sangju-si, Gyeongandbuk-do	March–October 2018; March–October 2019	trap	20	53
Korea	Yanggu-gun, Gangwon-do	April–October 2017; February–October 2020	trap	5–10	27
Korea	Inje-gun, Gangwon-do	April–October 2017; May–August 2019	trap	5–12	25
Korea	Chuncheon-si, Gangwon-do	March–September 2018; February–April 2020	trap	3–5	29
Korea	Boryeong-si, Chungcheongnam-do	April–October 2013	trap	45	118

[1] The attractants of funnel trap are alcohol with α-pinene or ipsenol + monochamol in China, and *Monochamus* longhorn beetles pheromone with α-pinene in Korea.

The known localities of *Hylurgus ligniperda* in Japan were gathered from literature, herbarium records, and observations.

The new collected beetles were firstly identified by comparing the external morphological features with published articles [11] and reference collection specimens from the Bark Beetle Academy (http://ambrosiasymbiosis.org/academy, accessed on 5 May 2021). Specimens from China and Korea are deposited at the University of Florida (USA), Shandong Agriculture University (China), and Research Institute of Forest Insect Diversity (Korea). The annotated maps were created in ArcMap 10.4.1.

Samples from China (Weihai and Yantai) and Korea (Buyeo-gun) were sequenced. DNA was extracted using the SDS-based DNA extraction method [14]. We amplified and sequenced the partial cytochrome oxidase I (COI) with the primers LCO 1490 and HCO 2198 [15], and the nuclear large ribosomal subunit (28S) using the primers S3690F and A4285R [16]. Reads were assembled using Geneious® 9.1.8 (www.geneious.com, accessed on 25 June 2021). Sequences were then used to search the databases GenBank (www.ncbi.nlm.nih.gov/genbank/, accessed on 25 June 2021). Also, the sequences for COI and 28S were deposited in GenBank (Supplementary material Table S1; MZ562956–MZ562958, MZ565500).

3. Results and Discussion

Eight new sites of the *H. ligniperda* in Asia were included, three from China and five from Korea (Table 1).

All three sites in China are located within Shandong province (Taian, Yantai, and Weihai city; Figure 1, Supplementary Material Table S1). The earliest record is from Culai Mt., Taian, Shandong in 2019. Bottle traps with lure (slash pine resin) were set in the pine plantation. The host plant of *H. ligniperda* in Culaishan Forest Farm, Taian is unclear as the beetles were only captured in lure traps. Whereas, there are four pine tree species present at the farm, *Pinus tabuliformis* Carrière, *P. thunbergii* Parl., *P. densiflora* Sieb. et Zucc., and *P. armandii* Franch. One more sample was found from an unknown fallen branch in nearby mountain in 2020 (Supplementary Material Table S1). In Yantai and Weihai, all samples were collected from stumps of *P. thunbergia*, which are all under the attack of pine wood nematode. More than 15 stumps were peeled and dissected, and all were infested by *H. ligniperda.* Reports of *H. ligniperda* attacking these native trees in Shandong constitute the first natural occurrences recorded in China.

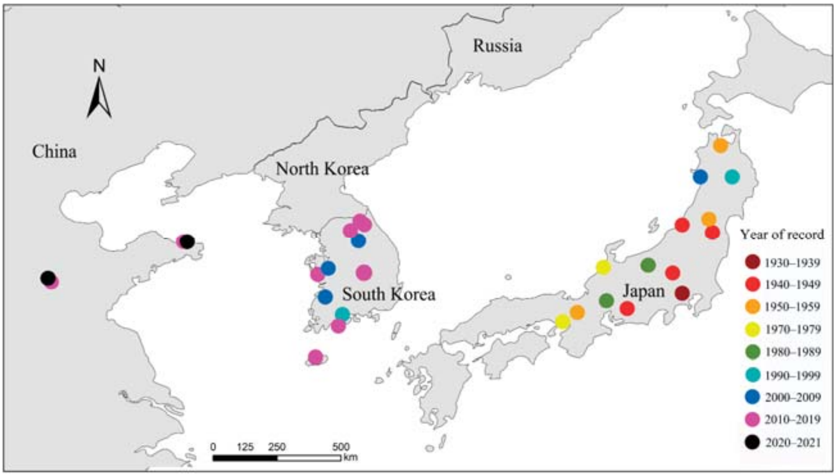

Figure 1. Invasion track of *Hylurgus ligniperda* in East Asia.

Park et al. [11] first recorded *Hylurgus ligniperda* in Korea based on deposited specimens in several institutes. After checking the survey results for wood-boring beetles, more samples were found from lure traps in five locations at various sites between 2013 to 2020

(Table 1, Supplementary Material Table S1). In areas such as Boryeong-si in 2013, Inje-gun in 2017 and 2019, Yanggu-gun in 2017 and 2020, Sangju-si in 2018–2019, and Chuncheon-si in 2018 and 2020, a total of 252 *H. ligniperda* specimens were captured. In South Korea, the suspected first individual was captured in Jeollanam-do in 1999 [11]. Before 1999, several forest surveys had been conducted with a focus on bark beetles in local fauna, but no *H. ligniperda* were found [17,18]. The lack of collections during this period is problematic. Possible scenarios include the beetle not being present, the beetle being undetected at low populations, or the survey methods may not be ideal for capture, such as seasonality or flight time. After 1999, *H. ligniperda* quickly made a spread to nearby regions in Korea, suggesting an expanding nascent population was present at this time.

In Japan, the first record of *H. ligniperda* was in "Yokoyama village, Tokyo" in 1935 [19]. After that, 13 other occurrences were recorded in Honshu Island (Figure 1). The occurrences and times were as follow: Niigata, 1948 [20]; Aichi, 1949 [20]; Fukushima,1949 [21]; Gunma, 1949 [21]; Aomori, 1950 [21]; Yamagata, 1953 [22]; Kyoto, 1954 [21]; Hyogo, 1970 [23]; Ishikawa, 1970 [24]; Gifu, 1984 [25]; Nagano, 1986 [26]; Iwate, 1992 [27]; and Akita, 2001 [28]. The high frequency and large scale of surveys conducted prior to 1940 give little doubt that the beetle is invasive in Japan [19,29–31]. Since the first record of *H. ligniperda* in 1935, it had spread to the north and south of Honshu by 1950. The host of *H. ligniperda* in Japan is *Pinus* spp., such as *P. densiflora* Siebold & Zucc, and *P. thunbergii* Parl. Takahashi [28] reported that *H. ligniperda* were captured by a lure trap (pinene and ethanol) in Japanese pear (*Pyrus pyrifolia* Nakai) orchards.

Because there are only limited reference sequences in NCBI and we only have sequences from Asian samples, we do not provide the genetic population analysis at this time. However, Chinese samples are 100% identical to Korean samples in COI with the closest matching sequence on NCBI being *H. ligniperda* from New Zealand (COI: HM002621; 99.75% similarity). Our results show that the populations in China and Korea are closely related to those in New Zealand, and all Asian populations may have the same origin.

Biotic invasions pose major threats to agriculture, forestry, natural environments, and public health. With the globalization of the world's economy and a drastic increase of human activities, the number of invasive species is also increasing [32]. Scolytines feature prominently among invasive species because they are easily transported through international trade inside wooden products and wood packaging materials in which they are concealed and protected [33,34]. Between 2003 to 2016, *H. ligniperda* was the second most frequently intercepted scolytine in ports of China, with 10,199 interceptions [35]. A worldwide survey of pine bark beetles on several continents also indicated that *H. ligniperda* was one of the most abundant and widespread invasive species [36]. Considering that there are several big ports managing timber import in Shandong, it is possible that *H. ligniperda* invaded China through international timber trade or contaminated wooden packages. However, it cannot be ruled out that it naturally spread to Shandong province through South Korea as the distance between both is only 310 km. The molecular tools in this survey show an identical match for COI between the China and South Korean samples. While important, it should be noted this result is based on a limited sample size (n = 5). More precise genetics methods, such as Single-nucleotide polymorphisms (SNPs) on specimens collected from the invaded areas and native ones, would help to clarify the invasive process and pathways.

Hylurgus ligniperda was first recorded in New Zealand in 1974. Three years later, it was found throughout New Zealand, including travelling between the two main islands [2]. To date, *H. ligniperda* has been one of the most highly abundant exotic wood borers and bark beetles in New Zealand [37]. Chase and Kelly [38] noted that the contributing factors to *H. ligniperda's* invasion success could be their effective long-distance dispersal, and it can mate with siblings before dispersal to increase the probability of colonization success. The rapid spread also occurred in other introduced areas, such as Japan, South Africa, and South America [12]. The survey data show that *H. ligniperda* spread rapidly in South Korea between 2010 and 2019. Unfortunately, the recent survey area of *H. ligniperda* in China is only limited to some parts of Shandong Province. The local government is eager to control and investigate it in more regions. Given the number of localities already occupied by the species in China, it can be considered established.

Wood and Bright [9] recorded *H. ligniperda* from "Manchuria" in China. Historically, "Manchuria" has often referred to Northeast China, including three provinces of Heilongjiang, Jilin, and Liaoning, as well as the current border area with Russia. "Manchuria" has also been called "Guandong" in Chinese. We speculate that this is the origin of the CABI's misquotation "Guangdong is present", because there is no record of the occurrence of *H. ligniperda* in "Guangdong" in the CABI reference list and other literature. Tsai and Li [39] recorded 86 species of Scolytinae in North China (include Northeast China) and no record of *H. ligniperda.* In Northeast China. Yin et al. [40] conducted many field investigations beginning in 1955, of which they published a monograph "Economic Insect Fauna of China Fasc. 29 Coleoptera: Scolytidae" in 1984, which recorded 165 species of Scolytinae in China and no record of *H. ligniperda*. Song et al. [41,42] conducted investigations in several provinces between 1982 and 1995 and recorded 98 species of Scolytinae in Liaoning. Similarly, no records of *H. ligniperda* were made. For other areas in China, there are three rounds of nationwide forest survey in China from 1979 to 2019. *H. ligniperda* was never reported. After reviewing the literature and inspecting the specimens in the National Animal Collection Resource Center [previously the National Zoological Museum of China, Institute of Zoology (IOZ), Beijing, China], we did not find any collection of *H. ligniperda* in China before 2019. Also, this beetle is not found in eastern Russia (Alex Petrov, personal communication). So, the occurrence record of *H. ligniperda* in "Manchuria" China [9] is likely erroneous due to difficulties in translation or a record of an adventive specimen intercepted at a port.

In recent years, several economically significant Scolytinae invaded China, such as *Dendroctonus valens* (LeConte, 1860), *Xyleborus affinis* (Eichhoff, 1868), *Xyleborus ferrugineus* (Fabricius, 1801), *Hypothenemus hampei* (Ferrari, 1867), *Ips calligraphus* (Germar, 1824), and *Ips grandicollis* (Eichhoff, 1868) [43–47]. Many of the recent invasions are attributed to the increased global trade in timber, as China has become the second largest timber importer in the world [48]. There are likely more invasive scolytines yet to be detected or intercepted in China. It is urgent to carry out a national surveillance program for invasive scolytines in China, which can provide the basis for subsequent prevention and control.

4. Conclusions

Investigation and analysis showed that the previous record of the occurrence of *H. ligniperda* in China was likely misplaced; new observations suggest it has recently invaded and successfully colonized parts of Shandong Province, China. In South Korea, it has spread rapidly in recent years, and its current distribution effectively covers the majority of South Korea. In Japan, its distribution has covered the entirety of Honshu Island due to decades of colonization. The reporting of the East Asian invasion by *H. ligniperda* has been confused by semantic issues. More measures should be taken to control the rapid spread of this pest in Asia.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/f12070950/s1, Table S1: Records of *Hylurgus ligniperda* in East Asia.

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Association of the Female Flight Ability of Asian Spongy Moths (*Lymantria dispar asiatica*) with Locality, Age and Mating: A Case Study from China

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Abstract: The spongy moth (formerly known as a gypsy moth), *Lymantria dispar L.* (Lepidoptera: Erebidae), is a polyphagous pest that defoliates various species of trees in the genera *Populus*, *Salix*, *Quercus*, *Acer* and *Pinus*. Egg masses of Asian spongy moths (*Lymantria dispar asiatica*) were obtained from five geographically distinct locations in China (Chengdu, Xifeng, Heilongjiang, Kuduer and Longhua regions) and were carried to the lab for incubation and rearing larvae up to adult emergence. The aim was to see how locality, age and mating status influenced the flying activity of female Asian spongy moths. The findings demonstrate that age significantly affects *L. dispar asiatica* flying activity, with flight period and distance decreasing dramatically as age increases. Locality also significantly impacts flight ability, and mating status does not affect the *L. dispar asiatica* female flight ability ($p > 0.05$). The maximum speed was observed for females from Xifeng (7.19 ± 0.34 km/h). The females from Heilongjiang were recorded as having the maximum average speed (2.35 ± 0.08 km/h). Females from Xifeng exhibited the maximum flight duration and distance (0.94 ± 0.04 h and 1.87 ± 0.18 km). Morphological features, i.e., thorax width (cm) and wing loading, significantly influenced the *L. dispar asiatica* flight ability ($R^2 = 0.93$ and $R^2 = -0.98$). The overall pattern observed for female *L. dispar asiatica* flight duration was Xifeng > Kuder > Chengdu > Heilongjiang > Longhua, and for flight distance, it was Xifeng > Longhua > Heilongjiang > Kuder > Chengdu. This research offers detailed information on the dispersion rate of Spongy moths in a known or newly introduced habitat, which can help us limit the spread of this polyphagous pest.

Keywords: *Lymantria dispar asiatica*; Asian spongy moth (ASM); female flight ability; flight mill; female age; female flight duration; female flight distance

1. Introduction

The spongy moth (formerly known as the gypsy moth) (*Lymantria dispar*) is proficient in triggering severe impairment to ecosystems and individual trees through repetitive defoliation [1]. This insect is polyphagous, and its caterpillars are particularly destructive defoliators, feeding on over 300 different types of trees [2,3] in coniferous and deciduous woodlands [2–5]. The preferred species genera are *Quercus*, *Populus*, Salix [6,7] *Pinus* and *Acer* [8,9]. This pest infests many fruit trees, ornamental trees and shrubs [10], i.e., *Alnus* spp., *Malus* spp., *Prunus* spp., *Pyrus* spp., *Tilia* spp., *Betula papyrifera*, *Crataegus* spp., *Corylus* spp., *Hamamelis* spp. [11,12], *Vaccinium* spp., *Viburnum* sp. and *Vitis* spp. [7]. Forests are a vital source of sequestering CO_2 from the atmosphere [13–15]; unlike many other forest pests that undermine commercial wood values [16], the spongy moth wreaks havoc on forests, urban forest habitats and inhabitants [17], causing significant losses to the carbon sink. Due to spongy moth attacks, more geographical areas are affected, which results in the decline of most economical tree species, such as pines and many fruit plants [18]. Furthermore, outbreaks are more likely in severely degraded forests and woods (canopy cover rate less than 25%) than in natural areas [19]. Based on females' ability to fly, where they come from and their mitochondrial DNA, three subspecies of *L. dispar* have been found [20,21], i.e., *Lymantria dispar dispar* [European spongy moth (ESM)], *Lymantria dispar asiatica* [Asian spongy moth (ASM)] and *Lymantria dispar japonica* [Japenese spongy moth (JSM)] [22]. The European spongy moth (ESM) is distributed throughout Eurasia and spreads from the west of Portugal to the eastern mountains of the Ural regions [23]. In contrast, *L. dispar asiatica* occupies much of continental Asia and the eastern areas of Russia, and *L. dispar japonica* is exclusively distributed in Japan [20] and the Kuril Islands of Russia [22]. *L. dispar asiatica* is found around the world in a variety of forest environments. Therefore, *L. dispar asiatica* may produce progeny with a broader host specificity than the initial populations [24].

The ESM invaded the US as early as 1869, and the *L. dispar asiatica* invaded in 1991 [25]. However, *L. dispar asiatica* has been decimated on at least 20 sites across the United States since its first invasion [25], but it must be considered that it has more evolved wings [26] and a higher probability of new invasion than the ESM. Adult females of *L. dispar* cannot fly for long periods, limiting the geographical distribution of egg masses [27]. In addition, compared to *L. dispar asiatica* with *L. dispar dispar* females, the European spongy moth cannot fly due to more significant abdominal muscles, smaller wings and poorly developed flight muscles [23,28]. Therefore, the subspecies *Lymantria dispar dispar* (ESM), introduced to North America, has flightless females [29].

A female spongy moth's flight capability and frequency are two of the chief criteria used to determine a spongy moth's proliferation [30]. Females that fly can deposit their eggs near sharp light sources or bright-colored surfaces [26,30]. On the other hand, virgin females fly less frequently than mated females because they must first find a partner for mating [31]. Instead of confining the investigation to a subspecies level, it is critical to look at flying capabilities at a population level [30]. Arakawa and Iwaizumi [32] and Iwaizumi et al. [33] related the female *L. dispar asiatica*'s flight capabilities collected from five locations in Japan. The Tomakomai population could fly the longest distance than the other four localities. Pogue and Schaefer [22] reviewed *Lymantria* and included two sub-species of *Lymantria dispar* (*Asiatica* and *Japonica*) and three different species in Japan (*Lymantria albescens* Hori and Umeno, *L. umbrosa* Butler and *L. postalba* Inoue), and they concluded that the Asian spongy moth could fly.

The attack of the *L. dispar asiatica* on forest land in China is severe. Every year, it destroys hundreds of hectares, resulting in thousands of dollars in losses. In the Sichuan basin, spongy moth infestations have caused harm to *Pinus massoniana* (pine species) and broadleaf species [34]. The spongy moth may be found across the arid river valley. Since 2002, forest health assessments and field inspections have seen it in all *Pinus radiata*, *P. tabulaeformis* and *P. armandii* stands [35]. To conserve forests from spongy moth invasions, it is necessary to study the features of this pest through morphological research, molecular research and flight research. To understand the spread rate of *L. dispar asiatica* globally and in China, the flight ability of female *L. dispar asiatica* must be studied.

We hypothesized that the age of moths and locality, by which morphological features differ, influence the flight ability of female *L. dispar asiatica* in China. We established the layout of the current study to obtained the desire objectives (Figure 1). To test this hypothesis, flight-related variables of female *L. dispar asiatica* from five different geographical locations were investigated in China. Therefore, this research study was conducted to analyze the impact of the locality factor, age effect and mating status on female Asian spongy moths (*Lymantria dispar asiatica*).

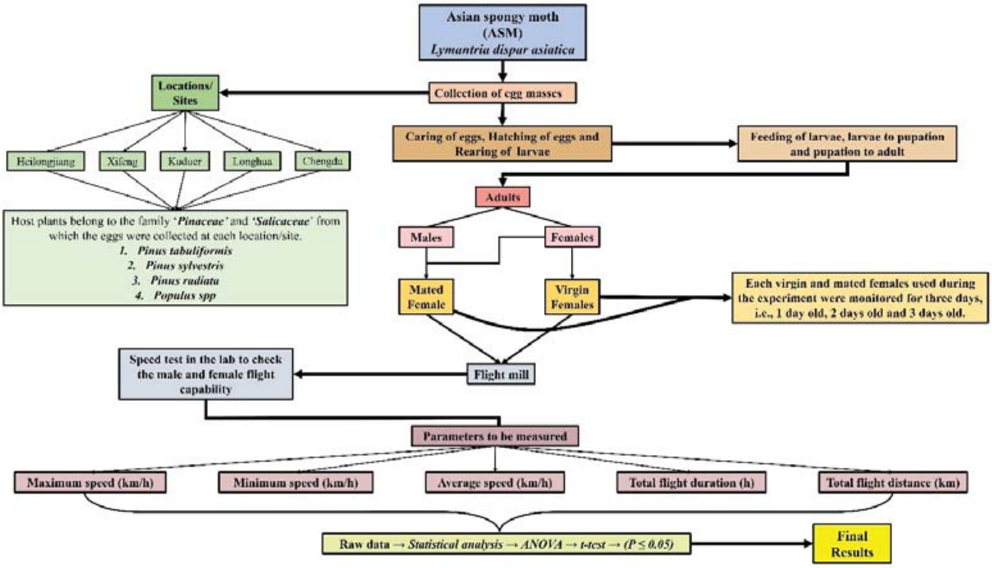


Figure 1. Conceptual framework of the present study.

2. Materials and Methods

2.1. Collection of Egg Masses

Lymantria dispar asiatica egg masses were collected from 5 different geographical locations in China, i.e., the Chengdu, Xifeng, Heilongjiang, Kuduer and Longhua regions [Figure 2, Table 1]. The preferred host trees are distributed all around these regions; aside from that, a large *L. dispar asiatica* population is present [28]. Therefore, we selected these geographical locations for our experimental study. Trees from which egg masses were collected belong to the family 'Pinaceae' (*Pinus tabuliformis*, *P. sylvestris*, *P. radiata*) and 'Salicaceae' (*Populus* spp) at each location. Egg masses were then placed into a small PVC container and were transported back to the invasive pest laboratory at Beijing Forestry University for rearing and caring for the insects.

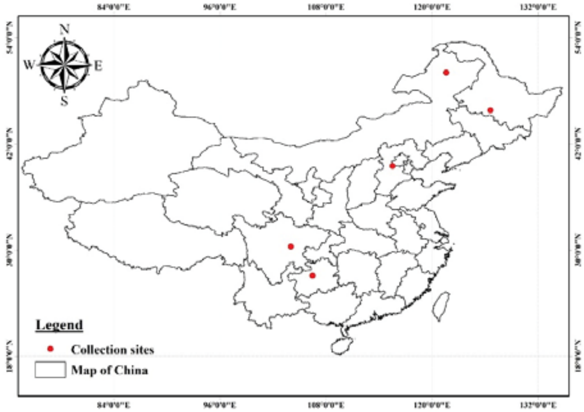


Figure 2. Map of China; red dots indicate collection sites of the Asian spongy moth (*L. dispar asiatica*) egg masses.

Table 1. Estimated locations (latitude, longitude and altitude) of five sampling areas of Asian spongy moths (*L. dispar asiatica)* in China.

Locations	Latitude	Longitude	Altitude(m)	Collection Date	No. of Collected Egg Masses
Chengdu	30.4000° N	104.0400° E	499	April 2017	25
Longhua	39.4854° N	115.5550° E	716	March 2017	14
Heilongjiang	45.78° N	126.61° E	122	August 2017	30
Kuduer	50.0259° N	121.6236° E	540	July 2019	21
Xifeng	27.11° N	106.49° E	1104	November 2018	14

2.2. Rearing of Insects

After arrival in a lab, all the egg masses were kept in an incubator at 4 °C until diapause (inside an incubator, chilling temperature was maintained at 4° to terminate the *L. dispar asiatica*; egg diapause took 10 days) was over. When the incubation ended, newly hatched larvae were raised in transparent plastic cups [Figure 3]. Each cup contained 30 mL of synthetic diet [36]. Two to four larvae were reared in each plastic cup until larvae pupated. Spongy moth larvae were kept in a laboratory under controlled conditions (at 25 °C), 40%–60% relative humidity (R.H.) and a light: dark (L:D) photoperiod (16:8 h). Before the moth's emergence, pupae were kept separately in 300 mL clear PVC cups. Moth wings were permitted to swell and stiffen before use. No moth with unexpanded or ruffled wings was used in the experiment.

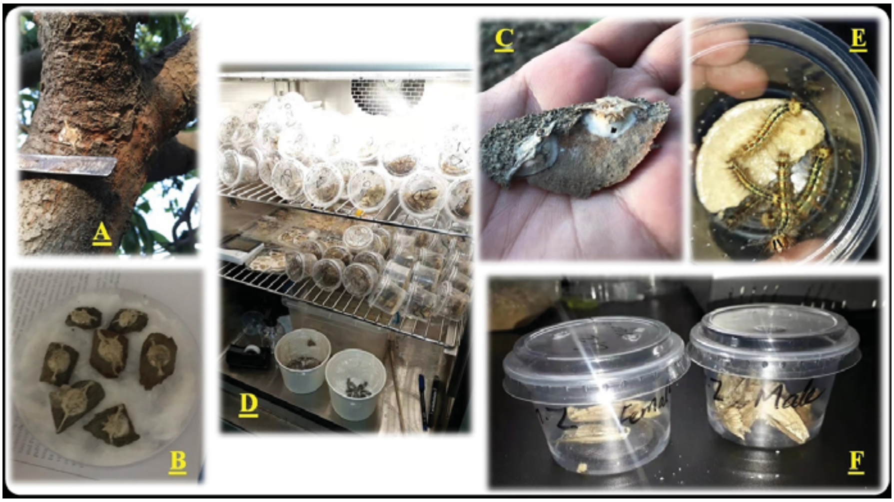
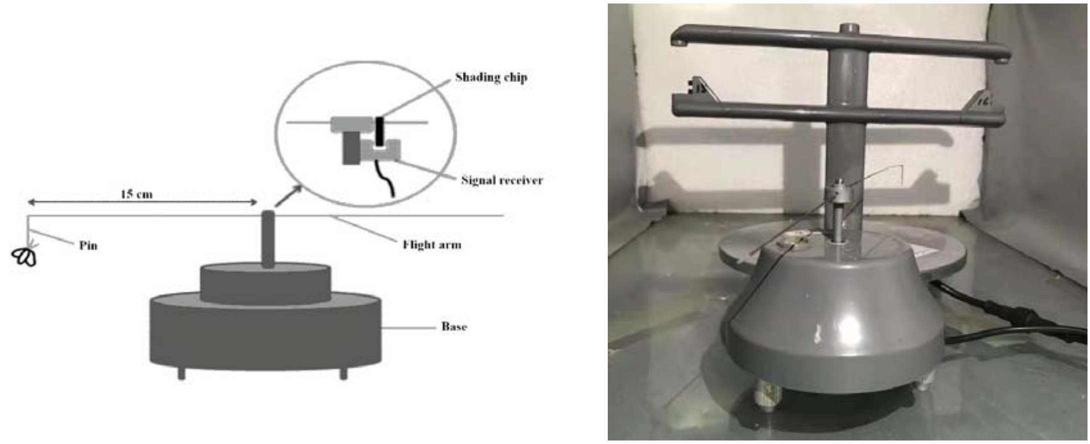


Figure 3. Images of Asian spongy moth (*L. dispar asiatica*) egg mass collection and rearing: (**A**) collection of egg masses from a tree trunk; (**B**) egg masses of *L. dispar asiatica*; (**C**) a piece of bark containing egg masses of *L. dispar asiatica*; (**D**) rearing of *L. dispar asiatica* larvae in the laboratory under controlled conditions; (**E**) a PVC container containing larvae of *L. dispar asiatica* feeding on an artificial diet; (**F**) PVC containers containing adult males and females of *L. dispar asiatica*.

2.3. Flight Activity Measurement

Adult female Asian spongy moth flight was tracked using a computer-linked flight mill to observe their flight activities (Figure 4]) (Jiaduo Company, Beijing Forestry University, Beijing, China), comparable to those defined by Briegel et al. [37] and Tanaka and Yamanaka [38]. Six flight mills were working simultaneously with controlled conditions of temperature and humidity.



(**a**) (**b**)

Figure 4. Flight mill (for checking the flight ability of spongy moths): (**a**) Details of flight mill; (**b**) Flight mill used during the experiment.

The flight mill consisted of four parts: the base, which serves as a support and the flight mill's balance (reducing gravity pull); two flight arms (one end used for fixing the insect and the other for balancing); and the flight mill itself. A computer chip was set at the base that measures the insect's movement (how many circles it completes, total distance and maximum speed). We put the Asian spongy moth females on a styrofoam plate and used insect pins to separate their wings from their bodies. With an ink brush, we tried to wipe them off gently. Then, we used super glue (cyanoacrylate) to attach a 2 cm-long piece of steel wire to the thorax of every female. The females were stationary on the foam plate for 3 to 4 minutes to permit the glue to set. Wire parts were linked to the arm of the flight mill with plasticizing, so they were linked to the opposite end of every rotor as counter masses.

Newly emerged moths were retrieved twice daily at 0900 and 1700 h, with age calculated as an average of nights post-eclosion. The influence of age on flying activity was investigated in virgin females 1–3 days following emergence (n = 30 moths per age class). Mating status's impact on flying behavior was investigated using age-matched unmated (n = 30) and mated (n = 30) females. All the females (unmated and mated) used during the experiment were 1 day old. The emerging females were maintained separately in PVC containers for the virgin treatment. To induce mating, freshly emerging females (30 per population) were housed in a net cage "50 cm × 50 cm × 50 cm" with 40 males for the mated treatment, and females did not start laying eggs until they completed their flight after mating or a scotophase [36]. A scotophase is a period of darkness, especially one that is artificially imposed (an artificial night). *L. dispar asiatica* females did not start laying eggs until they completed their flight after mating or beginning the dark period (night). After mating was observed, the mated females were employed. Every moth was engaged in a single flight mill run (i.e., every moth was only measured once). The moths in the age effects study were not the same as those in the mating status test. Overall flight investigations were carried out at a temperature of 25 °C. The number of spins of the flying arm every 5 s was recorded and utilized to determine the maximum flight speed, minimum flight speed, average flight speed, total flight duration and total flight distance.

2.4. Morphological Features (Thorax and Wing) Measurement

Following the flying test, female wings were cut, unfurled on a horizontal plane and snapped with a camera (Nikon D90) to calculate wing loading [wing loading is a measurement that compares an insect's mass to its entire wing area. The wing-area-to-body weight proportion is expressed in milligrams per square centimeter (mg/cm^2)]. Insect body mass is usually calculated from body length. Each insect body length was calculated using two methods: (i) a vernier caliper and (ii) digital imaging. In our case, we computed the body length with wing size to calculate wing loading. ImageJ (version 1.37 for Windows; National Institutes of Health, Bethesda, MD, USA) was used to draw the wing outline in the digital picture, and the wing areas were transformed from pixels on the inside of the wing outline to square centimeters using a conventional conversion factor. A vernier caliper was used to measure the thorax width.

2.5. Data Analyses

Data were calculated using 30 replications for each location (10 replications for day 1, 10 replications for day 2 and 10 replications for day 3). In total, 5 response variables were calculated, i.e., maximum speed (km/h), minimum speed (km/h), average speed (km/h), flight duration (h) and flight distance (km). Residual data from all five response variables were subjected to a normality probability test (QQ Plot) to check the normal distribution of the data and to a chi-squared test for homogeneity of variance. A two-way ANOVA with two main effects, i.e., location and age, was used to determine the significance of locality and age on the flight activity of the Asian spongy moth in China. Tukey's HSD all-pairwise comparison tests were employed to compare different parameters at a significance level of $p < 0.05$. The mean value was calculated for each variable separately. Using a one-way ANOVA, the significance of each variable related to different localities/sites was determined separately. Mean values of day one, day two and day three of each response variable were calculated individually for each locality to calculate the effect of age on *L. dispar asiatica* flight. Using a two-way ANOVA, the significance of each response variable related to age and different localities/sites was determined separately. A *t*-test was performed using each response variable's mean values to check the flight status of unmated and mated females. The mean values of flight distance (km), wing loading (mg/cm^2) and thorax width (cm) were calculated for each locality separately; a linear regression model was used to check the association of body size with the flight ability of female *L. dispar asiatica*. All statistical work was conducted using SPSS Statistics (version 26, IBM Corporation, Armonk, NY, USA), MS-Excel Professional Plus (version 2016, Microsoft, Redmond, WA, USA) and Origin Pro (version 2016, OriginLab Corporation, Northampton, MA, USA).

3. Results

This study examined the association between age, mating status and flying ability in female *L. dispar asiatica* from five distinct localities. According to a two-way ANOVA, locality and age significantly impacted all the variables associated with the flight ability of *L. dispar asiatica*, i.e., maximum and minimum speed, average speed, flight duration and flight distance (Table 2). Females from Xifeng were observed fastest with a mean maximum speed of 7.19 ± 0.34 km/h (Figure 5a). Females from Chengdu were the slowest, with a mean minimum speed of 0.56 ± 0.03 km/h (Figure 5b). The females from Heilongjiang had the highest values for mean average speed (2.35 ± 0.08 km/h) (Figure 5c). Females from Xifeng exhibited the maximum flight time (0.94 ± 0.04 h) (Figure 5d). Moreover, females from Xifeng flew the maximum distance (1.87 ± 0.18 km) (Figure 5e).

Age significantly impacted flight activity metrics such as speed, flight duration and distance (Table 3). At 1 day old, all females achieved their longest flying distance, with an average flight distance of 2.64 ± 0.32 km. Later, between 2 and 3 days of age, the flight distances of females dropped. The population from all five localities followed a similar flight speed and duration (Table 4, Figure 6). The flight speed of *L. dispar asiatica* was the maximum on the first day and decreased on the second and third days (Table 3). According to the observed results, the mean maximum speed of the female Asian spongy moths was 8.99 ± 0.49 km/h when the adults were 1 day old, and minimum of 3.76 ± 0.87 km/h was observed when the adults were 3 days old. Similarly, the mean minimum speed was higher on the first day (0.84 ± 0.09 km/h) and the lowest on the third day (0.46± 0.11 km/h).

The mean average speed was more (2.51 ± 0.43 km/h) when the females were 1 day old and decreased to 1.34 ± 0.31 km/h when it reached the age of 3 days. The mean flight time of the first-day adult Asian spongy moth was 1.09 ± 0.14 h greater than that of the 3rd-day adults (0.54 ± 0.11 h). The mean total flight distance of the Asian spongy moth was 2.64 ± 0.32 km on the first day, which was much greater than the distance covered by an adult having an aging period of 3 days old (0.81 ± 0.24 km) (Table 3).

Table 2. The impact of age on the flight ability of tested female *Lymantria dispar asiatica* assessed by a two-way ANOVA for maximum/minimum/average speed, total flight time and fight distance.

Response Variables	Independent Variables	Two-Way ANOVA		
		df	*F*	*p*-Value
Maximum speed (Km/h)	Location	5	83.34	<0.0001 **
	Age	2	4042.99	<0.0001 **
	Location × Age	10	23.24	<0.0001 **
Minimum speed (Km/h)	location	5	28.04	<0.0001 **
	Age	2	410.56	<0.0001 **
	Location × Age	10	3.4	<0.001 **
Average speed (Km/h)	Location	5	102.98	<0.0001 **
	Age	2	1028.54	<0.0001 **
	Location × Age	10	80.42	<0.0001 **
Total flight time (h)	Location	5	36.13	<0.0001 **
	Age	2	599.27	<0.0001 **
	Location × Age	10	3.65	<0.001 **
Total flight distance (km)	location	5	95.31	<0.0001 **
	Age	2	4514.17	<0.0001 **
	Location × Age	10	51.13	<0.0001 **

Note: ** indicating the level of significane at $p < 0.01$.

Table 3. Effectiveness of age on female Asian spongy moth (*L. dispar asiatica*) flight speed, time and distance. Values are presented as Mean ± SE.

Age	Maximum Speed (Km/h)	Minimum Speed (Km/h)	Average Speed (Km/h)	Total Flight Time (h)	Total Flight Distance (km)
1 day old	8.99 ± 0.49 ***	0.84 ± 0.09 ***	2.51 ± 0.43 ***	1.09 ± 0.14 ***	2.64 ± 0.32 ***
2 days old	6.89 ± 0.64 **	0.69 ± 0.10 **	1.98 ± 0.46 **	0.83 ± 0.12 **	1.78 ± 0.21 **
3 days old	3.76 ± 0.87 *	0.46 ± 0.11 *	1.34 ± 0.31 *	0.54 ± 0.11 *	0.81 ± 0.24 *

Note: ***, ** and * indicate the highest, middle and lowest level, respectively.

Table 4. The impact of age on different parameters concerning female Asian spongy moths' (*L. dispar asiatica*) flight aptitude from five locations in China. Values are presented as Mean ± SE.

Age	Location	Maximum Speed (Km/h)	Minimum Speed (Km/h)	Average Speed (Km/h)	Total Flight Time (h)	Total Flight Distance (km)
1 day old	**Chengdu**	8.709 ± 0.32^{cd}	0.745 ± 0.06^{bcd}	1.664 ± 0.22^{ef}	1.116 ± 0.05^{ab}	2.442 ± 0.13^{d}
	Longhua	9.028 ± 0.25^{bc}	0.799 ± 0.09^{abc}	2.574 ± 0.13^{bc}	0.861 ± 0.12^{d}	2.806 ± 0.13^{b}
	Heilongjian	8.246 ± 0.27^{d}	0.876 ± 0.06^{a}	2.400 ± 0.07^{c}	1.024 ± 0.07^{bc}	2.626 ± 0.17^{c}
	Kuduer	9.012 ± 0.10^{bc}	0.881 ± 0.07^{a}	2.850 ± 0.10^{a}	1.132 ± 0.08^{ab}	2.113 ± 0.09^{e}
	Xifeng	9.338 ± 0.10^{ab}	0.890 ± 0.07^{a}	2.791 ± 0.07^{ab}	1.235 ± 0.06^{a}	3.060 ± 0.05^{a}
2 days old	**Chengdu**	6.183 ± 0.07^{f}	0.576 ± 0.05^{f}	1.904 ± 0.05^{d}	0.841 ± 0.04^{d}	1.402 ± 0.12^{h}
	Longhua	7.663 ± 0.06^{e}	0.688 ± 0.08^{cf}	1.549 ± 0.16^{f}	0.669 ± 0.10^{e}	1.934 ± 0.14^{f}
	Heilongjian	6.373 ± 0.86^{f}	0.841 ± 0.09^{ab}	2.777 ± 0.35^{ab}	0.859 ± 0.18^{d}	1.763 ± 0.08^{f}
	Kuduer	6.647 ± 0.12^{f}	0.640 ± 0.07^{def}	2.073 ± 0.05^{d}	0.911 ± 0.05^{cd}	1.950 ± 0.04^{ef}
	Xifeng	7.295 ± 0.10^{e}	0.685 ± 0.06^{def}	2.101 ± 0.08^{d}	0.906 ± 0.06^{cd}	1.808 ± 0.05^{f}

Table 4. *Cont.*

Age	Location	Maximum Speed (Km/h)	Minimum Speed (Km/h)	Average Speed (Km/h)	Total Flight Time (h)	Total Flight Distance (km)
3 days old	**Chengdu**	2.636 ± 0.14 ^{f}	0.370 ± 0.03 ^{d}	1.321 ± 0.08 ^{ef}	0.479 ± 0.05 ^{f}	0.440 ± 0.02 ^{ef}
	Longhua	3.320 ± 0.40 ^{ef}	0.407 ± 0.12 ^{ef}	1.404 ± 0.12 ^{f}	0.434 ± 0.05 ^{g}	0.849 ± 0.18 ^{f}
	Heilongjian	3.255 ± 0.13 ^{f}	0.614 ± 0.07 ^{ef}	1.881 ± 0.09 ^{de}	0.518 ± 0.10 ^{f}	1.191 ± 0.09 ^{d}
	Kuduer	4.611 ± 0.16 ^{f}	0.471 ± 0.08 ^{def}	1.430 ± 0.10 ^{f}	0.600 ± 0.10 ^{ef}	0.870 ± 0.08 ^{f}
	Xifeng	4.936 ± 0.61 ^{cd}	0.493 ± 0.05 ^{ef}	1.036 ± 0.07 ^{f}	0.681 ± 0.06 ^{e}	0.727 ± 0.05 ^{f}

Note: 'a', 'b', 'c', 'd,' 'e' and 'f' indicate the highest to the lowest significance level at the significance level of $p < 0.05$.

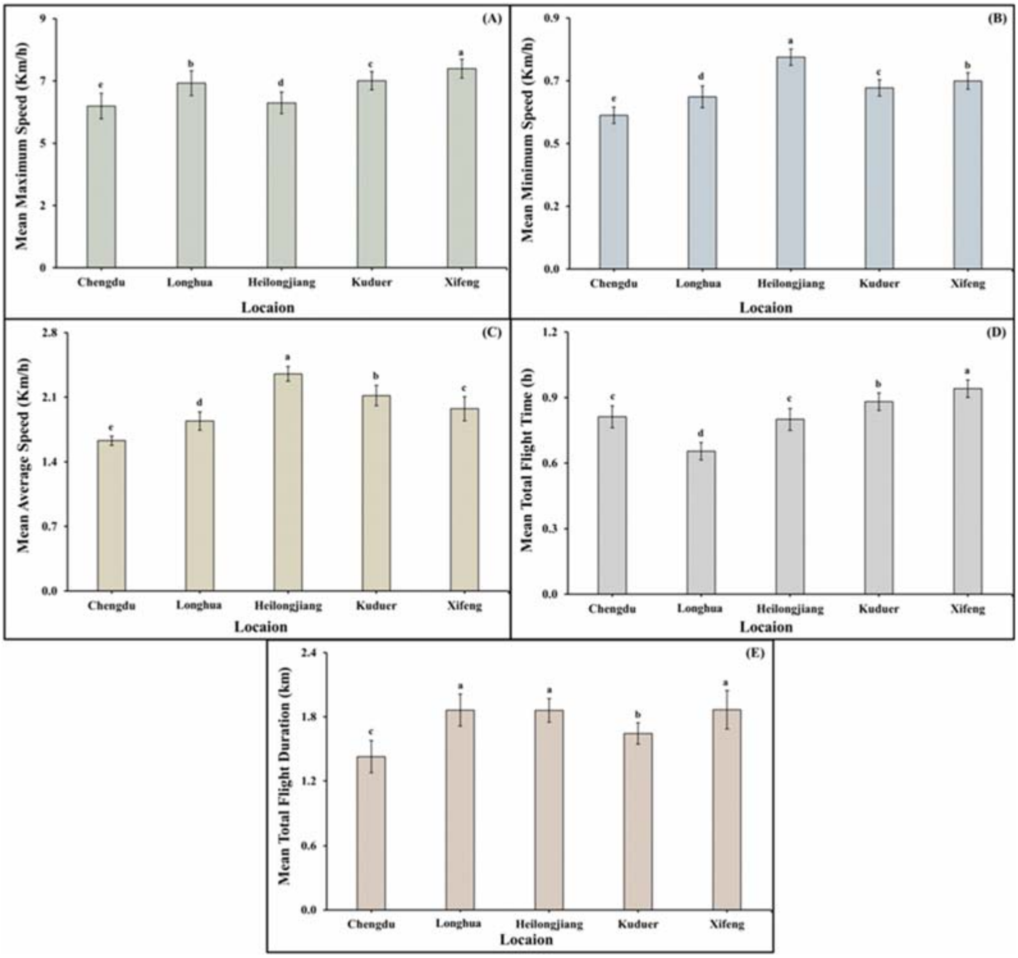


Figure 5. Parameters regarding the flight capability of female Asian spongy moths (*L. dispar asiatica*) from five different locations in China: (**A**) Mean maximum speed of females from five different sites; (**B**) Mean minimum speed of females from five different sites; (**C**) Mean average speed of females from five different sites; (**D**) Mean total flight time of females from five different sites; (**E**) Mean total flight distance of females from five different sites. A level of significance of $p < 0.05$ was used. Plots are with SE.

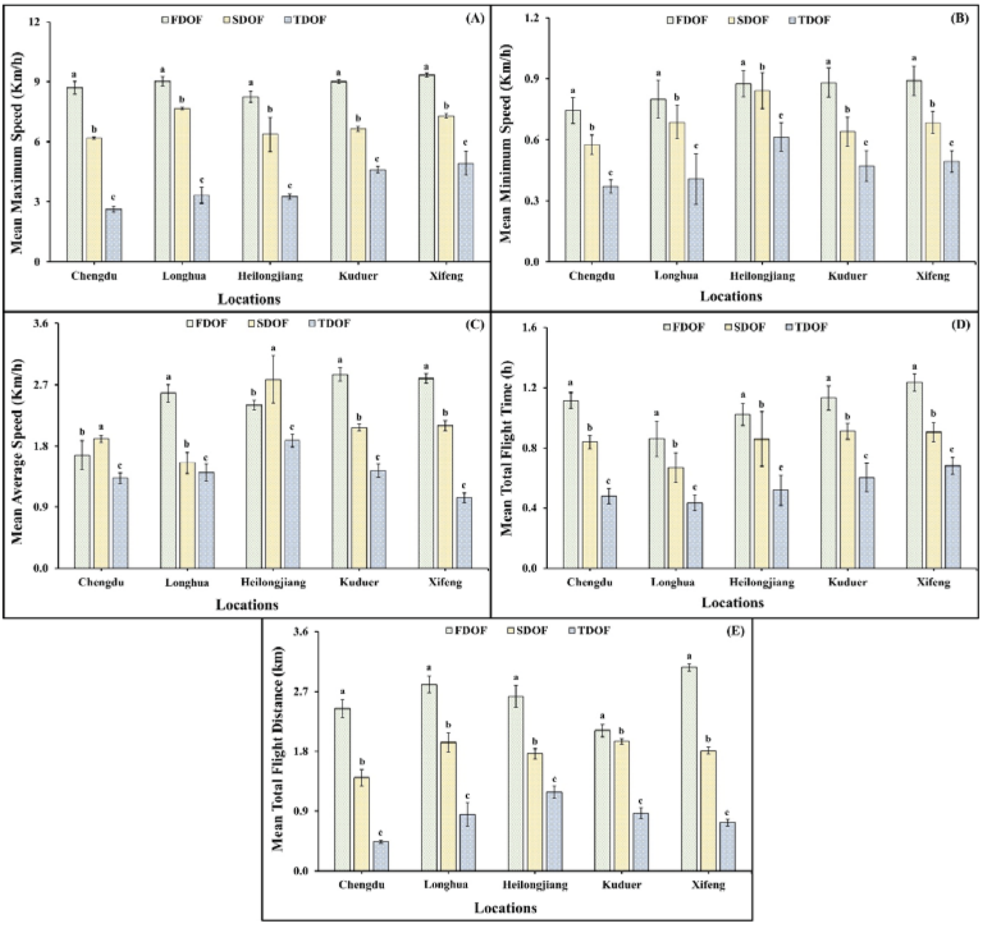


Figure 6. Effect of age on different parameters regarding the flight capability of female Asian spongy moths (*L. dispar asiatica*) from five different geographical locations in China: (**A**) Effect of age on the mean maximum speed of females from five different sites; (**B**) Effect of age on the mean minimum speed of females from five different sites; (**C**) Effect of age on the mean average speed of females from five different sites; (**D**) Effect of age on the mean total flight time of females from five different sites; (**E**) Effect of age on the mean total flight distance of females from five different sites. Letters (a,b,c) indicate the variation in flying capability with respect to female age for each locality. A level of significance of $p < 0.05$ was used. Plots are with SE.

There was no significant difference found among the flight variables of unmated and mated females, i.e., average speed ($p = 0.12$), flight duration ($p = 0.06$) and flight distance ($p = 0.14$) (Table 5). Unmated and mated female *Lymantria dispar asiatica* from all five sites reached their maximum average speed, flight time and flight distance on the first day of flight, and they gradually decreased with age (Figure 6; Table 4). On the first day of flight, the highest mean maximum speed was 9.62 ± 0.18 km/h for females from Longhua. On the second day, it was 7.66 ± 0.06 km/h for females from Xifeng, and on the third day, it was 4.94 ± 0.61 km/h for females from Kuder (Figure 6a). The mean minimum speed decreased as the age increased.

The highest value for the first day was 0.89 ± 0.07 km/h for females from Xifeng; for the second and third days, it was 0.84 ± 0.09 km/h and 0.61 ± 0.07 km/h for the females from Heilongjiang (Figure 6b). On the first day, the flight mean average speed was the maximum with the highest value (2.85 ± 0.10 km/h) for the females from Kuder, which gradually reduced on the second and third days of flight, with the highest values of 2.78 ± 0.35 km/h and 1.88 ± 0.09 km/h for the females from Heilongjiang (Figure 6c). The highest mean total flight time was observed (1.24 ± 0.06 h) for the females from Xifeng on the first day of the flight. On the second and third days, flight time reduced with maximum values of 0.91 ± 0.06 and 0.91 ± 0.05 h, and it was 0.68 ± 0.06 h for the females from Kuder and Xifeng (Figure 6d). The age of females limited the mean total flight distance as well. The highest value of the distance traveled was 3.06 ± 0.05 km for the females from Xifeng, which was eventually observed to decrease on the second and third days of flight, with the highest values of 1.95 ± 0.04 km and 1.19 ± 0.09 km for the females from Kuduer and Heilongjiang (Figure 6e).

Table 5. Impact of mating status on the flight speed, duration and distance of female Asian spongy moth (*L. dispar asiatica*) populations from five different geographical locations in China. Values are presented as Mean ± SE.

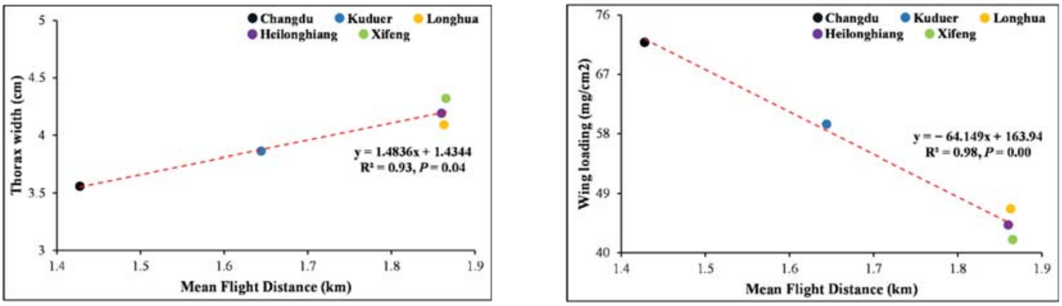
Site	Average Flight Speed (km/h)		Total Flight Time (h)		Total Fight Distance (km)	
	Unmated	Mated	Unmated	Mated	Unmated	Mated
Chengdu	1.63 ± 0.05	1.61 ± 0.06	0.81 ± 0.05	0.78 ± 0.05	1.43 ± 0.15	1.41 ± 0.13
Longhua	1.84 ± 0.1	1.86 ± 0.11	0.65 ± 0.04	0.66 ± 0.05	1.86 ± 0.15	1.84 ± 0.14
Heilongjiang	2.35 ± 0.08	2.31 ± 0.09	0.8 ± 0.05	0.76 ± 0.05	1.86 ± 0.11	1.85 ± 0.12
Kuduer	2.12 ± 0.11	2.09 ± 0.09	0.88 ± 0.04	0.85 ± 0.04	1.64 ± 0.10	1.65 ± 0.11
Xifeng	1.98 ± 0.13	1.95 ± 0.12	0.94 ± 0.04	0.92 ± 0.06	1.87 ± 0.18	1.86 ± 0.16
Variance	0.07	0.07	0.01	0.01	0.04	0.04
df	4		4		4	
p	0.12		0.06		0.14	

Thorax width and wing loading were also calculated for the females from five different geographical locations (Table 6). The female population from Xifeng had a maximum thorax width of 4.32 ± 0.22 cm with a minimum wing loading of 41.99 ± 5.09 mg/cm^2, and the female population from Chengdu had a minimum thorax width of 3.56 ± 0.17 cm, with a maximum wing loading of 71.87 ± 5.64 mg/cm^2 (df = 9, f = 74.98, p = 0.0001; $p < 0.05$). Furthermore, there was a significant linear relationship between body size and female flight ability (Figure 7). There was positive linear relationship between female thorax width and female flight distance ($y = 1.4836x + 1.4344$, $R^2 = 0.93$, $p = 0.04$; $p < 0.05$). Females from Xifeng flew a maximum distance of 1.87 ± 0.18 km due to having more thorax width (4.32 ± 0.22 cm), and females from Chengdu flew the minimum distance (1.43 ± 0.15 km) due to having less thorax width (3.56 ± 0.17 cm). There was negative linear relationship between female wing loading and female flight distance ($y = -64.149x + 163.94$, $R^2 = 0.98$, $p = 0.00$; $p < 0.05$). The females from Chengdu with the maximum wing loading (71.87 ± 5.64 mg/cm^2) flew a shorter distance, whereas females from Xifeng with the minimum wing loading (41.99 ± 5.09 mg/cm^2) flew the longest distance.

Table 6. Evaluation of body size between female Asian spongy moth (*L. dispar asiatica*) populations from five different geographical locations in China. Values are presented as Mean ± SE.

Population	*n*	Thorax Width (cm)	Wing Loading (mg/cm^2)
Chengdu	20	3.56 ± 0.17 ^{c}	71.87 ± 5.64 ^{a}
Longhua	20	4.09 ± 0.20 ^{e}	46.67 ± 5.51 ^{c}
Heilongjiang	20	4.19 ± 0.21 ^{b}	44.21 ± 5.29 ^{d}
Kuduer	20	3.86 ± 0.19 ^{d}	59.41 ± 5.58 ^{b}
Xifeng	20	4.32 ± 0.22 ^{a}	41.99 ± 5.09 ^{e}

Note: Letters (a,b,c,d,e) indicate the variation in morphological features within females from different localities.



(**A**) (**B**)

Figure 7. Association of body size with the flight ability of female *L. dispar asiatica*: (**A**) Correlation between female thorax width and female flight capability; (**B**) Correlation between female thorax width and female flight capability.

4. Discussion

This research study aimed to demonstrate a comprehensive approach to investigating the effect of age, locality, mating and morphological features on the flight ability of *L. dispar asiatica* that could be implemented globally. The results of this research study significantly verified that age and locality influence the abilities of female *L. dispar asiatica*. Moreover, female populations from different locations have distinct morphological features.

During the experiment, what we have found was significant enough to conclude that age significantly impacted the flight duration and flight ability of female *L. dispar asiatica*. Flight activity peaked when the females were one day old and gradually decreased (Table 4). The species present in the Xifeng location observed the highest maximum speed (9.34 ± 0.10 km/h) on the 1st day, and it continually reduced on 2nd and 3rd days. However, the population from Chengdu showed the lowest range of maximum speed (2.64 ± 0.14 km/h) on the 3rd day (Figure 6). Yang et al. [39] found that flight activity peaked when the females of AGM were one day old and then decreased gradually. Chen et al. 2011 [40] reported that *Dendroctonus armandi* (Tsai et Li) flight decreases with aging. Muhammad et al. [41] reported drop-in flight activity with the age of diamondback moths (*Plutella xylostella* Linnaeus).

The physical atrophy of the flight muscles and decreasing enzymatic activity may be linked to the drop-in flight activity as adults age. Our results indicated that the flight ability of female *L. dispar asiatica* is directly associated with thorax width and wing loading. Females with wider thoraxes had less wing loading, which considerably improved their capacity to fly, whereas females with the shortest thoraxes had more wing loading, which had an adverse effect on flying (Figure 7). We also found prominent differences in the morphological features of female *L. dispar asiatica* adults (Table 6). Females from the Xifeng population had the longest flight due to their maximal thorax widths and lower wing loading (Figure 7). As per our research results, females from Xifeng were capable fliers with a maximum speed of 7.19 ± 0.34 km/h and a total flight distance of more than 1.8 km. Light wing loading (41.99 ± 5.09 mg/cm^2) (Table 5) helped females from Xifeng to fly long distances. Shirai [42] investigated the link between flight activity variables and forewing length in *Plutella xylostella* and discovered that flight time and distance were proportional to forewing length. Muhammad et al. [41] revealed that small adults with long forewings of *Plutella xylostella* flew more vigorously than large adults with short forewings. Casey [43] discovered that, with the decrease in the body size, wing stroke frequency rose; hence, smaller insects of similar species exhibited more flying activity. Taylor et al. [44] discovered that, in *Agrilus planipennis* (Fairmaire), the correlation of flying speed with weight load was negative. Shi et al. [28] conducted a morphometric study on 821 spongy moths from eight multiple strains and reported that average wing loading was much lower, but average wing area was significantly greater for females from flight-proficient populations.

We found no changes in average flight speed, flight duration and flight distance of mated and virgin *L. dispar asiatica* females from all five geographically different locations (Table 5), which is in line with Yang et al. [39], who reported that mating did not affect the flying attributes of female *L. dispar asiatica*.

Geographical locality significantly impacted the flight capability of *L. dispar asiatica*. We observed a clear difference between the flight duration and distance among the *L. dispar asiatica* population from five different geographical locations in China. The population from Xifeng exhibited a mean maximum flight duration and distance of 0.94 ± 0.04 h and 1.87 ± 0.18 km, the population from Longhua exhibited a mean minimum flight duration of 0.64 ± 0.04 h and the population from Chengdu exhibited a mean minimum flight distance of 1.43 ± 0.15 km. Arakawa and Iwaizumi [32] and Iwaizumi et al. [33] investigated the female *L. dispar asiatica* flight capabilities collected from five different geographical locations in Japan. They stated that, out of the five locations, the female population from Tomakomai could fly the longest distance compared to those from the other four localities. Iwaizumi et al. [33] monitored the flying ability of female *L. dispar asiatica* using a video camera from five different geographical locations. They stated that the flight distance of Asian spongy moth females from Japan was restricted to 1 km on average. Aside from that, the female population from Tomakomai had a strong flight ability. Baranchikov and Sukachev [45] reported that female *L. dispar asiatica* flew 3–5 km. Yang et al. [38] stated that the *L. dispar asiatica* females from seven different geographical regions had differences in their flight capability. Chen et al. [46] investigated the flying aptitude of *L. dispar asiatica* females from seven nations, finding that 72 percent of the 567 females examined could fly for an extended period, whereas the remainder drifted within a confined spectrum.

We successfully predicted higher flight ability for the female population of *L. dispar asiatica* from Xifeng compared to those in Kuder, Chengdu, Heilongjiang and Longhua. We also predicted that age and locality would influence female moth flight ability, as morphological features differ with locality. Therefore, we accept our hypothesis and conclude that the flight capability of female *L. dispar asiatica* is age- and locality-dependent, and the morphological features of *L. dispar asiatica* are locality-dependent. In addition, mating did not influence female flight ability.

The flight periods perceived in this research study should be measured as maximum probable values. According to our research, adult life spans were no more than 3 days, and only a few lived to the 4th day [47]. The impromptu conflict was not feasible, and the flight was driven by the absence of a landing base [48]. The anchored flight distance may not be similar to that of moths in the field. Tethering was predicted to impact flight mill functionality [44]. As a result, the flight mill system employed in the present research may not describe actual flight patterns. The flight lengths found in this study should be considered optimum values.

Spreading due to flight is an important aspect of the spongy moth life cycle; it is an essential transition from the birth host to a new reproductive host. Spongy moths are economically significant. Therefore, understanding this vital life history feature is essential for calculating the distribution and for managing the tree-killing spongy moth species. However, for females with the ability to fly, these small populations may be higher in numbers outside of commonly infected areas and further away from the edge of infection [30]. As a result, more research on the *L. dispar asiatica* flying behavior in its natural environment is required to comprehend its translocation pathways completely.

Early monitoring and deduction are highly advised. Methods such as predicting potential distributions via a model-based approach [49] should be used to precisely indicate the suitable habitat for *L. dispar asiatica* to develop. Pheromone traps baited with disparlure, cis-7,8-epoxy-2-methyl-octadecane, as used by Wallner et al. [50], should be used to capture a significant amount of *L. dispar asiatica* adults in Beijing and the surrounding areas, and emamectin benzoate (EMB) should be used, as recommended by Zhe at al. [51], which induces digestive dysfunction and nutrition metabolism disease, which hinders the development of the spongy moth.

5. Conclusions

Asian spongy moth (*Lymantria dispar asiatica*) females are strong fliers that can fly long distances. Our experiment showed that the female Asian spongy moth from the Xifeng region in China has significant potential for quick dispersal because of its flying range. We also found that 1-day-old females travel faster and have more potential to fly strong than 2- and 3-day-old adult females. In conclusion, age is the most important factor affecting an insect's flight, predominantly for Asian spongy moths. Humidity, temperature and muscle strength also affect insect dispersal rate, particularly for flying insects. Even though the average flight distance is less, flight capacity may hasten the expansion of this population. As a result, it is advised that spongy moths in Xifeng be closely monitored. In a future scenario, our study will help understand the dispersal ability and rate of Asian spongy moths in China and worldwide.

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Potential European Geographical Distribution of *Gnathotrichus materiarius* (Fitch, 1858) (Coleoptera: Scolytinae) under Current and Future Climate Conditions

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Abstract: *Gnathotrichus materiarius* (Fitch, 1858) is an alien ambrosia beetle from North America, that has been spreading across Europe since the 1930s. The species infests coniferous trees, excavating galleries in sapwood. However, to date very few studies have predicted changes in ambrosia beetle habitat suitability under changing climate conditions. To fill that gap in the current knowledge, we used the MaxEnt algorithm to estimate areas potentially suitable for this species in Europe, both under current climate conditions and those forecasted for the years 2050 and 2070. Our analyses showed areas where the species has not been reported, though the climatic conditions are suitable. Models for the forecasted conditions predicted an increase in suitable habitats. Due to the wide range of host trees, the species is likely to spread through the Balkans, the Black Sea and Caucasus region, Baltic countries, the Scandinavian Peninsula, and Ukraine. As a technical pest of coniferous sapwood, it can cause financial losses due to deterioration in quality of timber harvested in such regions. Our results will be helpful for the development of a climate-change-integrated management strategy to mitigate potential adverse effects.

Keywords: ambrosia beetle; bark beetle; MaxEnt; insect pest; alien species; niche modelling; biological invasions

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1. Introduction

Biological invasions are among the most important phenomena affecting not only biodiversity, ecosystem functioning, but also the economy [1–3]. Invasive species can lead to shifts in species composition, affecting the course of succession, as well as modifying nutrient cycling, carbon sequestration, and water balance in ecosystems [4]. In terms of the impact on economy in the last 50 years, invasive species have been responsible globally for financial losses of over 1.288 trillion USD [5].

Bark beetles and ambrosia beetles are arboreal organisms, and are represented in large numbers among alien species due to introduction events in numerous regions [6,7]. These insects are associated with wood, where they develop. Microsites under the bark of round wood and wood products protect insects against unfavourable conditions during transport. Therefore, these insects can survive long journeys and become introduced far from their natural ranges [8]. Global trade development, leading to increases in the quantity and speed of long-distance transport, has been the main cause of accidental introductions of bark and ambrosia beetles worldwide [6,9–11], classified as a contaminant pathway [12]. Europe is a target area of bark and ambrosia beetle introductions, mainly from Asia and North America [10,13].

Ambrosia beetles (Coleoptera, Scolytinae, and Platypodinae) are a polyphyletic group covering numerous species from the Scolytinae subfamily, mainly Xyleborini and Xyloterini tribes, and from the Platypodinae subfamily [14]. Ambrosia beetles differ from bark beetles in foraging type: bark beetles feed on phloem, while ambrosia beetles feed on fungi growing in tunnels created within a host plant [15–17]. The wide range of host plants and the ability to carry fungal spores in their mycangia (unique structures on the insects' bodies adapted for the transport of symbiotic fungi) are life-history traits that are particularly important for the success of their naturalisation and spread. Furthermore, the possibility of asexual reproduction of Xyleborini, allowing individual females to produce offspring outside a natural range where their population was previously absent or very sparse and scattered, is a crucial aspect in promoting the spread of this group [14]. Alien species from the Scolytinae subfamily naturalized in Europe represent over 12% of all European Scolytinae, of which most are ambrosia beetles [6,10,13]. These species usually infect weakened or dead trees and rarely kill healthy plants [18]. However, fungi carried in their mycangia might be pathogenic to particular host species, leading to economic losses [19–23]. *Gnathotrichus materiarius* (Fitch, 1858) (Figure 1) is one of the alien ambrosia beetles spreading across Europe [11,24,25]. In Europe, it was recorded for the first time in France in the 1930s [26]. It has since spread eastward and currently occurs in Austria [27,28], Belgium [29], Czechia [30], Finland [31], Spain [32], Netherlands [33], Germany [34], Poland [35], Slovenia [36], Switzerland [24], Sweden [37], Great Britain [11], and Italy [38]. The species was also reported during phytosanitary inspections of products imported to New Zealand [39]. *Gnathotrichus materiarius* (Coleoptera, Scolytinae) is an ambrosia beetle from the Corthylini tribe [27]. It is a monogamous species, with a sex ratio of 1:1, with no sibling mating nor asexual reproduction, which distinguishes *G. materiarius* from the Xyleborini tribe species [25,40–42]. Larvae and imagines of *G. materiarius* feed on a symbiotic fungus *Ambrosiozyma monospora* (Saito) van der Walt (1972) (syn. *Endomycopsis fasciculata* Batra) [43–45], recorded both in the native and secondary range of *G. materiarius* [43,46]. Imagines of the studied species occur during the whole growing period, with flight culmination in May or June [38,45,47]. *Gnathotrichus materiarius* is a technical pest of coniferous wood in the USA and Europe [38,48,49]. It prefers *Pinus*, while also infesting numerous genera of conifers: *Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, *Thuja*, or *Tsuga* [25,33,50]. Due to excavation galleries in the lower part of the trunk, it is of economic importance as a pest, decreasing the technical quality and economic value of affected timber [25,51,52]. Moreover, fungi growing in the tunnels can cause wood staining [25,31], although this phenomenon is not very common. So far, *G. materiarius* has not caused significant losses to forest management in areas beyond its natural range, as evidenced mainly by the relatively limited attention devoted to this species in scientific studies of these regions.

The progression rate of climate change intensifies its impact on ecosystems [31,52–56]. One of the predicted impacts of climate change on forest ecosystems is the increasing frequency and intensity of insect outbreaks, including alien species [57]. Due to their short life cycles and their strict dependence on temperature, insects are exceptionally responsive to climate change [58,59]. Moreover, increasing temperatures and drought intensity lead to physiological stress in trees, decreasing their resistance to bark and ambrosia beetle infestation [60–63]. The poor overall condition and lack of vigour among host plants favour the naturalisation and spread of alien species and increases their population size [18].

Previously published models predicting the future distribution of Scolytinae under changing climate conditions were created primarily for phloeophagous bark beetles of high economic importance [64–69]. In contrast, among alien ambrosia beetle species occurring in Europe, such models have been developed only for *Xylosandrus compactus* and *X. crassiusculus* [70]. For other parts of the world, data on their distributions are scarce [70,71]. Due to information deficiency and differences in the biology and ecology of bark and ambrosia beetles [49], the need to develop species distribution models is urgent and increasingly pressing.

Figure 1. Dorsal and lateral view of an adult male *Gnathotrichus materiarius* (R. Witkowski).

Gnathotrichus materiarius differs from two *Xylosandrus* spp. for which distribution models have already been developed. *Xylosandrus compactus* and *X. crassiusculus* infest broadleaved as well as coniferous tree species, and have different sex determination systems. *Gnathotrichus materiarius* is a diploidal species, while *Xylosandrus* spp. are haplodiploid inbreeders, with beetles sib-mating before the new generation of females emerges from the gallery system [40–42]. Xyleoborini males do not cover long distances and sometimes do not even leave galleries [40]. The mating behaviour of outbreeding *G. materiairus* with a balanced sex ratio is different. Males and females leave galleries and spread by active flight to find both a mate and substrate to infest [41]. Thus, *G. materiarius* is an ideal candidate for a model species, exemplifying trends for other invasive ambrosia beetles [72–74], and our results can serve as a proxy for other species sharing similar life-history traits, either already present or potentially appearing in Europe in the future. Therefore, we aimed to develop a *G. materiarius* distribution model to assess which areas will be suitable for its spread under various climate change scenarios. We hypothesized that (1) the current climatic conditions support further spread of *G. materiarius* in Europe (unsaturated climatic niche), and (2) under climate change scenarios for the 2050s and 2070s, the area of climatic suitability for *G. materiarius* (climatically suitable area) will expand.

2. Materials and Methods

2.1. Data Collection

We compiled all available records of *G. materiarius* from public databases [47,75,76] containing data from peer-reviewed publications [77–91], peer-reviewed publications not included in datasets [11,25,35,92,93], and previously unpublished communications included in a PhD dissertation [94], as along with observations collected by the authors of this paper between 2017 and 2020, (Supplementary Table S1). After data compilation, we excluded observations with incorrect coordinates or metadata suggesting a mismatch between locality description and coordinates. After excluding duplicated coordinates, we obtained 1448 data points. To reduce over-sampling in some regions and under-sampling in others, we randomly selected only one occurrence from each 0.25° grid raster cell [95]. This stratified downsampling prevented overestimation of species occurrence in regions with higher sampling effort and decreased spatial autocorrelation of data. As a result, we obtained 807 single observations that were used for analysis.

We downloaded a dataset of 19 bioclimatic variables from the WorldClim 2.1 database [96]. These variables, derived from monthly temperature and precipitation records, are widely used when modelling species distributions [97–99]. To avoid including highly correlated predictors, we checked the Pearson correlation coefficients for the pairs of bioclimatic variables. We removed the variables that were most strongly correlated with others, assuming $r > |0.7|$ as the threshold value. The final set of variables used for model development included six bioclimatic variables (Table 1).

Table 1. Bioclimatic variables used in the study.

Abbreviation	Parameter
BIO2	Mean Diurnal Range (Mean of monthly (max–temp–min temp)) (°C)
BIO7	Temperature Annual Range (BIO5-BIO6) (°C)
BIO11	Mean Temperature of Coldest Quarter (°C)
BIO15	Precipitation Seasonality (Coefficient of Variation: mean/SD × 100) (%)
BIO16	Precipitation of Wettest Quarter (mm)
BIO17	Precipitation of Driest Quarter (mm)

We used future climate projects provided by the IPCC 6th Assessment Report, based on Shared Socioeconomic Pathways (SSPs) [100]. These scenarios reflect uncertainties in possible trajectories of climate change mitigation. We chose four scenarios available in the WorldClim 2.1 database [96]: SSP126 (sustainability, the most optimistic scenario reflecting RCP2.6 from the fifth report), SSP245 (middle of the road, moderate scenario reflecting RCP4.5), SSP370 (regional rivalry, not used in the fifth report) and SSP585 (fossil-fuel based development or business-as-usual, reflecting RCP8.5). We used each SSP outcomes for four global circulation models (GCMs): IPSL-CM6A-LR (France), MRI-ESM2-0 (Japan), CanESM5 (Canada), and BCC-CSM2-MR (China), representing half of eight available GCMs for all the four SSPs. We prepared predictions for two timelines: 2041–2060 and 2061–2080. We decided to use these timelines as they are the most common frameworks for species distribution models [101–103].

2.2. Modelling Species Distribution

Before analyses, we divided the datasets into a training set (80% of observations), used for model development, and an independent validation set (20% of observations). The use of an independent dataset for model evaluation prevents model overfitting, which could limit our conclusions to the dataset range. Due to the presence-only character of distributional data, we used the MaxEnt algorithm to develop species distribution models. MaxEnt has been developed to process presence-only data [104,105]; in contrast to parametric models and other classification tools, it does not need absence data in the theoretical assumptions, instead using background data (the so-called pseudoabsences). We used default MaxEnt settings. For the species, we randomly selected 10,000 pseudoabsence points (background points). Thus, MaxEnt analysed patterns of presence distinct from the background data. Therefore, the prevalence of background points makes the model more conservative, requiring a stronger signal than would be the case for equal proportions of presences and pseudoabsences [105]. We assessed model quality using the area under the receiver operator curve (AUC) as a metric depending on true positive and true negative rates (i.e., positive and negative rates overlapping the real and predicted occurrence). The output of the MaxEnt model is the probability that a particular species can occur in a particular raster cell. To obtain presence–absence information, we calculated the threshold—the probability value with the highest sum of sensitivity (true positive rate) and specificity (true negative rate). Such an approach balances false negatives and false positives [106]. The MaxEnt model also provides information about variable importance, expressed as per cent contribution to the model, as well as response curves, showing how the model output changes along with studied variables. We used the "dismo" package [107] for MaxEnt model development, and the "raster" [108] and "sf" [109] packages for spatial data processing. According to the model, we calculated potential range saturation as a proportion of sampled points and the number of pixels suitable for particular species occurrence.

We applied models to maps of current and future climatic scenarios to obtain predictions of *G. materiarius* distribution. For each SSP we averaged predicted species occurrence probability across the four GCMs, to reduce uncertainty connected with particular GCMs [110–112]. Then, maps with threshold values (true/false) were used to estimate the changes in the potential range, changing the values on maps with the future potential range from 1 to 2 [113]. As a result of the calculations presented below, four different variants of change in the range of the species were estimated: (i) areas still unsuitable for colonisation ($0 - 2 \times 0 = 0$)—no changes, the species was not present, and the prevailing conditions will remain unsuitable for colonisation; (ii) range expansion ($0 - 2 \times 1 = -2$)—areas potentially suitable for colonisation; (iii) range contraction ($1 - 2 \times 0 = 1$)—areas where the species is currently present but will fall outside the future optimal climate; (iv) persistence ($1 - 2 \times 1 = -1$)—areas where the species is currently present and will remain within the optimal climate [113].

3. Results

3.1. G. materiarius Distribution Model

The Maxent models produced reliable *G. materiarius* distributions with a very high AUC value (0.98). The predicted threshold of presence was 0.2548. Precipitation of the driest quarter (bio17) was the most important predictor, with an average contribution of 58.7%, whilst less critical were the annual temperature range (bio7) and the precipitation seasonality (bio15) (Figure 2). Individual response curves of the two strongest bioclimatic variables showed that the predicted probability of the presence of *G. materiarius* was positively correlated with these factors (Figure 2).

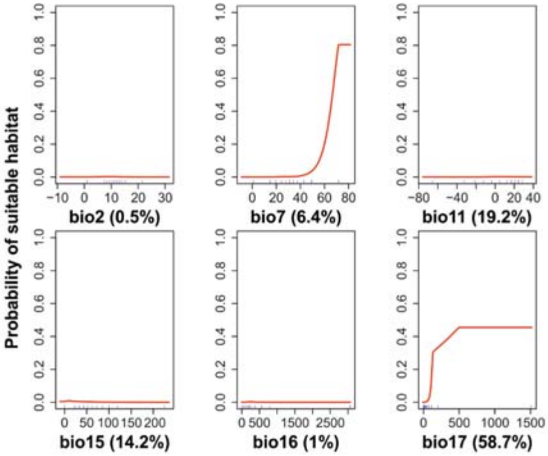

Figure 2. Response curves present how predicted niche suitability for *Gnathotrichus materiarius* changed along particular predictor gradients. Numbers in parentheses indicate the relative importance of variables. Variable importance, i.e., the proportion of variation, explained by particular variable based on AUC [113] gain for a single feature.

3.2. Current Potential Distribution

The MaxEnt model developed for current climate conditions reported habitat suitability for 98% of localities where *G. materiarius* was already documented. Furthermore, it identified areas where *G. materiarius* was not noted, but climate conditions allow for its development. In Europe, such highly suitable areas are concentrated in the Balkans (Albania, Bulgaria, Bosnia and Herzegovina, Montenegro, Romania, Serbia, Slovenia), the Black Sea and Caucasus region (Armenia, Georgia, Turkey, Russia), the Baltic countries (Lithuania, Latvia, and Estonia), the Scandinavian Peninsula and Ukraine (Figure 3). Our model indicated that currently *G. materiarius* can find suitable climate conditions across 13.1% of Europe.

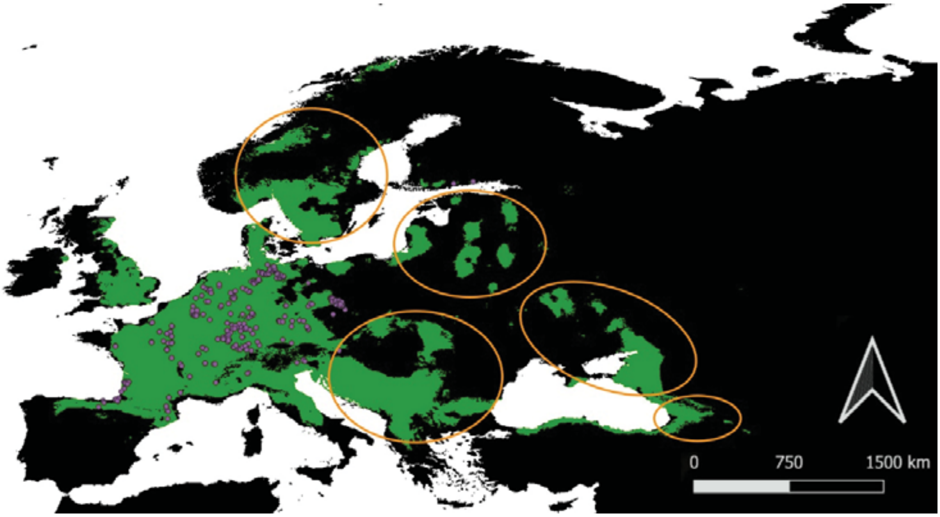

Figure 3. Predicted habitat suitability for *Gnathotrichus materiarius* under current climate conditions (green area), localities of known occurrence records (violet points), and crucial new potential sites that have not been colonised yet.

3.3. Predicted Range Shifts

The model predicted an increase in habitat suitability for *G. materiarius* in European countries for all the scenarios in both periods (Figure 4.). For each subsequent SSP, from the most optimistic (SSP126) to the least (SSP585), an increase was obtained in the number of cells where *G. materiarius* can find suitable conditions (Table 2). In the period 2041–2060 predicted habitat suitability increases to approx. 20.4%–25.2%, while in the period 2041–2060 it increases to ca. 20.8%–31.2% of the area in Europe. Predictions for the most pessimistic SSP585 in the period 2061–2080 indicate that with changing climate, the range of the species will increase mainly eastward to the Ural Mountains and northward almost to the Arctic Circle on the Scandinavian peninsula. Only slight shifts in the western and southern range of the species were forecasted.

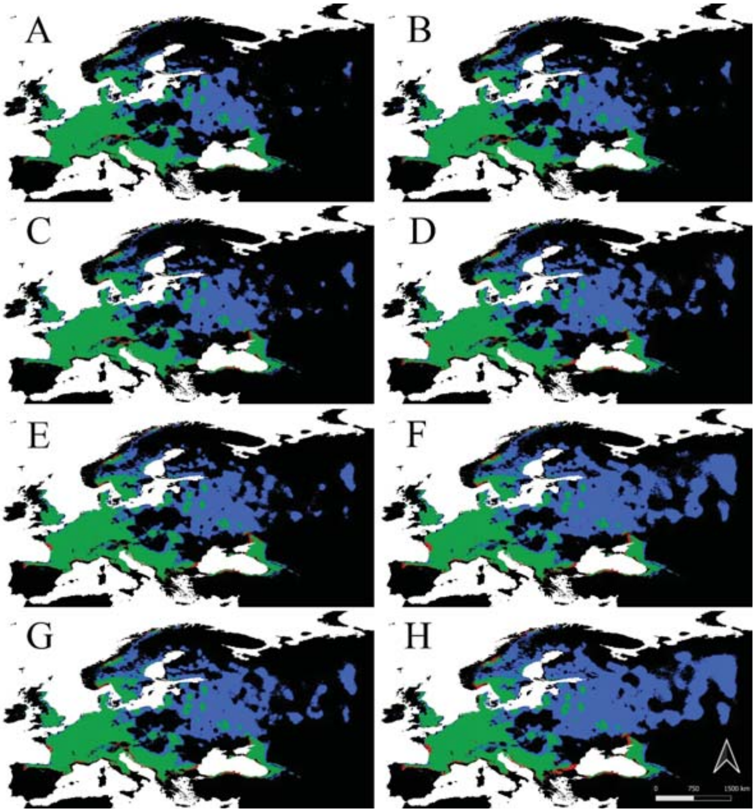

Figure 4. Maps of predicted shifts of *Gnathotrichus materiarius* climatic suitability, averaged across four GCMs in Europe for SSP126 (**A**,**E**) SSP245 (**B**,**F**) SSP370 (**C**,**G**) SSP585 (**D**,**H**) in two timelines: 2041–2060 (**A**–**D**), 2061–2080 (**E**–**H**). Green—persistence, blue—range expansion, red—range contraction.

Table 2. Predicted shifts of *Gnathotrichus materiarius* range size under analyzed SSP and timelines related to actual habitat suitability.

Shared Socioeconomic Pathway (SSP) and Timeline	Range Expansion (Absent/Present) (%)	Persistence (Present/Present) (%)	Range Contraction (Present/Absent) (%)	Net Shift (Expansion–Contraction)
SSP126 2041–2060	7.53	11.35	0.25	7.28
SSP245 2041–2060	9.09	11.27	0.32	8.77
SSP370 2041–2060	10.39	11.15	0.45	9.94
SSP585 2041–2060	12.53	11.13	0.47	12.06
SSP126 2061–2080	8.04	11.29	0.31	7.73
SSP245 2061–2080	11.88	11.14	0.46	11.43
SSP370 2061–2080	16.27	11.05	0.55	15.72
SSP585 2061–2080	18.86	10.87	0.72	18.14

4. Discussion and Conclusions

Our results indicate that the species has not yet already reached its maximum extent within the climatically suitable area. The study indicates that the species can spread and reach more sites, because a low level of niche saturation is typical of the beginning of the second stage of invasion—the 'log phase', which comes after the 'lag phase' [114]. Our prediction developed for current climate conditions (Figure 1) pointed out the most likely areas where the species can spread. The Balkans and the Baltic countries are highly suitable regions situated a short distance from the known locations of *G. materiarius*, and there are no natural barriers that would prevent the colonisation of this area. Although the Black Sea region is a considerable distance from these places, the natural spread of *G. materiarius* from the Balkans along the coastline to the Caucasus cannot be excluded. It should be remembered that trade plays an essential role in the spread of species, and was the probable source of the appearance of *G. materiarius* in Europe. Another factor that supports our prediction is a wide range of host tree species for *G. materiarius*, which would not limit the colonization process [115].

Climate change has a natural effect on the distribution of species and can also affect some aspects of their biology. Shortening the development cycle of beetles and thus increasing the number of generations during the year may have serious economic effects, as has already been observed in the case of other bark and ambrosia beetles [58,116]. In south-western Poland, where the species has been present since 2015 [35], it causes economic losses as a technical pest. However, additional studies are necessary to determine the exact scale of the phenomenon. Changes in precipitation patterns can significantly impact trees, triggering stress-induced ethanol accumulation [117]. Therefore, higher susceptibility to ambrosia beetle infestations and thus an increase in their economic importance may be reliably inferred.

We have sparse occurrence data including coordinates for most alien bark and ambrosia beetle species present in Europe. The distribution of *G. materiarius* is one of the better-documented, and has allowed us to develop a high-accuracy model. Developing such models is economically crucial because results can be used to design more detailed surveys in future, and thus facilitate better planning for the usage of limited funds and human resources. Furthermore, such models can be used to estimate the rate and direction of invasions of ambrosia beetle species with similar ecological habitats, but with less well recognised distributions.

However, it should be taken into account that climate change affects species and their populations directly (temperature, precipitation) and indirectly (by affecting antagonistic and symbiotic organisms or food bases) [118,119]. Therefore the effects of climate change are difficult to predict precisely. Moreover, the studied species has not colonised all suitable habitats within the study area. Consequently, it is not in equilibrium, which also affects the reliability of estimations for the studied species. In order to improve the model it will be necessary to collect coordinates of the places of occurrence, and make these available in the databases. Additionally, especially in high-risk areas, monitoring with dedicated or wide-range lures [41,120] is indicated for the rapid identification of threats, which may be crucial to ensure their reduction or control.

Our study provided a species distribution model based on climatic variables and presence-only data. Due to its correlative nature, our model explains patterns in data and does not reflect physiological processes [105,106]. Moreover, our model allows only conclusions for the predicted climatic niche, not actual occurrences that would be affected by other factors, especially dispersal limitation and land-use patterns.

The presence of a suitable host plant is also essential for beetle development. Models for *Pinus sylvestris* and *Picea abies* predict shifts to the north in their ranges, with potential contraction in the south [118]. However, it is hard to anticipate which species will replace them. So far, such models have been developed only for a subset of native and a few alien tree species, neglecting those with relatively small ranges. However, such species can become widespread and increase in economic importance [121]. The climate is the primary determinant of species distributions on a large spatial scale, affecting other interactions [122]. Another source of uncertainty in our model is the dependence on GCMs used in the study [110–112]. We decreased this uncertainty by averaging model predictions for four different GCMs.

Nevertheless, the results obtained in the study indicate which regions are more vulnerable to the occurrence of *G. materiarius* (Figure 4.), and thus the area where phytosanitary services need to focus to this species. Such actions could slow down the spread of the species in Europe through transported wood and wood products, which have been the major source of alien bark and ambrosia beetle invasions [123]. Phytosanitary measures including heating and fumigation may reduce the probability of invasions, although due to the depth of the galleries, which may cover the entire sapwood, they do not guarantee the neutralisation of all specimens. Another threat is related to ornamental plants, mainly Thuja and Tsuga, which are transported alive, thus limiting the range of the methods used for treating raw wood (e.g., heating and drying).

Our study provides the first assessment of *G. materiarius* potential current and future ranges in Europe. This study, together with models obtained for two *Xylosandrus* species, are the only predictions of the potential spread of ambrosia beetles. Therefore, our study provides quantitative foundations for spread prevention and risk assessments necessary for conservation biogeography [124].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13071097/s1, Table S1: Coordinates used to develop the model of the occurrence of *Gnathotrichus materiarius*.

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Infestation of Early- and Late-Flushing Trees by Spring Caterpillars: An Associational Effect of Neighbouring Trees

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Abstract: In temperate forests within Europe, early-flushing (EF) deciduous trees are often heavily infested by early spring leaf-eating Lepidoptera, while late-flushing (LF) trees are better protected in a phenological manner against such heavy infestations, as spring moth larvae begin to appear before their bud burst. The associational effects of EF trees on LF ones are only poorly known. We studied whether or not the infestation of LF trees by spring Lepidoptera can be affected by EF ones if they grow in the immediate vicinity. We compared spring assemblages of leaf-eating larvae of Lepidoptera on LF *Quercus cerris* L. with those on EF *Q. pubescens* Willd. in several microhabitats in Slovakia, Central Europe. Larvae were collected from mature and young trees. Mature trees sampled were growing: (1) in a closed-canopy forest; (2) in small groups; or (3) as a lone tree. Forest and tree groups are both constituted by oak species. Tree groups and lone trees were 20–50 m distant from forest edges. Young trees were growing (1) under mature *Q. pubescens* trees in a forest or (2) as a lone tree within forest gaps or near the edges. In the closed-canopy forest where LF trees (*Q. cerris*) were surrounded by EF ones (*Q. pubescens*), the caterpillars on mature LF trees were in abundance, almost as on mature EF ones. The species composition of larval assemblages on the two oak species was similar. In contrast, on small groups and on lone trees, the lepidopteran larvae were significantly less abundant on LF trees than EF ones. In the case of young trees, the abundance of larvae and their composition assemblages on both oaks were comparable in the forest. In the open habitat, LF trees were less infested by larvae than EF ones and the assemblages of moth larvae differed between the two. Our results reveal the effect (associational susceptibility) of EF trees on LF ones when growing in a close vicinity. It means that the phenological protection of LF trees may not be sufficient if they grow close to or are surrounded by EF ones.

Keywords: associational susceptibility; *Quercus*; forest protection; phenological synchrony; *Operophtera brumata*; *Agriopis leucophaearia*; bud burst; herbivory

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1. Introduction

In temperate European forests, many early-flushing (EF) deciduous trees host numerous species of spring-feeding caterpillars of Lepidoptera, among them well-known *Operophtera brumata* (Linnaeus, 1758), *Erannis defoliaria* (Clerck, 1759), *Agriopis* spp., *Tortrix viridana* (Linnaeus, 1758), *Lymantria dispar* (Linnaeus, 1758), etc. [1–12]. They often cause defoliation of woody plants in forests or fruit trees.

The successful development of these Lepidoptera depends on synchrony between the hatching of caterpillars and the bud burst of host trees [13–21]. Neonates from folivorous caterpillars of early spring Lepidoptera have access to suitable food if they hatch or activate after overwintering inside the narrow phenological window occurring right after the bud burst of their hosts. If they hatch too early—before the bud burst, they starve because flushing buds and young foliage are lacking [14,19,22]. Spring caterpillars can resist starvation only for a few days [14]. On the other hand, if they do it too late—relatively long after the bud burst, they also suffer from a lack of suitable food (i.e., young foliage) on host trees because the increased content of non- or low-digestible compounds and protective chemicals in maturing and mature leaves have a negative impact on caterpillar performance [19,23–29].

Caterpillars hatching early and late, which miss the window of opportunity for feeding on young foliage on the primary host tree (the one where they are born) need to locate food, usually on another tree. Baby caterpillars can achieve long-distance dispersal through ballooning—they use a silk thread or long setae on their bodies to float on air currents or to be borne by wind [30–38]. Caterpillars can leave unsuitable places also by lowering on a silk thread or dropping [31,39–41], or by walking from one tree to another where they touch each other [42].

The effects of woody plant diversity on herbivory in forests have often been studied with variable results [43–49]. Neighbouring trees can increase or decrease insect attacks to those that are at the centre (associational susceptibility or resistance) [50]. It is generally accepted that insect herbivory is lower in mixed forest stands than in pure stands, i.e., associational resistance e.g., [51–53]. However, many authors emphasise that beyond tree diversity *per se*, these effects are strongly dependent on the traits of host trees as well as the specificity of insect phytophages e.g., [51,53–57].

There is a little knowledge on fine-scale mechanisms leading to the associational effects of neighbouring plants on those that are at the centre [45,46,48,57–59]. For example, herbivores can move from one host plant to another neighbouring one [50]. There are few data on how EF trees affect the occurrence and abundance of spring caterpillars on late-flushing (LF) ones. Nealis and Régnière [60] recorded redistribution of late-instar caterpillars belonging to *Choristoneura fumiferana* Clemens, 1865, from damaged EF to undamaged LF host trees. Schafellner et al. [61] supposed that older caterpillars of *Lymantria dispar* left primary food places on *Quercus petraea* (Matt.) Liebl. trees and moved to young foliage on LF *Q. cerris* L. trees. Wesołowski and Rowiński [7] stated that LF oaks (*Quercus robur* L.) co-occurring with EF trees were visibly defoliated only during an outbreak.

Mixed deciduous forests comprising trees with various stages of bursting are widespread in temperate Europe [62–67]. We assume, therefore, that neighbourhood of early- and late-flushing co-occurring trees can significantly affect the infestation of the latter by spring Lepidoptera.

Studies on defoliators in fragmented forests or, in general insect herbivores in fragmented habitats, have brought conflicting results [47,68–71] which reflect the specific conditions of habitats (fragment size and quality, degree of insulation) and the characteristics of studied organisms (host plants and insect phytophages) [70,72–78]. To our knowledge, there is no study published about the impact of spring caterpillars, from EF trees on LF ones in small forest fragments.

Oaks (*Quercus* spp.) are among the most infested deciduous trees by early spring Lepidoptera in Central Europe [4,5,79,80]. From all oak species in this region, *Q. cerris* is the last one in terms of bud bursts [81] and, compared with other oaks, this delay is approximately two weeks [61,82]. The *Quercus cerris* often grows with EF deciduous woody species in mixed forests and is also frequent in forest fragments [66,83,84].

Quercus cerris and *Q. pubescens* Willd. are well adapted to moderate drought stress in summer, and due to a climate change, their increasing importance is expected in temperate European forests [85–91] as well as in Central Europe [92].

We studied (1) whether the infestation of mature LF trees by caterpillars can be affected by neighbouring mature EF ones in the forest interior and at a distance from it—in small mixed tree groups 20–50 m away from the edges, and (2) whether the infestation of young LF trees by caterpillars can be by neighbouring mature EF ones. We focused on early spring leaf-chewing caterpillars belonging to the group of “brumata-viridana complex” [10] on two co-occurring oak species—the LF Turkey oak (*Quercus cerris*) and the EF pubescent oak (*Q. pubescens*). The caterpillar assemblages, usually dominated by the well-known pests *Operophtera brumata* and *Tortrix viridana* (hence the name “brumata-viridana complex”), comprise numerous lepidopteran species occurring first in spring and being synchronised with the bud burst of EF host trees [10].

We suppose that conditions for the development of neonate (first instar) caterpillars on *Q. cerris* are unfavourable due to late bud burst. Consequently, in early spring, the caterpillar abundance on LF *Q. cerris* would be lower than on EF *Q. pubescens.* Later, when the young leaves of *Q. cerris* unfold, caterpillars can switch their hosts to *Q. cerris* trees and profit from the food with higher nutritional quality occurring on this oak species [61]. The probability of *Q. cerris* being infected by caterpillars from *Q. pubescens* would be the highest in the case of trees in close neighbourhood belonging to both oak species [63,93]. We assume, therefore, that the caterpillar abundance on mature LF *Q. cerris* in a closed-canopy forest surrounded by EF *Q. pubescens* will be higher than the one on LF *Q. cerris* located out of the forest—in small mixed tree groups and solitary trees. Similarly, caterpillar assemblages on young LF (low) *Q. cerris* growing under the crowns of mature EF *Q. pubescens* are expected to be enriched by caterpillars from those mature crowns, unlike the young trees in open space [94]. Thus, mature and young LF trees that are more isolated from EF ones should be less infested by caterpillars.

Our study is clarifying one little-known aspect of a fine-scale mechanism leading locally to the tree diversity effects on herbivory cf. [48,59,74].

2. Materials and Methods

2.1. Studied Area

The research was carried out in Krupinská planina plateau (Southern Slovakia; 48°10′0.19″ N, 18°59′46.08″ E) in the southern part of the Western Carpathians Mts, at altitudes between 265 m and 330 m a.s.l. The study area belongs to a warm region with mean annual air temperatures 8–9 °C and mean annual precipitations 600–700 mm [95]. It is covered with xeric and thermophilous vegetation consisting of an oak forest and a forest-steppe. The share of *Quercus pubescens* in the forest was accounting for about two thirds of trees, and that of *Q. cerris* about one third. There are other tree species much less frequent and less abundant (up to 2%), such as *Acer campestre* L., *A. tataricum* L., *Tilia* spp., *Sorbus torminalis* (L.) Crantz., *Crataegus* spp., *Carpinus betulus* L., *Pyrus* spp. and *Ulmus* spp. The average height of mature oaks is 8–12 m and their age is about 70 years. Young trees and saplings are scattered in the forest understory. The adjacent forest-steppe constitutes a grassland with small tree patches and groups, and solitary trees. Like in the forest, EF *Q. pubescens* and LF *Q. cerris* trees are the most frequent here. Several shrub species such as *Rosa* spp., *Crataegus* spp., *Ligustrum vulgare* L. and *Prunus spinosa* L. also grow in tree patches. The study area was approximately 100 ha (50 ha of forest, 50 ha of forest steppe).

2.2. Data Collecting

Caterpillars were obtained from oaks by beating tree branches over a beating tray with 1.0 m diameter [96], i.e., samples originated from one-metre-long branches or one-metre-long terminal parts of them. Collections were carried out during the non-outbreak period in the first week of May in years 2015 and 2016.

In the first year, caterpillars were collected from mature LF *Q. cerris* and EF *Q. pubescens* growing in three types of microhabitats: (1) in a forest; (2) in small mixed tree groups (3–6 trees) composed of both oak species growing in a forest-steppe out of a dense forest; and (3) as solitary (lone) trees also out of it. Regarding the forest, caterpillars were sampled in the forest interior (canopy 80%) from mature trees located at a distance of 30–50 m from the edges, and at least 20 m from each other.

Every LF *Q. cerris* tree on which the caterpillars were collected was surrounded by EF *Q. pubescens* with crowns touching those of *Q. cerris* trees. Also crowns of *Q. cerris* within the mixed groups touched those of *Q. pubescens* trees. Mixed tree groups and solitary trees selected for this study were at a distance of 20–50 m from forest edges, other trees or groups. From each oak species in every microhabitat (i.e., forest, mixed tree groups, and lone trees), 11 samples of caterpillars were obtained, which are 66 (2 × 3 × 11) if taken together. One sample represented caterpillars beaten from three branches (up to 3 m from the ground level), from one mature tree.

In the following year, caterpillars were collected from young LF *Q. cerris* and EF *Q. pubescens* (each tree up to 2 m high) growing in two types of microhabitats: (1) in the forest interior under the crowns of mature *Q. pubescens* at a distance of 30–50 m from the edges and (2) in open forest glades or edges, as solitary (lone) trees. These solitary young trees were located at 3–5 m from the crowns of mature trees belonging to any species, including *Q. pubescens*, and at a distance of at least 10 m from other young trees (Figure 1). From each oak species in every microhabitat (i.e., in a forest under the crowns of *Q. pubescens*, and out of forest in open glades or edges), we collected 15 samples of caterpillar assemblages, which are 60 (2 × 2 × 15) if taken together. One sample represented caterpillars beaten from two branches, from one young tree. Since the mixed groups of young trees composed of LF *Q. cerris* and EF *Q. pubescens* altogether were scarce in the study area, we did not include that kind of microhabitat in our research.

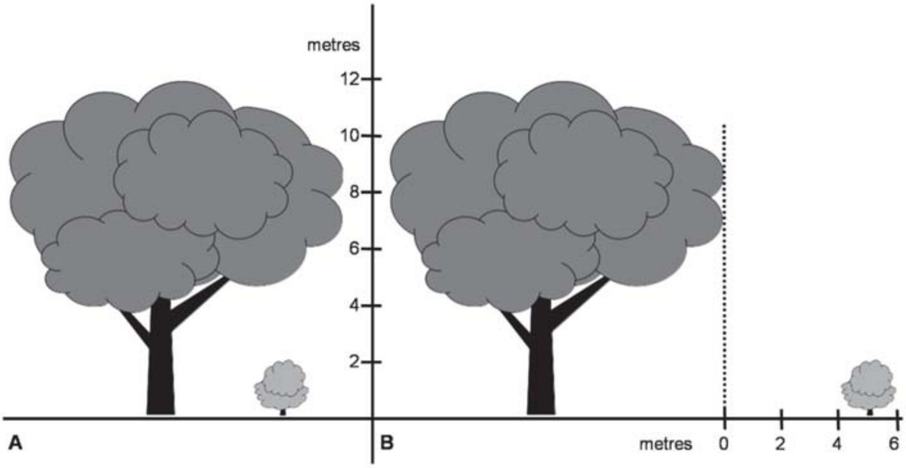

Figure 1. Position of young trees selected to sample caterpillars (mature trees—*Quercus pubescens*; young trees—*Q. cerris* or *Q. pubescens*; (**A**)—in the forest interior; (**B**)—in open forest glades or edges [as a solitary tree]).

Caterpillars were preserved in 75% ethanol and identified in the laboratory by using guides [2,82,97,98]. The nomenclature of lepidopteran species follows Pastorális et al. [99]. Two species, *Agriopis marginaria* (Fabricius, 1776) and *A. aurantiaria* (Hübner, 1799), which caterpillars are difficult to distinguish from each other according to their external morphology, were considered as a single taxon in the analyses.

2.3. Data Analyses

The caterpillar total abundance, and the abundance of *A. leucophaearia* (Denis and Schiffermüller, 1775) and *O. brumata*, were statistically compared using analysis of deviance (ANODEV), for mature and young LF *Q. cerris* and EF *Q. pubescens* separately. The ANODEV model with a negative binomial error distribution and a log-link function was used to test the effect of tree species and forest structural combinations on the abundance. Permutational multivariate analysis of variance (perMANOVA) was employed for testing the effect of tree species and forest structural combinations on the composition of species assemblages [100]. Data on species abundance were log(x + 1) transformed and the Bray-Curtis dissimilarity index [101] was used. The results were presented using the non-metric multidimensional scaling ordination technique (NMDS) [102].

The significant level of 0.05 was applied. Statistical analyses and graphical outputs were made in R [103] package boot [104], also using ggplot2 [105], MASS [106], multcomp [107] and vegan [108].

3. Results

3.1. Caterpillars on Mature Trees

Abundance (Figure 2). Caterpillars on LF *Q. cerris* were abundant only in the forest interior and this differed significantly from that on *Q. cerris* in mixed tree groups (z = −4.280, $p < 0.001$) and on lone trees (z = −4.796, $p < 0.001$). In contrast, caterpillars on EF *Q. pubescens* were in abundance in all microhabitats without differences between forest and non-forest environment. A difference between *Q. cerris* and *Q. pubescens* within the forest was considerable but not significant (z = 2.413, $p = 0.088$).

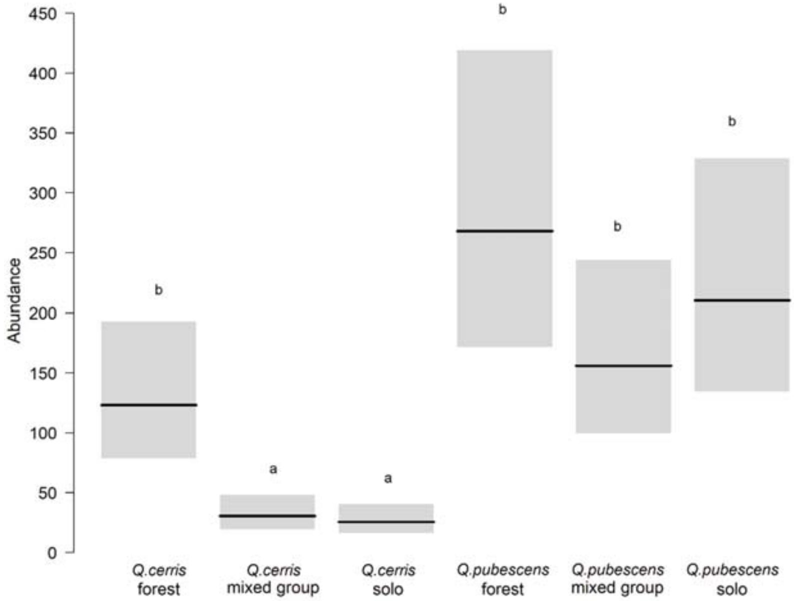

Figure 2. Abundance of caterpillars (number of individuals on three branches, each 1 m long) on mature LF *Quercus cerris* and EF *Q. pubescens* located in the forest interior (forest) and out of it, in mixed tree groups composed of *Q. cerris* and *Q. pubescens*, and on lone (solo) trees. A horizontal line denotes the mean, and bars the 95% confidence intervals. Distinct letters above columns indicate a significant difference.

Species composition (Figure 3). Caterpillar assemblages on LF and EF trees did not differ between each other in the forest interior (F = 2.094, $p = 0.105$) but they significantly did in a non-forest environment—in mixed tree groups (F = 11.258, $p < 0.001$) and on lone trees (F = 12.639, $p < 0.001$). Assemblages on *Q. cerris* in microhabitats out of the forest had a similar composition (F = 0.487, $p > 0.100$) but they were different from those in forest (mixed groups: F = 4.660, $p = 0.003$; lone trees: F = 5.836, $p < 0.001$). The same was found for assemblages on *Q. pubescens* (mixed groups vs. lone trees: F = 2.104, $p > 0.05$; mixed groups vs. forest: F = 4.331, $p = 0.001$; lone trees vs. forest: F = 4.709, $p < 0.001$).

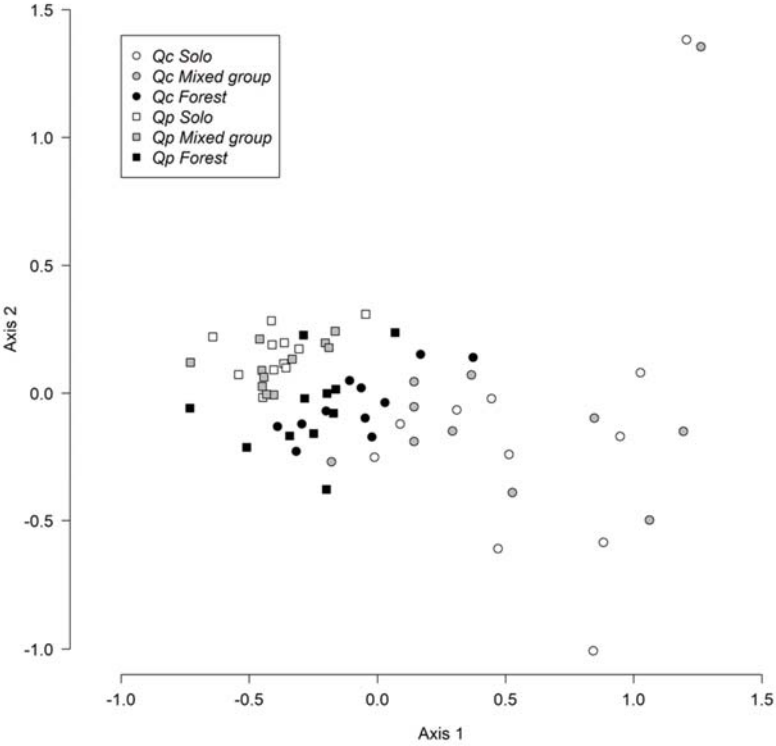

Figure 3. Non-metric multidimensional scaling of caterpillar assemblages on mature LF *Quercus cerris* and EF *Q. pubescens*. (Qc—*Q. cerris*, Qp—*Q. pubescens*, Solo—lone trees, Mixed group—mixed tree groups composed of *Q. cerris* and *Q. pubescens*, Forest—trees growing in the forest interior).

Agriopis leucophaearia (Figure 4). This species was predominant in all microhabitats on both oak species (LF *Q. cerris*—dominance in forest: 87%, in mixed tree groups: 72%, on lone trees: 78%; EF *Q. pubescens*—in forest: 91%, in mixed groups: 72%, on lone trees: 69%), significantly influencing the abundance of caterpillar assemblages. On *Q. pubescens*, this moth was abundant in all microhabitats. In contrast, on *Q. cerris* it was abundant only in the forest interior and much less abundant in mixed tree groups ($z = -4.111$, $p < 0.001$) and on lone trees ($z = -4.342$, $p < 0.001$).

Operophtera brumata (Figure 5). The second most abundant moth also appeared on the two oak species (LF *Q. cerris*—dominance in forest: 4%, in mixed tree groups: 2%, on lone trees: 2%; EF *Q. pubescens*—in forest: 3%, in mixed groups: 11%, on lone trees: 19%). Its caterpillars on both oaks were similarly abundant in the forest interior ($z = 1.577$, $p > 0.100$). On *Q. cerris,* they were significantly less in abundance out of it—in mixed tree groups ($z = -3.708$, $p < 0.001$) and on lone trees ($z = -4.072$, $p < 0.001$). In contrast, on *Q. pubescens*, they were more abundant in mixed tree groups ($z = 2.621$, $p = 0.034$) and especially on lone trees ($z = 5.258$, $p < 0.001$) when compared with the forest interior.

3.2. Caterpillars on Young Trees

Abundance (Figure 6). Caterpillars were abundant on both oak species growing under mature EF *Q. pubescens* in the forest interior and did not differ significantly between each other ($z = 1.275$, $p > 0.100$). Those on LF *Q. cerris* were less abundant on lone trees than in the forest interior ($z = -3.520$, $p = 0.002$) while caterpillars on EF *Q. pubescens* were also in abundance on lone trees, and this did not differ from that within the forest ($z = -1.296$, $p > 0.100$).

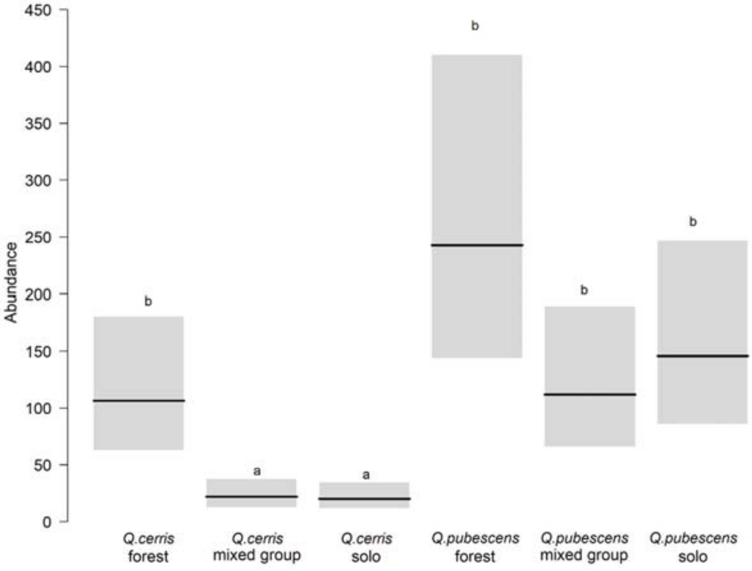

Figure 4. Abundance of *Agriopis leucophaearia* caterpillars (number of individuals on three branches, each 1 m long) on mature LF *Quercus cerris* and EF *Q. pubescens* located in the forest interior (forest) and out of it, in mixed tree groups composed of *Q. cerris* and *Q. pubescens*, and on lone (solo) trees. A horizontal line denotes the mean, and bars the 95% confidence intervals. Distinct letters above columns indicate a significant difference.

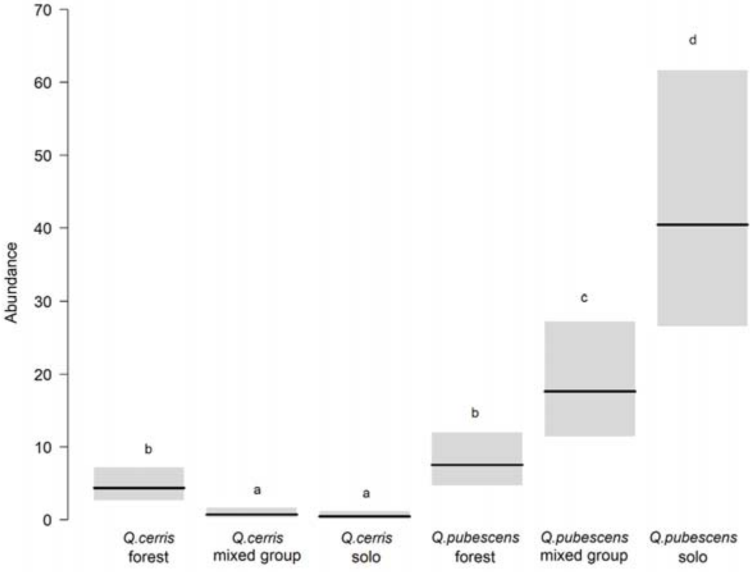

Figure 5. Abundance of *Operophtera brumata* caterpillars (number of individuals on three branches, each 1 m long) on mature LF *Quercus cerris* and EF *Q. pubescens* located in the forest interior (forest), and out of it, in mixed tree groups composed of *Q. cerris* and *Q. pubescens*, and on lone (solo) trees. A horizontal line denotes the mean, and bars the 95% confidence intervals. Distinct letters above columns indicate a significant difference.

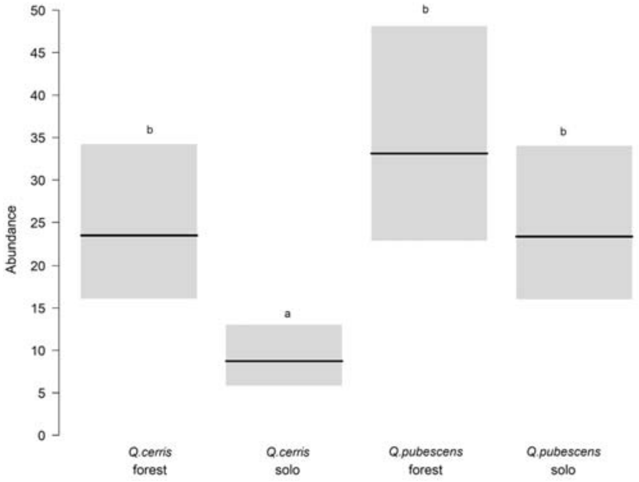

Figure 6. Abundance of caterpillars (number of individuals on two branches, each 1 m long) on young LF *Quercus cerris* and EF *Q. pubescens* located in the forest interior and on lone (solo) trees in open forest glades or edges. A horizontal line denotes the mean, and bars the 95% confidence intervals. Distinct letters above columns indicate a significant difference.

Species composition (Figure 7). Caterpillar assemblages on LF *Q. cerris* and EF *Q. pubescens* did not differ between each other in the forest interior (F = 2.586, p = 0.082) but they significantly did in a non-forest environment, i.e., on lone trees (F = 3.100, p = 0.014). In the case of *Q. cerris*, assemblages on lone trees were different from those within the forest (F = 3.136, p = 0.014) while for *Q. pubescens*, they were similar on lone trees and in the forest interior (F = 0.778, p > 0.1).

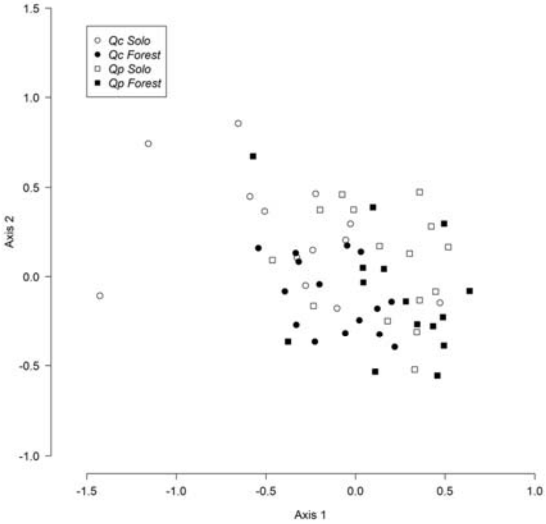

Figure 7. Non-metric multidimensional scaling of caterpillar assemblages on young LF *Quercus cerris* and EF *Q. pubescens*. (Qc—*Q. cerris*, Qp—*Q. pubescens*, Solo—lone trees growing in open forest glades or edges out of the crowns of mature *Q. pubescens*, Forest–trees in the forest interior under the crowns of mature *Q. pubescens*).

Agriopis leucophaearia (Figure 8). It was a predominant species also on young LF *Q. cerris* (dominance in the forest interior: 83%, lone trees: 77%) and EF *Q. pubescens* (within the forest: 70%, lone trees: 67%). In the forest interior, there was a non-significant difference between caterpillar abundances on *Q. cerris* and *Q. pubescens* ($z = 0.559$, $p > 0.100$). On lone trees, their abundance on *Q. cerris* was significantly lower than that in the forest interior ($z = -3.390$, $p = 0.005$) while on *Q. pubescens* it was relatively high, and the difference between abundances, in both microhabitats, was not significant ($z = -1.309$, $p > 0.100$).

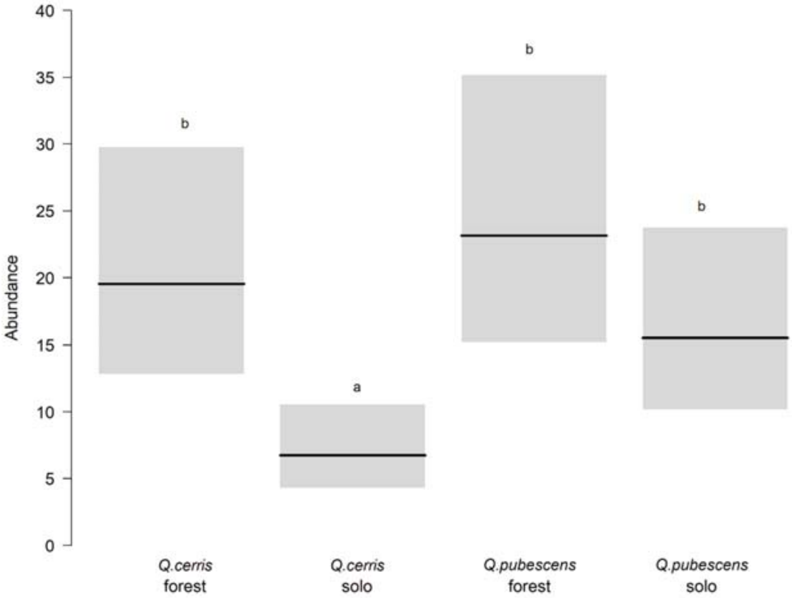

Figure 8. Abundance of *Agriopis leucophaearia* caterpillars (number of individuals on two branches, each 1 m long) on young LF *Quercus cerris* and EF *Q. pubescens* located in the forest interior and on lone (solo) trees in open forest glades or edges. A horizontal line denotes the mean, and bars the 95% confidence intervals. Distinct letters above columns indicate a significant difference.

4. Discussion

Our research has revealed that caterpillar assemblages on LF *Q. cerris* and EF *Q. pubescens* were similar in the closed-canopy mixed forest composed of both LF and EF trees and different on those growing out of it. In the forest, the caterpillar abundance on mature LF trees almost reached that on mature EF trees (insignificant difference). In microhabitats out of the forest—on small mixed tree groups composed of both species and on lone trees, caterpillars on LF oaks were significantly less abundant than on EF ones. Moreover, the species composition of their assemblages on LF *Q. cerris* and EF *Q. pubescens* was similar in the forest interior but different in microhabitats out of it. It suggests an increased infestation by caterpillars (measured as a caterpillar abundance) of LF trees surrounded by EF ones in forest (associational susceptibility). This latter effect could be the result of a spill-over where herbivores move from neighbouring primary host trees (*Q. pubescens*) onto secondary host trees (*Q. cerris*) at the centre.

Early spring Lepidoptera, as recorded, can develop on the leaves of both studied oak species [5,9,10,79,109,110], but caterpillars in Central Europe hatch synchronously with opening buds of EF *Q. pubescens* [5]. Previous studies have reported low abundances of caterpillars on *Q. cerris* [9,10,31,111] and J. Liška, pers. comm. These low abundances observed without taking into account the possible effect of neighbouring trees may reflect environmental conditions which are harsher for first-instar caterpillars on this LF oak species than on other oaks. Our results have also confirmed the significantly lower abundance of caterpillars on LF *Q. cerris* than on EF *Q. pubescens* for solitary growing trees.

We studied the associational effect of neighbouring trees on spring caterpillar assemblages feeding on two oak species. The abundance and composition of these assemblages were mostly determined by two dominant moth species, *A. leucophaearia* and *O. brumata*, occurring frequently and in abundance on Central European oak species [2–5,9–12]. As for other tree species and their caterpillar assemblages, further research is needed.

Females of many early spring Lepidoptera oviposit at times different from those over which their offspring develops in. It means that these females do not experience environmental conditions their caterpillars are going to live next spring. Thus, there is a poor chance for them to select the best food resources for their future offspring [112]. Non-selective oviposition has been documented in common geometrids *O. brumata* and *Epirrita autumnata* (Borkhausen, 1794) [36,113,114]. Also, Tiberi et al. [115] recorded a similar number regarding eggs of *Tortrix viridana* on two oak species, *Q. cerris* and *Q. pubescens*. The oviposition of remaining species from the "brumata-viridana complex" has not been studied yet, but it is highly probable that the females place their eggs on both oaks.

The majority of caterpillars that hatched on LF oaks need to find new feeding places. Only some neonates among them can stay on this host tree—either those hatching later or those which are lucky to do it on a branch or tree with unusually early flushing buds. Previous studies suggest that small caterpillars leave relatively often their primary places to feed [18,31,32,113,116,117], however, inclination to disperse is a species-specific trait [14]. Baby caterpillars that were dispersing through ballooning can reach neighbouring or close-growing trees quite easily [31,113], and can increase caterpillar abundance on EF trees. The question of an extent to which neonate caterpillars being dispersed from LF *Q. cerris* affect neighbouring EF *Q. pubescens* trees remains still unknown. Similarly, the knowledge of dispersing late-instar caterpillars between trees is insufficient; this phenomenon has been most studied marginally, and only in some species so far [60,61,93,118,119].

There are plenty of abiotic and biotic stimuli inducing caterpillars to leave their feeding places (e.g., low quality or lack of food, a contact with a predator or parasitoid, physical stimuli caused by weather conditions, etc.) [4,60,61,94,120–126]. Many caterpillars can be seen as they climb tree trunks to get into crowns after rainstorms or strong winds (pers. observations of authors). Dispersing larvae have a better chance to survive when finding suitable feeding places near their primary host trees. It is assumed that the impact of those caterpillars on other trees is predominantly local [63,93,127,128].

The presence of EF *Q. pubescens* in isolated small mixed tree groups seems to be insufficient to increase considerably the caterpillar abundance or affect the composition of their assemblages on LF *Q. cerris*. Dispersal of neonate caterpillars (at least some species) may easily overcome distances between microhabitats we have studied (20–50 m) ([30–33] and references therein). However, ballooning is of little importance for the redistribution of caterpillars onto *Q. cerris* trees as their buds are still mostly closed at a time when they are hatching. Older caterpillars have limited ability to spill over, from EF primary host to neighbouring LF trees growing in small isolated groups. Hanging on silk threads or falling on the ground in such a microhabitat, they may easily miss neighbouring trees, and get lost in open space. Similarly, we suppose that solitary growing trees are highly improbable to be reached by older caterpillars from a forest or other trees being several tens of metres away. Consequently, in microhabitats out of forest, adults that originated from caterpillars living on LF *Q. cerris* are less abundant than those on EF *Q. pubescens*. Thus, the abundance of females laying eggs on *Q. cerris* is also lower and only a small part of offspring (i.e., caterpillars having hatched later) complete its development on trees belonging to this LF oak species. To summarise it, the small number of eggs and the few possibilities to enrich their assemblages from more infested EF *Q. pubescens* contribute together in low caterpillar abundances on LF *Q. cerris* trees.

Very low caterpillar abundance on LF *Q. cerris* in small fragments distant several tens of metres from a continuous forest suggests that no specialised lepidopteran population is genetically adapted on these trees unlike some known cases [18,129,130]. In mixed forests where moth adults and caterpillars move between trees, interbreeding of individuals that develop on different plants occurs. Both sexes or at least males, and some neonate caterpillars can overcome short distances between a forest and its fragments [31,131,132] is what inhibits a genetically determined specialisation on trees with specific phenology [19].

The caterpillar abundance on mature EF *Q. pubescens* in small forest fragments, i.e., mixed tree groups and solitary trees, almost reached that in the forest interior but the composition of their assemblages in these microhabitats differed from those in forest. On oaks, a small or positive effect of forest fragmentation on herbivory has been recorded e.g., [47,77]. Caterpillars living on solitary trees and those in small groups out of forest are influenced by distinct conditions present within the forest [133–136] as well as the different quality of leaves, as their food plays an important role when compared with these in the forest interior [137–140]. The above-mentioned conditions together with the preference of certain habitats varying among species [5,141–143] determine the assemblages of herbivores.

Agriopis leucophaearia, the most dominant species recorded in caterpillar assemblages, on mature EF *Q. pubescens*, was in abundance, almost as in microhabitats out of forest as within it (differences were not significant), thus, it follows that the moth does not prefer any of the specific environments given. This species was abundant as well on *Q. pubescens* in open-canopy forest [10]. In contrast, another dominant species, *O. brumata*, appeared on *Q. pubescens* in higher abundance in microhabitats out of forest than in a continuous forest. It could be caused by its different habitat preference, since this moth is also abundant in fruit orchards [93,113,114,144–147], parks and urban alleys of trees [142,148,149] where the latter or shrubs do not grow close to each other. Van Dongen et al. [68] and van Dongen & Scott [72] studied *O. brumata* in patches, larger and more isolated in comparison with those in our research, and they recorded the negative effect of patch isolation on this moth.

On mature LF *Q. cerris*, caterpillar assemblages on small tree groups and on solitary trees differed from those in the forest interior. Although *A. leucophaearia* and *O. brumata* were dominant on these oaks in the studied small forest fragments, being very low abundant suggest that *Q. cerris* is not a suitable host for them in such microhabitats. Other lepidopteran species occurred there in low abundance, too.

We have also recorded associational susceptibility in young LF *Q. cerris* growing in close vicinity (i.e., right under the crowns) of mature EF *Q. pubescens* within the forest. These young LF trees were infested by caterpillars more significantly than young ones in open space—in open forest glades or edges. In forest, caterpillar assemblages on young LF and EF trees were similar. These results suggest that a close distance between young LF trees and mature EF ones is crucial because dispersing caterpillars reach rather closely trees in growth. Saplings and other plants in the forest understory are known to be infested by caterpillars that descend on silk threads or fall from the forest canopy (e.g., in searching for food) [5,39,94,121,141]. The frequency of the movements made by caterpillars between forest strata has not been studied so far. The reported abundance of the dominant species *A. leucophaearia* suggests that young LF *Q. cerris* growing out of the close range from mature EF *Q. pubescens* be less suitable than trees in forest under the crowns of mature *Q. pubescens*, for the larval development of this moth. We did not statistically analyse other lepidopteran species due to their low abundances.

Effects of associational susceptibility on secondary host trees are known mainly during outbreaks when previously unsuitable or suboptimal hosts were also infested [4,7,45,60,150]. We have recorded positive associational effects of EF trees on LF ones at reduced (non-outbreak) herbivore abundance. Our results show that the effect of EF trees on LF ones is manifested only in close vicinity of trees in forest. So, tree density and forest fragmentation can modify the strength of the associational effect trees with different phenology have. The high caterpillar abundance on LF *Q. cerris* in closed-canopy forest seems to be the result of the tree-to-tree movement of older larvae and probably of high numbers of moth eggs laid on *Q. cerris* (comparable with those on *Q. pubescens*). Also, other studies describing the effects of associational susceptibility on woody plants are linked to forests or dense stands of trees [4,7,39,45,60,61,94,150,151]. The importance of the close vicinity around early- and late-flushing trees for associational effects was indicated by the results of our previous research. In a sparse forest (an open-canopy forest) dominated by both studied oak species, mature and young LF *Q. cerris* were significantly less infested by early spring caterpillars than EF *Q. pubescens* [10].

The composition of caterpillar assemblages on both oak species was similar in forest but differed in microhabitats out of it. It suggests that the associational effect of EF oaks on LF ones is related to many lepidopteran species. The most abundant moth, *A. leucophaearia*, can feed on broad-leaved tree species preferring oaks [10,80,152]. The second in abundance, *O. brumata*, is a typical generalist [31,80,82,114]. The studied type of associational susceptibility can develop when both plants at the centre and in the surroundings are palatable for these herbivores [50]. In case of phylogenetically related and palatable hosts (in our study, *Q. cerris* and *Q. pubescens*), herbivores can have a broad [4,51,150] as well as a narrow diet breadth [45,60].

Except for well-known species of early spring Lepidoptera that are considered pests, *A. leucophaearia* should also be added to the list of forest pests, since it was abundant in our study area and other Central European regions, too [10,12,82,153,154].

The method used (branch beating) is appropriate and advantageous for collecting externally feeding leaf-chewing caterpillars of early spring Lepidoptera [155]. In case of mature trees, these were only obtained from the lower part of tree crowns (up to 3 m from the ground) but for the comparison of caterpillar assemblages between two oak species it was sufficient. All comparable microhabitats in this study were located in the same biogeographical area and landscape structure. In each microhabitat examined, the same (or very similar) abiotic (e.g., climatic) and biotic (e.g., predators and parasitoids) factors acted on caterpillars on both oak species (*Q. cerris* and *Q. pubescens*). There were differences only between conditions associated with host species (different phenology and food quality). This enabled us to acquire new knowledge on fine-scale mechanisms leading to the increased caterpillar infestation of LF trees in the immediate vicinity of EF ones.

Our results suggest that EF trees influence the surrounded LF ones regularly (every season). Such influence can be modified by other factors. For example, the content of chemical substances in oak leaves is species-specific [156,157], so we cannot rule out differences in the palatability of *Q. cerris* and *Q. pubescens* leaves for early spring caterpillars. Severe defoliation can induce resistance against leaf-chewing insects in the following year [158]. On the other hand, trees which usually few caterpillars feed on, i.e., those with low resistance (such as *Q. cerris*) could be sporadically heavily infested by herbivore insects, for example by *Lymantria dispar* L. [159]. In addition, global warming can disrupt the synchrony of EF oak and spring Lepidoptera phenology [160] and affect the abundance of caterpillars on trees. Associational effects of EF trees on LF ones can be connected with forest health deterioration, as weakened trees are often infested by various insect pests [5]. Moreover, young leaves produced by damaged oaks are often damaged by oak powdery mildew [161]. Further studies should address these issues in detail.

Our findings are important for silvicultural practices. The establishment of mixed forest stands with distinct tree species or cultivars using a different time for bursting can increase the susceptibility of certain trees to the attack of herbivores. These trees can be considered resistant if growing in monocultures or with other species with similar phenology. For example, in monocultures of LF *Q. cerris* in Central Europe, Lepidoptera within the "brumata-viridana complex" occur in small abundances (J. Liška, pers. comm.). The seedlings of resistant species or cultivars planted under mature trees with different phenology may also be attacked by herbivores more strongly than when they are in open space. Before the establishment of any forest or park stand, we recommend, therefore, to take into account traits of trees and the local fauna of potential pests.

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Article

Evaluation of Attractant Composition, Application Rate, and Trap Type for Potential Mass Trapping of *Ips typographus* (L.)

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Abstract: This study focused on elucidating the possibilities of improving current trapping methods for *Ips typographus* (Linnaeus, 1758). Three field experiments were conducted simultaneously in one study area in the German federal state of Saxony. A comparison of six different commercial attractants revealed a significant superiority of Typosan®, especially for adult beetles after hibernation in the phase of their first swarming. It also attracted fewer individuals of *Thanasimus* spp. than the other highly attractive products Pheroprax® and IT Ecolure Extra®. Increasing the Pheroprax® application rate by using four instead of one dispenser in a single trap increased the total catch of *I. typographus* only by 15.5%. In contrast, *Thanasimus* spp. catch increased by 195.5% when four dispensers were used. A test of different trap types showed a species-specific catching capability, with the 12-funnel WitaTrap® being the most effective in catching *I. typographus*. The quantity of *Thanasimus* spp. bycatch in multiple-funnel traps demonstrated the necessity of a selective mechanism to minimize impacts on predator populations. Although we were not able to identify new milestones towards mass trapping, this study contributes to necessary improvements of current trapping methods. Especially in future stands with a smaller share of Norway spruce (*Picea abies* Karsten, 1881) the weakened beetle population in spring could be effectively reduced by properly conducted mass trapping.

Keywords: *Ips typographus*; *Thanasimus* spp.; bark beetles; Norway spruce; mass trapping; attractants; release rate; trap type; integrated pest management

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1. Introduction

Climate change impacts on forests are a worldwide phenomenon [1–3]. For Europe, an increase in extreme weather events is predicted to cause physiological stress for trees and forests as well as promoting the reproduction of forest insects with high damage potential [4,5]. Consequently, millions of hectares of forest land are annually damaged by insects and pathogens in Europe [6]. The eight-toothed spruce bark beetle (*Ips typographus* Linnaeus, 1758) particularly benefits from more frequent droughts, storms, and rising temperatures [1,7,8]. The species is able to undergo eruptive population outbreaks and is known as the most destructive pest in forests of Norway spruce (*Picea abies* Karsten, 1881) where it has killed extensive areas in recent years [7,9,10].

Enormous amounts of suitable breeding material following disastrous storms, a drought-induced raised susceptibility of trees and high temperatures accelerating the development of *I. typographus* increase the probability of outbreaks [1,11–13]. These factors coincided in Central Europe from 2017 to 2020. Six devastating storm events [14–19] and unprecedented hot and dry summers [20] gave rise to a perennial large-scale outbreak of *I. typographus*. In such situations, the species is able to overcome the defense mechanism of healthy trees and not depend on susceptible stands [7,21–23]. As a result, large amounts of calamity wood accumulated, especially in Germany, the Czech Republic, and Austria [24–26], which exceeded the capacity of the regular forestry and wood industry, thus resulting in severe logistical problems [9,27]. For example, in the German federal state of Saxony, unplanned wood logging due to bark beetle infestations alone added up to 2.2 million m^3 in 2019, which is almost as much as the annual mean for Europe from 1950 to 2000 with 2.9 million m^3 [26,28]. Historically, bark beetle induced calamities are a frequent phenomenon. A total of 7000 hectares of spruce forest were killed during an outbreak of *I. typographus* from 1781 to 1786 in the Harz mountains [29]. Several other eruptive population outbreaks followed in the 19th and 20th century in Europe [28,30,31].

Even though *I. typographus* is considered to be a crucial ecosystem engineer and keystone species in natural forests, its management in forests, serving ecological, social, and economic purposes, is often inevitable to sustain a multifunctional forestry [8]. Trap-based monitoring programs are an important part of managing spruce forests [13,32]. Since trap trees were replaced by artificial traps after the discovery of the species-specific aggregation pheromone of *I. typographus* [33,34], trapping methods have been constantly developed and improved [35–39]. Salvage logging and sanitation felling are based on the monitoring data and are still the most effective measures in bark beetle management [11,13,32]. Consequently, the enormous amount of unplanned logging caused a drop in wood prices due to a saturated wood market [9]. The preventive application of pheromone traps to protect spruce stands and avoid calamity logging has been, however, discussed intensively. Various studies have proven that effective trap-based management can prevent attacks by *I. typographus* and decrease unplanned logging [40–45]. In contrast, many studies exist in which no significant reduction in population sizes and protection of living trees were achieved by trapping despite great effort [30,46,47], or where the cause for the decline in damage cannot be explicitly assigned to mass trapping [31,45]. The critical factor for success or failure appears to be the stage of population development in which mass trapping was conducted [13,42]. The increasing restrictions for the application of insecticides in forests due to strict formalities of the European Union [48] and the side effects of calamity logging draw interest to mass trapping options without insecticides. Such methods aim to reduce the population density of the target species in a way that secures the ecological, social, and economic objectives of the forest.

In our study we conducted alterations of current trapping methods for *I. typographus* to improve the state of knowledge for future mass trapping as part of bark beetle management. With regard to the "surveillance with the intent to control" approach by Vité (1989) [34], and in consideration of important antagonists, three main components of current monitoring techniques were tested concerning their potential for mass trapping of *I. typographus*. These are:

(1) attractant composition,
(2) application rate, and
(3) trap type.

2. Materials and Methods

2.1. Study Site

The study took place in Germany in the federal state of Saxony in the Tharandt forest from the 6 April 2020 to the 29 June 2020. Three different clearings were selected in the forest district Bärenfels between the villages of Grillenburg and Naundorf, north and south of the state road S194 and within a radius of 1700 m to ensure similar climate conditions. The study sites are located at an altitude of 400 to 430 m above sea level and are categorized as humid lower mountainous region. The mean annual temperature is 9.8 °C, long-term precipitation mean is 843 mm, but was distinctly lower in 2018 (496.4 mm), 2019 (676.1 mm), and 2020 (644.7 mm). The distance between the three clearings was at least 100 m to avoid interdependence.

In the study area, a mass outbreak of *I. typographus* started in 2018 and has caused large amounts of calamity wood. The clearings selected for this study are the result of sanitation felling or salvage logging. The adjacent stands consist mainly of Norway spruce, 40 to 70 years old and colonizable by *I. typographus*. Scots pine (*Pinus sylvestris*), European larch (*Larix decidua*), and European beech (*Fagus sylvatica*) are secondary tree species. Silver birch

(*Betula pendula*), common ash (*Fraxinus excelsior*), sycamore maple (*Acer pseudoplatanus*), and European alder (*Alnus glutinosa)* are present as single trees.

2.2. Species Identification

I. typographus, *Thanasimus femoralis* (Zetterstedt, 1828), and *T. formicarius* (Linnaeus, 1758) were defined as target species, which are considered to be two of the most important antagonists of *I. typographus* [49,50]. In the process of further analyses, *T. femoralis* and *T. formicarius* were regularly grouped as *Thanasimus* spp. *Pityogenes chalcographus* (Linnaeus, 1761), another important bark beetle on Norway spruce [51–53] was additionally included in the trap trial analysis to study species-specific behavior within the group of bark beetles. A stereomicroscope Zeiss Stemi 508 was used for species identification with keys [54–57].

I. typographus was identified and counted individually at least in the first collection of all three trials. With increasing numbers, individual counting became too time-consuming and catches were quantified using a mean weight value. For its determination, catches were dried and 50 individuals of each trap in each collection were weighed using a precision balance (Kern EW 150-3M) to derive a trap-specific mean beetle weight. Individuals of the genus *Thanasimus* were always identified and counted individually.

2.3. Study Design

2.3.1. General Design

The experiments were based on the trap island methodology [58], which was also applied in other field trials with bark beetles [59,60]. For each of the three trials five trap islands were set up with a minimum distance of 50 m to each other. Within a trap island the different test variants were arranged depending on the number of variants: an isosceles triangle for three, a square for four, and a circle for more than four variants. The circular arrangement enables the integration of more than four variants while ensuring that each trap has only two adjacent traps. Traps on the same trap island were spaced at least 10 m from each other. The distance between traps was set in a way to both guarantee a targeted approach of the insects to the respective variants to avoid mutual interference due to too narrow spacing [61] and to reduce the probability of catching different beetle populations on the same trap island [58]. Traps were activated on the 6 April 2020 by attaching the attractants inside the trap analogous to Miller (2013) [62]. Trap catches were then collected six times in a 14-day interval beginning with the first on the 20 April 2020. Saturated benzoic acid solution with a drop of dishwashing detergent to lower surface tension was used as killing and preservative agent and renewed at every collection date. The attractants were replaced with a new attractant according to the specifications given by the manufacturer (Table 1).

2.3.2. Attractant Composition Trial

Since the first commercially produced attractant Pheroprax® in 1979 [34], various alternative attractants have been developed, differing in release rate and composition. Out of these, six were selected and tested regarding their attractivity to *I. typographus* as well as its antagonists *T. femoralis* and *T. formicarius* with Theysohn® slot traps (FLÜGEL GmbH, Lower Saxony, Germany). All products have been included in several other studies. Pheroprax® was specifically examined [63] and has been the standard attractant in many other experiments [41,61,64,65]. It is also part of the standardized bark beetle monitoring applied in the study area [26]. The attractants IT Ecolure classic®, IT Ecolure Extra®, and IT Ecolure Mega® are also known from literature [44,66,67] as well as Ipsowit® [68–71] and Typosan® [70–72]. Table 1 summarizes the duration of attractivity, release rate and composition of the tested products. The release rate has been determined by weekly weighing the products with a precision balance (Kern EW 150-3M), while the composition of the attractants was obtained from literature [71], where only information for IT Ecolure Extra® was available. Since it had the same manufacturer as IT Ecolure classic® and IT Ecolure Mega®, information was transferred. However, it seems that only the pheromonal components were specified [71]. *Cis*-Verbenol was mentioned as the only constituent for the Ecolure products, but since it is crystalline and solid in pure state [73], it needs a solvent, which was not listed.

Table 1. Composition of products tested in the attractant composition trial modified after Sramel et al. (2021) [71].

Dispenser	**IT Ecolure Classic®**	**IT Ecolure Extra®**	**IT Ecolure Mega®**	**Ipsowit®**	**Pheroprax®**	**Typosan®**
Duration of attractivity (weeks)	8–10	6–8	18–20	6–8	6–8	10
Release rate (mg/d)	36.5	70.4	69.5	16.2	33.8	30.0
Composition	(*S*)-*cis*-Verbenol	(*S*)-*cis*-Verbenol	(*S*)-*cis*-Verbenol	*S*-Ipsdienol, (*S*)-*cis*-Verbenol	*S*-Ipsdienol, (*S*)-*cis*-Verbenol 2-Methyl-3-buten-1-ol	(*S*)-*cis*-Verbenol, 2-Methyl-3-buten-1-ol
Dispenser type	blotter (aluminum foil)	blotter (aluminum foil)	blotter (aluminum foil)	blotter (membrane foil)	ampoule	blotter (membrane foil)
Producer	Fytofarm	Fytofarm	Fytofarm	Witasek	BASF	Sintagro AG
Country of production	Slovakia	Slovakia	Slovakia	Austria	Germany	Switzerland

2.3.3. Application Rate Trial

In this experiment, the attractivity of different dosages of Pheroprax® was tested. In addition to single-baited Theysohn® slot traps the dispenser was applied twice and four times within one trap. The attractants were individually distributed in the center of the trap over the entire trap width.

2.3.4. Trap Type Trial

The development of trap types began parallel to the first commercial attractants in the late 1970s [34] resulting in a variety of existing types, two of which were tested in this study. WitaTraps® (WITASEK PflanzenSchutz GmbH, Kärnten, Austria) designed after Lindgren (1983) [74] with 8, 12, and 16 funnels were compared with the Theysohn® slot trap [75], in terms of trapping efficacy. By reducing the number of funnels from the standard 12 to 8 and increasing it to 16, we wanted to examine whether the size of the trap surface affects its trapping efficacy. All tested trap types were baited with one dispenser of Pheroprax®. *P. chalcographus* individuals were only identified and counted for the first two collections.

2.4. Data Analysis

Statistical analysis was performed using the R software, version 4.1.1 with the ggplot2, ggpubr, PMCMRplus, pgirmess, plyr, tidyverse, and readxl packages [76–82]. First absolute numbers of caught beetles were transformed into relative ones. This approach has the advantage of compensating different population densities as well as varying trapping periods during the study [58–60]. The relative values represent the percentage of caught individuals of one species in one variant per trap island and collection date. The numbers of trap islands and collections were used as replications, assuming that new individuals emerge every week [58–60]. The data was then tested for normal distribution using the Shapiro–Wilk test and for homogeneity of variances using the Levene test. If these conditions were not met, a Kruskal–Wallis test was applied as a nonparametric test for independent samples. Subsequently pairwise Iman–Conover tests located the significant differences between the variants. A p value of 0.05 was applied as threshold of significance.

3. Results

During the study period, a total of 1,245,835 individuals of *I. typographus* were caught in the traps on the three study sites, with most individuals in the application rate trial and the fewest in the attractant composition trial. Figure 1 shows the numbers during the course of the study. A first maximum was reached at the collection on the 4 May, a second more distinctive one followed six weeks later.

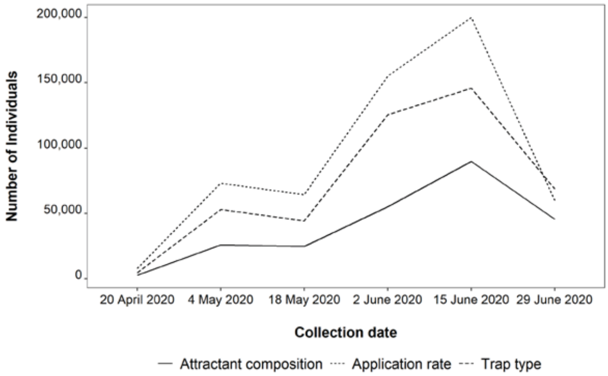
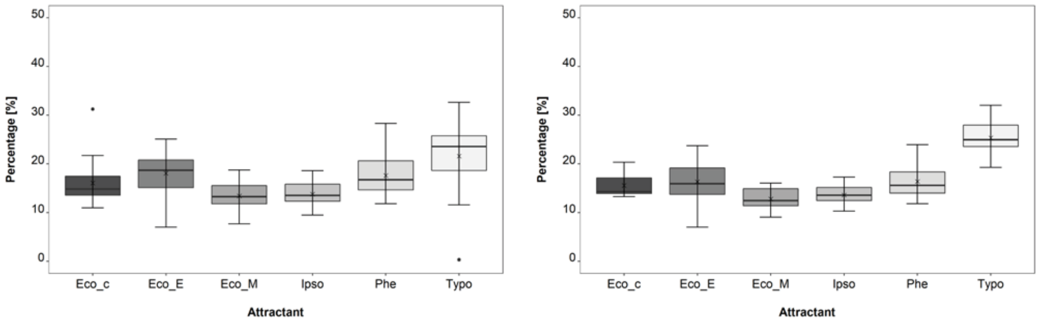


Figure 1. Total number of *I. typographus* caught in the three trials within the study period.

3.1. Attractant Composition Trial

A total of 243,491 individuals of *I. typographus* were caught in this trial. Additionally, 211 specimen of *T. formicarius* and 163 specimen of *T. femoralis* were present. In terms of attractivity to *I. typographus* the products can be distinguished in two groups: a group with higher trapping numbers consisting of Typosan® with a median of 23.5%, IT Ecolure Extra® with a median of 18.7%, Pheroprax® with a median of 16.7%, a group with lower trapping results consisting of IT Ecolure classic® with a median of 14.8%, Ipsowit® with a median of 13.5%, and IT Ecolure Mega® with a median of 13.3% (Figure 2a). All products of the first group caught significantly more individuals than Ipsowit® and IT Ecolure Mega® (Table A1 in Appendix A), while IT Ecolure classic® took a middle position. Within the first group Typosan® stood out from the other two products with significant higher catching numbers than Pheroprax®.



(**a**) (**b**)

Figure 2. Mean percentage of *I. typographus* catches in Theysohn® slot traps baited with different attractant products per trap island and collection date in the study period (**a**) 6 April 2020 to 29 June 2020, $n = 30$; (**b**) 6 April 2020 to 18 May 2020, $n = 15$; Eco_c = IT Ecolure classic®, Eco_E = IT Ecolure Extra®, Eco_M = IT Ecolure Mega®, Ipso = Ipsowit®, Phe = Pheroprax®, Typo = Typosan®.

Typosan®, Pheroprax®, and IT Ecolure Extra® also showed superior results in terms of absolute trapping numbers. Their total catches of 44,357, 45,542, and 47,378 individuals of *I. typographus* represent increases of 29.9%, 33.4%, and 38.8% compared to the least effective product Ipsowit®, which caught 34,146 individuals. Although the absolute number of individuals for Typosan® was lower than for Pheroprax® and IT Ecolure Extra®, a significantly higher attractivity of Typosan, considering relative numbers, is given. This is a result of the first three trapping periods displayed in Figure 2b, in which Typosan® is clearly superior and caught significantly more individuals of *I. typographus* than any other product (Table A2 in Appendix A), which is also reflected in the absolute numbers for this period. In total, catches of *I. typographus* were distinctively lower during the first three collections with 53,119 individuals than in the second half of the trial, when 190,372 individuals were caught. However, during the second half only three significant differences were verifiable (Table A3 in Appendix A).

Regarding the trapping results for *T. femoralis* and *T. formicarius* all three Ecolure products showed a high attractivity for both species (Figure 3). Together they caught 82.9% of all 374 *Thanasimus* spp. individuals in this trial, with IT Ecolure classic® and IT Ecolure Extra® attracting significantly more individuals than Ipsowit® and Typosan® (Table A4 in Appendix A).

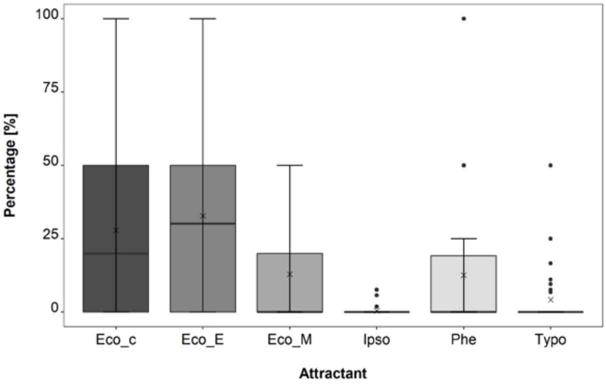


Figure 3. Mean percentage of *Thanasimus* spp. catches in Theysohn® slot traps baited with different attractants per trap island and collection date in the study period from 6 April 2020 to 19 June 2020, Eco_c = IT Ecolure classic®, Eco_E = IT Ecolure Extra®, Eco_M = IT Ecolure Mega®, Ipso = Ipsowit®, Phe = Pheroprax®, Typo = Typosan®, $n = 30$.

3.2. Application Rate Trial

In this trial a total of 560,598 individuals of *I. typographus* were caught, whereas *T. femoralis* and *T. formicarius* were present with 474 and 599 individuals, respectively.

Increasing the number of Pheroprax® dispensers per trap led to an increase in attractivity for *I. typographus* (Figure 4). Thus, the median of traps with a single Pheroprax® was 31.8%, with two Pheroprax® 32.5% and the fourfold application 34.7%. The statistical analysis showed significantly higher trapping percentages of the variant with four Pheroprax® compared to the variants with a single or two Pheroprax® (Table A5 in Appendix A), whereas no significant difference existed between the single and double application variants. However, increasing the application of Pheroprax® by four times only resulted in a 15.5% increase in absolute numbers of caught *I. typographus* compared to the regular single application.

Applying four dispensers of Pheroprax® in one trap resulted in a significant increase in *Thanasimus* spp. bycatch by 195.5% from 199 to 588 individuals (Table A6 in Appendix A). Double application of Pheroprax® increased trap attractivity for the considered antagonists as well (Figure 5), however, this was not statistically significant.

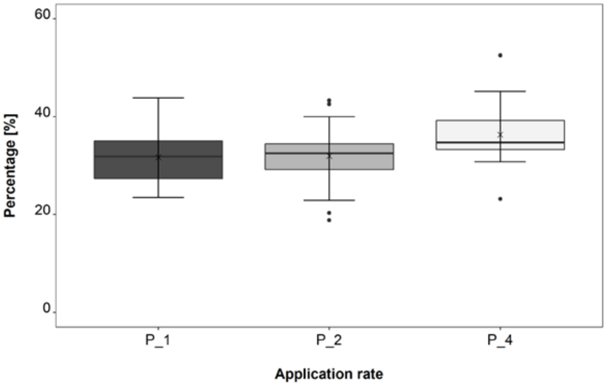


Figure 4. Mean percentage of *I. typographus* catches in Theysohn® slot traps baited with different numbers of Pheroprax® dispensers per trap island and collection date in the study period from 6 April 2020 to 29 June 2020, P_1 = one Pheroprax®, P_2 = two Pheroprax®, P_4 = four Pheroprax®, *n* = 30.

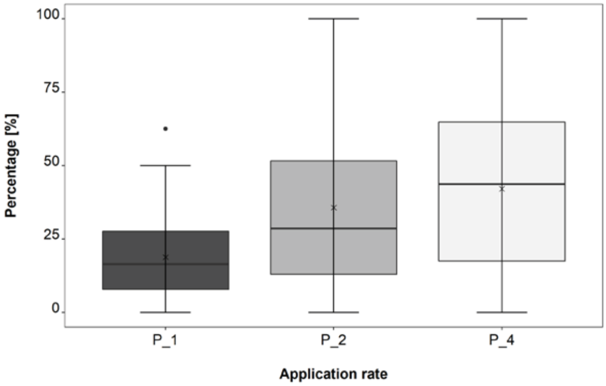


Figure 5. Mean percentage of *Thanasimus* spp. catches in Theysohn® slot traps baited with different numbers of Pheroprax® dispensers per trap island and collection date in the study period from 6 April 2020 to 29 June 2020, P_1 = one Pheroprax®, P_2 = two Pheroprax®, P_4 = four Pheroprax®, *n* = 30.

3.3. Trap Type Trial

A total of 441,746 individuals of *I. typographus*, 492 individuals of *T. femoralis* and 490 individuals of *T. formicarius* were caught in this trial comparing trapping efficacy of different trap types.

The tested trap types showed clear differences in their trapping efficacy for *I. typographus* (Figure 6) and revealed a significant superiority of the 12-funnel WitaTrap® (Table A7 in Appendix A) with a median of 30.4%, whereas the median for the 16-funnel WitaTrap® was 23.9%, for the Theysohn® slot trap 22.1%, and for the 8-funnel WitaTrap® 20.4%. Both increase and reduction in the number of funnels led to a decrease in trapping numbers compared to the standard 12-funnel WitaTrap®. With regard to absolute numbers, the 12-funnel WitaTraps® caught 45.5% more individuals than Theysohn® slot traps.

The results for *P. chalcographus*, with 7225 individuals, the second most frequent species in the first two collections, differed from those of *I. typographus* (Figure 7). With a total of 3012 individuals the Theysohn® slot trap was the most effective trap type for this species (Table A8 in Appendix A).

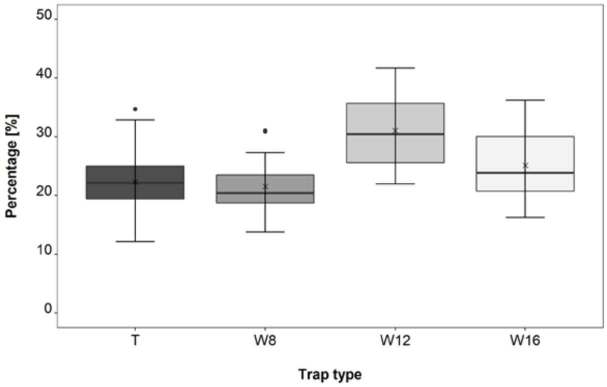


Figure 6. Mean percentage of *I. typographus* catches in different trap types per trap island and collection date in the study period from 6 April 2020 to 29 June 2020, T = Theysohn® slot trap, W8 = 8-funnel WitaTrap®, W12 = 12-funnel WitaTrap®, W16 = 16-funnel WitaTrap®, *n* = 30.

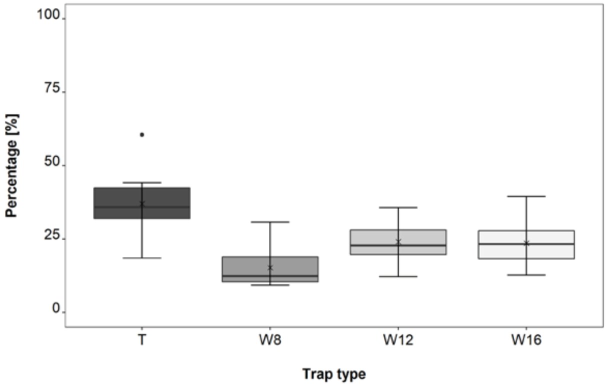


Figure 7. Mean percentage of *P. chalcographus* catches to different trap types per trap island and collection date in the study period from 6 April 2020 to 29 June 2020, T = Theysohn® slot trap, W8 = 8-funnel WitaTrap®, W12 = 12-funnel WitaTrap®, W16 = 16-funnel WitaTrap®, *n* = 30.

The majority of *Thanasimus* spp. showed a preference for the WitaTrap® types (Figure 8), whereas the Theysohn® slot trap caught the fewest individuals of these antagonists with a median of 8.2%. Within the three WitaTrap® types, the more funnels were installed, the more individuals of *Thanasimus* spp. were caught. The median of the 8-funnel WitaTrap® was 18.9%, of the 12-funnel WitaTrap® 24.2%, and of the 16-funnel WitaTrap® 37.8%, with the latter catching significantly more individuals than any other tested trap type (Table A9 in Appendix A).

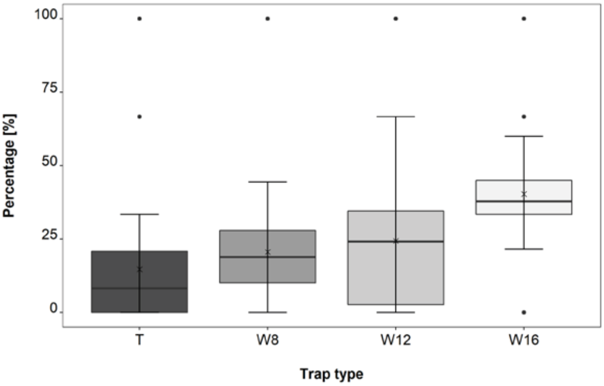


Figure 8. Mean percentage of *Thanasimus* spp. catches in different trap types per trap island and collection date in the study period from 6 April 2020 to 29 June 2020, T = Theysohn® slot trap, W8 = 8-funnel WitaTrap®, W12 = 12-funnel WitaTrap®, W16 = 16-funnel WitaTrap®, *n* = 30.

4. Discussion

4.1. Attractant Composition Trial

After the development of the first commercial pheromone attractant for *I. typographus* in 1979 [34], the manufacturing of other products with varying compositions and release rates followed. According to the results of this study, they also differ significantly in their attractivity to *I. typographus*. The compositions of the attractants are based on the components of the species-specific aggregation pheromone. Based on findings on olfactory communication of *Ips confusus* (LeConte, 1876) [83–85], the attractivity of the three genus-specific components Ipsenol, Ipsdienol, and *cis*-Verbenol was proved under natural conditions [86]. The existence of an aggregation pheromone was also demonstrated for *I. typographus* and further components were discovered [87–89]. After advances in the knowledge on the complex chemical communication of bark beetles [90], 2-Methyl-3-buten-1-ol was identified as the species-specific pheromonal component [33]. The variation in trapping results among the tested products illustrates the potential of attractant composition to optimize the trapping efficacy for *I. typographus*.

All products tested in this study contain merely a part of the entire known bouquet of attractive components of the *I. typographus* aggregation pheromone (Table 1). The reason for this is their main application in the monitoring of the species [13,32,71,91], which only requires the attraction of a sufficient number of beetles. The best trapping results were obtained by Typosan®, which is the only product containing nothing more than the synergistically acting pheromone components *cis*-Verbenol and 2-Methyl-3-buten-1-ol. While the former acts as long-range orientation component of the aggregation pheromone, the latter has been indicated as landing stimulus [92]. In contrast, Pheroprax® and Ipsowit® contain Ipsdienol, which is known to trigger a reaction on receptor neurons [93] and has an aggregating effect [94]. However, the addition of Ipsdienol to a combination of *cis*-Verbenol and 2-Methyl-3-buten-1-ol does not increase attractivity [61,95,96], which is supported by the results of this study. *Cis*-Verbenol is the only pheromone component in the Ecolure products. It might be the absence of 2-Methyl-3-buten-1-ol that causes a lower attractivity of these products for *I. typographus*, which was particularly apparent for IT Ecolure classic® and IT Ecolure Mega®. The high efficacy of IT Ecolure Extra®, on the other hand, could be explained by its high release rate, which is the highest in the group of most attractive products. *I. typographus* catches tend to increase with increasing release rate of pheromones [34,61,64,67,97]. Apparently, the lower release rate of Typosan® and Pheroprax® compared to IT Ecolure Extra® was compensated by a more suitable attractant composition.

The aforementioned high efficacy of Typosan® was manifested particularly in the first half of the trial. Assuming a start of swarming at an air temperature of 16.5 °C [98,99], the first three collections consisted of overwintered adult beetles from the previous year. Since favorable conditions allow a complete development from egg to emerging young beetle within 29 days [100], the last three collections consisted partly of individuals of the first generation. This hypothesis is supported by the PHENIPS model [101] based on the climate data of the station at Dippoldiswalde-Reinberg located close to the study site. The combination of *cis*-Verbenol and 2-Methyl-3-buten-1-ol in Typosan® seems to have the most attractive effect on overwintered adult beetles, whereas the emerging young beetles of the first generation appear to be much less selective when approaching the traps. Such intraspecific variability in olfactory perception is already known for *I. typographus*. A distinction between primary and secondary attraction referring to different attack phases of *I. typographus* was established [90]. Moreover, an increased attractivity of monoterpenes for pioneer beetles was assumed [102], as well as specific primary attractants favoring the initial selection of a breeding habitat were described [93]. Even during colonization of a host tree the composition of produced pheromones varies considerably [103]. The overwintered generation, weakened by winter mortality of approximately 50%, continues to be exposed to high mortality during the first swarming, which is more intense than the flight of subsequent generations [7,104]. The increased risk possibly leads to an increased olfactory sensitivity of overwintered adult beetles compared to the vital young beetles of the first generation.

In contrast to the high attractivity of Typosan® to *I. typographus* a low attractivity to its antagonists *T. femoralis* and *T. formicarius* was found, thus emphasizing its suitability for potential mass trapping. The reciprocal behavior to *I. typographus* seems unusual at first, since *Thanasimus* spp. responds to similar volatiles as its prey, so similar results were expectable [63,105–107]. However, for *Thanasimus* spp. as generalists [108] genus-specific semiochemicals such as Ipsdienol, Ipsenol, or *cis*-Verbenol [86] are more important than species-specific attractants such as 2-Methyl-3-buten-1-ol [105]. *Thanasimus* spp. do not have olfactory receptor cells for the latter, triggering no reaction as single component for these species [105,106]. Furthermore, *cis*-Verbenol elicits a lower antennal response in *Thanasimus* spp. than Ipsenol and Ipsdienol [105]. Both *cis*-Verbenol and 2-Methyl-3-buten-1-ol are pheromone components in Typosan® and explain its low attractivity to *Thanasimus* spp. However, the Ecolure products containing solely *cis*-Verbenol showed the highest attractivity. This does not seem to be a behavior induced by attractant composition but by higher release rates. The results of the application rate trial show that *Thanasimus* spp. reacts strongly to a higher attractant concentration. This behavior can be transferred to the results of the attractant composition trial as cause for the high catches of *Thanasimus* spp. with the Ecolure products characterized by the highest release rates and low catches with Ipsowit®, which has the lowest release rate of the tested products.

Similar results regarding the trapping of *I. typographus* with Typosan® can be found with the evidence of its higher attractivity compared to Pheroprax® [72] and its relatively low number of bycatch [71]. However, Pheroprax® is considered to be an effective attractant for *I. typographus* when compared with different products in other studies [44,71,109]. IT Ecolure Mega® was previously described as comparatively less attractive for *I. typographus* [110] according to the results of our study. On the contrary, numerous studies contradict our results. In Šramel et al. (2021) the lowest trapping numbers of *I. typographus* were achieved with Typosan® compared to Pheroprax®, IT Ecolure Extra®, Ipstyp®, and Ipsowit® [71]. In Otto (2005), Typosan® performed significantly worse than Pheroprax® [70]. IT Ecolure classic® achieved higher trapping results in Nakládal et al. (2013) than IT Ecolure Mega® [110], whereas no significant differences between both products were detected in our study. Zahradník and Zahradníková (2014) described IT Ecolure Mega® as the most efficient attractant [109], while in this study it belongs to the group of less effective products. In studies by Pfister (1997, 1998), Ipsowit® showed similar trapping results as Pheroprax® [68,69], which could not be confirmed in our study. The reasons for these differences in trapping results of various products are likely to be found in differing methodological approaches. In the above-mentioned studies trapping was conducted over the entire activity phase of *I. typographus*, while this study only considered twelve weeks of its flight starting with the first swarming of overwintered adult beetles in spring. Moreover, the experimental design used in this study [58,59] was not applied in the other studies.

4.2. Application Rate Trial

The increased release rate by the application of more than one Pheroprax® dispenser resulted in higher trapping numbers of *I. typographus*, which is consistent with other studies [61,64,67]. However, while a proportional increase in trapping numbers when increasing the release rate was observed in previous studies [61], the fourfold application of Pheroprax® in our study only resulted in an increase of 15.5% with regard to absolute numbers. Instead of a proportional relationship between application rate and attractivity, trapping numbers followed the principle of the Weber–Fechner law, which states that "linear increments in sensation are proportional to the logarithm of stimulus magnitude" [111]. A behavior according to the Weber–Fechner law was observed for several insect species, such as *Drosophila melanogaster* (Meigen, 1830), *Hylobius abietis* (Linnaeus, 1758), *Trypodendron lineatum* (Olivier, 1795) [112–114], and also *I. typographus*. For this species the number of caught beetles cannot be increased perpetually by increasing the release rate of an attractant. Instead it asymptotically approaches a saturation level, since very high release rates do not exert relevant effects on behavior [34,92].

Thanasimus spp. exhibited a stronger reaction to a high application of Pheroprax® than *I. typographus*. This behavior is consistent with their general ecology, since such predators aggregate at sites of increased prey abundance. They follow an aggregation reaction that describes an increase in local population densities of predators due to increased prey abundance [115,116]. Such a response was observed for *Nemozoma elongatum* (Linnaeus, 1761) as predator of *Taphrorychus bicolor* (Herbst, 1793) [117] and applied in the form of allochthonous kairomones as part of a nature-based bark beetle management [58]. In this context, the increased release of pheromonal components in our trial simulated a higher abundance of *I. typographus*. The antennal receptors of *Thanasimus* spp. have the same olfactory sensitivity as their potential prey [63,105–107]. Thus, they are able to perceive the semiochemicals emitted by Pheroprax® and aggregate at sites of higher concentrations.

4.3. Trap Type Trial

The results of this trial showed that trapping numbers of *I. typographus* can be significantly influenced by the choice of the trap type. The previously known superiority of the multiple funnel trap with 12 funnels over the Theysohn® slot trap [38,39] was confirmed. The reduction to eight funnels caused a decline in trapping efficacy, since trap surface and trapping numbers correlate positively [34,39]. Correspondingly, increasing the trap surface by adding more funnels should result in higher numbers of caught individuals. However, this effect only occurred when comparing the 8-funnel with the 12-funnel WitaTrap®, while trapping efficacy declined when using the 16-funnel WitaTrap®. This can be explained with the increasing trap height by adding additional funnels. Insects are then able to spread their wings within the trap and leave it before reaching the trapping container like discussed in other studies [74,118]. The trap design also affects the emission of semiochemicals causing higher or lower release rates into the environment [32,62,118,119]. Multiple-funnel traps probably allow a higher emission of the used attractants due to the larger gaps between funnels compared to the Theysohn® slot trap. Even the position of the dispenser within the trap can affect trapping efficacy [62,119], which is why attractants in this trial were always placed in the same position.

In contrast to *I. typographus* we found a significant preference of the Theysohn® slot trap for *P. chalcographus*. A species-specific trapping efficacy of different trap types was described in many other studies [39,118,120–123]. With regard to *I. typographus* and *P. chalcographus* a possible explanation is the visual similarity of trap design and preferred beetle habitat, since both species use visual cues for orientation [124,125]. *I. typographus* primarily colonizes the trunk below the crown of trees, while *P. chalcographus* usually breeds in the canopy [126]. On one hand, multiple-funnel traps better resemble a tree trunk than the Theysohn® slot trap due to their vertical, elongated appearance and are preferred by *I. typographus*. On the other hand, the Theysohn® slot trap has similarities to the silhouette of a tree canopy due to its rectangular, planar shape, and is therefore more attractive to *P. chalcographus*.

Thanasimus spp. showed similar preferences as *I. typographus* favoring WitaTraps®. The higher trapping numbers of antagonists in multiple-funnel traps are consistent with the results of other studies [39,127]. The critical factor seems to be the visual orientation used by *Thanasimus* spp. to locate its prey [125]. Since *I. typographus* is the more important prey, the trap that more closely resembles its habitat is also more attractive to *Thanasimus* spp. In addition, a potentially better attractant emission from WitaTraps® might contribute to the higher numbers of *T. femoralis* and *T. formicarius* in these traps. In contrast to *I. typographus* *Thanasimus* spp. is apparently not able to spread its wings and escape before dropping into the trapping container, resulting in higher numbers the more funnels were installed. The amount of caught antagonists emphasizes the necessity of having more selective trap types, which has also been mentioned in former studies [44], and has already been developed for *Ips sexdentatus* [128]. *Thanasimus* spp. is essential for the natural regulation of bark beetle populations [49,50,129] requiring highly selective mass trapping devices to minimize the impacts on predators [130].

5. Conclusions

The data of our study prove that an optimization of the analyzed three-directional approach to mass trapping results in a significant increase in catches of *I. typographus*. The attractant composition seems to be the most powerful tool to reach further improvements, especially since neither of the tested attractants contains all known pheromone components. Increasing the application rate had an unwanted side effect on antagonists similar to some of the tested trap types. Improving the selectivity of traps is required if mass trapping programs are to be implemented. Furthermore, trap types showed a species-specific efficacy exacerbating the chances of a universally applicable mass trapping technology for bark beetles.

The high efficacy of Typosan® for hibernated *I. typographus* shows a promising time dependent reaction to volatiles. Further research such as gas chromatographic analysis of attractants and electroantennogram responses are crucial to understand the differences in olfactory receptors of hibernating adult beetles and their offspring. It is desirable to develop a mass trapping attractant specifically designed for the first swarming period of *I. typographus* when the population size is at its lowest level due to high winter mortality. During early spring pheromone traps are highly attractive to individuals in search for breeding sites, meaning it is the best time for mass trapping. A temporary increase in the application rate for this first swarming period could contribute to a more pronounced reduction of population sizes, while trap types with a high selectivity would spare antagonists. This would increase the chances to sustainably reduce the population of *I. typographus* to a level not harmful for standing trees or at the least mitigate the peak of a mass outbreak. Hence, mass trapping of *I. typographus* without insecticides might be applicable in the future. Considering the forest conversion in Central Europe from monocultures towards mixed stands, a reduction in local outbreaks and protection of smaller spruce stands has higher chances to succeed than under current circumstances. In future stands, it might be economically sensible to protect rare spruce wood by traps. It remains disputable if mass trapping can cause an abrupt end to ongoing eruptive population outbreaks in pure spruce stands on a large scale, even though successful small-scale operations are known. However, the significant increase in total trap catches achieved by the three-directional approach of this study does not seem sufficient to reduce the number of beetles in an outbreak situation effectively. Thus, the replacement of salvage logging and sanitation felling as most effective treatments in bark beetle management by mass trapping is not yet an option.

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Appendix A

Table A1. Significance table of the results of pairwise comparisons with the Iman–Conover test for the attractant composition trial from 6 April 2020 to 29 June 2020 for *I. typographus*, $n = 30$, Eco_c = IT Ecolure classic®, Eco_E = IT Ecolure Extra®, Eco_M = IT Ecolure Mega®, Ipso = Ipsowit®, Phe = Pheroprax®, Typo = Typosan®, $0.05 \geq * > 0.01 \geq ** > 0.001 \geq ***$.

Pairwise Comparison	*p* Value	Significance Code
Eco_E–Eco_M	2.5×10^{-5}	***
Eco_E–Ipso	0.0001	***
Phe–Eco_M	0.00045	***
Phe–Ipso	0.00156	**
Typo–Eco_c	0.00013	***
Typo–Eco_M	2.3×10^{-10}	***
Typo–Ipso	1.4×10^{-9}	***
Typo–Phe	0.04111	*

Table A2. Significance table of the results of pairwise comparisons with the Iman–Conover test for the attractant composition trial from 6 April 2020 to 18 May 2020 for *I. typographus*, $n = 15$, Eco_c = IT Ecolure classic®, Eco_E = IT Ecolure Extra®, Eco_M = IT Ecolure Mega®, Ipso = Ipsowit®, Phe = Pheroprax®, Typo = Typosan®, $0.05 \geq * > 0.01 \geq ** > 0.001 \geq ***$.

Pairwise Comparison	*p* Value	Significance Code
Eco_c—Eco_M	0.0413	*
Eco_E–Eco_M	0.0098	**
Phe–Eco_M	0.0135	*
Typo–Eco_c	9.4×10^{-6}	***
Typo–Eco_E	5.9×10^{-5}	***
Typo–Eco_M	1.0×10^{-11}	***
Typo–Ipso	5.3×10^{-10}	***
Typo–Phe	4.0×10^{-5}	***

Table A3. Significance table of the results of pairwise comparisons with the Iman–Conover test for the attractant composition trial from 19 May 2020 to 29 June 2020 for *I. typographus*, $n = 15$, Eco_c = IT Ecolure classic®, Eco_E = IT Ecolure Extra®, Eco_M = IT Ecolure Mega®, Ipso = Ipsowit®, Phe = Pheroprax®, Typo = Typosan®, $0.05 \geq * > 0.01 \geq ** > 0.001 \geq ***$.

Pairwise Comparison	p Value	Significance Code
Eco_E–Eco_M	0.0052	**
Eco_E–Ipso	0.0022	**
Phe–Ipso	0.0277	*

Table A4. Significance table of the results of pairwise comparisons with the Iman–Conover test for the attractant composition trial from 6 April 2020 to 29 June 2020 for *Thanasimus* spp., $n = 30$, Eco_c = IT Ecolure classic®, Eco_E = IT Ecolure Extra®, Eco_M = IT Ecolure Mega®, Ipso = Ipsowit®, Phe = Pheroprax®, Typo = Typosan®, $0.05 \geq * > 0.01 \geq ** > 0.001 \geq ***$.

Pairwise Comparison	p Value	Significance Code
Eco_c–Ipso	1.6×10^{-5}	***
Eco_c–Typo	0.0013	**
Eco_E–Ipso	1.9×10^{-7}	***
Eco_E–Phe	0.0441	*
Eco_E–Typo	2.8×10^{-5}	***
Eco_M–Ipso	0.03	*

Table A5. Significance table of the results of pairwise comparisons with the Iman–Conover test for the application rate trial from 6 April 2020 to 29 June 2020 for *I. typographus*, $n = 30$, P_1 = one Pheroprax® applied, P_2 = two Pheroprax® applied, P_4 = four Pheroprax® applied, $0.05 \geq * > 0.01 \geq ** > 0.001 \geq ***$.

Pairwise Comparison	p Value	Significance Code
P_4–P_1	0.0059	**
P_4–P_2	0.0108	*

Table A6. Significance table of the results of pairwise comparisons with the Iman–Conover test for the application rate trial from 6 April 2020 to 29 June 2020 for *Thanasimus* spp., $n= 30$, P_1 = one Pheroprax® applied, P_2 = two Pheroprax® applied, P_4 = four Pheroprax® applied, $0.05 \geq * > 0.01 \geq ** > 0.001 \geq ***$.

Pairwise Comparison	p Value	Significance Code
P_4–P_1	0.0052	**

Table A7. Significance table of the results of pairwise comparisons with the Iman–Conover test for the trap type trial from 6 April 2020 to 29 June 2020 for *I. typographus*, $n = 30$, W8 = 8-funnel WitaTrap®, W12 = 12-funnel WitaTrap®, W16 = 16-funnel WitaTrap®, T = Theysohn® slot trap, $0.05 \geq * > 0.01 \geq ** > 0.001 \geq ***$.

Pairwise Comparison	p Value	Significance Code
W12–T	6.1×10^{-8}	***
W12–W8	6.7×10^{-10}	***
W12–W16	0.00046	***
W16–W8	0.02047	*

Table A8. Significance table of the results of pairwise comparisons with the Iman–Conover test for the trap type trial from 6 April 2020 to 29 June 2020 for *P. chalcographus*, n = 10, W8 = 8-funnel WitaTrap®, W12 = 12-funnel WitaTrap®, W16 = 16-funnel WitaTrap®, T = Theysohn® slot trap, $0.05 \geq * > 0.01 \geq ** > 0.001 \geq ***$.

Pairwise Comparison	p Value	Significance Code
T–W8	1.0×10^{-5}	***
T–W12	0.044	*
T–W16	0.028	*
W12–W8	0.041	*

Table A9. Significance table of the results of pairwise comparisons with the Iman–Conover test for the trap type trial from 6 April 2020 to 29 June 2020 for *Thanasimus* spp., n = 30, W8 = 8-funnel WitaTrap®, W12 = 12-funnel WitaTrap®, W16 = 16-funnel WitaTrap®, T = Theysohn® slot trap, $0.05 \geq * > 0.01 \geq ** > 0.001 \geq ***$.

Pairwise Comparison	p Value	Significance Code
W16–T	1.6×10^{-6}	***
W16–W8	0.0009	***
W16–W12	0.0186	*

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Article

Changes in Forest Stand and Stability of Uropodine Mites Communities (Acari: Parasitiformes) in Jakubowo Nature Reserve in the Light of Long-Term Research

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Abstract: The current study has been conducted for over 40 years (between 1981 and 2022) in a natural forest reserve in Jakubowo (western Poland). The material for the analysis was collected in three permanent monitoring ground plots with different vegetation cover, humidity and degree of shade. The major aim of the study was to analyze the changes in the species composition and abundance in uropodine (Acari: Parasitiformes) mite communities that occurred in the three ground plots in Jakubowo over 40 years. The second goal was to assess the stability of the species composition and the number of Uropodina mites in the examined communities. The most important phenomenon observed during the research period was a considerable decrease in the abundance of Uropodina in ground litter and soil, and the second was the loss of stenotopic and rare species. Similar observations are also presented in previous studies, which embraced 36 years of research period (between 1978 and 2013). Now, this unfavorable trend, which is caused by anthropogenic disturbances in the environment, has become permanent.

Keywords: anthropogenic disturbances; environmental monitoring; forest reserve; long-term research; natural succession; oak-hornbeam forests; stability of mite communities; Uropodina

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1. Introduction

Many years of acarological observations in one location focusing only on Uropodina (Acari: Parasitiformes) mites, which have been carried out for over 40 years in Jakubowo (oak-hornbeam nature reserve) in western Poland, are undoubtedly unique in this kind of research on a global scale. The research in this nature reserve had been conducted since April 1979 on three permanent monitoring plots with different vegetation cover, humidity and degree of shade [1–4]. From the beginning until now, the supervision over the course of these observations was carried out by the initiator of the research (the first author). The research was and still is carried out all the time with the same methods, and the extraction of the fauna takes place with the same devices. At the same time, photographic documentation of the examined plots is prepared, which allows for a more precise assessment of the changes that have occurred in a given plot and then to draw conclusions about the impact of these changes on the species composition and the number of mites from the suborder Uropodina. In addition to mites, in Jakubowo, changes in communities of terrestrial snails were also monitored [5]. However, most publications concerning Jakubowo focus on mites from the suborder Uropodina [1–4,6,7]. The current article is a continuation of previous research because it takes into account the subsequent years of research against further changes in the vegetation cover on the examined plots.

Over the course of 43 years, the research plots have undergone changes resulting from both the natural succession of plant cover and changes in the management of dead wood in nature reserves in Poland. Until the end of the 1970s, dead wood was removed from nature reserves. Thus, the mites were deprived of one of the most important microenvironments, the presence of which determines the high biodiversity of this group of mites in forests [2,8,9]. Leaving dead trees in the reserve considerably changed the conditions for the functioning of the entire ecosystem, significantly expanding the range of niches for all fauna, including Uropodina. The increase in the amount of dead wood in the area of this reserve has been observed especially in recent years (since 2000), and it seems to be one of the results of the natural reconstruction of the forest stand caused mainly by felling of old monumental beech trees due to windbreaks. These changes for each monitoring area are described in more detail later in this article.

The major aim of the research presented in the current study was to analyze the changes in the species composition and abundance of uropodine mite communities that occurred in three ground plots in Jakubowo over 40 years. The second goal was to assess the stability of the species composition and the abundance of Uropodina communities in the analyzed period in the selected areas of the reserve.

2. Materials and Methods

The samples from Jakubowo nature reserve were collected in an oak-hornbeam forest, in three selected monitoring ground plots (each of them covered an area of 625 m^2). In each research period, the mites were collected with the same methods: they were mainly litter and soil samples collected with a biocenometer from a depth of 10 cm. In each of the selected plots, a series of 10 samples were collected once at two-week intervals (between April and November in the period 1981–1982) or monthly in subsequent years of the study (i.e., 2005, 2006, 2012, 2014, 2016 and 2022). In 2012 and 2014, no samples were collected from plot J-II, focusing solely on the remaining plots. The mesofauna was extracted with Tullgren funnels for 3 or 5 days, depending on the degree of humidity of the samples, and the specimens were then preserved in 75% ethyl alcohol. The identification of the Uropodina is based on the original description keys [10–13]. In each phase of the research project, all specimens were verified by the first author. The specimens were deposited in the Natural History Collections (Faculty of Biology at Adam Mickiewicz University in Poznań).

The study rests upon the metadata stored in the Soil Fauna Bank in AnalizaTor 2.0 software by Desmodus (Poznań, Poland) (Natural History Collections). The full list of mite species found there is given in the publication [7].

2.1. Data Analysis

Due to the fact that the number of collected samples is different for each period, the dynamics of the changes in the analyzed mite communities is illustrated with a scale of dominance (D) and frequency (F) of occurrence [1,14]. The scale has the following classes: dominance D5 eudominants (>30%), D4 dominants (15.1%–30.0%), D3 subdominants (7.1%–15.0%), D2 recedents (3.0%–7.0%) and D1 subrecedents (<3%); frequency F5 euconstants (>50%), F4 constants (30.1%–50%), F3 subconstants (15.1%–30.0%), F2 accessory species (5.0%–15.0%) and F1 accidents (<5%) [1]. Statistically significant differences between the abundance of all Uropodina in two research periods were established with the Mann–Whitney U-test, and statistically significant differences between the abundance of four most frequent species in the examined plots in each research period were established with the (ANOVA) Kruskal–Wallis rank test.

The community similarity of the species composition for Uropodina mites inhabiting each ground plot was calculated by means of the Marczewski–Steinhaus species similarity index: $S = c/(a + b - c)$, where c is the number of species present in both compared communities, and a and b stand for the total number of species in each community [14]. The full joining analysis, which uses the most distant neighbors, was used to draw the dendrogram. The analyses were calculated with AnalizaTor 2.0 software (Poznań, Poland).

2.2. Changes in Plant Cover in Monitored Ground Plots

Jakubowo nature reserve, which covers an area of 4.22 ha, is located in western Wielkopolska (Greater Poland) (52°48′17″ N 16°28′67″ E) (Figure 1). A more detailed description of the reserve is presented in earlier studies [1,4]. On the examined ground plots, natural reconstruction of the stand was observed as a result of the loss of the oldest beech trees caused by windbreaks and the development of the undergrowth.

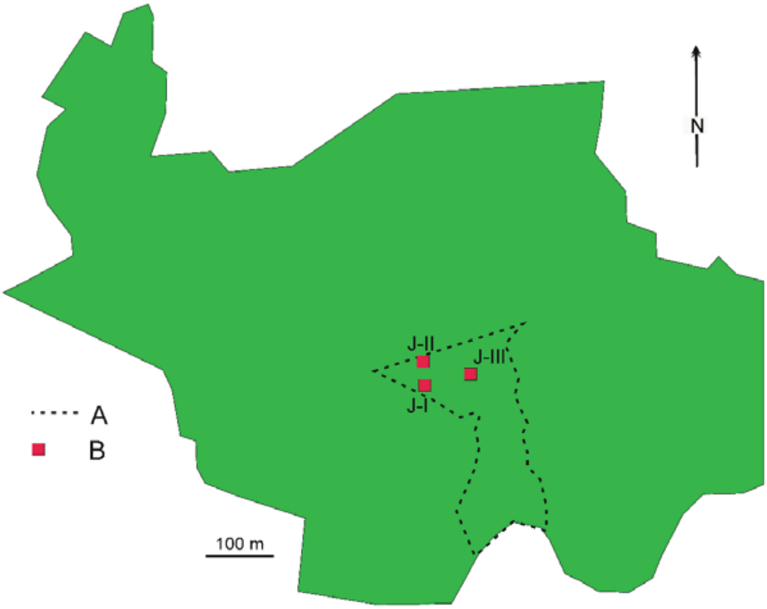

Figure 1. Exact location of examined ground plots J-I, J-II, J-II (red square) in Jakubowo nature reserve against whole forest area of the reserve.

The photographs presented below show the changes in the vegetation in the examined ground plots in the period between April 1979 and February 2022, i.e., over 43 years. The characteristics of each surface describe the extent of these changes. The areas differed not only in the type of the plant cover but also in the dynamics of stand succession over the years of research.

Plot J-I (52°48′28″ N 16°28′52″ E) (Figure 1) is a typical oak-hornbeam forest area (*Galio-sylvatici-Carpinetumstachyetosum* var. with *Fagus sylvatica*). In the first period of research (1981–1982), this area was adjacent to a large cut-over area. This plot was the driest area, with considerable daily and seasonal temperature fluctuations. The stand consisted mainly of old monumental beech (*Fagus sylvatica* L.), with a slight mixture of hornbeam (*Carpinus* L.) and oaks (*Quercus* L.). There was no undergrowth (Figure 2A). The ground flora comprised mainly grass (95%) with a slight mixture of sedge (Figure 3A). The litter contained mainly old beech leaves (75%) and oak, hornbeam and sedge leaves.

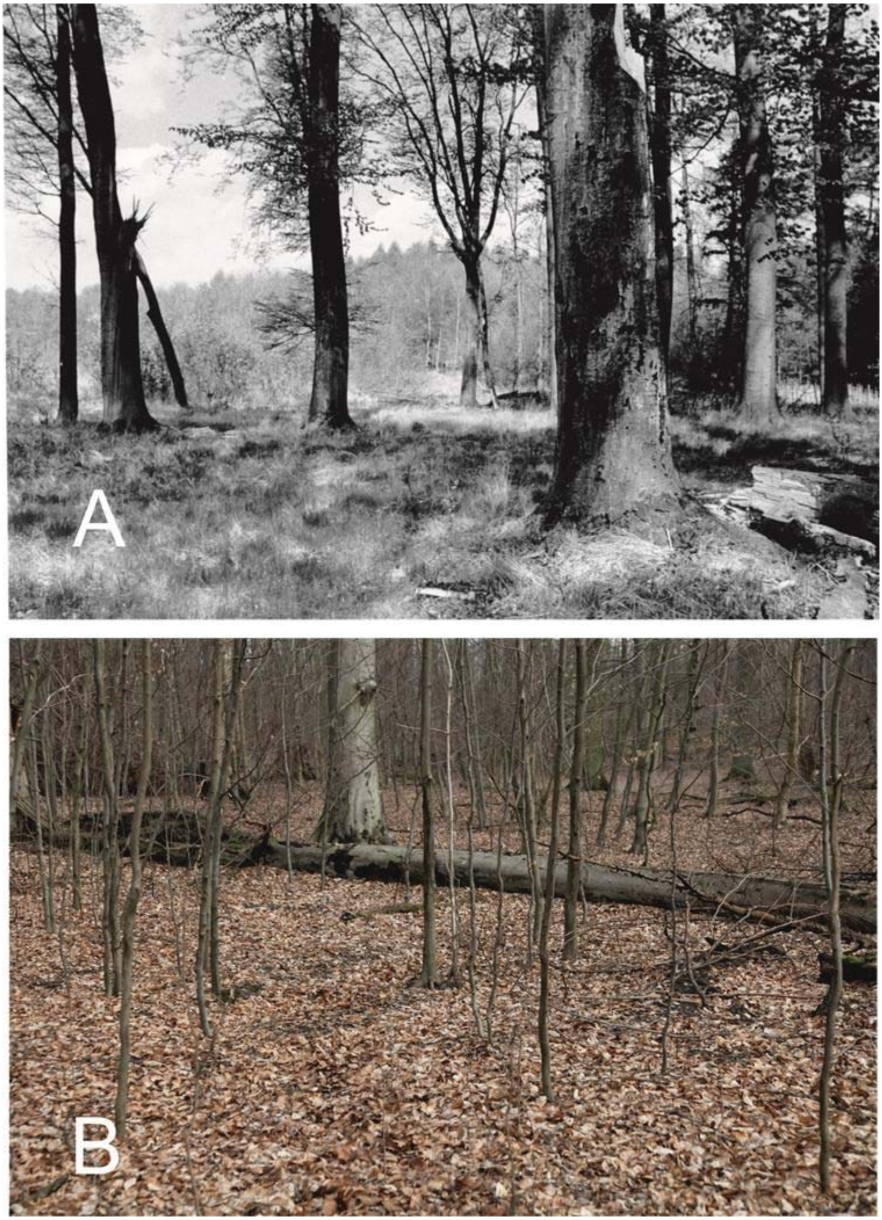

Figure 2. Changes in ground flora in plot J-I: (**A**)—In 1979, (**B**)—Present (2022).

After 1985, in the cut-over area, new beech trees began to grow, which are now over 40 years old. Due to windbreaks, in this ground plot, the old beeches started to collapse one by one. In the resulting gaps, a gradual renewal of beech trees can be observed, which currently form a fairly dense undergrowth (Figure 2B). This, in turn, causes an increase in shade and therefore increases the soil moisture. The increase in shade has eliminated the grassy undergrowth. Currently, the litter consists mainly of a thick layer of fallen beech leaves (Figure 3B).

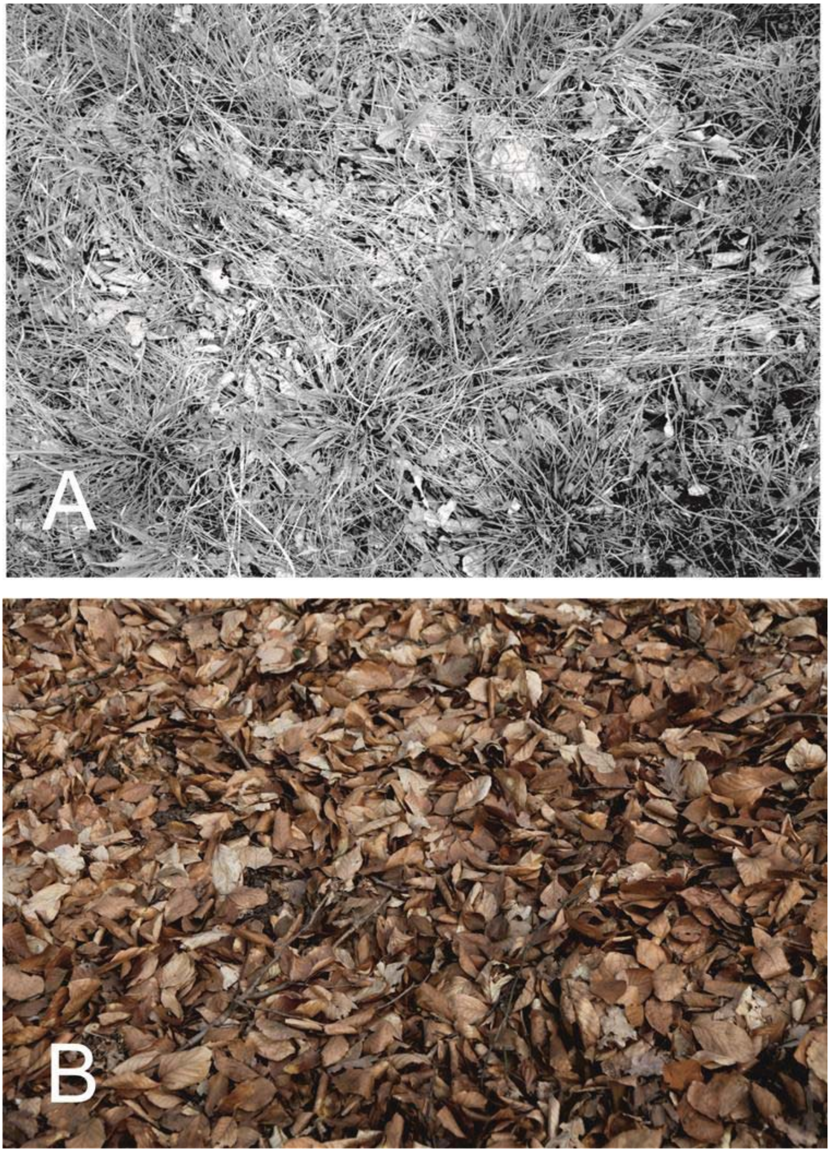

Figure 3. Changes in ground cover in plot J-I: (**A**)—Grass and sedge (1979); (**B**)—Now only a thick layer of fallen beech leaves (2022).

Plot J-II (52°48′28″ N 16°28′56″ E) (Figure 1) is an area mainly with oak-hornbeam forest with beech trees (*Galio-sylvatici-Carpinetum stachyetosum* var. with *Fagus sylvatica*/*Caricetum acutiformis*). It is located at a shallow terrain hollow, where water used to accumulate during rainy periods. The last surface flooding in this area was observed in 1982. There are also hazel bushes (*Corylus* L.), and earlier, there was quite dense common dogwood (*Cornus sanguinea* L.) and not too much grass. At present, however, in this area, there is almost no ground flora (Figure 4A).

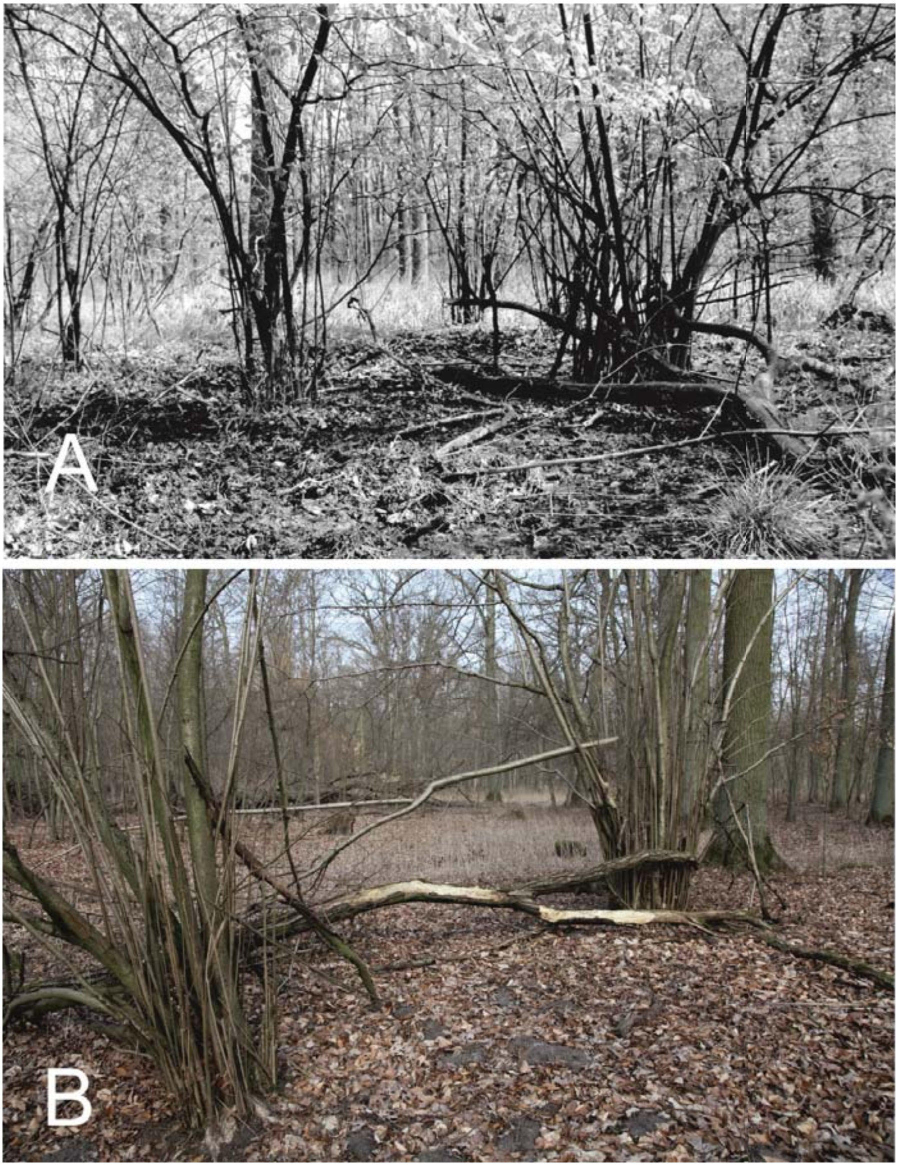

Figure 4. Changes in ground flora in plot J-II: (**A**)—In 1979, (**B**)—Present (2022).

In this area, only the bushes of the hazel (*Corylus avellana* L.) are still present, whereas the common dogwood has been lost completely. Additionally, the sporadic tufts of the sedge (*Carex*) are not present now (Figure 4B).

Plot J-III (52°48′29″ N 16°28′57″ E) (Figure 1) is a moderately humid area of an oak-hornbeam forest with beech trees (*Galio-sylvatici-Carpinetum stachyetosum* var. with *Fagus sylvatica*) (Figure 5A). The main changes that have taken place in this area are the higher percentage of dead wood matter and the presence of new dense undergrowth, consisting mainly of young beech trees (Figure 5B).

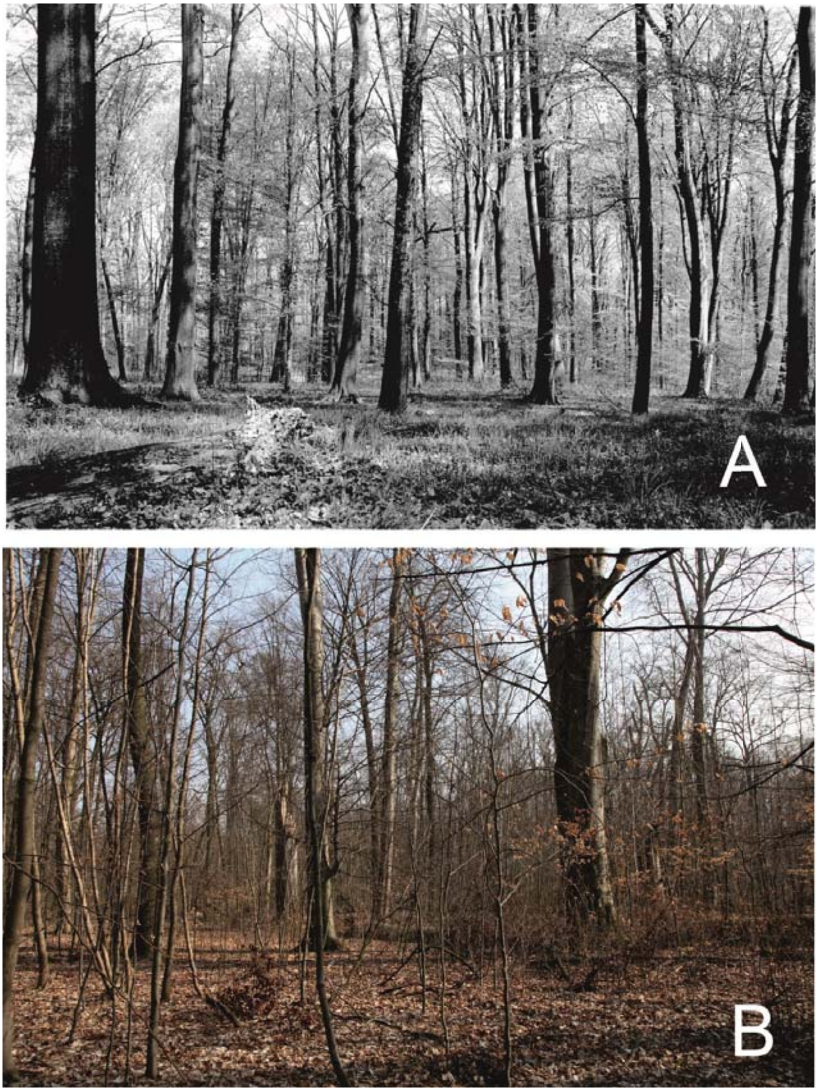

Figure 5. Changes in ground flora in plot J-III: (**A**)—In 1979, (**B**)—Present (2022).

3. Results

3.1. Changes in the Species Composition and Abundance of Uropodina Communities in Jakubowo Nature Reserve in Subsequent Years

The analysis of the communities in the subsequent years of the research project (1981–2022) revealed that out of the 17 Uropodina species found in total in the examined reserve, only 4 occurred regularly in the communities throughout the whole period: *O. minima*, *T. aegrota*, *T. pauperior* and *U. pannonica* (Table 1). The number of Uropodina species forming the communities in the consecutive periods of research varied from 6 to 12. The most species-rich community was found in 2006; it consisted of 12 species (i.e., 71%) of all identified species.

Table 1. Changes in species composition of Uropodina communities in Jakubowo nature reserve, 1981–2022.

Species	1981	1982	2005	2006	2012	2014	2016	2022
Olodiscus minima (Kramer, 1882)	+	+	+	+	+	+	+	+
Trachytes aegrota (C. L. Koch, 1841)	+	+	+	+	+	+	+	+
Trachytes pauperior (Berlese, 1914)	+	+	+	+	+	+	+	+
Urodiaspis pannonica Willmann, 1952	+	+	+	+	+	+	+	+
Polyaspinus cylindricus Berlese, 1916			+	+	+	+	+	+
Urodiaspis tecta (Kramer, 1876)	+	+	+	+	+			+
Oodinychus ovalis (C. L. Koch, 1839)		+	+	+	+			+
Cilliba rafalskii (Błoszyk Stachowiak et Halliday, 2008)	+	+	+	+				
Dinychus perforatus Kramer, 1882		+	+			+	+	
Dinychura cordieri (Berlese, 1916)			+	+				
Phaulodiaspis rackei (Oudemans, 1912)				+		+		
Discourella modesta (Leonardi, 1889)							+	
Uroobovella pyriformis (Berlese, 1920)				+				
Pulchellaobovella pulchella (Berlese, 1904)				+				
Neodiscopoma splendida (Kramer, 1882)						+		
Oodinychus obscurasimilis (Hirschmann et Z.-Nicol, 1961)						+		
Trachytes lamda Berlese, 1903		+						
Number of species	6	9	10	12	7	9	7	7
% of species	35.29	52.94	58.82	70.59	41.18	52.94	41.18	41.18
Number of samples	120	120	288	360	120	120	120	120

Since 2005, *P. cylindricus* has been regularly found in the reserve, while *U. tecta* was not found in the period 2014–2016. Additionally, *O. ovalis* was only sporadically found in the reserve. The absence of these species in the analyzed samples does not indicate their complete disappearance, but it may also suggest a low abundance of the specimens, which made it impossible to catch them using the sampling methods used in this research. This is also relevant in the case of two other species, e.g., *C. rafalskii* and *D. perforatus*. The former was regularly found in the years 1981–2006, but after this period, it did not occur in the collected samples. The latter occurred in two periods, 1982–2005 and 2014–2016. Only in two research periods was the presence of *D. cordieri* and *Ph. rackei* recorded. The other species occurred extremely rarely in the reserve during the conducted research.

Figure 6 shows the observed changes in the abundance of four most numerous Uropodina species (i.e., *T. aegrota*, *T. pauperior*, *O. minima* and *U. pannonica*), which formed the community. Since 2012, a considerable decrease in the number of Uropodina has been observed in the analyzed samples. The differences in the average number of specimens per sample of these species have turned out to be statistically significant ((ANOVA) Kruskal–Wallis rank test H(7, N = 792) = 53.14, $p < 0.001$). They were observed in the following periods: 1981–2005 ***, 1981–2012 ***, 1981–2016 ***, 1982–2012 **, 2006–2012 **, 2012–2014 * and 2012–2022 ** (Table 2).

The average abundance of Uropodina in the reserve during the research periods remained at the level of 5400 specimens per m^2. In general, since 2006 in Jakubowo, one can observe a decrease in the abundance of Uropodina mites. Statistically significant differences in the average number of specimens per sample in the whole community of Uropodina in research periods 1981–2006 and 2021–2022 were observed ($p < 0.01$ Mann–Whitney U-test = 10.805, z = 2.88) (Table 3, Figure 7). Abundances below the average were observed in the years 1981, 2016, 2022, while abundances above the average were observed in the period 2005–2006. In the other periods, the abundance of Uropodina mites remained similar to the average (Figure 8).

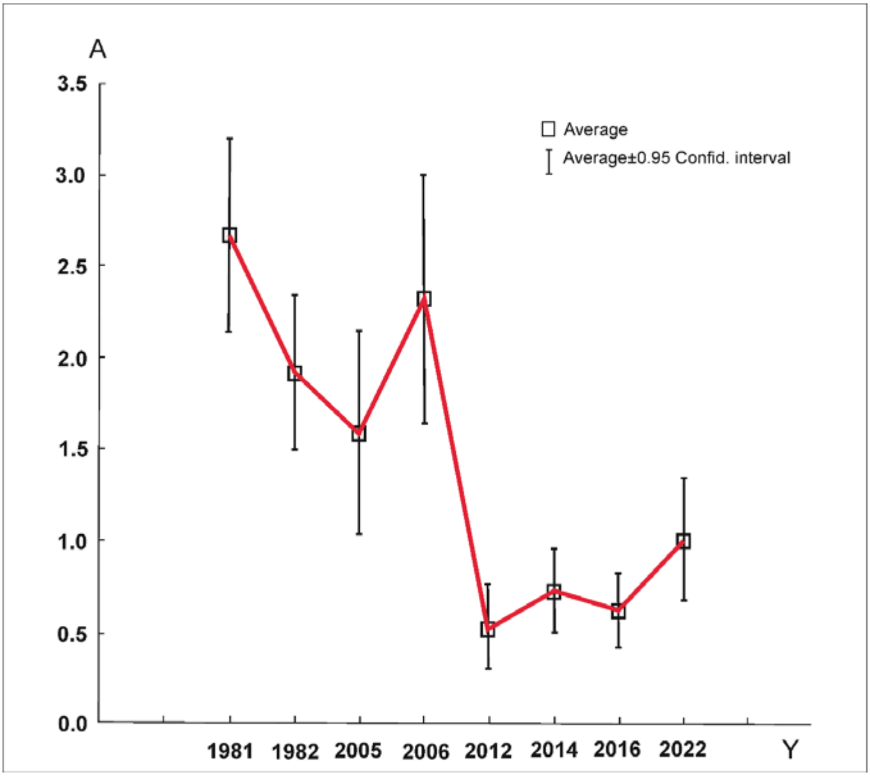

Figure 6. Dynamics of abundance (A) (specimen per sample) of four most numerous Uropodina species found in Jakubowo during consecutive research periods (Y).

Table 2. Statistical differences between the abundance of four most frequent species of Uropodina (i.e., *T. aegrota*, *T. pauperior*, *O. minima* and *U. pannonica*) during consecutive research periods. N—number of observations, X—average, SD—standard deviation, Med.—median.

		N	**X**	**SD**	**Med.**	**X Rang**
1	1981	99	2.67	2.61	1	505.54
2	1982	99	1.92	2.13	1	425.34
3	2005	99	1.59	2.79	1	333.8
4	2006	99	2.31	3.37	1	421.41
5	2012	99	1.05	1.42	1	303.05
6	2014	99	1.45	1.24	1	405.2
7	2016	99	1.23	1.18	1	358.08
8	2022	99	1.73	1.68	1	419.58

Table 3. Statistical differences between the abundance of Uropodina in two research periods (1981–2006 and 2021–2022). N—number of observations, X—average, SD—standard deviation, Med.—median.

	N	X	SD	Med.	X Rang
1981–2006	255	3.25	5.82	2	190.63
2012–2022	105	1.78	1.59	1	155.90

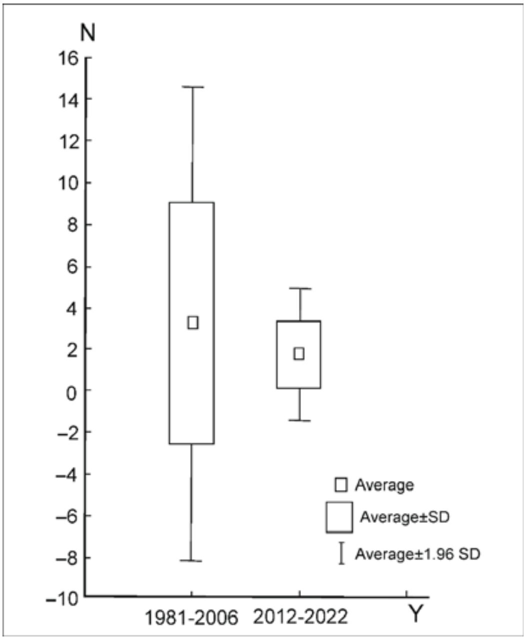

Figure 7. Average number (N) of Uropodina specimens in positive samples in the Jakubowo reserve in two research periods (Y) (1981–2006 and 2021–2022).

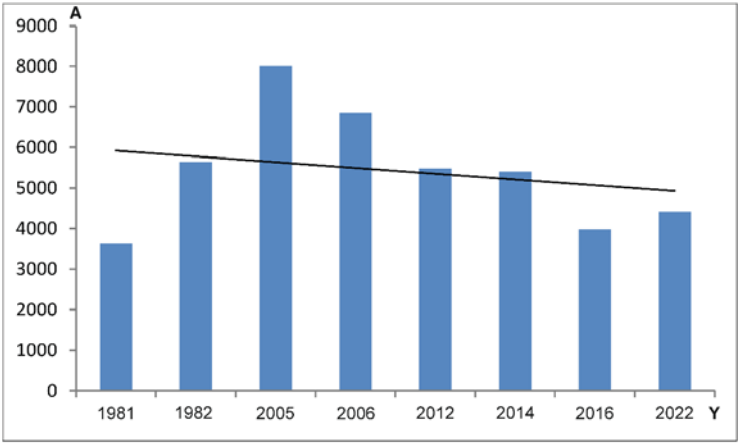

Figure 8. Changes in average abundance (A) (specimens per m^2) of Uropodina mites in Jakubowo over research years (Y).

The analysis also focuses on the changes in abundance of *T. aegrota*, *T. pauperior*, *O. minima* and *U. pannonica* (Figure 9), which were the four species with regular occurrence in the examined communities.

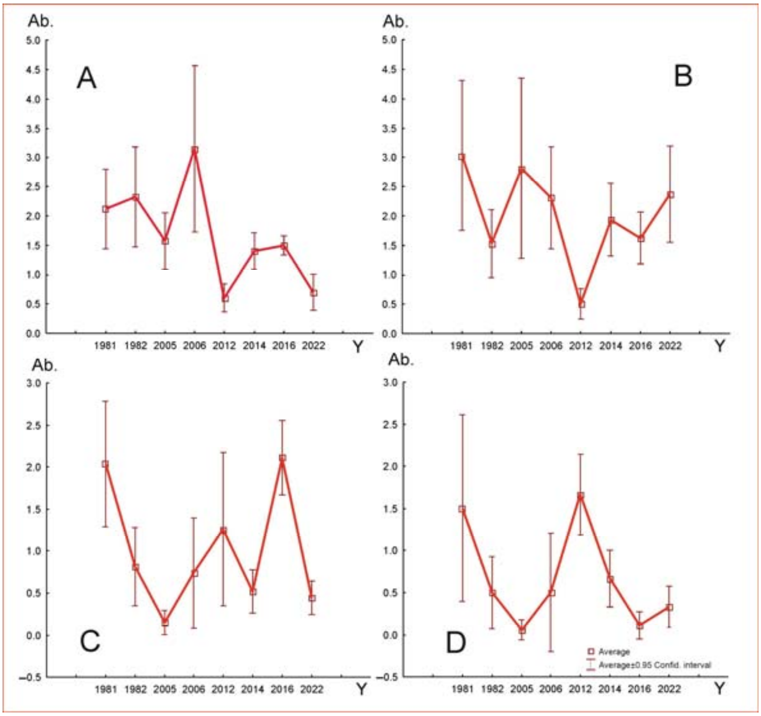

Figure 9. Changes in abundance (Ab.) over research years (Y) of four species, which regularly occurred in examined Uropodina communities in Jakubowo: (**A**)—*T. aegrota*, (**B**)—*O. minima*, (**C**)—*T. pauperior*, (**D**)—*U. pannonica*.

As shown in Figure 8, the curves indicate fluctuations in the abundance of the four regularly occurring Uropodina species in Jakubowo. The differences between the average values in the consecutive research periods for all species turned out to be statistically significant:

O. minima—(ANOVA) Kruskal–Wallis rank test H(7, N = 256) = 34.45; $p < 0.00$. In the case of this species, the average number of specimens was considerably different in the samples collected in the following periods: 1981–2012 ***, 2005–2012 **, 2006–2012 ***, 2012–2014 ***, 2012–2016 ** and 2012–2022 *** (Table 4).

Table 4. Statistical differences between the abundances of *O. minima* during consecutive research periods. N—number of observations, X—average, SD—standard deviation, Med.—median.

		N	X	SD	Med.	X Rang
1	1981	32	3.03	3.55	1	149.59
2	1982	32	1.53	1.59	1	119
3	2005	32	2.81	4.27	1	131.02
4	2006	32	2.31	2.43	1	145.66
5	2012	32	0.5	0.72	0	61.25

Table 4. *Cont.*

		N	X	SD	Med.	X Rang
6	2014	32	1.94	1.7	1	140.88
7	2016	32	1.63	1.24	1	132.81
8	2022	32	2.38	2.27	1	147.8

T. aegrota—(ANOVA) Kruskal–Wallis rank test H(7, N = 320) = 49.33; $p < 0.001$. A significant difference in the average number of specimens was observed in the following periods: 1981–2012 **, 1981–2022 **, 1982–2012 ***, 1982–2022 **, 2005–2012 *, 2005–2022 *, 2006–2012 ***, 2006–2022 ***, 2012–2014 *, 2012–2016 ***, 2014–2022 * and 2016–2022 *** (Table 5).

Table 5. Statistical differences between the abundances of *T. aegrota* during consecutive research periods. N—number of observations, X—average, SD—standard deviation, Med.—Median.

		N	X	SD	Med.	X Rang
1	1981	40	2.13	2.13	1	182.85
2	1982	40	2.33	2.66	1	183.96
3	2005	40	1.58	1.52	1	165.92
4	2006	40	3.15	4.44	1	195.74
5	2012	40	0.6	0.74	0	98.05
6	2014	40	1.4	0.98	1	169.25
7	2016	40	1.5	0.51	1.5	187.25
8	2022	40	0.7	0.97	0.5	100.98

T. pauperior—(ANOVA) Kruskal–Wallis rank test H(7, N = 216) = 57.81; $p < 0.001$. The average number of specimens per sample was considerably different in the following periods: 1981–2005 ***, 1981–2006 **, 1981–2014 *, 1981–2022 *, 1982–2016 **, 2005–2016 ***, 2006–2016 ***, 2012–2016 ***, 2014–2016 *** and 2016–2022 *** (Table 6).

Table 6. Statistical differences between the abundances of *T. pauperior* during consecutive research periods. N—number of observations, X—average, SD—standard deviation, Med.—median.

		N	X	SD	Med.	X Rang
1	1981	27	2.04	1.89	1	151.94
2	1982	27	0.81	1.18	0	104.07
3	2005	27	0.15	0.36	0	66.24
4	2006	27	0.74	1.65	0	90.11
5	2012	27	1.26	2.31	0	99.2
6	2014	27	0.52	0.64	0	94.76
7	2016	27	2.11	1.12	2	169.94
8	2022	27	0.44	0.51	0	91.72

U. pannonica—(ANOVA) Kruskal–Wallis rank test H97, N = 144) = 40.72; $p < 0.001$. The average number of specimens per sample was considerably different in the following periods: 1981–2005 *, 1982–2012 **, 2005–2012 ***, 2006–2012 ***, 2012–2016 *** and 2012–2022 ** (Table 7).

Table 7. Statistical differences between the abundance of *U. pannonica* during consecutive research periods. N—number of observations, X—average, SD—standard deviation, Med.—median.

		N	X	SD	Med.	X Rang
1	1981	18	1.5	2.23	1	90.89
2	1982	18	0.5	0.86	0	67.64
3	2005	18	0.06	0.24	0	47
4	2006	18	0.5	1.42	0	59.53
5	2012	18	1.67	0.97	1	118.67
6	2014	18	0.67	0.69	1	81.28
7	2016	18	0.11	0.32	0	50.5
8	2022	18	0.33	0.49	0	64.5

3.2. Dynamics of Species Composition and Abundance in Uropodina Communities in Examined Ground Plots in Jakubowo Nature Reserve

Different biotic and abiotic conditions prevailing in the examined ground plots resulted in the formation of Uropodina communities with different species composition and structure (Table 8). During the research project, out of 17 Uropodina species found in the reserve, only 7 of them were present in all three monitored plots. They were the following species: *O. minima*, *T. aegrota*, *T. pauperior*, *U. pannonica*, *U. tecta*, *C. rafalskii* and *O. ovalis*. The species found only in plot J-I were *T. lamda* and *O. obsurasimilis*. On the other hand, in plot J-III, there were such species as follows: *N. splendida*, *D. modesta*, *U. pyriformis* and *D. cordieri*. No exclusive species were found in plot J-II.

The highest index of species similarity (S = 62%) was recorded for plots J-II and J-III, whereas the most divergent species composition (S = 44%) was revealed in plots J-I and J-II (see Figure 10).

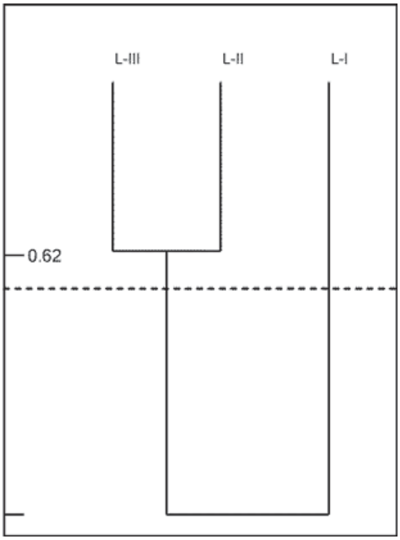

Figure 10. Species similarity (S) in examined ground plots (I, II, III) in Jakubowo nature reserve.

Table 8. Structure of dominance (D%) and frequency of occurrence (F%) of the found Uropodina species in monitored ground plots (J-I, J-II, J-III) in Jakubowo nature reserve during research periods. Bold – dominant and the most frequent species.

Species	1981		1982		2005		2006		2012		2014		2016		2022	
	D%	F%	D%	F%	D%	F%	D%	F%	D%	F%	D%	F%	D%	F%	D%	F%
J-I																
O. minima	**57.89**	25.0	**39.34**	22.50	**54.06**	**57.29**	**46.74**	38.33	17.14	12.50	**54.76**	33.33	26.67	**60.00**	**63.08**	46.67
T. aegrota	22.81	20.0	**45.90**	32.50	**36.11**	46.88	29.35	30.83	**31.43**	25.00	23.81	20.00	23.33	**50.00**	3.08	6.67
U. pannonica	10.53	7.5	1.64	2.50	4.73	8.33	8.42	9.17	**42.86**	28.13	7.14	6.67	3.33	10.00	4.62	10.00
T. pauperior	7.02	7.5			1.13	4.17	4.89	6.67	2.86	3.13	2.38	3.33	**43.33**	**50.00**	4.62	10.00
C. rafalskii	1.75	2.5	1.64	2.50												
U. tecta			4.92	5.00	2.08	7.29	7.61	10.83							6.15	10.00
O. ovalis			4.92	2.50	0.57	1.04	0.27	0.83							4.62	6.67
T. lamda			1.64	2.50												
P. cylindricus					1.13	6.25	2.17	5.00	5.71	6.25	7.14	6.67	3.33	10.00	13.85	23.33
D. cordieri					0.19	1.04										
P. pulchella							0.27	0.83								
Ph. rackei							0.27	0.83			2.38	3.33				
O. obscurasimilis											2.38	3.33				
Number of species	5		7		8		9		5		7		5		7	
J-II																
T. pauperior	**46.51**	17.50	16.67	5.00	4.66	6.25	5.59	4.17					25.00	10.00	11.11	4.76
T. aegrota	23.26	**17.50**	**33.33**	7.50	23.83	26.04	16.78	15.83	No data							
O. minima	18.60	17.50	**33.33**	10.00	**54.40**	38.54	**50.35**	31.67					**75.00**	30.00	**88.89**	28.57
U. tecta	4.65	5.00			12.95	9.38	17.48	10.83								
U. pannonica	4.65	5.00			1.04	2.08	2.10	1.67								
C. rafalskii	2.33	2.50	8.33	2.50	0.52	1.04	3.50	3.33								
D. perforatus			8.33	2.50	0.52	1.04										
O. ovalis					2.07	3.13	4.20	5.00								
Number of species	6		5		8		7						2		2	
J-III																
T. aegrota	**35.63**	45.00	**54.95**	45.00	**64.78**	42.71	**63.71**	**51.67**	4.00	3.33	**48.65**	33.33	23.53	**50.00**	24.00	38.10
O. minima	**32.18**	37.50	18.92	32.50	14.95	26.04	12.79	24.17	8.00	6.67	21.62	20.00	**44.12**	**60.00**	**54.00**	**52.38**
T. pauperior	17.82	32.50	18.02	27.50	3.65	9.38	2.61	7.50	**64.00**	13.33	16.22	16.67	14.71	30.00	4.00	9.52
U. pannonica	10.92	15.00	8.00	7.21	1.99	5.21	1.83	5.00			8.11	10.00				
U. tecta	2.87	10.00	0.90	2.50	5.98	9.38	12.01	21.67	8.00	6.67					4.00	4.76
C. rafalskii	0.57	2.50														
P. cylindricus									4.00	3.33			11.76	20.00	12.00	23.81
O. ovalis					8.64	13.54	6.53	13.33	12.00	3.33					2.00	4.76
D. perforatus											2.70	3.33	2.94	10.00		
N. splendida											2.70	3.33				
D. modesta													2.94	10.00		
U. pyriformis							0.26	0.83								
D. cordieri							0.26	0.83								
Number of species	6		5		6		7		6		6		6		6	

Figure 11 shows the changes in the abundances of the three most numerous Uropodina species in the reserve during the research periods in plots J-I and J-III. In the case of *O.*

minima and *T. pauperior*, one can observe an increase in the abundance, while in the case of *T. aegrot*, the long-term diagram shows a decrease.

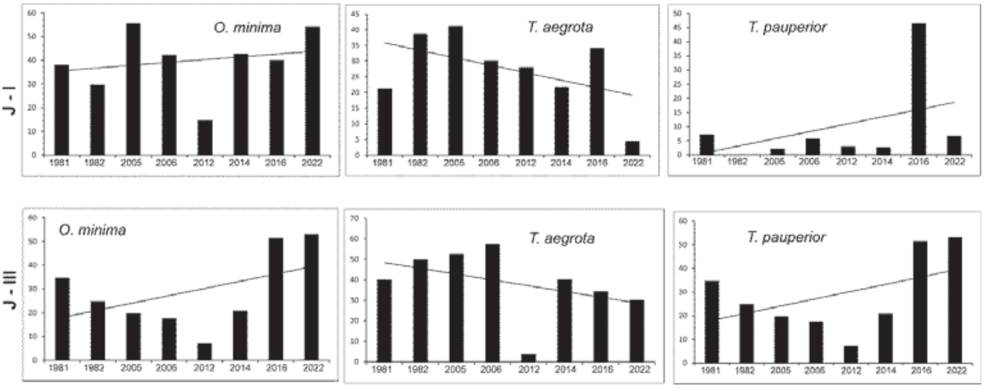

Figure 11. Dynamics of changes in abundances of three most abundant species (average number of specimens per sample) in consecutive research periods (horizontal lines indicate trends) in examined ground plots (J-I, J-III) in Jakubowo nature reserve.

4. Discussion

One of the characteristics of local Uropodina communities in such small forest complexes is the low number of species [3,4]. Out of the 33 species found so far in the oak-hornbeam forests in Wielkopolska, [3], as many as 17 species (51%) were found in the examined location, with the maximum number of species found only in one year, which was 12 species (36%). Thus, one can conclude that the area of Jakubowo nature reserve, despite its small size (4.22 ha), offers relatively favorable living conditions for soil mite communities from this group. The examined communities inhabiting the reserve were dominated by parthenogenetic and eurytopic soil species: *O. minima*, *T. aegrota*, *T. pauperior* and *U. pannonica* [1,15]. These species can be considered a permanent element of the whole community, as they were present throughout all research periods. The occurrence of other species was associated with specific conditions concerning humidity and temperature, resulting from the diversity of the plant cover or, in some cases, the presence of specific micro-environments inhabited by these species. One of these species is associated with the mole's nests, which occasionally appeared in the examined ground plots, i.e., *Ph rackei* [1,8,16], which was found twice during the research. Moreover, in 2006, the presence of *U. pyriformis* and *P. pulchella*, which inhabit dead wood and hollows, was also recorded [1,8,9], probably due to the presence of an increasing amount of dead wood in the areas under scrutiny.

The natural succession of plant cover observed in each ground plot, especially in plot J-I and J-II, undoubtedly has a bearing on the changes in the communities of the discussed group of mites, both in the species diversity and abundance [4]. The observed trend of an increase in the number of *O. minima* and *T. pauperior*, i.e., species with higher moisture requirements [1], in the reserve is most likely caused by the formation of an undergrowth layer, which causes additional shading and helps maintain higher soil moisture. These species compete with *T. aegrota*, which causes a decreasing trend in the abundance of this species. It is noteworthy that the percentage of *O. ovalis*, which is currently one of the most numerous species of Uropodina in Poland [17], is apparently low here. Furthermore, the global warming effect may have been responsible for the appearance in the reserve in 2014 of two species not previously listed here, i.e., *O. obscurasimilis* and *N. splendida*. Perhaps their presence is the result of broadening of the ranges of these species. The former is a species, which occurs in the Carpathian Mountains, and its range extends to the north along the Vistula line, whereas the latter has a south-European and boreal-mountain range of occurrence [1]. However, this phenomenon requires further research.

To sum up, one can conclude that the conducted research confirmed the previous observations on the functioning of the Uropodina communities in Jakubowo nature reserve over the course of 30 years [4]. The obtained results corroborate both the permanent loss of stenotopic and rare species, such as *C. rafalskii* and *T. lamda*, which were not recorded even once after 1982 [4]. Both species are rare and sparse: *Trachytes lamda* Berlese, 1903, has the CR category, according to the Red List for Uropodina of Poland, while *C. rafalski* has the EN category [18]. *Trachytes lamda* was recorded in the study area in the late 1970s but was no longer recorded in the 1980s [1]. It is a species typical for oak-hornbeam forests and this area, as it was still numerous in the 1980s in the nearby area; however, it has not been recorded there since 2005 [1,4]. Moreover, the previously observed decline in the total number of Uropodina in the litter and soil in the examined areas [4] can now be considered a well-established and unfavorable trend, which has been observed for almost 10 years now. These unfavorable changes are undoubtedly the result of the progressive negative anthropogenic processes observed in the examined location.

5. Conclusions

The results obtained from the conducted research project clearly show the importance of constant monitoring of changes taking place in the soil environment of forests. Only in this way is it possible to determine the impact of various factors, both global (climate changes) and local (environmental contamination, plant succession changes, etc.), on the soil environment and the soil fauna that inhabits them to plan any necessary protective measures.

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Article

Diversity and Distribution of Xylophagous Beetles from *Pinus thunbergii* Parl. and *Pinus massoniana* Lamb. Infected by Pine Wood Nematode

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Abstract: The vectors of pinewood nematode of *Bursaphelenchus xylophilus* (Steiner & Bührer, 1934) are mainly known as xylophagous beetles. Understanding the composition and distribution of these xylophagous beetles in host pine trees infected by PWN is critical to control the spread of PWN. In this study, we investigated the community structures of the xylophagous beetles in two main host trees in Fujian and Shandong, *Pinus massoniana* Lamb. and *Pinus thunbergia* Parl., in different stages of infection. All beetles were collected by dissecting the whole pine trees and then identified by their morphological characteristics and COI genes. The results showed that the diversity of xylophagous beetles was different not only between the two host pine trees but also among the different infection stages. The diversity of *P. massoniana* xylophagous beetles was significantly higher than that of *P. thunbergii*, and there were also significant differences in the different stages of PWN infection. In total, Scolytinae was the most common (53.70%), followed by Curculionidae (18.26%), Cerambycidae (16.31%), and Cleridae (6.04%). *Monochamus alternatus*, the most important vector of PWN, occupied a large niche and showed different aggregation positions during the three infection stages in both host trees. This result might be related to the resistance of bark beetles to host trees and competition with other xylophagous beetles. The community diversity of xylophagous beetles was jointly affected by both the infection stages of PWN and the spatial niche of xylophagous beetles. Knowledge of the diversity and competitive relationships among xylophagous beetles might help regulate the population dynamics of these beetles.

Keywords: xylophagous beetles; distribution; *Bursaphelenchus xylophilus*

1. Introduction

Pine wilt disease (PWD) is an internationally recognized destructive disease of pine trees caused by pinewood nematode (PWN). Infected pine trees quickly lose water and wilt to death in a short time [1,2]. Due to its strong pathogenic ability and rapid transmission, PWD is difficult to cure once pine forests are infected, which poses a great threat to China's forest ecosystem [3,4]. This disease has spread to 726 county administrative regions among the 18 provinces in China, covering an area of 1.8092 million hectares and producing a cumulative number of dead pine trees close to 19.47 million [5]. *Pinus massoniana* (Lamb, 1803) and *Pinus thunbergii* (Parl, 1868) are important afforestation trees in the north and south of China, respectively, and are also the main host trees of *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934). Therefore, to protect these pine forests, it is urgent to control the transmission of *B. xylophilus*.

Pine forests have long suffered a complex disease caused by pine wood nematode, carrier insects, other xylophagous beetles, associated fungi, bacteria, and forest abiotic factors [6,7]. The transmission of diseases mainly depends on vector insects carrying pathogen and transferring them to trees [8]. Although all reported vectors of *B. xylophilus* are currently *Monochamus* spp., *Orthotomicus erosus* (Wollaston, 1857) was previously reported to be a carrier of *Bursaphelenchus teratospicularis* (Kakuliya, 1966) and *Bursaphelenchus sexdentati* (Braasch, 2001), while *Pityogenes* sp. was reported to be a carrier of *Bursaphelenchus leoni* (Baujard, 1980) [9,10]. Therefore, in addition to *M. alternatus*, other xylophagous beetles might also be potential carriers of *B. xylophilus*.

The natural transmission of disease is tightly linked with the life history of its insect vectors and spread through the dispersal of adult beetles [11,12]. Therefore, the spatial distribution and dynamic variation of xylophagous beetles on diseased trees are important indicators of the disease [13]. Interestingly, the colonization of xylophagous beetles is often accompanied by competition [14,15], including competition for space and nutrient resources [16]. Xylophagous beetles colonizing pine trees encounter interspecific and intraspecific competition from conspecifics, other cerambycids, and other wood-inhabiting insects that can alter species distributions within trees [17]. In addition, most beetles are more likely to invade weakened pine trees; and the community structure of xylophagous beetles on pine trees was greatly changed after the colonization of pine wood nematode [18]. One of the most important factors causing a decline in pine tree vigor is the colonization of *B. xylophilus* and xylophagous insects [19–22]. Therefore, the diversity was also not only affected by the species of the host trees, but also the tree vigor. Study of biological ecological regulation of the xylophagous insects population plays an important role in successful implementation of chemical or biological control to vectors of PWN. However, the coexistence and competition relationship of these wood-inhabiting beetles was not clear enough in infected pines.

In this research, to understand the colonization sites (phloem and xylem) and colonization sequences of xylophagous beetles on pine trees, we investigated the species richness and spatial distribution of these beetles along PWN-infected tree boles during different stages of the disease. The analysis results of the spatial dynamic patterns of populations and the interspecific relationships between xylophagous beetles at different infection stages after PWN infection could provide valuable insights into the mechanisms of their change dynamics, which would help determine potential carriers of PWN. Understanding the interspecific relationships would also provide a basis for controlling the population of xylophagous beetles [23,24].

2. Materials and Methods

2.1. Study Area

From July to November 2019, we randomly selected pines infected with PWN in a *P. massoniana* forest of Fujian province and a *P. thunbergii* forest of Shandong Province. The infection stages were identified according to the growth of the pine trees infected by PWN, the colors of the pine needle leaves, the number of bite marks and wormholes, and the isolation of *B. xylophilus* [25–27]. We divided the *P. massoniana* and *P. thunbergii* infected by *B. xylophilus* into three stages: the initial stage of infection (weak wood); the medium stage of infection (dying wood); and the terminal stage of infection (a withered tree). In the initial stage of infection, some pine needles were yellow and withered, and the bole had insect bite marks, but the tree still had vitality and water, along with greater pine-resin exudation. In the medium stage of infection, the pine needles turned red and withered over a large area, the tree basically withered, the bole was dry, the borers increased, and the exudation of pine resin decreased. At the terminal stage, the needles largely fell off, the bark was dry, there were pits and insect excreta in the phloem, the tree lost its water completely, and no resin exudation was observed (Figure A1).

2.2. Sampling Design

We cut trees close to the ground without damaging the tree crowns, and the residual root pile did not exceed 5 cm. We then measured and recorded the tree height and DBH. Low-speed drills were used to collect wood at 1.5 m from the base of the bole and at the crown, and pine wood nematodes were collected from wood samples using the Baermann funnel method and identified by the PCR test methods described in Wei et al. and morphological characteristics to determine whether the pine was infected with pine wood nematode disease [28,29]. Primers for partial ribosomal-DNA-large-subunit D2/D3 (LSU D2/D3) were forward-primer D2a (5′-ACAAGTACCGTGAGGGAAAGT-3′) and reverse-primer D3b (5′-TGCGAAGGAACCAGCTACTA-3′) [29]. The identification of *B. xylophilus* was based on diagnostic morphological characteristics. The whole pine was divided into three equal positions according to the height: the upper part, the middle part, and the bottom part. The felled boles were divided by chain saw into one-meter sections. We recorded the number of cavities on the felled bole; then, all insect samples were manually obtained directly from galleries of the infested pine trees using fine forceps; placed into centrifugal tube and store in 96-well frozen storage box; and labeled with the host pine, infection stage, feeding site, and location of the log. Three infected pines were sampled for each stage, and three replicates were used at each sampling site. A total of 27 infected pine trees was collected per site. We then calculated the volume of the truncated wood pine and counted the insect population in each meter of wood. We recorded the species, number, and distribution of insects in the bole. The xylophagous beetles were stored at 4 °C until use.

2.3. Xylophagous Beetle Identification

Morphological identification of the collected xylophagous beetles was accomplished under a stereomicroscope based on external morphological characteristics. These samples were preliminarily identified to the genera and species levels [27]. The cytochrome oxidase subunit I (COI) gene was used for molecular identification of the xylophagous beetles, as described in previous studies [30,31]. We extracted total DNA from 3–5 of each beetle according to morphology and placed each head separately in a centrifuge tube. Then, we used the CTAB method to extract DNA. Next, the purity and concentration of DNA were determined and the DNA was stored at −25 °C. The primers were LepF (5′-ATTCAACCAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGAC CAAAAAATCA-3′). The PCR reactions were conducted in a final volume of 50 μL containing 25 μL of 2 × Taq PCR Master Mix, 2.5 μL of 0.5 μmol/L of each primer, 10 μL of nuclease-free water, and 10 μL of the DNA template [30]. The PCR products were electrophoretically detected on agarose gel with a concentration of 1%. The PCR products were sequenced at the Boshang sequencing company of Fuzhou, and the sequences were deposited in GenBank (MT811991-MT812011). The homologous sequences were screened and the phylogenetic tree was constructed using the MEGA version 5.05 (Mega Limited, Auckland, New Zealand, 2011). For molecular identification, we used NCBI database to perform blast alignment on the obtained sequences, and the morphological identification was based on the described in ”Insect Taxonomy” [32]. The PCR test methods described above was applied in the detection of pine wood nematode in xylophagous beetles collected in this research; thirty insects were detected in each species. All samples were detected for those insects with a low number not reaching thirty [30].

2.4. Functional Diversity

Functional diversity was calculated based on the locations and diets of the beetles using Rao’s diversity index (Rao, 1982; Ricotta, 2005). To account for differences in the number of individuals collected at each site, species richness was rarefied using the Vegan package, version 1.15-0, implemented in the R-statistical environment, version 2.7.1 (Lucent Technologies, Murray Hill, NJ, USA, 2008).

2.5. Statistical Analyses

To compare the species and abundance of xylophagous beetles under different forest types, the effects of different infected *P. massoniana* and *P. thunbergii* trees were analyzed through a one-way ANOVA test and an independent-sample T-test. The number of species (richness) and individual number (abundance) of xylophagous beetles on *P. massoniana* and *P. thunbergii* trees in different infection stages were calculated according to the positions of bole colonization by xylophagous beetles, and the Shannon diversity indices were calculated for the different infection stages. The calculation formulas were as follows:

$$\text{Niche breadth} : B_i = 1/\left(n\sum {P_{ik}}^2\right)\text{a} = 1, \tag{1}$$

where B_i is the niche width of the ith species, n is the value of the niche resource levels, and P_{ik} is the proportion of the individuals of the ith species among the total number of individuals in the total resources.

$$\text{Nicheoverlap} : Q_{ij} = \sum (P_{ik}P_{jk}) / \sqrt{(\sum {P_{ik}}^2 \sum {P_{jk}}^2)} \tag{2}$$

where Q_{ij} is the niche overlap values of species i and species j, and P_{ik} and P_{jk} are the proportion of individuals using the kth resource unit of species i and species j, respectively, compared to the total number of corresponding species in the total resources.

We adopted the dominance index of Berger–Parker (1970):

$$d = N/N_t \tag{3}$$

where d is the dominance index, N is the number of individuals in a group, and Nt is the number of all species.

$$H' = -\sum p_i \log p_i \tag{4}$$

where H' is community diversity, and p_i is the probability of the occurrence of species i. Community evenness was then calculated and analyzed using the formula proposed by Pielou (1966):

$$E = H'/\ln S \tag{5}$$

where E is community evenness, H' is community diversity, and S is species richness. In the case of insect communities, species richness S is the number of species in the community.

3. Results

3.1. Identification, Composition, and Diversity of Xylophagous Insects in the Forests of P. thunbergii and P. massoniana

The results of investigating *P. massoniana* and *P. thunbergii* infected with PWN were as follows: the average DBH of *P. massoniana* was 17.3 cm (14 m higher on average); the average DBH of *P. thunbergii* was 12 cm (12.3 m higher on average) (Table A1).

According to molecular identification of the COI gene and comprehensive identification of the morphological characteristics, we identified 20 species of insects. In this experiment (Figure 1), a total of 12,272 xylophagous beetles from 16 species with high richness were selected for analysis. Among them, nine species were taken from the *P. massoniana* forest and nine species were taken from the *P. thunbergii* forest. These species included six species in the family Curculionidae, subfamily Scolytinae, Ipidae, including *Xyleborus hawaiiensis* (Perkins, 1900), *Orthotomicus laricis* (Fabricius, 1792), *Orthotomicus erosus* (Nordl, 1888), *Pityogenes trepanatus* (Nördlinger, 1848), *Pityokteines spinidens* (Reitt, 1894), and *Cyrtogenius luteus* (Blandford, 1894), accounting for 53.70%; four species in the family Cerambycidae including *M. alternatus*, *Arhopalus rusticus* (Linnaeus, 1758), *Arhopalus* *unicolor* (Gahan, 1906), and *Cephalallus oberthuri* (Sharp, 1905), accounting for 16.30%; three species in the family Elateridea including *Homotechnes brunneofuscus* (Nakane, 1954), *Dalopius vagus* (Brown, 1934), and *Tetrigus lewisi* (Candèze, 1873), accounting for 4.19%; two species in the family Curculionidae including *Shirahoshizo patruelis* (Voss, 1937) and *Hypposipalus gigas* (Fabricius, 1775), accounting for 18.26%; and one species in the family Cleridae (*Trichodes leucopsideus* Olivier, 1795), accounting for 18.26%. Other species belonged to Carabidae (1), Scarabaeidae (1), and Buprestidae (1).

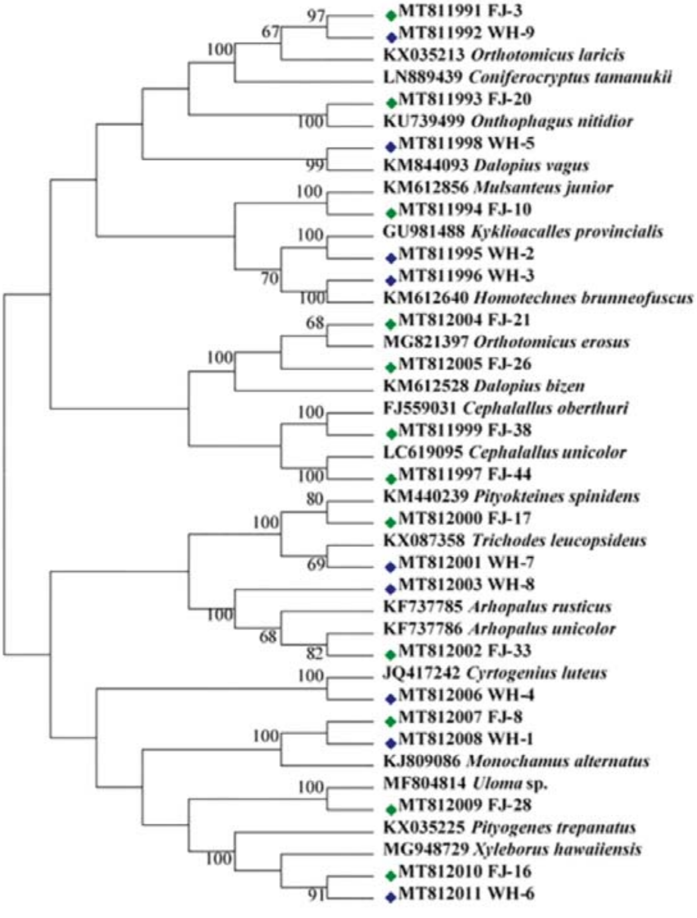

Figure 1. Phylogenetic tree based on the COI sequences of 20 xylophagous beetles collected from pine infected with PWN. FJ1–FJ12 labeled on green dots indicates xylophagous beetles collected from *P. massoniana* Lamb.; WH1–WH9 labeled on blue dots indicates xylophagous beetles collected from *P. thunbergii* Parl.; others indicate the reference sequences.

3.2. Rarefaction Curves Presenting the Relationship between the Number of Samples and Insect Species Richness in P. massoniana and P. thunbergii Forests

In this study, we analyzed a dilution curve constructed by the Shannon index (Figure 2). The results showed that the Shannon index at the species level increased with an increase in the amount of sampled data. When the amount of sampled data reached a certain value, the curve increased until becoming smooth. The sampling depth was sufficient, the amount of sampled data was reasonable, and most insect samples were covered.

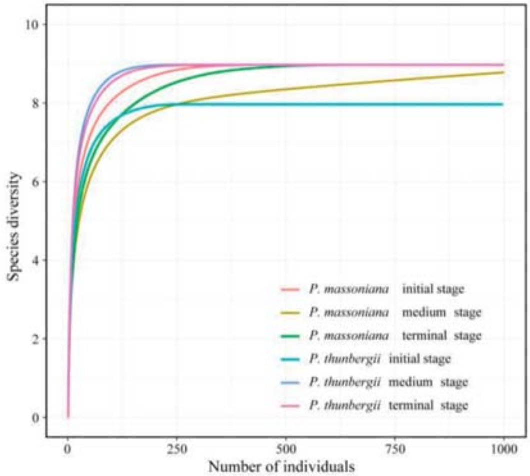

Figure 2. Rarefaction curves of the species richness level of *P. massoniana* and *P. thunbergii* at different affected stages. The red, yellow, and green curves represent the *P. massoniana* samples in three stages; the cyan, blue, and purple curves represent the *P. thunbergii* samples in three stages. Broken lines represent the 95% confidence interval for each curve.

3.3. Community Structure of Xylophagous Beetles at Different Infection Stages in the Forests of P. massoniana and P. thunbergii

In the *P. massoniana* forest, 11 insect species were identified: three species in the family Scolytinae, accounting for 54.52%; one species in the family Curculionidae, accounting for 24.29%; three species in the family Cerambycidae, accounting for 15.09%; one species in the family Cleridae, accounting for 3.15%; one species in the family Elateroidea, accounting for 0.93%; and other insects accounting for 2.01% (Figure 3).

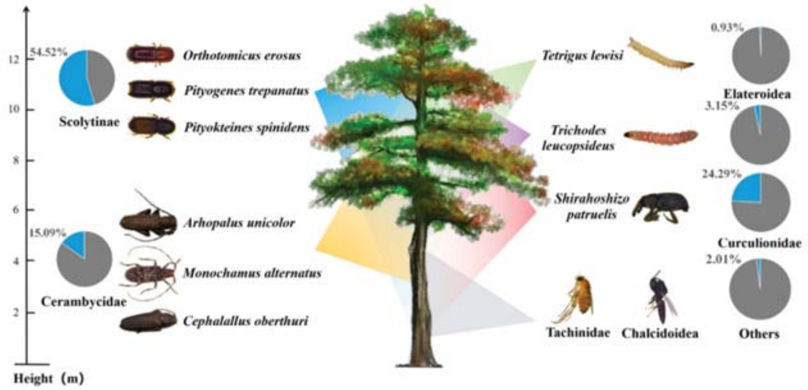

Figure 3. Diversity and distribution of xylophagous beetles and other insects in infected *P. massoniana.* The pie chart shows the proportion of xylophagous beetles, and the projection shows the distribution of xylophagous beetles.

In the *P. thunbergii* forest, nine insect species were identified: three species in the family Scolytinae, accounting for 51.43%; two species in the family Cerambycidae, accounting for 19.75%; one species in the family Cleridae, accounting for 14.14%; two species in the family Elateroidea, accounting for 13.33%; and one species in the family Curculionidae, accounting for 1.36% (Figure 4).

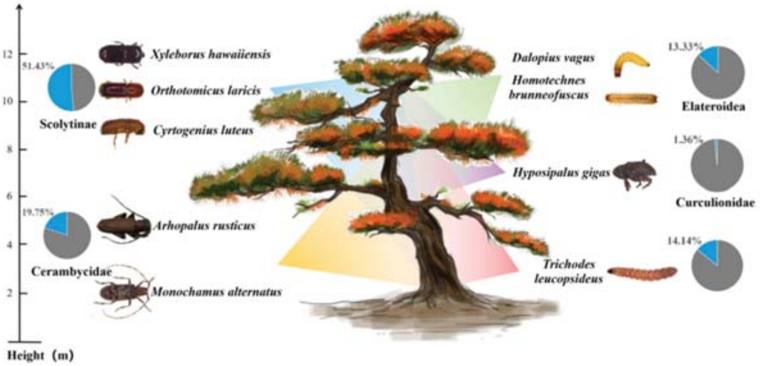

Figure 4. Diversity and distribution of xylophagous beetles in infected *P. thunbergii*. The pie chart shows the proportion of xylophagous beetles, and the projection shows the distribution of xylophagous beetles.

In the *P. massoniana* forest, *S. patruelis* and *O. erosus*, the two largest populations, were distributed in different stages of pine infection, while *M. alternatus*, an important vector of pine wood nematode disease, was enriched in the upper part of the initial stage of pine infection and the middle and bottom parts of the terminal stage of infection. In the *P. thunbergii* forest, *M. alternatus* was clustered in the bottom part of the bole during the medium and terminal stages of the disease (Figure 5).

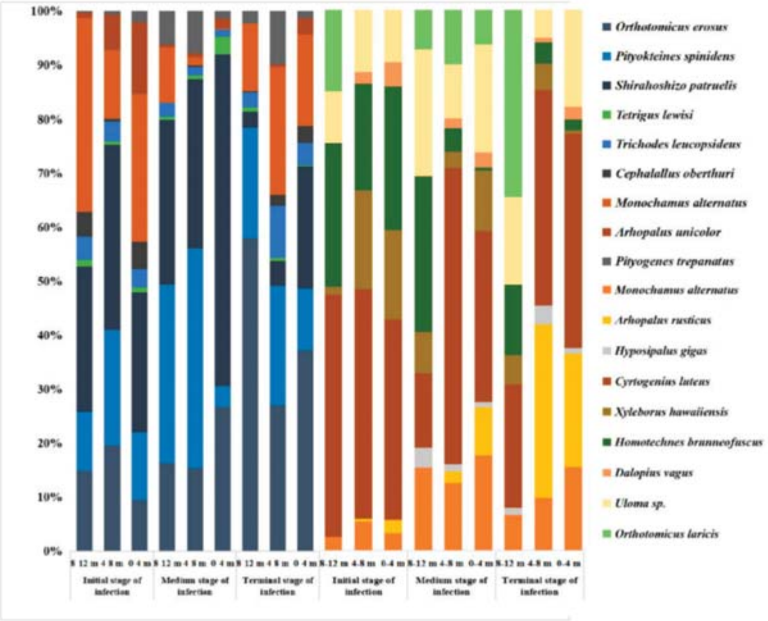

Figure 5. Composition of xylophagous beetles in the upper, middle, and bottom positions of *P. thunbergii* and *P. massoniana* infected by PWN at different stages.

3.4. Identification of Dominant Insect Species and Division of the Functional Groups

The dominant insect species of *P. massoniana* and *P. thunbergii* in different infection stages were analyzed according to the dominance index. The species were divided into three different functional groups: woodboring beetles, bark beetles, and predators. In the *P. massoniana* and *P. thunbergii* forests infected with PWN, bark beetles dominated, with a dominance diversity of 0.8043 and 0.6611, respectively. *Orthotomicus* sp. was the dominant species, with a dominance index (*d*) of 0.3544 and 0.1261 in *P. massoniana* and *P. thunbergii*, respectively. Woodboring beetles mainly lived in weak trees infected with pine wood nematode, and their dominance indexes were 0.1510 and 0.1975, respectively. In this functional group, *M. alternatus* presented the largest dominance diversity of 0.8297 and 0.5416 in *P. massoniana* and *P. thunbergii*, respectively. The dominance of predators was 0.0617 and 0.1414, respectively, in the two forests (Tables 1 and 2).

The composition of xylophagous beetles in the two forests changed significantly in the different infection stages. The diversity of xylophagous beetles in the forest increased gradually with an increase of the infection stage in the *P. massoniana* forest (from 0.69 to 0.79, Table 3). According to the Shannon index, the diversity of xylophagous beetles was reduced in the terminal stage of infection in the *P. thunbergii* forest (0.66) and continued up to the terminal stage. In the medium stage, the diversity of xylophagous beetles in the *P. thunbergii* forest (0.76) was similar to that in the *P. massoniana* forest (0.79) (Figures A2 and A3 and Table 3).

Table 1. Function groups of insects and their dominance index (*d*) in *P. thunbergii* Parl.

Function Groups	*d* (Functional Group)	Dominant Insect Species	*d* (Insects)
woodboring beetles	0.1975	*Monochamus alternatus*	0.5416
		Arhopalus rusticus	0.4584
bark beetles	0.6611	*Hyposipalus gigas*	0.0206
		Cyrtogenius luteus	0.5570
		Xyleborus hawaiiensis	0.0947
		Homotechnes brunneofuscus	0.1777
		Dalopius vagus	0.0239
		Orthotomicus laricis	0.1261
predators	0.1414	*Trichodes leucopsideus*	1.0000

Table 2. Function groups of insects and their dominance index (*d*) in *P. massoniana* Lamb.

Function Groups	*d* (Functional Group)	Dominant Insect Species	*d* (Insects)
woodboring beetles	0.1510	*Monochamus alternatus*	0.8297
		Arhopalus unicolor	0.1055
		Cephalallus oberthuri	0.0648
bark beetles	0.8043	*Orthotomicus erosus*	0.3544
		Pityokteines spinidens	0.2863
		Shirahoshizo patruelis	0.3082
		Pityogenes trepanatus	0.0511
		Monochamus alternatus	0.8297
predators	0.0617	*Tetrigus lewisi*	0.1536
		Trichodes leucopsideus	0.5210

Table 3. Community characteristic indexes of xylophagous beetles in *P. massoniana* and *P. thunbergii* at different infection stages.

Infected Stages	*P. massoniana*			*P. thunbergii*		
	H'	E	S	H'	E	S
Initial stage	0.69 ± 0.06	0.35 ± 0.02	7	0.79 ± 0.07	0.36 ± 0.04	9
Medium stage	0.79 ± 0.03	0.38 ± 0.02	8	0.76 ± 0.03	0.37 ± 0.06	8
Terminal stage	0.79 ± 0.02	0.38 ± 0.01	8	0.66 ± 0.06	0.30 ± 0.06	9

Note: Data are means ± standard error (SE), $n = 3$.

3.5. Interspecific Competition among Xylophagous Beetles at Different Infection Stages in P. thunbergii and P. massoniana Forests

By comparing the niche breadth of the nine species of xylophagous beetles with the greatest population richness in each forest, we found that *D. vagus* occupied the main niche in the *P. thunbergii* forest (0.993). In the *P. massoniana* forest, *M. alternatus* had the largest niche breadth (0.909) (Table A2). In the *P. thunbergii* forest, *T. leucopsideus* and *C. luteus* firmly occupied the middle and bottom positions of the bole in the medium and terminal stages of the infected pine trees, spatially following *M. alternatus*, while *O. laricis* (Q = 0.971) competed with *M. alternatus* in the middle part of the bole at the specific medium stage of the disease and showed obvious aggregation behavior in the upper part of the bole at the initial and terminal stages of the disease. The niche overlap index of *O. laricis* and *M. alternatus* gradually increased over the duration of infection. Niche overlap also existed among *P. spinidens*, *O. erosus*, and *T. lewisi* (Q > 0.969) (Figure 6, Table A3). In addition, *O. laricis* and *A. rusticus* showed the largest niche overlap index, which mainly occurred in the middle and bottom parts of the bole in the terminal infection stage.

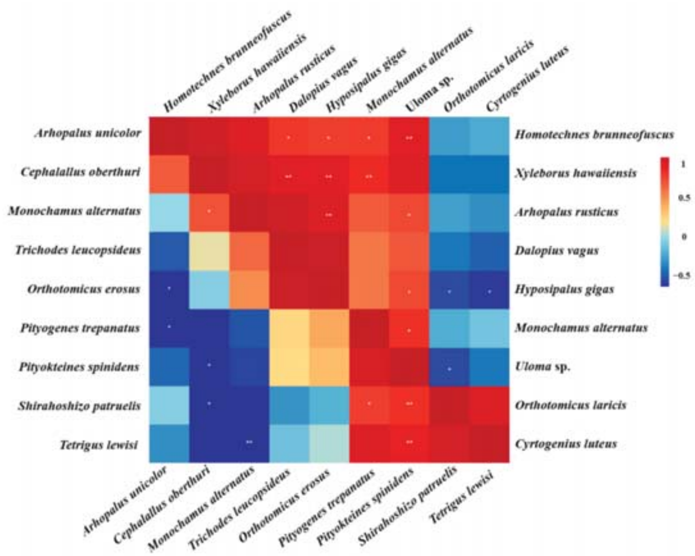

Figure 6. Correlation heat map analysis of xylophagous beetles in *P. massoniana* and *P. thunbergii*. The red color of the grid in the figure indicates that the niche overlap index between vertical and horizontal species is larger, and the blue color indicates that the niche overlap index between species is smaller; and * indicates significant ** indicates extremely significant.

3.6. Identification of PWN in Xylophagous Beetles

According to the result of the PCR test and morphological characteristics, *B. xylophilus* was isolated from *M. alternatus* in both *P. massoniana* and *P. thunbergia*. No *B. xylophilus* was isolated from other xylophagous beetles and other insects in this research.

4. Discussion

In pine forests infected by *B. xylophilus*, the complex invasions of xylophagous beetles accelerate the death of pine trees, reduce the resistance of pine trees to *B. xylophilus*, and provide intermediate hosts for the transmission of PWN [33]. In our study, the composition and distribution of xylophagous beetles were surveyed in *P. massoniana* and *P. thunbergia* trees in different infection stages. Nine species of xylophagous beetles were identified in *P. massoniana* and *P. thunbergii*, respectively. In addition, two parasites were identified in *P. massoniana*. These parasites shared two of the same taxa: *M. alternatus* and *T. leucopsideus*

M. alternatus and Cleridae were also reported in *Pinus yunnanens* infected by PWN [34]. Moreover, in the present study, no *B. xylophilus* was isolated from these xylophagous beetles except for *M. alternatus*. However, *O. erosus* and *Pityogenes* sp. were reported as carriers of *Bursaphelenchus* spp. [35]. More studies are needed to determine if there are other carriers of *B. xylophilus* besides *Monochamus*.

The composition and structure of xylophagous beetles were different between the two host trees species in different infection stages. The diversity of insects was affected by multiple biotic and abiotic factors, such as climate, topography, soil, host plants, and natural enemies [36]. Together, these complex factors influenced the distribution and population of xylophagous beetles in the two forests. Pine trees infected by *B. xylophilus* have different weakening cycles when observed in different forests, different stand ages, and different management modes, each showing different attraction effects to xylophagous beetles. For example, Blatt et al. [37] caught greater numbers of several *Monochamus* species in Christmas tree plantations than in adjacent forests.

Interestingly, the population of *M. alternatus*, an important vector of pine wood nematode disease, was enriched in the upper part of *P. massoniana* during the initial infection stage, while the middle and bottom parts were enriched in the terminal infection stage. In *P. thunbergii*, *M. alternatus* also tended to clustered in the bottom part during the medium and terminal infection stages. This phenomenon might be related to the host tree's utilization of nutritional resources via vertical migration [22]. *T. leucopsideus* and *C. luteus* were also enriched in the middle and bottom sections of the bole in the medium and terminal stages, possibly because the complex colonization of other xylophagous beetles increased the pressure of nutritional competition in the crown layer during the medium and terminal infection stages. Additionally, during the terminal stage of PWN infection, pine trees lost a great deal of water and nutrients. The canopy of the pine tree then became unsuitable for xylophagous beetles, so they migrated to the bottom part of the bole [38]. Akbulut et al. [17] also believed that the loss of water from pine bole might reduce the survival rate of xylophagous beetles.

The colonization order of insect functional groups living on pine trees also followed a certain law (from health to infection to death) for the pine wood nematode. The relative richness of *O. erosus* was found to be the highest in xylophagous beetles in *P. massoniana.* Moreover, *O. erosus* was reported to be a pioneer species [39] able to reduce the resistance and tree potential of the host pine. Subsequently, other xylophagous beetles and predators gradually enter the tree. In the different stages of infection with pine wood nematode disease (until death), insects with different functional groups occupy the dominant position. According to the dynamic analysis of the niche, *M. alternatus*, as a secondary pest, damaged the weakened pine trees after colonization with pioneer bark beetles such as *C. luteus* and *O. erosus.* Thus, colonization with *M. alternatus* might benefit from bark beetles. The presence of exit holes, galleries, and tunnels made by the primary colonizers subsequently has a positive effect on the species richness and abundance of secondary colonizers and their predators [40,41]. It was previously reported that bark beetle reaches its niche load in terms of its trophic niche during the medium stage of infection, which limits increases in the *M. alternatus* population [42]. Therefore, the colonization of other xylophagous beetles might affect the distribution of the *M. alternatus* population.

In the initial stage of infection, *M. alternatus* was found to be mainly in competition with Scolytidae, which was able to overcome the resistance of the host pine tree and further reduce the tree's vigor. In the terminal stage of infection, competition with *M. alternatus* was mainly provided by species such as Elateridae. At this stage, xylophagous beetles in the bole mainly played a role in accelerating the decline of the pine trees [43]. We suspect that intraspecific competition, cannibalism, and resource quality affected the survival of *M. alternatus*; otherwise, the species without a competitive advantage would be eliminated early [44].

The niche overlap between *O. laricis* and *M. alternatus* accompanied the entire infection cycle and peaked at the terminal stage. Scolytinae had a certain inhibitory effect on the canopy of *M. alternatus*, which together limited the population growth of *M. alternatus*. Research on xylophagous beetles in *P. massoniana* in Zhejiang also found that *Tomicus piniperda* (Scolytinae) inhibited population growth of the *M. alternatus* [18]. The population dynamics of *M. alternatus* might be related to the resistance of bark beetles to host trees and competition with other xylophagous beetles. In the *P. massoniana* forest, *O. erosus* and *T. leucopsideus* showed an obvious spatial migration phenomenon, suggesting that *T. leucopsideus* might be a predator of *O. erosus*. Ultimately, understanding the spatial distribution and interspecific relationships of xylophagous beetles in pine trees infected with *B. xylophilus* provides a basis for regulating the population of PWN vectors to control the transmission of PWD.

5. Conclusions

The diversity of xylophagous beetles was different not only between the two host pine trees but also among the different infection stages. *M. alternatus* and *T. leucopsideus* were investigated in both pines. *M. alternatus*, as the only vector of *B. xylophilus* in our research, occupied a large niche and showed a similar population fluctuation during the three infection stages in both host trees. It might be related to the cooperation and competition of other xylophagous beetles to the host defension and nutritional utilization.

Author Contributions: Data curation, X.C. and Q.M.; Investigation, X.C.; Methodology, X.H.; Project administration, F.Z.; Resources, X.H.; Supervision, M.Y., G.L. (Guoqiang Li), J.L., G.L. (Guanghong Liang), S.W., R.W. and X.H.; Writing—original draft, X.C. and Q.M.; Writing—review & editing, Q.M. and X.H. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The data presented in this study are openly available in Zenodo at 10.5281/zenodo.5235663.

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

Appendix A

Figure A1. Three stages of pine infected with pine wood nematode disease. From left to right are the initial stage, medium stage, and terminal stage of the disease.

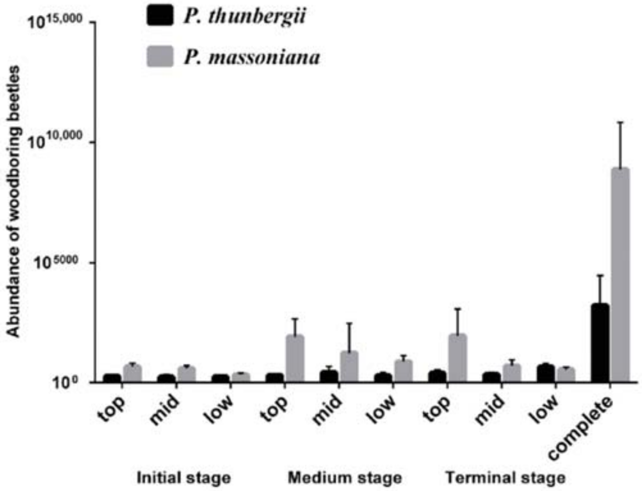

Figure A2. Abundance of xylophagous beetles in different infected stage.

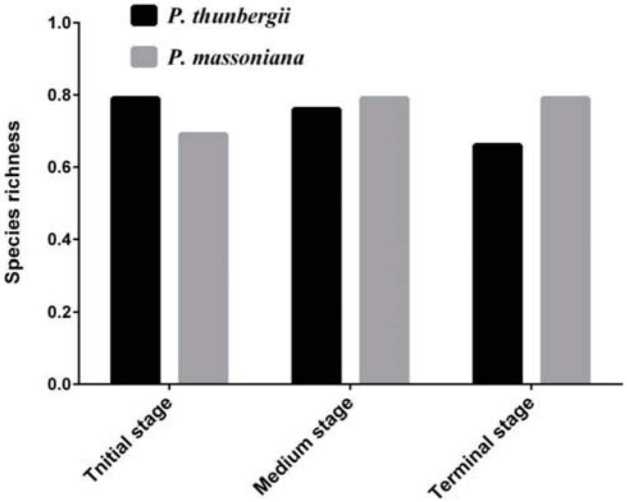

Figure A3. Species richness of xylophagous beetles in different infected stages.

Table A1. The average height, diameter at breast height (DBH), and annual ring of the sample pine trees in the infected *P. thunbergii* and *P. massoniana*.

Host Tree	Forest Composition	Infected Stage	Average DBH (cm)	Average Hight (m)	Annual Ring (Year)
Pinus thunbergii	Pure forest	Initial stage	9.26 ± 3.61	10.25 ± 2.523	18.7 ± 4.2
		Medium stage	11.50 ± 1.53	12.01 ± 2.650	20.3 ± 2.5
		Terminal stage	16.31 ± 2.65	15.24 ± 2.082	26.7 ± 4.0
Pinus massoniana	Mingled forest	Initial stage	15.44 ± 2.52	12.63 ± 2.659	15.0 ± 2.9
		Medium stage	17.58 ± 3.51	13.52 ± 2.009	20.0 ± 1.1
		Terminal stage	20.19 ± 4.51	17.17 ± 2.520	20.1 ± 3.6

Table A2. Niche breadth of xylophagous beetles in the infected *P. thunbergii* and *P. massoniana*.

Beetles in *P. thunbergii*	**Niche Breadth**	**Beetles in *P. massoniana***	**Niche Breadth**
Monochamus alternatus	0.777	*Monochamus alternatus*	0.909
Cyrtogenius luteus	0.967	*Orthotomicus erosus*	0.713
Homotechnes brunneofuscus	0.879	*Pityokteines spinidens*	0.761
Orthotomicus laricis	0.831	*Shirahoshizo patruelis*	0.630
Arhopalus rusticus	0.446	*Pityogenes trepanatus*	0.653
Hyposipalus gigas	0.661	*Tetrigus lewisi*	0.729
Xyleborus hawaiiensis	0.927	*Trichodes leucopsideus*	0.871
Dalopius vagus	0.993	*Cephalallus oberthuri*	0.703
Trichodes leucopsideus	0.892	*Arhopalus unicolor*	0.716

Table A3. Niche overlap of xylophagous beetles in the infected pine trees.

Beetles in *P. thunbergii*	**Label**	**Beetles in *P. massoniana***	**Label**
Monochamus alternatus	1	*Monochamus alternatus*	A
Cyrtogenius luteus	2	*Orthotomicus erosus*	B
Homotechnes brunneofuscus	3	*Pityokteines spinidens*	C
Orthotomicus laricis	4	*Shirahoshizo patruelis*	D
Arhopalus rusticus	5	*Orthotomicus erosus*	E
Hyposipalus gigas	6	*Tetrigus lewisi*	F
Xyleborus hawaiiensis	7	*Trichodes leucopsideus*	G
Dalopius vagus	8	*Cephalallus oberthuri*	H
Trichodes leucopsideus	9	*Arhopalus unicolor*	I
Niche overlap index			
Q12	0.933	Qab	0.881
Q13	0.681	Qac	0.726
Q14	0.971	Qad	0.576
Q15	0.812	Qae	0.661
Q16	0.990	Qaf	0.704
Q17	0.741	Qag	0.960
Q18	0.900	Qah	0.925
Q19	0.986	Qai	0.804
Q23	0.878	Qbc	0.811
Q24	0.970	Qbd	0.566
Q25	0.788	Qbe	0.807
Q26	0.521	Qbf	0.736
Q27	0.899	Qbg	0.976
Q28	0.994	Qbh	0.647
Q29	0.980	Qbi	0.460
Q34	0.735	Qcd	0.936
Q35	0.446	Qce	0.992
Q36	0.578	Qcf	0.990
Q37	0.991	Qcg	0.826
Q38	0.923	Qch	0.465
Q39	0.785	Qci	0.559
Q45	0.945	Qde	0.910
Q46	0.959	Qdf	0.975
Q47	0.767	Qdg	0.628
Q48	0.939	Qdh	0.381
Q49	0.986	Qdi	0.624
Q56	0.849	Qef	0.969
Q57	0.452	Qeg	0.795
Q58	0.628	Qeh	0.366
Q59	0.812	Qei	0.446
Q67	0.643	Qfg	0.777
Q68	0.841	Qfh	0.475
Q69	0.959	Qfi	0.624

Table A3. *Cont.*

Beetles in *P. thunbergii*	Label	Beetles in *P. massoniana*	Label
Q78	0.939	Qgh	0.784
Q79	0.829	Qgi	0.642
Q89	0.960	Qhi	0.994

Note: 1–9 represents nine species of xylophagous beetles in the *P. thunbergii* forest, A–I represents nine species of xylophagous beetles in the *P. massoniana* forest.

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