

# Search the canopies and you will find new species of insects

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## Abstract

Arthropods were collected by fogging the canopy of Scots pine *Pinus sylvestris* selected from a 2 km<sup>2</sup> boreal forest area in Sigdal, Norway with the overall purpose to examine whether there were faunal differences in the representation of arthropods among mature and old trees, and specifically for this paper, the biting midges (Ceratopogonidae). Target trees were chosen as pairs, one mature (70-110 years) and one old (250 years or older) tree from six different stands. All knock-down treatments were performed in June and July 1999, before dawn and after a dry and windless night. Knocked-down arthropods were collected in plastic funnels placed systematically on the ground. Funnels remained in place for circa one hour after treatment. Among the 61 species records new to Norway, the most frequently encountered taxon of invertebrates was Diptera, and the family of biting midges, Ceratopogonidae, comprised 30 of 61 (49%) of all new records, compared with the overall species numbers showing 40 biting midges of 193 recorded species (21%). Among the Ceratopogonidae new to Norway, two species new to science and two first records from Europe were found. Coleman rarefaction curves were constructed by running 500 iterations without replacements using EstimateS and showed that there were significantly more new records of Diptera in old trees in comparison with mature trees. A similar pattern of significance (by comparing standard deviations estimated by EstimateS) was found for Diptera when Ceratopogonidae was excluded. New species records of Ceratopogonidae were more common in old trees than in mature trees, although not significantly so. No predominance of new records in old trees was found for arthropods other than Diptera. Old trees are rare and may provide a variety of resources (e.g. resting sites, places to over-winter, hiding places, sites for oviposition, larval habitat, etc.) that are rarely found in younger trees. Thus, the high number of new species records probably result from studying a whole arthropod taxon (Diptera) in a part of a forest ecosystem (canopies) with a suite of microhabitats (old pine trees) that in combination has been poorly investigated earlier.

## Zusammenfassung

In einem 2 km<sup>2</sup> großen borealen Waldgebiet in Sigdal, Norwegen, wurden die Baumkronen von Waldkiefern (*Pinus sylvestris*) benebelt, um die Unterschiede in der Zusammensetzung der Fauna erwachsener bzw. alter Bäume herauszuarbeiten. Für die vorliegende Arbeit wird hier besonders die Zweiflüglerfamilie der Ceratopogonidae herangezogen. Benebelt wurden die Bäume von sechs verschiedenen Standorte und jeweils paarweise, d.h. jeweils ein erwachsener (70-110 Jahre alt) und ein alter Baum (250 Jahre oder älter), die Benebelungen fanden im Juni und Juli 1999 vor Sonnenaufgang und nach einer trockenen, warmen Nacht statt. Die

herab fallenden Arthropoden wurden während ca. 1 Std. in Plastiktrichtern am Boden aufgefangen. Die 61 für Norwegen neuen Artnachweise enthielten vor allem Zweiflügler, und 30 der 61 Neunachweise waren dabei Ceratopogonidae (49%), mit zwei neuen Arten für die Wissenschaft und zwei Arten neu für Europa. Statistische Analysen zeigen, dass in den alten Bäumen signifikant mehr Neunachweise zu finden waren als in der jüngeren Vergleichsbäumen. Dies gilt auch für die übrigen Diptera, ohne die Ceratopogonidae. Als Gründe für die hohe Artenzahl in den alten Bäumen wird das hohe Angebot an Ressourcen wie Larvenhabitate, Versteckmöglichkeiten, Totholzstrukturen etc. angeführt, die in jüngeren Bäumen noch selten sind oder fehlen.

## Introduction

The methods of gaining access to the forest canopy developed for the tropics have stimulated a biodiversity and faunistical renaissance in the exploration of temperate forest canopies. Numerous studies from tropical forests have shown that forest canopies contain a wide and diverse fauna of arthropods which, in many cases, is endemic to the canopies. To a certain level, this also applies to temperate forest canopies (e.g. Borkowski 1986, Winchester and Ring 1996a, b, Aakra 2000, Hagan et al. 2000, Szadziewski and Hagan 2000, Thunes et al. 2003, 2004), and the present paper is a further attempt to determine patterns of specialisation among arthropods in the canopy environment.

The aim of this paper is to examine whether certain taxa of invertebrates found in pine canopies in Norway can be said to be associated with the canopies to a greater degree than to the conventional sampling area of the forests, the forest floor and lower vegetation. By that, we presuppose that the unusually high number of new records found in comparison with faunal studies made elsewhere and with conventional sampling techniques [e.g. the All Taxa Biological Inventory, in the Great Smoky Mountains National Park (Reeves et al. 2004)], indicates that some (if not most) of these new records are actual canopy associates. Moreover, we also investigate whether there are historical factors which have resulted in differences in the canopy fauna by comparing the faunas found in mature and old pine trees.

## Material and methods

The study was performed in one of the permanent plots established as a part of the biodiversity programme 'Miljøregistrering i Skog', which was initiated in 1997 and funded by the Norwegian Ministry of Food and Agriculture. The study area of Heimseteråsen in Sigdal municipality, county of Buskerud (60°03'N, 9°25'E), comprise approximately 2km<sup>2</sup> of boreal forest, dominated by *Pinus sylvestris* L. intermixed with *Picea abies* (L.) Karst. (both Pinaceae) and *Betula pubescens* Ehrh. (Betulaceae). The study area was protected in 2002 as a part of the Trillemarka and Heimseteråsen nature reserves and is included in the proposed protection plan for Trillemarka - Rollag Østfjell (Fylkesmannen i Buskerud 2005).

For the purpose of this study, twelve pines were treated with a motorised fogging device (Swingfog 50) applying a synthetic pyrethroid (PySekt) at a concentration of 1%, which was dissolved in non-aromatic white spirit. All knock-down treatments were performed between June 19<sup>th</sup> and July 17<sup>th</sup> 1999 before dawn and after a dry and windless night in order to avoid breakdown of the insecticide and to minimise drift of the emitted mist of insecticide. Knocked-down arthropods were collected in 300mm diameter plastic funnels which were systematically placed on the ground following a trapping-web pattern, i.e. funnels were placed in circles under the crown. Within a circle, the funnels were spaced 1m apart from each other and the distance between each circle was 0.5m. This setup covered about 25% of the area below the canopy. The funnels remained on the ground for approximately one hour after treatment. Further

sampling details can be found in Thunes et al. (2004).

The target trees were chosen as pairs, six mature *versus* six old trees that were never further apart from each other than 50m. Their ages were determined by taking increment core samples. A tree was regarded mature when the age was determined to be within the age of a harvestable tree, i.e. between 70 and 110 years. The definition of a tree as old was more arbitrary, selecting trees older than 250 years old. The oldest tree that was sampled was 330 years old.

Thunes et al. (2003), that reported results from both Sigdal and Kvam study areas, found clear indications that species new to the Norwegian fauna were more commonly encountered in old trees. Here, we concentrate on the Sigdal samples from 1999 only, not only to avoid spatial and temporal differences, but also because the representation of new species records was greatest at this site. Moreover, the number of sampled trees was greater in Sigdal (twelve *versus* six in Kvam), thus giving more robust data. In this study we wanted to investigate whether that difference was constant over the entire fauna of new records or if the observed differences were related to the appearance of new records within a particular taxon. In order to analyse the data collected, rarefaction curves were constructed by running 500 iterations with EstimateS, version 7.5 (Colwell 2005) without replacements. Furthermore, the rarefaction curves of mature *versus* old trees were directly comparable when the number of sampled specimens was plotted along the x-axes and when non-overlapping standard deviations were used as an indicator of significance.

**Results and Discussion**

Approximately 27000 specimens of arthropods were collected from the 12 pine trees. The total catch was 332 species, excluding large taxa such as Chironomidae, parasitoid Hymenoptera and mites other than Oribatiidae. All taxa where new species records were

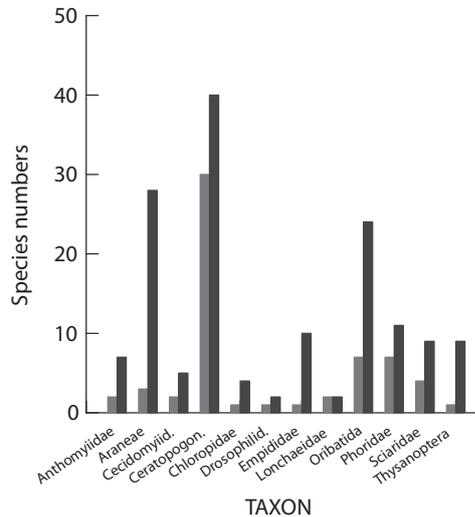


Fig. 1: Number of new species records (grey columns) and total number of species (black columns) sampled in Sigdal in 1999 for various Diptera taxa and spiders, oribatid mites and thrips. No new species were recorded for other taxa.

taken in Sigdal 1999 are quantitatively illustrated in Fig. 1 and detailed information can be found in Thunes et al. (2003, 2004).

Of the 96 new species records presented in Thunes et al. (2004) from both east and west Norway, the 61 new records encountered in Sigdal in 1999 alone are shown in Tab. 1. Even though not greatest in abundance, by far the greatest number of both species and new species records were recorded for Diptera. Fifty new species records of Diptera were collected in Sigdal in 1999 (Tab. 1). In particular, thirty species of Ceratopogonidae (biting midges) were new to Norway, including two species new to science (see Szadziewski and Hagan 2000 for their description) and two first records from Europe. Most Diptera, together with the majority of Hymenoptera, are poorly documented insect orders in Norway. Due to lack of taxonomical expertise, the Hymenoptera sampled in this study remained almost entirely unidentified, along with a few other species rich taxa of Diptera, e.g. the Chironomidae. The high proportion of new species records in Diptera does not neces-

Tab. 1: New species records of invertebrates taken from twelve pine canopies in 1999 in Sigdal municipality, Norway. Total number of specimens are shown with the number of specimens from old trees in parentheses.

higher taxon	family	species	new to:	NSpms (Old)	
Araneae	Theridiidae	<i>Theridion pinastri</i> Koch, 1862	Norway	1 (0)	
		<i>Dipoena torva</i> (Thorell, 1871)	Norway	1 (1)	
Acari	Linyphiidae	<i>Entelecara flavipes</i> (Blackwall, 1834)	Norway	2 (2)	
	Ceratozetidae	<i>Diapterobates humeralis</i> (Hermann, 1804)	Norway	34 (21)	
		<i>Paralieuus leontonycha</i> Travé, 1960	Norway	10 (7)	
	Oribatulidae	<i>Oribatula sp.n.</i>	Science	5 (3)	
		<i>Phauloppia coineaui</i> Travé, 1961	Norway	23 (20)	
		<i>P. saxicola</i> Travé, 1959	Norway	8 (7)	
	Phthiracaridae	<i>Phthiracarus sp.n.</i>	Science	2 (1)	
Micreremidae	<i>Micreremus brevipes</i> (Michael, 1888)	Norway	2 (1)		
Thysanoptera	Aeolothripidae	<i>Aeolothrips vittatus</i> (Haliday, 1836)	Norway	12 (10)	
Diptera	Sciaridae	<i>Corynoptera trepida</i> (Winnertz, 1867)	Norway	2 (2)	
		<i>Ctenosciara hyalipennis</i> (Meigen, 1804)	Norway	143 (107)	
	Cecidomyiidae	<i>Bradysia brevispina</i> Tuomikoski, 1960	Norway	1 (0)	
		<i>Epidapus gracilis</i> (Walker, 1848)	Norway	4 (4)	
		<i>Lestodiplosis sp.n.</i>	Science	4 (4)	
		<i>Porricondyla fuscostriata</i> Panelius, 1965	Norway	8 (7)	
		Ceratopogoni- dae	<i>Culicoides albicans</i> (Winnertz, 1852)	Norway	85 (70)
			<i>C. clintoni</i> Boorman, 1984	Norway	197 (148)
			<i>C. comosioculatus</i> Tokunaga, 1956	Norway	3 (3)
			<i>C. grisescens</i> Edwards, 1939	Norway	2 (1)
			<i>C. sphagnumensis</i> Williams, 1955	Norway	5 (3)
			<i>C. scoticus</i> Downes & Kettle, 1952	Norway	1 (1)
	<i>C. vexans</i> (Staeger, 1839)		Norway	2 (1)	
	<i>Alluaudomyia quadripunctata</i> (Goetghebuer, 1934)		Norway	21 (13)	
	<i>Brachypogon norvegicus</i> Szadziewski & Hagan, 2000	Science	19 (18)		
	<i>B. nitidulus</i> (Edwards, 1921)	Norway	32 (28)		
	<i>B. perpusillus</i> (Edwards, 1921)	Norway	1 (1)		
	<i>B. vitiosus</i> (Winnertz, 1852)	Norway	5 (4)		
	<i>Schizohalea leucopeza</i> (Meigen, 1804)	Norway	2 (2)		
	<i>Bezzia affinis</i> (Staeger, 1839)	Norway	1 (0)		

higher taxon	family	species	new to:	NSpms (Old)
		<i>B. bicolor</i> (Meigen, 1804)	Norway	6 (6)
		<i>B. rhynchostylata</i> Remm, 1974	Europe	17 (15)
		<i>Atrichopogon lucorum</i> (Meigen, 1818)	Norway	9 (6)
		<i>Forcipomyia acidicola</i> (Tokunaga, 1937)	Norway	1 (1)
		<i>F. albostyla</i> Remm, 1979	Norway	2 (2)
		<i>F. ciliata</i> (Winnertz, 1852)	Norway	2 (1)
		<i>F. fuliginosa</i> (Meigen, 1818)	Norway	1 (1)
		<i>F. hygrophila</i> Kieffer, 1925	Norway	3 (3)
		<i>F. kaltenbachii</i> (Winnertz, 1852)	Norway	8 (7)
		<i>F. monilicornis</i> (Coquillett, 1905)	Norway	6 (5)
		<i>F. nigra</i> (Winnertz, 1852)	Norway	24 (22)
		<i>F. nigrans</i> Remm, 1962	Norway	328 (221)
		<i>F. palustris</i> (Meigen, 1804)	Norway	1 (1)
		<i>F. titillans</i> (Winnertz, 1852)	Norway	13 (11)
		<i>Dasyhelea biunguis</i> Kieffer, 1925	Norway	3 (3)
		<i>D. ledi</i> Remm, 1993	Europe	4 (2)
	Empididae	<i>Rhamphomyia poplitea</i> (Wahlberg, 1844)	Norway	7 (5)
	Phoridae	<i>Megaselia spinigera</i> (Wood, 1908)	Norway	2 (2)
		<i>M. emarginata</i> (Wood, 1908)	Scandi- navia	3 (3)
		<i>M. aculeata</i> (Schmitz, 1919)	Scandi- navia	2 (2)
		<i>M. fusciclava</i> Schmitz, 1935	Norway	1 (0)
		<i>M. nigriceps</i> (Loew, 1866)	Norway	5 (3)
		<i>M. cothurnata</i> (Schmitz, 1919)	Scandi- navia	1 (1)
		<i>Menoziola obscuripes</i> (Schmitz, 1927)	Norway	1 (0)
	Lonchaeidae	<i>Lonchaea laxa</i> Collin, 1953	Norway	31 (27)
		<i>L. stackelbergi</i> Czerny, 1934	Norway	1 (1)
	Drosophilidae	<i>Scaptomyza teinoptera</i> Hackman, 1955	Norway	1 (1)
	Chloropidae	<i>Conioscinella sordidella</i> (Zetterstedt, 1848)	Norway	1 (1)
	Anthomyiidae	<i>Alliopsis brunneigena</i> (Schnabl, 1915)	Norway	2 (2)
		<i>Anthomyia mimetica</i> (Malloch, 1918)	Norway	1 (1)

sarily imply that these species are rare or that they are strictly arboreal, as a plausible explanation may be that they simply have been overlooked in earlier field studies.

In agreement with the findings of Thunes et al. (2003) the new species records were significantly more common in old than in mature trees in Sigdal 1999. Fifty-six 'new' species were collected from the old trees, whilst only 37 'new' species were collected from the mature trees. A paired student's t-test on raw data showed that there were highly significantly more individuals of new species records at greater abundance in the old trees in comparison with mature trees (Student's  $t = 3.34$ ,  $df = 60$ ,  $p < 0.002$ ). The number of individuals of each species per funnel (corrected for tree crown size), revealed a significantly greater number of individuals of new species records in the old trees ( $t = 1.97$ ,  $df = 60$ ,  $p = 0.05$ ), although less so than the figure for abundance.

Coleman rarefaction curves showed that the entire Diptera complex showed a significantly greater number of new records (Fig. 2a) in old than in mature trees (see also Thunes et al. 2003). This resolution was not evident for the Ceratopogonidae (Fig. 2b), but curves of other dipterous taxa combined (Fig. 2c) separated statistically at about 4 000 specimens indicating that the most important group(s) of Diptera giving the pattern in Fig. 2a were not the Ceratopogonidae. Unfortunately, the number of new records from Diptera, other than Ceratopogonidae, were too low to give reliable accumulation curves, since only 231 specimens were collected (Tab. 1). In contrast, when the new records of taxa other than Diptera were analysed, the rarefaction curves seem to suggest more new species in mature trees than in old trees (Fig. 2d). This pattern was, however, not statistically significant.

To summarise, when differences in sampling effort, size of trees and site characteristics were accounted for (described in Thunes et al. 2003), the tendency was clear: there were more new species records in old trees than in mature trees (Fig. 2a). Furthermore, when divided into different taxa, the results

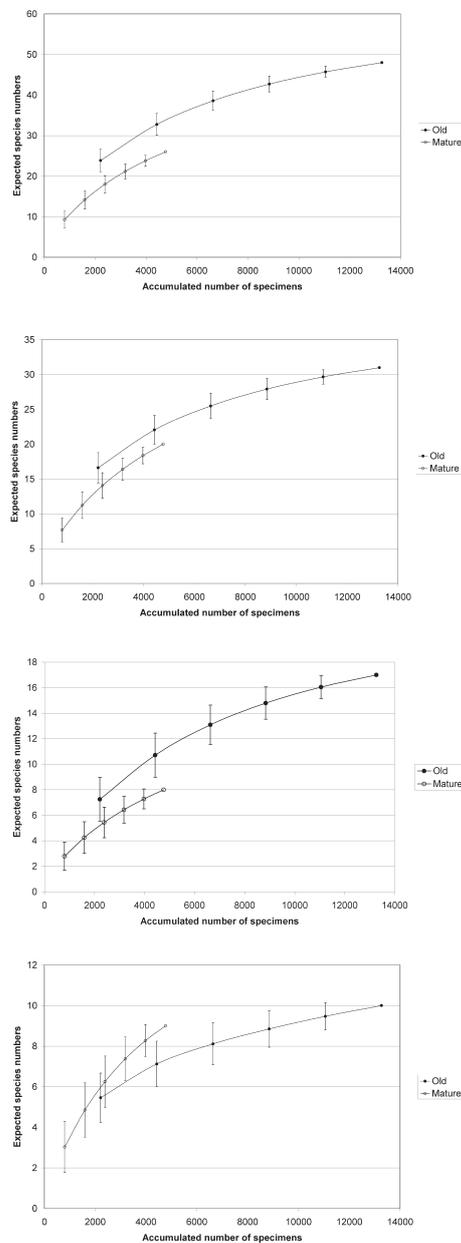


Fig. 2: Coleman rarefaction curves comparing accumulated number of new species records (y-axis) with overall number specimens collected from pine canopies of twelve trees (x-axis) in old (closed) and mature (open) trees. a) All Diptera, b) Ceratopogonidae, c) Other Diptera, d) New records except Diptera.

showed that this pattern was due to the predominance of new species records of Diptera in old trees.

The greater number of new species records in old trees in comparison with mature trees may have an explanation which is related to a successional change of species composition over time, as described by e.g. Southwood et al. (1982). In general, different species have different habitat requirements. Some species are mainly associated with old-growth characteristics and are restricted to old substrates (e.g. trees). Old trees are rare in the Norwegian forest landscape and so are specific old-tree resources, such as coarse woody debris, highly structured bark, etc. In addition, species associated with such resources might be more common in the canopy, and therefore rarely collected by conventional sampling from the ground. Older trees might be expected to provide more moderation of the micro-climates available for insect use (Willmer 1986). Warren and Key (1991) noted that there are particular assemblages of species associated with the pioneer, intermediate and climax phases in wood succession. Elton (1966) indicated that in lowland temperate mature woodland, the number of species of invertebrates present may run to several thousands; he summarised the most important micro-habitats in this temperate forest to include: bark, serving as resting perches; rugosities, providing niches in bark; sap runs, moisture sources; water-filled rot holes, larval habitat, etc. Because specific old-growth micro-habitats are rare, species strictly associated with these sites are rare. Consequently, it is probable that the high number of records of new species from the canopies of old trees is related to both facts that canopies are poorly investigated and that old trees are rare.

Specifically, the Diptera collected may use pine crowns as resting sites and for weather protection. The abundance of pine pollen may provide a source of protein, lipid and carbohydrate foods for adult flies. With reference to the differences in species composition, the old tree patches provide different micro-habitats through moss and lichen growth on the limbs and branches. The forest debris under

the canopies varies with tree age, and the debris from an old tree is of another quality than the debris from a young or mature tree, and these may represent different habitats for e.g. over-wintering organisms. Consequently, forest debris from old trees would be expected to provide important over-wintering habitat for larvae or adult flies, and may increase species richness at the landscape scale.

#### *The Ceratopogonidae*

Of the Diptera collected in Sigdal in 1999, Ceratopogonidae constituted 58.9% (i.e. 1997 of a total of 3393 Diptera specimens) and 27.0% of the species (i.e. 40 of 148 Diptera species totally). From all nine families of Diptera analysed, the greatest number of new records were found in the Ceratopogonidae (Tab. 1). Almost one-half of all new records (30 of 61) and 71.5% of the collected individuals belonged to this family.

The biology of many species within Ceratopogonidae is poorly known, but many of the species are known to be associated with moist seepages or rotting material as larvae and others are dung saprophages as described above. The larvae of biting midges are common inhabitants of various types of aquatic, semi-aquatic, and terrestrial habitats (Wirth and Grogan 1988, Szadziewski et al. 1997). In typically terrestrial habitats, for example in animal dung or rotting fungi, the larvae of *Culicoides scoticus* Downes and Kettle, 1952 have been found, whilst the larvae of all species of *Forcipomyia*, as well as *Atrichopogon lucorum* (Meigen, 1818) have been found in rotting wood, bark, kindling, plant debris or similar habitats.

Two species, *Culicoides clintoni* Boorman, 1984 and *Forcipomyia nigrans* Remm, 1962 were particularly abundant in our fogging samples, constituting nearly 47% of the total number of specimens. These two species have considerably different ecological requirements. Females of the rare *C. clintoni* are haematophages (as are most other species in this genus), probably feeding on birds or mammals. Females of *F. nigrans* do not take a blood meal and, like males, can visit flowers for nectar. Larvae of *C. clintoni* live

in peat bogs (Glukhova 1989) whilst those of *F. nigrans* live under rotting bark, logs, twigs and branches of different trees (e.g. *Pinus sylvestris*, *Betula* spp. or *Alnus* spp.) and among mosses (Gilka 1996). Both males and females were identified for these two species and the ratio of males/females (*C. clintoni*: 1.32, *F. nigrans*: 0.15) indicates clearly that the canopies are being used as swarming sites. This is corroborated by comparing the findings from 1999 with the field data from 1998 (Thunes et al. 2004). It is known that adults can treat an individual pine tree as a swarm marker. Swarms consist almost entirely of males. However, females view similar objects that males use as swarm-markers and it is probable that females are attracted to such objects (Downes 1955). Often, cone-like apices of either low or high conifers are used as orientation markers for swarming. In the field, biting midges appear to prefer coniferous trees and bushes to broad leaved plants. The mean air temperature is a key environmental factor to trigger midge swarming and since the sampling period was earlier in 1998 than in 1999, we assume that the midges had not started to mate. Whilst 195 specimens of *C. clintoni* were collected in 1999, none were collected in 1998. Similar numbers for *F. nigrans* are 326 and 2, respectively.

### Conclusion

Flies comprise one of the most abundant insect orders in temperate forest canopies but, due to the minute size of many species, the difficulty of gaining access for sampling and the lack of long term systematic studies, little information has been available on their diversity, abundance and distribution within forest canopies. With the development of insecticidal collection methods and other techniques to collect canopy arthropods, a clearer picture of the species composition and assemblage patterns of arboreal arthropods is emerging. Jolivet (1998) termed the forest canopies, the "enchanted" part. Grimly, he noted that, at the current rate of species extinction, many species may be lost before the taxonomic and ecological studies of the forest fauna, "the last

biotic frontier", are completed. Our study of arthropods from pine trees demonstrates how many species can be expected in the canopy and emphasises the lack of knowledge of the basic biology (e.g. bionomics, larval habitats, phenology, food preferences, etc.) of many common insect species.

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# Canopy Arthropod Research in Europe

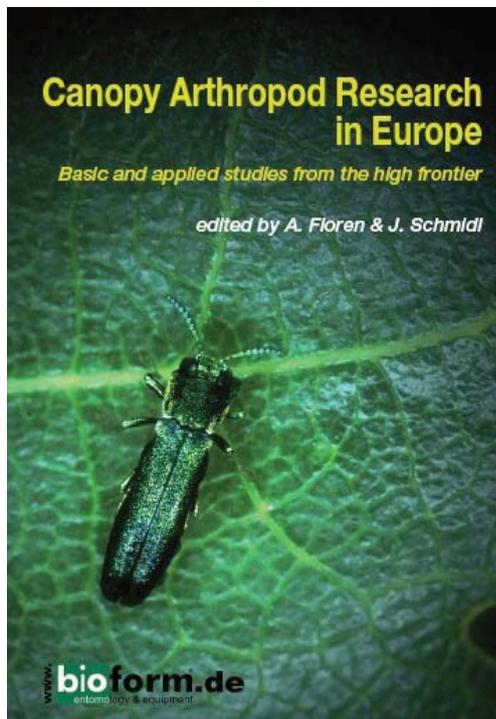
**Basic and applied studies from the high frontier**

Edited by **Andreas Floren (Univ. Würzburg) & Jürgen Schmidl (Univ. Erlangen-Nuremberg)**

Foreword by K.E. Linsenmair, University of Würzburg, Dep. of Animal Ecology and Tropical Biology

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Aims & Scope: In contrast to tropical ecosystems, in temperate zones the importance of canopy ecology is underestimated and underrepresented in science projects. Recent surveys and studies show that also in temperate forest canopies a diverse arthropod fauna exists, containing specialized and endangered species and even species new to science. Species and guild compositions of canopy arthropods in European forests are not yet described sufficiently, and many functional aspects of temperate forests still are not understood or studied.

The present volume tries to reduce this gap by summarizing studies and papers dealing with canopy arthropods in Europe. Aspects of diversity, function, structure and dynamics of canopy arthropod as well as aspects of nature conservation and transmission of scientific results into forestry and management practice are central aims of this book.

Contents & Chapters: Foreword • Introduction • General forest ecological aspects • Arthropod diversity, guilds and structure related communities • Stratification and distribution of arthropods in tree habitats • Anthropogenic and natural disturbance structuring arthropod communities • Canopy research and its impact on forestry and nature protection practice.

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# Canopy Arthropod Research in Europe

*Basic and applied studies from the high frontier.*

*Edited by Andreas Floren and Jürgen Schmidl*

*"As the global community comes to realise that our climatic future is intimately tied up with the health of our forests so canopy studies take their rightful place in the forefront of forest science. This book will ensure that studies of temperate forest canopies no longer remain the 'poor cousins' of tropical canopy studies. The research described will stimulate new and exciting activities in temperate canopy studies as well as giving the newcomer to the field an invaluable insight into what has gone before."* Roger Kitching, Professor of Ecology, Griffith University, Brisbane

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