BALTIC FORESTRY
http://www.balticforestry.mi.lt
ISSN 1392-1355
eISSN 2029-9230
Baltic Forestry 2021 27(1): 80–85
Category: research article
https://doi.org/10.46490/BF538

Diversity of the Bibionomorpha and Tipulomorpha (Diptera) from dead ash and aspen wood in the forests of Lithuania

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Gorban, I. and Podėnienė, V. 2021. Diversity of the Bibionomorpha and Tipulomorpha (Diptera) from dead ash and aspen wood in the forests of Lithuania. *Baltic Forestry* 27(1): 80–85. https://doi.org/10.46490/BF538.

Received 3 November 2020 **Revised** 12 April 2021 **Accepted** 17 April 2021

Abstract

The aim of this study was to investigate the biodiversity of nematoceran flies associated with dead wood in a forest ecosystem. Although wood is a primary habitat for a vast amount of Diptera species, no effort has previously been made to study saproxylic flies in Lithuania. During this research, emergence traps were used on aspen (*Populus tremula*) and ash (*Fraxinus excelsior*) trees in Dūkštų Ąžuolynas forest and Būda Botanical-Zoological Reserve during the period 2014–2019. In total, 672 individuals of the Bibionomorpha and Tipulomorpha collected from fallen tree trunks were identified to species. Seventy-four species represented nine families, with the Sciaridae, Anisopodidae and Mycetophilidae being most abundant. The Sciaridae family has barely studied in Lithuania and its diversity is still poorly known. During the research 23 nematoceran species, of which 19 belonging to the Sciaridae family, are first reported from Lithuania.

Key words: nematoceran, saproxylic species, emergence traps

Introduction

According to Lithuania State Forest Service data (2020), forests cover 33.7% of the land surface in Lithuania; however, forest management has reduced the number of old growth trees in the forest ecosystem, including the amount of dead wood, which is affected by clearance of branches, stumps and fallen trees during logging. Today, approximately 9 m^3/ha of dead wood is found in Lithuanian forests, although the suggested amount is 20–30 m^3/ ha, which should reduce the risk of biodiversity loss (Merganičová et al. 2012). Dead wood in forest ecosystems plays an important role as it serves as a long-term source of organic matter, food and shelter for many lichen, fungi and animal species, especially insects. Species that depend on dead wood can be collectively called saproxylic (Speight 1989). The concept of saproxylic insects includes various relationships with wood such as wood-feeders, fungivores or saprophagous species, as well as insects using dead wood as a wintering site (Grove 2002).

Saproxylic insect species together with rot fungi help to decompose fallen trees and release organic nutrients which enriches the soil. Fungi begin the decomposition process, which breaks down the cellulose, hemicellulose and lignin, and through this process the wood becomes a food source for insects (Stokland 2012). The largest orders of insects found in dead wood are Coleoptera and Diptera, however saproxylic dipterans are less studied and many new species are discovered each year, even though it can be the most abundant and species-rich group found in dead wood (Mlynarek et al. 2018).

Most larvae of saproxylic nematocerans (Diptera) can usually be found under fallen tree bark where larvae develop and some adults overwinter (Halme et al. 2012). The moist environment under tree bark is one of the most important factors for larval development (Hövemeyer and Schauermann 2003). Most species larvae are mycetophagous or saprophagous and feed on fungi or decaying wood penetrated with fungal mycelia. Most abundant mycetophagous dipterans belong to the Bolitophilidae, Ditomyiidae, Keroplatidae, Sciaridae and Mycetophilidae families, which are collectively called fungus gnats (Jakovlev 2011). They can be found not only in fruiting bodies of fungi but also in rotten dead wood. Most crane flies (Tipuloidea) are associated with microhabitats with high moisture content as it is an important factor in their development, and some species are found in moist dead wood (Halme et al. 2012).

Various studies were performed to elucidate what factors may be important for the diversity and distribution

of saproxylic nematocerans in wood. Studies on the importance of environmental factors on diversity of different saproxylic Diptera species and their succession in beech wood were carried out in Germany. It showed moss cushion, water content, trunk diameter, and litter cover are main factors influencing species diversity (Hövemeyer and Schauermann 2003). It has been found out that the diversity of saproxylic insects is greater in beech branches than in the trunk (Schiegg 2001). Species of tree can be very important for diversity of insects as well. Aspen, being of low economic value, is characterized by great diversity of saproxylic insects and is associated with rare species (Polevoi et al. 2018). Study of saproxylic Diptera in Scotland (Rotheray et al. 2001) showed that aspen and ash had the highest diversity of Diptera (7 tree species were studied). Evidence of aspen as an important tree species for saproxylic insect diversity has also been provided by Finnish scientists (Halme et al. 2012). Although the diversity of aspen wood has been studied more extensively, there is a great lack of research on ash wood. The aim of this paper is to present species occurring in dead wood and supplement the information on Lithuanian Dipteran biodiversity, as well as to compare saproxylic Diptera biodiversity in ash and aspen as information about saproxylic species in these trees is insufficient.

Materials and methods

This study was conducted in two locations of central Lithuania – Dūkštų Ąžuolynas forest in Neris Regional Park (Vilnius district) and Būda Botanical-Zoological Reserve (Kaišiadorys district). Būda Botanical-Zoological Reserve is in the Natura 2000 network and has conservation value due to rare species of plants and animals. Forest is dominated by oak, ash and alder tree species. Dūkštų Ąžuolynas forest is the largest and one of the oldest natural oak forests in Lithuania. Although dominated by oak trees, other deciduous tree species occur.

Trunk-emergence traps were designed to enable the study of the biodiversity of dead wood. Similar traps were used in another saproxylic insect study (Halme et al. 2012). Modified traps for easier placement on the trunks were made using transparent and air permeable polyester fabric (Figure 1). Each trap covered 1 meter of tree trunk, which ensured that comparable sections were used in the research. Tent-like traps were built using ropes and were attached to standing trees nearby to make sure there were no folds in the fabric. The front ends were tightly wound around the fallen tree trunk with a rope, making sure no gaps were left so the insects were trapped inside. One front wall of each trap was 20 cm higher than the others so the insects would fly towards that end. On the higher end of each trap a plastic container with 70% alcohol was attached.

For the research two tree species – aspen (*Populus tremula*) and ash (*Fraxinus excelsior*) – were chosen. During the period 2014–2019, emergence traps were set on middle parts of 12 fallen tree trunks (Table 1). Year 2018–2019 traps were set on the same fallen trees. Fallen trees had to be raised above the ground for easier placement of the emergence traps and the trunks were 20–35 cm diameter. The Stokland (2001) classification table of live trees and dead wood decay stages was used. Dead trees were visually analysed and assigned to the second decay stage, as tree bark was still attached to the trunks and decay penetrated less than 3 cm into the wood. Similar trunk size and decay stage can help to eliminate additional factors which could have effect on species diversity. Emergence traps were emptied every 14 days. All hatched and caught insects were preserved in alcohol and brought to the laboratory for further investigation. All collected specimens were sorted and nematocerans were used for further identification. Most of material was identified by the first author. Some species of the Mycetophilidae family were checked by J. Rimšaitė (Nature Research Centre, Lithuania), the Tipulidae and Limoniidae families by S. Podėnas (Vilnius University, Lithuania) and the Trichoceridae family by A. Petrašiūnas (Vilnius University, Lithuania). Small species of Sciaridae and some Mycetophilidae male genitalia were slide mounted and analysed using a microscope. Some specimens of the Sciaridae family were identified by K. Heller (Senckenberg German Entomological Institute, Germany). All further analyses were based on the Tipulomorpha and Bibionomorpha (except Cecidomyiidae) specimens. Only males of the Sciaridae and Mycetophilidae were included in analyses due to their females are difficult to identify. The material is deposited in the Zoological Museum of Vilnius University, Lithuania. For declaring new species, multiple resources were used (Pakalniškis et al. 2006, Kurina et al. 2011, Dvorak et al. 2019).

Figure 1. Emergence trap set on fallen tree trunk

The SPADE (Species Prediction and Diversity Estimation) software package was used to analyse assemblage in ash and aspen tree species (Chao and Shen 2010).

Table 1. List of emergence traps with information on their location, tree species and research year

Site No.	Locality	Coordinates		Tree species
Year 2014. 04.23–11.05				
1	DAF	54°50'56.9"N	24°58'21.3"E	Aspen *Populus tremula*
2	DAF	54°50'56.4"N	24°58'07.0"E	Ash *Fraxinus excelsior*
Year 2016. 05.11–11.08				
3	BBZR	54°52'52.7"N	24°21'20.6"E	Aspen *Populus tremula*
4	BBZR	54°52'52.9"N	24°21'30.1"E	Ash *Fraxinus excelsior*
5	BBZR	54°52'53.1"N	24°21'32.2"E	Ash *Fraxinus excelsior*
6	BBZR	54°52'53.0"N	24°21'25.1"E	Ash *Fraxinus excelsior*
7	BBZR	54°52'56.4"N	24°21'19.2"E	Ash *Fraxinus excelsior*
Year 2018. 05.11–11.11; 2019. 05.16–10.13				
8a, 8b	BBZR	54°52'54.3"N	24°21'30.7"E	Ash *Fraxinus excelsior*
9a, 9b	BBZR	54°53'00.1"N	24°21'32.9"E	Ash *Fraxinus excelsior*
10a, 10b	BBZR	54°53'01.7"N	24°21'51.9"E	Aspen *Populus tremula*
11a, 11b	BBZR	54°52'58.5"N	24°22'04.4"E	Aspen *Populus tremula*
12a, 12b	BBZR	54°52'59.8"N	24°22'15.8"E	Aspen *Populus tremula*

Note: DAF denotes Dūkštų Ąžuolynas forest; BBZR denotes Būda Botanical-Zoological Reserve. Characters a and b next to the site no. represent the years 2018 and 2019, respectively.

Community composition was compared using non-metric multidimensional scaling (NMDS) based on Bray-Curtis distances using XLSTAT statistical software for MS Excel. The analytical software package STATISTICA was used for non-parametric statistics. The Sign test was used to measure if species composition in ash and aspen trees were different.

Results

A total of 672 specimens representing 74 species of the Tipulomorpha and Bibionomorpha were identified to species level and used in further analyses (Table 2). The most abundant families were Sciaridae (203 specimens), Anisopodidae (175 specimens) and the Mycetophilidae (129 specimens). The highest diversity was observed in the Mycetophilidae (34 species) and Sciaridae (19 species) families, together representing 71.2% of all species collected in dead wood. The Anisopodidae family was represented by only one, but very abundant species *Sylvicola cinctus*, which together with two other very abundant species *Gnophomyia viridipennis* (Limoniidae) (93 specimens) and *Scatopsciara calamophila* (Sciaridae) (74 specimens) represented 50.8% of all specimens. Of all collected species, 48.6% were represented by only one individual.

The composition of species collected in ash and aspen dead wood were compared (Table 3). More species were reared from ash wood. Ash wood also was characterized by a greater number of the least abundant species, which were represented by only one or a few individuals. Of the collected material in aspen wood, 89.8% consisted of abundant species, while in ash wood this figure was only 68.7%. Comparing nematoceran species assemblage, only 11 (14.9%) species were observed in both ash and aspen trees.

Non-metric multidimensional scaling (NMDS) ordination shows dipteran communities being different in two tree species (Figure 2). Results demonstrate aspen tree community composition being more variable than that from ashes, however quite high stress level (Kruskal's stress = 0.249) not allow to make strong assumptions.

The Sign test in STATISTICA package showed that the difference between species composition in ash and aspen dead wood is statistically significant ($p < 0.002$).

Table 3. Basic data count in ash and aspen wood

	Fraxinus excelsior	*Populus tremula*
Number of samples	9	8
Number of observed species	54	31
Number of observed individuals	300	372
Number of observed individuals for least abundant species	94	38
Number of observed species for least abundant species	45	24
Number of observed individuals for abundant species	206	334
Number of observed species for abundant species	9	7

Note: Cut-off point, k = 10; the "least abundant" species group – frequency counts up to the cut-off point; "abundant" species group – frequencies beyond the cut-off point.

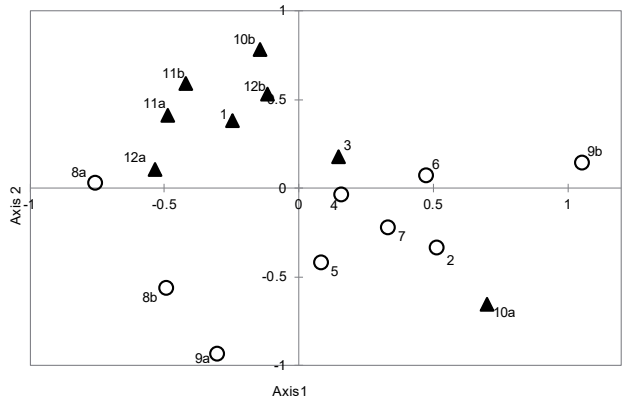

Figure 2. Non-metric multidimensional scaling (NMDS) ordination of dipteran community structure in ash and aspen deadwood (Kruskal's stress = 0.249)

Table 2. List of species reared from ash and aspen. Numbers in the table represent total count of specimen

Species	Aspen *Populus tremula*	Ash *Fraxinus excelsior*	Total
ANISOPODIDAE			
**Sylvicola cinctus* (Fabricius, 1787)	156	19	175
BIBIONIDAE			
Bibio nigriventris (Haliday, 1833)	1	-	1
Bibio reticulatus (Loew, 1846)	-	3	3
DITOMYIIDAE			
Ditomyia fasciata (Meigen, 1818)	19	-	19
Symmerus nobilis (Lackschewitz, 1937)	-	2	2
KEROPLATIDAE			
**Neoplatyura flava* (Macquart, 1826)	1	1	2
LIMONIIDAE			
Dicranomyia modesta (Meigen, 1818)	-	3	3
Gnophomyia viridipennis (Gimmerthal, 1847)	93	-	93
Idioptera pulchella (Meigen, 1830)	-	1	1
Limonia nubeculosa (Meigen, 1804)	-	1	1
Limonia trivittata (Schummel, 1829)	-	3	3
Metalimnobia quadrimaculata (Linnaeus, 1760)	11	-	11
Rhypholophus varius (Meigen, 1818)	0	2	2
MYCETOPHILIDAE			
Allodia lugens (Wiedemann, 1817)	-	1	1
Allodia subpistillata (Sevcik, 1999)	-	1	1
Brachypeza armata (Winnertz, 1864)	1	-	1
Brevicornu serenum (Winnertz, 1863)	1	-	1
Coelophthinia thoracica (Winnertz, 1864)	-	1	1
Cordyla brevicornis (Staeger, 1840)	1	-	1
Cordyla fissa (Edwards, 1925)	-	1	1
Cordyla pusilla (Edwards, 1925)	1	-	1
Dynatosoma reciprocum (Walker, 1848)	26	-	26
Exechia confinis (Winnertz, 1864)	-	1	1
Exechia dizona (Edwards, 1924)	-	11	11
Exechia dorsalis (Staeger, 1840)	-	2	2
Exechia exigua (Lundstrom, 1909)	-	1	1
Exechia fusca (Meigen. 1804)	1	6	7
Exechia nigroscutellata (Landrock, 1912)	-	1	1
Exechia parvula (Zetterstedt, 1852)	-	23	23
Exechia repandoides (Caspers, 1984)	-	1	1
Exechia seriata (Meigen, 1830)	-	2	2
Exechia spinuligera (Lundstrom, 1912)	-	1	1
Exechia unifasciata (Lackschewitz, 1937)	-	3	3
Exechiopsis clypeata (Lundstrom, 1911)	-	1	1
Exechiopsis fimbriata (Lundstrom, 1909)	-	10	10
Leia bilineata (Winnertz, 1863)	-	2	2
**Leptomorphus forcipatus* (Landrock, 1918)	1	-	1
Mycetophila alea (Laffoon, 1965)	-	2	2
Mycetophila uliginosa (Chandler, 1988)	1	-	1
Mycomya permixta (Vaisanen, 1984)	1	-	1
**Notolopha cristata* (Staeger, 1840)	-	1	1
Rymosia bifida (Edwards, 1925)	2	16	18
Rymosia fasciata (Meigen, 1804)	-	1	1
Rymosia placida (Winnertz, 1863)	-	1	1
Saigusaia flaviventris (Strobl, 1894)	1	-	1
Sciophila limbatella (Zetterstedt, 1852)	1	-	1
Sciophila lutea (Macquart, 1826)	-	1	1
SCIARIDAE			
**Bradysia strenua* (Winnertz, 1867)	1	-	1
**Bradysia pectoralis* (Staeger, 1840)	-	1	1
**Bradysia placida* (Winnertz, 1867)	2	-	2
**Corynoptera dentata* (Bukowski and Lengersdorf, 1936)	-	5	5
**Corynoptera deserta* (Heller and Menzel, 2006)	-	3	3
**Corynoptera furcifera* (Mohrig and Mamaev, 1987)	1	-	1
**Corynoptera irmgardis* (Lengersdorf, 1930)	-	3	3
**Corynoptera polana* (Rudzinski, 2009)	-	2	2
**Corynoptera subtilis* (Lengersdorf, 1929)	4	8	12
**Cratyna nobilis* (Winnertz, 1867)	4	23	27
**Epidapus detriticola* (Kratochvil, 1936)	1	2	3
**Epidapus lucifuga* (Mohrig, 1970)	1	-	1
**Leptosciarella rejecta* (Winnertz, 1867)	-	1	1
**Scatopsciara atomaria* (Zetterstedt, 1851)	2	20	22
**Scatopsciara calamophila* (Frey, 1948)	4	70	74
**Scatopsciara pusilla* (Meigen, 1818)	-	12	12
**Zygoneura bidens* (Mamaev, 1968)	15	2	17
**Zygoneura sciarina* (Meigen, 1830)	14	1	15
**Xylosciara heptacantha* (Tuomikoski, 1960)	1	-	1
TIPULIDAE			
Nephrotoma quadrifaria (Meigen, 1804)	-	1	1
Tipula autumnalis (Loew, 1864)	-	12	12
Tipula humilis (Staeger, 1840)	-	1	1
Tipula irrorata (Macquart, 1826)	-	2	2
Tipula variicornis (Schummel, 1833)	3	-	3
Tipula varipennis (Meigen, 1818)	-	1	1
TRICHOCERIDAE			
Trichocera forcipula (Nielsen, 1920)	-	3	3
Trichocera inexplorata (Dahl, 1967)	-	1	1

Note: * – species first recorded in Lithuania.

Discussion and conclusion

This is the first comprehensive study on diversity of the saproxylic Bibionomorpha and Tipulomorpha insects in ash and aspen wood. Due to the lack of data on saproxylic insects in ash wood, it is not possible to compare the results of our studies with results of other studies. Comparing the diversity of these groups of insects in aspen wood, it was found out that in Finland (Halme et al. 2012), using 14 traps of the same type, 76 species of fungus gnats and crane flies were caught on aspen of second-degree decay. In our study, there were only 8 traps on aspen that caught 31 species of the Bibionomorpha and Tipulomorpha, including 5 of the same species as in the Finnish study (in Finland, four species were caught on the aspen, while in our study – from ash). In Russian Karelia, 16 aspen logs were investigated with the same type of traps and 51 nematoceran species were collected, of which 9 were present in our study too (Polevoi et al. 2018).

Our results show various nematoceran species associated with ash and aspen dead wood. Most abundant species, which occurred in dead wood like *Sylvicola cinctus* (Anisopodidae), *Scatopsciara calamophila* (Sciaridae) and *Gnophomyia viridipennis* (Limoniidae), are well known saproxylic species. *S. cinctus* usually found in rotting organic material but has also been reported in fungi and tree sap (Ševčík 2006). During this research, this species was reared from both tree species, but it was the most abundant species in aspen dead wood. *G. viridipennis* was associated only with aspen wood. It is one of the first species to colonise dying trees (Hancock 2008). *S. calamophila* was the most abundant species from ash dead wood and known as a species that develops in young stumps and logs (Irmler et al. 1996). Usually, it is one of the most abundant species found in dead wood. It has previously been reported as developing in oak, beech or other broad-leaved woodlands, and also to have been reared from spruce bark (Menzel et al. 2006).

The Mycetophilidae was the most diverse family during this research. Most species collected during this research belong to the mycetophagous trophic group, which larvae are found in fungi fruiting bodies. However, the high number of species occurring in dead wood shows that they can be associated with rotten wood, as it is penetrated with fungal mycelia. The most abundant mycetophilid *Dynatosoma reciprocum,* which was collected from aspen wood, has previously been reported in white-rot affected wood (Jakovlev 2011) as well as fungi fruiting bodies (Jakovlev et al. 2008). Larvae of all species of genus *Exechia,* as well as other mycetophilid species *Exechiopsis fimbriata* and *Rymosia bifida* larvae develop in fungi fruiting bodies (Ševčik 2006). All these species were mostly reared on ash wood different from study in Finland in which most species were collected from aspen.

Even though the Sciaridae family is very diverse and abundant, data on their biology are severely lacking. During our research, species of genus *Scatopsciara* were one of the most abundant in ash dead wood with just few specimens collected from aspen wood. Very little is known about biology of this family, but species like *S. atomaria, S. pusilla* are common in forests. *S. atomaria* can be found in various types of forest; however, *S. pusilla* is more associated with broadleaf woodlands (Köhler et al. 2014). It is known that *S. pusilla* was very abundant in beech wood (Hövemeyer and Schauermann 2003). Genus *Scatopsciara* have been reported to be associated with fruit bodies of several fungi, as well as the wood decomposing fungi (Krivosheina 2006). *Zygoneura bidens* and *Z. sciarina* were reared on aspen dead wood. *Z. bidens* was very common in aspen logs in Russian Karelia (Polevoi et al. 2018). The larvae of *Z. sciarina* has been reared from xylophilic and young beech logs (Irmler 1996).

Species identified in this study belonging to the Sciaridae family are new to Lithuanian fauna. Although sciarids are one of the most abundant families found in forest ecosystems, due to difficulty in identification they have attracted little interest in Lithuania. Only 21 species of this family have been recorded from Lithuania to date (Pakalniškis et al. 2006, Kurina et al. 2011).

Two species of the Ditomyiidae were collected from dead wood. *Dytomyia fasciata* once was reared on aspen tree and few *Symmerus nobilis* specimens were reared on ash wood, but it is known that the larvae of this family are associated with tree fungal fruiting bodies (genus *Ditomyia*) or occur in rotten wood (genus *Symmerus*) and are mycetophagous. The only species of family Keroplatidae – *Neoplatyura flava* – was reared on dead wood of both tree species and it is known that this species associated with tree fungal fruiting bodies (Ševčík 2006, Jakovlev 2011).

Larvae of such families as the Bibionidae, Tipulidae and Trichoceridae feed on leaf litter and are called phyto-saprophages. They could be associated more with moss or litter cover on fallen tree bark than with fungi or rotten wood (Hövemeyer 1998). Some groups from the Tipulidae family (subfamily Ctenophorinae, subgenus *Tipula* (*Dendrotipula*)) are xylophagous, but during our research they were not found, because they are associated with later wood decay stages.

Findings of the Tipulidae (except *Tipula irrorata*) and some Limoniidae (*Idioptera pulchela. Dicranomyia modesta*) in the dead wood emergence traps were accidental. Larvae of *T. autumnalis* develop in rich organic mud (Podėnienė 2001). Larvae of *I. pulchella* develop in swamps, under moss cushion and larvae of *D. modesta* in mud in marshy areas (Podėnienė 2003, Podėnienė 2004). The reason for these species being collected in the trap might be associated with the fact that the trap was installed on very damp ground with water on the surface.

Our research shows that dead wood is an important habitat for larval development for many groups of Diptera. Although many species were represented by only one or a few specimens, it shows that more research could help gain a better understanding of saproxylic nematoceran diversity and their preferred habitat. Although it was already known that aspens are very important for the diversity of saproxylic Diptera, our study showed that ash wood is also of not less diversity. As the similarity of Diptera communities is small between ash and aspen deadwood, we can assume that a greater diversity of saproxylic species requires a greater diversity of dead wood tree species. Species emergence patterns are uneven during research period and to describe it more research needs to be done.

Acknowledgments

We would like to thank Kai Heller for helping with identification of family Sciaridae and providing basic knowledge of working with this family. We are very grateful to Dr. S. Podėnas, Dr. J. Rimšaitė and Dr. A. Petrašiūnas who helped with some other families and their species. We would like to express our gratitude to two anon-

ymous reviewers for their suggestive comments which allowed improving text of the manuscript in no small measure.

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