

VILNIUS UNIVERSITY
NATURE RESEARCH CENTRE

SVETLANA ORLOVSKYTĖ

INSECTS INHABITING THE TRAP-NESTS FOR HYMENOPTERA
AND EFFECTS OF ANTHROPOGENIC FACTORS
ON THEM

Summary of doctoral dissertation
Biomedical sciences, ecology and environmental science (03 B)

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Scientific supervisor – assoc. prof. dr. Eduardas Budrys (Nature Research Centre, biomedical sciences, ecology and environmental science – 03B).

The doctoral dissertation will be defended at the Council of Ecology and Environmental Research:

Chairman – prof. habil. dr. Vincas Būda (Nature Research Centre, biomedical sciences, ecology and environmental science – 03B).

Members:

prof. dr. Virginijus Sruoga (Lithuanian University of Educational Sciences, biomedical sciences, zoology – 05B);

prof. habil. dr. Jonas Rimantas Stonis (Lithuanian University of Educational Sciences, biomedical sciences, ecology and environmental science – 03B);

dr. Virmantas Stunžėnas (Nature Research Centre, biomedical sciences, zoology – 05B);

prof. dr. Jason M. Tylianakis (University of Canterbury, New Zealand, biomedical sciences, biology – 01B).

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VILNIAUS UNIVERSITETAS
GAMTOS TYRIMŲ CENTRAS

SVETLANA ORLOVSKYTĖ

PLĖVIASPARNIŲ LIZDAVIETĖSE-GAUDYKLĖSE
APSIGYVENANTYS VABZDŽIAI IR JŲ PRIKLAUSOMYBĖ NUO
ANTROPOGENINIŲ VEIKSNIŲ

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Nariai:

prof. dr. Virginijus Sruoga (Lietuvos edukologijos universitetas, biomedicinos mokslai, zoologija – 05B);

prof. habil. dr. Jonas Rimantas Stonis (Lietuvos edukologijos universitetas, biomedicinos mokslai, ekologija ir aplinkotyra – 03B);

dr. Virmantas Stunžėnas (Gamtos tyrimų centras, biomedicinos mokslai, zoologija – 05B);

prof. dr. Jason M. Tylianakis (Kenterberio universitetas, N. Zelandija, biomedicinos mokslai, biologija – 01B).

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INTRODUCTION

Theme relevance

Hymenoptera is one of the dominant groups of insects by species richness. This order has reached the top of evolutionary complexity in development of social species, reproductive castes and endoparasitism (Austin, Dowton, 2000).

Bees, being one of the main pollinators of entomophilous plants, occupy a significant position in the food webs of many terrestrial ecosystems (Vanbergen, 2013). Along with social species, solitary bees and wasps pollinate both naturally growing and cultivated plant species (Sjödin, 2007; Sobek *et al.*, 2009). They are becoming increasingly important not only because of the rapid loss of honey bees (Neumann, Carreck, 2010) in the temperate latitude of the Northern hemisphere, but also because they are more efficient in pollinating some plant species, when compared to honey bees (Michener, 2000).

Many solitary wasps are important predators regulating the populations of some herbivorous insects, such as leafroller caterpillars (Lepidoptera: Tortricidae), leaf beetles (Coleoptera: Chrysomelidae), weevils (Coleoptera: Curculionidae) (Jennings, Houseweart, 1984; Harris, 1994; Klein *et al.*, 2004; Sobek *et al.*, 2009). Other Hymenoptera species from the superfamilies Chalcidoidea, Ichneumonoidea, Proctotrupoidea are parasitoids, widely used in biological control of pest insects (DeBach, Rosen, 1991; Austin, Dowton, 2000).

The cavity-nesting solitary bees and wasps, along with other Hymenoptera species, compose the community of the insects inhabiting trap-nests for Hymenoptera. In addition to the use in agriculture and biological control, this community has been certified as a suitable bioindicator for measuring the condition and changes of ecosystems, landscapes, habitats (Tscharntke *et al.*, 1998; Sjödin, 2007; Budrys *et al.*, 2010) due to several reasons: it has a quite small number of species for comfortable handling; numerous enough for the research of its composition and interactions; sensitive to environmental changes; abundant in various habitats. In addition, trap-nesting bees and predatory wasps are dependent on a diverse set of resources (Tscharntke *et al.*, 1998): dead wood with cavities for nests, nesting materials, nutrients for offspring and imagoes, mating places. Not only their species richness and abundance, but also the ecological functions and interactions, such as pollination, predation, parasitism, are used in bioindication (Tscharntke *et al.*, 1998; Tylianakis *et al.*, 2004).

The aim and main tasks

The aim of this research is to establish the structure of the insect community inhabiting trap-nests for Hymenoptera in Lithuania and to assess the effects of the anthropogenic impact on it. To achieve this aim, the following objectives were set:

1. Determining the species composition of the trap-nesting community in Lithuania;
2. Clarifying the taxonomy of one of its abundant but poorly studied cleptoparasitic group – the *Chrysis ignita* complex;
3. Assessing the structure of this assemblage's food web;

4. Evaluating the effect of anthropogenic factors at different spatial scale (increasing agricultural areas, woodland loss and fragmentation, shift toward earlier successional stages, forest exploitation effects) on the parameters of this community;
5. Determining the species of the community, suitable for bioindication of ecosystem naturalness;
6. Evaluating the impact of anthropogenic factors on the trophic link diversity within this community.

Scientific novelty of the research

- The species composition of the European trap-nesting community has been revised, 6 bee and predatory wasp species, and 11 species of their natural enemies have been found for the first time in the trap-nests for Hymenoptera in Europe;
- New molecular and morphometric characters of the sibling species of the *Chrysis ignita* complex have been discovered, original identification keys have been composed;
- 28 new *COI* haplotypes of 10 sibling species of the *Chrysis ignita* complex have been detected;
- 20 new primers have been constructed for the analysis of mitochondrial and nuclear DNA of the *Chrysis ignita* complex;
- 15 mitochondrial DNA (*CO2*, *ATP8*, *ATP6*, *CO3*, *ND3*, *CytB*, 9 tRNAs), 3 nuclear rDNA (18S, 5.8S, 28S rRNAs), and 2 internal transcribed spacers (*ITS1*, *ITS2*) of cuckoo wasps have been analysed for the first time;
- Mitochondrial markers (*ATP8*, *ATP6*, *CO3*, *ND3*), characterized by the biggest molecular variability and suitability for the differentiation of cryptic cuckoo wasp species, have been determined;
- 2 new species have been described: *Chrysis horridula* Orlovskytė, 2016 and *C. parietis* Budrys, 2016; a description of a new species of *Ancistrocerus* is being prepared for publication;
- The food web structure of the community has been clarified, 80 new trophic links among the natural enemies and their hosts of the European community have been discovered;
- New patterns of the effect of anthropogenic factors on the community have been estimated;
- The species of the community, suitable for bioindication of forest ecosystem naturalness, has been determined.

Scientific and practical significance

- New data on the species composition and the food web structure of the community may be useful in further research of trap-nesting Hymenoptera and their natural enemies;
- The compiled molecular and morphometric identification keys of North European sibling species in the *Chrysis ignita* complex complement the existing keys and thus facilitate the differentiation of these species;
- The reconstruction of phylogeny of the *Chrysis ignita* complex provide more knowledge about the phylogenetic relationships among the species of this genus and may be used as a basis for further research on the taxonomy of this group;

- Newly constructed primers and determined DNA sequences allow usage of larger marker set in the studies of molecular taxonomy and phylogeny of Chrysidoidea;
- Newly established effects of anthropogenic factors on the parameters of the trap-nesting Hymenoptera insect community may be applied in practical bioindication of terrestrial habitats and further biodiversity studies.

Hypotheses

- Anthropogenic factors affect the nesting probability of trap-nesting Hymenoptera species and their brood abundance in the trap-nests;
- Anthropogenic factors affect the sex ratio and the average weight of prepupae of the trap-nesting Hymenoptera species;
- Anthropogenic impact reduces the trophic link diversity within the trap-nesting Hymenoptera community and simplifies its trophic network.

Defended statements

- A relatively large species richness of the trap-nesting community is found in Lithuanian forest and agricultural ecosystems; this community contains insects of 4 trophic ranks and 5 guilds: bees, predatory wasps, cleptoparasites, parasitoids, and predators;
- The *Chrysis ignita* complex consists of 15 independent sibling species;
- Specialists prevail among the natural enemies of the trap-nesting species;
- The studied anthropogenic factors (increasing agricultural areas, woodland loss and fragmentation, shift to earlier successional stages, effects of forest exploitation) affect the parameters of the trap-nesting community;
- Some species of this community are sensitive to anthropogenic factors and are suitable for bioindication of ecosystem naturalness.

Approbation of the results

The results of the research were presented in 4 scientific articles and 4 conference abstracts. In total, 2 oral and 2 poster presentations have been made on the subject of the thesis at conferences that took place in Lithuania and the USA.

Structure and volume of the dissertation

The dissertation consists of the following chapters: Definitions, Introduction, Literature Review (4 subchapters), Materials and Methods (4 subchapters), Results and Discussion (3 subchapters), Conclusions, References (211 sources), List of Publications, Supplementary Material (8 tables). The dissertation consists of 162 pages, with 129 pages of the main text, 7 tables, 22 figures. The dissertation is written in Lithuanian with the summary in English.

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LITERATURE REVIEW

This study investigates the community of insects inhabiting trap-nests for Hymenoptera. The food web of this community consists of the trophic interactions between cavity-nesting solitary Hymenoptera – bees and predatory wasps from 3 trophic ranks and their natural enemies belonging to 4 trophic ranks (Fig. 1).

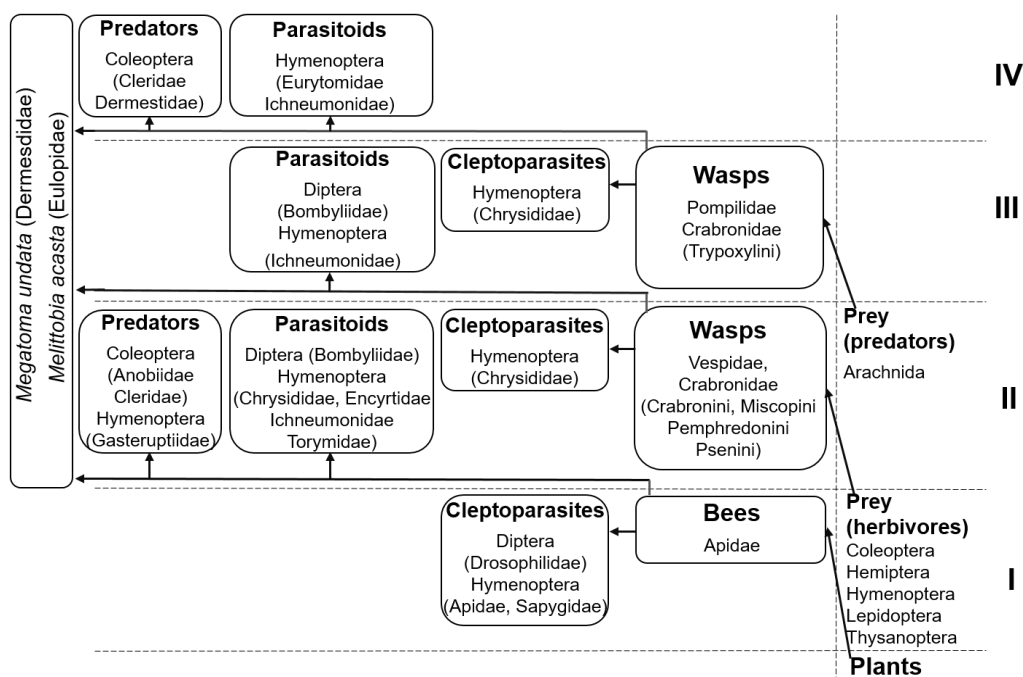


Fig. 1. The food web of the trap-nesting Hymenoptera community. Roman numbers on the right mark trophic ranks.

Previous research of the composition and biology of the community, as well as the effects of anthropogenic factors on it, are reviewed in 4 subchapters:

1. The species composition of the trap-nesting community (90 sources);
2. Taxonomic study of the cryptic cuckoo wasp species (68 sources);
3. Trap-nests for Hymenoptera and its use in the research of the community (55 sources);
4. The effects of anthropogenic factors on the trap-nesting community (38 sources).

MATERIALS AND METHODS

Trap-nests

The material was mainly reared from the trap-nests for Hymenoptera (Budrienė, 2003; Budrys *et al.*, 2010). This method allows obtaining data about the species composition of the studied community and the characteristics of their nests (the diameter and length of the cavity selected by Hymenoptera, the depths of brood cells), their prey (its systematic status, the number of individuals in a brood cell), trophic interactions between trap-nesting species and their natural enemies (their systematic status, body weight, width of the head capsule of a prepupa). The samples of trap-nests (usually 5 trap-nests in a sample) were exposed in the woodland habitats in May. Trap-nests of the same sample were located at least 25 m from each other and were attached to the thickest trunks of different tree species, if available. In September, the collected trap-nests were dismantled, the reed internodes with the Hymenoptera nests were split and studied. The length of a nest and the depth of brood cells were measured to the nearest 1 mm. The diameter of a nest and the width of the head capsule of a prepupa were measured using an ocular micrometer on a stereoscopic microscope MBS-10 to the nearest 0.1 mm. Prepupae were weighed using electronic scales Kern ABJ at 0.1 mg precision. They were placed to a climatic chamber at +4°C for reactivation for 3–5 months. Afterwards, prepupae were placed at +25°C until hatching of imagoes. The latter were weighted, identified and stored in 96% ethanol, at +4°C in the collection of the Nature Research Centre (Vilnius, Lithuania).

Sampling

The exposure of trap-nests was carried out in 2012–2015 in 31 sites of 15 Lithuanian districts. In addition to this, data, collected using the same method in 2003–2011, were added. In total, data from 117 sites of 28 districts of Lithuania and 2 Polish sites were included (Fig. 2). 377 collected samples were composed of 1586 trap-nests.

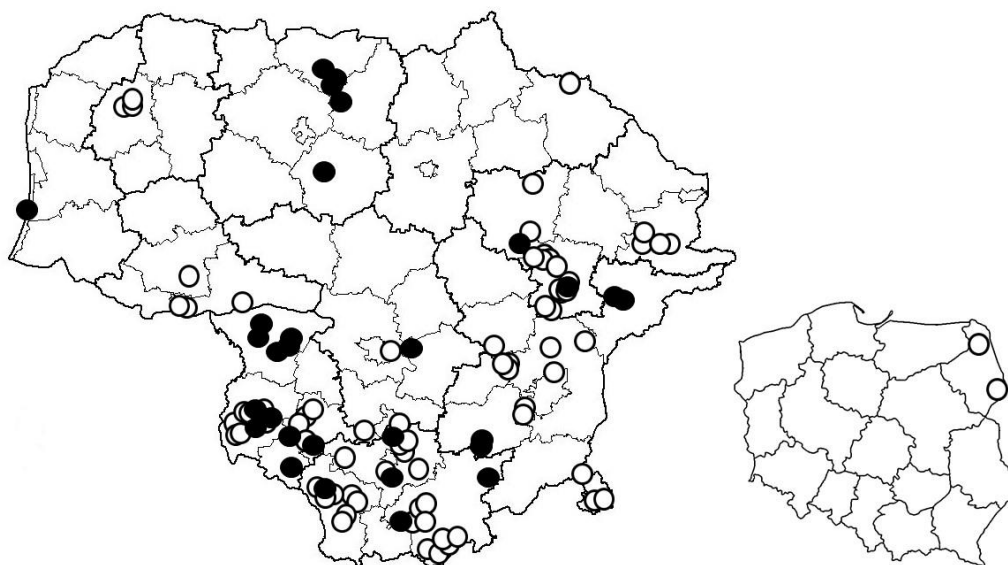


Fig. 2. Maps of trap-nest exposure sites (on the left – in Lithuania, on the right – in Poland (smaller scale)). Black circles indicate the sites, where the material has been collected during PhD studies (in 2012–2015), white circles indicate the sites, used in 2003–2011.

1465 specimens of the cuckoo wasps of the *Chrysis ignita* (Linnaeus, 1758) complex (Hymenoptera: Chrysididae) were used in the taxonomic study. 1240 of them have been reared from trap-nests, the remaining 225 specimens were collected using an entomological net (the exact sampling information is given in S. Orlovskytė *et al.*, 2010, 2016, V. Soon *et al.*, 2014). The studied specimens are deposited in the collections of the Nature Research Centre (Lithuania), Vilnius University (Lithuania) and the Natural History Museum of the University of Tartu (Estonia).

Taxonomic study of the cleptoparasitic cryptic species of the community

Molecular analysis

The molecular variation was analysed using the 297 specimens of 15 sibling species of the *Chrysis ignita* complex: *C. angustula* Schenck, 1856, *C. brevitarsis* Thomson, 1870, *C. corusca* Valkeila, 1971, *C. horridula* Orlovskytė, 2016, *C. ignita*, *C. impressa* Schenck, 1856, *C. leptomandibularis* Niehuis, 2000, *C. longula* Abeille de Perrin, 1879, *C. mediata* Linsenmaier, 1951, *C. parietis* Budrys, 2016, *C. pseudobrevitarsis* Linsenmaier, 1951, *C. schencki* Linsenmaier, 1968, *C. solida* Haupt, 1956, *C. subcoriacea* Linsenmaier, 1959, *C. terminata* Dahlbom, 1854. The analysis of the 750 bp-long part of the *COI-5'* gene, widely used as a "barcoding" sequence (Hebert *et al.*, 2003), was performed to check the discrimination of the cryptic species. The study of the remaining part of the *COI* gene (*COI-3'*) was used to establish additional variation within the haplotypes of the *COI* gene.

40 specimens, representing 39 different *COI* haplotypes of 15 sibling species of the *Chrysis ignita* complex and 1 haplotype of *C. fulgida* Linnaeus, 1761, used as an outgroup in phylogeny reconstructions, were selected for the further analysis of the contiguous region of the mitochondrial genome from the partial methionine tRNA gene to the partial aspartate tRNA gene, involving complete sequences of isoleucine tRNA (*tRNA-ile*), cysteine tRNA, tyrosine tRNA, *COI*, *CO2*, lysine tRNA, aspartic acid tRNA, ATP synthase 8 and 6 subunits (*ATP8* and *ATP6*), 3rd cytochrome c oxidase subunit (*CO3*), glycine tRNA, *ND3*, alanine tRNA, and arginine tRNA (*tRNA-arg*). In addition, partial sequence of the mitochondrial cytochrome b (*CytB*) was analysed. Moreover, partial sequence of the nuclear 28S rRNA gene (*28S*) and the rRNA region (*18S-ITS2* sequence), covering the partial sequence of the 18S rRNA (*18S*), the internal transcribed spacer *ITS1*, the complete sequence of the 5.8S rRNA and the internal transcribed spacer *ITS2*, were included. These 40 specimens and the rest 67 specimens, characterized by different *COI* haplotypes, were included in the phylogenetic reconstruction of the *C. ignita* complex.

Total genomic DNA was extracted from 96% ethanol-preserved thoracic muscles in accordance with either the protocol of "GeneJet Genome Purification Kit" (Thermo Fisher Scientific) or the extraction with the Tris-borate-EDTA (TBE 1×) buffer (Stunžėnas *et al.*, 2011). The PCR reaction mixture in a volume of 50 µL consisted of 25 µL of 2× PCR buffer (Thermo Fisher Scientific), 5 µL of 10 pmol of each primer (MacroBio), 14 µL of deionised water and 1 µL of genomic DNA. New primers were constructed using the mtDNA sequence of *Primeuchroeus* Linsenmaier, 1968 sp. (Castro *et al.*, 2006) and a web tool for primer construction (Rozen, Skaletsky, 1998).

PCR conditions were as follows: depending on the primer set, thermal cycling was initiated by denaturation at 80–95°C for 2–5 min; this was followed by 35–40 cycles of denaturation at 94–95°C for 30–40 s, annealing at 45–52°C for 30–60 s, and extension at 68–72 for 1–1.5 min with a final extension at 68–72°C for 5–10 min. Amplified products were analysed by electrophoresis on 1.5% agarose (Thermo Fisher Scientific) gel. The excess of primers and dNTPs were removed following the standard Exonuclease I/Shrimp Alkaline Phosphatase protocol (Thermo Fisher Scientific). The PCR products were precipitated with 96% ethanol and were sent for sequencing in Macrogen Inc. (South Korea, Seoul) with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequences were manually aligned with the BioEdit 7.2.5 software (Hall, 1999). They were converted from Fasta to Nexus format using Mesquite 3.04 software (Maddison, Maddison, 2015).

The discrete species characters were retrieved from the sequences of the *COI-5'* gene studied in this research and received from V. Soon (the sequences of *Chrysis borealis*, *C. clarinicornis* Linsenmaier, 1951, *C. subcoriacea*, *C. vanlithi* Linsenmaier, 1959 species) using the "Characteristic Attribute Organisation System" (CAOS) algorithm (Sarkar *et al.*, 2002, 2008; Bergmann *et al.*, 2009). The online CAOS-Barcode software was applied to detect the "simple pure" (sPu) characters of each species, discriminating it from the remaining studied species of the *C. ignita* complex. The number of the sPu characters per 100 bp in a mtDNA gene was considered as one of efficacy parameters of the molecular marker in the discrimination of these species.

The comparison of the nucleotide substitution models and model selection was carried out using the MEGA 6 software (Tamura *et al.*, 2013). The substitution patterns of the studied mtDNA sequence were best described by the general time reversible model with Gamma distribution evolutionary rates (5 discrete categories were applied) and the fraction of invariable sites (GTR+G+I). MEGA 6 was also used for the assessment of pairwise distances between the analysed sequences, the mean distances within and between species, for manual construction of trees, necessary for the CAOS analysis, and for conversion of the trees to the Newick format. The Newick trees were transferred to the Nexus data format using Mesquite 3.04.

The pairwise distances (p-distances) in the analysed sequences were calculated by dividing the number of nucleotide substitutions from the total number of nucleotides (Nei, Kumar, 2000). The suitability of the studied markers in the discrimination of cryptic species and their relative evolutionary rate were reflected by the correlation of the p-distances between these markers and the mtDNA *COI-5'* sequence. The regression coefficient b and the determination coefficient r^2 of such dependence were calculated using the StatSoft Statistica 8.0 software.

The final versions of phylogenetic trees were obtained using the Bayesian inference method, implemented in the MrBayes 3.2.3 software (Ronquist, Huelsenbeck, 2003). The analysed mtDNA sequences were grouped into the protein and the tRNA coding sections. The GTR+G+I (5 discrete gamma categories) nucleotide substitution model was applied to both, though the Nucmodel parameter was set to "codon" for the first section and to "4×4" for the second one; the number of substitution types (nst) was set to

"mixed". The Markov Chain Monte Carlo analyses were run for one million generations, with tree sampling every 1000 generations.

Morphometric analysis

Morphometric analysis was performed using 558 (375♀, ♂183) specimens of 16 sibling species of the *Chrysis ignita* complex: *C. angustula* (30♀, 35♂), *C. borealis* Paukkunen, Ødegaard & Soon, 2014 (5♀, 1♂), *C. brevitarsis* (16♀, 1♂), *C. corusca* (14♀, 6♂), *C. horridula* (30♀), *C. ignita* (34♀, 7♂), *C. impressa* (21♀, 11♂), *C. leptomandibularis* (25♀, 2♂), *C. longula* (30♀, 23♂), *C. mediata* (52♀, 25♂), *C. parietis* (3♀, 2♂), *C. pseudobrevitarsis* (21♀, 1♂), *C. schencki* (30♀, 17♂), *C. solida* (30♀, 35♂), *C. subcoriacea* (10♀, 1♂), *C. terminata* (24♀, 16♂). 17 morphometric measurements were made for each specimen (Table 1) using an ocular micrometer of a stereoscopic microscope MBS-10 at magnification 32–56×. The measurements were transformed to millimeters, averages and standard errors were calculated by the Microsoft Excel 2010 program.

Table 1. Morphometric characters used in the present study, their abbreviations, and the measured aspect.

Abbreviation	Aspect	Character	Source
BOD	Anterodorsal	brow-ocellar distance	D. Morgan (1984) as BOL
F1	Lateral	length of the 1 st flagellomere	D. Morgan (1984)
F2	Lateral	length of the 2 nd flagellomere	D. Morgan (1984)
LF	Frontal	length of face	E. Budrys (1996)
LMP	Dorsal	length of mesoscutellum and propodeum	D. Morgan (1984) as MPL
LPN	Dorsal	length of pronotum laterally	this study
OOD	Dorsal	oculo-ocellar distance	D. Morgan (1984) as OOL, E. Budrys (1996), J. van der Smissen (2010)
PT1	Dorsal	largest punctures on the 1 st tergite	D. Morgan (1984), W. Linsenmaier (1997), J. van der Smissen (2010)
PT2	Dorsal	largest punctures on the 2 nd tergite	D. Morgan (1984), W. Linsenmaier (1997), J. van der Smissen (2010)
POD	Dorsal	post-ocellar distance	J. van der Smissen (2010)
WT3	Dorsal	width of the 3 rd tergite basally	this study
WH	Dorsal	width of head	E. Budrys (1996)
WMB	Lateral	width of mandible basally	O. Niehuis (2000)
WMM	Lateral	width of mandible in its middle part	O. Niehuis (2000)
WPN	Dorsal	width of pronotum	E. Budrys (1996) as PRN
WPP	Dorsal	width of propodeum	D. Morgan (1984) as PPW
WRB	Posterodorsal	width of apical rim of the 3 rd tergite basally	this study

Morphometric measurements were used for the construction of discriminant equations. The best classification functions for 2 groups of specimens at each step of the dichotomous identification key were obtained using backward or forward stepwise discriminant analysis and manual adjustment of classification probabilities until the best congruence between the predicted and the observed classification was achieved. The coefficients of the 2 classification functions were pairwise subtracted to obtain the parameters of a linear discriminant equation; afterwards the parameters were divided by the constant of the equation, simplifying the latter to 1. To estimate the morphometric similarity among the species, squared Mahalanobis distances between the species measurement group centroids were used. They were calculated using a standard discriminant analysis, involving all 17 morphometric measurements. The distance matrix was visualised as a tree diagram applying cluster analysis, the unweighted pair-group average (UPGA) linkage method. The discriminant and cluster analyses were performed with the StatSoft Statistica 8.0 software.

Analysis of trophic specialization

The host preference was estimated using 1240 cuckoo wasp specimens reared from the nests of their hosts, built in the trap-nests for Hymenoptera. The Manly's index was used: $\alpha_i = (r_i / n_i) / \sum (r_j / n_j)$, $j = 1, \dots, m$ (Krebs, 1999), where α_i = the index of the preference for the host species i ; m = the total number of the trap-nesting wasp species observed being hosts of all species of the *Chrysis ignita* complex; r_i = the proportion of the brood cells of the host species i among the brood cells of all trap-nesting hosts exploited by the particular species of the *C. ignita* complex; n_i = the proportion of the brood cells of the host species i among the brood cells of all trap-nesting hosts of all species of the *C. ignita* complex.

The analysis of the structure of the community

The structure of the trap-nesting Hymenoptera community was estimated using the data set of 1586 trap-nests. The species of trap-nesting bees, predatory wasps, and their natural enemies were divided into dominance classes (Tischler, 1949) in accordance with the abundance of their brood cells in the trap-nests.

The analysis of anthropogenic impact on the community

The impact of anthropogenic factors on the trap-nesting Hymenoptera community was assessed on the base of 377 collected trap-nest samples. Parameters of the community were considered as the dependent variables. They were as follows:

- The species diversity of trap-nesting bees and predatory wasps. The Shannon's diversity index was used: $H' = -\sum p_i \log_2 p_i$, $i = 1, \dots, S$ (Heip *et al.*, 1998), where H' = diversity index; S = the species richness of trap-nesting bees and predatory wasps; p_i = the relative abundance of a species in the sample. The variable considered continuous;
- The relative abundance of natural enemies – the proportion of the brood cells occupied by all natural enemies and the total number of brood cells. The variable considered continuous;
- The relative species richness of natural enemies – the proportion of the number of the natural enemy species and the number of trap-nesting host species. The variable considered continuous;
- The diversity of trophic links. The Shannon's diversity index was used: $H' = -\sum p_i \log_2 p_i$, $i = 1, \dots, S$ (Heip *et al.*, 1998), where H' = diversity index; S = the number of trophic links, p_i = the proportion of the occupied brood cells by a natural enemy species and the occupied cells of all species of natural enemies. The variable considered continuous.

The remaining parameters of the community were assessed only for the species of trap-nesting bees and predatory wasps, found in at least 10 samples of trap-nests (28 species in total):

- The nesting probability of the species in a sample. The variable considered dichotomous;
- The abundance of the brood cells in a sample. The data were transformed according to the formula $x = \log_{10} (\text{abundance} + 1)$. The variable considered continuous;

- The sex ratio of a species – the share of female offspring among the brood cells with known sex in a sample. The variable considered continuous;
- The relative weight of a prepupa – the average rate of the weight of a prepupa in a sample to the average weight of a prepupa of the same species and sex. The variable considered continuous.

The effects of anthropogenic impact on the parameters of the trap-nesting Hymenoptera community were assessed at different spatial scale, such as increasing agricultural areas, woodland loss and fragmentation, shift toward earlier successional stages, and effects of forest exploitation. The factors of these drivers were considered as the independent variables:

- The increasing agricultural areas was assessed using the share of agricultural areas in the total area of a district. The data were taken from the Lithuanian Official Statistics Portal (<http://osp.gov.lt>) and Eurostat (<http://ec.europa.eu>). The variable considered continuous;
- The woodland loss and fragmentation was assessed using the \log_{10} size of woodland fragments. The variable considered continuous;
- The shift to the earlier successional stages was assessed using a late successional stage index, calculated as $y = 1 \times (\text{share of mid-successional tree species in the forest stand}) + 2 \times (\text{share of climax tree species in the forest stand})$. The climax species were considered oak and lime; the mid-successional were considered fir, elm, maple, hornbeam, ash. The remaining dominant tree species (alder, birches, aspen, willow, alder, pine) were considered pioneer. The data on the composition of forest stand in the studied woodland habitats were taken from the National Forestry Cadastre (www.amvmt.lt). To normalise the variable of the late successional stage index, it was transformed according to the formula $x = \log_{10}(y + 1)$. The variable considered continuous;
- Forest exploitation was estimated by the age of forest stands based on the data of the National Forestry Cadastre (www.amvmt.lt). The data were transformed according to the formula $x = \log_{10}(\text{age})$. The variable considered continuous;
Presence of upright dead wood was considered as another habitat parameter related to lacking or sustainable forest exploitation. At least one trap-nest was exposed on a dead trunk in the case of the presence of dead wood in the habitat. The variable considered dichotomous.

All studied anthropogenic factors are driven by the same human activities, thus they naturally correlate with each other. The highest correlation, found between the shift toward earlier successional stages and the age of the forest stand, was less than 30% (determination coefficient $r^2 = 0.27$). On the other hand, all these factors have different spatial and temporal scales; therefore, we assessed them as independent.

Logistic binomial regression was used to quantify the dependence of dichotomous dependent variables (the nesting probability) on the independent variables (share of agricultural areas, woodland fragment size, late successional stage, forest stand age, presence of dead wood). The logistic regression formula was used: $p_i = \exp\{z(x_i)\} / 1 + \exp\{z(x_i)\}$, $z(x_i) = a + b_1x_{1i} + b_2x_{2i} + \dots + b_kx_{ki}$ (Čekanavičius, Murauskas, 2002), where p_i – the probability of nesting of the i^{th} species; a , b_1 , b_2 , ..., b_k – coefficients; x_{1i} , x_{2i} , ..., x_{ki} – independent variables.

General linear models (GLM) were applied to assess the differences in the dependence of brood cell abundance of trap-nesting Hymenoptera, relative abundance of natural enemies, sex ratio, relative weight of a prepupa, species diversity of trap-nesting Hymenoptera, relative species richness of natural enemies, and diversity of trophic links on the anthropogenic drivers at different spatial scale.

Using both logistic binomial regression and the analysis of general linear models, the backward stepwise selection of the most appropriate model was applied in turn rejecting the independent variables with the the least reliable values ($p > 0.05$). Afterwards, the significance levels of the values of dependent variables, which were analysed separately for each species (nesting probability, brood cell abundance, sex ratio, relative weight of a prepupa), were adjusted with Bonferroni correction dividing 0.05 by the number of studied species (28). As a result, only the values with $p < 0.0018$ were considered significant.

The principal component analysis (PCA) was used to transform continuous independent variables (share of agricultural areas, woodland fragment size, late successional stage, forest stand age) into generalized factors. The impact of 2 major factors on the brood cell abundance of trap-nesting bees and predatory wasps was estimated using correlations.

The data were analysed with the StatSoft Statistica 8.0 software, the modules GLZ, GLM, and PCCA.

RESULTS AND DISCUSSION

The structure of the trap-nesting community in Lithuania

Representatives of Hymenoptera, found in the trap-nests in Lithuania, belong to bees (Apidae) and predatory wasps (Crabronidae, Pompilidae, Vespidae). In total 57 species of trap-nesting bees and predatory wasps were found, representing 41.3% of the total number of Hymenoptera species (138 species), detected in trap-nests in other European countries.

Insects from the 1st trophic rank, which feed their brood with a mixture of pollen and nectar, belong to 15 species of bees (Apidae: Colletinae, Megachilinae) (Fig. 3). 2 species of them, *Hylaeus bisinuatus* Foerster, 1871 and *H. rinki* (Gorski, 1852), were found for the first time in trap-nests for Hymenoptera in Europe.

Predatory wasps from the 2nd trophic rank, which feed their brood with phytophagous insects, dominate within the trap-nesting Hymenoptera by both the species richness and their brood abundance. 34 species of predatory wasps (Vespidae: Eumeninae; Crabronidae: Pemphredoninae: Pemphredonini, Psenini, Crabroninae: Crabronini, Miscophini) (Fig. 4) were detected; 3 of them were found for the first time in the European trap-nests: *Pemphredon baltica* Merisuo, 1972, *Crossocerus megacephalus* (Rossi, 1790) (Crabronidae), and an undescribed cryptic *Ancistrocerus* Wesmael, 1836 species, closest morphologically and genetically to *A. trifasciatus* (Müller, 1776) (it is preliminary named *A. balticus* sp.n.).

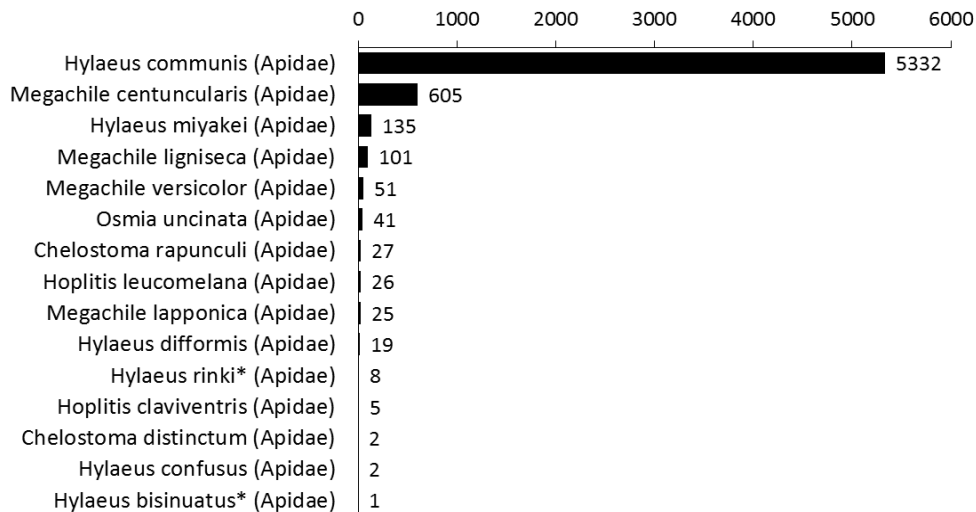


Fig. 3. Bees from the 1st trophic rank and the number of their brood cells in 1586 trap-nests. * marks the species, found for the first time in trap-nests in Europe.

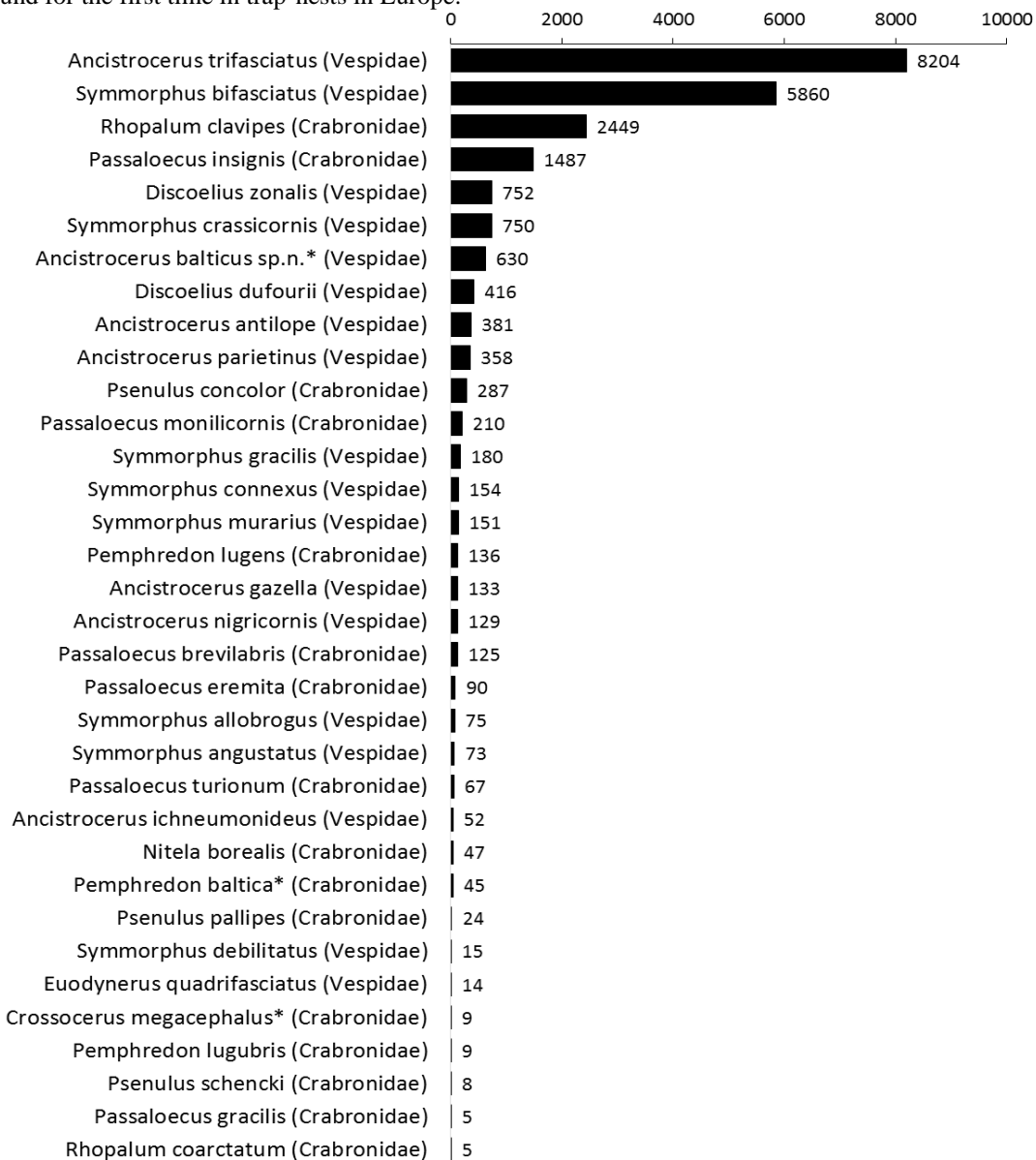


Fig. 4. Predatory wasps from the 2nd trophic rank and the number of their brood cells in 1586 trap-nests. * marks the species, found for the first time in trap-nests in Europe.

8 species of predatory wasps (Crabronidae: Crabroninae: Trypoxylini; Pompilidae: Pompilinae, Pepsinae) from the 3rd trophic rank, which feed their brood with spiders, were found in trap-nests (Fig. 5). Of them, *Dipogon vechti* Day, 1979 was detected for the first time in trap-nests for Hymenoptera in Europe.

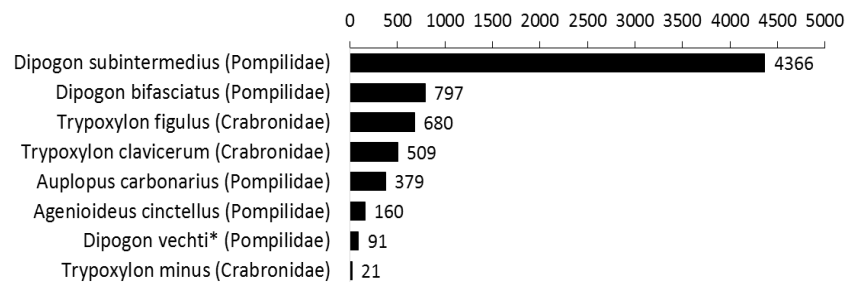


Fig. 5. Predatory wasps from the 3rd trophic rank and the number of their brood cells in 1586 trap-nests. * marks the species, found for the first time in trap-nests in Europe.

The trap-nesting community contains 37 species of natural enemies (predators, parasitoids, and cleptoparasites) in Lithuania (Fig. 6). 11 species of them were found for the first time in the European trap-nests. The detected natural enemy species represent 50.7% of all species of natural enemies found in trap-nests for Hymenoptera in Europe (73 species).

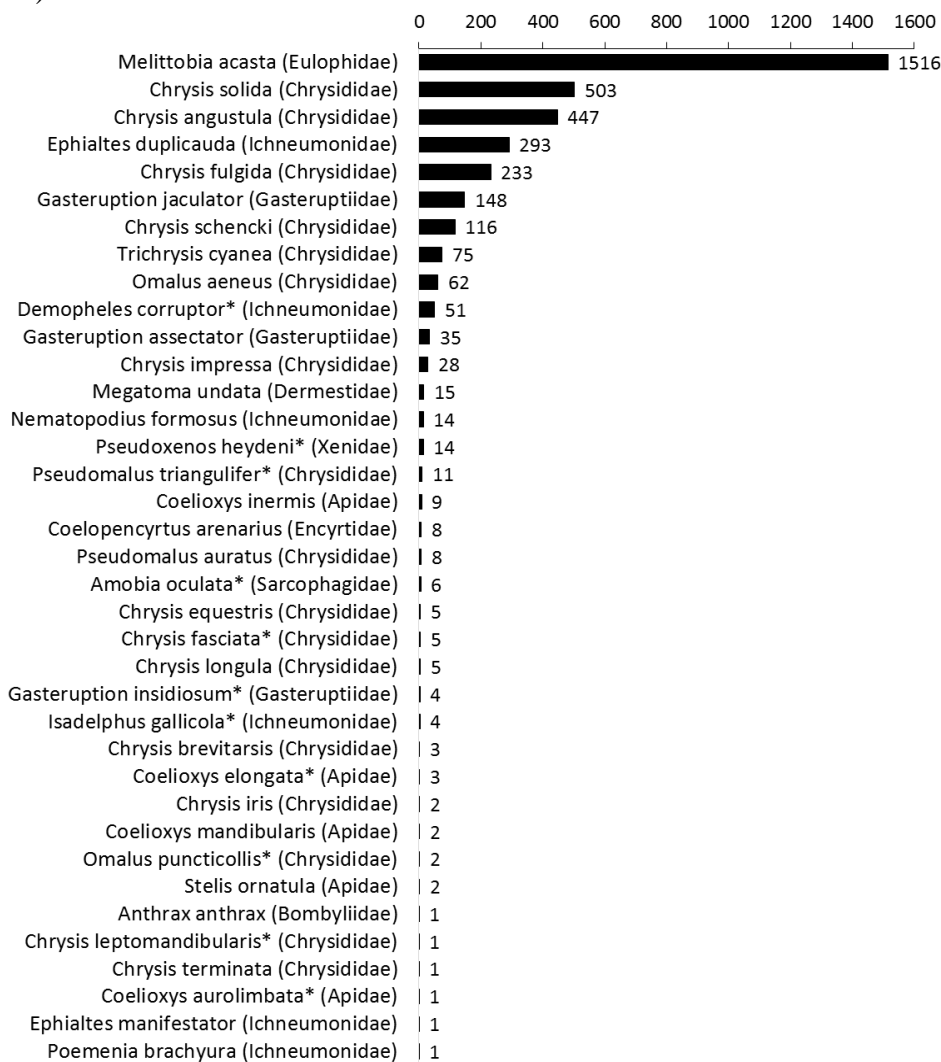


Fig. 6. Natural enemies of trap-nesting bees and predatory wasps, and the number of occupied brood cells by them in 1586 trap-nests. * marks the species, found for the first time in trap-nests in Europe.

It was determined that natural enemies are linked to 43 species of trap-nesting bees and predatory wasps by trophic interactions. In total, 126 trophic links represent 67.4% of the total host and its natural enemy species interactions, detected in trap-nests in Europe (187 trophic links); of them, 80 links were found for the first time in the European trap-nests.

After the examination of trophic links among the species of the community, it was established that most of the natural enemies (28 of 37 species) are specialists (insects, feeding on the species from a single genus), while the remaining 9 are classified as generalists. The most abundant natural enemy, found in trap-nests, is the omnivorous parasitoid *Melittobia acasta* (Walker, 1839) (Hymenoptera: Eulophidae), which occupies approximately 41.7% of cells attacked by all natural enemy species of this community. Nearly half of the remaining species of natural enemies (17 species of 38) are cuckoo wasps (Hymenoptera: Chrysididae), 12 of which belong to the *Chrysis* Linnaeus, 1761 genus. With exception of the generalist parasitoid *M. acasta*, this genus represents 63.6% of brood cells occupied by all species of natural enemies found in trap-nests. A significant part of *Chrysis* species comprises the *C. ignita* complex that includes numerous sympatric cryptic species.

Cleptoparasitic sibling species of the *Chrysis ignita* complex

Molecular markers and phylogenetic relationships

The molecular variability of the sequences of the mtDNA *COI* gene was successfully studied using 295 specimens of all 15 species of the *Chrysis ignita* complex. Different haplotypes were found for 106 specimens; these and other sequenced fragments were placed in the GenBank database (<http://www.ncbi.nlm.nih.gov/genbank/>).

37 haplotypes of 14 *C. ignita* complex species and 1 *C. fulgida* species were smoothly studied, except *ND3*, *CytB*, and nuclear markers of 2 haplotypes of *C. subcoriacea*. As a result, the obtained mtDNA sequence of *tRNA-ile-tRNA-arg* and *CytB* was approximately 4850 bp-long with small variation in length. This sequence was supplemented by the mitochondrial 12S rRNA (*12S*), valine tRNA, 16S rRNA (*16S*) genes, obtained in earlier study (Soon *et al.*, 2014); in total, we got the approximately 7400 bp-long mtDNA sequence. The region of the nuclear DNA, consisting of the *18S-ITS2* and *28S* genes, was approximately 3880 bp-long. Eventually, the data set of mitochondrial and nuclear sequences was 11200 bp-long.

Using the CAOS algorithm, the discrete diagnostic molecular characters of each species sequence were retrieved, i.e. the bp states of a species distinctive from the remaining studied species. The result is a molecular identification key, using the characters of the *COI-5'* for the 18 Northern European species of the *Chrysis ignita* complex (Orlovskyy *et al.*, 2016). The key considers the intraspecific variability of the cryptic species following the study of 78 *COI-5'* haplotypes. 13 studied species had at least one simple pure (sPu) character discriminating them from the remaining species. Among the studied mitochondrial markers, the highest evolutionary rates, estimated using comparison of p-distances, and the highest density of simple pure molecular characters (sPu) were found in the genes *ATP8*, *ATP6*, *CO3*, and *ND3*. The estimated

densities of the sPu might drop after further studies of intraspecific haplotype variation across the distribution areas of the species.

The overall mean distance between 7400 bp-long mtDNA sequences, including the *tRNA-ile-tRNA-arg*, *CytB*, and *16S-12S*, was $6.9\pm 0.2\%$. The intraspecific evolutionary divergence of the species, i.e. the average p-distances between the haplotypes of a species, was estimated using the studied mtDNA sequences, ranged from $0.03\pm 0.02\%$ in *Chrysis ignita* to $0.21\pm 0.03\%$ in *C. solida*, $0.23\pm 0.04\%$ in *C. longula*. Among the studied genes, the highest intraspecific variation was found in the *ATP8* (0.7% in *C. angustula* and *C. terminata*). The interspecific average p-distance between the pairs of the studied species, estimated using the same mtDNA sequence, varied from 1.4% to 11.5% , with 2 exceptions: it was $0.28\pm 0.05\%$ between *C. mediata* and *C. solida*, and $0.18\pm 0.05\%$ between *C. ignita* and *C. impressa*. The best molecular markers for the separation of the species pair *C. mediata* – *C. solida* were the *ATP6* (p-distance $0.67\pm 0.26\%$) and the *CO3* ($0.62\pm 0.21\%$). For separation of *C. ignita* – *C. impressa*, the best marker was the "barcoding" *COI-5'* (p-distance $0.53\pm 0.27\%$). The overall mean distance within the data set estimated using the studied nuclear *18S-ITS1-5.8S-ITS2-18S-28S* sequence was considerably smaller than that estimated using the mtDNA sequence: $1.6\pm 0.1\%$. The interspecific p-distances between the nuclear sequences varied from 0.4% to 5.6% , excepting the species pairs *C. horridula* – *C. parietis*, where it was $0.21\pm 0.01\%$, and *C. mediata* – *C. solida*, where it was only $0.15\pm 0.04\%$.

Estimating the relative evolutionary rate of the molecular markers and summarising their utility for the discrimination of cryptic species, the p-distances were compared between the studied haplotypes calculated using these markers, with the p-distances calculated using the 750 bp long "barcoding" *COI-5'* sequence (Fig. 7). The evolutionary rate of *COI-3'*, *CO2*, and *12S* was rather close to that of the *COI-5'* (Fig. 7 A, B, H, regression coefficient b (here and after: b) = $1.033-1.096$); the rate of *CO3*, *ND3*, *ATP6*, and *CytB* was higher (Fig. 7 C, D, E, G, b = $1.23-1.47$), up to twice as high in *ATP8*, as compared to the *COI-5'* (Fig. 7 F). In contrast, *16S*, tRNAs, *ITS1*, and *ITS2* were evolutionarily more conservative than *COI-5'* (Fig. 7 I, J, K, L, b = $0.52-0.88$); the nuclear *18S* and *5.8S* were identical in most studied cryptic species, the only exception being *Chrysis leptomandibularis*, having a few substitutions in the V7 area to that typical for the group *18S* sequence (Fig. 7 M). The evolutionary rate of the hypervariable D2 and D3 loops of the *28S* sequence was close to that of the mitochondrial DNA markers (b = $0.97-1.29$). However, the evolutionary divergence between the studied specimens estimated using the *28S* sequence had surprisingly low correlation with the divergence estimated using mitochondrial markers (Fig. 7 N, O, r^2 = 0.04 , p = 0.6 , in relation to the *COI-5'*).

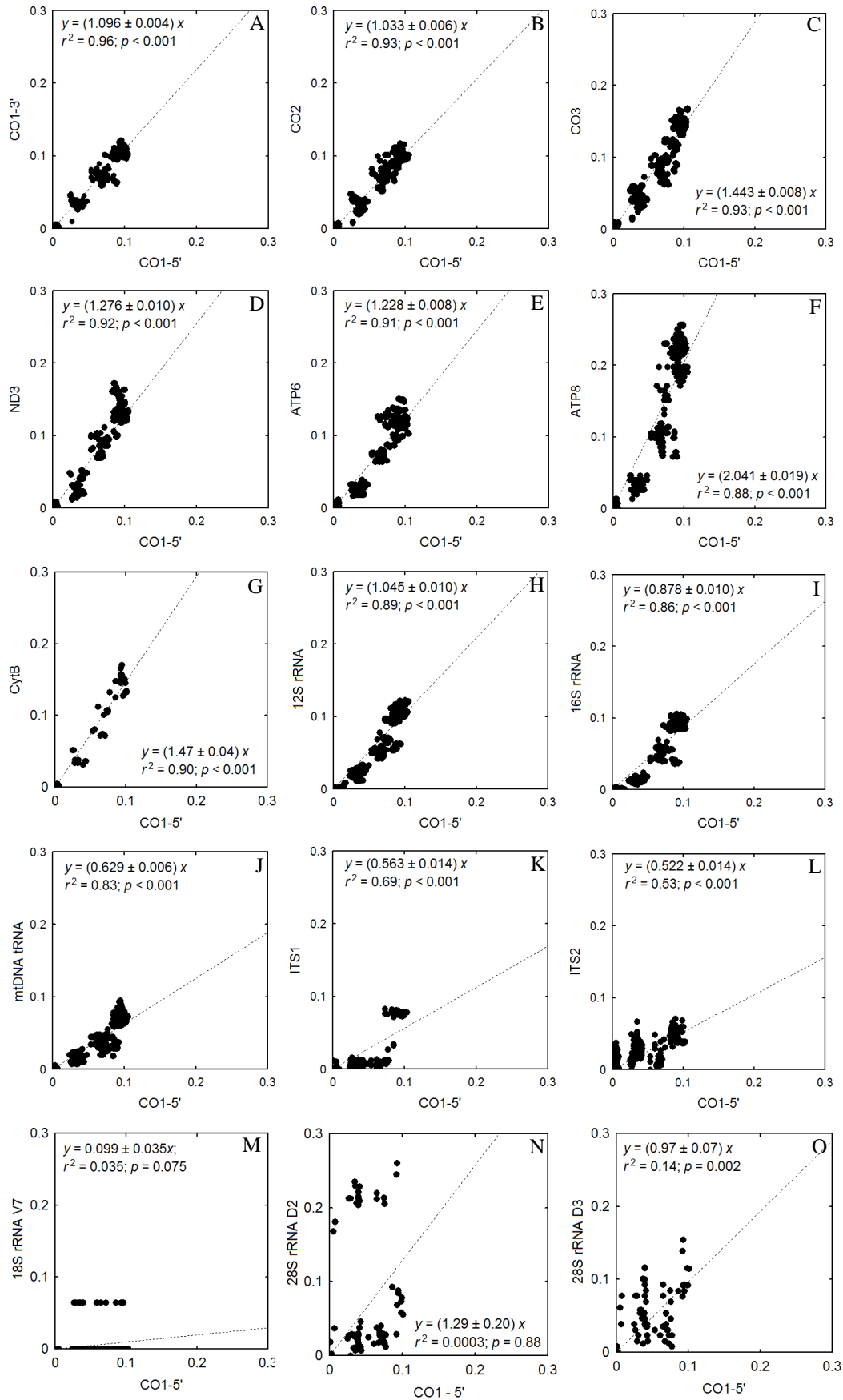


Fig. 7. Uncorrected p-distances between the pairs of haplotypes of the *Chrysis ignita* sibling species complex, estimated using the studied molecular markers (Y axis), compared to the p-distances, estimated using the "barcoding" 5'-end 750 bp part of the *COI* gene (X axis).

This comparison of molecular markers demonstrates that the mitochondrial genes generally have a higher evolutionary rate and seem to have a higher resolution power in separation of the groups of phylogenetically young species than nuclear markers, as it has been demonstrated in some earlier studies (e.g., Nieuwerkerken *et al.*, 2012), although combination of both marker types and further studies of nuclear markers are recommended. Among the studied mitochondrial markers, the highest evolutionary rates, estimated using comparison of p-distances, and the highest density of simple pure molecular characters (sPu) were found in the genes *ATP8*, *ATP6*, *CO3*, and *ND3*. The estimated densities of the sPu might drop after further studies of intraspecific haplotype variation across the distribution areas of the species.

The strongly supported topology of the phylogenetic tree reconstructed using the 11200 bp-long data set of the mitochondrial and nuclear sequences (Fig. 8) was similar to the results of the study based on the 2187 bp mtDNA sequence, combined of the "barcoding" *COI-5'*, the *12S*, and the *16S* (Soon *et al.*, 2014). However, our reconstruction resolved the position of *Chrysis schencki* and *C. parietis* as a sister clade of the *C. ignita* – *C. impressa* clade (ML bootstrap value 98, Bayesian posterior probability 1.00) and supported the monophyly of the *C. angustula* – *C. longula* clade (ML bootstrap value 77, posterior probability 1.00), *C. corusca* being a sister clade of it.

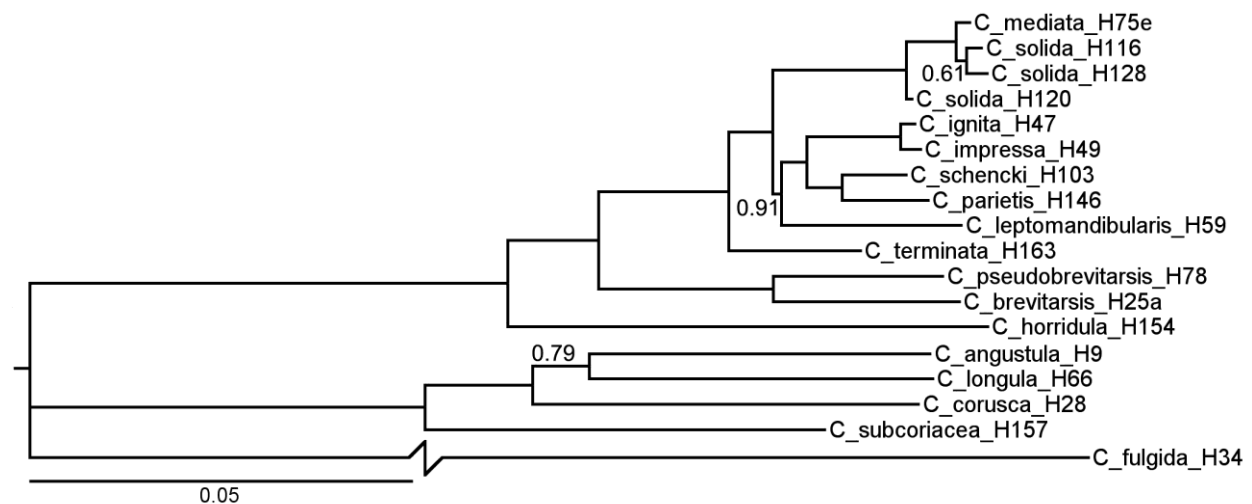


Fig. 8. Bayesian reconstruction of phylogeny of the *Chrysis ignita* sibling species complex, using the combined 7420 bp-long mtDNA sequence (complete *CO1*, *CO2*, *ATP8*, *ATP6*, *CO3*, *ND3*, 9 tRNAs, *12S*, *16S*, partial *CytB* genes) and approximately 3880 bp nuclear DNA sequence (*18S*, *ITS1*, *5.8S*, *ITS2*, *28S*). Posterior probabilities of branches indicated, if lower than 1.00; *C. fulgida* included as an outgroup.

Chrysis solida was the only species in which the haplotypes did not form a monophyletic group. An additional phylogenetic analysis of the *C. mediata* – *C. solida* clade revealed that the results of the reconstruction of relationships among their haplotypes depended on the selected marker set. In the Bayesian tree based on the sequences of the genes *CO1* and *CO2* (2289 bp) both species occurred paraphyletic (Fig. 9 A). The application of a combined sequence of the *ATP8*, *ATP6*, *CO3*, and *ND3* (2015 bp) resulted in a strong monophyly of *C. mediata* and paraphyly of *C. solida* (Fig. 9 B). Finally, the application of the combined *CO2*, *ATP8*, and *ATP6* sequences (1504 bp) resulted in monophyly of both species, despite a rather low posterior probability of the *C. solida* clade (Fig. 9 C).

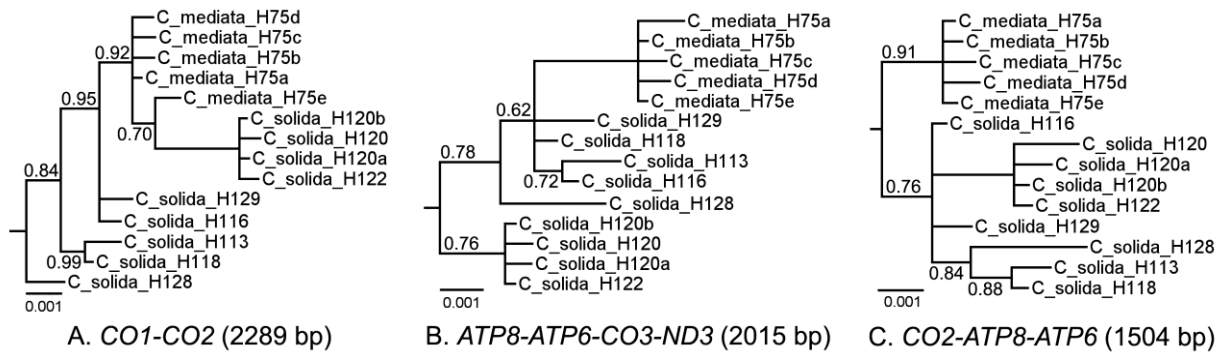


Fig. 9. Bayesian reconstructions of phylogenetic relationships among the studied haplotypes of *Chrysis mediata* and *C. solida*, using three combinations of mitochondrial gene sequences. Posterior probabilities of branches indicated if lower than 1.00.

Morphometric characters

Discriminant equations based on the morphometric measurements could separate the sibling species of the *Chrysis ignita* complex with a reasonable (90–100%) probability. The presented identification key with these equations (Orlovskytè *et al.*, 2016) can be used to complement the illustrated identification key of cuckoo wasps of the Nordic and Baltic countries compiled by J. Paukkunen *et al.* (2015), when identification using the usual morphological characters appears uncertain or impossible.

The phenogram, reflecting the similarity of body proportions (Fig. 10), did not have any common features with the reconstructed phylogenetic tree (Fig. 8): the closely related species pairs *Chrysis mediata* – *C. solida* and *C. ignita* – *C. impressa* were morphometrically more or less far separated, while the phylogenetically distant *C. horridula* and *C. parietis* were extremely similar in their body proportions. The species of the little related *comta* (*C. angustula*, *C. corusca*, *C. longula*, *C. subcoriacea*) and *ignita* clades (remaining species) were interlaced and scattered across the morphometric similarity cluster. Such mismatch implies that the body proportions represent an adaptive and evolutionarily flexible character set, poorly connected to the phylogenetic relatedness of the species.

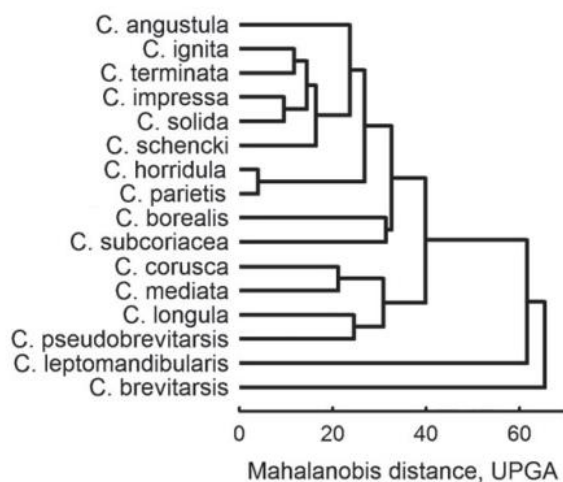


Fig. 10. Morphometric similarity of the sibling species of the *Chrysis ignita* complex. The Mahalanobis distances between species group centroids were estimated using the standard discriminant analysis of 17 morphometric measurements (mm); their explanations are presented in Table 1.

Trophic specialization

10 species of the *Chrysis ignita* complex were reared from the nests of 16 cavity-nesting, xylicolous potter wasps (Hymenoptera: Vespidae: Eumeninae) from the genera *Ancistrocerus*, *Discoelius* Latreille, 1809, *Euodynerus* Dalla Torre, 1904, *Symmorphus* Wesmael, 1836 (Fig. 11). The only exception is *C. mediata*, exploiting ground-nesting eumenines from the genus *Odynerus* Latreille, 1802. Compared with Europe, the data collected during this research revealed 18 new trophic interactions between cuckoo wasps and their hosts.

Most species of *Chrysis* have the main trophic link to a single or a few related host species, sometimes in particular habitats, as for *C. schencki*, and supplementary trophic links to a few other hosts, usually other species of the same wasp genus as the main host (Fig. 11). These results are confirmed by the findings of the study of *Chrysis* inhabiting trap-nests (Pärn *et al.*, 2015).

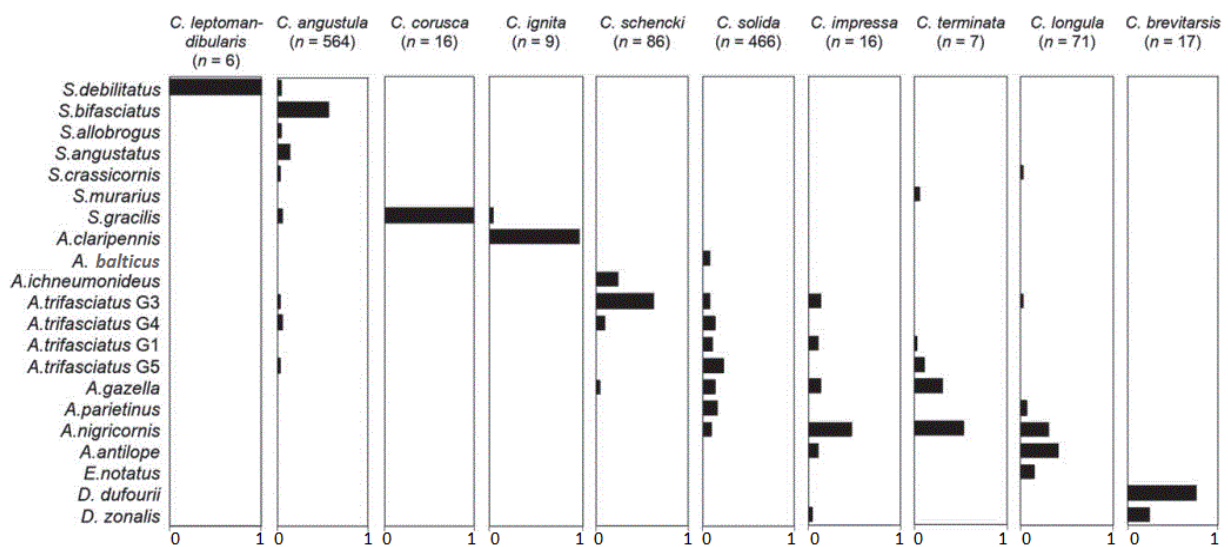


Fig. 11. Manly's preference index (α) of 10 sibling species of the *C. ignita* complex. Hosts: eumenine wasps of genera *Symmorphus*, *Ancistrocerus*, *Euodynerus* and *Discoelius*. For the most abundant and widespread host species, *A. trifasciatus*, the data are divided by the EUNIS habitat class (G1 – broadleaved deciduous woodland; G3 – coniferous woodland; G4 – mixed woodland; G5 – lines of trees in agricultural landscape, small anthropogenic woodlands).

In accordance with the results of the molecular, morphological, and trophic specialization analyses, we can confidently state that the cleptoparasitic *Chrysis ignita* complex consists of 15 independent sibling species. Taking into account the different host and habitat choice of the species, caused by completely different nesting behaviour of their hosts, *C. mediata* and *C. solida* should be considered as distinct species. This hypothesis is supported by the presence of molecular characters (cPu) and reliable morphometric differences, although a weak molecular differentiation proves their relatively recent divergence.

Within the framework of mitochondrial DNA markers and morphometric characters, 2 new species of the *Chrysis ignita* complex, *C. horridula* and *C. parietis*, were described (Orlovskytè *et al.*, 2016).

Effects of anthropogenic impact on the trap-nesting community in Lithuania

Effects of anthropogenic factors on the nesting probability

The effects of anthropogenic impact at different spatial scale (increasing agricultural areas, woodland loss and fragmentation, shift toward earlier successional stages, effects of forest exploitation) on the nesting probability of Hymenoptera was studied using the binomial logistic regression analysis. The effects of anthropogenic impact on the nesting probability were tangible in 13 species of 28 (Table 2).

Table 2. Dependence (logistic regression coefficient \pm standard error) of the nesting probability of trap-nesting Hymenoptera species (n – the number of samples) on increasing agricultural areas, woodland loss and fragmentation, shift toward earlier successional stages, effects of forest exploitation. Only values significant after Bonferroni correction are presented ($p < 0.0018$).

Species	Share of agricultural areas	Log-woodland fragment size	Log-late successional stage	Log-forest stand age
I trophic rank <i>Hylaeus communis</i> (n=250) <i>Megachile centuncularis</i> (n=25) <i>M. ligniseca</i> (n=12)		-0.27 \pm 0.08 -0.58 \pm 0.16		-1.91 \pm 0.34
II trophic rank <i>Discoelius dufourii</i> (n=30) <i>Passaloecus insignis</i> (n=85) <i>Pemphredon lugens</i> (n=14) <i>Symmorphus bifasciatus</i> (n=188) <i>S. crassicornis</i> (n=45) <i>S. murarius</i> (n=12)	2.19 \pm 0.68 3.43 \pm 0.93	1.54 \pm 0.46 -0.27 \pm 0.07 -0.40 \pm 0.11		-1.22 \pm 0.33 -1.56 \pm 0.42
III trophic rank <i>Auplopus carbonarius</i> (n=34) <i>Dipogon bifasciatus</i> (n=57) <i>D. subintermedius</i> (n=229) <i>Trypoxylon clavicerum</i> (n=50)	4.17 \pm 1.07 2.95 \pm 0.85	0.35 \pm 0.08	3.87 \pm 0.91	-0.97 \pm 0.24 0.81 \pm 0.22

Of them, bees from the 1st trophic rank prefer trap-nests, exposed in smaller woodland fragments (*Megachile ligniseca* (Kirby, 1802) (Fig. 12 A, the logistic regression coefficient (here and after: log. regr. coef.) = -0.58 \pm 0.16, $p < 0.001$), *Hylaeus communis* Nylander, 1852 (log. regr. coef. = -0.27 \pm 0.08, $p < 0.001$)) with younger forest stands (*M. centuncularis* (Linnaeus, 1758) (Fig. 12 B, log. regr. coef. = -1.91 \pm 0.34, $p < 0.001$)).

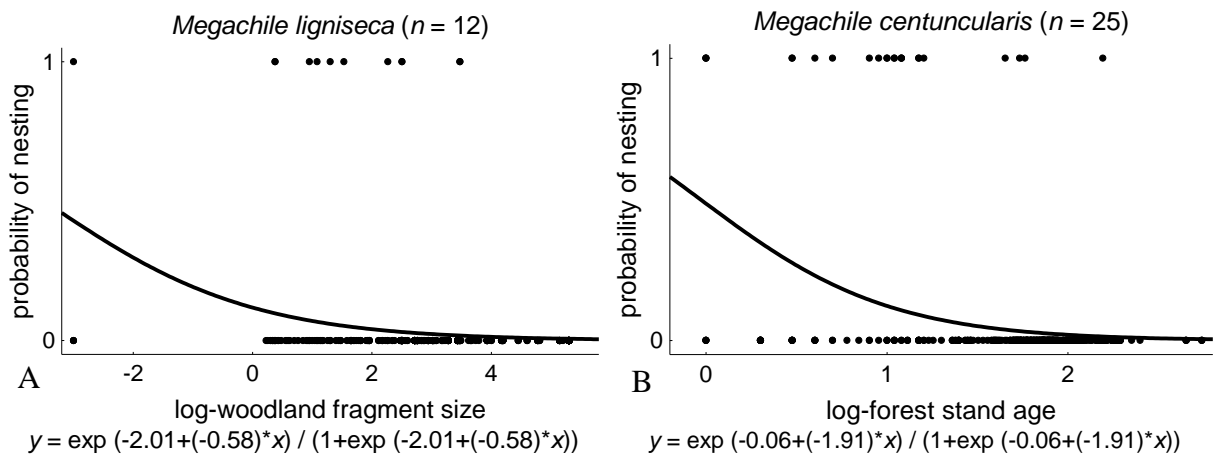


Fig. 12. Dependence of the nesting probability of bees (n – the number of samples) on woodland fragment size (A), forest stand age (B).

These habitats satisfy the description of forest glades in fragmented agricultural landscapes with crop fields. Bee association with them is caused by the large quantity of resources, mainly pollen rich entomophilous annual plants, used by bee offspring and found both in forest glades and crop fields. These findings contradict the results of previous studies (e.g., Steffan-Dewenter, 2003), where a negative effect of decreasing habitat areas on the species richness of trap-nesting bees was determined. This mismatch can be explained by the different types of explored habitats (many previous studies have been focused on natural grasslands, not woodland habitats) and the different geographic areas (the level of the anthropogenic impact on the environment is significantly lower in Lithuania than in the countries of Central Europe (Germany, Switzerland, etc.), where the majority similar research have been carried out).

Predatory wasps from the 2nd trophic rank respond differently to anthropogenic factors (Table 2): some species depend on them positively, e.g., the probability of nesting of potter wasp *Symmorphus murarius* (Linnaeus, 1758) in the trap-nests is higher in younger forest stands (Fig. 13 A, log. regr. coef. = -1.56 ± 0.42 , $p < 0.001$). While other species of the same trophic rank prefer unfragmented woodland habitats, e.g., crabronid wasp *Pemphredon lugens* Dahlbom, 1842 (Fig. 13 B, log. regr. coef. = 1.54 ± 0.46 , $p < 0.001$).

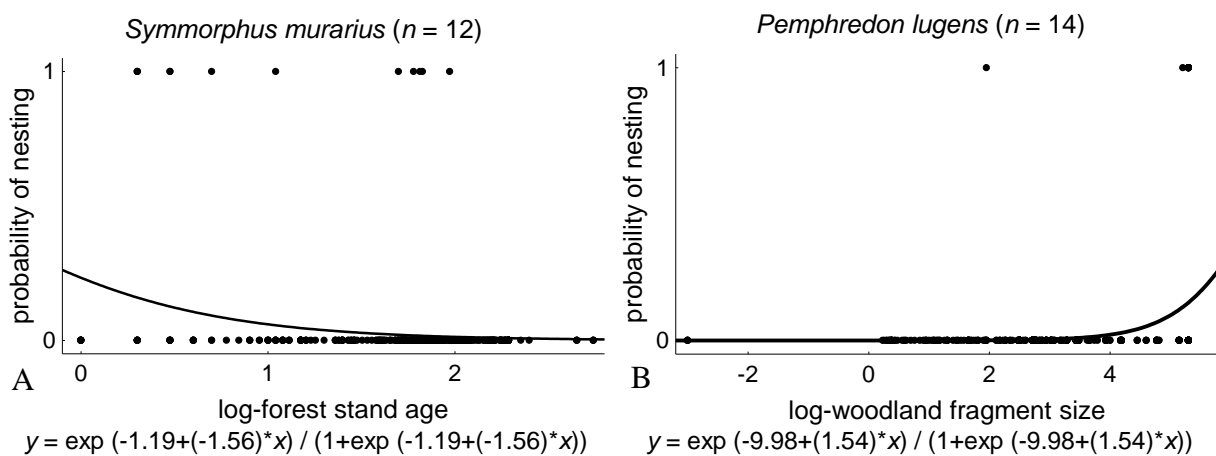


Fig. 13. Dependence of the nesting probability of the predatory wasps from the 2nd trophic rank (n – the number of samples) in a sample on forest stand age (A), woodland fragment size (B).

The nesting probability of insects from the 3rd trophic rank also varies depending on a species (Table 2). Some wasps demonstrate the preference to habitats affected by anthropogenic impact: spider wasp *Auplopus carbonarius* (Scopoli, 1763) and crabronid wasp *Trypoxylon clavicerum* Lepeletier & Serville, 1828 are found more frequently in landscapes with a larger share of agricultural areas (*A. carbonarius* log. regr. coef. = 4.17 ± 1.07 , $p < 0.001$; *T. clavicerum* log. regr. coef. = 2.95 ± 0.85 , $p < 0.001$); *Dipogon bifasciatus* (Geoffroy, 1785) – in habitats with younger forest stands (log. regr. coef. = -0.97 ± 0.24 , $p < 0.001$). The most abundant species of the same rank, spider wasp *D. subintermedius* Dahlbom, 1842, responds to anthropogenic factors contrarily: its preference to natural habitats has been determined on the base of even 3 anthropogenic factors: it is found more frequently in larger woodland fragments (log. regr. coef. = 0.35 ± 0.08 , $p < 0.001$), with forest stands of later successional stage (log. regr. coef. = 3.87 ± 0.91 , $p < 0.001$) and older tree age (log. regr. coef. = 0.81 ± 0.22 , $p < 0.001$).

Consequently, the relative abundance (a species parameter, usually used in bioindication) of *D. subintermedius* in trap-nests is suitable for bioindication of the naturalness of forest ecosystems.

The differences between the nesting probability of the predatory wasps from the 2nd and the 3rd trophic ranks in the habitats affected by different anthropogenic drivers can be explained by the fact that these species are mostly dependent on the distribution of their prey – insect and spider species (Klein *et al.*, 2004, 2006).

According to all analysed factors, anthropogenic impact on the nesting probability of species is different (Table 2):

- The probability to find bees *Hylaeus communis*, *Megachile centuncularis*, *M. ligniseca*, potter wasps *Discoelius dufourii* Lepeletier, 1841, *Symmorphus bifasciatus* (Linnaeus, 1761), *S. crassicornis* (Panzer, 1798), *S. murarius*, crabronid wasps *Passaloecus insignis* (Vander Linden, 1829), *Trypoxylon clavicerum*, and spider wasps *Auplopus carbonarius*, *Dipogon bifasciatus* in habitats affected by anthropogenic impact is higher than in natural habitats. These species represent 84.6% of 13 species, whose nesting is caused by anthropogenic impact;
- Other species, such as crabronid wasp *Pemphredon lugens* and spider wasp *Dipogon subintermedius*, demonstrate a preference to natural habitats. These species represent 15.4% of 13 species, affecting by anthropogenic impact.

Effects of anthropogenic pressures on species abundance

Effects of anthropogenic impact on the abundance of trap-nesting bees and predatory wasps was analysed using the principal component analysis (PCA). On the base of the main PCA factors calculated by anthropogenic factors, it has been found that spider wasp *Dipogon subintermedius* is strongly associated with natural habitats – large forests with old stands of late successional stages, while the remaining species exhibit various response to anthropogenic impact (Fig. 14).

In order to find out the details of anthropogenic impact on the brood abundance of trap-nesting Hymenoptera, and, in addition, on the relative abundance of their natural enemies, the general linear models (GLM) were applied. 5 species of trap-nesting predatory wasps, whose differences in the brood abundance had been affected by anthropogenic impact, were detected (Table 3), e.g., potter wasp *Ancistrocerus trifasciatus* prefer smaller woodland fragment size ($b = -0.09 \pm 0.02$, $p < 0.001$), while the abundance of *Discoelius dufourii* from the same trophic rank decrease as a result of anthropogenic effects: the biggest number of its brood cells is found in trap-nests, exposed in landscapes with a smaller share of agricultural areas ($b = -0.99 \pm 0.26$, $p < 0.001$).

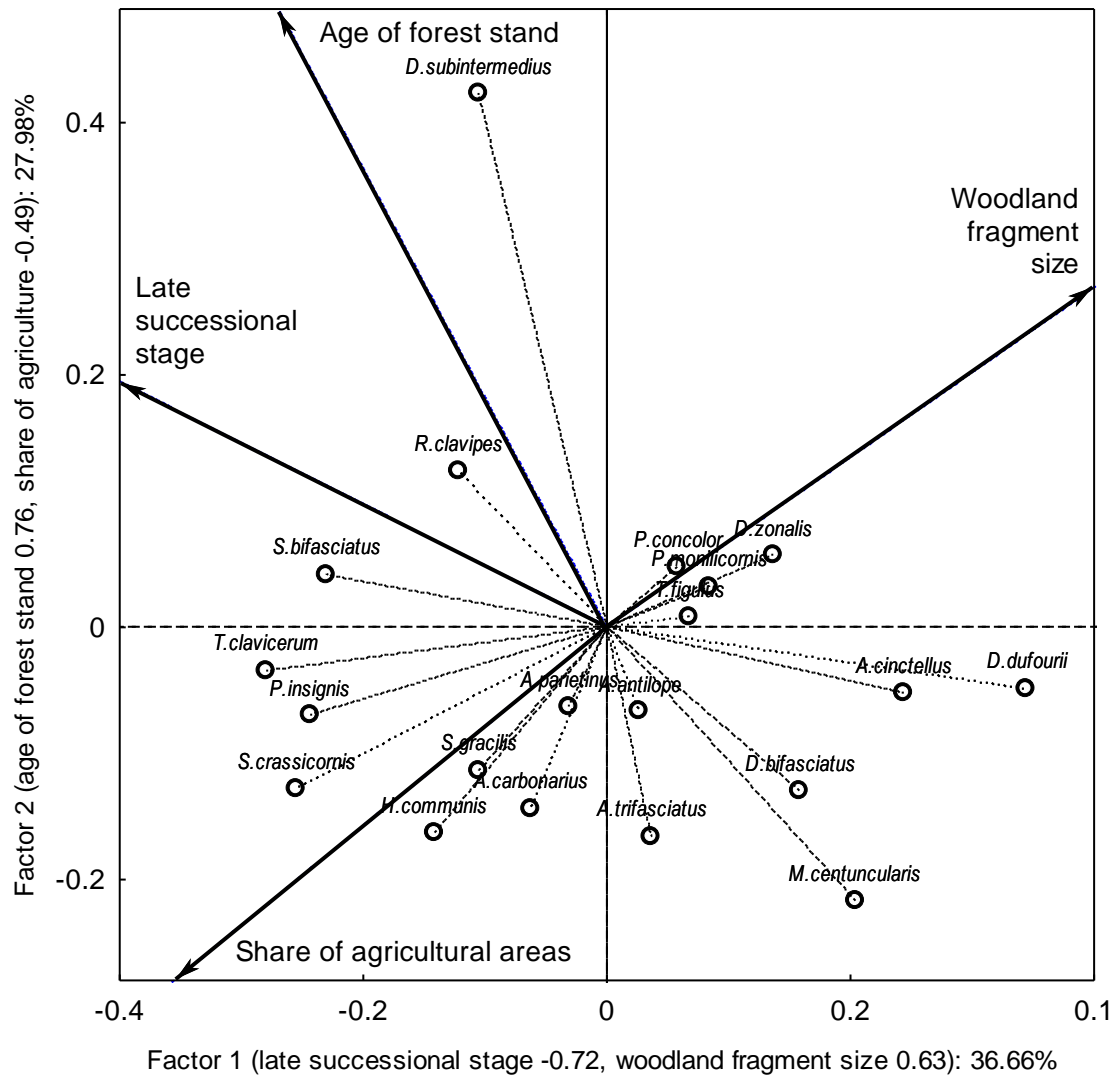


Fig. 14. Dependence of the brood abundance of the most abundant trap-nesting Hymenoptera species on the 2 main PCA factors calculated on the base of share of agricultural areas, woodland fragment size, late successional stage, forest stand age. Species abundances were included in the analysis as additional variables.

Table 3. Differences (regression coefficient $b \pm$ standard error) of the brood abundance of the trap-nesting Hymenoptera and relative abundance of their natural enemies (n – the number of samples) depending on increasing agricultural areas, woodland loss and fragmentation, shift toward earlier successional stages, effects of forest exploitation. Only values of the brood abundance of the trap-nesting Hymenoptera significant after Bonferroni correction are presented ($p < 0.0018$); the values of relative abundance of natural enemies considered to be significant if $p < 0.05$.

Species	Share of agricultural areas	Log-woodland fragment size	Log-late successional stage	Forest exploitation		r^2	df	F
				Log-stand age	Presence of dead wood			
II trophic rank								
<i>Ancistrocerus trifasciatus</i> (n=291)	-0.99±0.26	-0.09±0.02		0.46±0.08		0.10	2	15.85
<i>Discoelius dufourii</i> (n=30)						0.35	1	15.13
<i>Symmorphus bifasciatus</i> (n=188)						0.18	3	13.05
III trophic rank								
<i>Dipogon bifasciatus</i> (n=57)			-1.06±0.30			0.18	1	12.43
<i>D. subintermedius</i> (n=229)				0.35±0.05		0.21	2	30.30
Relative abundance of natural enemies			-0.09±0.04		0.01±0.01	0.03	2	4.93

The anthropogenic factors had weak ($r^2 = 0.03$) but still significant effect on the relative abundance of natural enemies: their attack rate is higher in the habitats with forest stands of earlier successional stages ($b = -0.09 \pm 0.04$, $p < 0.05$) and with dead wood ($b = 0.01 \pm 0.01$, $p < 0.05$) (Table 3). Forest stands of early successional stages are characterized by the large quantity of pioneer plant species, investing more nutrients in flowers, in comparison with perennial plants from later successional stages. Nectar is the main food resource not only for imagoes of trap-nesting bees and predatory wasps, but also for their natural enemies. Meanwhile, positive impact of the presence of dead wood in habitats on the trap-nesting Hymenoptera is confirmed by previous research (Loyola, Martins, 2008; Westerfelt *et al.*, 2015), showing that habitats with dead wood (stumps, windfalls, dry trunks) are characterized by the higher species richness and abundance of trap-nesting Hymenoptera. The frequency of interactions between natural enemies and their hosts often depends on the host species richness and abundance instead of spatial parameters (Steffan-Dewenter, 2003); therefore, habitat characteristics, such as the presence of dead wood, have a positive effect on the brood abundance of not only trap-nesting bees and predatory wasps, but also their natural enemies.

On the base of the results obtained during this study (Table 2, 3), the 1st hypothesis, according to which anthropogenic factors affect the nesting probability of trap-nesting Hymenoptera species and their brood abundance in the trap-nests, is confirmed.

Effects of anthropogenic impact on sex ratio and prepupal weight

The effects of anthropogenic impact on the sex ratio and relative weight of a prepupa were studied using the general linear models (GLM). Differences of the sex ratio affected by anthropogenic impact were tangible in 2 of 28 studied species, while the effects of anthropogenic pressures on the relative weight of a prepupa were determined for 6 species (Table 4).

Table 4. Differences (regression coefficient $b \pm$ standard error) of sex ratio of species and relative weight of a prepupa (n – the number of samples) depending on increasing agricultural areas, woodland loss and fragmentation, effects of forest exploitation. Only values significant after Bonferroni correction are presented ($p < 0.0018$).

Species	Share of agricultural areas	Log-woodland fragment size	Forest exploitation		r^2	df	F
			Log-stand age	Presence of dead wood			
Sex ratio							
I trophic rank							
<i>Hylaeus communis</i> (n=250)			0.18±0.04		0.12	2	15.10
II trophic rank							
<i>Ancistrocerus trifasciatus</i> (n=291)			0.16±0.03		0.07	1	20.94
Relative weight of a prepupa							
I trophic rank							
<i>Hylaeus communis</i> (n=250)		-0.02±0.01			0.09	2	10.91
II trophic rank							
<i>Ancistrocerus balticus</i> sp.n. (n=52)			0.15±0.04		0.25	1	16.62
<i>A. parietinus</i> (n=32)	-0.58±0.13				0.42	1	10.69
<i>A. trifasciatus</i> (n=291)			0.09±0.02		0.09	1	25.00
<i>Rhopalum clavipes</i> (n=141)				-0.18±0.04	0.14	1	14.23
<i>Symmorphus bifasciatus</i> (n=188)			0.10±0.02		0.09	2	8.23

In all cases, the highest share of females in population was found in habitats with older forest stands (Table 4), e.g., potter wasp *Ancistrocerus trifasciatus* (Fig. 15 A, $b = 0.16 \pm 0.03$, $p < 0.001$). Old forest stands are characterized by the high biomass of dead wood – the main nesting places for the studied community of insects. It confirms the hypothesis of sex allocation dependency on the resources (Charnov, 1979; Ulbrich, Seidelmann, 2001; Royle *et al.*, 2012), according to which a share of females is higher in the habitats, which are rich of resources – food, nesting places, building materials, mating sites, etc.

Older forest stands are characterized not only by the highest share of females in a population, but also by the largest relative weights of prepupae (Table 4, Fig. 15 B). These data are confirmed by the studies of bee *Osmia rufa* (Linnaeus, 1758) (Hymenoptera: Apidae) (Ulbrich, Seidelmann, 2001), when a direct link between the brood sex and the weight of the mother bee has been proved (larger females produce more female offspring). Consequently, natural habitats, unaffected by anthropogenic impact, are also able to supply trap-nesting Hymenoptera with a sufficient quantity of food resources, necessary for the development of offspring. The advantages of natural, heterogeneous landscape for the studied community have as well been determined by earlier studies (e.g., Gathmann, Tschardtke, 2002; Holzschuh *et al.*, 2009, 2013).

On the other hand, in our study the maximum relative weight of a prepupa has been also found in smaller woodland fragments and younger forest stands. As a result, the 2nd hypothesis, according to which anthropogenic factors affect the sex ratio and the average weight of prepupae of the trap-nesting Hymenoptera species, is confirmed.

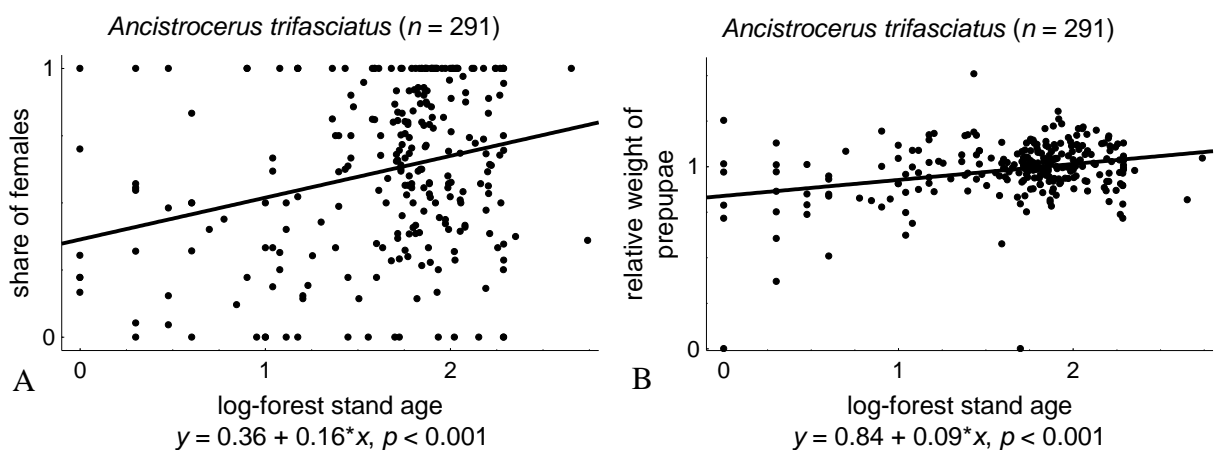


Fig. 15. Differences of share of females (A) and relative weight of prepupae (B) of potter wasp *Ancistrocerus trifasciatus* (n – the number of samples).

Anthropogenic impact on the diversity

The general linear models (GLM) were applied to assess relative species richness of natural enemies, species diversity (Shannon's H') of trap-nesting bees and predatory wasps, and diversity (Shannon's H') of trophic links depending on the anthropogenic impact. Significant differences between both the relative species richness of natural enemies and the species diversity of trap-nesting Hymenoptera were not determined.

Meanwhile, the dependence of anthropogenic factors on the differences between the trophic link diversity has been assessed (Table 5). The highest diversity of trophic links between the trap-nesting Hymenoptera and their natural enemies is found in smaller woodland fragments ($b = -0.06 \pm 0.03$, $p < 0.05$) with dead wood ($b = 0.10 \pm 0.04$, $p < 0.01$). Larger trophic link diversity in habitats with dead wood once again confirms the necessity of this resource for the community. On the other hand, the positive effect of the woodland loss and fragmentation on the diversity denies the results of previous research (Steffan-Dewenter, 2003), according to which the decreasing of woodland fragment size should decrease the species richness and the abundance of the trap-nesting community, which obviously should lead to lower trophic link diversity. However, due to a very weak, although reliable, correlation ($r^2 = 0.02$), it is not possible to confirm or reject the 3rd hypothesis, according to which anthropogenic impact reduces the trophic link diversity within the trap-nesting community and simplifies its trophic network. For more accurate results, it is necessary to carry out more detailed research including additional data from different habitats.

Table 5. Differences (regression coefficient $b \pm$ standard error) of the trophic link diversity (Shannon's H') (n – the number of samples) depending on woodland loss and fragmentation, and presence of dead wood. Only significant ($p < 0.05$) values are presented.

	Log-woodland fragment size	Presence of dead wood	r^2	df	F
Diversity of trophic links	-0.06±0.03	0.10±0.04	0.02	2	4.46

Summarising the results, the community of trap-nesting bees and predatory wasps, and their natural enemies is affected by the combination of anthropogenic factors at different spatial scale – increasing agricultural areas, woodland loss and fragmentation, shift toward earlier successional stages, the effects of forest exploitation. It is confirmed by the studies of this community in Europe and in the world (Tscharntke *et al.*, 1998; Gathmann, Tscharntke, 2002; Steffan-Dewenter, 2002; Klein *et al.*, 2004, 2006; Holzschuh *et al.*, 2009, 2013; Szczepko *et al.*, 2013; Pereira-Peixoto *et al.*, 2014, etc.).

CONCLUSIONS

1. The trap-nesting community in Lithuania is comprised of 94 species of bees, wasps, and their natural enemies, that represents 44.5% of all insect species found in the trap-nests for Hymenoptera in Europe.
2. Molecular, morphological and trophic differences between the studied species of the cleptoparasitic *Chrysis ignita* complex confirmed the independence of 15 sibling species.
Mitochondrial markers *ATP8*, *ATP6*, *CO3*, and *ND3* are more suitable for the differentiation of cryptic cuckoo wasp species, in comparison with other mitochondrial (*CO1*, *CO2*, *CytB*, 9 tRNAs), nuclear rDNA (18S, 5.8S, 28S rRNAs) genes, and the sequences of internal transcribed spacers (*ITS1*, *ITS2*).
3. 126 trophic links, discovered between trap-nesting Hymenoptera and their natural enemies in Lithuania, represent 67.4% of all trophic links, which had been detected in trap-nests for Hymenoptera in Europe.
Specialist species prevail among the natural enemies of trap-nesting bees and predatory wasps.
4. All explored anthropogenic factors affect the nesting probability and the brood abundance of trap-nesting Hymenoptera. Some species (13 of 28) optionally nest in habitats affected by anthropogenic impact; 84.6% of them prefer exclusively habitats affected by anthropogenic disturbance, while 15.4% of species avoid them.
Old forest stands are characterized by the largest share of females and the heaviest prepupae of some trap-nesting species, that is likely a result of the larger quantity of necessary resources in these habitats.
5. Spider wasp *Dipogon subintermedius* is the species most sensitive to anthropogenic impact in the studied trap-nesting community; therefore, its relative abundance in trap-nests is suitable for bioindication of ecosystem naturalness.
6. Anthropogenic factors weakly affect the trophic link diversity within the community; additional studies are necessary for evaluation of the effects of anthropogenic impact on it.

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1. Radzevičiūtė R., Budrienė A., Budrys E., **Orlovskytė S.**, Turčinavičienė J., 2012. A panel of microsatellite markers developed for solitary trap-nesting wasp *Ancistrocerus trifasciatus* (Müller, 1776) by cross-species amplification. *Ekologija*, 58(1): 1–7.
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Plėviasparnių lizdavietyse-gaudyklėse apsigyvenantys vabzdžiai ir jų priklausomybė nuo antropogeninių veiksnių

Santrauka

Pavieniui gyvenančios, negyvos medienos ertmėse lizdas įrengiančios bitės, vapsvos ir jų gamtiniai priešai sudaro plėviasparnių lizdavietyse-gaudyklėse įsikuriančių vabzdžių bendriją, pasižymingą augalėdžių biologinės kontrolės, augalų apdulkinimo bei bioindikacijos funkcijomis. Šio darbo tikslas – ištirti Lietuvoje lizdavietyse-gaudyklėse įsikuriančių vabzdžių bendrijos sudėtį ir įvertinti antropogeninių veiksnių įtaką jai. Ištyrus 377 lizdavietyse-gaudyklėse būvusių iš 117 miško vietovių duomenis, nustatyta bendrijos sudėtis iš 94 lizdas įrengiančių bičių, vapsvų ir jų gamtinių priešų rūšių bei 126 mitybinio tinklo sąveikos. Įvertinus 1465 gamtinių priešų auksavapsvių *Chrysis ignita* komplekso individų molekulinis, morfologinius ir mitybinius skirtumus, patvirtintas 15 tirtų rūšių antrininkų savarankiškumas. Iš molekulinės žymenų tinkamiausi šių rūšių atskyrimui yra mtDNR *ATP8*, *ATP6*, *CO3* ir *ND3* genai. Taikant dvinarę logistinę regresiją, bendrųjų tiesinių modelių ir pagrindinių komponentų analizės nustatyta, jog visi skirtingo erdvinio masto antropogeniniai veiksniai (žemės ūkio naudmenų plotų didėjimas, miško buveinių plotų mažėjimas ir fragmentacija, ankstyvesnių sukcesijos stadijų išsigalėjimas, miško eksploatacija) turi poveikį dalies tirtų bendrijos rūšių apsigyvenimo tikimybei ir gausumui. Iš jų išsiskyrė voravapsvė *Dipogon subintermedius*, savo santykinu gausumu tinkamiausia natūralių miško ekosistemų bioindikacijai. Didžiausia bendrijos rūšių patelių dalimi ir santykinai didžiausiu lervų svoriu pasižymėjo seni medynai, kas tikėtina atspindi didesnę bendrijai būtinų išteklių kiekį juose ar gretimose buveinėse.

CURRICULUM VITAE

Name: Svetlana Orlovskytė

Date and place of birth:

18 March 1987, Vilnius, Lithuania

Address:

Nature Research Centre

Akademijos Str. 2, LT-08412 Vilnius, Lithuania

Phone: +370 678 36860

E-mail: s.orlovskyte@gmail.com

Education:

2012–2016 – PhD studies at the Nature Research Centre, Vilnius, Lithuania.

2010–2012 – Master's degree in Zoology, Vilnius University, Lithuania.

2006–2010 – Bachelor's degree in Biology, Vilnius University, Lithuania.

Professional experience:

Jan. 2017–present – junior researcher at the Nature Research Centre, Vilnius, Lithuania.

2011–2016 – biologist at the Nature Research Centre, Vilnius, Lithuania.

2009–2011 – technical assistant at the Nature Research Centre, Vilnius, Lithuania.