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SAPROXYLIC COLEOPTERA (INSECTA) FROM THE REPUBLIC OF MOLDOVA: TAXONOMY, ECOLOGY, ZOOGEOGRAPHY AND IMPORTANCE

SPECIALTY: 165.04 - ENTOMOLOGY

Abstract of the Thesis of the Doctor Habilitatus in Biological Science

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The defense of the thesis will take place on March 11 2024, at 13^{00}, within the meeting of the Thesis Public Defence Commission within the Moldova State University, Institute of Zoology, Chisinau, 1 Akademiei str., hall no. 352 (http://www.usm.md).

The doctor habilitatus thesis and the summary can be consulted at the Library of the Moldova State University, the National Library of the Republic of Moldova and on the website of ANACEC: (http://www.cnaa.md/) and on the MUS website (http://usm.md).

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CONTENT

CONCEPTUAL MARKINGS OF THE RESEARCH

Actuality and importance of the approached topic

The saproxylic beetles are a major group in forest ecosystems and have both biocenotic and economic importance, being involved in the decomposition of dead wood and its recirculation, improving the soil quality [1; 38; 39]. They also serve as a trophic source for some groups of invertebrates and vertebrates, they are good pollinators, but they also participate in the spread of pathogenic fungi that attack living trees [2; 6; 40], they bore holes in the wood diminishing its commercial quality and damage heritage objects [23]. The saproxylic species depend on the wood of old, weakened and dead trees, of hollow trees that are becoming rarer [20], are associated with fungi that colonize dead wood, or depend on other saproxylic species [39]. In the last 200 years, forest ecosystems have suffered from long-term droughts, as well as from diseases and pests, which had a negative impact on biodiversity. Due to poor management of forest resources, the saproxylic beetles have become rare and threatened with extinction [10], or may contaminate and attack forests in the absence of competition [41].

Saproxylic beetles are very sensitive to disturbances, that is why they have become the object of study regardin the health status of many forests in the world [15; 35; 36]. Obligatory saproxylic coleopteran species from primary forest ecosystems are used as bioindicators for conservation planning of forest ecosystems with optimal value and functional resistance to climate change [12; 33]. Taking into account the vital importance of saproxylic coleopterans in the decomposition and circulation of substances in nature, and the fact that due to climate change, global warming, the reduction of forest areas and the planting of monocultures, biodiversity is reducing, the international scientific community has emphasized the need to preserve and conserve the saproxylic species and their habitats [18].

Overview of the research situation and identification of research problems

The first studies of the coleopteran fauna in the Republic of Moldova date from the beginning of the 20th century and belong to the researchers Yatsenkovskii E. (1912), Miller E. and Zubovskii N. (1917), Medvedev S. and Shapiro D. (1957). Another period rich in ecological-faunistic research was recorded between 1963 and 1983, a stage in which many specialists activated: M. Gilyarov (1963); S. Plugaru (1963, 1970); A. Striganova (1968); A. Apostolov (1970); N. Popov (1970); N. Seryi (1972); R. Stepanov and S. Antonovich (1970); A. Topchiev (1970); N. Filippov and F. Zhdankin (1970); V. Ostafichuk (1970a, 1970b); B. Vereshchagin, V. Ostafichuk, A. Podubnyi (1984); V. Ostafichuk (1990); Z. Neculiseanu et al. (1992a, 1992b); followed by the works published after 2000 by the authors A. Andreev et al. (2001); A. Poyras (1992, 1998); A. Poiras et al. (2003); B. Vereșceaghin, V. Ostaficiuc and A. Poiras (2003) [4].

The actual research of saproxylic coleopterans in the Republic of Moldova was initiated only in 2002 by Neculiseanu Z. et al., when 41 species of saproxylic invertebrates have been identified [27].

In order to identify the widest possible spectrum of saproxylic coleopteran species, starting from 2008 the studies were carried out in various forest ecosystems of the Republic of Moldova using numerous collection methods, at the same time the entomological collections in the country were checked for the presence of saproxylic coleopteran species, as well as literature sources that mentioned the species dependent on dead wood. The performed study is a continuation of the research started 20 years ago, which completed the list of saproxylic coleopteran species from 41 to 342. Their identification was possible by using flight interception methods, checking the fungi that grow on dead wood and the method of flotation, which were used for the first time in the collecting of coleopteran fauna from the Republic of Moldova.

The aim of the work was the comprehensive assessment of the taxonomic diversity of saproxylic species of beetles from the order Coleoptera, their current state, trends, zoogeographical distribution, ecological features and significance in the context of increasing of the anthropic impact and present climate changes in the forest ecosystems of the Republic of Moldova.

Research objectives:

- ✓ establishing the composition of saproxylic coleopterans in natural and anthropogenic forest ecosystems, as well as of the species stored in entomological collections of the Republic of Moldova;
- ✓ identification of new taxa of saproxylic beetles in the fauna of the country;
- ✓ analysis of trophic groups and zoogeographic distribution of beetles living in dead wood of forest ecosystems;
- ✓ identification of indicator species of old forests and of rare species for the fauna of the republic;
- ✓ identification of morphologically identical species using molecular genetic methods;
- ✓ assessment of the role of saproxylic coleopterans in natural and anthropogenic forest ecosystems;
- ✓ identification of vector species and of saprophytic fungi spread by them;
- ✓ creation of the first collection of saproxylic beetles;
- ✓ establishing the influence of forest management on the structure and richness of saproxylic beetle species.

The methodology of scientific research. The fundamental researches [6; 9; 38; 39], that studied the diversity of saproxylic beetles in natural and managed forest ecosystems in Europe served to carry out the study. The methodological works [7; 30] served for the inventory of saproxylic coleopterans; the rare species and indicators of forest ecosystems with a large amount of dead wood were identified [3; 22; 28; 33; 36]. Xylophagous coleopterans harmful to forest ecosystems were identified in accordance with specialized works [30; 43]. According to the studies in the field, coleoptera vectors have been identified in the transmission of fungal infections [5; 43]. The trophic groups were determined in accordance with the works in the field [29; 33], zoogeographical distribution [44], preference for tree species [24], invasive species [17], those harmful to forest ecosystems [43] and to

heritage objects [23], vector species in the transmission of fungal infections and saprophagous pathogenic fungi of deciduous trees [2; 5], the role of saproxylic coleopterans in natural and anthropogenic forest ecosystems [15; 38; 39] and the impact of forest management upon the biodiversity [7; 38].

Novelty and scientific originality. The work includes original results regarding the study carried out on the 342 species of saproxylic beetles identified in the forest ecosystems of the country. For the first time for the Republic of Moldova, 5 families, 47 genera and 89 species were identified. Through the molecular genetic method, 18 species from 8 families were identified, the sequences of which were deposited in GenBank. For the identified species the phylogeographic analysis was performed to establish the spread of phylogenetic lineages at the European level based on the grouping of haplotypes. Most of the sequences from samples collected in forest ecosystems in the country, from a phylogeographic perspective, are groupped into private haplotypes. Only the species *Gyrophaena manca* presents an exception, with two haplotypes identified, one of which is spread in Finland and Germany, and one is private, i.e. found only in the Republic of Moldova. The genetic data obtained contribute to the completion of databases that store genetic information, becoming available for future phylogeography, phylogeny and conservation biology studies at the European level. 18 species of saprophagous fungi associated with xylomycetophagous coleopteran species have been identified, of which the species *Alternaria alternata* and *A. tenuissima* can act as phytopathogenic agents for herbaceous and woody plants.

Scientific problem solved. The species diversity of saproxylic beetles in the country's forest ecosystems was established, which is composed of 342 species, 236 genera and 47 families. The first collection of saproxylic beetles in the country was developed. 78 bioindicator species of old forests were confirmed. 12 rare and endangered species, 33 harmful xylophagous species, 10 species xylophagous/mycetophagous harmful to forest ecosystems and 4 species harmful to heritage objects were identified. For the first time for the country, the rarity categories were assigned to saproxylic species from the Tenebrionidae family. The host tree species of saproxylic coleopterans identified in the period 2008-2023 were established. New methods of collecting coleoptera were used, among which the flotation method, used for the first time to identify saproxylic coleoptera species, 17 species were collected for the first time in the fauna of the country.

Theoretical significance: A new concept was developed regarding the current structure of saproxylic coleopteran species in forest ecosystems, their vertical, horizontal and regional distribution under the conditions of anthropogenic and climate change. Area limits were established for the species *Abdera quadrifasciata*, *Aesalus scarabaeoides*, *Diaclina testudinea*, *Neoclytus acuminatus*, *Eledonoprius armatus* and *Sunius fallax*, for which the Republic of Moldova is the South-Eastern border of the Central European distribution. Two rare species *Dirrhagofarsus attenuatus* and *Prostomis mandibularis* dependent on the dead wood of old forests, which have been preserved only fragmentarily in Europe, will be proposed for the 4th edition of the Red Book. The species identified for the first time in the fauna of

the country expand the level of knowledge of their zoogeographic area and ecology. For the first time in the Republic of Moldova, the saprophytic and parasitic microscopic fungi from forest ecosystems were identified, whose dispersal vectors are saproxylic coleopteran species. Knowledge of saprophytic and pathogenic fungi is important in planning afforestation and forest protection activities. The global gene bank was completed with sequences of 18 species of saproxylic coleopterans from the entomofauna of the Republic of Moldova.

Applicative value: The data obtained on the diversity and ecology of saproxylic coleopterans, allow their use as bioindicators in planning the conservation of forest ecosystems with optimal value and functional resistance to climate change. The results can be useful to forestry workers, scientific researchers in the fields of biology, ecology, teachers and environmental authorities in the protection of biodiversity, the realization of courses, monitoring, control of xylophagous and invasive species. The results obtained are part of the scientific themes of fundamental and applied research carried out within the Institute of Zoology between 2008-2023. The research falls within the requirements of the National Strategy and Action Plan for Biological Diversity, the Laws on the animal kingdom, the fund of natural areas protected by the state, on the protection of the environment, of the Conventions and the Directive on the study and protection of biodiversity.

Implementation of scientific results: The collection of saproxylic beetles is a scientific and educational support for researchers and students in the field. The list of saproxylic beetles is a scientific source, which completes the faunal information for the area of South-Eastern Europe. The species of saproxylic beetles from the Stapylinidae family identified in the period 2008-2023, were used to prepare the Catalog of Palearctic Coleopterans. The scientific results are included in 7 acts of implementation of relevant organizations and institutions: the State University of Moldova, the Scientific Reserves "Plaiul Fagului", "Pădurea Domnească", „Prutul de Jos" and the municipal enterprise "Association for the Management of Green Spaces", which used the results in the educational process and in the annual scientific reports, the silver medal was obtained at the Inventica salon in 2023.

Approval of scientific results. The results of the research were presented and approved at 26 scientific forums, including 10 national and 16 international ones.

Publications on the topic of the thesis. Based on the research carried out, 97 scientific papers were published, including a monograph as single author, 4 monographs with co-authors, 2 brochures and 3 guides, 4 articles in scientific journals from the Web of Science and SCOPUS databases; 1 article in journals from databases accepted by ANACEC; 12 articles in Category B journals; 6 articles in Category C journals; 1 – in the works of scientific events included in the Web of Science and SCOPUS databases; 29 – in the works of scientific events included in other databases accepted by ANACEC; 27-in the works of scientific events included in the Register of materials published on the basis of scientific events organized in the Republic of Moldova; 6 abstract in scientific materials from international scientific conferences (abroad); 1 patent issued by the State Agency for Intellectual Property.

The volume and structure of the thesis. The thesis is written in Romanian on 216 pages and consists of: introduction, seven chapters including the history of saproxylic coleoptera research, research materials and methods and five chapters with results and discussions, general conclusions and practical recommendations, bibliography with 537 titles, 8 appendices, 37 figures and 50 tables.
Key words: saproxylic coleopterans, taxonomy, diversity, distribution, forests, ecology, importance.

1. HISTORY OF SAPROXYLIC COLEOPTERAN RESEARCH

1.1. History of saproxylic coleoptera research in the world

The international term "saproxylic" appears for the first time in the studies of the author Dajoz [11], for the insects that lived in decaying wood. The actual study of saproxylic coleopterans was initiated in 1989 by Speight [38], who renamed the term saproxylic in the definition "saproxylic", which are those invertebrates that depend during their life, or a part of their life cycle, on the wood of dead or dying trees on stands or logs, or by wood-dwelling fungi, or by the presence of other species. Modern forestry that does not accept dead wood in managed ecosystems has caused in recent centuries an ecological degradation of forests and even the conversion of some natural deciduous forests into coniferous forests and the loss of connectivity between habitats. For this reason, the saproxylic beetles have become among the most threatened invertebrate groups in European forests [28; 38]. Taking into account the rarity status of many taxa and their vital role in the decomposition of dead wood, the scientific community has extrapolated the importance of conserving saproxylic beetles and their habitats which are constantly reducing [18]. In order to restore and protect the diversity of these species, the old, primary forests with deciduous trees and a lot of dead wood in various stages of degradation must be protected [16]. At the moment, the saproxylic coleopteran fauna of most European countries is extensively studied, the available information confirms the researchers' concern and the implementation of the results. For these reasons, in order to fill the gaps in the territory of the Republic of Moldova, the study of saproxylic coleopterans was deepened in our country as well.

1.2. The history of saproxylic coleopteran research in the Republic of Moldova

The study of saproxylic invertebrates in the Republic of Moldova was initiated in 2002 by Neculiseanu Z. et al. [27] who published the results in the works: Saproxylic invertebrates and forests of international importance from the Scientific Reserves "Pădurea Domnească" and "Plaiul Fagului"; Saproxylic beetles (Insecta, Coleoptera) from secular forests [25]; Coleopteran diversity (Insecta: Coleoptera) in the "Telita" Landscape Reserve [26] et al. and the author's work: Saproxylic Coleoptera from the "Codrii Tigheci" Landscape Reserve. The authors identified 41 species of saproxylic invertebrates from the old forests of the country ("Plaiul Fagului", "Pădurea Domnească", "Telita" and "Codrii Tigheci"). These studies are important, but the materials come only from direct collections. In order to identify

the diversity of saproxylic coleoptera species in the country, during the period 2008-2023 research was carried out using various methods, which allowed the collection of 240 species. Also, the entomological collections were studied: of the National Museum of Ethnography and Natural History dating from 1901-1939, the Museum of the Institute of Zoology - with the first records from 1911 to the present, the Institute of Genetics, Physiology and Plant Protection - 1957 -1989 and of the State University of Moldova – 1952-2005.

2. THE NATURAL ENVIRONMENT AND RESEARCH METHODS OF SAPROXYLIC BEETLES

2.1. The natural environment of the Republic of Moldova

In this subchapter, information is briefly presented regarding the location of the territory of the Republic of Moldova on the European map, with a description of the types of relief, soils, climate, precipitation and vegetation.

2.2. Characterization of the studied forest ecosystems

General information is presented on the type of vegetation of the Scientific Reserves: "Pădurea Domnească", "Plaiul Fagului", "Codrii" and "Prutul de Jos"; The "Cobîleni" and "Pădurea Condrița" forest natural reserves; Landscape reserves: "Vila Nisporeni", "Telita", "Pohrebeni", "Tipova", "Saharna", "Codrii Tigheci", "Dolna", "Rudi-Arioneşti", "Pădurea din Hîncești", "Căpriana-Scoreni"; Lower Dniester National Park, Orhei National Park, Valea Morilor urban park in Chisinau, Natural forest in Zabriceni village, Edineț district; The acacia forest plantation in Sadaclia village, Basarabeasca district; The acacia forest plantation in Troița Nouă village, Anenii Noi district; The protection strip of the waters of the Prut River in the village of Măcărești, Ungheni district, from which the saproxylic beetles were collected in the period 2008-2023.

2.3. Description of collecting methods, mounting, identification and preservation of coleopterans

Saproxylic coleopterans were collected from the dead wood of logs or old trees attacked by fungi or pests using transect or fixed point methods. Among the methods used are: manual collection from dead wood, from mushrooms growing on trunks; the use of trunk traps; of window and baited traps (beer); the entomological net; the entomological vacuum cleaner; flotation method; the screening method using entomological sieves; film shaking method of tree fungi and bark; the ultraviolet light trap; camera pictures. The verification time of each plot of the investigated forest ecosystem was a minimum of 2 hours for visual examination, so that the data could be compared with each other. Flytraps were operated for 14 to 20 days using a 1:10 salt solution as a preservative.

The collected specimens were mounted according to the techniques used in entomology. As a rule, beetles are kept by two methods: mounted on entomological needles and placed on entomological mats. For the small specimens, difficult to identify by the external morphology, preparations of the genital armature were

made. The collected materials were cleaned of impurities, mounted, determined and treated against bacteria and dermestids.

The species of saproxylic coleopterans were identified according to the external morphological characters present in the keys for the identification of the taxa of the coleoptera order with the help of the LEICA 2500 microscope and Meiji Techno using the following keys: [19; 42], or according to the keys of xylobiont coleopterans [43; 45]. The nomenclature was made according to the authors Lobl and Smetana [21; Boushard et al., [8] and the GBIF website 14]. The identification of saproxylic species was carried out with the help of specialists in the field from the "Grigore Antipa" National Museum of Natural History in Bucharest, the Scientific-Practical Center for Bioresources of the National Academy of Sciences of Belarus; Museum and Institute of Zoology, Polish Academy of Sciences. The specimens are kept in the the provisional Collection of the Museum of Entomology of the Institute of Zoology of the Moldova State University. The mounted materials are in quarantine, after this period they will be placed in the collection of the Entomology Laboratory of the Institute of Zoology.

2.4. Statistical analysis

When processing the collected material, ecological indices were used: abundance, dominance, constancy, ecological significance index. The list of saproxylic coleoptera species was statistically processed with the help of biodiversity indices such as: Shannon diversity index, Simpson diversity index and equitability. Biodiversity indices are used to measure and compare the diversity of saproxylic species in a community through mathematical calculations.

2.5. The use of saproxylic coleopteran species in the identification of old forests

The species of saproxylic beetles are divided into obligate saproxylic and facultatively saproxylic [13]. Obligate saproxylic coleopteran species from old forest ecosystems are used as indicators in the assessment of forest characteristics important for the management and protection of forest biodiversity. The study of biodiversity indicators based on saproxylic beetles was carried out by numerous researchers including Speight [38], Schmidl and Bussler [33], Mazzei et al. [22], Eckelt et al. [12] et al.

The methodology for identifying biodiversity indicators consists in investigating the fauna of dead wood in different stages of decay, of different tree species, different thicknesses, trees with different microhabitats (hollows, broken branches, partially peeled stems, trees colonized by lignicolous fungi or molds, colonized by ants). The dead trees resulting from natural events (storms, fires) or anthropogenic (stumps and trunks resulting from the cutting of damaged trees, affected by pests) from old deciduous and coniferous forests were studied by various methods: direct collection, collection with traps flight interception (window, with intersecting vanes), Barber traps for hollows, sifting, shaking off mushrooms, etc.

Based on the obligate/indicator saproxylic coleoptera species, the forests of European importance were subsequently identified.

When establishing the indicator saproxylic coleopteran species, the criteria proposed by Speight [38] were taken into account: to be associated with the dominant old trees in European forests (beech, oak, spruce); to be spread over the European continent; have large and medium sizes and be easy to detect; to be relatively easy to determine.

2.6. Methods for identification of saproxylic beetles from fragments based on DNA barcodes

Damaged saproxylic beetles that could not be identified based on external morphological characters, beetle fragments found in dead wood and some beetle larvae were identified from fragments based on "DNA barcodes" by sequencing a fragment at the gene level which codes for cytochrome C oxidase 1 and comparing the sequence obtained with international databases such as GenBank (NCBI) and BOLD (Barcode of Life System) [31]. The technique of "DNA barcodes" is used for the identification of species but also for the discovery of new species, at the same time it is also of major importance for the recognition of the phylogeographic and evolutionary pattern of living organisms. DNA isolation was carried out in the Department of Molecular Biology of the "Grigore Antipa" National Museum of Natural History in Bucharest.

2.7. Methods for the isolation and cultivation of saprophagous fungi associated with saproxylic coleopterans

The beetles, after being cleaned of impurities by washing with saline solution, were placed in eppendorfs, left to macerate in 1.0 ml of sterile distilled water, then stacked and the mixture was vortexed. Decimal dilutions were made from the obtained suspension, and 0.05 ml were inoculated on the surface of Petri dishes with malt extract agar medium (Millipore), pH adjusted 5.6. Petri dishes were incubated in a thermostat for 10 days at 25-26°C and examined on the 3rd, 7th and 10th day. The isolation in pure culture of the grown microorganisms was performed on Petri dishes with MEA medium by pricking (fungi) or by the exhausted loop technique (yeasts). In laboratory conditions, microorganisms are kept on MEA and Sabouraud media (Sigma-Aldrich) at +4°C. Fungal isolation was carried out in the Soil Microbiology Laboratory of the Institute of Microbiology and Biotechnology, Technical University of Moldova.

2.8. Identification by molecular methods of saprophagous fungi associated with saproxylic coleopterans

For a more precise identification of the microscopic fungi associated with the saproxylic coleoptera extracted from the decaying wood, molecular analyzes were carried out using the PCR (Polymerase Chain Reaction) technique. The ZR Fungal/Bacterial MiniPrep™ kit (Zymo Research, USA) was used for DNA extraction, according to the manufacturer's instructions, which allows simple and

rapid isolation of genetic material from various microbial samples. The ITS1-5.8S-ITS2 regions were subsequently amplified with ITS1/ITS4 universal primers to identify yeast isolates to the species level. The identification of the fungi was carried out through DNA isolation, amplification and analysis, at the University of Agronomic Sciences and Veterinary Medicine in Bucharest.

3. TAXONOMIC STRUCTURE OF SAPROXILIC COLEOPTERANS

3.1. Taxonomic structure and chronological analysis of materials from entomological collections in the country

The abundance and faunal diversity of the saproxylic coleopterans in the Republic of Moldova is due to the presence of old natural forests with a lot of dead wood of various tree species.

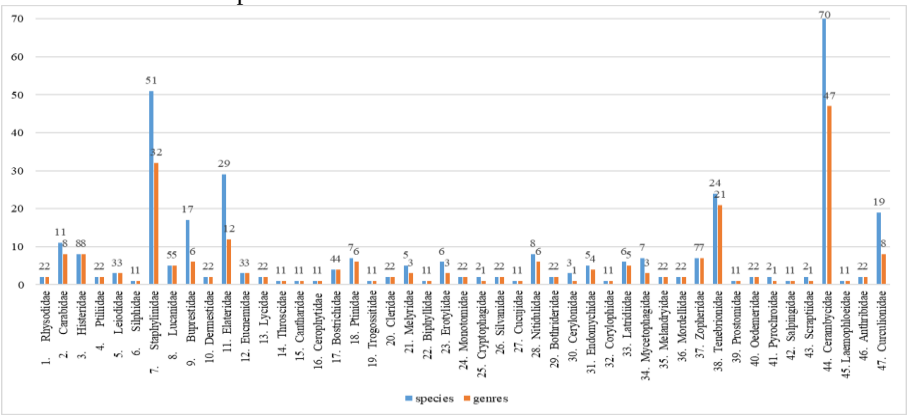


Figure 3.1.1. Numerical share of saproxylic coleopteran taxa within the families identified in the period 1901-2023 from the forest ecosystems of the Republic of Moldova

The specimens studied and included in the work are kept in 4 entomological collections belonging to the National Museum of Ethnography and Natural History, the Entomology Museum of the Institute of Zoology, the Museum of the State University of Moldova and the collection of the Institute of Genetics, Physiology and Plant Protection.

These specimens belong to 47 families, 236 genera and 342 species. The majority of the species belong to the family Cerambycidae – 70 species and 47 genera, followed by the families Staphylinidae – 51 and 32, Elateridae – 29 and 12, Tenebrionidae – 24 and 21, Curculionidae – 19 and 8, Buprestidae – 17 and 6, Carabidae – 11 and 8, Histeridae – 8 and 8, Nitidulidae – 8 and 6, Zopheridae – 7 and 7, Ptinidae – 7 species and 6 genera, Mycetophagidae – 7 species and 3 genera, Latridiidae – 6 species and 5 genera, Erotylidae – 6 species and 3 genera, Lucanidae – 5 species and 5 genera, Endomychidae – 5 species and 4 genera, Melyridae – 5 and 3 and Bostrichidae with 4 species from 4 genera. Other families were represented by a small numbers of species, between 3 and 1 (Figure 3.1.1).

Taxonomic analysis of saproxylic coleopterans from the entomological collections of the Republic of Moldova

The MNEIN collection contains 137 species belonging to 102 genera and 23 families (Carabidae, Silphidae, Staphylinidae, Lucanidae, Buprestidae, Elateridae, Bostrichidae, Trogossitidae, Cleridae, Melyridae, Erotylidae, Silvanidae, Endomychidae, Nitidulidae, Latridiidae, Mycetophagidae, Zopheridae, Tenebrionidae, Oedemeridae, Pyrochroidae, Scraptiidae, Cerambycidae and Curculionidae), the materials come from collections created in the period 1901-1939 (Fig. 3.1.2); 123 species from 86 genera and 20 families are stored in the MEIZ collection (Carabidae, Histeridae, Staphylinidae, Lucanidae, Buprestidae, Elateridae, Bostrichidae, Ptinidae, Trogossitidae, Melyridae, Erotylidae, Silvanidae, Cucujidae, Nitidulidae, Mycetophagidae, Tenebrionidae, Oedemeridae, Pyrochroidae, Cerambycidae and Curculionidae), the materials come from collections made from 1911 to the present (Fig. 3.1.2). The collection of the Museum of the State University of Moldova preserves 7 species from 6 genera and a Cerambycidae family, collected in the period 1952-2005 (Fig. 3.1.3); 32 species, 25 genera and 10 families (Staphylinidae, Lucanidae, Buprestidae, Elateridae, Bostrichidae, Trogossitidae, Mycetophagidae, Tenebrionidae, Cerambycidae and Curculionidae) are preserved in the IGFPP collection, the materials come from materials collected between 1957-1989 (Fig. 3.1.3).

Chronological analysis of saproxylic beetles from analyzed entomological collections

In the collection of the Museum of Entomology of the Institute of Zoology there are specimens collected during 43 years and determined by the researchers R. Stepanov, S. Plugaru, B. Vereșceaghin, V. Ostaficiuc, A. Poiras, Z. Neculiseanu, A. Dănilă, E. Baban, S. Bacal, V. Chyubchik and I. Mihailov. In some years multiple collections were made, showing numerous species, but there are also years in which only one species was collected. Personal collections have been undertaken from 2008 to the present.

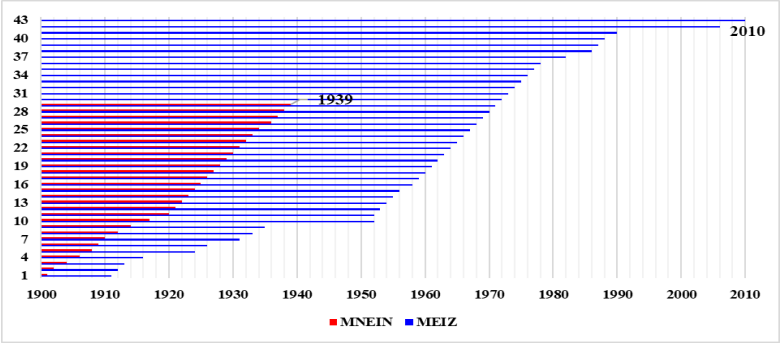


Figure 3.1.2. Years of collection for saproxylic coleopteran species deposited in the MEIZ and MNEIN collections

In the collection of the National Museum of Ethnography and Natural History, the specimens were collected and determined by Zubowsky during 29 years of research. The collection contains specimens collected since the beginning of the 20th century, they are practically the oldest specimens collected, stored and preserved in the country. In the collection of the Institute of Genetics, Physiology and Plant Protection, materials collected during 16 years are preserved. The specimens were collected by the employees of the institution B. Adashkevich, L. Mațiuc, E. Verlan, V. Danilov, V. Talitschii, V. Egorov, A. Tiurganova, A. Poiras, O. Krîjanovschi and S. Medvedev. The collection of the Museum of the State University of Moldova includes a small number of saproxylic species, collected during 7 years.

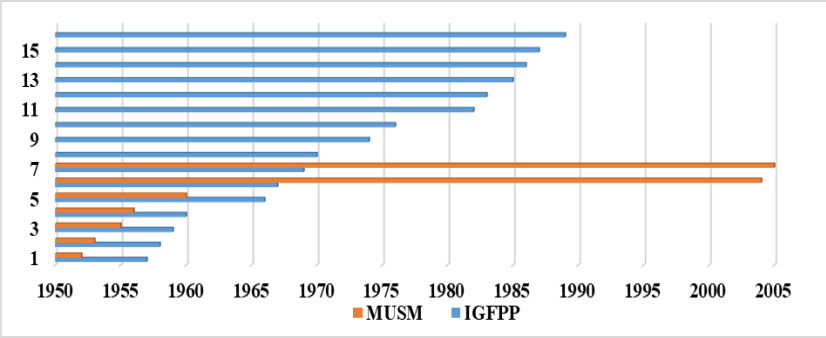


Figure 3.1.3. Collection years for saproxylic coleopteran species deposited in the IGFPP and MUSM collections

3.2. Identification of morphologically identical species by molecular genetic methods

The species of saproxylic beetles, difficult to identify on a traditional morphological basis due to damage, were determined using the DNA barcode technique. This involves sequencing a fragment of the gene that codes for cytochrome C oxidase I and comparing the sequence obtained with international databases such as GenBank (NCBI) and BOLD (Barcode of Life System) [31]. 56 samples collected from the scientific and landscape reserves of the country were analyzed, of which 22 samples were successful, from which 18 species were identified. DNA isolation was performed using the ISOLATE II Genomic DNA @Bioline kit, following the manufacturer's specifications. The amount of genomic DNA isolated from each sample was later used to amplify a fragment from the 5' end of the gene for cytochrome C oxidase I, a gene extensively used in the identification of animal species (Hebert et al., 2003). The mentioned fragment was amplified using universal primers COI HCO2198 (5'-TAAACTTC AGGGTGACCAAAAAATCA-3') and LCO1490 (5'- GGTCAACAAATCATA AAGATATTGG-3') (Folmer and Black 1994) marked with tail M13.

The PCR reaction was performed in a total volume of 50 µl containing genomic DNA matrix, 1X Green GoTaq® Flexi Buffer, 2.5 mM MgCl2, each dNTP

at 0.1 mM, 0.5 μM of each primer and 1.5 units of GoTaq® DNA polymerase (Promega, Madison, USA). The amplification products were isolated from samples that showed clear and visible bands on agarose gel stained with 0.5 μg/ml ethidium bromide. Bands of interest were excised from the gel and amplification products isolated using the Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan), following the manufacturer's specifications. Macrogen services (Seoul, South Korea) were used for sequencing. The raw sequences were manually aligned and edited in CodonCode Aligner version 3.7.1 (CodonCode Corporation, Dedham, MA, USA), after which the GenBank and BOLD Systems databases were queried. The GenBank database has its own interface called nucleotide BLAST (Basic Local Alignment Search Tool), which finds the similarity between sequences. The interface compares the nucleotide sequences with the sequences stored in its own database and calculates a statistical significance. The identity ratio calculated by BLAST represents the percentage of nitrogenous bases that are similar between our subject sequence and the sequences found in the database. BLAST also calculates a degree of overlap which is the percentage of alignment between our sequence of interest and the reference sequences stored in GenBank. Molecular identification established using the GenBank database was confirmed by querying the BOLD Systems database. The species identified by the DNA barcoding method, the sequences of which were deposited in GenBank were *Batrisodes unisexualis, Trichonyx sulcicollis, Sepedophilus bipunctatus, Sepedophilus pedicularius, Gyrophaena manca, Scaphisoma agaricinum, Stenus ochropus, Euconnus fimetarius, Sepedophilus testaceus, Anaspis frontalis, Dyschirius globosus, Agathidium nigripenne, Cidnopus pilosus, Brassicogethes aeneus, Scolytus multistriatus, Xyleborus dryographus, Xyleborinus saxesenii* and *Rhopalocerus rondanii*.

To perform a basic phylogeographic analysis and to have an accurate picture of how the phylogenetic lineages are spread at the European level, all COI sequences corresponding to the 18 identified coleopteran species were downloaded from the databases. The Mega7 program was used for sequence alignment. DnaSP v. 5 software was used to calculate the number of haplotypes. The network of haplotypes identified for each individual species was reconstructed under a Median Joining algorithm implemented in PopART v 1.7.

For the species *Batrisodes unisexualis* 7 COI sequences were analyzed, six of them coming from specimens collected in Belgium and Germany. The haplotype identified in the Republic of Moldova is a distinct one. The other two haplotypes, one common for Belgium and Germany and one distinct for Germany differ by a single mutation, while the one from the Republic of Moldova has accumulated a number of 4 additional mutations (substitutions) (Fig. 3.2.1).

For the species *Trichonyx sulcicollis* the number of COI sequences identified/downloaded from international databases is low. Two sequences from Belgium were analyzed, grouped in the same mitochondrial haplotype which is different from the one present in the Republic of Moldova, in the Scientific Reserve "Pădurea Domnească" by 15 mutations (Fig. 3.2.2).

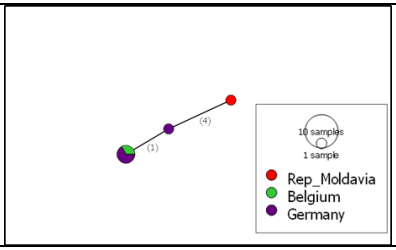


Figure 3.2.1. Haplotype network for the species *Batrisodes unisexualis*

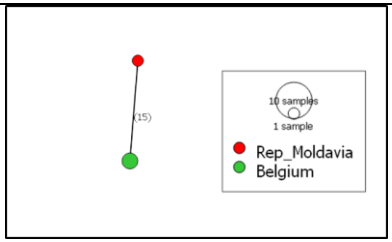


Figure 3.2.2. Haplotype network for the species *Trichonyx sulcicollis*

For the species *Sepedophilus bipunctatus*, 4 haplotypes were identified in 16 analyzed COI sequences. Two of the haplotypes are common to Belgium and Finland, while distinct haplotypes appear in the Republic of Moldova and Belgium. The number of mutations by which they differ from each other is reduced (1 mutation) (Fig. 3.2.3).

For the species *Sepedophilus pedicularius* in the 6 analyzed sequences, which came from Finland, Germany and the Republic of Moldova, 3 haplotypes were identified, two of which are private for the Republic of Moldova and Finland and one common in which sequences from Germany and Finland are grouped. All three haplotypes identified in the samples differ by a single mutation (Fig. 3.2.4).

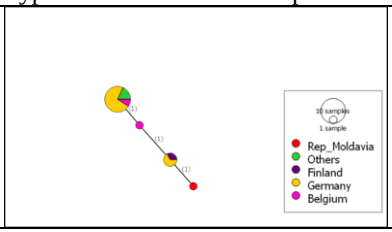


Figure 3.2.3. Haplotype network for the species *Sepedophilus bipunctatus*

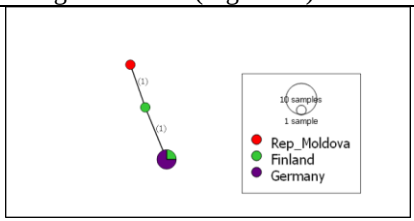


Figure 3.2.4. Haplotype network for the species *Sepedophilus pedicularius*

Seven haplotypes were identified in 13 sequences *Gyrophaena manca*. In the Republic of Moldova, two haplotypes were identified, one of which is common, also found in samples from Germany and Finland, while the second is distinct and differs by 2 mutations from the common one. The most distant is the haplotype from Finland, at a distance of 7 mutations from the common one (Fig. 3.2.5).

Seven haplotypes were identified in 19 COI sequences analyzed for *Scaphisoma agaricinum*. 3 private haplotypes are present in samples collected from Germany. A private haplotype is present in Finland, while the samples from the Republic of Moldova from "Pădurea Domnească" and "Plaiul Fagului" Reserves are grouped into two private haplotypes. A common haplotype is found in samples from Germany and Finland (Fig. 3.2.6).

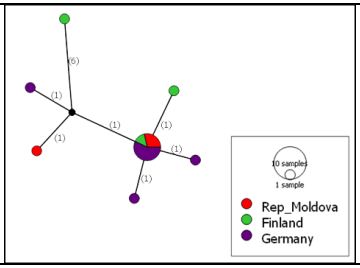


Figure 3.2.5. Haplotype network for the species *Gyrophaena manca*

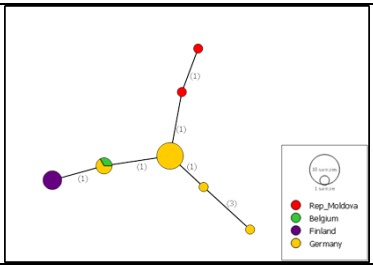


Figure 3.2.6. Haplotype network for the species *Scaphisoma agaricinum*

For the species *Stenus ochropus* 9 COI sequences with a length of 658 bp were analyzed and grouped into 5 haplotypes. The analyzed sequences come from countries such as: Germany, Iran, Norway and the Republic of Moldova with one specimen collected in Vila Nisporeni. The haplotype spread in the Republic of Moldova was also identified in the samples collected in Germany (Fig. 3.2.7).

To describe the species *Sepedophilus testaceus*, 67 COI sequences with a length of 667 bp were analyzed and grouped into 7 haplotypes. The analyzed sequences come from such countries as: Canada, the USA, Germany, Finland, France and the Republic of Moldova with one specimen collected in "Plaiul Fagului". The haplotype spread in the Republic of Moldova is a central one with a global distribution, also identified in samples collected in Canada, USA, Germany and Finland (Fig. 3.2.8).

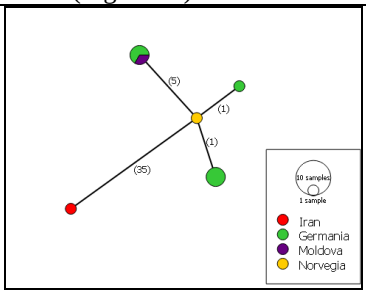


Figure 3.2.7. Haplotype network for the species *Stenus ochropus*

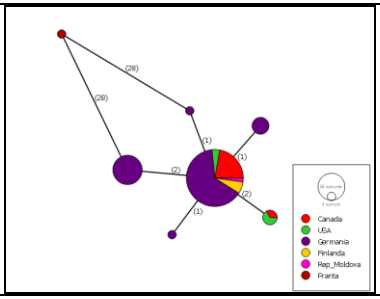


Figure 3.2.8. Haplotype network for the species *Sepedophilus testaceus*

For the species *Euconnus fimetarius* 13 COI sequences with a length of 658 bp were analyzed and grouped into 6 haplotypes. The analyzed sequences come from countries such as: Germany, Finland and the Republic of Moldova with one specimen collected in the Lower Prut.

The haplotype spread in the Republic of Moldova, the Lower Prut, is also one identified in samples collected in Germany and Finland, so it has a wide European distribution. Two haplotype networks were built for this species: based on the geographical location of the sequences, so we can see that the haplotype spread in

the Republic of Moldova is also one identified in samples collected in Germany and Finland (Fig. 3.2.9a); based on the GenBank and BOLD identification of the downloaded sequences and it can be easily seen that the morphological identification leads to the finding of a central haplotype in which both species are grouped. The haplotype found in the Republic of Moldova clearly groups with *Euconnus fimetarius* (Fig. 3.2.9b).

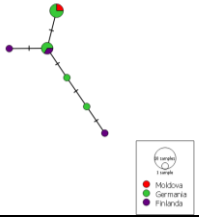


Figure 3.2.9a. Haplotype network constructed with the localization of the sequences of *Euconnus*

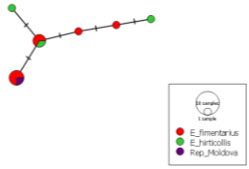


Figure 3.2.9b. Haplotype network constructed using the two species of *Euconnus*

For the species *Anaspis frontalis* 33 COI sequences with a length of 658 bp were analyzed and grouped into 9 haplotypes. The analyzed sequences come from such countries as: Estonia, Poland, Germany, Sweden, Finland and the Republic of Moldova with a specimen collected in the "Pădurea Domnească". The haplotype widespread in the Republic of Moldova is also found in Estonia, being one mutation away from a central haplotype comprising 11 samples and which is widespread in Germany (Fig. 3.2.10).

For the species *Dyschirius globosus* 32 COI sequences with a length of 650 bp were analyzed and grouped into 13 haplotypes. The analyzed sequences come from countries such as: Germany, Canada, Sweden, Finland, Belgium, France as well as the Republic of Moldova with a specimen collected in the "Pădurea Domnească". The haplotype occuring in the Republic of Moldova is a private one at a distance of 4 mutations from another semi-central haplotype spread in Germany and Finland (Fig. 3.2.11).

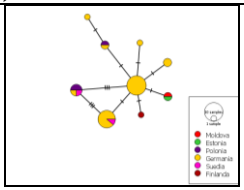


Figure 3.2.10. Haplotype network for the species *Anaspis frontalis*

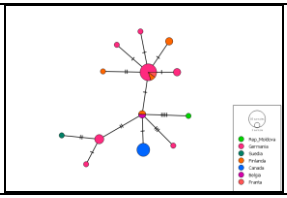
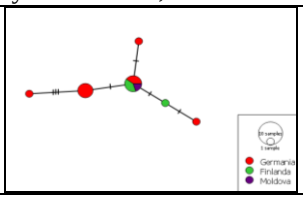


Figure 3.2.11. Haplotype network for the species *Dyschirius globosus*

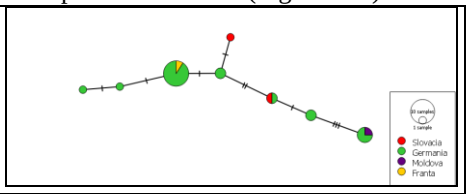
For the species *Agathidium nigripenne*, 13 COI sequences with a length of 658 bp were analyzed and grouped into 6 haplotypes. The analyzed sequences come from countries such as: Germany, Finland as well as the Republic of Moldova with a specimen collected in the "Pădurea Domnească". The haplotype widespread in the

Republic of Moldova is one also identified in samples collected in Germany and Finland, so one with a wide European distribution (Fig. 3.2.12).

To describe the species *Cidnopus pilosus*, 24 COI sequences with a length of 700 bp were analyzed and grouped into 8 haplotypes. The analyzed sequences come from the countries of Germany, Slovakia, France as well as from the Republic of Moldova with a specimen collected in the "Pădurea Domnească". The haplotype widespread in the Republic of Moldova is one also identified in samples collected in Germany and Finland, so one with a wide European distribution (Fig. 3.2.13).



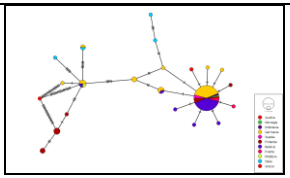
Figură 3.2.12. Haplotype network for the species *Agathidium nigripenne*



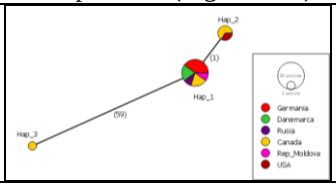
Figură 3.2.13. Haplotype network for the species *Cidnopus pilosus*

For the species *Brassicogethes aeneus*, 79 COI sequences with a length of 577 bp were analyzed and grouped into 22 haplotypes. The analyzed sequences come from countries such as: Austria, Norway, Indonesia, Germany, Sweden, Finland, Belarus, France, Italy, Greece and the Republic of Moldova with a specimen collected in the "Pădurea Domnească". The haplotype spread in the Republic of Moldova is one also identified in samples collected from Germany and Belarus (Fig. 3.2.14).

For the species *Scolytus multistriatus*, 14 COI sequences with a length of 658 bp were analyzed and grouped into 3 haplotypes. The analyzed sequences come from the countries of Germany, Denmark, Russia, Canada, the USA as well as the Republic of Moldova with a specimen collected in the "Pădurea Domnească". The haplotype spread in the Republic of Moldova is also found in Germany, Denmark, Russia, Canada and is one mutation away from another haplotype spread in Canada and the USA. Also, a third haplotype, but which is at a distance of 59 mutations from the central and widespread haplotype is located in Canada, but in this case it may also be an error in the determination of the control specimen (Fig. 3.2 .15).



Figură 3.2.14. Haplotype network for the species *Brassicogethes aeneus*



Figură 3.2.15. Haplotype network for the species *Scolytus multistriatus*

To describe the *Xyleborus dryographus* species, 10 COI sequences with a length of 646 bp were analyzed and grouped into 5 haplotypes. The analyzed sequences come from countries such as: Germany, Portugal, France and the Republic of Moldova with a specimen collected in the "Pădurea Domnească". The haplotype spread in the Republic of Moldova is a common one for France and Germany and central from which the two haplotypes from Portugal are derived (Fig. 3.2.16).

To describe the species *Xyleborinus saxesenii*, 198 COI sequences with a length of 611 bp were analyzed and grouped into 7 haplotypes. The analyzed sequences come from countries such as: Canada, USA, China, Germany, Portugal, South Africa, Egypt, France, Belarus, Georgia and the Republic of Moldova with a specimen collected in the "Pădurea Domnească". The haplotype spread in the Republic of Moldova is common globally, being also identified in samples collected in Germany, South Africa, China, etc. (Fig. 3.2. 17).

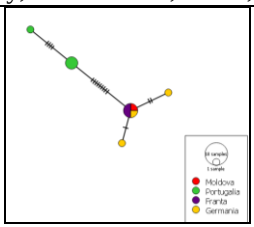
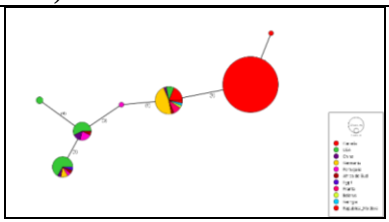


Figura 3.2.16. Rețeaua de haplotipuri pentru specia *Xyleborus dryographus*



Figură 3.2.17. Rețeaua de haplotipuri construită pentru specia *Xyleborinus saxesenii*

To describe the species *Rhopalocerus rondanii*, 2 COI sequences with a length of 658 bp were analyzed, one of them coming from a specimen collected in Germany. The haplotype identified in the Republic of Moldova is a distinct one. The two haplotypes, one distinct for Germany and the one from the Republic of Moldova, differ from each other by a number of 4 mutations (substitutions).

From a phylogeographic point of view, most sequences from the samples collected from the reserves of the Republic of Moldova are grouped into private haplotypes. Only the species *Gyrophaena manca* is an exception, because for it two haplotypes have been identified, one of which is widespread in both Finland and Germany and only one is private, i.e. found only in the Republic of Moldova.

3.3. Fungal dispersal in saproxylic coleopterans and their importance to forest ecosystems

From the forest ecosystems of the "Plaiul Fagului" Scientific Reserve, 5 species of xylophagous coleopterans *Dryocoetes alni*, *Scolytus carpini*, *Stereocorynes truncorum*, *Platypus cylindrus* and *Xyleborus monographus* were identified, involved in the dispersion of microscopic fungi from infected to healthy trees. The material was collected from *Quercus petraea* with an average age of 80 years.

From the insect samples, they were isolated and identified by the PCR technique up to the genus (4 strains) or species (20 strains) of microscopic fungi. According to the sequencing results of the ITS1-5,8S-ITS2 region, 6 strains of the genus *Alternaria* sp. were identified isolated from 4 species of beetles D. *alni*, *S. carpini*, *S. truncorum* and *X. monographus*. Among them were *Alternaria infectoria* - ubiquitous in different ecosystems and potentially pathogenic for humans; *Alternaria alternata* and *Alternaria tenuissima* - conditionally pathogenic for many herbaceous and woody plant species.

Fungi of the genus *Cladosporium* sp. were isolated from all species of saproxylic beetles. The most widespread were *Cladosporium herbarum*, followed by *Cladosporium cladosporioides*. These fungi are ubiquitous saprophytes, but can also be conditionally pathogenic agents for plants.

It should be mentioned the *Querciphoma carteri* strain, isolated from *D. alni*, known as a pathogen for oak trees; the strains isolated from P. *cylindrus*: *Peniophora cinerea* - pathogen for black walnut (*Juglans nigra*) and *Botritys* sp. – saprophyte and pathogen of gray rot in many plant species.

An important species with biocontrol potential of various plant pathogens, *Aureobasidium pullulans* was isolated from *P. cylindrus* and *X. monographus*.

The saprophyte *Lophiostoma*, which grows on herbaceous and woody plants, has been isolated from *D. alni* and *S. truncorum*. Fungal strains were identified, isolated from only one species of saproxylic coleoptera: *Filobasidium magnum* – isolated from *X. monographus* samples; *Penicillium citreonigrum* – isolated from *S. truncorum*; the saprotroph *Parathyridaria flabelliae* - from samples of the coleopteran *D. alni*; strain *Acrodontium salmoneum* and the representative of the genus *Metschnikowia* from the samples of *S. carpini*. The micromycete molecularly identified as *Sarocladium bacillisporum*, saprophagous of decaying plant material, was isolated only from *P. cylindrus*. The representative of the genus *Myrmecridium* was extracted from the samples isolated from *X. monographus*. The isolate which was identified as *Fomes fomentarius* (basket), an inedible, saprophytic and parasitic fungus that grows on tree trunks, was isolated from samples of *S. truncorum*. Fomes fomentarius is a microhabitat and trophic source for some species of saprotrophic beetles.

Thus, the research demonstrated that the species of xylophagous coleoptera collected from oaks (*Quercus petraea*) are vectors of dispersion of saprophytic fungi from infected trees to healthy ones, some of them being pathogenic.

3.4. Species of saproxylic coleopterans at the first report in the fauna of the Republic of Moldova

The research carried out in Scientific Reserves, Landscape Reserves, National Parks, artificial forest plantations and protective forest strips from the republic allowed the identification of 5 families Biphyllidae, Bothrideridae, Corylophidae, Prostomidae and Throscidae, 47 genera (underlined) and 89 species of new saproxylic coleopterans in the fauna of the Republic of Moldova. The species at the first signal are: *Calodromius spilotus* (Illiger, 1798), *Plegaderus dissectus* Erichson,

1839, *Nossidium pilosellum* (Marsham, 1802), *Ptenidium formicetorum* Kraatz, 1851, *Agathidium nigripenne* (Fabricius, 1792), *Amphicyllis globus* (Fabricius, 1792), *Achrotona fungi* (Grav, 1806), *Anthobium atrocephalum* (Gyllenhal, 1827), *A. fusculum* (Erichson, 1839), *Atheta marcida* (Erichson, 1837), *Batrisodes unisexualis* Besuchet, 1988, *Dinaraea aequata* (Erichson, 1837), *Euaesthetus bipunctatus* (Ljungh, 1804), *Geostiba circellaris* (Gravenhorst, 1806), *Gyrophaena joyi* Wendeler, 1924, *G. manca* Erichson, 1839, *Heterothops niger* Kraatz, 1868, *Hypnogyra angularis* (Ganglbauer, 1895), *Lordithon trinotatus* (Erichson, 1839), *Lathrobium longulum* Gravenhorst, 1800, *Medon rufiventris* (Nordmann, 1837), *Mycetoporus forticornis* Fauvel, 1875, *M. eppelsheimianus* Fagel, 1968, *M. baudueri* Mulsant & Rey 1875, *Oxypoda abdominalis* Mannerheim, 1830, *Quedius ochropterus* Erichson, 1839, *Q. suturalis* Kiesenwetter, 1845, *Scaphisoma agaricinum* (Linnaeus, 1758), *Sepedophilus bipunctatus* (Gravenhorst, 1802), *S. constan*s (Fowler, 1888), *S. immaculatus* (Stephens, 1832), *S. littoreus* (Linnaeus, 1758), *S. marshami* (Stephens, 1832), *S. obtusus* Luze, 1902, *S. pedicularius* (Gravenhorst, 1802), *Siagonium humerale* Germar, 1836, *Sunius fallax* (Lokay, 1919), *Tachinus rufipes* (Linnaeus, 1758), *Tachyporus transversalis* Gravenhorst, 1806, *Trichonyx sulcicollis* (Redtenbacher, 1816), *Aesalus scarabaeoides* Panzer, 1794, *Megatoma undata* (Linnaeus, 1758), *Attagenus punctatus* (Scopoli, 1772), *Cardiophorus discicollis* (Herbst, 1806), *C. ruficollis* (Linnaeus, 1758), *Xylophilus testaceus* (Herbst, 1806), *Dirrhagofarsus attenuatus* (Mäklin, 1845), *Lopheros rubens* (Gillenhal, 1817), *Aulonothroscus brevicollis* (de Bonvouloir, 1859), *Malthinus balteatus* Suffrian, 1851, *Biphyllus lunatus* Fabricius, 1792, *Axinotarsus ruficollis* (Olivier, 1790), *Triplax aenea* (Schaller, 1783), *T. collaris* (Schaller, 1783), *Tritoma bipustulata* Fabricius, 1775, *Monotoma longicollis* (Gyllenhal, 1827), *Rhizophagus bipustulatus* Fabricius, 1792, *Placonotus testaceus* (Fabricius, 1787), *Cryptarcha strigata* (Fabricius, 1787), *C. undata* (Olivier, 1790), *Glischrochilus quadriguttatus* (Fabricius, 1776), *Bothrideres bipunctatus* (Gmelin, 1790), *Oxylaemus cylindricus* (Creutzer in Panzer, 1796, *Endomychus armeniacus* Motschulsky, 1835, *Mycetina cruciata* (Schaller, 1783), *Symbiotes gibberosus* (Lucas, 1846), *Sericoderus lateralis* (Gyllenhal, 1827), *Corticarina minuta* (Fabricius, 1792), *Dienerella filum* (Aube, 1850), *Enicmus rugosus* (Herbst, 1793), *E. testaceus* (Stephens, 1830), *Latridius hirtus* Gyllenhal, 1827, *Mycetophagus fulvicollis* Fabricius, 1792, *Triphyllus bicolor* (Fabricius, 1777), *Abdera quadrifasciata* (Curtis, 1829), *Dircaea australis* Fairmaire, 1856, *Mordellistena neuwaldeggiana* (Panzer, 1796), *Synchita undata* Guérin-Méneville, 1844, *Rhopalocerus rondanii* (Villa & Villa, 1833), *Nosodomodes diabolicus* (Schaufuss, 1862), *Pycnomerus terebrans* (Olivier, 1790), *Corticeus fasciatus* (Fabricius, 1790), *Eledonoprius armatus* (Panzer, 1799), *Mycetochara flavipes* (Fabricius, 1792), *Palorus depressus* (Fabricius, 1790), *Platydema dejaeni* Laporte de Castelnau & Brullé, 1831, *Prostomis mandibularis* (Fabricius, 1801), *Stictoleptura scutellata* (Fabricius, 1781) and *Neoclytus acuminatus* (Fabricius, 1775).

4. DIVERSITY OF SAPROXYLIC BEETLES FROM THE STUDIED FOREST ECOSYSTEMS

4.1. Saproxylic beetles detected in the Scientific Reserves

In the Scientific Reserve "Pădurea Domnească" the materials were collected by the direct method, with the entomological vacuum cleaner, with the entomological net, by shaking off tree mushrooms, the flotation method and with the help of trunk traps. 602 specimens belonging to 84 species, 73 genera and 36 families were collected. 13 species were distinguished by more than 10 specimens, and 61 species between 10 and one specimen. Among the rare and protected species in the fauna of the Republic of Moldova *Cucujus cinnaberinus* and *Lucanus cervus* were reported in the reserve (Red Book, 2015). There are other rare species that are protected in some European countries and that require increased attention in our country as well. Among them can be mentioned *Triphyllus bicolor, Dirrhagofarsus attenuatus, Prostomis mandibularis* and *Uloma culinaris*. At the same time, xylomycetophagous species that cause damage to the trees weakened and affected by natural factors were also collected. The harmful species include *Platypus cylindrus, Scolytus multistriatus, Xyleborus dryographus, Xyleborus monographus* and *Xyleborinus saxesenii*. From the "Pădurea Domnească" Reserve 24 species from 16 families were identified for the first time in the fauna of the Republic of Moldova and 20 species are indicators of old forests.

In the Scientific Reserve "Plaiul Fagului" the materials were collected using all the collection methods mentioned in the methodology chapter. 693 specimens belonging to 123 species, 107 genera and 37 families were collected. 16 species were distinguished by more than 10 specimens, and 107 species had between 10 specimens and one specimen. Among the rare and protected species in the fauna of the Republic of Moldova, the species *Carabus intricatus, Cerophytum elateroides, Cucujus cinnaberinus, Lucanus cervus, Morimus asper funereus* and *Rosalia alpina* were identified. Other rare species protected in Europe that also require protection in our country are *Corticeus fasciatus, Platydema dejeanii, Prostomis mandibularis, Uloma culinaris, Rhysodes sulcatus, Oxylaemus cylindricus, Neatus picipes* and *Aesalus scarabaeoides*. Among the xylophagous and xylomycetophagous pest species there were registered *Saperda populnea, Xylotrechus rusticus, Xylotrechus antelope, Phymatodes testaceus, Platypus cylindrus, Scolytus carpini, Xyleborus dispar, Xyleborus monographus* and *Xyleborinus saxesenii*. 38 species of saproxylic coleoptera from 16 families, new for the fauna of the Republic of Moldova, were identified from "Plaiul Fagului". 28 indicator species of old forests were reported.

In the Scientific Reserve "Codrii" the materials were collected by the direct method, with the entomological vacuum cleaner, with the entomological net, by shaking off tree mushrooms and the flotation method. 173 specimens belonging to 46 species, 38 genera and 21 families were collected. 4 species stood out with more than 10 specimens, and 17 species with less than 10 specimens. Among the rare protected species, the following species were identified: *Carabus intricatus, Cucujus cinnaberinus* and *Lucanus cervus*. From this reserve, 4 species of saproxylic coleoptera from 4 families, new for the fauna of the Republic of Moldova, were

identified. In the Scientific Reserve "Codrii" 19 indicator species of old forests were registered.

In the Scientific Reserve "Prutul de Jos" the materials were collected by the direct method, with the entomological vacuum cleaner, with the entomological net and by the flotation method. 15 specimens belonging to 13 species, 13 genera and 8 families were collected. Very few specimens were collected during the 5 expeditions carried out in the territory of the reserve, due to the lack of dead trees and logs. Materials were collected from 2020 to 2022.

Among the rare species protected in the fauna of the Republic of Moldova the following species were collected: *Aromia moschata* and *Lucanus cervus*. The sp4ecies *Neoclytus acuminatus* from the Cerambycidae family could be a potential pest in the future for various deciduous species, being a polyphagous species. From the Scientific Reserve "Prutul de Jos" 2 new species for the fauna of the country from 2 families were identified. In the Scientific Reserve "Prutul de Jos" 5 indicator species of the old forests were detected.

4.2. The saproxylic beetles recorded in the researched Landscape Reserves

In the "Telita" Landscape Reserve, the materials were collected by the direct method, with the entomological vacuum cleaner, with the entomological net, by shaking the tree fungi and by the flotation method in the laboratory. 80 specimens belonging to 13 species, 13 genera and 11 families were collected. 3 species were represented by more than 10 specimens each, and 9 species with less than 10 specimens each. 3 indicator species of old forests were detected. Due to the extraction of dead wood from the reserve, the saproxylic species can't find favorable microhabitats, have reduced their numbers and are threatened with extinction.

In "Țapova" Landscape Reserve the materials were collected by the direct method, with the entomological vacuum cleaner and the entomological net. 18 specimens belonging to 5 species, 5 genera and 4 families were collected. The species *Uleiota planata* stood out with more than 10 specimens, and the other 4 species with fewer specimens. Only one indicator species was reported, which proves that dead wood is removed from the forest, and saproxylic species have no habitat.

From the "Codrii Tigheci" Landscape Reserve, the materials were collected by the direct method, with the entomological net, with the entomological vacuum cleaner, by the tree fungus shaking method and by the flotation method carried out in the laboratory. 57 specimens belonging to 15 species, 15 genera and 9 families were collected. Among the rare and protected species in the fauna of the Republic of Moldova in the "Codrii Tigheci" Landscape Reserve, only the species *Lucanus cervus* was reported. Among the indicator species of the old forests, the species *Teretrius fabricii*, *Lucanus cervus*, *Uloma culinaris* and *Aegosoma scabricorne* were registered.

From the dead wood of the "Cobîleni" Landscape Reserve, the materials were collected by the direct method and with the entomological vacuum cleaner. 35 specimens belonging to 3 species, 3 genera and 2 families were collected. The

species *Rhagium inquisitor* was represented by 32 specimens. Among the indicator species of the old forests, only the species *Ampedus elegantulus* was detected, due to the extraction of dead wood, the saproxylic species have no environment to develop.

From the "Vila Nisporeni" Landscape Reserve, the materials were collected by the direct method, with the help of the entomological vacuum cleaner, by shaking the tree fungi, the flotation method and with the entomological net. 66 specimens were collected, belonging to 20 species, 17 genera and 8 families. Among the rare and protected species in the fauna of the Republic of Moldova the following species were identified: *Carabus intricatus* and *Lucanus cervus* (Red Book, 2015). Among the harmful xylophagous species, the species *Rhagium inquisitor* was detected that attacks weakened trees. 4 indicator species of the old forests were detected in the "Vila Nisporeni" Landscape Reserve.

4.3. Saproxylic beetles recorded in the „Lower Dniester" and „Orhei" National Parks

In the Lower Dniester National Park, the materials were collected by the direct method, with the help of the entomological vacuum cleaner, with the entomological net, by shaking the tree fungi and the flotation method. 159 specimens belonging to 42 species, 33 genera and 12 families were collected. Among the rare protected species, the species *Lucanus cervus* and *Ischnodes sanguinicollis* were identified. At the first report in the fauna of the country, there were 15 species from 10 genera and one family. In the Lower Dniester National Park, 4 indicator species of the old forests were detected.

In the Orhei National Park, the materials were collected by the direct method, with the entomological vacuum cleaner, by shaking the tree fungi and by the flotation method in the laboratory. 94 specimens belonging to 20 species, 18 genera and 12 families were collected. 4 species stood out with more than 10 specimens each, and 16 species with less than 10 specimens each. Among the rare and protected species in the fauna of the Republic of Moldova, the species *Morimus asper funereus* and *Lucanus cervus* were reported. There are other rare species that are protected in Europe. Among them we mention *Biphyllus lunatus*, *Bothrideres bipunctatus* and *Uloma culinaris*. For the protection of rare species, dead wood must be kept in its natural habitat. Within the park, 3 new species for the country's fauna and 3 indicator species of the old forests were identified.

4.4. Saproxylic beetles registered in natural forests, forest plantations and urban parks in the country

From natural forests, forest plantations and urban parks, the materials were collected most frequently by the direct method, or with the help of the entomological vacuum cleaner, sometimes the species that visit the flowering plants were collected with the entomological net, the species that inhabit the tree fungi were shaken from the mushrooms on a white cover from which they were sucked, and some wood fragments were examined for the presence of saproxylic species in the laboratory by the flotation method. 252 specimens were collected, belonging to 53 species, 46

genera and 17 families. Among the rare and protected species in the fauna of the country *Cucujus cinnaberinus* - collected in the Valea Morilor park in Chisinau and *Lucanus cervus* - collected in the Valea Morilor park and in the natural forest from Zabriceni were identified. Among the indicator species of old forests detected in the natural forests, forest strips and urban parks can be mentioned *Anthaxia millefolii*, *Cucujus cinnaberinus*, *Aegosoma scabricorne* and *Lucanus cervus* detected in the Chisinau parks; *Tenebrio opacus* and *Neatus picipes* in the forest strip from Măcărești; *Ampedus elegantulus* in the forest plantation at Lopatna; *Mycetophagus fulvicollis* in the "Dolna" Landscape Reserve and in the natural forest from Vulcănești; *Uloma culinaris* in the "Saharna" Landscape Reserve and *Silvanus unidentatus* in the "Bularda" Landscape Reserve. The indicator and rare saproxylic species that are in the parks of the city of Chisinau and the town of Codru demonstrate the presence of secular, native trees and the fact that in the municipality of Chisinau there is a clean environment that must be protected and maintained in good condition.

The diversity indexes Simpson, Shannon and eveness were calculated to quantify the biodiversity in the investigated forest ecosystems. The greatest diversity of saproxylic beetles was established in the Scientific Reserves "Plaiul Fagului", "Pădurea Domnească" and "Codrii" (Tab. 4.4.1).

Table 4.4.1. The number of species and individuals identified in the studied forest ecosystems and the diversity indexes

Ecosystem	Species	Individuals	Simpson	Shannon	Eveness
"Pădurea Domnească"	84	602	0,1233	**3,069**	0,6927
"Plaiul Fagului"	123	693	0,0494	**3,931**	0,8169
"Codrii"	46	173	0,0598	**3,243**	0,847
"Prutul de Jos"	13	15	0,01905	2,523	**0,9837**
"Nistrul de Jos"	42	159	0,05899	3,21	0,8588
"Orhei National Park"	20	94	0,08213	2,616	**0,8731**
"Telița"	13	80	0,1484	2,093	0,816
"Țâpova"	5	18	**0,5163**	0,9609	0,597
"Codrii Tigheci"	15	57	0,1034	2,35	0,8678
"Cobîleni"	3	35	**0,8353**	0,3471	0,3159
"Vila Nisporeni"	20	66	0,2005	2,13	0,7111

The Schannon diversity index recorded the highest values for the "Plaiul Fagului" Reserve, which has the best conditions, a great diversity of microhabitats, dead wood in various stages of decay belonging to various tree species. The eveness index recorded the highest values for the Scientific Reserve "Prutul de Jos" and the Orhei National Park, which presented an almost similar number of individuals for all captured species. The analysis of the Simpson index shows the lowest diversity in the "Cobîleni" and "Țapova" landscape reserves, the recorded values approaching the maximum limit.

5. RARE AND BIOINDICATORS SAPROXYLIC COLEOPTERANS OF OLD FORESTS

5.1. Rare saproxylic coleopterans and their protection

The saproxylic beetles are protected at European level by the Berne Convention (1997) on the Conservation of European Wildlife and Natural Habitats. The species *Cucujus cinnaberinus*, *Cerambyx cerdo* and *Rosalia alpina* are included in Appendix II. The species *C. cerdo*, *C. cinnaberinus*, *Lucanus cervus*, *Morimus funereus*, *Rhysodes sulcatus*, *R. alpina* are listed in Annex II of the Habitats Directive 92/43/EEC of the European Council of 21 May 1992 on the conservation of natural habitats and species of wild fauna and flora, in Annex IV of the same Directive the species *C. cerdo*, *C. cinnaberinus* and *R. alpina* are indicated. Coleopterans are also protected by the European Red List for the conservation of saproxylic species [10; 28]. At the national level, the rare species are protected by the Law of Animal Kingdom (No. 439 of 27-04-1995), in which the species of saproxylic beetles *Cerophytum elateroides*, *Elater ferrugineus*, *Ischnodes sanguinicollis*, *Porthmidius austriacus*, *L. cervus*, *C. cerdo*, *M. asper funereus* and *R. alpina* are mentioned.

The current European Red List of the International Union for Conservation of Nature provides an assessment for 693 species of saproxylic beetles, of which 436 species published by Nieto and Alexander [28] and 257 species published in 2018, based on a study performed in 2017-2018. There are over 3500 species of saproxylic beetles in Europe.

Among the saproxylic coleopteran species protected in the fauna of the Republic of Moldova, there are 12 species from 6 families. The protected species are *Carabus intricatus* recorded in the Scientific Reserves "Plaiul Fagului", "Codrii" and in the Landscape Reserve "Vila Nisporeni"; *Lucanus cervus* was registered in Nature Reserves and in urban parks; *Elater ferrugineus* reported only in the "Codrii" Nature Reserve; *Ischnodes sanguinicollis* reported in "Plaiul Fagului" and in the Lower Dniester National Park; *Cerophytum elateroides* reported in the "Plaiul Fagului" Scientific Reserve; *Porthmidius austriacus* Orhei National Park; *Cucujus cinnaberinus* in Scientific reserves "Plaiul Fagului", "Pădurea Domnească", Landscape Reserve "Dolna" and Chisinau Valea Morilor urban park; *Aromia moschata* in Orhei National Park and Lower Dniester National Park; *Cerambyx cerdo* reported in the Scientific Reserve "Codrii" and Orhei National Park; *Morimus asper funereus* reported in the Scientific Reserves "Codrii" and "Plaiul Fagului", Orhei National Park, Lower Dniester National Park and the forest in the village of Cobîlea, Șoldănești district; *Purpuricenus kaehleri* detected in the "Plaiul Fagului" and "Codrii" Reserves; *Rosalia alpina* recorded in 2019, 2021 and 2022 in the "Plaiul Fagului" Scientific Reserve.

In order to protect the saproxylic beetle species in forest ecosystems there is a need to preserve a sufficient amount of dead wood, including trunks and branches of different thicknesses and to protect old mixed forests with a high diversity of forest species and, of course, to maintain damaged and hollow old tree in habitats [15].

5.2. Saproxylic beetles indicators of old forests

In 1999, the saproxylic quality index based on saproxylic beetle species was proposed in Great Britain. A tool capable of assessing the importance of intact conservation of forest ecosystems for saproxylic beetle species. Studies were carried out with the aim to identify the list of saproxylic coleopterans dependent on dead wood. Numerous species of coleoptera were identified, some of which were obligatory saproxylic, others facultatively saproxylic. Some species depended on thick trunks, others needed only thin branches for development, some species depended on dead trees exposed to the sun, others on the shady exposure of the tree, on standing trees, or on the ground, some species depended on stumps, on filamentous fungi or fruiting bodies. For some species of coleoptera the biology of the species is known, for other species the presence in dead wood has been confirmed in international specialized studies. Based on the saproxylic quality index, the habitats/forests were evaluated for their conservation.

The indicator saproxylic species help to assess forest characteristics important for the management and protection of their biodiversity. The most relevant species as an indicator of biodiversity, which also reveals a good state of conservation of the habitat, is the species *Cucujus cinnaberinus* (Scop., 1763) [22], to detect the species it is necessary to examine the cracks in the stems, or remove the dry bark.

The saproxylic coleopterans from natural and anthropogenic forests of the Republic of Moldova have been reflected in a limited number of studies. The data have been presented sporadically in faunal and ecological works, but have not been represented for certain ecosystems entirely. Some saproxylic species are common throughout the territory of the republic, others are quite rare and strictly related to a certain microhabitat and depend on the species of trees, on the wet decaying wood, on the fungi that decompose the dead wood. Saproxylic beetles are specific to the age of the trees. Thus, some species invade weakened or dead trees between 40-80 years old, trees with an average age of 80-120 years and old trees over 120 years old. Some obligately saproxylic coleopteran species are used according to the standard methodology proposed by Schmidl and Bussler [33] in the identification of landscape ecology, or according to the authors Eckelt et al., [12], in the identification of old trees in old forests (relicts) with a conservation value throughout Central Europe.

Among the 342 species of saproxylic beetles identified – 78 species, from 72 genera and 26 families are indicators of old trees and good ecological condition of forests. Of these indicator species – 43 are quite rare, some with only one record. The obtained scientific results represent a valuable information base that can be used to establish the status of important habitats for the protection and conservation of diversity. The analysis of the indicator saproxylic species for old forests with a good ecological status, revealed 49 species belonging to 20 families - which depend on the old dead wood of old trees; 17 species from 5 families depend on the wood of freshly cut trees; of the fungi growing on the dead wood – 8 species from 5 families and of the cavities/scurvy formed in the dead wood of old trees – 7 species from 5 families. Based on saproxylic coleoptera species, 7 important forest ecosystems

were identified at the regional, national and international level. Among these internationally important forests, 2 forests were highlighted: "Plaiul Fagului" with 123 saproxylic species and 31 indicator species and "Pădurea Domnească" with 84 saproxylic species and 20 indicator species. Nationally important forests are the forest ecosystems of the Lower Dniester National Park with 42 saproxylic species, of which 5 are indicators, and the "Codrii" Scientific Reserve with 46 saproxylic species, of which 19 are indicator species. Orhei National Park with 20 saproxylic species, of which 5 species are indicators of old forests, and "Vila Nisporeni" Landscape Reserve with 20 saproxylic species, of which 4 species are indicators of old forests.

5.3 Estimation of the threat degree of saproxylic species form the family Tenebrionidae

The saproxylic beetles from the family Tenebrionidae are represented in the fauna of the Republic of Moldova by 19 species belonging to the subfamilies: Alleculinae, Diaperinae and Tenebrioninae. The species were analyzed according to version 3.1 (IUCN, 2001) according to the rarity categories proposed by the International Union for Conservation of Nature (IUCN) assigned at the European level: A – population in decline, B – narrow distribution and habitat fragmentation, decline or fluctuations, C – small size and population fragmentation, decline or fluctuations, D – very small population or very restricted distribution, E – quantitative analysis of extinction risk for example, population viability analysis [3].

In the Republic of Moldova, the area covered by forest ecosystems is quite small, so the species were analyzed according to category B with some modifications: 1) data deficient category (DD) – species cited in specialized literature at the beginning of the 20th century, from our data, the species occurs in collections in only one location, could not be evaluated due to lack of data; 2) critically endangered category (CR) – species reported in 1-2 locations, according to analysis data, reconfirmed over 90 years; 3) endangered category (EN) – species reported from 2-6 locations, data analysis places it in the range from 50 to 90 years; 4) vulnerable category (VU) – species reported in 6-13 locations in a small number of individuals, in the interval from 30 to 50 years between the first reports; 5) near threatened category (NT) – species detected in 13 – 32 locations reported in the 30-10 years interval; 6) non-threatened category (LC) – species detected in more than 32 locations and reported up to 10 years after the first detection (Table 5.3.1).

Table 5.3.1. The values of the degree of threat by the number of locations and the period of occurrence in years

Rarity criteria location/years	CR	EN	VU	NT	LC	DD
Numver of locations	1-2	>2-6	>6-13	>13-32	>32	citations
Occurrence in years, between the first reports, according to data from the collections	>90	50-90	30-50	10-30	<10	>90

In order to assign rarity criteria to tenebrionid species from the Republic of Moldova, they were monitored in their natural habitats for 17 consecutive years, at the same time the museum collections in the country, the national and international entomological literature were analyzed. The species *Platydema dejeani* was collected only once during the study period, the species *Neatus picipes*, *Mycetochara flavipes*, *Tenebrio opacus*, *Pseudocistela ceramboides*, *Cryphaeus cornutus* and *Hypophloeus bicolor* were confirmed twice in the fauna of the country, they could receive the status of critically endangered (CR). Three species *Bolitophagus reteticulatus*, *Prionychus ater* and *Platydema violaceum* were identified from 3 locations each, *Hymenalia rufipes* was detected in 4 locations, thus, the 4 species can receive the status of endangered (EN). *Diaclina testudinea* was collected from 7 locations, *Uloma culinaris* was recorded in 8 locations, *Diaperis boleti* was reported from 11 locations, these species can receive the status of vulnerable (VU). The diversity of saproxylic tenebrionids identified in the fauna of the Republic of Moldova, including the presence of the rare and protected species in Europe *N. picipes, M. flavipes, D. testudinea, T. opacus, H. bicolor, D. boleti* and *U. culinaris* demonstrates the ecological value of microhabitats in the natural reserves "Plaiul Fagului", "Pădurea Domnească", of the Orhei National Park and the "Telita" Landscape Reserve. Some studied forest ecosystems are severely affected anthropogenically, the biodiversity of saproxylic coleopterans is drastically threatened. In order to protect the species of saproxylic coleopterans from the Tenebrionidae family, the microhabitat of these species must be preserved.

6. ECOLOGICAL AND ZOOGEOGRAPHIC CHARACTERISTICS OF THE SAPROXYLIC BEETLE SPECIES IN THE INVESTIGATED TERRITORY

6.1. Preferences of saproxylic beetles for the tree species

The analysis of saproxylic coleopteran species collected from the investigated forest ecosystems highlighted the host tree species. Thus, 240 species belonging to 184 genera and 46 families were collected and determined from dry or drying trees on legs or logs, with trunk circumference from 53 cm to 2 m 73 cm. Saproxylic beetles depend not only on the tree species, but also on the succession of wood decay, its position, the volume of the wood and the presence of monocellular or multicellular fungi. The analysis of saproxylic coleoptera species in association with the hosts - tree species, revealed that the most numerous were the coleoptera collected from native tree species. 122 species of saproxylic beetles were collected from oak trees (*Quercus robur, Q. petraea, Q. pubescens*), of which 122 species also appeared on other tree species, and 96 species were collected only from on the oak tree. 49 species were collected from poplar (*Populus* sp.), of which 31 species appeared only on poplar, and 18 species also on other tree species. The beech (*Fagus sylvatica*) was populated by 23 species of coleoptera, of which 12 only on the beech and 11 species common to other tree species as well. Birch trees (*Betul*a sp.) were populated by 7 species of saproxylic beetles. Ash (*Fraxinus excelsior*) was populated by 17 species (11 only from ash, 6 common to other tree species as well).

The willow trees (*Salix* sp.) were populated by 3 species, one of which is also common on ash, another also on oak and one only on willow.

The linden (*Tilia* sp.) was also populated by 2 species, of which the first species is also common to beech, and the second characteristic to linden. The elm trees (*Ulmus* sp.) were populated by 7 species, of which one is also common to oak and ash, another is also characteristic of beech and 5 are characteristic of elm. A single species was collected from cherry (*Prunus avium*), maple (*Acer platanoides*) and hornbeam (*Caprinus betulus*) trees. 48 saproxylic species were collected from deciduous stumps and logs in an advanced state of decomposition without establishing the tree species. Allochthonous tree species pine (*Pinus* sp.) and acacia (*Robinia pseudoacacia*) were populated by 2 species of saproxylic beetles each (Figure 6.1.1).

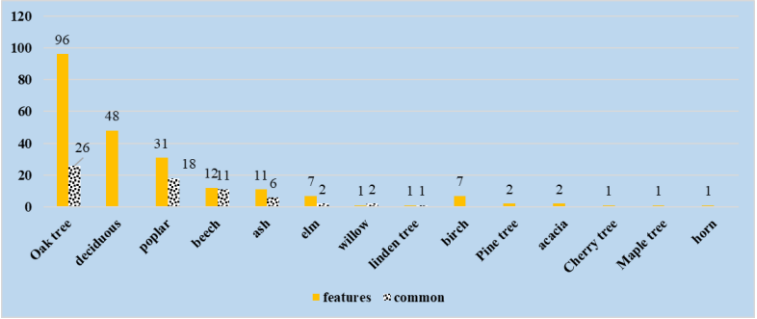


Figure 6.1.1. The number of species of saproxylic beetles collected from various tree species in the period 2008-2023

The obtained results confirm that 43.0% of saproxylic coleopteran species are dependent on the dead wood of oak trees, as the largest area of land in the country is occupied by oak forests. 17.3% of the collected species depended on the dead wood of the poplar trees. Another 16.9% of saproxylic coleoptera species develop on various deciduous species, the stage of wood degradation and its volume being more important.

The saproxylic beetle species were dependent on beech wood - 8.1%, on dead ash wood - 6.0%, on birch - 2.5%, on elm - 2.5% and 0.7% of each species were dependent of the dead wood of willow, linden, acacia and pine trees. 0.4% of the studied species were dependent on decaying wood of cherry, maple and hornbeam. Due to the extraction of dead wood from Scientific, Landscape Reserves and forest plantations, the saproxylic beetles are threatened.

6.2. Analysis of saproxylic coleopterand in vertical aspect

In the "Pădurea Domnească" Scientific Reserve, the collecting by the trunk trap method was performed in 2022. 2 traps were mounted on 7 weakened, drying and dead trees. On the date of collection, the tree species on which the trunk traps were mounted at 2 and 4 meters from the ground and the circumference of the trees were recorded in the field register. The collected materials were cleaned of impurities and

identified in the laboratory. 40 species belonging to 35 genera and 21 families were collected. The most species collected by this method belonged to the families Cerambycidae with 8 species from 8 genera and Curculionidae – 5 species, 4 genera. A smaller number of species were collected from the other families, namely the Erotylidae family was identified by 3 species, belonging to 2 genera; the families Melyridae, Ptinidae and Eucnemidae had 2 species from 2 genera each; the families Mycetophagidae, Endomychidae and Nitidulidae were represented by 2 species each, from a single genus; the families Leiodidae, Staphylinidae, Lycidae, Cantharidae, Dermestidae, Cucujidae, Latridiidae, Mordellidae, Tenebrionidae, Prostomidae, Scraptiidae and Anthribidae with a single species each.

The use of this method of collecting entomological material allowed the identification for the first time in the fauna of the Republic of Moldova of 10 new species among which *Dirrhagofarsus attenuates, Xylophilus testaceus, Lopheros rubens, Malthinus balteatus, Megatoma undata, Axinotarsus ruficollis, Triplax collaris, Enicmus rugosus, Mycetochara flavipes* and *Prostomis mandibularis.* Some species have also been reconfirmed in the fauna of the country. The trunk trap method allowed the identification of the rare and endangered species *Dirrhagofarsus attenuatus* in the fauna of Europe, which is characteristic fpr primary forests with large amounts of dead wood. Among the pests, we can mention the species of the Curculionidae family *Platypus cylindrus, Scolytus multistriatus, Xyleborus dryographus, Xyleborus monographus* and *Xyleborinus saxesenii*. Most species were collected from oak (1.47 m circumference), although the girth of the tree was smaller than one of the poplar trees (2.16 m) and the elm (1.60 m). On the 3 poplar trees on which 2 flight intersection traps were mounted, each collected 11 species, from the ash – 6 species, from the elm 4 – species and from the willow – 3 species. More important in the population of dead wood was the decay stage of the wood. Figure 6.2.1 graphically represents the altitude at which the taxa were collected in the "Pădurea Domnească" Reserve. Common to both heights were the species *Dasytes niger, Dacne bipustulata, Triplax lepida, Cucujus cinnaberinus, Meligethes aeneus, Mycetophagus quadripustulatus, Tomoxia bucephala, Mycetochara flavipes, Anaspis frontalis* and *Xyleborus monographus*.

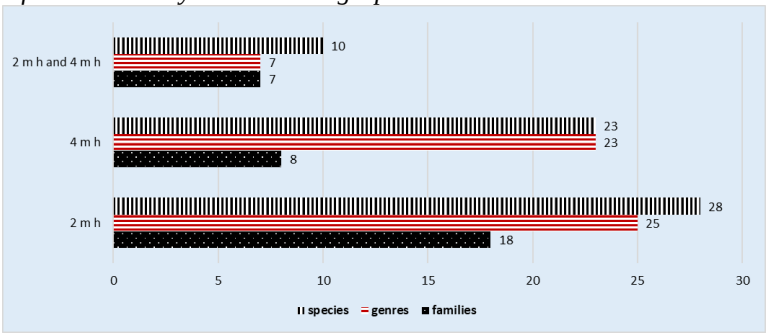


Figure 6.2.1. The number of taxa collected by the vertical trunk trap method from the "Pădurea Domnească" Reserve in 2022

From the "Plaiul Fagului" Scientific Reserve, saproxylic beetles were collected by the trunk trap method at 2, 4 and 6 meters from the ground in sector 1 and 2, and 4 meters from the ground in the strictly protected area. Through the method of trunk traps placed 2, 4 and 6 meters from the ground, 72 species were collected, belonging to 67 genera and 30 families. From sector 1 in the years 2022 and 2023, 51 species, 47 genera and 22 families were collected. The most species belonged to the families Cerambycidae with 9 species from 8 genera and Curculionidae with 8 species from 7 genera. These were followed by the families Ptinidae with 4 species from 4 genera. The families Zopheridae and Tenebrionidae were represented by 3 species from 3 genera each, Nitidulidae by 3 species from 2 genera. The families Histeridae, Erotylidae, Latridiidae, Mordellidae had 2 species from 2 genera each; the Scraptiidae family was represented by 2 species from a single genus; and the families Lucanidae, Buprestidae, Eucnemidae, Bostrichidae, Elateridae, Trogossitidae, Melyridae, Silvanidae, Bothrideridae, Mycetophagidae and Pyrochroidae were all represented by a single species and a single genus. The use of this method of collecting entomological material allowed the identification for the first time in the fauna of the Republic of Moldova of 11 species: *Tritoma bipustulata, Cryptarcha strigata, Cryptarcha undata, Glischrochilus quadriguttatus, Oxylaemus cylindricus, Enicmus testaceus, Latridius hirtus, Pycnomerus terebrans, Corticeus fasciatus, Nosodomodes diabolicus* and *Mordellistena neuwaldeggiana*. Of these, 2 species have been reconfirmed in the entomofauna of the country. Traps were set on 3 oak trees that had the largest trunk circumference (1.18 m; 1.08 m and 0.92 m), they allowed the collection of 45 species. 8 species were collected from the beech, it had a trunk circumference of 0.86 m, 2 species were collected from the poplar, it had a trunk circumference of 0.69 m, one species was collected from the ash, it had a trunk circumference of 0 .94 m. In 2022, in sector 1 of the "Plaiul Fagului" Scientific Reserve, 18 trunk traps were installed on 6 affected trees. 3 traps on each trunk at 2, 4 and 6 meters from the ground, and 12 traps at 2 and 4 meters from the ground in 2023. For each tree, the circumference of the trunk was measured at chest level and the tree species was noted.

Most species were collected from the traps placed at a height of 2 and 4 meters from the ground. The number of species collected from the height of 6 meters has been halved. Common for traps placed at 2, 4 and 6 meters above the ground were 2 species *Phymatodes testaceus* and *Xyleborus monographus* (Figure 6.2.2).

In the strictly protected area in the year 2023 of the Scientific Reserve "Plaiul Fagului"29 species belonging to 28 genera and 19 families were collected. The most species were from the family Tenebrionidae - 4 species from 4 genera, followed by the family Mycetophagidae with 3 species from 2 genera. From the families Dermestidae, Erotylidae, Silvanidae, Nitidulidae and Curculionidae, 2 species from 2 genera each were collected. The families Staphylinidae, Eucnemidae, Throscidae, Ptinidae, Monotomidae, Cucujidae, Laemophloeidae, Endomychidae, Latridiidae, Zopheridae, Salpingidae and Cerambycidae were represented by a single species each. 2 traps were mounted on 5 trees at a height of 2 and 4 meters from the ground.

20 species were collected in the traps mounted 2 meters from the ground and 18 species in the traps mounted 4 meters.

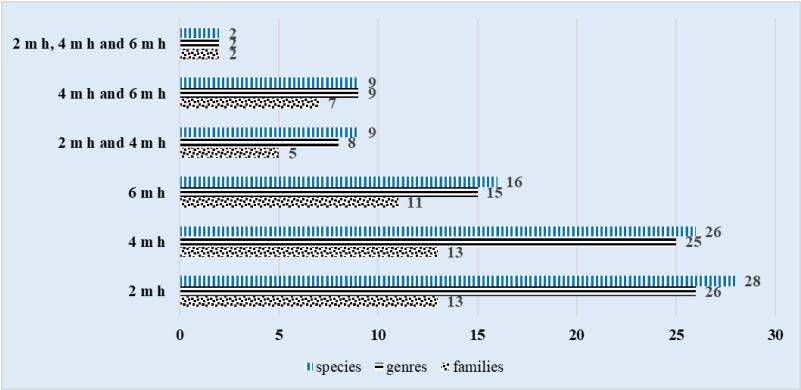


Figure 6.2.2. The number of taxa collected by the method of vertical trunk traps from "Plaiul Fagului" in 2022-2023, from sector 1

The use of this method of collecting entomological material in the strictly protected area allowed the identification for the first time in the fauna of the Republic of Moldova of 11 species, among which *Siagonium humerale, Aulonothroscus brevicollis, Attagenus punctatus* and *Eledonoprius armatus* form strictly protected area exclusively, while the species *Glischrochilus quadriguttatus* (Nitidulidae), *Megatoma undata* (Dermestidae), *Triplax aenea* (Erotylidae), *Rhizophagus bipustulatus* (Monotomidae), *Placonotus testaceus* (Laemophloeidae), *Symbiotes gibberosus* (Endomychidae) and *Enicmus rugosus* (Latridiidae) have been reconfirmed for the fauna of the republic. By this method only 2 xylophagous pest species were identified – *Xyleborus monographus* and *Xyleborinus saxesenii*. Among the indicator species of the old forests can be mentioned *Eledonoprius armatus, Cucujus cinnaberinus* and *Melasis buprestoides*. The highest number of species were collected from the oak, having the largest circumference (2.73 m), but also the best conditions for populating. The bark was visibly perforated and shows sap on the stem on the western side. No species was collected from the hornbeam (2.55 m), it being in the initial phase of drying, the branches in the crown were partially dry, the stem shows the adventitious branches. From beech (2.18 m), ash (1.90 m), and linden (1.46 m), 3, 5 and 2 species were collected, respectively. The trees had been dry for several years, but the bark was well attached to the trunk and no other symptoms such as traces of mycelia or sap on the stems were reported.

20 species were collected with the traps mounted 2 meters from the ground, and 18 species at 4 meters. Common to the traps at 2 and 4 m from the ground were the species *Siagonium humerale, Attagenus punctatus, Glischrochilus quadriguttatus, Rhizophagus bipustulatus, Uleiota planata, Cucujus cinnaberinus, Mycetophagus quadriguttatus* and *Mycetophagus quadripustulatus* (Figure 6.2.3).

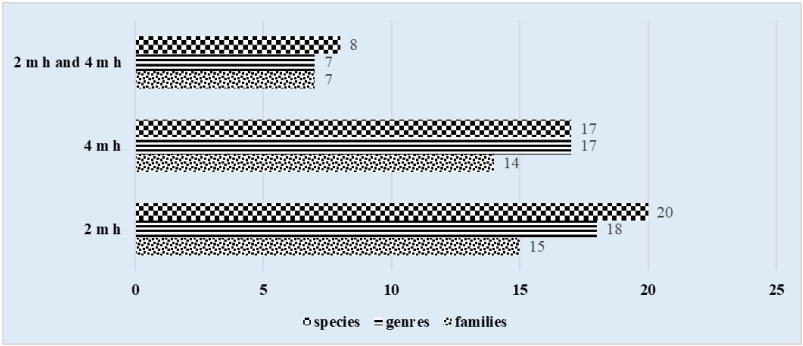


Figure 6.2.3. The number of taxa collected by the vertical trunk trap method from "Plaiul Fagului" in 2023, from the strictly protected area

6.3. Dependence of saproxylic coleopterans on microhabitat: hollows, fungi and fully decayed wood

In „Plaiul Fagului" reserve the species *Prionychus ater, Platysoma compressum, Sepedophilus testaceus, Ischnodes sanguinicollis, Cerophytum elateroides, Siagonium humerale, Attagenus punctatus* and *Latridius hirtus* were collected from the hollows of studied trees. In „Pădurea Domnească" reserve the species *Megatoma undata* was regfistered. In Măcărești forest belt the species *Neatus picipes* and *Tenebrio opacus* and in Orhei National Park *Ampedus pomorum* were collected. From the total decomposed wood of the 5th degradation class, as well as from the remains under the bark of the trunks from the 3rd and 4th stages of degradation, the following species were collected only by the flotation method: *Plegaderus dissectus, Triphyllus bicolor, Latridius hirtus, Corticarina minuta, Dienerella filum, Enicmus rugosus, Sericoderus lateralis, Bothrideres bipunctatus, Gyrophaena joyi, Dinaraea aequata, Atheta marcida, Scaphisoma agaricinum, Sunius fallax, Anisotoma humeralis, Agathidium nigripenne, Nossidium pilosellum* and *Ptenidium formicetorum.* Only from the mushrooms with the soft fruiting body or the lignicolous ones that grow on the wood of old trees were collected at the first mention in the fauna of the country 3 species *Scaphisoma boleti*, *S. agaricinum* and *Biphyllus lunatus*. Other species collected from the mushrooms were *Diaperis bolete, Scaphidium quadrimaculatum, Dacne bipustulata, Litargus connexus* and *Endomychus coccineus* known in the fauna of the country.

6.4. Trophic analysis of saproxylic coleopteran species

For saproxylic beetles, the dead wood, as well as unicellular fungi and those of the Polyporaceae family constitute an important source of food. Other invertebrate species inhabiting dead wood are food for zoophagous saproxylic coleopterans. Also, the organic remains (exuvia, dead invertebrates) decomposing in the attacked or dead wood are a source of food for some saproxylic saprophagous coleoptera. From the point of view of the trophic regime, saproxylic coleopterans can be xylophagous, mycetophagous, zoophagous and saprophagous. Various scientific

papers have been studied for the trophic analysis of saproxylophagous coleopterans [1; 22; 32; 42; 43]. Saproxylic beetles are trophically associated with deciduous and coniferous trees. Most species are xylophagous – 86 species, followed by mycetophagous – 76 and zoophagous – 73 species, xylophagous/saprophagous included 50 species. There are 9 polyphagous species, 26 saprophagous species and only 10 saprophagous-phytophagous species including those that consume tree sap (Figure 6.4.1).

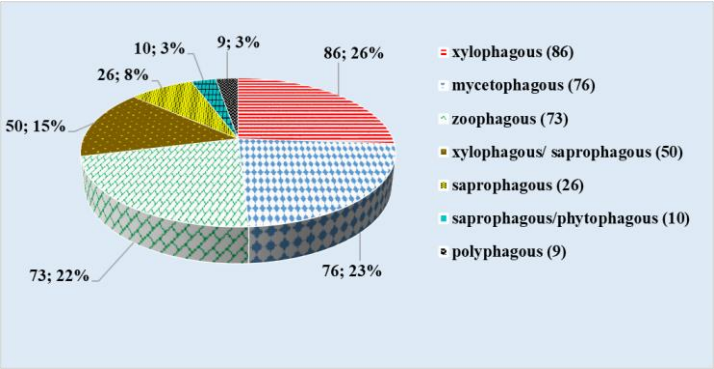


Figure 6.4.1. Trophic analysis of saproxylic coleopteran species from the fauna of the Republic of Moldova

6.5. Zoogeographical analysis of saproxylic coleopteran species by region

The zoogeographical analysis was carried out based on specialized works [14 and 42]. The fauna of saproxylic beetles in the territory of the republic is part of 13 zoogeographical groups: European - 107 species, followed by Palearctic species - 78 species, West-Palearctic - 47, Trans-Palearctic - 23, Holarctic - 19, Euro-Siberian - 15, Euro-Asiatic – 8, Euro-Mediterranean 12, Euro-Caucasian – 11, cosmopolitan – 9, East-Palearctic – 5, Euro-Turanic – 4, Mediterranean – 4 species (Figure 6.5.1). The group of European elements is delimited by the Ural Mountain Massif which includes species dependent especially on moist forest ecosystems. The Palaearctic element group is represented by species with a wide range of distribution that includes both mesophilic and xerophilic species. The group of West-Palearctic elements is represented by mesophilic, hygrophilic and xerophilic species. The group of Trans-Palearctic elements is mainly represented by mesophilic species. The groups of Holarctic, Euro-Siberian and cosmopolitan elements are represented by species characteristic of humid habitats. The other groups include a smaller number of species with a restricted area of distribution.

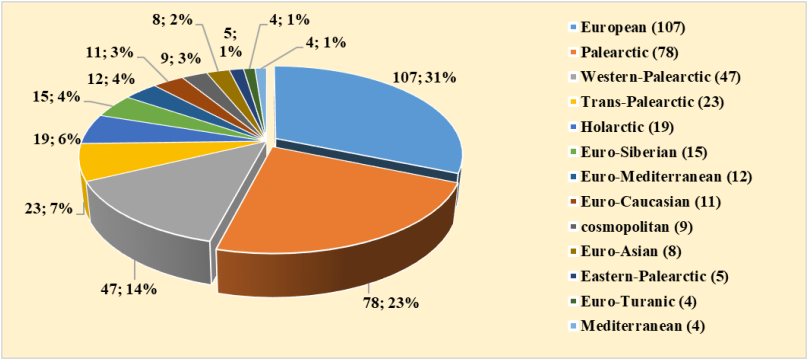


Figure 6.5.1. Zoogeographic analysis of saproxylic coleopteran species from the fauna of the Republic of Moldova

7. IMPORTANCE OF SAPROXYLIC BEETLES TO FOREST ECOSYSTEMS AND DAMAGE TO HERITAGE PIECES AND FORESTS

7.1. The role of saproxylic coleopterans in forest ecosystems

Saproxylic beetles are among the basic components of forest fauna due to their diversity and their particularly important role in ecosystem services, such as dead wood decomposition and nutrient recycling [39]. Forests cover approximately 30% of the planet's land surface and are home to 80% of the planet's terrestrial species [39]. Forests perform many ecological services, such as oxygen production, carbon sequestration, but also wood production [37]. About a quarter of the species inhabiting forest ecosystems in European countries depend on dead wood [35]. Due to the irrational management of forests, by removing dead wood and replacing deciduous trees with coniferous, which grow faster, the saproxylic fauna suffered a lot [34]. In the past (until 1989), the conservation of biodiversity was not part of the silvicultural objectives in the management of the forest fund. Old and mature trees affected by natural calamities or biotic factors were selectively harvested from the forest fund with the aim of protecting the forest from pests. The lack of ecosystem heterogeneity with old trees affected by fungi, or dead mature trees and the presence of a low amount of dead wood, put many species of saproxylic beetles at risk.

The conservation of some saproxylic species can be achieved by implementing good practices to stop the loss of species' habitats and by increasing the connectivity between habitats. The saproxylic beetles are used in forest conservation actions, the presence or absence of threatened species in the investigated samples is basic information widely used in the elaboration and implementation of conservation actions. The rate at which dead wood decomposes depends on a host of climatic and biological factors, including how quickly the substrate is populated by xylophagous beetles. Depending on these factors, the time for complete decomposition of dead wood is from 5-7 to 100 years or more. The decomposition of dead wood depends on precipitation, temperature, the position of the wood on the ground or vertical, its thickness, the tree species, the age and class of the wood, the xylophagous fungi and then the xylophagous coleopters of the subfamily Scolitinae (Curculionidae). These

are among the first insects that populate dead wood. The scolitins bore holes in the bark, facilitating the access of other xylophagous organisms. The saproxylic beetles are important trough theie contribution to the control of pests in forest ecosystems, for example the action of saproxylic zoophages on xylophagous beetles of the subfamily Scolytinae. Adult pollen-consuming saproxylic beetles also play an important role in the pollination of flowering plants. Saproxylic beetles are bioecological indicators of old forests [12; 33]. The saproxylic coleopterans are very important for forest ecosystems because they also interact with other groups of organisms and are an important source of food.

7.2. Harmful and invasive saproxylic coleopteran species for the investigated ecosystems

The saproxylic beetles also have a negative role from an economic point of view. Bark beetles and wood caries are dangerous pests of forests [43] because they reduce the quality of wood and infest it with fungi. Most saproxylic species of the families Buprestidae, Bostrichidae, Cerambycidae, Curculionidae and Ptinidae are xylophagous, and some species of the families Buprestidae and Cerambycidae even depend on the wood of living trees. The xylophagous species harmful to the researched forest ecosystems are *Agrilus biguttatus, Agrilus laticornis, Agrilus viridis, Agrilus sulcicollis, Chrysobothris affinis* and *Dicerca aenea* – from family Buprestidae*; Bostrichus capucinus* from family Bostrychidae*; Anobium punctatum, Oligomerus brunneus, Ptilinus pectinicornis, Xestobium rufovillosum* – from family Ptinidae*; Xylotrechus rusticus, Hylotrupes bajulus, Plagionotus arcuatus, Plagionotus detritus, Saperda octopunctata, Saperda punctata, Saperda scalaris, Saperda populnea, Tetropium fuscum, Rhagium inquisitor, Callidium violaceum, Chlorophorus varius, Isotomus speciosus* and *Monochamus sutor* – from family Cerambycidae*; Xyleborus monographus, Xyleborus dryographus, Xyleborus dispar, Xyleborinus saxesenii, Platypus cylindrus, Scolytus carpini, Scolytus multistriatus* and *Scolytus scolytus* – from family Curculionidae.

The wood stored and untreated against forest pests, but used in the manufacture of furniture and other wooden objects, may contain xylophagous species such as *Anobium punctatum, Ptilinus pectinicornis, Xestobium rufovillosum, Oligomerus brunneus* etc., which will destroy the wood over time.

The most abundant pest species in the extracted samples were *Xyleborus monographus* and *Platypus cylindrus*. They live in symbiosis with ascomycete fungi (Ascomycota) of the genera *Ceratostomella* and *Raffaelea* for *Platypus* and *Raffaelea* for *Xyleborus*, respectively.

The xylophagous saproxylic beetles, although considered harmful to the wood industry, in nature are beneficial because they participate in the decomposition of dead wood and the recycling of organic substances.

At the same time, saproxylic species are a source of food for other groups of organisms, regulators of the numbers of some invertebrate species, consumers and disseminators of xylophagous fungi useful in the degradation of dead wood.

Among the biological substances effective in combating harmful species are the bactericidal preparations obtained on the basis of *Bacillus thuringiensis kurstaki*. Preparations based on mushrooms are those that contain the fungus *Beauveria bassiana*. Preparations with mycelium in nature do not always show good results because the fungus needs high humidity 92-94% for germination, which is difficult to ensure (infestation of xylophages). Entomophagous insects are very useful in combating harmful xylophages. Ants, zoophagous coleopterans (Cleridae, Histeridae, Tenebrionidae, Zopheridae, Carabidae), birds and insectivorous mammals are quite important in combating harmful xylophages.

Among the alien saproxylic coleopteran species for the fauna of the Republic of Moldova are *Xyleborinus saxesenii*, *Monochamus sutor* and *Neoclytus acuminatus*. Of these, only the species *N. acuminatus* is invasive, it being originally from North America, currently it occurs in Europe with a relatively wide distribution. It attacks ash and oak, but also other hardwood species and sometimes shrubs and vines. The larvae feed on the sapwood of weakened or dead trees. Feeding larvae can cut off sap flow and weaken the trunks of young trees, making them vulnerable to strong winds.

7.3. The damage caused by the xylophagous coleoptera to heritage objects

The xylophagous beetles are consumers of dry dead wood or contaminated with xylophagous fungi. Under favorable conditions, several generations can develop on the same substrate, but usually some individuals migrate in search of other sources of food and mates. The number of generations of beetles living inside the food source (icons, old furniture) will be maintained as long as the conditions are favorable and the food persists. It is difficult to combat them because they have a cryptic way of life, and their detection occurs many years after infecting, once the damage caused is ascertained.

Through the Natura journal between February and May 2023, information was collected about saproxylic beetles from the wood of old icons. The information was sent to the editorial office of the journal and directly to the organizers. The qualitative images of the received coleopteran adults allowed the identification of the species *Anobium punctatum* from Bălți and Covurlui (Leova), *Xestobium rufovillosum* and *A. punctatum* from Rădenii Vechi; *Oligomerus brunneus*, from Sociteni (Ialoveni) and *Ptilinus pectinicornis* and *X. rufovillosum* from Sadova (Călărași) and Chișinău. The species *Lyctus linearis*, *Priobium carpini*, *Bostrichus capucinus* and *Cacotemnus rufipes* are present in the collections in the country. Even though some species of beetles are harmful because they destroy our wooden goods, in nature xylophagous beetles are useful, they together with xylophagous fungi decompose the dead wood and turn it into nutrients for plants.

7.4. Impact of forest management on the structure and species richness of saproxylic beetles

In the natural forests of Europe, the amount of dead wood constitutes up to 25% of the amount of wood in the forest. Such forests are rare, but they provide the

widest conditions for the diversity of saproxylic species. Contemporary forest ecosystems are characterized by "induced forest sanitation", or the clearing of forests of dry wood. Defective management of forests leads to the reduction of forested areas, to the fragmentation and loss of connectivity between habitats, to the decrease in the amount of dead wood in forests [35], as well as its quality, as a result the populations of saproxylic beetles are decreasing or even threatened with extinction.

A natural forest, over 450 years old, for example the Bialowieza Forest, contains about 3.3 m^3 of dead wood (a vigorous old tree). In reality, due to climate change, more and more trees are dying every year. Saproxylic beetles are more abundant in old than in young forests, may actually be more naturally abundant in unmanaged forests, and less abundant in long-term managed forests. For monocultures the abundance is even lower. Saproxylic species in northern Europe have been endangered by intensive forest exploitation, which has made these species rarer and threatened with extinction [36]. The substitution of forest species results in a change or even a decrease in the composition of saproxylic coleopteran species. Removal of saproxylic beetle species along with dead wood negatively influences the level of decay of the remaining wood and affects energy flows in the habitat. Saproxylic beetles need thick stems on foot, but also logs with thick and thin branches. It is very dangerous to leave the cut logs for a season in the forest and then extract them for fire. Also, the peeling of forest logs has a negative impact on species whose larval development depends on tree bark.

Many species of saproxylic beetles are considered "pests" to trees because their outbreaks weaken trees and lead to the development of fungal infections, usually resulting in the death of infested trees and/or the loss of large areas of forest. Wood-boring beetles are considered to be one of the most serious problems in wood production and are the reason for many studies aimed at finding methods to prevent or mitigate such outbreaks. Forest management neglects the natural role of these common saproxylic species. In long-term managed forests, the diversity of saproxylic coleopterans decreases [35; 38]. Irrational forest management puts saproxylic coleopterans at risk of extinction [28], or diminishes their diversity, some of which end up being included in the International Red List for Nature Conservation (IUCN), or national lists. In Europe, saproxylic coleopterans are becoming increasingly endangered due to the reduction of habitats, their fragmentation and the extraction of dead wood [38]. The conservation of saproxylic beetle species requires the creation of strictly protected areas or at least substantial changes in forest management practices that result in reduced exploitation and the preservation of large numbers of dead trees and large amounts of rotten wood in forests. Currently forest management practices attempt to vitally restore fully functioning ecosystems by incorporating dead wood into managed habitats. The natural process of restoring the habitat with a sufficient amount of dead wood required by the saproxylic fauna can take more than a hundred years.

The rational management of forests can be a solution in stopping the loss of biodiversity. In the Republic of Moldova, the conservation of saproxylic species can

be carried out in nature reserves. For saproxylic species, the amount of dead wood stored in forest ecosystems is very important, the threshold for maintaining most saproxylic species ranges from 20 m^3/ha to 50 m^3/ha, while some species require more than 100 m^3/ha. The preservation of large dead wood in forests is very important for large coleopteran species [15], but no less important for saproxylic coleopterans is the presence of thin branches and trees weakened on the leg. Dead wood plays an important role in forest ecosystems, contributing to carbon storage, building and enriching soil with nutrients, retaining water, reducing soil erosion, providing shelter for numerous invertebrates and vertebrates, and serving as a food source for various species, including for saproxylic coleopterans [15; 38; 39]. The greatest richness of saproxylic species was found in the old forest ecosystems of the Scientific Reserves "Pădurea Domnească", "Plaiul Fagului", "Codrii" and the Lower Dniester National Park. In the Republic of Moldova the of old forest stands are very rare, practically there are no ecosystems from which dead wood is not extracted. However, the largest amount of dead wood, according to assessments made during the material collection period, was in the strictly protected areas of the Scientific Reserves "Plaiul Fagului", "Pădurea Domnească" and "Codrii".

The practice of extracting dead wood from forests is an inefficient one, in the absence of natural predators that depend on dead wood, harmful xylophagous species multiply en masse and damage weakened trees. Currently, this phenomenon is also favored by climate changes, namely high temperatures and lack of precipitation, the trees becoming weaker and easier to attack.

Conservation of saproxylic beetles requires large areas of intact primary forest and conscientious forest management. The decrease in the amount of wood, entails the decrease in the number of all species that depend on it. Various species of plants, mosses, lichens and fungi grow in the dead wood. Some species of mammals (bats) and amphibians (frogs, salamanders) hibernate in dead wood. In the dead wood, some species of mammals build their burrows (wild cat, squirrel, marten), or some birds (woodpeckers, owls) nest. All the mammals and birds mentioned also feed on wood-dwelling insects (https://en.wikipedia.org/wiki/Lucanus_cervus). The dead wood accumulates water, influencing the microclimate below the tree canopy. At the same time, it is the host of numerous species of useful zoophagous invertebrates, which can control the population of some xylophagous species. Xylophagous beetles are not essentially harmful as foresters confirm and there are few initiator species in the colonization of weakened trees or dead wood. Removing dead wood from forests lacks the ability to effectively eradicate pest populations. Without dead wood, forest ecosystems become less resistant to natural disasters, which will therefore also affect people's quality of life.

In order to protect saproxylic coleopterans from the fauna of the Republic of Moldova, a positive attitude towards their habitats must be shown - natural forests, old trees, logs and wood fallen on the ground to be maintained until reintegration into the natural circuit. Stopping the extraction of dead wood from forests is a fact that must be brought to the attention of the competent authorities and the entire society. The study based on saproxylic coleopterans reflects the state and

functioning of the biodiversity of forest ecosystems in the Republic of Moldova. An effective forest management requires special training, involvement, and constructive collaboration on the part of researchers, forest sector managers, as well as local and national authorities.

GENERAL CONCLUSIONS

1. For the first time in the fauna of the Republic of Moldova, an analysis of the diversity of saproxylic coleopterans was carried out, highlighting 342 species, 236 genera and 47 families. The data were chronologically analyzed and provided information about the saproxylic coleoptera stored in the entomological collections in the country, establishing the presence in the MNEIN collection of 137 species from 102 genera and 23 families, dating from 1901 to 1939; the MEIZ collection preserves 303 species from 215 genera and 47 families, dating from 1911 to the present; the IGFPP collection stores 32 species from 25 genera and 10 families, collected from 1957-1989 and the MUSM collection stores 7 species, 6 genera and one family, collected from 1952 to 2005.
2. Using the "DNA barcoding" technique, 18 species of saproxylic beetles were determined from damaged specimens of adults and larvae (*Agathidium nigripenne, Anaspis frontalis, Batrisodes unisexualis, Brassicogethes aeneus, Cidnopus pilosus, Dyschirius globosus, Euconnus fimetarius, Gyrophaena manca, Rhopalocerus rondanii, Scaphisoma agaricinum, Scolytus multistriatus, Sepedophilus bipunctatus, S. pedicularius, S. testaceus, Stenus ochropus, Trichonyx sulcicollis, Xyleborinus saxesenii* and *Xyleborus dryographus*), the sequences of which have been deposited in GenBank.
3. Using the molecular method, the following microscopic fungi were identified for the first time in the fauna of the Republic of Moldova: *Acrodontium salmoneum, Alternaria alternata, A. infectoria, A. tenuissima, Aureobasidium pullulans, Cladosporium herbarum, C. cladosporioides, Filobasidium magnum, Botrytis* sp., *Fomes fomentarius, Lophiostoma* sp., *Metschnikowia pulcherrima, Myrmecridium* sp., *Parathyridaria flabelliae, Penicillium citreonigrum, Peniophora cinerea, Querciphoma carteri* and *Sarocladium bacillisporum*, isolated from the body of xylophagous species *Dryocoetes alni, Scolytus carpini, Stereocorynes truncorum, Platypus cylindrus* and *Xyleborus monographus*. The species *A. alternate, A. tenuissima, Q. carteri, C. herbarum, A. salmoneum* and *P. cinerea* under certain conditions can act as phytopathogenic agents for numerous plant species. The species *F. fomentarius* is an important food source for some species of fungal coleopterans, but for trees it is a saprophytic and parasitic fungus that damages trees.
4. At the first report in the fauna of the Republic of Moldova, 89 species of saproxylic coleoptera are mentioned, assigned to 47 genera and 5 families. For the species *Abdera quadrifasciata, Aesalus scarabaeoides, Diaclina testudinea, Neoclytus acuminatus, Eledonoprius armatus* and *Sunius fallax*, it was established that the Republic of Moldova is the limit of the South East area of

Central Europe, except for the invasive species *Neoclytus acuminatus*, the other 5 species are rare and depend on old forests with a large amount of dead wood and affected by fungi.

5. It was established that trophically the fauna of identified saproxylic coleopterans corresponds to the groups xylophagouss – 86 species, mycetophagous – 76, saprophagous (detritophagous, xylosaprophagous, which consume sap) – 86, zoophagous – 73 and polyphagous – 9. According to the distribution area, there are 107 of European species, 78 – Palearctic, 47 – West-Palearctic, 23 – Trans-Palearctic, 19 - Holarctic, 15 – Euro-Siberian, 12 species – Euro-Mediterranean, 11 – Euro-Caucasian, 9 – cosmopolitan, 8 – Euro-Asian, 5 – East-Palearctic, 4 – Euro-Turanic and Mediterranean species each.
6. 78 species indicators of the good ecological status of the investigated forests were identified, assigned to 72 genera and 26 families. The presence of 12 rare and endangered species belonging to 12 genera and 6 families was confirmed in the forest ecosystems of the republic. The possible degree of rarity was established for 14 saproxylic species of the Tenebrionidae family, which especially includes useful mycetophagous saproxylic species used in habitat quality assessment.
7. The particularly important role for forest ecology of saproxylic saprophagous coleopterans, involved in dead wood decomposition and nutrient recycling, has been demonstrated; of zoophages in the control of xylophagous pests; in the pollination process; important link of the food chain. At the same time, 33 species present a negative role from an economic point of view, affecting live trees, including processed wood. The xylophagous species *Anobium punctatum*, *Xestobium rufovillosum*, *Ptilinus pectinicornis* and *Oligomerus brunneus* were confirmed in the heritage objects in the country.
8. The analysis of the saproxylic coleoptera species in association with the host trees, revealed that the most numerous were the species dependent on the native trees. 122 species were identified from the oak; poplar – 49; beech – 23; ash – 17; birch and elm 7 species each; willow, pine, acacia and linden 2 species each; one species each was reported on cherry, hornbeam and maple. However, the stage of wood decay is more important than the diameter or tree species. The rare species *Megatoma undata*, *Tenebrio opacus*, *Ischnodes sanguinicollis*, *Neatus picipes* and *Cerophytum elateroides* reported in "Plaiul Fagului" and the water protection strip from Măcărești were identified from the tree barks.
9. The use of trunk traps at varuious level allowed the identification of rare species for local and regional entomofauna. Among them, the species *Lichenophanes varius* and *Oxylaemus cylindricus* collected only at a height of 6 meters from the ground, *Xylophilus testaceus* – at 4 and 6 m and *Stenagostus rhombeus* – at 2, 4 and 6 m stand out; the species *Dirrhagofarsus attenuates*, *Trichoferus pallidus, Lopheros rubens, Melasis buprestoides* and *Eledonoprius armatus* – at 4 m from the ground. The species *Siagonium humerale*, *Attagenus punctatus* and *Hypophloeus bicolor* – at 2 and 4 m; the species *Pycnomerus terebrans*,

Nosodomodes diabolicus, Corticeus fasciatus, Hedobia imperialis and *Mesosa curculionoides* – at 2 m high on trunks per foot.

10. The collection of saproxylic coleoptera as part of the faunal heritage was elaborated, which includes 240 species assigned to 184 genera and 46 families mounted in 12 entomological boxes. The materials have scientific value in local, regional and international aspects, educational value for students and pupils and museum value for the country's heritage.
11. It was found that only in the Scientific Reserves "Plaiul Fagului" and "Pădurea Domnească" is the limit of 20 m^3/ha and more of dead wood is permanently provided until the integration into the soil. Rare saproxylic beetles are dependent on successive stages of wood decay and connectivity between habitats, they serve as bioindicators for conservation planning of forest ecosystems with optimal value and functional resistance to climate change.

PRACTICAL RECOMMENDATIONS

1. In forest ecosystems affected by pests, to increase the abundance and diversity of useful saproxylic coleopteran species, it is necessary to enrich the habitat with freshly cut wood, which attracts many useful species and contributes to changing their composition, which will diminish over time phytophagous and xylophagous species- mycetophagous.
2. Dry forest sectors must be checked for the presence of pests, which, if left unchecked, can affect the entire ecosystem. The presence of pests or symptoms of attack requires individual measures of action. Most commonly, the wood of live or weakened trees that are attacked by pests has exit holes, resin, sawdust or sap flowing on the stem. To control the multiplication of xylophagous pests, the affected trees must be peeled, in the absence of moisture the pests die and the spread of pathogenic fungi associated with beetles is limited.
3. Xylophagous beetles due to their cryptic way of life are difficult to detect, the use of flight interception traps placed on the trunk are the most effective in detecting pests. Some species attack the trees in the crown, which makes it necessary to examine the attack with the help of drones.
4. In the case of establishing outbreaks of pests in managed and semi-managed sectors, specific methods can be applied to the detected group. Trees that are dry and infested with xylophagous pests must be extracted from the forest and dried in special ovens, or burned immediately, in order not to allow the pests to mature, survive and spread to new territories. Or the trees should at least be stripped to expose the slime to the sun, to dehydrate both the eggs, larvae and pupae thus stopping the spread of the pest. Nocturnal xylophagous species can be caught using light traps. For species that are attracted by pheromones, pheromonal traps can be used during the mating period.
5. Insecticides are not effective in forest ecosystems to combat xylophagous coleopterans and their application is not recommended because they will destroy mostly the useful fauna. Biological preparations based on microorganisms can be

successfully used. They are formed on the basis of viruses, bacteria and fungi. Biological control methods, however, require certain conditions: temperature, optimal humidity and time, but they are non-pathogenic for entomophages.

6. The entomophagous species are very useful in keeping xylophagous ones at a minimum level of damage. Among the most useful entomophages for forest ecosystems are the ants, the coleopteran species that in the larval and adult stages consume xylophagous larvae (Cleridae, Histeridae, Tenebrionidae), the parasitoid hymenopterans that lay eggs in the larvae of xylophagous coleopterans on which hymenopteran larvae feed, birds and insectivorous mammals.

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Ghiduri metodice/metodologice (aprobate de consiliul metodic, consiliul facultăţii)

1. BACAL, S. Coleopterele saproxilice şi rolul lor pentru ecosistemele forestiere. Ghid ştiinţifico-metodic. Chişinău, 2023, S.R.L. Căpăţînă-Print. 94 p.
2. BACAL, S.; COCÎRȚĂ, P.; MUNTEANU, N. Metode şi echipament de colectare a artropodelor. Ghid ştiinţifico-practic. Chişinău, 2014.Tipografia AȘM. Chişinău, 2014, 88 p.
3. BUȘMACHIU, G.; ENCIU, E.; BACAL, S. ș.a. Metode de cercetare a colembolelor şi insectelor: Ghid metodologic. Universitatea de Stat din Moldova, Institutul de Zoologie. Chişinău. 2023, S.R.L. Căpăţînă-Print. 74 p.

ADNOTARE

Bacal Svetlana „Coleopterele saproxilice (Insecta) din Republica Moldova: taxonomie, ecologie, zoogeografie și importanță", teză de doctor habilitat în științe biologice, Chișinău, 2024.

Structura tezei: introducere, șapte capitole, concluzii generale și recomandări practice, bibliografia include 537 de titluri, 8 anexe, 216 pagini text de bază, 37 figuri, 50 tabele. Rezultatele cercetărilor sunt publicate în 97 de lucrări științifice.

Cuvinte-cheie: coleoptere saproxilice, taxonomie, diversitate, distribuție, ecosisteme forestiere, ecologie, importanță.

Scopul lucrării: evaluarea complexă a diversității taxonomice a speciilor saproxilice din ordinul Coleoptera, stării actuale, tendințelor, răspândirii zoogeografice, particularităților ecologice și importanței lor în condițiile intensificării acțiunii antropice și schimbărilor climatice actuale asupra ecosistemelor silvice din Republica Moldova.

Obiectivele cercetării: stabilirea componenței speciilor de coleoptere saproxilice din ecosistemele forestiere naturale și antropizate și a speciilor din colecțiile entomologice din Republica Moldova; identificarea taxonilor noi de coleoptere saproxilice în fauna țării; analiza grupelor trofice și a distribuției zoogeografice a coleopterelor saproxilice asociate cu lemnul mort din ecosistemele forestiere; identificarea speciilor indicatoare a pădurilor bătrâne și a celor rare pentru fauna republicii; identificarea speciilor morfologic identice prin metode molecular genetice; evaluarea rolului coleopterelor saproxilice în ecosistemele forestiere naturale și antropizate; identificarea speciilor vectori și a fungilor saprofagi; crearea primei colecții de coleoptere saproxilice; stabilirea impactului managementului pădurilor asupra structurii și bogăției speciilor de coleoptere saproxilice.

Noutatea și originalitatea științifică: Lucrarea cuprinde rezultate originale privind 342 de specii de coleoptere saproxilice identificate în diverse ecosistemele forestiere din țară. În premieră pentru Republica Moldova, au fost identificate 5 familii, 47 de genuri și 89 de specii, 18 specii au fost identificate prin metoda molecular-genetică, secvențele cărora au fost depuse în GenBank. A fost realizată analiză filogeografică a 18 specii saproxilice. Datele genetice obținute contribuie la completarea bazelor de date genetice, studiile de filogenie devenind disponibile la nivel european. Au fost stabilite în premieră în Republica Moldova 18 specii de fungi saprofagi transmiși de coleopterele xilomicetofage, din care speciile *Alternaria alternata* și *A. tenuissima* pot fi agenți patogeni ai arborilor.

Rezultatele principale: Au fost identificate 342 de specii, care fac parte din 236 de genuri și 47 de familii de coleoptere saproxilice din diverse ecosistemele forestiere din țară. A fost creată colecția de coleoptere saproxilice care include peste 2 mii de exemplare. Au fost confirmate 78 de specii bioindicatoare a pădurilor bătrâne, identificate 12 specii rare și periclitate, 33 de specii xilofage dăunătoare, 10 xilofage/micetofage dăunătoare pentru ecosistemele forestiere și 4 specii dăunătoare obiectelor de patrimoniu. În premieră pentru țară, a fost propus un sistem de clasificare a speciilor saproxilice din familia Tenebrionidae pe categorii de raritate.

Semnificația teoretică: A fost elaborată o concepție nouă privind structura actuală a speciilor de coleoptere saproxilice în ecosistemele forestiere, distribuția lor pe verticală, orizontală și în plan regional în condițiile modificărilor antropice și climatice. A fost întocmită în premieră listă a 342 de specii, identificate preferințele lor față de arborii gazdă autohtoni și invazivi, stabilite relațiile lor trofice, evidențiate speciile vectori ai fungilor saprofagi și identificați agenții patogeni ai arborilor. La prima semnalare în fauna țării sunt 89 de specii de coleoptere saproxilice, care extind nivelul cunoașterii distribuției și ecologiei lor. În premieră pentru Republica Moldova au fost identificați fungii microscopici saprofiți și paraziți din ecosistemele silvice, ale căror vectori de dispersie sunt speciile de coleoptere saproxilice. Cunoașterea fungilor saprofiți și patogeni este importantă la planificarea activităților de împădurire și protecție a pădurilor.

Valoarea aplicativă: Datele obținute privind diversitatea și ecologia coleopterelor saproxilice, permit utilizarea lor ca bioindicatori ai pădurilor bătrâne, planificarea conservării și managementul durabil al ecosistemelor forestiere cu o valoare optimă și rezistență funcțională la schimbările climatice. Rezultatele obținute sunt parte componentă a temelor științifice de cercetări fundamentale și aplicative realizate în cadrul Institutului de Zoologie, USM din perioada 2008-2023. Rezultatele sunt utile lucrătorilor silvici, cercetătorilor din domeniile biologie, ecologie, cadrelor didactice și autorităților de mediu în protecția biodiversității, monitoring, controlul xilofagilor și speciilor invazive.

Implementarea rezultatelor științifice: Rezultatele obținute au fost publicate în 97 de lucrări științifice, o monografie monoautor, coautor la 4 monografii, 2 broșuri și 2 ghiduri de specialitate. Colecția de coleoptere saproxilice este un suport științific și educațional pentru cercetători și studenți din domeniu. Lista întocmită a coleopterelor saproxilice completează informația faunistică pentru spațiul Europei de Sud-Est. Coleoptere saproxilice din familia Stapylinidae au fost utilizate la întocmirea Catalogului Coleopterelor Palearctice. Rezultatele științifice sunt incluse în 7 acte de implementare a organizațiilor și instituțiilor de profil: Universitatea de Stat din Moldova, Rezervațiile științifice Plaiul Fagului, Pădurea Domnească, Prutul de Jos și Întreprinderea municipală „Asociația de Gospodărire a Spațiilor Verzi", care au utilizat rezultatele în procesul educațional și în rapoartele științifice anuale.

АННОТАЦИЯ

Бакал Светлана «Сапроксильные жёсткокрылые (Insecta) Республики Молдова: Таксономия, экология, зоогеография и значение», диссертация на соискание учёной степени доктора хабилитат биологических наук, Кишинёв, 2024.

Структура диссертации: введение, 7 глав, общие выводы и практические рекомендации, список литературы из 537 наименований, 8 приложений, 216 страниц основного текста, 37 рисунков и 50 таблиц. Полученные результаты опубликованы в 97 научных работах.

Ключевые слова: сапроксильные жесткокрылые, таксономия, разнообразие, зоогеографическое распространение, лесные экосистемы, экология, значение.

Цель исследования: комплексная оценка таксономического разнообразия сапроксильных видов жуков отряда Coleoptera, их современного состояния, тенденций, зоогеографического распространения, экологических особенностей и значения в условиях усиления антропогенного воздействия и современных климатических изменений лесных экосистем Республики Молдова.

Задачи исследования: установление состава сапроксильных видов жесткокрылых природных и антропогенных лесных экосистем, а также видов, хранящихся в энтомологических коллекциях Республики Молдова; выявление новых таксонов сапроксильных жуков в фауне страны; анализ трофических групп и зоогеографического распределения жуков обитающих в мёртвой древесине лесных экосистем; выявление видов-индикаторов старых лесов и редких для фауны республики; идентификация морфологически идентичных видов молекулярно-генетическим методом; оценка роли сапроксильных жесткокрылых в природных и антропогенных лесных экосистемах; идентификация видов-переносчиков сапрофитных грибов; создание первой коллекции сапроксильных жуков; установление влияния лесопользования на структуру и богатство сапроксильных видов жесткокрылых.

Научная новизна и оригинальность: Работа включает оригинальные данные по 342 видам сапроксильных жуков, выявленных в различных лесных экосистемах страны. Впервые для страны идентифицировано 5 семейств, 47 родов и 89 видов, молекулярно-генетическим методом определены 18 видов, генетический материал которых депонирован в GenBank. Проведен филогеографический анализ 18 европейских сапроксильных видов жесткокрылых. Полученные генетические данные способствуют пополнению генетических баз данных, а исследования филогении являются доступными на европейском уровне. Впервые для страны установлены 18 видов грибов-сапрофагов, переносимых жесткокрылыми ксиломицетофагами, из них виды *Alternaria alternata и A. tenuissima* могут быть возбудителями болезней для растений.

Основные результаты: Выявлено 342 вида сапроксильных жесткокрылых, принадлежащих к 236 родам и 47 семействам из различных лесных экосистем страны. Создана коллекция сапроксильных жесткокрылых, насчитывающая более 2 тысяч экземпляров. Подтверждено присутствие 78 видов-биоиндикаторов старовозрастных лесов, выявлено 12 редких и находящихся под угрозой исчезновения видов, 33 вредных видов-ксилофагов, 10 видов-ксилофагов/мицетофагов, вредных для лесных экосистем и 4 вида вредителей объектов наследия. Впервые для страны предложена система классификации сапроксильных видов семейства Tenebrionidae по категориям редкости.

Теоретическая значимость: Разработана новая концепция современной классификации сапроксильных видов жесткокрылых лесных экосистем, их вертикального, горизонтального и регионального распределения в условиях антропогенного прессинга и климатических изменений. Составлен список из 342 видов, выявлены их предпочтения к аборигенным и инвазионным деревьям-хозяевам, установлены их трофические взаимоотношения, выделены виды-переносчики грибов-сапрофагов и идентифицированы древесные патогены. Впервые в фауне страны выявлены 89 видов сапроксильных жесткокрылых, что значительно расширяет знания об их распространении и экологии. Впервые для Республики Молдова идентифицированы сапрофитные и паразитические микроскопические грибы древесины лесных экосистем, векторами распространения которых являются сапроксильные виды жуков. Знание сапрофитных и патогенных грибов важно при планировании лесонасаждений и лесозащитных мероприятий.

Прикладное значение: Полученные данные о разнообразии и экологии сапроксильных жесткокрылых позволяют использовать их в качестве биоиндикаторов старых лесов, природоохранного планирования и устойчивого управления лесными экосистемами с оптимальной ценностью и функциональной устойчивостью к изменению климата. Полученные результаты являются частью научных тем фундаментальных и прикладных исследований, проводимых ИЗ, ГУМ в 2008-2023 гг. Результаты будут полезны работникам лесного хозяйства, исследователям в области биологии, экологии, преподавателям и природоохранным органам при защите биоразнообразия, мониторинга, борьбе с ксилофагами и инвазивными видами.

Внедрение научных результатов: Полученными результаты опубликованы в 97 научных работах: 1 авторской монографии, 4 монографий в соавторстве, 2 брошюрах и 2 методических пособиях. Созданная коллекция сапроксильных жуков послужит научным и образовательным пособием для учёных и студентов биологов и экологов. Составленный список сапроксальных жесткокрылых пополнит фаунистические сведения для Юго-Восточной Европы. Выявленные сапроксильные жуки семейства Stapylinidae были использованы для составления Каталога жесткокрылых Палеарктики. Научные результаты внедрены в работу 7 организаций и учреждений: ГУМ, Научных заповедников «Plaiul Fagului», «Pădurea Domnească», «Prutul de Jos» и муниципального предприятия «Asociația de Gospodărire a Spațiilor Verzi», которые используют результаты в учебном процессе и в годовых научных отчетах.

ANNOTATION

Svetlana Bacal „Saproxylic Coleoptera (Insecta) from the Republic of Moldova: taxonomy, ecology, zoogeography and importance”, habilitation thesis in biological sciences, Chișinău, 2024.

Thesis structure: introduction, 7 chapters, general conclusions and practical recommendations, bibliography of 537 titles, 8 appendices, 216 pages of main text, 37 figures and 50 tables. The obtained results were published in 97 scientific papers.

Keywords: Saproxylic Coleoptera, taxonomy, diversity, zoogeographical distribution, forest ecosystems, ecology, importance.

The aim of the work: a comprehensive assessment of the taxonomic diversity of saproxylic species of beetles of the order Coleoptera, their current state, trends, zoogeographical distribution, ecological features and significance in the context of increasing anthropogenic impact and modern climate changes in the forest ecosystems of the Republic of Moldova.

Research objectives: establishing the composition of saproxylic species of beetles in natural and anthropogenic forest ecosystems, as well as species stored in entomological collections of the Republic of Moldova; identification of new taxa of saproxylic beetles in the fauna of the country; analysis of trophic groups and zoogeographic distribution of beetles living in dead wood of forest ecosystems; identification of indicator species of old forests and rare species for the fauna of the republic; identification of morphologically identical species using molecular genetic methods; assessment of the role of saproxylic Coleoptera in natural and anthropogenic forest ecosystems; identification of vector species of saprophytic fungi; creation of the first collection of saproxylic beetles; establishing the influence of forest management on the structure and richness of saproxylic beetle species.

Novelty and scientific originality: the work includes original data on 342 species of saproxylic beetles identified in various forest ecosystems of the country. For the first time for the Republic of Moldova, 5 families, 47 genera and 89 species were identified, 18 species were identified using the molecular genetic method, genetic material of which was deposited in GenBank. A phylogeographic analysis of 18 European saproxylic beetle species was carried. The resulting genetic data contribute to the development of genetic databases, and phylogeny studies are available at European level. For the first time in the Republic of Moldova, 18 species of saprophagous fungi transmitted by xylomycetophagous beetles have been identified, of these, the species *Alternaria alternata* and *A. tenuissima* can be pathogens for plants.

Main results: A total of 342 species of saproxylic Coleoptera have been identified, belonging to 236 genera and 47 families from various forest ecosystems of the country. A collection of saproxylic Coleoptera has been created, numbering more than 2 thousand specimens. The presence of 78 bioindicator species of old-growth forests was confirmed, 12 rare and endangered species, 33 harmful xylophagous species, 10 xylophagous/mycetophagous species harmful to forest ecosystems and 4 species of heritage site pests were identified. For the first time in the country, a classification system for saproxylic species of the family Tenebrionidae according to rarity categories has been proposed.

Theoretical significance: A new concept of modern classification of saproxylic beetles species from the forest ecosystems, their vertical, horizontal and regional distribution under conditions of anthropogenic pressure and climate change has been developed. A list of 342 species has been compiled, their preferences for native and invasive host trees have been identified, their trophic relationships have been established, vector species of saprophagous fungi and tree pathogens were identified. For the first time, 89 species of saproxylic Coleoptera have been revealed in the country's fauna, which significantly expands knowledge about their distribution and ecology. For the first time for the Republic of Moldova, saprophytic and parasitic microscopic fungi from forest ecosystems were identified, whose dispersal vectors are saproxylic coleopteran species. Knowledge of saprophytic and pathogenic fungi is important in planning afforestation and forest protection activities.

Application value: The obtained data on the diversity and ecology of saproxylic beetles allow their use as bioindicators of old forests, environmental planning and sustainable management of forest ecosystems with optimal value and functional resilience to climate change. The research carried out are part of fundamental and applied research conducted by the IZ, SUM in 2008-2023. The results will be useful to forestry workers, researchers in the field of biology, ecology, teachers and environmental authorities in protecting biodiversity, conducting courses, monitoring, combating the xylophages and invasive species.

Implementation of scientific results: The results were published in 97 scientific papers: 1 author's monograph, 4 co-authors monographs, 2 brochures and 2 methodological ghides. The created collection of saproxylic beetles will serve as a scientific and educational tool for scientists and students of biological and environmental faculties. The compiled list of saproxylic Coleoptera species will supplement the faunal information for South-Eastern Europe. The identified saproxylic beetles of the family Stapylinidae were used to compile the Catalog of Coleoptera of the Palaearctic. Scientific results are implemented in the work of 7 organizations and institutions: State University of Moldova, Scientific Reserves „Plaiul Fagului”, „Pădurea Domnească”, „Prutul de Jos” and the municipal enterprise „Asociația de Gospodărire a Spațiilor Verzi”, which use these results in the educational process and in annual scientific reports.

MOLDOVA STATE UNIVERSITY

INSTITUTE OF ZOOLOGY

BACAL SVETLANA

SAPROXYLIC COLEOPTERA (INSECTA) FROM THE REPUBLIC OF MOLDOVA: TAXONOMY, ECOLOGY, ZOOGEOGRAPHY AND IMPORTANCE

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