

RESEARCH ARTICLE – ARTICLES DE RECHERCHE

**NEW SPECIES OF STAPHYLINIDAE (COLEOPTERA) FROM THE REPUBLIC OF MOLDOVA REVEALED BY DNA BARCODING AND MORFOLOGICAL ANALYSIS**Svetlana BACAL¹, Galina BUSMACHIU¹, Oana Paula POPA²¹Institute of Zoology, State University of Moldova, Chisinau, Republic of Moldova²Grigore Antipa National Museum of Natural History, Bucharest, Romania

Corresponding author: Galina Busmachi, e-mail: bushmakiu@yahoo.com

DOI: 10.38045/ohrm.2024.2.02

CZU: 595.763:577.212(478)

Keywords: Staphylinidae, barcoding, DNA, newly recorded species, saproxylic species.

Introduction. Saproxylic beetles are important components of biodiversity and are used for monitoring the quality of old-growth forests. Due to the substantial reduction of forested areas and low availability of dead wood, saproxylic beetles are becoming threatened and many small coleopteran species risk disappearing before they are even discovered.

Material and methods. The research was carried out in one natural forest, three natural and two landscape reserves. Coleoptera species were identified using morphological characters and DNA barcoding. Molecular identification established using the GenBank database was confirmed by querying the BOLD Systems database.

Results. The work includes 9 species of coleoptera: *Batrisodes unisexualis*, *Trichonyx sulcicollis*, *Sepedophilus bipunctatus*, *S. constans*, *S. pedicularius*, *Gyrophaena manca*, *Scaphisoma agaricinum*, *Medon rufiventris* and *Hypnogyra angularis* newly identified for the fauna of the Republic of Moldova. Obtained COI sequences were analysed and compared with European species stored in GenBank. From a phylogeographic point of view, the majority of the sequences from the Republic of Moldova are grouped into private haplotypes, that are distributed only in the analysed region. The species *Gyrophaena manca* has 2 haplotypes, one is present in Finland and Germany, another in the Republic of Moldova.

Conclusions. As a result of the research 9 species of saproxylic coleoptera from the studied natural forests were identified as new for the fauna of the Republic of Moldova. Obtained genetic data are available for future phylogeny, phylogeography and conservation biology studies carried out at European level.

Cuvinte-cheie: Staphylinidae, barcodare, ADN, specii noi înregistrate, specii saproxilice.

SPECII NOI DE STAPHYLINIDAE (COLEOPTERA) DIN REPUBLICA MOLDOVA IDENTIFICATE PRIN BARCODAREA ADN-ULUI ȘI ANALIZA MORFOLOGICĂ

Introducere. Coleopterele saproxilice sunt un component important al biodiversității și sunt utilizate pentru monitorizarea stării pădurilor seculare. Din cauza reducerii substanțiale a suprafețelor împădurite și a volumului redus de lemn mort în păduri, coleopterele saproxilice sunt amenințate și multe specii de dimensiuni mici riscă să dispară, chiar înainte de a fi descoperite.

Material si metode. Cercetarea a fost efectuată într-o pădure naturală, trei rezervații naturale și două peisagistice. Speciile de coleoptere au fost identificate folosind caractere morfologice și prin barcodarea ADN-ului. Veridicitatea identificării moleculare cu GenBank a fost confirmată prin interogarea BOLD Systems.

Rezultate. Lucrarea include nouă specii de coleoptere noi pentru fauna Republicii Moldova: *Batrisodes unisexualis*, *Trichonyx sulcicollis*, *Sepedophilus bipunctatus*, *S. constans*, *S. pedicularius*, *Gyrophaena manca*, *Scaphisoma agaricinum*, *Medon rufiventris* și *Hypnogyra angularis*. Secvențele COI obținute au fost analizate și comparate cu cele europene stocate în GenBank. Din punct de vedere filogeografic, majoritatea speciilor secvențiate din Republica Moldova se grupează în haplotipuri private, care sunt distribuite doar în regiunea analizată. Specia *Gyrophaena manca* are două haplotipuri – unul esre prezent în Finlanda și Germania, iar altul în Republica Moldova.

Concluzii. În urma studiului realizat au fost identificate nouă specii de coleoptere saproxilice noi pentru fauna Republicii Moldova. Genele obținute au fost depozitate în GenBank și sunt disponibile pentru studiile de filogeneză, filogeografie și conservare a biodiversității la nivel european.

INTRODUCTION

Saproxylic beetles are important components of biodiversity and are used for monitoring the quality of old-growth forests (1, 2, 3). Due to the substantial reduction of forested areas and low availability of dead wood, saproxylic beetles are becoming threatened (4), and many small coleopteran species risk disappearing before they are even discovered.

The research was carried out on the dead wood found in several protected areas, in order to provide new data on the fauna of saproxylic staphylinids in the Republic of Moldova. Dead wood is a preferred microhabitat for predatory and mycetophagous staphylinids and it harbors many small and very agile species from which many are still unknown.

In the fauna of the Republic of Moldova, a total of 262 species of staphylinids, belonging to 13 subfamilies were known until now: Aleocharinae, Habrocerinae, Omaliinae, Oxyporinae, Oxytelinae, Paederinae, Piestinae, Proteininae, Pselaphinae, Scaphidiinae, Staphylininae, Steninae and Tachyporinae (5).

To date, 24 species of staphylinids were identified from dead wood (6, 7, 8). Of these, 21 species belonging to the subfamilies Aleocharinae (3 species), Piestinae (1), Scaphidiinae (2), Staphylininae (7), Tachyporinae (8) are considered saproxylic. The following identified species are saproxylic: *Abemus chloropterus* (Creutzer, 1796), *Astrapaes ulmi* (Rossi, 1790), *Atrecus affinis* (Paykull, 1789), *Dinaraea aequata* (Erichson, 1837), *Gabrius splendidulus* (Gravenhorst, 1802), *Gyrophana joi* Wendeler, 1924, *Gyrophana nana* (Paykull, 1800), *Milichilinus decorus* (Erichson, 1839), *Othius punctulatus* (Goeze, 1777), *Scaphidium quadrimaculatum* Olivier, 1790, *Scaphisoma boleti* (Panzer, 1793), *Sepedophilus immaculatus* (Stephens, 1832), *Sepedophilus littoreus* (Linnaeus, 1758), *Sepedophilus marshami* (Stephens, 1832), *Sepedophilus obtusus* Luze, 1902, *Sepedophilus testaceus* (Fabricius, 1793), *Siagonium humerale* Germar, 1836, *Velleius dilatatus* (Fabricius, 1787), *Tachinus corticinus* Gravenhorst, 1802, *Tachyporus hypnorum* (Fabricius, 1775) and *Tachyporus nitidulus* (Fabricius, 1781) (9).

The aim of the present study was to expand the knowledge regarding the composition of the spe-

cies of saproxylic coleoptera in the Staphylinidae family in the Natural and Landscape Reserves of the country with the identification of new species.

MATERIAL AND METHODS

The research was carried out in natural and landscape reserves, which have an important role in the conservation and regeneration of rare species of flora and fauna in the country.

Prutul de Jos Nature Reserve (45.584188N 28.258056E) is a protected area that includes Lake Beleu and the surrounding floodplains, which preserve and restore the species of flora and fauna in this area. The territory of the reserve is mainly occupied by swamp and floodplain vegetation and the waters of Lake Beleu (10).

Pădurea Domnească Nature Reserve (47.609722N 27.393611E) is a natural floodplain forest dominated by oak, poplar and willow species, located in the Prut River floodplain (11). Currently, floodplain ecosystems are rare and threatened in Europe (3). For the protection of these forest ecosystems, a correct management that will also contribute to the ecological recovery of the floodplain ecosystems is necessary.

Plaiul Fagului Nature Reserve (47.291111N 28.054444E) is covered by natural oak forest in combination with sessile oak, ash, hornbeam and beech. The nature reserve is one of the most representative, well preserved and managed forest ecosystems in the central area of the Republic of Moldova (12).

Pohrebeni Landscape Reserve (47.562778N 28.8875E) is a protected area where the vegetation consists of forests of sessile oak, pedunculated oak and maple. The most widespread forest phytocenoses are sessile with linden and ash, which usually are found at higher altitudes, while sessile associations with hornbeam are more common at lower altitudes (10).

Vila Nisporeni Landscape Reserve (47.009444N 28.175651E) consists of sessile oak, downy oak and pedunculated oak forests (10). Currently it is still heavily affected by natural calamities of the spring of 2017, which broke trees under the weight of the snow.

Natural forest near Vulcănești, Nisporeni district (47.153611N 28.191111E), consists of ses-

sile oak, pedunculate oak, maple, hornbeam and linden species (10).

The material was collected from under the bark of dead and decaying trees that was colonized by ants, affected by molds and fungi that grow on dead wood. The extraction was carried out with the help of the entomological exhaustor or fragments of dead wood were examined in the laboratory using a modified flotation method (13).

Coleoptera species that were difficult to identify based on morphological characters were analysed using DNA barcoding. This technique, which revolutionized the study of biodiversity (14), involves sequencing a fragment of the gene for cytochrome C oxidase I and comparing the obtained sequences to those included in the international databases such as GenBank (NCBI) or BOLD (Barcode of Life System) (15). This approach can lead to the identification of species and the discovery of new species, but it is also an important tool in revealing the phylogeographic and evolutionary models of different organisms.

In the present study, a number of 56 samples (whole larvae or fragments, or damaged specimens) collected from the mentioned Natural and Landscape Reserves were analysed using DNA barcoding. DNA isolation was performed using the ISOLATE II Genomic DNA Kit (Bioline meridian BIOSCIENCE®, London, UK), following the manufacturer's specifications. The amount of genomic DNA isolated from each sample was subsequently used to amplify a fragment from the 5' end of the cytochrome C oxidase I gene, a gene extensively used for animal species identification (16).

The fragment was amplified using the universal COI primers HCO2198 (5'-TAAACTTCAGGGTGA CCAAAAAATCA-3') and LCO1490 (5'-GGTCAA CAAATCATAAAGATATTGG-3') (17) marked with M13 tails. The PCR reaction was performed in a total volume of 50 µL containing genomic DNA template, 1X Green GoTaq® Flexi Buffer, 2.5mM MgCl₂, each dNTP at 0.1 mM, 0.5 µM from each primer and 1 unit of DNA polymerase GoTaq® (Promega, Madison, SUA). 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). The corrected sequences were used to interrogate the GenBank and BOLD Systems online databases, in order to identify the species. The GenBank database has a proprietary interface called nucleotide BLAST (Basic Local Alignment Search Tool) that uses an algorithm based on sequence similarity. The interface compares submitted nucleotide sequences with sequences stored in its own database and calculates a statistical significance.

The identity ratio calculated by BLAST represents the percentage of nucleotides that are similar between the subject sequence and the sequences found in the database. BLAST also calculates a degree of overlap which is the percentage of alignment between the 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.

In order to perform a basic phylogeographic analysis and to have an accurate picture of how the phylogenetic lines are spread at the European level, all COI sequences corresponding to the six staphylinid species for which we obtained DNA data were downloaded from online databases. The alignment was made in Mega7 (18) and DnaSP v.5 was used to calculate the number of haplotypes (19). The haplotype network identified for each species was reconstructed under a Median Joining algorithm implemented in PopART v1.7 (20).

Several keys were used to identify the coleopteran species according to morphological characters: (21, 22, 23, 24).

RESULTS

As a result of the investigations, this study has reported 9 species of coleoptera new for the fauna of the Republic of Moldova which are grouped as follows: subfamily Tachyporinae with 3 species, family Pselaphinae with 2 species and subfamilies Aleocharinae, Paederinae, Scaphidiinae and Staphylininae with one species each.

In the analysed samples, the molecular identification detected species from the Staphylinidae fa-

mily, subfamilies: Pselaphinae, Tachyporinae, Aleocharinae and Scaphidiinae. The species from the subfamilies: Tachyporinae, Paederinae and Staphylininae were identified using morphological characters.

Family Staphylinidae Latreille, 1802

Subfamily Pselaphinae Latreille, 1802,

Tribe Batrisini Reitter, 1882

Genus *Batrisodes* Reitter, 1881

Batrisodes (Batrisodes) unisexualis Besuchet, 1988

Identification: DNA barcoding.

Collection data and material examined: 1 ex., 20.V.2022, Pădurea Domnească.

Ecology and biology: collected from decaying wood along with ants from genus *Lasius*. Adult sizes 2.3-2.6 mm (22).

Occurrence: Palaearctic. In Europe: Austria, Czech Republic, Continental France, Germany, Poland,

Slovakia, Sweden, Switzerland, the Netherlands and Ukraine (25).

For *Batrisoides unisexualis* species, seven COI sequences were analysed, six of them mined from GenBank, obtained from specimens collected in Belgium and Germany (tab. 1). 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 Moldova has accumulated four more additional mutations (substitutions) (fig. 1). Four of the observed nucleotide substitutions were synonymous and did not determine differences in the amino acid sequence. Only one causes the change of Alanin (Ala) present at the selected position, in all the samples from Germany and Belgium, to Tyrosine (Tyr).

Table 1. Provenance of the analysed *Batrisoides unisexualis* sequences.

Species	Sequence	Voucher	Place of collection
<i>Batrisodes (Batrisodes) unisexualis</i> Besuchet, 1988	OQ883684	SV5_PD	Republic of Moldova, Pădurea Domnească
	HQ954034	BC_ZSM_COL_01295	Belgium, Blanden, BR Meerdaalbos
	KM439991	BFB_Col_FK_4127	Germany: Rhineland Palatinate, Neuburg, Altrheine
	KM447949	BC_ZSM_COL_00421	Germany: North Rhine-Westphalia, Worringer, Bruch
	KM448359	BFB_Col_FK_8080	Germany: North Rhine-Westphalia, Bergsee
	HQ954018	BC_ZSM_COL_01274	Belgium, Leuven, Blanden, BR Meerdaalbos
	KM444179	BFB_Col_FK_7409	Belgium: West-Vlaanderen, Leuven, BR Meerdaalbos

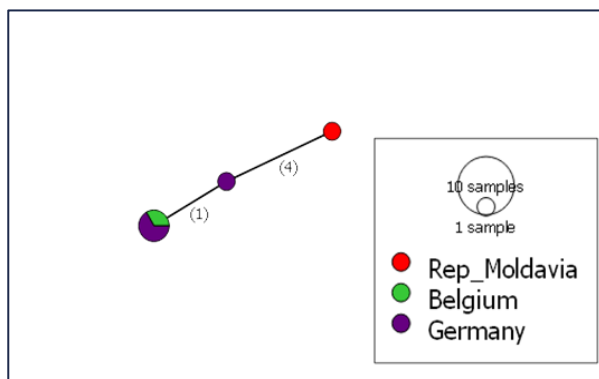


Figure 1. Network analysis for *Batrisodes (Batrisodes) unisexualis*.

Tribe Trichonychini Reitter, 1882

Genus *Trichonyx* Chaudoir, 1845

Trichonyx sulcicollis (Redtenbacher, 1816)

Identification: DNA barcoding.

Collection data and material examined: 1 ex., 20.V.2022, Pădurea Domnească.

Ecology and biology: collected from decaying wood populated by ants using exhauster. Adults are predators. The species occur in dry wood or under the bark of dead stumps, together with ants from genera *Lasius* and *Formica* (25). Adult sizes 2.5-3.5 mm.

Occurrence: In Europe: Austria, Belarus, Belgium, Bulgaria, Central European Russia, Croatia, Czech Republic, Danish mainland, French mainland, Germany, Greek mainland, Hungary, Italian mainland, Lithuania, Norwegian mainland, Poland, Romania, Slovakia, Slovenia, Sweden, Switzerland, The Netherlands, Ukraine and UK (22).

In the case of *Trichonyx sulcicollis*, the number of COI sequences identified/downloaded from international databases is low (tab. 2). Two sequences from Belgium were analysed and they grouped in the same mitochondrial haplotype that is different from the one present in the Re

public of Moldova, in the Pădurea Domnească Reserve by 15 mutations (fig. 2). All observed nucleotide substitutions were synonymous and did not have differences in amino acid sequence.

leotide substitutions were synonymous and did not have differences in amino acid sequence.

Table 2. Analyzed sequences COI of *Trichonyx sulcicollis*.

Species	Sequence	Voucher	Place of collection
<i>Trichonyx sulcicollis</i> (Redtenbacher, 1816)	OQ883692	SV6_PD	Republic of Moldova, Pădurea Domnească
	HQ954018	BC ZSM COL 01274	Belgium, Blanden, BR Meerdaalbos
	KM444179	BFB_Col_FK_7409	Belgium, Blanden, BR Meerdaalbos

In the case of *Trichonyx sulcicollis*, the number of COI sequences identified/downloaded from international databases is low (tab. 2). Two sequences from Belgium were analysed and they grouped in the same mitochondrial haplotype that is different from the one present in the Republic of Moldova, in the Pădurea Domnească Reserve by 15 mutations (fig. 2). All observed nucleotide substitutions were synonymous and did not have differences in amino acid sequence.

Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Danish mainland, Finland, French mainland, Germany, Greek mainland, Hungary, Italian mainland, Latvia, Near East, East, North and South European Russia, Norwegian mainland, Poland, Romania, Slovakia, Sweden, Switzerland, The Netherlands, Ukraine, UK and former Yugoslavia.

In the case of *Sepedophilus bipunctatus*, four haplotypes were identified in 16 COI sequences analysed (tab. 3 and fig. 3). Two of the haplotypes are common to Germany and Finland, while distinct haplotypes appear in the Republic of Moldova and Belgium. These haplotypes differ from each other only by one mutation (fig. 3). The amino acid sequence obtained for the Moldova sample exhibits one silent mutation and three others that change the amino acid order: Tyrosine replaces Histidine – this change is present in our samples from Moldova, but also in samples from Belgium, Germany and Finland; Serine replaces Proline, change that is particular to our samples; and Alanine substitutes Tyrosine not only in our samples, but also in samples from Finland and Germany.

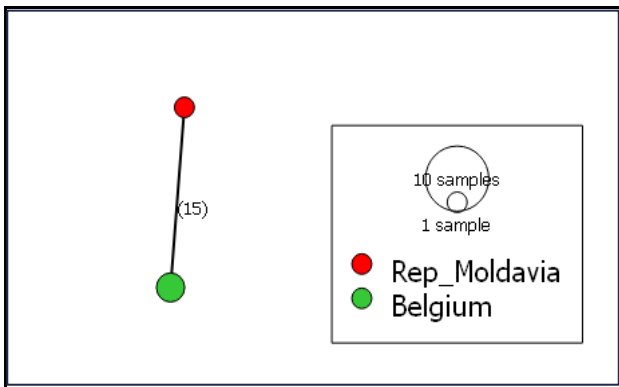


Figure 2. Network analysis for *Trichonyx sulcicollis*.

Subfamily Tachyporinae MacLeay, 1825
Tribe Tachyporini MacLeay, 1825
Genus *Sepedophilus* Gistel, 1856
Sepedophilus bipunctatus (Gravenhorst, 1802)
Synonym: *Tachyporus bipunctatus* Gravenhorst, 1802
Identification: DNA barcoding.
Collection data and material examined: 1 ex., 23.III.2022, Prutul de Jos.

Ecology and biology: species were collected from under damp and mold-affected willow bark using the exhaustor. Corticolous, saproxylic, mycetophagous species, it feeds on the hyphae of fungi that develop on the dead wood of deciduous and coniferous trees (26). Adult sizes 2.0-2.5 mm (25). Occurrence: Palaearctic (25). In Europe: Austria,

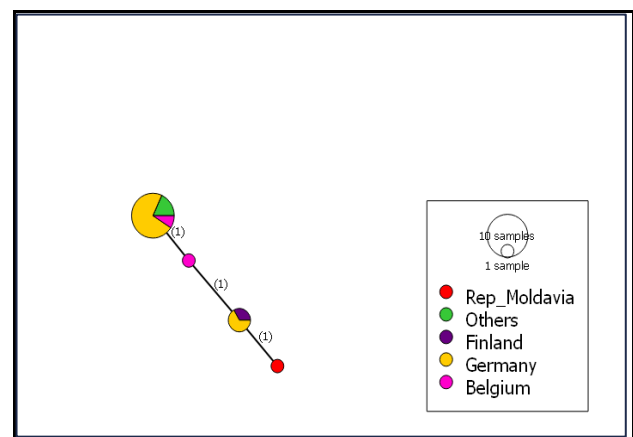


Figure 3. Haplotype network for the species *Sepedophilus bipunctatus*.

Table 3. Analysed sequences COI of *Sepedophilus bipunctatus*.

Species	Sequence	Voucher	Place of collection
<i>Sepedophilus bipunctatus</i> (Gravenhorst, 1802)	OQ883690	SB11_Prutul de Jos	Republic of Moldova, Prutul de Jos
	NC_028611	-	-
	MZ659744	ZMUO.024139	Finland: Ostrobothnia ouluensis, Poellaennokka
	MW259859	ZFMK-TIS-2573928	Germany: North Rhine-Westphalia, Klosterholz
	MW259566	ZFMK-TIS-2573927	Germany: North Rhine-Westphalia, Klosterholz
	KU919616	ZFMK-TIS-2500597	Germany: Thuringia, Wartburgkreis, Neuroth
	KU915356	ZFMK-TIS-2522848	Germany: Saxony-Anhalt, Timmenrode
	KU913802	ZFMK-TIS-5858	Germany: Saxony, Leisnig
	KU911498	ZFMK-TIS-2500628	Germany: Thuringia, Wartburgkreis, Neuroth
	KU911387	ZFMK-TIS-21603	Germany: Rheinland-Pfalz, Thuer
	KU907881	ZFMK-TIS-2500553	Germany: Thuringia, Wartburgkreis, Nesseufer
	KU906618	ZFMK-TIS-2500613	Germany: Thuringia, Wartburgkreis, Neuroth
	KT780676	BMNH 845956	-
	KM446485	GBOL_Col_FK_6206	Germany: Rhineland Palatinate, Scheibenhardt
KM443565	BFB_Col_FK_7477	Belgium: Vlanderen, Leuven, BR, Meerdaalbos	
HQ953492	BC ZSM COL 00539	Belgium, Sint-Genesius-Rode, BR Zonienwoud	

Sepedophilus constans (Fowler, 1888)
 Synonym: *Conosoma constans* Fowler, 1888
 Identification: morphological method.
 Collection data and material examined: 1 ex., (♀), 31.III.2022, Vulcănești (Nisporeni), leg. S. Bacal, det. M. Stan. Ecology and biology: saproxylic species, collected with exhauster from wood of dead oak, mycetobiont, mycetophagus (27). Adult sizes 3.9-4.9 mm (22).
 Occurrence: Austria, Czech Republic, Finland, Germany, Hungary, Italy, Northern European Russia, Norway, Poland, Romania, Slovakia, Sweden, Ukraine and UK.

Sepedophilus pedicularius (Gravenhorst, 1802)
 Synonyms: *Tachyporus pedicularius* Gravenhorst, 1802, *Conosoma pedicularium* (Gravenhorst, 1802), *Conosomus pedicularius* Gravenhorst, 1802, *Tachyporus truncatellus* Gravenhorst, 1806)
 Identification: DNA barcoding.
 Collection data and material examined: 1 ex., 23.II.2022, Vila Nisporeni.
 Ecology and biology: collected using exhauster

from decaying wood. Adult sizes 2.5-3.0 mm.
 Occurrence: Palaearctic. In Europe: Austria, Belarus, Central, North and East European Russia, Czech Republic, Danish mainland, Estonia, Finland, French mainland, Germany, Ireland, Italian mainland, Latvia, Liechtenstein, Northern Ireland, Norwegian mainland, Poland, Romania, Slovakia, Sweden, Switzerland and UK.

In the case of *Sepedophilus pedicularius* in the six analysed sequences which came from Finland, Germany and the Republic of Moldova, three haplotypes were identified, two of which are specific for the Republic of Moldova and Finland, respectively, and one common haplotype in which the sequences from Germany and Finland are grouped. All three haplotypes identified in the samples differ by a single mutation (tab. 4 and fig. 4). In our analysed sample from Vila Nisporeni, one nucleotide substitution is synonymous and did not induce any differences in the amino acid sequence, and another substitution which replaces Methionine with Valine is shared with a sample from Finland.

Table 4. Analysed sequences COI of *Sepedophilus pedicularius*.

Species	Sequence	Voucher	Place of collection
<i>Sepedophilus pedicularius</i> (Gravenhorst, 1802)	OQ883691	SV19_VN	Landscape Rezerve Vila Nisporeni
	KJ962555	ZMUO<FIN>:001221	Finland: Aland Islands, Finstroem, Attboele
	KJ966871	ZMUO<FIN>:005649	Finland: Alandia, Lemland, Bathusfjaerden
	KM448438	GBOL02411	Germany: Bavaria, Schiessplatzheide-Sued
	KU912252	ZFMK-TIS-2521952	Germany: Kiesgruben und Sumpfstellen
	KU915117	ZFMK-TIS-2524706	Germany: Thuringia, Walterslebener Grund

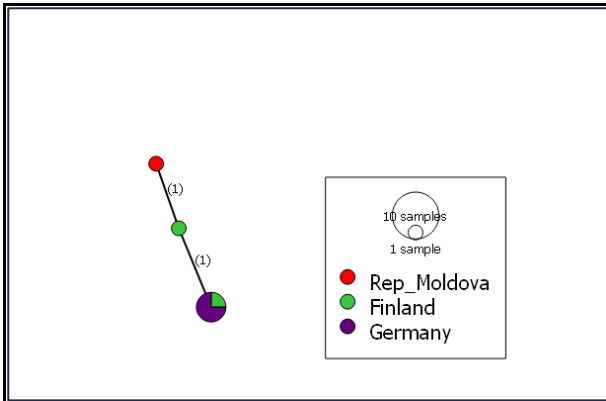


Figure 4. Haplotype network for the species *Sepedophilus pedicularius*.

Subfamily Aleocharinae Fleming, 1821
 Tribe Homalotini Heer, 1839
 Genus *Gyrophæna* Mannerheim, 1830
Gyrophæna manca Erichson, 1839
 Synonyms: *Aleochara angustata* Stephens, 1832, *Gyrophæna angustata* (Stephens, 1832), *Gyrophæna manca* Erichson, 1839, *Gyrophæna puncti-collis* (Hochhuth, 1872)
 Identification: DNA barcoding.

Collection data and material examined: 5 ex., 16.II.2022, Pohrebeni; 2 ex., 20.V.2022, Pădurea Domnească.
 Ecology and biology: mycetophagous species collected from mushrooms growing on decaying dead wood using exhaustor. Adult sizes 1.3-1.6 mm
 Occurrence: Palaearctic (22).

Seven haplotypes were identified in the 13 *Gyrophæna manca* sequences analysed to generate the network presented in Figure 5. In the Republic of Moldova, two haplotypes were identified, one of which is common, also found in samples from Germany and Finland, while the other is distinct and differs by two mutational steps from the common one. The most distant from the common haplotype is the one from Finland (seven mutations) (tab. 5 and fig. 5). From three analysed samples, only one collected in Pădurea Domnească exhibits two mutations in the amino acid sequence, as follows: Proline (Pro) replaces Leucine (Leu), change that is common with a sample from Germany, and one modification private to our sample is the substitution of Glycine (Gly) with Proline (Pro).

Table 5. Analysed sequences COI of *Gyrophæna manca*.

Species	Sequence	Voucher	Place of collection
<i>Gyrophæna manca</i> Erichson, 1839	OQ883685	SV10_Poh	Landscape Reserve Pohrebeni
	OQ883686	SV13_PD	Republic of Moldova, Pădurea Domnească
	OQ883687	SV48_PD	Republic of Moldova, Pădurea Domnească
	MZ660144	ZMUO.028867	Finland: Regio aboensis, Pohja, Fiskars
	MZ657487	ZMUO.028868	Finland: Regio aboensis, Pohja, Fiskars
	MZ656527	ZMUO.028869	Finland: Regio aboensis, Pohja, Fiskars
	KU907280	ZFMK-TIS-2556315	Germany Mecklenburg, Nationalpark, Mueritz
	KM448754	GBOL_Col_FK_0877	Germany: Bavaria, Lusen- und Boehmstrasse
	KM447407	GBOL_Col_FK_0809	Germany: Rhine-Westphalia, NWZ Altwald Ville
	KM443621	GBOL_Col_FK_0832	Germany: Bavaria, Diensthuettenstrasse
	KM441816	GBOL_Col_FK_0825	Germany: Bavaria, Schwarzachstrasse
	KM440835	BC ZSM COL 02540	Germany: Rhineland Palatinate, Lautermuendung
	HQ953314	BC ZSM COL 00312	Germany

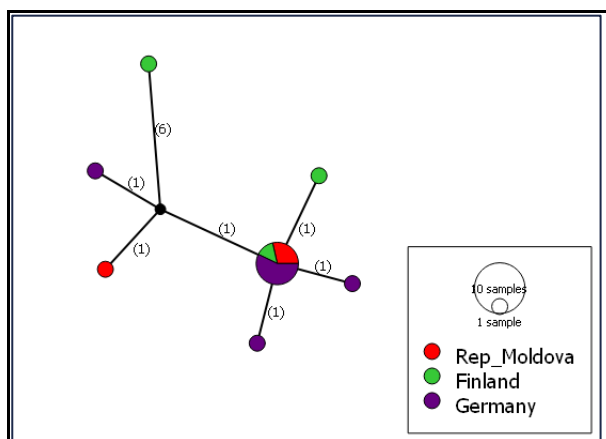


Figure 5. Haplotype network for the species *Gyrophæna manca*.

Subfamily Scaphidiinae Latreille, 1806
 Tribe Scaphidiini Latreille, 1806
 Genus *Scaphisoma* Leach, 1815
Scaphisoma agaricinum (Linnaeus, 1758)
 Synonym: *Silpha agaricinum* Linnaeus, 1758
 Identification: DNA barcoding
 Collection data and material examined: 1 ex., 12.I.2022, Plaiul Fagului; 1 ex., 20.V.2022, Pădurea Domnească.

Ecology and biology: species were collected in the Plaiul Fagului, from under mouldy oak bark. It can be found on mushrooms. The specimens were obtained by the flotation method applied in the laboratory. Adults range in size from 1.7-2.0 mm. Occurrence: Eurasia (25). In Europe: Albania, Austria, Belarus, Belgium, Bosnia and Herzegovina, UK, Bulgaria, Central and East European Russia, Corsica, Croatia, Czech Republic, Danish mainland, East Palaearctic, Estonia, Finland, French mainland, Germany, Greek mainland, Hungary, Ireland, Italian mainland, Latvia, Liechtenstein, Lithuania, Macedonia, North European Russia, Northwest European Russia, Norwegian mainland, Poland, Romania, Slovakia, Slovenia, South European Russia, Spanish mainland, Swe-

Switzerland, The Netherlands, Ukraine and Yugoslavia.

Seven haplotypes were identified in 19 COI sequences analysed for the *Scaphisoma agaricinum*. Three private haplotypes are present in samples collected from Germany. Another private haplotype is present in Finland, while the samples from the Republic of Moldova from Pădurea Domnească and Plaiul Fagului, are grouped into other two private haplotypes. A common haplotype is found in samples from Germany and Belgium (tab. 6 and fig. 6). For our analysed specimens of *S. agaricinum*, all the observed nucleotide substitutions were synonymous and did not induce differences in the amino acid sequence.

Table 6. Analysed sequences COI of *Scaphisoma agaricinum*.

Species	Sequence	Voucher	Place of collection
<i>Scaphisoma agaricinum</i> (Linnaeus, 1758)	OQ883688	SV7_PD	Republic of Moldova, Pădurea Domnească
	OQ883689	SV12_PF	Republic of Moldova, Plaiul Fagului
	HQ953834	BC ZSM COL 00929	Belgium, Sint-Genesius-Rode, Zonienwoud
	KJ963540	ZMUO<FIN>:004481	Finland: Regio aboensis, Turku, Ruissalo
	KJ964524	ZMUO<FIN>:004480	Finland: Regio aboensis, Turku, Ruissalo
	KJ965088	ZMUO<FIN>:004038	Finland: Ostrobotnia kajanensis, Tuohilamminkangas
	KJ966647	ZMUO<FIN>:005909	Finland: Ostrobotnia borealis pars australis, Oulunsalo, Lassilanranta
	KM443518	GBOL_Col_FK_1344	Germany: Rhineland Palatinate, Oberrhein, Altrheine
	KM447364	BFB_Col_FK_5740	Germany, North Rhine-Westphalia, Altwald Ville
	KU906446	ZFMK-TIS-2500635	Germany: Thuringia, Wasungen, Bonndorf, Werra-Aue
	KU908177	ZFMK-TIS-2532952	Germany: Saxony, Klosterbuch/Mulde, NSG Maylust
	KU908615	ZFMK-TIS-2532966	Germany: Saxony, Klosterbuch/, NSG Maylust
	KU908786	ZFMK-TIS-2537173	Germany: Saxony, Klosterbuch, Maylust
	KU911799	ZFMK-TIS-13947	Germany: Thuringia, Beichlingen, Wurmberg
	KU914296	ZFMK-TIS-2519525	Germany: Saxony-Anhalt, Huy, Paulskopf
	KU915341	ZFMK-TIS-6042	Germany: Rhine-Westphalia, Siebengebirge
	KU916539	ZFMK-TIS-13941	Germany: Thuringia, Beichlingen, Wurmberg
	KU917062	ZFMK-TIS-2537172	Germany: Saxony, Klosterbuch, Maylust
	KU919452	ZFMK-TIS-2519522	Germany: Saxony-Anhalt, Huy, Paulskopf

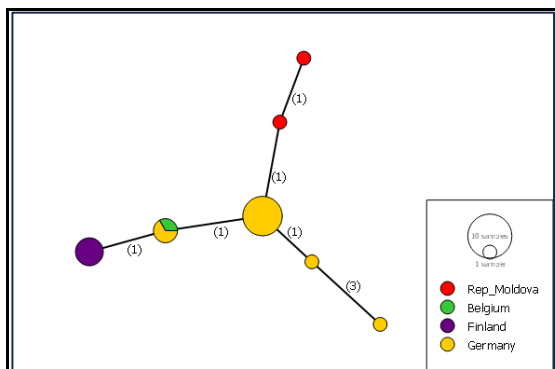


Figure 6. Haplotype network for the species *Scaphisoma agaricinum*.

Paederinae Fleming, 1821
 Tribe Paederini Fleming, 1821
 Genus *Medon* Stephens, 1833
Medon rufiventris (Nordmann, 1837)
 Synonyms: *Lathrobium rufiventris* Nordmann, 1837, *Medon incertus* Mulsant & Rey, 1878
 Identification: morphological method.

Collection data and material examined: 2 ex., (1♂, 1♀), 31.III.2022, Vulcănești (Nisporeni), leg. S. Bacal, det. M. Stan. Identified by the aedeagus, sternite VII and VIII.

Ecology and biology: species collected with exhaustor from under the bark of a rotting ash tree. A specimen was extracted in the laboratory by the floatation method. Saprophagous species, feeds on decaying plant matter, quite rare (28). Species associated with dead wood (29). Adult sizes 3.5-4 mm (22).

Occurrence: Austria, Bosnia and Herzegovina, Croatia, Czech Republic, France, Germany, Greece, Hungary, Italy, Lithuania, Middle East, North Africa, Poland, Romania, Slovakia, Slovenia, Spain, Ukraine.

Staphylininae Latreille, 1802

Tribe Xantholinini Erichson, 1839

Genus *Hypnogyra* Casey, 1906

Hypnogyra angularis (Ganglbauer, 1895)

Synonyms: *Xantholinus angularis* Ganglbauer, 1895, *Staphylinus glabra* Gravenhorst, 1802, *Gyrohypnus rotundicollis* Stephens, 1833, *Xantholinus flavipennis* Redtenbacher, 1849, *Lepidophallus bernaboi* Coiffait, 1980, *Hypnogyra bernaboi* Coiffait, 1980, *Hypnogyra flavipennis* Redtenbacher, 1849, *Hypnogyra glabra* (Nordmann, 1837), *Hypnogyra rotundicollis* Stephens, 1833, *Phalacrolinus angularis* (Ganglbauer, 1895), *Phalacrolinus bernaboi* (Coiffait, 1980), *Phalacrolinus flavipennis* (Redtenbacher, 1849), *Phalacrolinus glabra* (Gravenhorst, 1806), *Phalacrolinus rotundicollis* (Stephens, 1833).

Identification: morphological method.

Collection data and material examined: 1 ex., (♂), 08-30.VI.2022, Pădurea Domnească, leg. S. Bacal, det. M. Stan.

Ecology and biology: species was collected using exhaustor, along with ants of the genus *Lasius* from dead oak, infested with mould. Adult sizes 6-7 mm (22).

Occurrence: Austria, Belgium, Bosnia and Herzegovina, Great Britain, Central European Russia, Croatia, Czech Republic, Denmark, Estonia, Eastern European Russia, Finland, France, Germany, Greece, Hungary, Italy, Lithuania, Middle East, Netherlands, North Africa, Poland, Romania, Slovakia, Spain, Sweden, Switzerland and Ukraine.

DISCUSSIONS

Today, the total number of saproxylic staphyli-

nids from seven subfamilies has reached 30 species present in the Republic of Moldova: Staphylininae (11 species), Tachyporinae (8), Aleocharinae (4), Scaphidiinae (3), Pselaphinae (2), Paederinae and Piestinae with only one species for each (9).

The species *Batrisodes unisexualis* was collected from Hungary in 2017 from under the bark of dead trees colonized by ants in the genus *Lasius* (25). Our research confirms the association between the coleopteran species and xylobiont ants.

In Great Britain, *Trichonyx sulcicollis* was collected from under the bark of deciduous trees, and was found in association with *Lasius* and *Formica* ants (25). In our case, the species was collected from dead wood colonized by ants.

The species from the genera *Sepedophilus* (*S. bipunctatus*, *S. constans* și *S. pedicularius*), *Gyrophaena* and *Scaphisoma* are mycetobionts and mycetophages (25, 27). In the present research, the species were collected from dead wood affected by mould and colonized by mushrooms.

Medon rufiventris is considered to be associated with dead wood, quite rare, and usually found in decaying plant matter (28). In the Republic of Moldova, the species was collected from the Vulcănești sessile oak forest in combination with pedunculate oak, birch and beech (10).

Hypnogyra angularis is considered an indicator species of old forests (30). In our research, the species was collected from the Pădurea Domnească natural floodplain forest that includes unique wet habitats in the Republic of Moldova, with old trees and dead wood in various stages of degradation.

From a phylogeographic point of view, most sequences from the Republic of Moldova are grouped into private haplotypes, that are distributed only in the analysed region. The only exception is represented by *Gyrophaena manca* for which two haplotypes have been identified, with one present also in Finland and Germany and the other one, private, found only in the Republic of Moldova.

The COI region was chosen as a barcode because it is sufficiently conserved at species level and is variable enough between species to enable a good taxon identification. Also, the COI barcode fragment is located at the core of energy production within cells and changes in amino acid sequences

that modify the protein structure may affect energy metabolism. In 2016, Pentinsaari and collaborators (31) have revealed that the beetles (Coleoptera) show more amino acid variation, indicating fundamental differences in patterns of molecular evolution in COI. In our analyses we identified some species (*Trichonyx sulcicollis* and *Scaphisoma agaricinum*) with silent mutations in the COI sequences with no differences in amino acid sequence after translation. Considering the

other four analysed species, the identified mutations generate changes in the translated amino acid sequences, from which one is a private variant for Moldova, while the others are shared between different European regions.

The newly identified species confirm the fact that the natural and landscape reserves of the Republic of Moldova are well preserved and rich in terms of biodiversity.

CONCLUSIONS

1. As a result of the research carried out on the saproxylic coleoptera from the dead wood of the Natural Reserves Pădurea Domnească, Plaiul Fagului, Prutul de Jos, the Landscape Reserves Pohrebeni and Vila Nisporeni and the natural forest from Vulcănești (Nisporeni), 9 species, belonging to seven genera and 6 subfamilies, were identified as new for the fauna of the Republic of Moldova: *Batrissodes unisexualis*, *Trichonyx sulcicollis*, *Sepedophilus bipunctatus*, *Sepedophilus constans*, *Sepedophilus pedicularius*, *Gyropaena manca*, *Scaphisoma agaricinum*, *Medon rufiventris* and *Hypnogyra angularis*.
2. The identification of new species of coleoptera, especially the damaged individuals, was possible thanks to the DNA barcoding, but it is also an important tool in revealing the phylogeographic and evolutionary models.
3. The majority of the sequenced coleopteran species from the Republic of Moldova are grouped into private haplotypes, that are distributed only in the analysed region. The only exception is the species *Gyropaena manca* with two haplotypes, one present also in Finland and Germany and the other one, found only in the Republic of Moldova.
4. The analysis of the obtained COI sequences and their comparison with stored in GenBank showed that the species identified from the Republic of Moldova are distributed in Europe and in the Palearctic.
5. Thus, as a result of this study, the number of Staphylinidae saproxylic species identified in the Republic of Moldova has reached 30, and they belong to the subfamilies Aleocharinae, Paederinae, Piestinae, Pselaphinae, Scaphidiinae, Scydmaeninae, Staphylininae and Tachyporinae.
6. The genetic data obtained in the present study will contribute to the enrichment of genetic databases, becoming available for future phylogeny, phylogeography and conservation biology studies carried out at European level.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors express their gratitude to Dr. Melanya Stan from the “Grigore Antipa” Museum, Bucharest, Romania for identifying the species based on morphological characters. The work

was carried out with financial support from projects 20.80009.7007.02, 22.00208.7007.05/PD1 “Saproxylic Coleoptera from the Republic of Moldova: taxonomy, ecology, zoogeography and importance” and 010701 “Evaluation of the structure and functioning of the animal world and aquatic ecosystems under the influence of biotic and abiotic factors in the context of ensuring ecological security and the well-being of the population”.

REFERENCES

1. Bussler H, Müller J, Dorka V. European Natural Heritage: The saproxylic beetles in the proposed National Park Defileul Jiului. *Analele ICAS*. 2005;48:3–19. Available at: https://www.researchgate.net/publication/238754464_European_Natural_Heritage_The_saproxylic_



- beetles_in_the_proposed_parcul_National_De-
fileul_Jiului [Accessed 01.02.2023].
- Davies ZG, Tyler C, Stewart GB, Pullin AS. Are current management recommendations for saproxylic invertebrates effective? A systematic review. *Biodiv. Conserv.* 2008;17:209–234. doi:10.1007/s10531-007-9242-y
 - Della Rocca F, Stefanelli S, Pasquaretta C, Campanaro A, Bogliani G. Effect of deadwood management on saproxylic beetle richness in the floodplain forests of Northern Italy: Some measures for deadwood sustainable use. *Journal of Insect Conservation.* 2014;18:121–136. doi:10.1007/s10841-014-9620-1
 - Grove SJ, Stork NE. An inordinate fondness for beetles. *Invertebr Syst.* 2000;14:733–739. doi:10.1071/IT00023
 - Mihailov I, Mocreac N. List of the rove beetles (Coleoptera, Staphylinidae) from the several museum collections from the Republic of Moldova. Muzeul Olteniei Craiova. *Oltenia. Studii și comunicări. Științele Naturii.* 2020;36(2):91-104. Available at: http://olteniastudiisicomu.nicaristi-intelenaturii.ro/cont/36_2/III.%20ANIMAL%20BIOLOGY%20III.a.%20INVERTEBRATES%20VA RIOUS/13Mihailov%20&%20 Mocreac.pdf [Accessed 01.02.2023].
 - Bacal S, Derunkov A. Rove beetles (Coleoptera, Staphylinidae) from the dead wood in the forests of Lower Dniester, Republic of Moldova. *Muzeul Olteniei Craiova. Oltenia. Studii și comunicări. Științele Naturii.* Craiova. 2009;20:111-113. Available at: <http://olteniastudiisicomunicari.stiintelenaturii.ro/cont/25/IZ15-Bacal.pdf> [Accessed 01.02.2023].
 - Bacal S, Derunkov A. Contributions to the knowledge of rove beetles (Coleoptera, Staphylinidae) from state nature reserve “Plaiul Fagului”, Republic of Moldova. *Travaux du Museum National d’Histoire Naturelle «Grigore Antipa».* 2010;LIII:217-221. doi:10.2478/v10191-010-0016-5
 - Bacal S, Mihailov I, Calestru L. Contributions to study of staphylinidae (Coleoptera, Staphylinidae) of dead wood in forest ecosystems of Moldova. State and prospects of plant protection. Materials of the International Scientific and Practical Conference dedicated to the 45th anniversary of the organization of the Republican Unitary Enterprise "Institute of Plant Protection", Minsk - Priluki, 2016:322-324. Available at: https://ibn.idsi.md/sites/default/files/imag_file/278-286%20%281%29.pdf [Accessed 01.02.2023].
 - Bacal S. Coleopterele saproxilice din Republica Moldova [Saproxylic coleoptera from the Republic of Moldova]. Chișinău, Căpățîna-Print. 2022:256. doi:10.53937/9789975347297
 - Postolache G, Lazu L. Ariile naturale protejate din Moldova [The protected natural areas of Moldova]. *Rezervații silvice.* 2018;3:212. Available at: <http://editurastiinta.md/ariile-naturale-protejate-din-moldova-vol-3-rezerva-ii-silvice> [Accessed 14.04.2023].
 - Postolache G. *Vegetația Republicii Molodva* [Vegetation of the Republic of Moldova]. Chișinău, Știința. 1995:340. Available at: <https://www.scribd.com/doc/212227937/Gheorghe-Postolache> [Accessed 23.03.2023].
 - Cuza P. Descrierea succintă a Rezervației „Plaiul Fagului” [Brief description of the "Plaiul Fagului" Reserve]. *Fauna Rezervației „Plaiul Fagului”. Nevertebrate.* 2021:9-15. doi:10.53937/9789975347792.01
 - Buşmachi G, Bedos A, Deharveng L. Collembolan species diversity of calcareous canyons in the Republic of Moldova. *ZooKeys.* 2015; 506:95-108. doi:10.3897/zookeys.506.8643
 - Dincă V, Dapporto L, Somervuo P. et al. High resolution DNA barcode library for European butterflies reveals continental patterns of mitochondrial genetic diversity. *Commun Biol.* 2021;4:315. doi:10.1038/s42003-021-01834-7
 - Ratnasingham S, Hebert PDN. The Barcode of Life Data System. *Molecular Ecology Notes.* 2007;7: 355-364. doi:10.1111/j.1471-8286.2007.01678.x
 - Hebert PDN, Cywinska A, Ball SL, deWaard JR. Biological identifications through DNA barcodes. Proceedings of the Royal Society. *Biological Sciences.* 2003;270:313–321. doi:10.1098/rspb.2002.2218
 - Folmer OM, Black M. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Phylogenet Evol.*, 1994;2:304–313. Available at: <https://pubmed.ncbi.nlm.nih.gov/7881515/> [Accessed 23.03.2023].
 - Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Med.* 2016;33:1870–1874. doi:10.1093/molbev/msw054
 - Rozas J, Ferrer-Mata A, Sánchez-Del Barrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Datasets. *Mol. Biol. Evol.* 2017;34:3299–3302. doi:10.1093/molbev/msx248
 - Leigh JW, Bryant D. Popart: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* 2015;6:1110–1116. doi:10.1111/2041-210X.12410
 - Kaefer der Welt. Available at: <https://www.kaefer-der-welt.de> [Accessed 23.03.2023].
 - Die Kafer Europas. Available at: <http://coletonet.de/coleo/index> [Accessed 23.03.2023].

23. Assing V, Schülke M. Freude-Harde-Lohse-Klausnitzer - Die Käfer Mitteleuropas. Band 4. Staphylinidae 1. Zweite neubearbeitete Auflage. – Heidelberg: Spektrum Akademischer Verlag. 2011. Available at: https://beckassets.blob.core.windows.net/product/toc/8938965/9783827416773_toc_001.pdf [Accessed 23.03.2023].
24. Kryzhanovskij OL. Opredelitel' nasekomyh Evropejskoj chasti SSSR. Zhestkokrylye i veerokrylye. 1965;2:560 pp. Available at: <https://www.zin.ru/animalia/coleoptera/rus/keyeur2v.htm> [Accessed 01.02.2023].
25. UK Beetles. Available at: <https://www.ukbeetles.co.uk> [Accessed 23.03.2023].
26. Gamarra P, de la Rosa J, Outerelo R. *Sepedophilus bipunctatus* (Gravenhorst, 1802), nueva especie para la fauna española (Coleoptera, Staphylinidae, Tachyporinae). *Archivos Entomológicos*. 2012;7:189-192. Available at: [https://www.academia.edu/106136668/Sepedophilus bipunctatus_Gravenhorst_1802_nueva_especie_para_la_fauna_espa%C3%B1ola_Coleoptera_Staphylinidae_Tachyporinae](https://www.academia.edu/106136668/Sepedophilus_bipunctatus_Gravenhorst_1802_nueva_especie_para_la_fauna_espa%C3%B1ola_Coleoptera_Staphylinidae_Tachyporinae) [Accessed 23.03.2023].
27. Schigel DS. Polypore-beetle associations in Finland. *Ann. Zool. Fennici*. 2011;48:319–348. Available at: <https://www.jstor.org/stable/23737096> [Accessed 02.03.2023].
28. Mokrzycki T, Borowski J, Byk A, Rutkiewicz A. Waloryzacja ekosystemów Leśnego Kompleksu Promocyjnego “Lasy Spalsko-Rogowskie” na podstawie struktury zgrupowań chrząszczy (Coleoptera) zasiedlających pniaki. *Studia i Materiały CEPL w Rogowie R*. 15. Zeszyt. 2013;35(2):48-81. Available at: https://www.researchgate.net/publication/334194454_Waloryzacja_ekosystemow_Lesnego_Kompleksu_Promocyjnego_Lasy_Spalsko-Rogowskie_na_podstawie_struktury_zgrupowan_chrzaszczy_Coleoptera_zasiedlajacych_pniaki_Valorisation_of_the_ecosystems_of_Forest_Prom [Accessed 23.04.2023].
29. Assing V. A revision of Western Palearctic Medon. VII. A new species from southern Turkey and additional records (Coleoptera: Staphylinidae: Paederinae). *Linzer biol. Beitr*. 2009; 41/2:1253-1268. Available at: https://www.zobodat.at/pdf/LBB_0036_2_0655-0662.pdf [Accessed 02.03.2023].
30. Whitehead PF. Gwent levels traditional orchard invertebrate study 2019-2021. Report on the invertebrates. Moor Leys, Little Comberton, Pershore, Worcestershire WR10 3EH. Available at: <https://www.gwentwildlife.org/sites/default/files/2022-04/P%20Whitehead%20Gwent%20Levels%20orchard%20report%202021.pdf> [Accessed 01.02.2023].
31. Pentinsaari M, Salmela H, Mutanen M, Roslin T. Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. *Sci. Rep*. 2016;6:35275. doi:10.1038/srep35275

Date of receipt of the manuscript: 22/01/2024

Date of acceptance for publication: 24/03/2024