Biodiversity Data Journal 10: e77661
doi: 10.3897/BDJ.10.e77661

Data Paper

Inventory and DNA-barcode library of ground-dwelling predatory arthropods from Krokar virgin forest, Slovenia

Žan Kuralt[‡], Urška Ratajc[§], Neža Pajek Arambašić[‡], Maja Ferle[§], Matic Gabor[‡], Ivan Kos[‡]

‡ University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia
§ National Institute of Biology, Ljubljana, Slovenia

Corresponding author: Žan Kuralt (zan.kuralt@gmail.com)

Academic editor: Gergin Blagoev

Received: 05 Nov 2021 | Accepted: 01 Feb 2022 | Published: 09 Mar 2022

Citation: Kuralt Ž, Ratajc U, Pajek Arambašić N, Ferle M, Gabor M, Kos I (2022) Inventory and DNA-barcode library of ground-dwelling predatory arthropods from Krokar virgin forest, Slovenia. Biodiversity Data Journal 10: e77661. https://doi.org/10.3897/BDJ.10.e77661

Abstract

Background

At a time of immense human pressure on nature and the resulting global environmental changes, the inventory of biota - especially of undisturbed natural areas - is of unprecedented value as it provides a baseline for future research. Krokar, an example of such an undisturbed area, is the largest virgin forest remnant in Slovenia. It is located in the Dinaric Alps, which are believed to harbour the most diverse fauna of soil invertebrates in Europe. Nevertheless, the soil fauna of the Krokar virgin forest has not been thoroughly studied. Moreover, modern taxonomic approaches often rely on genetic information (e.g. DNA-barcodes), while extensive reference libraries from the Dinaric area are lacking. Our work, therefore, focused on addressing this lack of faunistic and genetic data from the Dinaric area.

New information

A total of 2336 specimens belonging to 100 taxa (45 spiders, 30 centipedes, 25 ground-dwelling beetles) were collected and deposited to GBIF. DNA-barcodes of 124 specimens belonging to 73 species were successfully obtained and deposited in GenBank and BOLD databases.

Keywords

Araneae, Chilopoda, Geophilomorpha, Scolopendromorpha, Lithobiomorpha, Coleoptera, Carabidae, faunistics, primary forest

Introduction

The European landscape is probably one of the most fragmented on the planet. Forests that once covered vast areas have undergone significant changes in the past and now exist only in relatively small fragments (Estreguil et al. 2013). Amongst them, the proportion of primary forests is vanishingly small, accounting for 0.7% of Europe's forest area (Sabatini et al. 2018). However, these forests are essential forest ecosystems that encompass all stages of forest development. They also provide habitat for a large number of fungi, plants and animals and serve as an extensive scientific resource (Navarro and Pereira 2012). Primary forests preserve natural ecological processes and are, therefore, resilient to natural disturbances (Thompson et al. 2009, Král et al. 2014).

European primary forests are mainly located in boreal and alpine regions (Sabatini et al. 2018). The virgin forest remnant Krokar (hereafter Krokar) is an example of the latter. It is located on the Borovec Mountain in southern Slovenia, in the Dinaric Mountains, which extend for 650 km from NW to SE and form an orographic barrier between the Adriatic Sea and the Pannonian Basin (Mihevc et al. 2010). The area served as a glacial refugium during the Pleistocene (Hewitt 2000, Brus 2010, Simaiakis and Strona 2015), its diverse landscape and relatively mild climate with high precipitation allowing for a diverse flora and fauna with high endemism (Griffiths et al. 2004).

In the face of climate change, however, the Dinaric Mountains are likely to be as vulnerable as other mountain regions of the world (Beniston 2003). The effects of global change on alpine ecosystems have been observed many times, affecting environmental morphology, vegetation and soils. Several studies have reported upward shifts in vegetation (up to 4 m per decade) and increased erosion (Pauli et al. 1996, Theurillat and Guisan 2001, Nearing et al. 2004, Gehrig-Fasel et al. 2007, Rounsevell and Loveland 2013, Chersich et al. 2015, Robinson et al. 2018). In addition, Pizzolotto et al. (2014) reported similar findings for Carabid beetles in the Dolomites. Knowledge of the current status of plant and animal communities is, therefore, of great importance and allows the assessment of changing climate and human impact (Tuf and Tufova 2008, Bauhus et al. 2009, Cluzeau et al. 2012, Burrascano et al. 2013, Bončina et al. 2017).

Whilst the structure and forest development of Krokar have been thoroughly studied (Diaci 2002, Kraigher et al. 2002, Kutnar et al. 2002, Piltaver et al. 2002, Diaci et al. 2008, Grce 2010, Bončina 2011, Nagel et al. 2012, Kamenik 2013), the diversity of ground-dwelling invertebrates is largely unknown. Nevertheless, some studies have already found a high diversity of predatory invertebrates, such as centipedes (Kos 1996, Griffiths et al. 2004, Grgič and Kos 2005, Ravnjak and Kos 2015, Simaiakis and Strona 2015, Bonato et al. 2017a, Peretti and Bonato 2018) in the Dinarics. Ground-dwelling invertebrates play an important role in forest soil processes (e.g. nutrient cycling, pedogenesis). Predators (e.g. spiders, centipedes and certain groups of beetles) play an important role in regulation and, thus, indirectly influence these processes (Lavelle et al. 2006). They respond rapidly to habitat changes and, because of their position as mesopredators in the trophic cascade, are also highly sensitive to changes at lower trophic levels (Maelfait 1996, Paoletti et al. 1996, Rainio and Niemelä 2003, Pearce and Venier 2006, Koivula 2011, Schreiner et al. 2012, Gerlach et al. 2013).

The main objectives of the study were: (1) to generate a checklist of soil and ground-dwelling predatory arthropods in the study area and (2) to build a DNA-barcode library of these taxa.

Sampling methods

Study extent: Krokar is located on Mount Borovec in the Dinaric Mountains in southern Slovenia (45.540333°N, 14.764737°E) and covers an area of 74.5 hectares at an altitude of 880 to 1190 m a.s.l. The dolomite bedrock of the northern part is gradually replaced by limestone towards the south, resulting in a diverse and rugged terrain. The average annual temperature is 5°C with 2000 mm of precipitation (Grce 2010). The predominant forest communities are Omphalodo-Fagetum, Isopryo-Fagetum and Orvalo-Fagetum (Bončina and Robič 1993). Krokar was excluded from management plans in 1885 (Hočevar et al. 1985) and declared a special purpose forest in 2005 under the Regulation of protective forests and forests with special purpose (Uradni list RS, št. 88/05, 56/07, 29/09, 91/10, 1/13 in 39/15 2005). Finally, it was declared a UNESCO natural heritage area in 2017 (UNESCO 2017).

Parallel sampling was conducted in an adjacent secondary forest (45.53891°N, 14.76478°E), located approximately 300 m west of the sampling sites in Krokar (see Figure 1), with similar geographic, geologic and climatic characteristics. The sampling sites there were located in sloping terrain with varying stages of forest development.

Sampling description: Collecting methods

We used a variety of non-selective sampling methods to minimise collector bias. The selected methods also allowed for efficient collection of both endogeic and ground-dwelling species (Bonato et al. 2017). Two sets of five pitfall traps were set in patches with different forest developmental stages (sapling, pole and sawlog). Similarly, six soil samples per developmental stage were collected.

Soil samples were collected approximately 15 cm deep in the soil using a soil corer with a diameter of 21 cm. Litter and fermentative layers were also collected. Macroinvertebrates were later extracted for one month using modified Tullgren funnels with a cooled funnel base and ethylene glycol as a preservative. The extracted animals were then sorted, identified and preserved in 96% ethanol at -20°C for molecular methods.

Leaf litter was sampled using a sieve with a diameter of 38 cm and a mesh size of 13×13 mm over a white cloth. They were then collected with an aspirator and forceps and preserved in 96% ethanol and later stored at -20°C.

Pitfall traps were set using white plastic cups with a diameter of 10 cm and transparent plastic rain cover, filled with ethylene glycol and set in a line of five traps 1 m apart. After 7–10 days, the contents of the traps were collected, sorted, preserved in 96% ethanol and stored at -20°C.

Specimen identification

Spider and centipede specimens were observed using an Olympus SZX7 stereomicroscope, while beetles were observed using an Olympus SZ61 stereomicroscope. Smaller centipedes were mounted on permanent microscopic slides and observed with an Olympus CX41 microscope.

Adult spiders were identified using standard identification keys (Roberts 1995, Nentwig et al. 2020, Oger 2020). If the morphology of the female epigyne was not discernible, the epigyne was dissected and macerated overnight in 15% potassium hydroxide (KOH) to remove soft tissue. For taxonomy and nomenclature, we followed the World Spider Catalog (World Spider Catalog 2021).

Centipedes were identified according toMatic (1966), Matic (1972), Koren (1986), Koren (1992), Stoev et al. (2010) for Lithobiomorpha; Brölemann (1930) and Lewis (2011) for Scolopendromorpha; ChiloKey (Bonato et al. 2014) for Geophilomorpha. For taxonomy and nomenclature, we followed ChiloBase 2.0 (Bonato et al. 2016).

Beetles were identified using the determination keys from “Die Käfer Mitteleuropas" by Freude et al. (1974) and the subsequent editions.

DNA extraction and sequencing

Genomic DNA was isolated from one of the legs or the whole animal (depending on the size of the specimen). DNA extraction was performed with the MagMAX DNA Multi-sample Kit (Thermo Fisher Scientific Inc., United States) used on a Microlab STAR (Hamilton, United States) pipetting robot. We used the KAPA2G Robust PCR Kit (Sigma-Aldrich, United States) to amplify the mitochondrial cytochrome oxidase I (COI) gene. A 650 bp long fragment of COI was amplified using primers LCO1490 and HCO2198 (Folmer et al. 1994). PCR began with initial denaturation for 3 min at 95°C, followed by 35 cycles of denaturation (30 sec at 95°C), annealing (30 sec at 48°C), elongation (60 sec at 72°C) and then final elongation for 3 min at 72°C. PCR products were purified with Exonuclease I and

FastAP (Thermo Fisher Scientific Inc., United States) according to the manufacturer's instructions. Each fragment was sequenced in both directions using PCR amplification primers from Macrogen Europe (Amsterdam, The Netherlands).

Using Geneious Prime software (Biomatters, New Zealand), we assembled forward and reverse reads, trimmed and manually inspected for possible base-calling errors. Finally, we translated the sequences using all six reading frame positions to ensure that no stop codons were present and generated consensus sequences. For verification, we performed BLAST searches to confirm the identity of all new sequences as either centipede, spider or ground-dwelling beetle barcodes, based on previously-published sequences (high identity values, very low E-values).

In order to investigate the relations amongst the DNA-barcoded taxa, we built a COI tree using Geneious Prime Tree Builder (Geneious version 2022.0 created by Biomatters). Distance matrix was calculated using Global alignment with free end gaps and 70% similarity (IUB)(5.0/-4.5) cost matrix, while the tree was built with Tamura-Nei genetic distance and the Neighbour-Joining tree build method.

Geographic coverage

Description: The study area includes Krokar virgin forest (74.49 ha) and an adjacent secondary forest. Both sites are situated on Borovec Mountain in the northern Dinaric Alps (Fig. 1).

Coordinates: 45.53630 and 45.55152 Latitude; 14.76796 and 14.78080 Longitude.

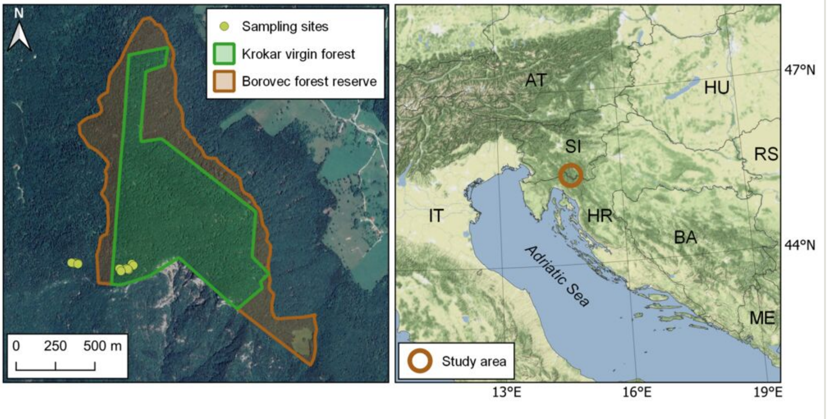

Figure 1. doi

Map on the left shows Borovec Forest Reserve and Krokar virgin forest where sampling was performed (Map data ©2015 Google). Map on the right displays a wider area of the study site location (Map tiles by Stamen Design, under CC BY 3.0. Data by OpenStreetMap, under ODbL).

Taxonomic coverage

Description: The database contains data on 2336 specimens we collected and identified (1079 spiders, 323 ground-dwelling beetles, 299 geophilomorphs, 386 lithobiomorphs, 249 scolopendromorphs). See Suppl. material 1 for list of specimens. The dataset was deposited to GBIF (https://doi.org/10.15468/72ytmh).

Taxa included:

Rank	Scientific Name	Common Name
order	Araneae	spider
class	Chilopoda	centipedes
order	Coleoptera	beetles

Temporal coverage

Notes: Collecting was conducted between October 2018 and August 2019 (see Table 1).

Table 1.

List of field excursions to Borovec Mountain. See Suppl. material 2 for full list of sampling events.

Date	Locality	Sampling method	No. of soil cores / pitfall traps
17.10.2018	Krokar, secondary forest	leaf litter sifting	
17.10.2018	Krokar, secondary forest	soil sampling	36
17.10.–25.10.2018	Krokar	pitfall traps	30
4.1.2019	Krokar, secondary forest	soil sampling	36
4.1.2019–16.1.2019	Krokar, secondary forest	pitfall traps	36
19.4.2019–7.5.2019	Krokar, secondary forest	pitfall traps	60
17.5.2019–28.5.2019	Krokar, secondary forest	pitfall traps	60
1.8.2019–9.8.2019	Krokar, secondary forest	pitfall traps	60

Collection data

Collection name: Ground-dwelling invertebrates of Krokar virgin forest.

Collection identifier: KROK-1819

Parent collection identifier: KROK

Specimen preservation method: 96% ethanol, some smaller centipedes are mounted on microscopic slides.

Usage licence

Data resources

Data package title: Soil and ground-dwelling predatory arthropods (Araneae; Chilopoda: Geophilomorpha, Lithobiomorpha, Scolopendromorpha; Coleoptera: Carabidae, Staphylinidae) of Borovec Mountain and Krokar virgin forest.

Number of data sets: 2

Data set name: Soil and ground-dwelling predatory arthropods (Araneae, Chilopoda, Carabidae) of Borovec Mountain and Krokar virgin forest.

Description: List of all collected and identified specimens. GenBank accession codes and BOLD process IDs of DNA-barcoded specimens are listed in the *GenBankAccession* and *boldSequenceID* columns.

Column label	Column description
eventID	An identifier of the sampling event, corresponding to the eventID in the "Sampling events" dataset.
order	The name of the order.
scientificName	The full scientific name, with authorship and date information, if known.
sex	The sex of the specimen, if applicable.
taxonRank	The taxonomic rank of the most specific name in the scientificName.
identifiedBy	A list (concatenated and separated) of names of people, groups or organisations who assigned the Taxon to the subject.
dateIdentified	The date on which the subject was identified as representing the Taxon.
basisOfRecord	The specific nature of the data record.
preparations	Type of preservative. Either AP (alcohol preparation) or MP (microscopic slide preparation)
GenBankAccession	GenBank accession code.
occurrenceID	Unique occurrence identifier.
lifeStage	Life stage of specimen. Either adult, subadult or juvenile.
boldSequenceID	Sequence identifier at boldsystems.com

Data set name: Sampling events

Column label	Column description
eventID	An identifier for the sampling event.
eventDate	Date of sampling event.
geodeticDatum	Coordinate reference system of coordinates.
habitat	Forest type, either virgin forest or secondary forest and forest development stage, either sapling, pole or sawlog.
decimalLatitude	The geographic latitude (in decimal degrees, using the WGS84 spatial reference system).
decimalLongitude	The geographic longitude (in decimal degrees, using the WGS84 spatial reference system).
minimumElevationInMetres	Elevation of the sampling site.
samplingMethod	The name of the sampling method used in sample collection.
coordinateUncertaintyInMetres	Uncertainty of coordinates in metres.
recordedBy	A list of names of people responsible for collecting of samples.
country	The name of the country in which the location occurs.

Additional information

Summarized results

The taxonomical structure of the dataset is represented by 100 different species - 72 species from Krokar, 80 from the secondary forest and 52 species from both sites. A total of 30 centipede species, 45 spider species and 25 ground-dwelling beetle species are included in the dataset. The most abundant centipede species were *Lithobius pygmaeus* (225 specimens), *Cryptops hortensis* (129), *Strigamia acuminata* (116) and *Cryptops parisi* (103) and, for spiders, *Inermocoelotes inermis* (202), *Harpactea lepida* (172), *Histopona luxurians* (154), *Microneta viaria* (133) and *Comaroma simoni* (105) and, amongst ground-dwelling beetles, *Aptinus bombarda* (125), followed by *Pterostichus burmeisteri* (71). DNA-barcoded specimens are listed in Table 2.

We collected an old-growth forest specialist *Carabus irregularis* and some Balkan/Dinaric endemics, namely *Carabus caelatus*, *Carabus croaticus*, *Dysdera adriatica*, *Amaurobius obustus*, *Histopona luxurians* and *Centrophantes roeweri*, *Harpolithobius gotcheensis*, *Lithobius anici* sp.n., *Lithobius carniolensis* and *Cryptops rucneri*.

A few of the spider species are considered rare according to the Spiders of Europe (Nentwig et al. 2020). These include *Amaurobius obustus* (rare), *Coelotes atropos* (rarely found), *Scotargus pilosus* (very rarely found) and *Walckenaeria simplex* (very rarely found). The finding of *Erigone autumnalis* and *Mermessus trilobatus*, both spiders of North American origin, in this remote area, indicates their alarming invasive potential and suggests a wider distribution than known or expected. Their impact on native (spider) fauna is also unknown and should be studied in the future.

Table 2.

DNA-barcoded specimens with GenBank accession codes and BOLD process IDs.

order	scientificName	GenBankAccession	boldSequenceID
Araneae	*Amaurobius obustus* L. Koch, 1868	OL874923	KROK134-20
Araneae	*Amaurobius obustus* L. Koch, 1868	MT994070	KROK058-19
Araneae	*Araneus diadematus* Clerck, 1757	OL874924	KROK136-20
Araneae	*Centromerus cavernarum* (L. Koch, 1872)	MT994077	KROK069-19
Araneae	*Centromerus cavernarum* (L. Koch, 1872)	OL874925	KROK143-20
Araneae	*Centrophantes roeweri* (Wiehle, 1961)	MT994146	KROK080-19
Araneae	*Ceratinella brevis* (Wider, 1834)	OL874926	KROK144-20
Araneae	*Ceratinella brevis* (Wider, 1834)	MT994078	KROK070-19
Araneae	*Clubiona terrestris* Westring, 1851	MT994081	KROK060-19
Araneae	*Clubiona terrestris* Westring, 1851	OL874930	KROK137-20
Araneae	*Coelotes atropos* (Walckenaer, 1830)	MT994082	KROK052-19
Araneae	*Coelotes atropos* (Walckenaer, 1830)	OL874931	KROK127-20
Araneae	*Comaroma simoni* Bertkau, 1889	MT994083	KROK059-19
Araneae	*Comaroma simoni* Bertkau, 1889	OL874932	KROK135-20
Araneae	*Dasumia canestrinii* (L. Koch, 1876)	MT994088	KROK061-19
Araneae	*Dasumia canestrinii* (L. Koch, 1876)	OL874946	KROK141-20
Araneae	*Diplocephalus picinus* (Blackwall, 1841)	MT994092	KROK072-19
Araneae	*Dysdera adriatica* Kulczynski, 1897	OL874949	KROK138-20
Araneae	*Dysdera adriatica* Kulczynski, 1897	OL874947	KROK139-20
Araneae	*Dysdera adriatica* Kulczynski, 1897	MT994096	KROK064-19
Araneae	*Dysdera adriatica* Kulczynski, 1897	OL874948	KROK140-20
Araneae	*Dysdera adriatica* Kulczynski, 1897	OL874950	KROK152-20
Araneae	*Dysdera ninnii* Canestrini, 1868	MT994097	KROK065-19
Araneae	*Dysdera ninnii* Canestrini, 1868	MT994095	KROK066-19

order	scientificName	GenBankAccession	boldSequenceID
Araneae	*Erigone autumnalis* Emerton, 1882	MT994098	KROK073-19
Araneae	*Hahnia pusilla* C. L. Koch, 1841	MT994103	KROK068-19
Araneae	*Haplodrassus silvestris* (Blackwall, 1833)	MT994104	KROK067-19
Araneae	*Histopona luxurians* (Kulczynski, 1897)	MT994106	KROK053-19
Araneae	*Histopona luxurians* (Kulczynski, 1897)	OL874953	KROK128-20
Araneae	*Histopona luxurians* (Kulczynski, 1897)	OL874952	KROK129-20
Araneae	*Histopona torpida* (C.L.Koch, 1837)	MT994107	KROK054-19
Araneae	*Histopona torpida* (C.L.Koch, 1837)	OL874954	KROK130-20
Araneae	*Inermocoelotes anoplus* (Kulczynski, 1897)	OL874955	KROK131-20
Araneae	*Inermocoelotes anoplus* (Kulczynski, 1897)	MT994108	KROK055-19
Araneae	*Inermocoelotes inermis* (L. Koch, 1855)	MT994109	KROK056-19
Araneae	*Inermocoelotes inermis* (L. Koch, 1855)	OL874956	KROK132-20
Araneae	*Maso sundevalli* (Westring, 1851)	MT994122	KROK074-19
Araneae	*Mermessus trilobatus* (Emerton, 1882)	MT994123	KROK075-19
Araneae	*Microneta viaria* (Blackwall, 1841)	MT994124	KROK077-19
Araneae	*Microneta viaria* (Blackwall, 1841)	OL874967	KROK145-20
Araneae	*Pardosa alacris* C.L. Koch, 1833	OL874968	KROK149-20
Araneae	*Pardosa alacris* C.L. Koch, 1833	MT994132	KROK085-19
Araneae	*Robertus lividus* (Blackwall, 1836)	MT994136	KROK089-19
Araneae	*Robertus lividus* (Blackwall, 1836)	OL874970	KROK153-20
Araneae	*Robertus lividus* (Blackwall, 1836)	OL874969	KROK154-20
Araneae	*Scotargus pilosus* Simon, 1913	MT994139	KROK078-19
Araneae	*Scotargus pilosus* Simon, 1913	OL874977	KROK146-20
Araneae	*Segestria senoculata* (Linnaeus, 1758)	MT994140	KROK088-19
Araneae	*Tegenaria silvestris* L. Koch, 1872	MT994145	KROK057-19
Araneae	*Tegenaria silvestris* L. Koch, 1872	OL874981	KROK133-20
Araneae	*Tenuiphantes flavipes* (Blackwall, 1854)	MT994147	KROK079-19
Araneae	*Tenuiphantes flavipes* (Blackwall, 1854)	OL874982	KROK147-20
Araneae	*Tenuiphantes tenebricola* (Wider, 1834)	MT994148	KROK082-19
Araneae	*Tenuiphantes tenebricola* (Wider, 1834)	OL874983	KROK148-20

order	scientificName	GenBankAccession	boldSequenceID
Araneae	*Trochosa terricola* Thorell, 1856	MT994150	KROK086-19
Araneae	*Trochosa terricola* Thorell, 1856	OL874984	KROK150-20
Araneae	*Walckenaeria antica* (Wider, 1834)	MT994151	KROK083-19
Araneae	*Walckenaeria mitrata* (Menge, 1868)	MT994152	KROK084-19
Araneae	*Zora nemoralis* (Blackwall, 1861)	MT994153	KROK087-19
Araneae	*Zora nemoralis* (Blackwall, 1861)	OL874986	KROK151-20
Coleoptera	*Abax ovalis* (Duftschmid, 1812)	MT994068	KROK008-19
Coleoptera	*Abax parallelepipedus* (Piller and Mitterpacher, 1783)	MT994069	KROK002-19
Coleoptera	*Carabus catenulatus* Scopoli, 1763	MT994072	KROK019-19
Coleoptera	*Carabus coriaceus* Linnaeus, 1758	MT994073	KROK006-19
Coleoptera	*Carabus creutzeri* Fabricius, 1801	MT994074	KROK011-19
Coleoptera	*Carabus croaticus* Dejean 1826	MT994075	KROK007-19
Coleoptera	*Carabus irregularis* Fabricius, 1792	MT994076	KROK020-19
Coleoptera	*Cychrus attenuatus* (Fabricius, 1792)	MT994087	KROK003-19
Coleoptera	*Dima elateroides* Charpentier, 1825	MT994091	KROK023-19
Coleoptera	*Licinus hoffmannseggii* (Panzer, 1803)	MT994111	KROK004-19
Coleoptera	*Molops piceus* (Panzer, 1793)	MT994126	KROK017-19
Coleoptera	*Molops piceus* (Panzer, 1793)	MT994125	KROK018-19
Coleoptera	*Molops piceus* (Panzer, 1793)	MT994127	KROK012-19
Coleoptera	*Molops striolatus* (Fabricius, 1801)	MT994128	KROK015-19
Coleoptera	*Nebria dahlii* Sturm, 1815	MT994129	KROK021-19
Coleoptera	*Notiophilus biguttatus* (Fabricius, 1779)	MT994131	KROK010-19
Coleoptera	*Platynus scrobiculatus* (Fabricius, 1801)	MT994133	KROK022-19
Coleoptera	*Pterostichus burmeisteri* Heer, 1837	MT994134	KROK005-19
Coleoptera	*Pterostichus oblongopunctatus* Fabricius, 1787	MT994135	KROK016-19
Coleoptera	*Stenichnus collaris* (Müller, P.W.J. & Kunze, 1822)	MT994142	KROK014-19
Coleoptera	*Trechus croaticus* Dejean, 1831	MT994149	KROK013-19
Geophilomorpha	*Clinopodes carinthiacus* (Latzel,1880)	MT994079	KROK025-19
Geophilomorpha	*Clinopodes carinthiacus* (Latzel,1880)	OL874927	KROK090-20
Geophilomorpha	*Clinopodes carinthiacus* (Latzel,1880)	OL874929	KROK098-20

order	scientificName	GenBankAccession	boldSequenceID
Geophilomorpha	*Clinopodes carinthiacus* (Latzel,1880)	OL874928	KROK100-20
Geophilomorpha	*Dicellophilus carniolensis* (C.L. Koch, 1847)	MT994089	KROK026-19
Geophilomorpha	*Dicellophilus carniolensis* (C.L. Koch, 1847)	OL874945	KROK091-20
Geophilomorpha	*Dicellophilus carniolensis* (C.L. Koch, 1847)	MT994090	KROK032-19
Geophilomorpha	*Eurygeophilus pinguis* (Brölemann, 1898)	MT994101	KROK027-19
Geophilomorpha	*Schendyla armata* Brölemann, 1901	OL874972	KROK092-20
Geophilomorpha	*Schendyla armata* Brölemann, 1901	OL874971	KROK102-20
Geophilomorpha	*Schendyla tyrolensis* Meinert, 1870	MT994138	KROK029-19
Geophilomorpha	*Schendyla tyrolensis* Meinert, 1870	OL874976	KROK095-20
Geophilomorpha	*Strigamia acuminata* (Leach, 1814)	MT994143	KROK030-19
Geophilomorpha	*Strigamia acuminata* (Leach, 1814)	OL874979	KROK096-20
Geophilomorpha	*Strigamia transsilvanica* Verhoeff, 1928	MT994144	KROK031-19
Lithobiomorpha	*Eupolybothrus grossipes* (C. L. Koch, 1847)	MT994099	KROK048-19
Lithobiomorpha	*Eupolybothrus tridentinus* (Fanzago, 1874)	MT994100	KROK035-19
Lithobiomorpha	*Harpolithobius gottscheensis* Verhoeff, 1937	MT994105	KROK036-19
Lithobiomorpha	*Harpolithobius gottscheensis* Verhoeff, 1937	OL874951	KROK103-20
Lithobiomorpha	*Lithobius anici* sp.n.	MT994141	KROK043-19
Lithobiomorpha	*Lithobius carinthiacus* Koren, 1992	MT994112	KROK044-19
Lithobiomorpha	*Lithobius castaneus* Newport, 1844	MT994113	KROK037-19
Lithobiomorpha	*Lithobius dentatus* C.L.Koch, 1844	MT994116	KROK038-19
Lithobiomorpha	*Lithobius dentatus* C.L.Koch, 1844	OL874961	KROK104-20
Lithobiomorpha	*Lithobius dentatus* C.L.Koch, 1844	MT994115	KROK046-19
Lithobiomorpha	*Lithobius forficatus* (Linnaeus, 1758)	MT994117	KROK047-19
Lithobiomorpha	*Lithobius latro* Meinert, 1872	OL874962	KROK105-20
Lithobiomorpha	*Lithobius latro* Meinert, 1872	MT994118	KROK039-19
Lithobiomorpha	*Lithobius latro* Meinert, 1872	OL874963	KROK109-20
Lithobiomorpha	*Lithobius pelidnus* Haase, 1880	OL874964	KROK111-20
Lithobiomorpha	*Lithobius tenebrosus* Meinert, 1872	MT994120	KROK041-19
Lithobiomorpha	*Lithobius tenebrosus* Meinert, 1872	OL874965	KROK108-20
Lithobiomorpha	*Lithobius validus* Meinert, 1872	MT994121	KROK042-19

order	scientificName	GenBankAccession	boldSequenceID
Lithobiomorpha	*Lithobius validus* Meinert, 1872	OL874966	KROK106-20
Scolopendromorpha	*Cryptops hortensis* Donovan, 1810	OL874934	KROK125-20
Scolopendromorpha	*Cryptops hortensis* Donovan, 1810	OL874933	KROK126-20
Scolopendromorpha	*Cryptops parisi* Brölemann, 1920	OL874941	KROK119-20
Scolopendromorpha	*Cryptops parisi* Brölemann, 1920	OL874940	KROK120-20
Scolopendromorpha	*Cryptops parisi* Brölemann, 1920	MT994086	KROK050-19
Scolopendromorpha	*Cryptops parisi* Brölemann, 1920	OL874939	KROK121-20
Scolopendromorpha	*Cryptops parisi* Brölemann, 1920	OL874942	KROK122-20
Scolopendromorpha	*Cryptops parisi* Brölemann, 1920	OL874943	KROK123-20

The specimens identified as *Lithobius* (*Sigibus*) *anici* sp.n. belong to an undescribed species that has already been recorded at various localities in the Dinaric parts of Slovenia and Bosnia and Herzegovina. Its currently known area of distribution suggests that the species is endemic to the Dinarics, although further studies are needed to confirm this claim.

Comprehensive voucher information, taxonomic classifications, DNA barcode sequences and trace files (including their quality) are publicly accessible through the public dataset “DS-KROK4BDJ” (Dataset ID: dx.doi.org/10.5883/DS-KROK4BDJ) on the Barcode of Life Data Systems (BOLD; www.boldsystems.org) (Ratnasingham and Hebert 2007). In addition, all new barcode data were deposited in GenBank.

The COI tree (Fig. 2) of DNA-barcoded taxa is showing a topology consistent with the current knowledge of relationships between the taxa included. There are, however, a few species with deep genetic differences, that could be explained by the fact that the area served as a glacial refugium during the Pleistocene, which resulted in high intraspecific genetic diversity or even cryptic species. For instance, two DNA-barcoded specimens of *Zora nemoralis* show deep genetic difference, although they were identified as such, based on genital and palpal morphology. Similarly, there is a deep genetic difference between two specimens of *Strigamia acuminata*. The specimens were placed into separate unique BINs - BOLD:AEB5728 and BOLD:AEG5654 with distances (p-dist) to nearest neighbour being 7.85% and 10.42%, respectively. Since the divergence of Western and Eastern Alps populations of *S. acuminata* was estimated to around 14 Ma (Bonato et al. 2017b), we could presume that the turbulent events of Neogene and Quaternary - especially Pleistocene - could lead to the observed cryptic diversity.

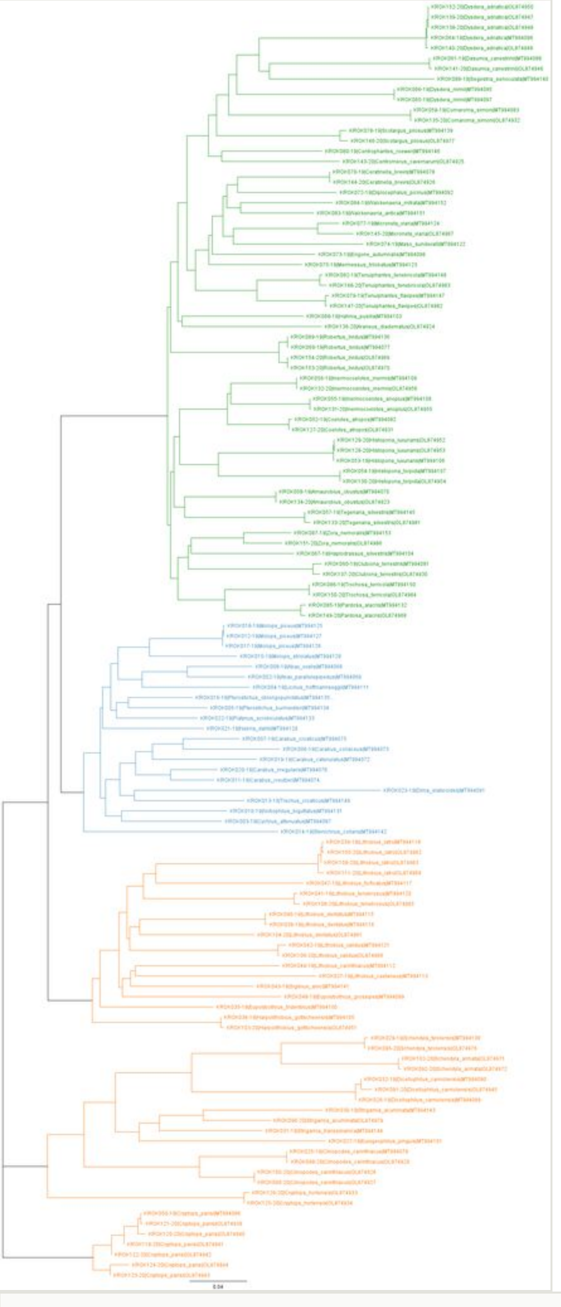

Figure 2. doi

COI tree of DNA-barcoded taxa. Tree branches and labels are coloured according to the predator group (green for spiders, blue for ground-dwelling beetles, orange for centipedes). The tree was constructed in Geneious Prime (Geneious version 2022.0 created by Biomatters).

Acknowledgements

We would like to thank to Manca Velkavrh, Mark Plut and Franc Kljun for their assistance during fieldwork. Lab work would be impossible without Barbara Boljte, Maja Jelenčič, Marjeta Konec and Špela Borko, we are truly grateful for your help. This study was supported by a PhD fellowship and P1-0184 research programme by the Slovenian Research Agency. A permision (340-29/2018/7) for sampling in Krokar virgin forest was granted by the Ministry of Agriculture, Forestry and Food.

Author contributions

ŽK collected the material, identified the spiders and contributed to the writing of the paper; UR identified the beetles and contributed to the writing of the paper; NPA & MF collected the material and identified the spiders; MG identified the beetles; IK identified the centipedes and contributed to the writing of the paper.

References

- Bauhus J, Puettmann K, Messier C (2009) Silviculture for old-growth attributes. Forest Ecology and Management 258 (4): 525-537. https://doi.org/10.1016/j.foreco.2009.01.053
- Beniston M (2003) Climatic change in mountain regions: A review of possible impacts. Climatic Change 59: 5-31. https://doi.org/10.1023/A:1024458411589
- Bonato L, Minelli A, Lopresti M, Cerretti P (2014) ChiloKey, an interactive identification tool for the geophilomorph centipedes of Europe (Chilopoda, Geophilomorpha). ZooKeys 443: 1-9. https://doi.org/10.3897/zookeys.443.7530
- Bonato L, Chagas Jr A, Edgecombe G, Lewis J, Minelli A, Pereira L, Shelley R, Stoev P, Zapparoli M (2016) ChiloBase 2.0 - A World Catalogue of Centipedes (Chilopoda). http://chilobase.biologia.unipd.it.
- Bonato L, Minelli A, Zapparoli M (2017a) Centipede communities (Chilopoda) of forest soils across Europe: abundance, species richness and species composition. Accademia Nazionale Italiana di Entomologia113-120.
- Bonato L, Bortolin F, Drago L, Orlando M, Dányi L (2017b) Evolution of Strigamia centipedes (Chilopoda): a first molecular assessment of phylogeny and divergence times. Zoologica Scripta 46 (4): 486-495. https://doi.org/10.1111/zsc.12234
- Bončina A, Robič D (1993) Report from international camp "Virgin Forest Slovenia '93": international camp in Borovec near Kočevska reka, from 17.7. to 28.7.1993. Ljubljana, 49 pp.
- Bončina A, Klopčič M, Simončič T, Dakskobler I, Ficko A, Rozman A (2017) A general framework to describe the alteration of natural tree species composition as an indicator of forest naturalness. Ecological Indicators 77: 194-204. https://doi.org/10.1016/j.ecolind.2017.01.039
- Bončina Ž (2011) Vpliv svetlobnih razmer na pomlajevanje v pragozdnem rezervatu Krokar. Univerza v Ljubljani, Biotehniška fakulteta

- Brölemann HW (1930) Éléments d'une faune des Myriapodes de France: Chilopodes. Imprimerie toulousaine.
- Brus R (2010) Growing evidence for the existence of glacial refugia of European beech (*Fagus sylvatica* L.) in the south-eastern Alps and north-western Dinaric Alps. Periodicum Biologorum 112 (3): 239-246.
- Burrascano S, Keeton WS, Sabatini FM, Blasi C (2013) Commonality and variability in the structural attributes of moist temperate old-growth forests: A global review. Forest Acology and Management 291: 458-479. https://doi.org/10.1016/j.foreco.2012.11.020
- Chersich S, Rejšek K, Vranová V, Bordoni M, Meisina C (2015) Climate change impacts on the Alpine ecosystem: an overview with focus on the soil. Journal of Forest Science 61 (11): 496-514. https://doi.org/10.17221/47/2015-JFS
- Cluzeau D, Guernion M, Chaussod R, Martin-Laurent F, Villenave C, Cortet J, Ruiz-Camacho N, Pernin C, Mateille T, Philippot L, Bellido A, Rougé L, Arrouays D, Bispo A, Pérès G (2012) Integration of biodiversity in soil quality monitoring: Baselines for microbial and soil fauna parameters for different land-use types. European Journal of Soil Biology 49: 63-72. https://doi.org/10.1016/j.ejsobi.2011.11.003
- Diaci J (2002) Gap disturbance patterns in a beech virgin forest remnant krokar in the mountain vegetation belt of Slovenia. Univerza v Ljubljani, Biotehniška Fakulteta
- Diaci J, Roženbergar D, Mikac S, Anić I, Hartman T, Bončina A (2008) Long-term changes in tree species composition in old-growth Dinaric beech-fir forest. Glasnik za Sumske Pokuse 42: 13-27.
- Estreguil C, Caudullo G, de Rigo D, San Miguel J (2013) Forest landscape in Europe: pattern, fragmentation and connectivity. EUR Scientific and Technical Research 25717.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3 (5): 294-299.
- Freude H, Harde KW, Lohse GA, Lucht W (1974) Die käfer mitteleuropas. Goecke & Evers
- Gehrig-Fasel J, Guisan A, Zimmermann NE (2007) Tree line shifts in the Swiss Alps: Climate change or land abandonment? Journal of vegetation science: official organ of the International Association for Vegetation Science 18 (4): 571-582. https://doi.org/10.1111/j.1654-1103.2007.tb02571.x
- Gerlach J, Samways M, Pryke J (2013) Terrestrial invertebrates as bioindicators: an overview of available taxonomic groups. Journal of Insect Conservation 17 (4): 831-850. https://doi.org/10.1007/s10841-013-9565-9
- Grce D (2010) Razvoj mladja na rastišču Isopyro-Fagetum v pragozdu Krokar: diplomsko delo (visokošolski strokovni študij). Univerza v Ljubljani, Biotehniška fakulteta
- Grgič T, Kos I (2005) Influence of forest development phase on centipede diversity in managed beech forests in Slovenia. Biodiversity and Conservation 14 (8): 1841-1862. https://doi.org/10.1007/s10531-004-1040-1
- Griffiths H, Kryštufek B, Reed J (2004) Balkan biodiversity: Pattern and process in the European hotspot. Springer Netherlands https://doi.org/10.1007/978-1-4020-2854-0
- Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405 (6789): 907-913. https://doi.org/10.1038/35016000
- Hočevar S, Batič F, Martinčič A, Piskernik M (1985) Preddinarski gorski pragozdovi: Trdinov vrh in Ravna gora na Gorjancih, Kopa v Kočevskem Rogu in Krokar na hrbtu

pogorja Borovška gora-Planina nad Kolpo: (mikoflora, vegetacija in ekologija). Inštitut za Gozdno in Lesno Gospodarstvo pri Biotehniški Fakulteti
- Kamenik K (2013) Razvojna dinamika v pragozdovih Šumik in Krokar: diplomsko delo-univerzitetni študij. Univerza v Ljubljani, Biotehniška fakulteta
- Koivula MJ (2011) Useful model organisms, indicators, or both? Ground beetles (Coleoptera, Carabidae) reflecting environmental conditions. ZooKeys287-317. https://doi.org/10.3897/zookeys.100.1533
- Koren A (1986) Die Chilopoden-Fauna von Kärnten und Osttirol. Verlag des Naturwissenschaftlichen Vereins für Kärnten
- Koren A (1992) Die Chilopoden-Fauna von Kärnten und Osttirol. Teil 2, Lithobiomorpha. Verlag des Naturwissenschaftlichen Vereins für Kärnten, Klagenfurt, 138 pp.
- Kos I (1996) Centipedes (Chilopoda) of some forest communities in Slovenia. Mémoires du Muséum National d'Histoire Naturelle, 635-646.
- Kraigher H, Jurc D, Kalan P, Kutnar L, Levanic T, Rupel M, Smolej I (2002) Beech coarse woody debris characteristics in two virgin forest reserves in southern Slovenia. Zbornik Gozdarstva in Lesarstva (Slovenia).
- Král K, McMahon SM, Janík D, Adam D, Vrška T (2014) Patch mosaic of developmental stages in central European natural forests along vegetation gradient. Forest Ecology and Management 330: 17-28. https://doi.org/10.1016/j.foreco.2014.06.034
- Kutnar L, Péter Ó, Dort Kv (2002) Vascular plants on beech dead wood in two Slovenian forest reserves. Zbornik Gozdarstva in Lesarstva 69: 135-153.
- Lavelle P, Decaëns T, Aubert M, Barot S, Blouin M, Bureau F, Margerie P, Mora P, Rossi J-P (2006) Soil invertebrates and ecosystem services. European Journal of Soil Biology 42 (Suppl. 1).
- Lewis JGE (2011) A review of the species in the genus *Cryptops* Leach, 1815 from the Old World related to Cryptops (Cryptops) hortensis (Donovan, 1810) (Chilopoda, Scolopendromorpha). International Journal of Myriapodology 4: 11-50. https://doi.org/10.3897/ijm.4.1116
- Maelfait J (1996) Soil spiders and bioindication. In: van Straalen NM, Krivolutsky DA (Eds) Bioindicator Systems for Soil Pollution. Kluwer Academic Publishing https://doi.org/10.1007/978-94-009-1752-1_14
- Matic Z (1966) Clasa Chilopoda: subclasa Anamorpha. Editura Academiei Republicii Socialiste România
- Matic Z (1972) Fauna Republicii Socialiste Romania: Classa Chilopoda, Subclassa Epimorpha. Academiei Republicii Socialiste Romania
- Mihevc A, Prelovšek M, Zupan Hajna N (2010) Introduction to the Dinaric Karst. Karst Research Institute at ZRC SAZU, Postojna. https://doi.org/10.3986/9789612541989
- Nagel TA, Diaci J, Rozenbergar D, Rugani T, Firm D (2012) Old-growth forest reserves in Slovenia: the past, present, and future. Schweizerische Zeitschrift fur Forstwesen 163 (6): 240-246. https://doi.org/10.3188/szf.2012.0240
- Navarro LM, Pereira HM (2012) Rewilding abandoned landscapes in Europe. Ecosystems 15 (6): 900-912. https://doi.org/10.1007/s10021-012-9558-7
- Nearing MA, Pruski FF, O'Neal MR (2004) Expected climate change impacts on soil erosion rates: A review. Journal of Soil and Water Conservation 59 (1): 43-50.
- Nentwig W, Blick T, Bosmans R, Gloor D, Hänggi A, Kropf C (2020) Araneae: Spiders of Europe (Version 06.2020). https://araneae.nmbe.ch/. Accessed on: 2020-6-17.

- Oger P (2020) Les araignées de Belgique et de France. https://arachno.piwigo.com. Accessed on: 2020-6-17.
- Paoletti MG, Bressan M, Edwards CA (1996) Soil Invertebrates as Bioindicators of Human Disturbance. Critical Reviews in Plant Sciences 15 (1): 21-62. https://doi.org/10.1080/07352689609701935
- Pauli H, Gottfried M, Grabherr G (1996) Effects of climate change on mountain ecosystems–upward shifting of alpine plants. World Resource Review 8 (3).
- Pearce JL, Venier LA (2006) The use of ground beetles (Coleoptera: Carabidae) and spiders (Araneae) as bioindicators of sustainable forest management: A review. Ecological Indicators 6 (4): 780-793. https://doi.org/10.1016/j.ecolind.2005.03.005
- Peretti E, Bonato L (2018) How many species of centipedes coexist in temperate forests? Estimating local species richness of Chilopoda in soil coenoses of the South-Eastern Prealps. European Journal of Soil Biology 89: 25-32. https://doi.org/10.1016/j.ejsobi.2018.10.001
- Piltaver A, Kosec J, Matočec N, Jurc D (2002) Macrofungi on beech dead wood in the Slovenian forest reserves Rajhenavski Rog and Krokar. Zbornik Gozdarstva in Lesarstva (Slovenia).
- Pizzolotto R, Gobbi M, Brandmayr P (2014) Changes in ground beetle assemblages above and below the treeline of the Dolomites after almost 30 years (1980/2009). Ecology and Evolution 4 (8): 1284-1294. https://doi.org/10.1002/ece3.927
- Rainio J, Niemelä J (2003) Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodiversity and Conservation 12 (3): 487-506. https://doi.org/10.1023/A:1022412617568
- Ratnasingham S, Hebert PN (2007) bold: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes 7 (3): 355-364. https://doi.org/10.1111/j.1471-8286.2007.01678.x
- Ravnjak B, Kos I (2015) The current knowledge on centipedes (Chilopoda) in Slovenia: faunistic and ecological records from a national database. ZooKeys (510)223-231. https://doi.org/10.3897/zookeys.510.8672
- Roberts MJ (1995) Spiders of Britain & Northern Europe. HarperCollins
- Robinson SI, McLaughlin ÓB, Marteinsdóttir B, O'Gorman EJ (2018) Soil temperature effects on the structure and diversity of plant and invertebrate communities in a natural warming experiment. The Journal of Animal Ecology 87 (3): 634-646. https://doi.org/10.1111/1365-2656.12798
- Rounsevell MDA, Loveland PJ (2013) Soil Responses to Climate Change. Springer Science & Business Media
- Sabatini FM, Burrascano S, Keeton WS, Levers C, Lindner M, Pötzschner F, Verkerk PJ, Bauhus J, Buchwald E, Chaskovsky O, Debaive N, Horváth F, Garbarino M, Grigoriadis N, Lombardi F, Marques Duarte I, Meyer P, Midteng R, Mikac S, Mikoláš M, Motta R, Mozgeris G, Nunes L, Panayotov M, Ódor P, Ruete A, Simovski B, Stillhard J, Svoboda M, Szwagrzyk J, Tikkanen O, Volosyanchuk R, Vrska T, Zlatanov T, Kuemmerle T (2018) Where are Europe's last primary forests? Diversity & Distributions 24 (10): 1426-1439. https://doi.org/10.1111/ddi.12778
- Schreiner A, Decker P, Hannig K, Schwerk A (2012) Millipede and centipede (Myriapoda: Diplopoda, Chilopoda) assemblages in secondary succession: variance and abundance in Western German beech and coniferous forests as compared to fallow ground. Web Ecology 12 (1): 9-17. https://doi.org/10.5194/we-12-9-2012

- Simaiakis SM, Strona G (2015) Patterns and processes in the distribution of European centipedes (Chilopoda). Journal of Biogeography 42 (6): 1018-1028. https://doi.org/10.1111/jbi.12463
- Stoev P, Akkari N, Zapparoli M, Porco D, Enghoff H, Edgecombe GD, Georgiev T, Penev L (2010) The centipede genus *Eupolybothrus* Verhoeff, 1907 (Chilopoda: Lithobiomorpha: Lithobiidae) in North Africa, a cybertaxonomic revision, with a key to all species in the genus and the first use of DNA barcoding for the group. ZooKeys 50: 29-77. https://doi.org/10.3897/zookeys.50.504
- Theurillat JP, Guisan A (2001) Potential impact of climate change on vegetation in the European Alps: A review. Climatic Change 50 (1-2): 77-109. https://doi.org/10.1023/A:1010632015572
- Thompson I, Mackey B, McNulty S, Mosseler A (2009) Forest resilience, biodiversity, and climate change. 43. Secretariat of the Convention on Biological Diversity, Montreal. Technical Series no. 43. 1-67.
- Tuf IH, Tufova J (2008) Proposal of ecological classification of centipede, millipede and terrestrial isopod faunas for evaluation of habitat quality in Czech Republic. Časopis Slezského Zemského Muzea, Opava, Série A 57: 37-44.
- UNESCO (2017) Ancient and primeval beech forests of the Carpathians and other regions of Europe (Albania, Austria, Belgium, Bulgaria, Croatia, Italy, Germany, Romania, Slovenia, Slovakia, Spain, Ukraine). https://whc.unesco.org/en/decisions/6879
- Uradni list RS, št. 88/05, 56/07, 29/09, 91/10, 1/13 in 39/15 (2005) Uredba o varovalnih gozdovih in gozdovih s posebnim namenom. http://www.pisrs.si/Pis.web/pregledPredpisa?id=URED3176#
- World Spider Catalog (2021) World Spider Catalog Version 22.5. Natural History Museum Bern. https://doi.org/10.24436/2

Supplementary materials

Suppl. material 1: Specimen list doi

Authors: Žan Kuralt, Urška Ratajc, Neža Pajek Arambašić, Maja Ferle, Matic Gabor, Ivan Kos
Data type: dataset
Brief description: List of specimens collected during field excursions to Mount Borovec and Krokar virgin forest.
Download file (272.89 kb)

Suppl. material 2: Sampling events doi

Authors: Žan Kuralt, Urška Ratajc, Neža Pajek Arambašić, Maja Ferle, Matic Gabor, Ivan Kos
Data type: dataset
Brief description: Field excursions to Mount Borovec and Krokar virgin forest.
Download file (10.63 kb)