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

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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, Samaïakis 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, Rousevell 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 to Matic (1966), Matic (1972), Koren (1986), Koren (1992), Stoew 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. [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

Usage licence: Creative Commons Public Domain Waiver (CC-Zero)

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 *Centrophanes roeweri*, *Harpolithobius gotcheensis*, *Lithobius anici* sp.n., *Lithobius camiolensis* 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 ninini Canestrini, 1868        | MT994097         | KROK065-19     |
| Araneae | Dysdera ninini 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 parallelepipeds* (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 creutzneri* 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 hoffmannsiegii* (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          | *Nebra 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 carinthiaca* (Latzel,1880) | MT994079         | KROK025-19     |
| Geophilomorpha      | *Clinopodes carinthiaca* (Latzel,1880) | OL874927         | KROK090-20     |
| Geophilomorpha      | *Clinopodes carinthiaca* (Latzel,1880) | OL874929         | KROK098-20     |
| order         | scientificName                          | GenBankAccession | boldSequenceID |
|--------------|----------------------------------------|------------------|----------------|
| Geophilomorpha | *Clinopodes carinhiacus* (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         | KROK029-20     |
| 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 | *Harpolthobius gottscheensis* Verhoeff, 1937 | MT994105         | KROK036-19     |
| Lithobiomorpha | *Harpolthobius 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     |
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. 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).
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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.

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Supplementary materials

Suppl. material 1: Specimen list [doi](https://doi.org/10.24436/2)

**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](https://doi.org/10.24436/2) (272.89 kb)

Suppl. material 2: Sampling events [doi](https://doi.org/10.24436/2)

**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.

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