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New bugs on the block: new records and first barcodes of rare and specialized ground beetle species from block scree slopes in Germany

(Coleoptera, Carabidae)

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This study reports new distributional records and DNA barcodes of rare ground beetles (Carabidae) resulting from a project on the block scree slope fauna of Germany. We provide and discuss first and new records of *Patrobus assimilis*, *Leistus piceus*, *Leistus montanus* and *Pterostichus negligens* for several federal states in Germany. *Pterostichus negligens* is reported for Saxony-Anhalt for the first time. Further, we generated the first barcode data for *Leistus piceus*, *Leistus montanus* and *Pterostichus negligens* and provide preliminary phylogenies based on the COI fragment. Our analysis of *Leistus* does not support the current systematics of this genus, suggesting the need of additional molecular work with further genetic markers. Our new records provide additional data for the unique faunistic communities of cold refuges in the German middle mountain ranges. In addition, we provide records for more common carabid species based on our field campaign.

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Introduction

Block scree slopes are one of the most special habitat types in Central Europe. Their specific microclimate is the prerequisite for many specialist invertebrate species, which are either endemic to this habitat type, e. g. *Acantholycosa norvegica sudetica* (Koch, 1875) or *Choleva lederiana lederiana* (Reitter, 1902), or are otherwise distributed in the Alps and Fennoscandia (Holdhaus & Lindroth 1939, Molenda 2000, Kastner et al. 2018, Růžička 2011). While detailed analyses of the block scree slopes have been performed in the Black Forest and in the Fichtel Mountains (Buse et al. 2020, Fritze & Blick 2010), there is still much to discover and the distributions of many of the specialized inhabitants of this habitat type remain incomplete (Buse et al. 2020). However, such data is important to understand the ecological requirements on the one hand, and to assess their conservation status on the other hand.

In order to close this gap we have started a larger trapping campaign including most of the middle mountains in Germany to understand the distributions of such specialized taxa. Besides, we generated DNA barcode data for the block scree slope specialists and use this data in order to pro-

vide some phylogenetic context for the species. Here, as part of this project, we focus on rare and little known ground beetles (Carabidae) detected in the trapping samples. Carabids, with currently 582 taxa in Germany (Schmidt et al. 2016), represent a diverse beetle family in Germany and many species are considered important bioindicators (Rainio & Niemelä 2003). As such, many species have specific habitat requirements and are hence only found in special habitat types.

Here, we report the presence of four extremely rare carabids in block scree slopes (*Leistus montanus* Heer, 1837; *Leistus piceus* Froelich, 1799; *Patrobus assimilis* Chaudoir, 1844; *Pterostichus negligens* Sturm, 1824) in Germany and provide assessments of the ground beetle assemblages of these habitats. While *L. montanus* and *Pt. negligens* are listed as extremely rare in Germany, *L. piceus* is considered threatened, and *Pa. assimilis* is even rated threatened by extinction in the recent Red List (Schmidt et al. 2016). We present new records and DNA barcodes of these species and confirm old findings to provide a more complete assessment of their distributions in Germany and discuss their conservation status.

Material and methods

Sampling sites and dates, and trapping method

A total of 250 pitfall traps (up to 21 at each location) were employed in block scree slopes across 15 locations in seven middle mountain regions across Germany (Table 1). In the absence of ecological questions, the traps were randomly placed at each site aiming at an equal distribution across the habitat. Clearly structured scree slopes consisting of source rock, slope body and base of the slope, vegetated and barren parts, including forested zones, received the same number of traps in each part. All sampling sites received 14–21 traps, which were diagonally distributed across the whole width.

The pitfall traps consisted of a plastic cup with a volume of 0.3 l or 0.5 l and a wooden board with a hole in which the cup was inserted. This construction was placed in the rocks aiming to provide a plain entry to the board from the rock (Fig. 1). If there was substrate between the rocks, we used the cups similar to a classical pitfall trap by inserting them directly in the ground. The traps were filled with 99 % propylene glycol (Bay-Wa AG, Munich, Germany) and some drops of detergent. Propylene glycol has been suggested as good DNA conservation fluid for pitfall traps in the past (Höfer et al. 2015), also providing the advantage of lower evaporation compared to ethanol and water based conservation fluids.

Sampling was performed between April 2018 and September 2019. All traps were employed for at least one year in the block scree slopes. Material was collected and conservation liquid was changed every two to three months. In winter, traps were not accessible. However, propylene glycol does not freeze above -60° C, and hence, the traps were active for the whole winter season under snow. Traps were changed, as soon as the snowmelt allowed save access to the traps.

Identification

The material was sorted at higher taxonomic levels (orders) and several taxa (e.g. Carabidae, Arachnidae) were sent to experts for identification. For several groups (e.g. Collembola, Diptera, larvae of diverse arthropod orders), specimens were pooled for further analyses. In some cases of especially interesting taxa,

Table 1. Sampling locations with the mountain range they are located in and associated coordinates.

Mountain range	Location	Lat.	Long.
Harz	Odertal	10.559160	51.736840
Harz	Mönchskappenklippe	10.462560	51.746760
Harz	Hammersteinklippe	10.450390	51.766560
Harz	Wolfswarte	10.502660	51.790650
Harz	Brocken	10.615771	51.801587
Fichtelgebirge	Backöfele	11.855402	50.050889
Fichtelgebirge	Große Kösseine	11.981343	49.987212
Fichtelgebirge	Ochsenkopf (north)	11.809026	50.031621
Thüringer Wald	Großer Beerberg	10.741893	50.665314
Thüringer Wald	Lütschetalsperre	10.773647	50.736390
Thüringer Wald	Jüschnitzgrund	10.816934	50.695493
Schiefergebirge	Steinheid	11.110036	50.467404
Rhön	Schaftstein	9.972061	50.503631
Erzgebirge	Kahleberg	13.729970	50.749047
Oberpfalz	Rauher Kulm	11.849721	49.828477

specimens were subjected to individual DNA barcoding to verify unsure identifications or to identify juvenile individuals of potential scree slope specialists. The Carabidae addressed in this study were identified by second author using the key provided by Müller-Motzfeld (2006).

Molecular analyses

For rare Carabidae detected in our samples we performed DNA barcoding (Hebert et al. 2004), as for these species barcodes so far were either absent or only single or few sequences were available. We generated individual barcodes for eleven specimens from four scree slopes. DNA was isolated non-invasively from whole individuals using the DNeasy Blood & Tissue Kit® (Qiagen, Hilden, Germany). After lysis, the specimens were retrieved and stored in 99.9 % ethanol.

The barcoding fragment of the mitochondrial *cytochrome c* oxidase subunit I gene (COI) was amplified using standard polymerase chain reaction (PCR) procedures with the primers LCO-1490 and HCO-2198 (Folmer et al. 1994). PCR was performed with the following setup: 5.7 µl PCR grade water, 2 µl 5x buffer, 0.5 µl of each primer, 0.2 µl dNTPs, 0.1 µl DreamTaq™ (Thermo Fisher) polymerase and 1 µl template. PCR conditions were as follows: activation at 95°C for 5 min, followed by 35 cycles of 30 s denaturation at 95°C, 1 min annealing at 50°C and 1 min elongation at 72°C. Finally, a 10 min final extension step was performed. PCR success was checked on 1 % agarose gels stained with GelRed and successful products were purified with an enzyme mix consisting of Exonuclease I and Shrimp-Alkaline Phosphatase (ExoSap). Amplicons were sequenced by Macrogen (Amsterdam, Netherlands).

Chromatograms were checked and trimmed in Geneious v. 9 (Kearse et al. 2012). We added published sequences for 22 *Leistus*, 57 *Pterostichus* and 37 *Patrobus* from BOLD (Ratnasingham & Hebert 2007) and NCBI (Sayers et al. 2021) to our dataset (Appendix 1). For all species, we used a maximum of five sequences in the analyses. For *Leistus* and *Patrobus*, we selected all species present in the databases. For *Pterostichus* we focussed on species which were recorded from Germany, because of the huge number of species included in this genus. *Nebria brevicollis* (KU907486) was added as outgroup to all alignments. MUSCLE (Edgar 2004) as implemented in Geneious was used to align sequences for each genus to obtain three separate alignments. The resulting alignments were trimmed to similar length (*Pterostichus* 551 bp, *Leistus* 562 bp, *Patrobus* 622 bp). All sequences are deposited at the BOLD database (Appendix 2). Information on BIN distances, nearest BIN and Neighbour from BOLD systems workbench is provided in Table 2.

Alignments were checked for saturation (Xia et al. 2003, Xia & Lemey 2009) with DAMBE v. 7 (Xia 2018). All Iss.c values were significantly larger than Iss values. We then generated phylogenetic trees using Bayesian inference performed with BEAST v. 2.6.2 (Bouckaert et al. 2019). The best substitution model was determined using the R package PHANGORN (Schliep et al. 2017) in mRAN v. 3.4. (Microsoft 2017) with RStudio v. 1.0.143 (Allaire 2012). The GTR+G+I was determined as best model for *Pterostichus*; HKY+G+I for *Leistus* and *Pa. trobus* Input files for BEAST were prepared with BEAUti v. 2 (Bouckaert et al. 2019). All analyses were run for 10 million generations sampling every 1000 iterations. Resulting log-files were checked with Tracer v. 1.7 (Rambaut et al. 2018) to confirm sufficient ESS (>200) and convergence of the analyses. In a last step, the MCC tree (maximum clade credibility tree) was annotated with TreeAnnotator v. 2 (Bouckaert et al. 2019) after excluding a burn-in of 10 % of samples; the final tree was visualized in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results and discussion

In our survey of ground beetles in the block scree slopes of the middle mountains of Germany, we recorded 31 species of carabids (Table 3). Besides many relatively common species, we detected four very rare specialist species, which have very few records from Germany. We found a total of five individuals of *Leistus piceus* from three mountain ranges

Table 2. BIN cluster, data and distances to nearest neighbours of presented rare species barcodes (created with BOLDsystems Workbench). All % of p-dist.

BIN (BOLD:)	Total Members	Taxa	Count in Project	Average Distance	Maximum Distance:	Distance to Nearest Neighbor:	Nearest BIN URI (BOLD:)	Nearest Member Taxonomy:
ABA1655	59	*Leistus montanus*	2	0.72 %	3.21 %	7.05 %	AAP8339	*Leistus rufomarginatus*
ABW5014	14	*Patrobus assimilis*	1	1.00 %	2.09 %	10.18 %	ADY8917	*Patrobus septentrionis*
ADL2230	5	*Pterostichus negligens*	3	0.39 %	0.66 %	2.75 %	ABZ9679	*Pterostichus tareumiut*
AEE7938	6	*Leistus piceus*	5	0.17 %	0.52 %	6.46 %	ACA9875	*Leistus ferruginosus*

Fig. 1. A pitfall trap between rocks consisting of a plastic cup with a volume of 0.3 l or 0.5 l and a wooden board with a hole in which the cup was inserted.

(Fichtel Mountains, Harz Mountains, Thuringian Forest), two individuals of *Leistus montanus* from the Harz Mountains, one *Patrobus assimilis* and five *Pterostichus negligens* from the Brocken in the Harz Mountains (Table 3, Fig. 2). All specimens of *Leistus montanus*, *Leistus piceus*, and *Patrobus assimilis* and four specimens of *Pterostichus negligens* are stored in the private collection of M.-A. Fritze. One specimen of *Pt. negligens* is stored in the private collection of K. Hannig. All material from the Harz, excluding the focal species, was discarded after determination. All further material is stored in the Natural History Museum Leipzig (Table 3).

Zoogeographic assessment

Pterostichus negligens reaches its western range limit in Germany. Towards the east, disjunct isolated occurrences of the montane to the high alpine distributed species are known as far as western Siberia with a core area in the High Tatras and the Krkonoše Mountains. The nominate subspecies occurs in the Czech Republic, Germany, Poland, Russia and the Ukraine. The second subspecies, *Pt. negligens patris* Smetana, 1950 is reported from Slovakia and the Ukraine (Růžička & Zacharda 1994, Růžička 1996, Molenda 2000, Löbl & Löbl 2017, Mossakowski 2017). The species is stenotopic, cold-adapted and outside of alpine regions depending on block scree slopes. Starting from the montane zone, it is also found next to snowfields (Molenda 1999, 2000). The species is difficult to record, as it only comes to the surface of the scree slopes when conditions are extremely humid; otherwise, it hides deep within the slope or in rocky grounds (Mossakowski 2017). So far, *Pt. negligens* was only known from Hesse, Lower Saxony, Rhineland-Palatinate and Thuringia (Molenda 1996, 1999, 2000, Trautner et al. 2014). In 2019, we caught five individuals of the species in the scree slope on top of the Brocken, representing the first record for Saxony-Anhalt.

Leistus montanus is patchily distributed in Europe, in the west from Ireland, Great Britain and northern Spain, in the east up to the western Ukraine. In Germany, it is largely restricted to block scree slopes (Fritze & Hannig 2012, Schmidt & Trautner 2016, Buse et al. 2020). Most occurrences in Germany are recorded from Baden-Wuerttemberg (Harry &

Table 3. All records of ground beetle species (Carabidae) generated in this study. Records marked with * are from meta-barcoding.

Genus	Species	Subspecies	#	Federal State	Mountain Range	Location
Abax	*parallelepipedus*	*parallelepipedus*	1	Saxony-Anhalt	Harz	Odertal
Abax	*parallelepipedus*	*parallelepipedus*	1	Saxony-Anhalt	Harz	Wolfswarte
Acupalpus	*meridianus*		1	Saxony-Anhalt	Harz	Odertal
Amara	*montivaga*		1	Saxony	Erzgebirge	Kahleberg
Bembidion	*lampros*		1	Hessen	Rhön	Schafstein
Calathus	*micropterus*		1	Saxony	Harz	Brocken
Calathus	*micropterus*		1	Saxony-Anhalt	Harz	Wolfswarte
Calathus	*micropterus*		1	Thuringia	Thuringian Forest	Großer Beerberg
Carabus	*auronitens**		na	Bavaria	Fichtel Mountains	Ochsenkopf (north)
Carabus	*sylvestris*	*sylvestris*	2	Saxony-Anhalt	Harz	Brocken
Carabus	*sylvestris*	*sylvestris*	1	Saxony-Anhalt	Harz	Odertal
Carabus	*hortensis*		1	Thuringia	Thuringian Forest	Jüschnitz
Carabus	*coriaceus*		1	Thuringia	Thuringian Forest	Lütschetalsperre
Carabus	*sylvestris*		1	Thuringia	Thuringian Forest	Großer Beerberg

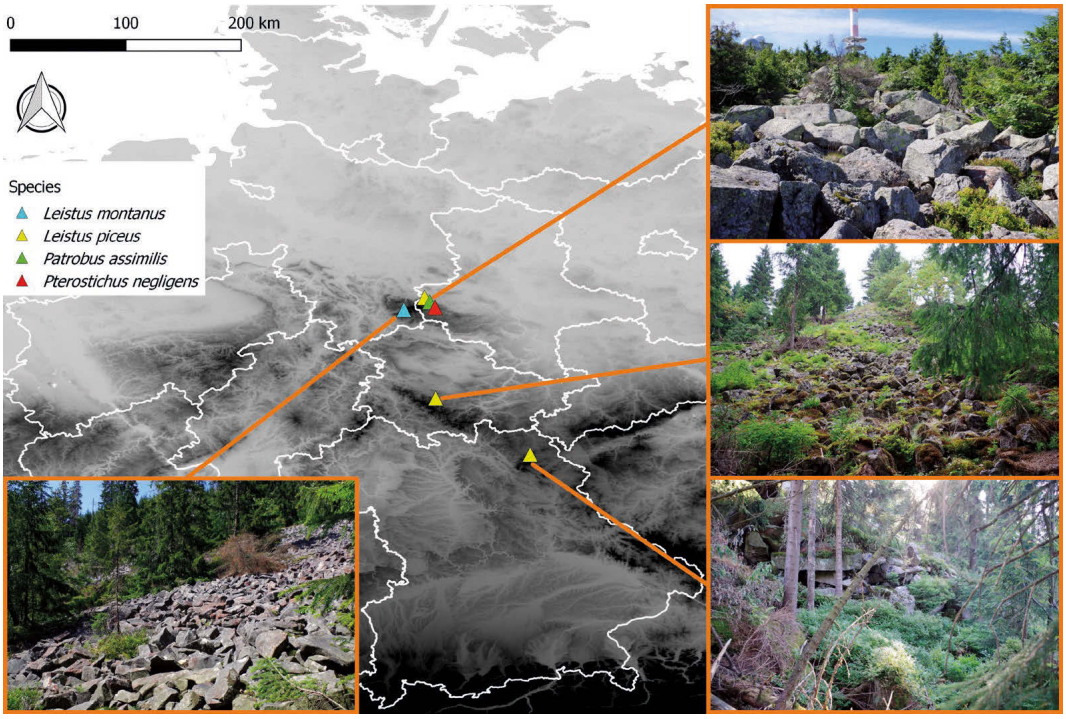

Fig. 2. Map displaying the sampling locations and locations of new records for *L. montanus* (▲), *L. piceus* (▲), *Pa. assimilis* (▲) and *Pt. negligens* (▲).

Trautner 2017). Further, the species has been reported from Bavaria, Lower Saxony, Rhineland-Palatinate, Saxony and Saxony-Anhalt (Fritze & Hannig 2012, Trautner et al. 2014). Habitat choice and ecology of *L. montanus* are comprehensively described in Buse et al. (2020) and Harry & Trautner (2017). We here report two individuals caught at Hammersteinklippe in the Harz Mountains (Lower Saxony) in 2019. They probably belong to the subspecies *kultianus*, which has also been found in Saxon Switzerland (Farkač & Fassati 1999). Our find confirms a 20 year old record of the species from the same location, which was not considered in the last Red List of Lower Saxony in 2003 (Assmann et al. 2003, Mossakowski 2017).

Period	Genbank ID	Collection	Collection ID
2018/2019	na	na	na
2018/2019	na	na	na
2018/2019	na	na	na
2018/2019	na	na	na
2019	na	Natural History Museum Leipzig	NML-i2021/4505
2018/2019	na	na	na
2018/2019	na	na	na
2018	na	Natural History Museum Leipzig	NML-i2021/4503
2018/2019	na	na	na
2018/2019	na	na	na
2018/2019	na	na	na
2019	na	Natural History Museum Leipzig	NML-i2021/4492
2019	na	Natural History Museum Leipzig	NML-i2021/4493
2019	na	Natural History Museum Leipzig	NML-i2021/4506

Table 3. continued

Genus	Species	Subspecies	#	Federal State	Mountain Range	Location
Carabus	*sylvestris*		1	Thuringia	Thuringian Forest	Großer Beerberg
Carabus	*sylvestris*		1	Thuringia	Thuringian Forest	Großer Beerberg
Carabus	*problematicus*		1	Thuringia	Thuringian Forest	Lütschetalsperre
Carabus	*sylvestris*		1	Thuringia	Thuringian Forest	Großer Beerberg
Carabus	*sylvestris*		1	Thuringia	Thuringian Forest	Großer Beerberg
Carabus	*sylvestris*		1	Thuringia	Thuringian Forest	Großer Beerberg
Carabus	*sylvestris**		na	Saxony-Anhalt	Harz	Brocken
Carabus	*arvensis*		1	Saxony	Erzgebirge	Kahleberg
Carabus	*intricatus*		1	Thuringia	Thuringian Forest	Lütschetalsperre
Cicindela	*campestris**		na	Bavaria	Fichtel Mountains	Backöfele
Cychrus	*caraboides*		1	Saxony-Anhalt	Harz	Mönchskappe
Cychrus	*caraboides*		1	Saxony-Anhalt	Harz	Odertal
Cychrus	*caraboides*		1	Hessen	Rhön	Schafstein
Cychrus	*caraboides*		1	Hessen	Rhön	Schafstein
Cychrus	*caraboides*		1	Thuringia	Thuringian Forest	Großer Beerberg
Harpalus	*laevipes**		na	Lower Saxony	Harz	Hammersteinklippen
Harpalus	*rubripes**		na	Bavaria	Fichtel Mountains	Backöfele
Leistus	*piceus*		1	Bavaria	Fichtel Mountains	Ochsenkopf (north)
Leistus	*piceus*		1	Bavaria	Fichtel Mountains	Ochsenkopf (north)
Leistus	*montanus*		1	Lower Saxony	Harz Mountains	Hammersteinklippe
Leistus	*montanus*		1	Lower Saxony	Harz Mountains	Hammersteinklippe
Leistus	*piceus*		1	Saxony-Anhalt	Harz Mountains	Brocken
Leistus	*piceus*		1	Thuringia	Thuringian Forest	Großer Beerberg
Leistus	*piceus*		1	Thuringia	Thuringian Forest	Großer Beerberg
Nebria	*brevicolis*		1	Saxony	Erzgebirge	Kahleberg
Nebria	*brevicollis*		1	Saxony-Anhalt	Harz	Mönchskappe
Patrobus	*assimilis*		1	Saxony-Anhalt	Harz Mountains	Brocken
Poecilus	*cupreus*		1	Bavaria	Fichtel Mountains	Backöfele
Pterostichus	*oblongopunctatus*		1	Bavaria	Fichtel Mountains	Backöfele
Pterostichus	*oblongopunctatus*		1	Bavaria	Fichtel Mountains	Backöfele
Pterostichus	*oblongopunctatus*		1	Bavaria	Fichtel Mountains	Backöfele
Pterostichus	*burmeisteri*		1	Lower Saxony	Harz	Hammersteinklippen
Pterostichus	*burmeisteri*		1	Lower Saxony	Harz	Hammersteinklippen
Pterostichus	*aethiops*		1	Saxony	Harz	Brocken
Pterostichus	*niger*		1	Saxony-Anhalt	Harz	Odertal
Pterostichus	*aethiops*		1	Saxony-Anhalt	Harz	Mönchskappe
Pterostichus	*burmeisteri*		1	Saxony-Anhalt	Harz	Mönchskappe
Pterostichus	*negligens*		1	Saxony-Anhalt	Harz Mountains	Brocken
Pterostichus	*negligens*		1	Saxony-Anhalt	Harz Mountains	Brocken
Pterostichus	*negligens*		1	Saxony-Anhalt	Harz Mountains	Brocken
Pterostichus	*negligens*		1	Saxony-Anhalt	Harz Mountains	Brocken
Pterostichus	*negligens*		1	Saxony-Anhalt	Harz Mountains	Brocken
Pterostichus	*niger*		1	Thuringia	Thuringian Forest	Steinheid
Pterostichus	*niger*		1	Thuringia	Thuringian Forest	Steinheid
Pterostichus	*niger*		1	Thuringia	Thuringian Forest	Steinheid
Pterostichus	*niger*		1	Thuringia	Thuringian Forest	Steinheid
Pterostichus	*niger*		1	Thuringia	Thuringian Forest	Großer Großer Beerberg
Pterostichus	*oblongopunctatus*		1	Thuringia	Thuringian Forest	Großer Großer Beerberg
Trichotichnus	*laevicollis*		1	Saxony	Erzgebirge	Kahleberg
Trichotichnus	*laevicollis*		1	Saxony	Harz	Brocken
Trichotichnus	*laevicollis*		1	Thuringia	Thuringian Forest	Großer Großer Beerberg

Period	Genbank ID	Collection	Collection ID
2019	na	Natural History Museum Leipzig	NML-i2021/4507
2019	na	Natural History Museum Leipzig	NML-i2021/4508
2019	na	Natural History Museum Leipzig	NML-i2021/4513
2018	na	Natural History Museum Leipzig	NML-i2021/4497
2018	na	Natural History Museum Leipzig	NML-i2021/4498
2018	na	Natural History Museum Leipzig	NML-i2021/4499
2018/2019	na	na	na
2019	na	Natural History Museum Leipzig	NML-i2021/4494
2019	na	Natural History Museum Leipzig	NML-i2021/4484
2018/2019	na	na	na
2018/2019	na	na	na
2018/2019	na	na	na
Summer 2019	na	Natural History Museum Leipzig	NML-i2021/4496
2019	na	Natural History Museum Leipzig	NML-i2021/4504
2018	na	Natural History Museum Leipzig	NML-i2021/4502
2018/2019	na	na	na
2018/2019	na	na	na
Summer 2018	MZ921412	M.-A. Fritze	na
Summer 2018	MZ921413	M.-A. Fritze	na
Summer 2019	MZ921411	M.-A. Fritze	na
Summer 2019	MZ921410	M.-A. Fritze	na
Summer 2018	MZ921414	M.-A. Fritze	na
Summer 2018	MZ921416	M.-A. Fritze	na
Summer 2018	MZ921415	M.-A. Fritze	na
Spring 2019	na	Natural History Museum Leipzig	NML-i2021/4495
2018/2019	na	na	na
Summer 2019	MZ921417	M.-A. Fritze	na
2019	na	Natural History Museum Leipzig	NML-i2021/4485
2019	na	Natural History Museum Leipzig	NML-i2021/4490
2019	na	Natural History Museum Leipzig	NML-i2021/4491
2018	na	Natural History Museum Leipzig	NML-i2021/4511
July 2019	na	Natural History Museum Leipzig	NML-i2021/4512
July 2019	na	Natural History Museum Leipzig	NML-i2021/4514
2018/2019	na	na	na
2018/2019	na	na	na
2018/2019	na	na	na
2018/2019	na	na	na
Summer 2019	MZ921418	M.-A. Fritze	na
Summer 2019	MZ921419	M.-A. Fritze	na
Summer 2019	MZ921420	M.-A. Fritze	na
Summer 2019	na	K. Hannig	na
Summer 2019	na	M.-A. Fritze	na
2019	na	Natural History Museum Leipzig	NML-i2021/4486
2019	na	Natural History Museum Leipzig	NML-i2021/4487
2019	na	Natural History Museum Leipzig	NML-i2021/4488
2019	na	Natural History Museum Leipzig	NML-i2021/4489
2018	na	Natural History Museum Leipzig	NML-i2021/4501
2019	na	Natural History Museum Leipzig	NML-i2021/4509
2018/2019	na	na	na
2018/2019	na	na	na
2019	na	Natural History Museum Leipzig	NML-i2021/4510

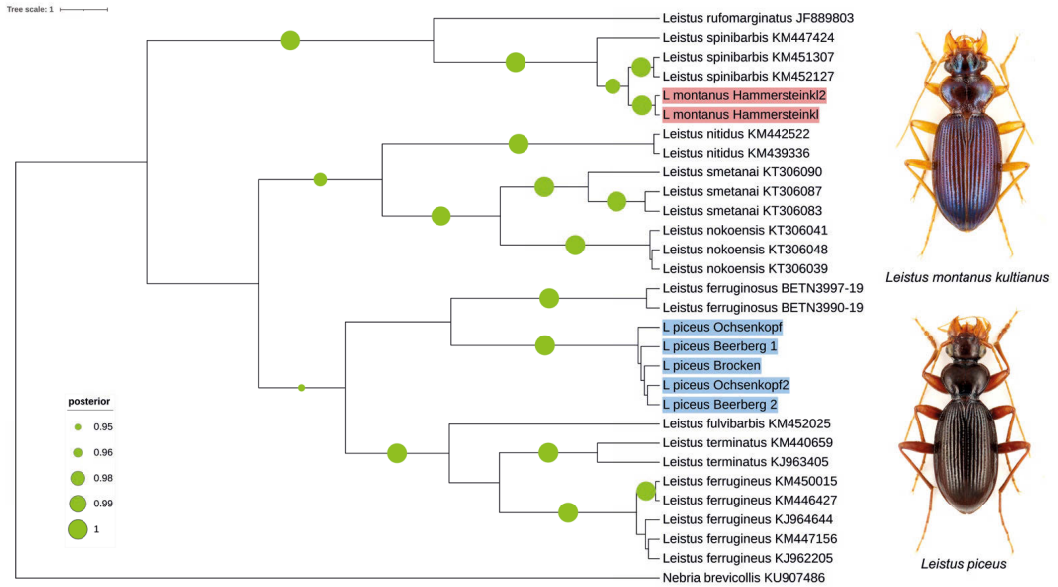

Fig. 3. Phylogenetic reconstruction of assorted species of the genus *Leistus* based on DNA barcodes (photos of *Leistus montanus kultianus* and *L. piceus* by Ortwin Bleich).

Leistus piceus is mainly distributed in the mountain ranges from northeastern France, across southern and central Germany and the Alps to Eastern Europe (Fritze & Blick 2012, Harry & Trautner 2017). In Germany, the species can be found scattered in most mountain ranges up to the Harz in the north. It occurs in montane forests, block scree slopes and near rocky outcrops. At higher altitudes in middle mountains and in the Alps, it can also be found in habitats without trees (GAC 2009, Luka et al. 2009). Habitat preferences and ecology of the species are comprehensively described in Fritze & Blick (2012) and Harry & Trautner (2017). In the current Red List of Saxony-Anhalt, *L. piceus* is listed as "extremely rare" with restricted geographic range (Schnitter et al. 2020). In this federal state, it is only known from the Brocken. Here, so far only single individuals have been reported few times from the open top plateau of the Brocken and from the mountainous spruce forests surrounding it (Schnitter 1999). We here report it once more from the top of the Brocken. A second find from our study comes from the Great Beerberg in the Thuringian Forest. In Thuringia, only few records of *L. piceus* are known from the mountainous parts of the federal state (Rapp 1933, Horion 1941, Liebmann 1955, Hartmann 2011). Here, the species is considered "threatened by extinction" (Hartmann 2011). We also could confirm the record from northern Bavaria, from the scree slope on top of the Ochsenkopf. In Bavaria, the species is mainly found in the Alps and the Bavarian Forest and is considered "endangered" (RL 3) (Trautner et al. 2014, Lorenz & Fritze 2020).

Patrobus assimilis is a boreo-alpine species (Holdhaus 1912, 1954). In Germany, Schnitter (1999) reports it as sporadically occurring in the northeastern German lowlands in raised bogs and in the areas of the postglacial lateral moraines; outside the north-east German range, only one confirmed find from the Bavarian Forest has been reported so far (Trautner et al. 2014). The species has historically and currently been found in the Harz Mountains (Petry 1914, Schnitter 1999) and was reported by the find of a single individual from the top of the Brocken. In the current Red List of Saxony-Anhalt, *Pa. assimilis* is considered "threatened by extinction" (RL 1). Besides the record from the Brocken, only a single further individual is known from Saxony-Anhalt from a fen in the lowlands (Schnitter et al. 2020).

DNA barcoding

In addition to the morphological assessment, we generated two barcodes for *L. montanus*, five for *L. piceus*, one for *Pa. assimilis* and three for *Pt. negligens*. These represent the first published barcodes for *L. piceus*, *L. montanus* and *Pt. negligens* and will help to further assess their distribution and provide important baseline data for molecular-based biomonitoring approaches in the future.

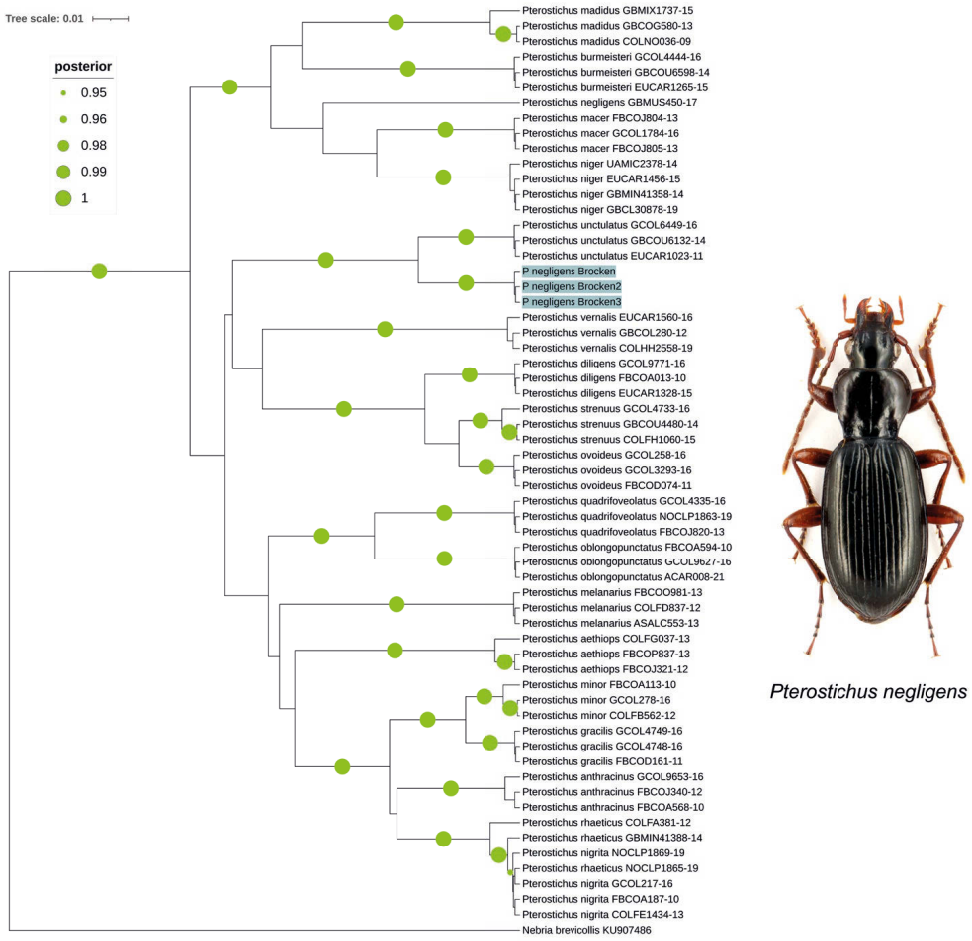

Fig. 4. Phylogenetic reconstruction of assorted species of the genus *Pterostichus* based on DNA barcodes (photo of *Pt. negligens* by Ortwin Bleich).

We used the sequence data to generate small preliminary phylogenies to provide some phylogenetic context for the focal species. For *Leistus*, the two species of our study represented independent clusters in the tree (Fig. 3). *Leistus nitidus* (Duftschmid, 1812), which is currently assigned to the subgenus *Leistus* s. str. (Farkač 2005, Löbl & Löbl 2017), clustered nearby *L. nokoensis* Minowa, 1932, *L. smetanai* Farkač, 1995, but *L. nokoensis* and *L. smetanai* belong to *Evanoleistus*. This might be caused by the limited taxon sampling of our study. *Leistus ferrogineus* is the type species of the subgenus *Neoleistus* and clustered in our tree within a clade (pp = 0.98) of *L. piceus*, *L. ferrugineus* (Linnaeus, 1758), *L. terminatus* (Panzer, 1793) and *L. fulvibarbis* Dejean, 1826, which in the current systematics are members of the subgenus *Leistus*. Kavanaugh et al. (2021), based on a multi-gene dataset suggested treating *Neoleistus* as junior synonym of *Leistus* s. str., as it is nested within this lineage. This suggestion finds support in our data. The subgenus *Pogonophorus*, which includes *L. montanus*, *L. spinibarbis* (Fabricius, 1775) and *L. rufomarginatus* (Duftschmid, 1812) seems to be stable in our tree. This clade is well supported (pp = 1).

Caused by the high number of species in the genus *Pterostichus* we decided for a tree, which just includes a few native German species (Fig. 4). Nevertheless, also this phylogeny pointed to some potential problems in the current systematics of the genus as also previously reported. *Pterostichus nigrita* Escherholz, 1823 clustered admixed with *Pt. rhaeticus* with high support, a result already referred by Raupach et al. (2010, 2020). For both species a close relationship within a species complex together with

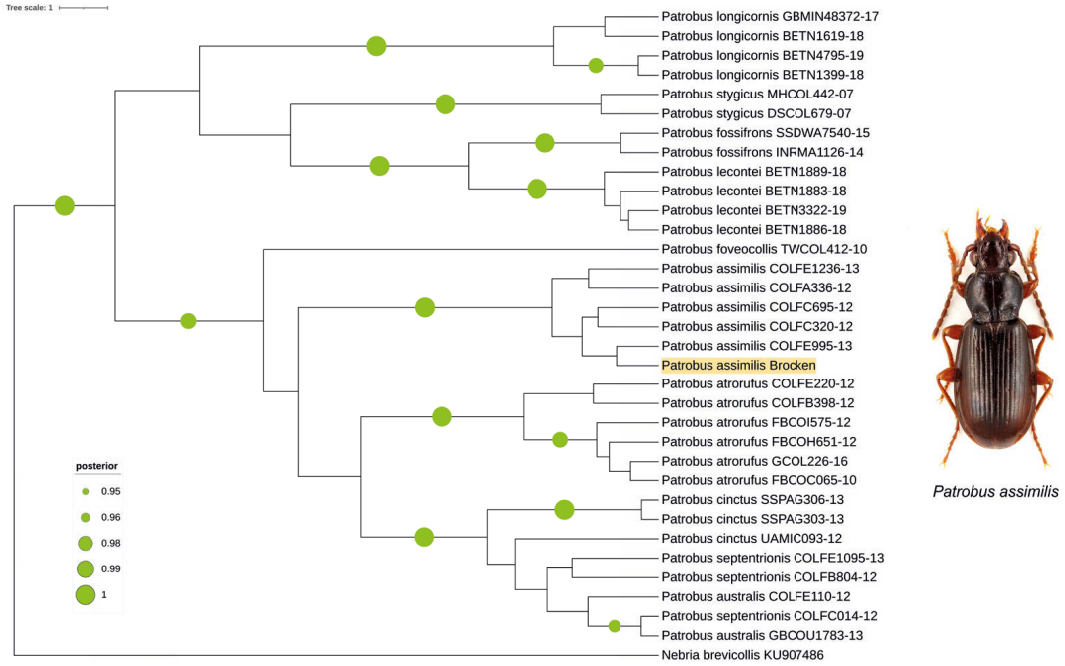

Fig. 5. Phylogenetic reconstruction of assorted species of the genus *Patrobus* based on DNA barcodes (photo of *Pa. assimilis* by Ortwin Bleich).

Pt. anthracinus had already been suggested; all three belong to the subgenus *Pseudomaesus* (Raupach et al. 2010). The sequence GBMUS450-17 (the taxonomic assignment in BOLD has been changed meanwhile) suggested as *Pt. negligens* was problematic; in contrast to our sequences of *Pt. negligens*, this sequence clustered in a distant position in the tree together with species of the subgenera *Adelosia*, *Cheporus*, *Melanius* and *Steropus*. The reason for this is clearly a misidentification of this specimen. The photo provided in BOLD, does not show *Pt. negligens*, but *Pt. rhilensis rhilensis* Rottenberg, 1874 of the subgenus *Pterostichus* (pers. communication with Teodora Teofilova and comparison to material of collection J. Trautner and M.-A. Fritze). *Pterostichus negligens* was never recorded for Bulgaria before (Hieke & Wrase 1988, Hieke 1989, Wrase 1991). Further, the quality of this sequence seems to be very low, as a high number of missing bases (N) within the sequence are present. Our sequences of *Pt. negligens* represent together with *Pt. unctulatus* (Duftschmid, 1812), another species of the subgenus *Cryobius*, a monophyletic and distinct cluster.

For *Patrobus*, we added representatives of all available taxa from BOLD and NCBI to our analysis (Fig. 5). Our sequence of *Pa. assimilis* joined a well-supported clade (pp = 1) of other sequences of *Pa. assimilis*. Not clear seemed to be a clade of *Pa. australis*, *Pa. septentrionis* and *Pa. cinctus*. Our phylogeny could not resolve the relationships between these species.

Conclusion

In this paper we provide new and additional records for four rare carabid beetles from block scree slopes in the middle mountains of Germany and the first record of *Pterostichus negligens* for Saxony-Anhalt. We further generated DNA barcode data for verification of the species identity. For *Leistus piceus*, *Leistus montanus* and *Pterostichus negligens* our data represent the first published DNA barcodes.

Remark

MAF and RK contributed equally.

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Appendix

Appendix 1. List of sequence IDs from BOLD.

Species	Seqence_ID (BOLD)	Species	Seqence_ID (BOLD)	Species	Seqence_ID (BOLD)
L. ferrugineus	KM447156	*Pa. atrorufus*	FBCOI575-12	*Pt. gracilis*	GCOL4749-16
L. ferrugineus	KM450015	*Pa. atrorufus*	GBCL32331-19	*Pt. macer*	FBCOJ804-13
L. ferrugineus	KM446427	*Pa. atrorufus*	GCOL226-16	*Pt. macer*	FBCOJ805-13
L. ferrugineus	KJ962205	*Pa. australis*	COLFE110-12	*Pt. macer*	GCOL1784-16
L. ferrugineus	KJ964644	*Pa. australis*	GBCOU1783-13	*Pt. madidus*	COLNO036-09
L. ferruginosus	BETN3997-19	*Pa. cinctus*	SSPAG303-13	*Pt. madidus*	GBCOG580-13
L. ferruginosus	BETN3990-19	*Pa. cinctus*	SSPAG306-13	*Pt. madidus*	GBMIX1737-15
L. fulvibarbis	DQ155974	*Pa. cinctus*	UAMIC093-12	*Pt. melanarius*	ASALC553-13
L. fulvibarbis	KM452025	*Pa. fossifrons*	INRMA1126-14	*Pt. melanarius*	COLFD837-12
L. nitidus	KM439336	*Pa. fossifrons*	SSDWA7540-15	*Pt. melanarius*	FBCOO981-13
L. nitidus	KM442522	*Pa. foveocollis*	SSBAD3551-12	*Pt. minor*	FBCOA113-10
L. nokoensis	KT306039	*Pa. foveocollis*	SSBAF7815-13	*Pt. minor*	COLFB562-12
L. nokoensis	KT306048	*Pa. foveocollis*	TWCOL412-10	*Pt. minor*	GCOL278-16
L. nokoensis	KT306041	*Pa. lecontei*	BETN1883-18	*Pt. niger*	GBMIN41358-14
L. rufomarginatus	KM446066	*Pa. lecontei*	BETN1886-18	*Pt. niger*	UAMIC2378-14
L. rufomarginatus	JF889803	*Pa. lecontei*	BETN1889-18	*Pt. niger*	GBCL30878-19
L. smetanai	KT306083	*Pa. lecontei*	BETN3322-19	*Pt. niger*	EUCAR1456-15
L. smetanai	KT306090	*Pa. longicornis*	BETN1399-18	*Pt. nigrita*	FBCOA187-10
L. smetanai	KT306087	*Pa. longicornis*	BETN1619-18	*Pt. nigrita*	COLFE1434-13
L. spinibarbis	KU907520	*Pa. longicornis*	BETN4795-19	*Pt. nigrita*	GCOL217-16
L. spinibarbis	KM447424	*Pa. longicornis*	GBMIN48372-17	*Pt. oblongopunctatus*	FBCOA594-10
L. spinibarbis	HQ164882	*Pa. longicornis*	RBINA338-13	*Pt. oblongopunctatus*	GCOL9627-16
L. spinibarbis	JQ689906	*Pa. septentrionis*	COLFB804-12	*Pt. oblongopunctatus*	ACAR008-21
L. spinibarbis	KM451307	*Pa. septentrionis*	COLFC014-12	*Pt. ovoideus*	FBCOD074-11
L. spinibarbis	KM452127	*Pa. septentrionis*	COLFE1095-13	*Pt. ovoideus*	GCOL258-16
L. terminatus	KM440659	*Pa. stygicus*	DSCOL679-07	*Pt. ovoideus*	GCOL3293-16
L. terminatus	KJ963405	*Pa. stygicus*	MHCOL442-07	*Pt. quadrifoveolatus*	NOCLP1863-19
Nebria brevicollis	KU907486	*Pa. stygicus*	SSKUB12561-15	*Pt. quadrifoveolatus*	FBCOJ820-13
Patrobus assimilis	COLFA336-12	*Pterostichus aethiops*	COLFG037-13	*Pt. quadrifoveolatus*	GCOL4335-16
Pa. assimilis	COLFC320-12	*Pt. aethiops*	FBCOP837-13	*Pt. rhaeticus*	NOCLP1865-19
Pa. assimilis	COLFC695-12	*Pt. aethiops*	FBCOJ321-12	*Pt. rhaeticus*	GBMIN41388-14
Pa. assimilis	COLFE995-13	*Pt. anthracinus*	FBCOA568-10	*Pt. rhaeticus*	COLFA381-12
Pa. assimilis	COLFE1236-13	*Pt. anthracinus*	FBCOJ340-12	*Pt. strenuus*	GCOL4733-16
Pa. assimilis	GBMNA36967-19	*Pt. anthracinus*	GCOL9653-16	*Pt. strenuus*	GBCOU4480-14
Pa. atrorufus	COLFB398-12	*Pt. burmeisteri*	GCOL4444-16	*Pt. strenuus*	COLFH1060-15
Pa. atrorufus	COLFE220-12	*Pt. burmeisteri*	EUCAR1265-15	*Pt. unctulatus*	GCOL6449-16
Pa. atrorufus	COLHH2559-19	*Pt. burmeisteri*	GBCOU6598-14	*Pt. unctulatus*	GBCOU6132-14
Pa. atrorufus	COLFB398-12	*Pt. diligens*	FBCOA013-10	*Pt. unctulatus*	EUCAR1023-11
Pa. atrorufus	COLFE220-12	*Pt. diligens*	GCOL9771-16	*Pt. vernalis*	COLHH2558-19
Pa. atrorufus	COLHH2559-19	*Pt. diligens*	EUCAR1328-15	*Pt. vernalis*	GBCOL280-12
Pa. atrorufus	FBCOC065-10	*Pt. gracilis*	FBCOD161-11	*Pt. vernalis*	EUCAR1560-16
Pa. atrorufus	FBCOH651-12	*Pt. gracilis*	GCOL4748-16		

Appendix 2. List of sequence IDs of new barcodes.

Species	Seqence_ID (Genbank)	Species	Seqence_ID (Genbank)	Species	Seqence_ID (Genbank)
Leistus montanus (Hammersteinkl)	MZ921411	*Leistus piceus* (Brocken)	MZ921414	*Pterostichus negligens* (Brocken)	MZ921418
Leistus montanus (Hammersteinkl2)	MZ921410	*Leistus piceus* (Ochsenkopf)	MZ921412	*Pterostichus negligens* (Brocken2)	MZ921419
Leistus piceus (Beerberg1)	MZ921416	*Leistus piceus* (Ochsenkopf2)	MZ921413	*Pterostichus negligens* (Brocken3)	MZ921420
Leistus piceus (Beerberg2)	MZ921415	*Patrobus assimilis* (Brocken)	MZ921417		