



First insights into the phylogeny of the subgenus *Cryobius* Chaudoir, 1838 (Coleoptera: Carabidae: *Pterostichus*)

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Abstract

The past climatic changes caused repeated distribution shifts within insect populations leading to a highly diverse fauna in the mountain regions, which have acted as a refuge for many groups. There, some taxa have adapted to high altitudes and cold climatic conditions. One of those is the highly diverse and Holarctic subgenus *Cryobius* Chaudoir, 1838 (Carabidae: *Pterostichus*) including both locally and widely distributed species. Isolated and morphologically divergent populations of the same species led to the description of many subspecies. Until now, there has been no comprehensive work concerning the phylogeny of *Cryobius*, and genetic data on this taxon are sparse. This study is the first to provide insights into the molecular phylogeny of this subgenus, focusing on species from the Pyrenean and Cantabrian mountain systems. *Cryobius* specimens were sequenced targeting mitochondrial and nuclear genes. A molecular phylogeny was then built, merging the new data with genetic data from online public databases. All species of *Cryobius* included in this study form a monophyletic clade within *Pterostichus*. The synonymy of the two former taxa *Pyreneorites* and *Haptoderus* with *Cryobius* is confirmed by this study. *Cryobius* of the Pyreneo-Cantabrian area are closely related. Moreover, several well-supported clades of local species were found. The results further indicate a relation between Nearctic and Eastern Palearctic *Cryobius*, in agreement with the theory of faunal and floral colonization of North America via the Bering land bridge.

Keywords

Haptoderus, Pyrenees, Cantabria, ground beetle, orophily

1. Introduction

The phylogenetic classification of insects is a process that is subject to constant changes, not least due to the introduction of molecular methods, which lead to major progresses. And still, for most of the groups, an in-depth knowledge is missing (Wiegmann et al. 2009; Trautwein et al. 2012; Misof et al. 2014). This is especially the case

among representatives of the highly diverse insect fauna of montane regions. It was found by various studies that species richness, not only of insects, is concentrated in mountains (Barthlott et al. 1996; Pryke and Samways 2010, Garrick 2011; Steinbauer et al. 2016; Polato et al. 2018). It is argued that repeated climatic fluctuations in



Figure 1. *Cryobius* specimens. **Left** *Pterostichus (Cryobius) colasi* (Jeannel, 1937) male, from the Pyrenees. **Right** *Pterostichus (Cryobius) pumilio* (Dejean, 1828) female, from Cantabria. Scale bars: 3 mm.

the Pliocene and Pleistocene caused radical landscape changes leading to shifts in species distribution (Zinovyev 2007; Ehlers et al. 2018). During glacial maxima, populations retreated to isolated glacial refuges and adapted in situ, whereas some later recolonized the re-exposed areas. This repeated process driven by climatic fluctuations led to an acceleration of speciation in those regions, as postulated in the so-called ‘Pleistocene species pump’ hypothesis (Knowles 2000; Schoville et al. 2012; Wallis et al. 2016). Another contributing aspect are the different environmental conditions along altitudinal gradients (Körner 2007). Ultimately, all of those factors facilitate population isolation and specialization, and therefore contribute to explain the species richness in montane habitats.

Similar effects are observed in ground beetle fauna (Coleoptera: Carabidae). There are many diverse taxa comprising species that are adapted to high altitudes and cold climatic conditions. This is especially true for some representatives of the tribes Carabini, Pterostichini, Nebriini, or Trechini (Jeannel 1928; Müller-Motzfeld 2004). Additionally, many high altitude ground beetle species are brachypterous (atrophied hindwings) (Kavanaugh and Ball 1985). Flightlessness leads to a reduced dispersal rate, which is likely to further promote isolation. Considering all those facts, the study of the phylogeny of highly diverse montane ground beetle taxa is of great interest. Besides morphological characters and ecological information, genetic data is nowadays fundamental for our understanding of lineage diversification. Individual taxa of high altitude ground beetles have already been analyzed in molecular phylogenetic studies (see e.g. Schmidt 2011; Schmidt et al. 2012; Weng et al. 2016, 2020). Still, some taxa remain underrepresented in this regard, as is the case for some species of the *Pterostichus* subgenus *Cryobius*

Chaudoir, 1838. This taxon was rearranged several times. The most important changes are summarized below.

Jeannel (1937) regarded *Cryobius* as a subgenus of *Haptoderus* Chaudoir, 1838. It was previously defined as a subgenus of *Pterostichus* Bonelli, 1810, but Jeannel regarded *Haptoderus* as a distinct genus. According to his views, *Cryobius* was a species-rich group distributed in the whole Arctic, North America, Asia and Europe, whereas *Haptoderus* was restricted to Europe and Central Asia (Jeannel 1937). In addition, Jeannel recognized that some *Haptoderus* species from the Pyrenees (e.g. *pumilio* (Dejean, 1828), *infimus* (Chaudoir, 1868), *amoenus* (Dejean, 1828)) differ in one morphological character (the punctuation of the metepisterna) from other species of *Haptoderus* and *Cryobius*. Therefore, he further divided the genus *Haptoderus* into three subgenera: 1. *Haptoderus* s.str., 2. *Cryobius* and 3. *Pyreneorites* Jeannel, 1937. Jeannel further stated that amongst those three subgenera, *Pyreneorites* and *Cryobius* would morphologically be very close to each other (Jeannel 1942). Interestingly, *Pyreneorites* exclusively included species that were described from the Pyrenees, but *Haptoderus* s.str. also comprised species restricted to the Pyrenees like *abaxoides* (Dejean, 1828), *colasi* (Jeannel, 1937) and *amaroides* (Dejean, 1828). Ball (1966) considered *Pyreneorites* as a synonym of *Haptoderus* and further proposed the subgeneric status of *Haptoderus* within *Pterostichus*. Finally, Bousquet (1999) synonymized *Haptoderus* with *Cryobius*. He chose to retain the name *Cryobius*, as the concept of this group includes both Palearctic and Nearctic species, in contrast to *Haptoderus*, which only includes Palearctic species (Bousquet 1999). The subgeneric status of *Cryobius* within the genus *Pterostichus* is nowadays consensual (Pupier 2011; Bousquet 2017).

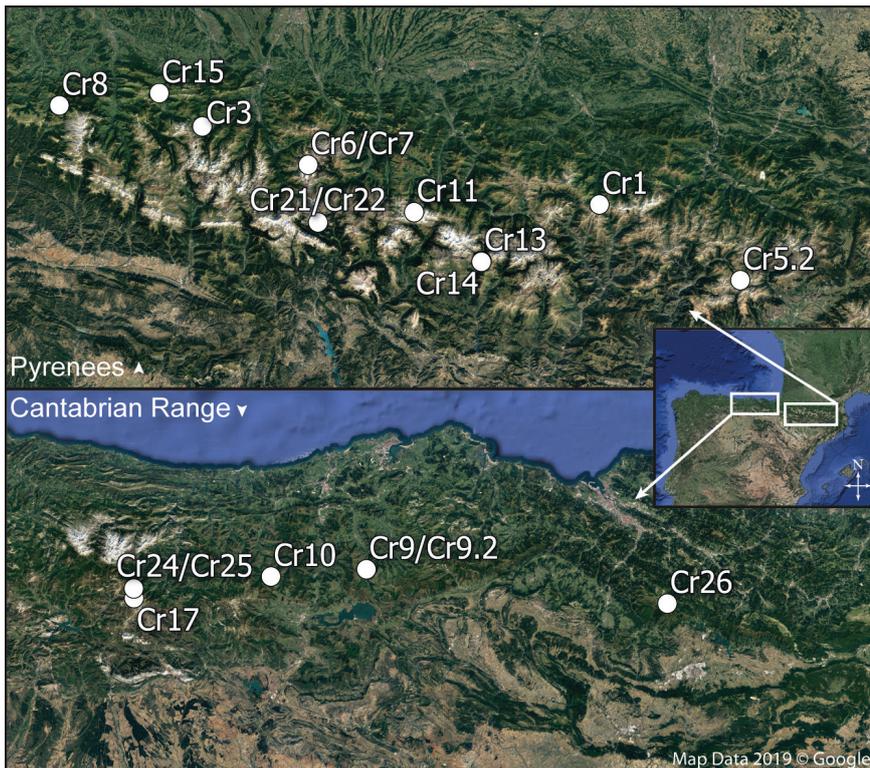


Figure 2. Sampling locations in the Pyrenees and the Cantabrian Range. Localities are labelled with the respective specimen codes. Google Maps layer edited with QGIS 3.16.6-Hannover (<https://qgis.org/de/site/>), edited with Adobe Illustrator v.26.0.3 (<https://adobe.com/products/illustrator>)

As its name indicates (Greek: cryos = cold, bios = life), *Cryobius* comprises many cold-adapted species. About 215 species are currently described (subspecies not included) that are present in the Palearctic and the Nearctic (Bousquet 2012, 2017). They are most exclusively endemic to montane regions and often occur at high elevations. Many species described from the Pyrenees and the Cantabrian Range can be found above 2000 m (Jeannel 1942; Jeanne 1969). In contrast to that, *P. pumilio* (Dejean, 1828) is a widespread species found at low altitude, with records between ca 300 m and 1200 m in Germany for instance (Scheurig et al. 1996; Rietze 2001; Müller-Kroehling 2013; Borchard et al. 2014). As is often the case for ground beetles, the subgenus *Cryobius* includes both very widely and very locally distributed species. Amongst those, there are several Holarctic species. One remarkable example is *P. brevicornis brevicornis* (Kirby, 1837) which ranges from the Kola Peninsula (Northwest Russia) eastward to Newfoundland (Ball 1966; Zubrii et al. 2022). For the central European region, the species with probably the largest distribution range is *P. unctulatus* (Duftschmid, 1812). It is reported from 16 countries, ranging from the Alps to the Carpathians. *Pterostichus pumilio pumilio* is another example with a range expanding from the Cantabrian Mountains to the Carpathians (Jeannel 1942; Coulon and Pupier 2014; Bousquet 2017; Trautner 2017).

The size of *Cryobius* species roughly ranges between 4 and 12 mm (Jeannel 1937, 1942, 1947; Ball 1966). The coloration of the imagoes varies from brown to black. A reduction of wings is reported for many species. For example, Jeannel (1942) described the former subgenus *Haptoderus* as apterous. Bousquet (1999) states that in *Cryobius* the “wings are markedly reduced in all species”. More re-

cent publications on *Cryobius* report only brachyptery (Ball and Currie 1997; Aßmann 1998; Strodl et al. 2007; Trautner 2017). In contrast to aptery, brachyptery merely describes the condition of atrophied hindwings that have no function as flight organs. It might be that Jeannel did not make this distinction when talking about *Haptoderus* species. Information on the wing formation is not available for all currently valid *Cryobius* species. However, it is likely that brachyptery is a common feature of this subgenus. In any case, there are no macropterous species described for this subgenus. Little is known about the biology and life cycles of *Cryobius* species. According to Bousquet (1999), in boreal forests specimens can be found under leaf litter and the bark of dead trees. Regarding the arctic and alpine tundra, they mainly live under rocks and in moss. Representative specimens of *Cryobius* from Western Europe are shown in Fig. 1.

Until now there has been no comprehensive work on the phylogeny of *Cryobius*. There are some publications discussing the relationships of several *Pterostichus* subgenera or relationships between the North American and European *Cryobius*. Still, all those works are mainly based on morphological characters (Jeannel 1937; Ball 1966). Molecular data on this subgenus are overall scarce. Some studies provide single mitochondrial or nuclear gene sequences of specimens that were included in general phylogenetic analyses of the genus *Pterostichus* or other subgenera than *Cryobius* (Will and Gill 2008; Sasakawa 2009; Raupach et al. 2010). Additional genetic material accessible was sequenced within data collection projects such as BOLD (www.barcodinglife.org).

The aim of this study is to provide a first insight into the molecular phylogeny of *Cryobius* by focusing on species inhabiting the Pyrenean and Cantabrian massifs, and

Table 1. Primers used in this study.

	Primer Name (Sense: forward F, reverse R)	Sequence	Reference
<i>cox1</i>	LCO 1490 (F)	5'GGTCAACAAATCATAAAGATATTGG3'	Folmer et al. (1994)
	HCO 2198 (R)	5'TAAACTTCAGGGTGACCAAAAAATCA3'	Folmer et al. (1994)
	K699 (F)	5'WGGGGGGTAAACTGTTTCATCC3'	Wahlberg (2009)
	RON (R)	5'GGAGCYCCWGATATAGCTTTCCC3'	Simon et al. (1994)
<i>rrnl + tRNA-Leu + nad1</i>	16Sar (F)	5'CGCCTGTTTAWCAAAAACAT3'	Simon et al. (1994)
	ND1A (R)	5'GGTCCCTTACGAATTTGAATATATCCT3'	Simon et al. (1994)
<i>LSU</i>	D1 (F)	5'GGGAGGAAAAGAACTAAC3'	Ober (2002)
	LS1R (R)	5'TTTCGGGKTCWCAGGTTTAC3'	Kanda et al. (2014)
	LS1F (F)	5'AGAGTTCAAGAGTACGTGAAACCG3'	Kanda et al. (2014)
	D3L (R)	5'GCATAGTTCACCATCTTTCCGG3'	Kanda et al. (2014)
<i>SSU</i>	18S5' (F)	5'GACAACCTGGTTGATCCTGCCAGT3'	Shull et al. (2001)
	18Sb5.0 (R)	5'TAACCGCAACAACCTTAAT3'	Shull et al. (2001)

to test the synonymy of the two subgenera *Haptoderus* and *Pyreneorites*. For that purpose, four gene fragments of several species were analyzed. A first molecular phylogeny was then built by combining these datasets with sequences publicly available at Genbank (www.ncbi.nlm.nih.gov/Genbank).

2. Methods

2.1. Taxon sampling

The specimens used for this work were mainly collected in the Pyrenean and Cantabrian mountain chains (Fig. 2). Further specimens included were from the Massif Central (France), Italy, Bosnia Herzegovina and Turkey. The respective collection sites are given in Table 2.

Directly after collection in field, the specimens were transferred to 2 ml plastic microtubes with sealed screw caps, filled with 95% ethanol to preserve the specimens. The tubes were later stored at -20°C . The specimens of each collection site were sorted by morphospecies. One individual of each morphospecies was used for DNA analysis. A code was then given to each of these specimens (e.g. 'Cr2', Table 2). These codes are referenced throughout this work. Spare specimens remained stored as described above. This procedure was carried out for all collection sites. In total, 26 specimens were sampled and processed for DNA analysis. The specimens are deposited at the Stuttgart State Museum of Natural History (SMNS).

2.2. DNA extraction, PCR amplification and sequencing

DNA extraction and purification were carried out with the DNeasy Blood & Tissue Kit (50) (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The DNA extraction was non-destructive and processed

with whole specimens. The areas between the head and the thorax and between the thorax and the abdomen were slightly opened to allow better digestion by the proteinase K. Overnight sample incubation for DNA extraction was performed with the Heating ThermoMixer MHL 23 (Ditabis, Pforzheim, Germany). Subsequently, the DNA concentration of each sample was measured using the NanoPhotometer® N60 (IMPLEN, München, Germany) to confirm a successful extraction.

Four gene fragments were targeted for sequencing including two mitochondrial and two nuclear genes: "cox1" cytochrome c oxidase subunit 1 – mitochondrial (CO1); "rrnl + tRNA-Leu + nad1" 5' end of the large ribosomal 16S unit + tRNA-Leucine gene + 3' end of the NADH dehydrogenase subunit 1 – mitochondrial (16S); "LSU" large ribosomal subunit – nuclear (28S); "SSU" small ribosomal subunit – nuclear (18S). The primers used are listed in Table 1. For some samples, the amplification of the whole fragment of CO1 and LSU fragments failed. In those cases, primer pairs targeting shorter but partially overlapping fragments were used. DNA amplification was carried out with the Taq PCR Master Mix Kit (250) (QIAGEN, Hilden, Germany) using the Labcycler Basic (SensoQuest, Göttingen, Germany). A standard program was first used for all PCRs. In case of an unsuccessful run (no-, insufficient- or ambiguous results shown in the control gel), the cycling programs were slightly altered to achieve better amplification results.

The PCR products were then controlled with a gel electrophoresis. Therefore a 1%-agarose gel (1:100 agarose / TAE buffer 1×) with GelRed® (Fremont, CA, USA) was run at 100 V in a Mupid® One Electrophoresis System, Advance (Mupid CO. LTD., Tokyo, Japan) for 25 min. The gels were then photographed under UV light using the Pentax TV Zoom lens 8–48 mm 1:1.0 (Ricoh Co. Ltd. Operations, Tokyo, Japan) and the BioDocAnalyze-Software (Analytik Jena GmbH, Jena, Germany). PCR product purification was conducted with the QIAquick PCR Purification Kit (250) (QIAGEN, Hilden, Germany). In preparation for sequencing, 5 µl of the purified PCR product and 5 µl of the respective primer were added to a 5 ml centrifuge tube. The same primer aliquots were used

Table 2. Sequenced specimens with localities, codes and GenBank accession numbers (new sequences in bold).

Subgenus (in ingroup) Genus (in outgroup)	Species	Locality	Code	COI	28S	16S	18S	Reference
Ingroup taxa								
<i>Cryobius</i> Chaudoir, 1838	<i>abaxoides</i> cf. <i>abaxoides</i> (Dejean, 1828)	Spain, Pyrenees, Huesca, Sierra Tendeñera, Biescas, 1983 m	Cr22	ON969265	ON979805			this study
<i>Cryobius</i> Chaudoir, 1838	<i>abaxoides</i> cf. <i>bigerricus</i> (Jeannel, 1937)	France, Pyrenees, Hautes-Pyrénées, Pic du Néouvielle, 2700 m	Cr6	ON969266	ON979804	ON979788		this study
<i>Cryobius</i> Chaudoir, 1838	<i>amoenus</i> (Dejean, 1828)	Spain, Pyrenees, Huesca, Sierra Tendeñera, Biescas, 2205 m	Cr21	ON969283	ON979806		ON979822	this study
<i>Cryobius</i> Chaudoir, 1838	cf. <i>anatolicus</i> Jedlička, 1963	Turkey, Black Sea region, Trabzon, Hamsiköy, 1550 m	Cr4	ON969268	ON979800		ON979816	this study
<i>Cryobius</i> Chaudoir, 1838	<i>apenninus</i> (Dejean, 1831)	Italy, Apennine Alps, Piemont, Biella, Santuario di Oropa (beech grove)	Cr2	ON969267	ON979798	ON979789	ON979823	this study
<i>Cryobius</i> Chaudoir, 1838	<i>aralarensis aralarensis</i> (Español & Mateu, 1945)	Spain, Cantabrian Range, Basque region, Monte Gorbea, Dolina	Cr26	ON969279	ON979808		ON979824	this study
<i>Cryobius</i> Chaudoir, 1838	<i>aralarensis asturicus</i> (Jeannel, 1969)	Spain, Cantabrian Range, Cantabria, Puerto de la Magdalena, Luena	Cr9	ON969284	ON979811	ON979786		this study
<i>Cryobius</i> Chaudoir, 1838	<i>barryorum</i> Ball, 1962	Canada, Nunavut, Bylot-Island	—	HQ938140				iBOL – direct submission
<i>Cryobius</i> Chaudoir, 1838	<i>brevicornis</i> (Kirby, 1837)	Canada, Nunavut, Cambridge Bay	—	MN670020				Pentinsaari et al. 2020
<i>Cryobius</i> Chaudoir, 1838	<i>cantabricus cantabricus</i> (Schaufuss, 1862)	Spain, Cantabrian Range, Asturias, Puerto de San Glorio	Cr17	ON969278				this study
<i>Cryobius</i> Chaudoir, 1838	<i>cantabricus cantabricus</i> (Dejean, 1828)	Spain, Cantabrian Range, Asturias, Puerto de San Glorio	Cr25	ON969271	ON979810	ON979787	ON979821	this study
<i>Cryobius</i> Chaudoir, 1838	<i>caribou</i> Ball, 1962	Canada, Manitoba, Churchill	—	KJ203835				Woodcock et al. 2013
<i>Cryobius</i> Chaudoir, 1838	<i>colasi</i> (Jeannel, 1937)	Spain, Pyrenees, Lleida, Vielha, Pania de Senet, Barranco de Salenca	Cr14	ON969264	ON979814		ON979818	this study
<i>Cryobius</i> Chaudoir, 1838	<i>empetricola</i> (Dejean, 1828)	Canada, Yukon Territory, Whitehorse	—	KR490739				Hebert et al. 2016
<i>Cryobius</i> Chaudoir, 1838	<i>infirmus</i> (Chaudoir, 1868)	Andorra, Pyrenees, Port d'Envalira, Pic Blanc	Cr5.2	ON969280	ON979802	ON979790	ON979825	this study
<i>Cryobius</i> Chaudoir, 1838	<i>kurosawai</i> Tanaka, 1958	Japan, Hokkaido, Daseitsu mountains	—		AB243485			Sasakawa 2009
<i>Cryobius</i> Chaudoir, 1838	<i>nivalis</i> (Sahlberg, 1844)	USA, Alaska, St. Matthew Island	—	KU876047				Sikes et al. 2016
<i>Cryobius</i> Chaudoir, 1838	<i>pinguedinensis</i> (Eschscholtz, 1823)	Canada, Manitoba, Churchill	—	HQ582359				iBOL – direct submission
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	Germany, Rhineland-Palatinate, Zweibruecken-Mauschbach	—	KM451184				Hendrich et al. 2014

Subgenus (in ingroup) Genus (in outgroup)	Species	Locality	Code	COI	28S	16S	18S	Reference
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	Germany, Bavaria, Freyung-Grafenau	—	KM444226				Hendrich et al. 2014
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	Germany, Thuringia, Fischbach/Rhoen	—	KU915690				Rulik et al. 2017
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	France, Pyrenees, Ariège, Couffens, Cirque d'Anglade	Cr1	ON969274	ON979813	ON979794		this study
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	France, Pyrenees, Hautes-Pyrénées, Arrens-Marsous, Pic du Gabizos, 2000 m	Cr3	ON969261	ON979809			this study
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	France, Pyrenees, Pyrénées-Atlantiques, Sainte-Engrâce, in front of La Verna cave	Cr8	ON969260				this study
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	Spain, Pyrenees, Lleida, Vielha, Panta de Senet, Barranco de Salenca	Cr13	ON969276		ON979795		this study
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	France, Pyrenees, Pyrénées-Atlantiques, Gères Belessten	Cr15	ON969277				this study
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	France, Central Massiv, Cantal, Le Lioran, way to the Font de Cère pass	Cr20	ON969281		ON979792	ON979820	this study
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	Spain, Cantabrian Range, Asturias, Puerto de San Glorio	Cr24	ON969262	ON979807	ON979793		this study
<i>Cryobius</i> Chaudoir, 1838	<i>pumilio</i> (Dejean, 1828)	France, Central Massif, Cantal, Albeipierre-Bredons, Prat de Bouc, 1279 m	Cr27.2	ON969282	ON979815			this study
<i>Cryobius</i> Chaudoir, 1838	<i>pusillus</i> (Dejean, 1828)	France, Pyrenees, Haute-Garonne, Oô, Portillon d'Oô, 2600 m	Cr11	ON969263	ON979801	ON979791	ON979819	this study
<i>Cryobius</i> Chaudoir, 1838	<i>pusillus pusillus</i> (Dejean, 1828)	France, Pyrenees, Hautes-Pyrénées, Pic du Néouvielle, 2700 m	Cr7	ON969275	ON979803			this study
<i>Cryobius</i> Chaudoir, 1838	<i>riparius</i> (Dejean, 1828)	Canada, British Columbia, Revelstoke	—	JF888281				iBOL-direct submission
<i>Cryobius</i> Chaudoir, 1838	<i>riparius</i> (Dejean, 1828)	USA, Montana, Flathead County	—		EU142445			Will & Gill 2008
<i>Cryobius</i> Chaudoir, 1838	cf. <i>subiasi</i> (Ortuño & Zaballo, 1992)	Spain, Cantabrian Range, Cantabria, Puerto de la Magdalena, Luena	Cr9.2	ON969285				this study
<i>Cryobius</i> Chaudoir, 1838	cf. <i>subiasi</i> (Ortuño & Zaballo, 1992)	Spain, Cantabrian Range, Cantabria, Bosque de Saja, Campoo de Cabu-erniga	Cr10	ON969270	ON979812			this study
<i>Cryobius</i> Chaudoir, 1838	<i>subsinuatus</i> (Dejean, 1828)	Austria, Upper Austria, Salzkammergut	—	KM441461				Hendrich et al. 2014
<i>Cryobius</i> Chaudoir, 1838	<i>unctulatus</i> (Dufschmid, 1812)	Austria, Carinthia, Gurktaler Alps	—	GU347340				Raupach et al. 2010
Outgroup taxa								
<i>Pterostichus</i> Bonelli, 1810	<i>brevis</i> (Dufschmid, 1812)	Bosnia Herzegovina, Igman, 1199 m	Cr18	ON969269	ON979799			this study
<i>Pterostichus</i> Bonelli, 1810	<i>burmeisteri</i> Heer, 1837	Germany, Thuringia, Tambach-Dietharz	—	KU917896				Rulik et al. 2017

Subgenus (in ingroup) Genus (in outgroup)	Species	Locality	Code	COI	28S	16S	18S	Reference
<i>Pterostichus</i> Bonelli, 1810	<i>tama</i> (Ménétriés, 1843)	USA, California, Sierra County	—				EU142281	Will & Gill 2008
<i>Pterostichus</i> Bonelli, 1810	<i>melanarius</i> (Illiger, 1798)	Germany, Schleswig-Holstein, Fehmarn	—	GU347302				Raupach et al. 2010
<i>Pterostichus</i> Bonelli, 1810	<i>niger</i> (Schaller, 1783)	Denmark, North Jutland, Skagens Gren	—			MN122874		DNAmark project – direct submission
<i>Pterostichus</i> Bonelli, 1810	<i>niger</i> (Schaller, 1783)	Schweden, Uppland	—				KT204329	Staudacher et al. 2016
<i>Pterostichus</i> Bonelli, 1810	<i>oblongopunctatus</i> (Fabricius, 1787)	Denmark, North Jutland, Byrum	—			MN122833		DNAmark project – direct submission
<i>Pterostichus</i> Bonelli, 1810	<i>oblongopunctatus</i> (Fabricius, 1787)	Germany, North Rhine-Westfalia, Haltern-Borkenberge	—	GU347327				Raupach et al. 2010
<i>Platyderus</i> Stephens, 1828	<i>magrini</i> Degiovanni, 2005	Italy, Tuscany, Arezzo, Eremo de Camaldoli	Cr16	ON969273	ON979796		ON979817	this study
<i>Platyderus</i> Stephens, 1828	cf. <i>pyrenaicus</i> Tempère, 1947	Spain, Cantabrian Range, Navarre, Urbasa, Bidoiza	Cr23	ON969272	ON979797			this study

to reduce the possibility of contamination after the PCR product was controlled with a gel. The samples were then sent to the MacroGen laboratory Europe B.V. (Amsterdam, Netherlands) for sequencing.

The raw sequences were processed with GENEIOUS PRIME® 2020.2.2 (<https://www.geneious.com>). The sequences were cleaned and aligned with „Geneious Alignment“ (global alignment with free end gaps, cost matrix: 65% similarity) and primer sequences were trimmed using the „trim primer“ function. Consensus sequences were aligned with MUSCLE v.3.8.425 (R. C. Edgar, www.drive5.com/muscle/). Additional sequences of *Pterostichus* specimens available at Genbank (www.ncbi.nlm.nih.gov/Genbank) were added to the alignments. All specimens included in this work are listed in Table 2. The alignments were then exported to BIOEDIT v.7.2.5 (Hall 1999). Single sequences within the alignment were brought to the same length. Therefore, parts of the 5’ or 3’ ends of some sequences were cut or filled with a placeholder character which was then recognized as missing information by the following programs used in the phylogenetic analysis.

2.3. Phylogenetic analysis

The cleaned alignments were exported in NEXUS format. For the combined analysis, the alignments were first assembled in a data matrix using SEQUENCE MATRIX v.1.8 (Vaidya et al. 2011). Tree reconstruction was performed by maximum likelihood analysis for all single genes as well as the combined matrix. W-IQ-TREE 1.6.12 (Trifinopoulos et al. 2016), available at the IQ-TREE web server <http://iqtree.cibiv.univie.ac.at>, was used for maximum likelihood analysis including ultrafast bootstrap (Hoang et al. 2017). The MODELFINDER tool was applied to determine the best fitting substitution model beforehand (Kalyaanamoorthy et al. 2017). The chosen models were TIM + F + I + G4 for COI, TPM2u + F + I + G4 for 28S, TIM2 + F + I for 16S, JC for 18S and GTR + F + I + G4 for the combined alignment (Jukes and Cantor 1969; Kimura 1981; Tavaré 1986; Posada 2003). In addition, a Bayesian inference was performed for the combined matrix using MRBAYES 3.2.7a (Ronquist and Huelsenbeck 2003) applying the Markov chain Monte Carlo algorithm (MCMC) altered by Geyer (1991). The output was visualized with FIGTREE v.1.4.4 (<http://github.com/rambaut/figtree/>) and edited using Adobe Illustrator v.26.0.3 (<https://adobe.com/products/illustrator>). For the combined analysis, a chimera was created for *P. riparius* (Dejean, 1828) (JF888281 + EU142445). The determination of the respective specimens was trusted. Only the topology of the combined analysis is shown in the results section (Fig. 3). For the single gene analyses, a summary is given in Table 3.

2.4. Morphological study

After DNA extraction the specimens were glued on rectangular cards for morphological study. For male individuals, the genitalia were removed beforehand and glued

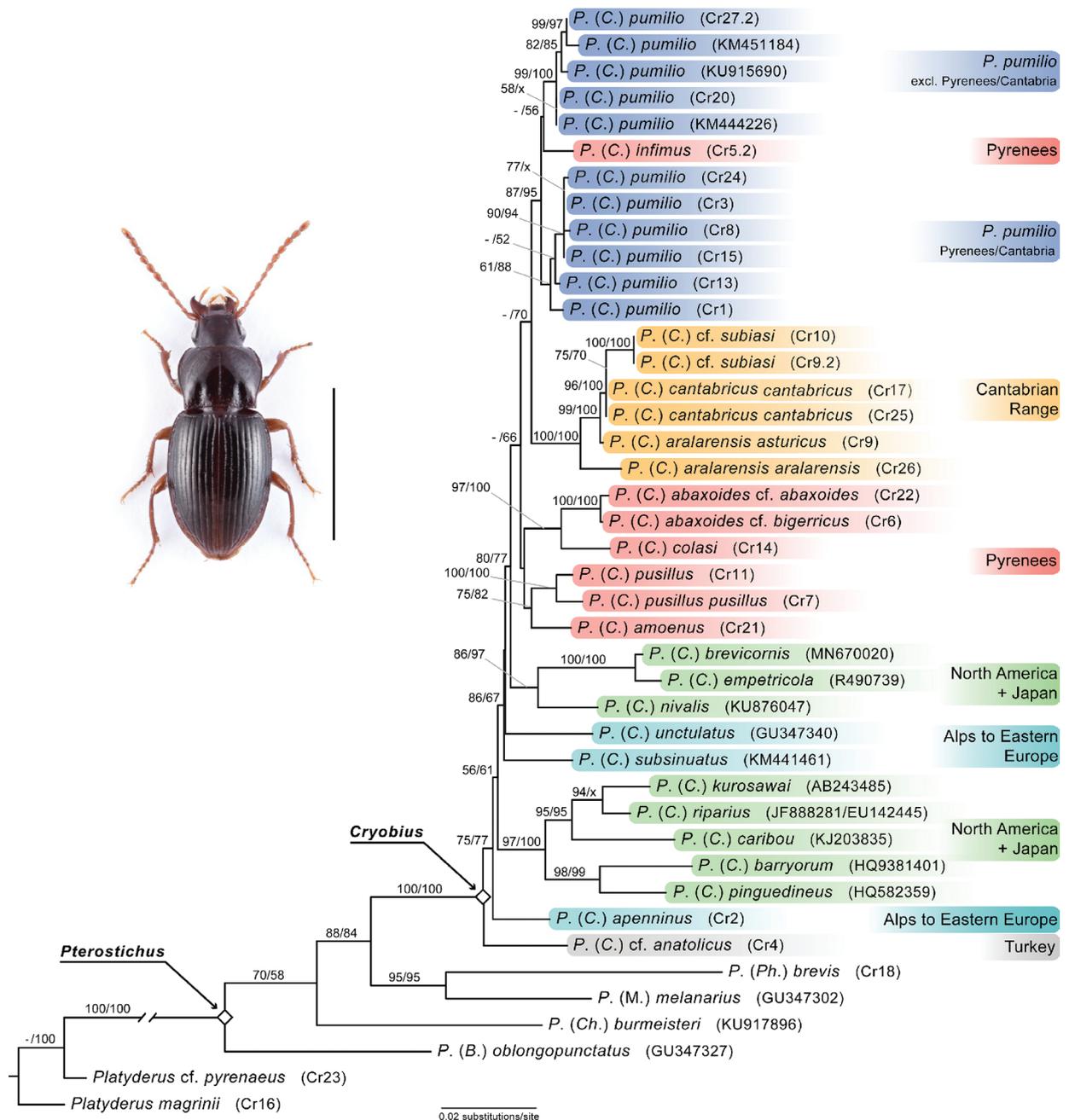


Figure 3. Combined tree of CO1, 28S, 18S and 16S sequences based on maximum likelihood (ML) analysis. Numbers in nodes indicate ML bootstrap value / Bayesian posterior probability (both: only when >50). Coloration indicates species distribution patterns of *Cryobius*. “-” poorly supported node (value <50), “x” node not recovered by Bayesian analysis. *P.* = *Pterostichus*, *C.* = *Cryobius*, *B.* = *Bothriopterus*, *Ch.* = *Cheporus*, *M.* = *Morphnosoma*, *Ph.* = *Parahaptoderus*. In brackets: specimen code (CrXX, this study) or GenBank accession number. *P. (C.) riparius*: chimera (JF888281+EU142445). Specimen: *Pterostichus pumilio*, scale bar: 3 mm.

beside the specimen. Species determination was conducted regarding the currently valid species list of *Cryobius* published in the “Catalogue of Palearctic Coleoptera V1” (Bousquet 2017). The identification of the Pyrenean and Cantabrian species was mainly performed using the dichotomic key published in the “Faune de France Vol. 95” (Pupier 2011) and the “Faune de France Vol. 40” (Jeannel 1942) for male genital characteristics. The Turkish specimen was tentatively identified using species publications with type locality near the collection site, as there is no determination key available that includes all Palearctic *Cryobius* species. Additional information provided by the

DNA analysis or distribution patterns of *Cryobius* species (Serrano 2013; Coulon and Pupier 2014; Bousquet 2017) was used to validate the morphological determination. When possible, determination was carried out to subspecies level. However, in some cases, the variability of external characters even within populations (Pupier 2011) or missing information concerning characters of female genitalia did not allow for an unambiguous classification at the subspecies level.

Species identification of non-*Cryobius* specimens was conducted with the “Käfer Mitteleuropas – Band 2, Adephaga 1” (Müller-Motzfeld 2004).

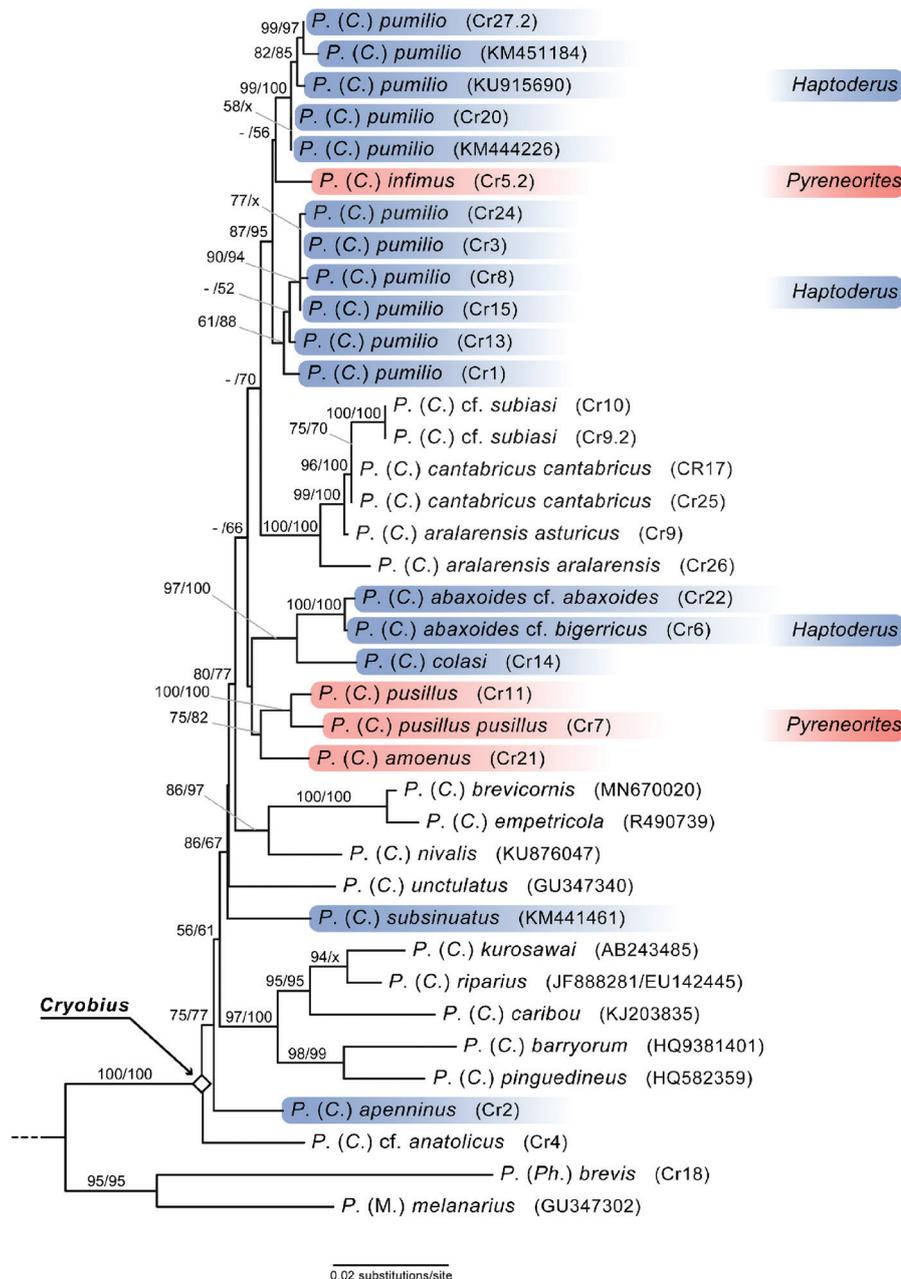


Figure 4. Phylogenetic position of the former subgenera *Haptoderus* (blue) and *Pyreneorites* (red), excerpt of the tree from combined analysis (Fig. 3) of CO1, 28S, 18S and 16S sequences based on maximum likelihood (ML) analysis. Numbers in nodes indicate ML bootstrap value / Bayesian posterior probability (only when >50), “x” node not recovered by Bayesian analysis. *P.* = *Pterostichus*, *C.* = *Cryobius*, *M.* = *Morphnosoma*, *Ph.* = *Parahaptoderus*. In brackets: specimen code (CrXX, this study) or GenBank accession number. *P. (C.) riparius*: chimera (JF888281 + EU142445).

3. Results

3.1. The subgenus *Cryobius* is recovered as a monophyletic group within *Pterostichus*

Our data support the monophyly of *Cryobius* with a bootstrap value (BV) of 100 and a Bayesian probability of 100 (BP) in the combined tree (Fig. 3). This monophyly is also recovered by the CO1 and the 16S single gene analyses (BV = 100 and 78, respectively; Table 3). The two conserved markers 28S and 18S do not show a dis-

tinct *Cryobius* clade. However, it must be said that testing the monophyly of *Cryobius* was not the scope of this work and the type species of *Cryobius* (*C. ventricosus* (Dejean, 1831)) was not included, although two species belonging to the *ventricosus* group sensu Ball (1966) (*C. caribou* Ball, *C. riparius* Dejean) were included. Also, a much more comprehensive sampling of *Pterostichus* subgenera and species would be required to achieve this aim.

No support was found for the synonymized subgenera *Haptoderus* and *Pyreneorites*. Neither the combined – nor the single gene phylogenies showed a distinct clade for either *Haptoderus* – or *Pyreneorites* species (Fig. 4, Table 3).

Table 3. Support for different clades according to the respective phylogenetic analysis. Single genes: results of maximum likelihood (ML), numbers indicate bootstrap value. “Combined”: result of ML analysis / result of Bayesian posterior probability. Note that less specimens were analyzed for 16S, 18S and 28S than for CO1. Abbreviations: excl. = excluding, subg. = subgenus.

Clade	CO1	28S	16S	18S	Combined
subg. <i>Cryobius</i>	100	x	78	x	100 / 100
Pyrenean <i>Cryobius</i>	x	x	x	x	x / x
Pyrenean <i>Cryobius</i> excl. <i>pumilio</i>	x	x	x	x	x / x
Cantabrian <i>Cryobius</i>	x	x	x	x	x / x
Cantabrian <i>Cryobius</i> excl. <i>pumilio</i>	96	74	81	x	100 / 100
<i>pumilio</i>	94	x	x	n.a.	x / x
<i>pumilio</i> + <i>infimus</i>	x	x	70	x	87 / 95
<i>pumilio</i> excl. Pyrenees/Cantabria	92	n.a.	n.a.	n.a.	99 / 100
Pyreneo-Cantabrian <i>pumilio</i>	x	49	52	n.a.	61 / 88
subg. <i>Haptoderus</i> (Chaudoir, 1838)	x	x	x	x	x / x
subg. <i>Pyreneorites</i> (Jeannel, 1937)	x	x	x	x	x / x
Alpine <i>Cryobius</i>	x	n.a.	n.a.	n.a.	x / x
Nearctic <i>Cryobius</i>	x	n.a.	n.a.	n.a.	x / x

Legend:	
	bootstrap value > 80
	bootstrap value 51 – 80
	bootstrap value ≤ 50
x	not recovered
n.a.	not available (0 – 1 specimen tested)

3.2. The Pyreneo-Cantabrian *Cryobius*

The Pyrenean and Cantabrian specimens form a monophyletic clade together with *P. pumilio*. The local Pyrenean species *P. infimus* is, regarding the combined phylogeny, grouped in one clade with *P. pumilio* (BV = 87, BP = 95). This arrangement is also found by 16S (BV = 70) and 18S, but here with low support. In the CO1 analysis, *P. infimus* is placed as sister to *P. pumilio* (BV = 79), which is not the case in the 28S phylogeny. According to the combined phylogeny, the remaining species with an exclusively Pyrenean or Cantabrian distribution are grouped in three separate clades.

3.3. *P. pumilio*

The widely distributed species *P. pumilio* does not, according to the combined tree, form a monophyletic group since the Pyrenean *P. infimus* belongs to the clade. A monophyly for *P. pumilio* is only recovered in the CO1 topology (BV = 94). The clade of *Pterostichus pumilio* is further divided into two subclades. The first is a clade of specimens from the Massif Central (Cr20 + Cr27.2) and Germany (BV = 99) and BP = 100 in the combined tree). The clade is also recovered and well supported within the CO1 tree (BV = 92) (Table 3: “*pumilio* excl. Pyrenees/Cantabria”). No statement concerning the support for this clade is possible for the three other markers taken independently (16S, 28S, and 18S) as for those gene fragments only one sequence was available for this group. The second clade comprises Pyrenean and Can-

tabrian *P. pumilio* with a BV of 61 and a BP of 88 in the combined tree (Table 3: “Pyreneo-Cantabrian *pumilio*”). It has low support in the 28S and 16S trees (not tested in 18S). The clade was not recovered in the CO1 phylogeny.

3.4. The *P. abaxoides*- and *pusillus* groups

Except for *P. infimus*, all *Cryobius* specimens with an exclusive Pyrenean distribution are arranged in two sister clades. The clade containing *P. abaxoides* and *P. colasi* is very well supported (BV = 97, BP = 100). The adjacent clade contains *P. pusillus* and *P. amoenus* with a relatively good support (BV = 75, BP = 82). Within those Pyrenean *Cryobius*, two subclades are well supported: one gathering all specimens of *P. abaxoides* (BV = 100, BP = 100) and one including all specimens of *P. pusillus* (BV = 100, BP = 100). Both are based on two specimens each.

3.5. The *P. cantabricus* group

Another well supported clade includes the *P. cantabricus* – and *P. aralarensis* groups (BV = 100, BP = 100), as well as two *P. cf. subiasi* (Ortuño et Zaballos, 1992) specimens (further discussed below). This clade will be referred to as the ‘Cantabrian clade’ (Table 3: “Cantabrian *Cryobius ex pumilio*”; including all Cantabrian specimens, *P. pumilio* excluded). The Cantabrian clade is also found in the CO1 tree (BV = 96), the 28S tree (BV = 74)

Table 4. Differences in the external morphology of *Pterostichus subiasi* and the specimens Cr9.2 and Cr10. Characters of *P. subiasi* are taken from Ortuño and Zaballos (1992). Abbreviations: incl. = including, post. = posterior.

	Character state <i>P. subiasi</i> (Ortuño and Zaballos, 1992)	Character state Cr9.2 / Cr10
Body length	6.3 – 6.8 mm	7.5 mm (Cr10), 8 mm (Cr9.2)
Head	eyes only slightly prominent	eyes prominent
Pronotum	front angles little pronounced	front angles pronounced and protruded
	posterior margin almost straight, slightly arched between hind angles	posterior margin straight towards the hind angles but concave in the middle
Elytra (each)	9 th interval wider than the others	9 th interval not wider than the others
	a seta near origin of 2 nd stria	no seta near origin of 2 nd stria

and the 16S tree (BV = 81). It is not recovered in the 18S single phylogeny.

Two Spanish specimens (Cr9.2: female, Cr10: male) included in the Cantabrian clade were determined as *P. (C.) cf. subiasi*. Based on their outer morphology and their position within the tree, it was assumed that they are the same species. The uncertainty of the determination at species level was due to several ambiguous clues. The morphology of the aedeagus of specimen Cr10 resembles that of *P. subiasi*. When compared to all Spanish *Cryobius*, the external morphological characters of Cr9.2 and Cr10 are also most consistent with the ones described for *P. subiasi*. However, some do not match, such as the body length, the size of the eyes, characters of the pronotum and the elytra (Table 4). Additionally, the collection sites of these two specimens are not matching the currently known distribution of *P. subiasi*. Morphology suggests both limited relatedness to *P. subiasi* and to *P. cantabricus*.

3.6. *Cryobius* from the Alps

The species from the Alps – *P. apenninus* (Dejean, 1831) (Apennine Alps), *P. subsinuatus* (Dejean, 1828) and *P. unctulatus* (Austrian Alps) – do not form a clade in the combined– or the CO1 phylogeny (Table 3: “Alpine *Cryobius*”).

3.7. The Nearctic and Eastern Oriental *Cryobius*

The sequences for the Nearctic *Cryobius* species were obtained from Genbank, most are CO1 except for one sequence of 28S for one specimen (*P. riparius*). A clade with all those species together was never recovered, neither in the combined tree, nor in the CO1-only tree. According to the combined phylogeny, the Nearctic species are divided into two separate but well supported groups, respectively, which are intermixed with Palearctic species.

One group includes two Canadian species (*P. brevicornis* + *P. empetricola*) and one from Alaska (*P. nivialis*). Within that group, the two Canadian species form a well-supported clade (BV = 100, BP = 100). The other Nearctic species are grouped with the Japanese *P. kurosawai* Tanaka, 1958 which is closest to *P. riparius*

(BV = 79, not recovered in the Bayesian inference). The relative position of these groups differs in the CO1 phylogeny, but the supports are lower.

3.8. *P. cf. anatolicus*

Pterostichus (Cryobius) cf. anatolicus Jedlička, 1963 from Northeast Turkey is placed at the base of *Cryobius* as a sister to all other species, but without support. In addition to the combined phylogeny, this basal position of *P. cf. anatolicus* is also recovered by the analyses of CO1 and 28S only. However, in all cases this position is not well supported. 16S and 18S were not sequenced for this specimen.

4. Discussion

4.1. The subgenus *Cryobius*

This study is the first to provide phylogenetic data on the *Pterostichus* subgenus *Cryobius*. However, *Cryobius* was not tested for monophyly, but first molecular support for a monophyly of *Cryobius* is provided. Subsequent studies with a more comprehensive sampling of the genus *Pterostichus* are needed to further address this issue. Such subsequent studies would be important to verify the current phylogenetic status of *Cryobius* which is based on morphological clues (Ball 1966).

4.2. *Haptoderus* and *Pyreneorites*

Within the phylogeny of *Cryobius*, no distinct clades of the former *Haptoderus* s.str. or *Pyreneorites* were recovered. Although not all species originally assigned to these two taxa were included in this study, the type species for *Haptoderus* (*P. pumilio*) and *Pyreneorites* (*P. pusillus*) were included.

In his revision of the genus *Haptoderus*, Jeannel (1937) distinguished the subgenera *Haptoderus* s.str. and *Pyreneorites* by the punctuation of the metepisterna, lacking in *Pyreneorites*, a character not mentioned by Ball (1966). Regarding the specimens of this study

(Fig. 4), this character was reliable except for *P. pumilio*, for which the punctuation was sparse or absent in many specimens. Only the individual Cr27 showed a clear punctuation. Considering that this character is not mentioned in current determination keys, it could be that the punctuation of the metepisterna is a varying and hence unreliable character within *Cryobius*. Furthermore, Jeannel (1937) only used morphological clues for his separation of the former three subgenera. This led to the fact that the species of *Pyreneorites* were not the only ones exclusively distributed in the Pyrenees, as several species of *Haptoderus* s.str. were, according to Jeannel, also restricted to the Pyrenees (Jeannel 1937). In light of this, the synonymy of *Pyreneorites* with *Haptoderus* by Ball (1966) appears reasonable.

Ultimately, the synonymy of *Haptoderus* and *Cryobius* by Bousquet (1999) is also supported by the molecular data obtained in this study, as the specimens of *Haptoderus* (incl. *Pyreneorites*) and *Cryobius* form a monophyletic group.

4.3. The Pyreneo-Cantabrian *Cryobius*

The focus of this study was on the Pyrenean and Cantabrian *Cryobius* species. The results show that there are three lineages in the Pyreneo-Cantabrian mountain massifs, (i) one made up by *P. (C.) pumilio-infimus*, (ii) one by members of the *P. (C.) cantabricus* group and (iii) that formed by species of the *P. (C.) abaxoides-amoenus* group. The addition of molecular data from other taxa inhabiting either the Pyrenees (e.g. *P. (C.) amaroides* (Dejean, 1828), *P. (C.) amblypterus* (Chaudoir 1868)) or the Cantabrian Mountains (*P. (C.) ehlersi* (Heyden, 1881)) may even show the existence of new lineages. It is expected that a complete taxon sampling will show the existence of a large monophyletic clade comprising all taxa from the Pyrenees and the Cantabrian Mountains, including the widely distributed species *P. (C.) pumilio*, which likely became secondarily adapted to montane and lowland forests of central Europe. Within this large clade, others are expected to be found including (i) taxa restricted to the Pyrenees or the Cantabrian Mountains, or (ii) others occupying both mountain systems as is the case of the *P. (C.) cantabricus* clade. This hypothesis agrees with that formulated by Ortuño and Zaballos (1992) about a progressive colonization of the north Iberian mountains by ancestral *Cryobius* coming from the Pyrenees, perhaps from the onset of the Pleistocene.

4.4. The Pyrenean *Cryobius*

In the combined tree, the Pyrenean species *P. infimus* is grouped with the widely distributed *P. pumilio*. This arrangement is not recovered in the COI, 28S and 18S single gene analyses. At species level, *P. infimus* is morphologically well characterized. Therefore, a false determination of the specimen is unlikely. *Pterostichus infimus* might be the sister species of *P. pumilio*. This would

have to be investigated further by adding more genetic data of other specimens and species. *Pterostichus infimus* comprises three subspecies with unreliable morphological characters (Pupier 2011; Bousquet 2017). Hence, a genetic analysis of those populations could help to clarify the status.

The other Pyrenean *Cryobius* form two sister clades. Within those, the clades of *P. abaxoides* and *P. pusillus*, each represented by two specimens, are well supported. For both species and most Pyrenean taxa, several subspecies have been described, as expected for alpine beetle populations with low dispersal power and reduced gene flow. The use of barcoding might help to assign specimens to described subspecies or to test the validity of these subspecies. Additionally, a broader sampling is necessary – including the type localities of all the described taxa – to precise the distribution of those populations. A question to answer would be if those subspecies form isolated populations across the Pyrenees or whether there is genetic exchange, in which case they are probably not valid and should be synonymized.

4.5. The Cantabrian clade

The Cantabrian species form a supported clade in which the position of the two *P. aralarensis* subspecies is unexpected as they are placed separately. The genetic differentiation between these two subspecies could be explained by the large geographic distance between the sampling locations. One corresponds to the occidental Pyrenees (*P. (C.) a. aralarensis*), the other to the Cantabrian Massif (*P. (C.) a. asturicus*), respectively. In this case, it seems that the morphological differentiation has occurred at a slower rate than the molecular one. This hypothesis deserves further in-depth investigation.

According to Pupier (2011), Jeanne reported *P. aralarensis* from the Pic d'Anie (Eastern Pyrenees) being far East from its Eastern distribution border in Spain (Aralar Range). However, this record is dubious (Coulon and Pupier 2014, Serrano pers. com.)

The two *P. cf. subiasi* specimens (Cr9.2 and Cr10), share several ambiguous clues compared to what is known about this species. Regarding the morphological characters, the larger size of the specimens, the form of the fore angles of the pronotum, and the lack of setae at the base of the second elytral stria in both specimens are the most noticeable. Also, the collection sites of the two specimens are about 200 km apart from the two only known distribution areas of *P. subiasi* in Northwest Spain: the Sierra de Los Ancares (Lugo) and the Sierra del Invernadero (Orense) (Serrano 2013). However, this species was described relatively recently (1992) compared to other *Cryobius* species. It is therefore likely that the current information about the distribution of *P. subiasi* is incomplete due to a lack of sampling. Still, the morphological ambiguity does not allow a clear assignment of Cr9.2 and Cr10 to *P. subiasi*. It could be that these specimens belong to an undescribed species possibly close to *P. subiasi*. To clarify this, more sampling in the respective distribution

areas and, most importantly, more genetic information would be needed.

4.6. The case of the widespread species *P. pumilio*

Pterostichus pumilio comprises two subspecies: *P. pumilio pumilio* and *P. pumilio nevadensis* (Jeannel, 1947). However, *P. pumilio nevadensis* is not considered in this study, as it is only described from the Sierra Nevada in southern Spain, a record which was questioned by Serano (2013).

A monophyletic clade for *P. pumilio* was only recovered in the CO1 tree, but with low support. This is due to the close relation with the *P. infimus* specimen that is discussed in paragraph 4.3. above. Still, two groups of *P. pumilio* are recovered in the phylogeny: “*pumilio* excl. Pyrenees/Cantabria” and the “Pyreneo-Cantabrian *pumilio*”. Interestingly, Jeannel (1949) discussed the presence of two forms for *P. pumilio*. First, the Central European form, which was described as *spadiceus* (Dejean, 1828). This form is distributed at lower altitudes in France but can be found above 1500 m in the Alps and the Massif Central. Second, the *pumilio* s.str. form which is typical for the Pyrenees (no mention of the Cantabrian Range) where it reaches very high altitudes. It is characterized by a minor size and differences in the shape of the pronotum and elytral striae. Given the collection localities, the two *P. pumilio* groups shown by the combined tree could reflect those two population groups. However, a morphological analysis of the nine *P. pumilio* specimens collected for this study could not confirm most of the characters described for *pumilio* and *spadiceus*. The absence of the parascutellar stria in the *pumilio* s.str. form could be confirmed for the Pyrenean specimens whereas the specimens from the Massif Central (Cr.20 and Cr.27.2) showed slight remains of this stria, as described for the *spadiceus* form. However this finding must be viewed with caution since Español and Mateu (1945) generally considered the parascutellar stria a non-reliable character.

The altitude of the collection localities between the two groups did not differ significantly. More sampling and sequencing of those two groups is needed to further investigate Jeannel’s hypothesis of two *P. pumilio* forms. The fact that the Cantabrian specimen Cr24 of *P. (C.) pumilio* is closely related to Pyrenean ones (Fig. 3) confirms that this species is an exception within *Cryobius* of these mountains, due to its dispersal and colonization abilities denoted by its wide distribution area. It should be noted that Cr24 also lacked the parascutellar stria, as found in Pyrenean specimens.

4.7. The Eastern *Cryobius*

The position of three species with an Alpine to Eastern European distribution (*P. apenninus*, *P. subsinuatus* and *P. unctulatus*) in the phylogeny does not allow statements concerning the lineage, as the supports are low and the

sampling is scarce. Though, the lack of molecular data on species from the Alps and other European mountain systems is a major limitation to investigate whether the Cantabrian Range and the Pyrenees were colonized via the Alps or vice versa.

4.8. The Nearctic *Cryobius*

The Nearctic *Cryobius* species did not form a monophyletic group but were intermixed with Palearctic species. The reason for this could be that these specimens were only based on CO1, except for the *P. riparius* chimera combining a CO1– and a 28S sequence (Table 3). It can be expected that an inclusion of more taxa, and genes with different levels of conservation, would change the arrangement of the Nearctic *Cryobius*. Yet, most of the publicly available sequences for Nearctic *Cryobius* are currently from CO1.

An interesting result is the grouping of two Nearctic species with the Japanese *P. kurosawai* (Hokkaido). Unfortunately, this specimen was represented by a 28S fragment only. In the combined tree, as well as in the 28S tree, it is closest to the North American *P. riparius*, a species that, according to Bousquet (2012), “ranges from central Alaska to eastern Alberta” (Canada). According to Morita (2002), *P. kurosawai* is reported from Western Russia (Primorskij Territory, Sakhalin Island) and Northern Japan (Hokkaido, Rishiri Island). This relation between Palearctic and Nearctic *Cryobius* is in line with several studies showing a disjunct distribution of Eastern Asian and Northern American flora and fauna (Krukkeberg 1983; Ball and Currie 1997; Zhou et al. 2012; Weng et al. 2016; Liu et al. 2017; Haas et al. 2020; Sugawara et al. 2021). This biogeographical pattern is often explained by migration events across the Bering land bridge between the Cretaceous and the Quaternary (Sanmartín et al. 2001). In fact, in his revision of *Cryobius*, Ball (1966) stated that the subgenus *Cryobius* would have no close relatives in North America but was morphologically similar to several Palearctic subgenera of *Pterostichus*. Therefore, he assumed that the North American *Cryobius* would have a Palearctic origin. Ball and Currie (1997) listed *Cryobius* species according to their geographic distribution assigning several species as either Beringian, Palearctic-Beringian or Nearctic-Beringian. Here, in contrast to Bousquet (2012), *P. riparius* is listed as a Palearctic-Beringian species.

5. Conclusion

This study provides a first insight into the molecular phylogeny of the subgenus *Cryobius*. A monophyletic origin of this taxon is suggested. The combined phylogeny also supports the current taxonomic state of *Cryobius* as a subgenus of *Pterostichus*. The investigation of the Pyrenean and Cantabrian *Cryobius* did not reveal separated

groups in general. Instead, shared lineages between both massifs might suggest that there could be a monophyletic clade comprising all taxa from the Pyrenees and the Cantabrian mountains, including widespread species as *P. pumilio*. The relationship between *pumilio* and eastern species remains to be tested. Although our sample of *Pterostichus* subgenera was limited, *Cryobius* sensu Ball and Bousquet is corroborated by our molecular data and that is well differentiated from similar lineages of the vast genus *Pterostichus*. Open questions concerning the origin of lineages, colonization routes, distribution patterns, and the validity of subspecies demand further investigation. This might also allow to test the impact of glaciations in the diversification of the group (Schoville et al. 2012). In this regard, the alpine *Cryobius* certainly represent a good model to study the impact of glacier retreat on high altitude biodiversity (Sommer et al. 2020).

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7. References

- Åbmann T (1998) A new subspecies of *Haptoderus* (*Iberoderus*) *cantabricus* Schaufuss 1862 (Coleoptera, Carabidae, Pterostichini). *Acta Coleopterologica* 14: 47–50.
- Ball GE (1966) A revision of the North American species of the subgenus *Cryobius* Chaudoir (*Pterostichus*, Carabidae, Coleoptera). *Opuscula Entomologica*, Lund, Supplementum 28: 1–166.
- Ball GE, Currie DC (1997) Ground beetles (Coleoptera: Trachypachidae and Carabidae) of the Yukon: geographical distribution, ecological aspects, and origin of the extant fauna. *Insects of the Yukon. Biological Survey of Canada (Terrestrial Arthropods)*, Ottawa: 445–489.
- Barthlott W, Lauer W, Placke A (1996) Global Distribution of Species Diversity in Vascular Plants: Towards a World Map of Phytodiversity (Globale Verteilung der Artenvielfalt Höherer Pflanzen: Vorarbeiten zu einer Weltkarte der Phytodiversität). *Erdkunde* 50: 317–327.
- Borchard F, Buchholz S, Helbing F, Fartmann T (2014) Carabid beetles and spiders as bioindicators for the evaluation of montane heathland restoration on former spruce forests. *Biological Conservation* 178: 185–192. <https://doi.org/10.1016/j.biocon.2014.08.006>
- Bousquet Y (1999) Supraspecific classification of the nearctic Pterostichini (Coleoptera: Carabidae). *Fabrerias, Supplément* 9: 1–292.
- Bousquet Y (2012) Catalogue of Geadephaga (Coleoptera, Adephaga) of America, north of Mexico. *ZooKeys* 245: 1–1722. <https://doi.org/10.3897/zookeys.245.3416>
- Bousquet Y (2012) Tribe Pterostichini Bonelli, 1810. In: Löbl I, Löbl D (Eds) *Catalogue of Palearctic Coleoptera: Archostemata-Myxophaga-Adephaga*. BRILL, 704–711.
- Coulon J, Pupier R (2014) Harpalidae. In: Tronquet M (Ed) *Catalogue des Coléoptères de France*. Association Roussillonnaise d'Entomologie, Perpignan, France, 156–157.
- Ehlers J, Gibbard PL, Hughes PD (2018) Chapter 4 - Quaternary Glaciations and Chronology. In: Menzies J, van der Meer JJM (Eds), *Past Glacial Environments* (Second Edition). Elsevier, 77–101. <https://doi.org/10.1016/B978-0-08-100524-8.00003-8>
- Español CF, Mateu SJ (1945) Coleópteros nuevos de la Sierra del Aralar (confines Navarra-Guipúzcoa). *EOS, Revista Española de Entomología* 21: 259–273.
- 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: 294–299.
- Garrick RC (2011) Montane refuges and topographic complexity generate and maintain invertebrate biodiversity: recurring themes across space and time. *Journal of Insect Conservation* 15: 469–478. <https://doi.org/10.1007/s10841-010-9349-4>
- Geyer CJ (1991) Markov chain Monte Carlo maximum likelihood. In: Keramidas EM (Ed.) *Computing Science and Statistics: Proceedings of the 23rd Symposium on the Interface*. Fairfax Station: Interface Foundation of North America. 156–163.
- Gobbi M. (2020). Global warning: challenges, threats and opportunities for ground beetles (Coleoptera: Carabidae) in high altitude habitats. *Acta Zoologica Academiae Scientiarum Hungaricae*, 66 (Suppl.), 5–20. <https://doi.org/10.17109/AZH.66.Suppl.5.2020>
- Haas GMS, Hoberg EP, Cook JA, Henttonen H, Makarikov AA, Gallagher SR, Dokuchaev NE, Galbreath KE (2020) Taxon pulse dynamics, episodic dispersal and host colonization across Beringia drive diversification of a Holarctic tapeworm assemblage. *Journal of Biogeography* 47: 2457–2471. <https://doi.org/10.1111/jbi.13949>
- Hall TA (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hebert PDN, Ratnasingham S, Zakharov EV, Telfer AC, Levesque-Beaudin V, Milton MA, Pedersen S, Jannetta P, deWaard JR (2016) Counting animal species with DNA barcodes: Canadian insects. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 371: 20150333. <https://doi.org/10.1098/rstb.2015.0333>
- Hendrich L, Morinière J, Haszprunar G, Hebert PDN, Hausmann A, Köhler F, Balke M (2015) A comprehensive DNA barcode database for Central European beetles with a focus on Germany: adding more than 3500 identified species to BOLD. *Molecular Ecology Resources* 15: 795–818. <https://doi.org/10.1111/1755-0998.12354>
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution* 35: 518–522. <https://doi.org/10.1093/molbev/msx281>
- Jeanne C (1969) *Les Haptoderus lusitaniens* (Coleoptera, Pterostichidae). *Miscelánea Zoológica* 2: 33–38.
- Jeannel R (1928) *Monographie des Trechinae. Morphologie comparée et distribution géographique d'un groupe de Coléoptères* (Troisième livraison). *Les Trechini cavernicoles*. *Abeille, Paris* 35: 1–808.
- Jeannel R (1937) Révision du Genre *Haptoderus* Chaudoir. *Revue Française d'Entomologie* 4: 9–23.
- Jeannel R (1942) *Coléoptères Carabiques. Deuxième partie. Faune de France* 40. Librairie de la Faculté des Sciences, Paris: 573–1173.
- Jeannel R (1947) Sur les *Haptoderus* des Pyrénées (Coleoptera, Pterostichidae). *Revue Française d'Entomologie* 14: 105–118.

- Jeannel R (1949) 51 Coléoptères Carabiques Supplément. Fédération Française des Sociétés de Sciences Naturelles: Office Central de Faunistique, 50 pp, 20 plates.
- Jukes TH, Cantor CR (1969) CHAPTER 24 - Evolution of Protein Molecules. *Mammalian Protein Metabolism* 3: 21–132. <https://doi.org/10.1016/B978-1-4832-3211-9.50009-7>
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haesler A, Jermini LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589.
- Kanda K, Pflug JM, Sproul JS, Dasenko MA, Maddison DR (2015) Successful recovery of nuclear protein-coding genes from small insects in museums using Illumina Sequencing. *PLOS ONE* 10: e0143929. <https://doi.org/10.1371/journal.pone.0143929>
- Kavanaugh DH (1985) On wing atrophy in carabid beetles (Coleoptera: Carabidae), with special reference to Nearctic *Nebria*. In: Ball GE (Ed.) *Taxonomy, Phylogeny and Zoogeography of Beetles and Ants*. Dr. W. Junks Publishers, Dordrecht, Netherlands, 408–431.
- Kimura M (1981) Estimation of evolutionary distances between homologous nucleotide sequences. *Proceedings of the National Academy of Sciences of the United States of America* 78: 454–458. <https://doi.org/10.1073/pnas.78.1.454>
- Knowles LL (2000) Tests of Pleistocene speciation in montane grasshoppers (genus *Melanoplus*) from the sky islands of western North America. *Evolution* 54: 1337–1348. <https://doi.org/10.1111/j.0014-3820.2000.tb00566.x>
- Körner C (2007) The use of ‘altitude’ in ecological research. *Trends in Ecology & Evolution* 22: 569–574. <https://doi.org/10.1016/j.tree.2007.09.006>
- Kruckeberg AR (1983) Temperate floras: The North Pacific Connection. *Annals of the Missouri Botanical Garden* 70: 591–596. <https://doi.org/10.2307/2398978>
- Liu P, Wen J, Yi T (2017) Evolution of biogeographic disjunction between eastern Asia and North America in *Chamaecyparis*: Insights from ecological niche models. *Plant Diversity* 39: 111–116. <https://doi.org/10.1016/j.pld.2017.04.001>
- Misof B, Liu S, Meusemann K, Peters RS, Donath A, Mayer C, Frandsen PB, Ware J, Flouri T, Beutel RG, Niehuis O, Petersen M, Izquierdo-Carrasco F, Wappler T, Rust J, Aberer AJ, Aspöck U, Aspöck H, Bartel D, Blanke A, Berger S, Böhm A, Buckley TR, Calcott B, Chen J, Friedrich F, Fukui M, Fujita M, Greve C, Grobe P, Gu S, Huang Y, Jermini LS, Kawahara AY, Krogmann L, Kubiak M, Lanfear R, Letsch H, Li Y, Li Z, Li J, Lu H, Machida R, Mashimo Y, Kapli P, McKenna DD, Meng G, Nakagaki Y, Navarrete-Heredia JL, Ott M, Ou Y, Pass G, Podsiadlowski L, Pohl H, Reumont BM von, Schütte K, Sekiya K, Shimizu S, Slipinski A, Stamatakis A, Song W, Su X, Szucsich NU, Tan M, Tan X, Tang M, Tang J, Timelthaler G, Tomizuka S, Trautwein M, Tong X, Uchifune T, Walz MG, Wiegmann BM, Wilbrandt J, Wipfler B, Wong TKF, Wu Q, Wu G, Xie Y, Yang S, Yang Q, Yeates DK, Yoshizawa K, Zhang Q, Zhang R, Zhang W, Zhang Y, Zhao J, Zhou C, Zhou L, Ziesmann T, Zou S, Li Y, Xu X, Zhang Y, Yang H, Wang J, Wang J, Kjer KM, Zhou X (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science* 346: 763–767. <https://doi.org/10.1126/science.1257570>
- Moret P, Aráuz M de los Á, Gobbi M, Barragán Á (2016) Climate warming effects in the tropical Andes: first evidence for upslope shifts of Carabidae (Coleoptera) in Ecuador. *Insect Conservation and Diversity* 9: 342–350. <https://doi.org/10.1111/icad.12173>
- Morita S (2002) Pterostichine carabid beetles of the subgenus *Cryobius* (Coleoptera, Carabidae) from North Japan. *Elytra* 30: 73–89.
- Müller-Kroehling S (2013) Zum Vorkommen moorspezifischer Laufkäfer (Coleoptera: Carabidae) und Schwimmkäfer (Dytiscidae) in Spirkenfilzen (FFH-Sub-LRT* 91D3) des Südschwarzwaldes als charakteristische Arten. *Mitteilungen Badischer Landesverein für Naturkunde und Naturschutz e.V.* 21: 283–302.
- Müller-Motzfeld G (2004) Die Käfer Mitteleuropas, Band 2: Adephega 1, Carabidae (Laufkäfer). Spektrum Akademischer Verlag, Elsevier.
- Ober KA (2002) Phylogenetic relationships of the carabid subfamily Harpalinae (Coleoptera) based on molecular sequence data. *Molecular Phylogenetics and Evolution* 24: 228–248. [https://doi.org/10.1016/S1055-7903\(02\)00251-8](https://doi.org/10.1016/S1055-7903(02)00251-8)
- Ortuño VM, Zaballos JP (1992) A new *Haptoderus* (Coleoptera: Caraboidea: Pterostichidae) from the Sierra de Ancares (Spain). *The Coleopterists Bulletin* 46: 337–342.
- Pentinsaari M, Blagoev GA, Hogg ID, Levesque-Beaudin V, Perez K, Sobel CN, Vandenbrink B, Borisenko A (2020) A DNA Barcoding Survey of an Arctic Arthropod Community: Implications for Future Monitoring. *Insects* 11. <https://doi.org/10.3390/insects11010046>
- 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: 1284–1294. <https://doi.org/10.1002/ece3.927>
- Polato NR, Gill BA, Shah AA, Gray MM, Casner KL, Barthelet A, Messer PW, Simmons MP, Guayasamin JM, Encalada AC, Kondratieff BC, Flecker AS, Thomas SA, Ghalambor CK, Poff NL, Funk WC, Zamudio KR (2018) Narrow thermal tolerance and low dispersal drive higher speciation in tropical mountains. *Proceedings of the National Academy of Sciences* 115: 12471–12476. <https://doi.org/10.1073/pnas.1809326115>
- Posada D (2003) Using MODELTEST and PAUP* to select a model of nucleotide substitution. *Current Protocols in Bioinformatics*: 6.5.1-6.5.14. <https://doi.org/10.1002/0471250953.bi0605s00>
- Pryke JS, Samways MJ (2010) Significant variables for the conservation of mountain invertebrates. *Journal of Insect Conservation* 14: 247–256. <https://doi.org/10.1007/s10841-009-9253-y>
- Pupier R (2011) Tribu Pterostichini – Subgen. *Cryobius* Chaudoir, 1838. In: Coulon G, Pupier R, Queinnee E, Ollivier E, Richoux P (Eds) *Faune de France 95 – Coléoptères Carabiques*. Faune de France, Paris, 443–455.
- Raupach MJ, Astrin JJ, Hannig K, Peters MK, Stoeckle MY, Wägele J-W (2010) Molecular species identification of Central European ground beetles (Coleoptera: Carabidae) using nuclear rDNA expansion segments and DNA barcodes. *Frontiers in Zoology* 7: 26. <https://doi.org/10.1186/1742-9994-7-26>
- Rietze J (2001) Zur Phänologie ausgewählter Laufkäfer in baden-württembergischen Wäldern. *Angewandte Carabidologie Supplement II Laufkäfer im Wald*: 105–115.
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Rulik B, Eberle J, Mark L von der, Thormann J, Jung M, Köhler F, Apfel W, Weigel A, Kopetz A, Köhler J, Fritzlär F, Hartmann M, Hadulla K, Schmidt J, Hörren T, Krebs D, Theves F, Eulitz U, Skale A, Rohwedder D, Kleeberg A, Astrin JJ, Geiger MF, Wägele JW, Grobe P, Ahrens D (2017) Using taxonomic consistency with semi-automated data pre-processing for high quality DNA barcodes. *Methods in Ecology and Evolution* 8: 1878–1887. <https://doi.org/10.1111/2041-210X.12824>
- Sanmartín I, Enghoff H, Ronquist F (2001) Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biological*

- Journal of the Linnean Society 73: 345–390. <https://doi.org/10.1111/j.1095-8312.2001.tb01368.x>
- Sasakawa K (2009) Phylogeny and genital evolution of carabid beetles in the genus *Pterostichus* and its allied genera (Coleoptera: Carabidae) inferred from two nuclear gene sequences. *Annals of the Entomological Society of America* 100: 100–109. [https://doi.org/10.1603/0013-8746\(2007\)100\[100:PAGEOC\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2007)100[100:PAGEOC]2.0.CO;2)
- Scheurig M, Hohner W, Weick D, Brechtel F, Beck L (1996) Laufkäferzönosen südwestdeutscher Wälder – Charakterisierung, Beurteilung und Bewertung von Standorten. *Carolina* 54: 91–138.
- Schmidt J (2011) Biogeographisch-phylogenetische Untersuchungen an Hochgebirgs-Laufkäfern. Ein Beitrag zur Umweltgeschichte des Himalaya-Tibet Orogens. PhD thesis, Philipps Universität Marburg, Marburg, Germany. Available from: <https://archiv.ub.uni-marburg.de/ubfind/Record/urn:nbn:de:hebis:04-z2011-0067>
- Schmidt J, Opgenoorth L, Höll S, Bastrop R (2012) Into the Himalayan exile: the phylogeography of the ground beetle *Ethira* clade SUPPORTS the Tibetan origin of forest-dwelling Himalayan species groups. *PLOS ONE* 7: e45482. <https://doi.org/10.1371/journal.pone.0045482>
- Schoville SD, Roderick GK, Kavanaugh DH (2012) Testing the ‘Pleistocene species pump’ in alpine habitats: lineage diversification of flightless ground beetles (Coleoptera: Carabidae: *Nebria*) in relation to altitudinal zonation. *Biological Journal of the Linnean Society* 107: 95–111. <https://doi.org/10.1111/j.1095-8312.2012.01911.x>
- Serrano J (2013) Nuevo Catálogo de los Carabidae (Coleoptera) de la Península Ibérica. Editum. Ediciones de la Universidad de Murcia, 130 pp.
- Shull GH (1948) What Is “Heterosis”? *Genetics* 33: 439–446.
- Shull VL, Vogler AP, Baker MD, Maddison DR, Hammond PM (2001) Sequence alignment of 18S ribosomal RNA and the basal relationships of aedeagid beetles: evidence for monophyly of aquatic families and the placement of Trachypachidae. *Systematic Biology* 50: 945–969.
- Sikes DS, Bowser M, Morton JM, Bickford C, Meierotto S, Hildebrandt K (2017) Building a DNA barcode library of Alaska’s non-marine arthropods. *Genome* 60: 248–259. <https://doi.org/10.1139/gen-2015-0203>
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651–701. <https://doi.org/10.1093/aesa/87.6.651>
- Sommer C, Malz P, Seehaus TC, Lippel S, Zemp M, Braun MH (2020) Rapid glacier retreat and downwasting throughout the European Alps in the early 21st century. *Nature Communications* 11: 3209. <https://doi.org/10.1038/s41467-020-16818-0>
- Staudacher K, Jonsson M, Traugott M (2016) Diagnostic PCR assays to unravel food web interactions in cereal crops with focus on biological control of aphids. *Journal of Pest Science* 89: 281–293. <https://doi.org/10.1007/s10340-015-0685-8>
- Steinbauer MJ, Field R, Grytnes J-A, Trigas P, Ah-Peng C, Attorre F, Birks HJB, Borges PAV, Cardoso P, Chou C-H, Sanctis MD, Sequeira MM de, Duarte MC, Elias RB, Fernández-Palacios JM, Gabriel R, Gereau RE, Gillespie RG, Greimler J, Harter DEV, Huang T-J, Irl SDH, Jeanmonod D, Jentsch A, Jump AS, Kueffer C, Nogué S, Otto R, Price J, Romeiras MM, Strasberg D, Stuessy T, Svenning J-C, Vetaas OR, Beierkuhnlein C (2016) Topography-driven isolation, speciation and a global increase of endemism with elevation. *Global Ecology and Biogeography* 25: 1097–1107. <https://doi.org/10.1111/geb.12469>
- Strodl M, Gereben-Krenn B-A, Krenn H (2007) In die dritte Dimension alpiner Lebensräume: Erfassung der Laufkäfer (Coleoptera, Carabidae) mit Subterraneanfallen. *Carinthia II* 197: 117. Jahrgang: 341–350.
- Sugawara Y, Ihara Y, Nakano T (2021) A new species of *Cybaeus* L. Koch, 1868 (Araneae, Cybaeidae) with simple genitalia from central Japan is the sister species of *C. melanoparvus* Kobayashi, 2006 with elongated genitalia. *Zoosystematics and Evolution* 97: 223–233. <https://doi.org/10.3897/zse.97.64473>
- Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences (American Mathematical Society)* 17: 57–86.
- Trautner J (2017) 1 Die Laufkäfer Baden-Württembergs. Eugen Ulmer, Stuttgart.
- Trautwein MD, Wiegmann BM, Beutel R, Kjer KM, Yeates DK (2012) Advances in insect phylogeny at the dawn of the postgenomic era. *Annual Review of Entomology* 57: 449–468.
- Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44: W232–W235. <https://doi.org/10.1093/nar/gkw256>
- Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180. <https://doi.org/10.1111/j.1096-0031.2010.00329.x>
- Wahlberg N (2009) The Nymphalidae systematics group. Available at: <http://www.nymphalidae.net/Molecular.htm>, Last accessed on the 20th December 2020.
- Wallis GP, Waters JM, Upton P, Craw D (2016) Transverse alpine speciation driven by glaciation. *Trends in Ecology & Evolution* 31: 916–926. <https://doi.org/10.1016/j.tree.2016.08.009>
- Weng Y-M, Yang M-M, Yeh W-B (2016) A comparative phylogeographic study reveals discordant evolutionary histories of alpine ground beetles (Coleoptera, Carabidae). *Ecology and Evolution* 6: 2061–2073. <https://doi.org/10.1002/ece3.2006>
- Weng Y-M, Veire BM, Dudko RY, Medeiros MJ, Kavanaugh DH, Schoville SD (2020) Rapid speciation and ecological divergence into North American alpine habitats: the *Nippononebria* (Coleoptera: Carabidae) species complex. *Biological Journal of the Linnean Society* 130: 18–33. <https://doi.org/10.1093/biolinnean/blaa014>
- Wiegmann BM, Trautwein MD, Kim J-W, Cassel BK, Bertone MA, Winterton SL, Yeates DK (2009) Single-copy nuclear genes resolve the phylogeny of the holometabolous insects. *BMC Biology* 7: 34. <https://doi.org/10.1186/1741-7007-7-34>
- Will KW, Gill AS (2008) Phylogeny and classification of *Hyperperes auctorum* (Coleoptera: Carabidae: Pterostichini: *Pterostichus*). *Annals of Carnegie Museum* 77: 93–127. <https://doi.org/10.2992/0097-4463-77.1.93>
- Woodcock TS, Boyle EE, Roughley RE, Kevan PG, Labbee RN, Smith ABT, Goulet H, Steinke D, Adamowicz SJ (2013) The diversity and biogeography of the Coleoptera of Churchill: insights from DNA barcoding. *BMC Ecology* 13: 40. <https://doi.org/10.1186/1472-6785-13-40>
- Zhou Z, Wen J, Li G, Sun H (2012) Phylogenetic assessment and biogeographic analyses of tribe Peracarpeae (Campanulaceae). *Plant Systematics and Evolution* 298: 323–336. <https://doi.org/10.1007/s00606-011-0547-7>
- Zinovyev EV (2007) A history of ground-beetle faunas of West Siberia and the Urals during the Late Pleistocene to Holocene. In: Back to the roots and back to the future. Towards a new synthesis amongst taxonomic, ecological and biogeographical approaches in carabi-

- dology. Proceedings of the XIII European Carabidologists Meeting, Blagoevgrad. 241–254.
- Zubrii NA, Filippov BY, Kondakov AV, Khruleva OA, Rybalov LB, Vikhreva DV (2022) DNA barcoding versus morphological variability of *Pterostichus brevicornis brevicornis* (Kirby, 1837) (Coleoptera, Carabidae) in the Arctic and Subarctic. *Insects* 13: 204. <https://doi.org/10.3390/insects13020204>