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Short Communication

Two new species of Cricetidae for the fauna of Albania

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Abstract: Albania represents an important area in Europe where zoological research brings interesting findings, even in a well-studied group such as small mammals. During field research in the central-south part of the country (Tomorr Mountains; Hellenides range) trapping of small terrestrial mammals was carried out. Using ten traps per night/locality, two specimens of family Cricetidae and one of family Muridae were trapped. The first two individuals were genetically and morphologically examined and identified as *Chionomys nivalis* (Martins, 1842) and *Microtus subterraneus* (de Selys-Longchamps, 1836). They represent unique mitochondrial haplotypes and the first records of these species in Albania. The third species, *Apodemus sylvaticus* (L., 1758), represents the first record of the species in this region and the highest recorded occurrence for the country.

Key words: *Chionomys nivalis; Microtus subterraneus; Apodemus sylvaticus;* cytochrome *b*; Balkan Peninsula; phylogeography; faunistics

Introduction

Albania is a country inside the Balkan Peninsula situated in one of the most important biodiversity hotspots, the Mediterranean Basin (Hewitt 2011). It is characterized by the combination of Mediterranean and continental climatic conditions, and by diverse geography comprising the Dinarides Mountain range in the north and Hellenides Mountain range in the eastern and southern parts (Bego & Koni 1999). Seventy percent of the country is mountainous with lowlands in the west along the Adriatic Sea. Based on the past zoological inventories, as well as recent molecular-biogeographic studies, this region probably hosted multiple glacial microrefugia and was probably part of the most important speciation center in the Balkans (Ljubisavljević et al. 2007; Ursenbacher et al. 2008; Jablonski et al. 2016; Marzahn et al. 2016). Approximately 780 species of vertebrates have been recorded there (Bego & Koni 1999; Deliany et al. 1999). Several of them are endemic to the south-western Balkans, as *Pelophylax shqipericus* (Hotz, Uzzell, Guenther, Tunner & Heppich, 1987), *Dinarolacerta montenegrina* (Ljubisavljević, Arribas, Džukić & Carranza, 2007), *Dinaromys bogdanovi* (Martino, 1922), *Microtus thomasi* (Barrett-Hamilton, 1903), *Alburnoides fangfangae* Bogutskaya, Zupančič & Naseka, 2010 or *A. devolli* Bogutskaya, Zupančič & Naseka, 2010 (Bego et al. 2008; Bogutskaya et al. 2010; Jablonski 2011).

Despite the importance of Albania for understanding zoogeography of small terrestrial mammals (STM, defined here as the species belonging to Soricomorpha and Rodentia) in the Balkans, the information on the recent distribution of this group remains incomplete. Recent studies have improved basic knowledge about STM distribution in the country (Bego et al. 2008, 2014; Paspali et al. 2013; Rogozi et al. 2013), however, there is a need for further research that would cover important regions of the whole country. The last updated checklist of STM in Albania includes 24 species. Therefore, discovery of other new species for Albanian fauna in the future is expected (Bego et al. 2008, 2014). The occurrence of some species is known only from mountain habitats along the Albanian border regions in Montenegro, Kosovo and F.Y.R.O. Macedonia (Bego et al. 2008). Currently, 18 species of Rodentia and six species of Soricomorpha are known to be from Albania and the records of other ten species of these orders are expected (Bego et al. 2008).

Material and methods

During the field trip carried out in September 2015 we studied the mountain habitats of Tomorr Mountains (Hellenides) in central-south Albania: 40.622° N, 20.174° E (2045 m a.s.l.), 40.627° N, 20.173° E (1998 m a.s.l.) and 40.621° N, 20.177° E (2028 m a.s.l.). The mountain consists of three different habitat types, i.e. alpine grassland, pine mixed forests and rocky moraine. Live traps (type Chmela) were used to catch the specimens, with oat flakes as bait. Ten traps per locality were exposed during one night and checked in the morning about 9 am. Body length (LC), tail length (LCd), feet length (LTp), weight (G), sex and sexual activity were measured and determined. The trapped individuals and habitats were photographically documented. The individuals were identified sensu Krištofík (2012a, b)

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Fig. 1. Trapped individual (A, B) and habitat (C) of *Chionomys nivalis*. The individual (D, E) and habitat (F) of *Microtus subterraneus*. All pictures from Tomorr Mts, Albania.

and basic distributional information were adopted from Shenbrot & Krasnov (2005).

Total genomic DNA was isolated from tail tips fixed in 96% ethanol. Isolation was carried out with Macherey-Nagel NucleoSpin® Tissue kit according to the manufactured protocol. The complete mitochondrial cytochtome *b* gene (cyt *b*) was amplified in a polymerase chain reaction (PCR) using L14727-SP and H-15195-SP primers (Jaarola & Searle 2002). The PCR protocol consisted of an initial 3 min denaturation step at 94 °C, 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and 3 min long extension at 72 °C and the final extension at 72 °C for 10 min. Sequences were checked and aligned using BioEdit 7.2.5 (Hall 1999). Obtained sequences were combined with available GenBank sequences (see Electronic supplementary file). Phylogenetic networks using statistical parsimony were constructed for particular species in TCS 1.21 (Clement et al. 2000). To infer possible connections to a network in cases of highly divergent haplotypes, a fixed connection limit at a higher number of steps allowing visualization of their likely connections was applied. New sequences (809 bp length fragment) were deposited in GenBank under accession numbers KY620210 and KY620211 (see Electronic supplementary file).

Results and discussion

During the trapping night (September 23th, 2015; cloudy, no rainfalls) two individuals of the family Cricetidae (Rodentia) were caught. They were morphologically identified as *Microtus subterraneus* (de Selys-Longchamps, 1836) (LC: 105 mm, LTp: 15 mm, LTp: 34 mm, G: 19 g, female, sexually active) and *Chionomys nivalis* (Martins, 1842) (LC: 103 mm, LTp: 19.5 mm, LCd: 47 mm, G: 24 g, male, sexually inactive). *Microtus subterraneus* was trapped in the low grassland place situated at the altitude of 2045 m a.s.l., *C. nivalis* was trapped in a rocky habitat (1998 m a.s.l.). Slope orientation was north-east. Genetic relationships of these individuals showed that both species represent unique haplotypes closely linked to other Balkan sequences in comparison with published data. They differ by five mutation steps in *C. nivalis* from Cn-mk1 haplotype and 12 steps in *M. subterraneus* from Ms-gr2 haplotype (Figs 2, 3, Electronic supplementary file).

Another rodent species, *Apodemus sylvaticus* (L., 1758) from family Muridae, was caught during the trapping night in low grassland habitat (LC: 102 mm, LTp: 21 mm, LCd: 100 mm, G: 20 g, female, sexually active). This individual was morphologically examined and represents the first evidence of the species in Tomorr Mts as well as the highest recorded occurrence for the country (Bego et al. 2008).

It is noteworthy that faunistic and phylogeographic research in the Balkans has usually omitted the territory of Albania due to previous political situations. The

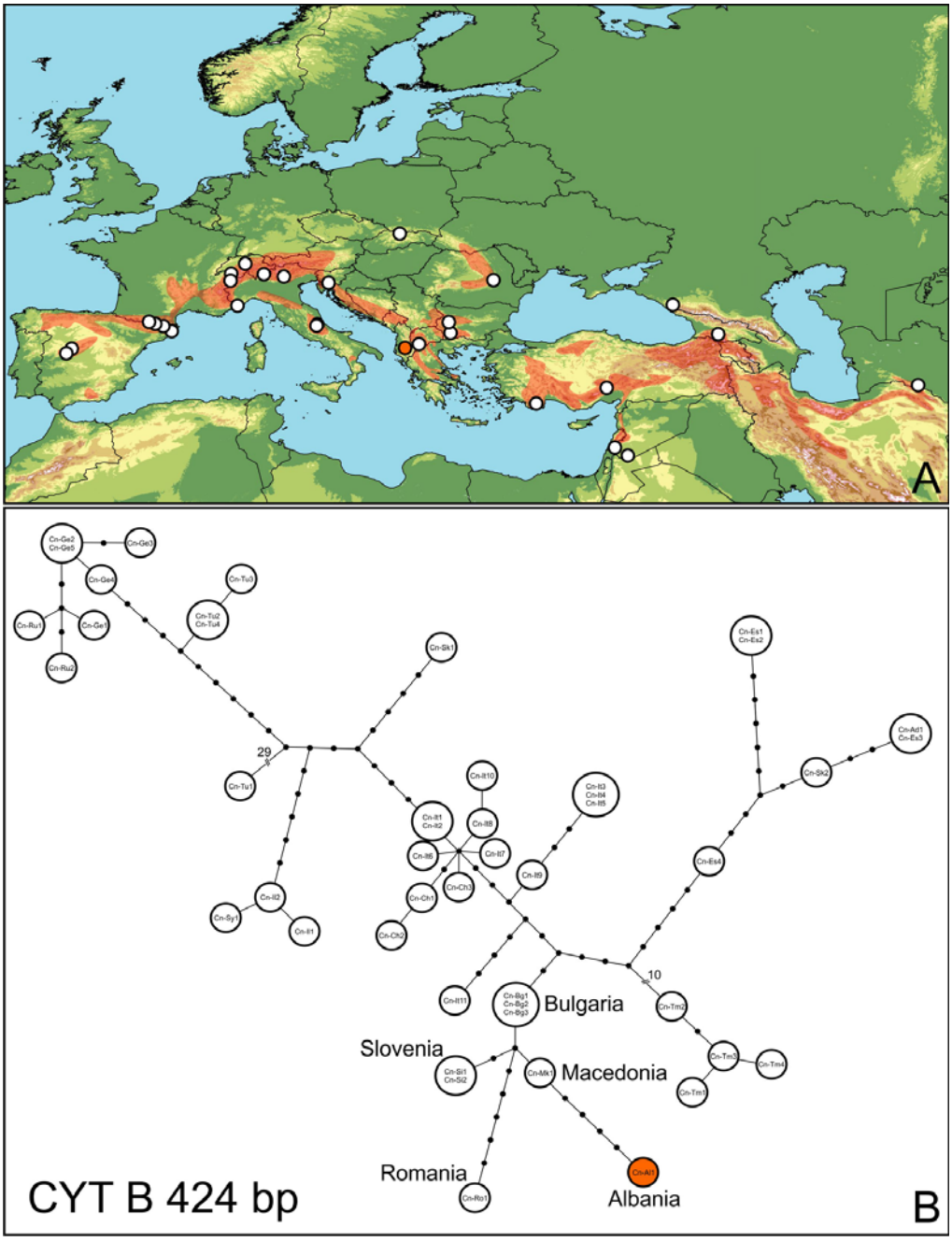

Fig. 2. A: Distribution of *Chionomys nivalis* in the Western Palearctic (orange layer) with localities used for haplotype affiliation (white circles); B: haplotype network based on 424 bp length dataset of sequences. The orange circle represents Tomorr Mts individual. Small black circles in the network indicate hypothetical haplotypes.

faunistic data were often deduced based on data from neighbouring (Bego et al. 2008). This fact is evident in the species richness map of mammals (Mitchell-Jones et al. 1999). Some species of STM were recorded in Albania just a few years ago (see Macholán & Vohralík 1997; Bego et al. 2008). Records of several other species, such as *Clethrionomys glareolus* (Schreber, 1780), *Crocidura leucodon* (Hermann, 1780), *Micromys minutus* (Pallas, 1771) and *Neomys anomalus* (Cabrera, 1907) are still based only on few individuals (Bego et al. 2008).

During the present study, the occurrence of two new species of STM for Albania is proved and thus fills the gap mentioned in Bego et al. (2008, 2014). These authors mentioned five rodents with potential distribution in Albania – *Arvicola amphibius* (L., 1758), *D. bogdanovi*, *C. nivalis*, *M. arvalis* (Pallas, 1778) and *M. subterraneus*, since they live in border mountain areas of surrounding countries. Bego et al. (2008) expected the findings of *C. nivalis* and *M. subterraneus* in the mountainous habitats of northern and eastern Albania. However, both present records are from central-south Albanian. This area is located approximately 100 and 200 km respectively from the closest recorded locations of these species in Kosovo (Mt. Žljeb), Macedonia (Mt. Šara, Mt. Jablanica) and Montenegro (Mt. Komovi;

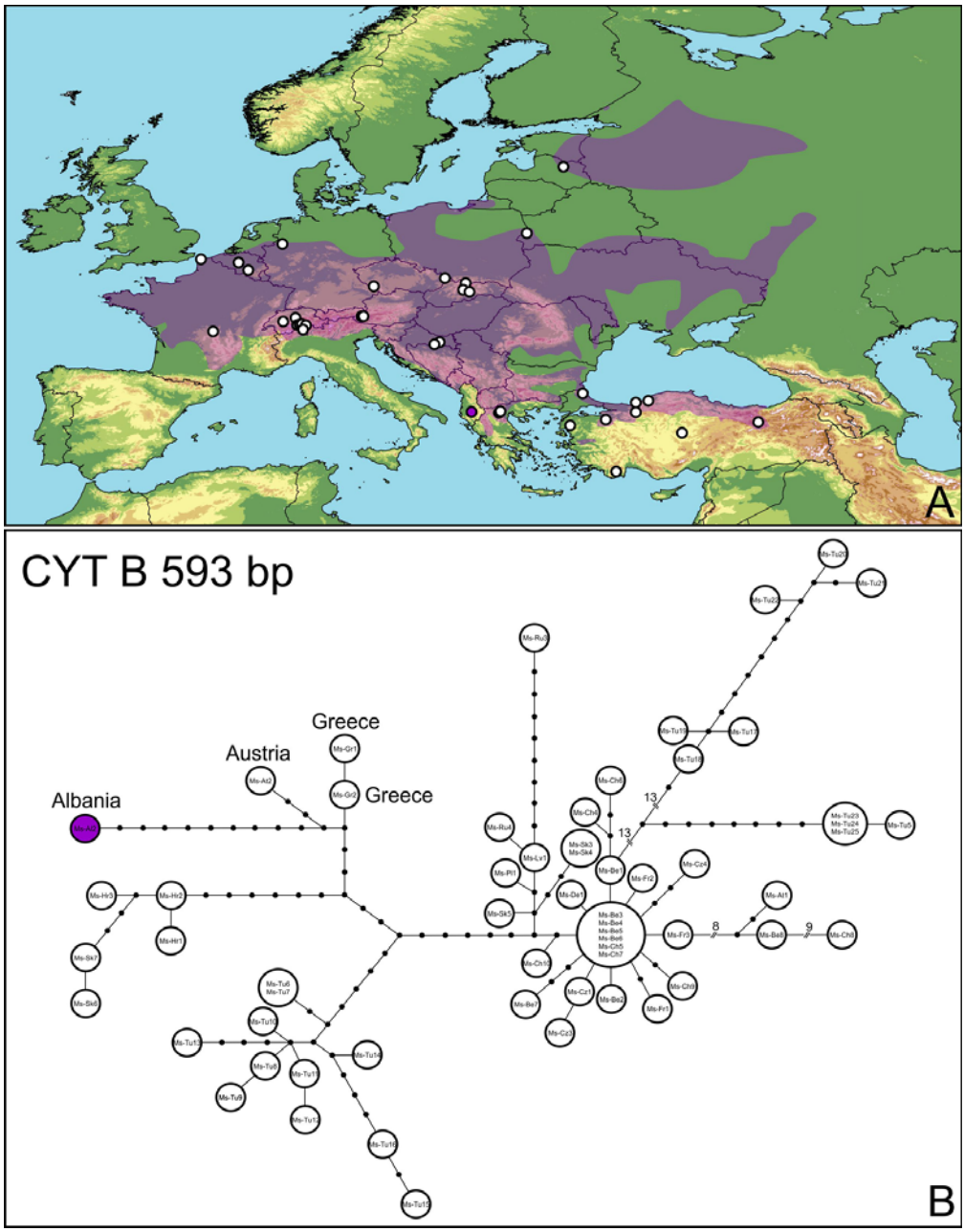

Fig. 3. A: Distribution of *Microtus subterraneus* in the Western Palearctic (violet layer) with localities used for haplotype affiliation (white circles); B: haplotype network based on 593 bp length dataset of sequences. The violet circle represents Tomorr Mts individual. Small black circles in the network indicate hypothetical haplotypes.

Kryštufek & Petkovski 2003; Bego et al. 2008; Castiglia et al. 2009).

Genetic analysis also suggests an interesting past population processes. The haplotype uniqueness and phylogeographical pattern of both individuals showed complex evolutionary history similar to the other species in the region (Kryštufek et al. 2007; Jablonski et al. 2016; Marzahn et al. 2016). Therefore, further investigations are needed to uncover diversity of STM in Albania.

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Electronic supplementary file. Dataset of available published sequences deposited in GenBank used for this study.

Species	Code	GenBank code	Country	Locality	N	E	Source
Chionomys nivalis	Cn-Ru1	JX440342	Russia	Adygea, Caucasian Reserve, Abago Mountain	43.900000	40.133333	Bannikova et al. (2013)
	Cn-Ru2	JX440341	Russia	Adygea, Caucasian Reserve, Abago Mountain	43.900000	40.133333	Bannikova et al. (2013)
	Cn-Bg1	GQ352464	Bulgaria	Vitosha Mountain	42.619444	23.383055	Bannikova et al. (2013)
	Cn-Bg2	JN244734	Bulgaria	Vitosha Mountain	42.619444	23.383055	Bannikova et al. (2013)
	Cn-Tu1	JN244707	Turkey	Central Taurus	37.721388	35.181388	Bannikova et al. (2013)
	Cn-Bg3	JN244706	Bulgaria	Pirin Mts.	41.828611	23.539722	Bannikova et al. (2013)
	Cn-Ro1	GQ352465	Romania	Eastern Carpathians, Breskul Mts.	45.781388	26.758611	Bannikova et al. (2013)
	Cn-It1	AY513845	Italy	Trento	46.069648	11.119663	Jaarola et al. (2004)
	Cn-It2	AY513846	Italy	Trento	46.069648	11.119663	Jaarola et al. (2004)
	Cn-Sk1	AY513847	Slovakia	Prvé Roháčske pleso lake	49.206417	19.745538	Jaarola et al. (2004)
	Cn-Sk2	AY513848	Spain	Queralbs, Girona	42.348977	2.160703	Jaarola et al. (2004)
	Cn-Sy1	AY513849	Syria	Saleh, As Suwayda	33.666073	36.733201	Jaarola et al. (2004)
	Cn-Tu2	GQ150786	Turkey	Ciglikara	36.557485	29.954559	Castiglia et al. (2009)
	Cn-Tu3	GQ150787	Turkey	Ciglikara	36.557485	29.954559	Castiglia et al. (2009)
	Cn-Tu4	GQ150788	Turkey	Ciglikara	36.557485	29.954559	Castiglia et al. (2009)
	Cn-Mk1	GQ150791	Macedonia	Mt. Pelister	41.005429	21.202474	Castiglia et al. (2009)
	Cn-Si1	GQ150792	Slovenia	Mt. Sneznik	45.588514	14.447509	Castiglia et al. (2009)
	Cn-Si2	GQ150793	Slovenia	Mt. Sneznik	45.588514	14.447509	Castiglia et al. (2009)
	Cn-Il1	GQ150789	Israel	Mt. Hermon	33.307936	35.772203	Castiglia et al. (2009)
	Cn-Il2	GQ150790	Israel	Mt. Hermon	33.307936	35.772203	Castiglia et al. (2009)
	Cn-It3	GQ150800	Italy	Marta Alpi Liguri	43.880462	7.658863	Castiglia et al. (2009)
	Cn-It4	GQ150794	Italy	Marta Alpi Liguri	43.880462	7.658863	Castiglia et al. (2009)
	Cn-It5	GQ150795	Italy	Val Masino	46.218527	9.637879	Castiglia et al. (2009)
	Cn-It6	GQ150796	Italy	Val Masino	46.218527	9.637879	Castiglia et al. (2009)
	Cn-It7	GQ150797	Italy	Valle d'Aosta	45.741012	7.133111	Castiglia et al. (2009)
	Cn-It8	GQ150798	Italy	Valle d'Aosta	45.741012	7.133111	Castiglia et al. (2009)
	Cn-It9	GQ150801	Italy	Valle d'Aosta	45.741012	7.133111	Castiglia et al. (2009)

	Cn-It11	GQ150802	Italy	Duchessa	42.443096	13.542080	Castiglia et al. (2009)
	Cn-Es1	AM392367	Spain	Sierra de Gredos	40.300337	-5.089904	Galewski et al. (2006)
	Cn-Tm1	HQ901806	Turkmenistan	Ashabad, Kopet-Dag	37.940391	58.391098	Yannic et al. (2011)
	Cn-Tm2	HQ901807	Turkmenistan	Ashabad, Kopet-Dag	37.940391	58.391098	Yannic et al. (2011)
	Cn-Tm3	HQ901804	Turkmenistan	Ashabad, Kopet-Dag	37.940391	58.391098	Yannic et al. (2011)
	Cn-Tm4	HQ901805	Turkmenistan	Ashabad, Kopet-Dag	37.940391	58.391098	Yannic et al. (2011)
	Cn-Ge1	HQ901803	Georgia	Pass of Tskhra-tskharo	41.708771	43.507489	Yannic et al. (2011)
	Cn-Ge2	HQ901799	Georgia	Pass of Tskhra-tskharo	41.708771	43.507489	Yannic et al. (2011)
	Cn-Ge3	HQ901801	Georgia	Pass of Tskhra-tskharo	41.708771	43.507489	Yannic et al. (2011)
	Cn-Ge4	HQ901802	Georgia	Pass of Tskhra-tskharo	41.708771	43.507489	Yannic et al. (2011)
	Cn-Ge5	HQ901800	Georgia	Pass of Tskhra-tskharo	41.708771	43.507489	Yannic et al. (2011)
	Cn-Es2	GU987115	Spain	Avila	40.648757	-4.691982	Fink et al. (2010)
	Cn-Ch1	GU954317	Switzerland	Not available	46.279502	7.217192	Fink et al. (2010)
	Cn-Ch2	GU954316	Switzerland	Not available	46.279502	7.217192	Fink et al. (2010)
	Cn-Ch3	AY332715	Switzerland	Not available	46.979469	8.254735	Pfunder et al. (2004)
	Cn-Ad1	JX457754	Andorra	Andorra	42.517713	1.529457	Barbosa et al. (2013)
	Cn-Es3	JX457753	Spain	Girona	41.950008	2.775882	Barbosa et al. (2013)
	Cn-Es4	JX457752	Spain	Sorpe	42.652157	1.078460	Barbosa et al. (2013)
	Cn-Al1	KY620211	Albania	Tomorr Mts.	40.627132	20.173909	This study
Microtus subterraneus	Ms-Tu5	AY513836	Turkey	Guzyurdu	39.918365	39.562218	Jaarola et al. (2004)
	Ms-Tu6	AY513835	Turkey	Ciglikara	36.557485	29.954559	Jaarola et al. (2004)
	Ms-Tu7	AY513834	Turkey	Ciglikara	36.557485	29.954559	Jaarola et al. (2004)
	Ms-At1	AY513833	Austria	Glocknerhaus	47.069522	12.768164	Jaarola et al. (2004)
	Ms-At2	AY513832	Greece	Seli	40.554073	22.025010	Jaarola et al. (2004)
	Ms-Lv1	GU291956	Latvia	Gulbene district	57.233345	26.349978	Baltrūnaitė (2010)
	Ms-Ch4	AJ717745	Switzerland	Val Piora	46.543289	8.688379	Tougard et al. (2008)
	Ms-Ru3	KR107034	Russia	Crenna lake, Novgorod	Not available	Not available	Baskevich et al. (2015)
	Ms-De1	JF318998	Germany	Not available	51.999742	7.335720	Schlegel et al. (2012)
	Ms-Ch5	AY332714	Swizerland	Pilatus OW	46.979469	8.254735	Pfunder et al. (2004)
	Ms-Sk3	FR869887	Slovakia	High Tatra Mts., Oravice	49.300091	19.749562	Martinkova et al. (unpublished)
	Ms-Sk4	FR869886	Slovakia	High Tatra Mts., Oravice	49.300091	19.749562	Martinkova et al. (unpublished)
	Ms-Sk5	FR869885	Slovakia	Muránska planina, Za Nihovom	48.771417	20.022561	Martinkova et al. (unpublished)
	Ms-Pl1	FR869884	Poland	Bialowieza	52.699965	23.867552	Martinkova et al. (unpublished)
	Ms-Pl2	FR869883	Poland	Bialowieza	52.699965	23.867552	Martinkova et al. (unpublished)
	Ms-Fr1	FR869882	France	Calais	50.951676	1.859597	Martinkova et al. (unpublished)
	Ms-Fr2	FR869881	France	Bussieres, La Cote	46.065837	2.657177	Martinkova et al. (unpublished)

Ms-Fr3	FR869880	France	Calais	50.951676	1.859597	Martinkova et al. (unpublished)
Ms-Fr4	FR869879	France	Calais	50.951676	1.859597	Martinkova et al. (unpublished)
Ms-Cz1	FR869878	Czech Republic	Kašperské hory Mts.	49.143185	13.557178	Martinkova et al. (unpublished)
Ms-Cz2	FR869877	Czech Republic	Kašperské hory Mts.	49.143185	13.557178	Martinkova et al. (unpublished)
Ms-Cz3	FR869876	Czech Republic	Not available	Not available	Not available	Martinkova et al. (unpublished)
Ms-Cz4	FR869875	Czech Republic	Frydek	49.678977	18.354226	Martinkova et al. (unpublished)
Ms-Ch6	FR869874	Switzerland	Ticino, All Acqua	46.475440	8.938653	Martinkova et al. (unpublished)
Ms-Ch7	FR869873	Switzerland	Gurnigel	46.730415	7.445969	Martinkova et al. (unpublished)
Ms-Ch8	FR869872	Switzerland	Gurnigel	46.730415	7.445969	Martinkova et al. (unpublished)
Ms-Ch9	FR869871	Switzerland	Ticino, Forcola	46.191783	8.744827	Martinkova et al. (unpublished)
Ms-Ch10	FR869870	Switzerland	Ticino, Bedretto	46.506191	8.441629	Martinkova et al. (unpublished)
Ms-Ch11	FR869869	Switzerland	Ticino, Nufenen	46.478030	8.386347	Martinkova et al. (unpublished)
Ms-Be1	FR869868	Belgium	Brussels, Waterloo	50.709596	4.398191	Martinkova et al. (unpublished)
Ms-Be2	FR869867	Belgium	Ardennes	50.202285	5.027798	Martinkova et al. (unpublished)
Ms-Be3	FR869866	Belgium	Ardennes	50.202285	5.027798	Martinkova et al. (unpublished)
Ms-Be4	FR869865	Belgium	Ardennes	50.202285	5.027798	Martinkova et al. (unpublished)
Ms-Be5	FR869864	Belgium	Ardennes	50.202285	5.027798	Martinkova et al. (unpublished)
Ms-Be6	FR869863	Belgium	Ardennes	50.202285	5.027798	Martinkova et al. (unpublished)
Ms-Be8	FR869861	Austria	Heiligenblut, Grossglockner	47.071588	12.709195	Martinkova et al. (unpublished)
Ms-Sk6	FR869860	Slovakia	Low Tatra Mts., Veľký Gápeľ	48.859554	19.626728	Martinkova et al. (unpublished)
Ms-Sk7	FR869859	Slovakia	Low Tatra Mts., Bystrá dolina	48.858883	19.605300	Martinkova et al. (unpublished)
Ms-Hr1	FR869858	Croatia	Nova Kapela	45.197236	17.634952	Martinkova et al. (unpublished)
Ms-Hr2	FR869857	Croatia	Migalovci	45.332379	17.998986	Martinkova et al. (unpublished)
Ms-Hr3	FR869856	Croatia	Not available	Not available	Not available	Martinkova et al. (unpublished)
Ms-Gr2	FR869854	Greece	Edessa, Seli	40.538903	22.013970	Martinkova et al. (unpublished)
Ms-Tu8	FR869853	Turkey	Bursa, Uludag	40.071767	29.221663	Martinkova et al. (unpublished)
Ms-Tu9	FR869852	Turkey	Bursa, Uludag	40.071767	29.221663	Martinkova et al. (unpublished)
Ms-Tu10	FR869851	Turkey	Bursa, Uludag	40.071767	29.221663	Martinkova et al. (unpublished)
Ms-Tu11	FR869850	Turkey	Bursa, Uludag	40.071767	29.221663	Martinkova et al. (unpublished)

	Ms-Tu12	FR869849	Turkey	Bursa, Uludag	40.071767	29.221663	Martinkova et al. (unpublished)
	Ms-Tu13	FR869848	Turkey	Bursa, Uludag	40.071767	29.221663	Martinkova et al. (unpublished)
	Ms-Tu14	FR869847	Turkey	Kirklareli, Demirkoy	41.822801	27.764966	Martinkova et al. (unpublished)
	Ms-Tu15	FR869846	Turkey	Balikesir, Mt. Ida	39.701266	26.835734	Martinkova et al. (unpublished)
	Ms-Tu16	FR869845	Turkey	Balikesir, Mt. Ida	39.701266	26.835734	Martinkova et al. (unpublished)
	Ms-Tu17	FR869844	Turkey	Abant	40.603859	31.279165	Martinkova et al. (unpublished)
	Ms-Tu18	FR869843	Turkey	Abant	40.603859	31.279165	Martinkova et al. (unpublished)
	Ms-Tu19	FR869842	Turkey	Zonguldak, Geris	41.236145	31.442491	Martinkova et al. (unpublished)
	Ms-Tu20	FR869841	Turkey	Seyfe	39.175792	34.410917	Martinkova et al. (unpublished)
	Ms-Tu21	FR869840	Turkey	Zonguldak, Geris	41.368819	32.109569	Martinkova et al. (unpublished)
	Ms-Tu22	FR869839	Turkey	Zonguldak, Geris	41.368819	32.109569	Martinkova et al. (unpublished)
	Ms-Tu23	FR869838	Turkey	Gumushane, Guzyurdu	39.914783	39.557630	Martinkova et al. (unpublished)
	Ms-Tu24	FR869837	Turkey	Gumushane, Guzyurdu	39.914783	39.557630	Martinkova et al. (unpublished)
	Ms-Tu25	FR869836	Turkey	Gumushane, Guzyurdu	39.914783	39.557630	Martinkova et al. (unpublished)
	Ms-Al2	KY620210	Albania	Tomorr Mts.	40.62249	20.17480	This study

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