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Authors: Germann, Christoph, Wyler, Sofia, and Bernasconi, Marco Valerio

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DNA barcoding of selected alpine beetles with focus on Curculionoidea (Coleoptera)

Christoph Germann^{1,2,3} *, Sofia Wyler⁴ & Marco Valerio Bernasconi¹

- ¹ Natur-Museum Luzern, Kasernenplatz 6, CH-6003 Luzern, Switzerland
- ² Naturmuseum Solothurn, Klosterplatz 2, CH-4500 Solothurn, Switzerland
- ³ Naturhistorisches Museum der Burgergemeinde Bern, Bernastrasse 15, CH-3005 Bern, Switzerland
- ⁴ SwissBOL, Université de Genève, Département de Génétique et Evolution, Quai Ernest Ansermet 30, CH-1211 Genève
 4, Switzerland
- * corresponding author, E-mail: germann.christoph@gmail.com

Abstract: Selected beetles, mainly weevils, from the Alpine Arc were barcoded. From 187 samples of 106 assigned species of the families Curculionidae (152 samples, mainly Entiminae, Cyclominae and Hyperinae), Carabidae (18), Apionidae (6), Chrysomelidae and Staphylinidae (each 1 sample), sequences from the COI (subunit 1 of the cytochrome oxydase gene) were obtained, with a success of more than 86% (162 samples). In the cases of Otiorhynchus pupillatus Gyllenhal, 1834, O. nodosus (O. F. Müller, 1764), O. meridionalis Gyllenhal, 1834, Dichotrachelus koziorowiczi Desbrochers des Loges, 1873, D. augusti F. Solari, 1946 and D. maculosus Fairmaire, 1869 more diversity was hidden than foreseen in the beginning, suggesting partly cryptic (not yet described) species. One name is thus resurrected from junior synonymy (O. civis Stierlin, 1861 stat. rev. from synonymy with O. meridionalis). In another case with strictly parthenogenetically reproducing populations of O. pupillatus and O. nodosus in the Swiss Alps, several lineages from hypothetical postglacial immigration events, or alternatively complexes of species in *statu nascendi* might explain the results observed. Moreover, some morphologically debated species-pairs/triples confirmed to be problematic too, even with our COI sequence data [Hypera nigrirostris (Fabricius, 1775) - ononidis (Chevrolat, 1863) - melarynchus (Olivier, 1807)]. On the other hand, in some cases the species' identity, based on the monophyly of the investigated populations, could be confirmed [Anthonomus rubi (Herbst, 1795), Polydrusus chaerodrysius Gredler, 1866, P. paradoxus Stierlin, 1859]. In the hyperdiverse genus Otiorhynchus Germar, 1822, some preliminary insights into the systematics at the subgenus-level could be made, suggesting that many changes of the present morphologically based systematic structure will be necessary.

Keywords: COI - endemic species - Alps - Switzerland - Apionidae - Carabidae - Chrysomelidae - Curculionidae - Staphylinidae.

INTRODUCTION

Genetic analyses of speciation promises to substantially enhance our knowledge on evolution. In particular, the vast climatic oscillations during the present epoch of the quaternary can be linked closely to speciation processes and corresponding genetic change. Investigating the impact of the recent glacial periods has thus become a productive field in evolutionary research (Avise, 2000; Hewitt, 2004).

Of all extant taxa of higher living organisms, the Coleoptera are the most versatile, adaptive and successful group in exploiting ecological niches. Their success is reflected in persistence and adaptability of a huge variety of ancient lineages (Hunt et *al.*, 2007). Coleoptera are by far the most diverse group worldwide with about 400 000

In Switzerland, Coleoptera comprise more than 7000 species (estimation based on Besuchet, 1985). Whereas smaller families are less investigated, more than half of the species are covered presently by up-to-date checklists as Carabidae (Luka *et al.*, 2009a; 520 species); Staphylinidae (Luka *et al.*, 2009b; 1421 species); Curculionoidea (Germann, 2010a; 1070 species); Elateridae and allies (Chittaro & Blanc, 2012; 152 species); Cerambycidae, Buprestidae, Cetoniidae, Lucanidae (Monnerat *et al.*, 2015; 293 species), or are presently under investigation

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described species (Hammond, 1992), thus representing one fourth of all animal taxa described. Since Hunt *et al.* (2007), a first comprehensive molecular phylogenetic reconstruction of the most diverse suborder Polyphaga exists.

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(e.g. Chrysomeloidea, Cleridae, Histeridae and smaller xylobiont families). About 16 coleopterists are currently working on the mentioned families in Switzerland, the vast majority employing morphological approaches only. Alpine beetles have traditionally been regarded as a model group for the elucidation of the history of dispersal and formation of species. Of outstanding interest have been the immobile, flightless and endemic species currently inhabiting the highest ranges of the Alps and other mountainous regions. A century of classical zoological research has delivered quite a comprehensive knowledge on the alpine beetle fauna, and how it was formed through the "ice age" (Holdhaus, 1954; Janetschek, 1956). A recent study employing genetic analysis of carabid beetles could confirm the hypothesis of immobile alpine beetle species having a complex phylogenetic history, and also was able to address more general phylogeographic questions concerning the location of glacial refugia in the southern Alps (Lohse et al., 2011).

The Superfamily Curculionoidea comprises globally more than 62000 species (Oberprieler et al., 2007), and hence form a superdiverse group within Coleoptera. Several attempts to unravel and explain the triggers for this diversity were made, either based on combined molecular and morphological data (Farrell, 1998; Wink et al., 1997; Marvaldi et al., 2002) or solely on molecular data using several genetic markers (McKenna et al., 2009; Hundsdoerfer et al., 2009). However, as Franz & Engel (2010) criticised, the results obtained by attempts of reconstruction of "big" phylogenies within Curculionoidea are ambiguous and inconsistent, and interpretations are built on weak grounds. More fruitful would be to address more specific questions, or questions concerning the classification at the genus, tribal or subfamily levels (Franz & Engel, 2010). Just very recently Haran et al. (2013) addressed such a question with the aid of next generation sequencing and provided well-supported new insights into weevil systematics at the subfamily level. Based on several traditional genetic markers, Astrin & Stüben (2008, 2010), Astrin et al. (2012) and Stüben et al. (2013) contributed substantially to the phylogenetic understanding within the subfamily Cryptorhynchinae, and Meregalli et al. (2013) investigated several Cyclominae. Similar promising insights could be done with other groups, where unresolved systematic questions at the genus and/or species level persist, as for e.g. Entiminae and Hyperinae, with many species living in restricted areas at higher altitudes.

In this study, we focus above all on relict populations of rare Alpine endemic (or potentially endemic) beetle species of the family Curculionidae and Carabidae with a particular interest in detecting possible cryptic diversity.

MATERIAL & METHODS

Taxon sampling

The present project includes 187 samples (see annex 1) belonging to more than 20 genera and representing about 100 recognised species of the families Curculionidae (representing 85% of all the samples used here), Carabidae (10%), Chrysomelidae, Apionidae, and Staphylinidae.

We are aware that many COI sequences of Coleoptera, including the families analysed here, are already available in a databank such as BOLD or GenBank. However, in the view of the extremely high number of existing sequences, we deliberately decided to confine our analysis to the Swiss alpine region, where samples are presently largely missing. Subsequent analyses, focusing on particular genera and subfamilies, will include all the needed sequence data to address the problem more in detail.

Before and after DNA extraction, all samples were and are stored in 90% Ethanol at minus 20°C and housed in the collection of the Nature-Museum Lucerne (NML). The extracted DNA is stored at minus 80°C and is currently deposited in the SwissBOL molecular platform at the University of Geneva.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted using the DNeasy® Blood & Tissue Kit (Qiagen). Individuals were entirely plunged in the digestion buffer for 4 hours and removed thereafter. This technique allows a DNA extraction which preserves the exoskeleton and is useful when the specimen must be kept intact. Remaining protocols followed the supplier's instructions. Part of the mitochondrial COX1 (COI) gene was then amplified using the forward primer C1-J-2183 5'CAACATTTATTTTGATTTTTTGG3' and the reverse primer TL2-N-3014 5'TCCAATGCACTAATCTGCCATATTA3' (Vahtera & Muona, 2006). PCRs were made in 20 µl total volume with 0.60U Taq (Roche), 2 µl of the 10X buffer containing 20 mM MgCl₂, 0.8 µl of each primer (10 mM), 0.4 µl of a mix containing 10 mM of each dNTP (Roche) and 0.8 µl template DNA of unknown concentration. The PCR program comprised an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 40 s, annealing at 42°C for 45 s and 72°C for 1 min, with a final elongation step at 72°C for 8 min. COI PCR products were then directly sequenced bi-directionally on an ABI 3031 automated sequencer (Applied Biosystems) using the same primers and following the manufacturer's protocol.

DNA sequence alignment and phylogenetic analyses

Sequence editing and generation of consensus sequences were accomplished using CodonCode Aligner (CodonCode Corporation). Alignments were automatically generated using Muscle (Edgar, 2004) as

implemented in Seaview program (Gouy et al., 2010) and verified manually. Alternatively, the COI sequences were also edited with the Lasergene program Editseq (DNAstar Inc., Madison, WI, USA). Alignment of gene sequences was performed using the ClustalW method as implemented in Megalign (DNAstar Inc.) with default multiple alignment parameters. The COI alignment was gap free. ForCon (Raes & Van de Peer, 1999), a software tool for the format conversion of sequence alignments, was further applied. Phylogenetic and molecular evolutionary analyses were conducted using MEGA (Molecular Evolutionary Genetics Analysis) version 6 (Tamura et al., 2013). Phylogenetic trees were obtained by applying the neighbour-joining (NJ) tree reconstruction method with Kimura 2-parameters (K2) as nucleotide substitution model and by using the Maximum Likelihood (ML) method based on the models selected by MEGA (i. e. GTR+I+G for the "Curculionoidea & Chrysomelidae" and Tamura-Nei+G for the "Carabidae & Staphylinidae"). To avoid misleading results when all data is combined in a single tree due to the lack of resolving power of the COI at higher systematic levels, we split the analyses in the two mentioned parts. The robustness of internal branches was assessed by bootstrapping. MEGA was also used for the visualisation and managing of the electropherograms and to calculate the genetic distances. The sequences of the gene analysed here have been deposited in BOLD (annex 1).

The results of the NJ tree are not depicted here, but they are available as electronically archived supplementary material (see Supp. 1 and Supp. 2 at the end of this publication).

RESULTS & DISCUSSION

Out of 187 extracted samples, 162 (more than 86%) could be used successfully to produce good and usable COI sequences (with an expected length of about 800 nucleotides). The by far biggest set of samples are from the weevils in the narrower sense, Curculionidae, with 152 samples of species from the subfamilies Entiminae (77 samples assigned to 43 described species), Hyperinae (23 samples assigned to 16 species), Cyclominae (27 samples assigned to 9 species), Curculioninae (7 samples assigned to 4 species) and Cryptorhynchinae (1 sample and species) in mostly several specimens from different populations. Six samples of Apionidae (genera Aizobius, Hemitrichapion, and Osellaeus), which are part of the weevils in the broader sense, were included. Furthermore 18 Carabidae and one sample each of Chrysomelidae and Staphylinidae were included as well. Phylogenetic relationships obtained by both ML and NJ methods are depicted in Figs 1-2, resp. Supp. 1-2. The overall topology of the ontained trees is very similar (Fig. 1 vs Supp. 1 and Fig. 2 vs Supp. 2, respectively). In particular, the groups recorded in one analysis are identified in the

tree generated by using the other tree reconstructing method as well, however with variable bootstrap support (see below). The following discoveries could be made, reported under the respective systematic groups.

Family Apionidae Schönherr, 1823 Genera *Aizobius* Alonso-Zarazaga, 1990 *Hemitrichapion* Voss, 1859 & *Osellaeus* Alonso-Zarazaga, 1990

The Apionidae group is only weakly (NJ) or insufficiently (ML < 50%) supported in our analyses (Fig. 1, Supp. 1) but, on the contrary, the monophyly of the genera (i. e. *Aizobius, Hemitrichapion,* and *Osellaeus*) found strong support in both the ML and NJ tree.

The genus *Osellaeus* is represented with three strictly subalpine-alpine taxa in the western alpine arch – *O. bonvouloirii baldensis* (Bellò, Meregalli & Osella, 1980) on Monte Baldo, *O. bonvouloirii* s. str. (Ch. Brisout, 1880) in the central and western Alps and *O. bonvouloirii occidentalis* Germann, 2010 in the Vercors (Germann & Szallies, 2011). We included three Swiss populations of the nominal subspecies, but the third one from the Valais did not produce a positive PCR. The one from Uri (Brisen) and the other from Fribourg (Kaiseregg) are from localities just 91 km distant from each other. As *O. bonvouloirii* is a flightless, and restricted to its alpine habitat and thus a very low mobile species, the detected differences (K2 distance: 0.059; Table 1) are well explainable.

Three other Apionidae were included, of which *Mesotrichapion punctirostre* (Gyllenhal, 1839) did not give a result. The species with the widest distribution reaching from Central Asia to France is *Aizobius sedi* (Germar, 1818). However, the species is restricted to xerothermic places and unable to fly, this may explain for the rather large intraspecific genetic distance (0.027) between the two samples taken 300 km from each other. The third species sampled is *Hemitrichapion waltoni* (Stephens, 1839), recorded from Hungaria to France. The samples taken at localities separated by a distance of 340 km, a species which has normally developed hind wings and is the most mobile of all species included and may therefore show the lowest genetic distance of all Apionidae included (0.011).

These results underline once more the importance of the need for conservation of isolated populations of flightless, ecologically highly specialised and thus low mobile endemic species.

Family Curculionidae Latreille, 1802 Subfamily Curculioninae Latreille, 1802 Genus *Anthonomus* Germar, 1817

The samples of the genus *Anthonomus* form a strongly supported monophyletic group in both our analyses

Entiminae



Fig. 1. Best Maximum Likelihood tree (-ln=13697.7060; GTR+I+G model as selected by MEGA) based on COI sequences of 142 samples of Curculionoidea (Apionidae and Curculionidae) and Chrysomelidae obtained by using MEGA 6. Values (over 50%) of bootstrap support from 100 pseudo-replicates are depicted above nodes.



DNA barcoding alpine beetles



(Fig. 1, Supp. 1). The Swiss populations of the speciespair Anthonomus rubi (Herbst, 1795) / brunnipennis Curtis, 1840 were investigated. There is some ambiguity about the status of A. brunnipennis in the Alps. The species shows a supposedly boreoalpine distribution (Germann, 2010b) and lives on Dryas octopetala, a boreoalpine cushion plant, and in northern Europe it lives also on Filipendula ulmaria L., Potentilla palustris L. and P. erecta L. Anthonomus rubi on the other side is a widespread species living on different Rosaceae, but also Cistaceae. Both species are very difficult to separate based on morphological traits, which overlap largely. The finds of brunnipennis from Switzerland were preliminarily termed as somewhat doubtful and a molecular re-investigation was suggested (Germann, 2010b, 2011a).

We here included a heterogeneous set of samples collected from the northern Alps, from Grisons and Ticino, and collected from either Dryas octopetala (sample 150 from Grisons; sample 144 northern Alps) being small and brownish and thus corresponding to A. brunnipennis, and from Helianthemum and Potentilla (sample 157 from nearby Italy and sample 152 from the northern Alps) being bigger and black and corresponding to typical A. rubi. However, the investigated COI sequences do not support the hypothesis that the specimens collected from Dryas octopetala are a sister-clade to the remaining supposedly "true" Anthonomus rubi (highest intraspecific variability of 0.046; range 0.002-0.046). This might indicate that A. brunnipennis does not occur in Switzerland, however this should be corroborated with specimens of typical A. brunnipennis from northern Europe. On the other hand, an incomplete lineage sorting and/or a too short speciation time being detected by our COI barcode marker might explain our outcome (see also the discussion about the Hypera nigrirostris-group below).

Family Curculionidae Latreille, 1802 Subfamily Cyclominae Schönherr, 1826 Genus *Dichotrachelus* Stierlin, 1853

The monophyly of the genus *Dichotrachelus* is strongly supported in both our analyses (Fig. 1, Supp. 1). Within this genus, there is definitely more hidden diversity in these relatively immobile typically alpine living species distributed from the Rif Mountains of Morocco to the Carpathians in the east, with a speciation centre in the arc of the Alps. The species are ecologically bound either to mosses ("old" lineages) or Saxifragaceae ("derived" lineages) (Meregalli *et al.*, 2015). Based on COI sequences, we found at least in three species considerable differences among the samples, promoting the hypotheses of existing cryptic species.

Data from *D. koziorowiczi* Desbrochers des Loges, 1873 from two localities on Corsica (one in the North at Col de Verghio; the other in the South on Monte Calva) show that two taxa (K2 distance: 0.067; Table 2) are likely to occur on this island, instead of one at present described species. Only the examination of the type specimen(s) will help to resolve this issue, as no precise type locality on the island has been given by Desbrochers (1873).

Similarly, with the *D. maculosus* Fairmaire, 1869 -species group, where specimens of *D. maculosus* from rather isolated populations in the Vercors, at the western border of the main distribution area, differ from those from the Swiss Prealps (K2 distance: 0.026).

Also in the D. augusti F. Solari, 1946 -species complex, more morphological diversity was discovered (see Germann, 2011b), here corroborated partly by the detected genetic diversity. The rather isolated population from the Saas Valley (sample 89) differs genetically considerably (K2 distance: 0.115!) from those of samples from the Grand St. Bernard and Col de Balme regions at the Swiss-Italian and Swiss-French border, which is indeed surprising, as it is surprisingly not reflected in their morphology, whereas D. sondereggeri Germann, 2011 shows differences, but solely results in a genetic distance of 0.016 compared with the western populations of D. augusti. Furthermore, the different forms of the penis (Germann, 2011b) detected in the western populations of D. augusti in turn are not supported by relevant differences in the COI (0.002). However, to definitely delimit and show more solid insights into the systematics of the D. augusti-species complex we would still have to include samples from the type locality of D. augusti from around Champoluc in Valle d'Aosta. Additionally, the highly specialised habitat demands of the D. augustispecies complex might explain for the genetic differences between geographically close populations: all species of this complex live in mosses growing in alpine scree slopes, an unusual and certainly underestimated habitat, less in Carabidae (where exciting discoveries have been reported e.g. Molenda, 1996; Molenda & Gude, 2003; Huber & Molenda, 2004), or Staphylinidae (Molenda, 1999), but more in weevils where hardly any research has been done, and a promising field for investigations lies idle (Nikolai Yunakov, personal comm.). The alpine scree slopes thereafter can be seen as islands for the populations of the D. augusti-complex, where gene exchange via migrating individuals across alpine grasslands and glaciers might be very limited. This specific case once more shows that samples from populations of a species, at least if we deal with low mobile species, should be chosen very carefully.

The samples of species assigned to the *D. rudeni*species group, based on a similar external morphology and male genitalia with a prolonged, laterally flattened tip of penis, also clustered together (*D. rudeni* Stierlin, 1853, *D. imhoffi* Stierlin, 1857 and *D. variegatus* Daniel & Daniel, 1898) and therefore support the outcomes from previous morphological investigations (Table 2). The samples of *D. rudeni* cluster all together with high bootstrap support (ML 99%, respectively NJ 98%),



Fig. 2. Best Maximum Likelihood tree (-ln= 3045.2577; Tamura-Nei+G model as selected by MEGA) based on COI sequences of 20 samples of Carabidae and Staphylinidae obtained by using MEGA 6. Values (over 50%) of bootstrap support from 500 pseudo replicates are depicted above nodes.

although there is some herogeneity in it with sample 109 from the eastern border of the distribution near Disentis (sample 109) differing most from the others (0.010 to 0.016).

Family Curculionidae Latreille, 1802 Subfamily Hyperinae Lacordaire, 1863 Genera *Brachypera* Capiomont, 1868 and *Donus* Jekel, 1865

The genera *Brachypera*, *Donus* and *Hypera* form a strongly supported monophyletic group in both our analyses (Fig. 1, Supp. 1), with both individuals of *Brachypera vidua* (Gené, 1837) placed within *Donus* samples, even if with insufficient bootstrap support (<50%).

Despite of recent efforts to unravel the relationships at genus-level based on morphology within Hyperini (Skuhrovec, 2013), we recovered an alternative hypothesis regarding *Donus* and *Brachypera*; where the latter at best represents a subgenus within *Donus*. Although, in our dataset *Brachypera* is solely represented by *Brachypera vidua*. However, these results are supported by those of Stüben *et al.* (2015), who included *Brachypera grandini* (Capiomont, 1868), *B. dauci* (Olivier, 1807) and *B. lunata* Wollaston, 1854, which clustered also paraphyletically in different clades within *Donus*. In our analyses, the bootstrap support for two separate clades (*Donus* s. l. vs *Hypera*) is surprisingly low and their monophyly could not be therefore definitively established based on our sequence data.

Genus Hypera Germar, 1817

Even at the species-level, we found no support for a monophyly of all the Hypera species investigated here based on our COI data (Fig. 1, Supp. 1). In particular, the recorded genetic distances (Table 3) were relatively low (from 0.003 to 0.012) for any of the three species of the H. nigrirostris group [nigrirostris (Fabricius, 1775), ononidis (Chevrolat, 1863) and melarynchus (Olivier, 1807)]. The morphologically weakly supported hypothesis of the species status for Hypera ononidis was already questioned by Stüben et al. (2015) in their barcode approach. Although, obvious ecological differences are evident (H. ononidis lives on Ononis spp. and occurs in a sub-area of H. nigrirostris, which accepts a wider range of Fabaceae). Therefore, a more recent speciation process (not yet detectable with the possibly too conservative COI-marker), and thus the evolution of eco-species at an early stage of differentiation might be an explanation for this circumstance. Interestingly also the morphologically close H. melarynchus - living on the Fabaceae Ononis ramosissima - clustered together with H. ononidis + nigrirostris. However, H. melarynchus shows several morphological characters (biggest species

of all three > 5 mm; rostrum long and slender, at least as long as pronotum; 7th article about as wide as club; elongate elytra parallel along middle; penis S-shaped in lateral view, tip elongate, tongue-like) that allow an unambiguous separation from *H. nigrirostris* and *H. ononidis*, and therefore the species status has never been questioned. This provides further evidence that the *nigrirostris*-species group might indeed represent a younger group where speciation is at an early stage with an incomplete lineage sorting and highlighting therefore the limited resolution power of the used barcoding marker (see Germann *et al.*, 2010 for a similar case in Diptera).

Family Curculionidae Latreille, 1802 Subfamily Entiminae Schönherr, 1823 Genus Otiorhynchus Germar, 1822 Subgenera Metopiorrhynchus Reitter, 1912 pars, Nihus Reitter, 1912, Eunihus Reitter, 1912

A large number of the specimens coped with this study belongs to the genus *Otiorhynchus* (annex 1, Fig. 1, Supp. 1). This genus is one of the most specious genera – if not the most specious – in weevils. More than 1500 species are presently assigned to this genus and the systematics is midly expressed rather chaotic. Based on our data, the monophyly of *Otiorhynchus* is supported by insufficient bootstrap values in both the NJ and ML analyses. However, some new insights into alpine subgenera could be gained even if the overall relationships among all the proposed subgenera within this large genus are not always strongly supported in our analyses based on a relatively short fragment of the COI gene.

It was Yunakov (2006) who proposed subgenus Metopiorrhynchus as synonym to Nihus Reitter, 1912, which was reinstated by Magnano & Alonso-Zarazaga (2013). [The type species of Metopiorrhynchus is O. singularis (Linné, 1767) – included in our samples, and O. carinatopunctatus in Nihus, a sample that remained negative] We here provide support to the former synonymy, where species of Nihus cluster together within the subgenus Metopiorrhynchus. Interestingly, the only representative (O. grischunensis Germann, 2010) of Eunihus, a subgenus which has temporarily been placed in synonymy with Nihus, but is actually accepted as proper subgenus (Magnano & Alonso-Zarazaga, 2013), does not cluster together with Nihus. Even if the position of Eunihus remains unclear since not supported by enough bootstrap values, our result underlines its self-standing position in relation to the typical Nihus representatives. On the other side, the Corsican endemic species (O. corsicus Fairmaire, 1859) at present assigned to the subgenus Phalantorrhynchus Reitter, 1912 results in the clade Metopiorrhynchus + Nihus (bootstrap support ML: 58; NJ: 61).

Schütte et al. (2013) and Stüben et al. (2015) already provided molecular evidence to a common clade of

Nihus + Metopiorrhynchus + Aranihus Reitter, 1912 [represented by the species *O. parvicollis* Gyllenhal, 1834 and *O. ligneus* (Olivier, 1807)] + *Edelengus* Reitter, 1912 (*O. atlasicus* Escalera, 1914; *O. allardi* Stierlin, 1872).

Genus *Otiorhynchus* Germar, 1822 Subgenera *Metopiorrhynchus* Reitter, 1912 pars and *Postaremus* Reitter, 1912

In all our analyses (Fig. 1, Supp. 1), all the samples belonging to Otiorhynchus pupillatus Gyllenhal, 1834 clustered together with high bootstrap support. Otiorhynchus pupillatus is a highly polymorphic species. It varies in many characters as size, proportions (rostrum, pronotum and elytra), vestiture (e.g. form of scales, density), size of teeth on femora, and (female) genital organs. It reproduces almost strictly parthenogenetically; males are only known from the junior synonym teretirostris Stierlin, 1866 in the Seealps (mentioned by Stierlin in the description, but never revised since). The validity of several of the synonymous names is highly debated, part of them were recently resurrected in Magnano & Alonso-Zarazaga (2013). Such synonyms are subdentatus Bach, 1854 (described from Thuringia, Germany), frigidus Mulsant & Rey, 1859 (from the western Alps), cyclopterus F. Solari, 1946 (Tirol, Italy/ Austria, Bayern, Germany) and the before mentioned teretirostris.

Describing every single population as a separate species cannot be the goal of studying biodiversity [in the cases of parthenogenetically reproducing populations (unfertilized eggs producing only females, and apomixis, where no meiosis is involved) we have mostly nearly identical genotypes (but see also last section of this part)]. We therefore included 15 samples of O. pupillatus, which resulted in four roughly separable genetic lineages, where three of them differ in few substitutions, and a single specimen from Grisons (sample 085) differs substantially from all others (K2 distances: 0.073-0.086). There is no morphological match with any of the before mentioned debatable species or morphotypes. The first clade comprises samples from the Central and Eastern Swiss Alps (samples 028, 054, 055, 065, 075, 084, 097, 124 from Valais, bordering Italy and Grisons), the second one a specimen from the Val Mustair (sample 106), the third specimens from the Bernese Alps and Lower Engadine (samples 007, 012, 042, 074, 088), and the fourth one (the most differing, as already mentioned), a single specimen from Central Grisons (sample 085). Well supported sister to all samples of O. pupillatus is O. difficilis, an amphigonic, also morpholologically close standing species from northern Italy, Ticino up to the Valais in the Simplon region.

The same discrepancy between morphology and genetic lineages (the retrieved clades do not include specimens

sharing the same set of characters) was observed in *Otiorhynchus nodosus* (O. F. Müller, 1764) belonging to the subgenus *Postaremus* (K2 distances 0.068-0.07, Table 4). Not less than 12 synonymous names belong to this highly variable, boreo-alpine species (colour of legs from black to red, shape of body, vestiture). As already mentioned for *O. pupillatus, O. nodosus* is also parthenogenetic in most of its area, and throughout the Swiss Alps.

In both species mentioned, the observed well separated clades may more likely mirror several post glacial immigration lineages. An alternative explanation would be that these asexually reproducing species represent complexes of species in statu nascendi in the sense of Dobzhansky & Spassky (1959). A phenomenon reported just recently from an identically parthenogenetically reproducing entimine weevil: Naupactus cervinus Boheman, 1840 in South America (Rodriguero et al., 2013). Thereby the presence of different evolutionary units correlating with faint morphological and ecological differences could be shown, driven by many well-known evolutionary forces as mutation, selection, drift going along with geographic isolation. Whatsoever, naming these purely genetically recognisable evolutionary units/ populations will not (yet?) make sense, and unnecessarily blow up the taxonomy of Otiorhynchus. To gain a more complete insight into these complexes, definitely more samples from a broader geographical range and additional nuclear markers are needed.

Genus Otiorhynchus Germar, 1822 s. str.

All species samples from this subgenus clustered together, although with insufficient bootstrap support (Fig. 1, Supp. 1), including *O. morio* Fabricius, 1781, type species (!) of the subgenus *Phalantorrhynchus* Reitter, 1912, but morphologically hardly separable from *Otiorhynchus* s. str. This might show, as already suspected by the span of morphological differing members, and species only weakly differing from *Otiorhynchus* s. str. (as e.g. *O. tenebricosus* versus *O. putoni* Stierlin, 1891), that *Phalantorrhynchus* is a polyphyletic construct which needs to be thoroughly re-analysed in future.

In the case of the two samples of *Otiorhynchus* (s. str.) *meridionalis* Gyllenhal, 1834 included, one comes from Switzerland, Bern (sample 126), the other from southern France, Var (sample 129) and corresponds to the junior synonym *O. civis* Stierlin, 1861. This result uncovers a synonymy proposed by the first author in Pelletier (2005: 111) and later implemented in Magnano & Alonso-Zarazaga (2013). The type specimens of *O. civis* in the Gustav Stierlin collection (conserved in the Deutsches Entomologisches Institut, Müncheberg, Senckenberg) were examined in 2005, and one male specimen with the following label data "Gall. mer." [Gallia meridionale = southern France] is selected, and is here designated as

lectotype, labelled with a red label: "LECTOTYPUS *Otiorhynchus civis* Stierlin 1861 des. C. Germann 2016". The selection of the lectotype is of special importance, as Stierlin (1861) erroneously mentioned "Griechenland" [Greece] as type locality of *O. civis*. In his collection there was, among other specimens from southern France, also a female specimen from Greece determined as "*O. civis* Stl.". However, *O. meridionalis* is not (yet probably, the species is currently spreading across Europe) known from Greece, and as already stated by Reitter (1913), the specimen from Greece is most likely mislabelled. Furthermore, it is a female specimen, whereas Stierlin (1861) clearly portrayed a male specimen in his description.

The examination of the penis, including the internal sac, did surprisingly not reveal any relevant differences in the two species (the main reason for the proposed synonymy in 2005), but the external morphology, supported here by the molecular data, allows a differentiation between the two species. Therefore *Otiorhynchus civis* Stierlin, 1861 **stat. rev.** is removed from the synonymy with *O. meridionalis* Gyllenhal, 1834. Figure 3 shows both species, the broad elytra and the rugose surface and the denser grey hairs on elytra of *O. civis* (Fig. 3A) allows a differentiation from *O. meridionalis*, where the elytra are more elongate oval and shiny (Fig. 3B; a differentiation already given by Reitter, 1913: 44). *O. civis* is – after present knowledge and specimens examined – still restricted to southern France, whereas *O. meridionalis* is recorded more and more from surrounding countries (details in Magnano & Alonso-Zarazaga, 2013 under *meridionalis*).

The third species of the *O. meridionalis*-species group in our data set is *O. aurifer* Boheman, 1842, is also included in our dataset and it is well separated (Table 5).



Fig. 3. (A) Otiorhynchus civis Stierlin, 1861 stat. rev. (France, Var, Bargème). (B) O. meridionalis Gyllenhal, 1834 (Switzerland, Bern).

Tables 1-8: COI Kimura 2-Parameter genetic distances for a set of selected samples used in the present study. See the main text for further details.

Table 1: Apionidae

	A. s. 155	A. s. 141	<i>H. w.</i> 156	<i>H. w.</i> 146	<i>O. b.</i> 147
Aizobius sedi 155					
Aizobius sedi 141	0.027				
Hemitrichapion waltoni 156	0.182	0.189			
Hemitrichapion waltoni 146	0.180	0.188	0.011		
Osellaeus bonvouloirii 147	0.170	0.174	0.196	0.192	
Osellaeus bonvouloiri bonvouloiri 153	0.163	0.163	0.187	0.187	0.059

Table 2: selected Dichotrachelus samples

	D. k. 182	D. k. 081	D. a. 062	D. a. 089	D. s. 023	D. m. 093
Dichotrachelus koziorowiczi 182						
Dichotrachelus koziorowiczi 081	0.067					
Dichotrachelus augusti 062	0.151	0.163				
Dichotrachelus augusti 089	0.165	0.159	0.115			
Dichotrachelus sondereggeri 023	0.145	0.155	0.016	0.103		
Dichotrachelus maculosus 093	0.169	0.141	0.108	0.106	0.098	
Dichotrachelus maculosus 073	0.173	0.156	0.109	0.122	0.099	0.026

Table 3: Hypera nigrirostris species group

	<i>H. n.</i> 016	Н. о. 098	H. n. 094	<i>H. m.</i> 091
Hypera nigrirostris 016				
Hypera ononidis 098	0.003			
Hypera nigrirostris 094	0.007	0.007		
Hypera melarynchus 091	0.010	0.010	0.012	
Hypera nigrirostris 019	0.012	0.009	0.010	0.013

Table 4: Otiorhynchus nodosus

	<i>O. n.</i> 027	<i>O. n.</i> 034	<i>O. n.</i> 041	<i>O. n.</i> 080	<i>O. n.</i> 107
Otiorhynchus nodosus 027					
Otiorhynchus nodosus 034	0.068				
Otiorhynchus nodosus 041	0.000	0.068			
Otiorhynchus nodosus 080	0.070	0.001	0.070		
Otiorhynchus nodosus 107	0.000	0.068	0.000	0.070	
Otiorhynchus nodosus 108	0.000	0.068	0.000	0.070	0.000

Table 5: Otiorhynchus meridionalis species group

	O. aurifer 170	O. meridionalis 126
Otiorhynchus aurifer 170		
Otiorhynchus meridionalis 126	0.142	
Otiorhynchus civis 129	0.132	0.093

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	P. chaerodrysius 143	P. chaerodrysius 103
Polydrusus chaerodrysius 143		
Polydrusus chaerodrysius 103	0.002	
Polydrusus paradoxus 032	0.048	0.048

 Table 6: Sibling alpine species Polydrusus paradoxus/Polydrusus chaerodrysius

Table 7: selected Nebria samples

	N. f. rhaetica 121	N. c. escheri 117	N. heri 114
Nebria fontinalis rhaetica 121			
Nebria cordicollis escheri 117	0.006		
Nebria heeri 114	0.011	0.018	
Nebria cordicollis tenuissima 123	0.010	0.016	0.002

Table 8: Oreonebria bremii vs Oreonebria bluemlisalpicola

	O. bluemlisalpicola 083	O. bluemlisalpicola 072	O. bremii 082
Oreonebria bluemlisalpicola 083			
Oreonebria bluemlisalpicola 072	0.015		
Oreonebria bremii 082	0.040	0.043	
Oreonebria bremii 071	0.040	0.043	0.000

Genus *Otiorhynchus* Germar, 1822 Subgenera *Nehrodistus* Reitter, 1912, *Misenatus* Reitter, 1912, *Melasemnus* Reitter, 1912

From subgenus *Nehrodistus* the four species *O. armatus* Boheman, 1842, *O. turca* Boheman, 1842, *O. obesus* Stierlin, 1861, and *O. pesarinii* Diotti, 2008 are included. These species did not form a monophyletic clade, and species of other subgenera e.g. *Otiolehus* cluster within (Fig. 1, Supp. 1). This may show that a natural group of relatives including species of *Nehrodistus* – mainly characterised by the teethed femora, the rugose pronotum, the spotty distributed scales on elytra, these deprived of hairs and the slender antennae with second article almost twice as long as first – may include species of other subgenera as well. However, the detailed relationships among these species are not supported by sufficient bootstrap values and remain therefore questionable with our COI sequence data.

In the case of *O. armatus* the sample from the Ligurian coast differed substantially (K2 distance: 0.077) from the one taken on Ischia island. Just recently Diotti (2008) revised the species close to *O. armatus* and described with *O. pesarinii* a new species from Basilicata. The subsequent comparison with a paratype specimen provided by the author, the con-specificity of the sample specimen from Ischia Island with *O. pesarinii* could be confirmed.

Interesting and surprising from the morphological point of view are *Otiorhynchus lugens* (Germar, 1817) and *O. ovalipennis* Boheman, 1842 as highly supported sister taxa (ML and NJ both 99). Where a species with a single tooth on the femora, a slender rostrum, eyes laterally standing, elytra dull and deprived of hairs, and robust legs (subgenus Misenatus) is sister to O. ovalipennis (Melasemnus) with several additional small rasp like teeth on fore femora, a short rostrum, dorsally oriented eyes, shiny elytra with hairs, and gracile slender legs may represent unreliable characters for morphological estimates on phylogenetic relationships. The differences regarding teeth on femora is also present in the – although in both our analyses moderately supported - clade of O. magnicollis Stierlin, 1888 + O. thaliarchus Reitter, 1914 (Choilisanus Reitter, 1912 with unarmed femora, versus Melasemnus with teeth, often even several on fore femora). Another example for the absence and/or presence of teeth is the clade Metopiorrhynchus (teeth present) + Aranihus (teeth absent, or minute and often overlooked as in O. ligneus!) + pars Phalantorrhynchus (teeth absent) + Nihus (teeth absent), however with lower support (ML: 58; NJ: 61).

Genus Polydrusus Germar, 1817

We included five samples of this genus belonging to 3 species out of 2 subgenera (*Piezocnemus* Chevrolat, 1869 and *Chlorodrosus* K. Daniel & J. Daniel, 1898). The species were not retrieved in a monophyletic clade (Fig. 1, Supp. 1), suggesting that the species concept of *Polydrusus* is also polyphyletic, which is not really a surprise, regarding the span of morphological variability.

In the case of the sibling alpine species *Polydrusus* paradoxus Stierlin, 1859 / chaerodrysius Gredler, 1866 the differences in the COI support the very subtle morphological characters; both species can be distinguished mainly by the form of the scales on their femora (Germann, 2012). Thus it can be stated that small morphological differences are mirrored by a considerable genetic distance (K2 distance: 0.048). Furthermore, although from apparently very isolated populations, the samples of P. chaerodrysius collected in Valchava GR (sample 103) and Schwarenbach BE (208 km from each other; sample 143 / sample 063) differ in solely 0.2 % (Table 6). More localities were not discovered at present, despite of several specific excursions inbetween. An explanation could be their parthenogenetical reproduction, where no gene-exchange as in sexual reproduction occurs.

species-pair Phyllobius pyri / vespertinus

The species status of Phyllobius vespertinus (Fabricius, 1792) was (and still is) highly debated (e.g. Dieckmann, 1979; Germann, 2011a; Alonso-Zarazaga, 2013) and recently regarded as synonym to P. pyri (Linné, 1758) (e.g. Colonnelli, 2003; Yunakov, 2013). While Phyllobius pyri lives mostly on arboreous Rosaceae and shows a more elongate body and a regularly coloured vestiture, P. vespertinus is more xerothermophilous, lives on various herbaceous plants, its body is more stout, the elytra often with a striped vestiture. We here included further specimens from the southern side of the Alps, where the characters of P. vespertinus are well pronounced [and from there (Monte Rosa, Val d'Entremont, St. Bernhard) once described as separate taxon artemisiae Desbrochers, 1873, junior synonym of P. vespertinus]. However, we provide further support that the taxa are not separable based on analyses of COI sequences (Fig. 1, Supp. 1), as already shown by Schütte et al. (2013). Similar to the Hypera nigrirostris-group, COI might be not sensitive enough to show differences, due to recent (ecological) separation of the taxa (i. e. incomplete lineage sorting), and/or genetical interchange (hybridisation) might still occur.

Family Carabidae Latreille, 1802 Subfamily Nebriinae Laporte, 1834 Genera *Nebria* Latreille, 1802, *Oreonebria* K. Daniel, 1903

Both *Nebria* and *Oreonebria* are monophyletic and cluster together with good (ML) to strong (NJ) bootstrap support (Fig. 2, Supp. 2). In the case of the high-alpine *Nebria cordicollis* Chaudoir, 1837 -group, we here included three taxa: *N. heeri* K. Daniel, 1903, recently raised to species level from a subspecies of *cordicollis* by Szallies & Huber (2013), *N. cordicollis escheri* Heer,

1837 from southeastern Switzerland, and *N. cordicollis tenuissima* Bänninger, 1925, the westernmost populations in the Swiss Alps. All species of the *cordicollis*-group, as well as *N. fontinalis rhaetica* K. & J. Daniel, 1890 show conspicuously low interspecific K2 distances (0.002-0.018; Table 7).

As already mentioned by the authors (Szallies & Huber, 2014) in their very recent description of *O. buemlisalpicola*, the included samples are clearly separate (K2 distances: intraspecific = 0.0-0.015; interspecific: 0.04-0.043; Table 8) and belong to the eastern distributed *Oreonebria bremii*, whereas the western ones belong to *O. bluemlisalpicola*.

CONCLUSIONS

Coleoptera comprise about 35% of the total endemic animal species listed in Switzerland and more than 45% of all the listed Swiss endemic arthropod species (Germann et *al.*, 2013). The present project focused above all on relict populations of rare Alpine endemic (or potentially endemic) beetle species belonging particularly to the families Curculionidae and Carabidae (respectively 85%and 10% of all the samples included here).

The relationships within the species-rich family Curculionidae and within its large genus *Otiorhynchus* were overall not strongly supported in our analyses based on a relatively short fragment of the COI gene. However, the COI gene portion used here as DNA barcode was very useful to detect and discriminate single nominal species. Moreover, some further essential considerations could be done, especially focusing at the relationships within the identified monophyletic groups (which generally correspond to the proposed subgenera or species complexes). In several cases, incertitude at the morphological level was mirrored in the results recorded at the molecular genetic level as well. Outstanding examples are

- i) the parthenogenetical *Otiorhynchus pupillatus* lineages with probably several independent immigrations,
- ii) the *Hypera nigrirostris*-species group with *H. nigrirostris, H. ononidis* and *H. melarhynchus* merged,
- iii) the Phyllobius pyri / vespertinus-species complex.

Also in several cases species could be delimited or preliminarily approved as i) the alpine *Anthonomus rubi*populations; *Otiorhynchus armatus / pesarinii*. Moreover, we found both, species with small morphological differences, associated with considerable genetic divergence (*Polydrusus paradoxus / chaerodrysius*), and morphologically accepted species (or subspecies) where only small differences were found in the investigated barcode sequences (*Nebria cordicollis* -group, *N. fontinalis*). In some cases, species considered as a single one, are in fact composed of two "cryptic species"

(Otiorhynchus civis / O. meridionalis, Dichotrachelus koziorowiczi, D. augusti).

We also provide support that in relatively immobile species and isolated populations definitely more diversity is detectable (Osellaeus bonvouloirii, Dichotrachelus spp.), an issue that should be addressed in future projects including further samples from restricted populations. Within several genera, where more species from partly different subgenera could be included (e.g. Otiorhynchus, Dichotrachelus, Hyperini), first preliminary insights of the systematics at genus/subgenus-level could be gained, together with insights on the phylogenetic value of certain morphological traits. In the traditional morphology, the presence or absence and the shape of teeth on femora in the genus Otiorhynchus is used as decisive character for discrimination of subgenera. Hence teeth (or no teeth) are used as traits providing a considerable phylogenetic signal. This is questionable after our results, and should be corroborated including nuclear markers and more key species from further subgenera.

Overall, thanks to this kind of DNA barcoding approach, it was definitely possible to reveal potential cryptic taxa and identify (genetically) isolated beetle populations. These results stimulate the re-thinking of relationships and enhance the formulation of new phylogenetic hypotheses, which should be corroborated, as usual, with morphological, ecological, and genetic data (with the promising inclusion of both mitochondrial and nuclear markers). For the near future, we plan to extend our data set with the addition of other key taxa, again with focus on the Alpine region.

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SUPPLEMENTARY DATA

Supp. 1. Neighbor-Joining tree (Kimura 2 parameter) based on COI sequences of 142 samples of Curculionoidea (Apionidae and Curculionidae) and Chrysomelidae obtained by using MEGA 6. Values (over 50%) of bootstrap support from 5 000 pseudo-replicates are depicted above nodes.

Figure available trough http://doi.org/10.5281/zenodo.153861

Supp. 2 Neighbor-Joining tree (Kimura 2 parameter) based on COI sequences for 20 samples of Carabidae and Staphylinidae obtained by using MEGA 6. Values (over 50%) of bootstrap

support from 10'000 pseudo-replicates are depicted above

nodes. Figure available trough http://doi.org/10.5281/zenodo.153861

x 1. The 178 beetle samples belonging to the families Curculionidae (152 samples), Carabidae (18), Apionidae (6), Chrysomelidae (study. Nr = NMLU-ENT000XXX. det. = determined by; Coordinates refer to the Swiss coordinates; leg. = collected by; Abbrev oph Germann; CH = Charles Huber; PS = Peter Sonderegger, AS = Alexander Szallies; MR = Miguel Richard; US = Ueli Schnep
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Nr.	Family/subfamily/species	det.		date		Locality	Coord	nates	leg.	BOLD-nrs
			q	ш	y		N	Ш		COI
	Apionidae, Apioninae									
141	Aizobius sedi	CG	10	5	2013	FR, Ardèche, Les Ollières-sur-Eyrieux			CG	KU982998
155	Aizobius sedi	CG	10	8	2010	SZ, Neuchâtel, Le Sordet	563286	206929	CG	KU982997
146	Hemitrichapion waltoni	CG	19	4	2011	FR, Vaucluse, Mt Ventoux, NW Sault			CG	KU983045
156	hemitrichapion waltoni	CG	б	7	2010	IT, Piemonte, V. Formazza, V. di Morasco, Riale			CG	KU983044
185	Mesotrichapion punctivostre	CG	8	7	2012	SZ, Valais, Termen, Fleschbode			CG	no data
147	¹ Osellaeus b. bonvouloirii	CG	14	9	2012	SZ, Isenthal, Brisen	677485	194549	CG	KU983075
153	N Osellaeus b. bonvouloirii	CG	1	4	2011	SZ, Plaffeien, Kaiseregg	590564	166604	CG	KU983076
159) Osellaeus b. bonvouloirii	CG	5	8	2009	SZ, Zermatt, Gornergrat	93655	626327	CG	no data
	Carabidae, Nebriinae									
112	. Nebria picea	\mathbf{AS}	11	7	2012	SZ, Gridone			CH	KU983074
117	1 Nebria cordicollis escheri	AS	8	8	2012	SZ, Alperschällilücke			\mathbf{AS}	KU983062
123	Nebria cordicollis tenuissima	CG	19	7	2012	SZ, Albristhorn			\mathbf{AS}	KU983063
121	Nebria fontinalis rhaetica	CG	14	8	2012	SZ, Rosenlaui-Gletscher			AS	KU983064
114	1 Nebria heeri	\mathbf{AS}	31	7	2012	SZ, Glärnisch			AS	KU983065
118	8 Oreonebria angustata	\mathbf{AS}	6	~	2012	SZ, Tambogletscher			\mathbf{AS}	KU983066
115	oreonebria angusticollis	\mathbf{AS}	18	7	2012	SZ, Cornettes de Bise			\mathbf{AS}	KU983067
72	Oreonebria bluemlisalpicola	CG	16	7	2013	SZ, Grindelwald, below Wildgärst	648704	171141	\mathbf{AS}	KU983069
83	N Oreonebria bluemlisalpicola	CG		7	2013	SZ, Grindelwald, Schrybershörnli			\mathbf{AS}	KU983068
71	Oreonebria bremii	CG	16	7	2013	SZ, Grindelwald, below Wildgärst	648704	171141	\mathbf{AS}	KU983070
82	Oreonebria bremii	CG		7	2013	SZ, Grindelwald, Schrybershörnli			\mathbf{AS}	KU983072
113	1 Oreonebria bremii	\mathbf{AS}	28	6	2006	SZ, Brienzer Rothorn			\mathbf{AS}	KU983071
120) Oreonebria castanea raetzeri	CG	5	7	2005	SZ, Jura, Les Verrières			\mathbf{AS}	KU983073
	Carabidae, Pterostichinae									
122	. Pterostichus morio peirolerii	CG	28	6	2006	SZ, Brienzer Rothorn			\mathbf{AS}	KU983152
116	herostichus panzeri	AS	8	٢	2012	SZ, Silberen			AS	KU983153
	Carabidae, Trechinae									
134	1 Trechus glacialis	AS	26	5	2011	SZ, Tierwies			\mathbf{AS}	no data
137	' Trechus laevipes	AS	23	10	2012	SZ, Baraghetto			AS	no data

Nr.	Family/subfamily/species	det.		date		Locality	Coordi	nates	leg.	BOLD-nrs
			p	ш	у		Z	Е		COI
133	Trechus pertyi	AS	14	8	2012	SZ, Rosenlaui-Gletscher			AS	KU983154
166	Trechus piazzolii	\mathbf{AS}	11	7	2012	SZ, done			AS	KU983155
135	Trechus schaumi	\mathbf{AS}	6	8	2012	SZ, Tambogletscher			AS	KU983156
138	Trechus schyberosiae	\mathbf{AS}	5	5	2011	SZ, Pilatus Oberhaupt			AS	no data
186	Trechus tenuilimbatus	\mathbf{AS}	7	8	2012	SZ, Graubünden, Avers, Piotgletscher			AS	KU983157
	Chrysomelidae, Chrysomelinae									
128	Chrysolina latecincta vallesiaca	CG	29	8	2013	SZ, Ulrichen, Nufenenpass, above Griessee, Mändeli	672090	146080	CG	KU983009
	Curculionidae, Ceutorhynchinae									
136	Ceutorhynchus hutchinsiae	CG	16	7	2013	SZ, Grindelwald, below Wildgärst	648704	171141	AS	KU983008
	Curculionidae, Cryptorhynchinae									
14	Echinodera samosa	CG	1	4	2010	GR, Samos Isl., Oros Ambelos, Lazarou			CG	KU983043
	Curculionidae, Curculioninae									
144	Anthonomus rubi	CG	10	7	2011	SZ, Amden, Mattstock	728655	225707	CG	KU983000
149	Anthonomus rubi	CG	25	9	2011	SZ, Kandersteg, Schwarenbach	615475	144084	CG	KU983003
150	Anthonomus rubi	CG	12	8	2011	SZ, Ftan, Piz Clünas	814100	188854	CG	KU983001
152	Anthonomus rubi	CG	С	6	2011	SZ, Erlenbach, Stockhorn			CG	KU982999
157	Anthonomus rubi	CG	ŝ	7	2010	IT, Piemonte, V. Formazza, V. di Morasco, Riale			CG	KU983002
181	Anthonomus rubi	CG	11	7	2009	SZ, BE, Stockhorn, Obere Walalp	606829	171711	CG	no data
151	Tychius tridentinus	CG	6	8	2011	SZ, Scuol, Foppas			CG	KU983158
	Curculionidae, Cyclominae									
62	Dichotrachelus augusti	CG	8	6	2011	SZ, Orsières, Val Ferret, Ferret	576207	83488	CG	KU983012
69	Dichotrachelus augusti	CG	17	7	2012	SZ, Trient, Col de Balme, below Les Grandes Otanes			CG	KU983010
89	Dichotrachelus augusti	CG	15	10	2011	SZ, Saastal, Saas Fee, Galu	638580	105655	CG	KU983011
38	Dichotrachelus imhoffi	CG	16	6	2010	SZ, Pso. del Bernina, Giuf, below Piz Campasc	799905	141715	CG	KU983013
81	Dichotrachelus koziorowiczi	CG	24	6	2011	FR, Corse, Zonza, Monte Calva			CG	KU983014
182	Dichotrachelus koziorowiczi	CG			2011	FR, Korsika, Col de Verghio			CG	KU983015
30	Dichotrachelus lepontinus	CG	4	7	2010	IT, V. Formazza, Vannino above Valdo, Cra della Dighetta	673154	137119	CG	KU983017
68	Dichotrachelus lepontinus	CG	14	6	2012	SZ, Berisal, S Bortelalp	650387	126518	CG	KU983016
36	Dichotrachelus maculosus	CG	1	4	2011	SZ, Plaffeien, Kaiseregg	590564	166604	CG	KU983023
59	Dichotrachelus maculosus	CG	25	9	2011	SZ, Kandersteg, Schwarenbach	615475	144084	CG	KU983020
70	Dichotrachelus maculosus	CG	16	7	2013	SZ, Grindelwald, below Wildgärst	648704	171141	AS	KU983018
73	Dichotrachelus maculosus	CG	16	7	2013	SZ, Grindelwald, Schrybershörnli			CG	KU983019
92	Dichotrachelus maculosus	CG	5	5	2013	FR, Drôme, Vercors, Col de la Bataille			CG	KU983022

Nr.	Family/subfamily/species	det.		date		Locality	Coordi	nates	leg.	BOLD-nrs
			p	m	y		Ν	Е		COI
93	Dichotrachelus maculosus	CG	5	5	2013	FR, Drôme, Vercors, Font d'Urle			CG	KU983021
119	Dichotrachelus maculosus	CG	19	٢	2012	SZ, Albristhorn			AS	KU983024
26	Dichotrachelus rudeni	CG	5	8	2009	SZ, Zermatt, Gornergrat	93655	626327	CG	KU983026
31	Dichotrachelus rudeni	CG	4	7	2010	IT, V. Formazza, Vannino above Valdo, Cra della Dighetta	673154	137119	CG	KU983027
33	Dichotrachelus rudeni	CG	4	7	2010	IT, V. Formazza, Vannino above Valdo, W Il Polmone	672940	136971	CG	KU983028
47	Dichotrachelus rudeni	CG	15	7	2011	SZ, Trient, Col de Balme	564299	97168	CG	KU983029
64	Dichotrachelus rudeni	CG	30	9	2012	SZ, Hérémence, Col des Roux	595754	102045	CG	KU983030
99	Dichotrachelus rudeni	CG	25	٢	2012	SZ, Furkapass, Furkastock, below	674959	158847	CG	KU983031
67	Dichotrachelus rudeni	CG	8	6	2011	SZ, Orsières, Val Ferret, Ferret	576207	83488	CG	KU983025
LL	Dichotrachelus rudeni	CG	15	10	2011	SZ, Saastal, Saas Fee, Galu	638580	105655	CG	KU983033
109	Dichotrachelus rudeni	CG	٢	7	2013	SZ, NW-Disentis, Val da Lag Serein	705310	175720	CG	KU983032
23	Dichotrachelus sondereggeri	CG	7	7	2009	SZ, Puschlav, ob. Cavaione, Corn dal Solcun	804489	126180	CG	KU983034
25	Dichotrachelus s.sulcipennis	CG	5	8	2009	SZ, Zermatt, Gornergrat	93655	626327	CG	no data
95	Dichotrachelus variegatus	CG		5	2013	IT, Lazio, Monti Lepini, Sempre-Visa			AS	KU983035
139	Dichotrachelus venturiensis	CG	18	4	2011	FR, Vaucluse, Mt. Ventoux, below peak, E-exp. slope			CG	KU983036
154	Dichotrachelus venturiensis	CG	18	4	2011	FR, Vaucluse, Mt. Ventoux, Chalet Reynard			CG	no data
	Curculionidae, Entiminae									
45	Barynotus obscurus	CG	23	7	2011	SZ, Brail, Prazet			CG	KU983004
132	Brachysomus samos	CG	1	4	2010	GR, Samos Isl., Oros Ambelos, Lazarou			CG	KU983007
21	Otiorhynchus alpicola	CG	4	9	2010	FR, Haut Jura, Gran Crêt	485675	126772	CG	no data
90	Otiorhynchus alpicola	CG	9	8	2011	SZ, Tarasp, Avrona			CG	KU983077
111	Otiorhynchus alpicola	CG	29	9	2013	SZ, Val Mora, below Piz dal Döss Radond	823323	162634	CG	KU983078
51	Otiorhynchus anthracinus	CG	23	7	2011	SZ, Brail, Prazet			CG	KU983079
40	Otiorhynchus armadillo	CG	9	8	2011	SZ, Tarasp, Avrona			CG	KU983082
100	Otiorhynchus armadillo	CG	б	8	2013	SZ, Rubigen			CG	KU983080
104	Otiorhynchus armadillo	CG	20	9	2009	SZ, Innertkirchen			CG	KU983081
110	Otiorhynchus armadillo	CG	28	9	2013	SZ, Valchava, Val Vau, W-Praveder	824192	162000	CG	KU983083
173	Otiorhynchus armatus	CG	25	6	2010	IT, Liguria, Savona, Finale Ligure, S. Bernardino			CG	KU983085
174	Otiorhynchus pesarinii	CG				IT, Isola Ischia			SU	KU983084
170	Otiorhynchus aurifer	CG	17	6	2011	FR, Corse, Corte, Val Restonica, Camping de Tuani			CG	KU983086
163	Otiorhynchus caudatus	CG				IT, Monteriggioni, Badesse			MR	KU983087
2	Otiorhynchus chrysocomus	CG	20	9	2009	SZ, Gadmen			CG	no data
145	Otiorhynchus corsicus	CG	18	6	2011	FR, Corse, Corte, Val Restonica, Lago Melu			CG	KU983088

Nr.	Family/subfamily/species	det.		date		Locality	Coordi	inates	leg.	BOLD-nrs
			q	m	у		Ν	Е		COI
171	Otiorhynchus crataegi	CG	20	9	2009	SZ, Innertkirchen			CG	KU983089
8	Otiorhynchus densatus	CG	5	8	2009	SZ, Zermatt, Gornergrat	93655	626327	CG	no data
35	Otiorhynchus densatus	CG	4	٢	2010	IT, V. Formazza, Vannino above Valdo, above II Polmone	672842	136328	CG	KU983090
53	Otiorhynchus desertus	CG	12	8	2011	SZ, Ftan, Piz Clünas	814100	188854	CG	KU983091
148	Otiorhynchus desertus	CG	9	8	2011	SZ, Tarasp, Avrona			CG	no data
125	Otiorhynchus dieckmanni	CG	З	6	2013	SZ, Bern, Naturhistorisches Museum, environments			CG	no data
187	Otiorhynchus difficilis	CG		٢	2011	SZ, Ticino, Lamone			CG	KU983092
130	Otiorhynchus ghilianii	CG	22	4	2011	FR, Var, NW Fayence, Bargème, Montagnes de Brouis			CG	KU983093
52	Otiorhynchus gredleri	CG	12	8	2011	SZ, Ftan, Piz Clünas	814100	188854	CG	KU983095
96	Otiorhynchus gredleri	CG	29	9	2013	SZ, Val Mora, below Piz dal Döss Radond	823323	162634	CG	KU983094
50	Otiorhynchus grischunensis	CG	10	8	2011	SZ, Ftan, Piz Clünas			CG	KU983096
86	Otiorhynchus intrusus	CG	19	6	2011	FR, Corse, Porto, E Calanche, les roches bleues			CG	KU983097
169	Otiorhynchus juvencus	CG	26	6	2011	FR, Corse, Ste. Lucie de P.V., Pinarellu			CG	no data
48	Otiorhynchus lepidopterus	CG	23	٢	2011	SZ, Brail, Prazet			CG	KU983098
160	Otiorhynchus ligustici	CG				SZ, Churwalden			RC	KU983099
161	Otiorhynchus lugens	CG				GR, Kerkyra			SU	KU983100
131	Otiorhynchus magnicollis	CG	1	4	2010	GR, Samos Isl., Oros Ambelos, Lazarou			CG	KU983101
126	Otiorhynchus meridionalis	CG	б	6	2013	SZ, Bern, Naturhistorisches Museum, environments			CG	KU983103
129	Otiorhynchus civis	CG	22	4	2011	FR, Var, NW Fayence, Bargème, Montagnes de Brouis			CG	KU983102
6	Otiorhynchus morio	CG	11	٢	2009	SZ, Stockhorn, Obere Walalp	606829	171711	CG	KU983104
105	Otiorhynchus muffi	CG	28	9	2013	SZ, Val Mora, Döss Radond	823289	161728	CG	KU983105
41	Otiorhynchus nododus	CG	9	×	2011	SZ, Tarasp, Avrona			CG	KU983106
107	Otiorhynchus nododus	CG	28	9	2013	SZ, Valchava, Val Vau, W-Praveder	824192	162000	CG	KU983108
108	Otiorhynchus nododus	CG	29	9	2013	SZ, Val Mora, below Piz dal Döss Radond	823323	162634	CG	KU983111
27	Otiorhynchus nodosus	CG	б	٢	2010	IT, Piemonte, V. Formazza, Lago di Morasco	673750	141390	CG	KU983109
34	Otiorhynchus nodosus	CG	4	٢	2010	IT, V. Formazza, Vannino above Valdo, above II Polmone	672963	136556	CG	KU983110
80	Otiorhynchus nodosus	CG			2011	SZ, Val Niemet, Ferrera			PS	KU983107
127	Otiorhynchus nubilus	CG	29	8	2013	SZ, Ulrichen, Nufenenpass, above Griessee, Mändeli	672090	146080	CG	KU983112
164	Otiorhynchus obesus	CG	9	4	2012	GR, Crete Island, Chania, Askifou-Plateau, Petres			CG	KU983113
172	Otiorhynchus ovalipennis	CG	31	3	2010	GR, Athen, Airport, environments			CG	KU983114
178	Otiorhynchus ovatus	CG	16	11	2012	SZ, Mörel, Salzgäb			CG	KU983115
177	Otiorhynchus pinastri	CG	26	9	2010	SZ, Thun, Aareufer			CG	no data
162	Otiorhynchus porcatus	CG				SZ, Churwalden			RC	no data

Nr.	Family/subfamily/species	det.		date		Locality	Coordi	inates	leg.	BOLD-nrs
			q	ш	у		Ν	Е		COI
1	Otiorhynchus pupillatus	CG	20	9	2009	SZ, Gadmen			CG	no data
9	Otiorhynchus pupillatus	CG	9	8	2009	SZ, Habkern, Grünenbergpass	631996	178264	CG	no data
7	Otiorhynchus pupillatus	CG	28	9	2009	SZ, Sigriswil, Sigriswilergrat, Alpiglen			CG	KU983130
11	Otiorhynchus pupillatus	CG	'	8	2009	SZ, Reutigen, Lengeberg	611546	171965	CG	no data
12	Otiorhynchus pupillatus	CG	23	8	2009	SZ, Grindelwald, Brandegg	643715	162095	CG	KU983129
28	Otiorhynchus pupillatus	CG	б	٢	2010	IT, Piemonte, V. Formazza, Lago di Morasco	673750	141390	CG	KU983127
42	Otiorhynchus pupillatus	CG	9	8	2011	SZ, Tarasp, Avrona			CG	KU983124
54	Otiorhynchus pupillatus	CG	12	8	2011	SZ, Ftan, Piz Clünas	814100	188854	CG	KU983120
55	Otiorhynchus pupillatus	CG	23	٢	2011	SZ, Brail, Prazet			CG	KU983119
65	Otiorhynchus pupillatus	CG	25	٢	2012	SZ, Furkapass, Furkastock, below	674959	158847	CG	KU983116
74	Otiorhynchus pupillatus	CG	16	٢	2013	SZ, Grindelwald, Schrybershörnli			CG	KU983117
75	Otiorhynchus pupillatus	CG	12	٢	2012	SZ, Kandersteg, Schwarenbach			CG	KU983118
84	Otiorhynchus pupillatus	CG	17	٢	2012	SZ, Trient, Col de Balme, towards Tête de Balme			CG	KU983121
85	Otiorhynchus pupillatus	CG	15	8	2012	SZ, Riom, in garden			RC	KU983122
88	Otiorhynchus pupillatus	CG	19	9	2011	SZ, Kandersteg, Gasterental			CG	KU983123
76	Otiorhynchus pupillatus	CG	٢	٢	2013	SZ, NW-Disentis, Val da Lag Serein			CG	KU983126
106	Otiorhynchus pupillatus	CG	28	9	2013	SZ, Valchava, Val Vau, W-Praveder	824192	162000	CG	KU983128
124	Otiorhynchus pupillatus	CG	29	8	2013	SZ, Ulrichen, Zum Loch			CG	KU983125
168	Otiorhynchus pupillatus	CG		6	2013	SZ, Noiraigue vers Creux du Van			CG	no data
176	Otiorhynchus salicicola	CG				SZ, Churwalden			RC	KU983131
175	Otiorhynchus scaber	CG	9	8	2009	SZ, Habkern, Grünenbergpass			CG	no data
102	Otiorhynchus singularis	CG	1	8	2013	SZ, Gstaad, Hintereggli, Mühlesteini			CG	KU983132
4	Otiorhynchus subcostatus	CG	7	٢	2009	SZ, Puschlav, above Cavaione, Corn dal Solcun	804489	126180	CG	no data
18	Otiorhynchus subcostatus	CG	4	9	2010	FR, Haut Jura, Montoisey	486364	127500	CG	no data
Э	Otiorhynchus tenebricosus	CG	20	9	2009	SZ, Gadmen			CG	no data
10	Otiorhynchus tenebricosus	CG	11	٢	2009	SZ, Stockhorn, Obere Walalp	606829	171711	CG	KU983135
58	Otiorhynchus tenebricosus	CG	19	9	2011	SZ, Kandersteg, Gasterental			CG	KU983133
76	Otiorhynchus tenebricosus	CG	12	٢	2012	SZ, Kandersteg, Schwarenbach			CG	KU983134
78	Otiorhynchus tenebricosus	CG	14	9	2012	SZ, Isenthal, Brisen	677485	194549	CG	KU983136
165	Otiorhynchus thaliarchus	CG	9	4	2012	GR, Crete Island, Chania, Askifou-Plateau, Petres			CG	KU983137
60	Otiorhynchus tirolensis	CG	9	8	2011	SZ, Tarasp, Avrona			CG	KU983138
167	Otiorhynchus turca	CG				BG, Sofia Plain, City area, Knyazhevo			SU	KU983139
158	Otiorhynchus uncinatus	CG	4	9	2010	FR, Haut Jura, Gran Crêt	485675	126772	CG	no data

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Nr.	Family/subfamily/species	det.		date		Locality	Coordi	nates	leg.	BOLD-nrs
			q	m	у		Ν	Е		COI
5	Otiorhynchus varius	CG	7	7	2009	SZ, Puschlav, above Cavaione, Corn dal Solcun	804489	126180	CG	KU983140
15	Phyllobius pyri	CG	12	5	2010	SZ, Satigny, Montfleury	493701	119317	CG	KU983141
179	Phyllobius pyri	CG				SZ, Churwalden			SU	KU983142
180	Phyllobius pyri	CG				SZ, Chur, Kalkofen			SU	KU983143
13	Phyllobius vespertinus	CG	22	5	2010	SZ, Val Blenio, Dongio	716000	144000	CG	KU983145
39	Phyllobius vespertinus	CG	24	4	2011	IT, Valle d'Aosta, ob. Aosta			CG	KU983146
61	Phyllobius vespertinus	CG	21	4	2011	FR, Var, E Le Muy, Bois de Malvoisin, Gorges du Blavet			CG	KU983144
4	Polydrusus amoenus	CG	23	7	2011	SZ, Prazet			CG	KU983147
63	Polydrusus chaerodrysius	CG	12	7	2012	SZ, Kandersteg, Schwarenbach			CG	KU983149
103	Polydrusus chaerodrysius	CG	28	9	2013	SZ, Valchava, Val Vau, W-Praveder	824192	162000	CG	KU983148
143	Polydrusus chaerodrysius	CG	25	9	2011	SZ, Kandersteg, Schwarenbach	615475	144084	CG	KU983150
32	Polydrusus paradoxus	CG	Э	7	2010	IT, V. Formazza, V. di Morasco, Riale	674420	142160	CG	KU983151
	Curculionidae, Hyperinae									
22	Brachypera vidua	CG	24	4	2010	SZ, Biel, Pavillon	584070	220480	CG	KU983005
56	Brachypera vidua	CG	21	4	2011	FR, Var, E Le Muy, Bois de Malvoisin, Gorges du Blavet			CG	KU983006
183	Donu cyrtus	CG	5	4	2010	GR, Samos Isl., Oros Kerkis, E-Vigla			CG	KU983037
37	Donus globosus	CG	26	6	2010	IT, Liguria, Savona, Finale Ligure, C. di Melogno, Bricco della Guardia			CG	KU983039
46	Donus globosus	CG	16	4	2011	FR, Vaucluse, Bedoin, Crillon-le-Brave			CG	KU983038
49	Donus globosus	CG	17	4	2011	FR, Vaucluse, Gorges de la Nesque, Monieux			CG	KU983040
20	Donus ovalis	CG	4	9	2010	FR, Haut Jura, Gran Crêt	485675	126772	CG	KU983041
66	Donus segnis	CG	28	9	2013	SZ, Valchava, Val Vau, Spi da Vau	826000	164000	CG	KU983042
29	Hypera arator	CG	27	9	2010	SZ, Neuchâtel, L'Ermitage	561800	205700	CG	KU983046
140	Hypera diversipunctata	CG	5	5	2013	FR, Drôme, Vercors, Col de la Bataille			CG	KU983047
142	Hypera gracilenta	CG	8	4	2013	PT, Loulé, Pena, Rocha da Pena			CG	KU983048
6L	Hypera melancholica	CG	21	4	2011	FR, Var, E Le Muy, Bois de Malvoisin, Gorges du Blavet			CG	KU983049
91	Hypera melarynchus	CG	12	4	2013	PT, W Sagres, Cabo S. Vicente, resthouse			CG	KU983050
43	Hypera miles	CG	23	7	2011	SZ, Brail, Prazet			CG	KU983051
16	Hypera nigrirostris	CG	12	5	2010	SZ, Satigny, Montfleury	493701	119317	CG	KU983054
19	Hypera nigrirostris	CG	4	9	2010	FR, Haut Jura, Gran Crêt	485675	126772	CG	KU983052
94	Hypera nigrirostris	CG	13	4	2013	PT, W Lagos, Budens Umgb			CG	KU983053
98	Hypera ononidis	CG	1	8	2013	SZ, Gstaad, Hintereggli, Mühlesteini			CG	KU983055
57	Hypera plantaginis	CG	10	7	2011	SZ, Amden, Mattstock	728655	225707	CG	KU983056

Nr.	Family/subfamily/species	det.		late		Locality	Coordin	nates	leg.	BOLD-nrs
			q	ш	у		Z	Е		COI
87	Hypera plantaginis	CG	16	11	2012	3Z, Mörel, Salzgäb			CG	KU983057
17	Hypera striata	CG	12	5	2010	3Z, Vallon de l'Allondon, Malval, Les Granges	488856	119489	CG	KU983058
24	Hypera venusta	CG	4	9	2010	⁻ R, Haut Jura, Montoisey	486364	127500	CG	KU983059
101	Hypera venusta	CG	29	9	2013	5Z, Val Mora, below Piz dal Döss Radond	823323	162634	CG	KU983060
	Staphylinidae, Aleocharinae									
184	Leptusa pilatensis	AS	3	5	2006	3Z, Neuchâtel, Villiers			AS	KU983061