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

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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 oxidase 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 (Avice, 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

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.

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

(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 62 000 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 *Osellaus*), 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 obtained 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 &** ***Osellaus* 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 *Osellaus*) found strong support in both the ML and NJ tree.

The genus *Osellaus* 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

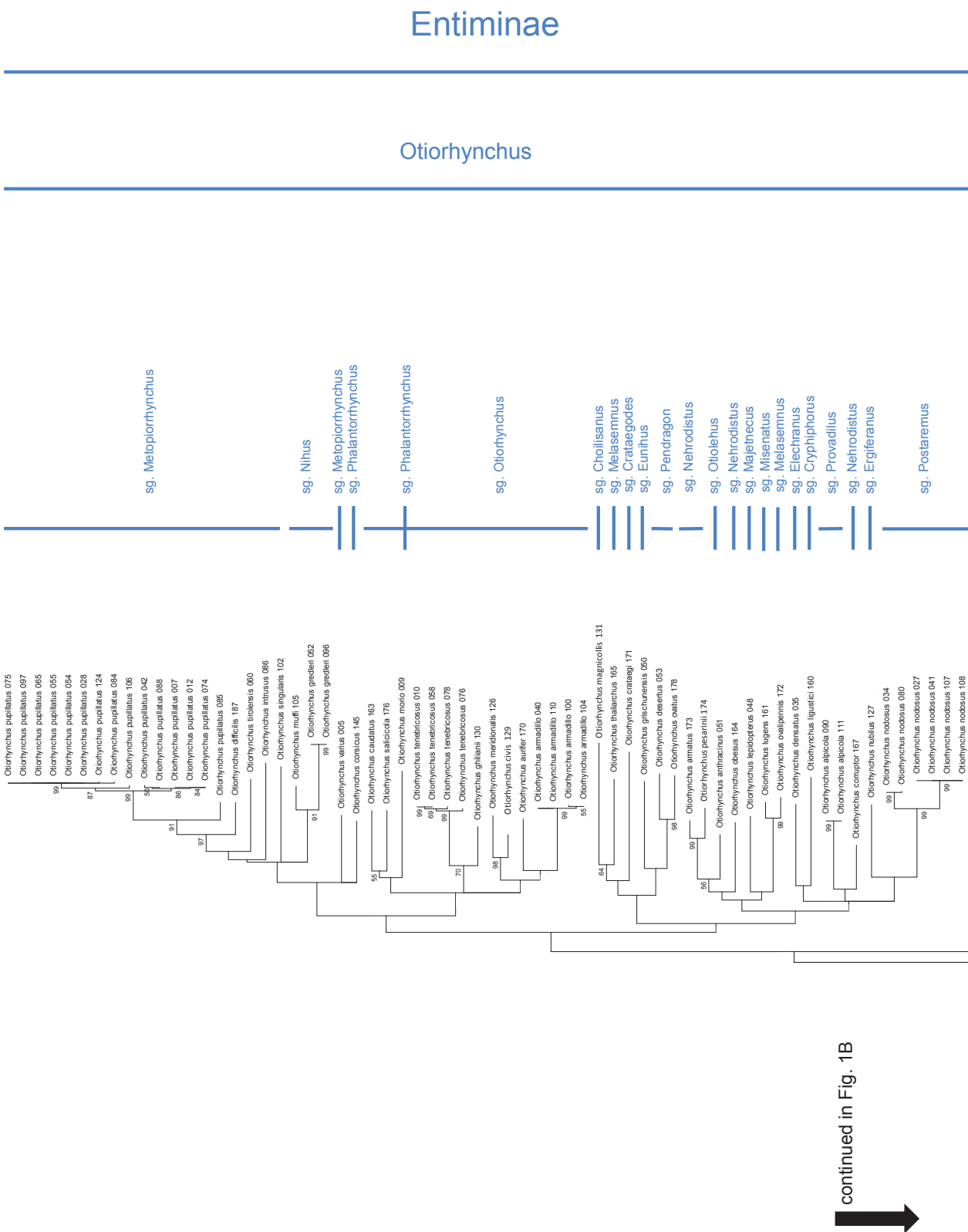
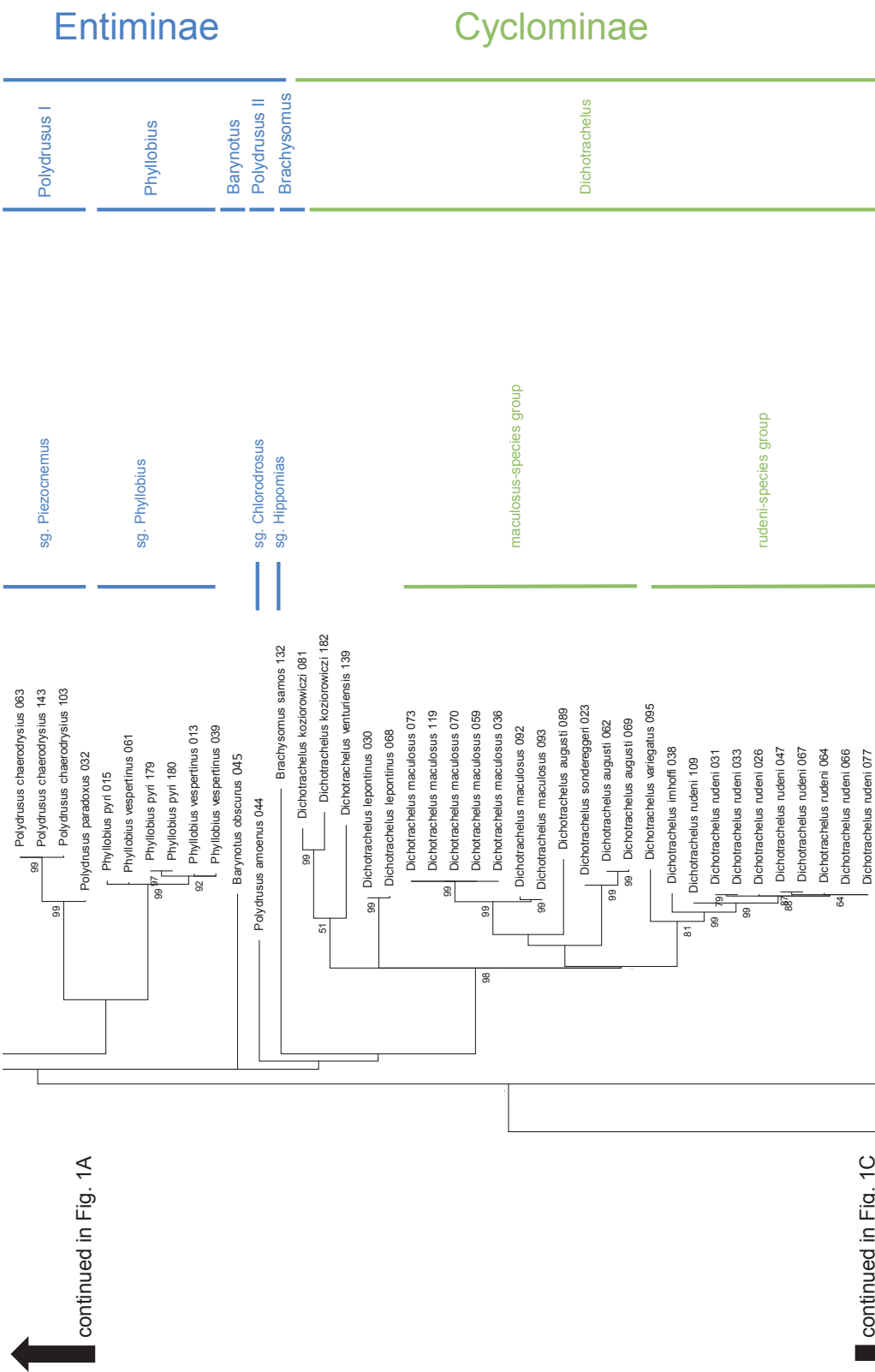
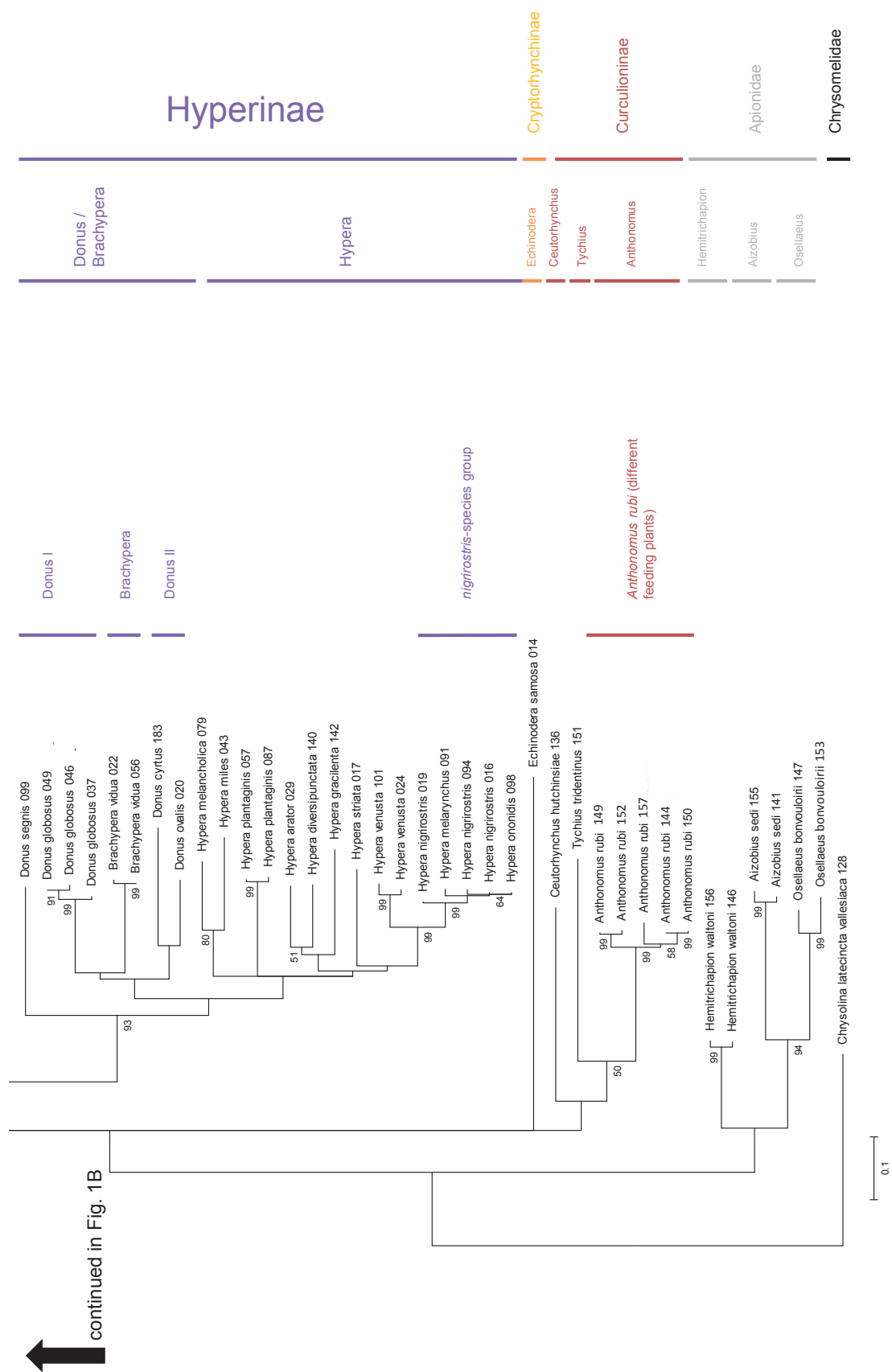


Fig. 1. Best Maximum Likelihood tree (-ln=13697.7060; GTR+I+G model as selected by MEGA) based on COI sequences of 142 samples of Curculionoidea (Apionidae and Curculionidae) and Chrysomelidae obtained by using MEGA 6. Values (over 50%) of bootstrap support from 100 pseudo-replicates are depicted above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





(Fig. 1, Supp. 1). The Swiss populations of the species-pair *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. augusti*-species 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%),

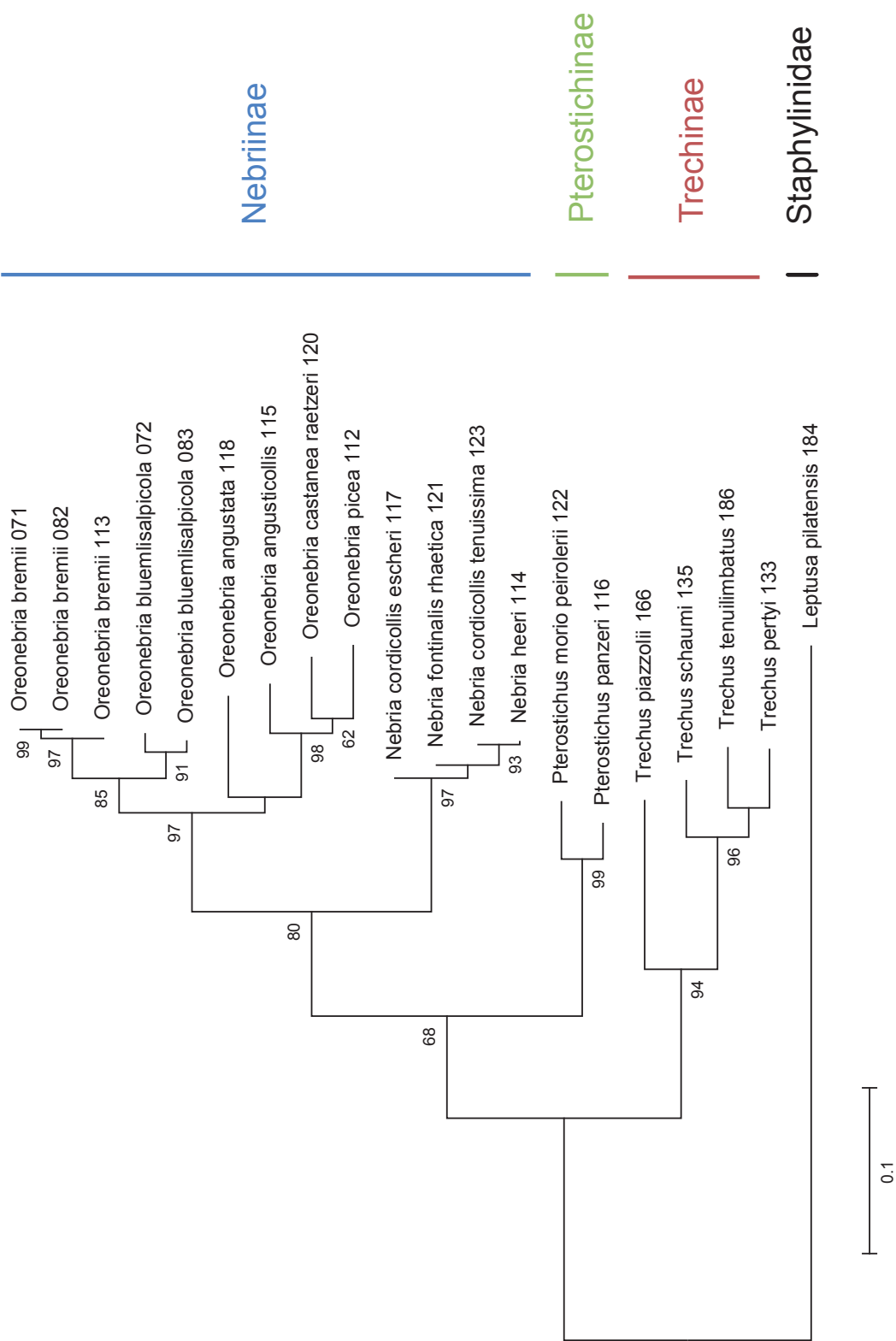


Fig. 2. Best Maximum Likelihood tree ($-ln = 3045.2577$; Tamura-Nei+G model as selected by MEGA) based on COI sequences of 20 samples of Carabidae and Staphylinidae obtained by using MEGA 6. Values (over 50%) of bootstrap support from 500 pseudo replicates are depicted above nodes.

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. atlasticus* 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 morphologically 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 entomine 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

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

Table 2: selected *Dichotrachelus* samples

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

Table 3: *Hypera nigrirostris* species group

	<i>H. n.</i> 016	<i>H. o.</i> 098	<i>H. n.</i> 094	<i>H. m.</i> 091
<i>Hypera nigrirostris</i> 016				
<i>Hypera ononidis</i> 098	0.003			
<i>Hypera nigrirostris</i> 094	0.007	0.007		
<i>Hypera melarynchus</i> 091	0.010	0.010	0.012	
<i>Hypera nigrirostris</i> 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
<i>Otiorhynchus nodosus</i> 027					
<i>Otiorhynchus nodosus</i> 034	0.068				
<i>Otiorhynchus nodosus</i> 041	0.000	0.068			
<i>Otiorhynchus nodosus</i> 080	0.070	0.001	0.070		
<i>Otiorhynchus nodosus</i> 107	0.000	0.068	0.000	0.070	
<i>Otiorhynchus nodosus</i> 108	0.000	0.068	0.000	0.070	0.000

Table 5: *Otiorhynchus meridionalis* species group

	<i>O. aurifer</i> 170	<i>O. meridionalis</i> 126
<i>Otiorhynchus aurifer</i> 170		
<i>Otiorhynchus meridionalis</i> 126	0.142	
<i>Otiorhynchus civis</i> 129	0.132	0.093

Table 6: Sibling alpine species *Polydrusus paradoxus*/*Polydrusus chaerodrysius*

	<i>P. chaerodrysius</i> 143	<i>P. chaerodrysius</i> 103
<i>Polydrusus chaerodrysius</i> 143		
<i>Polydrusus chaerodrysius</i> 103	0.002	
<i>Polydrusus paradoxus</i> 032	0.048	0.048

Table 7: selected *Nebria* samples

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

Table 8: *Oreonebria bremii* vs *Oreonebria bluementalis*

	<i>O. bluementalis</i> 083	<i>O. bluementalis</i> 072	<i>O. bremii</i> 082
<i>Oreonebria bluementalis</i> 083			
<i>Oreonebria bluementalis</i> 072	0.015		
<i>Oreonebria bremii</i> 082	0.040	0.043	
<i>Oreonebria bremii</i> 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 toothed 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 (*Cholisanus* 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 in-between. 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. bluemelispicola*, 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 breinii*, whereas the western ones belong to *O. bluemelispicola*.

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 through <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 through <http://doi.org/10.5281/zenodo.153861>

Annex 1. The 178 beetle samples belonging to the families Curculionidae (152 samples), Carabidae (18), Apionidae (6), Chrysomelidae (1), and Staphylinidae (1) sequenced in our study. Nr = NMLU-ENT000XXX. det. = determined by; Coordinates refer to the Swiss coordinates; leg. = collected by; Abbreviations: RC = Regula Cornu; CG = Christoph Germann; CH = Charles Huber; PS = Peter Sonderegger, AS = Alexander Szallies; MR = Miguel Richard; US = Ueli Schneppat. States are shortened following Löbl & Smetana (2013).

Nr.	Family/subfamily/species	det.	date			Locality		Coordinates		leg.	BOLD-nrs
			d	m	y			N	E		COI
Apionidae, Apioninae											
141	<i>Aizobius sedi</i>	CG	10	5	2013	FR, Ardèche, Les Ollières-sur-Eyrieux				CG	KU982998
155	<i>Aizobius sedi</i>	CG	10	8	2010	SZ, Neuchâtel, Le Sordet		563286	206929	CG	KU982997
146	<i>Hemitrichapion waltoni</i>	CG	19	4	2011	FR, Vaucluse, Mt. Ventoux, NW Sault				CG	KU983045
156	<i>Hemitrichapion waltoni</i>	CG	3	7	2010	IT, Piemonte, V. Formazza, V. di Morasco, Riale				CG	KU983044
185	<i>Mesotrichapion punctirostre</i>	CG	8	7	2012	SZ, Valais, Termen, Fleischbode				CG	no data
147	<i>Osellaeus b. bonvouloirii</i>	CG	14	6	2012	SZ, Isenthal, Brisen		677485	194549	CG	KU983075
153	<i>Osellaeus b. bonvouloirii</i>	CG	1	4	2011	SZ, Plaffeien, Kaiseregg		590564	166604	CG	KU983076
159	<i>Osellaeus b. bonvouloirii</i>	CG	5	8	2009	SZ, Zermatt, Gornegrat		93655	626327	CG	no data
Carabidae, Nebrinae											
112	<i>Nebria picea</i>	AS	11	7	2012	SZ, Gridone				CH	KU983074
117	<i>Nebria cordicollis escheri</i>	AS	8	8	2012	SZ, Alperschälliücke				AS	KU983062
123	<i>Nebria cordicollis tenuissima</i>	CG	19	7	2012	SZ, Albristhorn				AS	KU983063
121	<i>Nebria fontinalis rhaetica</i>	CG	14	8	2012	SZ, Rosenlauri-Gletscher				AS	KU983064
114	<i>Nebria heeri</i>	AS	31	7	2012	SZ, Glärmisch				AS	KU983065
118	<i>Oreonebria angustata</i>	AS	9	8	2012	SZ, Tambogletscher				AS	KU983066
115	<i>Oreonebria angusticollis</i>	AS	18	7	2012	SZ, Cornettes de Bise				AS	KU983067
72	<i>Oreonebria bluenlisalpicola</i>	CG	16	7	2013	SZ, Grindelwald, below Wildgärst		648704	171141	AS	KU983069
83	<i>Oreonebria bluenlisalpicola</i>	CG		7	2013	SZ, Grindelwald, Schrybershörnli				AS	KU983068
71	<i>Oreonebria breinii</i>	CG	16	7	2013	SZ, Grindelwald, below Wildgärst		648704	171141	AS	KU983070
82	<i>Oreonebria breinii</i>	CG		7	2013	SZ, Grindelwald, Schrybershörnli				AS	KU983072
113	<i>Oreonebria breinii</i>	AS	28	9	2006	SZ, Brienzer Rothorn				AS	KU983071
120	<i>Oreonebria castanea raetzeri</i>	CG	5	7	2005	SZ, Jura, Les Verrières				AS	KU983073
Carabidae, Pterostichinae											
122	<i>Pterostichus morio peirolerii</i>	CG	28	9	2006	SZ, Brienzer Rothorn				AS	KU983152
116	<i>Pterostichus panzeri</i>	AS	8	7	2012	SZ, Silberen				AS	KU983153
Carabidae, Trechinae											
134	<i>Trechus glacialis</i>	AS	26	5	2011	SZ, Tierwies				AS	no data
137	<i>Trechus laevipes</i>	AS	23	10	2012	SZ, Baraghetto				AS	no data

Nr.	Family/subfamily/species	det.	date			Locality	Coordinates			leg.	BOLD-nrs
			d	m	y		N	E			COI
133	<i>Trechus pertyi</i>	AS	14	8	2012	SZ, Rosenlaui-Gletscher			AS		KU983154
166	<i>Trechus piazzolii</i>	AS	11	7	2012	SZ, done			AS		KU983155
135	<i>Trechus schauinslandi</i>	AS	9	8	2012	SZ, Tambogletscher			AS		KU983156
138	<i>Trechus schyberosiae</i>	AS	5	5	2011	SZ, Pilatus Oberhaupt			AS		no data
186	<i>Trechus tenuilimbatus</i>	AS	7	8	2012	SZ, Graubünden, Avers, Piotgletscher			AS		KU983157
Chrysomelidae, Chrysomelinae											
128	<i>Chrysolina lateincta vallesiaca</i>	CG	29	8	2013	SZ, Ulrichen, Nufenenpass, above Griessee, Mändeli	672090	146080	CG		KU983009
Curculionidae, Ceutorhynchinae											
136	<i>Ceutorhynchus hutchinsiae</i>	CG	16	7	2013	SZ, Grindelwald, below Wildgärst	648704	171141	AS		KU983008
Curculionidae, Cryptorhynchinae											
14	<i>Echinodera samosa</i>	CG	1	4	2010	GR, Samos Isl., Oros Ambelos, Lazarou			CG		KU983043
Curculionidae, Curculioninae											
144	<i>Anthonomus rubi</i>	CG	10	7	2011	SZ, Amden, Mattstock	728655	225707	CG		KU983000
149	<i>Anthonomus rubi</i>	CG	25	6	2011	SZ, Kandersteg, Schwarenbach	615475	144084	CG		KU983003
150	<i>Anthonomus rubi</i>	CG	12	8	2011	SZ, Ftan, Piz Clüinas	814100	188854	CG		KU983001
152	<i>Anthonomus rubi</i>	CG	3	9	2011	SZ, Erlenbach, Stockhorn			CG		KU982999
157	<i>Anthonomus rubi</i>	CG	3	7	2010	IT, Piemonte, V. Formazza, V. di Morasco, Riale			CG		KU983002
181	<i>Anthonomus rubi</i>	CG	11	7	2009	SZ, BE, Stockhorn, Obere Walalp	606829	171711	CG		no data
151	<i>Tychius tridentinus</i>	CG	9	8	2011	SZ, Scuol, Foppas			CG		KU983158
Curculionidae, Cyclominae											
62	<i>Dichotrachelus angusti</i>	CG	8	9	2011	SZ, Orsières, Val Ferret, Ferret	576207	83488	CG		KU983012
69	<i>Dichotrachelus angusti</i>	CG	17	7	2012	SZ, Trient, Col de Balme, below Les Grandes Olanes			CG		KU983010
89	<i>Dichotrachelus angusti</i>	CG	15	10	2011	SZ, Saastal, Saas Fee, Galu	638580	105655	CG		KU983011
38	<i>Dichotrachelus imhoffi</i>	CG	16	9	2010	SZ, Pso. del Bernina, Giuf, below Piz Campasc	799905	141715	CG		KU983013
81	<i>Dichotrachelus koziorowiczi</i>	CG	24	9	2011	FR, Corse, Zonza, Monte Calva			CG		KU983014
182	<i>Dichotrachelus koziorowiczi</i>	CG			2011	FR, Korsika, Col de Verghio			CG		KU983015
30	<i>Dichotrachelus lepointinus</i>	CG	4	7	2010	IT, V. Formazza, Vannino above Valdo, Cra della Dighetta	673154	137119	CG		KU983017
68	<i>Dichotrachelus lepointinus</i>	CG	14	9	2012	SZ, Berisal, S Bortelalp	650387	126518	CG		KU983016
36	<i>Dichotrachelus maculosus</i>	CG	1	4	2011	SZ, Plaffeien, Kaiseregg	590564	166604	CG		KU983023
59	<i>Dichotrachelus maculosus</i>	CG	25	6	2011	SZ, Kandersteg, Schwarenbach	615475	144084	CG		KU983020
70	<i>Dichotrachelus maculosus</i>	CG	16	7	2013	SZ, Grindelwald, below Wildgärst	648704	171141	AS		KU983018
73	<i>Dichotrachelus maculosus</i>	CG	16	7	2013	SZ, Grindelwald, Schrybershornli			CG		KU983019
92	<i>Dichotrachelus maculosus</i>	CG	5	5	2013	FR, Drôme, Vercors, Col de la Bataille			CG		KU983022

Nr.	Family/subfamily/species	det.	date			Locality	Coordinates			leg.	BOLD-nrs
			d	m	y		N	E			COI
93	<i>Dichotrachelus maculosus</i>	CG	5	5	2013	FR, Drôme, Vercors, Font d'Urle			CG		KU983021
119	<i>Dichotrachelus maculosus</i>	CG	19	7	2012	SZ, Albristhorn			AS		KU983024
26	<i>Dichotrachelus rudeni</i>	CG	5	8	2009	SZ, Zermatt, Gornegrat	93655	626327	CG		KU983026
31	<i>Dichotrachelus rudeni</i>	CG	4	7	2010	IT, V. Formazza, Vannino above Valdo, Cra della Dighetta	673154	137119	CG		KU983027
33	<i>Dichotrachelus rudeni</i>	CG	4	7	2010	IT, V. Formazza, Vannino above Valdo, W Il Polmone	672940	136971	CG		KU983028
47	<i>Dichotrachelus rudeni</i>	CG	15	7	2011	SZ, Trient, Col de Balme	564299	97168	CG		KU983029
64	<i>Dichotrachelus rudeni</i>	CG	30	6	2012	SZ, Hérémence, Col des Roux	595754	102045	CG		KU983030
66	<i>Dichotrachelus rudeni</i>	CG	25	7	2012	SZ, Furkapass, Furkastock, below	674959	158847	CG		KU983031
67	<i>Dichotrachelus rudeni</i>	CG	8	9	2011	SZ, Orsières, Val Ferret, Ferret	576207	83488	CG		KU983025
77	<i>Dichotrachelus rudeni</i>	CG	15	10	2011	SZ, Saastal, Saas Fee, Galu	638580	105655	CG		KU983033
109	<i>Dichotrachelus rudeni</i>	CG	7	7	2013	SZ, NW-Disentis, Val da Lag Serein	705310	175720	CG		KU983032
23	<i>Dichotrachelus sondereggeri</i>	CG	2	7	2009	SZ, Puschlav, ob. Cavaione, Corn dal Solcun	804489	126180	CG		KU983034
25	<i>Dichotrachelus s.sulcipennis</i>	CG	5	8	2009	SZ, Zermatt, Gornegrat	93655	626327	CG		no data
95	<i>Dichotrachelus variegatus</i>	CG		5	2013	IT, Lazio, Monti Lepini, Sempre-Visa			AS		KU983035
139	<i>Dichotrachelus venturiensis</i>	CG	18	4	2011	FR, Vauluse, Mt. Ventoux, below peak, E-exp. slope			CG		KU983036
154	<i>Dichotrachelus venturiensis</i>	CG	18	4	2011	FR, Vauluse, Mt. Ventoux, Chalet Reynard			CG		no data
Curculionidae, Entiminae											
45	<i>Barynotus obscurus</i>	CG	23	7	2011	SZ, Brail, Prazet			CG		KU983004
132	<i>Brachysomus samos</i>	CG	1	4	2010	GR, Samos Isl., Oros Ambelos, Lazarou			CG		KU983007
21	<i>Otiorynchus alpicola</i>	CG	4	6	2010	FR, Haut Jura, Gran Crêt	485675	126772	CG		no data
90	<i>Otiorynchus alpicola</i>	CG	6	8	2011	SZ, Tarasp, Avrona			CG		KU983077
111	<i>Otiorynchus alpicola</i>	CG	29	6	2013	SZ, Val Mora, below Piz dal Döss Radond	823323	162634	CG		KU983078
51	<i>Otiorynchus anthracinus</i>	CG	23	7	2011	SZ, Brail, Prazet			CG		KU983079
40	<i>Otiorynchus armadillo</i>	CG	6	8	2011	SZ, Tarasp, Avrona			CG		KU983082
100	<i>Otiorynchus armadillo</i>	CG	3	8	2013	SZ, Rubigen			CG		KU983080
104	<i>Otiorynchus armadillo</i>	CG	20	6	2009	SZ, Innertkirchen			CG		KU983081
110	<i>Otiorynchus armadillo</i>	CG	28	6	2013	SZ, Valchava, Val Vau, W-Praveder	824192	162000	CG		KU983083
173	<i>Otiorynchus armatus</i>	CG	25	9	2010	IT, Liguria, Savona, Finale Ligure, S. Bernardino			CG		KU983085
174	<i>Otiorynchus pesarinii</i>	CG				IT, Isola Ischia			US		KU983084
170	<i>Otiorynchus aurifer</i>	CG	17	9	2011	FR, Corse, Corte, Val Restonica, Camping de Tuani			CG		KU983086
163	<i>Otiorynchus caudatus</i>	CG				IT, Monteriggioni, Badesse			MR		KU983087
2	<i>Otiorynchus chrysocomus</i>	CG	20	6	2009	SZ, Gadmen			CG		no data
145	<i>Otiorynchus corsicus</i>	CG	18	9	2011	FR, Corse, Corte, Val Restonica, Lago Melu			CG		KU983088

Nr.	Family/subfamily/species	det.	date			Locality	Coordinates			leg.	BOLD-nrs
			d	m	y		N	E			COI
171	<i>Otiorhynchus crataegi</i>	CG	20	6	2009	SZ, Innetkirchen				CG	KU983089
8	<i>Otiorhynchus densatus</i>	CG	5	8	2009	SZ, Zermatt, Gornergrat	93655	626327		CG	no data
35	<i>Otiorhynchus densatus</i>	CG	4	7	2010	IT, V. Formazza, Vannino above Valdo, above Il Polmone	672842	136328		CG	KU983090
53	<i>Otiorhynchus desertus</i>	CG	12	8	2011	SZ, Ftan, Piz Clünas	814100	188854		CG	KU983091
148	<i>Otiorhynchus desertus</i>	CG	6	8	2011	SZ, Tarasp, Avrona				CG	no data
125	<i>Otiorhynchus dieckmanni</i>	CG	3	9	2013	SZ, Bern, Naturhistorisches Museum, environments				CG	no data
187	<i>Otiorhynchus difficilis</i>	CG		7	2011	SZ, Ticino, Lamone				CG	KU983092
130	<i>Otiorhynchus ghilianii</i>	CG	22	4	2011	FR, Var, NW Fayence, Bargème, Montagnes de Brouis				CG	KU983093
52	<i>Otiorhynchus gredleri</i>	CG	12	8	2011	SZ, Ftan, Piz Clünas	814100	188854		CG	KU983095
96	<i>Otiorhynchus gredleri</i>	CG	29	6	2013	SZ, Val Mora, below Piz dal Döss Radond	823323	162634		CG	KU983094
50	<i>Otiorhynchus grischunensis</i>	CG	10	8	2011	SZ, Ftan, Piz Clünas				CG	KU983096
86	<i>Otiorhynchus intrusus</i>	CG	19	9	2011	FR, Corse, Porto, E Calanche, les roches bleues				CG	KU983097
169	<i>Otiorhynchus juvenus</i>	CG	26	9	2011	FR, Corse, Ste. Lucie de P.V., Pinarellu				CG	no data
48	<i>Otiorhynchus lepidopterus</i>	CG	23	7	2011	SZ, Brail, Prazet				CG	KU983098
160	<i>Otiorhynchus ligustici</i>	CG				SZ, Churwalden				RC	KU983099
161	<i>Otiorhynchus lugens</i>	CG				GR, Kerkira				US	KU983100
131	<i>Otiorhynchus magnicollis</i>	CG	1	4	2010	GR, Samos Isl., Oros Ambelos, Lazarou				CG	KU983101
126	<i>Otiorhynchus meridionalis</i>	CG	3	9	2013	SZ, Bern, Naturhistorisches Museum, environments				CG	KU983103
129	<i>Otiorhynchus civis</i>	CG	22	4	2011	FR, Var, NW Fayence, Bargème, Montagnes de Brouis				CG	KU983102
9	<i>Otiorhynchus morio</i>	CG	11	7	2009	SZ, Stockhorn, Obere Walalp	606829	171711		CG	KU983104
105	<i>Otiorhynchus mufi</i>	CG	28	6	2013	SZ, Val Mora, Döss Radond	823289	161728		CG	KU983105
41	<i>Otiorhynchus nododus</i>	CG	6	8	2011	SZ, Tarasp, Avrona				CG	KU983106
107	<i>Otiorhynchus nododus</i>	CG	28	6	2013	SZ, Valchava, Val Vau, W-Praveder	824192	162000		CG	KU983108
108	<i>Otiorhynchus nododus</i>	CG	29	6	2013	SZ, Val Mora, below Piz dal Döss Radond	823323	162634		CG	KU983111
27	<i>Otiorhynchus nododus</i>	CG	3	7	2010	IT, Piemonte, V. Formazza, Lago di Morasco	673750	141390		CG	KU983109
34	<i>Otiorhynchus nododus</i>	CG	4	7	2010	IT, V. Formazza, Vannino above Valdo, above Il Polmone	672963	136556		CG	KU983110
80	<i>Otiorhynchus nododus</i>	CG			2011	SZ, Val Niemet, Ferrara				PS	KU983107
127	<i>Otiorhynchus nubilus</i>	CG	29	8	2013	SZ, Ulrichen, Nufenenpass, above Griessee, Mändeli	672090	146080		CG	KU983112
164	<i>Otiorhynchus obesus</i>	CG	6	4	2012	GR, Crete Island, Chania, Askifou-Plateau, Petres				CG	KU983113
172	<i>Otiorhynchus ovalipennis</i>	CG	31	3	2010	GR, Athen, Airport, environments				CG	KU983114
178	<i>Otiorhynchus ovatus</i>	CG	16	11	2012	SZ, Mörel, Salzgäb				CG	KU983115
177	<i>Otiorhynchus pinastri</i>	CG	26	6	2010	SZ, Thun, Aareufer				CG	no data
162	<i>Otiorhynchus porcatus</i>	CG				SZ, Churwalden				RC	no data

Nr.	Family/subfamily/species	det.	date		Locality		Coordinates		leg.	BOLD-nrs
			d	m	y		N	E		COI
1	<i>Otiorynchus pupillatus</i>	CG	20	6	2009	SZ, Gadmen			CG	no data
6	<i>Otiorynchus pupillatus</i>	CG	6	8	2009	SZ, Habkern, Grünenbergpass	631996	178264	CG	no data
7	<i>Otiorynchus pupillatus</i>	CG	28	6	2009	SZ, Sigriswil, Sigriswilergrat, Alpigen			CG	KU983130
11	<i>Otiorynchus pupillatus</i>	CG	-	8	2009	SZ, Reutigen, Lengeberg	611546	171965	CG	no data
12	<i>Otiorynchus pupillatus</i>	CG	23	8	2009	SZ, Grindelwald, Brandegg	643715	162095	CG	KU983129
28	<i>Otiorynchus pupillatus</i>	CG	3	7	2010	IT, Piemonte, V Formazza, Lago di Morasco	673750	141390	CG	KU983127
42	<i>Otiorynchus pupillatus</i>	CG	6	8	2011	SZ, Tarasp, Avrona			CG	KU983124
54	<i>Otiorynchus pupillatus</i>	CG	12	8	2011	SZ, Ftan, Piz Clüinas	814100	188854	CG	KU983120
55	<i>Otiorynchus pupillatus</i>	CG	23	7	2011	SZ, Brail, Prazet			CG	KU983119
65	<i>Otiorynchus pupillatus</i>	CG	25	7	2012	SZ, Furkapass, Furkstock, below	674959	158847	CG	KU983116
74	<i>Otiorynchus pupillatus</i>	CG	16	7	2013	SZ, Grindelwald, Schrybershörli			CG	KU983117
75	<i>Otiorynchus pupillatus</i>	CG	12	7	2012	SZ, Kandersteg, Schwarzenbach			CG	KU983118
84	<i>Otiorynchus pupillatus</i>	CG	17	7	2012	SZ, Trient, Col de Balme, towards Tête de Balme			CG	KU983121
85	<i>Otiorynchus pupillatus</i>	CG	15	8	2012	SZ, Riom, in garden			RC	KU983122
88	<i>Otiorynchus pupillatus</i>	CG	19	6	2011	SZ, Kandersteg, Gasterental			CG	KU983123
97	<i>Otiorynchus pupillatus</i>	CG	7	7	2013	SZ, NW-Disentis, Val da Lag Serein			CG	KU983126
106	<i>Otiorynchus pupillatus</i>	CG	28	6	2013	SZ, Valchava, Val Vau, W-Praveder	824192	162000	CG	KU983128
124	<i>Otiorynchus pupillatus</i>	CG	29	8	2013	SZ, Ulrichen, Zum Loch			CG	KU983125
168	<i>Otiorynchus pupillatus</i>	CG		9	2013	SZ, Noiraigue vers Creux du Van			CG	no data
176	<i>Otiorynchus salicicola</i>	CG				SZ, Churwalden			RC	KU983131
175	<i>Otiorynchus scaber</i>	CG	6	8	2009	SZ, Habkern, Grünenbergpass			CG	no data
102	<i>Otiorynchus singularis</i>	CG	1	8	2013	SZ, Gstaad, Hintereggli, Mühlesteini			CG	KU983132
4	<i>Otiorynchus subcostatus</i>	CG	2	7	2009	SZ, Puschlav, above Cavaione, Corn dal Solcun	804489	126180	CG	no data
18	<i>Otiorynchus subcostatus</i>	CG	4	6	2010	FR, Haut Jura, Montoisey	486364	127500	CG	no data
3	<i>Otiorynchus tenebricosus</i>	CG	20	6	2009	SZ, Gadmen			CG	no data
10	<i>Otiorynchus tenebricosus</i>	CG	11	7	2009	SZ, Stockhorn, Obere Walalp	606829	171711	CG	KU983135
58	<i>Otiorynchus tenebricosus</i>	CG	19	6	2011	SZ, Kandersteg, Gasterental			CG	KU983133
76	<i>Otiorynchus tenebricosus</i>	CG	12	7	2012	SZ, Kandersteg, Schwarzenbach			CG	KU983134
78	<i>Otiorynchus tenebricosus</i>	CG	14	6	2012	SZ, Isenthal, Brisen	677485	194549	CG	KU983136
165	<i>Otiorynchus thaliarchus</i>	CG	6	4	2012	GR, Crete Island, Chania, Askifou-Plateau, Petres			CG	KU983137
60	<i>Otiorynchus tirolensis</i>	CG	6	8	2011	SZ, Tarasp, Avrona			CG	KU983138
167	<i>Otiorynchus turca</i>	CG				BG, Sofia Plain, City area, Knyazhevo			US	KU983139
158	<i>Otiorynchus uncinatus</i>	CG	4	6	2010	FR, Haut Jura, Gran Crêt	485675	126772	CG	no data

Nr.	Family/subfamily/species	det.	date			Locality	Coordinates			leg.	BOLD-nrs
			d	m	y		N	E			COI
5	<i>Otiorhynchus varius</i>	CG	2	7	2009	SZ, Puschlav, above Cavaione, Corn dal Soleun	804489	126180	CG		KU983140
15	<i>Phyllobius pyri</i>	CG	12	5	2010	SZ, Satigny, Montfleury	493701	119317	CG		KU983141
179	<i>Phyllobius pyri</i>	CG				SZ, Churvalden			US		KU983142
180	<i>Phyllobius pyri</i>	CG				SZ, Chur, Kalkofen			US		KU983143
13	<i>Phyllobius vespertinus</i>	CG	22	5	2010	SZ, Val Blenio, Dongio	716000	144000	CG		KU983145
39	<i>Phyllobius vespertinus</i>	CG	24	4	2011	IT, Valle d'Aosta, ob. Aosta			CG		KU983146
61	<i>Phyllobius vespertinus</i>	CG	21	4	2011	FR, Var, E Le Muy, Bois de Malvoisin, Gorges du Blavet			CG		KU983144
44	<i>Polydrusus amoenus</i>	CG	23	7	2011	SZ, Prazet			CG		KU983147
63	<i>Polydrusus chaerodrysius</i>	CG	12	7	2012	SZ, Kandersteg, Schwarenbach			CG		KU983149
103	<i>Polydrusus chaerodrysius</i>	CG	28	6	2013	SZ, Valchava, Val Vau, W-Praveder	824192	162000	CG		KU983148
143	<i>Polydrusus chaerodrysius</i>	CG	25	6	2011	SZ, Kandersteg, Schwarenbach	615475	144084	CG		KU983150
32	<i>Polydrusus paradoxus</i>	CG	3	7	2010	IT, V. Formazza, V. di Morasco, Riale	674420	142160	CG		KU983151
Curculionidae, Hyperinae											
22	<i>Brachypera vidua</i>	CG	24	4	2010	SZ, Biel, Pavillon	584070	220480	CG		KU983005
56	<i>Brachypera vidua</i>	CG	21	4	2011	FR, Var, E Le Muy, Bois de Malvoisin, Gorges du Blavet			CG		KU983006
183	<i>Donu cyrtus</i>	CG	5	4	2010	GR, Samos Isl., Oros Kerkis, E-Vigla			CG		KU983037
37	<i>Donus globosus</i>	CG	26	9	2010	IT, Liguria, Savona, Finale Ligure, C. di Melogno, Bricco della Guardia			CG		KU983039
46	<i>Donus globosus</i>	CG	16	4	2011	FR, Vaucluse, Bedoin, Crillon-le-Brave			CG		KU983038
49	<i>Donus globosus</i>	CG	17	4	2011	FR, Vaucluse, Gorges de la Nesque, Monieux			CG		KU983040
20	<i>Donus ovalis</i>	CG	4	6	2010	FR, Haut Jura, Gran Crêt	485675	126772	CG		KU983041
99	<i>Donus segnis</i>	CG	28	6	2013	SZ, Valchava, Val Vau, Spi da Vau	826000	164000	CG		KU983042
29	<i>Hypera arator</i>	CG	27	6	2010	SZ, Neuchâtel, L'Ermitage	561800	205700	CG		KU983046
140	<i>Hypera diversipunctata</i>	CG	5	5	2013	FR, Drôme, Vercors, Col de la Bataille			CG		KU983047
142	<i>Hypera gracilentia</i>	CG	8	4	2013	PT, Loulé, Pena, Rocha da Pena			CG		KU983048
79	<i>Hypera melancholica</i>	CG	21	4	2011	FR, Var, E Le Muy, Bois de Malvoisin, Gorges du Blavet			CG		KU983049
91	<i>Hypera melarynchus</i>	CG	12	4	2013	PT, W Sagres, Cabo S. Vicente, resthouse			CG		KU983050
43	<i>Hypera miles</i>	CG	23	7	2011	SZ, Brail, Prazet			CG		KU983051
16	<i>Hypera nigrirostris</i>	CG	12	5	2010	SZ, Satigny, Montfleury	493701	119317	CG		KU983054
19	<i>Hypera nigrirostris</i>	CG	4	6	2010	FR, Haut Jura, Gran Crêt	485675	126772	CG		KU983052
94	<i>Hypera nigrirostris</i>	CG	13	4	2013	PT, W Lagos, Budens Umgb			CG		KU983053
98	<i>Hypera ononidis</i>	CG	1	8	2013	SZ, Gstaad, Hintereggi, Mühlesteini			CG		KU983055
57	<i>Hypera plantaginis</i>	CG	10	7	2011	SZ, Amden, Mattstock	728655	225707	CG		KU983056

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			d	m	y		N	E		
87	<i>Hypera plantaginiis</i>	CG	16	11	2012	SZ, Morel, Salzgäb			CG	KU983057
17	<i>Hypera striata</i>	CG	12	5	2010	SZ, Vallon de l'Allondon, Malval, Les Granges	488856	119489	CG	KU983058
24	<i>Hypera venusta</i>	CG	4	6	2010	FR, Haut Jura, Montoisey	486364	127500	CG	KU983059
101	<i>Hypera venusta</i>	CG	29	6	2013	SZ, Val Mora, below Piz dal Döss Radond	823323	162634	CG	KU983060
	Staphylinidae, Aleocharinae									
184	<i>Leptusa pilatensis</i>	AS	3	5	2006	SZ, Neuchâtel, Villiers			AS	KU983061