First record of the hairy snail *Trochulus clandestinus* (Hartmann, 1821) in Austria in the course of an ABOL excursion in Vorarlberg

Michael Duda, Julia Schindelar, Oliver Macek, Anita Eschner & Luise Kruckenhauser

During an excursion of the Austrian Barcode of Life (ABOL) pilot project molluscs the north-west alpine distributed hairy snail *Trochulus clandestinus* (HARTMANN, 1821) was recorded for the first time in Austria. Two living specimens were found in Vorarlberg on the 11.07.2016 at the Rimsbach near Bezau. For both individuals sections of the genital tract were prepared, especially to analyse the detailed penial morphology, and DNA barcodes were generated. Both approaches, when compared with published data, confirmed the identity of these two specimens as *T. clandestinus*. The habitat of the Austrian location resembles those of natural populations in Switzerland. Nevertheless, for the time being it cannot be clearly stated whether the species colonized Vorarlberg in postglacial times or whether the current finding is a result of recent anthropogenic introduction. The high genetic similarity of all investigated specimens of *T. clandestinus*, is in contrast to other species of the genus *Trochulus* and indicates a quick expansion to the recent distribution area which might have occurred under human influence, however, postglacial colonization cannot be ruled out.

Keywords: Molluscs, Austrian barcode of life, DNA barcoding.

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Routine agricultural pathogen and pest identification by barcoding at the Department for Molecular Diagnostics of Plant Diseases (AGES)

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Beside the use of molecular techniques such as endpoint and realtime PCR, sequencing of generically amplified gene loci (barcoding) has been making its way into the diagnosis of plant pathogens and pests since a couple of years. The application of DNA barcoding in the field of plant health can be multifaceted and has become a standardized identification method for selected regulated pests (EPPO standard). This generic diagnostic method is used for confirmation of species identification, but also to identify pathogens and pests which are not in a developmental stage to be easily determined by classical morphological and biochemical characteristics. If using species or lower taxa specific molecular detection tools, like pathovar specific PCRs, the target can easily be missed eventually leading to an increased number of tests without a satisfactory result. At our department, barcoding is applied to confirm species identification as well as to identify plant pathogenic bacteria, phytoplasmas, fungi, viruses and viroids, but also insects and nematodes. The quality controlled confirmation of the identity of microorganisms and other pests like plant parasitic nematodes and arthropods by DNA barcoding has been established to enhance the quality of our in-house reference collections.

The rapid identification of harmful insect larvae in imported consignments by DNA barcoding is essential for minimizing the impact on trade traffic, giving more confidence that organisms under EU-quarantine are hindered from entering the territory. Since 2008, our department has been participating in several international projects (e.g. in the frame of EUPHRESCO, ERA-Net) establishing and validating barcoding techniques including training for the identification of plant pathogens and pests in following taxonomic groups: viroids, viruses, phytoplasmas, bacteria, fungi, insects, nematodes and invasive plants.

In the field of plant health, barcoding has become a very valuable tool for the detection and identification of pathogens and pests and has been evolved into a key pillar supporting the diagnostic process of diseases and pests affecting agriculture worldwide.

Keywords: Plant pathogens, bacteria, phytoplasmas, fungi, viruses, viroids.

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ABOL pilot project vertebrates – what we have achieved so far

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Vertebrates are certainly the native animals best known to laypeople. According to the most recent Red Lists Austria is home to 460 species of vertebrates (84 fishes, 20 amphibians, 14 reptiles, 241 breeding birds, and 101 mammals), a number that does not include the many bird species that visit Austria as winter guests or vagrants and the ever increasing number of exotic fish and reptile species. Despite being the best-studied animal group, hardly any DNA barcodes of Austrian vertebrates were available prior to ABOL. Hence, the ambitious goal of this pilot project is not only to generate DNA barcodes of as many vertebrate species as possible, but also to optimize barcoding of highly degraded museum specimens and conduct in-depth analyses of problematic taxa. Here, we provide a brief overview of what has been achieved in the first $\sim 2\frac{1}{2}$ years of the ABOL pilot project on vertebrates: i) development of a new primer set for DNA barcoding highly degraded bird and mammal specimens from historic museum collections, ii) DNA barcodes for all Austrian reptiles and most Austrian amphibians, iii) DNA barcodes for $-\frac{3}{4}$ of the Austrian fish species and a detailed multi-gene analysis of the gudgeon genus *Gobio*, and iv) DNA barcodes for several bird and mammal species. All these barcodes will be made available in a public database and may be used for a wide range of basic and applied research.

Keywords: DNA barcoding, birds, mammals, reptiles, amphibians, fish, historic specimens, primer.

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Application of mt-DNA Barcoding for fish larvae identification - Selected spatial and seasonal pattern of fish larvae drift in the Danube in Vienna

Paul Meulenbroek, Silke-Silvia Drexler, Susanne Krumböck, Christian Stauffer & Herwig Waidbacher

Heavily modified river systems often provide functioning habitats for a variety of life stages of fish. Investigating the fish larval dispersal over a stretch of 20 km in Vienna, drift nets were exposed at 13 different sites; including four riprap sections and three gravel bars. These habitats have been constructed at least 15 years ago when the impoundment of the Danube hydropower station Vienna/Freudenau was established. In the whole ongoing investigations 25.000 fish larvae were trapped. Identification in the early life history of fish has always been done by morphological characters. However, morphology can quickly change significantly during development from preflexion larvae to postflexion and to the pre-juvenile stage and rare species are very likely to be misidentified. Therefore a sample of 1011 larvae was analysed with mt-DNA barcoding to species level. 32 different species have been detected, including eight species listed in Annex II of the European Flora-Fauna-Habitat Directive. Regarding the spatial distribution and the species composition within the samples the dominance of cyprinids in downstream sites of gravel bars becomes visible, while in riprap sections the majority of the larvae consists of Cottidae and invasive Gobiidae. However, clear species related seasonal patterns with peak densities are developed and multiply spawning of same species may occur - visible by repeated drifting peaks.

Keywords: Modified river systems, drift nets, DNA barcoding, Cyprinidae, Cottidae, Gobiidae.

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Evaluating how prey and predator identity and meal size affect prey DNA detection in bat faeces: a feeding experiment

Petra Schattanek

Bats occupy a vast diversity of niches, inhabit various climate zones and use a wide range of different food resources. About 70% of all bat species are feeding, either obligate or facultative, on arthropods. Molecular examination of faeces has increasingly being used to identify feeding habits of bats and their role in food webs. Despite the widespread use of DNA-based prey identification, little is known about how specific factors such as gut transition time, bat and prey identity and meal size affect the detection success of prey DNA in bat faeces. This, however, is the prerequisite to correctly interpret the data received from molecular analyses from faeces samples collected in the field. The project "Evaluating how prey and predator identity and meal size affect prey DNA detection in bat faeces: a feeding experiment" directly addresses these knowledge gaps by evaluating within a feeding trial how prey and bat identity, gut transition time and meal size affect post-feeding prey DNA detection intervals. The outcomes of this experiment will represent an important basis for the interpretation of data collected in the field.

Keywords: Arthropods, gut transition time, DNA barcoding.

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Poster-Abstract

Pilotprojekt Schmetterlinge (Lepidoptera): Subprojekt Noctuiodea

Benjamin WIESMAIR & Peter HUEMER

In der ersten Phase von ABOL wurden die Lepidoptera als eine der 4 Zielgruppen für ein Pilotprojekt ausgewählt. Mit mehr als 4000 in Österreich nachgewiesenen Arten gehören diese zu einer der artenreichsten Tierordnungen. Um einzelne Gruppen möglichst weitgehend abdecken zu können wurde u.a. innerhalb der Lepidoptera das Subprojekt Noctuiodea definiert. Diese umfassen in Österreich 684 Arten und teilen sich auf die Familien der Notodontidae, Nolidae, Erebidae und Noctuidae auf.

Ziel dieses Subprojektes ist es, mindestens vier Barcodes jeder einzelnen Art der Noctuiodea aus Österreich zu generieren. Um eine geografische Abdeckung Gesamtösterreichs zu gewährleisten, wurde das Land grob in drei Teile untergliedert. In Nordostösterreich (Oberösterreich, Niederösterreich, Wien, Burgenland), Westösterreich (Vorarlberg, Nordtirol, Salzburg) und Südösterreich (Osttirol, Kärnten, Steiermark). Um eine Art erfolgreich abgeschlossen zu haben, musste ein Barcode aus jedem Teilbereich Österreichs, in welchem die Art nachgewiesen wurde, erhalten werden. Bis heute konnten insgesamt 2206 Barcodes von 540 Arten generiert werden. Insgesamt konnten 32% des Artenbestandes (218 spp.) erfolgreich abgeschlossen werden. Von weiteren 47% (322 spp.) ist aktuell mindesten ein Barcode vorhanden. Von 21% (144 spp.) ist noch kein Barcode aus Österreich sequenziert worden. Im Rahmen des Subprojekts wurden einige interessante Fragestellungen bezüglich kryptischer Diversität aufgeworfen. Ein Beispiel hierfür ist *Hoplodrina octogenaria*, von welcher zwei Cluster in Österreich vorkommen. Diese können überdies anhand der äußeren Morphologie und auch der Genitalmorphologie unterschieden werden.

Keywords: DNA barcoding, cryptic diversity, Austria.

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Poster Abstract

New tools for arctic research: DNA barcoding the entire communities of plants and animals in Zackenberg, NE-Greenland

Helena Wirta, Gergely Várkonyi, Claus Rasmussen, Riikka Kaartinen, Niels Martin Schmidt, Paul D. N. Hebert, Miroslav Barták, Gergin Blagoev, Henry Disney, Siegrun Ertl, Peter Gjelstrup, Dariusz J. Gwiazdowicz, Larry Huldén, Jari Ilmonen, Jevgeni Jakovlev, Mathias Jaschhof, Jere Kahanpää, Tuomas Kankaanpää, Paul Henning Krogh, Renee Labbee, Christian Lettner, Verner Michelsen, Søren Achim Nielsen, Tore R. Nielsen, Lauri Paasivirta, Stephanie Pedersen, Jaakko Pohjoismäki, Jukka Salmela, Pekka Vilkamaa, Henry Väre, Michael von Tschirnhaus & Tomas Roslin

DNA sequences offer powerful tools for addressing questions of community ecology: not only do they allow us to identify and describe the community members, but also to reveal the biotic interactions between them. Here, we present a recent study (Wirta et al. 2016) offering a comprehensive library of DNA barcodes for a terrestrial site, the Zackenberg region in Northeast Greenland. This library includes almost all macroscopic animals and vascular plants known from the region. A total of 403 terrestrial animal and 160 vascular plant species were recorded by morphology-based techniques. DNA barcodes were created with high sequencing success using standard gene regions (CO1 for animals; rbcLa and ITS2 for plants). 92% of the animal taxa were assigned to unique Barcode Index Numbers (BINs) and 93% to monophyletic clusters. For the flora, the discriminatory power was lower, with 54% of the plant species forming monophyletic clusters based on information combined from barcode regions rbcLa and ITS2. In evidence of the utility of the library created, we applied it to community samples of arthropods from Malaise traps, thus resolving compositional turnover in space and time. Nearly 20 000 arthropod individuals were identified from Malaise trap catches, revealing 122 BINs not detected by previous sampling and DNA barcoding. The insect community proved to be dominated by a few highly abundant taxa, with Diptera being the most abundant order, and Chironomidae the most abundant family. Thus, the DNA barcode library established for the Zackenberg region now offers scope for ecological explorations, for the detailed dissection of interspecific interactions throughout the community, and for long-term monitoring. Importantly, the development, application and curation of the library rely heavily on constant collaboration and feedback between ecologists and taxonomists.

Keywords: Community ecology, Barcode Index Numbers, monophyletic clusters, Malaise traps, Diptera, Chironomidae.

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Poster Abstract

Which and how many species of gudgeons (genus *Gobio*) are there in the Austrian Danube system? Insights from molecular data

Lukas Zangl, Daniel Daill, Wolfgang Gessl, & Stephan Koblmüller

Gudgeons of the genus *Gobio* are small elongate bottom-dwelling cyprinid fishes that are found in rivers and lakes throughout Eurasia. The genus includes numerous, often very closely related species, several of which have been described only recently. It is still not clear which *Gobio* species inhabit(s) the Austrian Danube system. Some sources state that the Austrian gudgeons are *G. gobio* whereas others suggest them to be *G. obtusirostris*, with a potential hybrid zone between the two species in the upper Danube. To clarify the status of the gudgeons in the Austrian Danube system, we sequenced two mitochondrial (COI & D-Loop) and one nuclear (RPS7) gene of the Austrian gudgeons and analysed them together with previously published *Gobio* sequences from throughout Europe. Our analyses showed that based on molecular data Austrian gudgeons cannot be assigned to a single valid species, but rather represent a mixture of three different species/lineages. *G. gobio, G. obtusirostris* and a lineage mainly distributed in the Balkans. Interestingly, and quite unexpected, this hybrid swarm is not confined to a particular geographic region but seems to be found throughout the (eastern) Austrian Danube system. Genetic distances among and within the major mitochondrial lineages indicate that they have diverged about one million years ago, survived the Pleistocene glaciations in separate refugia and came into secondary contact only recently (most likely after the last glacial maximum).

Keywords: Fish, DNA barcoding, mitochondrial lineages.

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