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WasserCluster Lunz Biologische Station, A-3293 Lunz am See Dr. Carl Kupelwieser Promenade 5

# ABSTRACTS

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# Phylogeography and population differentiation of *Clausilia dubia* across Europe

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The door snail species *Clausilia dubia* is widespread across Europe from the Pyrenees to the Carpathian Mountains and from the Alps to Scandinavia. It is usually found on moist, shaded rocks or on trees. In most of its range, the morphological variability of *Clausilia dubia* is low. Two widespread subspecies are often distinguished. However, several morphologically differentiated population groups can be found in the Eastern Alps. These forms differ in shell characteristics (e.g., shell dimensions, sculpture, features of the closing apparatus) and were regarded as endemic subspecies differentially adapted to specific habitats such as exposed calcareous rocks compared to the widespread mesophilic populations. In previous studies, these taxa were found to be non-monophyletic in mitochondrial gene trees (COI, 16S), highlighting the need to analyze genome-wide multilocus data. Using ddRAD sequencing, we obtained 3535 loci from 161 individuals of 34 populations. With this dataset we assess the phylogeography and evolutionary history of *Clausilia dubia* across Europe and provide new insights into the population differentiation in the Eastern Alps.

# The mollusc fauna of the Bisamberg hill and "Alte Schanzen" area (Vienna, Austria)

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In 2021 and 2022, qualitative studies on the species spectrum of the mollusc fauna were carried out at the Bisamberg hill and the "Alte Schanzen" (rests of a historical military fortification) up to the Marchfeldkanal (an artificial channel for soil irrigation), which is partly located in the Vienna area for "rural development". Besides the assessment of the species inventory, the aim of these investigations was to assess the current value of the area for nature conservation and landscape management. A total of 81 species, 51 land snails, 19 aquatic snails and 11 mussels, were recorded. The high proportion of dry grassland in the area is habitat for several xerothermophilous species endangered and protected in Austria, e.g., Granaria frumentum and Zebrina detrita. Concerning the latter one, the investigated area harbors more than half of the currently known populations within Vienna. At one locality Chondrula tridens, which is extremely rare in Central Europe and worldwide classified as "Near Threatened", was found alive. This is the first clearly documented evidence of this species alive for Vienna. The results point out, that the dry grasslands and vineyard banks in the area are of high conservation value. The few forests in the area tend to have fewer species that are typical for this habitat. On the other hand, the first clear evidence of Aegopinella minor for Vienna should be emphasized. Few invasive species have also been recorded in the terrestrial habitats. For the aquatic habitats, the occurrence of Viviparus contectus, which is highly endangered in Austria, in an artificial pond is particularly worth mentioning, as well as the second record of Bathyomphalus contortus in the Viennese part of the Marchfeldkanal. In summary, the study area can be considered as very important for conservation, especially because of its dry, open habitats.

# *Cornu aspersum* (O. F. Müller 1774) - eine Bereicherung für die österreichische Molluskenfauna – oder?

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#### Zusammenfassung:

Es wird über die Ausbreitungsgeschichte in Österreich, insbesondere in Wien und dem südlichen Marchfeld berichtet. Weiters über Auswirkungen auf Privat- sowie Erwerbsgärten und die landwirtschaftliche Gemüseproduktion aber auch über die Rolle der Schnecken als Parasitenwirte. Auch wird das Spannungsfeld zwischen Naturschutz und Bekämpfung sowie der kommerziellen Züchtung erörtert.

#### Abstract:

The history of their distribution in Austria, especially in Vienna and the southern Marchfeld, is reported. Impacts on private and commercial gardens and agricultural vegetable production are discussed, as well as the role of snails as parasite hosts. The tensions between conservation and control and commercial breeding are also discussed.

# Resolving evolutionary relationships in the Epitoniidae using phylogenomics and historical museum specimens

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The Epitoniidae is a family of marine snails with a long evolutionary history dating back to at least the early Cretaceous. Approximately 785 species of epitoniid are currently recognized from the intertidal to depths of more than 1,000 meters, but delimiting genera and understanding evolutionary relationships has remained difficult due to convergence in shell characters. We estimated a molecular phylogeny of the family using recently collected and historical museum samples using a combination of PCR of several loci (COI, 16S, 28S, 18S, and H3) and genome skimming of samples with degraded DNA. Mitochondrial DNA sequences were obtained from 960 specimens, with a focus on the taxa in the Indo-West Pacific. This dataset includes approximately 380 species and representatives of at least 28 currently recognized genera, most of which have not been previously studied in a molecular phylogenetic framework. In addition, selected specimens were utilized for target capture of ultraconserved elements (UCEs) and exons, to help resolve the deep evolutionary relationships within the phylogeny. We used a new universal probe set designed for caenogastropods to target 3,075 loci and recovered an average of 1,460 loci per specimen. A robust, well-supported phylogeny estimated from this data suggests the polyphyly and paraphyly of several diverse genera including Epitonium, Cirsotrema, and Opalia, and indicates a need for significant taxonomic revision. Analyses of deep sea nystielline genera indicate that some are deeply divergent from other epitoniids, but that the subfamily is not monophyletic. The analysis of dried, historical museum specimens contributed significantly to the broad taxon sampling of this study, and emphasizes the importance of museum collections in evolutionary studies of non-model taxa.

# In search of the glow – Histochemical and morphological investigations on the gland system of *Latia neritoides* (Mollusca; Gastropoda; Hygrophila)

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Bioadhesives encompass a wide range of applications as attachment, predation, or defence. *Latia neritoides*, a species found exclusively in the freshwater streams of New Zealand's North Island, has developed a special defence mechanism: when disturbed, it discharges large quantities of luminous, adhesive mucus. Although some data on the chemical composition of the luminescent elements of the defensive mucus are available, we have no information on the adhesive mechanisms associated with the gland system of this species. The defence mucus may originate from two glandular areas: the lateral foot region and/or the mantle cavity, as the secretion is expelled through the pneumostome. Histochemical and morphological investigations on both areas show the presence of two distinct types of glandular cells in the lateral region. However, due to gland density and discharge location, it appears that the luminescent component is primarily produced by glands located within the mantle cavity. To determine the possible presence of mucus reservoirs and explore alternative glandular cell structures,  $\mu$ -CT and HREM stacks were used for a reconstruction of the whole animal. However, there were no mucus reservoirs in the examined specimens.

A comparison of the protein compositions of the defence and trail mucus shows considerable variations in overall protein concentration, and in the number and physicochemical properties of proteins identified through techniques such as SDS-PAGE and two-dimensional electrophoresis. Thirty-three gel pieces, obtained from electrophoretically separated bands or spots, were treated with trypsin to cleave proteins into peptides. The resulting peptides were then identified through LC-MS/MS analysis and matched with transcriptomic data. Several hitherto unidentified proteins appear to be specific for the defence mucus.

# Molluscan Nephridia, a Story of Underlying Synapomorphies

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Classic zoology clearly distinguished between ectodermal protonephridia with ciliated terminal cells and mesodermal metanephridial systems, where ultrafiltration takes place via podocytes at various positions into various coelomatic cavities. Ultrafiltration in adult Mollusca typically occurs from the lumen of the heart auricle (or branchial hearts) through podocytes into the pericardial cavity. However, since the 1980es all these nephridial conditions are considered as an evolutionary continuum with homologous ultrafiltration cells. Mollusca uniquely show a diagnostic, solitary cell-type with ultrafiltration triggered by endocytosis, the rhogocyte (pore cell, brown cell), which play a central role in recycling respiratory proteins (hemocyanine, haemoglobin), in calcium ion and in heavy metal metabolism in general. General data of the last 30 years and certain special cases recently studied provide evidence for this latter scenario:

- (1) Recent molecular and evo-devo studies have provided unequivocal evidence that the ultrafiltration sieve apparatus in protonephridial terminal cells, metanephridial podocytes (including vertebrates), arthropod nephrocytes and molluscan rhogocytes are indeed homologous and thus underlying synapomorphies of all ultrafiltration systems.
- (2) All molluscan trochophore-like larva show a pair of protonephridia.
- (3) In cases of a lack of heart and pericardium (e.g. the enigmatic gastropod *Rhodope*, certain sacoglossan gastropods, or progenetic, small Solenogastres) the former metanephridial system is modified to pseudo-protonephridia.
- (4) Early juvenile Polyplacophora show a long functional overlap of retained larval protonephridia and the developing metanephridial system.
- (5) The development of the bivalve (*Mytilus*) metanephridial releasing system shows ectodermal origin and a transitional protonephridial stage.
- (6) Monoplacophora with their multiplied excretory organs (3 to 7 pairs) are suspected to represent a case of combination of retained protonephridia (the most anterior pair) and serial metanephridia (all other pairs), which are added from posterior to anterior and may or may not become connected with the pericardial cavity or the gonads.

Implications of these findings towards a general theory on the evolution of bilaterian excretory organs are outlined.

## Developing a mussel sensor for an aquatic biohybrid system

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As part of the EU project Robocoenosis, a biohybrid system is being developed that will enable autonomous and automatic analysis of water quality in freshwater bodies. The so-called biohybrid entity combines an electronic core, with parts such as environmental sensors and power supply, and observation of organisms, some of which will live in and on the biohybrid. One of the sub-projects of Robocoenosis is the refinement of valvometry through miniaturisation and adaptation to the natural living conditions of bivalves. This method is used to infer water quality from the shell movement of bivalves, as bivalves react differently to a variety of environmental stressors (e.g., salts, heavy metals, various plant toxins and fertilisers, and detergents). For this purpose, improved electronics in combination with 3D printed components are currently being developed and different energy sources are being tested. At the same time, experiments with *Dreissena polymorpha* mussels are already being carried out in an established laboratory colony and in the field. At the moment, primarily the behaviour and adaptations of the mussels to the prototypes are being investigated. First results show that the mussels adapt very quickly to the new environment and show a modified behaviour in the laboratory compared to their natural habitat.

# Photographing mollusk shells, how to do it right

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Images are an important element of the description of mollusk shells, as they contribute to the understanding of the text and provide additional information that cannot be adequately expressed verbally alone. Nevertheless, the images in original descriptions of new species are still often of such poor quality that they do not do properly serve these purposes. The reason for this is often not (only) insufficient equipment, but quite avoidable mistakes.

Based on examples from the literature, I want to point out the most frequent shortcomings and mistakes and, based on my many years of experience in microphotography of mollusks, point out what can be done to avoid them and give practical tips on how to get high-quality images that best serve the purposes mentioned above.

### Slugs and snails in my garden: counting, cutting, cooking

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Slugs and snails are important components of ecosystems in undisturbed nature as well as in cultivated land and private gardens. Although they are inspiring and fascinating to many people - especially children - most gardeners see snails mainly as pests. Especially the high abundances of the (very voracious) so called Spanish Slug (Arion vulgaris) has led gardeners to various control measures.

This report presents data and experiences with snails in a small garden at the eastern periphery of Vienna, Austria. As of 2019, just the number of Spanish Slugs (A. vulgaris) were noted, and in order to decimate them, killed consequently by cutting with a pruning shear. Beginning in 2020, beside the Spanish Slug, also other snail species were surveyed numerically, including size/age and reproductive data, and accompanying environmental factors.

Here the list of species is presented, and the counts of the more abundant species are compared over the months and years. Some biological data about growth and courtship during the seasons are presented.

The invasive Common Garden Snail (Cornu aspersum), first seen sporadically in this garden in 2017, proliferated explosively in subsequent years. In view of this rapidly increasing population densities and the resulting massive damage to numerous flowers and vegetable plants, the adults were regularly collected and prepared as food since 2022. The preparation of the snails followed known recipes, which, in addition to killing, slime removal and seasoning, includes several hours of cooking, which is also effective against possible transmission of parasites of humans and pets (e.g., lungworms, which use snails and slugs as intermediate hosts). Since the removal of the adult animals had no noticeable effect on the population increase, subadult animals were also collected and killed starting with May 2023.

In contrast to A. vulgaris and C. aspersum, populations of other snails in the garden were much more harmless from a gardener view. These species, including Roman Snail (Helic pomatia, White Lipp Banded Snail (Cepaea hortensis), Leopard Slug (Limax maximus) were not combated in this garden. The population of the Roman Snail (Helix pomatia) and the other species recorded stayed more or less stable despite the population explosion of the garden snail. They continue to be fascinating and inspiring objects of nature observation, as well as important components of the ecosystem.

# Emergent questions in (cephalopod) mollusk genomics

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The primary interest of our team is the investigation of animal genome evolution and how changes in animal genome "architecture" can result in the evolution of novel gene regulation and eventually phenotypes. Recent developments in genome assembly technologies and high-throughput genome topology and regulatory landscape profiling enable us to look deeper into what makes up the genome "architecture" and how different animal clades retain ancient and invent novel "architectural" genome modalities. One of the species groups we are interested in are the cephalopod mollusks, in particular the experimentally tangible Hawaiian bobtail squid Euprymna scolopes. Our research has revealed that coleoid cephalopod (squid, cuttlefish, octopus) genomes show strikingly different genomic organization compared to other mollusks and invertebrates in general. In this talk, we will introduce the approaches to identify evolutionary transitions in genomes and what insights they give into cephalopod evolution, the emergence of novel regulatory regions as predicted by our analyses, and approaches to test them. Beyond cephalopods, we will discuss what changes in genome "architecture" can be observed in mollusks and spiralians in general and what predicted impact on gene regulation they may have. Considering ever emerging high-quality genomic resources for many molluskan species, we can outline strategies to better define (evolutionary) genomic signatures behind particular types of gene regulation.