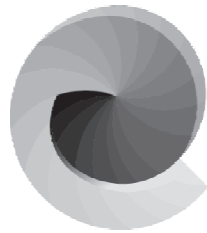


# Book of abstracts

Edited by:

Adam M. Ćmiel, Anna Lipińska, Katarzyna Zając,  
Tadeusz Zając, Robert A. D. Cameron



# euromal

8th European Congress of Malacological Societies

**10–14 September 2017, Kraków, Poland**

Hosted by:

**Association of Polish Malacologists**



**STOWARZYSZENIE  
MALAKOLOGÓW  
POLSKICH**

Co-organizer:



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# 8th Congress of the European Malacological Societies

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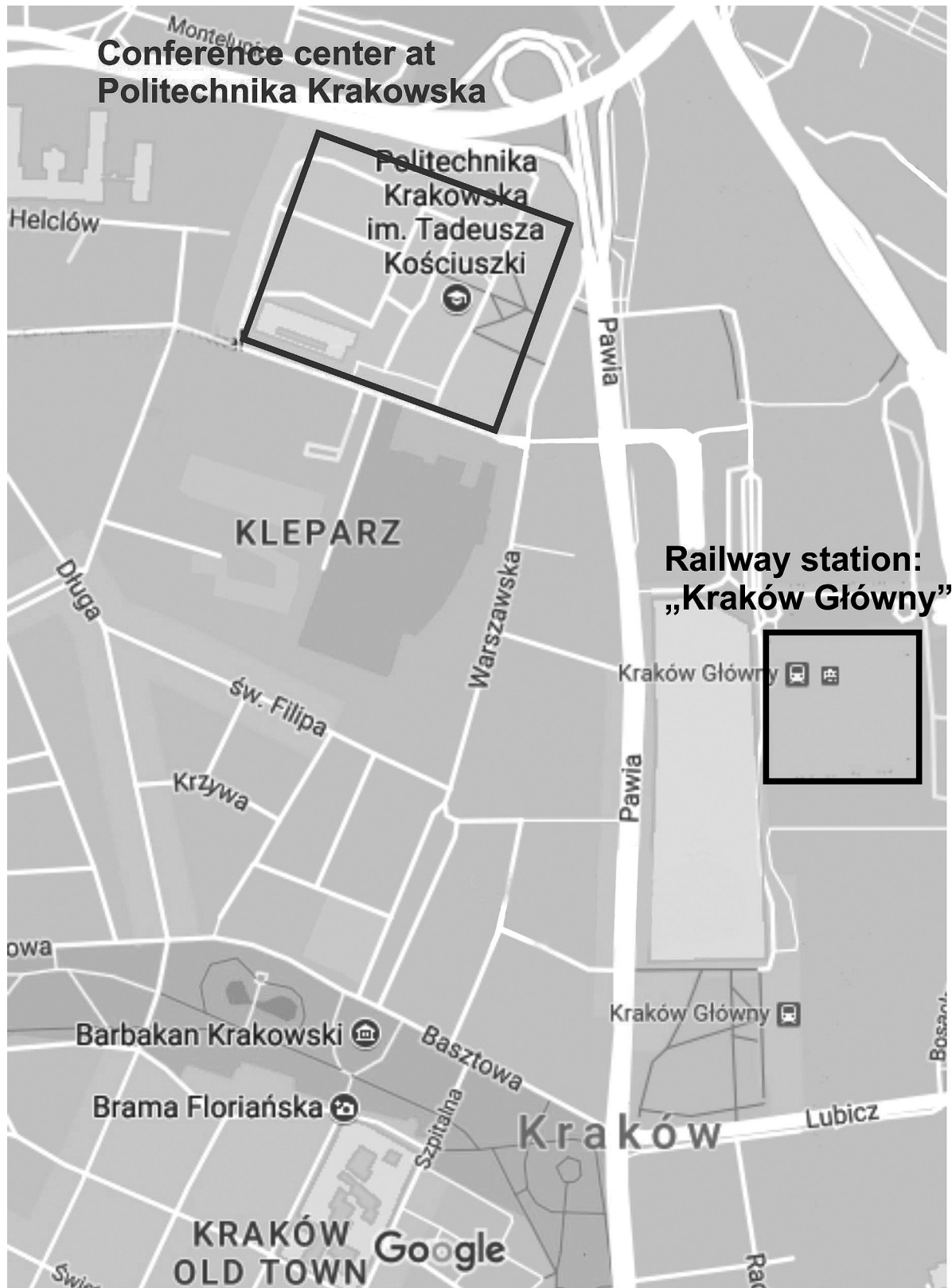
## Maps and directions

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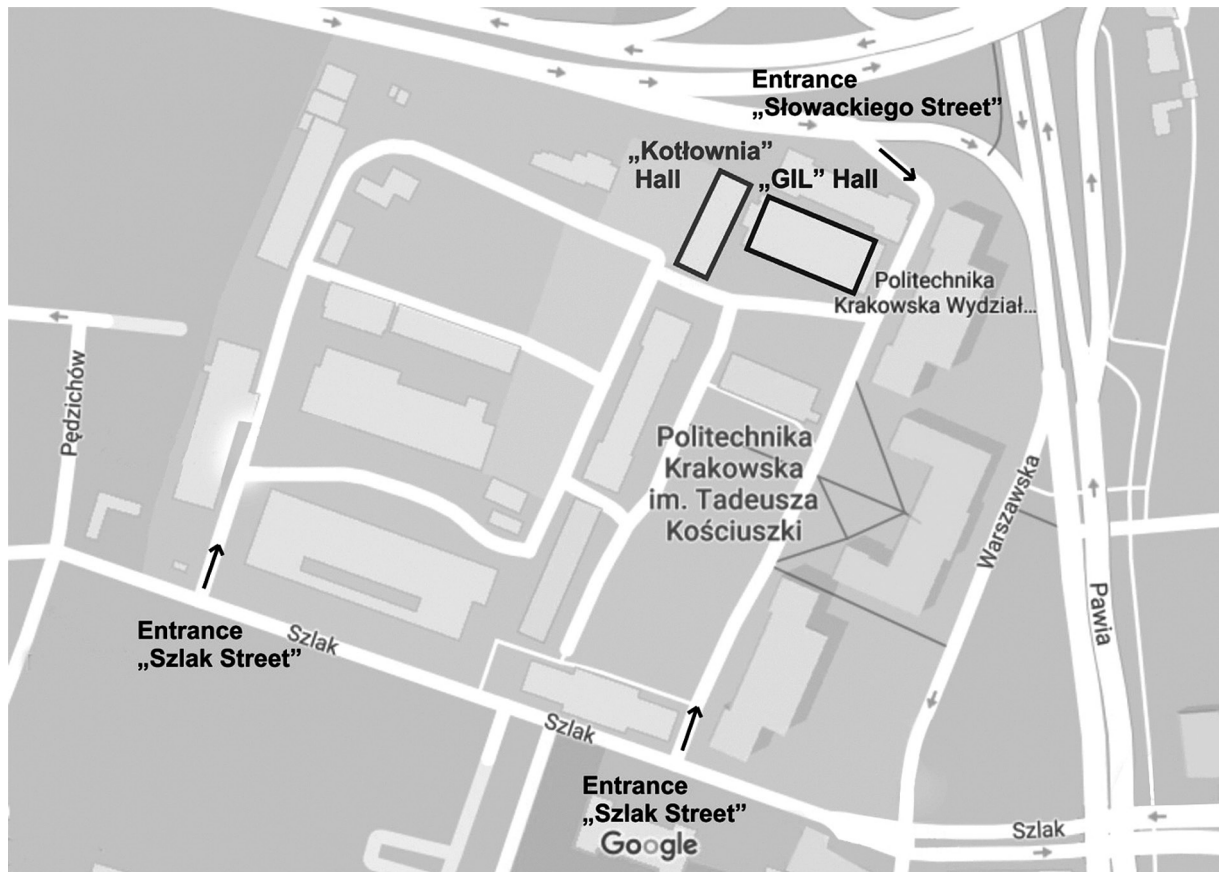
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# Maps and directions

The sessions will be held in the "Kotłownia" hall and the "Gil" hall located at the Politechnika Krakowska building code 10-06



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**ABSTRACTS**

**ORAL  
PRESENTATIONS**



## **Theory can explain a lot of life history diversity in mollusks**

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Why do some animals weigh a fraction of a milligram and others many tons? Why do some animals mature after a few days and others need several years? Why do some animals grow and then reproduce without growing, while others continue grow-

ing after maturation? Why are growth curves so often well approximated by von Bertalanffy's equation? Why do some animals produce myriads of tiny eggs and others produce only a few large offspring?

## What life history traits can tell on population dynamics and structure in threatened snail species *Vertigo moulinsiana* (Dupuy, 1849)

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In organisms with restricted dispersal abilities, such as small, terrestrial snails, population dynamics are usually based on simple ratio of reproduction vs mortality, and dynamics of such populations are not analyzed in detail. However, they can have unusual spatiotemporal patterns, which are difficult to explain on the basis of simple models. The more detailed analysis of their life history traits can help both in explaining observed patterns but also to identify life history traits or transition points, strongly influencing population dynamics, which is obviously important for conservation of threatened species or explaining invasions patterns.

The results of five years studies on the *Vertigo moulinsiana* population dynamics revealed the very characteristic pattern of within- and between-year fluctuations and increase in population abundance between consecutive years. In each season a repeatable pattern of population abundance was observed:

the number of snails grows from May with maximum abundance in August, whereas it decreases in September and October. Assuming that the basic life history parameters, obtained in the field and laboratory studies, like timing of transition points during ontogeny, variation in fecundity and survival during lifetime, are responsible for specific patterns of snail population dynamics within the season, a mathematical model of influence of these phenomena on population dynamics was constructed.

The results of simulations of *V. moulinsiana* population dynamics, based on its life history traits showed a pattern reflecting the pattern of within- and between-year fluctuations observed in the field. Numerical analyses revealed, that the most detrimental effects to the population are caused by variation in adult survival, while life history traits related to fecundity, juvenile survival and winter survival need very strong decrease to influence population stability.

## Who wins in the weaning process? Juvenile feeding morphology of two freshwater mussel species

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The global decline of freshwater mussels can be partially attributed to their complex life cycle. Their survival from glochidium to adulthood is like a long obstacle race, with juvenile mortality as a key critical point. Mass mortality shortly after entering into a juvenile state has been reported in both wild and captive populations, thus weakening the effective bivalve population. A similar phenomenon occurs during metamorphosis in natural and hatchery populations of juvenile marine bivalves. Based on a morphological analysis of newly formed juveniles of the freshwater species *Margaritifera margaritifera* (Margaritiferidae) and *Unio mancus* (Unionidae), we show that a second metamorphosis, consisting of

drastic morphological changes, occurs that leads to suspension feeding in place of deposit feeding by the ciliated foot. We hypothesise that suspension feeding in these two species develops and improves gradually due to several morphological features including the contact between cilia of the inner gill posterior filaments, the inner gill reflection, the appearance of the ctenidial ventral groove and the formation of the pedal palps. Regardless of the presence of available food, a suspension feeding mode replaces deposit feeding, and juveniles unable to successfully transition morphologically or adapt to the feeding changes perish.

## Differential disease resistance patterns in eastern Pacific Haliotids

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Many marine host-pathogen systems are poorly understood, and even less is known about how global climate change will affect the mechanisms of these systems. In the abalone and Rickettsiales-like organism (RLO) system, the RLO infects abalone digestive tissues and leads to extreme starvation and a characteristic “withering” of the gastropod foot (WS). First identified in black abalone in California after an El Niño event, the withering syndrome-causing RLO (WS-RLO) has been seen in various sites around the world, and has been found in at least low levels in all abalone species examined, yet not all express WS. Some abalone species appear to be highly resistant to the disease, unless held at extremely high temperatures. This suggests that the resistant species possess some physiological resistance to the effects of high temperature. Our goal is to develop a detailed understanding of the abalone digestive system and the mechanisms for differential resistance across the *Haliotis* (abalone) genus. In order to clarify differen-

tial susceptibility in geographically neighboring species (such as the highly susceptible red abalone *H. rufescens* and relatively resistant green abalone *H. fulgens*), we created a robust phylogeny of California’s *Haliotis* species to determine whether certain branches are more susceptible to WS. This phylogenetic tree was created using concatenated data from two mitochondrial (16s and cytb) and three nuclear genes (h3 and its1/2), and will result in the most robust phylogeny of eastern Pacific Haliotids to date. Interestingly, the two most resistant species, *H. fulgens* and *H. corrugata*, are sister taxa that form a clade separate from the other California taxa. To clarify the mechanisms for differential resistance, we are analyzing gene expression in pinto abalone, *Haliotis kamtschatkana*, that underwent a WS infection course lasting 7 months. With this data, we are identifying gene expression patterns in abalone digestive tracts that lead to WS resistance.

## Biological clock in the freshwater mussels *Unio tumidus* – daily and seasonal patterns in serotonin levels in haemolymph and tissues, and clock gene daily expression profiles

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Light is regarded as the strongest Zeitgeber for biological rhythms in organisms. The body of bivalves is usually light protected by their shell; furthermore, unionids live buried within the sediments, nevertheless they have the photoreceptors within their tentacles. Photoreceptors of organisms drive the light information to the nervous system, eg. by the serotonergic innervation. Serotonin (5-HT) levels in haemolymph of the marine gastropod *Aplysia* are regulated by light-dark conditions. Little is known about how unionids transfer the light signals and have a light or clock-depending rhythmicity in serotonin levels. Knowledge is poor also regarding the molecular mechanism of the biological clock in unionids.

*Unio tumidus* were kept in situ in lake littoral in natural photoperiod (LD) and constant darkness (DD) conditions in spring, summer, autumn and winter (12:12h, 16:8h, 12:12h and 8:16h light:darkness); every 4 hours haemolymph was isolated from 5 adults, tissues were immediately preserved in a fixative, or – in autumn – frozen for RNA. The level of serotonin was assessed by the ELISA method; the distribution of the 5-HT in tissues – by immunohistochemistry; molecular procedures required RT-qPCR.

We observed an endogenously driven circadian rhythm of serotonin level in haemolymph in *U. tumidus*; nevertheless, the influence of light on it was visible. We observed lower levels of 5-HT during the light phase of the day, and higher levels in the darkness in autumn and summer. During summer we confirmed that the daily changes of serotonin level in haemolymph are sustained in DD; however, the results from LD conditions show a strong influence of the long day light on *U. tumidus* physiology. During winter's long night we observed a continuous, generally higher level of the haemolymph serotonin in LD conditions and continuous low levels in DD. In spring we observed constant medium values of 5-HT in haemolymph in both LD and DD.

No daily and seasonal changes in 5-HT levels in bivalve tissues were observed.

We identified sequence of *Bmal1* clock gene and described its daily expression profiles in different tissues of *Unio tumidus*.

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## Presence and persistence of the neurotoxin domoic acid in cephalopod brains

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Domoic acid (DA) is a neurotoxin that causes degenerative damage to brain cells and induces permanent short-term memory loss in highly developed animals. In cephalopod mollusks, although DA is known to accumulate primarily in the digestive gland, there is no knowledge of DA accumulation in their brains. Here we report, for the first time, the presence of DA in the brains of the common octopus (*Octopus vulgaris*) and the European cuttlefish (*Sepia officinalis*), and the absence in the brains of several

squid species (*Loligo vulgaris*, *L. forbesi* and *Todarodes sagittatus*). We argue that such species-specific differences are related with their different life strategies (benthic/nektobenthic vs pelagic) and feeding ecologies. Additionally, the temporal persistence of DA in octopus' brain reinforces the notion that these invertebrates can selectively retain this phycotoxin, or be exposed to low toxin concentrations for long periods of time during and after the decay of DA-producing diatom blooms.

## Invasive bivalves in European fresh waters: impacts from individuals to ecosystems

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Invasive bivalves may cause great ecological, evolutionary and economic impacts in freshwater ecosystems. Species such as *Corbicula fluminea*, *Dreissena polymorpha*, *Sinanodonta woodiana*, among others, are widely distributed hyper-successful invaders in Europe. These species can affect hydrology, biogeochemical cycling and biotic interactions through several mechanisms, with impacts ranging from individuals to ecosystems. In this communication, and through the presentation and discussion of several

case studies, we will show that freshwater invasive bivalves can create no-analogue ecosystems, affecting several groups of organism from bacteria to mammals and posing serious difficulties for management. Although knowledge about the ecology of these bivalves has increased considerably in the last two decades, several fundamental gaps still persist and we will end this communication suggesting new research directions that are worth exploring in the near future.



## Risk assessment and management of invasive non-native molluscs

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Invasive non-native species represent one of the greatest threats to global biodiversity and have become a priority for management. Bivalve molluscs are a particular concern because they often reach high abundances and serve as 'keystone species', driving dramatic ecosystem-level shifts. However, not all non-native species represent an equal risk and not all geographies are equally vulnerable to a particular invader. The identity of future potential invaders can be assessed through formal horizon-scanning exercises. The potential distribution of high risk species

can then be predicted through species distribution modeling. Rapid response plans can be written for those species of greatest concern, with control actions based on a robust evidence base. In some cases it may be possible to control or eradicate invasive molluscs. Examples will be given for the assessment of invasive non-native bivalve molluscs in Europe, with a particular focus on the impacts and control of zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena rostriformis bugensis*).

## FRESHCO: Multiple implications of invasive species on freshwater mussel decline and coextinction processes

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Freshwater mussels (FM) are among the most threatened faunistic groups worldwide. FM depend on fish to complete their life cycle, where mussel larvae (glochidia) use a specific range of fish hosts. This trait makes them particularly threatened by invasive alien species (IAS) since changes on the host fish populations may eventually lead to the decline or co-extinction of the dependent species. The Iberian Peninsula holds a high level of threatened freshwater species, including fish and mussels. Furthermore, Iberian freshwater systems are among the most heavily invaded ecosystems in the world. Under those considerations, the FRESHCO project aims to estimate the direct and indirect impacts of selected highly IAS belonging to distinct functional groups (from plants to vertebrates) on the native Iberian FM and its fish host fauna. Five tasks (T) will be executed: T1 will use field and lab experiments to assess the possible competition between the IAS *C. fluminea* and native FM; T2 will be composed of a series of

lab experiments to assess host fish suitability of native FM; T3 will use molecular and classical detection tools to analyze the diets of selected IAS and assess their predation on FM larvae, juveniles and adults; T4 will use a battery of field and lab experiments to assess the impact of massive annual die-offs of the invasive aquatic plant *E. crassipes* on the native FM; and in T5, 150 sites of the Douro river basin will be surveyed where mussel and fish assemblages and their habitats will be characterized, in order to evaluate and distinguish the impacts of IAS and habitat degradation on the native fauna. From an ecological point of view, expected results from this project would help to elucidate the real impacts of the most prominent IAS on the threatened native FM and fish species in Iberia. From a management point of view, this project will facilitate a more effective allocation of resources spent on both native species conservation and IAS management.

## Potential impacts of the invasive species *Corbicula fluminea* on the recruitment of native freshwater mussels

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Freshwater mussels are one of the most threatened faunal groups globally; the introduction of invasive species being a possible major threat. The Asian Clam *Corbicula fluminea* is one of the most invasive species in aquatic ecosystems due to its opportunistic behaviour. Its high filtration capacity can be responsible for negative impacts in the recruitment of native freshwater bivalves; however, possible effects remain speculative and to date very few studies have addressed this issue quantitatively. Therefore, manipulative laboratory studies were performed to assess *C. fluminea* filtration impacts in the survival rates of larvae (glochidia) of different freshwater native

bivalve species (*Anodonta anatina*, *Potomida littoralis* and *Unio delphinus*). Through the glochidia counting and water toxicity analysis (ammonia) over 48h, we determined the mortality in different containers with different *C. fluminea* densities. Our results suggest that the mortality of glochidia and water toxicity (level of ammonia) increased with the density of *C. fluminea*. This study is one of the first showing the direct impact of *C. fluminea* on native freshwater mussels and this information should be taken in account in future management initiatives devoted to the conservation of freshwater mussels.

## Control of the invasive Gulf wedge clam, *Rangia cuneata*, using microencapsulated BioBullets

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Human activities facilitate the spread and establishment of invasive non-native species, which cause adverse ecological and economic impact. Once an aquatic invader has become established, control has rarely been attempted because traditional eradication programs are usually not cost-effective and often cause negative impacts to the native biota. An established population of Gulf wedge clam, *Rangia cuneata*, which has been recognized as invasive by the IUCN Invasive Species Specialist Group, has been discovered recently in Great Britain. In this study, we

investigated the control of the Gulf wedge clam in a low-cost and environmentally friendly way by using the newly developed microencapsulated BioBullets. Our results indicated that a single dose of 10–30 mg/L BioBullets in a closed environment could achieve 90% mortality after 30 days of the first exposure. Given the early stage of its invasion in Great Britain, and the restricted distribution of the species, eradication of the invasive Gulf wedge clam should be considered, especially by using this novel, cost-effective and targeted method.

## The role of molluscs alien to Europe in trematodes transmission

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Molluscs, and snails in particular, play an important role in trematode transmission. In the case of Digenea, they are the place for the production of tens of thousands of cercariae – larvae of parasites that actively infect both vertebrates and invertebrates. Additionally, molluscs play the role of second intermediate hosts, cumulating metacercariae transported in the food chain to the final hosts – including farm animals and humans. Some species may also be the site of sexual reproduction of parasites, although this variant is relatively rare.

The key role of molluscs in the life cycles of Digenea is due to a high specificity in the host-parasite system. This fact means that the spread of host molluscs determines the extent of parasites. Widening the boundaries of molluscs' range has been one of the most debated topics in recent decades. Most research on alien species in native ecosystems is devoted to their interactions with the free-living elements of biocoenosis – competition and predation in particular. Interest in the interaction between alien molluscs and parasites in invaded area in the short term perspective has not produced fruitful re-

sults. However, the detailed search for the traces of new associations allows for a better understanding of the long-term effects on biocoenosis.

We are studying natural and experimental invasions of trematodes to molluscan species alien in Europe: *Potamopyrgus antipodarum*, *Sinanodonta woodiana*, *Dreissena polymorpha*, *Dreissena bugensis* and *Corbicula fluminea*. Of these, only in *Dreissena bugensis*, and in *Corbicula fluminea* did we fail to find parasites. The remaining species were invaded by *Aspidogastrea* or *Digenea* species. The infected molluscs were first or second intermediate hosts and in some cases were infected with pre-adult form of parasites. Also some experimental attempts to infect alien species of molluscs succeeded. Only the use of echinocercariae – less specific to the second intermediate host resulted in an effective invasion.

The results show a potential and real possibility of building associations between alien species of molluscs and native parasitofauna. Getting to know the long-term effects of new associations requires the parasitic web to be included in environmental monitoring studies.

## Enemy release hypothesis: comparison of parasites infection in native and invasive species in Lake Maggiore

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In the last decade, in Lake Maggiore (Italy), the dominant native mussel (*Unio elongatulus*) decreased on average by 75%, and was gradually replaced by the invasive *Corbicula fluminea*. Could the enemy release hypothesis explain this heavy invader impact? The base-case scenario assumes the introduction of an alien invasive species (AIS) to a new region leads to a decrease in the regulation by its natural enemies (predators, parasites and pathogens), resulting in its rapid increase in distribution and abundance. Did parasites play a powerful role in the shift from a native dominated mussel assemblage to an invasive one? We compared the prevalence and intensity of infestation in the native and in the invasive species in an area colonized since 2010. Data from 2016 showed that *C. fluminea* is completely parasite-free, whereas *U. elongatulus* is heavily infested with tissue-dwelling watermites (91–94% prevalence) and sterilizing trematodes (*Rhipidocotyle campanula* – 72% prevalence). Although the results are consistent with the enemy release hypothesis, they are not enough to assess whether *C. fluminea* simply has taken advantage of native mussel disease, or whether it has

affected the rates of parasitism by weakening the resistance of native bivalves to infections. Since physiologically compromised mussels are more susceptible to parasitism, we hypothesized that the arrival of an alien species caused an increase in the parasite load contributing with other stresses (drought, heat waves) to weaken mussels' resistance to infection. If the hypothesis is true, the rate of parasitism in *U. elongatulus* should be lower before *C. fluminea* arrival, than after. By contrast, the comparison with samples collected in 2002–2006 in the same area, surprisingly revealed that the native population was heavily infested even before the invasion. However, before the invasion, the density of *Unio* was roughly constant with 46 ind/m<sup>2</sup>, but it declined to about 2–3 ind/m<sup>2</sup> after *Corbicula*'s establishment. Hence, in agreement with the enemy release hypothesis: 1) the alien invasive species did not affect the parasite load, 2) AIS was advantaged by being parasite-free, while the native reproductive capacity and physiological status were lowered by heavy infection; 3) the invaders' competition exacerbated the effects of parasites on the native population.

## Effects of globalisation – new arrivals of mainly tropical land snails in European warm houses

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Arrival of new species in warm houses might be the first step of colonisation, although currently they neither yet established nor invasive in the wild. Additionally, these species are already a direct threat to horticulture and some might adapt to the new and possibly changing climatic conditions.

While some species like *Hawaiiia minuscula* (Binney, 1840) (Pristilomatidae), *Opeas hannense* (Rang, 1831), *Allopeas clavulinum* (Potiez & Michaud, 1834) and *Subulina octona* (Bruguière, 1789) (all Subulinidae) are well established in greenhouses, a cursory search in Central European warm houses revealed a surprising additional diversity of mainly tropical land snails. This included places like the tropic house of

the Zoological Garden Schönbrunn, Vienna, which appeared well-studied and became malacologically “famous” by the description of even a new genus (*Schwammeria* Schileyko, 2010) based on snails established there.

Findings of exotic snails also often involves problems in identification because usually the origin is unknown and/or even if so, the respective groups are not well studied in the original area of occurrence, e. g. small tropical Euconulidae. New records of selected species and related taxonomic problems will be presented. In the overall species assemblage, a shift from mainly American and African species towards an increasing number of Asian species is recognised.



## Predicting the distribution of *Helix lucorum* Linnaeus, 1758 – a silent invader snail

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The Turkish snail *Helix lucorum* is a large helicoid land snail with Mediterranean–Black Sea basin distribution. However, the species is steadily extending its range northward and in other areas of the world. In some regions, the Turkish snail can act as a significant pest while in many countries it is an important food resource. Although there is wide public interest in this species, species, distributional data, invasion status and distribution trends are rather limited and scattered. In this work, we assessed the modern distribution of the Turkish snail throughout its range and developed predictive models based on climate change scenarios to see its climate-related invasion potential. In addition, we used modern distribution data to project past (mid-Holocene) distribution of

the Turkish snail and to study temporal dynamics of range variation on a geological timescale. Results show that the snail has probably achieved its maximum distribution extent in Europe under the supposed climate change assumptions; however, other parts of the world are still susceptible for invasion. In a historical perspective, the Turkish snail shows a rather strong range extension since the mid Holocene (ca. 6000 years BP) from south to northern European regions indicating strong climate related distribution of this species. In addition, human assisted distribution and high propagule pressure can result in occurrence of populations of the Turkish snail in rather harsh environmental conditions.

## Evolution of higher gastropods – important fossil calibration points

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‘Higher gastropods’ comprises the highly diverse gastropod subclasses Caenogastropoda and Heterobranchia and therefore the great majority of living gastropod species. They are sister-groups according to most phylogenetic analyses. Heterobranchia can be identified by a change in shell coiling direction during ontogeny (larval heterostrophy). By contrast marine caenogastropods have orthostrophic protoconchs: larval shell and teleoconch have the same coiling direction. In principle, the minimum age of the split of both groups can be recognized by the co-existence of ortho- and heterostrophic larval shells. The oldest evidence for larval heterostrophy has been reported from the Early Devonian but seems not to be entirely unequivocal due to poor preservation. In the Late Palaeozoic, high-spired heterobranchs with flatly sinistral larval shells are globally distributed (Donaldinidae, Streptacididae). Some of them closely resemble extant forms such as Ebalidae and Murchisonellidae. In the earliest Triassic, the first architectibranchs (or opisthobranchs) appeared representing the family Tubiferidae and at the same time the earliest mathildoids appear indicating a ma-

JOR turnover in Heterobranchia after the end-Permian mass extinction event.

The oldest modern-type caenogastropods with preserved orthostrophic larval shells of the planktotrophic type have been reported from the Early Carboniferous but are probably much older (protoconch preservation is rare in the Palaeozoic). High-spired slit-bearing gastropods (Murchisonia-like) from the Carboniferous have a larval shell resembling that of some modern cerithioids. The first undoubted member of Cerithioidea combining an anterior siphonal canal with a bi-carinate larval shell has a Middle Permian age. Stromboidea originate with Aporrhaidae in the Early Jurassic. The stromboids with narrow apertures appear in the Late Jurassic. The oldest possible neogastropod has an Early Jurassic age. Cypraeidae appear in the Late Jurassic. Highly diverse parasitic families such as Triphoroidea originate in the Late Cretaceous.

These and other first occurrences represent both true originations and preservational windows. Therefore, these dates must be used with caution for the calibration of phylogenetic hypotheses.

## Are rudists cardiids? Cardioidea, Hippuritida and their potential relationships

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Although not entirely focused on the origins of the modern Cardiidae, this study sheds light on the early days of one of the most prominent Cenozoic bivalve clades, the Cardioidea. The first unequivocal cardioids appeared in the Late Triassic of the Tethys (*Tulongocardium*). Until the Late Cretaceous cardioid diversity remained low, and, apart from *Protocardia*, cardioids were rather uncommon. However, they may actually have been more abundant than generally thought. Several Jurassic cardioids have not been recognized as such, or were not included in previous phylogenies and revisions of the group. Some species certainly have been misclassified as *Pachyrismatidae*. Moreover, I propose that *Pachyrisma* itself presum-

ably is a cardioid. This is based on newly discovered features of ornamentation and hinge dentition in *Pachyrisma grande*, the type species of the genus, which support an origin in the Cardioidea rather than the *Megalodontidae* for this taxon. If, as previously hypothesized, *Pachyrisma grande* is the last known ancestor of the Hippuritida, then, rudist bivalves are actually cardiids. Several arguments in favor of this hypothesis will be presented – as will be the caveats that remain. Moreover, a general account of Mesozoic Cardioidea will be given, including those taxa that gave rise to the impressive radiation of this bivalve group during the Cenozoic.

## The ancient origin and long fossil record of neomphalid and provannid gastropods from chemosynthesis-based ecosystems

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Neomphalids and provannids are the most typical taxa of gastropods thriving around deep-water hydrothermal vents. Both are endemic to chemosynthesis-based ecosystems and they commonly occur in significant numbers. Several species cultivate chemosynthesizing bacteria as a main source of nutrition. For many years both groups were either considered to be ancient or, in contrast, very modern evolutionarily. The intensive research on ancient hydrothermal vents, vertebrate/wood falls, and especially hydrocarbon seeps has revealed that they are old indeed. The oldest record of provannids is known from the Albian (113–100 Myr old) while oldest representative of neomphalids is known now from the Toarcian (183–174 Myr old). Both records are from ancient hydrocarbon seeps, which are relatively common in the fossil record. In contrast, the fossil record of hydro-

thermal vents is extremely scarce; however, one of the few known vent deposits from Cyprus (Turonian, 91 Myr old) yields numerous shells of pyritized provannids. The origin of the clade Neomphalida remains a mystery. It appears with Retiskenea-like species in the Early Jurassic and their ancestors are unknown. On the other hand, Provannidae are probably paraphyletic with at least two distinct groups, which appeared already in the Cenomanian (100–94 Myr old). They form with non-seep Abysochrysoidea a clade Abysochrysoidea. Most likely extinct Paskentanidae and Hokkaidoconchidae (both endemic to seeps and vents) also belong to this clade. The ancestors of entire group probably stem from Zygopleuroidea, which are an extinct clade highly diversified in the Mesozoic times.

## Paleocene faunal migration between northern Pacific and Arctic regions

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It is believed that the Arctic basin was isolated from other oceans during the early Paleogene. Recently, the Paleocene astartid bivalves and aporrhaid gastropods have been recovered from the Paleo-western Pacific margin deposit of Katsuhira Formation, eastern Hokkaido, Japan. The aporrhaid gastropods flourished in the Cretaceous and suffered from severe extinction at the K/T boundary. The Katsuhira species, *Kangilioptera inouei* is similar to *K. ravni* from the Paleocene deposit of western Greenland. From Spitsbergen, the Paleocene apor-

rhaid has been found. In the northern Pacific, the Paleocene *Astarte* is very small in size and has been recorded from Hokkaido and Sakhalin. These species are similar to *A. trigonula* from the Paleocene of Denmark. Moreover, the Paleocene *Conchocele conradii* has been found in the Paleocene of Spitsbergen. Species of this genus are common in the Eocene and Oligocene of the northern Pacific area, and are common in cold seeps of that age. Based on these criteria, the Arctic fauna had some connection with the northern Pacific one.

## Land snails of the British Paleogene

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Land snails occur frequently in the British Lower Tertiary. A few specimens have been recovered from marine sediments of the London Basin, where they are represented mostly by internal pyritized casts from the London Clay. The richest assemblages, however, occur in the Hampshire Basin and especially in various freshwater limestone formations of late Eocene (Priabonian) age on the Isle of Wight. Preservation is best in the Hatherwood Limestone, where the shells are still composed of aragonite, allowing the examination of fine details of shell microsculpture, such as hair pits. Colour banding even survives in some specimens from this limestone. The younger Bembridge Limestone has yielded the most diverse land snail faunas from the British Paleogene, with

over 50 taxa known. Preservation in the Bembridge Limestone is generally inferior, where the shells are mostly preserved as calcite. At some localities, the shells occur as internal casts, which allow detailed study of any internal dentition. Micro-CT scanning provides a new, non-destructive, way to examine the internal architecture of critical specimens. The land snail fauna of the Bembridge Limestone contains a range of species from forest and marshland environments. Many of the genera still occur in mainland Europe but some (e.g. *Craspedopoma*) are now restricted to Atlantic islands or are known living exclusively in the New World (e.g. *Proserpina*). Some species have been hard to place in existing genera or families.

## Empty bivalve shells: importance of heterogeneity and the role of invasive species in riverine systems

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Bivalve shells are a relevant ecological attribute that can introduce complexity and heterogeneity into the benthic environment creating a habitable substrate for several species. Nowadays a gradual replacement of the native fauna by the non-native is occurring in many freshwater systems. Abundant and dominant invasive bivalves can contribute to the accumulation of large quantities of shells of the same species (i.e. homogenization). Therefore, it is fundamental to evaluate the influence of the gradual changes in the substrate, provided by the increasing densities of some invasive shells, to the associated fauna. Empty shells of two native (*Potomida littoralis* and *Unio delphinus*) and one non-native (*Corbicula fluminea*) species were used to evaluate different substrate composition in the colonization of the as-

sociated fauna. Seven treatments were made, three of them consisting in homogeneous substrates, in which only shells of one species was used, and four of them in heterogeneous substrates, in which shells of more than one species were used. The composition of the associated fauna was significantly different in the comparison between treatments (PERMANOVA: Pseudo-F=2.52,  $p < 0.01$ ). Treatments with more than one species showed high density of individuals, richness and diversity. The results of the present study suggest that the associated fauna optimal level of abundance and species richness are related to the increase in the substrate heterogeneity provided by the presence of different shells, and that native bivalve shells play an important role in maintaining the optimal level.



## Genetic diversity used to investigate the colonisation and spread in Europe of the invasive clam *Rangia cuneata* (Bivalvia: Mactridae)

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The transcontinental invasion of the Gulf Wedge Clam *Rangia cuneata* (G.B. Sowerby I, 1831) from North America to Europe, occurred recently. The species was first observed in Europe from Antwerp, Belgium in 2005 and shortly afterwards the Netherlands in 2007. Further discoveries occurred from Baltic sites in Russia and Poland in 2010 and 2011, from Germany in 2013 and eastern England in 2015 with dead shells recorded in Estonia in 2016. We examined the genetic diversity of *R. cuneata* populations in Europe (invasive) and North America (native and invasive) to analyse its invasion history. Most invasive populations were characterized by a

high diversity of the mitochondrial cytochrome oxidase I (COI) gene fragment. We suggest that the clam's invasive history can partially be explained by the 'invasive bridgehead effect' whereby invasive populations serve as a source for further colonization. We consider that the first successful European *Rangia* populations in Belgium and the Netherlands served as a base for secondary invasions. Our results also support multiple introductions into Europe from at least two sources, these being the Gulf of Mexico and the estuaries of the North American east coast. Additionally, ecological data is included on studies of the only known English *Rangia* population.

## Comparative functional responses to explain the success of invasive species – a study of sympatric zebra and quagga mussels

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Invasive species management currently lacks tools that can be reliably applied across a range of environments to predict invasive species success. A comparative functional response approach may offer considerable insight and predictive power regarding invader impacts. This could prove valuable in the management of invasive species that drive losses in ecosystem function and biodiversity. In order to explore this, we conducted feeding experiments in the laboratory to evaluate the use of functional responses in predicting the relative success of two sympatric invasive species of dreissenid mussel; zebra and quagga (*D. polymorpha* and *D. rostriformis bugensis* respectively). Feeding rates and functional responses

were calculated for each species under a range of algal concentrations and temperatures; the different 'contexts'. We found a strong difference in feeding rates for both species between the 4°C and 24°C experiments, which is likely attributable to reduced metabolism. Results also show revealed that both species exhibit type I functional responses across all conditions studied. Thus, competition and exclusion between the study species does not appear to be driven by disparities in resource use. We conclude that functional responses should be adapted as both a diagnostic tool for understanding species interactions and as a predictive tool for ecological forecasting.

## What explains shell size variation in snails of recent and extinct long-lived lakes?

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Here we present the results of a recent study investigating shell size variation among gastropod faunas of fossil and recent long-lived European lakes and discuss potential underlying processes. Based on a dataset of 1412 species of lacustrine gastropods from 23 Miocene to recent long-lived lakes, we assessed differences in shell size in terms of characteristics of the faunas (i.e., species richness, degree of endemism, differences in family composition) and the lakes (i.e., surface area, latitude and longitude of lake centroid, distance to closest neighboring lake) using multiple and linear regression models. Because of a strong species-area relationship, we applied a resampling approach to determine whether any of the observed correlations is driven by that relationship.

The regression models indicate size range expansion rather than unidirectional increase or decrease

as the dominant pattern of size evolution. The multiple regression models for size range and maximum and minimum sizes are statistically significant, the model with mean size is not. Individual contributions and linear regressions indicated species richness and lake surface area as best predictors for size changes. Resampling analysis revealed no significant effects of species richness on the observed patterns. The correlations are comparable across families of different size classes, suggesting a general pattern.

Although the most outstanding and attractive examples for size evolution in lacustrine gastropods derive from lakes with extensive durations (e.g., Lake Pannon), shell size appears to be independent of the duration of the lake as well as longevity of a species.

## Molluscs in palaeoclimatic investigation: Holsteinian palaeolake record from Eastern Poland

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The typical approach of applying molluscs to palaeoclimatic studies is based on the structure and composition of mollusc assemblages. The climatic preferences of mollusc species and their modern distribution across Europe may be used for a general characterisation of climatic conditions. Warmth-demanding species usually point to interglacial conditions, whereas cold tolerant taxa may be indicative of cold periods. In this study,  $\delta^{18}\text{O}$  composition of mollusc shells was applied for quantitative estimation of past water temperatures of four Holsteinian (MIS 11c) palaeolakes from eastern Poland: Ortel Królewski, Ossówka-Hrud, Roskosz and Szymanowo Lakes. The  $\delta^{18}\text{O}$  shell values of aquatic gastropods *Viviparus diluvianus* and *Valvata piscinalis* were converted for the absolute growth-season past water temperatures with a use of carbonate thermometry equation. The calculation was based on  $\delta^{18}\text{O}$  shell values and the  $\delta^{18}\text{O}$  water assumed for the Holsteinian from modern oxygen isotope composition of precipitation and expected amount of evaporative enrich-

ment. The mean oxygen isotope palaeotemperatures of Ortel Królewski lake waters were in the range of 18.1–21.9°C covering both the *Taxus* and *Pinus-Larix* pollen zones. Ossówka-Hrud and Roskosz Lakes had mean temperatures of 17.4–21°C during the climatic optimum of interglacial (*Carpinus-Abies* zone), whereas the temperature of Szymanowo lake waters was estimated at 20.6–21.7°C at that time. These values were concordant with the pollen-inferred July air temperatures noted during the Holsteinian in eastern Poland. Relatively high values of ~25°C in the post-optimum noted at Szymanowo may be connected with the water depth rather than with significant climate change indicating shallow, warm waters of an isolated bay. Despite some speculation about past water  $\delta^{18}\text{O}$  composition, the high integrity of estimated palaeotemperatures with other proxy records makes the isotope signature of aquatic mollusc shells prospective for quantitative climatic interpretations of Pleistocene interglacials.

## Quaternary malacology in Czechia and Slovakia

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Modern approaches in Quaternary malacology started in the 50s and 60s of the last century, culminating in the book by Vojen Ložek: *Quartärmollusken der Tschechoslowakei*. Recently, more than 600 localities with Holocene and Pleistocene mollusc fossil records have been sampled, being successively processed and radiocarbon dated. We just start with use of the Holocene part of this worldwide unique dataset.

It seems that the general trend of postglacial development consists of interacting patterns of specific and partly independent local histories. We believe that the time is now ripe for more locally focused studies on the development of particular landscapes, with the aim of elucidating the biotic processes behind the emergence of general biogeographic patterns. Several mollusc successions covering the variability of particular landscape were studied. Five

existing palaeofaunistic studies of five and more successions per landscape clearly show specific developments in studied areas within relatively small part of central Europe. Areas of prehistoric agriculture settlement consist of mosaic of woodland, open country habitats during the whole Holocene and fully developed canopy-forest communities never developed. On the contrary, in the area of the Moravian Karst, where religious use of landscape predominates, fully developed woodland assemblages remain to recent times. Mountain successions of mollusc communities were continuous, but differ in the Bohemian Massif and Western Carpathians. While Holocene mollusc assemblages of the Bohemian Massif seem to be relatively poor, Western Carpathians are probably a source of local endemism but not a source of postglacial colonization of Central Europe.

## **Ecosystem services provided by freshwater mussels**

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Ecosystem services are the benefits that people derive from ecosystems. Freshwater mussels perform many important functions in rivers and lakes, which can in turn be viewed as the ecosystem services that they provide. Ecosystem services provided by freshwater mussels include regulating services such as biofiltration, nutrient recycling and storage; supporting services such as structural habitat, substrate modification, environmental monitoring, and food for other organisms; and provisioning and cul-

tural services, including tools, jewelry, and spiritual enhancement. Ecosystem services provided by mussels are declining because of large, global declines in mussel abundance. Propagation can be used to restore biomass of common mussel species and their services. Research on the value and magnitude of ecosystem services provided by mussels, across species, habitats, and environmental conditions, and scaled up to whole watersheds is needed.

## Using environmental DNA metabarcoding for freshwater bivalves biodiversity assessment

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Freshwater ecosystems are the most threatened ecosystems worldwide and freshwater bivalves rank amongst the most threatened animals in the world. The survey of freshwater bivalves is challenging. First because the freshwater ecosystems themselves are challenging for the biologist: deepness, turbidity, current and sometimes navigation make them difficult to access. Second because freshwater bivalves are difficult to sample. Some live buried in the sediment, or are covered with algae or mud making them hard to distinguish, some species are rare and scarce, some species are minute, with shells length measuring about a millimeter. For all these reasons, we lack efficient methods to sample all freshwater bivalve species. Moreover, freshwater bivalve species are difficult to identify in the field, leading to a lack of taxonomic expertise. Indeed, species determinations rely on few diagnostic characters, with a high variability. Shell plasticity in unionoids is documented by a plethora of classical and modern literature. Consequently, and despite their worrying conserva-

tion status, freshwater bivalves are often overlooked by conservation policies and environmental impact studies.

The use of environmental DNA (eDNA) has been shown to be a valuable survey technique for amphibians, fishes and other aquatic organisms. Among freshwater molluscs, some experiments with gastropods and bivalves have proven to be successful. However, these were aiming at only one species; or at most one genus. Metabarcoding is based on dedicated primers calibrated to amplify all the haplotypes of a focused gene in a group of species. First results with eDNA metabarcoding of freshwater organisms were obtained recently for fishes and amphibians.

Here, we present the first metabarcoding approach for all freshwater bivalves of France, and compare eDNA surveys to traditional survey methods. The results show that eDNA metabarcoding is a reliable tool, it allows highlighting unnoticed species such as newly introduced ones and it is a revolutionary tool for freshwater bivalves' conservation.

## Defensive behaviour in the sandy beach gastropod *Olivella semistriata* (Olivellidae, Caenogastropoda): variability, ecological context, and efficiency in predator-prey interactions

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The intertidal communities of sandy beaches of the Panamic faunal province (tropical pacific coast of the American continent) are often dominated by small gastropods, *Olivella semistriata* (Olivellidae) in Central America and *O. columellaris* in South America. The animals are suspension-feeders in the backwash of the beach, and perform tidal migrations to follow the movement of the backwash zone through the tidal cycle. Due to the open nature of their habitat, their behaviour and trophic interactions lend themselves to direct observation and study.

We here discuss the complex defensive behaviours applied by *O. semistriata* in response to its main predator, the gastropod *Agaronia propatula* (Olividae). *O. semistriata* responds to olfactory and – unusual for a gastropod – mechanical stimuli originating from its predator. When encountering a recent track of *A. propatula*, or when exposed to water-borne scents of *A. propatula*, *O. semistriata* will ‘freeze’ and remain motionless for prolonged periods. This olfactorily-induced response is highly efficient in the context of the sensory capabilities of hunting *A. propatula*,

which under certain circumstances respond almost exclusively to mechanical input. On the other hand, *O. semistriata* shows active flight responses that can be elicited by purely mechanical stimuli. Depending on the direction of the stimulus and other conditions, these responses include ‘jumping’ from the burrow in the sand, active swimming, a curious ‘somersaulting backwards’ to turn away from the stimulus, and an accelerated mode of stepwise locomotion that was mentioned but described incorrectly in the older literature. A critical evaluation of several hundred predation events in the wild, which we documented over the last seven years, together with behavioural field experiments demonstrated the effectiveness of the defensive flight behaviour in avoiding capture by *A. propatula*. On a more general level, the research presented here confirms our previous conclusion that certain neotropic intertidal communities represent particularly accessible systems for the study of the complex behavioural and sensory physiological basis of trophic networks among mollusks.



## Relationship status: “it’s complicated”. Responses of the zebra mussel *Dreissena polymorpha* to conspecifics

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Many papers have indirectly assumed that the zebra mussel creates dense 3-dimensional colonies due to its preferences for conspecifics. However, none of them clearly demonstrated relations between conspecifics and their presumed preference for one another. Looking at the complicated structure and high density of a *Dreissena polymorpha* colony, we can suppose that the life in a mussel colony is a compromise between the protection from negative environmental factors (e.g. predators) and deteriorating environmental conditions. We hypothesised that zebra mussels may not exhibit such a high affinity for conspecifics as it had been assumed. Our aim was to check reactions of *D. polymorpha* to conspecifics. We observed vertical locomotion (experiment 1) and valve movement (experiment 2) of tested mussels in the presence of other individuals (physically separated, in different densities). In experiment 1 we put tested mussels in plastic pipes which were placed in the centre of 1.5L tanks, above the bottom covered by

a 1-cm layer of mussels or without it. In experiment 2, mussels were video-recorded and behaviour analysing software was applied to follow the movements of a colour mark on the shell. In the presence of conspecifics, tested mussels more often moved upwards, spent more time with open valves and opened their valves more widely. Probably, zebra mussels avoid a direct contact with conspecifics, whose scent stimulates the escape behaviour to the upper part of a colony. The fact that adult zebra mussels in a colony are fouled by younger conspecifics makes this assumption more likely. The longer opening time may indicate some kind of competition among crowded mussels for food resources, making them intake water more intensely than in the absence of conspecifics.

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## Parasites – the enemies of (native) freshwater mussels

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Enemy Release Hypothesis (ERH) has been proposed to explain the success of biological invasions. ERH suggests that invasive species are ‘released’ from their enemies – such as parasites – when they leave their home range. Thus, in the new, invaded range they do not need to pay the cost of parasitism, giving them an advantage in competition with the native species that are suitable hosts for the parasites present at the new, invaded habitat. Prerequisite for ERH is that parasites are prevalent and harmful. I will present results, based on my own studies and literature, (i) on the prevalence and abundance of parasitism in freshwater mussels, and (ii) on the effects of parasites on their mussel hosts. A variety of parasite taxa infect freshwater mussels including ciliated protozoans, ergasilid copepods, oligochaetes, chi-

ronomids, nematodes, watermites, trematodes and fishes. For example, watermites are very common in practically all mussel species. Larval watermites encysted in the mussel tissues can occur in thousands of individuals per mussel. Sporocysts of bucephalid trematodes can decrease the growth, reproduction and survival of mussels, or completely sterilize the mussel host. Prevalences of bucephalid infections are usually less than 10% but can be as high as 70–90%. In general, the evidence that is available about parasitism in freshwater mussels points to a potential negative impact and frequent occurrence of parasites – a necessary condition for ERH. Therefore, if invasive mussels are released from parasitism, as suggested by ERH, parasitism may favor the invasive mussels and contribute to their success.

## The role of local adaptation and fish resistance in shaping freshwater mussels host relationships

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Bivalves of the order Unionida (freshwater mussels) critically depend on freshwater fish to host their parasitic larvae; however, the role of coevolution in shaping freshwater mussel host relationships remains poorly understood. We used an East Asian bivalve, *Sinanodonta (Anodonta) woodiana* (Lea 1834), and its potential fish hosts to study the mechanisms shaping host specificity. *Sinanodonta woodiana* is invasive worldwide and its expansion is characterized by a generalist use of fish hosts. We tested whether generalist host use of *S. woodiana* is pertinent to native host species, with a special attention to bitterling fishes (Acheilognathinae) that are characterized by a mutual association with freshwater mussels, including *S. woodiana*. We also tested whether the pattern of the parasite-host association varies temporally (between areas of ancient and recent sympatry) and spatially (at a sub-basin level in its native range). Laboratory experimental and field-collected data revealed the ability of *S. woodiana* to widely ex-

ploit non-bitterling fish host at a global scale. In contrast, the ability of *S. woodiana* to exploit closely associated bitterling fishes was low in its native range (with ancient sympatry). In areas of recent sympatry (non-native *S. woodiana* range in Europe), *S. woodiana* glochidia were demonstrated to readily parasitize local, evolutionarily naive bitterling species at high density. The results of a population-level experiment with three native populations of *S. woodiana* and rose bitterling, *Rhodeus ocellatus*, from various sub-basins of the River Yangtze confirmed that mussel populations vary in their compatibility with particular host populations. However, there was no evidence of population-specific adaptive coevolution. This study contributes to the understanding how species-level host resistance and population-level variation shape compatibility between glochidia and their fish hosts.

The research was funded by the Czech Science Foundation (13-05872S).

## Encystment of parasitic freshwater pearl mussel (*Margaritifera margaritifera*) larvae coincides with increased metabolic rate and haematocrit in juvenile brown trout (*Salmo trutta*)

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Gill parasites on fish are likely to negatively influence their host by inhibiting respiration, oxygen transport capacity and overall fitness. The glochidia larvae of the endangered freshwater pearl mussel (FPM, *Margaritifera margaritifera* (Linnaeus, 1758)) are obligate parasites on the gills of juvenile salmonid fish. We investigated the effects of FPM glochidia encystment on the metabolism and haematology of brown trout (*Salmo trutta* Linnaeus, 1758). Specifically, we measured whole-animal oxygen uptake rates at rest and following an exhaustive exercise protocol using intermittent flow-through respirometry, as well as haematocrit, in infested and uninfested trout. Glochidia encystment significantly affected whole-animal metabolic rate, as infested trout exhibited higher standard and maximum metabolic rates. Furthermore, glochidia-infested trout also had

elevated levels of haematocrit. The combination of an increased metabolism and haematocrit in infested fish indicates that glochidia encystment has a physiological effect on the trout, perhaps as a compensatory response to the potential respiratory stress caused by the glochidia. When relating glochidia load to metabolism and haematocrit, fish with low numbers of encysted glochidia were the ones with particularly elevated metabolism and haematocrit. Standard metabolic rate decreased with substantial glochidia loads towards levels similar to those of uninfested fish. This suggests that initial effects visible at low levels of encystment may be countered by additional physiological effects at high loads, e.g. potential changes in energy utilization, and also that high numbers of glochidia may restrict oxygen uptake by the gills.

## Can invasive alien fish species act as effective hosts of native freshwater mussels (Unionidae) in Iberia?

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The biodiversity of Mediterranean areas, particularly in freshwater ecosystems, is severely endangered. Affiliate species are particularly threatened by invasive alien species leading to biotic homogenization. It is known that freshwater mussels (Bivalvia, Unionoida) have an obligatory parasitic phase of a short-term larval stage on host fish gills and fins and this relationship can be sometimes highly specific. In Iberia, the identification of effective host fish for Unionidae species, and the impact of invasive alien fish species remain unclear. In fact, recent negative human impacts, like river regulation, dam constructions, water pollution and general habitat degradation, are drastically diminishing the native fish and mussel populations and conservation measures are necessary. However, basic information such as the identification of host fish is still to be obtained. In this study, in situ and laboratory studies were developed to evaluate host fish in Iberia for *Anodonta anatina* (Aa), *Unio delphinus* (Ud) and *Potomida littoralis* (Pl), considering both native and non-native fish

species. The results showed, for in situ experiments developed in the Douro basin (Northern Portugal), an effective but wide range of infestation for native (*Luciobarbus bocagei*, *Squalius carolitertii*, *Squalius alburnoides*, *Pseudochondrostoma duriense*) but also for non-native (*Lepomis gibbosus*, *Alburnus alburnus*, *Gambusia holbrooki*, *Gobio lozanoi*) fishes. The highest infestation values obtained for native fishes were registered for *L. bocagei* (Ud: I > 3; FO > 50%; Pl: I > 0,3; FO > 16%; Aa: I > 0,5; FO > 27%), and for *L. gibbosus* (Ud: I > 1; FO > 30%; Pl: I > 0,05; FO > 5%; Aa: I > 1; FO > 50%;). In laboratory experiments, significant differences were also found. In fact, for *Unio delphinus* and *Potomida littoralis* only native fish (mainly endemic cyprinids) were confirmed as effective hosts, while *Anodonta anatina* showed a more generalist behavior, with a wide range of native (endemic cyprinids of north and south Iberia) and non-native (e.g. *Australoheros facetus*, *Alburnus alburnus*, *Oncorhynchus mykiss*) fish species.

## Glochidial infestation of fish by the endangered thick-shelled river mussel *Unio crassus*

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Freshwater mussels (Unionoida) are globally threatened. The key to the development of effective conservation strategies for this group is to unravel their complicated life cycle, including the obligatory larva, which parasitises the external tissues of fish. Larvae of the endangered mussel *Unio crassus*, was used to study infestation intensity by *U. crassus* glochidia in three fish species: the common nase (*Chondrostoma nasus*), European chub (*Squalius cephalus*), and common barbel (*Barbus barbus*).

*U. crassus* glochidia are considered parasites of fish gills; however, this study revealed that the number of glochidia attached to *S. cephalus* and *C. nasus* fins was four times higher than the number of glochidia attached to gills. *U. crassus* glochidia formed cysts on

the fins of all of the tested host species. The infestation prevalence was higher for *C. nasus* and *S. cephalus* than for *B. barbus*. Infestation intensity was the highest for *C. nasus*, but was also dependent on fin type (higher for fins used for active movement). The number of attached glochidia demonstrated a nonlinear relationship with fish body length. Inspection of dissected fish guts revealed that intestines of *C. nasus* significantly less frequently contained eaten glochidia than in the case of *S. cephalus* and *B. barbus*. The results imply that the intensity of infestation is related with the ecological niche of the fish species, mainly the host foraging ecology. These factors should be taken into account when elaborating best conservation practices, both in the field and in captivity.

## Effect of infection by *Margaritifera margaritifera* on growth of host fish

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The freshwater pearl mussel (*Margaritifera margaritifera*) has an obligate parasitic stage, glochidia, which is attached to the gills of salmonid fish host for up to 11 months. Glochidia take shelter and nutrition from the fish host by attaching to the host's gills for a longer period. Thus, glochidia may have a negative effect on the host's growth during their parasitic stage. We tested this by infecting juvenile brown trout (1+ year) with *M. margaritifera* glochidia in late August 2015, while a control group was kept as uninfected. Later, 2-week post infection, fish were tagged with Passive Integrated Transponder (PIT) and divided randomly into 3 high food tanks and 3 low food tanks with both infected and uninfected fish in every

tank. Growth of fish was monitored throughout the parasitic period by measuring the length (mm) and weight (g) in September, November, March, May and July. Infected fish ( $5422 \pm 1951$ , number of glochidia) weighed slightly less than the control fish throughout the experiment. Mean growth rate was also higher in control fish than the infected group of fish. However, the feeding regime or period had no significant effect on the difference. On average, the proportional increase in weight for control individuals was 11% higher than that for infected during the experiment. To conclude, results indicate that *M. margaritifera* glochidia parasitism has a negative effect on the fish host's growth.



## **The species concept for freshwater Mollusca: from Bourguignat to the present day**

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The history of development of the species concept(s) in 'freshwater' malacology is reviewed. The first attempts to determine what species of freshwater Mollusca is may be dated to the second half of the 19th century (W. Kobelt, C.A. Westerlund, A. Locard, C.T. Simpson). The French malacologist J.-R. Bourguignat was, probably, the most peculiar of these authors. He affirmed that a new species may be surely established if an animal is found to differ from all others by at least three conchological characters. Such a method led Bourguignat and his associates (a network of researchers known as the 'Nouvelle École') to discriminate a mammoth number of nominal species on the basis of extremely slight differences, usually in shell shape and proportions. Most contemporaries of the Nouvelle École strongly criticized such practice as leading to the taxonomical and nomenclatorial overload and did not accept the overwhelming majority of these 'new species'. An over-conservative approach to freshwater mollusk species discrimination arisen in the first half of the 20th century, perhaps as a reaction against this over-splitting movement. The extensive usage of anatomical data in taxonomy, especially for systemati-

zation of aquatic pulmonate snails, seemed to serve as a remedy against the extremities of the Nouvelle École. In the middle of the 20th century most researchers followed a conservative approach and tend to accept "a characteristically small number of species, almost world-wide in distribution, but with a high degree of infraspecific interpopulation variation" (Russell-Hunter, 1964). The anatomical and genetic (chiefly obtained by means of disc electrophoresis) data became the standard tool for species delineation in the 1950–1990s, though more bizarre methods (such as Starobogatov's comparatorial method) were also in use. The present situation in the field may be characterized by several statements. 1. No universally accepted species concept in taxonomy of freshwater Mollusca exists; 2. The molecular techniques and statistically based methods of species delimitation dominate, though the so called 'integrative taxonomic approach' is often viewed as the most proper one; 3. The molecular taxonomy often operates with intraspecific parataxonomic entities such as MOTUs, phylogroups and similar. Their significance for taxonomy needs to be clarified.



## In the speciation race, can molecules lag behind? Looking into morphological and anatomical patterns in *Oxychilus* (*Drouetia*) (Gastropoda: Pulmonata) from the Açores

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Molecular analyses have become indispensable in the quest for species determination, usually testing morphological data, often replacing them. The overwhelming heuristic power of molecular tools amply justifies this preponderance. Among the currently used molecules, cytochrome oxidase I (COI) occupies a prevalent place, mainly due to its generalized usage in barcoding. The various cases in which COI has been deemed insufficient for species determination have not seriously affected its general efficacy in taxonomy; however, such cases have called attention to the necessity of a holistic approach to the wide-ranging processes of speciation. The incongruence of molecular and morphological/anatomical data is herein dealt with in a case study of the speciation in *Drouetia* (Gude, 1911).

The oxychilid subgenus *Drouetia*, endemic to the Açores, is spread throughout the archipelago and contains the highest number of species (8) of

any of the islands' gastropod genera. Recent studies have shown that about a dozen more await description. This is the case with the islands of Flores and Corvo, where 6 taxonomic units have been identified. Statistical analysis of morphological/anatomical parameters has shown that these units differ significantly, thus providing grounds for specific differentiation; however, preliminary molecular research with COI has failed to provide clear support for such differences. Comparison with *Drouetia* in other islands has brought about evidence of different patterns of congruence between molecular and morphological/anatomical data. In an attempt to interpret the dynamics of speciation of *Drouetia* in the Açores, attention was paid to the geological/vulcanological history of the islands. This study illustrates the necessity of a holistic approach to the taxonomic work, if we are to understand the multi-faceted ways in which evolution works.

## Contributions of 3D geometric morphometrics to integrative species delimitation: the case of *Pyrenaearia* (Pulmonata: Hygromiidae)

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Since species are considered as fundamental units of biology, accurate delimitation of species boundaries is especially important. The variety of speciation processes are not equally reflected in different data types (e. g. morphological, molecular or ecological characters) and, thus, integrating multiple character types is an indispensable approach for rigorously delimiting species. The development of geometric morphometrics along with advances on 3D technology, has brought the possibility to combine genetic data and morphology comprehensively in groups where correctly handling morphological variation was not possible before, such as gastropods shells. We applied an integrated approach combining multilocus coalescent species delimitation methods with 3D geometric morphometrics on the shell to delimit the species within the endemic land snail genus

*Pyrenaearia*. Our results recovered a new taxonomic scheme for the genus identifying ten species. Two of the nominal species were within the range of phenotypic plasticity and the occurrence of hitherto an unrecognized cryptic species was revealed. Our findings show the importance of combining multiple lines of evidence since several species were overlooked if just one of the characters was used. Furthermore, joining morphological and molecular data we get insights into the evolution of biological forms and the factors involved. A strong evolutionary allometry was found suggesting that allometry plays an important role in the shape of mollusks shells. According to the new taxonomy and phylogenetic reconstruction, passive dispersal is an important driver of speciation within the genus, along with the glacial cycles of the Pleistocene.

## Phylogeny vs taxonomy of the Cretan *Albinaria* taxa

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The genus *Albinaria* is the most diversified taxon among all the other Cretan animal taxa, and one of the most cited of the malacofauna of Greece.

During the last decade two major revisions from H. Nordsieck and F. Welter-Schultes attempted to resolve the taxonomical problems of the Cretan taxa, but without a general agreement; species number ranged from 27 to 37, and subspecies from 10 to 47.

In this study, double digest Restriction Associated DNA sequence (ddRADseq) data, which provide thousands of Single Nucleotide Polymorphisms (SNPs) suitable for estimating high-resolution phylogenetic trees, were obtained from 98 specimens

representing 34 Cretan and 4 non-Cretan *Albinaria*'s species were analyzed through several phylogenetic (Maximum Likelihood and Bayesian Inference) and species delimitation (mPTP) methods, aiming to evaluate the taxonomic status of the focal species and describe their new distributional patterns.

Our phylogenetic analyses were based on 11,771 loci and produced a fully resolved tree that supported the existence of 15–20 distinct clades (potential species) clustered in three major groups, i.e., Western Crete, Central and Northern Crete and Eastern and Southern Crete.

## Novel diversity of breathing-device refines the taxonomy of Alycaeidae (Caenogastropoda: Cyclophoroidea)

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Many cyclophoroideans have evolved diverse shell devices for their gas exchange while closing the aperture by an operculum. The family Alycaeidae is distributed from western India through the Himalaya to Japan in the east, Korea in the north and Indonesia to the south, and also in Madagascar. It has been puzzling that the sutural tube of Asian alycaeids has no external opening. We found that their last-whorl surface with the sutural tube has narrow tunnels (diameter ca. 16  $\mu\text{m}$ ) which open near the umbilicus and runs along the growth line into the sutural tube perpendicularly. We confirmed that this enemy-free breathing system is commonly found in alycaeid genera that possess the sutural tube. On the other hand, the Madagascan genus *Boucardicus* and Asian genus *Laotia* lack the sutural tube exceptionally in this family. We discovered that they instead form a dif-

ferent microtunnel system in the last-whorl surface. *Boucardicus* and *Laotia* are distinct from each other and from the other alycaeid genera in structure of the breathing system. Without knowing these elaborate features with ecologically definite functions, members of this family were described at the end of the 19th and beginning of the 20th centuries, and also in the past two decades. Based on the morphology of protoconch sculpture and breathing microtunnel system, we propose a new taxonomic system of Asian Alycaeidae with three subfamilies. *Boucardicus* belongs to one new subfamily, *Laotia* to another, and all the other alycaeids form the third subfamily. The phylogenetic legitimacy of this morphology-based system is being tested by means of molecular phylogeny.

## Global patterns of diversity and conservation status of freshwater bivalves

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Freshwater bivalves provide important ecosystem functions and services, yet many of their species are highly threatened by human activities, and recently their global decline has been causing conservational and social concern. In this presentation, we review the diversity patterns, the research efforts and conservation status assessment of freshwater bivalves across the globe. A great bias exists in research effort, with much more information available for bivalve species belonging to the Unionida in comparison to other groups. The same is true for the origin of these studies, since the publishing pattern does not

always correspond to the hotspots of biodiversity but is concentrated in the northern hemisphere mainly in North America, Europe and Russia, with regions such as Africa and Southeast Asia being quite understudied. Also, the IUCN Red List has increased profoundly its coverage in this faunistic group, but still many areas of the world are poorly assessed. In the present lecture, we also review all major threats and constraints for conservation and highlight priority research efforts and conservation needs in the distinct bioregions of the world.

## Conservation genetics of the Central European thick shelled river mussel (*Unio crassus*, Philipsson 1788)

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The thick-shelled river mussel, *Unio crassus* Philipsson 1788, was formerly one of the most abundant and widely spread freshwater bivalves among the order of Unionida in Europe, but there is still a lack of information on its genetic diversity and spatial genetic structure. We characterised the genetic constitution of 18 *Unio crassus* populations in Germany and Sweden originating from six major drainage systems using a set of nine microsatellite markers. *Unio crassus* populations from Northern Germany and Sweden revealed similar genetic constitution with high genetic diversity, whereas populations from Southern

Germany showed higher proportions of common ancestors and a stronger host-dependent diversification. The structured spatial genetic patterns suggest two glacial refugia and a strong north–south differentiation. The Elbe river basin was likely an important source for recolonization northwards to Sweden, and populations from the low mountain range in the Danube–Rhine systems acted as a source for recolonization southwards to the Alps after retreating glaciations. We identified three major conservation units for the analysed *Unio crassus* populations which should be considered in future conservation efforts.

## On reintroduction of the endangered thick-shelled river mussel *Unio crassus*: does river slope matter?

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To restore the full range of the population of *Unio crassus* in the Biała River (S-Poland), the species was re-introduced into a series of “stepping stones”, joining two remnant populations. First, relationships between the abundance of *U. crassus*, physical habitat and water quality were studied to assess re-introduction success. The mussel abundance in its main population was negatively related with the altitude, channel slope, percent of organic parts in suspended matter and pH, whereas positively so with silt contents in bottom samples, water conductivity, and concentrations of HCO<sub>3</sub><sup>-</sup>, Ca and NO<sub>3</sub><sup>-</sup>. During reintroduction, adult individuals were introduced into three functional habitats: shallow fine sediment bars,

deep silted bays, and ripraps. The largest number of juveniles was found in the lower course of the restored range, and recruitment was negatively related to channel elevation and the channel slope, forming very rapid change in recruitment at a channel slope of 1.8‰. The recruitment was positively related to the silt content, and an optimum relationship occurred with suspended matter concentration. The host fish species did not show relations to abiotic habitat features within the studied reach. The number of juveniles was strongly related to the number of the fish host, *Chondrostoma nasus*, recorded at the re-introduction site. The fish host was related to the same functional habitat as the mussel.

## Comparison of two semi-natural breeding methods for freshwater pearl mussel (*Margaritifera margaritifera*)

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All over Europe breeding programs for the endangered freshwater pearl mussel (*Margaritifera margaritifera*) are in action. The semi-natural breeding method is used in several projects, where mussels are raised in different breeding containers which are exposed to their native streams. However, even though it is known that mussels can be raised successfully in each of the breeding containers, they have not yet been systematically compared. In our experiment, we compared two common breeding containers, hole plates/buddensiek plates versus sediment boxes with respect to survival and growth rates of juvenile mussels and maintenance effort. We picked 10 study sites in 4 freshwater pearl mussel streams and installed 3 hole plates and 3 sediment boxes at each site. Every breeding container was stocked with 30 one-year-old juveniles. Starting in June 2016, survival and shell length were investigated at the end of the growing season and in the following spring. At each sampling step, the time for cleaning and analysis of each breeding container was recorded. During

the complete study period physicochemical parameters were measured regularly. Mean survival rates differed significantly between hole plates and sediment boxes with 70% and 47%, respectively, whereas mean growth rates were similar with 140% and 136%, respectively. Only in one study stream survival rates were comparable between sediment boxes and hole plates with 70% and 71%, respectively. Growth rates were comparable across streams in both breeding containers. However, in the study stream Ilz growth in hole plates was significantly higher than in the other streams. The results in this study confirm that both tested breeding containers are suitable for freshwater pearl mussel breeding. However, stream specific habitat conditions decide which breeding container is to be preferred at a certain locality. Furthermore, project aims as well as manpower and the number of juvenile mussels may influence the choice of the preferable breeding container, as it is more time-consuming to take care of the same number of mussels in hole plates than in sediment boxes.



## How many mussels? A mark and recapture study of the freshwater pearl mussel *Margaritifera margaritifera*

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The freshwater pearl mussel *Margaritifera margaritifera* lives in shallow clear water streams that allow sampling strategies to rely on visual searches. Population estimates usually rely on different sampling approaches, but the potential impact of the detectability of individuals in the outcome of the estimates are rarely considered. We used a mark and recapture approach to estimate the number of mussels using as a model a low-density population from northern Portugal. We registered the location of all mussels in the population that could be located by sight over four consecutive years. Each mussel was tagged using a unique alphanumeric code and its maximum shell length registered. Failure to detect

a mussel in a given year was attributed to mortality, cryptic behavior (burrowing), cryptic passive (non-visible individuals under rocks, roots or leaves) and sampling error (including tag loss). The data was analyzed using MARK<sup>®</sup> software. Data was analyzed considering different size classes to evaluate potentially different detectability according to age and possible recruitment of juveniles that remain buried during the first years of their life. The results show that a considerable part of the population may not be detected at any given year, up to 40% of the total estimate. Population assessments must take this into account, as a single sampling moment may grossly underestimate it and inflate its threaten level.

## Molecular phylogeny and biogeography of *Melanopsidae* (Caenogastropoda: Cerithioidea)

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*Melanopsidae*, as currently understood, are an important component of the freshwater fauna of the subtropical to southern temperate regions of the Western Palearctic and are also reported from the Zelandian microcontinent, viz. New Zealand and New Caledonia, which is an unparalleled disjunction among freshwater animal taxa. We sequenced nuclear and mitochondrial markers of a representative sample of species and of all genera covering the entire geographic range of the group to reconstruct its phylogeny. The results of our phylogenetic analyses indicate that *Melanopsidae* are not monophyletic. *Holandriana*, mainly from the Balkan Peninsula, is more closely related to *Pleuroceridae* (eastern North America) and *Semisuclospiridae* (western North America and eastern Asia) in our analyses than to the remaining melanopsid genera. The Zelandian taxa were recovered as a deeply divergent sister group of a clade including *Melanopsis*, *Microcolpia* and *Esperiana*, the latter of which branching of first in the phylogenetic trees. *Microcolpia*, including *M. acicularis* and the extinct *M. parreyssi*, was recovered as a moderately well-supported sister group of

*Melanopsis* s. str, which was comprised of three distinct lineages: one from the western Mediterranean region, a second from the eastern Mediterranean region and the Near East and a third endemic to thermal springs in a small region of southern Tuscany (Italy). While the Italian populations show little variability with regard to the shell, both the eastern and western clades of *Melanopsis* exhibit an extraordinary degree of variation in the form and/or sculpture of the shell. Whether this variability is indicative of more than one species within the eastern and western clade, respectively, can currently not be answered with certainty. However, in a number of cases morphologically distinct taxa, were not recovered as monophyletic units, which may indicate that these taxa are not completely reproductively isolated. *Melanopsidae* have an extraordinary rich fossil record in Europe. We compare the timeframe for the evolution of major melanopsid lineages obtained from a fossil calibrated phylogeny with geologic events and elucidate scenarios that may have shaped the distribution patterns observed today.

## Multilocus phylogeny of the land snail family Geomitridae (Boettger, 1909)

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Gomitridae is one of the most diverse families of land snails in the western Mediterranean region, being also present in the Macaronesian region. So far, the classification of the family is mainly based on the auxiliary copulatory organs, including the number, morphology, and structure of the dart sacs, accessory sacs and digitiform glands. We present a multilocus molecular phylogeny based on two mitochondrial genes (COI and 16S rRNA), the rDNA region (ITS1-5.8S-ITS2-28S) and two nuclear markers (60SL13 and 60SL17) obtaining a total of 3820 bp. In this work, at least one species of each known genera belonging to the family were included.

The two subfamilies currently recognized, Geomitrinae and Helicellinae, were recovered mono-

phyletic, being well supported. The Helicellinae family was divided into two biogeographical main groups, the western and the central-eastern clades. The monophyly of several genera were not supported indicating that the structure of the auxiliary copulatory organs showed some cases of homoplasy in the family, including parallel and convergent evolutionary trends. In this way, *Candidula* species were distributed into seven groups requiring generic status, whereas *Xerotricha* was recovered in at least five different lineages. Finally, according to this new molecular analysis a new subfamily reassignment is proposed for this family.

## Taxonomic revaluation of the Austrian endemic *Helicopsis austriaca* (Gastropoda: Eupulmonata: Hygromiidae) in the light of Natura 2000

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Inconsistencies in (sub)specific classification among land snails might not only lead to taxonomic confusions, but can also interfere with conservation and environmental policy. In the presented case, the taxonomic state of the protected Austrian endemic *Helicopsis austriaca* changed several times since its first description. Originally described at species level, it was later treated as a subspecies of *H. striata* or even considered synonymous with this taxon. Despite its sometimes unclear taxonomic assignment, *H. austriaca* is listed in the Annex II of the Flora-Fauna-habitat directive. The unclear taxonomic situation and the lack of traits delimiting it from its congeners caused some confusion concerning the definition of protected areas and conservation measures for *H. austriaca*. To resolve these uncertainties, an integrative study was started comparing genetics, morphology and biogeography of *Helicopsis* sp. in Eastern Austria and adjacent areas. The results show a clear genetical and morphological separation between *H. austriaca*, *H. striata* and *H. hungarica* in Eastern Austria and ad-

jacent areas. Mitochondrial and nuclear genes show a clear disjunction between all three taxa confirming *H. austriaca* as a separate clade. Concerning its distribution range, *H. austriaca* was confirmed to be an endemic of Lower Austria. Furthermore, we could show that *H. austriaca* is subdivided into two subpopulations. Specimens from the type locality and other – predominately edaphic – steppe land areas of the Viennese Basin are characterized by smaller shell dimensions and can be clearly separated from *H. striata* and *H. hungarica* without dissection. The other subpopulation harbors mainly primarily treeless rock steppe in the Northern Calcareous Alps and shows some overlap with *H. striata* in shell dimensions. Detailed sections of the penis papilla allow a strict and unambiguous separation of *H. austriaca* to its congeners. Therefore, we reevaluate *H. austriaca* to species level as it was intended in its first description. The new awareness concerning taxonomic state and distribution of *H. austriaca* now enables to establish concrete conservation actions.

## A parapatric contact zone between sibling species of *Deroceras*

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Along the Czech–German border in the hills south of Dresden, two sibling species of *Deroceras* slug occur, *D. juranum* and a subspecies of *D. rodnae*. At a coarse scale the distributions interdigitate, but the two species very rarely co-occur at the same site even though they share a similar range of habitats. A transect along a stream valley, for example, can repeatedly yield samples exclusively of one species, then yield samples only of the other. We have mapped several contact zones between the species; they are parapatric with co-occurrence over only a few hundred metres, even in the absence of any sharp habitat discontinuity. There is no sign of morphological intermediates in these contact zones and the consis-

tent agreement of genital morphology with mtDNA sequence also indicates no hybridisation. This fits with observations of mating behaviour in the laboratory: although interspecific couples willingly court, sperm exchange is prevented by *D. rodnae* everting its penis upwards and *D. juranum* downwards. We propose that this may also explain the narrow contact zone; whichever is the rarer species is at a disadvantage as it more often courts the wrong species, losing opportunities to mate successfully (the Satyr Effect). However, we recently have observed one contact zone shifting some hundreds of metres over a few years, so it may be that *D. rodnae* is an invader outcompeting *D. juranum*.

## Multilocus molecular phylogeny of the genus *Chondrina* (Gastropoda, Pulmonata, Chondrinidae) in the Iberian Peninsula

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*Chondrina* is a highly diverse genus of terrestrial mollusks currently including 37 species with about 25 subspecific taxa. It is distributed through central and southern Europe, from Portugal in the West to the Caucasus and Asia Minor in the East. Approximately 75% of species are endemic to the Iberian Peninsula, constituting its center of dispersion. The study of the phylogenetic lineages of the genus *Chondrina* will provide relevant data for the conservation of its biodiversity.

This genus includes a multitude of microendemisms (some of them not yet described) confined to limestone habitats (being strictly rock-dwelling species), distributed along rocky outcrops up to 1800 m a.s.l. It is a genus of conical-fusiform snails that differs mainly in shell characters and in the number and position of teeth in their aperture. So far, molecular studies on *Chondrina* have been based exclu-

sively on the mitochondrial COI gene fragment. This study gave a first view of the phylogeny of the genus but many inner nodes were not statistically supported. So, further studies are needed to progress in the knowledge of this genus.

This work aims to advance in the resolution of the taxonomy and phylogeny of the genus *Chondrina* on the Iberian Peninsula, using multilocus molecular analysis. Mitochondrial DNA sequences of the Cytochrome Oxidase subunit I region (COI – 623bp), 16S rRNA (1012bp) and internal transcribed spacer 2 (ITS2 – 1038bp) were obtained for 176 individuals, representing more than 80% of the extant *Chondrina* species from the Iberian Peninsula. The purpose of this study is to contribute to a better understanding of the determinants and mechanisms that drive the diversification of species as well as to identify cases of non-adaptive radiation patterns.

## The last 20 years of IUCN Red Listing of Molluscs

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In the period prior to 1994 only threatened species were listed by IUCN. These lists were very subjective, lacking any published documentation to support the category of listing. In 1994 IUCN (1994) introduced the new IUCN categories and criteria, based on Mace & Lande (1991) and Mace *et al* (1992) providing quantitative thresholds to measure decline of the species listed, and the new Categories and Criteria (version 2.3) was used to transfer the previous listings to the new system with the first IUCN Red List using these categories published in 1996. Between 1996 and 2000 there was a review of the application of the new categories and criteria, resulting in a revised IUCN categories and criteria (version 3.1) which has been used for Red List assessments from 2001 to 2017. To accompany this system there are now comprehensive guidelines to aid with understanding on how to apply the categories and criteria. In this period we have increased from 671 molluscs listed as threatened and 157 extinctions to a comprehensively documented system

containing over 7,267 molluscs which contains images, maps and downloadable PDF documents with conservation and research actions. The latest version recognised 297 Extinctions, 1984 Threatened species, 533 Near Threatened and 1953 Data Deficient species. Now species accounts are edited directly into an online database (SIS), instead of requiring compilers to enter data into the old access database. This means that at any point in time some 30–40 users may be working on Molluscan species accounts in the system.

In this talk I will review results from the datasets from different regional assessments for covering freshwater molluscs, from Africa, Asia, the Mediterranean and Canada and review the different threats operating within the different regions. This talk will provide data that complements talks by Bohm *et al* on the SRLI project Lopes-Lima *et al* on Freshwater Bivalves and Neubert *et al* on the European project.



## The global status of freshwater molluscs: a sampled assessment of threat

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With the biodiversity crisis continuing unchecked, it is vital to establish levels of risk and predominant threat processes affecting the world's species, to develop targeted conservation actions in a timely manner. Freshwater ecosystems are consistently among the most threatened on earth, with high proportions of threatened species. To date, assessments have been comprised of better known groups of species, resulting in an incomplete view of biodiversity change. To add to knowledge on freshwater systems and the threats they face, we carried out a sampled assessment on 1,500 randomly selected freshwater molluscs as part of the Sampled Red List Index project. This project aims to provide estimates of the global status and trends in extinction risk for a large number of previously under-represented species groups. In addition, the assessment allows us to analyse the main threats associated with high extinction risk. Our data show that 29% of species in our sample are threatened with extinction, with levels of threat being highest in the Palearctic and Nearctic realms (34% and 30% threatened, respectively) and among

gastropods (30% threatened). Threat levels were estimated to be higher in lotic systems (29%) compared to lentic systems (19%). Overall, 26 species were classified as extinct, of which seven are bivalves and 19 are gastropods, mostly from the Nearctic realm. Identifying predominant threat processes leading to threatened status and higher extinction risk in freshwater molluscs is vital in order to design effective conservation strategies. Pollution and the modification of natural systems (e.g. through damming and water abstraction) were the most frequently reported threats to freshwater molluscs. However, using a combination of statistical tests and machine learning techniques for more in depth analyses, we show that damming and water abstraction, as well as human disturbance from recreational activities were consistently associated with higher threat levels in freshwater molluscs, while pollution was primarily associated with lower levels of threat. We discuss the implications of our findings for freshwater mollusc conservation as well as next steps to deliver a trend in freshwater mollusc extinction risk over time.



## A spatially explicit approach to prioritize protection areas using endangered freshwater mussels

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Freshwater ecosystems are amongst the most endangered ecosystems in the world. Yet, only a fraction of lakes and streams including their catchments are currently covered by conservation areas. To identify these gaps, conservation ecologists explore new methods assessing spatial distribution of biodiversity in relation to conservation areas. This study focused on evaluating the protection status of habitats of two endangered freshwater mussel species, *Margaritifera margaritifera* and *Unio crassus*, in the Federal State of Bavaria, Germany, using a gap analysis approach.

In a first step, ecological niche models (ENMs) were calculated for both mussel species based on presence-only data in order to identify suitable habitats. Binary maps served as species distribution layers for a subsequent gap analysis. We calculated the length of protected suitable habitats. In particular, we distinguished between different categories of protected areas, including 'nature conservation areas' and 'special areas of conservation' and 'protected landscapes'.

Results of ENMs for *M. margaritifera* showed a spatially restricted distribution of the species with significant model performance (AUCtest =  $0.936 \pm 0.020$  SD), distribution of *U. crassus* was wider, extending to calcareous areas, and the model performance was significantly weaker (AUCtest =  $0.677 \pm 0.045$  SD). Calculation of the protection state of potential habitats of *M. margaritifera* showed, that a higher percentage of suitable habitat (86.7%) is already under protection, whereas for *U. crassus* only half of the suitable habitats (50.5%) are under any sort of protection. However, large fraction (67.7% for *M. margaritifera*; 31.5% for *U. crassus*) is in areas with low protection (landscape protection areas).

In conclusion, our results suggest that suitable habitats of both species are not sufficiently protected. Therefore, it is necessary to put a higher fraction of suitable habitats under protection for the conservation of those freshwater mussel species.

## Redlisting the molluscs of Europe – insights to the conservation situation of the malacofauna of a continent

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Continental Molluscs have been the target of red list activities for almost 3 decades, with sporadic and taxonomically scattered entries from the 1970's. Since 2009, the complete continental malacofauna of Europe has been screened using the knowledge of almost 40 experts and threat status assessed against the standard categories and criteria of the IUCN Red List (IUCN version 3.1). Today, we can present data on the current conservation status of approximately 3000 species of terrestrial and freshwater molluscs. These data will be made visible through the IUCN Red List database in November 2017 ([www.iucnredlist.org](http://www.iucnredlist.org)), presenting a lot of hitherto unpublished information on the species including as a novelty a distribution map for almost all species. The taxonomic backbone is based on a fundamentally revised version of the Fauna Europaea list, which will be also

used in the upcoming MolluscaBase project. For the terrestrial molluscs, there is good news: of the ca. 2.500 species, only 19.5% have a threatened category, and slightly more than 50% are considered Least Concern. Unfortunately, whilst the freshwater mollusc were assessed in 2011, these assessments were not updated (so recently described species are not assessed), so the latest numbers stay with ca. 40% of the species having a threatened category, contrasted by only 22% of Least Concern species. As could be expected, the Balkans can be considered the area with the highest biodiversity, whilst, the Macaronesian Islands with all the small-range species show a very similar pattern in species richness. The major threats identified against terrestrial molluscs in Europe are grazing, increased incidence of fires, deforestation, agriculture and encroaching urbanisation.

## From the Pacific through the Arctic to the Atlantic and then again: the phylogeography of the family Dendronotidae (Gastropoda: Nudibranchia)

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Nudibranch molluscs represent a diverse and successful group of marine gastropods. They are distributed worldwide and can be found in the most marine ecosystems with the highest diversity in the Indo-Pacific region. Since nudibranchs are shell-less molluscs, the study of their evolution and speciation process always was problematic due to absence in the fossil record. In this study, we focused on the phylogenetic relationships of species of the genus *Dendronotus* (Nudibranchia: Dendronotida). These molluscs demonstrate a high variability in morphological characters and represent the highest diversity in boreal and Arctic waters instead of in the tropics, which is an uncommon distribution pattern within Nudibranchia. A phylogenetic hypothesis based on molecular markers reveals six major clades; each clade represented by species with Atlantic and Pacific

distribution and possessing similar radula morphology. The ontogenetic changes of radula through the late ontogenesis are a species-specific feature and stand in close interdependence with the feeding modes and the specialization on different feeding objects. A model of morphological evolution of the radula strongly corresponds with molecular phylogenetic hypothesis. Our data indicate the North Pacific origin of boreal *Dendronotus* species with fast radiation by adaptation to different feeding objects. Further speciation relates to migration and allopatric speciation during periodical opening and closing of Beringia during Miocene-Pliocene and early Pleistocene.

This study was supported by Russian Foundation for Basic Research (grants #16-34-00955 and #15-04-02580).

## **More than what meets the eye: a first morphological assessment of Chinese and Himalayan Carychiidae (Eupulmonata, Ellobioidea)**

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Although thirty-five species of minute terrestrial snails currently comprise the ellobioid genus *Carychium*, less than one third have been described from Asia. Half of all carychiid descriptions date from the 19th Century. Only two species are known from China and two have so far been recorded south of the Himalayas. Considering the richness of overall biodiversity reported in recent publications from the Tibetan Plateau and China, this paucity of carychiid finds reflects the whims and travel itineraries of early collectors rather than the actual situation. Increasing access to remote regions, the interconnectivity of global scientists and the use of computer tomog-

raphy (CT) to penetrate and interpret the shells of these tiny snails have enabled immense insights and perspectives into this family. New finds are surfacing, whereby three distinct morphological tendencies are becoming clear for the first time for Chinese and Himalayan species. We aim to clarify these tendencies as we scratch the surface of this broad expanse of hitherto unknown territory for carychiid snails. Computer tomography and SEM reveal the columellar and lamella configuration, the degree of lamellar sinuosity, radular dentition and superficial shell texture.

## Is the true identity of *Trochulus coelomphala* (Gastropoda: Hygromiidae) possible?

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Recognition of many *Trochulus* species using shell morphometry is often problematic because of their great phenotypic plasticity. Species delimitation on a genetic basis is also hindered by the large ancestral polymorphism of *Trochulus* populations or ongoing gene flow between them. Therefore, detailed analyses are required to understand the complicated evolutionary history of these snails. Here, we examined three similar species, *T. coelomphala*, *T. hispidus* and *T. striolatus*, which co-occur in the same area in Austria and Germany. They differ in certain shell features and reproductive system traits. However, in a limited zone in Germany, we detected shell phenotypes intermediate between *T. coelomphala* and *T. hispidus*, which simultaneously displayed the genital morphology of *T. coelomphala*. Mitochondrial COI gene sequences of these morphological intermediates were distributed in four clades and grouped with sequences assigned

to *T. coelomphala* or *T. hispidus* complex in phylogenetic trees. Almost 83% of these intermediate forms were grouped in one clade separated from others. The results imply that the intermediate shell morphology between *T. coelomphala* and *T. hispidus* can evolve independently in different genetic lineages and can be associated with the big phenotypic plasticity of *Trochulus* resulting from an environmental influence. Taking into account that these intermediate forms have the genital morphology of *T. coelomphala* and their sequences group with this species, we can assume that they also belong to this taxon. The results put into question validity of recognition of *Trochulus* species using shell morphometry.

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## Taxonomy and phylogeny of the family Rissoellidae (Mollusca: Gastropoda: “Lower heterobranchia”) in Hokkaido, Japan

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Although the family Rissoellidae Gray, 1850 has a worldwide distribution and its presence in Japanese waters is known, it has been little studied in Japan and there is a dearth of molecular information available. In the current work, five species of rissoellids were collected from Hokkaido, Japan. Two of these species have been previously described, *Rissoella elatior* Golikov, Gulbin and Sirenko, 1987 and *Rissoella elephantina* Golikov and Gulbin, 1978; three of these species appear to be novel. This study focused on shell, head-foot, radula and reproductive system morphology and body color patterns, as a metric for differentiating species. The new species “*Rissoella* n. sp. 1” and “Gen. 1 n. sp. 1” showed similar characteristics in the shell and radula morphology to the Russian species *Jeffreysina violacea* Golikov and Kussakin in Golikov and Scarlato, 1971 and *Jeffreysina*

*golikovi* Gulbin, 1979, respectively. Considering the morphological difference in the radula, which included three teeth, as well as the absence of seminal receptacle, we concluded that Gen. 1 n. sp. 1 likely represents a new genus; this result was supported in a molecular phylogeny reconstructed from cytochrome c oxidase subunit I (COI). “*Rissoella* n. sp. 2” had a similar reproductive system to other rissoellids, in that it contained a seminal receptacle, but had a different radular morphology, containing 7 teeth; the protoconch was striated (contained three lines). Further histological work and the inclusion of other gene markers (18S, 28S, and 16S) from samples isolated in new geographic regions will help resolve issues related to species diversity and biogeographic patterns in this group of marine gastropods.

## **Pioneers from the continents – evolution and phylogeography of the Vitrinidae of the Macaronesian Islands**

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The study of isolated islands, considered as biological hotspots and evolutionary engines, was always of distinguished interest to biogeographers. The Canary Islands represent an excellent environment to study island biogeography due to the high degree of endemism and habitat uniqueness that exists on these volcanic islands. The relationship between Macaronesian and the nearby Mediterranean basin fauna implies interesting questions for the endemic terrestrial snail fauna of the Canary Islands, notably questions on dispersal abilities as well as colonization mechanisms. Our project focuses on the terrestrial pulmonate gastropod family, the Vitrinidae. The Vitrinidae represent a curiosity among the terrestrial molluscs: being half slugs with translucent shells, consisting of widespread species, but also none the less fewer endemics living in extreme climatic con-

ditions throughout the European/African Palearctic realm. Here we show the first molecular phylogeny of the Canarian and Madeiran Vitrinidae opposed to the East African members of the family, generated by a five-gene marker approach, which represents a novelty. It is the first stepping stone to the future exploration of the biogeographical aspects of the Macaronesian islands colonisation patterns, emigration, dispersal and situation of source populations using the family Vitrinidae. In future projects, we will try to reveal the evolutionary life history of the family from the Macaronesian and Mediterranean area, resolving questions of potential immigration pathways through Europe covering a timescale of 68 ma, which represents the maximum age of colonisable land patches in the area.

## **Who, where, what and why: some basic questions in land mollusc diversity**

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Over the last 25 years or so there has been great progress in our understanding of phylogeny (the who), and of the where (distribution at many scales) of land molluscs, so that big how or why questions about, for example, island biogeography, phylogeography and the effects of environmental change on speciation and faunal composition have been addressed. But the what? questions have been less satisfactorily answered: the nature of niches and the assembly rules, if any, that govern both the composition and richness of local faunas over and above the constraints of availability and powers of dispersal. Can we identify the traits that enable survival in particular cases? To what extent do such traits reflect

common adaptations to the same environment, or differentiation reflecting different roles within the local assemblage? The picture is confusing. There is often a mismatch between the easily measured features of shell morphology and the less comprehensively studied traits related to diet, physiology, life history and behaviour. We rely on proxies of uncertain reliability for a holistic view of why species are found here, but not there. Some cases give us useful clues, but what works in one case may not do so in another. We are far from answering all the why and how questions. In particular, the question of whether there are upper limits on local diversity, and if so, what determines them, remains open.



## Biogeography of deep-sea mollusks: a network perspective

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Deep-sea hydrothermal vents and methane seeps host unique ecosystems that are often dominated by mollusks. Both habitat types are inhabited by members of the same higher taxa but share few species, thus scientists have long sought habitats or regions of intermediate character that would facilitate connectivity among these habitats. Here a network analysis of 79 vent, seep, and whale-fall communities with 121 genus-level taxa identified sedimented vents as a main intermediate link between the two types of ecosystems. Sedimented vents share hot, metal-rich fluids with mid-ocean ridge type vents, and soft sed-

iment with seeps. Such sites are common along the active continental margins of the Pacific Ocean, facilitating connectivity among vent/seep mollusks in this region. In contrast, sedimented vents are rare in the Atlantic Ocean, offering an explanation for the greater distinction between its vent and seep mollusks compared to those of the Pacific Ocean. The distribution of subduction zones and associated back arc basins, where sedimented vents are common, likely plays a major role in the evolutionary and biogeographic connectivity of vent and seep mollusks.

## Predator-prey interactions as the trigger of prey species divergence

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Predator-prey interactions are recognised as the major factor promoting phenotypic evolution. However, it remains unclear how predation causes phenotypic diversity in prey species and how it might lead to speciation. We show that substantial divergence in the phenotypic traits of prey species has occurred among subarctic *Karatohelix* land snails (Camaenidae) in northeast Asia as a result of adaptation to predation. Two alternative defence behaviours against predator attacks by malacophagous beetles were documented in these snails: passive defence and active defence. Each behaviour was clearly associated with differences in shell morphology among each species. Furthermore, molecular phylogenetic

analyses indicated that these alternative strategies against predation arose independently on the islands (Hokkaido Is., Japan) and continent (Russian Far East, Russia). The divergence of *Karatohelix* land snails in northeast Asia are unlikely to be caused by interspecific resource competition, because distinctive morphotypes generally coexist in the same regions. These results suggest that anti-predator adaptation is a major cause of phenotypic divergence of these snails. Here, we suggest the potential speciation of *Karatohelix* snails as a result of the divergence of defensive strategies into passive and active behaviours and the possibility of species radiation due to anti-predatory adaptations.

## Range-constrained co-occurrence simulation reveals little niche partitioning among rock-dwelling *Montenegrina* land snails (Gastropoda: Clausiliidae)

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Taxon co-occurrence analysis is commonly used in ecology, but it has not been applied to range-wide distributional data on partly allopatric taxa because existing methods cannot distinguish between distribution-related effects and taxon interactions. Our first aim was to develop a taxon co-occurrence analysis method that is also capable of taking into account the effect of different ranges and can handle faunistic records from museum databases or biodiversity inventories. Our second aim was to test the independence of taxon co-occurrences of rock-dwelling gastropods at different taxonomic levels, with special focus on the subfamily Alopinae of the family Clausiliidae, and in particular the genus *Montenegrina*. We introduced a taxon-specific metric that characterizes the occurrence probability at a given location. This probability was calculated as a distance-weighted mean of the taxon's presence and absence records at all sites. We applied corrections

to eliminate the distorting effects of varying sampling intensity in our data set. Then we used probabilistic null-models to simulate taxon distributions under the null hypothesis of no taxon interactions and calculated pairwise and cumulated co-occurrences. Independence of taxon distributions was tested by comparing observed co-occurrences to simulated values. Significantly fewer co-occurrences among species and intra-generic lineages of *Montenegrina* were observed than expected under the assumption of no taxon interaction. This indicates that species divergence preceded niche partitioning and suggests a primary role for non-adaptive processes in the speciation of rock-dwelling gastropods. The method can account for the effects of distributional constraints in range-wide datasets of records, making it suitable for testing ecological, biogeographical or evolutionary hypotheses where interactions of partly allopatric taxa are in question.

## Non-marine snails of Sint Eustatius (Lesser Antilles), surprisingly unknown and diverse

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A two weeks survey of the tiny (21 km<sup>2</sup>) Dutch Caribbean island of Sint Eustatius (Statia) in 2015, organised by the Naturalis Biodiversity Center, Leiden, supplemented by brief visits in 2016, more than doubled the number of non-marine gastropod species known, including at least one undescribed species. About 35 morphospecies are presently recognised, including one freshwater species. Even though the island lies in a relatively well explored and accessible part of the world, surprisingly many taxonomic problems were encountered, and the taxonomic position of many species are as yet more or less unclear. Cytochrome Oxydase-1 sequences were of limited use for species identification, since precious few sequences of Caribbean land snails, even of common species, are available from databases like Genbank and Bold. Sint Eustatius lies rather close to Saint Kitts and Nevis, and all three islands were part of a single island in the past. A recent publication

(2016) reported only 26 land snails for St Kitts and 22 for Nevis, and 33 for both islands, so the much smaller island of Statia seems to have disproportionately high species diversity. The faunas of the three islands are not identical. Various species reported from St Kitts and Nevis have not been found on Statia, and even some large and conspicuous common taxa on Statia are not known from the two neighbouring islands. Also in comparison to other Lesser Antilles islands, Statia's fauna appears diverse. This is likely to be a reflection of the serious shortage of collecting efforts and taxonomic expertise in the region. A significant proportion of land snail species on Statia has probably been introduced in recent times by man, and this process continues to the present day (e.g. *Lissachatina fulica*). Attention is drawn to the presence of pre-human deposits containing land snails, which potentially allows to distinguish between naturally present taxa and human-introduced ones.

## Freshwater mollusks communities' changes in the lower Danube River triggered by human pressure and alien invasive species: case study – the Iron Gates Natural Park

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The Iron Gates is a gorge delimiting upstream and westward the lower sector of the Danube River. Since the Iron Gates Hydroelectric Power Stations were built at the exit of the gorge, the whole sector has become a huge reservoir and lentic ecological systems have replaced the former rapid flow features of the Danube. Based on references and on personal field surveys done between 1998 and 2016, we aim to give an overview of the freshwater mollusk community dynamics during the last decades. The community structure is characterized and reported in time, space, and along different environmental gradients (depth, dominant bottom type, flow etc.). Ordination analysis and other mathematical models are used in order to track the mollusk community changes in relation to environmental factors. While the dams and other human constructions have triggered some obvious and expected broad changes, other alterations in community structure were subtle and caused by an array of factors, with the main driver being most likely represented by the establishment of invasive alien mollusk species. Among these

we highlight *Sinanodonta woodiana*, present in the area by the end of the last century, followed later by *Corbicula fluminea*, and a few years since by *Dreissena rostriformis bugensis*. These species have invaded the area in a differentiated manner, selecting more or less distinct habitats, and modifying the community structure. The invasives are most abundant in the Danube's riverine gulfs and the soft bottom habitats placed closer to the riverbanks. In contrast, the tributaries of the Danube are less impacted: *C. fluminea* was found only in the lower sector of one tributary (Nera River), while the alien gastropod *Potamopyrgus antipodarum* seems to prefer the peculiar geochemical conditions of the Cerna River, in which thermal springs and brooks are discharged. During the two-week field survey accomplished in 2016 we learned that the alien mollusc species have become more abundant, different species being dominant in different categories of habitats, while several native species have undergone population declines, some being possibly driven to extinction by altered environmental factors and competition with the invasive species.

## Co-occurrence and hybridisation of three morphotypes of *Arion rufus* and *A. ater* in Eastern Saxony, Germany

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There has been much uncertainty about the identities, nomenclature and natural range of *Arion rufus* and *Arion ater* in different parts of Europe. A number of subspecies have been described based on genital morphology, but the quantity and geographical range of the material studied is unclear, and subsequently these taxa have been largely ignored. The uncertainty about the taxonomic status of different populations is accentuated by the morphological diversity within each form and by the difficulty of defining the morphology of the ligula, a complex genital structure. Based on mtDNA, British *A. rufus* were recently established as a separate species (*A. rufus* s.s.) from Continental populations, for which the name *A. empiricorum* was used. The northern *A. ater* was confirmed as a separate species, an opinion currently widely accepted by Continental authors even though hybridisation is well known at contact zones between *A. rufus* s.s. and *A. ater* in Britain. We have established a method to describe the genital morphology, particularly the ligula, and applied this to

populations from Eastern Saxony, Germany. The material included freshly collected slugs and museum specimens up to 40 years old. These were compared with specimens from elsewhere in Europe, including other parts of Germany, Sweden, Britain, France, and Lithuania. Morphology and mtDNA indicated the mixed occurrence of three commonly occurring forms: the British *A. rufus* s.s., Continental *A. empiricorum*, and *A. ater*. We found morphological intermediates indicating hybridisation between all three forms. British and Continental *A. rufus* s.l. mainly, but not exclusively, live in synanthropic habitats. *Arion ater* was limited to natural woodland, but morphological intermediates with the other forms were collected in synanthropic habitats. Our data suggest an early introduction of at least one, but probably both, forms of *A. rufus* s.l. in the study area and call the species status of all three forms into question. A more recent, complicating factor is the interbreeding of these three forms with the invasive *Arion lusitanicus* auct. non Mabilie.

## Correlation of shell morphology and color with spatial and environmental variables: the case of Subtidal Whelks (*Buccinum undatum*) in a shallow sub-arctic bay

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Geographical patterns in body form, behaviour and life history can arise from direct environmental control of physiological processes and body shape, differential adaptation to changing biological surroundings or random changes in distinct populations. Mollusc shells exhibit a wide variation of easily measurable phenotypic traits that can be used to study the causes of these patterns. Variability in shape, thickness and color of gastropod shells has been linked to environmental factors such as wave action, temperature and presence of predators using model species (e.g. *Littorina saxatilis* and *Nucella lapillus*). The aim of the project was to evaluate the spatial variation of the shell morphology and color of the subtidal common whelk (*Buccinum undatum*), a species with limited dispersal ability, and whether it is affected by environmental factors. Whelks were sampled at 8

sites in Breiðafjörður bay, west Iceland, which hosts the densest whelk population in the country. Shell shape was analysed by applying geometric morphometrics to the shells while the color and lightness of the shells were analysed using visual and digital analysis, respectively. Environmental variables measured were depth and substrate. Differentiation in shell shape, color and lightness of the common whelk in Breiðafjörður increased significantly with increased geographic distance between sites, suggesting different subpopulations. Shell shape and color was not correlated to sex ratio, while depth and substrate type were important factors. The next steps in the project are the analysis of genotypic variation of *Buccinum undatum* and a common garden experiment including shell trait ontogeny, as well as detailed analysis of life history traits.



## Shell variation and reproductive mode in the Clausiliid *Idyla bicristata*

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Clausiliid shell features are adaptations not only to the environment, but more indirectly, to reproductive mode. Such an unusual character was found in life-bearing *Idyla bicristata* where the high-vaulted cervix of the ultimate whorl possibly evolved to accommodate the passing of large embryos through apertural barriers. Here, we present findings concerning the life history and population dynamics of this clausiliid together with morphometric analysis of its shell and aperture.

We performed quadrat random sampling to estimate population density and dispersion indices of the population at the Nemea Archaeological Site, Greece. Samples were taken every month from October 2015 until October 2016 (excluding 3 aestivation months, i.e. June–August). The number of quadrats in each sampling occasion was set to 20 and the size of each quadrat was 50×50 cm. Mean population density was found to be 0.56 ind./0.25 m<sup>2</sup>. There were found ordinary density fluctuations and immature specimens were almost always fewer than matures (except May). Concerning Dispersion Indices we found always high aggregation, with higher values on April and May.

We analysed annual reproductive activity of *I. bicristata* at the same site. The highest proportion of

snails retaining embryos in the reproductive tract occurred in October. Number of embryos ranged from 1 to 10 (mean 4.0; SD 2.4). Embryonic shell height reached on average 2.2 mm, shell width – 1.2 mm. Under laboratory conditions (constant high humidity, temperature 20–25°C, natural lighting) snails reproduced twice a year delivering 3–4 neonates at one occasion. The time required for juvenile shell development ranged from 2.5 to 3.5 months and reproductive maturity was obtained after 6.5–7.0 months from birth.

We used geometric morphometric analysis to examine adult size and shape variation (1) among monthly samples and (2) between specimens without embryos vs. specimens with embryos. Considering shell size and aperture size we did not find (as expected) any significant differences between months. Yet, we found marginally significant size differences between specimens without embryos vs. specimens with embryos. However, surprisingly, specimens with embryos were on average smaller. For shell and aperture shape we also found a clear discrimination between specimens without embryos vs. specimens with embryos.



## The problem of excessive variation within species: phylogeny and morphometry in cryptic species of *Zospeum* (Eupulmonata, Ellobioidea, Carychiidae)

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*Zospeum* Bourguignat, 1856 is a terrestrial genus of minute cave-dwelling Ellobioidea, which are known to live throughout the Balkans, the Southeastern Alps of Austria and Slovenia, the Alps of Northeastern Italy and the Cantabrian and Pyrenees Mountains of Northern Spain. The taxonomic framework of this genus was defined in the seventies of the last century mainly by Jože Bole based on shell morphology and in use, with minor additions and changes, until recently. Recent molecular and internal conchological studies via computer tomography, however, couldn't fully support this classification. The biggest differences between the morphological and the genetic approach were found in the hitherto considered, widespread species *Zospeum isselianum* Pollonera 1887, which was found to be a polyphyletic complex of cryptic lineages. Some of them are genetically identical to species that were earlier considered to be morphologically different from *Z. isselianum*, while others cannot be assigned to any already described taxa. A significant issue is that most genetic data of the *Z. isselianum* complex and the genus *Zospeum* in general, comes from Slovenia. Molecular data from the rest of those

in this complex as well as throughout *Zospeum*'s patchy pan-European distribution is very scarce. The goal of this study is to specifically enhance our knowledge of this complex and of the genus *Zospeum* in general. This is achieved first by adding new genetic data from Croatia, Northeastern Italy and Bosnia-Herzegovina. Here, forms are considered that are mostly morphologically close to or within the *Z. isselianum* complex, reducing the geographic sampling bias towards Slovenia in the overall genetic data. Secondly, the unresolved morphology is addressed by adding several morphometric methods to the classically-used morphological characters such as lamella and ribbing configuration. Eight shell measurements were taken and a Principle Component Analysis (PCA) was conducted using these. The same set of measurements was also used with a script based on the Linear Discriminant Analysis (LDA), which extracts the two ratios that are most suitable for separating two previously defined groups. Subsequently, a set of 14 landmarks was taken and analysed with a PCA and a Canonical variates analysis (CVA).

## Improving productivity of bivalve mollusc aquaculture using microencapsulated products

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Aquaculture is the fastest growing food sector, feeds over 1 billion people, and has a pivotal role to play in global food security. Bivalve mollusc aquaculture makes up 25% of production and is worth US\$19 billion. However, current bivalve production is limited by several key factors. Feeding broodstock and juvenile bivalves requires growing lipid-rich algal cultures which equate to over 30% of the total production cost, are highly subject to contamination and have variable nutritional value. Bivalve hatcheries also suffer high losses due to frequent outbreaks of infectious disease.

I am using new advances in microencapsulation technology to provide an innovative and effective way to tackle these limitations during my PhD. Investigations so far provide strong evidence that microencapsulated products are successfully ingested by bivalve molluscs including *Mytilus edulis* and *Ostrea edulis*. Key nutrients are being encapsulated to provide dietary supplements many orders of magnitude more nutritional than the best algae. These may allow the development of better quality bivalve broodstock, greater numbers of larval offspring, and more rapid juvenile growth, increasing production efficiency and reducing costs in hatcheries.

## Coat for every occasion: periostracum of selected land snails – a preliminary report

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Calcareous layers (ostracum) of mollusc shells are covered by thin organic layer (periostracum) which protects them against environmental influences. Land snails, being adapted to various conditions, inhabit a wide range of habitats. Such adaptations include variation in the microarchitecture of the shell wall.

We compare closely related species (mainly members of families Vertiginidae and Valloniidae), which differ in their ecological requirements. Periostracum of species living in wet and acid habitats (e.g. genus *Columella*, some members of genus *Vertigo* and *Acanthinula aculeata*) is usually two-layered and thicker

than that of calciphilous or mesic species. In extreme cases (e.g. *Columella aspera* and *Zoogenetes harpa*), periostracum reaches one third of total shell thickness and possess multi-layered structure. On the other hand, species living in dry and/or calcium rich environment have one-layered and relatively thin periostracum. The periostracum of some *Vallonia* species and *Vertigo pygmaea* reaches only two or three percent of total shell wall thickness.

Still, microstructural shell characters together with the inner structure of the periostracum remain understudied and represent unknown land snail adaptation for various habitat condition.

## The first records of androgenic hybrid *Corbicula* lineages in Extreme North of Russia

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Increases in human activity and climate change are factors that are expected to augment the number of introductions and impact of alien species in the northern regions. Here, we report the discovery of a population of Asian *Corbicula* clams in the Extreme North of Russia near the Arctic frontier (64°N, 40°E). This population contains two distinct forms that are living in sympatry in a man-made channel of a thermal power plant. The first form belongs to the morphotype Rlc by shell morphology but reveals the FW5 mtDNA haplotype (COI & 16S rRNA genes), which was typically recorded in the morphotype R. The second form reveals the shell morphological pattern of the morphotype R but has the FW17 mtDNA haplotype, which usually occurs in specimens of the morphotype S. Both identified forms, based on conchological and anatomical features and differences in mtDNA haplotypes, reveal the same 28S rDNA sequences but show intragenomic nDNA variability

with two different variants of the 28S rDNA gene within each individual (allelic sequence heterogeneity). The discrepancy between mtDNA data and shell morphology could be explained by the well-known mechanism that is associated with androgenesis together with “egg parasitism” (mitochondrial genome-capture) in invasive *Corbicula* clams. The intragenomic 28S rDNA variability may be due to a hybridization event which can join different alleles from the two diploid (or polyploid) parental taxa to an allopolyploid descendant. Isolated *Corbicula* populations in artificially heated or geothermal reservoirs may therefore be primary sources for a broad invasion to seasonal waterbodies of the high-latitude regions in the future as climate warming progresses.

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**ABSTRACTS**

**POSTERS**

## On the glacial refugia of Czech members of the family Helicidae

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While for narrow-range endemics it can be expected that they survived the glacial cycles where they live now, finding glacial refugia of widely distributed taxa is a demanding task. Several representatives of the family Helicidae achieved large distribution ranges postglacially, expanding from various refugia. We summarize the current knowledge on the glacial refugia and colonization routes of helcid species native in Czechia, and present new data for *Caucasotachea*

*vindobonensis*, *Helix pomatia* and *Helix thessalica*. We show that Bosnia is a potentially important area for long-term survival of Central-European snail species. Also, we reveal common patterns of expansion from refugia in the Balkans. At present, better data are available for species of eastern origin; however, precise location of refugia remains in some cases elusive even for the studied taxa.

## Land snails of Rhodes, Symi and Chalki islands (SE Aegean)

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There are more than 7,500 islands in Greece; the majority of them is found in the Aegean Archipelago. For the last 40 years we have studied the malacofauna of the islands and islets of Greece. Within this framework we present data on three islands of the southeastern Aegean, namely Rhodes Isl. (1,401 km<sup>2</sup>), Symi Isl. (57 km<sup>2</sup>) and Chalki Isl. (26 km<sup>2</sup>).

The first survey was in 1992 in Chalki and Symi. During the past 3 years we recollected on Symi and Rhodes. As a result of our studies is that we doubled the known species for Chalki and Symi, while

we added a few more species to the malacofauna of Rhodes.

In total, there are 68 species in the 3 islands, 5 of them being single island endemics. The dominant chorotype is the Mediterranean. The fauna of Chalki, the smallest and closer to Rhodes Island, is almost a subset of Rhodes.

The relations and the interpretation of the land snail fauna among the three islands, and other Aegean islands will be presented.

## Effects of climatic, geographic and evolutionary variables on the global species richness distribution of the family Hydrobiidae *s.str.* Stimpson, 1865 (Caenogastropoda)\*

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Global species-richness patterns of highly diverse freshwater invertebrates, such as gastropods, are still poorly understood. This is of particular concern, since freshwater gastropods have an important role in ecosystem function. The family Hydrobiidae Stimpson, 1865 is one of the largest families of continental aquatic gastropods, but due to their small size and high endemism, the recognized number of species might be underestimated. In this work, we compiled geographic information (i.e., number of species per freshwater ecoregion) of Hydrobiidae *s.str.* species to investigate: i) the species richness, endemism and threat distribution; ii) the risk among hydrobiid species; iii) the ecological and evolutionary factors that explain the observed species richness distribution in one of the most diverse groups of freshwater organisms. Overall, this family presents a Nearctic-Palearctic distribution, although some species also occur in South Africa and South America. We found congruence among species richness, endemism and threats hotspots, which are located across the Mediterranean Basin and the Great Basin and the

Lahontan valley in North America. According to our dataset, about one third of the species were classified under a threat category of the IUCN, whereas ~15% were catalogued as least concern and near threatened species. Approximately half of the species were considered as data deficient. Moreover, we conducted generalized linear models to assess the influence of 14 ecological and evolutionary factors which, with an  $R^2=0.53$ , identified several variables, such as connectivity of ecoregions, annual range of temperature and latitude as determinant of regional richness. These evolutionary and ecological factors have also explained the distribution of the species richness in other freshwater organisms. This work also aims at identifying priority areas or habitats of high diversity for development of conservation strategies. Of interest are, for example, isolated springs at temperate latitudes, with a marked temperature seasonality and medium precipitation.

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## Patterns in the species richness and composition of the snail assemblages in acidic, neutral and alkaline forest ponds

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In recent years, the number of areas remaining under the influence of acidity has increased. At all levels of ecosystems, biodiversity decreases upon acidification due to elimination of species that are most sensitive to low water pH. Forest ponds belong to a specific group that varies in location, huge amount of leaf litter and isolation from other aquatic environments. They are crucial in industrial landscapes with well-developed industry and human activity. The aim was to investigate the relative importance of water chemistry in explaining snail assemblages' composition and species richness in forest ponds with a contrasting pH. Patterns in gastropod communities were determined from analysis in 26 forest ponds of contrasting pH with multivariate gradient analysis. Ponds ranged in a base mean pH from 3.0–9.0. pH has been found to be an important factor influencing gastropod fauna. Neutral ponds support diverse communities, typical of small water bodies. In acid-

ic pond types snail fauna was different. Among the species characteristic for acidic ponds (pH <6) were *Anisus spirorbis* and *Aplexa hypnorum*. The greatest distinctions characterise alkaline ponds with numerous appearance of alien *Physa acuta*. The most diverse gastropod fauna was found in neutral ponds whereas the lowest degree of diversity in ponds with the lowest pH. Among the different parameters of the water, pH has direct and interactive effects on the general chemical regime and snail populations. Current knowledge of pH-associated changes in aquatic ecosystems is still incomplete because anthropogenic acidification is a recent phenomenon, and may help to better understand the real impact of pH on gastropod diversity. It is extremely important in forest habitats since they react more intensively to climatic factors and are often used in landscape management and planning.

## Demographic analyses and population models of the land snail *Iberus gualtieranus* (Gastropoda: Helicidae) in southern Iberian Peninsula

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Knowledge about demography and population dynamics is fundamental to establish management actions in threatened species. *Iberus* Monfort 1810, is a land snail genus endemic to the Iberian Peninsula that presents critical conservation issues. In addition to common habitat loss problems, several *Iberus* species are traditionally harvested for gastronomic purposes reducing drastically their populations. One of the most threatened species of the genus is *I. gualtieranus* (Linnaeus, 1758), that is listed as endangered by the IUCN. It is necessary for collection of field data to know correctly the current demographic situation of the species. Hence, we present the first demographic analyses for *I. gualtieranus*, based on capture-recapture models. Two populations in Sierra Elvira (southern Iberian Peninsula) were visited monthly during two years and all specimens found in each visit were marked with an identification code and assigned to

juvenile or adult state. By applying multistate capture-recapture models to encounter data, we could estimate monthly survival and recapture probabilities for each state and year of study as well as juvenile-to-adult transition probabilities. Models results showed differences between juveniles and adults in both monthly survival and recapture probabilities. Mean adult survival ( $0.905 \pm 0.013$ ) was higher than that of juveniles ( $0.861 \pm 0.021$ ), with slightly higher values in the second year. Recapture probabilities of adults remained always above 50% but did not change between years, arguably because of similar recapture effort. Finally, a 14% of juveniles developed into adults every month during the 1<sup>st</sup> year but not in the 2<sup>nd</sup> year.

Thus, a long-period monitoring program of these species is necessary for correct conservation purposes.

## Natura 2000 land snail species in Romania: an overview

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Five of the land snail species listed in Annex II of the EU Habitat Directive (92/43/EEC) were previously known to be present in Romania: three of the whorl snail species – *Vertigo moulinsiana*, *Vertigo genesii* and *Vertigo angustior*, *Drobia banatica* and *Kovacsia kovacsi*. Due to an error, *Kovacsia kovacsi* is not included in our national species list and is not subject to national regulation, despite the evidence of its presence in Romania, the Romanian Carpathians being the center of its distribution. Among the species of *Vertigo* genus, recent information confirms the presence of the narrow mouthed whorl snail, *V. angustior*, apparently less uncommon than previously considered. *Vertigo moulinsiana* was recently reported by Sarkany-Kiss from the Sâncrăieni swamp, but three years later we could not find it. We checked all the sites recorded in the literature by Grossu and also other potential characteristic habitats, but *V. moulinsiana* and *V. genesii* were not found. Two hypotheses are possible, either the habitat modifications have led to their disappearance, or they have been misidentified from the start in some localities. In the case of

*V. genesii* the second hypothesis may be confirmed by the species description given by Grossu that is closer to *V. geyeri*. Therefore, the records from Romania have to be considered as uncertain, without excluding the possibility of their presence. *Drobia banatica* has a present range limited to Romania, Hungary and Ukraine, being introduced in several European countries. Historically, in Romania the species inhabits the western and southern Carpathians and sub-Carpathian area. In the southern part of the Meridional Carpathians it descends following the river valleys and reaches the sub-Carpathian area, while in the northern part it reaches the plateau area. The Carpathian populations seem to be well preserved, but in the hilly and plateau areas the intense anthropic disturbance, by deforestation, development of infrastructure, rural and urban areas, agricultural practices and changes in river flow regime, leads to severe habitat loss. The most critical is the loss of the riparian forests, the most important green corridors in agricultural areas.

## Three protected species of *Vertigo* in Ireland – losses, gains and the status quo in recent years

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Three of the eight *Vertigo* snail species which occur in Ireland are protected under the EU Habitats Directive – *V. angustior*, *V. geyeri* and *V. moulinsiana*. As in other EU countries, Ireland carries out national monitoring surveys in order to report on the conservation status of these species at six-yearly intervals. The most recent survey, a four-year monitoring project commissioned by the National Parks and Wildlife Service (NPWS), is due for completion at the end of this year. It involved visiting and surveying in detail a suite of 60 sites which support at least one of the three species. At each site, habitats and species were surveyed following and building upon a methodology set out by an initial survey (2008–2010), and so results can be compared. There have been losses and gains, and in some cases, the status quo stands. However, there are many pitfalls in the

monitoring process, making concrete statements on the outcomes difficult for some sites. For example, changes may be subtle and hard to detect, or conversely, changes may be obvious to the ecologist, but not always picked up with the chosen survey methods. Additionally, a site may appear to be in good condition, but yet fail on some assessment criteria. The natural world is complicated, and thus designing robust and useable monitoring protocols poses a huge challenge. This presentation will touch on some of the direct experiences from a large-scale and long-running ecological monitoring study on *Vertigo* snails, as well as presenting findings on the current conservation status of each of the three species in the Republic of Ireland. Additionally, mention will be made of survey work taking place in Northern Ireland, along with results.

## Conservation and management action plan for the recovery and expansion of *Vertigo angustior* and *V. moulinsiana* species in the Banyoles lake system (NE-Iberian Peninsula)

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*Vertigo moulinsiana* and *V. angustior* are two of the four species belonging to the genus *Vertigo* (Gastropoda: Vertiginidae) included in the Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora, otherwise known as the “Habitats Directive”. Since 2014 a LIFE Nature project “LIFE Potamo Fauna (LIFE12 NAT/ES/001091) is being carried out at the Banyoles lake system which includes some plan actions with the aim of the recovery and long-term conservation of these two *Vertigo* species in their habitat. The main objective defined within this project for these species is the “Expansion and consolidation of the populations of *Vertigo moulinsiana* and *V. angustior* in the Natura 2000 site Estany de Banyoles (ES5120008) via translocations from within the site”. For that, the following specific objectives are being developed: To delimitate the area of occupancy of both species across the lake and ponds; To determine the species

abundance, population structure and habitat requirements; To asses sampling methods for each species; To establish measures to ensure the conservation and management of both species; To devise a protocol for monitoring each species that is being used during the period 2014–2017. In this work we present the preliminary results obtained for each of these objectives. Nowadays there is no a standardized methodology for monitoring and recovering natural populations of both species in the Iberian Peninsula. Thus, the development of a package of protocols and methodologies with this purpose are of great interest to define the guidelines to be followed in future work of monitoring and management of these two species of Community interest. To *V. moulinsiana* and *V. angustior* this is the first major project developed in the Iberian Peninsula focused on their conservation and management, and it will allow consolidating some of the main Iberian populations.

## Biodiversity, distribution and conservation of freshwater molluscs of Georgia

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Freshwater molluscs are one of the most sensitive groups of animals to the environmental changes. Unfortunately, there is not enough information on diversity and distribution about freshwater molluscs to help in effective conservation. This problem is especially striking in Georgia, which is one of the world's

biodiversity hotspots. Here we present preliminary results and plans of an ongoing project concerning the diversity, distribution and conservation challenges of freshwater molluscs of Georgia based on extensive fieldwork and modern laboratory methodology.

## Issues concerning the monitoring and conservation status assessment of freshwater mollusk species in Romania: an overview

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One decade ago Romania became an EU member and has since largely developed and invested in nature conservation projects, especially those related to the EUHSD (92/43/EEC 1992), also known as the Habitats Directive. Consequently in 2007 and 2011 new laws were adopted aiming at the conservation of natural habitats, of wild fauna and flora. Some freshwater mollusk species were listed in their annexes, as requiring special attention and protection. Monitoring and conservation projects have been developed and implemented, guides and other materials have been published and it was expected that within ten years of joint efforts, the status and problems related to protected species would be revealed, but this expectation was only partially met. Without denying the results obtained by sound research at the national level, we attempt to draw attention to the problems with different causes that have diminished the quality of outcomes. Incomplete or erroneous information has been produced and propagated, because of a wide, complex, array of causes. The issues are many: we highlight the lack of specialists,

the reduced interest in malacology and field research, as well as in ecology and taxonomy, among the young but also senior generations. The species list in the laws also faces criticism: in our opinion the species should be subject to future amendments. However, the positive effect has been that attention and resources were attracted, among other issues, towards mollusk conservation and research on a larger scale and in an organized manner. The opposing effects were the exaggerated attention drawn by a few taxa and the correlated ignorance of others, the opportunistic raising of specialists, writing and reporting data erroneously, and others. Finding these species in certain areas is rewarding for scientists, managers and local inhabitants, but especially for politicians, therefore the subject became of political and social interest, surpassing the evidence of data and common sense. Political ambiguity, ignorance, opportunism, and other issues are discussed in our contribution. Finally we also present a synthesis concerning the knowledge and conservation status of some threatened freshwater mollusks from Romania.



## EU Life IP project FRESHABIT: Aiming to prevent extinction of the two main remaining *Margaritifera margaritifera* populations of southern Finland

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The freshwater pearl mussel (*Margaritifera margaritifera*, FPM) is endangered throughout the range of its distribution in Europe. There are over 120 rivers inhabited by FPM in Finland, most of them being located in the north. Only two major FPM rivers are found in southern Finland – River Ähtävänjoki/Essen (flowing to the Bothnian Bay, Baltic Sea) and River Mustionjoki/Svartå (flowing to the Gulf of Finland, Baltic Sea). Here we report results of monitoring of these two important FPM populations, in terms of population size and reproductive status, since the 1990s, and the efforts to save these populations. Estimated numbers of FPM, investigated by SCUBA diving covering the main mussel beds have declined alarmingly throughout the study period in both rivers. The total estimated FPM population size of River Ähtävänjoki was 50.000, 10.000, 3.500 and 500 in 1987, 2003, 2010 and 2016, respectively. Reproduction of FPM was evident in River Ähtävänjoki in 1990–93, 1997–98, 2004 and 2006 as viable glochidia were obtained from the mussels.

However, in 2015–16 FPM of River Ähtävänjoki did not release glochidia when kept in buckets, and no glochidia were found from salmonids caged in the river. In River Mustionjoki, also no glochidia were found to be released when mussels were monitored in buckets of river water in 2016, and no glochidia were attached to salmonids caged in the river in 2010–11 and 2016. Thus, populations are in a drastic decline and glochidia production of the mussels has stopped. Thus, actions have been undertaken in the EU Life IP project FRESHABIT (2016–2021) to prevent extinction of these last remaining southern Finnish FPM populations. First, to resume glochidia production, adult mussels have been transported to a breeding to enjoy artificial feeding. After that, captive breeding techniques will be used to produce juvenile FPM. Meanwhile, bottom quality will be improved to support introduced juveniles. Finally, to restore the natural migration and reproduction of salmonid hosts, fishways will be constructed.



## Culture methods of *Margaritifera margaritifera* (L., 1758): Comparison between plastic boxes, aquariums and Buddensiek's cages

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Conservation aquaculture involves recovery of endangered aquatic species using the complex and tested techniques of productive aquaculture. In this sense, captive breeding of threatened fish and/or crustaceans have evolved in Europe, nevertheless, only a few scientific studies have focused in the culture of naiads of the Unionoid order. These freshwater bivalves are one of the most threatened animals worldwide. Moreover, their unique life cycle, characterized by a temporary parasitic larval stage on fish, hinder the artificial propagation programmes through low reproductive success. In addition, the management of both host fish and wild mussel populations worsen this challenging culture.

*Margaritifera margaritifera* is categorized as Critically Endangered species by IUCN, it is linked to salmonid fish by their life cycle and it has become an imperative goal in the European conservation policies. Thus, several *ex situ* captive breeding programs were performed, however, during this process, mussel juveniles suffer from high mortalities. These early stages are also a critical point in wild populations, demonstrated by the extreme lack of recruit-

ment. Consequently, in order to optimize the mussel culturing, it is needed to standardize protocols that increase the survival and growth during the most critical stages.

Galicia (NW Spain) is a crucial territory for conservation of this mussel in south Europe. Concurrently, this region hosts the unique rearing facility of this species in the Iberian Peninsula. It was funded in 2013 by the Life Margal Ulla Project and nowadays it is maintained active by the Fundación Biodiversidad (Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente).

This work aims to compare three different rearing techniques applied to *M. margaritifera* juveniles. Therefore, one-year-old juveniles were reared in plastic boxes, aquariums and Buddensiek's cages for one year. Periodically, to calculate juvenile survival and growth rate, individuals were inspected and photographed by Image J software. Survival rate was significantly higher in plastic boxes than aquariums, by the other hand, juvenile growth was significantly more intense in aquariums than plastic boxes.

## Cephalopods preyed upon by the Loggerhead turtle *Caretta caretta*, in the Mediterranean

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Cephalopods are important elements of marine food webs, both oceanic and coastal. Their predator list is quite long and comprises members of several taxa, including Crustacea, Cephalopoda, Osteichthyes, Chondrichthyes, Mammalia and, to a minor extent, Reptilia, namely turtles. The loggerhead, *Caretta caretta*, which is the most frequent Mediterranean marine turtle, has been shown to have a carnivorous all-embracing diet, its main limits being prey swiftness. In the present work, cephalopod remains from 16 loggerheads were examined. They came from 23 cephalopods belonging to three species: *Sepia officinalis* (Sepiida) (19 specimens), *Histioteuthis reversa* (Teuthida) (2) and *Octopus vulgaris* (Octopoda) (2). Most prey remains were retrieved from the turtle intestine and consisted of beaks from all three prey

items and fragments of cuttlebone from *S. officinalis*. The identification of prey remains contributes to understand where the examined loggerheads had fed: *S. officinalis* and *O. vulgaris* are demersal species inhabiting coastal waters, whereas *H. reversa* is oceanic and dwells in the water column. Moreover, despite the cephalopod taxonomic diversity – they belong to three different orders – they share the feature of being comparatively slow moving; in particular *H. reversa* is an ammoniacal squid which, thanks to its neutral buoyancy, can stand suspended in the water thus becoming an easy prey for turtles. In one case, a loggerhead intestine contained remains of both the latter squid and cuttlefish, which suggest that it had moved in a short lapse of time from a deep water area to a shallow coastal zone.

## A proposed method for standardization of growth estimations of tropical and subtropical molluscs

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Following a particular cohort by analysing length-frequency distributions (*LFD*), originating from a timeconsuming quantitative sampling, is a common technique among ecologists to estimate the population growth of a single species. In this method, a given year class is followed, and the change in the average size of the mode is equivalent to average growth. Nevertheless, *LFD* analysis only works well in molluscs with a very narrow reproductive or recruitment period, which is normally the case of temperate and polar species. On the contrary, since in tropical and subtropical molluscs the recruitment pe-

riod is usually extended or growth rates are variable, *LFD* analysis has limited application and may not be useful to estimate growth for species of this climatic zone. On this account, the present study summarizes the available information on growth estimations of tropical and subtropical molluscs and recommends short time tagging-recapture experiments, using the *in situ* fluorescent marking (*IFM*) method, for measuring absolute growth as an appropriate standard method for future studies of economically and ecologically important molluscs, inhabiting tropical and subtropical climate areas.

## A mollusc in the tube instead of the shell: the first investigation of Cladobranch sea slug associated with Annelids

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The recent study of symbiotic communities of two *Chaetopterus* species in the Dam Bay, Southern Vietnam led to an unexpected discovery of a nudibranch living inside the annelid tube. Six specimens of nudibranch molluscs, belonging to the family Fionidae, were found inside the tubes of undescribed *Chaetopterus* sp. This case is the first example of symbiotic association between a mollusc and an annelid host among cladobranch sea slugs. The present study is focused on taxonomic identity of the discovered nudibranch, and on its position in revised Fionidae phylogeny. Molecular data, including partial sequences of mitochondrial *COI*, *16S* and nuclear *H3* genes, indicates this species is distinct from other members of the genus *Tenellia* as well as from other

fionids. The external morphology of this species suggests its adaptation for living inside the worm's tube: flattened body, laterally directed cerata, snuggled rhinophores and wide foot. This species is close to the coral-feeding fionids of the genus *Tenellia* (formerly in genus *Phestilla*) in several morphological characters such as general radula morphology, absence of cnidosacs and flattened body shape, as well as by molecular data. The unique biology of this symbiotic nudibranch implicates possible diversity previously overlooked by nudibranch faunistic studies.

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## Population dynamics of *Xerolenta obvia* (Gastropoda: Hygromiidae)

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*Xerolenta obvia* (Menke, 1828) is medium-sized (6–8 mm high, 12–17 mm wide) land snail of Pontic origin. It has wide ecological amplitude in terms of macroclimate as its distribution ranges from Asia Minor to the Balkans, the Carpathians, and along the Baltic coast, in the Mediterranean region to the southeast of France. In Poland it lives in sunny open habitats, dry grassy slopes, also fallows, gravel pits, railway embankments and road margins, often estimating in large numbers in the low vegetation.

The study was carried out from May 2015 till May 2017 in Piotrkowiczki, SW Poland. Seasonal changes in the size structure were traced based on regular samples (visual search) being taken monthly. On each occasion snails were collected during 1 hour, from an area of 25 m<sup>2</sup>. Growth rate and longevity estimated in the field were based on monthly marking with nail varnish (each month a different colour). Marking consisted in painting a narrow stripe on the shell, just next to the aperture margin. For each recaptured individual, the whorl increment

since the last marking was noted, then the individual was marked again and released. The growth rate was expressed by whorl increment. For size frequency histograms six size classes were distinguished.

Our results show that the smallest snails (up to 3.0 whorls), were present from April to August, suggesting that *X. obvia* lays eggs in spring and summer. Although, egg lying was only observed directly in the field in autumn (October). The eggs were nearly spherical, milky white, partly calcified (1.4–1.5 mm). The youngest juveniles, which probably hatched in spring, reached ca four whorls in the same growing season. Most snails wintered with the shells of 4.1–5.5 whorls. The final size is reached in the same or next year. Snails of the three oldest classes, present during the whole study, were most common from August to November. The mean whorl increment was different in both seasons. It was significantly slower in the first year of the study than in the second. During the first four months the mean whorl increments were 1.25 and 1.8, respectively.

## Cessation of grazing – the effects on land snail communities in farmed grassland, scrub and woodland habitats in the Burren region in the west of Ireland

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This study investigates the effects that the cessation of grazing might have on biodiversity, focusing on vascular plants and land snails. Few studies have used grazing exclosures in order to investigate the effects of changes in land management practices on molluscan communities. A network of twelve fenced exclosures (20 × 20 m) was set up across the region in 2006. The fences prevent access by large grazers (mainly cattle and goats) and were placed in three habitats: rough grasslands, areas with low or scattered hazel scrub, and hazel woodlands. Beside each fenced area is an unfenced plot of similar size which acts as a control. No mollusc species were lost or gained between the two sampling periods, although many species exhibited changes in abundance. The mean number of snails collected per quadrat increased by almost 50% inside the fenced plots between the first and second sampling periods (i.e. between 2006 and 2008), and there was only a very small change in the control plot numbers (a decrease of 3%). The largest and most consistent changes

were seen in the grassland sites, with significant increases in the numbers of snails collected across all quadrats. Analysis showed a definite shift in the species composition of the mollusc communities over the study period. The main factors associated with this shift were found to be cover of litter and vegetation height, both of which increased substantially in the absence of grazing. In conclusion, when grazing was removed experimentally from grassland habitats in the Burren, the snails seemed to benefit from the longer vegetation and denser litter which resulted. It is likely that the litter provides extra food, shelter and moisture for the snails, and thus conditions have improved (at least for certain species) within the fenced exclosures at the grassland sites. These findings contrast with the decreases in vascular plant richness and diversity which were seen in the grasslands, and thus they highlight the importance of assessing a suite of taxa when investigating the effects of changes in management practices on biodiversity.

## Two distinct forms of the hairy snail *Trochulus hispidus* (Linnaeus, 1758) show great phenotypic plasticity and no barriers in reproduction

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Morphological variation of snails from the genus *Trochulus* is so huge that their taxonomy is complicated. The greatest variability is shown by *hispidus* and *sericeus/plebeius* forms, which are often considered as separate species. To study the species barriers, we carried out crossbreeding experiments between these two sympatric morphs. We found that the two *Trochulus* morphs showed no reproductive barriers. The fecundity rates, the mean clutch size, and  $F_1$  viability observed for all crosses were not significantly different. In hybrid crosses of  $F_2$  generation, we also recorded the reproduction compatibility, similar fecundity, and hatching success as in their parents. Accordingly, the sequences of mitochondrial markers from these different morphs showed high similarity, and phylogenetic analyses grouped these sequences in a significant cluster. Comparison of shell morphology between wild and laboratory samples showed

that various characters are highly plastic. The average shell shape of the *hispidus* morph changed significantly from flat with wide umbilicus to elevated with narrower umbilicus such as in the *sericeus/plebeius* morph. All these findings indicate that the examined morphs do not represent separate biological species and their evolutionary differentiation is not advanced enough to separate their genetic pool. The two shell morphs can evolve independently in separate phylogenetic lineages under the influence of local environmental conditions. Nevertheless, the forms showing great phenotypic plasticity and polymorphism can be on on-going process of speciation and represent emerging species.

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## How well do you know your snails?

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Scanning Electron Microscopy (SEM) enables us to provide unusual insight into generally well-known objects. This poster presents such a view on periostracal structures of nine common European land snails (*Acanthinula aculeata*, *Aegopinella pura*, *Cochlodina lami-*

*nata*, *Isognomostoma isognomostomos*, *Monachoides incarnatus*, *Punctum pygmaeum*, *Pupilla pratensis*, *Vertigo substriata* and *Zoogenetes harpa*). Periostracal structures of these species differ substantially, being taxonomically as well as ecologically specific.



## More evidence for passive dispersal of land snails via birds

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Passive dispersal is a fundamental mode of land snail dispersion as their ability of active dispersal is very limited. Birds are known to be important vectors of snail dispersal. However, any direct evidence of this process is rare and mostly anecdotic. Snails can be dispersed both attached on a bird externally (ectozoochory) or being swallowed by bird and then expelled alive (endozoochory). The possibility of endozoochory has been studied only for few species, all of them minute (>4 mm). We examined an ability of larger species (up to ca 17 mm in maximum shell dimension) to survive the passage through digestive tract of various bird species. Live *Alinda biplicata*, *Cochlodina laminata* (both Clausiliidae) and *Discus rotundatus* (Discidae) were offered to 10 bird species

(Corvidae, Turdidae, Sturnidae and Columbidae) in 14 experimental trials. Out of 720 snail individuals offered, 14 were found intact in the faeces. Nine of them were alive (eight clausiliids, one *Discus rotundatus*) which represents more than 1% of offered snails. If the shell of clausiliids passed bird's digestive tract intact, more than 60% of specimens were alive. The possibility of endozoochory for other combinations of snail and bird species will be examined in our current project. An ability of *Alinda biplicata* and *Cochlodina laminata* to remain attached on bird legs (*Gallus gallus* cf. *domestica*) during simulated flight conditions was tested in additional experiments. Snails attached by pedal adhesion resisted oscillations of maximum rate, which is normally achieved during a flight.

## The impact of salinity on the biodiversity of mollusk communities in anthropogenic water bodies in a coal mining region (Upper Silesia, southern Poland)

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The study was conducted in 2016 in 9 anthropogenic water bodies with different salinity level. According to the classification of Hammer, 3 ponds belong to freshwaters ( $\text{TDS} < 0.5 \text{ g L}^{-1}$ ), 3 to subhaline waters ( $\text{TDS} = 0.5\text{--}3.0 \text{ g L}^{-1}$ ) and 3 to hypohaline waters ( $\text{TDS} = 3.0\text{--}20.0 \text{ g L}^{-1}$ ). The study showed the presence of 18 mollusk species: 12 gastropod species and 5 bivalve species. Among them three gastropods, alien in Polish fauna, were recorded – *Potamopyrgus antipodarum*, *Physa acuta* and *Ferrissia fragilis*. In freshwater ponds 14 mollusk species were found, in subhaline waters 12 species and in hypohaline waters only *P. antipodarum* occurs. Species diversity of mollusk communities expressed by Shannon-Wiener index  $H'$  was significantly higher in freshwater and subhaline water bodies compared to communities in hypohaline water bodies. The share of alien species in mollusk assemblages was very

high in hypohaline and subhaline waters and significantly lower in freshwaters. No mollusks were found in waters with TDS higher than  $17.1 \text{ g L}^{-1}$ . Canonical correspondence analysis (CCA) showed that TDS, total hardness, pH, phosphates, nitrates, nitrites and ammonium were the parameters most associated (statistically significant) with the distribution of mollusk species. *P. antipodarum* was associated with high concentration of TDS, whereas other species avoided waters with high salinity. *Lymnaea stagnalis*, *P. acuta*, *Radix auricularia*, *F. fragilis*, *Gyraulus crista*, *G. albus* and *Unio pictorum* were associated with a higher concentration of nutrients. The results of this study showed that the anthropogenic water bodies that are located in an industrial area may contribute to the dispersion of alien species, in particular, ponds with high water salinity may play an important role in the dispersion of *P. antipodarum*.

## Host *specificity* in the bivalvia-trematoda system: analyses based on rDNA sequences and phylogeny of European flukes representing Gorgoderidae, Bucephalidae and Allocreadiidae

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European freshwater bivalves of the families Unionidae, Sphaeriidae and Dreissenidae are used as a first intermediate host in the life cycles of trematode parasites of three families: Bucephalidae, Allocreadiidae and Gorgoderidae. Adult trematodes of these families are well-known parasites of teleost fish and, more rarely, of amphibians. Depending on species of Gorgoderidae, the first intermediate host is a sphaeriid or unionid clam, or *Dreissena polymorpha*. Only three species of Bucephalidae are known in Europe: two *Rhipidocotyle* species use unionid clams as first intermediate host, *Bucephalus polymorphus* is parasite of *D. polymorpha*. The family of Allocreadiidae is strongly concentrated in sphaeriid bivalves. We summarized newly obtained and other available data of molecular studies on larvae collected from bivalvia and representing the three trematode families. More than 30 species of the family Sphaeriidae are found in Europe, however only several species were recorded as an intermediate host for flukes representing Allocreadiidae and Gorgoderidae. Large geographical ranges of *Sphaerium corneum* and *Pisidium amnicum* provide an opportunity to collect

these clams from distinct Europe regions. Molecular results demonstrate that these clams are the hosts for larval trematodes of several species of both families. The world-wide distributed small sphaeriid clam *Pisidium casertanum* appeared as the first host for three species of Allocreadiidae. *Sphaerium nitidum* serve as host for flukes of the *Crepidostomum* spp. in North Europe. Only few molecular evidences were obtained about the association of other sphaeriid species with Trematoda: *Pisidium hibernicum* is host for *Phyllodistomum umblae*, *Sphaerium rivicola* – for *Bunodera lucioperca*. Molecular evidence proved that *Bucephalus polymorphus* is specific for *D. polymorpha*, while *Rhipidocotyle* species were found exclusively in unionids; *Unio* spp. can serve as host of *R. campanula*; *Anodonta anatina* is used by two species, *R. campanula* and *R. fennica*; and by *Cercaria duplicata* (larva of *Phyllodistomum* sp.). This study provides molecular evidence that different host specificity in the Bivalvia-Trematoda system is independent of host and parasite phylogeny.

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## Life cycle of *Bulgarica cana* (Held, 1836)

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The life cycle of *Bulgarica cana* (Held, 1836) (Gastropoda: Pulmonata: Clausiliidae) was observed between 2005 and 2014 with varying intensity partly in the laboratory and partly in its natural habitat. Field observation was carried out in Romincka Forest (NE Poland).

*Bulgarica cana* is oviparous. Its freshly laid eggs are oval, snow-white and partly calcified. The mean number of eggs per batch is 8.4 (SD = 3.97). Eggs are always laid in damp, drought-protected places. The mean incubation time at room temperature is 16.2 days (SD = 3.09). In laboratory conditions, the hatching success was 86.3%. Hatchling shells have the mean of 2.63 whorls (SD=0.32), adult shells – 12.42 whorls (SD=0.49). In the laboratory growth to adult size (completed shell growth) took on average 6.8 months (SD=2.1). The mean growth rate

was 0.75 to 2.67 whorls/month. In natural conditions juveniles grew (built their shells) only during the four warmest months of the year: from June till September. The mean growth rate in the summer was 0.71 whorl/month. The whole-year growth rate of the snails observed during one year or longer was 0.21 whorl/month. At this rate, the shell growth completion takes at least 40 months (more than 3 years). Sexual maturation was delayed in relation to shell growth completion by at least 3.5 months. The duration of reproduction varied widely among the pairs. The longest observed reproductive period in the laboratory was more than 4 years. The pairs of snails produced on average 60.74 eggs per year. No uniparental reproduction was observed. *B. cana* is a long-lived species. Its maximum lifespan in both laboratory and natural conditions was 9 years.

## The influence of human disturbances on the appearance of alien species in a small lowland river

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Environmental disturbances, especially those resulting from human activity, increase the vulnerability of water biocoenoses to the appearance of alien species to a greater or lesser degree. Disturbances of their new habitat, the presence of uninhabited niches and food resources, which are not fully utilised by native fauna, may facilitate the creation of their permanent populations. The aim of our research was to determine the impact of the anthropogenic transformations of the lower course of the Ruda River (Southern Poland, Upland Silesia, the right-bank tributary of the Odra River), as well as to determine a source of pollution possibly related to the occurrence of alien species. The samples were collected using standard quantitative methods, using a quadrat frame (0.25 m<sup>2</sup>), at seven sampling sites: three selected in the regulated part of the river and three along its natural course. In order to demonstrate the impact of the source of the infiltration of pollutants, one of the sampling sites was selected above their inflow. The results were converted to 1

m<sup>2</sup> of the bottom area. The mean density of benthic invertebrates was higher in the regulated part of the Ruda River, which was influenced by the presence of alien species, including snails, which were constant elements during the collection – *Potamopyrgus antipodarum* and *Physa acuta* (66.7% and 37.5% of the total material, respectively). In the regulated section of the river, where bottom sediments were covered with concrete slabs, mud and algae, the average density of *P. antipodarum* was 4080 ind./m<sup>2</sup>, while in its natural parts – 564 ind./m<sup>2</sup>. Studies have shown an alternating dominance of both of these alien species of snails. The source of pollution has a negative influence on the density and diversity of native benthos fauna and an increase of the density of *P. antipodarum* (18,498 ind./m<sup>2</sup>). Studies have shown that the source of the discharge of pollution, similar to a riverbed's hydro-technical development, creates favourable conditions for the occurrence of alien species and that an inflow of sewage limits the possibility for the occurrence of native fauna.

## The dispersion of *Corbicula fluminea* (O. F. Müller, 1774) (Mollusca: Corbiculidae) in the Upper Oder River (Southern Poland)

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*Corbicula fluminea* (O. F. Müller, 1774) is a south-eastern Asiatic species that has been recorded in Poland since 2003. To date, it has been found in the lower and middle course of the Oder River and in the Vistula River. The aim of the study was to evaluate the possibility of the further spread of the *C. fluminea* upstream of the Oder River.

The study was carried at three sampling sites of the Upper Oder River – in Dobrzeń Mały, Zdzieszowice and Bierawa in 2016. *C. fluminea* was found in two of them (Dobrzeń Mały and Zdzieszowice). The new localities are the southernmost records of this species in the Oder River, which indicates that it is spreading upstream.

During the study, 62 live individuals and 22 empty shells of *C. fluminea* were collected from sandy-gravel and sandy-muddy bottoms. The shells of *C. fluminea* have relatively small dimensions (mean height:  $12.1 \pm 3.4$  mm, mean length:  $13.9 \pm 3.7$  mm, mean width:  $9.0 \pm 2.6$  mm), which indicates that the population consists of young individuals. This fact is typical for young, growing populations in the initial stage of invasion.

The present occurrence of *C. fluminea* in Poland confirms the spread of this species, and therefore, its new localities should be monitored in order to determine the impact on native molluscs, especially due to the fact that there are many ways in which it is dispersed.

## The possibility to use invasive species in biological early warning systems

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The diversity as well as abundance of freshwater mussels are sorely decreasing globally. Almost half of the species are currently threatened. The possibility of using a native species for fresh water monitoring may be problematic in the near future. Hence, the ability to use an invasive species as indicator species should be taken into account. In many regions of the world one of the most problematic among exotic species is *Sinanodonta woodiana*. It is widely acknowledged that *S. woodiana* can increase their abundance faster than other native mussels. Based on notification about the spread of this species in temperate

countries, it may be suspected that *S. woodiana* will become common species in the near future. This research focuses on the use of *S. woodiana* in water quality monitoring. The behavioral reaction for substances such as nitrates, sulfates and phosphates in limiting concentrations for native species were checked. Based on our observations, it was found that *S. woodiana* show a similar (but not the same) range of tolerance to our native species to some pollutants. The specific behavior reaction – valve movement was different in both cases.



## Invasive chinese pond mussel *Sinanodonta woodiana* impact physiology and induce cross-resistance of host fish

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Geographic range extensions expose *Sinanodonta* (*Anodonta*) *woodiana* (Unionidae, Bivalvia) to novel communities of native fish hosts and their affiliate bivalves. Our research focuses on the impacts of *S. woodiana* parasitic stage (glochidium) on host fish physiology and the role of priming infections by the invader on parasitization success of native mussels. We report for the first time that glochidia of *S. woodiana* cause an unexpected reduction in the condition factor of parasitized native fish species. The reduction in the body mass and condition factor of experimentally infested European chub (*Squalius cephalus*) was associated with changes in several physiological parameters measured in host fish plasma. Ion concentrations (potassium, chloride) and enzymes activities (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase) were significantly affected. Results reveal the complex effects of non-native glochidia on the homeostasis of the individually tested fish in environmentally relevant infestation intensities (mean

of  $3.02 \pm 0.51$  glochidia gram<sup>-1</sup>). We also tested the capacity of European *A. anatina* to parasitize *Squalius cephalus* after previous infections with *S. woodiana*. We compared the dynamics of initial glochidia attachment, the length of the parasitic period and the transformation success rate of *S. anatina* glochidia among treatments. Our results document a strong cross-resistance between the invasive and native species as the transformation success rate of *S. anatina* was significantly reduced on primed hosts in comparison to naïve. Such cross-resistance is expected to significantly decrease the quality of host resources available to native mussels in natural habitats. The results provide novel insights on the competitive mechanisms between unionid bivalves and describe novel process affecting the invasion potential of invasive affiliate species such as *S. woodiana*. We argue for more careful consideration of potential multiple life-stage effects of *S. woodiana* and of other invasive alien species as well, as different life stages can have highly specific impacts on native communities.



## Parasitism of the exotic mudsnail *Potamopyrgus antipodarum* (Gray, 1843) (Mollusca: Caenogastropoda: Tateidae) in the Mont-Saint-Michel Bay (France)

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*Potamopyrgus antipodarum*, native to New Zealand, has invaded streams and lakes of most continents. Its successful spread is partly explained by high fecundity, ovoviviparity, parthenogenesis, wide physiological tolerances (e.g., euryhalinity), efficient dispersal, and enemy release. In New Zealand, *P. antipodarum* is first intermediate host for at least 20 species of trematodes. In contrast, *P. antipodarum* is rarely parasitized in introduction area as in Europe where it is infected as first intermediate host by only one aporocotylid species. Recent molecular identification demonstrated that this trematode (Aporocotylid sp. I) is originated to New Zealand, and then, an introduced species as its snail host.

The prevalence (%) of trematodes has been studied in gastropod communities from four sites varying in salinity (from  $1.00 \pm 0.61$  to  $13.28 \pm 5.27$ ‰) in the Mont-Saint-Michel Bay.

*P. antipodarum* was the dominant species whatever the site and represented 80.2% of the 7,218 gastropods collected from all sites, followed by *Radix peregra* (12.2%), *Physa acuta* (5.7%), *Planorbis planorbis* (1.9%), *Anisus leucostoma* (0.01%) and *Armiger crista* (0.01%).

Gastropod richness and abundance decreased with increasing salinity, and only *P. antipodarum* was recorded at the highest salinity site.

Larval trematodes (sporocysts/rediae and cercariae – no metacercariae) were observed in 1.16% of all sampled snails. The trematode prevalence per host population varied from 0 to 12.31% depending on site/salinity and host species. No trematodes were found at the highest salinity site but Aporocotylid sp. I was recorded in *P. antipodarum* from the three other sites (salinity  $\leq 10.60 \pm 1.34$ ‰), with a prevalence varying from 0.32 to 4.07%. The introduced aporocotylid was never found in the other gastropod species. Moreover, despite the occurrence of diverse trematodes in these latter (in particular in both native *R. peregra* and *P. planorbis*) at the lowest salinity site, no parasites were transferred to *P. antipodarum*.

Further studies are needed to identify the final host of Aporocotylid sp. I (fish) in both native and introduced range, and to trace the history of parasite introduction in Europe. The absence of infected *P. antipodarum* at highest salinity site may be indicative of the tolerance limit of the fish host species.

## Current distribution of *Sinanodonta woodiana* (Lea 1834) in Poland

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Invasive alien species have been recognized as one of the greatest direct threats to European nature. One of the alien species in Poland that spreads on a large scale is the Chinese pond mussel *Sinanodonta woodiana* (Lea, 1934) which departing from its natural range in Asia appeared in Europe and in America due to transport of farmed fishes.

On 9th September 2011, an order was issued by the Ministry of Environment in Poland that concerns the list of plants and animals of alien species which can threaten native species or natural habitats if released in the environment. Among 52 species, the Chinese mussel clam is also specified. Chinese pond mussels in Poland appeared in the 1980's and has expanded in Poland significantly and quickly since then. Each year new reports are published about the

occurrence of the Chinese pond mussel in Poland. It has found here appropriate conditions for living and settlement.

We summarize the current knowledge about the Polish population of the Chinese mussel clam. Including literature survey and our own research data comprising new, yet unreported sites as well. At least 35 locations of the Chinese pond mussel have been confirmed in Poland. *S. woodiana* is mostly present in fish ponds (64%) but in the last few years it has been discovered in lowland rivers, canals and wetlands, too. Even with these facts the current data about the distribution of *S. woodiana* seem to be underestimated. The data indicates that the species is spreading quickly and the monitoring should be still conducted.

## Alien species in the mollusc communities in selected abiotic types of rivers with different degrees of hydromorphological transformation (Upper Silesia and adjacent areas, Southern Poland)

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Upper Silesia (Southern Poland) constitutes one of the most industrialised and urbanised regions in Europe. The degradation of most of the rivers of this region includes hydromorphological transformations and excessive concentrations of chlorides, sulphates, total dissolved solids, nutrients and heavy metals in the water. The study was carried out in three abiotic types of rivers with different hydromorphological transformations in Upper Silesia and adjacent areas in 2016–2017. The objectives of the survey were to analyse the structure of the mollusc communities in three abiotic types of river and to determine the most important environmental factors that have a significant influence on the occurrence of molluscs. A greater number of species, density and the values of the Shannon-Wiener index  $H'$  were recorded at the sampling sites that had a higher conductivity, hardness, alkalinity and concentration of nutrients (abiotic type no 6: mid-altitude calcareous streams with a fine-particulate substratum on loess). *Potamopyrgus antipodarum*, an invasive alien species, was eudominant in these mollusc communities (density up

to 15,744 individuals/m<sup>2</sup>), whereas *Physella acuta*, another alien species, was recedent (density up to 384 individuals/m<sup>2</sup>). The Kruskal-Wallis one-way ANOVA test revealed significant differences between the medians of mollusc density in particular abiotic types of rivers with different degrees of hydromorphological transformations.

The results of canonical correspondence analysis (CCA) showed that the concentration of dissolved oxygen, conductivity, hardness and the concentration of organic matter in the bottom sediments were the environmental variables that were most associated (statistically significant) with the distribution of molluscs including alien and invasive species. Significant changes in the water quality can lead to a decline in a river's biodiversity and may favour alien species over native species in the fauna of the country. For example, *Potamopyrgus antipodarum* was the only mollusc species that occurred in the most degraded river sections that had a conductivity of up to 7,160  $\mu\text{S}/\text{cm}$ .

## Establishment of the alien species *Dreissena polymorpha* Pallas, 1771 in a man-made reservoir undergoing restoration (Pławniowice reservoir, Southern Poland)

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During the study of a man-made reservoir that was undergoing restoration (Pławniowice reservoir, Southern Poland), the alien species *Dreissena polymorpha* was first recorded in 2011, seven years after the start of restoration measures. To date, *D. polymorpha* has only been found in a few localities in southern Poland. The aim of the study was to assess the spatial distribution of zebra mussels, their settlement preferences and the dynamics of their population in Pławniowice reservoir.

The study was conducted over a five-year period (2011–2015). Samples were taken from the near-shore area (at a depth of up to 1 m) and along six transects at depths of 2, 4, 6, 8 and 10 m.

The density and biomass of *D. polymorpha* were low and ranged from 349 specimens m<sup>-2</sup> in 2011 to 545 specimens m<sup>-2</sup> in 2015 (density) and from 196.1 g m<sup>-2</sup> in 2011 to 447.5g m<sup>-2</sup> in 2015 (biomass), respectively. The fluctuations in population densities and biomass were not statistically significant. Colonies of zebra mussels were found to a depth of 6 m. The

density and biomass of *D. polymorpha* were higher in the near-shore area compared to deeper sites.

In Pławniowice reservoir, zebra mussels had mostly settled on stones (from 20% in 2013 to 66% of all of the collected zebra mussels in 2015), attached to large conspecific individuals (from 11% in 2015 to 31% of all of the collected zebra mussels in 2013) and attached to native unionoid mussels (from 15% in 2015 to 37% of all of the collected zebra mussels in 2012). The intensity of the infestation of native unionoid bivalves decreased in the subsequent years (from an average of 28 individuals on unionid shells in 2011 to an average of three individuals on unionid shells in 2015).

During the study, juveniles and moderate adult groups strongly dominated the age structure of the population (>70%) and the percentage of the largest adults was low (only up to 2%).

The recreational use of Pławniowice reservoir, its connection with other waters via the Toszecki Stream may be the reasons for the further dispersion of *D. polymorpha* into new localities in southern Poland.

## Long-term study on the occurrence of invasive and alien mollusc species in the mining subsidence reservoirs of industrial areas impacted by coal mine output (Upper Silesia, Southern Poland)

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Upper Silesia (Southern Poland), the most urbanised and industrialised region, constitutes one of the biggest coal basins in the world. This area has no natural water bodies, but reservoirs of an anthropogenic origin, including mining subsidence reservoirs are common. The study was carried out in 4 mining subsidence reservoirs that are supplied with flowing water from surface waters and with waters from a mine dewatering system and in 3 reservoirs that have been isolated from the mine dewatering system since 1993. The part of the Mleczna River that is connected with the mining subsidence reservoirs was also investigated. Since 1993 the conductivity of the water has constantly increased from 710 up to 3550  $\mu\text{S}/\text{cm}$  in the reservoirs and in the Mleczna River as a result of coal mine waters that have been pumped out and breached the geological strata during the hard coal mining. In total, 24 mollusc species were recorded in the mining subsidence reservoirs: 19 gastropod species and 5 bivalve species. Beginning with the initial dispersal in 1997, *Potamopyrgus antipodarum*, an invasive species originating in New Zealand, has been

eudominant in the mollusc communities with a maximum density of 7800 individuals/ $\text{m}^2$ . *P. antipodarum* is able to reproduce in the mining subsidence reservoirs throughout the year. The maximum number of embryos in a brood pouch per female was 59, which is lower than the maximum number of embryos of the specimens that occur in New Zealand (over 100 embryos). The maximum density of the two other alien species, i.e. *Physella acuta* and *Ferrissia wautieri* amounted to 297 and 58 individuals/ $\text{m}^2$ , respectively. The results of canonical correspondence analysis (CCA) showed that hardness, conductivity, alkalinity and pH were the environmental variables most associated (statistically significant) with the distribution of mollusc including alien and invasive species. *P. antipodarum* and *P. acuta* were positively influenced by increasing values of conductivity and hardness. The results of the survey showed that the mining subsidence reservoirs of the industrial areas and rivers of Upper Silesia play an essential role in the dispersal of invasive and alien species.

## Continuous reproduction of *Sinanodonta woodiana* (Lea, 1824) females: an invasive mussel species in a female-biased population

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The reproductive activity of females of the Chinese pond mussel (*Sinanodonta woodiana*) was investigated in a European population inhabiting cooling water. The study used histological and stereological methods to determine gonad structure, changes in reproductive follicles during gametogenesis, oocyte development, glochidia structure, and brooding periods. Water in the channel of the “Dolna Odra” power plant did not freeze during the winter, and its mean annual temperature was 18.4°C. The population sex ratio was female biased ( $\chi^2 = 25.70$ ,  $df = 1$ ,  $P < 0.0001$ ). Ovaries in mussels were formed by reproductive follicles. Previtellogenic and vitellogen-

ic oocytes were attached to the follicle wall via the cytoplasmic stalk, and mature ovulated oocytes were present in the follicle lumen. Individuals with mature oocytes in gonads were present over the whole two-year study period, which indicates the continuous activity of gonads. Hooked glochidia had a larval thread. Females incubated the offspring in gill marsupia of outer demibranchs and were characterized by multiple tachytictic brooding periods. All gravid individuals presented mature and spent stages of gonadal development. The study provides the first direct information about the reproductive dynamics of this invasive species outside its original Asiatic range.

## *Potamopyrgus antipodarum* (Gray, 1843) – protective shield against swimmers' itch

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The source of transmissions dangerous larvae of *Trichobilharzia* spp. for human are snails belonging to the Lymnaeidae. Inside infected snails, the miracidium transforms to a mother sporocyst, which produce daughter sporocysts, then thousands of invasive furcocercariae. The similarity between some lipid components in the coatings of a human and a bird body leads to the penetration of the skin of people swimming in the water. The symptom of this invasion is skin lesions known as "swimmers' itch". The inspiration for the research was limited precision of miracidia transmission to hosts, as well as received results for study on the biological control for human schistosomiasis that use alien and even invasive species of snails. The main objective of the presented research was to check whether *P. antipodarum* (Gray, 1843) – an alien snail species in the European waters can be a protective shield against the invasion of the first larval stages of bird schistosomes to specific intermediate hosts. We conducted experimen-

tal infection of *R. balthica* by *T. regenti* miracidia in the presence of increasing density of *P. antipodarum*, and attempt to infect this alien snail species by the species of bird schistosome. Our results indicate that the presence of increasing density of *P. antipodarum* in the neighborhood of *R. balthica* can affect parasite transmission success. Furthermore, this alien snail species is not a source of invasive cercariae of bird schistosomes for vertebrate hosts. There are several important reasons for research on biological possibilities of disturbing the life cycle of bird schistosomes: (i) the common occurrence of snails (intermediate hosts) emitting *Trichobilharzia* spp. cercariae, (ii) the presence of numerous waterfowl (final hosts) in recreation areas, (iii) frequent cases of *dermatitis* (especially in children) along with a number of additional symptoms, (iv) the lack of clear data on the fate of penetrating human skin cercariae, and (iv) the lack of a safe and effective method of protection against "swimmers' itch".



## Selected food products, as the attractants for the invasive slugs *Arion vulgaris* and *Limax maximus* – field and laboratory study

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*Arion vulgaris* and *Limax maximus* were tested in terms of their preference for commonly accessible food products. Samples of fermented raspberry juice, fermented apples, fermented milk (3.2% fat), fermented oatmeal, and multiflorous honey, and water as the reference sample, were used in the study. Experiments were performed using six-arm olfactometer. Thirty slugs were placed singly at the olfactometer arena, to chose either any attractant smell introduced to five arms of the olfactometer, or water in sixth arm. The olfactory preferences were determined after 12 hours of the test. Additionally, field tests were performed using home-made traps containing attractants or water, distributed in randomly selected places. Each test took one day and was performed in 24 repetitions. The results were subjected to a qualitative and quantitative analysis. Moreover, chromatographic analysis of the studied attractant volatile fractions (GC-MS) was carried out using the SPME technique. Classification of compounds was made using the NIST database 08. The attractant substances were ranked according to their attractiveness to slugs in field and laboratory tests, and according

to mean relative concentration of volatile substances. The relationship between those variables was analysed using the Spearman rank correlation test. The Bernoulli scheme was used to check out whether in the laboratory tests volatile fractions of attractants were chosen by the slugs randomly. Both tested slug species were characterised by similar olfactory preferences. They selected products in the following order: water (40% *A. vulgaris* and 30% *L. maximus*), honey (26.7 and 30.0%), raspberry juice (13.3 and 13.3%), apple fruits (26.7 and 30.0%), milk (6.6 and 3.3%) and oatmeal (3.3 and 10.0%). Similar preferences were observed in the field tests. The honey was selected most frequently (by 29.5% of 61 *A. vulgaris* individuals. and 35.3% of 34 *L. maximus* individuals). Remaining products were selected in the following order: raspberry juice (23.0 and 14.7%), apple fruits (21.3 and 26.5%), milk, oatmeal and water (16.4 and 14.7, 4.9 and 5.9 and 4.9 and 2.9%, respectively). Lauric acid and  $\alpha$ -ionene were responsible for the attractiveness of honey for both studied species. These results were consistent with our previous studies using beer as attractant.



## Testing the enemy release hypothesis on Polish and Italian populations of mussels

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Research on biological invasions has grown over the past years, yet ecologists still pursue general questions such as why some communities are more invaded than others, or why particular species become widespread and abundant. The enemy release hypothesis (ERH) explain the success of alien species after introduction into new areas and predict that invasive species will receive less damage from enemies, compared to co-occurring native and noninvasive exotic species in their introduced range. However, while the assumptions of the hypothesis were widely accepted by scientists, it has not been thoroughly studied yet and there are still some doubts. Results of some research are support the ERH but others reveal some weaknesses of the ERH assumptions.

The present study aimed at assessing the occurrence of parasites and the frequency of shared

parasites in invasive and native bivalve species. In four sites the prevalence of parasites in coexisting European native mussels (*Unio elongatulus*, *U. tumidus*, *U. pictorum*, *Anodonta cygnea* and *A. anatina*) and invasive species (*Sinanodonta woodiana*, *Corbicula fluminea*, *Dreissena polymorpha*) were determined. 30 specimens per each mussel species in each site were dissected to detect the presence and quantify the number of parasites stages in the different animal organs. The following parasites were detected and counted: trematodes (*Rhipidocotyle campanula*, *R. fennica*, *Bucephalus polymorphus*, *Aspidogaster conchicola*), oligochaetes (*Chaetogaster* sp.) water mites (*Unionicola* spp.) and bitterlings (*Rhodeus* spp.). Native species showed the highest parasite prevalence – while invasive alien species showed a lower to null prevalence – in both in Italian and Polish water bodies.

## Zebra mussel versus quagga: changes in population structure of two non-indigenous dreissenids in the Szczecin Lagoon (River Odra estuary, southern Baltic Sea)

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The zebra mussel (*Dreissena polymorpha*) and the quagga (*D. rostriformis bugensis*) are freshwater Ponto-caspian dreissenid bivalves which have colonised waters in Europe and North America. Expansion of *D. polymorpha* to Europe occurred much earlier than that of the quagga (at the turn of the 19th century), while the latter began spreading in Europe in the 1940s. In the Szczecin Lagoon, the zebra mussel has been featuring in species lists since the onset of studies in the area (19th century), the quagga being first recorded in 2014 when it was found co-occurring with the zebra mussel. To assess possible relationships between the two species, they have been sampled monthly since May 2015 at two sites in the Szczecin Lagoon, in the northern and the south-eastern part. Population structure of the two species was analysed in terms of abundance, biomass, and size distribution. The data show the expansion of the new immigrant to have proceeded from the north to the south, as in summer 2015 the quagga was markedly dominating over the zebra mussel – both in the abundance and biomass – at the northern site, the quagga in-

dividuals being much larger than those of the zebra mussel. At the south-eastern site, the zebra mussel was still dominating in terms of abundance, but the larger size of quagga individuals rendered the species a biomass dominant. In summer 2016, however, the quagga abundance at the northern site diminished and that of the zebra mussel increased. Thus the abundance dominance structure was reversed in favour of the zebra mussel, but the quagga – still represented by larger individuals – retained its domination in biomass. In 2016, the size distribution of *D. rostriformis bugensis* showed no clear peaks indicating the appearance of juveniles. The proportion of empty quagga shells at the northern site increased in summer 2016 compared to that at the initial stage of the immigration, which may evidence inhibited population growth of the newcomer, at least at the northern site.

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## Identification key for swiss molluscs via mobile application software

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The aim of this project is to develop a mobile application that identifies all mollusc species found in Switzerland. The last available dichotomous identification key was published in 2005, is taxonomically outdated, and did not include the freshwater bivalves. The primary focus is to give the interested public, answers at their fingertips, whereby molluscs can be determined quickly to the species level. The app will be handy, especially for those who have no prior knowledge of this invertebrate group. The key will be designed using the free software Xper3 It is based on photographs of shells from the mollusc voucher collection at the Natural History Museum of Bern and covering the 281 species recorded for Switzerland. Distribution maps can easily be checked via a link to

the CSCF webpage (Centre Suisse de Cartographie de la Faune). An extra plus is that one's own field observations can be reported directly to the CSCF. This feature will help to keep distribution records up to date while providing important information for applied malacology such as for IUCN red lists and data mining for environmental impact studies. The app includes tips on collecting and discovering molluscs while the glossary provides information towards understanding the essential characters and character states needed for successful species determination. Finally, it aims to fascinate people and distinctly engage public awareness for this animal group, of which 40% of the species can be found on the IUCN red list and thus, are in dire need of protection.

## MoFA – the newly founded Society for Molluscan Research in Austria

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We are pleased to announce the foundation of the society “Mollusken Forschung Austria – MoFA” (“Mollusc Research Austria”), which was authorised by public authority in August 2016. MoFA is a society based in Austria aiming to promote activities of Austrian malacologists and encourage the communication with national and international professional working groups and initiatives. Another task of the society is the enhancement of information exchange and coordination of scientific projects and studies.

The society was happily welcomed by several mollusk-related working groups and led already to two small meetings with interesting discussions. More information about MoFA, about molluscs in general and links concerning related initiatives and platforms are provided on our new webpage [www.molluskenforschung.at](http://www.molluskenforschung.at). At the moment MoFA counts 26 members and everyone interested in malacological research in Austria is welcome to join our society.

Contact: [team@molluskenforschung.at](mailto:team@molluskenforschung.at)

## Phylogeny of widespread Indo-tropical genera highlights the ancient connections between the largest river basins of Indochina

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Indochina represents a high biodiversity sub-region of Unionoida with numerous endemic and threatened species. However, this unique fauna is still poorly studied regarding systematics, ecology and phylogeny. It is interesting that freshwater mussel biodiversity in Indochina was historically formed by different geological and climate events during Cenozoic.

The multi-locus fossil-calibrated phylogeny of three Indo-tropical genera as *Contradens*, *Trapezoideus* and *Physunio* (tribe Contradentini) has revealed evolutionary relationships between them and has enabled us to examine the history of freshwater basin interactions across Indochina. Phylogeny of Contradentini has demonstrated that each studied river basin (Mekong, Irrawaddy, Salween and Sittaung) represented an evolutionary hotspot with

endemic species. According to a phylogenetic model, the Unionodae clade most likely originated in the Jurassic. Contradentini MRCA evolved in the middle of the Cretaceous. The endemic clade of the Mekong basin is assigned to Late Cretaceous. The Irrawaddy+Salween+Sittaung connection likely exists from Early Eocene to Middle Miocene. Our findings also show that the Mekong can be considered a long-lived river existing throughout the entire Cenozoic. Biogeographic scenario (vicariance vs dispersal) was tested using three alternative methods: S-DIVA, DEC and S-DEC.

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## Different expression pattern of genes encoding VDAC in terrestrial and freshwater gastropods

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It is known that adaptational processes result in molecular signatures at every level of the cellular organization. Therefore we determined transcripts differing in expression levels between terrestrial and freshwater gastropods by application of next-generation sequencing of transcriptomes obtained for *Helix pomatia* Linnaeus, *Arion vulgaris* (Moquin-Tandon), *Lymnaea stagnalis* (Linnaeus) and *Planorbarius corneus* (Linnaeus). We noticed that terrestrial (*H. pomatia* and *A. vulgaris*) and freshwater (*L. stagnalis* and *P. corneus*) gastropods differed remarkably in expression levels of voltage-dependent anion channel (VDAC) encoding sequences. VDAC, known also as mitochondrial porin, is the most abundant protein of the mitochondrial outer membrane and is regarded as crucial for mitochondrial functioning. Besides being a major channel allowing passage of inorganic ions and metabolites between the intermembrane space of mitochondria and the cytosol, the protein also interacts directly and indirectly with many cellular proteins. The channel may be present as isoforms encoded by separated genes, showing different channel-forming activities and probably playing various roles. In mammalian mitochondria, as in the case of other vertebrates, three isoforms of VDAC

(VDAC1-VDAC3) have been identified. However, in invertebrates mostly only a single VDAC isoform has been so far described for few species. In molluscs only putative VDAC coding sequences have been deposited in the GenBank for *Crassostrea gigas* (Thunberg), *Biomphalaria glabrata* (Say), *Aplysia californica* (Cooper), *Octopus bimaculoides* (Pickford et McConnaughey), *Haliotis diversicolor* Reeve, and *L. stagnalis* (L.). We have identified two distinct VDAC transcripts encoding orthologs of vertebrate VDAC in the tested gastropod species. We termed them VDAC1 and VDAC2 although the orthologs cannot be compared to the vertebrate isoforms in term of homology because they evolved independently. Furthermore, we observed domination of VDAC1 transcript over VDAC2 one in terrestrial gastropods and opposite quantitative relation of the transcripts in freshwater gastropods. These results suggest a relationship between animal habitat and VDAC expression profile as well as the protein possible contribution to adaptation of gastropods to the environment.

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## Mollusc bycatch from the Brazilian bottom trawling industrial fishery in the Amazonian continental shelf

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The north coast of Brazil is part of one of the most important shrimp banks in the world, where are located the exploitation areas of penaeids shrimps and demersal fish from the 70's to the present day. One of the main problem of the bottom trawl fisheries is the bycatch of organisms that are very diversified in this region, mainly of fishes, crustaceans, molluscs, echinoderms, testudines, porifers, cnidarians and marine macroalgae. Incidental capture is considerably more abundant than the capture of the target species. The study area is called the Amazon Gulf and is situated between the latitudes 00°00'S and 00°11'S; an area characterized by a gigantic estuarine deltaic complex, submitted to river and marine processes. A total of 30 bottom trawls were analysed on board of a fishing vessel of the Brazilian bottom trawling industrial fishery, monitoring the latitude, longitude and the depth of each trawl. To separate molluscs from other taxonomic groups, which all are part of the bycatch, on-board sorting was carried out, coding

each sample with trawl information and storing it in the refrigerating chamber of the vessel, before being sent to the Federal University Rural of the Amazon for subsequent analyses. In the laboratory, collected molluscs were identified with the help of specialized literature and data were recorded for analysis of the catch frequency. The catalogued species were: *Pteria sterna*, *Caryocorbula swiftiana*, *Aequipecten opercularis*, *Pinctada imbricate*, *Atrina seminuda*, *Plicatula* sp., *Musculus lateralis*, *Anachis lyrata*, *Stramonita floridana*, *Stramonita rustica*, *Naticarius canrena*, *Semicassis granulata*, *Lolliguncula (Lolliguncula) brevis* and Columbellidae ident. A total of 2,845 mollusc were sampled, distributed in three classes, 10 families and 14 species. The bivalve *P. sterna* was noted as the most abundant species in bottom trawls. From the data collected on the mollusc bycatch from the bottom trawling industrial fishery in the Amazonian continental shelf, it can be concluded that most representative were bivalves, followed by gastropods and cephalopods.



## The mysterious origin of Neogastropoda

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Since their emergence in the Palaeozoic time gastropods occupy an important place in the marine ecosystems. Changes in their abundance and taxonomic diversity reflect therefore the general trends in evolution of marine ecosystems. Emergence of carnivorous Neogastropoda and their rapid and extremely successful adaptive radiation was a significant part of reorganizing benthic fauna in the Mesozoic times known as Mesozoic Marine Revolution. For many years the origin of this group was a mystery. In spite of implementing new research methods – especially SEM examination of larval and juvenile shells – the research progressed relatively little. The fossil record shows that the rapid radiation of neogastropods took

place in the early Late Cretaceous and in the late Late Cretaceous (Campanian-Maastrichtian) all but one major neogastropod groups are already present, while their earlier record remains still elusive. The oldest published record of a juvenile neogastropod comes from the Valanginian of Poland (early Early Cretaceous) and a new possible neogastropod has been recently found in the latest Jurassic hydrocarbon seep deposits in Spitsbergen. These findings support earlier accounts that the origin of Neogastropoda took place within the Boreal Realm during the Late Jurassic–Early Cretaceous on the soft bottom of relatively deep seas and only afterwards they migrated towards the tropical regions.



## Shell thickening leading to apical occlusion in gastropods and its implications for paleoclimatology and the interpretation of the fossil record of gastropod diversity

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We routinely study tiny fossil internal molds (steinkerns) of Paleozoic gastropods. Normally one expects bulbous protoconch molds at the apex of unbroken specimens but we often find sharp terminations instead. These sharply terminated molds also show more open coiling toward the apex.

The reason for these oddly shaped micro-steinkerns is that helically coiled gastropods thicken their shells from the inside for strength, and this shell thickening leads to the internal occlusion of the apex. Thin larval shells of gastropods lack the strength to endure impacts that might regularly befall larger individuals. To avoid potentially fatal injury to the shell, internal accretionary thickening is a necessity. We studied shell thickening and apical occlusion by sectioning a variety of modern and fossil gastropods along the axis of coiling. All helically coiled gastropods examined so far show shell thickening and apical occlusion with ontogeny. This includes species that remodel the interior of the shell by resorption as well as species that do not.

Despite the universality and easy observability of shell thickening, we have been unable to find any

published accounts of the phenomenon. Perhaps it is considered a trivial observation?

However, apical occlusion can have profound effects on the shapes and diagnostic features of fossil gastropods, which often are preserved as steinkerns only. Researchers unaware of the apical occlusion process may mistake growth stages for different species. Moreover, secondary shell thickening may affect recent attempts at reconstructing paleoenvironments from fossil and subfossil gastropod shells (often in archeological contexts). Gastropod shells are sampled for isotopes (principally oxygen) by a series of micro drill holes that cross growth lines around the whorls in an attempt to sample a few years of seasonal change in isotopic composition. The assumption is that each successive drill hole samples an older part of the shell. This assumption is violated if the shell is constantly thickened by internal deposits, so that the deeper parts of drill holes will sample successively younger internal shell layers. As the drill holes progress to older whorls, the original shell becomes relatively thinner, and a greater proportion of the shell becomes younger.

## Thyasirid bivalves from Cretaceous and Paleogene cold seeps

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We have studied thyasirid bivalves from Cretaceous to Oligocene seep carbonates from around the world. Eleven species of thyasirid bivalves are identified, which belong to three genera: *Conchocele* Gabb, 1866; *Maorithyas* Fleming, 1950 and *Thyasira* Lamarck, 1818. Two species are new: *Maorithyas humptulipsensis* sp. nov. from middle Eocene seep carbonates in the Humptulips Fm., Washington State, USA, and *Conchocele kiritachiensis* sp. nov. from the late Eocene seep deposit of Kiritachi, Hokkaido, Japan. Two new combinations are provided: *Conchocele townsendi* (White, 1890) from Maastrichtian rocks of the James Ross Basin, Antarctica, and *Maorithyas folgeri* (Wagner and Schilling, 1923) from Eocene rocks in California,

USA. Three species are left in open nomenclature. We show that thyasirids have Mesozoic origins and appear at seeps before appearing in “normal” marine environments. This data is interpreted as a record of seep origination of thyasirids, and their subsequent dispersal to non-seep environments. We discuss the age of origination of thyasirids in the context of the origin of modern deep-sea fauna and conclude that thyasirids could have been deep-water origins. This hypothesis is supported by the observed lack of influence of the Cretaceous and Paleogene Oceanic Anoxic Events on the main evolutionary lineages of thyasirids, also shown by the remaining deep-sea fauna.

## Freshwater mollusk communities from the Oligocene Lake Nanning (Guangxi, southern China): insights into the evolution of an endemic lake fauna

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Discovered in the 1930s, the Oligocene freshwater mollusk fauna of the Nanning Basin in southern China has received little attention, although it is proof of one of the most spectacular fossil endemic lake radiations. The 25 species of macro-mollusks recorded so far include large, heavily ornamented Viviparidae and thick-shelled Unionidae. The spines, nodes and raised costae covering their shells are interpreted as adaptations to avoid predation by shell-crushing durophagous carps. Numerous species of micro-gastropods comprise several enigmatic forms of unclear taxonomic assignment, but also a species-flock of Stenothyridae, showing the polymorphism typical for lake radiations of truncatelloid gastropods. The iconic muricid-shaped cerithimorph *Sinomelania leei* occurs almost through the entire Lake Nanning

succession. Extensive urbanization during the last decade has created numerous temporary outcrops, providing access to fresh sediments of the Yongning Formation, which yielded thousands of fossil shells. From several localities spread over a large area of the former lake, we have identified four distinct, successive mollusk communities. Here, we provide an account of the fauna, half of the taxa being new to science, and present some of the most peculiar morphologies and their palaeoecological interpretation. We specify the four communities encountered and speculate on their evolutionary context. We further introduce the presumed top predator of the large mollusk fauna of Lake Nanning, and provide quantitative data on predation pressure on Viviparidae.

## The umbonal musculature of the Middle Triassic putative unionids from Poland

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Unionida, the freshwater pearly mussels, are today widely distributed. They originate from a trigoniid ancestor and are known from freshwater habitats from at least the early Late Triassic; however supposed unionids were also described from the Early Triassic of Karoo Basin, Africa.

Unionids maintained the plesiomorphic status of main groups of muscle attachment scars visible on the inside of their shells: scars of adductors, pedal retractors and protractor, and mantle attachment scars. Specific for the unionids is a group of minute muscles attached to the shell wall within the umbonal cavity. The most widespread pattern of their location both in recent and the fossil species is on the anterior slope of the umbo, near the main hinge tooth in a longitudinal row diverging slightly forward. They usually differ in number and disposition in left and right valves. The evidence that this pattern is ancient comes from the early Late Triassic fossils from Silesia (Poland), where the umbonal muscle scars occur more or less irregularly over the anterior and interior walls of the umbonal cavity. Unionids originate from the marine trigoniids, which have a single pedal ele-

vator scar in each umbo. Nevertheless at least since the Permian a split elevator appears in some species, and in Ladinian (Middle Triassic) marine deposits of Rottweil, a shell mould was found with split muscle scars visible in the umbonal cavity – the mould of a possible juvenile trigoniid *Trigonodus*, which line is considered as the likely ancestor of the unionids.

In the freshwater deposits of the late Olenekian-early Anisian (Early to Middle Triassic) age from the Holy Cross Mountains (Poland) several specimens of bivalve nuclei were found. These bivalves morphologically resemble the unionids, however the unionids have morphology that is rather generalized. Nevertheless on the surface of these nuclei the distribution of muscle attachment scars is preserved – with the minute umbonal scars well distinguishable. Scars are not fully separated, arranged more-or-less linear, on interior slope, near the top of umbo. We believe that both the age and morphology of the Early-Middle Triassic freshwater bivalves from Poland indicate the primitive status in evolution of umbonal musculature in Unionida.

## *Pupilla muscorum densegyrata* Ložek, 1954 – a missing link in *Pupilla* history

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Two wetland *Pupilla* taxa – *P. alpicola* (Charpentier, 1837) and *P. pratensis* (Clessin, 1871) – occur recently in Central Europe differing in morphology, in geographic distribution and slightly in ecological requirements. Despite this, these two forms share genetic variation and seem to be one species. The fossil records of both forms are very rare when compared with other central European *Pupilla* species. *Pupilla muscorum densegyrata* was described by V. Ložek in 1954 as one of extinct *Pupilla* taxa in the loess material from Central Europe. This taxon is now known

from 113 previously studied last glacial mollusc assemblages. Based on the surprising resurrections of some *Pupilla* species in Central Asia that were considered to be extinct, we decided to compare extinct *Pupilla m. densegyrata* with recent *Pupilla* snails. The most promising design attempts to fill the gap in morphological characters of *P. alpicola* and *P. pratensis*. Focused on shell measurements and shell shape analysis of these three *Pupilla* taxa we suppose that extinct ancestor of these wetland taxa commonly occurred the loess steppes of Central Europe.

## **Anomalodesmatan bivalves from the Jurassic of Poland – an ancient group in its prime**

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Extensive fieldwork in various facies (pelitic limestones, clays, sandstones to conglomerates) of Jurassic in central Poland resulted in over 2,100 specimens of anomalodesmatans from 67 localities. The preservation was mostly good, in many cases allowing one to investigate fine anatomical details of the shell (microornament or microstructure). The material enabled the recognition of 47 species representing nine genera belonging to six families (Pholadomyidae, Pleuromyidae, Ceratomyidae, Myopholadidae, Laternulidae and Thraciidae) within three superfamilies (Pholadomyoidea, Ceratomyoidea and Pandoroidea). Pholadomyoidea is an ancient superfamily, very diverse in Jurassic, but now rel-

ic; their extant representatives are poorly known. Ceratomyoidea are extinct, with most representatives in the Jurassic. In contrast Pandoroidea (traditionally divided into Pandoroidea and Thracioidea), which are an important living anomalodesmatan group, have their oldest representatives not much older than Jurassic. The vast majority of investigated taxa were known already from other parts of Europe and only a few are new and seemingly endemic. Additionally many specimens displayed pathological features, e.g., mud blisters, blister pearls, and some other structures of apparently parasitic origin. Only a few specimens bear traces of predation.

## An overview of recent studies of lymnaeid snails from geothermal habitats of Northern Palearctic

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In recent years a considerable amount of work has been done in the study of the fauna and ecology of molluscs living in the hot springs of different parts of the world. But the available data on this issue are fragmentary. On this occasion, we aimed to analyze some features and taxonomic status of lymnaeid snails inhabiting thermal springs. We studied the lymnaeid populations of different geothermal springs of North Eurasia and revised some taxa of species rank on the basis of the integrative taxonomic approach, using newly collected topotypes from different geothermal springs and specimens sampled from other sites (cold waterbodies and rivers). Species identification was performed using macromorphological and molecular data. In addition, the type series of *Lymnaea* spp. from collection of the Zoological Institute of the Russian Academy of Sciences, (St. Petersburg, Russia) were examined. We used data inferred from the mitochondrial cytochrome oxidase subunit I

(COI) and the nuclear internal transcribed spacer 2 (ITS2) genes, because the molecular analysis of the nuclear and mitochondrial markers provides the most reliable taxonomic results. As a conclusion, we have confirmed that not less than 5 widespread lymnaeid species could successfully invade the extreme warm environments of different geothermal sources, e.g., *Radix auricularia* and *R. kamtschatica* in Siberia and the Russian Far East; *R. lagotis* in North-East Europe and *R. balthica* in Iceland, *Ladislavella tumrokensis* from Kamchatka Peninsula.

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## Species boundaries, geographic distribution and evolutionary history of the Western Palaearctic freshwater mussels *Unio* (Bivalvia, Unionidae)

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Delimiting species boundaries is a fundamental yet challenging task that has been especially problematic in morphologically conserved lineages. The use of genetic markers and phylogenetic approaches has proven of paramount importance to resolve these cases. Using the genus *Unio* as a case study, we have analysed two mitochondrial markers, COI and 16S, phylogenetically and phylogeographically to explore species limits and evolutionary lineages within the group. We followed two different approaches to define species boundaries: the Generalised Mixed Yule Coalescence model in a Bayesian framework (bGMYC) and the Poisson Tree Process (PTP) model. Phylogenetic relationships among species and divergence times were also assessed using relaxed mo-

lecular clocks analyses. Taken together, we provide a sound systematic framework for Western Palaearctic *Unio* species, addressing phylogenetic relationships, and the taxonomy, biogeographic patterns, and evolutionary history of this group in this region. The *Unio* clade from the studied area showed a clear phylogenetic structure with four robust lineages that include the following species: 1) *U. durieui* + *U. gibbus*, 2) *Unio tumidus*, 3) *U. pictorum*, *U. delphinus* + *U. foucauldianus*, *U. elongatulus*, *U. mancus*, *U. ravoisieri*, *U. tigridis*, and 4) *U. tumidiformis* + ((*U. bruguierianus* + (*U. crassus* + *U. ionicus*))). Deeper analyses, including filling gaps in distributions, should be conducted to disentangle the *U. crassus*, *U. pictorum*, and *U. tigridis* species complexes.

## **Chromosome numbers of families of Georgian terrestrial molluscs (Mollusca: Gastropoda)**

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The results of the chromosome studies of Georgian (Caucasus region) terrestrial molluscs are summarized. The chromosome numbers of the species of five Stylommatophoran and one Caenogastropod families were established. The basic chromosome number  $n=27$  was registered in the family Clausiliidae Gray, 1855. Two different values of basic chromosome number  $n=26$  and  $n=27$  were recorded for family Helicidae Rafinesque, 1815. The different numbers  $n=23$  and  $n=26$  were found also in the family Hygromiidae Tryon, 1866. Approximately  $2n=60-62$  somatic chromosome number was ob-

served in Limacidae Lamarck, 1801. The number  $n=26$  was recorded in Oleacinidae H. Adams & A. Adams, 1855 and  $n=13$  for Caenogastropod family Pomatiidae Newton, 1891 (1828). The chromosome numbers registered in the investigated families of Georgian snails and slugs are within the ranges of chromosome numbers described for these families of world malacofauna. It is supposed, that  $n=23$ ,  $n=26$ ,  $n=27$  and ca.  $2n=60-62$  are characteristic chromosome numbers for Caucasian terrestrial molluscs, the Georgian ones included.

## One species or two? Testing gene flow between the subspecies of *Trochulus oreinos* (Gastropoda: Pulmonata: Hygromiidae)

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*Trochulus oreinos*\* is an Austrian endemic land snail species occurring in the Northeastern Calcareous Alps at elevations above timber line. Two subspecies have been distinguished: The westerly distributed subspecies *T. o. scheerpeltzi*, is characterized by a groove beneath the shell keel, whereas the easterly distributed *T. o. oreinos* features an additional penial fold. Genetic analyses of the nuclear marker sequence ITS2 (internal transcribed spacer 2) as well as mitochondrial marker sequences (cytochrome c oxidase subunit 1 gene (COI), 16S rRNA gene, 12S rRNA gene) indicated a high genetic divergence between the two taxa. Analyzing an extended sample set from the potential contact zone, the Haller Mauern mountain range, a clear geographic break was found. Samples of all western sites were part of the clade representing *T. o. scheerpeltzi* and almost all samples from the Natterriegel sites eastwards clustered with *T. o. oreinos*. However, at the two sampling sites near the Natterriegel, one of the mountain peaks in the Haller Mauern, a few individuals possessed a COI

sequence matching the *T. o. oreinos* clade, whereas at the ITS2 locus they were heterozygous possessing the alleles of both taxa. Based on these results suggesting historical and/or ongoing hybridization, no decision could be made on whether to consider the two taxa as separate species. Therefore, in a next step, the amount of gene flow between the two subspecies of *T. oreinos* within the Haller Mauern contact zone was investigated using Amplified Fragment Length Polymorphisms (AFLPs), a DNA fingerprinting technique. We investigated 200 individuals including samples from the whole distribution range. The AFLP results confirmed a clear separation of the two taxa, congruent with the mitochondrial data. Although they occur on the same mountain range, no indication of ongoing gene flow between the two taxa was found.

\*In the recently published revision of Hygromiidae, Neiber and colleagues suggested the genus name *Noricella* for *T. oreinos*.

## Current knowledge and problems in *Metafruticicola* (Gastropoda, Hygromiidae)

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The genus *Metafruticicola* (Gastropoda, Hygromiidae) is distributed in North-eastern Mediterranean region from the Aegean Archipelago to coastal Anatolia, Cyprus and the Levantine coast south to Israel, and also in South Albania and Ipeiros, but it is absent from the rest of the Greek mainland. Currently, 24 species are recognized (two polytypic), half of them present in the Aegean islands. There are two areas with high endemism, the island of Crete and the area around Antalya in southern Turkey with 6 and 8 endemic species respectively.

In the Aegean Archipelago it is one of the most common land snail genera, distributed in the majority of islands and islets and in ecosystems, from sea-side to the subalpine zone in the case of Crete.

The taxonomy within the genus (subgenera, species, subspecies) is mainly based on the microsculpture of the shell alone and in some cases the anatomy of the genitalia, and in particular the structure of the penial papilla, is used. In addition, scarce results of phylogenetic analyses based on mitochondrial and nuclear genetic loci raise concerns and doubts about the current taxonomy.

Thus, we discuss the current knowledge and the different characters being used so far in the taxonomy of the genus and its place among Hygromiidae, as well as future plans in an attempt to unravel the systematics of *Metafruticicola* taxa.

## Molecular phylogeny of the land snail subfamily Leptaxinae (Gastropoda: Helicoidea: Hygromiidae)

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The Leptaxinae Boettger, 1909 is enclosed within the highly diverse land snail family Hygromiidae Tryon, 1866. Without a neat diagnostic morphological differences, the subfamily status is currently based solely on molecular information and it includes three disjunctly distributed tribes, Leptaxini, Cryptosaccini and Metafruticicollini. However, the phylogenetic relationships between the tribes are not fully resolved and, besides, the clustering of some of the genera to the tribes is not statistically supported. To solve the relationships within Leptaxinae and their position within Hygromiidae, we reconstructed their phylogeny using two mitochondrial genes and eight nuclear genes. The phylogeny was further calibrated to infer the biogeographic history of the group. We restricted Leptaxinae to Cryptosaccini

and Leptaxini. Metafruticicollini, conformed by the genera *Hiltrudia*, *Cyrnotheba* and *Metafruticicola*, was elevated to subfamily level (Metafruticicolinae) sister to the rest of Hygromiidae. The Lusitanian genus *Portugala* was transferred to Leptaxini tribe, previously containing uniquely the Macaronesian genus *Leptaxis*. All the genera grouped in Cryptosaccini are endemic to the Iberian Peninsula. Moreover, a new genus strictly confined to Sierra de la Cabrera must be considered within this tribe. According to our phylogenetic reconstruction, Leptaxinae was originated in the Iberian Peninsula from where the Macaronesian Islands were colonized. Madeira archipelago was colonized first and, then, the Azorean *Leptaxis* arose from a single Madeiran group.

## DNA barcoding of Austrian molluscs

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In the course of the initiative Austrian Barcode of Life (ABOL), molluscs were chosen for several reasons as group for a three-year pilot study. There are important indicator species, suitable for evaluation of habitat quality. This leads to a high risk of extinction for many molluscs: about 35% of the snail and 37% of the mussel species are endangered. Also the number of endemics is quite impressive (19.3%). Approximately 30% of the 400 native species are divided into subspecies. Genetic investigations in land pulmonates showed extreme high intraspecific diversity. Hence there is no standard value for genetic distances, which marks taxonomic delimitations. Due to the overlap of intra- and interspecific variation often no barcoding gap can be found. Previously collected data and experiences from past and running projects on snail species in Austria provide excellent pre-conditions for successful DNA-barcoding. Up to now approximately 250 Austrian mollusc species are

available. This includes material collected and preserved for DNA analyses during concerted field trips to different parts of Austria, but also older material from the collections of the Natural History Museum Vienna and the Biology Centre of Linz. DNA from museum material is often fragmented and of low concentration, therefore it is only chosen, when no other material from is available (approx. 35 species). Until now, from 185 different species 548 DNA-barcodes with all relevant metadata were established. From our results we find cryptic species, but also different morphologically described species that cluster in one barcoding BIN. This project provides DNA-Barcodes for the challenging group of molluscs. Data are sampled in a relatively small geographic scale, which is essential for taxa with low dispersal capacity. The DNA-Barcodes will facilitate determination, which is often difficult and vague in molluscs and hence their use in evaluations of nature conservation issues.

## Intraspecific diversity in the hyper-diverse rock-dwelling land snail *Montenegrina*

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Intraspecific diversity is an important measurement to evaluate taxonomy and phylogenetic hypotheses and to confirm or deny the taxonomic status of a species. In general, the amount of intra- as well as interspecific diversity varies with the distribution range. To test the intra- and interspecific diversity and its congruency with the current taxonomic assignment, we studied the obligate rock-dwelling land snail genus *Montenegrina*. It is a hyper diverse genus with 29 known species and 106 subspecies, the distribution range, though, is restricted to the western part of the Balkan Peninsula. The current systematics of *Montenegrina* is mainly based on conchological traits. Thus we wanted to test the congruency with molecular data based on short fragments of three mitochondrial genes *cytochrome c oxidase subunit 1*, *16S rRNA*, *12S rRNA* (*COI*, *16S*, *12S*) and a short section of two nuclear histone genes (*histone 3* and *histone*

*4*), including the spacer region (*H3–H4*). We included nearly 800 individuals assigned to 113 different taxa from over 350 localities, covering the whole distribution range. Phylogenetic trees were calculated and compared to the current system of *Montenegrina*. Furthermore, the diversity, based on average p-distances, was calculated between and within species as well as between and within populations and clades. The results show a high concordance between traditionally gained taxonomy and the phylogenetic tree. Only in few cases (around 5%) big discrepancies were found. This study revealed high genetic diversity within this rock-dwelling door snail genus and indicates that the large number of taxa, which mostly have very narrow distribution ranges, are not the result of a taxonomic over-splitting, but reflects mostly actual phylogenetic relationships.



## Taxonomy and distribution of the molluscan genus *Boreocingula* in the Arctic and Subarctic waters (Gastropoda: Rissoiidae)

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Diversity of the gastropod family Rissoiidae in the Arctic is extremely low compare with boreal and tropical environments. A few genus-level taxa with predominantly Arctic distribution are poorly studied in the respects of taxonomical composition, morphology, and distribution. Taxonomical revision of the genus *Boreocingula* Golikov et Kussakin, 1974, based on the morphology of shell and soft body is presented here. The study is based mainly on the material, stored in the Zoological Institute of Russian Academy of Sciences (Saint-Petersburg, Russia). As a result, five species are suggested to be members of the genus, three of them (*B. martyni* (Dall, 1887), *B. alaskana* (Bartsch, 1912) and *Boreocingula* sp.n.) distributed in the extremely north of the Pacific Ocean and the adjacent Siberian Seas, whereas two others (*B. castanea* (Møller, 1842) and *B. globulus* (Møller,

1842)) are known from the Arctic parts of the Atlantic Ocean, including the Davis Strait and the Barents Sea. Representatives of the genus are unique in having of pitted protoconch, upper oviduct gland, divided into small partitions. Morphology of radula, unusual for the Rissoiidae is found on *B. globulus* and *B. sirenkoi*. Imposex females are described for *B. martyni*. Species of *Boreocingula* are known almost exclusively in the shallow-waters (down to several tens of meters), including the littoral zone, which is uncommon for the typical Arctic macrobenthic organisms.

The study was supported by the Russian Foundation for Basic Research (Grant No. 16-34-00372 mol\_a). Work with the scanning electronic microscope was performed at the Research Park of Saint-Petersburg State University Interdisciplinary Center for Nanotechnology.

## Two new species of *Aldisa* (Gastropoda, Nudibranchia) from southern Mozambique

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The genus *Aldisa* is relatively poorly studied. Up to now no species have been described from the Western Indian Ocean (WIO). Two new species of *Aldisa* are described from Zavora (Mozambique). Both species are characterized by having two oval depressions on the dorsum, a red mantle with yellowish-white patches and red rhinophores. Moreover, one of them has large round tubercles on the dorsum tipped in black and a large flattened oral glan-

dular mass, while the other has tan gills, rounded red tubercles, branchial and rhinophores sheaths distinctively serrated and a large oral gland mass with a semi-spherical shape. Partial sequences of mitochondrial (COI and 16S) and nuclear (H3) markers of both species are provided. Both morphological and molecular data confirm that these species are different from other known species of the genus.

## *Okenia* in the Mediterranean Sea: what are we facing?

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Nine species of the small nudibranch genus *Okenia* Menke, 1830 have been reported from the western Mediterranean: *Okenia aspersa* (Alder & Hancock, 1845), *Okenia cupella* (Vogel & Schultz, 1970), *Okenia elegans* (Leucjart, 1828), *Okenia hispanica* Valdés & Ortea, 1995, *Okenia impexa* Er. Marcus, 1957, *Okenia leachii* (Alder & Hancock, 1854), *Okenia mediterranea* (Ihering, 1886), *Okenia pulchella* (Alder & Hancock, 1854) and *Okenia zoobotryon* (Smallwood, 1910). However, except for *O. hispanica* and *O. mediterranea* originally described from the Mediterranean

Sea, most of the remaining records are doubtful. In this contribution we study and discuss several specimens of *Okenia* collected in La Grande Motte (France), Sabaudia Lake (Lazio, Italy) and Gallipoli (Lecce, Italy), in order to try to clarify their taxonomic status. All of them were initially identified as *O. zoobotryon* but, after a detailed anatomical, molecular and ecological study and comparison with the neotype of this species designated by Pola (2015), it is clear that we are dealing with two other species, both different from *O. zoobotryon*.

## The female and male complete mitochondrial genomes of the freshwater mussel *Unio tumidus* (Bivalvia: Unionidae)

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Freshwater bivalves of the order Unionoida represent the largest bivalve radiation in freshwater. They are divided into 6 families (Etheriidae, Hyriidae, Iridinidae, Margaritiferidae, Mycetopodidae and Unionidae), 181 genera, and about 840 species. The largest and most widespread of the six families of Unionoida is the Unionidae, with 674 species, that occur in all geographic regions. In Europe there are 14 species of Unionidae, of which three are considered endangered and are protected by law (*Unio crassus*, *Anodonta cygnea* and *Pseudanodonta complanata*). Almost all European species are native, except for *Sinanodonta woodiana*, originating from South-East Asia. The species chosen for analysis in this study, *Unio tumidus*, is native to central and western Europe. It is relatively common in Poland, forming numerous and stable populations in rivers and lakes. Therefore, it is not expected to show genetic signs of recent rapid demographic processes such as bottleneck and founder effects.

Moreover, *U. tumidus* has a unique system of mitochondrial DNA inheritance called doubly uniparental inheritance (DUI). Under DUI two types of

mitochondrial DNA are present: haplotype F (female genome), inherited from mother and haplotype M (male genome), inherited from fathers to male offspring. The F genome occurs in eggs and in somatic tissues of both sexes whereas the M genome is present in male gonads and gametes. We characterized three M and three F mitochondrial genomes of *Unio tumidus*. The length of these genomes were 15,769–15,770 bp for F type and 16,607 bp for M type. In both genomes the typical for Unionidae set of 38 genes was identified, including 37 metazoan genes and gender-specific ORFs. The non-coding sequences constituted only 5.2 and 3.5% of F and M genome, respectively. Both genomes were similarly high in average AT content (65–66%) but intraspecific nucleotide diversity among the three M genomes of *U. tumidus* was four times lower than among the three F genomes. The patterns of polymorphisms across the seven closest relatives mitogenomes confirmed that the M genomes accumulate more substitutions and the conserved regions within one lineage are usually conserved in the other.

## ***Sphaerium nitidum* Clessin in Westerlund, 1876 (Bivalvia, Sphaeriidae): genetic characterization and micromorphology with notes on associated digeneans**

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The genus *Sphaerium* Scopoli, 1777 is represented in Europe by 7 species. The Arctic fingernail-clam *Sphaerium nitidum* Clessin in Westerlund, 1876 (Bivalvia, Sphaeriidae) has a Holarctic distribution and is more of a mountain dweller, living in cold lakes, both in oligotrophic and eutrophic habitats. This species is found only in the northern part of Europe and does not occur in the Baltic countries.

Specimens for investigation were collected in sub-Arctic Lake Kykkelvatnet and Lake Songsvaten near Oslo (Norway). *Sphaerium nitidum*, as the first intermediate host, was infected by *Crepidostomum* spp. with extensiveness of invasion 18%. Shell microstructure of *S. nitidum* was studied by scanning electron microscopy (SEM). SEM studies have revealed that inner shell porosity of *S. nitidum* isn't dense, ~970 pores in mm<sup>2</sup>. The genus *Sphaerium* is characterized by an extreme karyotypic diversification, with mitotic chromosome numbers varying from 28 (*S. nucleus*) to 260 (*S. rivicola*). Chromosomes of mitotic metaphases of *S. nitidum* were studied using

cell suspension techniques, a modal number of chromosomes revealed was ~ 230. 16S and ITS1 rDNA sequences of *S. nitidum* were obtained to clarify its phylogenetic position. The obtained rDNA sequences of *S. nitidum*, together with available sphaeriid molecular data were included into phylogenetic analysis. A placement of *S. nitidum* in both phylogenetic trees, based on mitochondrial 16S and nuclear ITS1 rDNA sequences, was identical. The rDNA sequences of *S. nitidum* clustered in one main clade together with rDNA sequences of *Sphaerium* spp., in one subclade with diploid *S. rhomboideum* and polychromosomic *S. occidentale*. These results not quite correspond with the phylogeny of the Sphaeriidae *sensu lato* based on the morphological analysis. *Sphaerium* s. str. clade in this phylogeny is constituted by *S. solidum*, *S. corneus*, *S. nucleus*, *S. rhomboideum* and *S. nitidum*. Four of these species (except *S. nitidum*) proved to be diploid.

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## New arrivals in Greece and Cyprus

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The terrestrial malacofauna of Greece is one of the richest in Europe and with a very high percentage of endemic species. Also land snails are one of the best studied invertebrate groups in Greece and Cyprus.

During our studies on the malacofauna of these countries the last decades, we found some unexpected species of snails. *Lehmannia valentiana* (Limacidae) was recorded on Skyros Isl. (Greece) in 2005, but since then we found it in more places in Greece. Apart, from the already known *L. valentiana* three

more species have been found, all of them around human settlements in Cyprus and Greece.

For the time being these species seem to be restricted in certain urban areas. However, we have to carefully and continuously record them, since on the one hand in other countries they are considered invasive, and on the other we are aware of other invertebrate groups, e.g. coleoptera that have invaded Greece and Cyprus causing a lot of damages.

## Genetic analysis of *Deroceras reticulatum* (O. F. Müller, 1774) in Poland based on mitochondrial and nuclear DNA

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*Deroceras reticulatum* (O. F. Müller, 1774) is widely distributed species in Europe, among terrestrial gastropods it is one of the most dangerous pests of cultivated plants, such as winter rape or winter wheat. In the presented study Polish populations of *D. reticulatum* were investigated. Confirmation of species identification was done by anatomical section and morphology of internal organs. In order to detect intra- and interpopulation genetic variability, analyses were performed on the basis of mitochon-

drial marker, cytochrome c oxidase subunit I (COI) and the nuclear marker, internal transcribed spacer (ITS-2). Genetic variation and comparison between and within populations, then phylogenetic analysis among these populations were conducted.

The results indicate crucial information about genetic variability between and within populations of *D. reticulatum*, which is widely distributed species exhibiting a relatively high genetic variability within Poland.



## Impact of non-indigenous macrobenthic species on structural and functional diversity in the Vistula Lagoon (southern Baltic Sea)

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The Vistula Lagoon is a shallow, brackish, coastal lagoon of the south-eastern Baltic Sea and extends along the Polish and Russian coast of the Gulf of Gdańsk. The study was carried out in the western (Polish) part of the Vistula Lagoon. All the available data on introductions of non-indigenous species, from the 19th onwards, were compiled and analysed to assess the impact of those species on the structural and functional diversity of the Lagoon's benthos. In the analyses, non-indigenous species inhabiting the inshore rooted vegetation (primarily reed belts) outside the water line, were disregarded. In the 19th century, large inputs of fresh water supplied with the River Vistula discharge was the major factor shaping life conditions of the macrobenthos. The macrozoobenthos consisted primarily of freshwater oligochaetes and chironomid larvae. In the end of 19th century, a canal guiding the Vistula discharge directly to the Baltic Sea was dredged, and sluices which substantially reduced the freshwater supply to the Lagoon were constructed. Therefore, seawater incursions into the Lagoon via the strait became the major factor affecting environmental conditions. The

first non-indigenous species to settle en masse on the bottom in the western part of the Lagoon (probably in 19th) was the zebra mussel *Dreissena polymorpha*, a freshwater bivalve. The zebra mussel was the first abundant suspension feeder in the sublittoral of the western part of the Lagoon. Another important factor affecting the environmental conditions in the Lagoon was the eutrophication, observed as of the 1960s. The associated environmental changes resulted in reduction of the zebra mussel and restriction of its occurrence mainly to submerged vegetation. In the late 1980s, the entire bottom of the Lagoon, both in its sandy and muddy part, was colonised by the polychaete *Marenzelleria*. The polychaete, both a detritivore and a suspension feeder, substantially altered the food web in the Lagoon's ecosystem. Another new immigrant which has been observed to very dynamically spread in the Lagoon since about 2010 and which has colonised the entire area is a mactrid bivalve *Rangia cuneata*. The bivalve has substantially increased the suspension feeders' contribution to the macrozoobenthos of the Lagoon.

## Zinc Oxide nanoparticles induce genotoxic effect and biological, histopathological change in *Biomphalaria alexandrina* snails

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The potential impacts of zinc oxide nanoparticles (ZnONPs) on aquatic ecosystems have attracted special attention due to their unique properties. The aim of this study was to assess genotoxicity induced by ZnO nanoparticles in *Biomphalaria alexandrina* snails by using the comet assay, and throw the light on the deleterious effects of ZnO nanoparticles on biological parameters of the *Biomphalaria alexandrina* snails. The cytotoxic potential of ZnO nanoparticles was also investigated through histopathological examination by light and electron microscope. The present results showed that there is marked increase in mortality rate of juvenile and adult snails exposed to ZnO NPs. The growth rate of juvenile snails was lower than that of control. In addition, the egg laying capacity of *B. alexandrina* snails was greatly reduced after continuous exposure to ZnO NPs. Moreover, there is no hatchability even with the smallest concentration. The present results showed that a statis-

tically significant induction in DNA damage was observed by the comet assay in hemolymph exposed to ZnO NPs for 3 weeks, and histopathological studies showed that nanoparticles caused severe damage in digestive and hermaphrodite gland after 3 weeks of exposure. Transmission electron microscopy (TEM) showed that lysosomal membrane permeabilization (LMP) can be considered as a mechanism for the induction of cell death in *B. alexandrina* snails, since the production of reactive oxygen species (ROS), may induce the permeabilization of lysosomes. It can be concluded that ZnO NPs induce genotoxicity through oxidative stress and finally lead to cell death. Further studies are required to assess the current environmental burden of nanoparticles in riverine ecosystems to determine whether there is an extant need to monitor and/or regulate the use and release of ZnO NPs in the Nile River Basin.

## Pulmonate snails from Konin Lakes System as Digenea hosts

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Konin Lakes over 40 years have been functioning in the circulation of Pątnów-Konin cooling power station. The consequence of the discharge of heated water to lakes is an increase in the temperature of lake water, which in winter never drops below 7°C, and in summer reaches over 30°C. Thermal pollution has a significant impact on both – gastropods species richness as well as on Digenea prevalence in molluscan hosts' populations.

The purpose of this study was to test the richness of Lymnaeidae and Planorbidae snail host species invaded by Digenea larvae in the warmest lakes of the Konin Lakes System (Gosławskie, Pątnów and Licheńskie lakes).

Malacological and parasitological studies were conducted from June to September 2016. In total, 807 pulmonate snail individuals representing 7 snail species were collected – *Lymnaea stagnalis*, *Radix* spp., *Stagnicola palustris*, *Planorbarius corneus*, *Menetus dilatatus*, *Anisus vortex* and *Gyraulus albus*. The 19 Digenea species were found inside molluscs under study. The percent of infected snails depended on host species and ranged from 0.7% (*M. dilatatus*) to 44% (*S. palustris*).

We hypothesize that the low rates of Digenea invasion in Lymnaeidae and Planorbidae representatives from the Konin lakes are due to both – low survival rates of invasive for snails miracidia in elevated water temperature, and low survival of infected snail hosts.

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