

## Description of the main research directions investigated by the institute

The mission of the Institute of Vertebrate Biology is to contribute to society through the pursuit of education and research of various aspects of vertebrate ecology and evolution at the highest international levels of excellence.

The Institute realizes diversified research of free-living vertebrates aimed to obtain original data about their evolutionary and ecological strategies, and the epidemiological importance with respect to humans and domestic animals. Research activities have been performed across of various taxa of vertebrates. They are primarily of a fundamental nature and focus on observation and experiments in order to gain a better understanding of the processes that affect the fascinating world of the animal kingdom. Research subjects of the Institute can be divided into three main domains: **evolutionary ecology**, **biodiversity**, and **pathogens and diseases**. Individual projects, however, can cover broader range of topics and researchers often use interdisciplinary approaches.

### *Evolutionary ecology*

Organisms adapt to shifting conditions. Historical and recent fluctuations in environmental and biotic conditions shape current adaptations and provide baseline for the ability to adapt to current anthropogenic modifications. Research in Evolutionary Ecology examines how ecological factors affects evolutionary change, and how evolutionary patterns are linked to ecological processes. Specifically, we study how reproductive biology and life histories evolve within and among species, how they respond to environmental change, and how organismal form coevolves with the function. Major research topics include:

- evolution of life histories and ageing;
- sexual selection, evolution of mating systems and reproductive behaviour;
- avian and fish brood parasitism
- ecology of predator-prey and host-parasite interactions;
- migration and dispersal biology in birds;
- thermal physiology and individual energetics.

### *Biodiversity*

The current period is considered a biodiversity crisis. The knowledge of distribution and evolution of biodiversity is a prerequisite for its successful conservation. The research performed in Biodiversity focuses mainly on evolutionary processes affecting diversity of vertebrates (as holobionts, i.e. host plus its symbionts) at species and intraspecific (genetic) levels, including factors responsible for its increase (e.g. speciation) and decrease (e.g. extinctions of populations, conservation genetics). Individual projects deal mainly with the following issues:

- speciation, hybridization;
- evolutionary history, phylogeography - origin of current genetic diversity and its spatial distribution;
- factors affecting holobiont diversity, e.g. microbiome structure;
- conservation ecology of endangered species, ecology of invasive species;
- biogeography, evolution of biodiversity hot-spots (especially in Africa);
- functioning of ecosystems (especially in agricultural landscape);
- genetic threats of contemporary populations, conservation genetics.

*Pathogens and diseases*

As a result of environmental and socio-economic changes as well as advancing globalization, new ecological challenges emerge. Probably the most important among them are newly emerging infectious diseases, changes in landscape structure and freshwaters, or climatic changes. The aim of research in Pathogen and diseases is an interdisciplinary and comprehensive study of ecological interactions among vertebrates and their environment, which includes both environmental and socio-economic drivers. The typical research topics focus on:

- the role of endothermic vertebrates and haematophagous insects in spreading of infectious diseases and the persistence of their natural foci;
- diseases ecology including biology of vectors and eco-epidemiology of zoonoses;
- implementation of the 'One health' concept for the study of emerging pathogens;
- relationship of pathogens and hibernating bats.

## Research activity and characterisation of the main scientific results

General research activities and their characterisation were described in form 3.1, therefore, only the main scientific results are presented in the following text. The results are divided into three main domains i.e. evolutionary ecology, biodiversity, and pathogens and diseases, which were studied at the Institute. These activities are both fundamental and applied nature and significance. In many cases are the results based on broad international collaboration but all selected results have important team's share.

### ***Evolutionary Ecology***

#### *Evolutionary processes forming life history and aging in annual killifish*

Annual killifish inhabit seasonal pools in Africa and South America. Their embryos are resistant to drought and hatch soon after pools fill with water following seasonal rainfall. Young fish mature as soon as in two weeks and can sustain viable populations in habitats existing only three weeks (Vrtílek et al. 2018a). Their naturally short lifespan is determined by the inundation time of their pools (Vrtílek et al. 2018b), which desiccate over the dry season. These fish also live short lives in the laboratory, with some members of the African genus *Nothobranchius* only living for up to 3-6 months (Reichard and Polačik 2019). Amongst other factors (e.g. small size and ease of reproduction), this short lifespan makes them especially valuable as a vertebrate model taxon (Reichard et al. 2015). They are now used for a wide range of biological applications, bridging traditional divide between biomedical and evolutionary research (Cellerino et al. 2016).

We studied life history evolution in natural and laboratory populations and showed that killifish growth is limited by density dependent factors (Vrtílek et al. 2019) and lifespan in the wild is too short to enable expression of reproductive aging demonstrated as a decline in reproductive success with age (Vrtílek et al. 2018c). We improved killifish welfare by incorporating information from natural populations (Žák et al. 2019), such as thermal requirements (Žák et al. 2018), into continuously updated breeding protocol (Polačik et al. 2016). We used multiple naturally-derived populations to test predictions from evolutionary theories of aging in the laboratory. We discovered that killifish from dry regions live shorter lives also in standardized laboratory conditions (Blažek et al. 2017), demonstrating parallel evolution of killifish lifespan and ageing and its origin in ongoing natural selection. We also discovered that genome-wide relaxed selection limits lifespan of populations from dry region by increasing mutation load (Cui et al. 2019). We determined a novel mode of transport in freshwater fish with a patchy distribution, in digestive tract of migrating birds (Silva et al. 2019). Finally, we have found that annual killifish, tightly associated with temporal dynamic of their ephemeral habitats, can satisfactorily cope with unusual climatic events (mid-season desiccation) (García et al. 2019).

#### *Coevolutionary dynamics in fish reproductive parasitic systems*

Reproductive parasitism is a strategy where-by parents relinquish all their care for the offspring onto other hosts, i.e. individuals that act as foster parents for the parasitic offspring. This represents a special case of host-parasite relationship which is relatively common between parents of the same species but rare between species. We studied reproductive parasitism in two complementary model systems. First, we studied bitterling fishes (*Cyprinidae*) that parasitize freshwater mussel by laying their eggs onto

mussel gills. Using recent introduction of a mussel species from East Asia we tested response of European bitterling to this novel host and quantified causes and consequences of population-specific responses to the new host species (Reichard et al. 2015). We extended this approach and tested coevolutionary arms races in the region of its native distribution for contrast with its invasive range (Douda et al. 2017). We used detailed population genetic data to model invasion history of the non-native mussel (Konečný et al. 2018), demonstrating that repeated admixture between non-native populations enabled evolution of invasive lineage. Freshwater mussels also possess parasitic stage and we quantified costs and benefits to mussels and bitterling (Methling et al. 2018, 2019) in this reciprocal relationship to better understand constraints in this system.

We started to use the only non-avian vertebrate brood parasitic system to understand broader roots and implications of brood parasitism for the host and the parasite. The cuckoo catfish parasitizes mouth-brooding cichlid fishes in Lake Tanganyika. Mouth-brooding cichlids incubate their offspring in the buccal cavity, which is widespread parental care strategy in Lake Tanganyika and other African lakes. The cuckoo catfish intrudes on a cichlid spawning event and lays its own eggs, which are mistakenly taken by the female cichlid for incubation. The cuckoo catfish hatch inside the host's mouth and feed on host's own offspring (Reichard 2019). In an experimental framework, we found that host evolutionary and individual experience with brood parasitism interact in the outcome of parasitism. Evolutionarily naive hosts are heavily parasitized while sympatric natural hosts can learn to mitigate the costs of parasitism by ejection of parasitic eggs (Blažek et al. 2018). We also demonstrated that cuckoo catfish can capitalise on exploiting strong maternal instinct to collect stray offspring (Polačik et al. 2019).

#### *Postcopulatory sexual selection and sperm biology in birds*

Social monogamy is the prevailing mating system in birds, yet various levels of genetic polyandry have been detected in many species (Lifjeld et al. 2019). Post-copulatory sexual selection emerges due to promiscuous female behaviour. Surprisingly little is known about factors affecting sperm fertilisation capacity and male fertility in birds and whether postcopulatory sexual selection contributes to speciation. We focused on several free-living songbird species to explore sperm variability (Omotoriugun et al. 2016, 2017), heritability (Edme et al. 2019), associations between sperm traits and individual condition, male ornamentation and fertilisation success (Svobodová et al. 2017, Støstad et al. 2019, Edme et al. 2016). In the captive Zebra finch (*Taeniopygia guttata*), we showed that male ornamentation serves as a signal of sperm quality (Tomášek et al. 2017). Careful experiments manipulating oxidative stress and carotenoid intake in males (Tomášek et al. 2016) revealed that investment in "male showiness" is traded-off against sperm resistance to oxidative challenge and confirmed an in vivo antioxidant capacity of carotenoids. In the same species, we demonstrated that inbreeding is associated with higher proportion of abnormal sperm in the ejaculate (Opatová et al. 2016). Interestingly, inbreeding also affected beak colouration which could provide information on the quality of a male's ejaculate to choosing females (Forstmeier et al. 2017).

Finally, our research revealed that there is yet another novel mechanism associated with quality of ejaculate and male fertilisation success in zebra finches (Knief et al. 2017). The sperm of males that are heterozygous for the inversion on the sex chromosome Z have the longest mid-pieces and the highest velocity ("super sperm"). Such males achieve the highest fertility and siring success, both within-pair

and extra-pair. Our results thus suggest heterozygote advantage as the mechanism that maintains inversion polymorphism, and hence variance in sperm design and fitness in zebra finches. Postcopulatory sexual selection may also promote evolutionary diversification and speciation. We found evidence that elevated levels of sperm competition were associated with more rapid phenotypic divergence in sperm size across passerine species (Rowe et al. 2015). Furthermore, in two recently diverging species of nightingales (*Luscinia*), we found a greater divergence between species in sperm morphology in sympatry than in allopatry which indicates that isolation could be strengthened in sympatry by reinforcement (Albrecht et al. 2019, Janoušek et al 2019).

#### *Sexual ornamentation, sexual promiscuity and mate choice in birds*

Barn swallows (*Hirundo rustica*) represent a textbook example of a bird species with elaborated sexual ornamentation. We studied the role of natural and sexual selection on population diversification in barn swallow populations by comparing the “isolation by adaptation” scenario for population divergence with the “isolation by distance” pattern. Controlling for isolation by adaptation, we found 42 % of genome-wide divergence was attributable to isolation by distance through pairwise differences in traits related to migratory behaviour and sexual signalling alone (Safran et al. 2016). We also assessed sexual selection pressure for multiple male visual signals across European and Middle East barn swallow populations, some of them migratory (Klvaňa et al. 2017), some sedentary, and demonstrated significant phenotypic differentiation in sexual signalling axes, despite very low genomic divergence (Wilkins et al. 2016, Pap et al. 2019). We found that the relationship between signal trait elaboration and an oxidative stress differs across closely related populations that have diverged in signal use and preferences (Vitousek et al. 2016). Barn swallows are sexually promiscuous, with certain levels of brood parasitism (Petrželková et al. 2015). Interestingly, sexual traits important for social mate choice do not affect male fertilization success but experienced males gain paternity at the cost of inexperienced young males (Micháľková et al. 2019).

Using deep pyrosequencing, we described the cloacal and gut microbiome composition in barn swallows (Kreisinger et al. 2015, 2017, 2018) and detected a significant correlation of cloacal microbiome within breeding pair members, consistent with the hypothesis that cloacal contact during copulation may promote the transfer of bacterial assemblages. We have developed a novel method to analyze testosterone and corticosterone feather concentrations in small passerine birds (Bílková et al. 2018) and verified it on the barn swallow model (Adámková et al. 2019). Finally, barn swallow data were used to evaluate the evidence for assortative mating in birds (Wang et al. 2019) and contributed to the conclusion that the apparent ubiquity of assortative mating reported in the literature is overestimated and may not be driven by mate choice or mating competition for preferred mates.

#### *Thermal ecology of ectotherms*

Our research has been focused on strategies of ectotherm (cold-blooded) organisms for coping with climate change which poses a serious challenge. Their response to changing temperatures involves a combination of adjustments, varying greatly in their speed and reversibility. To understand such complex strategies, we applied a cross-disciplinary approach to tailed amphibians, newts and salamanders.

Phylogenetic comparative analyses revealed that thermal physiology traits do indeed evolve very slowly in this group (Gvoždík 2015). Results from a semi-natural

experiment showed that predator-induced selection on thermal sensitivity of burst speed is much lower than on body size in newt larvae (Gvoždík and Smolinský 2015). In addition, individual repeatability of thermal dependence of behavioural traits was negligible in juvenile newts (Baškiera and Gvoždík 2019), which suggest that this trait cannot be targeted by natural selection. Hence, our findings support the evolutionary conservative character of thermal physiology in amphibians, i.e. thermal adaptation cannot keep the pace with current climate change in these vertebrates. Among physiological adjustments, our results revealed between-species variation in thermal acclimation to low temperatures during winter (Podhajský and Gvoždík 2016). Current climate change increase winter temperatures, and so species-specific ability to reduce their mandatory energy requirements may be crucial for spring reproductive performance in temperate and mountain taxa.

We firstly quantified behavioural thermoregulation in aquatic ectotherms, which showed diverse thermoregulatory strategies (Balogová and Gvoždík 2015, Piasečná et al. 2015). We experimentally proved that newts modify thermoregulatory precision to maximize their factorial metabolic scope (Gvoždík and Kristín 2017). This shows the link between behavioural thermoregulation and aerobic energy metabolism in ectotherms. Reproductive female newts modify their thermoregulatory behaviour for their own benefit (Toufarová and Gvoždík 2016), but not for its offspring, which falsifies the maternal manipulation hypothesis. We experimentally proved interspecific competition between newt larvae and metamorphosed juveniles (Hloušková et al. 2018, Janča and Gvoždík 2017), and the effect of social interactions between competitors on behavioural thermoregulation (Winterová and Gvoždík 2018). These results highlight the importance of biotic factors for predicting the impact of climate change on ectotherm population dynamics. Finally, we reviewed the meaning of “thermal niche” in current literature and proposed a new framework, which provides a guide for using the term “thermal niche” in ecological literature and identifies key areas for further research (Gvoždík 2018).

#### *Coevolution between a brood parasite and its hosts*

The most striking coevolutionary arms race between avian brood parasites and their hosts takes place after the laying of parasitic eggs, when hosts attempt to identify alien eggs in the nest. In the case of the common cuckoo, the host faces a tricky challenge as cuckoo eggs are mimetic, i.e. they are very similar to the host's own eggs. As birds have very different visual systems than humans, allowing them to see ultraviolet (UV) light, we found that warblers more often rejected model eggs that had dissimilar UV reflectance than their own (Šulc et al. 2016) hence UV signals are likely to be useful to the host for recognising alien eggs in the nest. Moreover, we found (Hanley et al. 2016) that the UV colour of cuckoo and host eggs remained stable during incubation, in contrast with other colours. This gradual colour change eventually meant that the quality of cuckoo egg mimicry decreased during the incubation period, suggesting that parasitic egg mimicry may be more dynamic than previously thought.

In study by Požgayová et al. (2018) we explored sex differences in the growth of cuckoo nestlings reared by two warbler hosts and found that both sexes exhibited similar mass after hatching and grew at a similar rate. Nevertheless, males achieved higher asymptotic mass than females, while fledging at a similar age as females. These findings imply that male nestlings may have higher costs than female nestlings. Apart from the normal begging vocalisation produced during host feeding visits, common cuckoo nestlings use a different type of vocalisation in the absence of their foster parents. We confirmed experimentally (Honza et al. 2018), for the first time, that host-

absent vocalisation of a parasitic chick may be interpreted by foster parents as a signal of hunger, thereby increasing host feeding frequency.

The fundamental brood parasite-host coevolutionary paradigm assumes that host care for the parasite is much more costly than care for host's own progeny. We quantified (Samaš et al. 2018, Samaš et al. 2019a,b) parasite-rearing costs in common redstarts (*Phoenicurus phoenicurus*) raising either common cuckoo or their own chicks throughout the complete breeding cycle using multiple cost parameters. Contrary to traditional assumptions, rearing the much larger parasite was not associated with an overall increase in physiological or physical costs to the host but was similarly demanding as rearing its own brood.

### *Birds migratory behaviour*

Regular seasonal movements of animals in space and time represent a complex and widespread biological phenomenon that has shaped many of the migratory species' life history traits. Migrating animals often travel across enormous distances and the different conditions encountered in disparate parts of the world can have far-reaching consequences (Koleček et al. 2018a). Long-distance migrants also influence distant ecosystems where they may play various ecological roles. In order to understand such large-scale ecological processes, it is important to quantify how many individuals and what biomass moves annually between each ecosystem. A major breakthrough in the study of small bird migration was achieved only recently when miniaturised light-level geolocators were successfully applied to passerines (Koleček et al. 2016, Brlík et al. 2018, Procházka et al. 2018). These devices enable detailed tracking of migration and the location of stopover and wintering sites, which are frequently unknown for individual populations. Using geolocators, we demonstrated not only the position of non-breeding grounds of individual populations (Koleček et al. 2016, Procházka et al. 2017), but also that many birds performed intra-tropical movements between distant parts or the non-breeding grounds, which probably coincided with the onset of the dry season from late October onwards (Koleček et al. 2018b). Furthermore, on a large multi-species dataset, we revealed high inter- and intra-specific variation of migrants' behavioural strategies when crossing major geographical barriers during both autumn and spring migration periods (Adamík et al. 2016, Jiguet et al. 2019). In contrast to the preferred and long-standing "intermittent hypothesis", we convincingly showed for the first time that these otherwise mainly nocturnal migrants regularly extend their flights into the day. At the multi-species level, we further studied differences in migration timing in male and female long-distance migrants and the temporal dependences within the entire annual cycle (Briedis et al. 2019).

### **Biodiversity**

#### *Complex speciation processes in Ethiopian rodents*

The Ethiopian highlands are the largest mountainous complex in Africa and, together with the Great Rift Valley, provide a wide spectrum of model situations for evolutionary studies. The highlands have one of the most striking elevational ecological gradients on Earth, exactly the kind of place where ecological speciation associated with complex genetic changes is expected. Researchers from the Institute, together with their Ethiopian and Russian colleagues, used a unique collection of rodents collected from across the Ethiopian highlands over the last three decades (Bryja et al. 2019a) to reconstruct phylogenetic relationships in selected genera through combined analysis of multiple nuclear and mitochondrial markers. Previous models proposed a simple scenario of allopatric diversification following the separation of the highlands into two

main parts by the Great Rift Valley. Our genetic data, however, provide evidence for a more complex speciation process, often in the form of so-called reticulate evolution that combines divergence and hybridisation (Bryja et al. 2018, 2019b, Kostin et al. 2019, Šumbera et al. 2018). During colder Pleistocene periods, Afroalpine ecosystems spread to lower elevations, allowing specialised high-elevation taxa to cross the Great Rift Valley where they then hybridised with local species, possibly facilitating the colonisation of high elevation mountains, e.g. through adaptive introgression of mitochondrial genomes already adapted to high altitudes and low oxygen concentrations. One implication of this finding is an inability to use mitochondrial DNA sequences for simple species barcoding, at least for Ethiopian rodents. A further important feature of Ethiopian rodent diversification is the important role of selection in extreme Afroalpine conditions, which is reflected in the evolution of significantly distinct forms adapted to life on high plateaus (Bryja et al. 2018, 2019b, Lavrenchenko et al. 2017, Meheretu et al. 2015, Šumbera et al. 2018). However, all these taxa are internal lineages of more widespread congeners and their morphological adaptations are the result of rapid adaptive processes caused by strong selection.

#### *Biodiversity of amphibians and reptiles*

Within the Afrotropics, we studied primarily anuran amphibians (frogs). In Cameroon, we focused a special attention on the diversity and conservation status of amphibians from Mount Oku, a unique conservation landscape with a high endemism (Doherty-Bone & Gvoždík, 2017). In cooperation with the McMaster University in Hamilton, Canada, we examined historical biogeography, evolution and systematics of African clawed frogs (*Xenopus*) using modern techniques of high-throughput sequencing and micro-computed tomography. This research resulted in a revalidation of one species and descriptions of six new species (Evans et al. 2015, Furman et al. 2015, Evans et al. 2019). We have been also involved in several research projects studying radiation and diversification processes in anuran model taxa including bufonid true toads (Liedtke et al. 2016), ptychadenid ridged frogs (Zimkus et al. 2017), and hyperoliid reed frogs (Portik et al. 2019). The results showed the important role played by forest refugia in driving intraspecific divergences in the Guineo-Congolian rainforests and Gulf of Guinea archipelago of Central Africa, particularly in tree-frog models such as *Hyperolius* species complexes (Bell et al. 2015, 2017), *Africalus* (Charles et al. 2018), or *Chiromantis* (Leaché et al. 2019). Additional studies focused on tadpole morphology (Penske et al. 2015), frog ecology (Badjedjea et al. 2019), and distributions of little-known amphibians and reptiles (Dolinay et al. 2018, Gvoždík et al. 2018, Ihlow et al. 2019).

Within the Western Palearctic, we studied evolution and biogeography of the hylid frogs, and gekkonid, anguid and lacertid lizards. In a study of the speciation history of the European *Hyla* tree frogs, we demonstrated that gene introgressions played an important role in their speciation (Gvoždík et al. 2015). Multilocus phylogenetic and species-delimitation analyses uncovered hidden diversity and cryptic species in a Mediterranean gecko, and a new species-level taxonomy was proposed (Kotsakiozi et al. 2018). New microsatellite loci utilizable in population genetics of the anguid genera *Anguis* and *Pseudopus* were characterized (Mikulíček et al. 2018). A comparative phylogeographic study of four slow-worm (*Anguis*) species of the Balkan Peninsula revealed the distribution of evolutionary lineages, their contact zones, likely locations of glacial refugia, and directions of colonisations after the Ice Age (Jablonski et al. 2016, Jablonski et al. 2017). The phylogeographic study of the European glass lizard (*Pseudopus*) uncovered its low genetic variation suggesting population



extinctions during unsuitable climatic periods in the past, followed by subsequent rapid population expansions (Jandzik et al. 2018). Within the Czech Republic, the only-known three isolated populations of the lacertid lizard *Podarcis muralis* were investigated in a phylogeographic framework (Jablonski et al. 2019). It was demonstrated that the populations are non-exotic, having genetic relationships to the Slovak populations, and thus deserve conservation attention.

*Reconstruction of the Plio-Pleistocene history of sub-Saharan biomes by comparative phylogeography of African small mammals*

Small terrestrial mammals represent an ideal model group for studying the interplay of historical climate change and geomorphology in forming contemporary ecosystems as they have short life spans, rapid reproduction cycles, low dispersal ability and respond quickly to environmental change. Despite this, previous studies of sub-Saharan small mammals have often been biased by restricted geographic sampling and poor information on fossils suitable for divergence dating. Our recent sampling, supplemented by the collections of our collaborators, now allows analysis at a pan-African scale. Using genetic/genomic data and reliable fossil constraints (Aghová et al. 2018), we can now test the respective roles of forest retraction/expansion, rivers, ecological gradients and anthropogenic factors in explaining the diversification of African mammals from the late Miocene onward.

Our results demonstrate the importance of both climatic fluctuation and physiographic vicariance in shaping the distribution of sub-Saharan biodiversity. The evolution of forest taxa (Jacquet et al. 2015, Bryja et al. 2017, Krásová et al. 2019, Mizerovská et al. 2019, Sabuni et al. 2018) primarily mirrors fragmentation of forests in the late Miocene, separating Guineo-Congolese forests from East African montane and coastal forests. Later, and particularly in the Pleistocene, climatic oscillations were the most important factor in allopatric diversification of forest taxa in fragmented forest refugia. On the other hand, the evolution in open habitats was primarily driven by genetic differentiation in fragmented savannahs during the humid Plio-Pleistocene periods, as suggested by evolutionary history of species living in savannah-like habitats (Aghová et al. 2017, 2019, Bryja et al. 2018, 2019, Mazoch et al. 2018, McDonough et al. 2015, Mikula et al. 2016, Petružela et al. 2018). However, other factors were also involved. In the Zambezian bioregion, the most important factor was probably the Zambezi-Kafue river complex, which effectively stopped dispersal following the return of conditions promoting the spread of savannah. In East African Somali-Masai savannahs, the palaeolakes in the bottom of the Great Rift Valley played similar role. These results also have important taxonomic (description of new species) and conservation (defining regions with high phylogenetic diversity) implications.

*Invasive gobiid fishes*

Since the 1990s, five Ponto-Caspian gobiid species have extended their distribution throughout Europe, principally through introduction by shipping at inland ports followed by population expansion. Abundant populations of non-native species have the potential to affect recipient systems. Contrary to expectations, our long-term research strongly suggests that invasive gobiids do not represent an immediate threat to native fish assemblages in Central European rivers (Janáč et al. 2016). While gobies have had a negative impact on local macrozoobenthos, river productivity appears high enough to prevent any competition with native fish species (Mikl et al. 2017a,b, Ondračková et al. 2019). Our results also suggest that their successful invasion of Central European rivers is related to exploitation of an under-utilised niche space, i.e.

rip-rap bank stabilisation, which provides an excess of shelter and spawning sites (Janáč et al. 2018). While gobies show high susceptibility to three local parasites, they appear not to represent a danger to native fish assemblages through introduced parasites or disease (Ondračková et al. 2015, Kvach et al. 2019). Gobiid impacts on ichthyofauna are region-specific, being driven by local idiosyncrasies of the invaded systems (Janáč et al. 2019).

The genetic studies have been widely used to assess gobiid invasion history in North America, but such studies are less common in Europe. We used both nuclear and mitochondrial markers to assess genetic diversity and structure in native and non-native populations of three gobiids, the Western tubenose goby (*Proterorhinus semilunaris*), round goby (*Neogobius melanostomus*) and bighead goby (*Ponticola kessleri*), sampled from the main areas of their joint distribution, i.e. the lower Danube, middle Danube and lower Rhine. Significant differences were observed in the invasion histories of these three species, despite their joint distribution. The results provided support for (i) a Danubian origin for all three goby species in the Rhine, (ii) lower genetic diversity in fish colonising non-navigable tributaries, and (iii) provided early genetic data for a newly established round goby population on the River Elbe (Roche et al. 2015, Janáč et al. 2017).

#### *Endangered and invasive species of mammals in Central Europe*

Within our research we used Eurasian lynx (*Lynx lynx*) and sika deer (*Cervus nippon*) as suitable model species to study eco-evolutionary processes influencing extinctions and invasions as opposite ends of a spectrum of ecological success.

Even though populations of large carnivores are expanding throughout Europe, the Eurasian lynx populations in Central and Western Europe are isolated, small-sized and threatened by the loss of genetic diversity due to the genetic drift and inbreeding. The main negative factors influencing lynx populations are poaching and landscape fragmentation. Our research uncovered the attitude of hunters towards lynx and their experience with poaching of lynx in the Czech Republic (Červený et al. 2019). The attitude of hunters towards lynx was not affected by hunting region, lynx population density or origin of the population, but we detected that the attitudes became more negative during the last decades and the majority of hunters believed that lynx had negative effects on other wildlife. Population modelling suggested that at least 25 % of the population might be poached annually and such rate significantly constrains population growth and further lynx expansion. In a unique research, we utilised genetic analysis of non-invasively collected samples from the Eurasian lynx to study the social structure of isolated population at the edge of distribution range in the West Carpathians. The results confirmed a low population density and a relatively high level of annual fluctuation in the number of individuals (Krojerová-Prokešová et al. 2019). Female offspring usually settled in or near the maternal home range, new lynx from outside participated in reproduction rarely and the breeding with close relatives significantly decreased the effective population size as well as population genetic variability.

At the begging of 20th century, sika deer were introduced into many countries across Eurasia, Australasia and North America and free-living invasive populations became established in many countries. The expanding Czech sika population represents the ideal model species for studying micro-evolutionary processes, especially when comparing source and founder populations. In cooperation with Japanese colleagues we detected a high level of genetic differentiation between native Japanese sika and the introduced Czech populations (Krojerová-Prokešová et al.

2017) but genetic variability was generally low in both areas. The results also showed that multiple sika introductions, rapid population growth and possible hybridisation with red deer appear to have helped the successful expansion of sika in Europe.

#### *Searching for effective measures for farmland biodiversity conservation*

Within the European Union, substantial resources are currently spent on conservation measures aimed at halting the decline of farmland biodiversity; however, the effects of these measures are usually not clear and several studies have even reported no positive effects. We found conservation measures promoting habitat heterogeneity, namely decreasing field size and increasing the availability of non-cropped elements may be most effective tools for conserving declining farmland biodiversity (Šálek et al. 2018a). There is also increasing evidence showing that a substantial number of rare and declining farmland taxa exclusively inhabit non-farmland habitats. We demonstrated that farmsteads, especially those with animal and plant production, are hotspots for farmland birds and key habitats for several species of conservation concern (Šálek et al. 2018b). These measures may be also beneficial for the Ortolan Bunting (*Emberiza hortulana*) whose European populations have suffered marked population declines during recent decades (Šálek et al. 2019a) which has the core area of the current distribution in the Czech Republic located within post-mining landscape. Our results also showed that effective conservation for Ortolan Bunting in this landscape should be aimed to prevent the loss and degradation of suitable habitats, particularly the patches in early stages of spontaneous succession. In contrast to technical reclamation represented by forest and agricultural reclamation, spontaneous succession provides greater topographic and structural habitat diversity (Šálek et al. 2019a). Agricultural intensification and subsequent loss of suitable foraging and nesting habitats (bottom-up effect) and predation (top-down effect) have also been previously identified as crucial factors in Little Owl (*Athene noctua*) and Barn Owl (*Tyto alba*) population declines. Our results strongly indicate that direct anthropogenic mortality, especially collision with vehicles and entrapment, may significantly contribute to these declines in the Czech Republic. In order to improve the population status of these owl species, in addition to foraging habitat restoration, the focus should be on the reduction of anthropogenic mortality in urbanized landscapes (Šálek et al. 2019b).

#### **Pathogens and diseases**

##### *WNS - biotic and abiotic interactions shaping host-pathogen relationships*

Hibernation is an adaptation of temperate zone bats that allows them to survive scarcity of alimentary resources in winter. With lowered body temperature, the animal's metabolic functions are suppressed which, together with lowered energetic costs for thermoregulation, makes hibernation a behavioural adaptation of energy metabolism. Parasite exposure during hibernation (Lučan et al. 2016, Zahradníková et al. 2018) shapes survival and subsequent reproductive success of organisms through directly draining the host resources and indirectly by contributing to pathogen transmission. To minimise the impact of parasites and pathogens, hosts can alter their behaviour to select environmental conditions limiting pathogen growth (Kovacova et al. 2018, Martínková et al. 2018) or evolve molecular mechanisms enabling the host to tolerate the infection (Flieger et al. 2016, Harazim et al. 2017). Hibernating bats infected with the fungal pathogen *Pseudogymnoascus destructans* can develop the white-nose syndrome (Bandouchova et al. 2015, Pikula et al. 2017, Zukal et al. 2016a) with large differences in mortality (Bandouchova et al. 2018, Flieger et al. 2016, Martínková et al.

2019, Pikula et al. 2017). An established evolutionary balance in the host-pathogen system utilizes the genetic adaptations to skin infection, with genes involved in skin integrity and down-regulation of the immune reaction being under positive selection, indicating that energy conservation and the protective role of bodily barriers act together to improve host survival (Harazim et al. 2018) and enables hibernating bats to tolerate the infection (Zukal et al. 2016a).

Infected bats with severe white-nose syndrome arouse more often during hibernation. The frequency of active euthermic periods during torpor is a key factor determining survival during hibernation as fat reserves are rapidly depleted with repeated arousals. The ability to adjust roosting position without body temperature elevation (Bartonička et al. 2017) and to resist disturbance from arousing neighbours (Blažek et al. 2019) represent stabilizing mechanisms that decrease energy expenditure during hibernation and facilitate physiological torpor-arousal rhythm (Zukal et al. 2016b). The ability to maintain low body temperature in torpor despite prevalent skin infection that leads to inflammation and skin necrosis (Zukal et al. 2016a, Pikula et al. 2017, Bandouchova et al. 2018) might be the key determinant of survival until spring. Our results suggest that hibernating bats have evolved skin disease tolerance through a set of genomic, behavioural and physiological adaptations.

#### *Primate parasite ecology and microbiome*

Wild chimpanzees and other African great apes live in regions where African sleeping sickness is endemic; yet, little is known about their trypanosome infections. We established a protocol allowing us to detect trypanosomes from faeces and revealed the presence of trypanosomes of the *Trypanosoma brucei* group in the faecal samples of wild chimpanzees (Votýpka et al. 2015). We did not detect any *Leishmania* parasites in analysed faeces; however, we revealed an unexpected diversity of free-living bodonids and parasitic trypanosomatids (Votýpka et al. 2018). In the Dzanga Sangha Protected Areas (DSPA) (the Central African Republic), we studied ecology and transmission of malaria infections in lowland gorillas and humans, and observed switching between two different *Plasmodium* spp. We confirmed the presence of the human malaria parasite *Plasmodium ovale wallikeri* in both gorillas and humans (Mapua et al. 2015; Mapua et al. 2018). In a follow-up study, we found marked differences in the prevalence of malaria parasites among free ranging chimpanzee populations living in different habitats (Mapua et al. 2016). Moreover, we confirmed that the bleeding caused by some strongylid nematodes cannot explain the presence of *Plasmodium* DNA in ape faeces (Mapua et al. 2017).

Syngamid strongylids of the genus *Mammomonogamus* are among the least known nematodes having zoonotic potential. We found two haplotypes in lowland gorillas and forest elephants in DSPA suggesting the potential for sharing *M. loxodontis* between phylogenetically distant hosts, co-habiting same ecosystem (Červená et al. 2017). Although we highlighted importance of habitat sharing between hosts for parasite transmission in primates (Červená et al. 2016, Kalousová et al. 2016), the sequences obtained from humans differ from those in the non-human primates (Hasegawa et al. 2015, Čepička et al. 2020) and ecology and spatial overlap among primates in the tropical forest does not always led to parasite exchange among even closely related hosts. We also designed novel high-throughput sequencing approach (metabarcoding) for strain-level identification of parasite communities, namely gastrointestinal strongylids and *Entamoeba* spp. in primates (Pafčo et al. 2018, Vlčková et al. 2018) and implemented it to study of sympatric primates in DSPA and the Dja Faunal Reserve (Cameroon). Our results indicate that the degree of habitat

sharing between hosts, together with mode of parasite transmission, is the most important factor for parasite spillover among primate species including humans (Pafčo et al. 2019) and supports our previous findings that habituation may not necessarily pose a greater risk of protist and helminth infections in gorilla groups (Pafčo et al. 2017). We also observed that some *Entamoeba* haplotypes are shared between humans and other primates, indicating zoonotic potential. Finally, we investigated fractal patterns in the behavioural activity of wild chimpanzees and domestic sheep in relation to strongylid infections. The complexity of chimpanzee feeding sequences and the intensity of infection with strongylid nematodes were positively related i.e. individuals with more intense infections exhibited more stochastic feeding sequences (Burgunder et al. 2017) but sheep treated with anthelmintics exhibited a higher complexity in their activity sequences (Burgunder et al. 2018).

We characterized faecal bacterial communities and, for the first time, gut metabolomes of four wild lowland gorilla groups and determined how these microbial communities respond to the host's external environment (Gomez et al. 2015). Our results suggest that dietary constraints triggered during gorillas adaptive radiations were potential factors behind the species-specific microbiome patterns observed in primates today (Gomez et al. 2016). We also explored the impact of host traits, parasite infections, stress and antibiotic treatment on microbiomes of wild lowland gorillas (Pafčo et al. 2019, Vlčková et al. 2016, Vlčková et al. 2018a,b). The microbiomes of hunter-gatherers and agriculturalists inhabiting DSPA reflected dietary gradients which have triggered loss of traditional microbes and increased carbohydrate and xenobiotic metabolism in humans (Gomez et al. 2016). We also showed that the taxonomic composition of the human gut microbiome exhibits increased compositional plasticity and revealed unexpected similarities between African monkeys and human populations (Gomez et al. 2019).

#### *Emerging zoonoses and their impact on human health: from surveillance to epidemiological preparedness and risk assessment*

In a monitored time frame, our team was successfully engaged in two large scale European projects (EDENext and VECTORNET) and our studies focused primarily on West Nile Virus (WNV), the most important mosquito-borne virus in Europe, which circulates in various ecosystems (Rudolf et al. 2015a,b, Čabanová et al. 2019, Rudolf et al. 2018 and 2019). We detected for the first time WNV in overwintering mosquitoes in Europe (Rudolf et al. 2017) and our data support the hypothesis of WNV persistence in mosquitoes throughout the winter season in Europe. As an epidemiological consequence, it can be assumed that lineage 2 WNV infections in Europe are sustained by virus persistence in mosquitoes followed by vertical transmission and maintenance of the mosquito-bird transmission cycle, without the necessity of virus re-introduction. Usutu virus (USUV), another mosquito-borne pathogen, represents another health threat to many bird species and human. Our new data contribute to knowledge about USUV genetic variability, distribution and spread in Central Europe (Hönig et al. 2019). In addition to studying mosquito vectors, we also examined arbovirus circulation in vertebrate hosts (Straková et al. 2015, Bakonyi et al. 2016, Hubálek et al. 2017, 2018 and 2019, Stejskalová et al. 2019). Interestingly, we monitored course of WNV infection in captive Nestor kea (*Nestor notabilis*), after becoming naturally infected with WNV lineage 2. The WNV RNA persisted and the virus evolved in the bird's brains, as demonstrated by phylogenetic analysis of the complete viral genome detected in kea euthanised between 2009 and 2014 (Bakonyi et al. 2016). We also examined common coots (*Fulica atra*) for antibodies against WNV

and USUV and our results indicated that both occur in common coots and that these birds may serve as “sentinel” species (Straková et al. 2015). Similarly, birds of prey (*Accipiter gentilis*) might serve as early indicators for WNV circulation (Hubálek et al. 2018 and 2019). Concerning human cases of arbovirus infections, the detection of WNV in a blood donation originating from an area with low human WNV prevalence emphasises the importance of WNV nucleic acid testing of blood donations (Kolodziejek et al., 2015).

Our “tick-borne research” was focused at prevalence studies of human pathogenic viruses (Crimean-Congo haemorrhagic fever virus, Tick-borne encephalitis virus), bacteria (*Rickettsia* spp., *Babesia* spp., *Neoehrlichia* spp.) and protozoans (*Hepatozoon* spp.) in a range of tick vectors (Hamšíková et al. 2015 and 2019, Venclíková et al. 2015 and 2016, Duscher et al. 2016, Rudolf et al. 2016, Hubálek and Rudolf 2017) and vertebrates (Kříž et al. 2015, Mertens et al. 2016), including eco-epidemiological aspects (Vayssier-Taussat et al. 2015, Rosa et al. 2018, Jaarsma et al. 2019). In case of rodent-borne diseases, we performed first molecular survey of pathogenic hantaviruses in rodents and humans in the Czech Republic and detected Dobrava-Belgrade and Puumala hantaviruses in humans and Dobrava-Belgrade, Tula and Sewis in rodents and provided epidemiological glue between virus circulating in rodents and reported human cases (Vrbovská et al. 2015, Zelená et al. 2019). Concerning additional wildlife borne diseases, we have shown that the wild boars act as a reservoir of hepatitis E virus (HEV) in the Czech Republic and that this virus has been circulating in the study area for more than 20 years (Straková et al. 2018).

#### *Evolution of parasites in host secondary contact zones*

Hybrid-zone studies of host-parasite systems have largely taken a host-centric viewpoint focussing on how parasitism might affect the outcome of host hybridisation by differentially impacting the fitness of host taxa vs. their hybrid descendants, the parasites being seen as a homogeneous entity with a single strong effect on hybrid vs. parental hosts. In contrast, the process creating secondary contact zones in hosts is also likely to give rise to secondary contact in their intimate parasites. These parasites will be two entities, each arising out of co-existence with a different host and having no clear singular effect on hybrids (Baird and Goüy de Bellocq, 2019). We showed that this is the case in the European house mouse (*Mus musculus*) hybrid zone with three different parasites, a DNA virus (the murine cytomegalovirus, MCMV), a fungus (*Pneumocystis murina*), and a nematode (*Syphacia obvelata*). These parasites have a host-specific genetic clustering, with 94% of the genome in the MCMV model showing host-specific genetic clustering while a small part of the genome encoding immune evasion genes shows maintenance of ancestral polymorphism (Goüy de Bellocq et al. 2015; Čížková et al. 2018). For the fungal and nematode parasites, we found clear host-specific genetic clustering, with presence of hybrid parasites at the centre of the host hybrid zone forming themselves a more abrupt hybrid zone than their hosts (Goüy de Bellocq et al. 2018). In contrast to these results we also investigated the genetic structure of the whipworm nematode *Trichuris muris* which has a less intimate relationship with its host mouse than the former parasites. We found no host-correlated structure (Wasimuddin et al. 2016). In this case, passage through alternate hosts seems sufficiently common to prevent/erase any genetic structure signal associated with particular host taxa.

To confirm the generalities of our findings across host/parasite systems we started investigating another secondary contact of a rodent species and their parasites in Tanzania using the model of the Natal multimammate mouse, *Mastomys natalensis*

and its arenaviruses. We first discovered a new arenavirus in this rodent species, the Gairo virus (Gryseels et al. 2015). We then investigated whether geographically and genetically distinct sub-taxa of *M. natalensis* carry distinct arenaviruses by detailed sampling across the contact zone of two of these sub-taxa. Ongoing hybridisation shows that individuals of the sub-taxa are in direct physical contact, in principle allowing viral exchange, yet neither of two arenaviruses (Gairo and Morogoro virus) were found to have crossed the zone (Gryseels et al. 2017). Such intraspecific genetic barriers to arenavirus spatial spread have important implications for our understanding of the geographic distribution of arenavirus pathogenic to humans such as Lassa virus in Western Africa.