- *2nd Asellus Symposium - synthesis* -




Highlights of the day (compiled by the organisers):

Below you find an abbreviated synopsis of the *2nd Asellus Symposium*. Whilst being probably biased, we hope this provides a holistic view of what the community works on and is interested in (but any bias is our fault, of course). We also highlight a few themes that, at least to us, seem to have a particular potential for future research avenues. Below you find:

i) a brief history leading to the symposium,
ii) a thematically structured synthesis based on the talks,
iii) a list of themes/methods that were identified during the symposium and warrant attention.

For reference, you find **the final program and recorded talks** here: https://www.asellus.org/news/second-asellus-symposium/

- **Favourite quote of the day:** "*Asellus* is a vacuum cleaner" (Terezia Horvatova)

i) A brief history leading up to the symposium - by Katja, Žiga, and Blake:

- In 2018, two separate *Asellus* meetings took place unbeknownst to each other: the "1st Asellus symposium" at Eawag, Switzerland, which initiated the local "*Asellus* consortium" working on surface populations, and the one in Romania with scientists working on cave adapted *Asellus*. In 2019, the paths started to merge after Katja, Elvira, and Moritz visited Slovenian colleagues to learn more about *Asellus* and to visit the fantastic caves. Since then many separate and interconnected lines have developed, including recent connections to researchers working on drinking water pipe *Asellus*.
- *Asellus* research really has a long history, as highlighted by Žiga, who gave a brief history on research on the surface-cave divergence, addressing early contributions from E. Racovitzai, C. Kosswig, B. Sket - and covering questions around taxonomy, phylogeography, neutral theory of regressive evolution, and ecological speciation.

- Blake introduced the more recent developments leading to the recent surface water *Asellus* research at Eawag, which benefitted from earlier work by Finnish and Swedish colleagues, and by 2019 culminated in studies of ecosystem feedbacks, plasticity and pigmentation by Moritz Luerig and host-microbiome responses to global change by Elvira Lafuente.

ii) A thematically structured synthesis based on the talks**:

***Note that the order does not reflect order of talks during the symposium, and is not a balanced reflection of full talk contents. For specifics, please have a look at the talks at https://www.asellus.org/news/second-asellus-symposium/ . This synopsis was drafted by Katja with help from Žiga. For any inaccuracies, Katja takes the blame. Please let her know if something needs to be corrected.*

N.B. If you have publications related to the talks and would like to have them linked to teh videos on the website, please contact Moritz.

Phylogeographical and phylogenetic analyses provide insight into colonization history and macroevolution:

- Based on the mtDNA analyses across 1500 individuals and 400 localities (presented by **Lidia**), we have a relatively good understanding of the phylogeography of *A. aquaticus* and its close relatives across most of its distribution range. *A. aquaticus* (sensu lato) has a miocene origin (Paratethys basin) and harbours high haplotype diversity. There is evidence for genetic exchange between two haplotype networks, and the data suggests that the Carpatian mountain range acted as a migration barrier, with a geographic range expansion ca. 200 000 yrs ago. Trace fossils from proglacial lake sediments further suggest that *Asellus* may have survived temporary freezing. Interestingly, in Ireland some specimens were found that belong to one of the Balkan clades, which may reflect anthropogenic introductions.
- Based on phylogenetic analyses of the Aselloidea (presented by **Tristan**), multiple independent transitions from surface to subterranean habitats are found throughout this superfamily, and subterranean species have larger genomes than surface ones.

Sexual selection can be a potent evolutionary force, although relatively little understood in Asellus:

- Based on detailed morphological analyses (presented by **Anna**), there are differences between surface and cave forms in several morphological traits, including antennae and pereopods. Evidence for habitat specific sexual dimorphism suggest that sexual selection differs between caves and surface habitats.
- **Žiga** highlights that nothing is known about cave-freshwater *Asellus* phenology. Yet fluctuating (surface) *vs.* stable (cave) environmental conditions and food availability (high *vs.* low) could select for different life-histories in surface and cave populations. New data hint that there is a partial mismatch in reproductive period of surface and cave populations, which might contribute to the overall reproductive isolation between the morphs (i.e. temporal assortative mating). *Asellus* are also a good model to test the Island and Rensch´s rules (in the latter sexual dimorphism varies predictably with body size), an idea originally put forth by Gabor Herczeg. In *Asellus* males are generally the larger sex in general, but in cave populations males are

relatively larger than in surface water populations (i.e. sexual dimorphism in body size is larger in caves). Sex-ratio seems more male-biased in caves but, interestingly, there seems to be temporal variation in the sex-ratio in both surface and cave populations. This could cause temporal variation in sexual selection (incl. male-male competition).

Pigmentation is one of the most variable and classically studied traits in A. aquaticus:

- Interestingly, size-pigmentation relationships appear to be similar in Japanese populations of *A. hilgendorfii* and in *A. aquaticus* (work with Osamu Kishida, flash talk by **Moritz**).
- In her plenary, **Meredith** provided insight to the genetic basis of cave-related traits (loss of pigmentation and eyes). Some key questions included: When and how do morphological differences arise during organismal development? Are same or different genes responsible for trait variation in different systems? Genomic data, combined with between-lineage crosses, and genotype-phenotype correlations, showed for certain loci that CC (homozygotes for cave allele) are unpigmented, while CS (heterozygotes) or SS (homozygotes for surface allele) are pigmented. Same pigmentation genes are likely already in the ancestral population (as standing genetic variation) and have then become fixed in caves (i.e. loss of pigmentation alleles).
- Based on genomic analyses of Gotland Island (Sweden) populations (surface and Lummelunda Cave populations), there is a QTL for pigmentation (presented by **Vid** and **Dominic**). The next goal is to verify the linkage by genome-wide-association studies (GWAS). This is a recent example including a draft genome assembly (10x), combining whole genome sequencing (WGS) of parents, RADseq of F2 and F4 offspring, phenotyping, genotype-phenotype associations and analyses of allele frequency changes (Bakovic et al. 2021; https://onlinelibrary.wiley.com/doi/10.1111/mec.15987?af=R).

In addition to pigmentation, behaviour is a trait that varies substantially in Asellus:

- In general, within species variation in behaviour could arise between stable *vs.* variable (daily or seasonal fluctuations) habitats and foraging innovation may facilitate colonisation of new environments.
- The Molnar-Janos Cave (Budapest, Hungary) provides insight to behavioural divergence and its potential to facilitate colonisation of new environments.This cave has thermal water with 23°C all year round and the *Asellus* population in this cave has been genetically isolated from the surface population for at least 60.000 years. There are no barriers to dispersal, but there is genetic isolation of *Asellus* populations.
  Gergely shared a video of Molnar-Janos Cave research: https://vimeo.com/208467571
    - Behavioural innovation (presented by **Gabor**) and aggregation/sheltering (presented by **Sara**) have been studied in this system. In the cave, only biofilms of chemoautotrophic bacteria are available as food (as no food sources form the surface enters the cave), suggesting that surface *Asellus* might have been able to colonise the cave due to their foraging innovation. However in behavioural assays, both cave and surface *Asellus* prefer leaves and it is possible that colonists prefer "unnatural" foods (e.g. cucumber).

    Aggregation and sheltering bring costs and benefits, and thus can vary in relation to predation risk (or other factors). In this system, the cave *Asellus* shelter less, and there is also divergence in aggregation behavior between populations but not structured by cave vs. surface morph.
    - Behavioural activity can also differ between sexes, but be habitat dependent (presented by **Hajiriz**). Foraging and mate searching behaviours affect fitness and it is predicted that males are more active than females. Analyses of divergence in behavioural activity among surface and cave populations and sexes suggests that there are population and gender specific responses to light regimes as well.
    - Behavioural consistency may be related to dispersal/colonisation, with potentially decreased exploration if selection is relaxed or if there are trade-offs (presented by **Gergely**). Caves may also act as dispersal traps. Surface *Asellus* appear to be more active during the day than cave *Asellus,* which might reflect an adaptive shift in circadian activity. Possibly coloniser individuals are more active in dispersal behaviour.
- Natural selection could also act on mean and variance, and hence differ between stable *vs.* fluctuating environments - such as seen in cave *vs.* surface habitats. **Simona** presented behavioural variation across hierarchical levels (from individual to population level) and potential for spatio-temporal variation in selection: divergence in general activity, between bolder *vs.* shyer individuals (i.e. risk prone *vs.* adverse), low *vs.* high inter-individual variation, low *vs.* high plasticity, high *vs.* low predictability, and individual level repeatability. Video based analyses showed, for example, that surface *Asellus* are more active, while cave *Asellus* are bolder.

Drinking water pipe studies could provide an opportunity for joint microevolutionary studies:

- *A. aquaticus* is surprisingly abundant in many drinking water pipes (presented by **Emmanuelle**), and becomes a societal problem as it is “not so much fun if you get *Asellus* and their poop out of your tab”. In addition, water pipe dwelling *Asellus* may facilitate pathogenic *Aeromonas* (presentation by **Claudia**). As habitats, drinking water pipes are cave-like: lightless, low in nutrients and rich in biofilm. In addition, there are no predators in these systems (*Asellus* is on top of the food chain) and *Asellus* can occur even in pipes with chlorinated systems. Population densities can be very high and sediment in the pipes accumulates, fluctuates seasonally, and consists upto 30% of *Asellus* faeces. It is not clear what allows high abundance of *Asellus* in the Dutch systems, but possibly high microbial growth is facilitated by decaying *Asellus* and faecal nutrients. Microevolutionary/developmental biology research potential is seen as *Asellus* tend to have wider gaps between their tergites in pipes than on surface. Further studies are needed to test if this may reflect early stages of divergence.

Biotic interactions (host-parasites and host-microbiomes) are strong selective agents and important mediators of ecological functions:

- *Asellus* is a host for acanthocephalan parasites, which can modify host behaviour. Behavioural comparisons indicate that cave animals are less active than surface forms, but infestation by the *A. aquaticus* specialised *Acantocephalus aguillae* has no effect on behavioural activity (presented by **Grega**). However, both surface and cave individuals spend less time sheltering when infested.

- The host associated microbiome can provide a fast response system (act as an extended phenotype). *Asellus* is tolerant to chemical pollutants and harbours microbes in its mid- and hindgut (presented by **Elvira**). Experimental studies on wastewater effects show that wastewater, and abundance of external microbes, affect the gut microbiome composition and that the microbiome composition differs among populations - suggesting that microbiome may provide rapid means of coping with pollution.
- In addition to gut microbiomes potentially providing benefits to the host, they may provide sources of new microbial compounds or be reproductive manipulators. **Terezia** suggested that actinobacteria might be a new source of microbial compounds against fungal pathogens. **Christoph** provided genetic evidence for the presence of two reproductive manipulators (which favour female production) in *Asellus*: *Wolbachia* and *Cardinium*. This offers potential to study the role of reproductive manipulators in host evolution.

A haphazard subset of questions that arose from the talks (and you may have the answer to):

- Has it been experimentally shown that *Asellus* can survive freezing?
- What is known of the dispersal ability of *Asellus* in nature?
- What is the genetic distance of *A. aquaticus* to terrestrial isopods?
- Are the different genetic lineages within *A. aquaticus* morphologically identical?
- How different is *A. kosswigi* from *A. aquaticus* (genetically and phenotypically)?
- Why do subterranean *Asellus* have larger genomes?
- What other reproductive isolation mechanisms are there other than temporal isolation in *Asellus*?
- What is known of male-male interactions?
- Is the ability to feed on the biofilm (i.e. foraging innovation) facilitated by the gut microbiome?
- To what degree is intraspecific variation in behaviour reflecting phenotypic plasticity or stochasticity, rather than responses to divergent selection?
- Would we expect a bias in what type of *Asellus* colonise caves or drinking water pipes? (e.g. those that explore more and are bolder?) Could this create a predetermined bias for population divergence between caves (or pipes) and surface forms?
- Does behavioural divergence come before morphological divergence in early stages of speciation?

iii) Themes and methods that warrant attention (collection of discussion points during talks and discussion sessions):

It is noted that there is limited knowledge on even some basic things and we need to build a better base to achieve better science (e.g. old literature needs some follow up). More integration across the community would therefore be beneficial. Based on presentations and discussions, for example the following aspects need attention:

Conceptual topics:

- Studies on **colonisation history** (cave *vs.* freshwater systems) and **phylogeography.** Potentially could use eDNA to detect *Asellus* in wider surveys. World database of Asellidae: backbone database GOTIT https://besjournals.onlinelibrary.wiley.com/doi/abs/10.1111/2041-210X.13307. Some areas of the distribution range are poorly resolved (see: https://www.frontiersin.org/articles/10.3389/fevo.2021.748212/full)
- *Asellus* as a putative **model for ecological speciation** → need formal tests of reproductive isolation (e.g. mating experiments are needed to test for phenotype based assortative mating and temporal isolation). Here one could also make use of the potential for the microevolution continuum (surface / waterpipe / caves of different ages)
- More studies in general are needed on **sexual selection** (e.g. in what form does it take place? male:male competition? female choice?).
- **Sex determination system** is poorly studied. Can individuals change sex? Strange genitalia observed by Elvira - are they *Wolbachia* infected? Would need to develop genetic markers for sex determination.
- Studies on the selective forces acting on **pigmentation** and relative contribution of genes versus environment. E.g. what is the role of UV (https://lucris.lub.lu.se/ws/portalfiles/portal/5494091/544961.pdf)? Is pigmentation influenced by sexual selection?
- *Asellus* is a keystone species with broad dietary use. More studies on **foraging ecology / nutritional ecology** needed (e.g. what is the role of fungi and microbial chitinase production, variation in fatty acids).
- **Life-history variation and divergence** is still poorly studied, yet there is evidence for example for variation in phenology in caves, water pipes and surface waters (from temporal data).
- **More intraspecific studies on behaviour** to understand innovativeness, predator responses (e.g. to chemical cues) and agents of selection acting on behaviour.
- **Eco-evolutionary studies on species interactions** from host-microbiome (e.g. role of the microbiome in colonisation in caves and drinking water pipes, role of reproductive manipulators), host-parasite (e.g. divergence in responses to acanthocephalan parasites), predator-prey interactions (*Asellus*-fish). What are possible experimental designs for testing the effect of acanthocephalan infection on *Asellus* behaviour or other phenotypic traits? More work needed on epibionts (rotifers, fungi etc…).
- Potential for using *Asellus* as a model for **urban evolution** (urban streams, drinking water pipes, drinking water reservoirs).
- Studies on rapid **evolution of plasticity**?
- **Applied use of *Asellus***: could it be used as a natural vacuum cleaner to remove microbes? Could its microbial associates provide a source of new antibiotics? To understand and, if needed, to control water pipe *Asellus* experimental manipulations would be needed.

Methods to develop / emerging:

- **-Omics resources:**
    - **Genomic resources:** In addition to developmental genetics and crossing approaches, a fully annotated genome is not yet available.
        - Several *Asellus* draft genomes seem to be available / in preparation in the community (e.g. Eawag, Switzerland: Katja Räsänen/Philine Feulner; Linköping Univ, Sweden: Dominic Wright; Romania: Serban Sarbu & team), but discussions at the symposium suggest that parallel efforts are done without knowing from each other. How could we connect?
        - Development of functional genomic tools seems possible (e.g. CRISPR)
        - How much DNA is needed for RADseq? Can this be done on a single leg?
    - **Microbiomic, metagenomic and metaproteomic** resources. Would transcriptomics help getting at the genetic basis of behaviours and pigmentation (as is used in studies of host-microbiome interactions)? Note that one can use beads (+ antibiotics) to feed to remove microbial contamination in the gut.
- Developing **standardised protocols for behaviour**. A potential resource could be a database of protocols for standardised classic behavioural experiments (what are best ways to measure basic behaviours?)
- Developing **standardised laboratory experimental protocols**, including a developmental time series for embryonic/juvenile stages, lab rearing protocols (including embryonic *in vitro* rearing).
- Sharing and developing **high throughput phenotyping tools** (advanced image, video, microscopy techniques). Creating an online repository of *Asellus* images would allow, for example, testing effects of environmental variation on *Asellus* phenotype (shape, pigmentation, etc.) at wider scales.
- **Long-term data sets** are useful for temporal analyses (life histories, phenology, temporal reproductive isolation, genomic responses to natural selection).