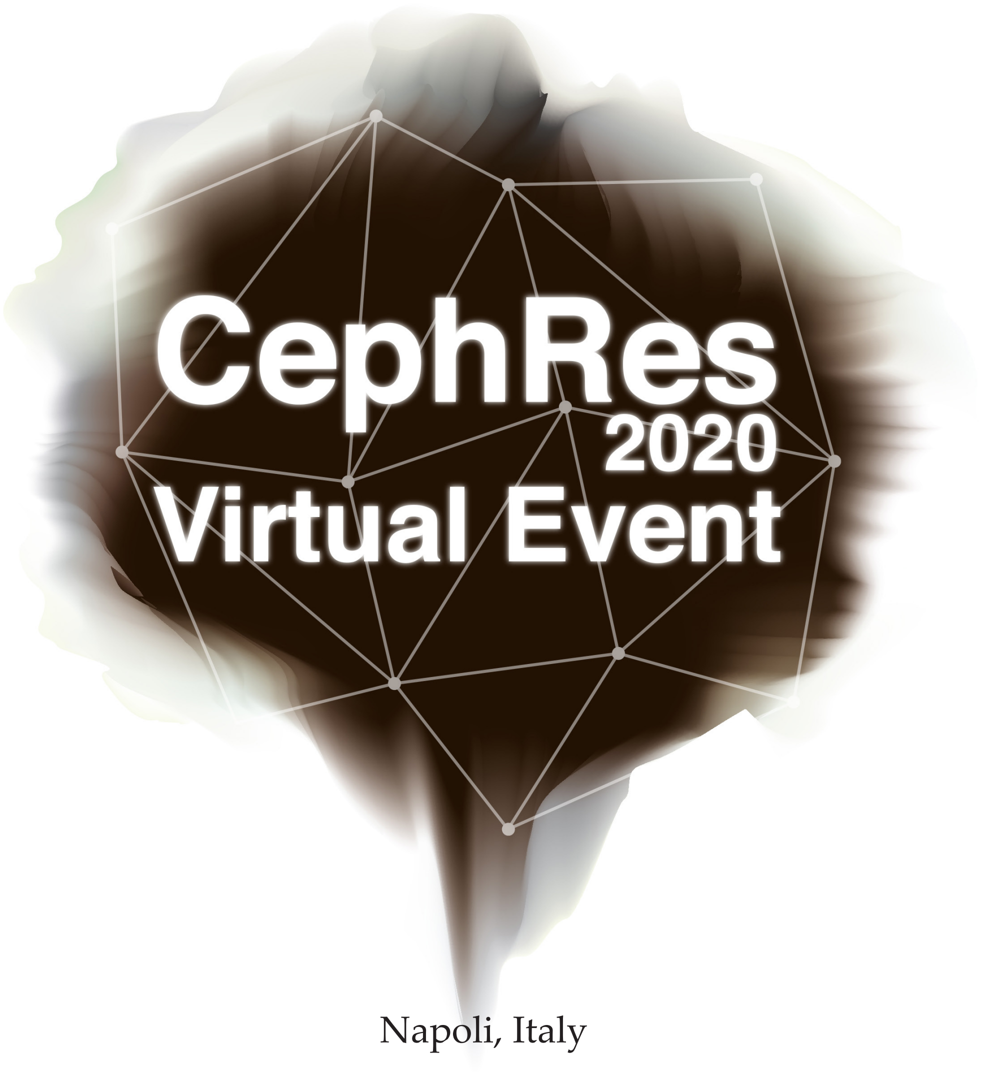
Biology and Life History of Cephalopods

an interim meeting of the international cephalopod community



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Making sense of alien genomes – synteny and regulation in cephalopods

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Cephalopods, with their sophisticated camouflage, extraordinary intelligence and highly modified body-plan can almost seem other-worldly. But not only their morphology and behaviour is fascinating, cephalopod genomes also show some unique features. Recent studies indicate large-scale genomic rearrangements before the split of Octo- and Decapodiformes. Local gene-order (synteny), which is widely conserved between many distantly related species, has largely been lost in cephalopods through this event. Interestingly, however, we can find hundreds of novel, cephalopod-specific gene clusters, which have been retained in different cephalopod species for the last 300m years. Chromosomal-scale analyses show that those novel syntenic clusters formed through a complex fission and fusion history of ancestral linkage groups. So far it is unclear if and how these cephalopod-specific micro-syntenies contributed to the formation of novelties in cephalopods. Through state-of-the-art methods including Hi-C and ATAC-seq we can now reconstruct the evolutionary history of the formation of recent cephalopod chromosomes, the emergence of novel, cephalopod specific-local gene clusters and their regulation during development. We find large topologically associated domains (TADs) of an average size of 2.5Mbp in the Hawaiian bobtail squid Euprymna scolopes. Both the organisation of cephalopod-specific micro-syntenies within these TADs and their co-expression profiles indicates that the clusters are functional, co-regulated units. Their function however seem to be more complex than a simple contribution to a particular cephalopod-specific tissues, indicating novel regulatory mechanisms associated with their emergence.

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Hannah Schmidbaur *Early Career*

Hannah is a PhD student at the University of Vienna in Dr Oleg Simakov lab, interested in the evolution of animal diversity. She wants to understand how morphological diversity is linked to molecular mechanisms using her background in genomics and zoology. Her current work focuses on regulation and synteny in cephalopods and how genome rearrangements can lead to the evolution of novelties.

Genome skimming clarifies the evolutionary relationship of bobtail squid

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Bobtail squid are small benthic, nektonic, or nektobenthic species; abundant in the Mediterranean Sea, the Indo-Pacific Ocean, and the Atlantic Ocean. However, their species relationship remains unclear. Here, we used low-coverage whole-genome sequencing to retrieve the complete mitochondrial genome and nuclear loci, from 24 different species that comprise the two genera within family Sepiidaridae and the three accepted subfamilies within the Sepiolidae, to resolve their phylogenetic relationship.

Our results based on mitochondrial genes support the sister relationship between the subfamilies Heteroteuthinae and Rossiinae, and indicate that some species need to be re-assigned, particularly within the Sepiolinae. The available reference genome of the Hawaiian bobtail squid *Euprymna scolopes* also proved useful to retrieve over 600 conserved loci. These conserved loci supported the relationship of subfamilies within the family Sepiolidae, similar to that based on mitochondrial genes alone, yet some relationships are in discordance.

Our study resolves the relationships of the main clades within the Sepiolidae and highlights the utility of low-coverage whole-genome sequencing to assess the relationship of cephalopods by using mitochondrial genes and nuclear loci, particularly when a reference genome of a closely related species is available.

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Deciphering cephalopods along south coasts of India using genetic markers

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Cephalopods are the third-largest group in Mollusca and the subclass Coleoidea, includes squids, octopuses and cuttlefish. There are about 60 species of cephalopods recorded from Indian waters. The annual fishery of cephalopods from Indian waters is estimated around 220,844 tonnes, with 6-7% of the total marine fish landing. There are a lot of taxonomic ambiguities within the Cephalopods and the accurate identification of species is essential for the sustainable fishery, ecosystem management and conservation measures. Though many studies are there elsewhere on molecular identification of cephalopods, from Indian region, molecular characterisation studies are very few. With a view to identify and genetically catalogue the Cephalopod species of Indian coasts, specimens were collected from landing centres of Southern Indian waters. Molecular characterization was done with partial sequence information of mitochondrial gene Cytochrome C oxidase-I (COI) gene. Genetic analysis showed, a total number of 14 species belonging to four families Loliginidae, Octopodidae, Sepidae and Sepiolidae. Phylogenetic analysis using maximum likelihood approach revealed that all 4 families are monophyletic. Interspecific distance between octopus, cuttlefish and squid was in the range 11-19%, 15-22% and 14-27% respectively. However, *Sepiella inermis* collected from East and West Coast showed 7% difference, hence warrants the need for finding any cryptic species available within the species. Unlike other loliginids, *Sepioteuthis lessoniana* (~20%) showed more genetic similarity with Sepidae family than loliginidae family, and this also needs further exploration to understand the evolutionary relationship among the cephalopods.

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Molecular tools are contributing to the discovery of new cephalopod species in the northern Gulf of Mexico

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Two programs focusing on assessing biodiversity for the midwater fauna of the Gulf of Mexico, the Offshore Nekton Sampling and Analysis Program (ONSAP, 2011) and the Deep Pelagic Nekton Dynamics of the Gulf of Mexico (DEEPEND, 2015-2019), have provided the research team with over 12,000 cephalopods ranging in size from paralarvae to adults. These samples have been used for numerous studies that presently include: trophic dynamics, contaminants (PAH's), vertical distribution patterns, and genetic connectivity to other ocean basins. One exciting outcome to this work has been the discovery of at least four new cephalopod species.

The team has been using both morphological and molecular tools to analyze potential new species. One of the surprising discoveries made was finding three new Bathyteuthid species to the Gulf of Mexico and north Atlantic Ocean which has been recently published in the Bulletin of Marine Science (April 2020).

The discoveries from these programs will be discussed in this presentation including a new description of a *Helicocranchia* species.

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Adaptive evolution at mRNA editing sites in soft-bodied cephalopods

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Adaptation of low-polymorphic populations is limited by their genetic variability, and heritable epigenetic modifications could be an additional source of selected variants due to the involvement of otherwise neutral mutations in the evolutionary process. One example of such modification is a widespread type of mRNA editing where adenine nucleotide is substituted with inosine, which is subsequently recognised by the cellular machinery as guanine. mRNA editing, while potentially important, very rarely alters the protein sequences in all lineages studied so far. An exception are soft-bodied cephalopods (coleoids), where the numbers of re-coding editing sites exceed those in other lineages by orders of magnitude, which is interpreted in terms of editing sites being useful for sophisticated phenotypic adjustments in complex coleoid nervous systems.

We propose, that, apart from conservative editing sites, which comprise up to 47% of sites and could bear a regulatory function, there are non-conservative sites, comprising more than 53%, which could temporarily mask beneficial adenine-to-guanine substitutions until an actual substitution to guanine happens, and, as coleoids are rather low-polymorphic, such scenario could be a way for them to promote adaptation. We show, that non-conservative edited adenines are indeed frequently substituted to guanines and for heavily edited sites there is positive selection acting on this transition, and, as we further show, that editing depends on mutations of the local context, we propose, that mutations enhancing editing could be indeed a source of variants needed for selection.

Our study provides the first transcriptome-wide example of an epigenetic process that contributes to the increased expressed genetic variability and hence facilitates adaptation.

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Architeuthis dux genome: a search into cephalopod gigantism and key-adaptations to deep-sea environments

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The giant squid (*Architeuthis dux*; Steenstrup, 1857) is an enigmatic giant mollusc with a circumglobal distribution in the deep ocean, except in the high Arctic and Antarctic waters. The elusiveness of the species makes it difficult to study, the lack of fresh samples hinders genetic studies, and the complexity of the cephalopod tissue creates technical challenges in the laboratory. Still, having a genome assembled for this deep-sea dwelling species will hopeffully allow unlocking several pending evolutionary questions.

I will present our strategy and describe how we produced a draft genome assembly that includes 200 Gb of Illumina reads, 4 Gb of Moleculo synthetic long-reads and 108 Gb of Chicago libraries, with a final size matching the estimated genome size of 2.7 Gb, and a scaffold N50 of 4.8 Mb. In addition, we sequenced the proteome of the same individual and RNA from three different tissue types from three other species of squid species (*Onychoteuthis banksii*, *Dosidicus gigas*, and *Sthenoteuthis oualaniensis*) to assist genome annotation. We annotated 33,406 protein coding genes supported by evidence and the genome completeness estimated by BUSCO reached 92%. Repetitive regions cover 49.17% of the genome.

This annotated draft genome of *A. dux* provides a critical resource to investigate the unique traits of this species, including its gigantism and key adaptations to deep-sea environments.

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see Invited Lecture - Early Career: H. Schmidbaur (Austria)
Making sense of alien genomes – synteny and regulation in cephalopods

Establishment of a Model Association: Investigating the Squid-*Vibrio* Symbiosis Research from 1970s

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Model organism approaches gained considerable attention by historians and philosophers of science as they have been very prominent in life sciences. One of the recent trajectories in historical and philosophical scholarship is looking beyond individual species and focusing on model systems research involving multispecies parallel to recent trends in biological research.

Here I argue that multispecies model systems are beyond the reach of 'model organism' and propose 'model association'. This work has two aims: ***1.*** A case study is developed on the squid-*Vibrio* model system from 1970s offering a detailed historical account of the development of this model association which the literature is lacking despite the important status and wide use of squid-*Vibrio* system in symbiosis research. **2.** Notion of model association inherently assumes a relationship between more than one organism, so the methodological analysis answers the critique of scientists regarding the neglect of the multi-organismic nature of life and their emphasis on the microbiome.

Accordingly, I first offer a historical description of how the partnership of the squid *Euprymna scolopes* endemic to the shallow coasts of Hawaiian archipelago and the bacteria *Vibrio fischeri* became a prominent model with the work of McFall-Ngai and colleagues. This draws on earlier challenges of introduction and adjustment of the squid into the laboratory and later establishment of the model system and the formation of a research community around it. Secondly, based on the case study, I identify the practical, philosophical, and social conditions turning a model system into a model association.

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“It just stares back at you!”. The early history of psychophysiological experimentation on Octopus

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By focusing on selected examples of experimental psychophysiology of *Octopus vulgaris* in the XX Century, this work will follow three main routes.
Firstly, I will consider the weight of casual observations and long-established “octopus myths” on the choice of this animal for conditioning and learning experiments.
Secondly, I will reconstruct the development and refinement of experimental settings, in relation to both the paradigm and the technical setup.
Finally, I will pose the question of the “contribution” of the octopus to psycho-physiological theories at different degrees of generality, as an exception, an example or a model. In this connection, special attention will be devoted to the framing of the octopus in a comparative perspective with vertebrates, in terms of both specific experimental paradigms and general theoretical interpretations.
The construction of the octopus as an “exceeding” mollusk will be put in relation with more recent debates about its legal status of protected animal in many countries.

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The Octopus and Consciousness: *What Can We Learn*?

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This talk presents philosophical insights that arise from empirical research on octopuses, focusing on those significant for the scientific study of consciousness. The results presented are the outcome of integrating empirical findings about the octopus nervous system with philosophical theories about the mind.

Due to the extent of decentralization of the octopus nervous system, and the ensuing anatomical distribution of cognitive substrates and routines, octopus consciousness may not exhibit a unified structure. This possibility is significant, as it challenges the received view that where consciousness exists, it is unified (i.e. what is experienced by the subject is a single, integrated field of consciousness). Consequently, the need arises for theorists to reevaluate presuppositions and other commonly held notions about the mind, such as the association of complex intelligence and behaviour with unified consciousness, or that the "default" or "normal" structure of consciousness is that it is unified.

The broader significance of philosophical investigations such as these is that they address the issue of how cognitive science can expand its explanatory toolkit to better accommodate forms of intelligence that are not as familiar as those of well-studied vertebrates.

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