Biology and Life History of Cephalopods

an interim meeting of the international cephalopod community

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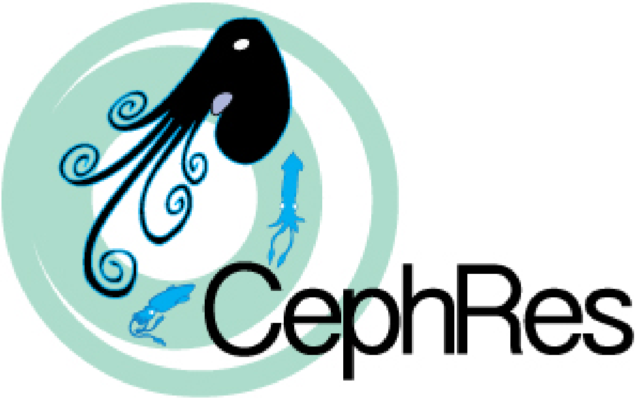

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Abstracts are paginated here in the order as they are presented in the sessions of the Day

A metabarcoding approach for assessing the diet of the glass squids (Oegopsida: Cranchiidae)

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Members of the family Cranchiidae Prosch, 1847, commonly known as glass or bathyscaphoid squids, range from small translucent species to the massive-sized colossal squid. Those cephalopods are widely distributed in all oceans, also representing one of the more diverse families of squids and, despite their ecological importance, their diet is almost unknown. Cranchiids represent a significant prey for many organisms in the mesopelagic zone, from fishes to marine megafauna, as sharks, swordfishes and whales. Their trophic role is mostly unknown and only few studies are available.
Aim: characterize for the first time the diet of cranchiid squids through DNA metabarcoding.
Methods: gut contents of 62 squids collected in the Atlantic, belonging to 10 genera were used to amplify a fragment of the mitochondrial gene cytochrome c oxidase subunit I, and sequenced in the high-throughput sequencing platform Illumina. Sequencing reads were identified using published sequences. Results: A preliminary bioinformatic analysis of the reads from the gut contents shows a wide diversity of eukaryotic sequences. The more represented reads belong to Malacostraca, Actinopterygii, Cephalopoda, Hydrozoa and Insecta.
Conclusion: Molecular assessment of the diet of 10 of the 13 described genera of cranchiids revealed the taxonomic spectrum of their ingested items, helping to understand the role of these squids in the mesopelagic realm.

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Enigmatic parasites from an uncommon habitat: Diversity of ciliates and mesozoan parasites of renal appendages of cephalopods

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The renal sacs of cephalopods are an uncommon habitat for two phylogenetically distant wormlike parasites, dicyemid mesozoa and ciliate chromidinids. These are organisms, which can reach a few millimetres in length and are attached to the renal epithelia by their anterior part. Information was mainly based on morphological criteria and little is known about the biodiversity and taxonomy of these enigmatic parasites. In this study, we have undertaken to study these two groups with regard to their biodiversity in cephalods from the Mediterranean Sea and the Atlantic Ocean. Cephalopods from sampling campaigns revealed a weak prevalence of the infection by chromidinids ciliates probably biased by the sampling method since sampled hosts were predominantly benthic, while chromidinids mainly infect pelagic cephalopods. Hence, the diversity is underestimated, even if we were able to describe two new species and obtain the first time molecular data from the 18S rDNA marker.

Phylogenetic analyses show that chromidinids belong to the apostome ciliates and to the Oligohymenophorea. Prevalence of the infection by dicyemids was shown to be more important. A major problem in the dicyemid systematics is that a large number of named morpho-species are based on incomplete morphological descriptions, causing ambiguities in their identification. We therefore redescribed all stages of the development and assigned an 18S rDNA marker sequences to the species of sampled dicyemids species.

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A comprehensive review of early life stages in Cirrata (Cephalopoda: Octopodiformes)

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Living cephalopods (Mollusca: Cephalopoda) exhibit a wide range of reproductive strategies, that can be classified into simultaneous terminal, polycyclic, multiple, intermittent terminal, as well as continuous spawning. While information about reproductive, and ultimately life history strategies are readily available for most neritic taxa, significantly fewer data has been gathered on deep-sea cephalopods. This applies in particular to the Cirrata (Octopodiformes: Octopoda), an enigmatic group of semi-gelatinous benthic, benthopelagic, and pelagic octopods that occur down to over 7,000 m depth. Within this taxon, females have an ostensibly derived reproductive biology, whereby the eggs are coated with a protective layer that hardens when in contact with water and thus also serves in attaching the embryo permanently to a wide range of surfaces.

Here we provide the first comprehensive overview of biogeographic, morphometric, and morphological data gathered for representative taxa from all eight extant cirrate genera. The insights derived from this comparative analysis are based on results obtained through a broad literature search, the application of non-invasive as well as invasive imaging techniques, and online database queries. The data obtained on cirrate eggs, embryos, hatchlings, and juveniles are compared with documented characters from selected octopodiform outgroup taxa and provide further evidence for the uniqueness of the cirrate reproduction strategy among cephalopods.

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Evolution of the hectocotylus in Sepiolinae (Cephalopoda: Sepiolidae), a sound base for generic revision

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The subfamily Sepiolinae (Mollusca: Cephalopoda: Sepiolidae), currently containing the genera *Sepiola*, *Euprymna*, *Inioteuthis*, *Rondeletiola* and *Sepietta*, is characterized by the hectocotylization of the left dorsal arm, i.e. its transformation into a copulatory organ due to modifications of some sucker/pedicel elements. The hectocotylus morphology varies to a great extent across genera and species. In particular, one to several pedicels in its proximal third are suckerless and highly and diversely modified in shape to constitute the copulatory apparatus. An evolutionary gradient was observed in copulatory apparatus morphology, from the simple modification into a papilla of one pedicel from the third element of the ventral sucker row (some nominal *Euprymna* species) to a quite complex structure involving several variously modified pedicels in both the ventral and dorsal sucker rows (*Inioteuthis*). In some species, elements in the distal portion of the hectocotylus may also be highly modified, such as the *Euprymna* columnar suckers. Nine groups of species that do not match the current generic subdivision of Sepiolinae can be distinguished on the basis of hectocotylian diversity. Additional morphological characters (number of sucker rows on arms, female bursa copulatrix, occurrence and shape of visceral light organs, etc.) corroborate the subdivision of Sepiolinae into nine subtaxa, i.e. genera. Accordingly, a cladogram may be drawn to describe the possible phylogenetic relationships among these nine clades. It corroborates the need to redefine all extant genera and describe some new ones.

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Effect of water turbidity on visual abilities of cuttlefish (*Sepia officinalis*)

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In the English Channel the level of turbidy changes seasonally and daily in seawater. As a consequence, most visual abilities based on intensity of contrast are useless. On the contrary, in such environment, polarization sensitivity may help individuals detecting preys and predators. Almost all studies use cuttlefish coming from breeding systems in clear water, but according to species history, one can expect that cuttlefish sensory systems are better adapted to turbid environment, for example with development of polarized light sensitivity. To tackle this question, hatchlings from eggs laid by wild cuttlefish were reared one month under three conditions: clear water, slightly turbid water (0.1 g/l of clay) and highly turbid water (0.5 g/l of clay). The visual capacities of the cuttlefish were tested at 7 days and 1 month with an optomotor apparatus in clear or turbid water, using three patterns (contrasting stripes, polarized stripes and uniform gray).

Preliminary results suggest that the breeding conditions had no significant effect on the visual performance of cuttlefish of both age. Surprisingly turbidity seems to reduce ability of all cuttlefish to respond to polarized patterns but have only few or no effect on contrasting patterns. Whether this result is due to species history or to individual experience needs further investigations. Our results may differ from previous literature because we use clay to obtain turbid water instead of fine sand. The size and shape of the particles can change light polarization pattern in the environment.

In conclusion, cuttlefish should probably use both polarization and intensity contrast depending on the property of light in water.

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CephDev - Cephalopod developmental biology

a CephRes2020 Virtual Event Focus on Session

Main Organizer: Prof. Eve Seuntjens (Belgium)
Co-Organizer: Dr Camino Gestal (Spain)

We aim to cover research within the broad field of cephalopod developmental biology, including the early larval stages. Given the technological advances in sequencing, genome editing and 3D imaging, a novel era of descriptive and functional developmental biology is expected to provide interesting novel insights into major developmental biology questions.
The session spans from genomic studies and novel tools such as CRISPR genome editing, organ development and embryonic to early larval physiology and behavior including different cephalopod species.

Within the physiological research, developmental biology is one of the most integrative fields, covering topic from genetics and epigenetics over stem cells and cell biology, and yielded several Nobel prizes. Novel technological break-throughs have the potential to open up cephalopod research to developmental biology, yet many hurdles still need to be taken. Bringing together researchers in this field is of utmost importance to boost this exciting cephalopod research domain.

[*abridged from the original proposal*]

Observing cephalopod embryonic development: from Naef to now

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In the beginning of the twentieth century, Adolf Naef described the embryonic development of cephalopods in great detail during two stays at the Stazione Zoologia Anton Dohrn in Napoli, Italy. Being a representative of the "idealistic morphologists" he carefully observed cephalopod development to develop his view on evolution, phylogeny and systematic morphology. His work became the work of reference for embryologists that followed his trace to study cephalopod development, and is -still today- a highly valued resource. Using Octopus vulgaris as an example, this short presentation will discuss the main characteristics of cephalopod development, based on Naef's data as well as on our own observations recently obtained using contemporary microscopy.

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Transcriptomic study of early developmental stages of *Octopus vulgaris*

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The common octopus *Octopus vulgaris* has been proposed as a good candidate for aquaculture operations due to its fast growth and short life cycle. Settlement of animals has been the main bottleneck to reach the adult stage under captivity. However, recent achievements allowed the obtaining of samples to decipher the molecular basis that regulates the intricate early stages in this species.

In this study, *O. vulgaris* samples at different stages were sequenced by RNA-seq for a massive *de-novo* transcriptomic analysis following the TRINITY protocol. Four main comparisons were carried out considering a mix of different embryonic stages and paralarvae at 0, 10, 20 and 40 days post-hatching (dph). A total of 2300 up-regulated transcripts were found in newly hatched paralarvae (0 dph) compared to embryo whereas the number of overexpressed transcripts reached 2336, 3013 and 2580 in 10, 20 and 40 dph paralarvae, respectively, compared to newly hatched animals.

Gene ontology enrichment analysis revealed common terms at all ages such as chitin metabolism and peptidase related activities. Terms related to proteolysis and catabolism were significantly more represented in 0, 10 and 20 dph paralarvae whereas mobilization of proteins into the nucleus was more represented at 40 dph. Neural development and response to stimulus and activation of immune response were also differently represented attending to the age of the animals.

Altogether these results represent an overview of the different biological functions at paralarvae stage and will help to decipher at what age relevant functions are activated.

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Generation of Transgenic Lines in Cephalopods: Development of CRISPR-based tools in *Euprymna* spp. early embryos

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Cephalopod and vertebrate lineages split about 600 million years ago. Despite this evolutionary distance, both lineages independently evolved complex nervous systems and behaviours. This makes cephalopods ideal non-vertebrate animals to study and compare the design features of complex nervous systems across phyla. Cephalopod neuroscience and behaviour would greatly benefit from the establishment of powerful genetic tools. However, no transgenic animals have been recorded in this taxon.

Our goal is to generate CRISPR-based transgenic lines in *Euprymna berryi*. Because of its small size, ease of culture, short reproductive cycle and transparent embryo, this species has emerged as a potential genetic model organism among cephalopods. To integrate a transgene in the genome successfully, we plan to use the CRISPR/Cas9 system to induce knock-in through homology-directed repair. Target endogenous genes are cephalopod orthologs of SNAP25, VGLUT and VGAT, while possible transgenes are eGFP and GCaMP6. Genome editing involves several processes, such as phylogenetic analysis, design of sgRNAs, microinjection of dyes, mRNAs and CRISPR solutions into early embryos, as well as phenotyping and genotyping. We are at an early stage of testing these components.

In parallel, we aim to solve several challenges related to the propagation of genetic lines, by improving the husbandry of embryos, paralarvae and adults, sexing, identification tagging, evaluation of anaesthesia and euthanasia agents, and live video monitoring.

Ultimately, this study will generate powerful molecular tools to produce stable and tractable transgenic lines in cephalopods, that express genetically encoded calcium indicators and optogenetic actuators to image and control neural circuits.

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The octopus' solution to building a complex brain

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The development of the cephalopod nervous system from neural cords eventually forming mature lobes has been described on the morphological level. It is however still unclear where the progenitor cells are located and what the sequence of neurogenesis (from stem cell to postmitotic neuron) precisely is.

We used histological techniques and molecular markers to label dividing cells, neural stem cells, intermediate progenitors and neuroblasts on tissue sections of *Octopus vulgaris* embryos. Our results indicate that progenitor cells are located outside the optic, cerebral and palliovisceral cords in the lateral lips adjacent to the eyes, leading to the hypothesis that newly formed neurons should migrate into the cords.

(Neural) migration is one of the most complex cellular behaviors in the animal kingdom. In the developing vertebrate nervous system, extensive migration over long-range paths allows neurons born in distinct regions to integrate in a network. This process is essential to establish proper circuits and brain function. Little evidence exists for such neural migration in invertebrates and reported trajectories are limited to few cell lengths. To test our hypothesis, we performed lineage tracing experiments. Using light sheet imaging at different time points after dye injection in the lateral lips, we found that neurogenic progenitors are indeed located in these lips and that they divide multiple times before integrating into the cords.

The findings that octopus neural progenitors divide multiple times and migrate over such long distances are reminiscent of vertebrate neurogenesis and emphasize the complexity of the octopus brain even more.

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Awareness of danger inside the egg: early behavioral competencies in the cuttlefish

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The embryos of many species are extremely sensitive to prenatal stress. This stress in particular has deleterious effects on the cerebral and behavioral development of the young. O'Brien et al. (2018) have shown that there were no such effects in cuttlefish *Sepia officinalis*. Marthy et al. (1976) suggested the existence of a tranquilizer in the perivitellin liquid (PVF) of the eggs of the squid *Loligo vulgaris*, another cephalopod. Indeed, the locomotor activity of squid larvae decreases when they are placed in PVF taken at the end of embryonic development. The squid's PVF also has a tranquilizing effect in crustaceans. The existence of such a tranquilizer in cuttlefish eggs could reduce the effects of stressors in the embryo. Adaptively, the presence of a tranquilizer would be particularly relevant in the cephalopod species that develop in transparent eggs. In the absence of parental protection, it would act as a buffer against a stressful environment (e.g. presence of predators) and reduce the movements of embryos decreasing the probability of being detected by visual predators or premature birth.

He we will review the development of the sensory systems in embryos of two cuttlefish species. We showed that embryos can innately respond to predatory cues and can learn to recognize an odour as a threat by classical conditioning. Last, we showed that embryos are not sensitive to direct artificial or natural stress although a maternal stress impairs juveniles behaviours.

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Molecular pathway of light photosensitivity in cuttlefish embryo

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Background: Cephalopods are well known for their performing eyes and visually guided behaviour. Extraocular photosensitivity have also been evidenced in several species of adult cephalopods: in the skin, luminous organ or stellate ganglia. Photosensitivity is allowed by photosensitive molecules belonging to the same conserved gene families: opsins and cryptochromes. Behavioural studies have demonstrated that photosensitivity appears during development before hatching.

Aim: We looked for the molecular systems implicated in photosensitivity during development. We determined in a first step the diversity of the photosensitive molecules in the cuttlefish, then tested their expression (level and localization) in eye and two extraocular tissues, skin and central nervous system (CNS).

Results: We identified six (6) opsins, two (2) cryptochromes and one (1) visual arrestin in *Sepia officinalis*, more than previously shown. In embryos, some of these genes are expressed only in the eyes but not in the skin or CNS. Expression in these extra-ocular tissues appears in juvenile and adult *S. officinalis*.

Conclusion: These results suggest that *1)* the molecules studied (possibly in association) could have a role in both visual and non-visual ability to perceive light, *2)* extra-ocular photosensitivity sets up after hatching, reflecting a complex maturation in light perception of the cuttlefish.

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Emergence of cephalopod genomic novelty in developmental and organismal expression modules

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Studies on cephalopod genomes have revealed various levels at which genomic novelty evolved in these fascinating animals. However, how such novelties translate to regulatory and ultimately organismal innovation remains answered.

Using comparative genomic and transcriptomic approaches, we studied how extensive rearrangements and the emergence of novel genes in cephalopod genomes have contributed to the organismal evolution during developmental transitions, from early embryogenesis to adult. We conducted developmental time-course RNAseq in the Hawaiian bobtail squid *Euprymna scolopes* and found that novel cephalopod genes are preferentially expressed at later developmental stages and contribute to specific sets of adult tissues (e.g., orphan genes in the accessory nidamental gland, GPCR cephalopod-specific duplicates in the eyes). We also found that the ancestral bilaterian and molluscan genomic regions still retained in cephalopods showed a depletion of novel genes. Conversely, novel cephalopod syntenies and non-syntenic regions had a higher amount of novel gene formation. This suggests a relaxation of selective pressure potentially aiding novel gene emergence. Moreover, to study regulatory innovations we reconstructed 38 putative co-expression modules active during development using a weighted gene correlation network analysis (WGCNA), characterized the number of novel and syntenic genes in those modules, and profiled their expression in adult tissues.

Altogether, these results give us first insights into the potential role of novel genes during cephalopod development and their importance for particular organ functions.

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