International Workshop on Chondrichthyan Development and Genomics 2024, Prato, Italy

DAY 1: Monday, Oct. 22nd - WELCOME & COCKTAIL RECEPTION		
17.30 to 19.00 hours		
Registration & Welcome Cocktails		
DAY 2: Tuesday, Oct. 23rd - SKELETAL SYSTEM & SOFT TISSUE DEVELOPMENT		
9.00 - 10.30a.m.		
9.00 - 9.45	Richard Dearden	Nothing but the tooth? A total evidence approach to the elasmobranch tree of life.
9.45 - 10.30	Gareth Fraser	Dental and Cranial diversity in chondrichthyan fishes : new jaws and odd faces
10.30 - 11.00a.m.		
MORNING TEA		
11.00 - 11.45	Michael Palmer	The genetic basis of a permanent cartilagenous skeleton
11.45 - 12.30	Catherine Boisvert **(via zoom)**	Imaging the complexity of the chimaeroid pelvic muscle morphology and development
12.30 - 13.00	Frank Tukenko	Evolution and vertebrate appendicular muscle patterning systems and implications for the fin to limb transition
13.00 - 14.00p.m.		
LUNCH		
14.00 - 14.45	Martin Cohn / Cohn lab	TBA
14.45 - 15.15	Rebecca Dale	Brood Colony Management and Embryo Staging Guide of the Epaulette Shark (Hemiscyllium ocellatum)
15.15 - 16.00p.m.		
AFTERNOON TEA		
16.00 - 16.30	Benoit Haerlingen	Off The Beaten Tracks: Exploring Heart Regeneration In Sharks
16.30 - 17.15	Peter Currie	Evolution of a specific stem cell niche cell drives distinct muscle growth strategies in the vertebrate lineage.
19.00 - 22.00		
DINNER - Il DEK RESTAURANT		
DAY 3: Wednesday, Oct. 24th - GENOMICS		
9.00 - 10.30a.m.		
9.00 - 9.45	Tetsuya Nakamura	The little skate genome illuminates the evolutionary emergence of exceptionally wide paired fins
9.45 - 10.30	Gavin Naylor	Leveraging Shark Genome Data for Conservation
10.30 - 11.00a.m.		
MORNING TEA		
11.00 - 11.45	Shigehiro Kuraku	Developmental gene landscape in shark and ray genome sequences
11.45 - 12.30	Shawn Burgess	Darwinian Genomics: Rapid advances in genome assembly can make any fish a model organism

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12.30 - 15.00		
LUNCH & POSTER SESSION		
	Virginia Panara	A molecular characterisation ofthe chondrichthyan vasculature
	Hannah Bryne	The evolution of Tesselated Calcified Cartilage (TCC) in chondrichthyans
	Nicolas Vidal-Vasquez	A single-nucleus RNA-sequencing atlas of the shark retina
	Anna Box	Diverse macroglia in the evolution of the vertebrate BBB
	Eva Candal	Expression of Hedgehog signalling genes in the juvenile shark retina suggests a role in postnatal neurogenesis
15.00 - 15.30p.m.		
AFTERNOON TEA		
15.30 - 16.15	Melanie Debias-Thibaud	The sensory shark-phenotypic, genomic and transcriptomic data for the small spotted catshark Scyliorhinus canicula
16.15 - 16.45	Ana Verissimo	An Ancestral MHC Organization in Cartilaginous Fish: Reconstructing MHC origin and evolution
17.30 - BUS DEPARTS (PIAZZA DELLA CARCERI)		
18.15 - WINE TASTING		
19.30 - DINNER - CAPEZZANA ESTATE		
22.00 - BUS RETURNS TO PRATO		
DAY 4: Tuesday, Oct. 25th - NERVOUS SYSTEM		
9.00 - 10.30a.m.		
9.00 - 9.45	Mikiko Tanaka **(via Zoom)**	Evolution of paired appendage-specific motor innervation
9.45 - 10.30	Idoia Quintana Urzainqui	The castharkembryo as a model to study the origin and evolution of the vertebrate brain
10.30 - 11.00a.m.		
MORNING TEA		
11.00 - 11.45	Jan Kaslin	Defining the evolutionary origin and diversity of macroglia in the vertebrate CNS
11.45 - 12.30	George Kafetzis	Unravelling the evolution of visual circuits through the eyes of sharks
12.30 - 14.00p.m.		
LUNCH		
14.00 - 15.00	Corinne Houart & Dana Fakhreddine	The shark embryonic forebrain –An ancestral version of mammalian organisation
15.00 - 17.00	All	Community session
17.00p.m.		
CONFERENCE ENDS		

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Abstracts

DAY 2: Tuesday, Oct. 23rd - SKELETAL SYSTEM & SOFT TISSUE DEVELOPMENT

Richard Dearden [1,2], Zerina Johanson[3], Martin Rucklin[1]

[1] Naturalis Biodiversity Center, Leiden, The Netherlands
[2] University of Birmingham, Birmingham, UK
[3] Natural History Museum, London, UK

Nothing but the tooth? A total evidence approach to the elasmobranch tree of life.

The long evolutionary history of elasmobranchs (sharks and rays) is recorded primarily by a rich fossil record of teeth. These teeth are powerful tools for studying the recent history of elasmobranchs, and show that living families' roots stretch well back into the Mesozoic. However, there are limits to how accurately they can date this history. While teeth are easy to match up with the distinctive morphologies of living elasmobranch taxa it is difficult to confidently place them on specific branches deeper in the tree, and early divergence events in the elasmobranch tree of life remain poorly constrained. The alternative is to use data from elasmobranch skeletons, which are cartilaginous and fossilize only rarely. In this talk I describe our ongoing work using a total-evidence approach to better understand the evolutionary history of elasmobranchs. We are building a phylogenetic dataset for elasmobranchs incorporating information from the skeletal morphology of elasmobranchs living and extinct, as well as publicly available mitochondrial gene sequences for extant taxa. In particular this is supported by the use of computed tomographic methods to reveal the three-dimensionally preserved skeletal morphologies and phylogenetic affinities of crown-group selachians from the Upper Cretaceous Chalk of the United Kingdom. These include close relatives of extant groups, like the stem-group parascylliid Pararhincodon, as well as the extinct problematic taxon Synechodus. With this work we aim to provide a new perspective on elasmobranchs' evolutionary history independent from and complementary to that provided by the tooth record.

Gareth J. Fraser

Department of Biology, University of Florida, USA

Dental and craniofacial diversity in chondrichthyan fishes: new jaws and odd faces.

Chondrichthyan fishes, as models for the study of evolutionary and developmental biology, offer a wealth of dental and craniofacial variation compared to many other vertebrate groups. Craniofacial variety appears to be prevalent in this class of vertebrates, in part, due to the cartilaginous chondrocranium and the developmental and ontogenetic changes this system may promote. Sharks, skates and rays (elasmobranchs) show extensive variation in craniofacial development e.g., the extended rostrum of sawsharks or the unusually wide and flattened head of the hammerhead sharks, however their dentition has been confined to more subtle variation, e.g., in tooth shape, patterning and regenerative rate. In contrast, the understudied subclass of chondrichthyans, the Holocephali, have vast diversity in both dental and craniofacial morphologies, and in addition have a unique sexual dimorphic component of the chondrocranium - the "head clasper" – which arises from developmental shifts unique to this group. We describe the development and ontogeny of this unusual tooth-covered, jaw-mimic - the tenaculum, a head clasper embedded in the forehead of male chimaeras. The evolutionary origins and how this tooth-covered modification of the chondrocranium develops could provide insights into the evolutionary transition of tooth-like structures in this obscure group of shark-relatives. Overall, the showcase of diversity in craniofacial and dental anatomy across chondrichthyan fishes offers incredible opportunities for further evo-devo study in this evolutionarily impressive group of vertebrates.

Michael A. Palmer[1], Andrew Gillis[1]

[1]Marine Biological Laboratory, Woods Hole, MA, USA

The genetic basis of a permanent cartilaginous skeleton

In mammals, cartilage is predominantly an embryonic tissue, forming a transient model of the developing skeleton. Most cartilage undergoes a morphological and biochemical transition called "hypertrophy" before being replaced by bone through the process of endochondral ossification. Cartilage persists in relatively few places

	within the adult skeleton (e.g. in joints, as articular cartilage). Chondrichthyan fishes, on the other hand, lack bone, and possess a skeleton that remains cartilaginous throughout life. We investigated cartilage development in the skate (Leucoraja erinacea) to discover gene expression correlates of this permanent cartilaginous skeleton. Using comparative skate and mouse single-cell RNA-sequencing, we find that adult skate cartilage cells (chondrocytes) are most transcriptionally similar to mouse growth plate and articular chondrocytes. This is consistent with paleontological evidence for loss of bone in chondrichthyans, and with evolution of the cartilaginous skeleton of chondrichthyans by premature arrest of mammalian endochondral ossification. We also find persistent expression in postembryonic skate chondrocytes of genes encoding secreted inhibitors of a key pro-osteogenic signaling pathway. This stands in contrast to mammals, where orthologous genes are expressed during early cartilage development but are downregulated with hypertrophy and ossification. We tested the impact of prolonged inhibition of this pathway on mammalian cartilage differentiation using a recombinant inhibitor with an in vitro model of mammalian endochondral ossification, and we found that prolonged inhibition leads to a significant reduction in chondrocyte hypertrophy. These observations suggest that modulation of this pathway may have contributed to the evolution of a permanent pre-hypertrophic cartilaginous skeleton in chondrichthyans.
Boisvert, C. A. [(1)], Pears, J, B. [(2)], Tillett, C. [(3)], Tahara, R. [(4)], Larsson, H. [(4)], Trinajstic, K. [(1)] [1] Curtin University, School of Molecular and Life Sciences, Perth, Western Australia, Australia [2] Imperial College London, Department of Life Sciences, Ascot, United Kingdom [3] Curtin University, School of Electrical Engineering, Computation and Mathematical Sciences, Perth, Western Australia, Australia [4] McGill University, Canada	**Imaging the complexity of the chimaeroid pelvic muscle morphology and development (VIA Zoom)** Pelvic girdles, fins and intromittent organs, evolved during the agnathan to gnathostomes transition but their origin and the mechanisms underlying their evolution are still unclear. Comparative musculo-skeletal anatomy has been used since the late 19th century to understand the evolution of such novelties but has been superseeded by developmental genetics. However, studying the development of muscles and cartilage, which have correlates in the fossil record allows for additional data to better understand transitions. Here, we used nano CT scanning of a growth series of elephant shark (Callorhinchus milii) embryos, to model and describe muscle development. C.milii is a holocephalan, the sister group of all other chondrichthyans, which has been understudied but is in an important phylogenetic position to understand the origin of paired pelvic fins. Literature from the 19th century and traditional dissections were used to describe the adult anatomy and the development was compared with the only other published study of appendicular muscles in chondrichthyans. We show the fin musculature is more complex and differentiates earlier than previously thought. We also found that the superficial muscles are more prominent relative to the deeper muscles which might indicate that the tetrapod morphology is plesiomorphic. Understanding the skeletal and muscular anatomy and development of extant organisms in critical phylogenetic positions is essential for performing comparisons with and interpreting fossil gnathostome taxa such as placoderms. Using the knowledge of model organisms from the historical literature in tandem with modern imaging technologies enables a comprehensive understanding of anatomy and development to inform these analyses and help elucidate the origins of these structures.
Frank Tulenko[1], Margo Montandon[1], Avnika Ruparelia[1,2], Peter Currie[1] [1] Australian Regenerative Medicine Institute, Monash University [2] Centre for Muscle Research, Department of Anatomy and Physiology, University of Melbourne	**Evolution of vertebrate appendicular muscle patterning systems and implications for the fin to limb transition** Extant tetrapods have a complex limb musculature that includes 40+ individual muscles required to drive a limb dominant locomotor strategy and support body weight in a terrestrial environment. In contrast, extant cartilaginous and basal ray-finned fish have a simpler arrangement of appendage musculature, generally consisting of two primary groups, a dorsal adductor and a ventral abductor, subdivided into deep and superficial layers. Thus, muscle complexity increased dramatically with the fin-to-limb transition. However, the mechanistic basis for this change is poorly understood. Models for limb muscle formation posit that lateral plate derived connective tissue (LMCT) forms a prepattern of distinct limb muscle

	groups. Naïve muscle precursors delaminate from their parent somite and migrate as mesenchymal cells into the limb bud where they mix extensively with LMCT and populate this prepattern, preconfiguring the position of adult muscle groups prior to myofiber differentiation. Here we characterize the developmental morphologies, mesodermal dynamics, and molecular profile of cell populations involved in early appendicular muscle formation in representative cartilaginous fish, basal ray-finned fish, and lobe-finned fish. In addition, we use time lapse imaging in zebrafish to characterize the interactions between myoblasts and lateral plate mesoderm as myoblasts first invade the fin and establish unique dorsal and ventral muscle groups. Together, these data fuel new hypotheses for the relationship between cell type evolution and the elaboration of appendicular muscle complexity in vertebrates.
Martin Cohn	TBA
Rebecca E. Dale[1], Frank J. Tulenko[1], Peter D. Currie[1,2] [1] Australian Regenerative Medicine Institute, Monash University, [2] EMBL Australia, Victorian Node, Monash University	**Brood Colony Management and Embryo Staging Guide of the Epaulette Shark (*Hemiscyllium ocellatum*)** Historically, the main model systems in developmental biology have been representatives from the osteichthyan clade, such as mice, chick, xenopus and zebrafish. Chondrichthyans form the sister group to osteichthyans and as the oldest group of extant jawed vertebrates, they occupy a key phylogenetic position for gaining insight into the evolution of vertebrate features. However, chondrichthyans remain relatively understudied, which is partly due to challenges around captive breeding and embryo acquisition. The epaulette shark is a small, oviparous shark, found in shallow tropical reefs off the coast of Australia and New Guinea. They are well known in public and private aquaria for their ability to thrive in captivity and breed year-round. At the AquaCore Facility at Monash University, we have built a breeding colony that houses 20-30 adult epaulette sharks (operational since 2014). Our breeding program contributes to expanding the resources for studying shark developmental biology and facilitates the establishment of new experimental tools and techniques, genomic sequence resources and a comprehensive epaulette staging guide. This presentation will briefly cover some key aspects of managing a captive breeding colony, animal husbandry, and embryo collection, as well as provide an overview of Epaulette staging using live images and MicroCT datasets. Together this highlights how the epaulette shark is a tractable model species for the laboratory environment. And having this representative Chondrichthyan provides a unique and ancient anchor for comparisons with traditional osteichthyan model species.
Benoit Haerlingen, Yasmin K. Alshoubaki, Frank Tulenko, Peter Currie [1] Australian Regenerative Medicine Institute, Monash University	**Off The Beaten Tracks: Exploring Heart Regeneration In Sharks** Heart regeneration allows some vertebrates to restore their heart to its full functionality following an injury. However, the capacity for heart regeneration varies greatly across vertebrates, with a decreasing general trend observed from fish to mammals. To date, the cellular and molecular reasons for this inter-species variation remain unclear, but recently, macrophages have been proposed as master regulators of this process. Specific macrophages subpopulations appear to regulate the shorter and milder initial inflammatory response and subsequently promote the regenerative phase observed in regenerative species. Notably, most heart regeneration studies have used a limited number of species, offering only a narrow evolutionary window on heart regeneration. To expand our evolutionary view and see whether macrophages play conserved roles across distant vertebrate species, we investigated heart regeneration in sharks. We first established the foundation of heart regeneration by adapting the cryo-injury method developed in zebrafish to pre-hatchling epaulette sharks. In this setting, we observed extensive tissue damage occurring rapidly after the cryo-injury. Subsequently, the different myocardium layers underwent significant rearrangements to close the wound and ultimately develop new functional tissue. During this process, we observed substantial macrophage infiltration, suggesting that they also play essential roles in sharks during heart regeneration. Here, we have successfully adapted, for the first time, an experimental setting for heart regeneration in sharks and

	demonstrated that they can regenerate their hearts similarly to zebrafish. This study provides novel insights into the evolution of heart regeneration and will help in identifying the universal characteristics of pro-regenerative macrophages.
Frank Tulenko [1#], Sam Keenan[1#], Catherine Boisvert[2] **Peter D. Currie[1,3*]** *#equally contributing * Presenting author* [1] Australian Regenerative Medicine Institute, Monash University, [2] School of Molecular and life sciences, Curtin University [3] EMBL Australia	**Evolution of a specific stem cell niche cell drives distinct muscle growth strategies in the vertebrate lineage.** Two modes of muscle development have been documented during vertebrate body axis formation. In amniote embryos, a transient somite-derived epithelial structure termed the dermomyotome co-ordinates trunk muscle formation prior to birth, with muscle growth postnatally occurring exclusively by increasing existing fibre size. By contrast, teleost embryos lack an epithelial dermomyotome, creating the earliest muscle, or myotome, directly from somitic cells. Muscle growth is consequently driven by the formation of immortal stem cell zone on the surface of the myotome, which generates new muscle fibres throughout life. These differences have led to the prevailing view that the teleost mode of muscle formation is ancestral, and that its alteration was critical to the adoption of terrestrial vertebrate locomotor strategies. However, this model lacks phylogenetic validation. Here we deploy developmental approaches utilizing chondricthyan species to reveal that the ancestral mode of muscle formation in vertebrates is surprisingly shared by basal gnathostomes and crown tetrapod species. This mechanism was dramatically altered in bony fish to generate the teleost muscle pattern. We further demonstrate that this pattern arose as a consequence of the evolution of a teleost-specific muscle cell type that acts to create a unique stem niche for continuous muscle growth. This study reveals how morphological innovation can be driven by the acquisition of specific stem cell niches within individual phylogenetic lineages.

DAY 3: Wednesday, Oct. 24th - GENOMICS

Tetsuya Nakamura and The Skate Genome Consortium Rutgers, the State University of New Jersey	**The little skate genome illuminates the evolutionary emergence of exceptionally wide paired fins** Batoids, including skates and rays, evolved exceptionally wide pectoral fins as well as a flat body to adapt to benthic habitats. The molecular underpinnings of this unique trait, however, remain mostly elusive. Here, we investigate the origin of this phenotypic innovation by a combinatorial approach of genome sequencing and embryonic experiments using the little skate Leucoraja erinacea as an experimental model. Analysis of a high-quality chromosome-scale genome sequence shows that the little skate genome shares many ancestral jawed vertebrate features with other fish genomes. Combining comparative genomics and regulatory landscape description, we found skate-specific genomic rearrangements that alter the 3D regulatory landscape of genes involved in the planar cell polarity (PCP) pathway. Functional manipulation of the PCP signaling resulted in a marked reduction of skate fin size, confirming this pathway as a major contributor to batoid fin development and evolution. Consistently with the previous data demonstrating redeployment of Hox gene expression in anterior pectoral fins, we also identified a fin-specific enhancer that interacts with 3' Hox genes and confirmed the potential of this element to activate transcription in the anterior fin using zebrafish reporter assays. Our findings highlight the central role of genome rearrangement and regulatory changes in the evolution of the batoid unique body, shedding light on the molecular origin of exceptionally wide paired fins. This work was accomplished by the Skate Genome Consortium and supported by multiple American federal grants (including NIH and NSF), European governmental grants (including ERC), and other funding (including MBL)
Gavin Naylor[1], Lei Yang[1], Pierre Lesturgie[2], Adrian Lee[1], Joe Miguez[1], Olivier Fedrigo[3], Stefano Mona[2]	**Leveraging Shark Genome Data for Conservation** We have been involved in assembling high quality reference genomes for representatives of all of the extant chondrichthyan orders as part of the Vertebrate Genome Project. While it has long been appreciated that high quality reference

[1] Florida Museum of Natural History, Gainesville, Florida [2] National Museum of Natural History Paris [3] Vertebrate Genome Project, Rockefeller University, New York	genomes are important for studies that focus on comparative developmental biology and gene expression, it is less widely appreciated that reference genomes can also be used to determine components of life history and population health that are important for the management and conservation of endangered species. I will present two applications based on the analysis of genome sequence data with which we have been involved (1) estimating population size change going back through time and (2) Estimating estimation of the age distribution of populations. Both of these parameters can be determined from blood samples and do not require lethal sampling.
Shigehiro Kuraku Molecular Life History Laboratory, Department of Genomics and Evolutionary Biology, National Institute of Genetics, Mishima, Japan	**Developmental gene landscape in shark and ray genome sequences** Sharks and rays, classified within the taxon Elasmobranchii, represent one of the long-established evolutionary lineages of vertebrates, yet they remain vastly understudied at the molecular level. This gap arises from challenges such as their elusive nature, low fecundity, and the complexity of their relatively large and repetitive genomes (reviewed in Kuraku, 2021. *Dev. Biol. 477: 262-272*). Previously, we provided initial insights into shark genomics, presenting interim findings from genome analyses of three species (brownbanded bamboo shark, cloudy catshark, and whale shark), alongside tissue-specific gene expression profiles and epigenomic data on the chromatin regulator CTCF (Hara et al., 2018. *Nat. Ecol. Evol. 2: 1761-1771*). More recently, through the collaborative efforts of the Squalomix consortium (https://github.com/Squalomix/info), we employed PacBio HiFi genome sequencing and Hi-C scaffolding to achieve chromosome-scale assemblies for multiple shark and ray genomes. These sequences were validated with karyotype data obtained from cell culturing (Uno et al., 2020. Commun. Biol. 3:652). Our growing dataset enables us to analyze genomic trends in developmental gene repertoires, sometimes shedding light on the patterns that were not observed in many other vertebrates. Particularly noteworthy in emerging shark genome sequences with enhanced continuity is the presence of Hox C genes previously thought to be absent in some elasmobranchs. These findings not only deepen our understanding of ancestral vertebrate genomes but also may offer insights into the phenotypic diversity of sharks and rays.
Shawn Burgess[1] National Human Genome Research Institute, NIH	**Darwinian Genomics: Rapid advances in genome assembly can make any fish a model organism** Fish are, by far, the most diverse group within the vertebrate lineage with extreme variations in physiology, morphology, and niche adaptation. For the study of evolutionary biology, developmental biology, physiology, population genetics, and many other disciplines, this vast collection of organisms represents an incredible opportunity, limited somewhat in the past by our ability to generate sulicient genomic information on a scale that would make these questions tractable. These limits historically are the reason why researchers focused on a small number of “model organisms” that were easy and inexpensive to raise in captivity. Resources were pooled to generate the needed genomic information which involved complex integration of DNA sequencing and incomplete mRNA data. In the past decade, new sequencing technologies, molecular biology techniques, and computational approaches have radically changed what is possible with a relatively “modest” investment in personnel and equipment when it comes to genome assembly and building gene models. I will give three examples of fish genome assembly, how the technologies have improved between each one, what kinds of data can be extracted by picking a specific fish model, and what the current state-of-the-art is for quickly generating new genomic data for a species of interest.
Korsching, S.I. [1]; Mazan, S. [2]; **Debiais-Thibaud, M.** [3] [1] Institute of Genetics, Faculty of Mathematics and Natural Sciences of the University at Cologne [2] Sorbonne Université [3] Institut des Sciences de l’Evolution de Montpellier	**The sensory shark-phenotypic, genomic and transcriptomic data for the small spotted catshark Scyliorhinus canicula** Here, we report reference genomic, transcriptomic and phenotypic data in the small spotted catshark Scyliorhinus canicula, and integrate these data to shed light on the evolution of sensory organs. Taking advantage of a dense sampling of transcriptomic data, we also identify gene signatures for all major organs, including chondrichthyan specializations, and evaluate expression diversifications between

	paralogs within major gene families involved in sensory functions. Finally, we combine these data with 3D imaging and in situ gene expression analyses to explore chondrichthyan-specific traits and more general evolutionary trends of sensory systems. This approach brings to light, among others, novel markers of the ampullae of Lorenzini electro-sensory cells, a duplication hotspot for crystallin genes conserved in jawed vertebrates, and a new metazoan clade of the Transient-receptor potential (TRP) family. These resources and results, obtained in an experimentally tractable chondrichthyan model, open new avenues to integrate epigenomics, single-cell and spatial transcriptomics, for the study of elasmobranchs and vertebrates.
Ana Veríssimo[1,2*], L. Filipe Castro[3,4], Antonio Muñoz[1,2], Tereza Almeida [1,2], Arnaud Gaigher[1,2], Fabiana Neves[1,2], Martin F. Flajnik[5], Yuko Ohta[5] [1] CIBIO-InBIO, Research Center in Biodiversity and Genetic Resources, University of Porto, [2] BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão [3] Department of Biology, Faculty of Sciences, University of Porto [4] CCIIMAR/CIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto [5] Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore	**An Ancestral MHC Organization in Cartilaginous Fish: Reconstructing MHC origin and evolution** Chondrichthyans are the oldest living jawed vertebrates with a mammalian-like adaptive immune system (AIS) based on immunoglobulins (Ig), T-cell receptors (TCR) and the Major Histocompatibility Complex (MHC). They are thus a key taxon to understand the evolution and origin of vertebrate adaptive immunity and to identify its ancestral and derived features. The evolution of the AIS is intimately tied to the emergence and evolution of MHC genes in the jawed vertebrates. Here, we perform comparative genomic analyses to gain insight into the Elasmobranch MHC to infer its gene composition and genomic architecture, in addition to a comparative genomic analysis of the MHC and paralogous regions across basal living jawed vertebrates to gain insights into the origin and evolution of the MHC. Using high quality elasmobranch genomes, we have reconstructed a stable and highly conserved core adaptive MHC region, including MHC-I and -II genes in addition to structurally and/or functionally-relevant genes for MHC protein structure and function, on a human chromosome (huchr) 6 precursor. Further analysis of MHC paralogous regions across early-branching taxa from all jawed vertebrate lineages revealed that Ig/TCR genes likely arose on a precursor of the huchr9/12/14 MHC paralog. These two ancestral chromosomes may have descended from a common proto MHC/AgR region at 1R bearing the major building blocks of antigen processing, presentation and receptor genes. In sum, extant cartilaginous fish exhibit a conserved and prototypical MHC genomic organization with features found in various vertebrates, reflecting the ancestral arrangement for the jawed vertebrates.

Poster Sessions:

Virginia Panara [1,2], Tatjana Haitina[1], Melanie Debiais-Thibaud[2] [1] Department of Organismal Biology, Uppsala University, Sweden [2] Institut des Sciences de l'Evolution de Montpellier, Université de Montpellier, France	**A molecular characterisation of the chondrichthyan vasculature** One of the key features of vertebrates is a closed vascular system, in which the blood transits from arteries to capillary beds, to veins, to then return to the heart. This system performs several fundamental functions, including delivering oxygen to the tissues and removing catabolites. Although the main vessels of the Chondrichthyes have been anatomically and functionally described, very little is known about their molecular signature, and how it compares to Osteichthyes. In this project, I will investigate the identity and heterogeneity of the different chondrichthyan vascular beds. First, the evolutionary history of key gene families for vascular development (such as for example the VEGF-receptor family) will be defined using phylogenetic and syntenic analyses. Then, their expression will be investigated by in situ hybridization and immunohistochemistry in different embryonic stages of S. canicula. This way, the conservation of vascular markers within Gnathostomes will be assessed, and a useful set of markers as the base for further investigations established. Moreover, this approach will reveal if the vasculature of Chondrichthyes is characterised by the same heterogeneity as in Osteichthyes, in which different compartments of the arterial and venous system have different molecular signatures. Overall, this project represents the first step in a more thorough study of the evolution of vessel types within Gnathostomata.

Hannah Byrne[1], Sophie Sanchez[2], Richard Dearden[1] Martin Rücklin[1]

[1] Vertebrate Evolution, Development and Ecology group, Naturalis Biodiversity Center, Leiden,
[2] Department of Organismal Biology, Uppsala University

The evolution of Tesselated Calcified Cartilage (TCC) in chondrichthyans

Tesselated calcified cartilage (TCC) is an evolutionary innovation exhibited in chondrichthyans, providing the skeleton with an optimum between lightness, stiffness and flexibility. Recently, high-resolution scanning techniques have enabled the 3D visualisation of the ultrastructure of TCC, but these methods have only been applied to a limited number of extant taxa so far. Consequently, our knowledge of TCC variation across extant chondrichthyans is minimal. Furthermore, we have a poor understanding of the evolution of TCC among extinct chondrichthyans, stifled by a lack of appropriate analysis techniques. Compared to hard tissues in other vertebrates, our comprehension of calcified cartilage in chondrichthyans is significantly behind. This gap impacts our grasp of cartilage calcification mechanisms, its potential relationship to bone, and our overall understanding of hard tissue evolution in vertebrates. For the first time, the ultrastructure of TCC in both extant and extinct chondrichthyans will be examined in 3D using synchrotron microtomography. This will enable a broader exploration of TCC variation among both modern and extinct crown-chondrichthyans, as well as the precursors of TCC in stem-chondrichthyans. Preliminary observations suggest that the evolution from bone to cartilage in stem-chondrichthyans was not linear. Additionally, within crown-chondrichthyans, there are notable differences in the TCC between elasmobranchs and holocephalans. These results will be of importance across multiple scientific fields: advancing our understanding of the evolution of hard tissues in vertebrates, detailed 3D information on structural properties of calcified cartilage relevant to material engineering, and insight into calcification mechanisms relevant to medical research on cartilage disorders in humans.

Nicolás Vidal-Vázquez[1,2], Ismael Hernández-Núñez[1], Pablo Carballo-Pacoret[1,3], Sarah Salisbury[3], Paula R. Villamayor[3,4], Francisca Hervas-Sotomayor[5,6], Xuefei Yuan[5], Francesco Lamanna[3], Céline Schneider[5], Julia Schmidt[5], Sylvie Mazan[7], Henrik Kaessmann[5], Fátima Adrio[1], Diego Robledo[3,8], Antón Barreiro-Iglesias[1,2], Eva Candal[1,2]

[1] Departamento de Bioloxía Funcional, Facultade de Bioloxía, Universidade de Santiago de Compostela,
[2] Aquatic One Health Research Center (ARCUS), Universidade de Santiago de Compostela
[3] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh
[4] Departamento de Zooloxía, Xenética e Antropoloxía Física, Facultade de Veterinaria, Universidade de Santiago de Compostela
[5] Center for Molecular Biology (ZMBH), DKFZ-ZMBH Alliance, Heidelberg University,
[6] INRAE, LPGP, Rennes, France
[7] CNRS, Sorbonne Université, Biologie Intégrative des Organismes Marins France
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A single-nucleus RNA-sequencing atlas of the shark retina

The basic cellular structure of the retina, consisting of five neuronal cell classes (photoreceptors, horizontal cells, bipolar cells, amacrine cells and retinal ganglion cells), and a main glial cell type (Müller glia), is vastly conserved throughout vertebrates. In recent years, single-cell RNA sequencing studies have allowed for the molecular characterisation of retinal cell types in several species, but data on cartilaginous fishes (sharks, rays and chimaeras) are lacking. Their phylogenetic position as the sister group to the rest of jawed vertebrates makes cartilaginous fishes a key group to understand cell type evolution in the retina. In this study, we performed a single-nucleus RNA sequencing analysis of three retinas from three female juvenile specimens of the shark Scyliorhinus canicula, also known as the small spotted catshark. The expression of known marker genes from other vertebrates allowed us to identify all major retinal cell types, except for cone photoreceptors, in accordance with previous reports of their absence in this species. Since retinal neurogenesis in fishes persists into postnatal life, we also identified populations of retinal progenitor cells. Thus, our dataset constitutes a valuable tool for the study of cell type evolution in the vertebrate retina, and will allow us to achieve a better understanding of postnatal neurogenesis in the shark retina.

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Diverse macroglia in the evolution of the vertebrate BBB

Macroglia (astroglia, oligodendroglia, ependymoglia etc.) occupy diverse roles in almost every aspect of health and disease of the vertebrate CNS, including in the formation and maintenance of the blood brain barrier (BBB). Despite this, the evolutionary origins of specific sublineages of macroglia in the vertebrate phylogeny remain obscure. In particular, how the specialised glia which contact the CNS vasculature and occupy roles in BBB formation and maintenance came to evolve over the course of vertebrate evolution is poorly understood. Most macroglial sublineages are thought to have evolved in either an ancestor to all vertebrates (approx. 520 million years ago) or an ancestor to all jawed vertebrates (approx. 450 million years ago). However, information on the extent to which the macroglia identified in evolutionarily basal vertebrates are similar in their levels of heterogeneity and function to amniote macroglia is lacking. Here we present evidence of diverse astroglial sublineages from various CNS regions of three anamniote species: the zebrafish (teleostei), the epaulette shark (elasmobranchii), and the elephant shark (holocephali). Using a combination of single cell RNA sequencing and investigations of BBB and glial markers, we show evidence of diverse astroglial sublineages participating in the neurovascular unit in sister lineages of chondrichthyan, suggesting the presence of diverse astroglial subtypes occupying a similar physiological niche to amniote astroglia in a crown gnathostome ancestor.

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Expression of Hedgehog signalling genes in the juvenile shark retina suggests a role in postnatal neurogenesis

In the postnatal fish retina, neurogenesis persists in two main neurogenic niches: the ciliary marginal zone (CMZ) and Müller glia. Neurogenesis also occurs in the retina of postnatal small-spotted catsharks (Scyliorhinus canicula), but mitotic activity becomes greatly reduced with age, being almost absent in adults. Bulk RNA-sequencing analyses comparing the retinal transcriptomes of juvenile and adult catsharks revealed a decrease in the expression of shh and gli2, as well as an increase in the expression of hhip in adult retinas, suggesting that Hedgehog signalling activity is reduced in the adult shark retina. Furthermore, single-nucleus RNA-sequencing data of the juvenile retina showed expression of key Hedgehog signalling genes (ptch1, ptch2, smo, gli2, gli3, hhip) in populations of retinal progenitor cells and Müller glia. To validate these findings, we studied the expression of shh and gli2 in the juvenile catshark retina by in situ hybridisation and immunohistochemistry, which showed expression of both shh and gli2 in proliferating cells of the CMZ. Taken together, our results suggest a role for Hedgehog signalling in the regulation of postnatal neurogenesis in the shark retina. We then performed intravitreal injections of cyclopamine (a Hedgehog signalling inhibitor) and Smoothened agonist (SAG) in juvenile catsharks. Quantitative PCR analysis comparing treated retinas with those from control eyes injected with DMSO showed changes in the expression of key Hedgehog signalling genes, confirming the effectiveness of the treatment in the catshark. Further analyses will allow us to determine the role of Hedgehog signalling in postnatal neurogenesis in the shark retina.

DAY 4: Tuesday, Oct. 25th - NERVOUS SYSTEM

Yuumi Yoshioka[1], Reiko Yu[1], Toru Kawanishi[1], Akane Kawaguchi[2], Wataru Takagi[3], Susumu Hyodo[3], Shigehiro Kuraku[2], **Mikiko Tanaka[1]**

(via Zoom)

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Evolution of paired appendage-specific motor innervation (via zoom)

The transition from fins to limbs in vertebrates required a novel organization of spinal motor neurons to coordinate limb muscle activation. This study explores the evolution of the developmental program that coordinates innervation towards paired appendages. Previous studies showed that in chicken and mice, Hoxc9 crucially shapes the organization of motor neurons by suppressing a lateral motor column (LPMC) fate, a function also conserved in Hoxa9. This repressive activity on Foxp1 expression is mediated by a conserved motif known as the Foxp1 expression was termed Foxp1 modulatory domain (MD). We demonstrate that motor neurons expressing high levels of Foxp1 are initially present at all rostrocaudal levels in both chicken and cloudy catshark embryos, but becomes selectively downregulated at thoracic levels as development progresses. In chickens, this downregulation correlates with the distribution of Hoxc9. Intriguingly, in cloudy catshark— where the Hoxc9 gene is absent—Foxp1 expression is suppressed in inter-fin motor neurons that express Hoxa9 instead. Moreover, the Foxp1 MD is conserved in the Hoxa9 across all examined chondrichthyan species. In contrast, Hoxc9 genes were identified in a subset of shark species and in all examined batoids except rajiform and holocephalan species. The Foxp1 MD was retained in Hoxc9 of all rays and holocephalans but has vanished from the genomes of sharks. These findings suggest that the novel motor innervation programs have distinctly evolved within the lineage leading to sharks.

Idoia Quintana-Urzainqui[1], Philip Adam Oel[1], Nikolaos Papadopoulos[2], Leslie Pan[1], Tobias Gerber[1], Gülce Serka[1], Ana Verbanac[1], Detlev Arendt[1]

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The cat shark embryo as a model to study the origin and evolution of the vertebrate brain

Sharks are direct descendants of an ancient lineage of vertebrates whose common ancestor with us lived around 450 million years ago, coinciding with the emergence of jaws and predatory behaviour in vertebrates. This intriguing transition in vertebrate history was likely accompanied by a rapid complexification of their brains. Modern cartilaginous fish offer us a unique window to reconstruct early vertebrate brains, identify developmental variations that led to brain complexification, and understand how developmental programs were subsequently modified across different vertebrate lineages. In my talk, I will show how our single-cell and spatial transcriptomic study in the embryonic brain of the catshark Scyliorhinus canicula: 1) is helping us reconstruct the developmental programs at the core of all vertebrate telencephalons; 2) revealed the presence of homologous cell types with important roles in the mammalian neocortex, whose presence in sharks is highly intriguing and carries significant evolutionary implications. I will touch on our CRISPR efforts in the shark embryo and explain why it is a fantastic model to answer deep fundamental questions about how vertebrate brains evolved.

Sebastian-Alexander Stamatis[1], Anna Box[1], Mitra Amiri Khabooshan[1], Alon M. Douek[1], Samuel Crossman[1], Rebecca Dale[1], Frank Tulenko[1], Ben Hogan[2], Peter Currie[1], **Jan Kaslin[1]**

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Defining the evolutionary origin and diversity of macroglia in the vertebrate CNS

Diverse glia make up a significant fraction of the vertebrate nervous system, in which they perform critical functions in maintaining CNS physiology. Despite their importance, we know very little about evolutionary development of glia in vertebrates. Astroglia are major constituents of the mammalian CNS but it is debated at which point in vertebrate evolution these cells emerged. This is in part due to their close interrelationship and high similarity, making it difficult to resolve glial diversification, and a lack of systematic studies of glia across non-mammalian vertebrate clades, which impedes our general understanding of their origin. To resolve outstanding questions regarding the evolutionary origin of different glial cell types in the vertebrate CNS we adopted a systematic analysis of glial cell markers, ultrastructural analysis, single-cell RNA sequencing and spatial transcriptomics in the spinal cord of various non-mammalian vertebrate models including primitive sharks. Here we demonstrate that parenchymal astroglia are major constituents of the ancient vertebrate CNS and likely existed at the advent of jawed vertebrates. In contrast, bony fish have relatively few astroglia. Thus, our findings shift current views of the astroglial origin in vertebrates with several hundred million years.

George Kafetzis, Marvin Seifert, Takeshi Yoshimatsu, Tom Baden School of Life Sciences, University of Sussex, United Kingdom	**Unravelling the evolution of visual circuits through the eyes of sharks** The basic blueprint of retinal organisation is highly conserved across all vertebrates. And yet, our detailed understanding of its functional properties have primarily come from only a handful of species that can not reflect the diversity of neither the lifestyle and behavioural repertoire of vertebrates nor the environments they inhabit. In search of ancestral in origin, universal principles of vertebrate visual processing, we explore the surprisingly understudied elasmobranch tree, the first branch of jawed vertebrates diverging 440 million years ago, with species known to possess elaborate optics but historically assumed of having poor vision. We characterise the retinal output of the catshark Scyliorhinus canicula by presenting a broad arsenal of visual stimuli and recording with multielectrode arrays simultaneously from hundreds of retinal ganglion cells. We demonstrate that a sophisticated and well-studied computation, namely direction selectivity, is an ancient feature of the vertebrate retina and confirm, in line with the general concept, the early segregation of visual information in opposite elementary channels: on and off, transient and sustained. Upon pharmacological manipulations however, and contrary to the other vertebrates, we unveil unexpected crosstalk between the different channels in the shark retina. We find that the picture of poor vision in sharks is far from the truth.
Corinne Houart, Dana Fakhreddine, Afnan Azizi [1]Centre for Developmental Neurobiology, Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK [2]Francis Crick Institute, London, UK [3]Australian Regenerative Medicine Institute, Monash University, Clayton, Australia	**The shark embryonic forebrain –An ancestral version of mammalian organization** The vertebrate forebrain is one of the most complex and evolutionary diverse brain structure responsible in decision making, cognitive behaviour, and communication. Developmental research in classical model organisms across different vertebrate clades identifies conserved early developmental processes that divide the forebrain anlage, the telencephalon, into ventral subpallial progenitor zones and a dorsal pallial progenitor region. However, the neuronal organisation and forebrain morphogenesis is widely varied across vertebrate species, the organisation of the mammalian forebrain perceived as the most evolved. In our research we leverage the emergence of chondrichthyans, the sister group to all other extant gnathostomes (jawed-vertebrates), as model organisms to deepen the understanding of the evolutionary origin of the vertebrate brain development. We use the epaulette shark (*Hemiscyllium ocellatum*) to investigate early embryonic patterning of the telencephalic tissue using advanced *in-situ* staining, imaging, and single-cell transcriptomics techniques. We analysed µCT images of epaulette shark embryos across different developmental stages to identify the temporal morphogenic variation in the telencephalon during development. Our observation led to the unexpected discovery of a morphological organisation closer to the mammalian forebrain than the one found in amphibians, reptiles or birds. To test the hypothesis of an original 'mammalian-like' embryonic blueprint, we performed multi-plex whole-mount *in-situ* hybridisation using the hybridisation chain reaction (HCR) method to identify progenitor territories and signalling centres in the developing telencephalon along the dorso-ventral axis across four developmental timepoints. We used single cell transcriptomics across these stages to decipher the identity of cell-types in the shark developing telencephalon. Progress on the comparison of these data with the ones we collected from other vertebrate species (chicken, mouse, and human) will be presented.