## PROGETTO E PIANO DELLE ATTIVITÀ

- **TITOLO DEL PROGETTO DI RICERCA:**
  Another brick in the wall: wedging in the molecular and structural basis of skin pigmentation in non-model elasmobranchs

- **TUTOR PROPONENTE:**
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- **ELENCO DEI PARTECIPANTI (incluso non strutturati) – SOLO AREA BIO**

| Cognome e nome | Ruolo nel progetto | SSD | Impegno previsto (mesi/uomo) |
|---|---|---|---|
| Cariani Alessia | Supervisor | BIO/05 ZOOLOGIA | 3 |
| Tinti Fausto | Supervisor | BIO/05 ZOOLOGIA | 3 |
| Da selezionare | Assegnista di ricerca | BIO/05 ZOOLOGIA | 12 |

- **BASE DI PARTENZA SCIENTIFICA ed OBIETTIVI** *(max 500 parole)*

Advances in sequencing technologies and computational tools led the study of genomics and transcriptomics to a more holistic scale. A comprehensive transcriptome annotation of *Raja miraletus* (L.), a non-model elasmobranch species that is widely distributed in the Mediterranean Sea and the Atlantic Ocean, is currently ongoing and relies on two customized bioinformatic pipelines consisting of computational tools and data input types developed and employed for RNA-Seq data analyses (Haas et al. 2013; Simão et al. 2015; Tang et al. 2015; Mitchell et al. 2015; Huerta-Cepas et al. 2016; Hart et al. 2020). A first functional annotation of the *Raja miraletus* transcriptome was initially produced from 26.572 transcripts. Among these, a total of 646 transcripts were related to pigmentary functions, in particular, 37 out of the 38 genes known to be involved in the melanogenesis biochemical pathway were identified in the target species (Zemella, 2021). Illumina cDNA reads libraries from three different integument areas (dorsal uniform matrix, pigmentary traits as spots, eyespots and white ventral skin) were retrieved from *R. miraletus* and other skate species, mapped and quantified using the annotated reference transcriptome (Ferrari, 2017). The subsequent differential gene expression (DGE) analysis between dorsal and ventral integumental tissues, both across and within species, revealed that dorsal and ventral integument tissues do not share the same genetic basis. Ten genes involved in melanin synthesis, chromatophore development and carotenoid metabolism are suggested as the candidates in determining the dorso-ventral colouration common to skates. At this stage, their functional role needs to be validated using Real-Time quantitative PCR (henceforth RT-qPCR) methods.
A further task will concern the investigation of skates' skin structure, in particular the ultrastructure of eyespots and other dorsal traits. The siblings *Raja miraletus* and *R. ocellifera* (Regan 1906) show one of the most interesting dorsal patterning among batoids: on the yellowish dorsal side of the body, a well-defined dark eyespot leaps out from the centre of each pectoral fin. The central portion of the eyespots is light blue, encircled by a black/dark blue inner and a yellow to orange outer rings. The blue colouration is probably the result of incoherent light scattering produced by an epidermal collagen layer. Instead, the pseudo-eyespots colouration in *R. asterias*

(Delaroche 1809), *R. clavata* (L.) and *R. straeleni* (Poll 1951) may depend on a different arrangement of melanophores in the integumental layers (Bagnara et al., 2007; Meyer & Seegers, 2012).

These are the hypotheses that will be tested during the one-year fellowship proposed, along with the measurement of the selection and the evolution of the candidate genes. This will be attempted through two main objectives:

1) The validation of the RNA-seq results, analysing the DEGs of interest that will be selected for RT-qPCR analysis.
2) The investigation of the histology and ultrastructure of the integumental chromophores in skates, by using light microscopy and transmission electron microscopy.

This approach will likely wedge in the molecular and structural basis of skin pigmentation in non-model elasmobranchs, adding a new tile to the knowledge of these peculiar and fascinating organisms.

**ARTICOLAZIONE DEL PROGETTO E TEMPI DI REALIZZAZIONE** (*MAX 1000 PAROLE PER AREA BIO; MAX 800 PER AREA GEO*)

TITLE (PROVISIONAL): In vitro assay of differentially expressed genes related to skates' pigmentary traits (MONTH 1-4)

As the most primitive living jawed vertebrates with paired appendages, elasmobranchs are an evolutionarily important model, especially for studies in body traits development (i.e., skin patterning) and evolution. To date, there are no reports of established suitable reference genes for RT-qPCR expression studies in sharks or skates' skin tissues. The RT-qPCR methodology currently represents a popular method for the validation of the mRNA gene expression analysis, because of its high specificity, sensitivity and reproducibility (Bustin et al., 2009). However, the suitability of particular reference genes depends on the system being investigated, the experimental conditions and the analytical approach must be assessed for the accurate profiling of gene expression (Piheler et al., 2010). Thus, the present task aims at developing a set of genes that can be used for RT-qPCR gene expression in *Raja miraletus*, testing ten genes involved in melanin synthesis, chromatophore development and carotenoid metabolism in three main tissues (dorsal uniform matrix, dorsal coloured spot and white ventral skin). In order to complete objective 1), total RNA will be extracted by using TRIZOL reagent (Invitrogen, Carlsbad, CA) from the same samples used for RNA sequencing, initially focussing on *Raja miraletus*. Then, first strand of cDNA will be synthesized using a reverse transcription kit and will be used for qRT-PCR quantification. Specific primers will be designed using e.g., Premier Primer 5 or other online tools (https://www.genscript.com/tools/real-time-pcr-taqman-primer-design-tool) and the PCR reaction conditions will be tested and optimized.

TITLE (PROVISIONAL): Ultrastructural analysis and skin composition in *Raja miraletus* L. (MONTH 5-12)

In order to complete objective 2) a comprehensive literature review will be carried out in order to design a sampling protocol and compare, with a "state of the art" approach, the current microscopy methods available and used for scanning electron microscopy. Secondly, fresh skin samples will be collected together with biological and collection data (i.e., total length, date of

catch, sex, maturity staging and geographical coordinates). A proper sampling protocol will be specifically defined in order to ensure the best tissue conservation, essential for downstream analyses. The sampling phase will be carried out during commercial fishery or scientific trawl surveys (e.g., Solemon – FAO Adriamed). A first histologic presentation of *Raja miraletus* skin section will be initially performed with haematoxylin and eosin staining. Then, the ultrastructure of the spot area will be observed by using light and transmission electron microscopy. According to the preliminary evidence reported in Bagnara et al. (2007), we expect the spot area to contain a collagen matrix without melanocytes, the dermis to contain very large and several electron-enriched melanosomes, while the dark ring will likely contain normal-sized melanophores both in the epidermis and dermis. Considering the differences of the pictorial motifs characterizing the common torpedo, *Torpedo torpedo* L. analysed in Bagnara et al. (2007) and *R. miraletus*, a different structural basis might underpin the yellow ring encircling the eyespot of our target. This task will allow to compare the skin structure of skates, including a subsequent analysis extended to the sibling *R. ocellifera* and other congenerics (*R. asterias, R. clavata* and *R. straeleni*) in order to test the relationship between the absorption of different wavelengths by dermal melanocytes and the epidermal collagen layer.

- **PROGRAMMA FORMATIVO (O PIANO DI ATTIVITÀ) DELL'ASSEGNISTA** *(MAX 1000 PAROLE PER AREA BIO; MAX 500 PAROLE AREA GEO)*

Along with the duration of the project, the research fellow will be handling the statistics, bioinformatics, sampling, lab work and other analyses requested to conclude each task. By the end of the fellowship, the postdoc will be submitting at least one manuscript in Scientific Journals with IF.

The postdoc will enhance and curate the collaborations with external Universities, Institutions and International Organizations (e.g., FAO, IUCN). In particular, the Research Fellow will be spending at least 2 months in contact with experts in the field of evolution and diversification of skin structure (e.g., the Department of Dermatology, Medical University of Vienna, Austria) for improving skills, learning and testing the ultimate technologies in microscopy and image analysis to accomplish objective 2.

The postdoc will participate at data analysis workshops in order to improve her skills, to learn and test new platforms, packages and software.

The postdoc will present the work at international meetings (oral contribution and posters).

The postdoc will be collaborating with the team where she will be inserted as a teaching tutor and co-supervisor for Masters' students.

REFERENCES

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