PROGETTO E PIANO DELLE ATTIVITÀ

☐ TITOLO DEL PROGETTO DI RICERCA:

Stabilizing selection in the sea: looking for evidence from comparative transcriptomics of skin pigmentation in non-model skates of the genus *Raja*

☐ TUTOR PROPONENTE:

Prof. Fausto Tinti (fausto.tinti@unibo.it)

☐ ELENCO DEI PARTECIPANTI (incluso non strutturati) – SOLO AREA BIO

<table>
<thead>
<tr>
<th>Cognome e nome</th>
<th>Ruolo nel progetto</th>
<th>SSD</th>
<th>Impegno previsto (mesi/umano)</th>
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<tbody>
<tr>
<td>Tinti Fausto</td>
<td>Project Manager</td>
<td>BIO/05 ZOOLOGIA</td>
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<td>Da selezionare</td>
<td>Assegnista di Ricerca</td>
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<td>Cariani Alessia</td>
<td>Supervisor</td>
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<td>Salzburger Walter</td>
<td>Supervisor</td>
<td>BIO/05 ZOOLOGIA</td>
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☐ BASE DI PARTENZA SCIENTIFICA ed OBIETTIVI (*max 500 parole*)

Approximately thirty years have passed since the DNA and RNA-based techniques were used for the identification of marine species and better understanding their ecology and evolution (Rummer & Munday 2017). With the progressive increasing of massive DNA and RNA-based studies, many living taxa have been demonstrated to be hidden under a single nominal species (Tornabene et al. 2015), hence defined as cryptic (Bickford et al. 2007). In cryptic speciation, the conservation of external morphology is one of the key-features resulting from the stabilizing effect of selection on important adaptive traits of species occupying similar ecological niches (Losos&Glor 2003). Among cartilaginous fish, the family Rajidae exhibits remarkable ecological stasis and strikingly conserved gross morphology (Aschilman et al. 2012) including peculiar dorsal pigmentation patterns (eyespots, pseudo-eyespots). Such eco-morphological stasis may be involved in cryptic speciation and correlated to phenotype stabilisation. Investigations on the genetic basis of skate’s dorsal pictorial traits and their adaptive role have just begun. The very recent application of RNA-sequencing technologies on skate non-model species enabled the setting of a robust groundwork (Ferrari 2017) represented by

i) the draft reference transcriptome for the non-model cryptic species *Raja miraletus*

ii) the transcriptome profiling of dorsal and ventral skin tissues from five sibling and sister, cryptic and non-cryptic species (*R. miraletus, R. ocellifera, R. clavata, R. straeleni* and *R. asterias*).
The functional annotation of the draft reference transcriptome of *R. miraletus* revealed more than 50 genes known for their involvement into the pigmentation machine (e.g. *ednrb*, *mitf*, *pmel*), while the Differential Gene Expression (DGE) analysis between skin tissues showed that similar pigmented traits share similar levels of expression (e.g. the dorsal tricolored eyespot characterizing *R. miraletus* and *R. ocellifera*) and very different trends when compared with other kinds of ornaments (tricolored eyespots vs. pseudo-eyespots). In addition, several families of proteins have also been identified and referred to typical skin functions (e.g. immune response), including pigmentation (keranocytes formation, transduction signalling).

This project deals with disentangling evidence of stabilizing selection on skate pigmentation, evaluating whether the same or alternative trait have been selected in parallel adaptations to similar environments or if these features are ancestral common characters stabilized after species divergence. To accomplish these objectives, the project will address

1) the improvement of *R. miraletus* transcriptome by processing sequencing data with other assemblers (e.g. MaLTA, Oases), comparing the outputs and applying different filtering to decrease contigs redundancy;

2) the enhancement of functional annotation by performing the GeneOntology analysis relying on available genomic data of cartilaginous and ray-finned fish (i.e. *Callorhincus milii*, *Rhyncodon typus*, *Danio rerio*);

3) a new DGE analysis to narrowing down the number of candidate genes for pigmentation that will need to be validated;

4) the extension of comparative transcriptome analysis of pigmentation to other non-cryptic and cryptic *Raja* species (e.g. *R. polystigma*, *R. montagui*, *R. microcellata*, *R. brachyura*, etc.).

The publication of a definitive reference transcriptome will be fundamental for mapping skates’ transcripts and will provide future insight on cartilaginous fish genome evolution, local adaptation and population genetics.
**Task 1. Release of a reference transcriptome and transcriptomic library of Raja miraletus (M1-6)**

This task will be accomplishing objectives 1) and 2) of the project using acquired bioinformatic skills. The Ion Torrent sequences used for the assembly of the draft version of the reference transcriptome (performed with Trinity v.2.3.2; Grabherr et al. 2011) will be implemented on different pipelines. For instance, MalTA_IsoEM (Mangul et al. 2014) will be tested as it was specifically designed for Ion Torrent RNA-Seq data to explore transcriptome structure, incorporate maximum likelihood model into a de novo assembly and to estimate transcript expression levels. The software package Oases (Schulz et al. 2012) will be also tested since it was programmed to assemble RNA-seq reads in presence of alternative isoforms. The read filtering and the robust identification of alternative splicing events make this package a good candidate to study the dynamic range of expression value characterizing complex and redundant transcriptomes as those of cartilaginous fish. The resulting assemblies will be compared considering the number, length and redundancy of contigs and the functional information derived from the BlastX and BlastP analyses. For these procedures, the available non-redundant data belonging to cartilaginous and bony fish species (*Callorhinchus milii, Rhyncodon typus* and *Danio rerio*) will be used.

The research fellowship will also participate to the scientific trawl survey scheduled in November 2017 in the frame of the SoleMon Project (FAO-Adriamed Project) to collect skate specimens that will be analysed in the last phase.

Once obtained a good quality reference transcriptome for the non-model *R. miraletus*, the annotation phase will follow and will be accomplished with GOseq tool (Young et al. 2012). At the end of this important task, the research fellowship will be able to make the transcriptome library available in form of assembled contig data (in .fasta and .gff format on https://www.ncbi.nlm.nih.gov) and related paper (at the end of the 6<sup>th</sup> month).

**Task deliverable:** a peer-reviewed paper tentatively entitled “The reference transcriptome and transcriptomic library for the Brown skate, *Raja miraletus* L.”. Candidate Journals: **BMC genomics IF 4.206** or Plos One IF 3.022 or Marine Genomics IF 1.883 or Molecular genetics and genomics IF 2.622.

**Task 2. Comparative transcriptomics of skin pigmentation patterns in RNA-sequenced skate species (M7-12)**

This task will be accomplishing objective 3) of the project. The Illumina sequences used for the first DGE analysis (performed with DESeq2 v.3.4; Love et al. 2014) will be mapped to the definitive and annotated reference transcriptome. A new DGE analysis will be performed to punctually identify the number of candidate genes involved in pigmentation. With the application of more strict parameters and the exclusion of un-desired reads (e.g. those identified by BlastX as related to muscle contraction), we expect to reduce the number Differentially Expressed Genes (DEGs) between the tissue types (less than the 1700 previously identified by Ferrari 2017). Once a narrowed list of candidate genes will be obtained, we will use a quantitative real-time PCR for genes validation. The experiment will include the sampling of at least one species (during SoleMon 2017 survey), the RNA extraction from three different skin tissues (dorsal eyespot, dorsal un-spotted skin and ventral white skin) and the design of primers for qPCR. We expect to find a subset of DEGs displaying a different expression pattern between the three kind of tissues. Based on the
sample availability (collected in 2014-2015) we will be able to validate genes on the five target species. We expect to find a subset of DEGs displaying an analogous expression pattern cryptic species (\textit{R. mirletus} and \textit{R. ocellifera}), suggesting that these genes are regulating the eyespot phenotype. Finally, we will analyse the expression patterns of the same gene subset in two species showing pseudo-eyespot instead of eyespots (\textit{R. clavata} and \textit{R. straeleni}), expecting that the expression levels in pseudo-eyespots and eyespots will be confirmed as distinct. The identified candidate genes will be a useful resource for future research on the diversification of skates’ dorsal ornament and its comparison with phylogenetic patterns, measuring how much these traits are conserved across species and to understand if their origin is independent rather than common.


**Task 3. Extending and improving transcriptome analyses of skate skin pigmentation (M13-24)**

This task will be accomplishing objective 4) of the project, extending the comparative transcriptome analysis of \textit{Raja} skates not previously considered. Research fellowship will be investigating the molecular basis of other types of ornaments (e.g. bars, stripes and blotches) and complete the picture of DEGs between congeneric non-cryptic and cryptic species (e.g. \textit{R. polystigma}, \textit{R. montagui}, \textit{R. microcellata}, \textit{R. brachyura}, etc.). Further experiments will be including the isolation of novel molecular markers (e.g. microsatellites, SNPs) for population genetics, the in-situ hybridization for pigmentation genes in dorsal tissues and the histology and ultrastructure of the integumental chromatophores in skates, by using light microscopy and transmission electron microscopy. Deeper details of activities of this task will be re-assessed and planned at the end of the first year of the project and based on the results obtained.

**Task deliverable:** two peer-reviewed papers to be defined

**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 bioinformatics, sampling, lab work and other analyses requested to conclude each phase. The Research Fellowship will enhance and curate the collaborations with external Universities (i.e. University of Basel), Institutions and International Organizations (e.g. FAO). In particular, Research Fellowship will be spending a total of 6 months at the Salzburger Lab (http://www.salzburgerlab.org) for completing the data analysis and set the experiments for accomplishing objective 4). The Research Fellowship will participate at data analysis workshops in order to improve skills, to learn and test new platforms, packages and software. The Research Fellowship will present carried work and results obtained at international meetings as oral communications or posters. The Research Fellowship will be collaborating with the team where it will be inserted as a teaching tutor and co-supervisor for Masters’ students.

**REFERENCES**


1. TITOLO DEL PROGETTO DI RICERCA: Stabilizing selection in the sea: looking for evidence from comparative transcriptomics of skin pigmentation in non-model skates of the genus *Raja*

2. TUTOR PROPONENTE: Prof. Fausto Tinti

3. PUBBLICAZIONI DEL TUTOR 2012-2016

<table>
<thead>
<tr>
<th>TITOLO</th>
<th>AUTORI</th>
<th>RIVISTA</th>
<th>ANNO</th>
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<tr>
<td>1. Genetic Structure of Bluefin Tuna in the Mediterranean Sea Correlates with Environmental Variables</td>
<td>G. Riccioni; M. Stagioni; M. Landi; G. Ferrara; G. Barbujani; F. Tinti</td>
<td>PLOS ONE</td>
<td>2013</td>
<td>3.534</td>
<td>A</td>
<td>-</td>
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<tr>
<td>2. Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (Merluccius merluccius)</td>
<td>I. Milano; M. Babbucci; A. Cariani; M. Atanassova; D. Bekkevold; G. R. Carvalho; M. Espiñeira; F. Fiorentino; G. Garofalo; A. J. Geffen; J. H. Hansen; S. J. Helyar; E. E. Nielsen; R. Ogden; T. Patarnello; M. Stagioni; F. Consortium; F. Tinti; L. Bargelloni</td>
<td>MOLECULAR ECOLOGY</td>
<td>2014</td>
<td>6.494</td>
<td>A</td>
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<tr>
<td>3. Molecular Identification of Atlantic Bluefin Tuna (Thunnus thynnus, Scombridae) Larvae and Development of a DNA Character-Based Identification Key for Mediterranean Scombrids</td>
<td>Gregory Neils Puncher; Haritz Arrizabalaga; Francisco Alemany; Alessia Cariani; Isik K. Oray; F. Saadet Karakulak; Gualtiero Basilone; Angela Cuittita; Salvatore Mazzola; Fausto Tinti</td>
<td>PLOS ONE</td>
<td>2015</td>
<td>3.057</td>
<td>A</td>
<td>-</td>
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<td>4. Misidentification of bluefin tuna larvae: a call for caution and taxonomic reform</td>
<td>Gregory Neils Puncher; Francisco Alemany; Haritz Arrizabalaga; Alessia Cariani; Fausto Tinti</td>
<td>REVIEWS IN FISH BIOLOGY AND FISHERIES</td>
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<td>5</td>
<td>Putting all the pieces together: integrating current knowledge of the biology, ecology, fisheries status, stock structure and management of yellowfin tuna (Thunnus albacares)</td>
<td>Pecoraro, C.; Zudaire, I.; Bodin, N.; Murua, H.; Taconet, P.; Diaz-Jaimies, P.; Cariani, A.; Tinti, F.; Chassot, E.</td>
<td>REVIEWS IN FISH BIOLOGY AND FISHERIES</td>
<td>2016</td>
<td>3.222</td>
<td>Valutata dal proponente in Classe A (15% percentile superiore) in JCR Incites, Subject Area FISHERIES, 2015 ranking: 3/52, JIF percentile 95.673</td>
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<td>6</td>
<td>Morphology and Species Composition of Southern Adriatic Sea Leptocephali Evaluated Using DNA Barcoding</td>
<td>Anibaldi, Alessandra; Benassi Franciosi, Claudia; Massari, Francesco; Tinti, Fausto; Piccinetti, Corrado; Riccioni, Giulia</td>
<td>PLOS ONE</td>
<td>2016</td>
<td>3.057</td>
<td>Valutata dal proponente in Classe A (15% percentile superiore) in Scimagojr, Subject Area AQUACULTURE AND BIOLOGICAL SCIENCES - MISCELLANEOUS, 2015 ranking: 24/217, SJR 1.395</td>
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<td>7</td>
<td>Population connectivity and phylogeography of the Mediterranean endemic skate Raja polystigma and evidence of its hybridization with the parapatric sibling R. montagui</td>
<td>Frodella, N; Cannas, R; Velonà, A; Carbonara, P; Farrell, Ed; Fiorentino, F; Follesa, Mc; Garofalo, G; Hemida, F; Mancusi, C; Stagioni, M; Ungaro, N; Serena, F; Tinti, F; Cariani, A</td>
<td>MARINE ECOLOGY PROGRESS SERIES</td>
<td>2016</td>
<td>2.361</td>
<td>Valutata dal proponente in Classe A (15% percentile superiore) in Scimagojr, Subject Area AQUATIC SCIENCES, 2015 ranking: 13/195, SJR 1.554</td>
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<td>9</td>
<td>Detection and characterization of pathogenic vibrios in shellfish by a Ligation Detection Reaction-Universal Array approach.</td>
<td>Cariani A.; Piano A.; Consolandi C.; Severgnini M.; Castiglioni B.; Caredda G.; Candela M.; Serratore P.; De Bellis G.; Tinti F.</td>
<td>INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY</td>
<td>2012</td>
<td>3.425</td>
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<td>10</td>
<td>Evaluating genetic traceability methods for captive-bred marine fish and their applications in fisheries</td>
<td>Bylemans, J; Maes, Ge; Diopere, E; Cariani, A; Senn, H; Taylor, Mi; Helyar, S; Bargelloni, L; Bonaldo, A</td>
<td>AQUACULTURE ENVIRONMENT</td>
<td>2016</td>
<td>1.985</td>
<td>Valutata dal proponente in Classe A (15% percentile superiore) in Scimagojr,</td>
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**TITOLO** | **AUTORI** | **RIVISTA** | **ANNO** | **IF**  
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1. Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (Merluccius merluccius) | I. Milano; M. Babbucci; A. Cariani; M. Atanassova; D. Bekkevold; G. R. Carvalho; M. Espiñeira; F. Fiorentino; G. Garofalo; A. J. Geffen; J. H. Hansen; S. J. Helyar; E. E. Nielsen; R. Ogden; T. Patarnello; M. Stagioni; F. Consortium; F. Tinti; L. Bargelloni | MOLECULAR ECOLOGY | 2014 | 6.494  
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NB: Elenco pubblicazioni 2012-2016 (massimo 10) con IF e classificazione dell'Osservatorio della Ricerca dell'Ateneo o VRA e indicazione delle tre pubblicazioni a più alto I.F. scelte dal proponente: