Position paper

Squalomix: shark and ray genome sequencing to analyze their diversity and evolution

Squalomix Project Consortium*

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Why sharks and rays?

Although usually recognized as a kind of 'fish', cartilaginous fishes (chondrichthyans) form a distinct class of vertebrates with more than 1,200 species, known mostly as sharks and rays (Nelson et al., 2016). Among vertebrates, they, as a taxonomic class, have the longest evolutionary history of about 400 million years, in terms of the divergence of extant members (Naylor et al., 2012). Whereas its diversity might not be widely recognized, different species in this taxon are characterized by unique traits including electromagnetic sensing (all cartilaginous fishes), electricity generation (electric rays), varying morphology sometimes with a flattened body (angelsharks and most rays) and/or a toothed rostrum (sawsharks and sawfishes). The highlight of their biological enigmas is in their reproductive modes with high plasticity between oviparity and viviparity, while they occasionally exhibit parthenogenesis and intersexuality (Penfold and Wyffels, 2019). Mainly because of overfishing, many cartilaginous fish populations are declining (Pacoureau et al., 2021), which necessitates the establishment of genomic platforms for evidence-based resource management and the archiving of genomic sequence information and evolutionary trajectory for individual species.

Despite these outstanding phenotypic and phylogenetic properties, modern genomic approaches have not been applied to this taxon until recently (reviewed in (Kuraku, 2021; Yamaguchi et al., 2020). The only exception is *Callorhinchus milii*, a member of the Holocephali (chimaeras and ratfishes, in the more species-poor chondrichthyan lineage) with a relatively small genome of about 1.9 Gbp (Venkatesh et al., 2014). In contrast, most elasmobranchs have genomes of more than 3 Gbp with abundant repetitive elements.

Project scope and organization

To sequence the genomes of chondrichthyans, the project Squalomix was launched in 2020. The project is conducted by the author's research group in RIKEN Kobe, Japan which harbors a DNA Analysis Facility and works mainly on vertebrate evolutionary genomics. It is funded by academic agencies as of February 2021 and is seeking more funding sources, especially from industrial groups oriented toward the conservation of biodiversity and marine environments. The Squalomix project aims to provide genomic sequences and other genome-wide data including transcriptomes and epigenomes, and its network of collaboration includes researchers with diverse backgrounds and locations.

Versatile sample collection featuring the local fauna

In Squalomix, sample collection is performed cautiously to minimize the sacrifice of wildlife—importantly, no sacrifice of species recognized as endangered. The collection is characterized by a rich marine fauna in Japan's neighboring temperate waters, with occasional sources from death stranding of elusive species. The project collaborates closely with local aquariums oriented toward academic science. Their contributions play indispensable roles in relaying offshore sampling and enable sustainable sampling of embryos and blood from live individuals, although the latter approach is limited to species that can be bred in captivity or are amenable to husbandry.

Another specialty of the Squalomix project is its expertise in laboratory solutions that are not confined to DNA sequencing but help consolidate its products. Access to fresh tissues from local aquariums facilitates embryological analysis, genome size quantification with flow cytometry, and karyotyping using cultured cells. Remarkably, cell culture in cartilaginous fishes, which was thought difficult because of their high body fluid osmolarity, was enabled by modifying the culture medium with balancing osmolytes (Uno et al., 2020). Our cytological expertise also allowed various epigenomic analyses that benefit from whole genome sequencing, on transcription factor binding and chromatin openness, in addition to long-range DNA interactions (Kadota et al., 2020; Onimaru et al., 2021). These techniques contributed to biological analyses based on the draft genome sequences of three shark species (Hara et al., 2018), which led to the launch of the Squalomix project.

Sequencing strategy and recent progress

The sequencing strategy in the Squalomix project is designed to accommodate particular genomic characteristics of cartilaginous fishes, mostly with large, repetitive genomes. In the standard protocol formulated in January 2021, we start by estimating genome size using flow cytometry and karyotyping as well as by 'survey' sequencing of transcriptomes, which serves for species identification with an assembled mitochondrial DNA sequence. These initial steps ensure sample authenticity and quality. We then proceed to genome sequencing, which employs both short-read and long-read high-fidelity sequencing platforms, together with Hi-C data production for chromosome-scale scaffolding based on three-dimensional interactions of DNA. The long-read data will be acquired by the use of HiFi reads obtained on the Sequel II platform (Pacific Biosciences) with a minimum coverage of x20. The outputs will be curated with reference to genome size and chromosomal organization obtained separately, and the finalized sequences will be released under the project gateway website (https://github.com/Squalomix/info). These validations allow us to scrutinize the inclusion of those genomic regions that are difficult to sequence and assemble, such as the Hox C genes that were previously thought to be missing in elasmobranchs but were retrieved by elaborate annotation (Hara et al., 2018). Complete genome assembly is also demanded by such an investigation as corroborating the scarcity of gene repertoires previously suggested for visual opsins and conventional olfactory receptors (Hara et al., 2018). The standard procedure outlined above has been applied to two pilot study species, the brownbanded bamboo shark (*Chiloscyllium punctatum*) for which a draft genome assembly was already released, as well as the zebra shark (*Stegostoma tigrinum*, formerly called *S. fasciatum*), one of the egg-laying species with the smallest known genome size among sharks.

Cooperation toward the global goals

The project Squalomix aims not only to sequence the genomes but also to tightly interact with other EBP-affiliated projects whose target species list includes cartilaginous fishes. To maximize mutual benefit among those projects, some animal samples from our collection could be provided for genome sequencing at other sites. The Squalomix project offers laboratory experiments for genome size quantification or chromosome analysis for species listed by other projects, provided that fresh cells are available. The sample transfer will be processed in accordance with the Nagoya Protocol and other relevant regulations. Inclusive cooperation respecting complementary expertise is expected to overcome the long-standing

difficulty in studying elasmobranchs sustainably and contribute to disentangling the marine ecosystems for effective conservation.

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