Reviewer No. 1

Originality & innovation

The project proposal entitled “Uncovering the molecular mechanisms of parasite-host interactions during myxozoan infection of tilapia” aims to uncover the myxozoan mode of action by combining different omics technologies, microscopy, and integrative data analysis. The proposal is very well written, contains a very clear background and shows preliminary results that support the current working hypothesis. The proposed model, that includes susceptible and resistant hosts, is a very valuable tool that will allow to unravel resistance mechanisms and the cross-talk between host and parasite. This level of combination of multi-omics techniques has not been applied to myxozoan parasites before, supporting the originality and innovation of the proposal.

Project importance and contribution to scientific knowledge

Myxozoan parasites are a wide group of parasites that affect many fish species worldwide, including economically important fish. Many studies have investigated the biology and strategies of these parasites and the host’s response, showing that many myxozoans have complex strategies to modulate the host immune system to facilitate infection. Most of these strategies remain to be elucidated. The approach proposed in this project has the potential to answer important questions about this host-parasite relationship and resistance mechanisms.

Adequacy of methods

The proposed methodology intents to achieve the highest amount of information from the experimental design. The methodology is well written, justified and perfectly aligns with the proposed aims. A very good integration of state-of-the-art methodologies is proposed. The biological model is very interesting, as information can be obtained from susceptible and resistant animals and compared. Availability of information from these species (genomes, infection transmission models, etc.) allow the application of the proposed methods. The work plan is highly adequate but presents one main weakness. The collection of serum samples will be performed in different animals than for the other tissues. To be able to integrate and compare all results, samples should be obtained from the same individuals. As fish used for serum collection will be sacrificed and sampled for diagnosis anyway, there is no real reason why not all samples can be collected from these individuals. The general risks of the proposed research are correctly identified. However, the use of only three biological replicates can present the risk of losing statistical power due to individual variation, which is common in fish, and this is not contemplated. The time allocated for each experiment is coherent and considers possible problems or deviations.

Suitability of investigators' scientific background to the project

The investigator clearly shows a high degree of experience in this field of research. Preliminary published and unpublished results from previous projects led by the applicant support the proposed work and increase the feasibility of successfully performing the tasks of the project and obtaining valuable results. The applicant shows good potential and experience to lead this line of research.

Summary (strengths / weaknesses of the proposal)

The proposal is very clearly written and the background and preliminary results presented support the hypothesis and work plan of the project.
The experimental model available (host-parasite) is highly suitable to tackle the research questions.
The research plan is clear and adequate in terms of scientific questions and timing.
Applicant expertise and available facilities and collaborations are very good to develop the project.
Aspects of the proposed methodology present some weaknesses or should have been better justified (i.e. use of data from different individuals for data integration, and low number of biological replicates).

Reviewer No. 2

After critically reading the proposal entitled with "Uncovering the molecular mechanisms of parasite-host interactions during myxozoan infection of tilapia" (although I suggested to revise the title to be specific for Myxobolus bejeranoi or gill-infecting Myxobolus" for different species of taxonomic position or different habitat-dwelling possibly have different interactions with host), I recommends strongly it to get granted based on the following 4 reasons:

1) To the best of my knowledge, this is the first comprehensive try in Myxozoan biology to combine transcriptomic, proteomic, metabolomic approaches to uncover the enigmatic molecular events during the myxosporean intrapiscine development processes which is of very importance to understand both the adaptive evolutionary mechanisms of myxosporeans and provide clues for the development of preventative methods and novel anti-myxosporean infection fish strains. Therefore, this project is original from this perspective. As a researcher engaging in myxozoa, I look forward very much to seeing the expected results of this project.
2) All of applied methods or techniques involved in this project have been developed several years and successfully applied to many research fields. Especially, the applicant is very professional, with the assistance of the listed cooperators on them. Many publications authored by the applicant can demonstrate her professionality. This is also why I strongly thought that the project is very feasible to get the expected results.
3) Prof. Lotan and her leading team has achieved much in molecular and evolutionary aspects of myxozoan biology during the past 10 years. And, she has hosted and finished several scientific funds with good publication records. So, I thought that she is competent to lead this project move forward smoothly.
4) Beside the suggestion on the project title, several incomplete references listed was suggested to be revised, although I totally thought that it is a proposal with good academic values and feasibility.

Reviewer No. 3

Originality & innovation:
The project elegantly illustrates an original and innovative research, aimed at comprehensively revealing the pathogenetic mechanisms of the myxozoan parasite (Myxobolus bejeranoi) infection in Tilapia. Considering the current knowledge on the dynamics of myxozoan infections in fish species, which is still low when compared to other fish parasites and other animal infections, the research topic proposed is highly innovative. Approaching the understanding of the mechanisms inducing differential fish host susceptibility (analyzing susceptible vs resistant tilapia phenotypes) to this parasitic infection will lead to novel management solutions that could benefit the aquaculture industry at local and global level. The use of multi-omics analysis tools is novel, both in this host-parasite interaction model and in the fish parasitology/immunology field.

Project importance and contribution to scientific knowledge:
The project is in line with the ISF program mission to support basic research proposals with the highest standards of scientific excellence. The project is of high relevance, when considering the importance of aquaculture in the world and in the region, thus the growing commercial value of Tilapia, and lastly when considering the issues caused by several myxozoan parasites to many fish species. Elucidating pathogenetic mechanisms using the integrative approach proposed in this project may guarantee that enough results can be generated, above all when dealing with the uncertainty of recently discovered and still poorly characterized fish pathogens.
I have particularly appreciated the working hypothesis 2 and 3. Despite clues have been provided for other myxozoan infections in fish, it is still unknown how these parasites may selectively induce systemic immune suppression to their susceptible host organism and species.

Adequacy of methods:
The project is designed with an adequate and cutting edge scientific methodology. Using a novel comprehensive approach, including transcriptomics, proteomics, and metabolomics, they will combine different layers of biological information to reveal the molecular mechanisms underlying host-parasite interactions, and to elucidate the myxozoan mode of action. This unique methodology combination will certainly improve the current knowledge on fish-myxozoan interactions, and based on these discoveries, allowing to propose new hypothesis for future research towards applied management strategies. The experiments are well designed and the methodologies indicated are adequate.
Please note I am unable to evaluate the adequateness of the requested budget because I am not familiar with the operational costs in Israel. Although the budget requested looks reasonable and will nicely integrate funding already achieved through other funding agencies.

Suitability of investigators' scientific background to the project:
Dr. Tamar Lotan has shown to possess the ability (unique in Israel) to carry out the well-articulated study proposed, taking advantage of existing infrastructure and research group. They will be moving forward from a great base of preliminary data already achieved and published in prestigious scientific journals. In addition, the scientist will be supported by a team of experienced colleagues, allowing the group to access new techniques and data analysis.

Summary:
The project is very strong. I am unable to find weaknesses of this proposal, apart for the expected difficulties in carrying out the novel techniques proposed and for combining data for the final comprehensive analysis. Although they have a great operating plan and the support of a great team of experts.

Reviewer No. 4

This proposal is very interesting, lending the opportunity to study host-pathogen interactions leading to different outcomes, disease or not. It has significance for both the basic understanding of myxozoa biology, fish biology (immunity) and myxozoa-fish interactions. Further, to fish health, mainly under aquaculture conditions.
In general, I find this proposal well written and experiments well described. There were also at least two papers already published, attesting that an important prerequisite, which is a disease challenge assay, was resolved. Also, other methodologies were developed and, in this respect, add to the solid and feasible state of the proposed research and to the competence of the PI with the support of the fish collaborators.
Nevertheless, I read the proposal, examined the preliminary data and read the published papers, and consequently had a few significant comments that I think require attention:

1. The expertise of the PI is in Myxozoa. The comments I have relate more to the host (fish) side of the proposal, which seems less attended to. Therefore, the proposal could have benefitted from a tighter collaboration with an expert on fish biology including application of the proposed "omics" methodologies.
2. Indeed, tilapia is important for aquaculture, as stated in the introduction, but the large majority of tilapia in aquaculture are close derivatives of Nile tilapia, the so called "resistant" species. Thus, it is not surprising that this new and specific parasite was identified where blue tilapia, the so called "susceptible" species and its hybrid are found. Host-parasite coadaptation. Thus, the significance to aquaculture is more limited to where blue tilapia and its hybrid are grown.
3. In the proposal, the goals include "omics" methods on both the fish and the parasite. However, it is not clear if the datasets will be obtained from the same joint samples or not (e.g., same gill sample for both host and parasite analysis). Because the ratio of tissue/cells in joint samples is heavily biased towards the fish, extracting the parasite data will be challenging. If the data for each will arrive from different tissues (e.g., parasites collected from gills and fish kidney samples) It will be less straightforward to determine host-pathogen direct interactions. The preliminary data, demonstrates this difficulty.
4. The transcriptomics data of the parasite will be limited. The parasite genome assembly is relatively fragmented. With this many scaffolds for such a small genome, the analysis will miss many of the genes. Indeed, the Busco analysis is around 50% of the genes, meaning that using this methodology is probably premature if the objective is to study pathways and mechanisms. If this is the case for transcriptomics, the situation for parasite proteomics might be even more premature.
5. Clearly, one of the advantages of this study is the possibility to compare susceptible vs. resistant hosts. Having this research system in hands is both valuable and rare and therefore, a potential significant advantage. However, I further wondered, what does it mean "susceptible" or "resistant". If one wants to understand the mechanism of resistance, one needs to define better the type of resistance based on simple measurements before shooting with the heavy "omics" cannons. Based on the preliminary data and publications, the level of parasite in Nile tilapia even after 33 days is comparable to day 0 (shown), and probably to uninfected fish (not shown). Neither RNA expression

nor cysts were found in Nile tilapia. Are Nile tilapia infection resistant, hence experience no disease because not infected, or are they disease resistant (infected but not "sick"). Obviously, the mechanisms would be very different between these two "resistance" types. If Nile tilapia are infection resistant, they do not "sense" the parasite, hence are like uninfected controls that has no host-pathogen interactions. It is easy to find out, for instance by analyzing basic responses, like qRT-PCR of the fish interferon response, which was elicited early in most diseases so far studied.

6. One of the common denominators of disease resistance mechanisms in animals is the improved function of the host's immune system (either innate or acquired, or both). However, one key factor of immunity is environmental temperature, since fish do not regulate their body temperature. Thus, outbreaks of fish disease are first and foremost related to density and stress, oftentimes by suboptimal temperatures. Nile tilapia is adapted to warmer waters (hence, why pure Nile tilapia is not performing well in countries like Israel). Blue tilapia is adapted to cooler climates and thus, stressed by warmer waters. It was well documented for many infectious diseases of fish that the temperature range for infection and disease progression is limited. Usually, it happens when the pathogen is well and active and the fish immunity is sub-optimal (too cold or too hot). The published data indeed show that the disease takes place mostly around 30C and declines to almost none at 25C. A fair hypothesis is then, that the difference between species is simply a reflection of their physiological (including immunity) activity/adaptation. Nile likes 30C and thus, uninfected, while blue likes 25C and stressed at 30C and thus, infected and sick. The temperature factor must be considered and controlled as a prerequisite to further characterizing multiple "omics". It would be a pity to find that Nile activates efficient immunity while blue fails due to just different temperature adaptations...