

Review of Ph.D. thesis for Ms Sneha Patra

Overall comments:

Clearly the candidate did a lot of work in the course of the various projects that make up this thesis. There are two papers (out of 6) in which the candidate is listed as first author, and as such I would have expected the description of her contributions to detail her involvement in **designing** the studies – I may have missed something, but her work appears more technical and descriptive in nature, rather than the “higher level” design that I expect of a Ph.D. candidate (with hypotheses, proposed tests, and comments RE support for hypotheses once tests are done. Hence my first question to the candidate –

Q1. Can you please provide details of how you contributed to the experimental design of the included papers – particularly papers 5 and 6 for which you are first author. Please comment on the underlying hypotheses for 5 and 6, if the results support these, and what new hypotheses impel future work.

The “General Introduction to the Myxozoa” is thorough and raises several current problems with the group. I find the summary tables very useful to a review both of the group and specific sub-clades e.g. *Sphaerospora s.s.*

I would ask the candidate to speculate on two points:

Q2. You state on page 2 that one of the structural properties of Malacosporea is soft spore valves. Are these actually any softer than *Kudoa* and *Ceratonova* myxospores? Can you speculate on the valve cell composition?

Comment: Pg 3. I feel the word “inacceptable” is too harsh when referring to sequencing in species descriptions. It is absolutely preferred, but I would still accept species descriptions from labs where sequencing is not possible/included. Indeed, a lack of sequencing should encourage collaboration between labs, to produce complete descriptions.

I like the supplementary summary tables, but have some questions RE the Malacosporea PCR results on Pg. 64:

Q3: The table shows that all of the marine fish kidneys were negative by PCR for Malacosporea. I would like you to give your thoughts on this point – were the primers too restrictive? i.e. given comments elsewhere in the papers, would you expect rates of evolution to render putative primitive marine Malacosporea too different to detect? If the kidney samples are truly negative, to what do you attribute the positive LM observations?

RE the Malacosporean table on Pg 66:

Q4. Did you make a conscious effort to sample invertebrates and fish from the same localities? And did you only sample fish? – please speculate on the possibility of other vertebrate hosts (or any other non-bryozoan hosts).

See below for line-specific remarks or corrections for the Introduction and the unpublished Paper 6. In general (and this is advice I give all writers) please refrain from wordiness – two specific examples: instead of “a total of XX” or just state “XX” and instead of “as well as” just state “and”

Pg 1: delete double space before “cnidarians” about half way down page

Pg.7 about half way down page “nuclei” should be “nucleus”

Pg. 13 at bottom I suggest “parasite antigens” instead of “parasitic antigen”

Pg. 14 near top; I suggest “in a race” instead of “is at race”

Pg. 19 middle; “bauplan” is good German, but you should use an English word.

Pg. 19 next line to remark above – delete “the”; should read “during evolution”

Pg. 20 title 3.1; “a short history” not “the”

Pg. 20 “of 3.7 kb” is better English than “with 3.7 kb”

A general comment RE inclusion of published works – please delete the double page numbers.

Pg. 146 Delete “the”: “sphaerosporid”, “comprehensive” and “host-parasite co-evolution”.

Pg. 146 at bottom – add “for” not just “24”

Pg. 147 “in shape” is redundant – delete.

Pg. 147 del. “of the genus Sphaerospora”

Pg. 148 I think this is the first mention of fish as “intermediate” hosts. I suggest adding the terms “definitive” and “intermediate” in the Introduction describing the life cycle.

Pg 148. How low was “low taxon sampling”? And how many taxa do you think you would need to make a difference?

Q5. Please define what **significant figures** are with respect to scientific data. Do the dimensions you report (e.g. on pg. 152) conform to this?

Q6. RE paper 6. Why not describe the new *Sphaerospora* spp. as novel species (and give them names)? What more data would you need to report?

I look forward to hearing discussion of these points!

Sincerely,
Stephen Atkinson



Review of Sneha patra's Ph.D. thesis:

Malacosporea and Sphaerospora sensu stricto: Myxozoan clades with unique biology and evolution

The thesis is well written and comprehensive, and consists of 6 papers, 5 of which are published. The candidate Sneha Patra is first author of two, one of which is published and one that is a manuscript ready for submission. A broad range of methods and analyses have been used, new species have been described, taxonomic revisions done and life cycle studies performed. There is also pioneering work on myxosporean motility. My impression is of meticulous high quality work, and this clearly represents a leap forward in our knowledge on the Malacosporea and the Sphaerosporidae. I really enjoyed the reading!

A few errors were noted, as must be expected in such a large work, but nothing serious as far as I could find (e.g. underestimation of bryozoan diversity p. 18, mono and disporic plasmodia p. 20). I have selected some questions that I believe are important here, may interest the audience and that could support a good discussion. Two of them are general in nature, and may concern other branches of zoology, one goes to the characters of some members of the Sphaerosporidae.

I recommend the thesis for defense.

Questions

Q1. In papers I, V and VI, very accurate measurements of spores and other stages are provided. Two decimals are given, on dimensions measured by light microscopy. What is the consequence of the resolution of normal light microscopes on the accuracy of measurements that can be reported in morphological descriptions?

Q2. In paper VI, the morphology of *Sphaerospora* spp. in samples from cyprinids (and other hosts) are compared with named species, including to *Sphaerospora leuciscusi* and *Sphaerospora ousei*. What are the requirements for a name to be valid, and hence available?

Q3. In 1991 Feist, Chilmonczyk and Pike redescribed the type species of *Sphaerospora*, *S. elegans* from sticklebacks. They noted large "bubble"-like structures associated with the posterior end of the spores. The same "bubble" appeared as a vacuole around spores inside pseudoplasmodia. *S. elegans* was also found to have a pitted spore surface. The same phenomenon is apparent in e.g. some images of *Polysporoplasma* spp. in Sitja-Bobadilla and Alvarez-Pellitero (1995) and Palenzuela et al. 1999 (as lumen), which also had pitted surface. In paper I, *Sphaerospora motemari* was described, and images (e.g. 1c) show a prominent

halo around the spores, not commented upon, and a nice SEM image clearly show pits into the valves.

Do you have an opinion of the nature of this "halo" around the posterior of the spores in members of some sphaerosporid clades, and a possible relationship with the pits?

Refs

- Feist SW, Chilmonczyk S, Pike AW (1991) Structure and development of *Sphaerospora elegans* Thélohan 1892 (Myxozoa: Myxosporea) in the sticklebacks *Gasterosteus aculeatus* L. and *Pungitius pungitius* L. (Gasterosteidae). *European Journal of Protistology* 27(3): 269-277.
- Massard, J.A. & G. Geimer, 2015. L'histoire de la recherche bryozoologique au Luxembourg (Phylactolémates et Gymnolémates d'eau douce). *Bulletin de la Société des naturalistes luxembourgeois* 116 : 373-379
- Palenzuela O, Sitjà-Bobadilla A, Alvarez-Pellitero P. (1999) Glomerular disease associated with *Polysporoplasma sparis* (Myxozoa) infections in cultured gilthead sea bream, *Sparus aurata* L. (Pisces: Teleostei). *Parasitology* 118: 245-256.
- Sitjà-Bobadilla A, Alvarez-Pellitero P. (1995). Light and electron microscopic description of *Polysporoplasma* n. g. (Myxosporea: Bivalvulida), *Polysporoplasma sparis* n. sp. from *Sparus aurata* (L.), and *Polysporoplasma mugilis* n. sp. from *Liza aurata* L. *European Journal of Protistology* 31(1):77-89



Egil Karlsbakk
Bergen 20 August 2017

Bergen 12. Sep. 2017

Regarding

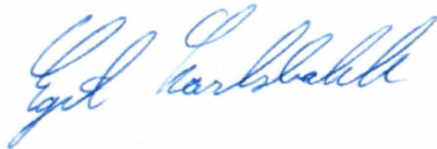
Thesis defence,

Sneha Patra 13 Sep. 2017

Confirmation,

I hereby declare that I forwarded 3 questions to the candidate Sneha Patra, which I have received her answers to, and her opinions on. I consider her defence or response's appropriate, and hence approve it.

I judge the thesis as excellent ('pass with honours').

A handwritten signature in blue ink, appearing to read 'Egil Karlsbakk', written in a cursive style.

Egil Karlsbakk

Professor, University of Bergen

Prof Oleg Ditrich

Head of the Committee for PhD studies in Parasitology

University of South Bohemia in České Budějovice

Dear Prof. Ditrich,

Please, find attached my comments on dissertation written by Sneha Patra with the title:
Malacospora and Sphaerospora sensu stricto: Myxozoan clades with unique biology and evolution.

Yours sincerely



Dr. Kálmán Molnár DSc

emeritus scientific adviser

Budapest, 21. 08. 2017

Reviewer's opinion

The task of the reviewer for evaluating this dissertation is not easy. This reviewer is a warm admirer of the research activity made in the Institute of Parasitology of the Academy of Sciences of the Czech Republic. This reviewer thought that after the death of Jiri Lom the high ranked research on myxosporeans cannot be continued with the same drive as before. It was a mistake and results proved that the reorganised research group continue to publish series of excellent works. The candidate Ms. Patra is an active participant of the research team. Into her dissertation she has by rights incorporated results of papers published on her name with co-workers. As these papers appeared in excellent journals after being reviewed by experts of the field, I accept data and appreciate them. My task is to evaluate Ms. Patra's contribution to these achievements. Ms Patra built up her dissertation from 6 papers. In two of them she was the first author and in four papers her name is found among co-authors. This proves that her co-workers admit her research.

I am satisfied with the form of the dissertation. I had often problems with dissertations written by Hungarian and other candidates, who after working in large scale co-operations, collected and bounded their published papers into a thesis without explaining their special role in those co-operations. In this respect Ms Patra's dissertation is correct as in special sections (Chapter 1 and 6) she tries to determine her role in the actual research.

The chapter **Introduction to Myxozoa** is well written. I enjoyed reading this chapter as the candidate gave a really good summary on the development and major achievements of myxozoan research with a special respect to her selected fields.

The author selected two rather far standing groups of myxozoan parasites for her subject. This seemingly surprising selection is quite acceptable if we regard the common occurrence of malacosporan stages and *Sphaerospora* developmental stages in fish blood. More over the

malacosporean research in fish started on the similarity of renal myxosporean stages and malacosporean spores. Morphological similarity of pseudoplasmodia of malacosporeans and sphaerosporids still indicates examining these relatively far standing groups within a single research. Difficulties in species identification with morphological methods still exist, and the only acceptable solution for identifying malacosporean developmental stages and species is using molecular methods. Most of the results came from the achievements of the research team in which the candidate was an active member. The cited literature includes relevant research papers of morphological, pathological and experimental sides of the malacosporean and myxosporean topics, although the candidate mastered mostly the morphology and development of these parasites. Figures and tables fit to the chapter.

The **objectives of the research** summarised in separate chapter is clear.

Published papers and a paper to be published incorporated into the dissertation show that several questions of these objectives were successfully solved during this ongoing research.

No special chapter deals with materials and methods but data in this respect can be found in papers attached.

Concerning this section, however I have to make some remarks. The authors examined several fish species for malacosporean and sphaerosporid infections. The number of examined fish and work made on them is really remarkable. Unfortunately, however, the age of the examined fish cannot be found among data. I know from my practice that age of the fish is very important, e.g. *Sphaerospora dykova* infection in some groups of fingerlings of common carp can achieve 100 %, while in older generations of fish this figure is very low. Data received in this respect, therefore are less reliable.

Results reported in this work are remarkable and they coincide mostly with experiences of the reviewer. Finding other **sphaerosporean and malacosporean stages in the blood** of SBI infected fish is really a new achievement and it makes researchers reconsidering their role in evolvement of SBI. In this respect, however I have a more conservative opinion. A pathologist has to make clear difference between heavy infection and disease. No matter *S. dykova* K stages by blocking capillaries cause heavy dysfunction of some organs. The question, however arise, whether small sized pseudoplasmodia of the, in number overwhelming, *S. molnari* and malacosporean stages can produce similar disorders in the affected organs, or not? My observations and experiments proved that *S. dykova* K-stages could evoke a disease in fish. No matter that intensive presence of *S. molnari* and malacosporean spp. have a non-negligible effect on fish, but their pathogenic effect has not been proved.

I want to call attention of the candidate that the question whether swimbladder inflammation of the common carp was caused by *S. dykova* was first forwarded in a paper (Molnár, K. (1980): Sphaerosporosis, a new kidney disease of the common carp. Published in: Fish Diseases, Third COPRAQ-Session. Ed.: by W. Ahne, Springer Verlag, Berlin-Heilderberg-New York, pp. 157-164).

In this respect I have a question: Has the candidate observed that two heavily infected periods might exist both in *S. molnari* and *S. dykova* infections, namely one in summer in fingerlings and another infection peak in April and May in 0+age groups of common carp?

If your answer is yes, do you think that this second infection was caused by persisting blood stages, or the new infections have been produced by the still unknown intermediate (primary?!) hosts?

Is it possible that in *Sphaerospora* infection a doubled cycle exists, namely fingerlings initiate an infection through intermediate hosts in elderly generations, and vice versa?

On page 33, you mention experimental transmissions of *Sphaerospora* blood stages. In these experiments it was proved that *Sphaerospora* blood stages could be transferred to uninfected hosts but they do not initiate spore formation. At the other hand *S. dykova*e second blood stages, developed to spores. Do you have any idea why the first blood stages only survived but stopped finishing development?

What concerns **Malacosporea**, this reviewer cannot add essential comments to the field, and he only congratulate to the pioneering work achieved on infection of cyprinid fishes.

At a similar way this reviewer appreciate very much that the candidate is a member of the research group which have an essential role in the better understanding of ***Sphaerospora* infections** of the common carp through examining and proving that other blood stages than *Sphaerospora* might play essential role in evolvement of swim-bladder inflammation, as well.

Molecular fingerprints of myxozoans proved the presence of **multiple agents in the etiology of SBI**. These agents could have been demonstrated by other, less complicated methods as well. I mean, in stained blood and impression smears *S. molnari* second blood stages (K-stages) could also be studied. I am especially interested, in the structure of these forms. In *S. dykova*e K-stages the bisporid nature of the formatting pseudoplasmodia is well observable. In a Giemsa stained preparation maybe the monosporid structure of *S. molnari* could also be demonstrated.

In page 163 and in other places of the dissertation the candidate refers that up to this time only two **histozoic *Sphaerospora* species**, *S. fugu* and *S. molnari* have been sequenced. It is true but I want to call her attention to another species, *S. carassii* which commonly occurs in gills of the goldfish and which is a good tool for being sequenced.

Summarising my opinion I have to conclude that

- 1) The dissertation written by Sneha Patra with the title: Malacosporea and *Sphaerospora* sensu stricto Myxozoan clades with unique biology and evolution, is a well-constructed, valuable work,
- 2) It proves that the candidate well knows the major problems of the myxozoan research.
- 3) She has made excellent studies in solving some of its basic problems.
- 4) She proved that she is able to perform autotelic research tasks.
- 5) She is worthy of being awarded by title PhD.



Dr. Kálmán Molnár DSc

emeritus scientific researcher