

# **Symonová Radka: Microanatomy and cytogenetics of non-marine ostracods – an insight into evolutionary biology of their reproductive modes**

## **Review of Ph.D. Thesis**

First reading of this dissertation presented by Radka Symonová indicates advanced original thesis (divided into 7 basic chapters, acknowledgement and references; together 80 pages) completed with 80 images in 12 plates and 2 images in the text, 8 graphs in 1 plate, 3 papers published in peer reviewed journals with impact factor, unpublished results (1 *in prep* paper), list of conferences actively attended during the PhD program, 2 posters and 4 abstracts of papers *in prep* resulting from the PhD program and some necessary forms.

During my review I will evaluate subsequently: individual chapters, images, and other appendices. Then I will present several questions concerning some details from this dissertation I would like to explain from Radka Symonová. Last but not least I will express my general evaluation of this Ph.D. Thesis.

I would like to claim now basic fact that I did not find essential mistakes here in presented dissertation. Some minor imperfections (mostly formal ) are present which I will note in the following parts.

First chapter is introductory. In the part „Rationale of the work and its objectives“ explain Radka her effort to contribute to general understanding and to fill in knowledge of ostracod biology. With her own studies or in collaboration. She is able to work well in both modes. Here she explain „heterogeneity“ and also „complexity“ of this thesis, which I consider as advantage. This whole part is very good introduction to evolutionary biology of reproductive modes in non-marine ostracods with general aspects of reproduction, overview of the reproductive modes in ostracods, model species and the framework of the SexAsex project. I found here small mistake on page 11- last sentence is not finished and thus probably important information is missing (maybe it concern more sentences?).

Second impressive chapter is „Material and methods“. In“Ostracods´ samples“ Radka Symonová describes species and their populations analysed in this study. I recommend to complete especially Tab.1 with basic taxonomic information (author and year of description) for better characterisation of individual species.

Rest of the chapter is divided into protocols (for histology, for cytogenetics, for cryo-embedding and immuno-histochemical detections) and information on PCR primers and their thermal profiles. I am very sufficient with the styles used here- all techniques is possible to reproduce on the basis of those precise descriptions. I recommend to add citation of author or place them where it were described several less known techniques. Anyway, I would like to accent such broad spectrum of method used here (together with techniques used in next chapters and papers).

Third chapter is called „Microanatomy“. In“Rationale and objectives of micro-anatomical part“ Radka Symonová explain strategy of the first stage of micro-anatomical part and future utilization of results (like here in female reproductive organ (FRO)). Radka accents cryo-sections as effective link between microanatomy and cytogenetics. She noted possible use of Technovit 9000 enabling immuno-histochemical detections at higher resolution. It is proof that Radka Symonová is able to plan addition of further methods to improve recent ones. In the part „Perspectives in the microanatomy“ Radka explain: „The complexity of FRO has resulted in a need of 3D reconstruction and visualization of this structure“. Simultaneously

she knows that „The accomplishment of this task will, however, require further data processing, 3D modelling and, ideally, also animation based on biomechanical principles“. Radka Symonová utilized 3D visualization in at least 2 publications together with R. Matzke-Karasz with help of synchrotron with holotomography (one paper has been published in Science together with supporting online material). They both contribute in such manner to „renaissance in the morphology“. It is in accordance to new trends discussed last year in Germany („New techniques have the potential to trigger a strong renaissance in the morphology of insects and other organisms. They will enable systematists to acquire large, well documented morphological data sets, which can be combined with extensive molecular data. Combined analyses will likely lead to new and important insights in insect phylogeny and evolution“ (Beutel R.G., Friedrich F., 2008: A renaissance of insect morphology –  $\mu$ -Ct and other innovative techniques. DGaaE-Nachrichten 22(1), 2008: 5-8)).

Regardless to big contribution of such quite new techniques (3D reconstruction) Radka Symonová remains realistic: „The results obtained during micro-anatomical part have rather opened new issues and brought new questions than answered our former questions“ (page 37). On the opposite, tomographical techniques generally are fully non-invasive. That fact is important for examination of rare specimen (for example valuable museum exhibits).

Fourth chapter concerns „Karyology and Cytogenetics“. It belongs together with microanatomy to most important parts of thesis. Radka Symonová solve here various cytogenetical problems like chromosomes of podocopid and myodocopid ostracods, karyotypic variability in model *E. virens* and other non-marine ostracod species (including levels and types of chromosome variability in this species), chromosomes in other non-marine European ostracod species karyotyped including variability in chromosome numbers. Then summarize chromosome numbers and morphology. Following are parts concerning molecular cytogenetics. Various techniques and problems like FISH with insect telomeric repeats (TTAGG)<sub>n</sub>, FISH with rDNA, CGH, GISH are presented. Meiosis in sexuals vs. asexuals is the last part of this chapter. Radka Symonová discuss here this problems including some controversies.

Fifth chapter is „Microorganisms inducing asexual reproduction“. It is known that some microorganisms are reason for induction of asexual reproduction in some invertebrates. *Wolbachia* –like microorganisms are in the centre of interest longer time. Also *Microsporidia* as intracellular parasites are widely spread among animals. Chapter fifth deals with those microorganisms and their relations to ostracods.

Chapter six has name „Giant spermatozoa“. Concerns with fact that sperm reveal diversity in size and structure. Radka evaluate them from the point of view of ostracodan evolutionary biology. She concentrates on giant filiform spermatozoa, especially on their evolution. Excellent are results concerning fossil spermatozoa published in Science and completed with supporting online material.

Chapter seven is „Final comments on aspects dealt with in this thesis (Discussions and conclusions)“. Radka summarize and discuss here important parts of thesis. The are „Giant sperm and FRO“ (Radka expect here more data for future comparison of ostracod situation with other groups of animals based on introduction of modern techniques used here also for them), „Histology“ (lot of new information including free cells, hepatopancreas etc.), several parts concerning chromosomes, karyotypes and cytogenetic data and pitfalls in ostracod research. Part ends with „Summary and overview of factors involved in distribution of reproductive modes“. Radka Symonová comments here in all parts of chapter seven individual results from thesis, summarize them and discuss various aspects of subjects. I consider that chapter as well written and intellectually strong.

In part eight „Acknowledgement“ Radka Symonová thanks to all co-authors and people who helped her with science.

Part „References“ represents all relevant publications concerning ostracod research and modern techniques of investigation. Those references are also completed with references in publications in next part of thesis. I found several formal mistakes in the text from page 5 to page 67. For instance Radka introduce publication of Horne et al 1998 on page 5, but in References are 2 references Horne et al 1998a and Horne 1998b. The similar situation is with Butlin et al, 1995 (page 12), but in References are Butlin et al 1998a and Butlin 1998b. On page 7 is Slobodchikoff, 1971, but in References are Slobodchikoff & Daly 1971. Flegr, 1992 (page 11 and 13), Pokorny, 1992 (page 11), Jurine, 1820 (page 15) and Siveter et al., 2007 (page 39) absent in References completely. Anyway, it seems it is formal mistake only and I notice it here to be correct for publication.

Chapter ten is „Appendices“. It begins with 80 images in 12 plates and 8 graphs in 1 plate. All images and graphics are of very good quality and completed with appropriate explanatory text. Following 3 papers were published in peer reviewed journals with impact factor (Hydrobiologia- Symonová is only author; Journal of Crustacean Biology and Science- Symonová is co-author) and represent top scientific paper. They very well complete text part of thesis. Unpublished results (1 *in prep* paper), list of conferences actively attended during the PhD program, 2 posters and 4 abstracts of papers *in prep* resulting from the PhD program also support text part of thesis. Especially paper on methods of visualization in the microscopic freshwater ostracod... (page 158) based for example on data processing with software Amira will bring new valuable information.

Some necessary forms and list of all publications close thesis.

### **Questions concerning some details from this dissertation for Radka Symonová**

It is written on page 7: „It is necessary to stress that the occurrence (or finding) of males in a species to a certain extent also reflects the level of investigation of that particular species (males were discovered after a more intensive investigation). Do you think that further deeper investigation could change our knowledge about reproduction of various groups of ostracods substantially?

You introduce on page 37 possibility to use Technovit 9000 for better preservation of tissues and better resolution. Would you briefly specify that fact?

Your set of various techniques used in paper reviewed is enough. But, it seems to me that addition of immuno-electron microscopy would increase value of information on particular subjects. Do you agree? What is reason for absence of such techniques in your recent investigation?

Theoretically- In the case of continuation of similar type of research- what other techniques you think to add, if any?

Plate 3, page 86- I noticed not very well visible difference between quality of paraffin sections (A,D) and „Spurr-medium embedded“ sections (in literature often „semithin sections“) (B,C); the same is on Plate 4 (B) vs (A) (page 88). Theoretically results from those both techniques could differ more. Could you comment it?

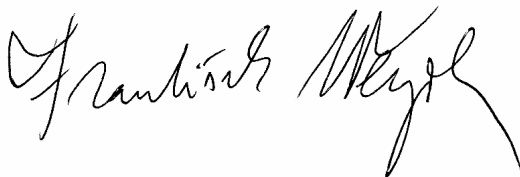
### **Summary**

This dissertation presented by Radka Symonová is full of very actual and valuable scientific data. I would like to mark out especially introduction of various new techniques like

synchrotron based holotomografie, 3D reconstruction and visualization. Ability for complex evaluation of data is scientifically very valuable. Results from investigation are published in premium journal including Science etc. Ability to investigate as a member of scientific team is in accordance with modern trends.

**Final evaluation:**

Radka Symonová presents in this Ph.D. thesis modern complex scientific data based on her own original research as well as on cooperation with scientists in the framework of various projects, domestic or international. It summarize recent state-of-art of ostracod research. Radka Symonová confirm sufficiently her ability to investigate as well as interpret complex scientific problems and present them in appropriate form. **I strongly recommend this Ph.D. thesis for defence.**

A handwritten signature in black ink, appearing to read 'František Weyda'. The signature is fluid and cursive, with a long, sweeping tail on the final letter.

doc.RNDr.František Weyda,CSc.  
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