

Submitted by Dr. ANETA KOSTADINOVA as evaluator of the doctoral thesis by ANNA FALTÝNKOVÁ, entitled

**Developmental stages of trematodes (Digenea) in freshwater molluscs in Central Europe**

supervised by Prof. RNDr. Tomáš Scholz, CSc.

**1. Evaluation of specific objectives and their relevance**

The thesis presents an excellent example of a state-of-the-art faunistic/systematic research and represents a substantial contribution to scientific knowledge on trematode life-cycles in Europe's freshwater environments. It fills a large gap in our knowledge on larval trematode morphology, identification and distribution by a thorough exploration of abundant larval material from Central Europe. Four study objectives are accurately identified following an exhaustive and critical review of the literature. These are fully justified in relation to previous research and provide a logically interconnected framework that integrates detailed morphological, taxonomical and ecological studies.

**2. Evaluation of methodology used in relation to the objectives proposed**

This large-scale study is well-designed and well planned. The parasite material used and the databases compiled are ample and adequate for the specific objectives. Sample processing and methods used are described in detail. All descriptive and comparative tables are detailed and provide clarification and summaries of the present and historical surveys. The identification keys are correctly structured and working. The beautiful illustrations (i) represent the best contemporary assemblage of drawings on such a wide range of taxa; (ii) are of excellent quality, and (iii) further enhance the identification keys proposed.

**3. Relevance of findings/results**

All findings of the present study are original and their interpretation provides important insights into the identification, morphology and distribution of larval trematodes in Europe, as well as on the ecological associations of their life-cycle strategies with birds. The thesis (i) resolves to a substantial extent the long-standing need for a critical evaluation of the existing knowledge on larval trematode systematics and distribution at an European level; (ii) sets a much needed unifying framework for further studies on freshwater mollusc host-parasite systems, and (iii) largely facilitates opening up new areas of research, *i.e.* ecological studies using larval trematode communities as response indicators reflecting the quality of Europe's freshwater environments and epidemiology of trematodes with veterinary and medical importance. The content of the thesis is already published in quality, peer-reviewed journals and a monograph. The data and results contained in the two manuscripts (under evaluation) are of high quality and I have no doubts that these will withstand peer review. In spite of the of multiauthorship of the publications (Ms Anna Faltýnková is the first author in all but the monograph) the present work demonstrates that she is able to: (i) define and implement independent research, and (ii) delineate its contribution to the advancement of scientific knowledge.

I would like to highlight Chapters 3.2. and 3.3. which represent the most exhaustive study on larval trematodes in Europe (based on more than 11,000 molluscs of 13 species) and more importantly, contains a critical survey of all previous findings. The latter are interpreted in the context of the modern comparative morphology data. The two papers will, without doubt, help speed up community studies on larval trematodes in an environmental context. I also find the Checklist of the trematodes of birds of the Czech and Slovak Republics an important contribution to the main body of the thesis. The life-cycle data in particular, provide a contemporary summary that will influence meta-analyses on European bird trematodes.

**4. Formal characteristics of the thesis**

The main core of the thesis, so-called compilation thesis, represents a summary of significant scientific publications already published (2) or submitted (2) in reputable peer-reviewed scientific journals, plus a monograph in press. The thesis is very well-organised, reads well and the English is very good. The reference section is exhaustive (Chapter 5, 197 refs, plus many more additional references in the papers, Chapter 3).

**5. Comments**

I have indeed very few criticisms, mainly editorial, that should be considered before the final submission of the MSs.

- Use 'unidentified' instead of 'undetermined'; 'terrestrial' instead of 'terrestic' and 'provisional' instead of 'provisoric'.
- Replace *Tylodelphys* with *Tylodelphys* (p. 25, 28 and 32).
- A total of 7826 molluscs .... WAS collected (p. 27).
- *Cathaemasia* is not a representative of the family Echinostomatidae (its placement in Cathaemasiidae is correct elsewhere in the text but in the keys to the cercariae from planorbid snails (p. 30).
- Add measurements to text ('x' on p. 31):

2 Metacercaria large (about x  $\mu\text{m}$  in diameter) ..... 3  
Metacercaria small (about x  $\mu\text{m}$  in diameter) ..... *Echinostoma* sp. (Fig. 7B)

and (p. 68)

7 Metacercaria large (200  $\mu\text{m}$ ), shape of stylet – see Fig. 5E .....  
..... *Opisthioglyphe ranae* (Fig. 6E)  
Metacercaria small (x  $\mu\text{m}$ ); shape of stylet – see Fig. 5G .....  
..... *Plagiorchis* sp. (Fig. 6F)

- Better use 'more or less' instead of +/- (p. 28 and elsewhere):

Ventral sucker +/- equatorial ..... 5

- Key on p. 65. Replace

1 Cercaria without tail (cercariaeum); intestine radial ..... *Cyclocoelum microstomum*  
Cercaria with tail; intestine of different shape ..... 2

with

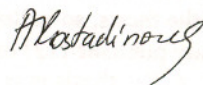
1. Cercaria without tail (cercariaeum); intestinal caeca form cyclocoel .....  
..... *Cyclocoelum microstomum*  
Cercaria with tail; intestinal caeca blind ..... 2

## 6. Conclusion

Overall, this is an excellent text and a pleasure to read. The large-scale study carried out by Anna Faltýnková covers parasite groups that are rather diverse and difficult to work with. The identification of trematode larval stages is complicated and there are indeed very few scientists worldwide, who are able to identify the much wider taxonomic range of trematode larvae co-occurring in natural freshwater ecosystems. This study, enhancing the identification of larval trematodes, is key for future studies on the relationships within this host-parasite system, and Anna Faltýnková has tackled the subject successfully. She is aware of an exceptionally wide range of studies covering the fauna, life histories and systematics of trematodes. The work represents a significant contribution to the field and is publishable. It fully deserves a high rating.

Signed at Sofia on 20<sup>th</sup> September 2006

Signature:



Dr Aneta Kostadinova  
Central Laboratory of General  
Ecology  
Bulgarian Academy of Sciences,  
2 Gagarin Street, Sofia 1113,  
Bulgaria

by Anna Faltýnkova:

**“Developmental stages of trematodes (Digenea) in freshwater molluscs  
in Central Europe”**

The Ph.D. thesis of dr A. Faltýnkova is composed of five separate parts, completed in collaboration with other researches. The contributions of Ms. Faltýnkova explained by her in the annotation to the thesis, are genuine and of major importance. Two parts have already been published: one in *Parasitology Research* („Larval trematodes in freshwater molluscs from the Elbe to Danube rivers (South-East Germany): before and today”), and the other in *Acta Parasitologica* (“Is *Radix peregra* a new intermediate host of *Fascioloides magna* (Trematoda) in Europe? Field and experimental evidence”). The next two manuscripts are ready for publication. They concern a survey of cercariae occurring in snails from the family Planorbidae and from *Lymnaea stagnalis* (Lymnaeidae) in Central Europe, and also provide keys to their determination. The separate fifth part is the checklist of the trematodes of birds of the Czech and Slovak Republics with added information on life cycles, compiled by Anna Faltýnkova based on original publications.

The thesis begins with an extensive introduction devoted to the history of investigations of trematode life cycles in Europe starting from 14th century. The author discusses the most important European literature on cercariae, and describes the progress in investigations of systematic and life cycle studies, as well as the difficulties that researchers of the life cycles are facing. This part is based on a carefully selected literature review and is of great value, especially for interested scientists.

Anna Faltýnkova presents the following research goals: investigating the occurrence of larval trematodes in aquatic molluscs in Central Europe; compiling the keys to identification the cercariae and metacercariae of planorbid and *Lymnaea stagnalis* snails; to compile a revised survey of literary data on cercariae found in Central Europe; and compiling the literature data on life cycles of birds trematodes recorded in the Czech and Slovak Republics. Ms. Faltýnkova also proposes compiling and revising often incomplete, incorrect or redundantly published data on trematodes in Europe. The latter is an important task, since because of these redundancies the previous list of trematodes was much longer than it should be.

Experienced in examination and description of cercariae in Czech and Slovak Republics, as well in South-East Germany, Poland, Hungary and Austria Ms. Faltýnkova

undertook an extremely laborious job that requires the proper knowledge of morphology of cercariae, their descriptions, life cycles and familiarity with extensive published data. The author's own examination of about 13,500 molluscs of different families show the scope of the work she carried out. During her research in South-East Germany (of 6403 snails from 28 species which she examined from 26 localities, 4.9 % were infected), she analyzed 23 trematode species at cercaria stage, 6 determined only as representative of families, and 7 metacercariae species. These species, compared with about 50 published papers, were the basis for establish the correct species names, synonyms, and identifying redundant, invalid or provisional names referring to the species recorded in this region. Some of cercariae which were unidentified, were included tin *species incerte sedis*. Thus, the big part of the fauna of cercariae and corresponding terminology was comprehensively verified and is now available as a proper starting point for other investigators.

The two manuscripts prepared for publication - based on Ms. Faltýnkova's own investigations in Austria, Czech and Slovak Republics, Poland, Hungary and also in South-West Germany - focused on snails of the family Planorbidae (12 species) and *Lymnaea stagnalis* (Lymnaeidae). Regarding Planorbidae - among 7826 specimens were examined, 11.5 % were infected with cercariae, while among 3628 *L. stagnalis*, 26.3 % were infected. Among Planorbidae, the most infected species was *Planorbarius corneus* (35.6 %), while *Planorbis planorbis* had the highest number of cercaria species (15). *L. stagnalis* harboured 19 cercaria species (6 of which were also at metacercarial stage) and 5 species of metacercariae only. These two papers are supported with a voluminous literature on cercariae occurring in these hosts, which warrant the proper verification of cercariae description as in previous paper dealing with cercariae of a South-West Germany (description of cercariae, provisional names, synonyms and *species inserte saedis*). The findings showed 28 cercariae species (one also at metacercarial stage) and 6 species of metacercaria.

The investigations presented above enabled Ms. Faltýnkova to develop the keys for determination the cercariae and metacercariae of previously verified species of trematoda. The correct determination is very important for researchers working on trematode life cycles and for the study of snail parasites. The advantage of these keys is the selection of mainly external characters, as the shape and size of body and tail, their relations, number, size and position of ventral sucker, the most visible components of spination (spines, stylets), pigmented or unpigmented eyes, and - in several families - also the flame cell formula and some other details of excretory system. Based on developed keys, the cercariae may be determined to the trematode genus and species. The only concern may be that this set of

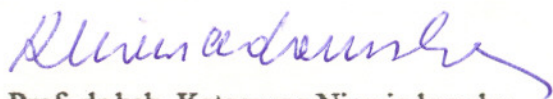
characters may be inadequate as a general key for all European cercariae. The keys are supplemented by high quality figures of all included species clearly showing morphological features of the species. The figures also demonstrate the author's excellent knowledge of them structure of cercariae and her scientific inquiry, especially when body spination and excretory system are studied. They indicate that Ms. Faltýnkova is an experienced investigator, smoothly navigating in the difficult-to-study world of cercariae.

The interesting supplement to the main idea of the Ph.D. thesis is short paper (published in *Acta Parasitologica*) discussing the possibility of a new intermediate host of *Fascioloides magna* – the lymnaeid snail *Radix peregra* apart from well known species *Galba truncatula*. The natural finding in *R. peregra* was confirmed experimentally. The authors received cercariae in snails experimentally infected with miracidia that develop inside eggs taken from adults *F. magna*.

The last part of the thesis: "Checklist of the Trematodes (Digenea) of birds of the Czech and Slovak Republics", was a joint paper with J. Sitko and T. Scholz. It is an important work, and a part of the contemporary trend in cataloguing living resources of our planet. The results are very interesting, as the number of trematodes of birds from both, Czech and Slovak Republics increased to 260 including 83 species, reported from the Czech Republic for the first time. Ms. Faltýnkova provided data on the known life cycles complementing existing general knowledge about the species.

In conclusion, I would like to stress that the research area chosen by Anna Faltýnkova is very important, but also difficult. Studying cercariae not only requires a lot of patience and precision in investigating features important for taxonomy, but it also requires a familiarity with the existing voluminous literature on subject, which is necessary for verification and organization of data, to obtained a set of cercariae with correspond to trematode species described on the basis of their adult stage. The contributions of Anna Faltýnkova documented in her thesis are genuine, important and undoubtedly advance her research area. I, therefore, wholeheartedly recommend that she be granted the Philosophy Doctor degree.

Warszawa, 28.09.2006



Prof. dr hab. Katarzyna Niewiadomska  
W. Stefański Institute of Parasitology  
Polish Academy of Sciences

## DEVELOPMENTAL STAGES OF TREMATODES (DIGENEA) IN FRESHWATER MOLLUSCS IN CENTRAL EUROPE

### *Reviewer's report*

The submitted Thesis summarizes the main results obtained by the candidate during her experimental studies that focused on free living stages of larval digenetic trematodes. It is compiled as a set of author's published papers as well as of manuscripts prepared for publication and all of them are focused on the studies of digenean Trematodes in various countries of Central Europe (Austria, Czech Republic, Germany, Hungary, Poland, Slovak Republic). The presented data deal with the distribution and description of trematode larval stages of various genera and species detected water snails in the area and include illustrated keys to identification of cercariae and metacercariae developing in planorbid snails and a great pond snails *Lymnaea stagnalis*. At least, the Thesis presents information devoted to host intermediate hosts of *Fascioloides magna* introduced to the area. To summarize the recent knowledge on fauna of digenean flukes in Central Europe a checklist of Trematodes in the Czech and Slovak Republics is presented.

The Thesis has classical structuring, including Preface, Introduction, Results, Conclusions, References and Appendix. Main part of the Thesis - Results is devoted to five independent parts: 1<sup>st</sup> - a survey of cercariae from South East Germany; 2<sup>nd</sup> and 3<sup>rd</sup> - surveys of cercariae from planorbid snails and the most common aquatic molluscs *Lymnaea stagnalis*, which are accompanied by the taxonomical keys, 4<sup>th</sup> - study on intermediate hosts of *Fascioloides magna* introduced from North America to Central Europe, and 5<sup>th</sup> represented by a checklist of adult trematodes of birds in the Czech and Slovak Republics. The chapter References is related to the Introduction and Conclusions, Appendix presents author's CV, List of her publications, including the information on scientific conferences participation.

One of the major positive of this Thesis is a complex methodological approach, which utilizes a number of methods ranging from parasitological examination of the trematode hosts to deep taxonomical studies. This approach enabled to examine potential links between the specific host-parasite interactions as well to prepare a taxonomical key facilitating the identification of trematode larval stages. In the presented papers the candidates describes all the methods and experimental protocols clearly and in sufficient details. Undoubtedly, the candidate was able to acquire all these methods and apply them successfully in her study.

On the beginning of the Thesis the candidate presents very valuable Introduction to the history of studies focused on trematodes and shows difficulties which accompany the investigations in this area till present, including the problems with explanations of the parasites life-cycles and, therefore, with taxonomical determinations of the larval stages. Well arranged and comprehensible text shows the past and present situation in each country from which the material originated.

In the chapter Results, the candidate presents parasitological findings in 8 species of planorbid snails and *Lymnaea stagnalis*, *Lymnaea truncatula* and *Bithynia tentaculata*. A total of 58 species of trematodes of 14 families were found in Central Europe, and the recent candidate findings are analysed with literature data.

The candidate obtained many valuable and novel results, which are sufficient for Ph.D. degree. The Thesis has been elaborated satisfactory, there are no substantial mistakes, incorrect statements, clumsy formulations or formal imperfections throughout the text.

*Specific comments:*

1) The candidate made a deep ecological and morphological studies using methods which are commonly applied in the research on trematode taxonomy. *Despite the fact the methods which were appropriate for her study, does the candidate confirmed her results by other investigations (e.g., on chaetotaxy, experimental infection in final hosts)?*

2) The candidate noted that most prevalent larval stages belong to *Opisthioglyphe ranae*, *Echinoparyphium aconiatum* and *Plagiorchis elegans*. Comparing the literature data, no changes were recorded in the prevalence of larval stages of the species *Echinoparyphium aconiatum*, *Echinostoma spiniferum*, *Opisthioglyphe ranae*, *Plagiorchis elegans*, *Trichobilharzia szidati*, *Bilharziella polonica*. On the other hand, she was not able to detect several trematodes found mainly in planorbis in the past (e.g. *Alaria alata*, *Neodiplostomum spathoides*, *Dendritobilharzia pulverulenta*, *Gigantobilharzia suebica*) and she consider their occurrence is rare, although (according to Sitko et al., 2006) some of them were recorded as adults in Central Europe. Last but not least, comparing the literature data, the lower spectrum of diplostomid and strigeid species found by the candidate in the snails is, probably, due to differences in the composition of fauna of migratory birds. *My question is: Are there any other factors which can explain the discrepance between the candidate findings in planorbid snails and other snails and the literature data? If yes, which other factors can be considered as well?*

3) Concerning the detection of *Trichobilharzia szidati* in *Lymnaea stagnalis*: Does the candidate performed other methods for distinct taxonomical subsumption of her findings? According to my view, these information has to be mentioned in the discussion of MS „*Larval trematodes (Digenea) of the great pond snails,.....*“ more in detail.

4) How the candidate can explain the relatively wide spectrum of intermediate hosts of *Fascioloides magna*?

4) To which scientific journals the candidate plan to submit the manuscripts?

5) Who is the author of presented pictures?

6) According to my view, it will be useful to publish independently in some scientific journal also the chapter Introduction presenting the history of research on digenetic trematodes.

**SUMMARY**

**Result obtained by the candidate and presented in the Theses are interesting, novel and valuable. They significantly extend our knowledge about the occurrence of digenetic trematodes in Central Europe. Therefore, RNDr. Anna Faltýnková can be awarded the degree Ph.D.**

Prague, 11 September 2006



doc.RNDr. Libuše Kolářová, CSc.  
Department of Microbiology  
Ruská 85, 100 05 Prague 10

CC: RNDr. Anna Faltýnková  
Prof. RNDr. Tomáš Scholz, CSc.