

Report on Thesis 'Revision of the paraphyletic 'Pseudophyllidea' (Eucestoda) with description of two new orders Bothriocephalidea and Diphyllbothriidea' by Roman Kuchta, University of South Bohemia

The first point to be made is that Roman has taken on a difficult task in trying to understand and bring order to the Pseudophyllidea. Although, he has had the advantage of the use of molecular methods to provide an outline for his classification, the acquisition of appropriate material for sequencing in this widespread group is not straightforward and, of necessity, many important taxa have not been available. Roman outlines eight practical problems involved in the study, and the fact that a comprehensible and convincing provisional classification has been produced is a testament to the industry and insight of the candidate.

The most important finding of the thesis is that the old order Pseudophyllidea can no longer be considered valid as it consists of two unrelated monophyletic taxa. It has long been realised that the order contained two distinctive subgroups, but with the arrival of molecular phylogenetic studies of the class Cestoda it gradually emerged that pseudophyllidean genera were distributed in two parts of the tree. The published molecular phylogeny (Appendix IV) and the further molecular studies reported in the thesis have shown convincingly that the two groups are unrelated and should be considered distinct orders. The names Diphyllbothriidea and Bothriocephalidea have been adopted for these taxa.

The bulk of the thesis is a detailed morphological study of all the genera of the Bothriocephalidea and a summary of the Diphyllbothriidea. The molecular coverage is not enough as yet to provide a detailed framework for the internal classification of the Bothriocephalidea, so the established four family, morphologically based, system is retained. A suite of 80 morphological and biological characters have been used to develop a character matrix, but the resulting trees were too weakly supported for use in classification. In the 'remarks' sections referring to each genus the relationships as revealed by molecules have been discussed, where known. Most generic diagnoses and remarks were based on observations of archival specimens, many of which are not in ideal condition for morphology and are not suitable for sequencing. The candidate has been indefatigable in seeking out specimens from a wide range of sources, but has found that in several cases material is not extant or at least not available.

The Diphyllbothriidea is not treated to the generic level. The valid families (3) and genera are listed and the order defined. A molecular phylogeny is presented, showing that there are two sister clades, one consisting of the families found in amphibians and reptiles and the other consisting of families from birds and mammals. The sample size is small, but the validity of some established genera is called into question.

Appendix IV contains five published papers, on four of which Roman is the senior author. Two are detailed descriptions of new bothriocephalidean genera in the *Journal of Parasitology*, two are reviews of major bothriocephalidean genera in the journal *Folia Parasitologica*, and the fifth is a molecular phylogenetic study in the *International Journal for Parasitology*. This latter is a major contribution to cestode phylogeny studies. All three journals are prestigious international peer-reviewed parasitology journals. Other relevant papers, such as a very interesting paper on *Eubothrium* eggs, have not been submitted as part of the thesis, but show that the

candidate has a wide interest and has contributed in various ways to our understanding of the 'pseudophyllideans'.

I'd like to congratulate Roman on his work in this thesis. He has been able to produce a comprehensible system from the confusion of the old order. Having attempted to do the same some years ago, I acknowledge that Roman has been much more successful, although admittedly he has had the advantage of molecular results. I would also like to acknowledge the generally good English in the study. Although I will suggest a few stylistic changes to him, I was able to comprehend nearly everything written.

There are, of course, some points I would like Roman to clarify and discuss.

Firstly, I would like to suggest that paraphyletic is not the best term to use for the relationships of the two new orders. There are many definitions on the web, but they all are similar. A useful one is: 'A group of taxa that includes an ancestral taxon but not all descendants of that taxon - eg "fish" is a paraphyletic group because it does not include the other vertebrates, which undoubtedly also derive from the ancestral fish species.' My interpretation would be that the two new orders are related polyphyletically. I would like Roman to discuss his thoughts on this point.

I notice that you do not include the matrix of morphological characters. I think it should be included as it may well be useful for mapping characters on to a molecular phylogeny. This would indicate what morphological characters are likely to be of phylogenetic value. The poorly resolved tree would also be interesting, to show what morphology can, and cannot, tell us.

I notice that in Appendix III you consider that the two species defined on biochemical characters, i.e. *Bothriocephalus funiculus* and *B. gregarius* are *nomina nuda*. Could you explain this decision. (see also p. 30).

Could you comment on the interpretation of the apical disc? For example *Probothriocephalus* (fig. 8C) and *Neobothriocephalus* (Fig. 8A) have a structure apically on the scolex. The interpretation of the neck also appears a bit problematical to me.

Another character which may be problematical is the pseudoscolex. For example in *Parabothriocephaloides* and *Parabothriocephalus*. If the scolex of the latter may be detached, is the pseudoscolex a good character for the former?

On p. 17 in the diagnosis of the Bothriocephalidea you say that the two bothria are always of different shape and depth. Is this invariably true?

There are a few points of interpretation of the molecular phylogenies I would like the candidate to comment on.

On p. 34 you stated that according to the molecular phylogenies *Echinophallus* is the sister to *Parabothriocephalus* and other Echinophallidae genera. My interpretation is that in the published paper it is the sister to a group of bothriocephalid genera and in the new study (Fig. 10) it is in a polytomy with some echinophallids and a bothriocephalid clade.

On p. 54 you state that *Ptychobothrium* has a tendency to group with *Penetrocephalus*. In Figure 10 it doesn't appear to be anywhere near *Penetrocephalus*.

On p. 82 you say that the basal position of the Cephalochlamydiae is strongly supported by molecular data. This is true for Brabec et al (2006), but on your own tree (Fig. 9) it forms a sister to the reptile-inhabiting genera.

In figure 10 the Bothriocephalida is not monophyletic. Can you explain the discrepancy between this and the published phylogeny? Why did you choose a trypanorhynch as the outgroup rather than one of the members of the sister group in the published phylogeny, i.e. Tetraphyllida/Lecanicephalida?

You make a convincing case for the polyphyly of *Bothriocephalus*. I hope that in your further studies you are able to divide it up.

I have just a couple of pedantic points I would like the candidate to consider.

On p. 55 you consider '*Polyonchobothrium* in part' as a synonym of *Senga*. Using this logic you could say that '*Taenia* in part' is a synonym of nearly all older cestode genera and '*Bothriocephalus* in part' is a synonym of *Andyceustus* and *Plicocestus*.

On p. 41 you form a new combination for *Neobothriocephalus mamaevi*, but later say it is probably conspecific with *N. aspinosus*. If this is the case, you have erected a new combination that is likely fall as a synonym.

There are other minor points that should be addressed, but need no formal discussion.

p. 10. The Order from elasmobranchs is Diphyllida van Beneden in Carus, 1863 not Mola, 1921.

p. 17, line 26. Diphyllidobothriids should be bothriocephalids, also line 29.

p. 18, 5b. What shape is fun?

p. 18, 6b. The couplet should lead to 9

There are unbalanced couplets on p. 18, couplet 4, p. 19, couplet 12, p. 21, couplets 15 and 18.

p. 28. If *Fissurobothrium* was never described should it be a *nomen nudum*?

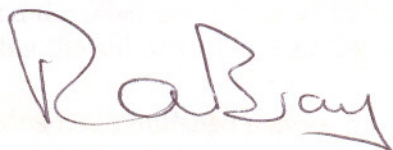
Figure 1. H and E. legends are transposed.

p. 55. How can the vitelline follicles be circumsegmental and separated medially?

Figure 10. It would be useful if the nominal families were indicated as in Brabec et al (2006).

Appendix IV. Why is there no 'type-host' column? (cf Appendix III)?

Report by Dr R A Bray





VILLE DE
GENÈVE

Roman Kuchta PhD report

Revision of the paraphyletic « Pseudophyllidea » (Eucestoda) with description of two new orders Bothriocephalidea and Diphyllbothriidea

Several recent works on the phylogenetic relationships of the Eucestoda based on morphological or molecular characters showed the presence of two unrelated clades and so a possible para- or polyphyly of the Pseudophyllidea van Beneden, 1863.

An accurate revision of the order Pseudophyllidea using morphological and molecular approach represented a nice subject for a PhD thesis, which Roman Kuchta undertook with enthusiasm and courage.

The present thesis is based on the morphological and/or molecular study of 93 taxa belonging to 62 genera. This work represents the most complete data set on "Pseudophyllidea" cestodes and an important step in the knowledge of this difficult group. Roman Kuchta chooses to not present the morphological revision of the order Diphyllbothriidea because of the lack of available comparative material. He revised the Bothriocephalidea, gave a key for the families and genera belonging to this order and updated the diagnosis of all the concerned taxa (4 families and 44 genera). For each genus, he makes numerous detailed and accurate morphological remarks, proposed three new genera, resurrected as valid genera three taxa and invalidated 5 genera.

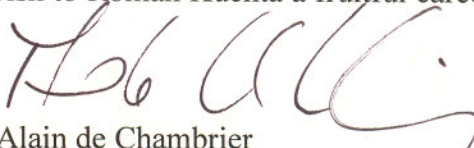
The manuscript is accurately presented, with good quality illustrations and in equilibrates number. The bibliography is complete and detailed. Various appendix in the end of the work show useful lists, particularly the appendix III, with the preliminary list of valid species within the order Bothriocephalidea with their synonyms.

The candidate, since the beginning, took contact with numerous specialists, and as soon as the first results obtained, published his preliminary results. He also took part in all the important recent meetings in parasitology, which shows his real interest for the subject.

I have some questions and remarks in a separate sheet.

I recommend the acceptance of the PhD thesis and wish to Roman Kuchta a fruitful career in Biology.

Geneva, May 17, 2007


Alain de Chambrier
Scientific Officer

Specific remarks

P. 13. It is not clear, for samples collected for DNA purposes, if it is for each individual specimen, a voucher fixed in hot formalin, is conserved for further identification or verification of specific status. Same remark for Brabec et al., 2006, p. 1536 for which Roman Kuchta wrote page 13, end of second paragraph, "for methodology – see Brabec et al. 2006" and within no information is given.

p. 17, end of second paragraph, last sentence, ... "indicate a very close relationships of Bothriocephalids", (not diphyllbothriids).

p. 85 point 2) Same remark as above, (in p. 13), precise the importance of comparison voucher when collecting for DNA. Each individual specimen must have a voucher for DNA in adequate fixative medium (for example, gravid proglottids) and the remaining part of the worm fixed in hot formalin for morphological studies (scolex, mature and pregravid part of the cestode)

Some orthographic corrections needs to be made:

p. 22, line 7 possible

p. 36 second paragraph, Gill de Pertierra, suppress the second l

p. 36, idem in line type- and only species

p. 70 figure 7, idem in line 7, twice

p. 79 line 26, "Artic", to Arctic

p. 82, last paragraph, line 3 "acquisiton"

Two questions:

- In p. 79, paragraph 5, you mentioned the quasi absence of bothriocephalids in freshwater fish in Amazon and Parana basin, which contrast with the extreme diversity of proteocephalideans in freshwater fish of the same region. Do you have an explanation?

- In p. 79, you mentioned that most of Diphyllbothriids and Bothriocephalids are stenoxenous, sometime euryxenous. As a more strict specificity is generally observed in the more evolved cestodes (i.e. Proteocephalids, Cyclophyllids), do you think that the type of specificity is related to the cestode primitive or derived position?



I have read the manuscript and I recommend the acceptance of the PhD thesis in the field of Biology.

I recommend the acceptance of the PhD thesis in the field of Biology.

May 17, 2007

REFeree'S REPORT

of the thesis presented by Roman Kuchta, MSc. (Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice): REVISION OF THE PARAPHYLETIC "PSEUDOPHYLLIDEA" (EUCESTODA) WITH DESCRIPTION OF TWO NEW ORDERS BOTHRIOCEPHALIDEA AND DIPHYLLOBOTHRIDEA.

The thesis presents a coherent study aimed at taxonomical re-evaluation and revision of the former cestode order Pseudophyllidea using approaches of classical taxonomy and molecular systematics. The work is presented in a form of booklet and comprises a general part presented on 89 pages, and Appendix composed of (i) four tables, (ii) list of published papers (Roman Kuchta is the first author of five papers and co-author of 15 others, all published in top-ranking parasitological journals), and (iii) full copies of five papers related to the topic of PhD thesis. The thesis itself encompasses six multi-part line drawings and two plates of SEM photomicrographs that properly illustrate the most important characters of species of all 44 revised genera belonging to the newly established order Bothriocephalidea. Additionally, two cladograms illustrating molecular phylogenetic analyses of Diphylobothriidea and Bothriocephalidea and the list of 227 relevant literature references are included.

GENERAL EVALUATION

The work of Roman Kuchta is based on a thorough morphological, biological and partially molecular study and documentation of a huge number of representatives of the former order Pseudophyllidea, which leads to suppression of this taxon and proposal of two new orders, Bothriocephalidea and Diphylobothriidea. The thesis includes redescriptions of type species of bothriocephalidean genera, a critical evaluation of their validity and original descriptions of three new genera. The work also provides the complete list of valid genera of the new tapeworm order Diphylobothriidea, however, their detailed re-evaluation is a task for future studies. The new or amended diagnoses of both new orders, four families and 44 genera of the order Bothriocephalidea are included. Morphology-based keys to the families and relevant genera are also presented. In general, the thesis embraces mighty taxonomy work that has been partially published yet and additional sound papers are expected.

CONTRIBUTION TO OUR KNOWLEDGE OF CESTODE SYSTEMATICS AND PHYLOGENY

It is beyond doubt that the results of the thesis represent a substantial contribution to the systematics and taxonomy of the Cestoda. I appreciate that the author has partaken in molecular phylogenetic studies which affirmed former suggestions of reorganization of the order Pseudophyllidea. The methods of formal taxonomy have been applied flawlessly, and the relevance of original descriptions of taxa new for science is obvious. I also highly appreciate that the thesis has been, and the future work of Roman Kuchta will be, focused on "tidying up" one of the most abundant and important cestode group and that this painstaking work will issue in clearly identifiable taxa that are confirmed in a reliable phylogenetic tree. From my point of view, lists of valid species of orders Bothriocephalidea and Diphylobothriidea (see Appendices III and IV) will be extremely useful after they are completed and published.

CRITICAL REMARKS

The quality of formal processing is good. Line drawings and SEM photos are excellent, however, the quality of photos has obviously been reduced by processing of the booklet. The thesis contains several typing errors (see p. 48 – which/witch, p. 78 – Scypocephalidae etc.).

Chapters are arranged logically, methods and techniques used are sufficiently described. Within the chapter Results, I would prefer bothriocephalidean genera to be assigned according to their families (not listed alphabetically). In such case, Figures would be easier to exploit for the rapid publication of the work. Moreover, I have awaited a section which would give information on molecular phylogenetic analyses of the Diphyllbothriidea and Bothriocephalidea shown in Figures 9 and 10, because there are new data in addition to the paper Brabec-Kuchta-Scholz: Int J Parasitol. 36 (2006) 1535-1541. These Figures could be more informative, if families are marked in vertical direction.

QUESTIONS AND COMMENTS ON THESIS ARE AS FOLLOWS:

- *I admire extensive range of used and listed references which include ancient books and papers with original descriptions of genera and species. How have you been able to collect all those rare sources and have you all the copies here in the Institute?*
- *Could you explain in more detail a reason why you consider the diphyllbothriidean family Scyphocephalidae to be valid?*
- *What is your opinion on the future completing the data matrix of morphological and biological characters for a cladistic analysis? Are you sure that the more characters used means the more reliable output?*
- *Do you mean that characters used in differential diagnoses and morphology-based keys should represent apomorphic characters? Do you think that cladistic analyses might result (or help) in a construction of keys?*
- *What is your attitude to recently promoted phylogenetic nomenclature expressed e.g. in PhyloCode?*


THE GENERAL EVALUATION

I state that the findings of Roman Kuchta presented in his thesis "REVISION OF THE PARAPHYLETIC "PSEUDOPHYLLIDEA" (EUCESTODA) WITH DESCRIPTION OF TWO NEW ORDERS BOTHRIOCEPHALIDEA AND DIPHYLLOBOTHRIIDEA" are original and that he has clearly demonstrated his scientific erudition in solving current problems of systematics and taxonomy. The thesis results are of great quality and the author has unambiguously demonstrated possessing the skills required for an independent scientific work. The proposed thesis fulfils all the requirements constituted by the law, therefore I fully

RECOMMEND

it for the acceptance and sequential defence.

Košice, May 11, 2007



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