

## REFeree'S REPORT

**on the habilitation thesis submitted by RNDr. Roman Kuchta, Ph.D. (Institute of Parasitology, Biology Centre CAS, České Budějovice): EVOLUTION, BIOLOGY AND SYSTEMATICS OF THE BOTHRIOCEPHALIDEA (CESTODA).**

The habilitation thesis, aimed at complex characteristics of Bothriocephalidea (one of the 19 current orders of Cestoda, Platyhelminthes), is comprised of seven publications of the candidate released from 2008 to 2017. Six papers published in top-ranking parasitological journals illustrate gradual gathering of information about bothriocephalid tapeworms during the period from the formal erection and subsequent revision of the order (Kuchta et al., 2008a, 2008b). The latest seventh comprehensive overview, which includes a list of valid bothriocephalid taxa, has been published as a book chapter on cestodes, representing the outcomes of the international collaborative project PBI funded by the United States National Science Foundation (Kuchta and Scholz, 2017 – Dr. Kuchta has actively participated in this project as one of leading experts throughout the duration of the project). This text is either used as the Introduction in the habilitation thesis or as a Summary in the thesis supplement, which is rather unusual.

### CONTRIBUTION TO OUR KNOWLEDGE OF SYSTEMATICS AND PHYLOGENY OF BOTHRIOCEPHALIDEA

It is beyond doubt that the results of the thesis represent a substantial contribution to the systematics and taxonomy of the order Bothriocephalidea. The emphasis is on the classical studies and descriptions or redescrptions of bothriocephalidean species and genera, a critical evaluation of their validity, new or amended diagnoses of the families and morphology-based keys to the families from various host groups. I highly appreciate the fact that the author has partaken in molecular phylogenetic study which has confirmed that only multi-method research is capable to reliably solve the systematics in the higher taxa. Moreover, a list of valid species of the order Bothriocephalidea is also extremely useful.

### CRITICAL REMARKS

The quality of formal processing is good, however, common page numbering is missing throughout the document and thus the orientation according to the introductory list is impossible. The IF is missing in paper No. 7 (Brabec et al., 2015). In the 19 lines of Introduction, there are some inaccuracies: erected orders (line 9), the candidate participated in preparation of 10 of 21 chapters (line 14), collaborative project (2008-2015) (line 16). Some minor bugs are also in the material Summary of habilitation work which comprises the identical copies of the List of works, Introduction and the chapter Kuchta and Scholz (2017); the copies are completed with the Slovak CV of Dr. Kuchta. Here we can follow a high-quality scientific career of the candidate including plenty of international collaborations and field research stays as well as his publication and citation reports and lecturing activities. Here my only critical notes are the following: the Czech grammar in the sentence "Spolupracovník mezinárodního projektu **podpořeného** NSF", and my incertitude in the management of three post-doctoral students (Bazsalovicsová, Hernández-Orts, Schaeffner).

My main objection lies in the fact that the habilitation work does not include at least a short passage written only by the candidate, evaluating broader outcomes and perspectives in cestode systematics. It would allow him to define own personal opinions, for instance about

his attitude to the recently promoted phylogenetic nomenclature expressed e.g. in PhyloCode etc.

#### THE GENERAL EVALUATION

I state that the research profile of RNDr. Roman Kuchta, Ph.D. presented in his habilitation thesis "EVOLUTION, BIOLOGY AND SYSTEMATICS OF THE BOTHRIOCEPHALIDEA (CESTODA)" is of high quality and has clearly demonstrated the scientific erudition of the candidate in solving problems of systematics and taxonomy of selected tapeworms. The thesis results are of great quality; however, the habilitation work does not give broader outcomes and perspectives in cestode systematics which would allow the author to define his own personal opinion.

Košice, September 11, 2017



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## **Reviewing of the habilitation thesis submitted by Roman KUCHTA**

For his request for habilitation, Roman Kuchta provides us with an overview from his work on fish tapeworms: Evolution, biology and systematics of the Botriocephalidea (Cestoda).

The presentation of his document, including the introduction, is based on 8 publications: 7 as first author and one as the last author out of a total of nearly 80 publications of which 62 referenced in Pubmed (results of crossing Kuchta-R AND parasites ), a choice not always easy among many papers.

Roman Kuchta propose a complete review on BOTHRIOCEPHALIDEA, a new order erected by himself and al. (Kuchta, Scholz, Brabec and Bray, 2008) as the result of the revision of the order PSEUDOPHYLLIDEA (Platyhelminthes: Eucestoda) in to new orders : BOTHRIOCEPHALIDEA mainly parasites of fishes and DIPHYLLOBOTHRIIDEA parasites of tetrapods mainly mammals. So the starting point of this review is strong precisely is how define this two new orders and after propose a revision of the order BOTHRIOCEPHALIDA. Of course this preliminary taxonomic revision suggest the possibility of the revision of tapeworms from hosts, the description of news species and the erection of new genera. The general view of this work is noteworthy for the various approaches used (morphological and molecular) and absolutely necessary for systematic, identification and study of the biodiversity.

All it perfectly resume by “ Newly collected material should include samples for molecular analyses, which should be clearly assigned to particular tapeworm specimen from which it has been taken. Such material can be easily obtained by preservation of a few segments from the very end of strobili in absolute ethanol. Importantly, host should be properly determined and whenever possible, photographed and its tissue sample should be preserved in absolute ethanol and deposited together with the parasite to allow ex post confirmation of its identity”. Actually it’s the only way to look to the future the characterization of parasites. In a global context of parasitism, I would add samples from the intermediate hosts for the knowledge of epidemiological situations.

It would be presumptuous of me to discuss the publications accepted in international journals (eg: International Journal of Parasitology) which have been peer-reviewed by

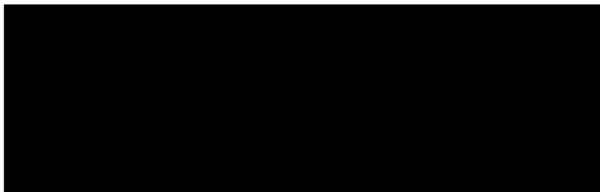
specialists of tapeworms and certainly from PBI participants. However I appreciate the quality of the iconography, drawings and pictures in SEM.

I have read with a big pleasure all the articles and I consider myself as not specialist of Cestoda and especially isolated from fishes. So I'm going to use for teaching the new concepts especially about the "Pseudophyllidea".

According to the Curriculum vitae, the present habilitation thesis is based on candidate's long-term research (fifteen years). During this time he realized many staying in worldwide laboratories specialist of Cestoda which is remarkable. He had also the opportunity to work with a large number of world renowned researchers and he acquired an extraordinary competence. That is why he actively participated at an international project funded by the National Science foundation (Planetary Biodiversity Inventory program) to map the cestode diversity globally and now as leading expert. The participation of Roman Kutcha in this project is very important and his contribution (9 chapters out of 21) shows a great knowledge of the group. As such it isn't difficult to recognize the exceptional quality of the scientific work provided and available for the scientific community. This may be due to the fact that it has been able to focus on a zoological group during a long time and so acquire a competence above the average for a young scientist.

In conclusion on the basis of the document, I give my agreement to the request of Roman Kutcha for habilitation in the field of Parasitology.

Reims, the 28<sup>th</sup> October 2017



H.FERTÉ

## **Review**

### **Evolution, biology and systematics of the Bothriocephalidea (Cestoda)**

Habilitation thesis of Dr Roman Kuchta

The thesis consists of an Introduction and six papers of which the candidate is a co-author. The candidate is the senior author of the Introduction and five of the selected papers.

The Introduction is in the form of a chapter in the book "Tapeworms from vertebrate bowels of the earth 2008-2016" published in 2017. The chapter titled "Bothriocephalidea", by Kuchta and Scholtz is a result of the candidate's participation since 2008 in the major international project "Planetary Biodiversity Inventory (PBI)". The Special Publication which was a part of this programme was designed to map cestode diversity globally. It is a measure of the candidate's standing in his chosen field of cestode biology that he was invited to participate in this prestigious project. The chapter serves as an extended summary of the results from the six publications that follow and compares the current status of our knowledge of different aspects of the biology and taxonomy of the Bothriocephalidea with that prior to the PBI project. It includes a useful table listing all the currently valid bothriocephalidean taxa with their type hosts. The chapter is well-written and presented and serves as an excellent introduction to the papers that follow.

The first paper is a Short Communication proposing the suppression of the long-established tapeworm order Pseudophyllidea and the proposal of the two new orders Bothriocephalidea and Diphylobothridea. The main source of evidence for the decision to establish the two new orders was a 2006 publication, of which the candidate was a co-author, which provided conclusive molecular evidence that the former order Pseudophyllidea consisted of two unrelated clades. This evidence supported and confirmed results from previous studies suggesting paraphyly or polyphyly of the order Pseudophyllidea. This paper includes a diagnosis of the two new orders, describes the four morphological features that distinguish them and their different definitive host groups and names the spectrum of definitive hosts used by them.

The second paper is the largest and perhaps most significant of the six selected. It consists of a complete revision of the order Bothriocephalidea with amended generic diagnoses and keys to families and genera. This is probably the most important contribution in recent times to the systematics and taxonomy of cestode parasites. In their section on the diagnoses of different genera, the authors chose to list the genera alphabetically irrespective of the family to which they belong. While I appreciate the reasons given for this arrangement, I would have preferred to have seen them arranged first according to family. Nevertheless, the amount of detail provided is impressive and the morphological descriptions, SEM micrographs and line drawings are excellent. The authors list the obstacles and difficulties associated with a work of this magnitude, including the non-availability and/or poor quality of the type material of many species, variations in methods of fixation, and the difficulty of obtaining specimens of some species, particularly from deep-water fish hosts. It is to

their great credit that they have produced such a comprehensive account in the face of these difficulties. In discussing the possible coevolution of bothriocephalids with their hosts, the authors refer to the presence of some basal taxa in ancient fish groups such as paddlefish and sturgeons. I would also have liked to have seen some discussion of another notable feature - the large number of genera and species reported from bathypelagic marine fish of the family Centrolophidae. Future studies will undoubtedly result in the identification of a number of new species within some currently recognized as a single species, such as *Bothriocephalus scorpii*, and in the synonymy of many species within genera such as *Senga*. It can thus be safely predicted that an updated revision of the Bothriocephalidea will be forthcoming in the near future as more parasite specimens become available for scrutiny and analysis.

The third selected paper provides descriptions of two new species of the genus *Bothriocephalus* from marine fish caught off South Australia and New Caledonia. The genus *Bothriocephalus* is the largest in the order Bothriocephalidea with more than 100 nominal species, many of which are probably invalid. Quoting some unpublished data, the authors stated that comparative genetic analyses of both new species with sequences of available species showed them to be most closely related to *B. scorpii*. As the latter species has been reported from a large number of hosts worldwide, it would have been informative to know which of its many host species the parasites sequenced came from. The morphological descriptions and illustrations of the new species are detailed and meticulous.

The fourth paper is a revision of bothriocephalids from lizardfish in the Indo-Pacific region based on newly collected material from type hosts and available type and voucher specimens. Only two species out of the nine listed in the literature are considered valid, and these are redescribed. The authors illustrate and stress the importance of using standard fixation methods of freshly collected cestodes for descriptive purposes. In particular they mention the loss of hooklets from the scolex in worms that have not been correctly processed. This is valuable information for researchers collecting material in the field. In the Discussion I noted the reference to marked seasonal variation in the prevalence of one bothriocephalid species, suggesting a life span of less than one year in the definitive host, and wondered if this is true for other bothriocephalid species. I was also interested to read about the presence of a *scolex deformatus* in two pairs of unrelated species in the families Bothriocephalidae and Triaenophoridae. Could this be related to the physical structure of the intestine and/or pyloric caeca of their hosts?

The fifth paper is a survey of bothriocephalideans parasitizing freshwater fish in Africa, based on examinations of type specimens and freshly collected material. This resulted in only seven species out of the 19 taxa listed in the literature being considered valid. One new species is described and a new genus proposed. Only species of the family Bothriocephalidae have so far been reported from African freshwater fish, but the authors point out that large areas of the African continent which harbour the majority of fish diversity remain virtually unsampled. There is thus no doubt that many more species await discovery. In the Discussion the authors make a number of important points regarding the use of appropriate fixation methods, adequate descriptions and the deposition of specimens in internationally recognized collections. They describe in detail the entire process from collection, fixation and preservation to full description and tissue sampling. This is essential

information for anyone handling such material from Africa or elsewhere. As with the previous publications, the descriptions in this paper are detailed and meticulous and the illustrations are informative and of excellent quality.

The final paper, published in 2015, reviews and assesses the relationships of bothriocephalideans using multi-gene molecular phylogenetic analyses. The results support many of the results from the 2008 revision (the second of the selected papers), but also challenges many of the traditional classifications based mainly on morphological characters. One family recognized in the 2008 revision is reduced to synonymy, while a formerly recognized genus is resurrected. Of considerable interest is the finding that host-parasite co-phylogeny has not played a major role in the evolutionary history of bothriocephalideans. The more primitive and ancient hosts are infected with some of the most derived taxa, while morphologically divergent groups of fish that inhabit the same geographical regions host phylogenetically closely related cestodes. The molecular results show that the Bothriocephalidea is composed of a larger number of well-defined clades than traditional views, based mainly on morphological features, would suggest.

In summary, this thesis represents an impressive body of work. It is in fact a continuation of the good work begun in the candidate's earlier master's and Ph.D. studies and establishes him firmly as an international authority on the biology and taxonomy of the Bothriocephalidea. Although many changes will be made to the detailed relationships within this order of cestodes, the basic classification formulated in the landmark 2008 revision is likely to remain unchallenged. Of course further updates are inevitable as new species are discovered, and it is to be hoped that researchers working on these species will heed the sound advice on the handling, preservation and description of these species given in these publications. Dr Kuchta has already made a significant contribution to our knowledge of this group of cestodes and no doubt will continue to do so. On the basis of this thesis I have no hesitation in commending him to you in support of his habilitation.



Ken MacKenzie, Ph.D., D.Sc.

26 October 2017