

June 18, 2012

Reviewer: Anindo Choudhury, St. Norbert College, 100 Grant Street, DePere, Wisconsin 54115, U.S.A.

**Review of Ph.D. thesis of Anirban Ash, School of Doctoral Studies in Biological Sciences
University of South Bohemia in České Budějovice Faculty of Science.**

Thesis Title: **Diversity of tapeworms (Cestoda) in freshwater fish of India**

General Comments:

This thesis contains descriptions and revisions of tapeworm taxa from freshwater fishes of India and Bangladesh, using freshly collected material as well as museum depositions where available. In addition, a portion of the work, - especially on *Gangesia* - extends the analysis using molecular data.

The work is of very high quality; the descriptions are thorough, accompanied by near exhaustive reference to museum collections around the world, supported by excellent illustrations, ideally prepared whole mounts, sectioned material and modern techniques (both molecular and electron microscopy). The work sets a new bar for taxonomy and systematics of tapeworms from the Indian subcontinent. I consider this pioneering work as a sort of second life of helminth systematics on the Indian subcontinent, in the same way that Woodland and his peers pioneered the first birth of the taxonomy of Indian helminths in the early part of the 20th century. The work is therefore of utmost merit and deserves to be recognized as such. Given the time frame and logistic issues involved in expeditions to the tropics and to developing countries, I can also appreciate the challenges and limitations.

Having complimented the work and pointed out its strengths, I can now turn to what I perceive as its weaknesses. First, given the title of the thesis, one would expect a much wider coverage of the Indian subcontinent. As it stands, all the described expeditions are in the same general geographical area - the northeastern part of the subcontinent. From the perspective of general geological history of the drainages and the fish fauna, there is little difference between Bangladesh and West Bengal. Assam is also contiguous with these areas and many of the drainages of this vast deltaic region flow through it. Entire drainages along the eastern seaboard, Mahanadi, Krishna, Kaveri, Goadvari and such are not represented, nor are the tributaries and distributaries of the Ganga in northern and northwestern India. One can and should also include Pakistan in any consideration of Indian subcontinent helminths. I would like to hear the candidate's thoughts on this at his defense.

Second, given the thoughts expressed in the introduction and references to the wider purpose of taxonomy and systematics, there is an opportunity to extend the revision work to bigger questions of evolution and particularly, biogeography. There are robust geological scenarios about the history of the Indian subcontinent, its origin and subsequent docking with the Eurasian plate. That history has had a profound and lasting impact on the fish fauna of India, its composition and indeed its parasite fauna. I share the author's sentiments that there is an extended purpose to the study of biodiversity, beyond the task of cataloguing species (which is no doubt important and critical). Given the unique and fascinating geological history of the region and its fish fauna (see Talwar and Jhingran's excellent two volume series 'Inland Fishes', and V.G. Jhingran's classic 'Fish and Fisheries of India'), there could have been some discussion, even in a limited and focused way, about the fish-cestode relationships in this region, at least in the eastern region. This could have been the place and time to make some predictions and craft some testable hypotheses, for future work. I would request the author to consider this in any future endeavor he undertakes.

In summary then, while every work can be critiqued and its limitations pointed out, one must evaluate it in terms of its overall contribution and merit. My perceptions of its limitations

notwithstanding, there is little doubt in my mind that the work, being a combination of truly outstanding depth, quality, and a pioneering effort, easily qualifies for the high scientific merit of a PhD thesis. I commend the candidate (and his advisor) for it. This tome, once bound will certainly be on my shelf and I predict will have to be first opened and read before anyone wishes to do any work on cestodes from the Indo-Malayan and neighboring biogeographical areas.

Specific comments

I have made several specific comments, mostly having to do with grammar, style, and typographic errors, on the manuscript itself, which I will pass on to the candidate at the defense. I will only confine myself to the more important issues here and also raise some questions for the candidate to consider.

1. I would suggest changing 'fish' to 'fishes' in your title because you are dealing with different species of fishes as well. It is more typical and the standard usage when referring to fish in this context. Generally, 'fish' is used to refer to them indiscriminately and collectively, for example in a net catch or fish on your plate.

Consider the following titles quoted randomly from books on my shelf'

Parasitology of Fishes – Dogiel
Synopsis of the Parasites of Fishes of Canada – Margolis & Arthur
Parasites of North American Freshwater Fishes – Hoffman
Parasites of Fishes in Wyoming – Mitchum
Parasitic Nematodes of Freshwater Fishes of Europe – Moravec
Metazoan Parasites of Salmonid Fishes of Europe – Moravec
South American Trematodes Parasites of Fishes – Kohn et al
Guide to the Parasites of Fishes of Canada – Volumes I – IV – various authors
Parasites of Puerto Rican Freshwater Sport Fishes – Bunckley Williams
etc.....

2. Page 8. "As Boero (2010) correctly stated – "it is much more 'scientific' to identify specimens with machines than doing it by simply looking at them", I am not sure what this even means. First of all, we look at specimens using machines, a cold field emission state of the art SEM can "see" in great detail the surface of a single bacterium, 250,000 times, a fluorescence scope (can pick out in vivid detail the cytoskeletal filaments inside a single cell that appears as big as your computer screen, a confocal microscope can bring in sharp focus hundreds of planes of view through a single cell (its like histology without sectioning) through it. So, I don't know what "simply looking at them" means, Ultimately, identifying a specimen involves evaluating information from several inputs, not looking at a sequence alone or a histological section or a scanning electron micrograph alone! And I would also draw attention to all the critiques of using molecular data, especially with barcoding.

3. Page 8. With regard to the criteria listed by Dayrat (2005), some of it is not always practical, but more importantly, there is a wider and deeper philosophical issue behind it here. There is a disconnect in trying to fit the Linnaean taxonomy espoused by the ICZN into the framework of biodiversity. A real understanding of biodiversity is not, in my opinion, about naming species and arguing about what is and is not a real species. As our taxonomy and morphological investigations become more refined, we recognise even smaller and more refined units of diversity by discerning morphological/genetic cohesion and consistency. In the absence of evidence for or against genetic isolation, these units of diversity may be called a species or any hierarchical level of organization below it such as subspecies, varieties, races, populations, etc. Whether we call such a group of morphologically unique individuals a 'population' of a more wide ranging species or a species itself does not alter the fact that this group of morphologically unique individuals maintain their morphological, and hence likely their genetic, cohesion through some mechanism that prevents gene flow. Therefore, we may view species

descriptions, synonymies and taxonomic rearrangements as hypothetical statements. This allows a more objective criticism of the hypothesis and may temper some of the authoritarian opinions that are often expressed regarding the validity of one taxonomic decision or another.

When we describe a new species, we are in fact making a hypothesis that this is an evolutionarily unique product. Like all hypotheses, the quality of the initial hypothesis depends entirely upon the quality of the data and observations used in constructing that hypothesis. Like all hypotheses, this one is also readily falsifiable.

Weak initial hypotheses, i.e. weak species descriptions, may be due to

- 1) small sample size
- 2) restricted sampling in few habitats
- 3) Sampling from a very restricted geographical area
- 4) Poor specimen preparation
- 5) Inappropriate comparisons with presumed related forms
- 6) Inadequate comparisons with other described species from its assumed larger inclusive group (genus)
- 7) Incorrect interpretation of the morphology

Or a combination of some or all of the above.

Species descriptions that do not suffer from these problems may still be falsified by other evidence, such as:

- 1) when characters that were thought to be unique for that species in fact show clinal variation through a geographic range until they merge with that of another previously described species described further away and otherwise morphologically indistinguishable from its congener.
- 2) Another form of evidence is intraspecific variation, such as when a character considered diagnostic for a described species is found to vary widely in that species without any discernible pattern or relation to geography.

What Dayrat proposes comes, at least partially, from a view that there is something concrete about describing and naming a new species, when in fact it is simply aspiring to be the best hypothesis about an evolutionary entity at the time.

So, the question for the candidate is this: What view of species and biodiversity guides your taxonomic and systematic work?

4. Some of the synonymies are quite extensive and cover a significant geographical range across drainages. Given the resolution we are now seeing with DNA prospecting (Perez-Ponce de Leon and Nadler), and its fruitful application in Mexico and elsewhere, is the candidate concerned that discrete evolutionary entities (species, etc.) may be getting lost in the synonymies? Especially where drainage isolation or host-shifting can influence diversification. Were any general criteria used for proposing these synonymies? Did drainage patterns and host associations influence the synonymies? In other words, while the candidate used the criteria of Dayrat in describing species, were there general guidelines for synonymizing species? Maybe these could be briefly mentioned in the introduction, right after the section on Dayrat.

5. The map of India should have the major drainages on it, especially given that the thesis is about cestodes of *freshwater fishes*.

6. With regard to the 'Particular Objectives', it seems that while the first three were undoubtedly achieved, the last two only partially so? Given the dismal state of taxonomy on the Indian subcontinent, it is difficult (if not impossible) to accurately clarify the host spectrum of the cestodes there. Also, there is the problem of sampling area covered. The last objective could also only be partially fulfilled given the material available, although the work on *Gangesia* is, in my opinion, superb.

Amade Chudley
06/22/12

Posudek doktorské disertační práce

Ash, A. (2012). Diversity of tapeworms (Cestoda) in freshwater fish of India.

The introduction to the thesis is well structured, informative and written with a clear language. I especially liked the introduction to past research on tapeworm taxonomy in India, the pitfalls and the clearly stated methodological improvements of the present study. I also liked the messy taxonomical situation which was the starting point for the author's research. Such a situation usually invigorates young scientists penetrating a difficult field and brings new interesting results. The author is to be commended for employing a multivariate methodological approach to his study that included strict rules for taxonomical (nomenclatural) statements, species determination, and reconstruction of phylogenetic relationships utilizing morphological, ultrastructural, and molecular data. The author also had during his doctoral studies the opportunity to be part of and to learn to lead research projects from their starting planning phase, through field work, a multitude of laboratory procedures, and as the publications demonstrate also the writing up of the results of his research. The thesis thus clearly fulfills all requirements for its successful defense.

As a non-parapsychologist I am limiting my review of the thesis to areas of general taxonomy/phylogenetics.

Specific questions/comments:

page 19 in general Methodology: The verification of closely related host species of the genus *Puntius* with the use of BLAST: Based on personal experience with BLAST I would urge against using BLAST as a tool for species identification, especially with a non-coding marker. A direct alignment and a phylogenetic analysis is always preferable. The same methodology was also used in paper 3. Why treating the host with a different methodology than the one used for the parasite?

Fig. 19 and analysis (paper 3): The used marker is unfortunately largely uninformative regarding the precise phylogenetic position and generic allocation of the new species since the Bayesian posterior probabilities at nodes in the polytomy including the new species are not robust (generally required to be > 95%). The long branch-length suggests indeed a separate species status, but the generic assignment of the species is difficult to judge because of the lack of significantly supported phylogenetic structure. I have not been able to find it in the paper, but I would like to know whether all sequences from all terminal taxa had the same length. If some sequences are markedly shorter than the branch lengths would be difficult to evaluate. I have not been able to find accession numbers for the sequences of the parasites to check the above speculation, while those of the host species are provided.

In my opinion as a reviewer the thesis it is a very valuable contribution based on through data analysis that will hopefully set a new standard for future studies on the very interesting and also important fauna of Cestoda of the Indian Subcontinent. I thus recommend the thesis and believe in its successful defense.

V Českých Budějovicích
18. 6. 2012
Mgr. Oldřich Říčan Ph.D.





June 1, 2012

THESIS REVIEW

Ph.D Thesis: Anirban Ash *Diversity of tapeworms (Cestoda) in freshwater fish of India.*

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RELEVANT BACKGROUND OF REVIEWER

Having been associated with the study of caryophyllid cestodes since 1955, with numerous publications including a couple of major reviews and a monographic key to genera, I am pleased to have an opportunity to review this major contribution dealing with the caryophyllid fauna of India, a country from which I collected specimens in 1979.

Thanks to an invitation from Dr. Tomáš Scholz in July, 2010, I visited České Budějovice for 10 days and interacted with Anirban Ash in the early stages of his study. I have been among the peer reviewers for publication papers 1, 2, & 3 of this thesis.

THESIS

Scientific value, Novelty of results, Fulfillment of Objectives

Given that taxonomic helminthology in India is generally not the most highly respected because of poor collection practices and species descriptions, questionable taxonomic decisions and procedures and unavailability of material for verification, major review of its helminth literature must be critically re-evaluated before its credibility can be judged. This study is clearly an important part of that re-evaluation and thus it has highly significant and important scientific value (as well as practical value; see section 1.1 of thesis) because it deals with basic questions of the helminth fauna (Cestoda) of freshwater fish of India, namely: (A) status of current knowledge as discerned through re-evaluation of published species descriptions, and (B) reassessment of that knowledge and addition of much new data and analysis through extensive supplementary collections. One can expect no more in establishing scientific credibility.

The scientific approach in this thesis is thorough and reflects the highest standards, as evident from Sections 3.3 (Methods) and Tables 1 & 2 (List of fish examined on three collection expeditions in India and Bangladesh). It therefore comes as no surprise that the results in this thesis are not so much "novel", given the shoddy nature of the original data from the literature, but more as accurately reflecting the true nature of the cestode fauna of freshwater fishes in India. And as evident from the results published in Papers 1 & 2, for the Caryophyllidea, (see Section 4) it is determined that there are only 8 valid species from 5 genera instead of 59 species from 15 genera, as originally reported in the literature!. The results with *Gangesia* of the Protocephalidea concluded that only 4 of 47 nominal species of *Gangesia* and *Siliurotaenia* are valid. Such results as these are astonishing and reemphasize the power of an enlightened, scientific approach.

Research on any subject is never completed. I think that Section 6 clearly reflects that the major objectives of the study, as stated in Section 2, have been achieved, but, not surprisingly, that there is much more to do in the future. What this impressive thesis has revealed is how much more has to be done to establish some credibility for the data on helminths, not only from fish, but I suspect from all vertebrate hosts in India. The future work is not solely restricted to collecting more data but, as the thesis points out (p.37) "The main emphasis should be to building a network of specialists all over India". If Anirban is successful in achieving even small success with this network, he will have contributed more to Indian helminthology than clarifying the status of any helminth group.

Strengths and Weaknesses (flaws and deficiencies)

The strengths of this thesis are many. In my view, the primary strength is incorporation of new data based on collections of fresh cestodes from suitable hosts over a wide geographical region of India (see Fig. 1). As I have found, and Anirban soon discovered, it is absolutely impossible to accurately assess the nature of the cestode fauna of freshwater fish, and probably other hosts in India as well, by simply relying on the information from the literature. One look at the nature of so much of this literature, as done in this thesis, quickly reveals that most is utterly worthless for critical taxonomic study. See Figure 2 of the thesis for some examples of the unusual poor quality of illustrations. The lack of a credible literature and other important factors, as covered in Section 4 have made it mandatory to study new, well-fixed material.

A second significant strength is the application of modern taxonomic techniques, rather than relying primarily on morphological characteristics visible only with the light microscope. As described in Section 3.3, and visible in all of the published papers 1, 2, 4 & 5 and unpublished ones, papers 3 & 6., excellent use has been made of surface ultra structure (see Fig. 2, Paper 2; Figs. 10-13, Paper 4; and Figs. 4-10, Paper 5 as examples) and especially molecular taxonomy techniques. The latter techniques, utilizing genomic DNA & RNA from ethanol preserved samples that could only be obtained from fresh material, are necessary for critical systematic and phylogenetic analysis, as visualized on the phylograms of the phylogenetic analysis of *Lobulovarium* (p.101) and subfamily Gangesiinae (p.164).

A third strength is the re-examination of Type-specimens and museum vouchers or specimens from private collections, as covered on pp. 15-17. Such work can be tedious and seem insignificant, when compared with modern molecular techniques, but it is necessary because it helps to give insight and gain perspective into the nature of the taxonomic process and how former investigators arrived at their conclusions.

A fourth strength is the exceptional high quality of the line drawing illustrations, of which there are many (see Paper 1 for prime examples of illustrations from whole mounts). They are of professional quality. Such a strength is not trivial because in taxonomic work, of which this thesis is a prime example, it is often the illustrations, rather than the descriptions, that will convey the identity of the species and therefore will be used most often in comparative taxonomy. In some cases, illustrations will be copied and republished, often in textbooks.

A fifth strength is the overall, high quality and thoroughness of the treatment of topics covered by the published papers, in strong contrast to the dismal state of past literature. Each of these papers provides such a wealth of pertinent detail with supporting data. Additionally, these papers have an unseen strength --- to serve for helminthologists in India as examples of how systematic helminthology should be done. In the absence of similar examples in the Indian literature, these papers on the Caryophyllidea and Proteocephalidea can serve as wonderful examples for the new generation of helminthologists of India who aspire to incorporating modern techniques into their taxonomic studies.

Few major theses of the depth and scope of this one are without weaknesses in the form of flaws and deficiencies. This thesis is no exception. I have not found any weaknesses, however, that are major ones and would invalidate any data presented. Despite the extensive review of the literature, I note that the full thesis of B.M. Murhar (1972) was not consulted, only the shorter synopsis, as indicated on p. 74 of the "Literature Cited" section of Paper 1 and commented on in the "Discussion" section of Paper 1. Because the Murhar thesis is one of the few, if not only Ph.D. thesis from India that is exclusively devoted to the Caryophyllidea, it is quite possible that it might have provided helpful information for the present thesis. The Murhar thesis has data from examination of 1468 fishes of 6 species with descriptions of 8 caryophyllid species from two of the most common host species.

Some of the text of the unpublished part of the thesis, though interesting, appears out of place or of doubtful importance, in my opinion. For example, much of the "Introduction" (pp. 2-7) dealing with a general overview of the morphology, biology, life cycles of aquatic forms, classification, etc. of cestodes, is the sort of information that one would find in a textbook or monograph of the Cestoda. In this regard, I think the last 3 paragraphs of the Section 1.2 should have been given a new section because it underlies the nature of modern taxonomy - as illustrated by this thesis.

I have not observed any serious grammatical errors but must call attention to the obvious and serious labeling error in Paper 6 of Table 2 on p. 170 of the thesis. The "Acknowledgments" have yet to be added to Paper 6, still in preparation.

Questions

Of the great many questions that come to mind after reading a thesis of this scope and detail, I have chosen three that deal with general aspects and personal aspirations -- rather than ones dealing with esoteric ,technical ones of cestode taxonomy.

This question is especially critical with any cestode species that has a wide distribution – *Lytocestus indicus* for example -- as described in Paper 1. Is it possible one of the reasons there is such an impressive synonymy for *L. indicus* (p.20 ,Table 1) of at least 25 other species names, or possibly *Gangesia bengalensis* (with at least 16 synonyms) is that there might be different subspecies or biotypes? As a general aspect with taxonomic decisions involving considerable variation in some species, how does one determine that different species are involved rather than variants, different biotypes, morphotypes or subspecies of a single species?

Considering the broad scope of this thesis, how do you account for the fact that you encountered caryophyllids almost exclusively from the family Lytocestidae and none from the family Caryophyllacidae?

From your Conclusions and Perspectives, and the last paragraph of your Discussion (p.36) in the thesis, it is clear that you hope to continue your interest in the helminth fauna of the subcontinent of India. Can you elaborate a little further not only on what goals, and priorities you would like to achieve in your future research on helminths in India , but also on how you hope to do so?

Comments

This thesis presents an enormous amount of data based on years of diligent research in the laboratory as well as the field. In the process , this research has involved acquiring a wide array of essential skills, among the more significant : interpreting the credibility and value of literature records, applying a wide variety of modern technological methods for cutting edge systematics, dealing with fellow scientists in planning of extensive field expeditions, interacting with colleagues , and unraveling a mass of confusing data and making difficult decisions on the taxonomy of a wide variety of cestodes. All of this in order to better understand the nature of the cestode fauna of freshwater fish of India. Without these skills --- and they *are* skills that take time to develop and hone --- the final product (Thesis) could not have been written.

In the years to come, the publications and thesis itself may join the ever growing ranks of forgotten literature that fill our libraries and now cyber space. But for the author of the thesis, the acquired skills will always remain -- to be put into service for the benefit of future students and, of course, science itself.

I have no doubt that this candidate for the Ph.D. degree has acquired and clearly demonstrated those all important skills that have the potential to help further significant, fruitful research in the future.

RECOMMENDATION

Based on my review of the Ph.D Thesis –*Diversity of tapeworms (Cestoda) in freshwater fish of India* – of Anirban Ash, I recommend that this thesis be accepted as part of the requirements for the Ph.D. degree.



John S. Mackiewicz Ph.D.
Emeritus Professor