

MASARYK UNIVERSITY  
FACULTY OF SCIENCE  
DEPARTMENT OF BOTANY AND ZOOLOGY

## REPORT

on Ph.D. thesis of **Carlos Alonso Mendoza Palmero**  
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University of South Bohemia in České Budějovice

„Species composition and phylogenetic relationships among monogenean parasites (Platyhelminthes: Dactylogyridae) of catfishes (Siluriformes) from the Amazon River basin“

### Background

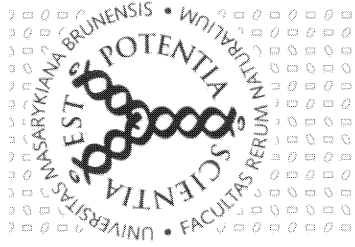
Monogenean parasites of the family Dactylogyridae (Platyhelminthes: Monogenea) are small ectoparasites infecting marine and freshwater fish. They exhibit an extraordinary morphological diversity which makes them an interesting object of study for the understanding of such intense diversification as a result of their evolution. The direct life cycle of these parasites, together with known host specificity, makes dactylogyrid monogeneans a suitable model for studying host-parasite co-evolutionary events. With regard to the high fish richness in the Amazonian region, the current knowledge on monogenean parasites is very negligible. The candidate focused on a formidable goal to contribute to the knowledge of monogenean parasite diversity of catfishes in the Neotropical region and to reveal their phylogenetic relationship to other known parasites of catfishes from different parts of the world.

### General evaluation

The present thesis brings unique results from a long-term sampling period and a big effort in the collecting of monogenean parasites of the family Dactylogyridae of catfishes from Peruvian Amazonia. The thesis is based on results presented in two published and one submitted paper together with one manuscript in preparation. In all four papers, the candidate is the first author with a high majority percentage in contribution in each of them and for all of them he is also the corresponding author. The candidate, while working on the thesis, has published an additional four articles of which two he served as first author.

### Positive points

- New insights into the systematics of Ancyrocephalinae as a result of phylogenetic analysis including a robust dataset of Neotropical monogeneans from catfishes.



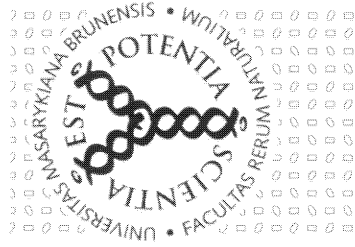
- Unquestionable evidence of unexplored diversity of monogeneans of the family Dactylogyridae on catfishes in the studied area as demonstrated by the records of 99 species of which 85 still represent undescribed species and genera.
- Persistence of the candidate to tackle with undoubtedly not easy difficulties faced during the work on the thesis.

### Critical remarks

- It's to understand that to summarize and present data of intensive and diverse sampling is not easy. But the including of field cods for fishes doesn't provide any reasonable value for reader in both Tables 1 and 2. Repeating names of localities for each fish specimens makes tables unnecessarily long. The use of abbreviations for localities would be eventually more suitable.
- As a part of material and methods a simple map showing all sampling spots would be helpful to give an idea about localities, the coordinates of the sampling spots are not mentioned. More information about the location of sampling spots can be found only later in paper IV.
- From the results it's not clearly understood how many fish specimens in total the candidate has examined and what percentage was found to be positive for the presence of parasites.

### Minor critical remarks

- Page 9 – the mentioning of overall number of localities sampled is lacking.
- Page 10 – for what reason are two *Demidospermus* names for type material studied cited in full name and the rest no?
- Page 12 – the name of locality Belén Market, is cited inconsistently, in Spanish and English forms.
- Page 14 – the list of sequenced monogeneans of the present study is presented in Table 2, not 1 as mentioned.
- Page 19 – A total number of 33 Ancyrocephalinae gen. sp. was recorded, as is evident from Table 4, not 29 as is mentioned within the text.
- Page 23 – there is a double line for table at the end the page.
- Page 57 – what is the current stage of the article? At the end of the first paragraph on discussion some references should be added.
- Page 67 – Please, concentrate on the format of the table. The part with gyrodictyloid parasites has a different format from the part containing dactylogyrids.



- Page 67 – *Iredactylus rivuli* is mentioned in the table twice, for the second time on page 70.
- Page 154 – the third paragraph, typing error *Urocleidus similis*.

### Questions

- Would the candidate be able to specify his estimation of the number of genera+species which represent those 33 Ancyrocephalinae gen. sp.?
- What percentage of newly collected material available in their collection are suitable for publication? What does he plan to do with all the other material?
- On page 57, as part of the manuscript in preparation, the candidate mentions richness of monogenean fauna of selected South American countries. Can you, please, specify those numbers?
- Could the candidate compare the species diversity of dactylogyrid monogeneans of catfishes from other regions? Based on the results of this thesis, we know the current status from the studied area, but no comparison with described species from the rest of the world has been mentioned or discussed.
- What are the future plans of the candidate after finishing his Ph.D.?

### Conclusions

I highly appreciate the capability of the candidate to handle such diverse data and after appeared difficulties to bring them in the final version of presented thesis. The contribution of candidate on articles and manuscripts shows his abilities in independent scientific work.

**I recommend Mr. Carlos Alonso Mendoza Palmero to be awarded by the degree of Doctor of Philosophy.**

Brno, 14th November 2014

  
Iva Prikrylová

## Re: REPORT ON THE Ph.D. THESIS of Carlos Alonso Mendoza Palmero

From: Walter A. Boeger, PhD

### *General observations:*

In general, the thesis presents the necessary quality to justify the accomplishment of this portion of the requirements to the title of Ph.D. I am satisfied with the quality of the taxonomic work presented herein, but this was already expected, as Carlos is already a respected taxonomist in the area. I am especially pleased to be part of his thesis reviewers committee. Descriptions are adequate and illustrations of high quality, something that has been widely neglected in recent publications on Dactylogyridae in the last years.

The uncover of the diversity of Monogeneoidea from Siluriformes has been clearly neglected by previous students of the group, including me. But it has been always clear to me and Dr. Delane Kritsky that the diversity was impressive and that the taxonomy would relatively complicated by the lack of large inventories, as Carlos attempted in this study. However, the effort is almost impossible for a single person (or a small group of persons and whatever limitations this study/attempt shows (and that I will likely comment below) reflects this. Large inventories, such as this, require the collaborations between many scientists and working groups. The task assumed by Carlos was complex and extensive...what he did if commendable and I congratulate both the Ph.D. committee and Carlos for the result.

Because it is an exciting subject for me, I will point some items that I would like to discuss and hear Carlos during the defense. But I advance that I am satisfied with the results and I think that it justifies the requirements for the title of Ph.D.

### *Specific comments on the study*

1. It appears to me that the objective described on Page 170 has not been achieved, but the reasons appear evident to me but I would like to discuss with Carlos about that during the defense. The methodology and analyses were not designed with the intention to "evaluate the diversity and taxonomic composition of the monogeneans infecting <or should it be infesting?> siluriforms..."

In my opinion this could have been done if adequate methodology was applied. A specific sampling methodology and the use of statistical tools would allow evaluate the diversity. The sampling in the study was largely opportunistic, sampling whatever was available. There could be a predefined method to cover a significant sample of all available clades of Siluriformes. For example, the sample of Loricariidae was extremely small and the absence of this family, at very least, hindered a better understanding of the diversity of monogeneids in this host group.

Also, the methodology of collecting, killing and fixing the animals was, in general, not adequate for the study of this group of monogenoids, with such morphological complexity. For instance, the use of GAP is largely inadequate for study of internal morphology, something that is required for the understanding of the taxonomic limits between genera (and suprageneric groups as well). I have worked in the Amazon for many years and it became obvious that any collection effort is greatly hindered by processing the specimens in the field. Further, although GAP is widely used by the community of scientists it often presses the hard structures so hard that distorts the morphology. The technique of digestion is far more adequate for this.

Because Carlos does such an exceptional job with morphology (just check the papers at the beginning of the thesis) I was expecting more from the phylogenetic analysis. But I have more comments on this later.

2. Page 14. Could you explain why did you choose 28S rDNA?
3. Page 15. Why did you not perform a parsimony analysis? Parsimony would provide additional support for some of the relationships recovered by Bayesian analysis. Instead of running Likelihood, I would use Parsimony AND Bayesian. Can you justify this decision?
4. Page 21. This table is very interesting. I am really impressed that you have not explored it since this would be your support to estimate the richness of species from Siluriformes in the Neotropics. Can you explore a little bit about this table and your results. Is there something that becomes evident from your study?
5. The list of species.  
On page 57, you forgot to add the number of species described from each country. There names of authors misspelled in the References and location names misspelled in the distributions.
6. On the section on phylogeny.  
In my opinion, a study such as this one should always do a parsimony analysis as well. Bayesian analysis may easily result in a wrong hypothesis as the resulting trees, considered for the definition of the majority rule consensus, may cause a significant reduction in the percentage of “true” or branches that otherwise would present higher branch/node support.

In fact, it appears that this actually happened. I have aligned the analysis on Muscle and submitted to the algorithm of Guidance (<http://guidance.tau.ac.il/overview.html>) and the result was a distinct topology and higher branch support. This supports the assertion I made above. The resulting cladogram depicts only two clades of species parasites of Siluriformes, possibly a single clade with an apparent host switch into different host groups followed by speciation and diversification. As I mentioned, the cladogram presented in this study depicts many unresolved (low support) branches (I think that is why you used “unsupported sister clades”), many required to support

some of the conclusions in this chapter. To base a discussion on “unsupported sister clades” is not robust.

Most of the conclusions should be re-considered if the resulting cladogram changes. There are some interesting inferences on coevolution and biogeography of the group...with higher branch support and a more robust alignment, in my opinion. Obviously, I have not explored the analysis deeply for a question of time, but this contradictory result indicates that the resulting cladograms derived from the alignment of this fragment of DNA needs special attention.

I also think that the discussion should not explore too much on morphology nor coevolution (e.g. “None of the tree major clades ... seems to share some obvious morphological synapomorphies nor clear patterns of host-associations..” ) if you did not perform any analysis about that. In fact, this is what I was expecting. Carlos is a good morphologist and he could have performed this analysis nicely. The result would be far more robust.

Now, considering that we may have a single clade of monogenoids from Siluriformes, the study strongly supports Susam Lim’s idea that these species likely represent members of a single subfamily. All species that do not occur in Siluriformes in this clade (See attached cladogram) could represent a event of host switch following the expansion of Siluriformes into North America (there should be studies reporting the biogeography of the Siluriformes in NA).

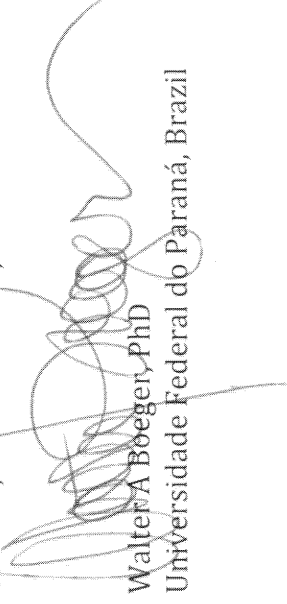
The absence of parasites of Loricariidae is a pity. Also, it would be most informative the inclusion of sequences of other marine siluriform species.

### ***Final conclusions.***

Despite the apparent criticism on the phylogenetic analysis above, it really reflects different view on methods and underlying theory of the analysis. The entire thesis is robust and Carlos has demonstrated that he is capable of exploring the subject (and others) even further on his professional life. It is my opinion that the thesis represents an important contribution to the knowledge on this poorly-known group of helminthes and that it represents an adequate evidence of Carlos capacity in developing research in the area in this future life. I am satisfied with the thesis and strongly recommend its approval, as the thesis is consistent with the degree sought.

I look forward to interact with Carlos during his defense.

Curitiba, November 14<sup>th</sup>, 2014



Walter A Boeger, PhD  
Universidade Federal do Paraná, Brazil

# OBSERVATOIRE OCÉANOLOGIQUE DE BANYULS

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Banyuls, 12 November 2014

**Report on the PhD of Carlos A Mendoza-Palmero, "Species composition and phylogenetic relationships among monogenean parasites (Platyhelminthes: Dactylogyridae) of catfishes (Siluriformes) from the Amazon River basin", University of South Bohemia in Ceske Budejovice, Czech Republic**

The subject of the PhD thesis of Carlos Mendoza-Palmero is the study of the diversity and evolution of monogeneans from catfishes (Siluriformes) in the Peruvian Amazonia. Monogeneans are an important group of Platyhelminth parasites that display a very high species diversity and intricate relationship with their aquatic hosts. This makes them a model of choice to address fundamental ecological and evolutionary questions, but these parasites may also have an impact on farmed fish hosts in aquaculture facilities, and it is important to gain as more knowledge as possible on this still poorly known group, in particular in understudied areas as is the case in the present work. The diversity of monogeneans from amazonian catfish is investigated here using morphological and molecular approaches, to obtain a detailed picture of the current knowledge on this host-parasite system. Six new monogenean species were described during this work, and published along with the revision of 3 other species. These species are part of the almost 100 monogenean species (from 42 fish host species) investigated during the course of this PhD. It must be noted that this impressive dataset was collected through numerous field trips, establishing Carlos Mendoza-Palmero as an experienced field biologist. In addition, a very important amount of data has been reviewed and organized to propose an updated checklist of all monogeneans from freshwater fishes in the Neotropical region.

This thesis also includes a more detailed study carried out on a subgroup of monogeneans from amazonian catfish: a phylogenetic analysis of the subfamily Ancyrocephalinae was performed using molecular data, allowing Carlos Mendoza-Palmero to carefully address the evolution of this group (including representative species of the subfamily Ancylodiscoidinae from Asia and Africa). I was impressed by the high quality of the phylogenetic reconstruction carried out. Carlos Mendoza-Palmero evidently masters the building of evolutionary tree using molecular data. A new taxonomic picture emerges from this analysis and is discussed in the thesis: Ancyrocephalinae seems paraphyletic and divided into 3 clades with no clear synapomorphies and geographical segregation, nor particular associations with hosts. This claims for a new taxonomic scheme and emphasizes the complex evolutionary history of monogeneans, in particular in the tropics.

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The manuscript of this thesis is well written and organized, with clearly stated objectives. The introduction is short but clear and easy to read, and the appropriate literature is cited. The thesis is organized around 4 scientific articles that form the "Results" section, and are completed by general Introduction and Discussion. On this 4 papers, 2 are already published in a very good journal, the *Journal of Parasitology*, another is submitted (in *Parasites and Vectors*), and the last one will be soon submitted to *Systematic Parasitology*. The high quality of these journals reflects the level of this PhD thesis. In addition, Carlos Mendoza-Palmero has already published 6 other papers (including 2 as first author), listed in an Appendix of the thesis, in good parasitology or systematic journals as *Parasitology Research*, *Journal of Parasitology* and *Zootaxa*. He also produced a book chapter as senior author. This impressive achievement already makes him a good specialist of the discipline, and he can already be considered as an expert in monogenean taxonomy. The huge amount of work he produced, especially the checklist of monogeneans of freshwater fishes from the Neotropical region, constitute an important material for future research, by himself or other parasitologists, ecologists and evolutionary biologists, for the investigation of large-scale patterns. As such, this work is an important contribution to science.

On a less positive side, I think this work could have been enlarged outside monogeneans, to encompass more general problematics, at least on parasites (e.g. specificity, biogeography, to remain on the main themes addressed in this thesis). The manuscript tends to only consider monogeneans, especially the monogeneans under study. That principally limits the interest of this otherwise impressive work to scientists specialist of these parasites. As a general criticism, my feeling is that this work lacks synthesis and general discussion. The main findings of this thesis could have been used for a broadened discussion about the ecology and evolution of monogeneans. In addition, I feel that the objectives (p. 8) could be more precisely defined. It would have been better to formulate them as questions and/or hypotheses to be tested. Even for a very descriptive work as this one, this would have been profitable and would have permit to obtain a more focused thesis. For example, the evolutionary origin of the monogeneans under study could have been more thoroughly discussed. Host biology is seldom addressed, and investigating it more closely may have shed light on the evolutionary patterns inferred for their parasites. Species status and definition are central to such taxonomical reassessment, but are never discussed here and it was a good opportunity to do so. These are not easy issues, but it should be made clear what criteria are used to define new species and discriminate them for polymorphism. At least the limitations and biases of the techniques used should be acknowledged. This could also have been addressed in a general discussion. The same thing could be pointed to the definition and measures of host specificity, which are central too here and not really discussed. The checklist could serve a more well-defined purpose, and even if time constraints may not give Carlos Mendoza-Palmero the possibility to carry that out during his PhD, that could have been described in the Perspectives part, which is too limited here. I am nevertheless aware that a PhD thesis is short, and that choices must be made during that period. These remarks are intended as suggestions to pursue this pioneering work, more



than criticisms! Moreover, these observations highlight that Carlos Mendoza-Palmero prefers to go directly to the point addressed and not to lose himself into digression and unnecessary discussions. This is a good quality for a scientist!

Considering the quantity and quality of the work carried out, I consider that this thesis fully meets the criteria required for a level of doctor in science, and I recommend the grade of PhD to be attributed to Carlos A Mendoza-Palmero as well as the public defense of the thesis.

A handwritten signature in black ink, appearing to read 'Yves Desdevises', written over a horizontal line.

Yves Desdevises  
Professor  
Université Pierre et Marie Curie