THE WINSTON CHURCHILL MEMORIAL TRUST OF AUSTRALIA

Report by Dr Haylee Weaver, 2011 Churchill Fellow

# THE AUSTRALIAN BIOLOGICAL RESOURCES STUDY CHURCHILL FELLOWSHIP TO EXAMINE THE SYSTEMATICS OF THE ACANTHOCEPHALA FROM AUSTRALIAN FISH.



Signed Haylee Weaver Date 8.v.2012

**Contents**

## Introduction

The aim of my Churchill Fellowship was to gain new skills and develop additional expertise in the field of molecular phylogenetics and systematics, in order to examine the systematics of Acanthocephala from Australian fish. There are very few Australian parasitologists who work on the Acanthocephala as a group. In 2010, I began a knowledge transfer program learning skills in morphological techniques for acanthocephalans from a retired expert. This was designed to increase research capacity for working with these parasites, and we are beginning to see success from this work, with one new species description being accepted for publication in the journal Comparative Parasitology, and another paper submitted to the Journal of Parasitology.

The reason why I applied for a Churchill Fellowship was because of the need to develop my ability to work with molecular data, in addition to using morphological techniques for dealing with acanthocephalans. This would bring an extra depth to the expertise I am gaining in morphological techniques for working with acanthocephalans. The integration of both molecular and morphological data can greatly increase the usefulness of a dataset, and these two methods can complement each other to produce more robust results. I had one set of these skills, but lacked the other.

I had the following objectives to achieve via the travel for my Fellowship:

- To examine material for taxonomic study from collections that I would otherwise not be able to see in Australia,
- To discuss research ideas with scientists and develop potential collaborative links for future research,
- To discuss how to increase the impact of taxonomic and systematic research on parasites, and
- To learn new techniques and protocols for using molecular data for the Acanthocephala and the application of such data for solving phylogenetics problems.

I came back to Australia with a book full of measurements, drawings, notes, ideas and protocols. I had very productive discussions with researchers on a range of topics associated with taxonomy, systematics, host-parasite ecology and parasitology in general, all of which will improve my ability as a researcher. The interesting thing about acanthocephalan research is that there are not many people in the world who work on these parasites. Therefore, making links with researchers is incredibly useful for developing collaborations and sharing research ideas. I hope that the collaborations I have founded will be productive and successful and enable me to develop my research expertise in the field.

**Executive Summary**

Dr Haylee Weaver
Research Associate,
Building 134, Research School of Biology
The Australian National University
Acton 0200, Australian Capital Territory.

**The Australian Biological Resources Study Churchill Fellowship to examine the systematics of the Acanthocephala from Australian fish.**

*Aim of Fellowship*

To learn new skills for working with the Acanthocephala and the use of molecular data to be applied to research on the systematics of Acanthocephala from Australian reef fish.

*Highlights*

- Learning techniques for integrating morphological and molecular data to improve research outputs.
- Viewing collections and specimens of acanthocephalans not able to be seen elsewhere, and learning the importance of using good preservation techniques.
- Developing new links and collaborations that will assist in increasing the research output for Acanthocephalan systematics in Australia.

*Lessons learned*

- Research on systematics alone can be integrated into wider ecological/ biological studies to give context to the life cycles and ecology of the group or species being studied.
- Data reference collections can never be big enough, however, specimens and data must be retained appropriately or they may degrade or get lost.
- Research ideas should be developed into strategic projects that aim to maximize return (i.e., publication outputs) and raise the profile of systematics and phylogeny as integral aspects of fundamental biology.
- Sharing of resources via collaboration is mutually beneficial, and important for disciplines that have limited funding opportunities.
- People are fascinated by parasites – therefore engagement with the public is a useful way to raise the profile of parasitology and systematics.

**Programme**

10 – 17 January 2012 – London, United Kingdom. Natural History Museum

- Examined the collection of Acanthocephala held at the Museum, focused on species from reef fish to compare with specimens held in Australia
- Discussed:
    - strategic research directions with researchers and
    - limits/barriers to access to collections with curatorial staff

18 – 25 January 2012 – Beltsville, Maryland, United States. National Parasite Collection, US Department of Agriculture

- Examined species of *Filisoma* and, *Corynosoma* and *Centrorhynchus* not able to be viewed elsewhere
- Discussed collaborative funding opportunities and schemes available in the US with researchers

26 January – 14 February 2012 – Mexico City, Mexico. Instituto de Biologia, Universidad Nacional Autonoma de Mexico

- Learned molecular techniques for working with Acanthocephala, hosted in the laboratory of Dr Martin Garcia Varela
- Gave a seminar on my research to the Instituto de Biologia (with introduction given in Spanish!)
- Discussed collaborative research opportunities with researchers

## Main Body

*What is the Acanthocephala?*

The Acanthocephala is a small phylum (~1200 species) of parasitic worms that infect all classes of vertebrates (Monks & Richardson 2011). This group of parasites resembles other parasitic groups, as they have a tegument (nutrition-absorbing ‘skin’) similar to cestodes (tapeworms, phylum Platyhelminthes), yet are worm-like in their appearance similar to nematodes (round worms, phylum Nematoda). Despite these similarities, acanthocephalans, as a group, are defined by their proboscis – a thorny prominence at the anterior end that is used to attach themselves to their host (Figure 1). They have a complex life cycle involving intermediate hosts, often smaller vertebrates or arthropods, and occur in all terrestrial and aquatic ecosystems. Detailed research on the Acanthocephala did not begin until the 1800s, despite knowledge of ‘worms with thorny hooks’ being known since 1684, when Redi provided the first written evidence of these worms (Amin 1985).

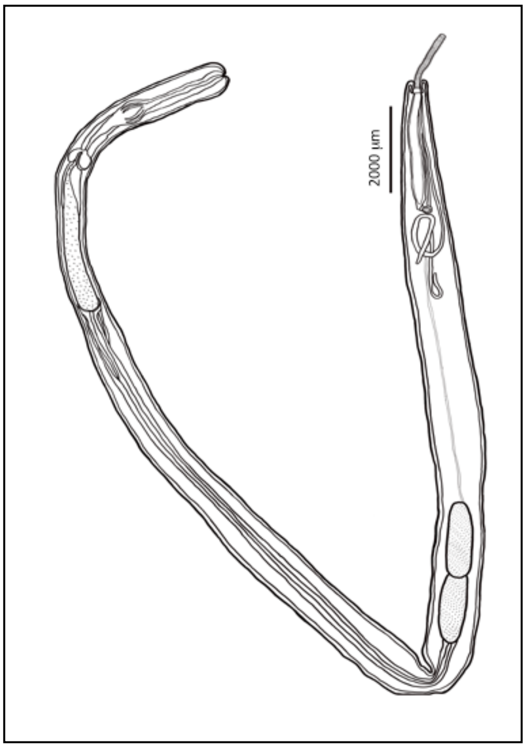


Figure 1. Diagrammatic representation of a male acanthocephalan. Note proboscis at anterior end (right hand side of diagram) (source: Weaver, unpublished data).

*History of taxonomy and systematics of the Acanthocephala*

The name Acanthocephala was given to the group in the late 1700s, to designate this clearly different group of parasites from others, but all known species were categorized into only one genus, *Echinorhynchus* (see Amin 1985). Taxonomic works describing new species increased in the 1800s, although many workers described new species that were eventually determined to be synonyms – a common error was to describe parasites as new if they occurred in an as-yet newly recorded host for the first time. Taxonomies were revised, including the first distinction of different genera from the original *Echinorhychus* and led to the erection of three families (Amin 1985). This formed the basis of modern systematics for the group. Work in the 1900s, initially led by Meyer and Van Cleave in the 1930s and 1940s, substantially revised the systematics of the group, beginning with the establishment of classes within the phylum – Archiacanthocephala, Eoacanthocephala and Palaeacanthocephala, which still remain valid, with the addition of the Polyacanthocephala in the 1980s (Van Cleave 1936; Monks & Richardson 2011).

The evolutionary and phylogenetic origins of the Acanthocephala were unclear, given their lack of sister groups or related taxa, and several cladistic studies during the mid 20$^{th}$ century suggested that the parasitic, metazoan Acanthocephala were actually most closely related to rotifers (phylum Rotifera), free-living organisms occurring in aquatic environments (Garey et al. 1996). The use of molecular techniques in the application of understanding phylogeny increased substantially from the 1980s onwards, and added a powerful new tool to reveal the relationships of taxa using their own genetic data. Molecular phylogenies of Acanthocephala using different genetic sequences have all, so far, provided support for the Rotifera/Acanthocephala relationship (for example, Garcia Varela et al. 2000, 2002; Garcia Varela & Nadler 2005; Verweyen et al. 2011).

There is now emerging consensus that a combination of both traditional morphological techniques be paired with newer molecular methods to generate an even more powerful data set to understand the relationships of taxa (Perkins et al. 2011). My personal expertise in parasite taxonomy and systematics is based on morphology, and by developing my ability to use and integrate molecular techniques in my research, I will be able to add an additional dimension to my research on Australian acanthocephalan parasites.

*Research on Acanthocephala in Australia*

Systematics, as a discipline, can often be overlooked or its importance misjudged. In the context of parasitology, systematics provides the fundamental information regarding the classification of a species and gives the basis for studying the biology and ecology of the parasite (Monis 1999). Australia currently lacks appropriate research capacity in the field of research on the Acanthocephala. This gap is not limited to taxonomy, systematics and phylogenetics, but also extends to ecology, host-parasite relationships and understanding the biodiversity of Australian acanthocephalans. Australian research on Acanthocephala has mainly utilized morphological methods for taxonomic and systematic research, without including molecular data to provide additional support for the data. Often it is simply not possible to include such data due to fixation of specimens, but this will be discussed in a later section. A key outcome from my Churchill Fellowship will be the acquisition of skills for working with acanthocephalans in the context of collecting and interpreting molecular data for use with morphological data in systematic and taxonomic research of Australian Acanthocephala.

Searches on the Web of Knowledge (http://apps.webofknowledge.com/) using the search terms “Australia” and “acanthocephala” reveal that in the past 20 years, 12 papers have been published on new species, or on systematic research. The majority of these papers were published by either Em. Prof. Lesley Smales (Warner) (6 papers) or Dr. Sylvie Pichelin (5 papers). One additional paper was authored by Omar Amin, from the United States. Dr. Pichelin is no longer working actively within the field, and Em. Prof. Smales is retired from academia, holding a post-retirement fellowship at the South Australian Museum. This large gap in expertise regarding the biology and ecology of acanthocephalans in Australia needs to be addressed, which is why making new links with international researchers within the field was an integral part of my Churchill Fellowship.

The overarching aim of my research and this Fellowship was to develop new links and new expertise for working with the Acanthocephala with a view to building research capacity in this field in Australia. Being able to share my ideas with others to develop collaborative projects is a key aspect of developing research capacity, as it allows workloads to be shared and resources to be pooled between collaborators. Learning new techniques for working with acanthocephalans enables me to produce high quality results for the work I do independently and as part of a collaboration.

*Morphology vs molecules*

As alluded to earlier, systematics and phylogeny are the cornerstones of understanding an organism's biology. The increasing use of molecular methods in the field of systematics, through the easy integration of DNA sequence data into phylogeny analysis programs, means that this method has quickly overtaken traditional morphological taxonomy as the standard method for generating phylogenies and understanding the systematics of different taxa (not just limited to parasites). The use of molecular data has been most useful in identifying cryptic species – those species that look morphologically similar and are treated as the same species using traditional morphological methods, but differ significantly at a molecular level enough to be differentiated as separate species.

Building phylogenetic analyses around morphological characters (i.e., the physical features or properties of a taxon) only can be problematic for several reasons:

- Character states can be subjective, and differ depending on who is looking at the specimens,
- Fixation of specimens can distort features which may result in them not being represented appropriately in character analysis, and

- Differentiation of characters for groups that are characterized by subtle differences in morphology can result in trees of low resolution due to the similarity of the characters.

The use of molecular data to build phylogenies and clarify the systematics of a group can alleviate many of the problems associated with the use of morphological characters alone. Furthermore, molecular analysis can reveal differences at the genetic level that are indistinguishable using morphology (i.e., cryptic species). The recommendations that combinations of morphological characters and molecular data be used for deep-level phylogeny can also be applied to revealing the phylogenetic relationships within families and genera. One of the aims of my research is to integrate these techniques in order to understand how groups of acanthocephalans are related to each other. Dr Martin Garcia Varela, of the Universidad Nacional Autonoma de Mexico, is using these methods, and being able to learn techniques directly from him was invaluable in extending and developing my set of skills for using molecular data.

*Understanding material*

In order to describe a new species, or revise the systematics of a group, one must examine as much available material as possible. Generally, specimens are held in museums, as the usual practice is that once species are described, they are then deposited in a museum of the author's choice – and accession numbers are provided in the descriptions to allow other workers to find and access the specimens. However, many specimens are submitted for deposition in museums without being identified, which provides researchers with unique opportunities to examine data from locations where they may not be able to collect specimens from. A key aspect of the deposition of specimens, described or not, is accurate record keeping. A highlight of my visit to the Natural History Museum was looking at specimens collected by Charles Darwin during his journey on *The Beagle* (Figure 2). It reminded me that all my taxonomic descriptions would be kept for posterity in the form of museum specimens for people to use in the future.

Figure 2. Subset of material collected by Charles Darwin, Natural History Museum, London.

Occasionally, it is not possible to provide accession numbers for everything submitted for deposition within collections. Obviously, specimens of new species are accessioned and entered into databases, but large collections of already documented species (= voucher specimens) can sometimes be not allocated accession numbers and their data subsequently not recorded on databases. This situation is not unique to any one particular institution, and is usually a result of limitations to funding and time available to museum staff. By not being able to access a full range of specimens, comprehensive study is limited. For Australian institutions, it is therefore essential that all collections of parasites be treated with the same level of importance as those for more glamorous invertebrate groups (e.g., insects). This also further highlights the importance of collaborations, as colleagues may have specimens or access to specimens that cannot be borrowed from museums or institutions.

Problems with material held in ethanol arise when workers wish to perform DNA extractions for molecular analysis. Acanthocephalans, and other softbodied parasitic worms, were often fixed (to preserve the morphological shape of the worm for taxonomic study) using formalin, which causes DNA molecules to stick together and makes extraction of useable DNA fragments extremely difficult. Since the advent of molecular methods for phylogeny and systematics, more workers have stopped using formalin as a fixative, but the trade-off exists between good-looking specimens for morphological study versus not-so-good-looking specimens for excellent molecular examination. When examining museum collections for suitable parasites for study, it is often the case that the right specimen has not been fixed correctly for the relevant research being conducted.

*Working with new material (molecular)*

Obtaining material to extract genetic data is potentially problematic, and storage conditions must be optimized to ensure that the material does not degenerate. My personal experience in collecting data for molecular analysis is limited, so the opportunity to examine how these data are dealt with in a museum setting was very useful, as was learning new methods for data entry and management. Key things I learned from discussing with researchers were:

- Freezers must be of an appropriate temperature: -20°C freezers are often not cold enough for long-term storage of genetic material. DNA can degrade anyway, so processing samples in a timely manner is important.
- Specimens must be collected fresh and stored in 96-100% ethanol. The best scenario is that the same specimens are used for both morphological and molecular analysis, for consistency of data, but for acanthocephalans this can be extremely difficult given the way in which they should be fixed for morphological analysis (in water) can degrade DNA if left for too long before being stored in ethanol.
- A reference collection can never be big enough, and in order to generate a good collection, collaboration with other parasitologists is essential. This is both in the context of simply collecting enough material, and in the analysis of data.

- All data must be accounted for. I learned a new way to handle data entry so that all steps of the DNA extraction process are accounted for and linked together. This streamlined method will be most useful in managing data for my own analyses.

*Collaborations*

A key aspect of working with the Acanthocephala is the establishment of international collaborations, because of the lack of research capacity in Australia. The number of researchers working globally on the systematics of the Acanthocephala is quite small. Not all these researchers investigate acanthocephalan biology and ecology, some focus only on taxonomy and systematics of the parasites. I believe that more can be achieved, from a strategic point of view, by broadening the field of research to include ecological information and understanding the interactions between acanthocephalans and other parasites in their hosts. The reason for this view is that the competitive nature of academia means that it is simply not enough to be a good taxonomist.

A common measure of success within the biological sciences is scientific output – how many peer-reviewed papers published in international journals, and the associated prestige of the journal as measured by the Impact Factor (IF). Unfortunately for taxonomy, the IF of a journal is calculated by the number of citations for the papers published in that journal over the preceding year. Further, a common index of productivity (or proxy for 'success as a researcher') is the H Index, which is a measure of the number of papers written by an academic against the number of citations of their papers over the preceding year. Papers in taxonomy and systematics tend to be cited infrequently, due to the long-standing nature of careful decisions made in description of new species and positioning of clades, which puts taxonomists at the disadvantage of not having their work cited frequently.

Therefore, I place very high value in establishing collaborations with productive researchers in the field who are also interested in investigating ecological aspects of acanthocephalans. Discussions in the US and Mexico were productive and will hopefully lead to several collaborative research projects (Figure 3). This serves the dual purpose of linking Australian researchers with international collaborators to produce high-quality results, and also to diversify the field of research on Acanthocephala within Australia and develop new projects and avenues for research. The inclusion of additional biological and ecological factors into research on the Acanthocephala will enable a wider range of papers to be published, enhancing the research profile of the scientists involved.

Figure 3. Taking a break from learning about fish habitats in Mexico City, with Dr Garcia Varela. We used a traditional flat-bottom barge to reduce disruption to the environment.

*Next steps*

The final step in putting together the new expertise into meaningful data collection, analysis and production of research outputs is to leverage funding to make it all possible. For the disciplines of taxonomy, systematics, wildlife parasitology and fundamental science in general, it can be incredibly difficult to attract funding for research projects. Data collection, in the form of collecting parasites, can take many months, even years, which causes further difficulties in maintaining research budgets.

The Australian Biological Resources Study (ABRS) is the only body in Australia to fund exclusively taxonomic and systematic projects. Their funding of my Fellowship has enabled me to establish the right connections with leading experts in acanthocephalan taxonomy and systematics. This has put me in a position to pursue new and exciting research on the Acanthocephala in Australia and to raise the profile of parasitology and taxonomy in the public eye.

Sharing resources between collaborators is one way in which limited funding for parasitology and taxonomy can be used constructively, and one method I hope to use in working with Dr Garcia Varela. I hope to develop strategic research opportunities with him and the researchers in his laboratory that require little funding, but result in high-quality research outputs. Access to an already well-developed reference collection (held by Dr Garcia Varela) will allow me to conduct research without the delay of having to first collect new sets of specimens, thus expediting the research process.

## Conclusions and Recommendations

The Acanthocephala is a relatively neglected group of parasites of vertebrates that is only studied by a small group of parasitologists worldwide. Australia currently lacks appropriate research capacity for studying these parasites, especially in the use and application of molecular data for analyses. Through the Churchill Fellowship, I was able to increase my experience in working with these parasites in the following ways:

- Developing my expertise and learning new skills in the use and application of molecular data
    - Includes DNA extraction methods, handling and storage of specimens for analysis, record keeping and data management of sequences, PCR products and host-parasite data.
- Discussion of the research priorities for acanthocephalan research, opportunities for funding and strategic career-building advice with Drs Martin Garcia Varela (UNAM), Eric Hoberg (US National Parasite Collection), Tim Littlewood and Ms Eileen Harris (Natural History Museum, London) and many others.
- Understanding the importance of developing linkages within the small acanthocephalan research community, for the purposes of sharing data and developing a research niche as a new scientist in the field.
- Learning techniques for maximizing return – in the context of publishing research papers that have a higher level of attraction for researchers, i.e., by including ecological and or biological research with systematics.

The long-term aim for this work is that I will be able to build the research capacity within Australia to work with acanthocephalan parasites. By fostering linkages with other researchers and developing my own expertise, I can teach new research students all the key information for working with this neglected group of parasites. Work needs to be done to raise the profile of taxonomy, systematics and phylogeny as important aspects of fundamental biology. Through my research and via interacting with organisations such as the ABRS, I hope to highlight the importance of the discipline and ensure that future generations of parasitologists value the contribution that systematics makes to improving our understanding of the world.

**References**

Amin, O. 1985. Classification. Pages 27-72 in D. Crompton, and B. Nickol, editors. Biology of the Acanthocephala. Cambridge University Press, Cambridge.

Garcia Varela, M., M. Cummings, G. Perez-Ponce de Leon, S. Gardner, and J. Laclette. 2002. Phylogenetic analysis based on 18S ribosomal RNA gene sequences supports the existence of the class Polyacanthocephala (Acanthocephala). Molecular Phylogenetics and Evolution **23**:288-292.

Garcia Varela, M., and S. Nadler. 2005. Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. Journal of Parasitology **91**:1401-1409.

Garcia Varela, M., G. Perez-Ponce de Leon, P. de la Torre, M. Cummings, S. Sarma, and J. Laclette. 2000. Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. Journal of Molecular Evolution **50**:532-540.

Garey, J., T. Near, M. Nonnemacher, and S. Nadler. 1996. Molecular evidence of Acanthocephala as a subtaxon of Rotifera. Journal of Molecular Evolution **43**:287-292.

Monis, P. 1999. The importance of systematics in parasitological research. International Journal for Parasitology **29**:381-388.

Monks, S., and D. Richardson. 2011. Phylum Acanthocephala Kohlreuther, 1771. Pages 234-237 in Z. Zhang, editor. Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. Magnolia Press, Auckland.

Perkins, S., E. Martinsen, and B. Falk. 2011. Do molecules matter more than morphology? Promises and pitfalls in parasites. Parasitology **138**:1664-1674.

Van Cleave, H. 1936. Recognition of a new order in the Acanthocephala. Journal of Parasitology **22**:202-206.

Verweyen, L., S. Klimpel, and H. Palm. 2011. Molecular phylogeny of the Acanthocephala (Class Palaeacanthocephala) with a paraphyletic assemblage of the orders Polymorphida and Echinorhynchida. PLoS One **6**:e28285.