# Progress Towards a Phylogeny of World Tachinidae

## Year 1

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#### INTRODUCTION

## by John Stireman (Figs. 1–2)

Developing a structural phylogenetic framework for the family Tachinidae has been an interest of mine since I first started working on tachinids as a graduate student. It was then that I developed a new phylogenetic perspective with which to view the world, and many of the questions I wanted to ask of tachinids depended on some level of phylogenetic knowledge of them. I was surprised at the time that no one had attempted a broad quantitative phylogenetic analysis of the family, and a publication from my thesis work on the Exoristinae (Stireman 2002) became the first such study that I know of. Ever since that initial work, I have been seeking ways to continue working towards the development of a broad and robust phylogeny of Tachinidae (e.g., Stireman 2005, 2010).

About ten years ago, Kevin Moulton (Univ. of Tennessee), developed the first full proposal for a molecular phylogeny of Tachinidae and invited me to participate as a postdoc. Unfortunately this, and a subsequent proposal, failed to garner interest from U.S. governmental funding agencies, and we put it "on the back burner" and pursued other research projects. Obtaining a professorship position in 2005 at Wright State University allowed me the resources and opportunity to begin collecting tachinid samples and sequence data



**Figure 1**. John Stireman at Anysberg Nature Reserve, South Africa.

"on the side" while conducting other work. We were not in a big rush to get rejected yet again, so we put off re-submitting a revamped proposal until 2010, when we invited Jim O'Hara (CNC) to be a collaborator. In this revised proposal, we sought to make the tachinid phylogeny project more of a collaborative effort as you can see from my 2010 article in The Tachinid Times exhorting aid from tachinid workers. This same year, Tachi and Shima (2010) published their paper focused on the phylogeny of Exoristinae, a vast improvement over my initial attempts. This paper demonstrated the potential insight that phylogenetic analysis of tachinids could provide, and spurred our efforts. Again, our proposal was rejected. But we were not so easily deterred; we revised it and resubmitted it twice more, each time expanding our goals and proposed products.



**Figure 2**. An *Archytas* sp. sitting on a leaf in Tar Hollow State Park, Ohio (USA).

We were finally awarded a grant in 2012 from U.S. National Science Foundation to pursue our goals of developing a broad phylogenetic framework for Tachinidae. This initiative, "The Phylogeny and Evolution of World Tachinidae", was designed to be a collaborative project, involving many tachinid researchers from around the world. The primary collaborators are John Stireman (me, Wright State Univ.), Jim O'Hara (CNC), Kevin Moulton (Univ. of Tennessee), Pierfilippo Cerretti (Univ. of Roma), and Isaac Winkler (who recently joined my laboratory as a postdoc working on the project). I also have a Ph. D. student, Zachary Burington, working in my lab on this project (see the Student News section of this newsletter for a note from Zach).

In addition, we have solicited the involvement of many additional tachinid researchers. Thus far, we have received advice and assistance from a number of colleagues, including but not limited to Monty Wood, Ashley Kirk-Spriggs, Dan Janzen, Daniel Whitmore, Takuji Tachi, and Hiroshi Shima.

I don't believe I need to sell the readers of this newsletter on the importance of Tachinidae or the benefits of an in-depth comprehensive phylogenetic study of the family. Nor do I probably need to impress upon you that the relationships among tribes and subfamilies of Tachinidae are obscure and the family is among the most taxonomically difficult of Diptera.

Thus, I can dispense with justification and get on with the main goals of our proposed research over the next several (many?) years, which include: 1) The robust reconstruction of phylogenetic relationships among major tachinid lineages (ca. 200–300 genera) using molecular (5+ genes) and morphological data.

 To use our phylogenetic findings to revise current classificatory schemes and produce a stable, predictive classification of Tachinidae.
More focused phylogenetic analyses of two biologically interesting and agronomically important groups, the tribe Blondeliini and the subfamily Phasiinae.

4) Analysis of the evolution of reproductive traits, oviposition strategy, and host associations and their effects on diversification and biological control success.

5) Dissemination of taxonomic and biological information on Tachinidae through the development of web resources and an interactive key to North American (for now) genera.

These goals are a little ambitious given the three year term of NSF grants (and the reduced budgets we were awarded), and to be realistic, I don't think it is all going to happen in this time frame. But, these are the goals we are working towards, and we seem to be making appreciable progress so far. I have invited each of the major collaborators to provide a brief overview of their recent efforts in relation to the overall goals of our project (below). We plan to provide continued news of our activities and progress in future issues of *The Tachinid Times*.

### MAJOR COLLABORATORS

## Jim O'Hara (Fig. 3)

first corresponded with John back in 1995. I think he had recently moved from Utah to Arizona and had spent the summer roaming around southern AZ in his pickup truck looking for tachinids before starting a Ph.D. on a tachinid ecology project at the University of Arizona in Tucson. I met John in person in 1999 during one of my field trips to Arizona. He led me to a remote canyon in the Huachuca Mountains to a spot he had christened "tachinid hole", which in a good year is rich in tachinids. Although distant by road from my best collecting spot in the Huachuca Mountains (the Hamburg Trail portion of Ramsey Canyon), it was only a couple of kilometers away as the crow flies.

John's interests extended beyond tachinid ecology to evolution, both in the sense of tachinid/ host interactions and tachinid phylogeny. Eventually these interests led to John's idea of putting a team together to seek funding from NSF to pursue the present study. The goals of this study actually incorporated some of the ideas put forth by Kevin Moulton in an earlier but unsuccessful attempt to obtain funding from the United States Department of Agriculture to study the phylogenetics of primarily Nearctic Tachinidae. We now hope that with funding in place and goals to achieve, we can make some progress towards understanding the relationships among world tachinid taxa above the generic level.

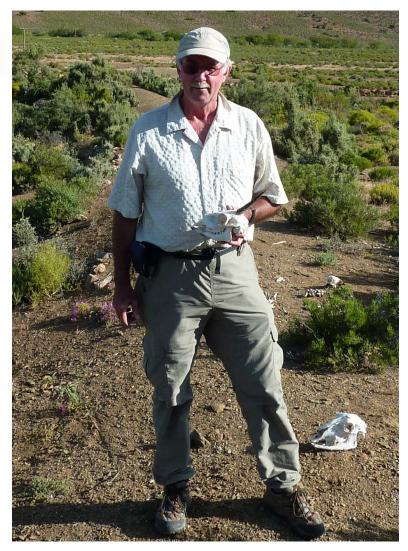


Figure 3. Jim O'Hara at Anysberg Nature Reserve, South Africa.

My contributions to this study are intertwined with my own research program funded by Agriculture and Agri-Food Canada (AAFC) that supports myself and my technician Shannon Henderson. My research over the past decade has shifted away from traditional taxonomic revisions to gathering together information for broader use; e.g., regional catalogues, an ongoing world taxonomic database, and involvement in the Tachinidae chapter of the Manual of Afrotropical Tachinidae (led by Pierfilippo Cerretti). If the present study leads to a better understanding of tachinid relationships and an improved classification, then this will be good for the projects I am pursuing on behalf of AAFC.

For the NSF project, Shannon and I will be expanding the coverage of the Tachinidae in the TachImage Gallery (http://www.nadsdiptera.org/Tach/Tachgallery/Tachgalleryhom.htm). I will be supplying Pierfilippo with specimens from the Canadian National Collection of Insects for use in an interactive key to the Tachinidae of America north Mexico and assisting with its development. I will participate in the interpretation of results and the writing of papers, and the collection of specimens for molecular analyses. The last is a vital part of the project and, from my perspective, one of the more enjoyable aspects of it. I started collecting specimens for molecular analyses several years ago in the hopes that the the project would be funded. The method I use for preserving material was described two years ago in this newsletter (*The Tachinid Times* 24: 11–14). Last year I collected specimens for this project in Arizona (Fig. 4) and New Mexico and also participated in the group trip to South Africa that we review elsewhere in this issue.



Figure 4. Unexplored hilltops near the Winchester Mountains in southern Arizona, USA.

### Kevin Moulton (Fig. 5)

yself and my Master's student Jeremy Blaschke (see the Student News section of this newsletter for a note from Jeremy) at the University of Tennessee, Knoxville are primarily responsible for collecting molecular data for the subfamily Phasiinae, collection of sequences for the gene MAC, and aiding Stireman's group in the collection of sequences for newly developed genes. The phasiines are an agronomically important group of heteropteran parasitoids and many genera have been used with varied success as biocontrol agents. From personal collecting trips and additional material sent from collaborators, we have been able to extract DNA from 35 phasiines representing 28 genera from 8 of the 9 collectable worldwide tribes. We are sequencing five different genes: CAD, MAC, LGL, MCS, and OPA or TUFT. These genes have varying evolutionary rates and when combined into a single data set will hopefully be able to reconstruct subfamilies, tribes, and beyond. We have sequences for CAD and MAC from all 35 available taxa, 33 for LGL, 16 for MCS, 8 for OPA, and 4 for TUFT.

As mentioned earlier, we are responsible for providing MAC, LGL, and MCS sequence data for a preliminary investigation of gene utility using a sparse though representative sample of genera. This initial study will consist of representative genera from important tribes within each subfamily as well as several outgroup taxa from each oestroidean family. We want to include around 32 taxa in this phylogeny and of those we have 32 for MAC, 30 for LGL, and 24 for MCS. We hope to have sequence data for all genes by the end of spring semester 2013. Some interesting notes from collecting efforts in 2012. We have been baiting flight intercept traps with pheromones from *Halyomorpha halys* (Stål) (brown marmorated stink bug). The pheromones have successfully doubled (sometimes more) our catch rate of several phasiine genera. We will be exploring heteropteran pheromone baiting in the upcoming collecting season. Lastly, we have discovered what might be two distinct morphotypes of the important biocontrol species *Trichopoda pennipes* (Fab.) in the eastern United States. Further morphological and molecular research will be done to determine if these entities are valid species.



**Figure 5**. Kevin Moulton collecting Blephariceridae at Brasstown Bald, Georgia, USA.

### Pierfilippo Cerretti (Fig. 6)

y focus on tachinid systematics dates back to 1998, when I first met Jim O'Hara and Monty Wood at the International Congress of Dipterology in Oxford (UK). Soon after the meeting Monty kindly introduced me to Benno Herting and Peter Tschorsnig (Stuttgart). Since 1999 I have visited the Tschorsnig lab several times and have learned a lot from Peter about tachinid systematics and biology. In more or less a decade I revised the whole Italian fauna to species level (Cerretti 2010), producing also

confident that a manuscript with preliminary but interesting results can be submitted in this spring), 2) to lead the development of an interactive key to Nearctic genera, and 3) to supply John's and Kevin's labs with specimens for molecular analyses. Of course, to study the evolution of morphological and biological traits over trees generated by molecular data will probably be the most exciting challenge for all of us!

an interactive key to Palaearctic genera in collaboration with Peter and Massimo Lopresti (Verona) (Cerretti et al. 2012). At the same time I started studying the Afrotropical fauna in order to revise the tribal and generic level classification and eventually extend the interactive key to this huge fauna. It is because of my interest in the African fauna that Ashley Kirk-Spriggs in 2009 invited me to lead the tachinid chapter of the upcoming Manual of Afrotropical Diptera (http://afrotropicalmanual.net/) that will be co-authored by Jim O'Hara, John Stireman and Monty Wood. In this project Jim is leading a new revised version of the Afrotropical tachinid catalogue.

Scoring morphological characters and developing interactive key applications is now one of my main activities and the reason why John Stireman and Jim O'Hara kindly asked me to join the "Phylogeny of World Tachinidae" project proposal in 2010. As mentioned already by John, the project has several ambitious goals and these are so interesting to me that I simply could not turn down their request to collaborate. In this context my main responsibilities are 1) to score morphological characters to infer the first phylogenetic reconstruction of the Tachinidae using a cladistic approach (we are



**Figure 6**. Pierfilippo Cerretti and shy tortoise at Anysberg Nature Reserve, South Africa.

#### Isaac Winkler (Fig. 7)

have been helping to coordinate and assemble the molecular data for the overall tachinid tree (except phasiines). We are focusing initially on getting sequence for CAD and 28S. Our student technician, Beth Stayrook, has also been doing a lot of lab work, and we now have some sequence data from 19 tribes and nearly 200 genera.

Also, we have some transcriptome sequence data now from eight tachinid species (two from each subfamily). These each consist of about 30 million short reads, assembled into sequences for about 20,000 genes. It has been interesting and fun learning how to assemble and analyze this genomic data. Within the next two months we should have a phylogenomic data set put together and analyzed. The data should also be helpful for designing primers, studying specific tachinid genes, and even for learning about genome evolution in tachinids.



Figure 7. Isaac Winkler hard at work in the lab.

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