

Comparative anatomy of wings and antennae in Trichoptera and Lepidoptera:
all dressed up but inordinately indifferent?

by

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A Paper Presented to the
Faculty of Mount Holyoke College in
Partial Fulfillment of the Requirements for
the Degree of Bachelors of Arts with
Honor

Department of Biological Sciences

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May 2010

This paper was prepared
under the direction of
Professor Stan Rachootin
for eight credits.

ACKNOWLEDGMENTS

For explaining and listening to many ideas, and for inspiring, criticizing, and supporting me, I am deeply indebted to my advisor Stan Rachootin. You opened my eyes to a “line of life” where I would be most content to spend my academic life.

I wish to thank Gary Gillis for giving me a glimpse of the world of biomechanics. It was exciting and fun. I want to thank Marian Rice for all of her help, without which I could not have documented most of my work. I would like to thank the Biology Department of Mount Holyoke College for funding and support. I also wish to thank Don Davis at the Smithsonian Institution, Ray Pupedis at Yale University, David Wagner at the University of Connecticut, and Rodger Gwiazdowski at the University of Massachusetts for opening their entomology collections to me, without which this would not have been possible. Special thanks to Addison Kemp, Annie Arbuthnot, Melissa Hartley, and Lenna Peterson for their advice, assistance, and support. This project would not have come to fruition without you.

I would like to thank my friends and family who sustained me throughout this process. I wish to express my perpetual gratitude to my parents Carol and Don for all of their encouragement and support—and for letting me loose with a “bug house” collecting jar when I was little.

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ABSTRACT

Trichoptera (caddisflies) are an order of small insects that are closely related to the Lepidoptera (moths and butterflies). Trichoptera and Lepidoptera together comprise the superorder Amphiesmenoptera. Lepidoptera may have evolved from Trichoptera, or they may have their most recent common ancestor in the superorder Amphiesmenoptera, which includes extinct insects that are neither clearly trichopteran or lepidopteran. The main differences between Trichoptera and Lepidoptera are that Trichoptera have hairy wings while Lepidoptera have scaly wings and that caddisfly larvae are generally aquatic and lepidopteran caterpillars are generally terrestrial. Another commonly held distinction has been that the M4 vein in the fore wings is present in Trichoptera but absent in Lepidoptera. In 1973, however, the M4 vein was found to be present in one lepidopteran group—the family Agathiphagidae¹. The discovery of the agathiphagids in 1952 has added some confusion to the basal phylogeny of the Lepidoptera.

There are two competing theories for the basal phylogeny of the Lepidoptera. Kristensen's theory is that the order of Lepidopteran evolution is Micropterigidae + (Agathiphagidae + (Heterobathmiidae + (Eriocraniidae + Coelolepida))). The alternative theory is Shields', which is Agathiphagidae + (Micropterigidae + (Heterobathmiidae + (Eriocraniidae + (Coelolepida))).² With this project, I aimed to improve our understanding of the relationship between Trichoptera and Lepidoptera and to analyze the importance of the line drawn between these orders.

¹ Common, I. F. B. 1973. A new family of Dacnonypha (Lepidoptera) based on three new species from Southern Australia, with a note on the Agathiphagidae. *J. Aust. ent. Soc.* 12: 11-23.

² Kristensen, N. P. 1999. *Lepidoptera, Moths and Butterflies. Volume 1: Evolution, Systematics, and Biogeography*. Walter de Gruyter: New York.

INTRODUCTION

What are the differences between Trichoptera (“hairy wings”, Figure 1), the caddisflies, and Lepidoptera (“scaly wings”, Figure 2), the moths and butterflies, and how important are these differences? These two orders of holometabolous insects make up the superorder Amphiesmenoptera (Figures 3, 4), which means “dressed up wings”. What are the diagnostic features that separate or define these orders? How meaningful are these features? For sexually reproducing organisms, species have biological meaning because their boundaries are the boundaries of genetic exchange and competition for representation in the next generation. Species are also a fundamental unit for ecological interaction. Higher level groups—genera, orders, and phyla—may just be convenient and useful organizational pieces that identify what we know about the branching history of the living world.

Here, I aim to address the significance of orders in classification, specifically the meaning of the lines we draw between them. In the case of Trichoptera and Lepidoptera, the line seems like more of a grey area. What is the justification for making Trichoptera and Lepidoptera separate orders of insects, rather than suborders within Amphiesmenoptera? What characters separate trichopterans and lepidopterans? Do the diagnostic characters play a part in the lives of caddisflies?

What can basal (primitive) moths (Lepidoptera) tell us about the differences between Trichoptera and Lepidoptera? By comparing primitive Lepidoptera (Agathiphagidae, Micropterigidae) and Trichoptera, we can learn about how close together the two orders are. Traits shared by primitive Lepidoptera and Trichoptera were most likely present in their common ancestor.



Figure 1. Adult trichopteran, or caddisfly (unidentified)
"Trichoptera." *Academic Dictionaries and Encyclopedias*. Web. 25 Apr. 2010.
<<http://en.academic.ru/dic.nsf/enwiki/747660>>.



Figure 2. Adult lepidopteran, or moth: *Hyalophora euryalus*
The antennae are bipectinate antennae, meaning that each side of each antenna has comb-like extensions. Males use the increased surface area of their antennae to sense pheromones emitted by females. Females bear unbranched antennae.
Bura, Veronica. *Hyalophora euryalus*. 2007. Photograph. *Lepidoptera and Associated Orders of British Columbia*. 24 Apr. 2007. Web. 20 Mar. 2010.
www.zoology.ubc.ca/bclepetal/



Figure 3: Cladogram of Endopterygota

Trichoptera and Lepidoptera are sister groups, that are next most closely related to Hymenoptera, Mecoptera, Diptera, and Siphonaptera. This cladogram does not include the names of any superorders, though the superorder Antliophora is made up of Mecoptera, Diptera, and Siphonaptera, and the superorder Amphiesmenoptera is made up of Trichoptera and Lepidoptera. Tree of Life Web Project. 1995. Endopterygota. Insects with complete metamorphosis. Version 01 January 1995 (under construction). <http://tolweb.org/Endopterygota/8243/1995.01.01> in The Tree of Life Web Project, <http://tolweb.org/>

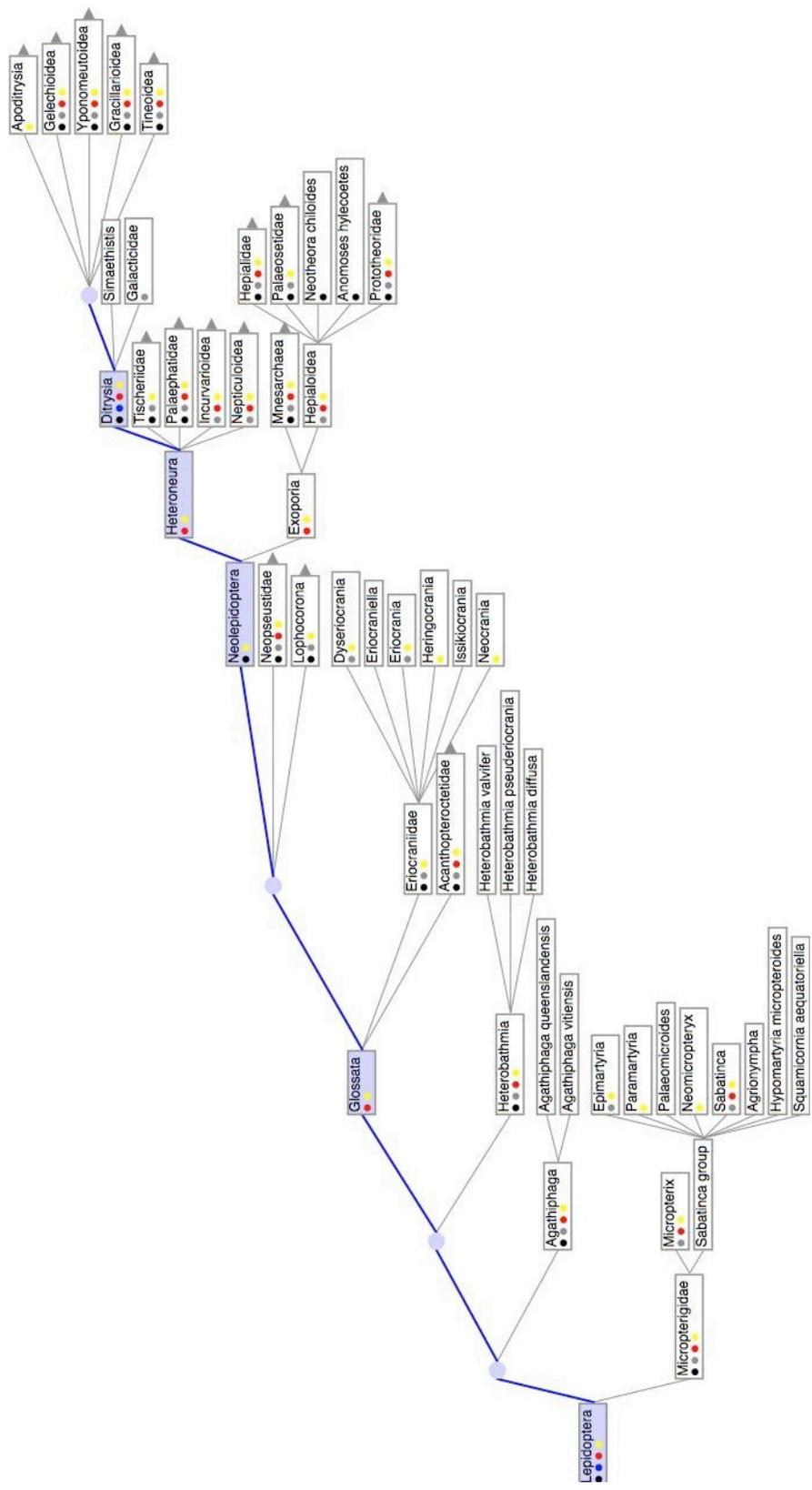


Figure 4. Cladogram of Lepidoptera

This cladogram is according to Kristensen's theory of basal lepidopteran evolution, from the Michael Cummings Laboratory of Molecular Evolution at University of Maryland and University of Maryland's Center for Bioinformatics and Computational Biology. The morphological work has predominantly been done by Kristensen and the molecular work has been done by Brian Wiegmann of North Carolina State University.

"Lep Taxon Tree | Leptree.net." *LepTree--Lepidoptera Phylogeny*. Web. 25 Apr. 2010. <<http://www.leptree.net/leptaxontree>>.

Comparing Trichoptera and Lepidoptera

While Trichoptera and caddisflies are synonymous, Lepidoptera are made up of moths, butterflies, and skippers. Scientifically, there is no real distinction between these three types of Lepidoptera, but there are some general differences. Male moths usually have feathery antennae (Figure 2) and are usually nocturnal. During pupation, they surround their bodies with cocoons made of silk and other materials (leaves, their own body hair, etc.). Butterflies have thin antennae with knobs on their ends. They are generally diurnal, and their pupae are naked and form chrysalises. Skippers are butterfly-like but they did not evolve from the immediate ancestor of the other butterflies (Bartlett, 2004). The order Lepidoptera is mainly moths. There are between 15,000 and 20,000 species of butterflies, and the other 100,000 or so species of Lepidoptera are moths (Sciencercay, 2009). There are just a few thousand more species of butterflies than there are Trichoptera, of which there are 13,000 species.

In his book *The British Caddis Flies*, Mosely (1939) wrote that the "rough and ready distinction [between caddisflies and moths] lies in the vestiture of the wings." He also admitted that this is an unimportant difference. If wing vestiture

were the main difference, it seems that this would not be enough to classify them as separate orders. Mosely (1939) goes on to say that the “real distinction. . . is the structural difference of the mouth-parts and the neuration of the wings”. The reference to these insects’ wing coverings in their names is, surprisingly, not in itself any “real” distinction.

Lepidopteran scales (Figure 33) and trichopteran hairs (Figure 5) are both macrochaetes. They are homologous. Their development and homology are discussed in Chapter 2, Section A. Certain Trichoptera have scales (*Protoptila*, Figure 19).

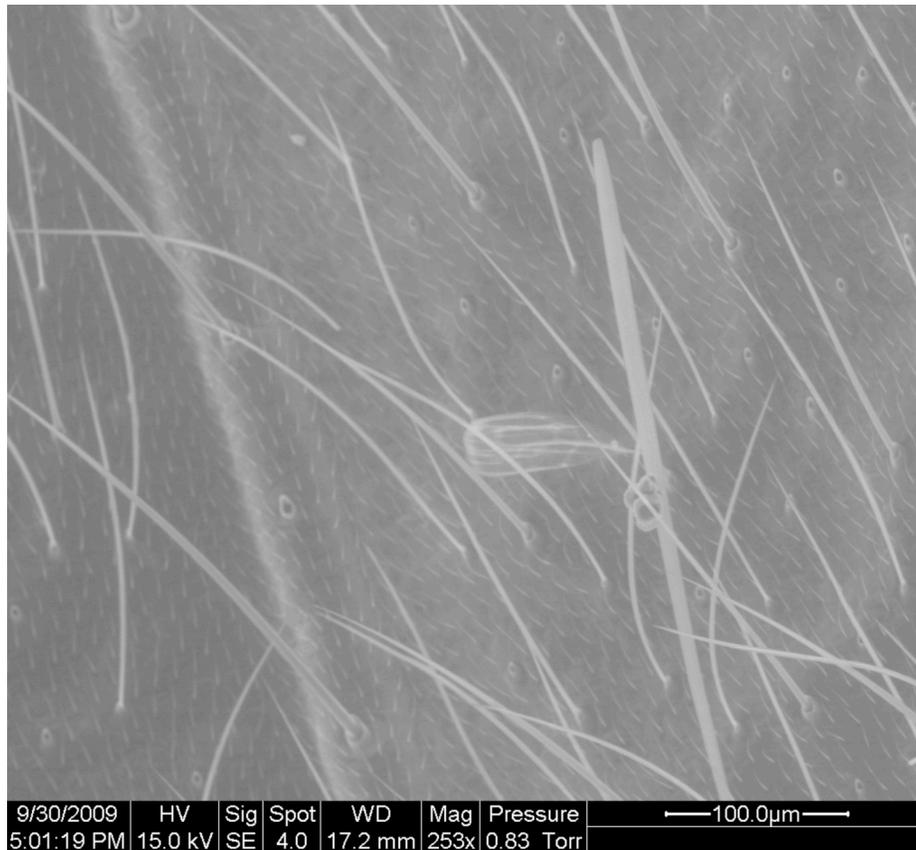


Figure 5. Trichopteran wing hairs

The very small hairs seen are microtrichia. The setae (macrochaetes) are the long (approximately 200 µm), thin hairs that appear white in this image. These setae are homologous to the scales of Lepidoptera. In the right half of the image, lepidopteran scales, one long and thick, can be seen. These were probably transferred to this specimen at the time of collection because some collecting jars were used for both Lepidoptera and Trichoptera. Specimen collected July 2009. Image taken using the scanning electron microscope at Mount Holyoke College.

While many moths play roles in the human environment as pests of tobacco, corn, tomato, wool, etc., the closest approach of caddisflies to humans is as a favorite food for trout. Artificial caddisfly larvae are commonly used by fly fishers as bait. Thus, one of the easily identifiable differences that is well-known to fly fishers is that trichopteran larvae are aquatic, while lepidopteran larvae, caterpillars, are terrestrial. The ecological distinction between Trichoptera and Lepidoptera is obvious and important. While there are exceptions, for instance, the terrestrial caddisfly larva of *Enoicyla* (Mosely, 1939) and the amphibious moth caterpillars of the genus *Hyposmocoma* (Figure 8; Rubinoff & Schmitz, 2010), this ecological distinction mostly holds true. Both trichopteran larvae and caterpillars can spin silk, and larval labial glands that produced silk are assumed to have been present in ancestral amphiesmenopterans (Kristensen, 2003). Some trichopteran larvae and lepidopteran caterpillars build cases that look similar (Figure 6, 7, 8). Trichopteran larvae build their cases out of what they find in their surroundings, much like tineoid caterpillars. Finally, both groups have well-developed mandibles as larvae.



Figure 6. Tineoid “case-bearing clothes moth” and its case (Lepidoptera)
Tineoid moths (bottom) build cases (top) out of what they find. This case resembles those of caddisflies.

Tinea Pellionella. 3 Mar. 2010. Photograph from web. 10 Apr. 2010.

<http://upload.wikimedia.org/wikipedia/commons/a/ab/Tinea_pellionella02.jpg>.



Figure 7. Caddisfly larva

This image is of an unidentified caddisfly larva in its case and underwater.

Wigney, Bev. *Large Caddis Tubes*. Photograph. *The Magick Canoe Website*. Bev Wigney. Web. 5 Mar. 2010. <magickcanoe.com/aquatics/caddis-tubes-large.jpg>.



Figure 8. Amphibious caterpillars of the genus *Hyposmocoma*
Three species of amphibious *Hyposmocoma* caterpillars with their cases: This genus has 12 species of moth with amphibious caterpillars that evolved independently in three lineages. A. Burrito-shaped case larva, attached to substrate with silk line. B. Cone-shaped case larva. C. Bugle-shaped case larva. Rubinoff & Schmitz, 2010.

The anatomical differences between adult trichopterans and lepidopterans hold to the pattern seen in larvae: almost all members of one order have features absent in the other, but then a few exceptions serve to remind us that taxa are diagnoses, not definitions. One of these important structural differences is their mouthparts (Figure 9). Almost all lepidopterans have a proboscis, an elongated mouthpart formed by well-developed galeae. The proboscis sucks up exudates, ranging from nectar to blood. Trichoptera, on the other hand, have a haustellum, or prepharynx. The haustellum can be thought of as a mouth mop and is used to absorb liquid from wet surfaces of mosses or lichens. Some primitive moths, however, including the tineoids and micropterigids, have neither a proboscis nor a haustellum. Micropterigidae, Heterobathmiidae, and Agathiphagidae have retained primitive biting-chewing mouthparts. Their mandibles are functional. Agathiphagids have large mandibles with a distal lobe articulation. They do not have incisor cusps, so it is not known if they can bite off pieces of food, or merely pick up particles, but they do use their mouthparts to break open the *Agathis* seed where they spend their larval and pupal stages. Other mandibulate moths use their mandibles for chewing on pollen (Scoble, 1992; Faucheaux, 2005; Krenn, 2010).

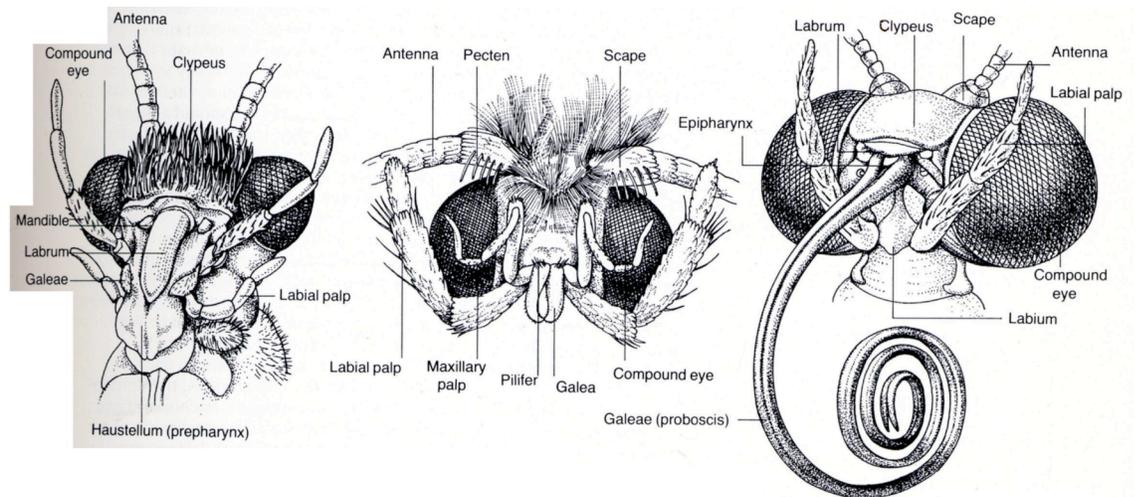


Figure 9. Mouthparts in Trichoptera and Lepidoptera

Mouthparts of a trichopteran, a primitive tineoid lepidopteran, and an advanced lepidopteran (left to right). Trichoptera have a haustellum (a mop), tineoids (Lepidoptera) have biting mouthparts, and advanced Lepidoptera have a proboscis (a coilable straw).

Sbordoni, 1985.

One of the most widely accepted differences between Trichoptera and Lepidoptera that was long thought to be a diagnostic trait is that Lepidoptera do not have the fourth median vein (M4) on the wing, while Trichoptera do (Figure 10). When this was discovered, it seemed to be the first characteristic that held true across Lepidoptera, even for the primitive moths, such as Micropterigidae (Lepidoptera), Eriocraniidae (Lepidoptera), and Tineoidea (Lepidoptera). However, the presence of an M4 vein in one family of Lepidoptera, the Agathiphagidae (Figure 11), has lessened the weight given to this trait. While M4 seemed a standard distinction between Trichoptera and Lepidoptera, which was needed, there is a great deal of variation in venation both within and between these groups. What is the functional or structural significance of this wing

venation difference? Does the M4 connect to the way caddisflies hold their wings or flex during flight?

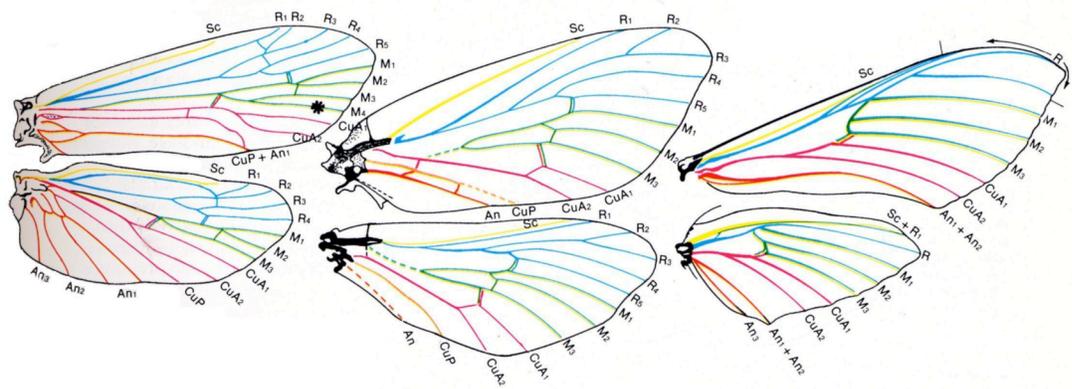


Figure 10. Wing venation in Trichoptera and Lepidoptera

This is the wing venation in a trichopteran, a heerialid primitive lepidopteran, and an advanced ditrysian lepidopteran (left to right). Trichoptera have an M4 vein, marked with an asterisk, which used to be a diagnostic trait of the order. Lepidoptera (except for Agathiphagidae, Figure 11) do not have the M4 vein. Sbordoni, 1985.

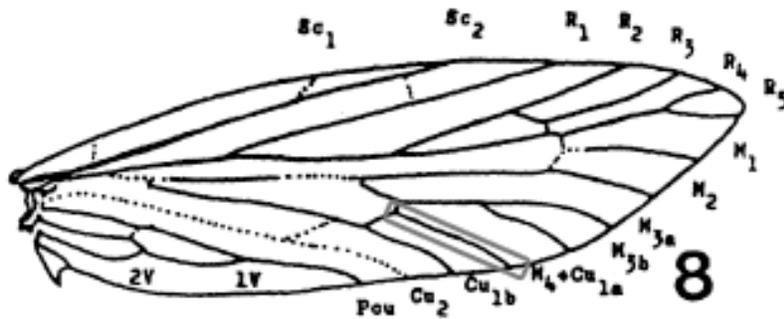


Figure 11. Wing venation in *Agathiphaga* (Lepidoptera: Agathiphagidae).

The M4 vein, in grey box, is present in this lepidopteran, but no other Lepidoptera.

From left to right starting on top left of image, veins are:

Sc₁, Sc₂, R₁, R₂, R₃, R₄, R₅, M₁, M₂, M₃, M₄ + Cu_{1a}, Cu_{1b}, Cu₂, P
Shields, 1988.

History of the classification of Trichoptera and Lepidoptera

According to Sharp (1909), Réaumur (18th century) thought Trichoptera are “practically Lepidoptera with aquatic habits.” In 1817, Leach linked Trichoptera and Lepidoptera together (Crampton, 1920). In 1896, Haeckel named this group Sorbentia. Most entomologists since Leach’s time have seen these two orders as very closely related. The micropterigid moths, a very primitive family of moths, have had an integral role in relating Trichoptera and Lepidoptera for ninety years. Speyer (1839) suggested that the Micropterygidae (now Micropterigidae) is a transitional group that *led to* the Trichoptera. Crampton (1920) asserts that subsequent investigations confirmed Speyer’s suggestions (1839) and the importance of the phylogenetic position of Micropterygidae. There were varied suggestions for the phylogeny of the groups and individuals within Micropterygidae, but eventually Packard (1895) placed the genus *Micropteryx* (Micropterygidae) into its own suborder, Paleolepidoptera, based on how different these insects are from Trichoptera or other Lepidoptera. Then in 1916, Chapman placed *Micropteryx* in its own order, Zeugloptera because he saw *Micropteryx* as so unusual and did not think that it was lepidopterous (Crampton, 1920).

In 1909, Sharp went so far as to assert that “unless it should be decided to transfer *Micropteryx* to Trichoptera, and then define Lepidoptera and Trichoptera as distinguished by the condition of the pupa, it would appear to be very difficult to retain the two groups as distinct” (Sharp, 1909). This represents the closeness

of these groups and the weight that has been given to the micropterigids for over a century. Their phylogenetic placement has inspired transitional scenarios for more than one hundred years. Other primitive moth families, in contrast, were not discovered until later. Actually, patterns of thought that arose in regard to micropterigids (Figure 12) were slow to change as new basal groups were discovered, notably the agathiphagids (Figure 13) in 1952 (Dumbleton, 1952).

Brauer was another biologist to emphasize the similarities between Trichoptera and Micropterigidae before the discovery of Agathiphagidae, and he thought that Trichoptera and Lepidoptera might be best placed in the same group. He found that trichopteran larval mandibles are

An important distinction: the pupa of *Micropteryx* has however been recently shown to be similar to that of Trichoptera, and then define Lepidoptera and Trichoptera as distinguished by the condition of the pupa, it would appear to be very difficult to retain the two groups as distinct (Sharp, 1909).

How is the pupa of this micropterigid moth (Lepidoptera: Micropterigidae: *Micropteryx*) similar to that of Trichoptera? If the only similarity he wrote about was the pupal jaws, then the presence of such jaws in other Lepidoptera indicates that the presence of pupal jaws in *Micropteryx* is not a strange occurrence in only one lepidopteran. The early importance placed onto the micropterigid moths may have clouded the judgment of lepidopterists to this day.



Figure 12. The micropterigid moth *Epimartyria auricrinella* (Lepidoptera: Micropterigidae)

This specimen of the micropterigid moth *Epimartyria auricrinella* was collected in Quebec in 1940. Yale University. Photographed using a portable eyepiece camera attached to dissecting microscope. Image edited in Adobe Photoshop.

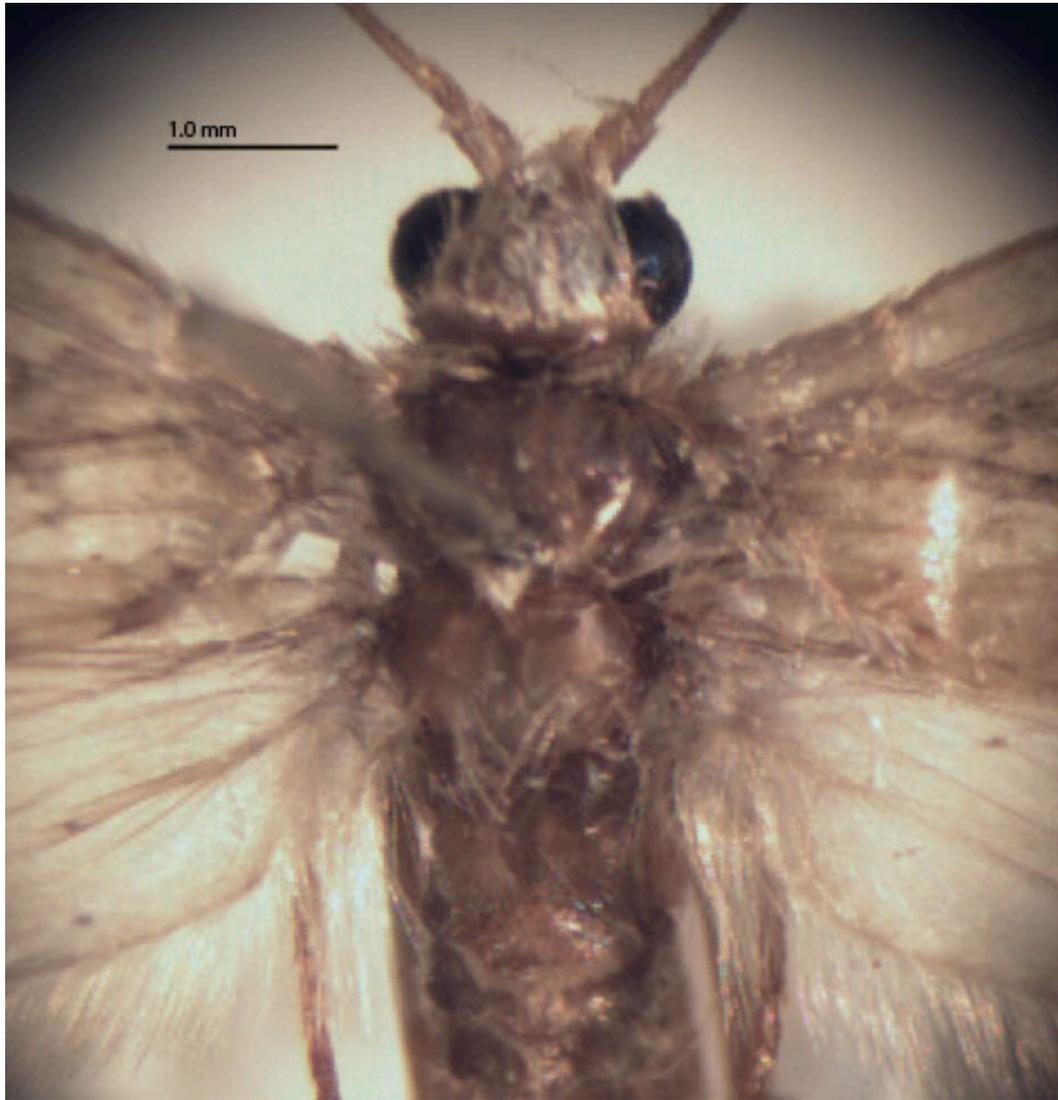


Figure 13. Body of *Agathiphaga vitiensis*

This *Agathiphaga vitiensis* specimen was collected in 1976 in Fiji. Smithsonian Institution. Photograph taken using a portable eyepiece camera attached to dissecting microscope. Image edited in Adobe Photoshop.

The ground plans of Amphiesmenoptera and Lepidoptera

The phylogeny of basal Lepidoptera is based on a number of characters that were catalogued by Kristensen and published in his article “Studies on the morphology and systematics of primitive Lepidoptera” (1984). In this article, he also wrote about autapomorphies of Amphiesmenoptera (Lepidoptera and Trichoptera combined) and Lepidoptera. There are twenty-six lepidopteran autapomorphies and twenty-one amphiesmenopteran groundplan characters (see Appendix A). One way to define a group of organisms (whether this be a genus, class, order, or superorder) is by its autapomorphies (derived traits unique to this group). These are the characters that are best used to understand what diagnoses amphiesmenopterans, and within this superorder, what diagnoses lepidopterans. What level of classification is more meaningful here, superorder or order? Does it mean more biologically if an insect is an amphiesmenopteran (whether trichopteran or lepidopteran) rather than a mecopteran or dipteran, or if an insect is a trichopteran or a lepidopteran? To answer these questions, I examined the literature on amphiesmenopteran and lepidopteran autapomorphies.

Of the twenty-one amphiesmenopteran autapomorphies cited by Kristensen, four are cytological; there are no lepidopteran cytological autapomorphies. The four cytological amphiesmenopteran autapomorphies listed by Kristensen (1984) are (1) female heterogamety, (2) apyrene sperm of usual occurrence, (3) spermatozoa with outer, accessory filaments very thick, filled with

proteinaceous and glycogen-like material, (4) chromosome number specialized (high) and chromosomes probably holocentric; oogenesis achiasmatic.

All in all, Kristensen includes one character (2) that is not an amphiesmenopteran autapomorphy, but he leaves out, by omitting or combining characters, two other characters (see Conclusion). There are five true cytological amphiesmenopteran autapomorphies (see Conclusion). These amphiesmenopteran autapomorphies should be given considerable weight when determining where the biologically important line should be drawn—whether between the orders Trichoptera and Lepidoptera or at the base of the superorder Amphiesmenoptera.

In addition to examining the details of the differences between Trichoptera and Lepidoptera, it is necessary to consider the basal evolution of Lepidoptera. This requires looking at Agathiphagidae (Lepidoptera) and Micropterigidae (Lepidoptera), the most primitive families of Lepidoptera, and the differences between these families and other Lepidoptera. There are eight traits that are found in all Lepidoptera except Agathiphagiade (but the state in Heterobathmiidae are unknown in 5 of these traits). These traits are listed and discussed in the Conclusion. The presence of traits in Agathiphagidae but no other Lepidoptera is not in itself proof that Agathiphagidae is the most basal lepidopteran family. However, traits that are shared by Agathiphagidae and Trichoptera, but no other Lepidoptera may shed light on basal lepidopteran evolution and the closeness of the orders Trichoptera and Lepidoptera.

There are ten traits known to be present in Agathiphagidae, Heterobathmiidae, and primitive Glossata, but not Micropterigidae (these are all primitive Lepidoptera). These are the ten morphological traits that are said to show that Micropterigidae is the most primitive extant lepidopteran family, according to Kristensen's theory. These are listed and discussed in the Conclusion.

Theories on basal lepidopteran evolution

These eighteen traits (see Conclusion) have led to two main theories on basal lepidopteran evolution. Kristensen (1999) proposed that the order of lepidopteran evolution was Micropterigidae (Figure 13), Agathiphagidae (Figure 12), Heterobathmiidae, Glossata. However, he had earlier proposed that the order was Micropterigidae, Heterobathmiidae, Agathiphagidae (1984). The change suggests that Agathiphagidae have been difficult to understand, and the understanding of them changed between Kristensen's publications. Shields (1988) proposed that the order of lepidopteran evolution was Agathiphagidae, Heterobathmiidae, Micropterigidae, Glossata. Kristensen's is the generally accepted theory (pers. comm., Davis, 5 March 2010). The main competing theory was first proposed by Shields (1988), and was later altered to the order of Agathiphagidae, Micropterigidae, Heterobathmiidae, Glossata (Kristensen, 1999).

Decades ago, before micropterigids (Lepidoptera) came to be understood as primitive moths, Micropterigidae was thought to be a transitional group

between primitive Trichoptera and primitive Lepidoptera (Friedlander, 1983). This is probably because Micropterigidae was discovered before several of the other primitive lepidopteran families, and it is such an odd group taken out of the context of other primitive lepidopteran families. Agathiphagid moths were not discovered until 1952 and because of the attention given to Micropterigidae, they were not considered important in the world of primitive Lepidoptera until Shields (1988) published his theory on basal lepidopteran evolution.

Personal communication between Malcolm Scoble, author of *The Lepidoptera: Form, Function, and Diversity*, and Niels Kristensen indicated that it is possible that either Micropterigidae or Agathiphagidae may occupy the most primitive phylogenetic position in the Lepidoptera (Scoble, 1992). At one point, it was hypothesized that Micropterigidae was the transitional group between Rhyacophilidae (Trichoptera) and other primitive Lepidoptera (Shields, 1988). Kristensen (1984) and Shields (1988) offered alternative hypotheses to the phylogenetic placement of Micropterigidae. Shields (1988) thought his theory best fit the studies done by Common (1973) and two by Kristensen in 1984, on the agathiphagid male genitalia and agathiphagid larval head. Kristensen (1999), of course, thinks his own theory is the best fitting theory.

Kristensen's theory was originally based on ten morphological traits that are present in all basal Lepidoptera except Micropterigidae (1984). He later came up with an ecological scenario that suggests Micropterigidae as the basal lepidopteran family (1997). Kristensen proposes that because micropterigid

(Lepidoptera) larvae are soil animals and live in moist habitats, their ecology likely does not differ much from that of their ancestral amphiesmenopteran. It should be remembered that exoporian larvae are also soil animals, though soil dwelling is not considered a retained plesiomorphic (primitive) trait in this group. Exoporia are a group of primitive Lepidoptera that is more recently evolved than Micropterigiade (Lepidoptera), Heterobathmiidae (Lepidoptera), Agathiphagidae (Lepidoptera), Eriocraniidae (Lepidoptera), and three other families of moths.

Kristensen (1997) proposes an ancestral amphiesmenopteran that was a generalized endopterygote insect. This possible ancestor would have had a primitive mouth, with a movable labrum with extrinsic retractors, mandibles with tentorial adductors, and a labium with distinct paraglossal lobes. These are the mouthparts in the micropterigid moths and in no other panorpoid endopterygotes. The ancestral amphiesmenopteran that Kristensen (1997) proposes is partially based on what is found in micropterigid moths, which assumes that Kristensen's (1984, 1997) own theory is correct. Larvae in basal lineages of Mecoptera, Siphonaptera, and Diptera can be broadly characterized as soil animals, though a group of scorpionflies, which are the sister group to all other Mecoptera, have aquatic larvae, which is here considered to be a specialization rather than a retained primitive character.

Kristensen (1997) also addressed the trichopteran lineage and noted that the shift from soil to an aquatic environment was the "key innovation" of Trichoptera. This was probably not a major step, because many soil habitats are

quite moist and might even be considered semi-aquatic. The aquatic nature of trichopteran larvae is permitted by their tracheal system. All extant trichopteran larvae have an apneustic tracheal system, including the trichopteran with a terrestrial larva, *Enoicyla*.

In 1988, Oakley Shields published his theory on the origin of Lepidoptera in “Mesozoic history and neontology of Lepidoptera in relation to Trichoptera, Mecoptera, and Angiosperms.” The most important conclusion Shields reached was that Agathiphagidae (Lepidoptera) is the oldest living family of Lepidoptera, not Micropterigidae (Lepidoptera). Shields supposedly traces the monophyletic Lepidoptera and Trichoptera back to their mecopteran ancestor. According to Shields (1988), the most primitive living trichopterans are those with free-living larvae—the Rhyacophilidae. Trichoptera evolved from the mecopteran Permochoristidae and Lepidoptera from Trichoptera.

While Micropterigidae (Lepidoptera) was long-thought to be the most primitive group of lepidopterans, Shields (1988) proposed that the first lepidopteran may have been a terrestrial agathiphagid (Lepidoptera) that evolved from an aquatic trichopteran of the Necrotauliidae. While this seems very unlikely because the loss of the apneustic tracheal system is seen in the terrestrial caddisfly larva of *Enoicyla*, though it is not seen in any Lepidoptera and if Agathiphagidae evolved from Trichoptera, then they would have to have lost the apneustic tracheal system. An ancestral amphiesmenopteran, rather than a primitive trichopteran, seems a more likely source of the lepidopteran lineage.

Shields' (1988) theory includes an ecological scenario for the evolution of Agathiphagidae from a trichopteran. At the Triassic-Jurassic boundary, there were a series of marked extinctions of periodic stress connected to extreme drought phases. He proposes that the evolution of Agathiphagidae happened as a result of an extreme drought at the end of the Triassic that dried up many streams. New niches opened up during this drought that paved the way for the evolution of Lepidoptera on land from an aquatic trichopteran ancestor, (Shields, 1988).

Adult Agathiphagidae are very similar to typical primitive Trichoptera, especially Rhyacophilidae, according to Shields (1988). The proposed early evolutionary history of Lepidoptera begins with Agathiphagidae, from which evolved the Heterobathmiidae, with the Micropterigidae derived from the Heterobathmiidae. Shields (1988) theory is based on morphological characters shared by Agathiphagidae and Trichoptera and fossil history. He then came up with an ecological scenario that fit his theory. He bases his theory on morphological characters that are shared by Agathiphagidae and Trichoptera: four large, free testicular follicles, an apical spur on the fore tibia, the M4 vein, and pupal claws. The continued presence of the M4 vein in this lepidopteran group may be important to the phylogenetic placement of the primitive Lepidoptera (Figures 11, 14-16; Chapter Three). There is no adaptive need for the M4 vein, and veins at the back of the wing, such as the M4 vein, are the most important for systematics because of this. These are the only Lepidoptera that share these characters with Trichoptera. Heterobathmiidae share several characters with

Agathiphagidae that more advanced Lepidoptera do not share with Agathiphagidae.

There are two species of *Agathiphaga*, *A. queenslandensis* in Queensland and *A. vitiensis* in Fiji, New Hebrides, the Solomons, and New Caledonia. The Agathiphagidae also have apodous larvae that mine in seeds of the gymnosperm *Agathis*, a genus that has been around since the Early Jurassic. Agathiphagidae are found in part of *Agathis*' range, and nowhere else. Because Shields believes that agathiphagids evolved from Trichoptera rather than from a common amphiesmenopteran ancestor, he theorizes that the agathiphagids' relationship with *Agathis* may have been what allowed these organisms to begin to live on land—agathiphagid larvae can diapause in a hard cell and spend four years inside an *Agathis* seed (Shields, 1988). *Heterobathmia* (Lepidoptera: Heterobathmiidae), on the other hand, feed on pollen on *Nothofagus* flowers as adults and leafmine as larvae. Heterobathmiid moths are one of the three most primitive moth families, the other two being Micropterigidae and Agathiphagidae. *Nothofagus*, the southern beech, is of later origin than *Agathis*, but it has a Gondwana distribution and is a host for other primitive lepidopterans. The ten species of this genus live from 600 to 1,400 m elevation in Argentina, Patagonia, and parts of Chile. The Sabatinca-group of Micropterigidae is in eastern Australia, New Caledonia, and New Zealand. The larvae feed on liverworts, mosses, and grasses while the adults feed on fern spores and angiosperm pollen.

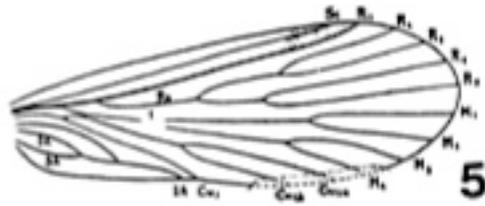


Figure 14. Wing venation of a fossil *Necrotaulius*
 The M4 vein is present in this extinct amphiesmenopteran.
 Shields, 1988.

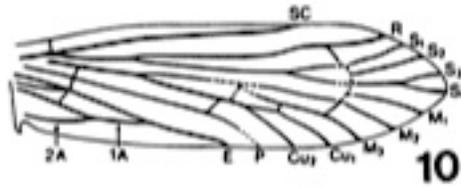
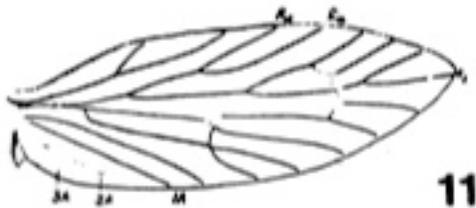


Figure 15. Wing venation of *Heterobathmia* (Lepidoptera: Heterobathmiidae)
 The M4 vein is not present in this primitive moth.
 Shields, 1988.



Amphiesmenoptera. Amphiesmenopteran fossils add confusion to the taxonomy of Trichoptera and Lepidoptera because they are so difficult to identify as either Trichoptera or Lepidoptera and because some amphiesmenopteran fossils have some of lepidopteran ground plan characters but lack others. Within the superorder Amphiesmentopera is the family Necrotauliidae and within Necrotauliidae is the genus *Necrotaulius*. The family Necrotauliidae can be confusing because some of their genera have been assigned to Trichoptera and some to Lepidoptera.

Necrotaulius and *Mesotrichopteridium* are stem group members of Amphiesmenoptera (Ansorge, 2002). Ansorge looked at specimens of the amphiesmentopteran *Mesotrichopteridium intermedium* and found that this species is larger than *Necrotaulius parvulus* and does not have hairs, among other differences between these species. It is curious that *Mesotrichopteridium* does not have hairs, because in Amphiesmenoptera, the lack of hairs is generally correlated with a presence of scales, which gets the species assigned to Lepidoptera.

Ansorge (2002) thinks that *Prorhyacophila* is a younger synonym of *Mesotrichopteridium* (Ansorge, 2002). Bending of the apical part of the CuP vein towards the wing margin usually diagnoses fossils as trichopteran.

Mesotrichopteridium intermedium and *Necrotaulius parvulus* do not have this wing venation trait (Ansorge, 2002). Not having the wing venation trait typical of trichopteran fossils or hairs (also typical of trichopteran fossils) means that

Mesotrichopteridium might have been an amphiesmenopteran that was (or was close to) a lepidoteran ancestor.

When I met with Don Davis of the Smithsonian Institution, he showed me a collection of amphiesmenopteran fossils that had yet to be identified. They had been sent to him for identification as either Trichoptera or Lepidoptera, but he had not had the time to do so. He admitted that identifying these fossils is very difficult (pers. comm., 5 March 2010). With the Amphiesmenoptera, especially the fossil specimens, we must ask, do the specimens define the characters or do the characters define the specimens?

Identification of fossil insects is often restricted to features visible on the wings (scales, venation), so it is helpful, if possible, to use the same features to identify extant Trichoptera (Ivanov, 2002). I would, however, argue that these features do not necessarily identify extant Trichoptera correctly. For Trichoptera, “the combination of the characteristic anal loop on forewings and absence of scales are usually enough to consider an insect a caddisfly” (Ivanov, 2002). But according to Don Davis (and common sense), the anal veins are the thinnest, least cuticularized, least visible, and worst preserved veins in fossil insect wings. This inclusive identification of Trichoptera contradicts the current cladistic thinking and tradition of separating ancestors as taxa (Ivanov, 2002). To define Lepidoptera in the fossil record, the presence of scales and three, rather than four median veins in the forewing are used as diagnostic characteristics (Ansong, 2002). This seems problematic to me because there are Lepidoptera with four

median veins, the Agathiphagidae. It also may be unlikely that scales are always preserved when they are present, especially in fossils of this age because these specimens are not preserved in amber, which would preserve scales.

Ivanov notes that some lepidopteran autapomorphies, such as leg epiphysis, leg-like maxillary palps, and a desclerotized abdominal base, are present in *Necrotaulius tener*, showing some of the first steps of the lepidopteran ground plan. Does this mean that Lepidoptera could have evolved from Trichoptera, or is it more likely that *Necrotaulius* is neither Trichoptera nor Lepidoptera, but related to their common ancestor, which also would have had these first steps of the lepidopteran ground plan? According to Ivanov (2002), “Family Necrotauliidae that time included early Trichoptera and Lepidoptera that yet did not acquire all synapomorphies of these orders, and the specialized by-side offshoots of the primitive Amphiesmenoptera.”

There are problems with rigid cladistic thinking when trying to differentiate Trichoptera and Lepidoptera. The strict holophyletic taxa that are required for fundamental cladistic thinking are contradictory to the natural process of one taxon evolving from another. This strict holophyly denies the existence of ancestors as taxa and makes ancestral taxa paraphyletic by definition. The common ancestor of Trichoptera and Lepidoptera and all its relatives is then neither attributed to recent Trichoptera nor Lepidoptera, nor as a separate order. Instead, the common ancestor and its relatives is a member of the superorder Amphiesmenoptera, along with caddisflies and moths (Ivanov, 2002).

The family Necrotauliidae includes both early Trichoptera and Lepidoptera that did not have all of the synapomorphies of Trichoptera or Lepidoptera. “At the early Mesozoic time the Lepidoptera were nothing more but specialized offshoot of Necrotauliid mainstream; the only way to distinguish them by wing remnants is the presence of wing scales” (Ivanov, 2002). The taxonomic position of the known Mesozoic families of Amphiesmenoptera is tentative: “Necrotauliidae is a heterogeneous family comprising the ancestors of Lepidoptera and extant Trichoptera together with the specialized by-side offshoots of the primitive Amphiesmenoptera phylum” (Ivanov, 2002). Trichoptera and Lepidoptera have been separated since the Jurassic.

The transition from the ancestral amphiesmenopteran to Lepidoptera should be more complicated than the transition from Amphiesmenoptera to Trichoptera because moths are more derived and possess numerous specializations. This could obscure or replace characters that would connect Lepidoptera to their near outgroups. My main question regarding the transition from the ancestral amphiesmenopteran to Lepidoptera is what changes provoked the evolution of the lepidopteran ground plan. The putative link is *Necrotaulius tener* of the Upper Jurassic or Lower Cretaceous of Siberia (Sukatcheva, 1990). *Necrotaulius tener* has certain lepidopteran ground-plan characters. The legs and mouthparts of lepidopteran ancestors probably became Lepidoptera-like before these ancestors developed scales. Scales may have been helpful for living among sticky surfaces, or for better thermal isolation of diurnal insects living in open

sunshine (Ivanov, 2002). The advantage of scales in certain Trichoptera is unclear. *Necrotaulius tener* was not a direct ancestor of Lepidoptera, but it does help us see the possible steps of lepidopteran evolution (Ivanov, 2002).

CHAPTER ONE
HAIRS AND SCALES IN TRICHOPTERA AND LEPIDOPTERA
SECTION A
INTRODUCTION TO WING VESTITURE

I began looking at trichopteran hairs or setae with a few questions in mind. It is commonly accepted that setae and hairs are homologous, but recognizing the basis for homology in these organs was one of my first concerns when addressing the details of the differences between Trichoptera and Lepidoptera. Then, I investigated whether there is any sign of patterning in setae, as there is in scales. Finally, I considered the effects of the differences between setae and scales on Reynolds number and what this may do to flight.

Scales are not universal among hexapods, but they are present on at least some species of Collembola, Archaeognatha, Thysanura, Psocodea, Coleoptera, Diptera, Trichoptera, and Lepidoptera (Kristensen, 2003). The dense covering of tiny, flattened scales on the lepidopteran wing is probably the most striking autapomorphy of the order (Simonsen, 2001). This is also the diagnostic trait used to identify the earliest known fossil lepidopteran, *Archeolepis*, which is from the Lower Jurassic (Simonsen, 2001).

There are many suggested functions for lepidopteran scales. The initial advantage of wing-scales at the time of their innovation may have been insulation (Simonsen, 2001). Wing scales are now known to function in insulation, mimiesis in derived Lepidoptera, and touch (Zhou et al., 2009). Another

advantage of wing scales is that they can come loose, and the insect can escape, leaving some scales behind. This escape mechanism would let the insect escape after contact with a spider web, much like lizards that lose their tails to escape predators. Lepidopteran scales also have close-set exposed surface ridges that allow rain drops to clean dust particles off of their wings (Kristensen, 2003).

According to Kristensen (2003), lepidopteran scales are thermoregulatory because of their absorption of solar radiation. They suggest that in taxa whose body temperature during flight is higher than the ambient temperature, insulating by trunk vestiture provides important protection against heat loss (Kristensen, 2003).

According to Simonsen (2001), scales and their resulting insulation may be one of the reasons that Lepidoptera are so successful as nocturnal insects, but Trichoptera are largely nocturnal and they have survived satisfactorily for millions of years.

The outer surfaces of animals mediate their interactions with the world. Today, the scales in Lepidoptera are involved in temperature regulation, visual, olfactory, and auditory communication with conspecifics, protection from rain and debris, and avoidance of predators, both at distance (mimicry, startle patterns, acoustic baffling) and close up (pulling away from spider webs, or birds that grab them by the wings). Which of these functions, if any, were the initial selective factor on the production of scales in the middle Mesozoic, is not known, and may well not be knowable. A closer study of the natural history of adult caddisflies

might reveal the extent to which these or other functions are served by their vestiture, and whether shared functions result from descent or parallelism.

CHAPTER ONE
HAIRS AND SCALES IN TRICHOPTERA AND LEPIDOPTERA
SECTION B
HOMOLOGY AND DEVELOPMENT OF HAIRS AND SCALES

Insect setae (Figure 17) are bristle- or hair-like processes of the cuticle that originate from certain hypodermal cells (Packard, 1898). Semper was the first to describe the development of hairs, which he observed in the pectinate (comb-like) antennae of the moth *Saturnia carpini*. Semper also observed the development of wing scales. He found that scales and hairs arise from large round cells in a cavity that send a long slender process out through the hypodermis and cuticle. This process becomes the hair or scale (Packard, 1898). He was likely the first to observe the similarities in the development of hairs and scales and to suggest that scales are modified hairs. He first noticed this in his observations of caterpillars, where he saw that in rare cases caterpillar hairs flattened and looked like scales (Packard, 1898). Semper did what he could to show that hairs and scales have the same mode of origin (Packard, 1898). Although Packard may not have known about the clear-winged butterflies (Figure 18), there do seem to be some scales at least on the edges of their wings, so Packard is not rendered incorrect on this matter.

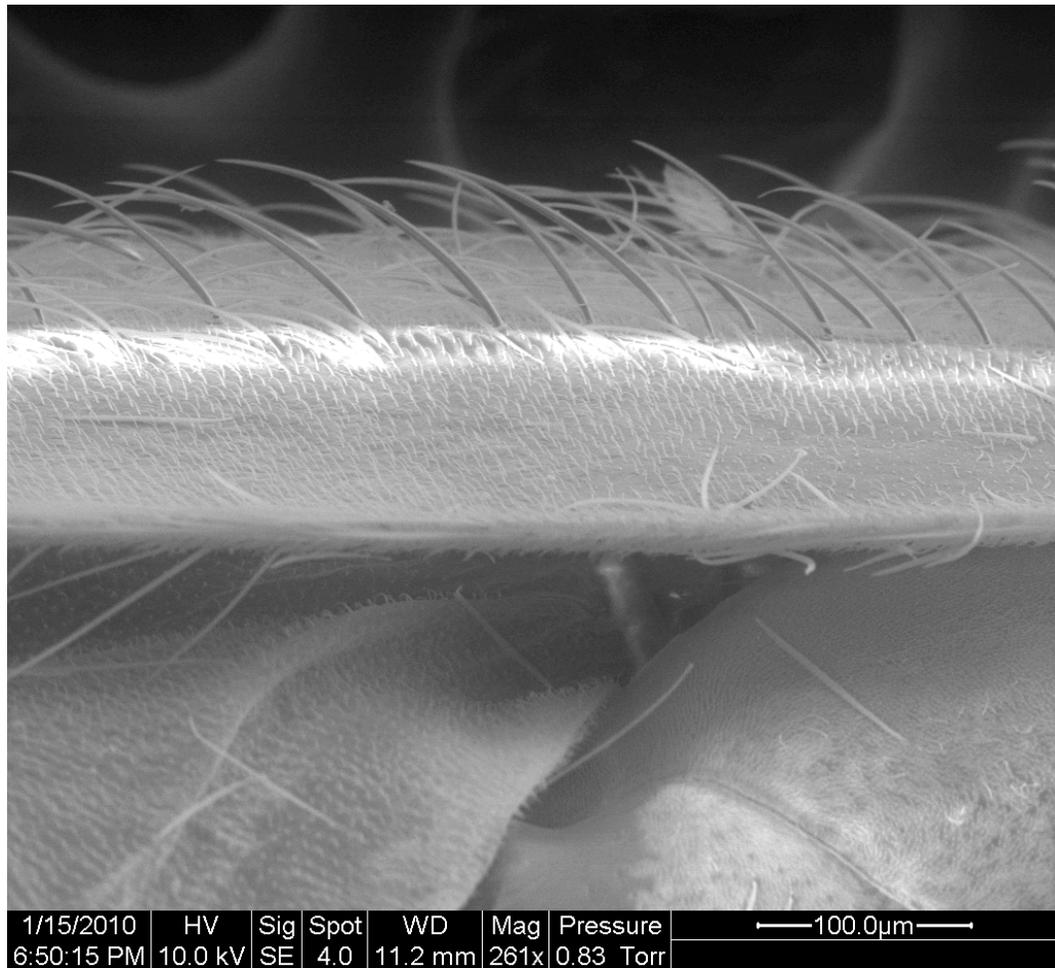


Figure 17. Surface of trichopteran wing
The setae (macrochaetes, measuring 150-200 μm) and microtrichia are shown. The specimen is oriented with the plane of the wing 90° to the stub inside the microscope, giving sideview of wing. Specimen collected in Hampshire County, Massachusetts, August 2009. Image taken using the scanning electron microscope at Mount Holyoke College.

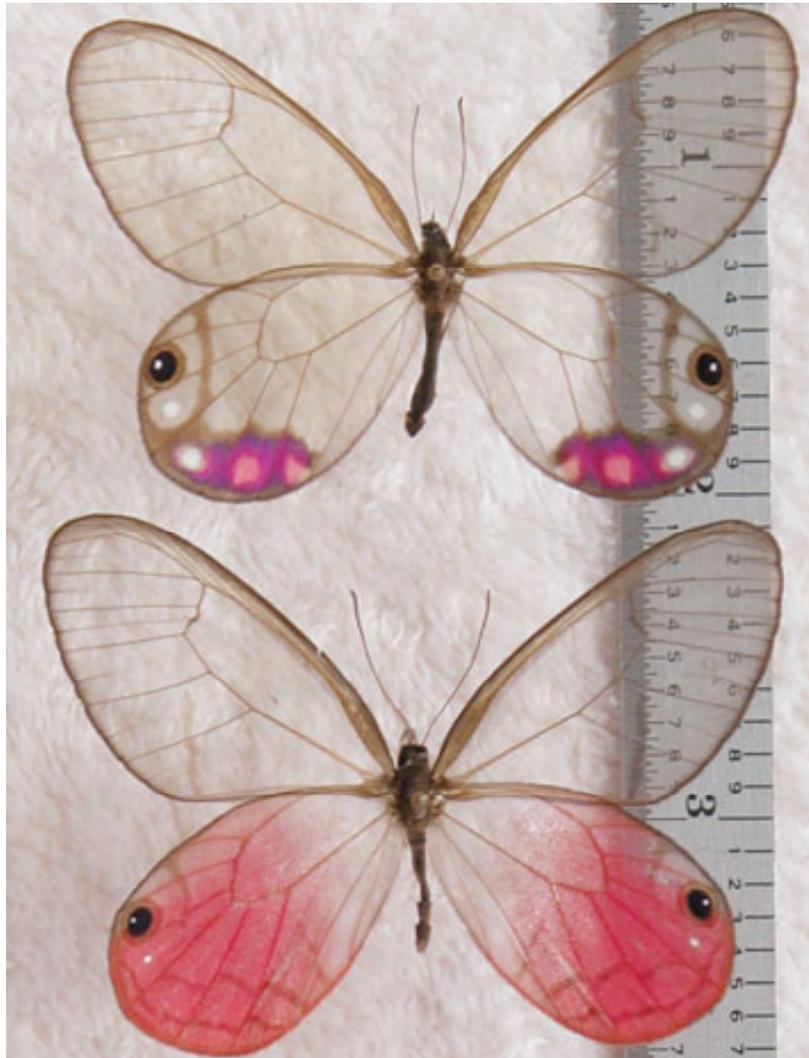


Figure 18. Clear winged butterflies

Clear winged butterflies have only a few scales, which are located around the edges of the wings or at the back of the hindwing.

"Clear Wing Butterflies." *Chuck's Butterfly Page*. Web. 26 Apr. 2010.

<<http://butterflies.aa6g.org/Butterflies/Tropical/clearwings.html>>.

Setae and scales can develop as different forms of the same structure, the macrochaete (Kristensen, 2003). All macrochaetes have common elements. The ultimate designation of their structure mostly depends on their shape. Thus, setae and scales are generally accepted as homologous. Macrochaetes differ from microtrichia and acanthae because macrochaetes have sockets formed by a tormogen cell that envelops the trichogen cell (Kristensen, 2003). The first stage of macrochaete development is the projection of an extension of the trichogen cell above the surface. Lepidopteran scales eventually develop longitudinal ridges and have bundles of actin filaments around the cell periphery. Only epicuticle is secreted at regions of close contact between the trichogen cell membrane and these actin bundles. When this trichogen cell dies, these regions open to form the pores of the scale. As the cell membrane withdraws from the epicuticle, procuticle is secreted. Ridges are then formed between the actin bundles and procuticle. The trabeculae of hollow scales are formed around invaginations of the cell membrane in the late phases of scale development (Kristensen, 2003).

Typically, lepidopteran scales are considered to be homologous to hairs or bristles of other insects. This includes lepidopteran scales and *Drosophila* bristles, based on the similarities in their cell lineages, but the underlying molecular mechanisms were largely unknown until recently (Zhou et al., 2009). In 2009, a group of collaborators at The Biotechnology Research Institute, The Sericultural Research Institute, and the State Key Laboratory for Biocontrol and Institute of Entomology, all in China, used the *scaleless (sl)* mutant of the silk moth *Bombyx*

mori, a model organism for lepidopterists, to learn about lepidopteran wing scale formation by figuring out what is amiss in this nearly scaleless moth. *Drosophila* bristle development is regulated by bHLH transcription factors in the AS-C (Aschaete-Scute Complex). There is a cluster of cells expressing *achaete* (*ac*) and *scute* (*sc*), and some of these develop into bristles (Zhou et al., 2009). Homologues of the Aschaete-Scute Complex (*ASH*) are expressed in developing lepidopteran wings (Zhou et al., 2009). The morphological differences between setae and scales may be because of differences in gene expression (Zhou et al., 2009). The formations of both fly bristles and butterfly wing scales are controlled by *AS-C* family gene expression (Zhou et al., 2009). Zhou and his collaborators were the first to show that closely related genes appear in equivalent cascades, and in Lepidoptera the endpoint of this cascade is scales. This shows that lepidopteran scales and dipteran bristles are homologous. Although molecular work has not been done on Trichoptera, the ties between the setae of this order and the phylogenetically distant dipteran bristles offers an implied homology of trichopteran hairs and lepidopteran scales.

CHAPTER TWO
SCALES IN TRICHOPTERA
WITH SPECIAL REFERENCE TO THE GENUS *LEPIDOSTOMA* AND THEIR
“SCALES”

Although Trichoptera are named for their hairy wings, just as Lepidoptera are named for their scaly wings, some Trichoptera have scales on their wings and other parts of their bodies. There are several possible functions of caddisfly scales. In many caddisflies with scales, the scales are present only in males not females, so they are thought to have some function in mating (Robertson & Holzenthal, 2008). They could serve as a visual cue to females. Scales also might function as androconial organs, glandular cells that produce aphrodisiac pheromones. Scales would provide a larger surface area for pheromone evaporation than hairs. The scales in some members of the trichopteran family Leptoceridae occur in both males and females, so it is possible that these scales are involved in intraspecific communication or protection from predators (Robertson & Holzenthal, 2008).

Huxley and Barnard (1988) found that lepidopteran and trichopteran scales are not homologous because lepidopteran scales' longitudinal ridges are fluted, but trichopteran scales' longitudinal ridges are not. Flutes, also known as microribs, run down the longitudinal ridges of lepidopteran scales (Kristensen, 2003). While the scales of Lepidoptera and Trichoptera may not be the same, this does not mean that they are not homologous as macrochaetes. The earliest known

account of scales in Trichoptera is in the limnephilid *Monocentra lepidoptera* (Robertson & Holzenthal, 2008). Scales are also found in certain members of the trichopteran groups Hydroptilidae, Sericostomatidae, Molannidae, Rhyacophilidae, and Leptoceridae, Conoesucidae, Goeridae, Helicopsychidae, Lepidostomatidae, Limnephilidae, and Glossosomatidae (Robertson & Holzenthal, 2008). The newly discovered glossosomatid *Protoptila diablita* (Figure 19) has scales covering most of its body.

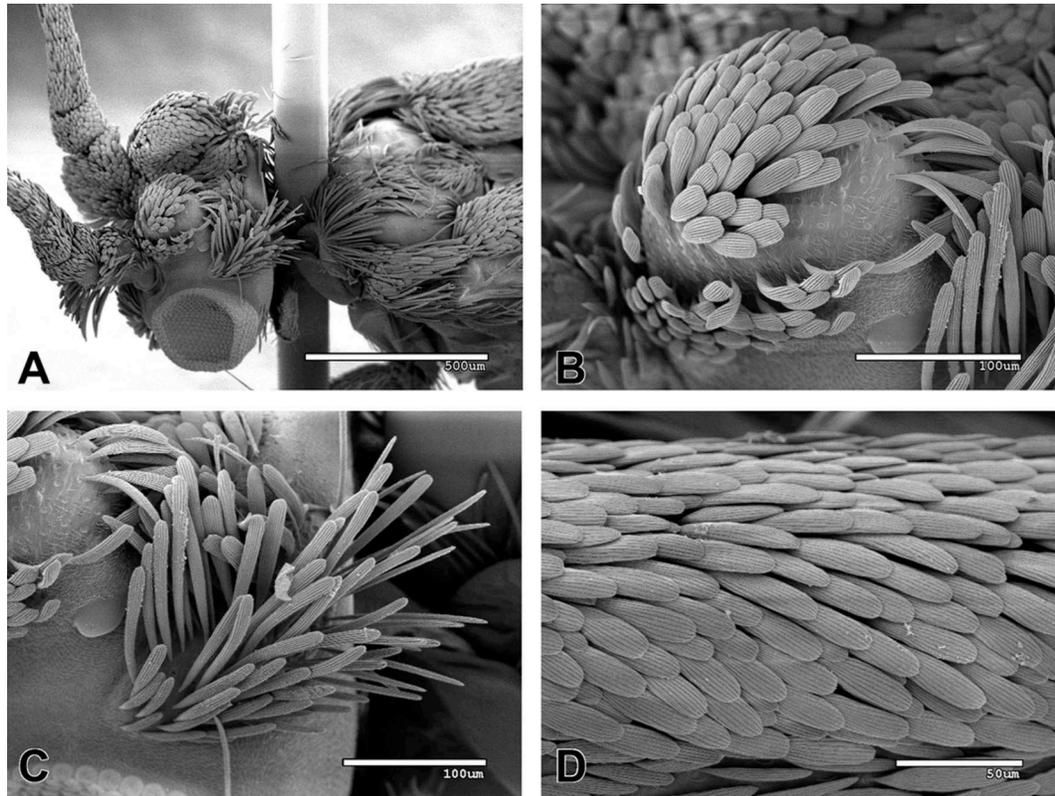


Figure 19. A scaly trichopteran, *Prooptila diablita* (Trichoptera)
This recently discovered species has scales covering most of its body, including antennae, head, thorax, base of wings (A). B and C show scales present on an enlarged setal wart on the head. D shows scales on the basal portion of the forewing. The scales are ribbed.
Robertson & Holzethal, 2008.

While there are many trichopterans with true scales, I examined one genus that is known for including some of the most unusual caddisflies, many with scales. McLachlan (1874) diagnosed this genus, *Lepidostoma*, as having scales. At the time of one of the first significant monographs on Trichoptera, the only discussed lepidostomids were described as being peculiar because of their black scales: “the scale-like clothing. . . are especial characteristics of this genus” (McLachlan, 1874). According to McLachlan (1874), the male *Lepidostoma* can have both scales and hairs, but those with scales have irregular venation. Female *Lepidostoma*, however, have regular venation and no scales. In Yale’s entomology collection, I observed Canadian and American lepidostomids. These included *Lepidostoma togatum* (from Canada), *L. griseum* and *L. pictile* (from Connecticut), on which scales were not observed. “Scales” were observed on an individual of the species *Lepidostoma carroll* (from Connecticut). There were 8 “scales” on one wing and one much larger scale on the other wing. The “scales” observed on *L. carroll* at Yale appeared similar to those on the wings of the specimens at UMass-Amherst. In UMass-Amherst’s Entomology Collection, I observed American specimens of *L. frosti* and *L. roafi* that had black “scales” (Figures 20-22).

I identified some specimens whose wings also seemed to be more or less as McLachlan described: “clothed with scattered black ‘scales’ regularly placed, almost without ordinary pubescence, and almost without hairs on the veins excepting about the sector and basal post-costal veins” (McLachlan, 1874).

Mosley (1939) also described lepidostomid scales: “wings of the male, plentifully strewn with scales” (Mosley, 1939). Mosley only cited Rambur’s *Hist. Nat. Nevr.*, (1842) and McLachlan’s *Rev. & Syn. Trich.*, (1876). McLachlan described lepidostomid scales no more than is demonstrated above. I could not find Rambur’s description of *Lepidostoma*. McLachlan’s is the only source that describes black ‘scales’ of this sort, that is, with scattered black scales, and so it seems that Mosley’s mention of these scales is based on McLachlan’s description of what he terms ‘scales,’ and no independent work on Mosley’s behalf.

As shown in Figures 20-22, these black spots on lepidostomids are not what we consider to be scales. Scales are defined as “modified hairs or setae, flattened and of various shapes so that they overlap one another forming a protective covering for the wings and body” (Jardine, 1914). They are neither uniform in shape nor size. They do not overlap in any way and are randomly strewn about the wing. They do not have the shape of any other hexapod scale. They are not all attached to the wing along the vein, as McLachlan (1874) also observed, though at least two black structures are (Figures 20, 22). I was unable to observe insertion points on the wing membrane. These “scales” are on the underside of the wing. It is difficult to ascertain what these are, but it can be certain that these are not scales comparable to lepidopteran or trichopteran scales. McLachlan’s finding of scattered black “scales” on lepidostomid wings has been repeated countless times, but I was unable to find any images of these “scales”, even in the publications which write of them. Much of the structural attention

given to the lepidostomid genus is to their scaly maxillary palps, though I was not looking at their maxillary palps. Interesting as they are, I find it striking that the repeated observation of scattered black scales has no images. Though many do write of this same phenomenon, the focus is on the maxillary palps, of which there are images.

Based on my observations, I determined that the “scales” that were first written about in McLachlan’s monograph (1874) and then redescribed in Mosely (1939) are not scales. While the presence of scales in many other trichopterans is confirmed, these structures should be rejected as scales. While it was not possible to obtain better images due to the conditions working with a portable camera and whatever dissecting microscopes were available, my conclusions on these black structures are based on my visual observations.

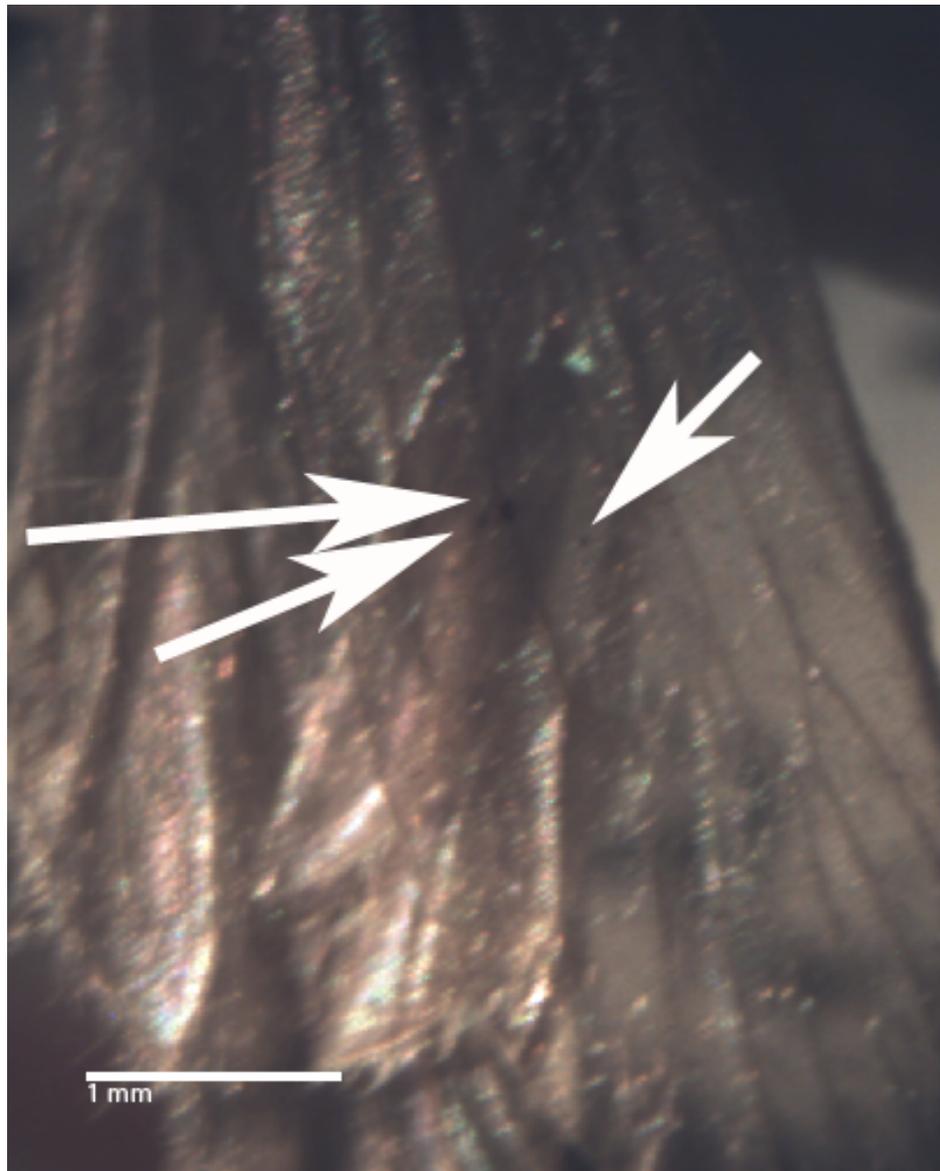


Figure 20. Lepidostomid “scales,” whole wing, (Trichoptera: *Lepidostoma*) This lepidostomid specimen is from University of Massachusetts-Amherst’s Entomology Collection. Two of these black structures are also attached along a wing vein. Same specimen seen in Figures 21, 22. UMass-Amherst. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.

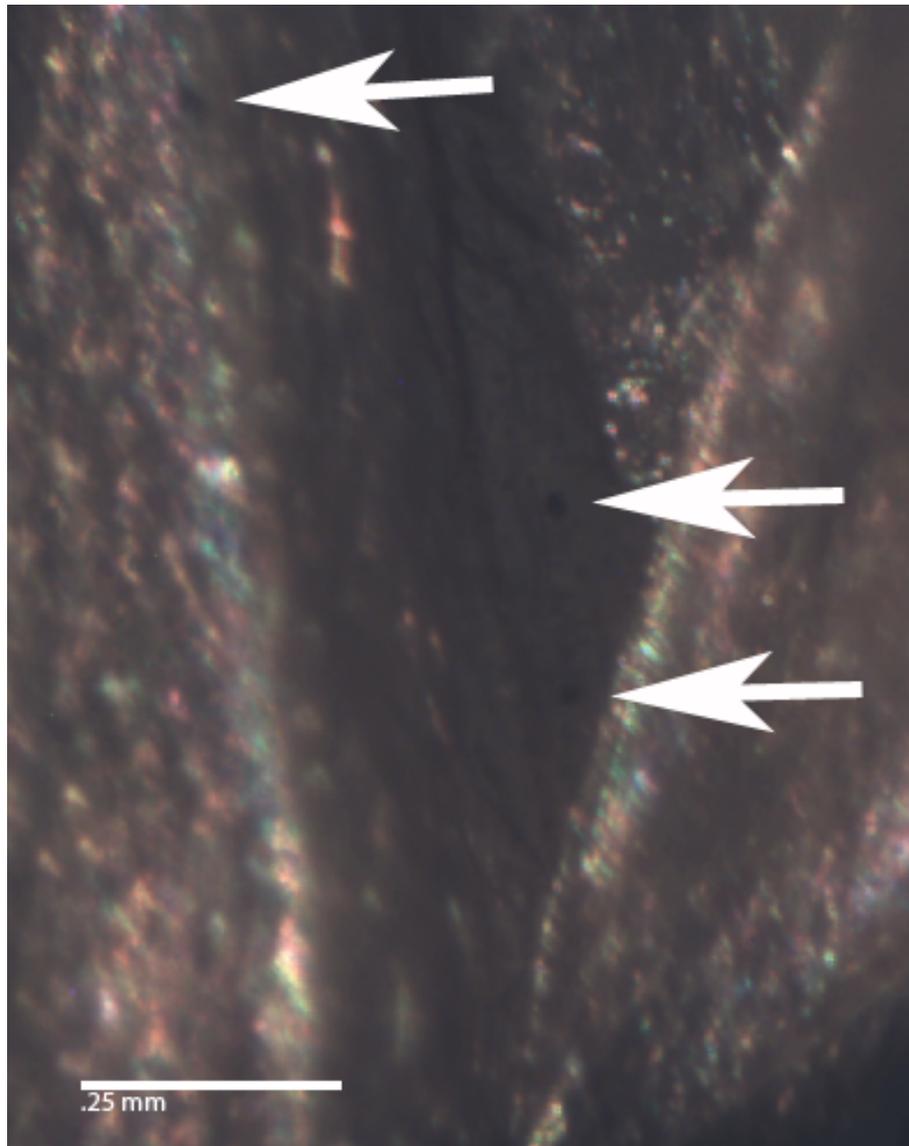


Figure 21. Lepidostomid “scales,” small “scales,” (Trichoptera: *Lepidostoma*) This lepidostomid specimen is from University of Massachusetts-Amherst’s Entomology Collection. These black structures are much smaller than others on the wing. Same specimen seen in Figures 20, 22. UMass-Amherst. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.



Figure 22. Lepidostomid “scales,” large “scales,” (Trichoptera: *Lepidostoma*)
This lepidostomid specimen is from University of Massachusetts-Amherst’s Entomology Collection. In this image, one can see that the black structures are on the underside of the wing membrane because the wing membrane continues over the black structures rather than under them, and the leftmost black structure in the image is partially obstructed by the wing vein. Two of these black structures are also attached along a wing vein. Same specimen seen in Figures 20, 21. UMass-Amherst. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.

CHAPTER THREE

VARIATIONS IN HAIRS AND SCALES AND THE EFFECTS ON FLIGHT

Packard (1898) was one of the first to discuss the variation in hairs and scales in Trichoptera and Lepidoptera.

Besides the scales, fine spinules occur on the thickened wing veins of the wings. . . in the Trichoptera, and in the more generalized Lepidoptera (Micropterygidae and Hepialidae), occur, as indicated by Spuler, delicate chitinous hollow spinules scarcely one-tenth as long as, and more numerous than, the scales, which sometimes form what he calls "Hatfields," or holding areas. These spinules have also been noticed by Kellogg, and by myself in *Micropteryx*; Kellogg and also Spuler, have observed them in certain Trichoptera (*Hydropsyche*). These also occur on the veins, and detached, ones near large one-jointed hairs, or hair-scales, said by Kellogg to be striated (Packard, 1898).

Hairs and scales and the variation in these structures are not only beautiful but also biologically interesting (Figures 23, 24). In some Lepidoptera, there are scales so thin that they look like hairs (Figure 25). According to Packard (1898), on the thickened wing veins of some Trichoptera, Micropterygidae (Lepidoptera), and Hepialidae (Lepidoptera), chitinous hollow spinules can be found. Some trichopterans and primitive lepidopterans, such as *Micropteryx*, have these chitinous hollow spinules (Packard, 1898). In Packard's time, Agathiphagidae had not yet been discovered, and perhaps Heterobathmiidae had not been discovered either. I identified longer, but similar structures in these two families (Figures 26- 30). These structures are probably the hairlike projections of setae

on the edges of wings of some insects that Dudley (2000) describes as increasing effective surface area in flight. Increase in surface area would aid these insects in flight. The presence of hairlike projections of setae in Trichoptera (Figure 24) and primitive Lepidoptera (Figures 26-32) is perhaps even more intriguing than the homology of their setae and scales.

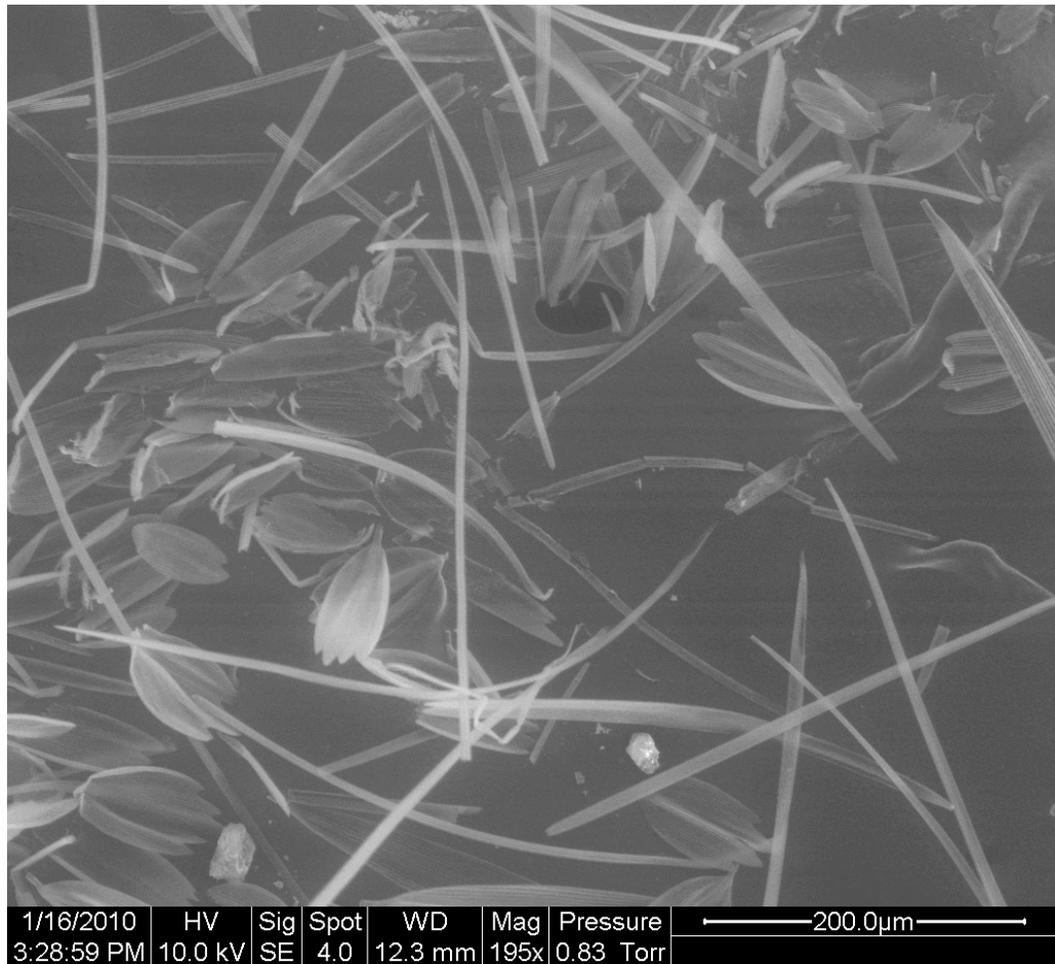


Figure 23. Trichopteran setae and lepidoptera scales
Scale variation is apparent in this image. There are both long and short scales, as well as trichopteran setae. Specimens collected in Hampshire County, Massachusetts, August 2009. Image taken using the scanning electron microscope at Mount Holyoke College.

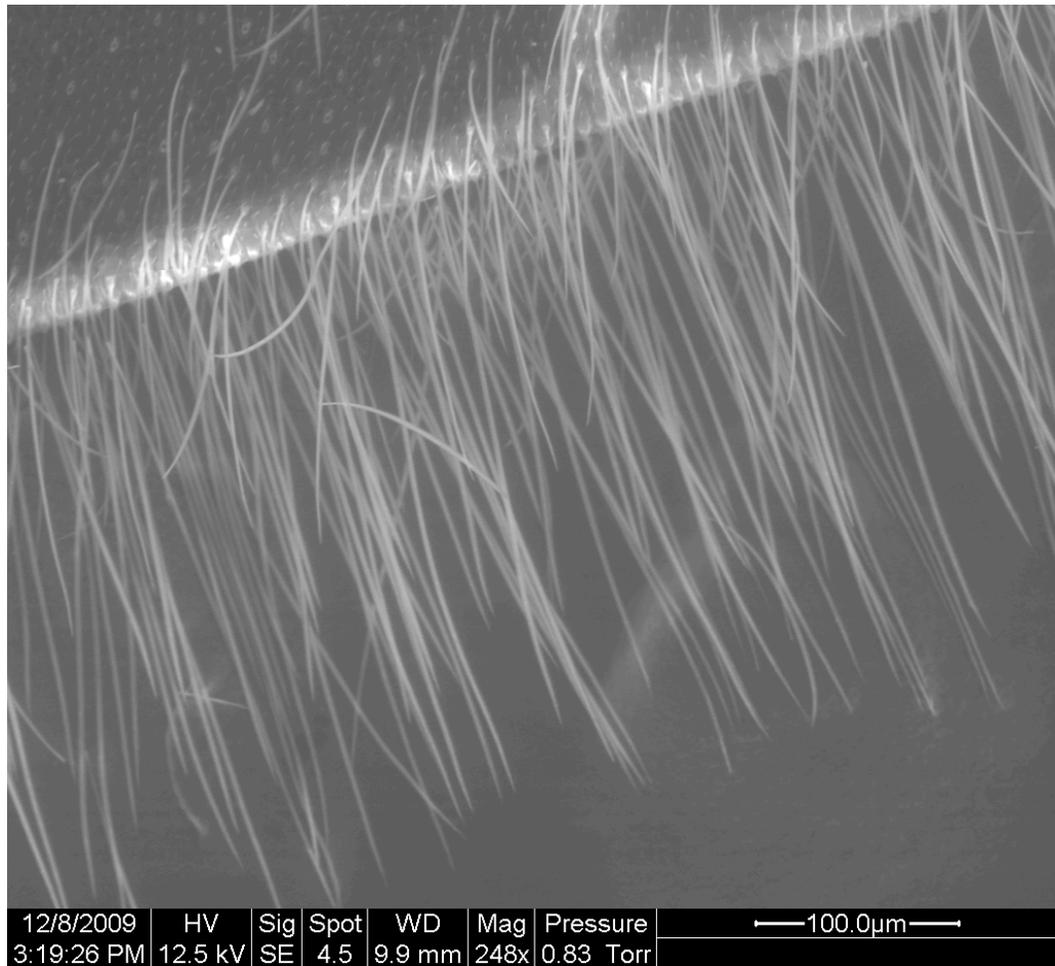


Figure 24. Trichopteran with setae on posterior edge of forewing
These setae (macrochaetes) are longer than the setae on the wing surface and may play a similar role in flight as the chitinous spinules of primitive Lepidoptera. Specimen collected in Hampshire County, Massachusetts, August 2009. Image taken using the scanning electron microscope at Mount Holyoke College.

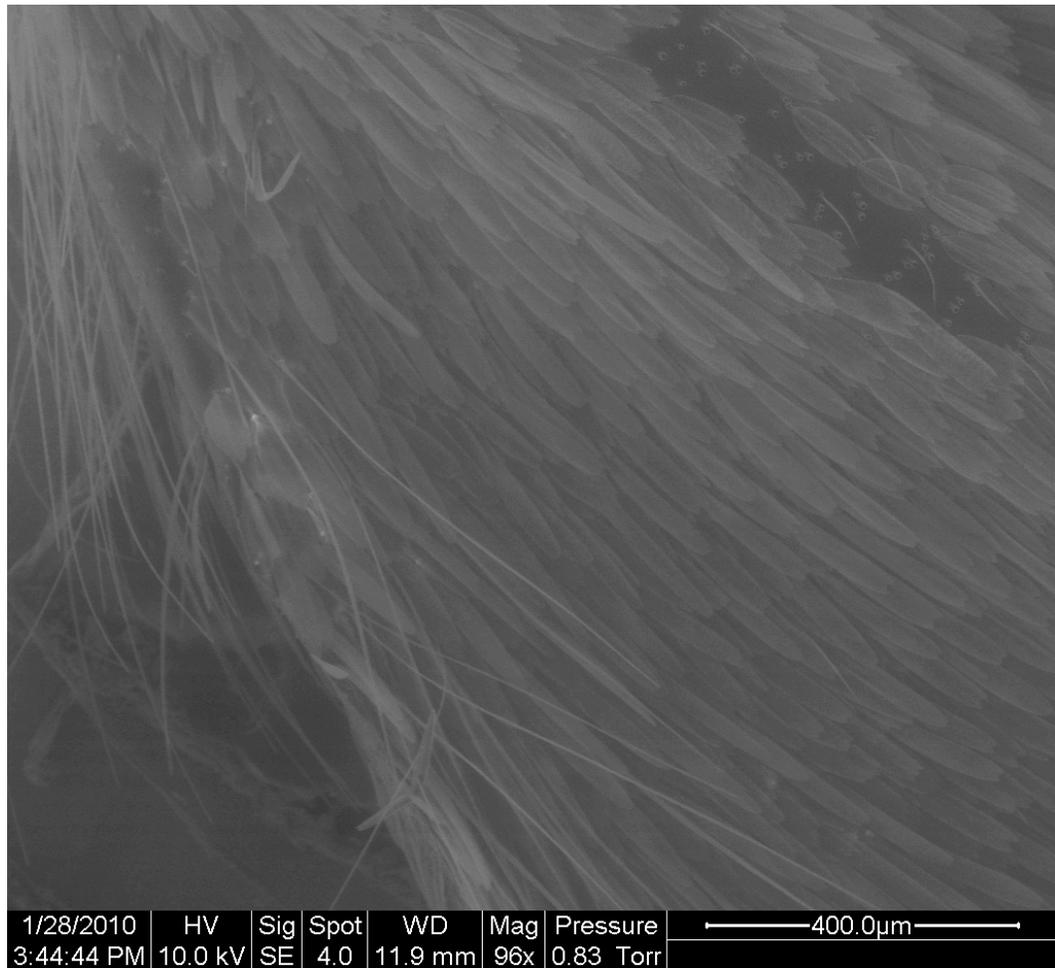


Figure 25. Moth wing with macrochaete variation (Lepidoptera)

On this lepidopteran wing, there are short scales, longer, thinner scales, and very thin long scales that look like hairs or setae. The thin hair-like structures in the left side of the image along the wing's edge are much smaller than the hairlike projections of setae seen in primitive Lepidoptera. Specimen collected in Hampshire County, Massachusetts, August 2009. Image taken using the scanning electron microscope at Mount Holyoke College.



Figure 26. Hairlike projections of setae on right hindwing of *Agathiphaga vitiensis* (Lepidoptera: Agathiphagidae)

There are hairlike projections of setae (Packard's chitinous, hollow spinules) along the edge of the hindwing and one of the veins on the hindwing. Smithsonian Institution. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.

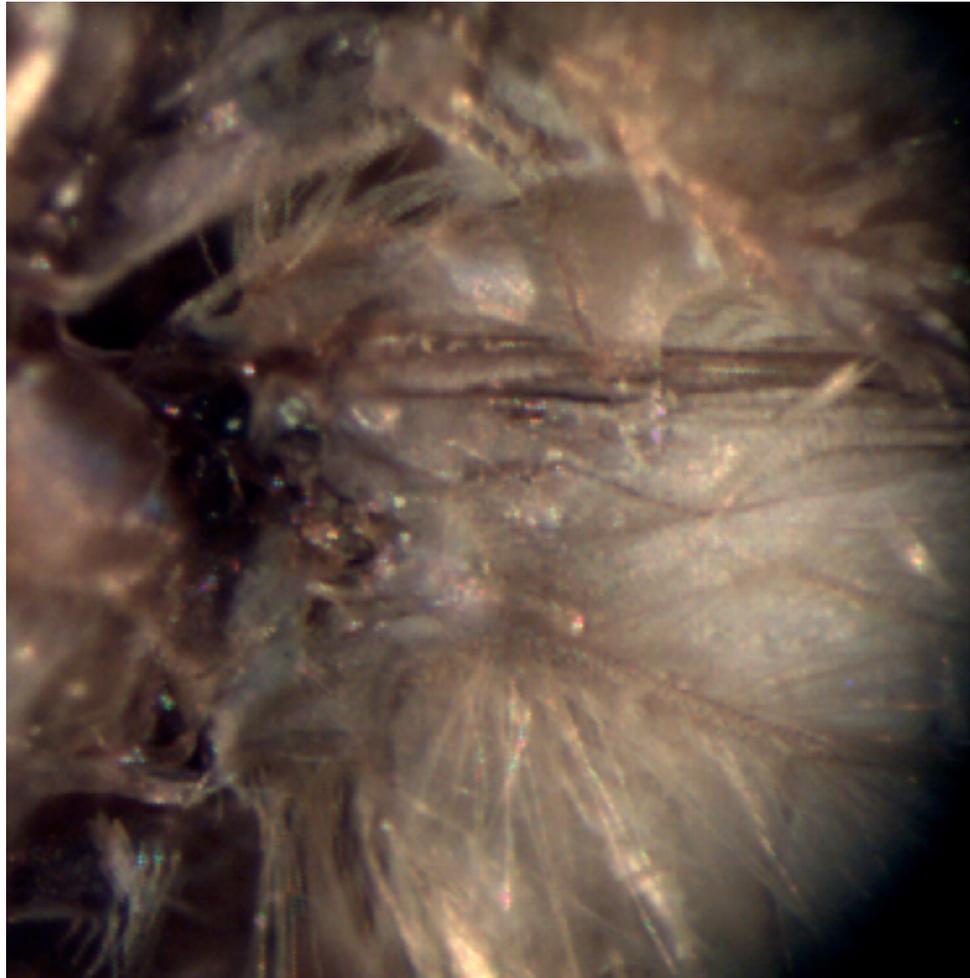


Figure 27. Hairlike projections of setae on right hindwing of an *Agathiphaga vitiensis* (Lepidoptera: Agathiphagidae)

This image is at a higher magnification than Figure 31. There are hairlike projections of setae (Packard's chitinous, hollow spinules) on one of the veins of the hindwing. Smithsonian Institution. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.



Figure 28. Hairlike projections of setae on wings of *Heterobathmia* (Lepidoptera: Heterobathmiidae)

There are hairlike projections of setae (Packard's chitinous, hollow spinules) along the edge of the hindwing. Smithsonian Institution. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.



Figure 29. Hairlike projections of setae on left wings of *Heterobathmia* (Lepidoptera: Heterobathmiidae)
There are hairlike projections of setae (Packard's chitinous, hollow spinules) along the edge of the hindwing. Smithsonian Institution. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.

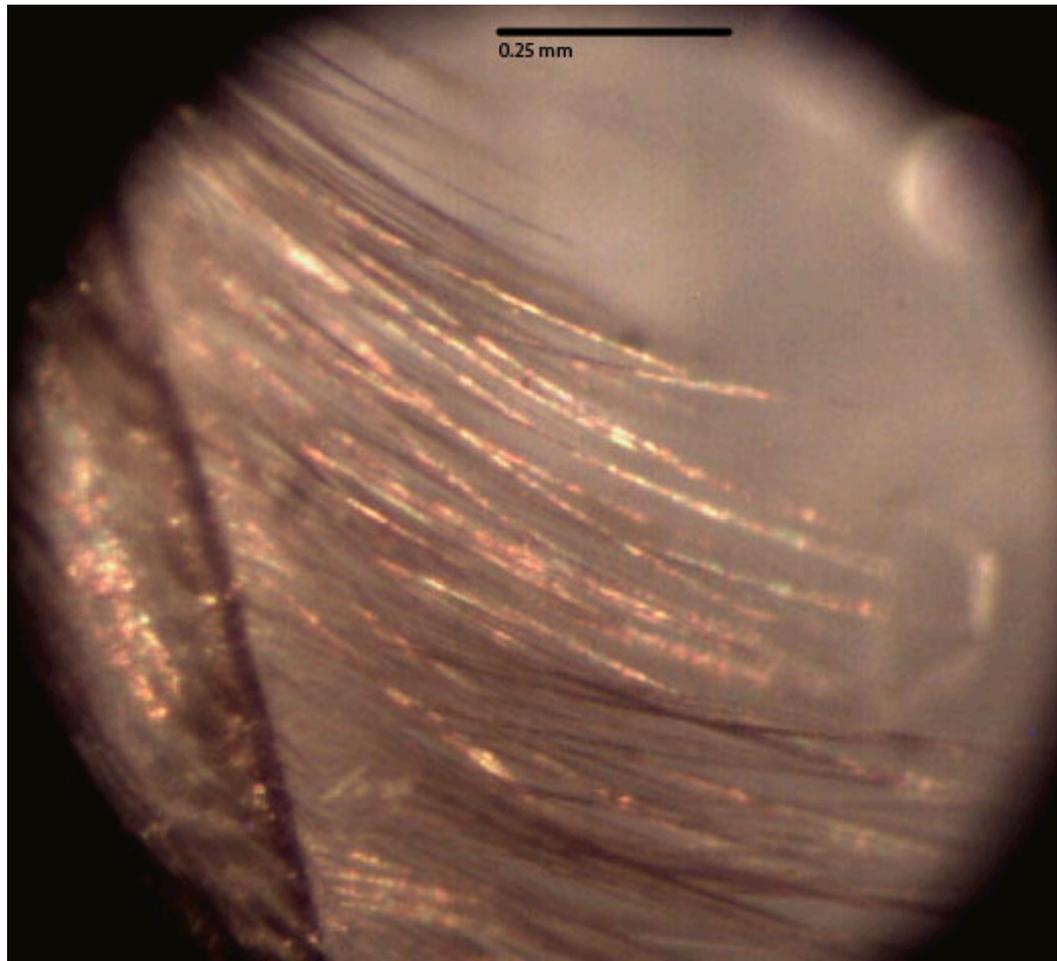


Figure 30. Hairlike projections of setae on posterior edge of left hindwing of *Heterobathmia* (Lepidoptera: Heterobathmiidae)
There are hairlike projections of setae (Packard's chitinous, hollow spinules) along the edge of the hindwing. Smithsonian Institution. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.



Figure 31. Hairlike projections of setae on left wings of *Epimartyria auricrinella* (Lepidoptera: Micropterigidae)

Hairlike projections of setae can be seen in this image on the edges of both fore and hind wings. Scales are sparse because this specimen was collected in 1940. Yale University. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.



Figure 32. Hairlike projections of setae on left hindwing of *Epimartyria auricrinella* (Lepidoptera: Micropterigidae)

Hairlike projections of setae can be seen in this image on the hind edge of the left hind wing. Scales are sparse because this specimen was collected in 1940. Yale University. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.

The differences between hairs and scales also affect Reynold's Number thus affecting flight of the animal. In Lepidoptera, wing scales are known to influence the aerodynamic properties of the wings (Dudley, 2000). According to Dudley (2000), the 3D surface of a wing has important aerodynamic consequences. The main factor of the 3D surface is the type of wing vestiture. In the orders Trichoptera and Lepidoptera, this means setae or scales and includes hairlike projections of setae. Many insect wings, including many of the primitive Lepidoptera, have hairlike projections of setae that look like a fringe at the wing's edge (Figures 26-32). These projections can increase effective surface area if there are enough of them (Dudley, 2000). Is it possible that these projections fostered the evolution of hairs to scales? Some Trichoptera have projections of setae on the posterior edges of their hindwings (Figure 24), but I have seen none with projections as long as those in primitive Lepidoptera. If there were an ancestral amphiesmenopteran with projections on the back of its hindwing like those seen in today's primitive Lepidoptera, these projections might have aided early Lepidoptera by increasing their effective surface area, making them much more efficient flyers.

Comparisons between the biomechanical effects of lepidopteran scales and trichopteran hairs have not been done, though they might shed light on whether there are adaptive differences between them. Although we lack comparisons between these orders, "the vertical projection of butterfly scales is low relative to the thickness of wing boundary layers (Martin and Carpenter, 1977), and their

aerodynamic properties might also be expected to be minimal” (Dudley, 2000). From simply observing scanning electron micrographs from these orders, it is clear that hairs project farther from the wing membrane than scales and probably have more effect on the wing boundary layers than scales do, in part because of the distance between hairs compared to the overlapping nature of scales.

I aimed to quantify the Reynold’s Number (during flight) of one trichopteran seta and compare it to that of a lepidopteran scale. I calculated the Reynold’s Number of a hair on the wing of a caddisfly during flight (see Appendix C). It would have been ideal to do Navier-Stokes calculations on scales and hairs. The Navier-Stokes equation is for motion and boundary conditions of a cylinder or multiple cylinders moving in fluid, like a seta or scale on a wing in flight (Cheer & Koehl, 1987). The Navier-Stokes equation has never been solved, and so calculating these velocity vector fields was not possible. While I intended to calculate Reynold’s Number for scales as well, the overlapping nature of scales (unlike setae, which are spread apart) made the Reynold’s Number of one scale irrelevant, because although these structures are wider than hairs, one wing scale does not interact with its environment without the presence of the overlapping scales nearby.

When scales are removed from a butterfly wing, lift is slightly reduced, but total drag is not very affected (Dudley, 2000). Lepidopteran scales have parallel ribs or ridges that run the length of the scale and contribute to drag and velocity changes in the turbulent boundary layer (Dudley, 2000). At a Reynold’s

number characteristic of butterfly wings, the three-dimensional shape of scales and the distance between scales can influence flow in the viscous sublayer of a turbulent boundary layer. Both ribs on wing scales and sequential arrangement on wing scales may influence flow patterns over lepidopteran wings (Dudley, 2000). There is some energetic cost in flying with scaly wings because they can be heavy relative to the wing membrane—heliconiine butterflies' scales weigh one-third the total wing mass (Dudley, 2000).

The scales of silverfish and of the trichopteran *Protophila diablita* are ribbed (Figure 19). However, silverfish are wingless, so here the ribbing is not improving flight aerodynamics. The ribs on silverfish and protoptilan scales are not the same as the flutes on the longitudinal ridges of lepidopteran scales. Flutes are on a much smaller scale than ridges. Other insects' scales do appear different from lepidopteran scales, but they are ribbed like lepidopteran scales and this is the relevant factor for drag, velocity, and boundary layer. The similarity between lepidopteran and protoptilan scales is striking. The close similarity of these structures does not seem to be applied to the closeness of these orders, though these details do reaffirm the similarity between Trichoptera and Lepidoptera.

It is possible that the aerodynamic effects of scales and hairlike projections of setae seen in primitive Lepidoptera were a significant improvement over the aerodynamics of the hairy, trichopteran wings. Despite the added weight of scales to the wing, the increased surface area of the hairlike projections of setae and the decreased height of projection above the turbulent boundary layer on a

scaly wing may make primitive lepidopteran wings much better for flight than trichopteran wings. There are much smaller setae making up a fringe at the posterior edge of some trichopteran wings, but these are not as striking as the setal projections of primitive Lepidoptera and would provide much less surface area than the projections on primitive lepidopteran wings.

CHAPTER FOUR HAIR AND SCALE ARRANGEMENT ON THE WING

Even in 1898, it was recognized by Packard that there was a significant and important difference between scale arrangement in primitive and advanced Lepidoptera:

The arrangement of the scales on the wings is, in the generalized moths, irregular; in the more specialized forms they are arranged in bands forming groups, and in the most specialized Lepidoptera they are more thickly crowded, overlapping each other and inserted in regular rows crossing the wings, these rows either uniting with each other or running parallel. (Spuler.) The scattered irregular arrangement seen in *Micropteryx* is also characteristic of the Trichoptera (Packard, 1898).

Trichoptera and the primitive lepidopteran families Agathiphagidae, Heterobathmiidae, Micropterigidae, and Eriocraniidae have a scattered, irregular arrangement of wing vestiture (setae and scales). To my knowledge, these four lepidopteran families are the only lepidopterans with such an unordered arrangement of scales. It is possible that the family Eriocraniidae has unordered scales as well, but I was unable to tell in the specimens that I observed. The unordered arrangement is in contrast to the ordered arrangement in higher Lepidoptera (Figure 33). It is easier to determine scale arrangement with a dissecting microscope when scales have come loose from the wing and fallen off or when the wing can be viewed using a scanning electron microscope. I have observed specimens from Trichoptera (Figure 34) the primitive lepidopteran

groups (Figure 35-37). The best images I was able to obtain of primitive Lepidoptera, given the difficult microscopy and photography conditions in museum and university collections, are of Micropterigidae. In Micropterigidae (Figure 37), the alveoles (where a scale or hair inserts onto the wing membrane) are not evenly spaced on the wing. This is also how I confirmed the unordered arrangement of setae in Trichoptera (Figure 34) using scanning electron microscopy.

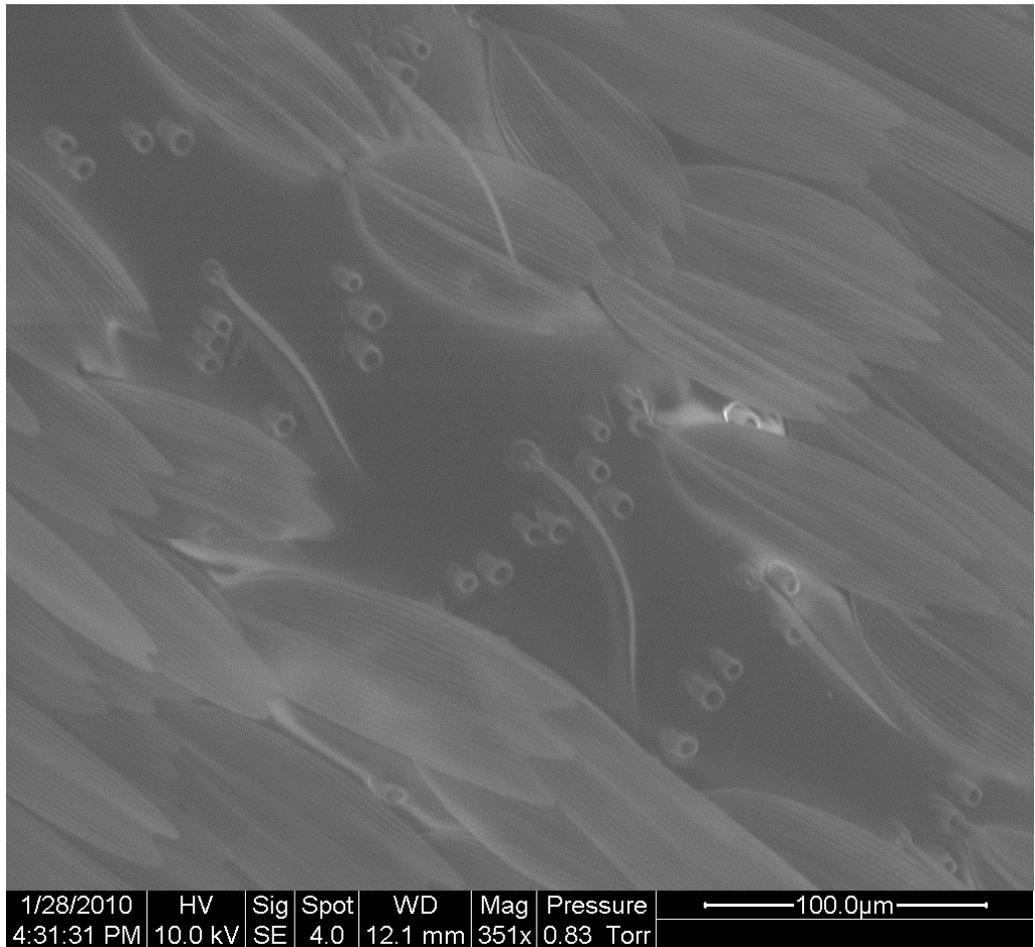


Figure 33. Wing scales of a higher lepidopteran, the moth *Haploa clymene*. The scales on this wing are arranged in rows. These rows are not in perfect lines, but they are in rows rather than unordered. Specimen collected in Hampshire County, Massachusetts, August 2009. Image taken using the scanning electron microscope at Mount Holyoke College.

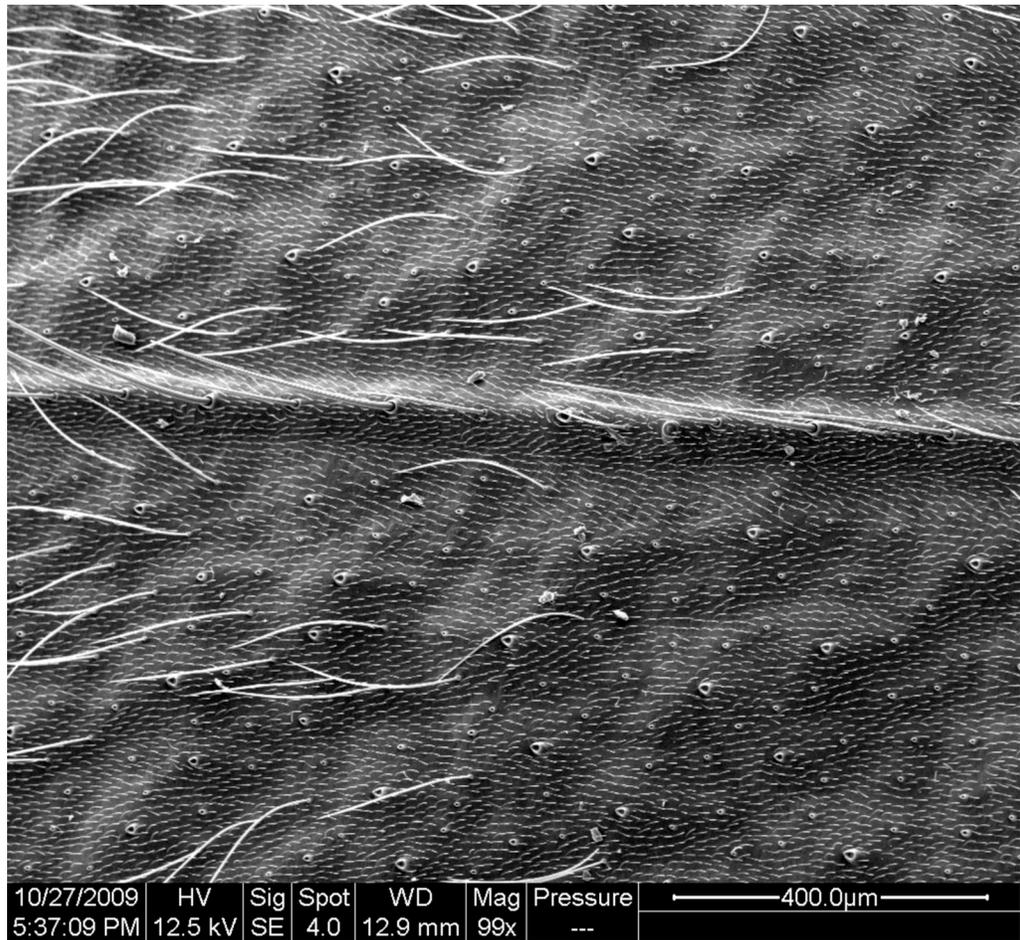


Figure 34. Trichopteran wing, showing setae and microtrichia. Setae, microtrichia, and alveoles (scale insertion points) are visible in this image. Setae are not arranged in rows. The setae are unordered. Specimen collected in Hampshire County, Massachusetts, August 2009. Image taken using the scanning electron microscope at Mount Holyoke College.

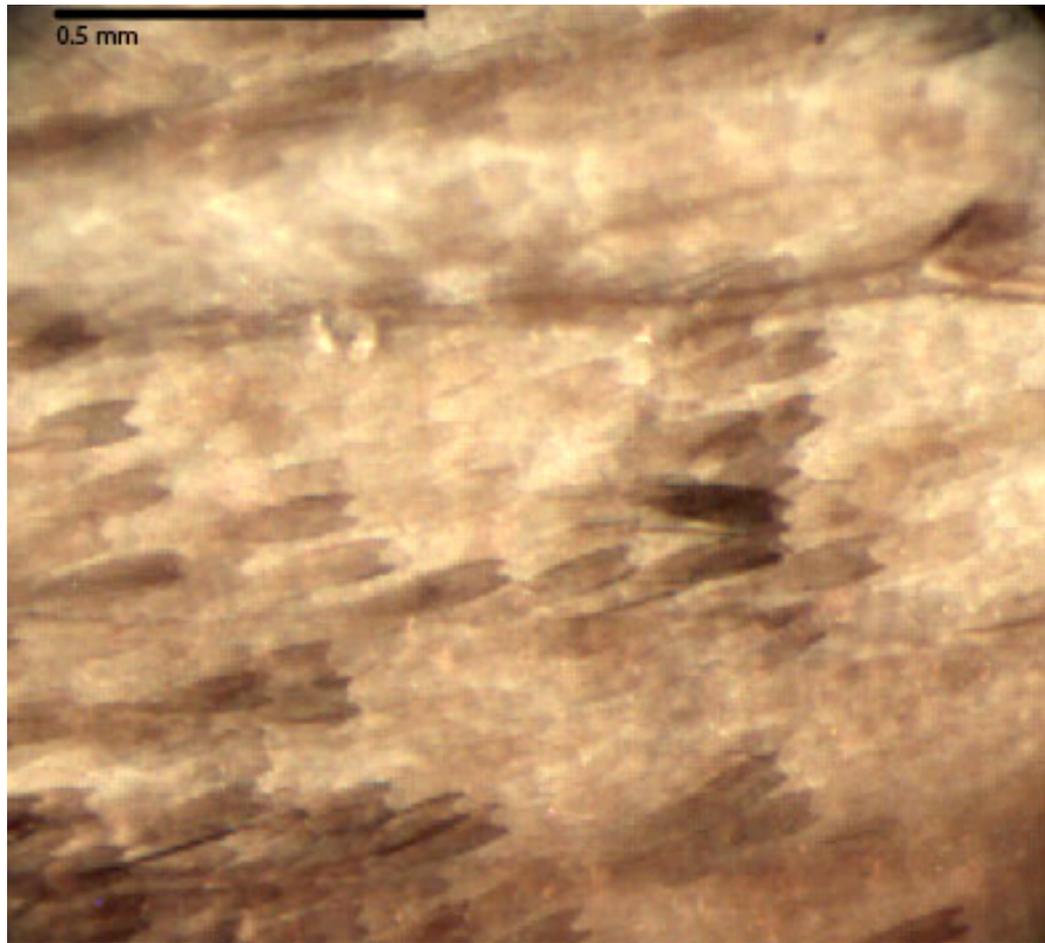


Figure 35. Scale arrangement of *Agathiphaga vitiensis* (Lepidoptera: Agathiphagidae)
Scales are not arranged in rows. The scales are unordered. Smithsonian Institution. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.



Figure 36. Scale alveoles of *Agathiphaga vitiensis* forewing (Lepidoptera: Agathiphagidae)

It cannot be seen in this image, but in Agathiphagidae, scales are not arranged in rows. These scales are unordered. Scale bar: 10 μm . Image taken using scanning electron microscope. Simonsen & Kristensen, 2001.

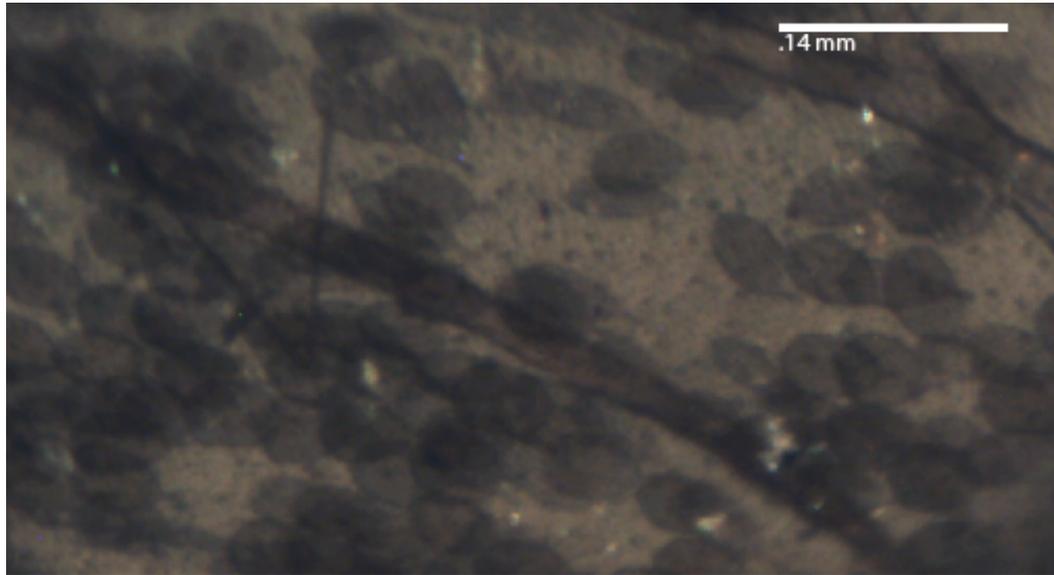


Figure 37. Scale arrangement in *Epimartyria auricrinella* forewing (Lepidoptera: Micropterigidae)
Scales are not arranged in rows. They are unordered. UMass-Amherst.
Photographed using a portable eyepiece camera attached to dissecting microscope. Image edited in Adobe Photoshop.

CHAPTER FIVE WING VENATION AND THE M4 VEIN IN TRICHOPTERA AND LEPIDOPTERA

Apart from, or perhaps associated with, their great adaptive significance, the wings of insects show so many characters of value in classification that they have been used more extensively than any other structure in comparative studies. Their importance is the greater, because they are usually the only remains of insects that are recognizably preserved in fossils, which often show essential detail, even down to trichiation, with remarkable clarity (Stark et al. 1999).

Differences in venation have been used to separate taxa for a long time, but in the second half of the 19th century, the first attempts were made at standardizing the interpretation of wing vein patterns across insect orders (Stark et al., 1999). According to Stark (1999), “reduction in wing venation is a common phenomenon in evolution” and “phylogeny is the fundamental determinant of venational patterns”.

The presence of the M4 vein in Agathiphagidae (Figures 38, 39) and no other Lepidoptera may be very important for the phylogenetic placement of this family. Some might argue that this vein is a red herring—a convenient character that turns out to be misleading, but I think this is not the case. For decades, the presence or absence of an M4 vein was the main character—the other being hairs or scales on the wing—used for determining whether an insect was a trichopteran or lepidopteran. In personal dialogue with Don Davis of the Smithsonian Institution, I asked Davis what wing veins are important. Davis said that the veins

at the back of the wing are the most important. I asked him why that is and if they are the most important for flight. Davis said that they do not influence flight very much at all, but they are the most important for phylogenetic placement, across the insect orders (pers. comm., Davis, 5 March 2010). The M4 vein is no exception. Importance of M4 vein in understanding basal lepidopteran evolution is discussed in the Conclusion.

There have not been many studies done on wing venation atavism, though those that have been done have focused on the model organism *Drosophila melanogaster*. In this species, “many aberrations in wing venation can be linked to genetic and molecular changes involving the regulatory hierarchies regulating wing formation” (Stark et al., 1999). In *Drosophila*, a disused prepatterning mechanism for vein formation was conserved for 100 million years (Palsson, 2000). *D. melanogaster* with a phenotype with the deficiency *Df(2R)Px* has six ectopic veins. These veins form where ancestral diptera wing veins are expected to have been. The atavism seen in these six ectopic wing veins indicates that the prepatterning mechanism of *Drosophila* wing veins was conserved even in the highly derived *D. melanogaster* (Palsson, 2000). Whether variation in regulatory gene expression affects modern day species is debated.

The authors confirmed that *Drosophila* wing veins are not fusions of ancestral veins. The lost veins were repressed and as a result, they fail to form at the boundaries of gene activity. The boundaries of gene activity exist in the derived *D. melanogaster*, as demonstrated by the six ectopic atavistic veins that

occur in the wing of *Df(2R)Px2* mutants. These atavistic veins are surprising because it is not clear why an unused prepatterning mechanism would be conserved over 100 million years (Palsson, 2000). It is possible that this prepatterning mechanism either patterns or is tied to something other than these six ancestral veins and that is why it has been conserved.

While it is possible that the M4 vein in Agathiphagidae is the result of atavism, this has not been suggested and this vein's presence in this family has gone unexplained by Kristensen and others. It seems that considering the evolutionary trend of venation (complex to simple), if Agathiphagidae is more advanced than other Lepidoptera without the M4 vein, then this vein in Agathiphagidae is an atavism. I acknowledge the possibility of an atavism, but it is not the best explanation of the M4 vein in Agathiphagidae. It seems unlikely that of all the ancestral veins that could reappear due to atavism, only the M4 vein reappeared. In Palsson's study, six veins that were in an ancestor 100 million years before were seen in the mutant *D. melanogaster*. It not ideal to draw conclusions on venation in a primitive moth with one possible atavism based on mutant *Drosophila melanogaster* with six ectopic veins.

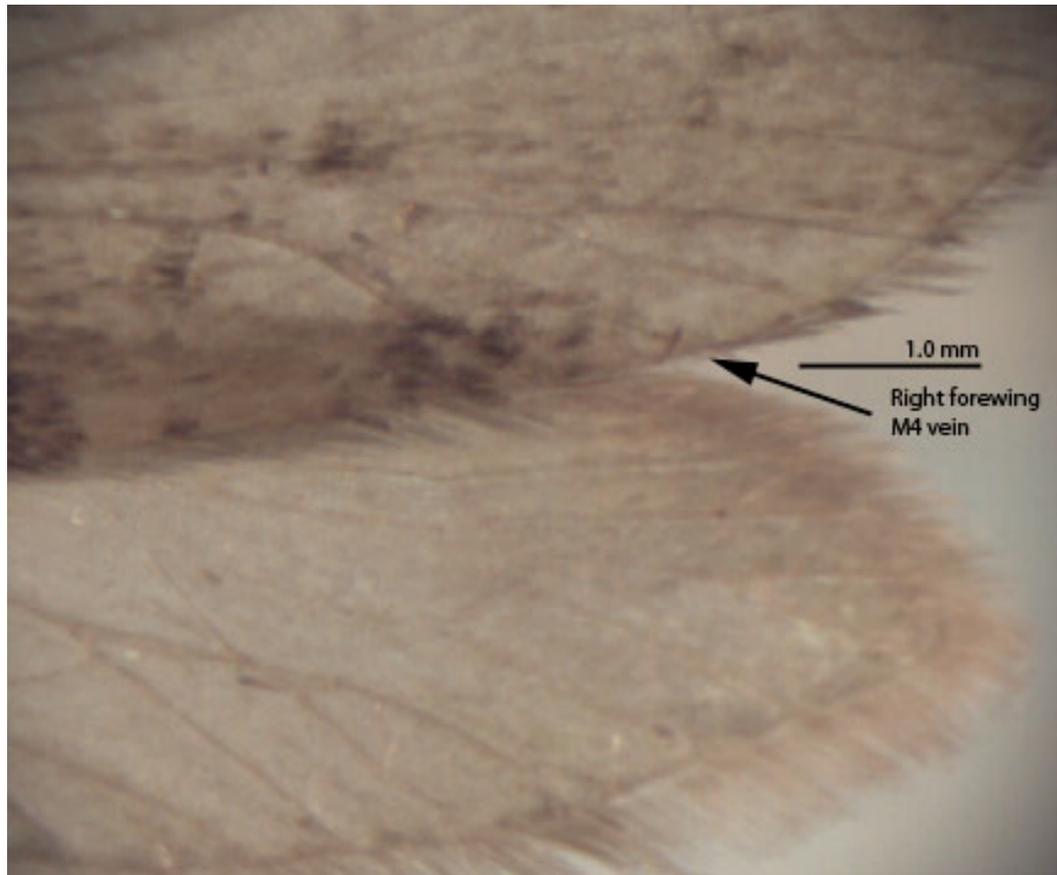


Figure 38. Right wings venation of *Agathiphaga vitiensis* (Lepidoptera: Agathiphagidae)
M4 vein is present. Smithsonian Institution. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.

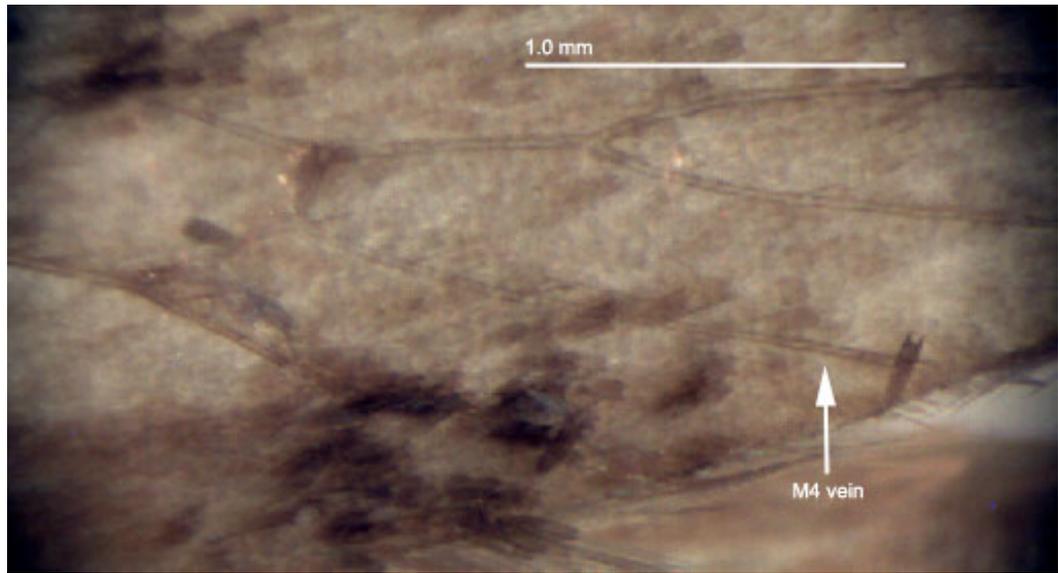


Figure 39. Right forewing venation of *Agathiphaga vitiensis* (Lepidoptera: Agathiphagidae)
M4 vein is present. Smithsonian Institution. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.

CHAPTER SIX TRICHOPTERAN ANTENNAE: IRREGULAR BENDING AND CHEMORECEPTORS

It is easy enough to forget how many ways the exoskeleton of an insect makes its life different from ours. One of these is as simple as chemical penetration of the body. Because the only openings through the exoskeleton of an insect are spiracles, all chemicals must either enter the body through a spiracle or by penetrating a thin exoskeletal layer to reach nerve endings (Frings & Frings, 1949). This affects how insects detect chemicals around them, which affects many things, including how they locate food.

Trichoptera have flagellar antennae that are composed of two basal segments, the scape and pedicel, from which a long flagellum extends. The flagellum protrudes from the insect's head and is the most noticeable part of an antenna. The muscles that move an antenna are only in the head and scape. Many insects use antennae as feelers. Schneider (1964) hypothesizes that slow-flying insects with long antennae use these appendages as passive stabilizers. This is what seems to occur in Megaloptera and Trichoptera, which have long antennae and clumsy flight. The moths, however, do not use these appendages in such a way. Could antennal use be an important difference separating Lepidoptera and Trichoptera? Lepidoptera can have much more ornate antennae with a different agenda—they offer more surface area for sense organs. There is sexual dimorphism in the antennae of some Lepidoptera. The surface area of

male pectinate and bipectinate antennae is much greater than that of female thread-like antennae. Both sexes in primitive moths, however, such as Agathiphagidae (Figure 40), have antennae that appear very similar to those of Trichoptera. All micropterigid specimens that I observed had similar thread-like antennae.

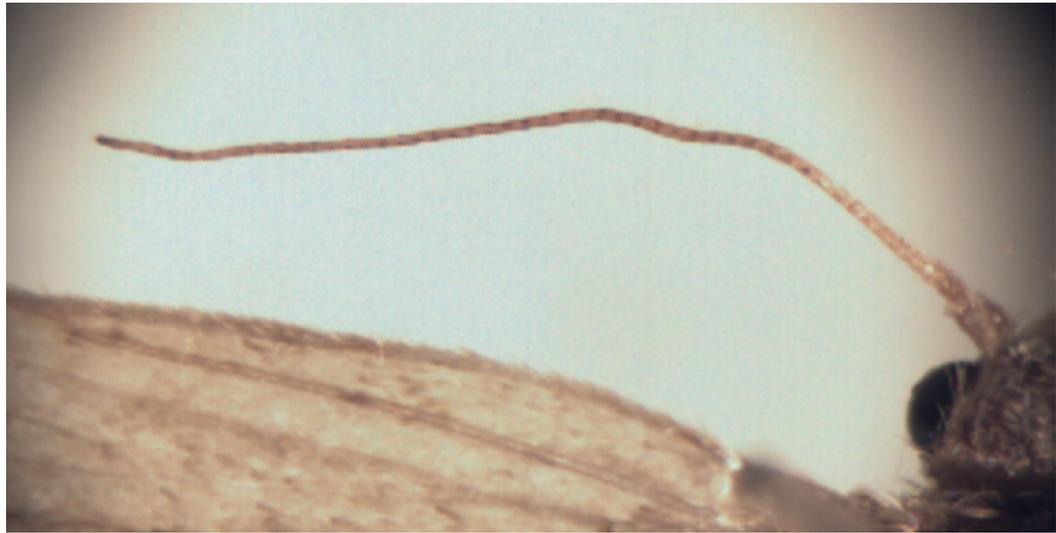


Figure 40. Antenna of *Agathiphaga vitiensis* (Lepidoptera: Agathiphagidae) *Agathiphaga* has a very thin antenna that superficially resembles those of Trichoptera. I did not observe any specimens with bipectinate antennae. Smithsonian Institution. Image taken with a portable eyepiece camera and a dissecting microscope. Image edited in Adobe Photoshop.

Flagellar antennae are controlled by two sets of muscles. The scape is moved by two to four muscles that originate in the head capsule and insert on the base of the scape (Schneider, 1964). There are two ways that the joints of an insect antenna can articulate. In a ball joint, two of the four muscles run on each side of the antennifer and incline the scape and exert torsions. The other two muscles only incline the scape (Schneider, 1964). In a hinge joint, there are two muscle groups moving the pedicel. These originate in the scape and insert on the pedicel (Schneider, 1964). The scape-pedicel joint is a hinge and the scape-head joint can be either a hinge or a ball joint. There is more freedom of movement in a hinge joint (Schneider, 1964). Trichoptera and Lepidoptera have one ball joint and one hinge joint. There are no muscles in the flagellum, but there can be movements in the flagellum (and in Lepidoptera, its branches). Flagellar movements can only be brought about by the flow of hemolymph. “The limiting factors are the elastic properties of the complicated intersegmental membranes and the membranes at the bases of all branches, and the extent and possible channeling of the blood stream” (Schneider, 1964).

Each insect antenna, as far as has been studied, has a blood vessel running from its base to its distal end (Schneider, 1964). A small accessory pulsating organ in the head capsule is connected to the sinus at the anterior end of the main body vessel and pumps blood into the antennal blood vessel. The nerves of the antennae are based in the deutocerebrum. From these nerves branch the motor branches that supply the antennal muscles. At the base of the antenna, in its

scape, the nerves divide into two main, approximately equal trunks/bundles.

These bundles go all the way to the distal end of the antenna. It was found in the fly that there is an extensive fusion of axons and axonal fusion is a common feature of the insect sensory system (Schneider, 1964).

With a number of high-speed videos that I made of Lepidoptera, Trichoptera, and Megaloptera, I found that some of the long-antennaed Trichoptera seemed to be able to perform localized bending of the tips of their antennae. I measured the angles of the bending antennae (Appendix B). What is surprising about this finding is that insects do not have true segments—segments have muscles that cause the joint between two segments to bend. Their antennae are a series of chitinous rings separated by flexible areas (Figure 41). Antennal cuticle is well-sclerotized except at the intersegmental membranes, where there is soft, flexible cuticle (Schneider, 1964). The antennae should not be able to perform localized bending. One would expect these Trichoptera only to be able to move their antennae in a whip-like manner. This localized bending was not seen in caddisflies with shorter antennae, but with a small sample size it is risky to make extrapolations about short-antennaed Trichoptera.

The localized bending in some trichopteran antennae may be due to the presence of chemoreceptors at the bending point. Glenn Richards (1952) found that the epicuticle on honeybee antennae varies with sclerotization, allowing for qualitatively different reactions on different parts of the antenna. He also found that “the cuticle over chemoreceptors cannot be assumed to have the same

penetration properties as cuticle on other parts of the body” (1952). Varying thickness in cuticle would allow for increased bending at that place in the antenna.

The presence of chemoreceptors in trichopteran antennae has not yet been shown. Their presence and location in some Lepidoptera has been shown, and similar studies need to be done in Trichoptera. Hubert and Mable Frings (1956b) found that trichopteran antennae lack chemoreceptors sensitive to sucrose, while the palpi have chemoreceptors. This is more reminiscent of the state in Diptera than in Lepidoptera (Frings & Frings 1956a). The absence of contact chemoreceptors on trichopteran antennae should be considered cautiously, however, because experiments with some Lepidoptera (Frings & Frings 1956a, 1959) showed that reactions mediated by the antennae can depend on the presence or absence of contact chemoreceptors elsewhere on the body. Frings and Frings (1956a) found in their study of feeding reactions in the lepidopterans *Ctenucha* and *Scepsis fulvicollis* that they did not react to antennal contact with sucrose solution when tarsi were intact. When two (in *Ctenucha*) or all (in *Scepsis*) of the tarsi, and the chemoreceptors on them, were removed from the organism, it reacted to antennal contact with sucrose solution as though its antennae had chemoreceptors. Frings and Frings (1959) readdressed this finding in the wood nymph butterfly *Cercyonis pegala*, and had similar findings. When all tarsi were intact, there were only responses to sucrose solution 5%-8% of the time that the antennae were stimulated. In contrast, when the legs were removed, the butterflies responded to 70%-80% of the antennal stimulations. In this same

study, they saw evidence that only the tips of the antennae are receptive. When a glass microneedle moved a droplet of sugar solution along the antenna, there was only a response when the droplet reached the tip of the antenna (Frings & Frings, 1959). With only the tips of the antennae being responsive, it is expected that these are the only articles of the antennae that have contact chemoreceptors on them. Frings and Frings (1959) concluded that the tips of antennae have chemoreceptors, and that until their absence in other butterfly species has been proven, it is best to assume these receptors are present. Homologous work, one might say, has not been done in Trichoptera, and the author expects that testing the trichopteran response to antennal contact when the tarsi have been removed would have similar findings.

It is also important to remember that the alleged lack of chemoreceptors is based on the insects indiscriminately reaching with their palpi towards brushes wet with either water or a sucrose solution, until they were sated with water. It is possible that the sensitivity to nearby water vapor represents some kind of receptor on the antennae. In bees, the contact chemoreceptors of the antennae are located on the eight terminal articles of the flagellum. Kunze (1933) found this by removing segments of the antennae one by one (Frings & Frings, 1949).

The hypothesized presence of chemoreceptors in the tips of trichopteran antennae, based on the location of these chemoreceptors in lepidopteran antennae, provides a reason to expect a difference in cuticle thickness at a location in the distal portion of the antennae that may account for the localized bending in

trichopteran antennae. I think that bending takes place at the boundary between the articles with chemoreceptors and the articles that are proximal to them.

A fossil trichopteran may have been preserved with an antenna bent (Figure 42) at such a point because of a difference in cuticle thickness, though the much higher viscosity of the liquid resin would have exaggerated any tendency to bend. The angle measurements of antennae taken from high-speed videos and images of a fossil specimen are located in Appendix B.

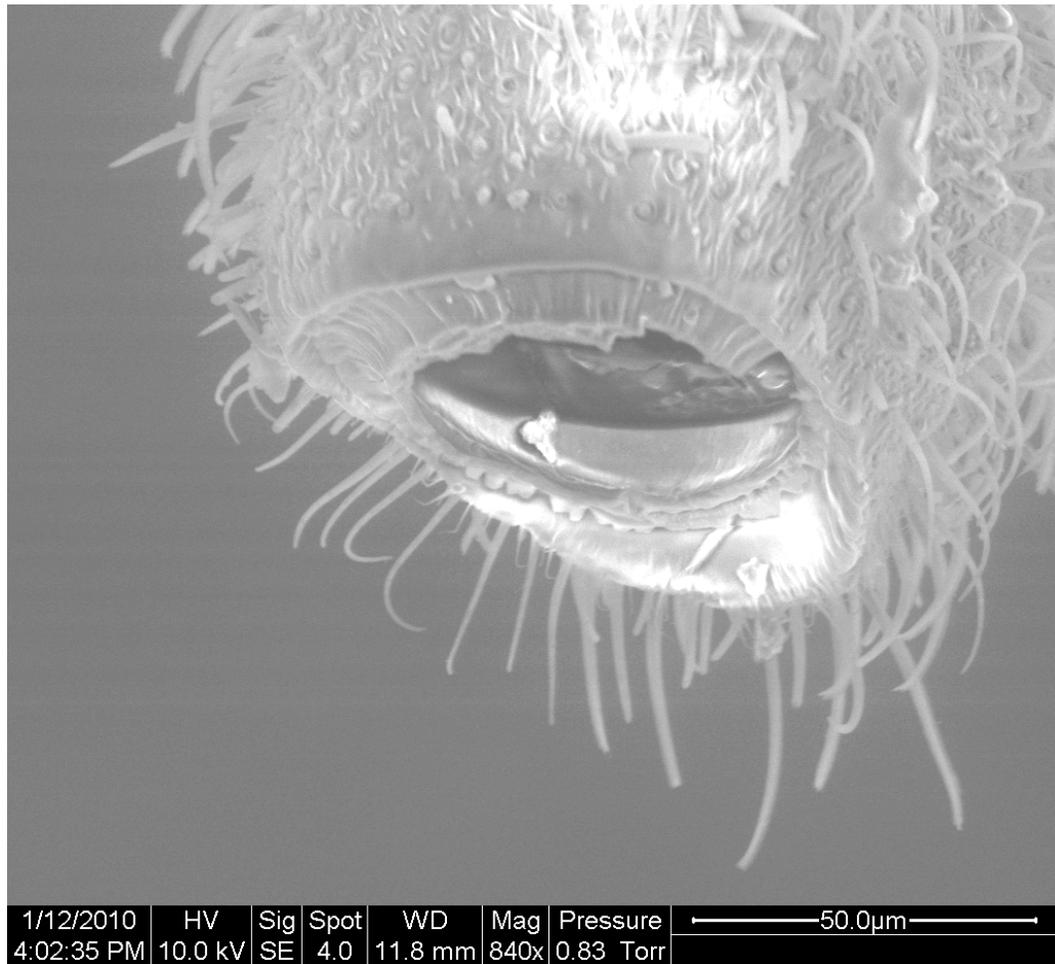


Figure 41. Inside a trichopteran antenna

This image shows what the inside of an antenna looks like between segments. Hairs of varying size can be seen on the surface of the antenna. Specimen collected in Hampshire County, Massachusetts, August 2009. Image taken using the scanning electron microscope at Mount Holyoke College.

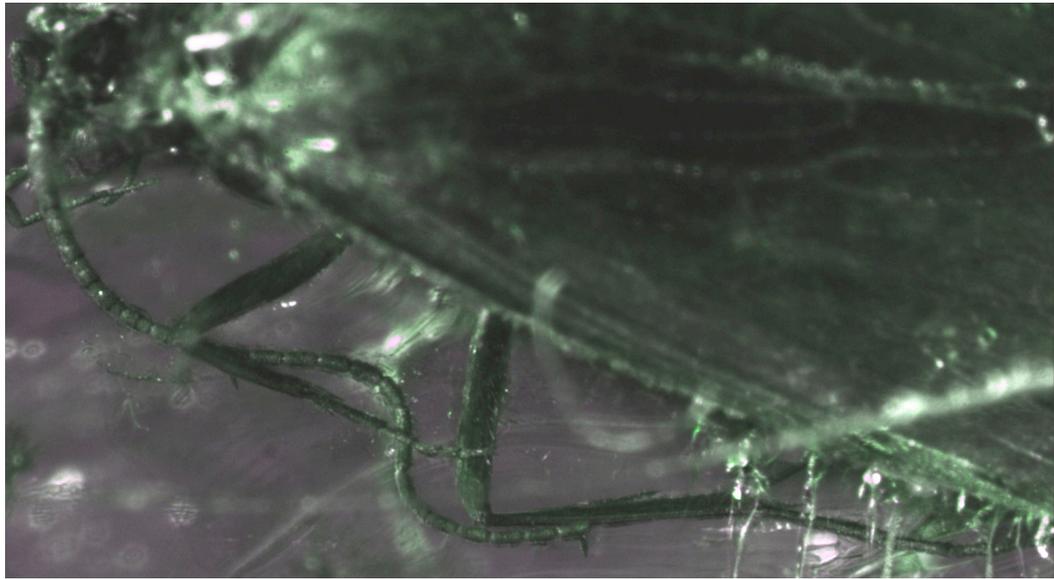


Figure 42. Fossil trichopteran in Baltic amber with bending at tip of antenna. Bending is preserved in amber. Bending preservation in amber indicates that localized bending may not be due to a flow of hemolymph but a difference in cuticle thickness. Image taken using dissecting microscope at Mount Holyoke College.

CONCLUSIONS ON THE ORDERS TRICHOPTERA AND LEPIDOPTERA AND BASAL LEPIDOPTERAN EVOLUTION

Perhaps more important than the differences between Trichoptera and Lepidoptera are their unifying characteristics. There are twenty-one amphiesmenopteran groundplan autapomorphies catalogued by Kristensen (1984). Autapomorphies (derived traits unique to a given terminal group) are one way to diagnose a group. These are listed in the Appendix. Four of the amphiesmenopteran autapomorphies are cytological, though none of the lepidopteran autapomorphies are cytological. While the meaning and weight of many morphological characters may be debatable, the cytological characters can affect genetics, sex, and cellular processes. These seem to be more far-reaching and more meaningful to an organism than a tiny island of cuticle between two sternites. The four cytological amphiesmenopteran autapomorphies listed by Kristensen (1984) are (1) female heterogamety, (2) apyrene sperm of usual occurrence, (3) spermatozoa with outer, accessory filaments very thick, filled with proteinaceous and glycogen-like material, (3) chromosome number specialized (high) and chromosomes probably holocentric; oogenesis achiasmatic.

Female heterogamety means that the female is the sex with chromosomes that differ in morphology—in other words, equivalent to XY in male mammals. The presence of female heterogamety in both Trichoptera and Lepidoptera has recently been re-examined (Taut *et al.*, 2007). Female heterogamety is shared by

Trichoptera and Lepidoptera, as Suomalainen (1966) found. Taut (2007) found that in all Lepidoptera more advanced than Micropterigidae, Agathiphagidae, and Heterobathmiidae, except where it has been lost, sex is determined by a WZ/ZZ (female/male) system, rather than the Z counting mechanism, Z/ZZ of Trichoptera and the very primitive Lepidoptera (Taut *et al.*, 2007). The mechanism of sex determination does not necessarily speak hugely to the biology of the organism and how it lives its life, but it is interesting that the change in sex determination mechanism that occurred in Lepidoptera happened above the basal Lepidoptera. Here, there is one autapomorphy at the amphiesmenopteran level and a change within the Lepidoptera, rather than at the boundary between Trichoptera and Lepidoptera. This shows how close together Trichoptera and Lepidoptera are, and that many “lep-y” features evolve within the Lepidoptera, not at the base of the Lepidoptera.

Apyrene sperm are sperm without a nucleus. Lepidoptera usually produce apyrene sperm, but Trichoptera do not. The presence of these dud sperm is likely due to competition between rival sperm that were deposited by other males (Silberglie *et al.*, 1984). Kristensen was incorrect about this trait, but he also did not do the investigation himself, he used the cytological characters that Suomalainen (1966) found. Kristensen is a morphologist, not a cell biologist. He does not check these cytological characters and one of them is incorrect. Presence of apyrene sperm is actually a cytological lepidopteran autapomorphy, not an amphiesmenopteran autapomorphy. This is a significant lepidopteran

autapomorphy and may say more about Lepidoptera, biologically, than many of Kristensen's morphological characters. Apyrene sperm are present in high numbers and are presumed to aid nucleate sperm from the same male in their competition with sperm from rival males (Silberglie et al., 1984). While apyrene sperm are not present throughout Amphiesmenoptera, they do draw an interesting line between Lepidoptera and Trichoptera. Agathiphagidae (Lepidoptera) have not been investigated (probably because they are so rare), but Micropterigidae (Lepidoptera) have been investigated and they have apyrene sperm. Apyrene sperm have been present in Lepidoptera since some of the most primitive extant moths. The presence of apyrene sperm in Lepidoptera may indicate that there is much more sexual competition between males and multiple matings in Lepidoptera than in the typically very short-lived adult Trichoptera.

The designation of a chromosome as holocentric or monocentric is based on its interaction with the spindle (Benavente & Volff, 2009). With holocentric chromosomes, spindle microtubules attach to the whole length or most of the length of the chromatid, yielding a non-localized centromere. With monocentric chromosomes, the spindle microtubules attach to the centromere (Benavente & Volff, 2009). How an organism's cells are constructed are important and it is biologically meaningful that there are holocentric chromosomes throughout this superorder.

The amphiesmenopteran autapomorphies listed by Suomalainen (1966) that Shields (1988) emphasizes are that the female is heterogametic, that no

chiasmata are formed in oogenesis, that chromatin elimination occurs in the first meiotic division in the egg, and that the chromosomes are holokinetic. Kristensen does not include when chromatin elimination occurs, but includes the others, although he only considers the oogenesis and chromosomal traits as one. There are five true cytological amphiesmenopteran autapomorphies, which should be given considerable weight when drawing the biologically important line between or below these groups. These are (1) female heterogamety, (2) spermatozoa with outer, accessory filaments very thick, filled with proteinaceous and glycogen-like material, (3) chromosome number specialized (high) and chromosomes holocentric (holokinetic), (4) achiasmatic oogenesis, and (5) chromatin elimination occurring in the first meiotic division in the egg (Suomalainen, 1966).

These 21 amphiesmenopteran autapomorphies along with 26 lepidopteran autapomorphies have been used to diagnose the superorder Amphiesmenoptera and the order Lepidoptera. The reasons, or in the case of phylogeny, the diagnostic characters that are present and useful for the division of these groups should not all be given equal weight, but determining which are given more weight is tricky. The original goal of this project was to examine the details of the differences between these two orders and whether they truly qualify as separate orders. Another possibility was what designation is more important in this case, the division between the superorder and its sister group or between the orders Trichoptera and Lepidoptera. Based on the significance of cytological characters, compared to the morphological characters (Appendix A), and the

details of the larger scale differences between these orders—setae and hairs, larval ecology, and wing venation, I propose that the emphasis on what is biologically meaningful should be on the superorder Amphiesmenoptera, not the division between the orders Trichoptera and Lepidoptera.

I spoke with Don Davis, who suggested that perhaps the acceptance of Kristensen's theory of basal lepidopteran evolution is supported by characters found in the larvae, because micropterigid larvae have what is interpreted as a plastron (what some aquatic larvae use to breathe underwater) (pers. comm., Davis, 5 March 2010). However, Davis (1987) himself says that this is because they live in areas that flood. Kristensen (2003) considers this plastron to be *analogous*, not *homologous* to the plastron in some aquatic insects. Homologous structures have common ancestry, but analogous structures do not. This implies that the formation of the plastron in micropterigid larvae is not due to the last common ancestor of Lepidoptera and Trichoptera having aquatic larvae, a scenario for which there is no other evidence. I know of no study of the development of the micropterigid plastron, or, for that matter, analogous structures in Trichoptera. Thus, the micropterigid plastron has no bearing on determining what lepidopteran family is most primitive. Davis also mentioned a primitive ovipositor in micropterigid moths, a feature I have not seen mentioned in the literature (pers. comm., Davis, 5 March 2010). My discussion with Davis did not leave me completely comfortable with the acceptance of Kristensen's

theory, so I re-examined the characters that the theories of basal lepidopteran evolution are based on.

In his article “Studies on the morphology and systematics of primitive Lepidoptera” (1984), Kristensen listed hundreds of characters, many of which are of primitive Lepidoptera and are meant to shed light on the early evolution of Lepidoptera. I first examined the literature about characters that are present in all Lepidoptera except Agathiphagidae, according to Kristensen (1984). I compared these characters to those in Trichoptera, because the lepidopteran group that shares the most characters with its sister group (Trichoptera) is likely to be the extant (living) group that branched off first within the Lepidoptera. The shared trichopteran and agathiphagid traits are (1) the M4 wing vein, (2) fore tibial spurs, (3) large, free testes follicles, (4) pupal claws, (5) more than one pair of accessory glands for male genital ducts, (6) more than five ovarioles per ovary, and (7) five or more abdominal ganglionic masses.

The fine print:

Three of the fundamental characters known to be in all Lepidoptera except Agathiphagidae are (1) the absence of the M4 wing vein (Kristensen, 1984), (2) the absence of true “spurs” on fore tibia (Kristensen, 1984), and (3) the absence of pupal claws (Shields, 1988). The M4 vein, fore tibial spurs, and pupal claws are present in Trichoptera (Shields, 1988). Because these characters are present in Trichoptera and Agathiphagidae, this provides evidence that these were characters of the last common ancestor of Trichoptera and Lepidoptera. It is more parsimonious for the M4 vein, fore tibial spurs, and pupal claws to have been lost at the splitting event between Agathiphagidae and the rest of the Lepidoptera than to think that they were lost and then regained in Agathiphagidae and then lost again in the rest of Lepidoptera. The numbers of fore tibial spurs

have been recorded for some caddisflies (University of Michigan Museum of Zoology, web).

There are five more characters that Kristensen addresses that are present in all Lepidoptera except Agathiphagidae (with their state in heterobathmiid moths unknown). The first of these characters, the fourth character in all Lepidoptera except Agathiphagidae, is (4) the absence of an “outer tergal remoter” of the mesocoxa. The “outer tergal remoter” is part of the endopterygote ground plan, but is not present in Antliophora (superorder containing Diptera, Siphonaptera, and Mecoptera), Trichoptera, or any Lepidoptera other than Agathiphagidae (Kristensen, 2003). The absence of the mesocoxal “outer tergal remoter” is considered a groundplan autapomorphy of the mecopteroid lineage. The presence of the “outer tergal remoter” of the mesocoxa in this one family of moths, the Agathiphagidae, does not aid the placement of this group, because this character is no more likely to be present whether Agathiphagidae is the most primitive, second most primitive, or third most primitive extant lepidopteran family. Because the trait is present in Agathiphagidae but not in any other Lepidoptera or Trichoptera, it does not help untangle early lepidopteran evolution. The fifth character that is present in all Lepidoptera except Agathiphagidae is (5) that the follicles in testes are small. Shields (1988) wrote that both Trichoptera and Agathiphagidae have four large, free testes follicles. Though all Lepidoptera have four follicles per testis, the size is not standard throughout the order. Some hepialid moths (also somewhat primitive, though not this primitive) have discrete and rather large follicles. All other Lepidoptera have very small and/or closely appressed testes follicles. Kristensen (2003) wrote “it is probably the ordinal ground plan state that the testes follicles are large and discrete”. With testes follicles, the lepidopteran groundplan state is the case in Agathiphagidae but not Micropterigidae. This is evidence that Agathiphagidae are more primitive than Micropterigidae. Despite Shields (1988) observation, the number of testes follicles does vary in Trichoptera, but four or five testes follicles are present in primitive Trichoptera (Rhyacophilidae) and three or four testes follicles are present in the related mecopterans (Kristensen, 2003). Although Shields (1988) did not know that there are some trichopterans with more than four testes follicles, the observation that trichopteran and agathiphagid follicles are large and free still stands. Large, free testes

follicles are a possible trait of the last common ancestor of Trichoptera and Lepidoptera, if Agathiphagidae is the most primitive extant lepidopteran family. The sixth character present in all Lepidoptera except Agathiphagidae is (6) the presence of only one pair of accessory glands for male genital ducts. Two have been recorded in some caddisflies and also in Agathiphagidae (Kristensen, 1984). I could not find other works with information on the male genital duct accessory glands in Trichoptera, but given the information in Kristensen (1984), it seems sufficient to consider this, too, a trait of the last common ancestor of Trichoptera and Lepidoptera. The seventh character is (7) having only five or fewer ovarioles per ovary in all Lepidoptera except Agathiphagidae, which have 40 or more ovarioles per ovary. Many Trichoptera have this many ovarioles per ovary, as well (Kristensen, 2003). Such high numbers may represent the lepidopteran ground plan condition (Kristensen, 2003), and perhaps occurred in the last common ancestor of Trichoptera and Lepidoptera. High numbers of ovarioles are also present in some Glossata, though this is considered an autapomorphy of that derived group within Lepidoptera. The final character that is assumed present in all Lepidoptera except Agathiphagidae is (8) fewer than five abdominal ganglionic masses. In contrast, Trichoptera can have six abdominal ganglionic masses (Kristensen, 1984). Micropterigidae and Glossata have fewer than five abdominal ganglionic masses (Kristensen, 1984), while Agathiphagidae have five abdominal ganglionic masses (Kristensen & Nielsen, 1981 in Kristensen, 2003), which Kristensen & Nielsen (1981) consider to be the primitive number for the Lepidoptera.

There are some relevant traits that Kristensen did not include in his “Studies on the morphology and systematics of primitive Lepidoptera” (1984). Some of these are addressed in his later publication, the *Morphology, Physiology, and Development* volume of the *Lepidoptera, Moths and Butterflies* volume of the *Handbook of Zoology* (2003).

(1) Within the Lepidoptera, there is a trend towards elongation of the median notal wing process and tergal fissure deepening (the tergal fissure is the pit between the two notal wing processes) (Kristensen, 2003). Because the tergal fissure is formed by the two notal wing processes, fissure depth is completely dependent on length of the notal wing processes and these should be treated as one trait. Ivanov found that the fissure of *Agathiphaga* is particularly small and consistent with the theory that Agathiphagidae is the most primitive extant lepidopteran family (Kristensen, 2003). The process is also small in hepialid moths (somewhat primitive moths, though they are glossatan, meaning that they have a proboscis), but I do not think that this excludes the small fissure of agathiphagids from being the primitive condition. The conditions in basal Trichoptera “do not lend unambiguous support to the agathiphagid condition being plesiomorphic in the Amphiesmenoptera” (Kristensen, 2003). Although it is not unambiguous, according to Kristensen, it is possible that this is the primitive state, though Kristensen does not provide any other hints to how he reached his conclusion or what the possible plesiomorphic conditions in Amphiesmenoptera may be. I could not find any other information about the tergal fissure and notal wing processes in Trichoptera, and Kristensen does not include what the conditions in basal Trichoptera are. While the agathiphagid condition is not unambiguously supportive of the basal-agathiphagid theory, the tergal fissure/median notal process trait was enough to convince Ivanov that Agathiphagidae is the basal lepidopteran family and this condition is a relevant morphological character. The adaptive significance, if any, of the tergal fissure of any depth is unknown, so it is difficult to know if convergence (likely to be the result of selection) is an option. This trait could also be a “spandrel” of some other feature that does matter. The presence of a small tergal fissure in hepialid moths does not necessarily exclude this trait from being considered. Including this character increases the number of morphological characters indicating that Agathiphagidae is the most primitive extant lepidopteran group.

(2) The venation of *Agathiphaga* is more typical of the venational ground plan of Lepidoptera because the forewing costa is a distinctive tubular vein and agathiphagid venation is overall very

generalized, though venation in other non-glossatans is only generalized at the wing base (Kristensen, 2003).

(3) Traits of the mesonotum are also indicative of the agathiphagid state being the lepidopteran ground plan: “In *Agathiphaga*, and perhaps in the ordinal ground plan the dorsal intersegmental groove between pro- and mesothorax is open throughout and medially membranous in the bottom, i.e., there is no phragma” (Kristensen, 2003). I do not know about the phragma of Trichoptera, but the trait in Agathiphagidae may be that of the lepidopteran ground plan, meaning that Agathiphagidae may be the most primitive lepidopteran family.

(4) The arrangement of malpighian tubules favors the basal-agathiphagid theory. The groundplan arrangement in Amphiesmenoptera was probably six simple tubes that each opened separately into the hindgut, and this is the condition in Agathiphagidae, Heterobathmiidae, Eriocraniidae, Neopseustidae, most Trichoptera, and most Mecoptera. In Micropterigidae and Neolepidoptera, the tubules are grouped into two bundles (Kristensen, 2003).

(5) The lepidopteran groundplan state of dististipes (a morphologically composite formation in the larval maxillolabium + hypopharynx, which are fused) was presumably that the lower/posterior surface of the dististipes is strengthened by a sulcus running between the lateral part carrying the palp and the medial part carrying the galeal proboscis. A sulcus like this is present in Micropterigidae, Heterobathmiidae, and Eriocraniidae, but not Agathiphagidae (Kristensen, 2003). Kristensen claims that this means that the groundplan state is that of Micropterigidae, but to my mind, it is another trait supporting the basal-agathiphagid theory.

(6) On the mesopleurosternum, the state of the tegular arm points to the agathiphagids as the basal lepidopteran family: “As in the Trichoptera (Matsuda 1970) there is no internal ‘arm’ on the pleural ridge near midheight, but a blunt apodemal process on the lower end of the ridge (such as is known from some other endopterygotes, Czihak 1965, Mickoleit 1967) is present at least in *Agathiphaga*” (Kristensen, 2003). Kristensen does not name the other endopterygotes with this character.

(7) The most primitive condition of interganglionic connectives is known in *Agathiphaga*; here all thoracic connectives are paired, but the paired condition is found again in Neopseustidae (Kristensen, 2003). The Neopseustidae are a rather odd primitive (though glossatan) family of Lepidoptera.

(8) The foreguts of Agathiphagidae and Trichoptera are very similar and are expanded into large, thinwalled ‘crops’ which extend into the abdomen. Other primitive Lepidoptera—Micropterigidae and Heterobathmiidae—have a short foregut that is a simple tube for the whole of its length and the foregut only extends into the mesothorax (Kristensen, 2003).

(9) The larval stomodaeum is a simple, posteriorly widened tube in basal Mecoptera, Trichoptera, Agathiphagidae, and Heterobathmiidae (Kristensen, 2003). Non-glossatans’ galea have a variably sclerotized base, or subgalea, and a membranous apex, or distigalea. Agathiphagidae, Heterobathmiidae, and Trichoptera have a somewhat larger distigalea than Micropterigidae (Kristensen, 2003).

(10) Agathiphagidae and Heterobathmiidae have a strong thickening of their epistomal sulcus, which delimits the clypeus. This is thought to be the lepidopteran groundplan state. The micropterigid sulcus has a high internal crest with a thickened margin (Kristensen, 2003). The groundplan state being in Agathiphagidae but not Micropterigidae favors the basal-agathiphagid theory, though there is little description of these structures in the literature so grasping the full meaning of the difference is difficult.

(11) The *fr-pha(antmed)* is the muscle that originates on the anteromedial frontal area of the face and inserts on the ceiling of the sucking pump behind the frontal ganglion connectives. This muscle is only present in *Agathiphaga*, though a counterpart is present in primitive Trichoptera—muscle 4 dlphy in *Rhyacophila* (Kristensen, 2003). This makes it seem likely that this muscle is in the last common ancestor of Trichoptera and Lepidoptera. The *fr-pha(antmed)* and 4 dlphy muscles are not named or described in Kristensen (2003) beyond what is provided here.

(12) The pleurofurcal muscle *fu2-plr2b* is present in *Agathiphaga* and *Eriocrania*, and no other Lepidoptera, though it is a plesiomorphy at the mecopteroid level (Kristensen, 2003). The

presence of this may either be a twice independently present atavism in these two lepidopterans, or it may be the retained presence of this mecopteroid trait in *Agathiphaga* and an atavism in *Eriocrania*. I do not think this muscle has been looked for in Trichoptera. It is not in any other literature, at least under this name. The presence or absence of this muscle in Trichoptera would help make sense of its presence in Agathiphagidae. This muscle is a pleurofurcal muscle in the larva. The description given by Kristensen (2003) does not make it any easier to understand what this muscle is or what it is doing.

(13) The *lip1-fu2* muscle “may pertain to the Lepidoptera ground plan, but so far has only been identified in *Agathiphaga*” (Kristensen, 2003). This muscle is not named or described in Kristensen (2003) beyond what is provided here.

(14) The *fu2-2lph* muscle is considered part of the lepidopteran ground plan and is present in *Agathiphaga*, but not *Micropteryx* (Kristensen, 2003). However, this muscle is also present in *Eriocrania* and *Hepialus*, which are thought to have evolved later than *Micropteryx*. This muscle is not named or described in Kristensen (2003) beyond what is provided here.

(15) The *plp-plp* muscles are larval muscles that are present as a pair of antagonistic muscles in the lepidopteran groundplan, the state of which is only found in *Agathiphaga*.

(16) The *t2-lcv* muscle is present in *Agathiphaga* and *Eriocrania*, and has homologues in Trichoptera and other endopterygotes, but it is not present in Micropterigidae (Kristensen, 2003). The presence of this muscle in *Eriocrania* complicates the question of the weight that can be given to this trait.

These traits are difficult to understand, but the important thing to take away from this list is that there are sixteen traits that are either part of the lepidopteran ground plan or demonstrate in some way that the basal-agathiphagid theory may be more appropriate than previously thought.

The phylogenetic implications of the muscle characters are difficult to determine. Take, for instance, the *ful-poev/tent* muscle on the anterior surface of the profurcopleural bridge. In *Micropteryx* (Lepidoptera: Micropterigidae), this muscle inserts laterally on the corporotentorium. This is also what was recorded in Mecoptera (Hepburn, 1970 in Kristensen, 2003). In *Agathiphaga* (Lepidoptera: Agathiphagidae) and Trichoptera this muscle inserts on the cranial margin below the posterior tentorial base (Maki, 1938). Both conditions appear in Glossata, so it is difficult to determine what the lepidopteran groundplan state is, though it does seem to be more in favor of *Agathiphaga* basal to *Micropteryx*.

There are ten traits that are shared by Agathiphagidae, Heterobathmiidae, and the primitive Glossata. If we follow Kristensen's theory that Micropterigidae is the most basal lepidopteran family, then these would be the traits that evolved at the splitting event between Micropterigidae and the rest of Lepidoptera. These are the morphological traits that have been used to claim the Micropterigidae as the most primitive extant lepidopteran family.

(1) Paraglossae (or superlinguae) are the lobes on either side of the hypopharynx. Paraglossae are lost (or absent) in Agathiphagidae, Heterobathmiidae, and the primitive Glossata. Micropterigidae do have paraglossae. The ancestral amphiesmenopteran is thought to have had paraglossal lobes (Krenn, 2007).

(2) The stem of the metafurca has an anterior process.

(3) In all Lepidoptera except Micropterigidae and Acanthopteroctetidae, the ductus spermathecae have thinwalled and thickwalled compartments. The ductus spermathecae of Micropterigidae may

be the primitive condition, but a similar simple ductus spermathecae is found in Acanthopteroctetidae, and so this state can clearly be reached by being secondarily simplified or the normal lepidopteran condition can be easily enough changed to the thinwalled and thickwalled ductus spermathecae condition (Kristensen, 2003). The biological significance of this character is unclear. Kristensen (2003) admits more histological work on the ductus spermathecae of basal Lepidoptera is needed. There is little literature on the spermathecae of Trichoptera, though in one family (Phryganeidae) has only thin-walled spermathecae (Wiggins, 1998). Based on what I could find in the literature, I concluded that Trichoptera and Micropterigidae do not share the same trait here, so this character is not unambiguously supportive of Kristensen's theory.

(4) The pupal mandible is hypertrophied and angularly bent. Pupal mandibles are present in Trichoptera, though they are reduced or lost repeatedly. According to Wiggins and Wichard (1989), we can infer from extant lepidopteran families that terrestrial pupal cocoons in primitive mandibulate families evolved to thinner cocoons as pupal mandibles were lost.

(5) The larval corporotentorium is slender.

(6) The medial labral retractors are lost in the larvae. Micropterigidae are the only Lepidoptera with larval medial labral retractors (Hinton, 1958). In the amphiesmenopteran ground plan, the extrinsic labral musculature comprises two pairs of extrinsic 'retractors', *fr-la,lat* and *fr-la,med*, the median labral retractors. An interesting component of the labral musculature is that the intrinsic labral compressor muscle, which is present in Micropterigidae (Kristensen, 2003), but not Agathiphagidae or Heterobathmiidae (with a solid-epipharynx-labrum (Kristensen 1984)). This muscle is present in higher Lepidoptera with a 'normal' labrum (Hinton, 1958). The presence of this intrinsic muscle indicates either that agathiphagids are basal in lepidopteran evolution, because Micropterigidae and Glossata have the same derived condition, or of this trait being highly adaptable and easily changed.

(7) Larvae have only one maxillary endite lobe.

(8) Larvae have a nonfunctional metathoracic spiracle. In Micropterigidae, the larval spiracles are functional, but this character seems to be unusable for the placement of Agathiphagidae because

the agathiphagid larval spiracles remain unstudied. In Heterobathmiidae and Glossata the mesothoracic spiracle is anteriorly displaced and situated on the prothorax (Kristensen, 2003).

(9) Larvae having lost the cranial flexor of dististipes, also known as the cranial ‘flexor lacinae’ or *cr-lac*. Hinton (1958) found the cranial flexor of dististipes to be present in Micropterigidae, and the absence of this muscle has been considered a putative synapomorphy of all other Lepidoptera (Kristensen, 2003). This is also known as the craniolacinalis of von K ler (1963), and despite Hinton’s report of this muscle being present in all Trichoptera and Micropterigidae, Vegliante (2005) did not find this muscle in the trichopteran larvae he investigated (genus *Sericostoma*). With this muscle not actually being present in all Trichoptera, and possibly not any, the weight of this muscle’s presence in Micropterigidae is significantly reduced.

(10) Larva having the dorsoventral cranial muscle laterally spanning the foramen magnum. This feature is not addressed in Kristensen (2003), it is only cited in Kristensen (1984).

Of the ten morphological traits that are supposed to diagnose basal lepidopteran evolution, four (3, 4, 8, 9) do not support Kristensen’s tree. These are the ten morphological traits that the accepted theory of basal lepidopteran evolution is based on. There are other relevant traits that Kristensen discusses in other publications of his (2003) that may support his theory.

(1) An abdominal apex in which segment IX is clearly separate from segment X is only encountered in Micropterigidae within the order and it may be part of the lepidopteran ground plan.

(2) The groundplan configuration of the midgut/hindgut transition may have resembled that of Micropterigidae. Micropterigidae have a sphincter muscle posterior to the openings of the Malpighian tubules. It is important to keep in mind, however, that the Malpighian tubules of

Micropterigidae are not considered to be the lepidopteran groundplan state. Micropterigidae and Heterobathmiidae conform to Kristensen's notion of a lepidopteran groundplan foregut, which differs from the condition shared by Agathiphagidae and Trichoptera. I think this character supports a basal-agathiphagid line of lepidopteran evolution. Why Kristensen thinks that the shared trichopteran-agathiphagid foregut is not the groundplan but micropterigid and heterobathmiid foreguts are, is unclear to me.

(3) Micropterigidae and many Trichoptera have a process that is sclerotized processus spermathecae projecting into the papilla/chamber lumen. This process may surround the duct opening on a papilla/chamber outpocketing.

(4) The ratio of the height of the head capsule above the compound eye : total height of the head capsule is modest in Micropterigidae, and Kristensen assumes this is the state of the lepidopteran groundplan.

(5) In some Micropterigidae and Heterobathmiidae, the median plate is laterally synscleritous with the valves, which become united by a sclerotized bridge. This is similar to the state in Trichoptera, so this is thought to be the lepidopteran groundplan state.

These five traits, however, were the only additional traits that I found in Kristensen (2003) that support his theory. In contrast, I found sixteen additional traits that may support the basal-agathiphagid theory.

The traits shared by Trichoptera and Agathiphagidae that are not present in Micropterigidae or any other Lepidoptera provide evidence that Kristensen's theory is no more compelling than Shields's theory, and, to my mind, is less so. It should be kept in mind that the worldwide community of primitive lepidopterists is very small, and while the majority of lepidopterists will probably still doubt a theory that is not Kristensen's, there are few people that have looked at the

characters themselves. It often happens in biology, especially in fields that are not as widely studied (like the realm of primitive Lepidoptera), that there are one or two world experts, and most of the biologists in or near the field defer to what the world experts say, rather than examining the albeit mysterious and confusing characters themselves. This may be what has happened with theories of basal Lepidoptera.

According to Kristensen (1984), there are ten morphological traits indicating that Micropterigidae are more primitive than Agathiphagidae, and seven traits indicating that Agathiphagidae are more primitive. The molecular study carried out by Wiegmann (2002) was partially based on Kristensen's (1984) 10 morphological traits in favor of Micropterigidae as the most primitive group, and Wiegmann (2002) provides no independent assessment of these traits. They are supposedly supported by 16 unambiguous ribosomal substitutions (Wiegmann, 2002). According to Wiegmann (2002), Shields (1993) uses traits that are shared by agathiphagids and a mecopteroid fossil to claim that Agathiphagidae is basal. While Wiegmann (2002) uses this to undermine Shields' ideas, here I used traits shared by Agathiphagidae and Trichoptera as evidence that Agathiphagidae is basal.

I will be the first to admit that I cannot judge some traits—there are often no figures that illustrate alternative states, and sometimes not all the relevant families and most appropriate outgroups have been described. But I think the

problems I had evaluating arcane characteristics would be just as much of a problem for the few lepidopterists with some interest in these issues.

It is difficult to identify where the division of ancestral Amphiesmenoptera to either Trichoptera or Lepidoptera occurred, especially when taking into account the extinct species. It is easier with extant species, but there are so many fossil amphiesmenopterans with characteristics of both Trichoptera and Lepidoptera that it is difficult to assign them to either order. I think the current state of knowledge should treat Amphiesmenoptera as the fundamental taxonomic distinction, rather than implying a strong distinction between Trichoptera and Lepidoptera. The mouthparts of primitive Lepidoptera tell more than one story, but it is interesting to note that Kristensen (1984) writes that the galea lobe of Agathiphagidae “is strikingly similar to the maxillary endite lobe in the primitive caddisfly *Rhyacophila*,” yet he takes the boundary between Trichoptera and Lepidoptera as clear, and argues against putting Agathiphagidae as the basal lepidopteran group.

I do not agree with the theories of either Kristensen (1984, 1997, 1999) or Shields (1988, 1993). Based on the characters presented by Kristensen and others, I propose that Agathiphagidae is the most primitive extant family of Lepidoptera. I do, however, disagree with Shields (1988) that this group evolved from a group of Trichoptera. It is commonly accepted that both Trichoptera and Lepidoptera evolved from a terrestrial amphiesmenopteran common ancestor. I think that the basal splitting event within the extant Lepidoptera was between Agathiphagidae and all other Lepidoptera. The extinct lepidopteran common

ancestor of Micropterigidae and Agathiphagidae, one of the earliest Lepidoptera, may have had biting mouthparts like those of Micropterigidae. The biting mouthparts were lost between the agathiphagid-micropterigid common ancestor and the Agathiphagidae, but were retained in Micropterigidae and Heterobathmiidae.

What is the importance of knowing what family of moths is most primitive? It may seem trivial in the grand scheme of things, but this has implications for the diagnoses of the orders Trichoptera and Lepidoptera and understanding how closely related these orders are. Knowing how closely related Trichoptera and Lepidoptera are has implications for how we assess cladistics—do we throw biological meaning to the wayside and just see what group is awarded the label “order” and proclaim that the important group distinction? Or is it important to assess groups and what they mean? Rethinking the boundaries between groups and what these really mean for a group is necessary to keep cladistics from ignoring the underlying biological theory. Although my examination of Kristensen’s characters suggests that some be shifted from favoring his phylogenetic hypotheses to another, and that other characters are not well enough documented to be useful, I do not think that the phylogeny of Lepidoptera is a question with a numerical answer. I think a survey of the characters in a broad sample of taxa, combined with accounts of their developmental trajectories and possible functions, is the best guide to their value in cladistic analysis. Today, huge numbers of nucleotide changes, analyzed

according to more and more sophisticated (and mysterious) algorithms, has replaced thinking about characters and their relation to the natural history of the organism. I think such trees should be tested against others that are connected to what the organism is and what it does.

Scales are one of the most striking lepidopteran characters, but the evolutionary advantages conferred on moths and butterflies by scales are still debated. Scales may have provided an evolutionary advantage to the insects that developed them by providing insulation (Simonsen, 2001) and thermoregulation (Kristensen, 2003), escape from predators, protection (Packard, 1898), colors and markings (Packard, 1898), and dust removal (Kristensen, 2003). Simonsen argued that the insulation provided by scales allowed for the incredible success of Lepidoptera as nocturnal insects (2001). However, Trichoptera and Mecoptera are largely nocturnal groups.

The independent evolution of scales in (some genera of) Trichoptera, Collembola, Archaeognatha, Zygentoma, Psocodea, Coleoptera, Diptera, and Lepidoptera indicates that the ability to develop and evolve scales is interesting but not an innovation unique to Lepidoptera. The ease of evolving scales is related to their close developmental similarity to hairs, which are ubiquitous in insects. The ability of Trichoptera to develop scales independently so many times indicates the ease with which this transition can take place, especially in the superorder Amphiesmenoptera. The impressiveness of lepidopteran scales is due to their persistence in this group for millions of years and correlation with

incredible species diversity, while scaled caddisflies persisted but did not diverge greatly or lead to any new lines of life. The relative ease of transition with which many insects have changed from hairs to scales points to the homology between these structures. This can lead us to the conclusion that the major difference between these orders is, obviously, ecological.

The aquatic world of trichopteran larvae is quite different from that of their terrestrial relatives, the caterpillars. The ecological difference between these worlds, rather than morphology, is what makes Lepidoptera and Trichoptera different. Lepidoptera have co-evolved with particular angiosperms. Trichoptera have established several guilds underwater and most are present wherever there is freshwater. And yet, despite these differences, there are species of both orders that have secondarily changed their larval ecological needs. *Eniocyba* is the classic example of the caddisfly with secondarily terrestrial larvae. *Eniocyba* is remarkable in a number of ways. Females of this genus are practically wingless and the larvae of *Eniocyba* live in moss at the roots of trees, not necessarily near water at all (Mosely, 1939). *Hyposmocoma*, a genus of 350 moth species, was recently discovered to have twelve species with larvae that can be aquatic. The surprise of these aquatic caterpillars is that there does not seem to be a plastron or gills. They do, however, live in fast-running water, which is highly oxygenated, and cannot survive in standing water. The ability to survive underwater developed independently in three lineages of moths (Rubinoff & Schmitz 2010). *Hyposmocoma* is successful at life in both aquatic and terrestrial environments.

Their larvae are able to be either terrestrial or aquatic or both and are not restricted to one or the other. This incredible plasticity has never before been recorded in insects. They can breathe, feed, and mature both above and below the surface of the water. The three lineages of amphibious caterpillars arose via parallelism more than 6 million years ago from terrestrial clades. These three independent invasions of water are more than have happened in any other genus of animals and they are the only insects that have been able to remain amphibious, developing either in or out of water. Amphibious caterpillars did not evolve because of an unexploited niche—there are Hawaiian Trichoptera, but there is less competition on these islands. In fact, the only damselfly with a terrestrial nymph (they are otherwise aquatic) is in Hawaii.

The closeness of Agathiphagidae (Lepidoptera) and Trichoptera demonstrates how even in extant species, these orders are shockingly close and share many features. Their possible common origin in the family Necrotauliidae and extinct species with some lepidopteran groundplan traits but not others also indicate the closeness of these orders. Trichoptera and Lepidoptera should be thought of as two subgroups of the Amphiesmenoptera, rather than as solidly separate groups. The characters that join Agathiphagidae and Trichoptera outnumber those joining Trichoptera and Micropterigidae, and they may be more meaningful. The difficulty in determining what primitive lepidopterans are closest to Trichoptera shows how hard it is to evaluate the differences between these orders (also indicated by the mysterious names of the muscles and structures

that are shared by Trichoptera and Agathiphagidae). Despite the differences between Trichoptera and Lepidoptera, the cytological characters uniting Trichoptera and Lepidoptera are more biologically meaningful than most of the characters separating these groups. There is no rule that orders have to be the most important level of classification. Combining two orders is a mess because the families, genera, and species are already sorted out, but there is no reason not to consider the significant clade here to be Amphiesmenoptera.

By reexamining things that seem so concrete—the details of the differences between Trichoptera and Lepidoptera, and the origin of Lepidoptera—I was able to uncover a great deal by simply questioning what was already “known.” Upon examination of the details, these orders are more similar than we think. In fact, if we took twelve of the 115,000 lepidopteran species and compared them to the 13,000 Trichoptera, these would probably not be separate orders.

Many ideas that we take as scientific facts are heavily influenced by historical accident. Here, the times of discovery of the primitive lepidopteran group Micropterigidae before Agathiphagidae might have “canalized” thinking about large patterns so that the later discovery of Agathiphagidae was slotted into a narrative that was already worked out, as opposed to re-evaluating the narrative. This bias can be detrimental to the fields we study if we ignore the elements that influenced those fields and never question our predecessors.

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Appendix A

Kristensen's (1984) 26 lepidopteran autapomorphies:

- (1) median ocellus lost
- (2) corporotentorium with posteromedian process
- (3) presence of an intercalary sclerite laterally in the membrane between the antennal scapus and pedicellus
- (4) maxillary palp with points of flexion between segments 1|2 and 3|4 with segment 4 as the longest segment of palp and no antagonistic muscles inserting on the base of any palp segment
- (5) presence of a slender craniostipital muscle running close to the craniocardinal muscle
- (6) the postlabium is an arched sclerite with piliform scales
- (7) terminal segment of labial palp with a group of chemoreceptors located in a depression
- (8) salivarium devoid of longitudinal dorsal muscle
- (9) labral nerve and frontal ganglion connective separate just from their origin on the tritocerebrum
- (10) nervus recurrens running within cephalic aorta until reaching retrocerebral complex
- (11) laterocervical sclerite with "hair plate" close to the anterior apex
- (12) prothoracic endoskeleton with a prominent free arm arising from the bridge between the sternum and the lower posterior corner of the pleuron
- (13) mesothorax with a "tergopleural apodeme", issued from the upper part of the pleural suture and accommodating the insertion of a tergopleural muscle
- (14) metathorax with a "prescutal arm"
- (15) fore tibia on inner surface with an "epiphysis"
- (16) wings with dense covering of broad scales
- (17) metathoracic spiracle with a single, anteriorly situated, external lip
- (18) tergum I extensively desclerotized and external layer of "short" dorsolongitudinal I|II muscles therefore lost
- (19) tergum I with paired lateral lobes extending downward/backward and articulating with the anterior corners of sternum II
- (20) male gonopod primarily undivided
- (21) protractor muscles of the male phallus originating within the gonopods
- (22) cerci lacking in both sexes
- (23) abdominal nerve cord with at most five ganglionic masses and with unpaired connectives
- (24) mesothoracic aorta curving upwards to dorsum, from the groundplan
- (25) pleurostome elongated, craniocardinal articulation far behind mandibular base
- (26) maxillary palp with less than five segments

Kristensen's 21 amphiesmenopteran autapomorphies (1984):

- (1) prelabium fused with hypopharynx
- (2) lower posterior corner of laterocervicale produced towards the prosternum
- (3) pronotum with paired setose "warts"
- (4) pterothoracic episterna with characteristic suture pattern of two sutures, with a common base, that extend forward from the pleural suture
- (5) secondary furcal arms in pterothorax fused with posterior margin of corresponding epimera
- (6) metathorax with a setose sclerite in the wing base membrane below or behind the subalare
- (7) pretarsus above the claws with a "pseudempodium", a strong seta on a socket
- (8) wings with extensive covering of setae (setae are further modified in Lepidoptera)
- (9) double-y configured anal veins in fore wing
- (10) one ventral neck muscle originating on the fore coxa
- (11) conical furcopleural muscle in the mesothorax having broad end on the pleural ridge
- (12) presence of paired glands opening on sternum V
- (13) male genital segment with tergum and sternum fused, forming a closed ring
- (14) anterior margin of female segments VIII and IX with long, rodlike apodemes accommodating the insertions of protractor/retractor muscles of the extensible ovipositor
- (15) ventral diaphragm muscles inserting on the nerve cord
- (16) female heterogamety
- (17) apyrene sperm of usual occurrence
- (18) spermatozoa with outer, accessory filaments very thick, filled with proteinaceous and glycogen-like material
- (19) chromosome number specialized (high) and chromosomes probably holocentric; oogenesis achiasmatic
- (20) In larvae, each stemma with one crystalline cone cell transformed into a primary pigment cell
- (21) in larvae, prelabium and hypopharynx fused into a lobe apically carrying the salivary (silk) gland orifice

Appendix B
Measurements of antennae bending

Caddisfly in amber: The fossil caddisfly (Figure 42) has a bent antenna. The measurements of the angle between the tip of the antenna and the articles between the distal and proximal portions of the antenna are the “distal bend” measurements. The angle between the proximal two portions of antenna are the “proximal bend” measurements. Measurements made with ImageJ.

Nautilus: Measurements taken from two different videos (Walk 9, Walk 3) of the caddisfly I named Nautilus. I translated the videos into frames and measured the angles in the frames. The “unbent” angle, or control angle is given at the end (unbent). Both left and right antennae angles are given where I could determine them. Videos taken with Photron and angles measured with ImageJ.

	distal bend	proximal bend
Amber	Blurry	132.019
Amber	131.881	133.919
Amber	123.781	out of view
Amber	128.126	130.192
Amber	125.538	130.691
Amber	124.17	133.668

Nautilus
Walk 9

frame	L antenna angle		R antenna angle	
1105	164.197		162.979	
1110	158.875, 142.765		146.449	
1115	142.765		147.529	143.973
1120	141.009		146.041	
1122	144.162	152.301, 148.570	139.764	142.943
1125	143.267		139.399	
1130	122.661		122.84	
1135	121.827		125.838	
1140	132.274	129.289, 135		
1145	135		143.13	
1149 (1150 blurry)	133.152, 161.565	149.036, 138.731		
1151	139.635	148.671, 135		
1154	127.875		136.507	
1155	120.964		136.507	
1598	141.189		137.49	

Nautilus

Walk 3

frame	L ant <		R ant <	
1460	135.0, 131.634		138.013	bent
1461	131.634, 130.815		131.987, 135	proximally
1462		131.987	131.987	compared to
1463		144.462	146.31	normal
1464	163.191, 155.25		166.724, 157.751	bending
1465		173.66	167.171	axis
87			124.796, 134.157	113.385
89				136.848
90			129.094, 137.490	124.695
91				119.932
92			117.150, 135	144.462
97				131.634
99				175.236
100	140.194, 131.055		144.462	177.879
101	108.435, 108.435		99.009	
102	127.504, 113.923		118.25	
103		120.67	112.751	159.146
104		112.62	111.532	147.995
105				145.905
110				140.194
111			127.875, 131.634	136.312
112				123.69
113				114.228
114				116.565
115				124.655
116				167.005
117				155.298
140		163.474	165.964	156.371
				150.255 (looks better)
				146.31
				146.31
				140.194
				119.876
				114.687
				110.956
				125.362
				161.565
				158.477
				156.161

Unbent

frame 291 L 157.166 R 161.565

Appendix C

Reynolds Number of trichopteran seta on anterior edge of forewing during flight

$$Re = (uL)/\nu$$

u = velocity

L = hair diameter

ν = kinematic viscosity of the fluid

Standard lab conditions are $25^{\circ}\text{C} = 298\text{K} = 72^{\circ}\text{F}$

Kinematic viscosity of air at $298\text{K} = 1.5952\text{E}-5$

$$\text{Hair diameter} = 0.0976 \mu\text{m} = 0.1 \mu\text{m} = 1\text{E}-7 \text{ m}$$

$$\text{Velocity at wing tip of flight} = (1.754 \text{ cm}/0.008 \text{ sec}) = 0.001754\text{m}/0.008\text{sec} = 0.21925 \text{ m/s}$$

$$Re = (0.21925\text{m/s} * 1\text{E}-7\text{m}) / (1.592\text{E}-5) = 0.00137719849$$

Glossary

Cast list – Groups

Agathiphagidae — also known as Aglossata, *Agathiphaga* sp., one of the most primitive families of Lepidoptera. A non-glossatan family. The most primitive family according to Shields (1988) and the second or third most primitive family of Lepidoptera according to Kristensen (1984, 1997, 1999). One of the three most primitive moth families.

Amphiesmenoptera — The superorder made up of Trichoptera, Lepidoptera, and some of their extinct relatives (extinct amphiesmenopterans).

Archaeognatha – Bristletails; a group of apterygote insects (*Tree of Life Web Project*. Web. 1 April 2010. <http://tolweb.org/tree>).

Coleoptera – Beetles; a group of endopterygotes (*Tree of Life Web Project*. Web. 1 April 2010. <http://tolweb.org/tree>).

Collembola – Springtails; they are not insects but they are Hexapods, a group to which insects also belong (*Tree of Life Web Project*. Web. 1 April 2010. <http://tolweb.org/tree>).

Diptera – The true flies; a group of endopterygotes (*Tree of Life Web Project*. Web. 1 April 2010. <http://tolweb.org/tree>).

Endopterygote — Those insects with complete metamorphosis, that is distinctive larval, pupal, and adult stages.

Glossata — A suborder of Lepidoptera that includes all the lepidopterans with a coilable proboscis.

Heterobathmiidae — Also known as Heterobathmiina, *Heterobathmia*. A family of non-glossatan, primitive moths. One of the three most primitive moth families.

Holometabolous — See Endopterygote (2).

Lepidoptera — An order of holometabolous insects; moths, butterflies, and skippers; sister group to Trichoptera; included in superorder Amphiesmenoptera.

Micropterigidae — A family of primitive moths that is made up of the Sabatinica group (including *Epimartyria*) and *Micropteryx*. Also known as micropterigid

moths, formerly known as Zeugloptera. The most primitive family according to Kristensen (1984, 1997, 1999) and the second or third most primitive family of Lepidoptera according to Shields (1988). One of the three most primitive moth families.

Psocodea — Parasitic lice, book lice, and bark lice (*Tree of Life Web Project*. Web. 1 April 2010. <http://tolweb.org/tree>).

Thysanura — Silverfish and Firebrats; a group of apterygote insects (*Tree of Life Web Project*. Web. 1 April 2010. <http://tolweb.org/tree>).

Trichoptera — An order of holometabolous insects; the caddisflies; sister group to Lepidoptera; included in superorder Amphiesmenoptera.

Props – Anatomical features

Cerci — Paired appendages on the rear-most segment of an insect.

Cranio-stipital muscle (*cr-st*) — a muscle autapomorphic for Lepidoptera that originates on the cranium and inserts with a tendon on the wall of the stipes (K's MP&D p 54).

Dististipes — a morphologically composite formation in the larval maxillolabium + hypopharynx (which are fused). In glossatan moths, there is a single extrinsic muscle for the dististipes, and that is the cranial flexor laciniae (2).

Fore tibial spurs — Spur is a spine that is not a process of cuticle but is implanted in it (1).

Chemoreceptor — An external chemical receptor.

Corporotentorium — the large tentorial bridge on a strongly developed tentorium (1).

Cranio-stipital muscle (*cr-st*) — A muscle autapomorphic for Lepidoptera that originates on the cranium and inserts with a tendon on the wall of the stipes (1).

Ductus spermathecae — connects the spermatheca to the genital chamber.

Foramen — A general name for any body opening (2).

Foramen Magnum — The occipital foramen (2).

Epiphysis — used for antennal grooming (2).

Epipharynx – the dorsal surface of the extra oral cavity of mandibulate insects (2).

Intercalary sclerite — The third segment of the head, a chitinous plate between the scape and pedicel of the antenna (2).

Labial palp — A jointed feeler originating on the labium.

Labium – The posterior median appendage of the mouthparts below the maxilla (2).

Labrum – The labium superius (2).

Macrochaete — A structure on an insect that is either a seta, bristle, or scale (1).

Malpighian tubules — Exceedingly fine glandular tubes for excretory purposes. They open into the food canal where the stomach joins the small intestine (2).

Maxilla — An unpaired appendage of the mouthparts in mandibulate insects (2).

Maxillary endite lobe — Inwardly directed lobe of first segments of maxillules, maxillae, and maxillipeds which may function as auxiliary mandibles (Stachowitsch, 1992).

Medial labral retractors — Middle of four muscles that move the labrum or the epipharynx (2).

Metafurca — the internal skeletal element of the metasternum built up of two principal parts (2).

Metanotum — The upper surface of the metathorax (2).

Metasternum — The entire ventral surface of the metathorax (2).

Metathoracic spiracle — It is situated on each side of the metanotum. See Tegula.

Metathorax — The fourth segment in insects (2).

Ocellus — A Simple, small, extra eye usually situated on the top of the head (Jardine 1914).

Paraglossae (or superlinguae) — The lobes on either side of the hypopharynx (2).

Pedicel — The second joint of the antenna (2).

Pleuron — a lateral sclerite of thoracic segment of an insect between the tergum and sternum (2).

Retrocerebral glands — Endocrine glands located behind the brain in insects (<http://www.answers.com/topic/retrocerebral-gland>).

Salivarium — A small pocket on the inside of the mouth of an insect, which contains the opening of the salivary duct.

Scape — The first joint of the antenna.

Setose warts — in lower moths, thought to be forerunners of stalked patagia in higher lepidopterans. This feature is termed a synapomorphy of Trichoptera and Lepidoptera.

Spermatheca – An organ within the reproductive tract of a female insect where sperm is deposited (2).

Tegula — A small tubercular plate immediately at the base of each fore-wing, which covers the metathoracic spiracle.

Tentorium- a semi-transparent chitinous septum of the complex inner skeleton in the head capsule formed by inpushing of the chitin, which gives attachment to the muscles used in moving the head and jaws.

Tritocerebrum — The insect brain has three pairs of fused ganglia. The tritocerebrum is the third pair of ganglia and it innervates the labrum and integrates the sensory inputs from the other two pairs of ganglia. It also links the brain with the rest of the ventral nerve cord and the stomodaeal nervous system that controls the internal organs.
(<http://www.cals.ncsu.edu/course/ent425/tutorial/nerves.html>)

Stage directions -- Theory

Autapomorphy — A derived trait that is unique to a terminal group.

(Glossary) Literature Cited:

(1) Kristensen, Niels P., Maximilian Fischer, Willy Kuhnenthal, Max Beier, Thilo Krumbach, and Jochen Niethammer. *Handbuch Der Zoologie: Eine Naturgeschichte Der Stammes Des Tierreiches*. Berlin [u.a.: De Gruyter, 2003. Print.

Stachowitsch, M. 1992. *The Invertebrates: An Illustrated Glossary*. Wiley-Liss, New York, 676 pp.

(2) Zombori, L., and Henrik Steinmann. *Dictionary of Insect Morphology*. Berlin: W. De Gruyter, 1999. Print.