



December 3, 2023

Review

Bulah Chia-hsiang Wu's PhD thesis

“Comparative Analysis of Silk Proteins and Discovery of Novel Sericin Gene in Lepidopteran Moths”

PhD thesis of Bulah Chia-hsiang Wu deals with the silk components of the Mediterranean moth, *Ephestia kuehniella*, and with the discovery of a novel silk gene, *P150/ser6*, in the silkworm, *Bombyx mori*, widely recognized model organism. Thesis analyzes and describes the cocoon silk components in both species. Wu's thesis is composed of two major papers: in the first one author combined transcriptomic, genomic, and proteomic approaches to identify silk proteins in *E. kuehniella*, and in the second paper he contributed to the discovery of gene *P150/sericin6* in *B. mori* based on microsynteny analysis.

It is widely known that silk, composed of complex biopolymers, is secreted by ectodermal glands in various species of invertebrate organisms including insects, arachnids and myriapods. Still it has long history of more than 4000 or even 5000 years when the silkworm *Bombyx mori* was domesticated and its silk was used for great variety of purposes including textile industry. Various insect species produce and utilize silk that is devoted to evolutionary specific functions such as cocoon formation, for ballooning for aerial dispersal, for constructing feeding tubes to facilitate larval foraging, to create an egg stalk that aids egg attachment to the leaves of the host plant *etc.* Among natural properties of silks are tensile strength, extensibility, sustainability, and biodegradability. All these reasons makes silk strongly attractive to be utilized not only in composite materials manufacturing, cosmetics industry but also by the arms industry. And it has growing potential also in biomedicine.

Bulah Wu has applied an integrated, multiomic approach combining genomic, transcriptomic and proteomic tools to systematically investigate the components of silk at different biological levels. Along these lines author characterized the cocoon silk of the Mediterranean moth *E. kuehniell*, then performed analysis of the cocoon silk of the greater wax moth *G. mellonella* and made intra-family comparisons. The *FibH* genes and encoded proteins of nine Pyraloidea species were compared and presented their conserved features.

In spite of fact that the silkworm *B. mori* is one of the most studied species in sericulture, there remained subset of unknown genes encoding the silk components. Bulah Wu conducted a microsynteny analysis and corrected an erroneous gene model for the current *B. mori* reference genome. This approach has led to the discovery of a novel silk gene *P150/ser6*, and finding that its expression pattern is similar in *B. mori* and two pyraloid moths, *E. kuehniella* and *G. mellonella*. Based on these findings and the difference in *P150/ser6* protein intensities observed in silk cocoons author implies that other genetic factors (for example, *cis*-regulatory elements) may be involved in these species-specific differences.

I am glad to summarize that these and numerous other results presented in the thesis are very nice and original, and document another good piece of scientific work from the laboratory of the mentor,

Michal Žurovec, that were profoundly contributed by PhD student Bulah Chia-hsiang Wu. In parallel, to my overall positive views on the thesis I have few questions to the author:

- (1) Can author provide explanation why there are only 30 major silk proteins in *E. kuehniella* and more than 50 in *G. mellonella* ?
- (2) Do you have technical explanation why majority of silk proteins in *E. kuehniella* were detected only by transcriptomic approach, and not by qPCR or proteomics ? And by contrast, why many of those which were identified by proteomics or qPCR were not found by transcriptomics ?
- (3) Why there is smaller number of sericins in *E. kuehniella* than in *G. mellonella*, and how it affects species' silk properties ?
- (4) Is anything known about function of P150 proteins (resembling both sericins and mucins) ? Are known any data from chemical or physical tests on P150 proteins ? Are there available any mutations to *p150* orthologues in other species ? If yes, what are the phenotypes ?
- (5) Can author explain what could be phylogenetic reasons why there are so significant differences between FibH proteins in terms of molecular weight, and amino acid composition between *Galleria*, or *Ephestia* (or *Bombyx*) on one side, and *Antheraea* or *Samia* on other side ? And how it reflects their role in the cocoon formation ?
- (6) Author stated that the sericins may serve as adhesives, lubricants or regulators of silk compactness and cross-linking. Is this cross-linking of chemical/covalent or mechanical in nature ?
- (7) By synteny analysis author found a region containing clusters of sericin genes and identified blocks of synteny between genomes of *E. kuehniella* and *G. mellonella*. The resulting microsynteny map allowed identification of duplication events in the sericin family. Can author speculate what were phylogenetic reasons (impulses) for these duplication events ?
- (8) Is it clearly identified or specified what are the functions of Muc1 (or mucins in general) in the moth silk ?
- (9) Do you have an explanation why some silk proteins of *E. kuehniella* are expressed in both anterior and also in rear MSG ? Does it make any physiological significance for silk ?

Based on above mentioned facts, contribution of the research data and obtained evidence, as well as variety of experimental methods used, the achievements of Bulah Chia-hsiang Wu meet all acceptable scholarly standards for PhD dissertation. Therefore, I can conclude that applicant fulfilled all major requirements, and I can gladly recommend the thesis for successful defense in the front of a PhD committee.

Dr. Robert Farkaš, CSc.
Head of the Laboratory of Developmental Genetics IEE BMC SAS



**INSTITUTE OF EXPERIMENTAL
ENDOCRINOLOGY**

**BIOMEDICAL RESEARCH CENTER
Slovak Academy of Sciences**

