SUMMARY

1. In this study, the phenotypic as well as molecular characterization of different morphs of *Antheraea assamensis* Helfer (Muga) and *Philosamia ricini* Hutt (Eri) silkworms were carried out. In addition, the phylogenetic relationships of these two species with other silkworm species were also investigated.

2. In recent years, DNA based marker systems have been increasingly employed in diverse areas of biology including phylogenetic studies, evolution, ecology, population genetics, population dynamics and genetics of complex traits in both plant and animal systems. Over a long time, significant contributions have been made in the field of insect systematics through morphometric traits, wherein a number of difficulties were encountered due to genotype-environment interactions. The limitations in using morphological, physiological and cytological markers for assessing genetic diversity have been largely circumvented by the developments in DNA-based markers as these are by nature are neutral to the stage of development, physiological status and environmental influences.

3. *A. Assamensis* Helfer, popularly known as Muga silkworm is endemic to Northeast India. It has three semi-domesticated morphs viz. green, blue and orange, and one wild morph. *P. ricini* Hutt, commonly known as Eri silkworm also has six morphs. These are namely white plain, white zebra, white spotted, blue plain, blue zebra and blue spotted. All the morphs of Eri silkworm are domesticated. These morphs were collected from different geographical
locations of Northeast India and used in this study. A total of 12 samples of Muga silkworm and 26 samples of Eri silkworm were collected from Khanapara, Mangaldoi, Udalguri, Howley, Kokrajhar, Barduar, Habim, Goalpara, Mendipathar, Tura, Nongpoh, Haflong, Titabor and North Lakhimpur.

4. The phenotypic characters like larval colour, larval weight, larval markings, cocoon colour, cocoon weight, shell weight, shell ratio and voltinism of different morphs of Muga silkworm were studied and found considerable variation. The colour of the matured larva of Muga silkworm was deep green, faint blue, orange and green in green, blue, orange and wild morphs, respectively. Irrespective of the morphs, the larva had no larval markings. The larval, cocoon and shell weights were recorded highest in the 5\textsuperscript{th} instar larvae of wild morph collected from Haflong (WM03) and lowest in orange morph collected from Howley (OM01). The cocoons in all the morphs were oval in shape. The color of the cocoon was mostly bright golden brown in wild, yellowish brown in orange, whitish golden brown in blue and golden brown in green morph. The shell ratio was highest in orange morph (OM01) and lowest in blue morph collected from Udalguri (BM02). The semi-domesticated morphs were non-diapausing and hence multivoltine. On the contrary, the wild morphs were diapausing and hence univoltine or bivoltine.

5. The larval colour of the morphs of Eri silkworm also showed considerable variation. The colour of the matured larva was white in white plain, white zebra and white spotted morphs, blue in blue plain, blue zebra and blue spotted morphs. Zebra markings were found on the larvae of white zebra and blue zebra,
and spotted markings were observed on white spotted and blue spotted morphs. White plain and blue plain morph had no larval markings. Except white plain morph collected from Kokrajhar (WP05), the cocoon colour was white in all the morphs. Brick red cocoon was produced by WP05. Irrespective of morphs, the cocoon shape was oval. The white plain morph collected from Barduar (WP04) was superior in larval and cocoon weights. The blue plain morph collected from Titabor (BP07) was inferior in terms of larval weight. The WP05 had highest shell weight and shell ratio. The blue morph collected from Goalpara (BP04) was found to be inferior in terms of cocoon weight, shell weight and shell ratio. All the morphs of Eri silkworm were multivoltine.

6. For molecular characterization, nuclear DNA, i.e., RAPD, ITS1 as well as mitochondrial DNA, i.e., 16S rRNA, 12S rRNA, CoxI and Cytb genes, and the non-coding CR were selected. These molecular markers were used to study the intraspecific genetic diversity and phylogenetic relationships of Muga and Eri silkworms. Additionally, mitochondrial DNA and ITS1 sequences were used to analyze the phylogenetic relationships of these two silkworms with other silkworm species. In RAPD analysis, a high degree of genetic polymorphism was observed among the morphs of Muga silkworm. The highest divergence was found between the green morph collected from North Lakhimpur (GM06) and wild morph collected from Haflong (WM03). On the other hand, the lowest genetic diversity was observed between the green morph (GM02) and blue morph (BM01) collected from Mangaldoi. The dendrogram generated based on
RAPD banding pattern divided the morphs into two clusters. One consisted of the semi-domesticated morphs and other comprised of the wild morphs.

7. The genetic diversity and phylogenetic relationships among the morphs of Muga silkworm were analyzed by using mitochondrial DNA. All the five mitochondrial loci showed higher ‘A’ and ‘T’ contents. The rate of nucleotide substitution and average genetic divergences was found to be highest in CR sequences and lowest in 12S rRNA gene sequences. It was observed that the morphs collected from same geographical area had identical 12S rRNA, 16S rRNA, CoxI and Cytb gene sequences. Moreover, the 12SrRNA and 16S rRNA gene sequences of some semi-domesticated and wild morphs collected from different geographical locations were found to be similar. In the phylogenetic trees generated based on the mitochondrial loci, mixing of some semi-domesticated and wild morphs was observed. The ITS1 sequences were devoid of AT biasness. The rate of nucleotide substitution and average genetic divergence were found to be more than that of mitochondrial DNA sequences. The phylogenetic tree produced based on ITS1 sequences discriminated the semi-domesticated and wild morphs of Muga silkworm forming two different groups.

8. In case of Eri silkworm, the genetic diversity based on RAPD data was less as compared to Muga silkworm. The minimum genetic distance was observed between white plain (WP01) and blue plain (BP01) morphs collected from Khanapara. The maximum genetic distance was found between blue zebra morph collected from Barduar (BZ01) and white plain morph collected from
Titabor (WP09). The morphs collected from same geographical area shared the same cluster in the dendrogram produced by using RAPD data.

9. The analysis of mitochondrial loci revealed a low genetic divergence among the domesticated morphs of Eri silkworm. The lowest polymorphism was observed in case of 16S rRNA gene sequences. While mitochondrial CR showed highest polymorphism. The mitochondrial DNA sequences of the morphs collected from same geographical area were found to be identical showing no variation. Moreover, some morphs of different geographical origin also shared similar sequences. The ITS1 sequences of the morphs of Eri silkworm collected from the same geographical area were found to be identical. The phylogenetic tree based on ITS1 sequences comprised of 3 major groups.

10. The sequences of the mitochondrial loci and ITS1 were used to study the phylogenetic relationships Muga and Eri silkworms among the other silkworm species. The sequences of other silk producing insects were obtained from GenBank to form a combined dataset. The phylogenetic trees based on the mitochondrial DNA and ITS1 sequences supported the monophyly for the silk producing families Saturniidae and Bombycidae. The trees formed of two major groups. The non-mulberry silkworms of Saturniidae were in one group and the mulberry silkworms of Bombycidae were in another group. A. pernyi, A. proylei and A. roylei shared a single group and occupied the crown of the trees. The Tasar silk producing species A. mylitta formed group with the geographically neighbouring species A. frithi. Muga silkworm made group with other Antheraea species and found to be closely related to A. mylitta, A. frithi and A.
yamamai. However, Eri silkworm clustered with Attacus atlas showing close relationship between them. B. mori and B. mandarina of the Bombycidae grouped together in all the trees.

11. The findings of this confirmed that RAPD, mitochondrial DNA and ITS1 sequences were suitable for resolving genetic diversity and phylogenetic relationships of Muga and Eri silkworms. From this study it was confirmed that the morphs not only differ in their phenotypic traits but also in their genetic makeup. However, the genetic variation was less in case of Eri silkworm though they show phenotypic variations. Among the molecular markers used in this study, RAPD and ITS1 sequences showed higher polymorphism as compared to mitochondrial DNA sequences. The results revealed in this study will help in formulating strategies to conserve the natural biodiversity present among these unique silkworms in Northeast India. In addition, this may be useful in identifying diverse morphs of Muga and Eri silkworms, which will help in effective breeding program. Much work has been done in case of mulberry silkworm but report on molecular characterization of non-mulberry silkworms is very scanty. Without this information effective conservation and breeding programs are not possible. Therefore, it is expected that the molecular characterization of these vast gene pool will generate some data which will help in boosting up the sericulture industry of this region.