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Generic and evolutionary view on domestic and wild silkworms through Cytochrome Oxidase subunit I genes

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Abstract

To study the genetic and evolutionary relationships of domestic and wild species of silkworms (Insecta: Lepidoptera), the mitochondrial DNA sequences of Cytochrome Oxidase subunit - I gene of thirteen wild silk worms and one domesticated silk worm were subjected to MEGA 5.1 and ProtParamExPASy tool. Phylograms were constructed using Maximum Likelihood, Maximum Parsimony and Neighbor-Joining methods. The results clearly revealed the genetic affinity and phylogenetic status of wild and domestic silk worms through phylogenetic analysis. The results were also confirmed with additional molecular parameters such as nucleotide composition, amino acid composition and evolutionary distance analyses.

Keywords: Mitochondria, Cytochrome Oxidase subunit I, Silkworm, Phylograms

1. Introduction

The silk producing insects belonging to Lepidoptera play a vital role in sericulture economy. Approximately, about 4310 silkworm strains are available worldwide (Goldsmith *et al* 2005)^[5]. They are divided into two major groups, i.e. mulberry and non-mulberry. Mulberry silk is produced by the domesticated silkworm, *Bombyx mori* and wild silkworm, *B. mandarina* (Bombycidae) and non-mulberry silks are from wild species belonging to family Saturniidae, i.e., tropical tasar (*Antheraea mylitta*), oak tasar (*A. frithi*, *A. pernyi*, *A. roylei* and *A. proylei*), muga (*A. assama*), eri (*Philosamia ricini*) and fagarina (*Attacus atlas*) and Shashe (*Gonometa postica*) of the family Lasiocampidae (Banno *et al.*, 2004)^[4]. Recently, Mitochondrial DNA sequences have been widely used as molecular markers to study the inter- and intra generic relationships of domesticated silk worms (Prasad *et al.*, 2005; Li *et al.*, 2005)^[12, 9]. In addition to that MEGA 5.1 and ExPASy tools have been used to study the Nucleotide, Amino acid composition and Evolutionary divergence of the species at subfamily level (Ambrose *et al.*, 2014)^[1]. Hence, here an attempt has been made to find out the genetic affinity and evolutionary relationships among domesticated and wild silkworms (Table 1).

2. Material and methods

The Cytochrome oxidase subunit I gene sequences of one domesticated and thirteen wild silkworms were retrieved from NCBI (Table 1). The following phylograms were constructed using MEGA 5. i.e., Maximum Likelihood, Neighbor-Joining and Minimum Evolution tree (Tamura *et al.*, 2011)^[13]. Pairwise distance analysis was carried out with gap opening penalty 15 and gap extension penalty 6.66 (Clustal W) (Thompson *et al.*, 1994)^[14]. The estimation of evolutionary distances in between species were made through the MEGA 5. The AT, GC composition and Amino acid analysis were performed with ProtParamExPASy tool. The entire analyses were performed from December 2014 to May 2015 at NIPHM (National Institute of Plant Health Management).

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Table 1: Mitochondrial DNA, Cytochrome Oxidase I gene sequences of fourteen silkworms subjected to molecular analysis.

Species	Distribution	Genbank accession No
<i>Antheraeaassama</i>	Yunnan, China	KJ146686.1
<i>Antheraeafriithi</i>	Assam, India	KJ676596.1
<i>Antheraeamylitta</i>	Mizoram, India	KC968964.1
<i>Antheraeapernyi</i>	Lioaning, China	EU532613.1
<i>Antheraeapolyphemus</i>	West Bengal India	AY605251.1
<i>Antheraeaproylei</i>	Andhra Pradesh, India	DQ415446.1
<i>Antheraearoylei</i>	Andhra Pradesh, India	DQ415448.1
<i>Antheraeayamamai</i>	Tsukuba, Japan	AB015864.1
<i>Attacus atlas</i>	Mizoram, India	KC968962.1
<i>Bombyxmandarina</i>	Tsukuba, Japan	AB737939.1
<i>Bombyxmori</i>	Tsukuba, Japan	AB649195.1
<i>Gonometapostica</i>	West Bengal India	AY605254.1
<i>Hyalophoracecropia</i>	Stevens point, USA	KM287195.1
<i>Samiaricini</i>	Assam India	KJ577700.1

Table 2: The A, T, G, C Composition of fourteen silkworms.

Species	T(U)	C	A	G	Total
<i>Antheraeaassama</i>	40.2	17.0	29.2	13.6	617.0
<i>Antheraeafriithi</i>	40.4	15.4	29.7	14.4	700.0
<i>Antheraeamylitta</i>	40.7	15.3	29.4	14.7	674.0
<i>Antheraeapernyi</i>	38.9	17.0	29.9	14.1	658.0
<i>Antheraeapolyphemus</i>	41.9	16.1	28.8	13.2	597.0
<i>Antheraeaproylei</i>	40.7	17.1	29.1	13.1	597.0
<i>Antheraearoylei</i>	41.0	16.6	29.1	13.2	597.0
<i>Antheraeayamamai</i>	40.2	15.7	29.7	14.4	714.0
<i>Attacus atlas</i>	37.0	17.4	29.7	15.9	656.0
<i>Bombyxmandarina</i>	37.1	15.1	33.6	14.1	714.0
<i>Bombyxmori</i>	38.7	14.5	33.2	13.6	737.0
<i>Gonometapostica</i>	38.9	15.6	32.2	13.4	597.0
<i>Hyalophoracecropia</i>	38.8	17.3	29.0	14.9	658.0
<i>Samiaricini</i>	40.9	16.5	27.8	14.8	514.0
Avg.	39.6	16.1	30.1	14.1	645.0

Table 3: The Amino acid composition of fourteen silkworms

Amino acids	<i>B. mandarina</i>	<i>B. mori</i>	<i>A. assama</i>	<i>A. mylitta</i>	<i>A. proylei</i>	<i>A. pernyi</i>	<i>A. yanamai</i>	<i>A. polyphemus</i>	<i>A. friithi</i>	<i>A. roylii</i>	<i>H. cecropia</i>	<i>G. postica</i>	<i>Samiaricini</i>	<i>Attacus atlas</i>
Alanine (A)	0	0	0	0	0	0	0	0	0	0	0	0	3.6	3.3
Arginine (R)	5.9	5.7	10.8	0	10.8	10.8	11.1	5.7	0	2.5	10.3	0	3.6	3.3
Asparagine (N)	8.8	5.7	5.4	3.6	8.1	8.1	8.3	14.3	2.1	5.0	3.4	0	7.1	6.7
Aspartic acid (D)	0	2.9	0	0	0	0	0	0	2.1	2.5	0	0	10.7	10.0
Cysteine (C)	5.9	5.7	8.1	0	8.1	8.1	8.3	8.6	0	7.5	3.4	0	0.0	0
Glutamine (Q)	0	0	0	7.1	0	0	0	0	6.4	0	0	8.3	3.6	3.3
Glutamic acid (E)	0	0	0	7.1	0	0	0	0	8.5	0	0	4.2	0.0	0
Glycine (G)	0	0	0	8.9	0	0	0	0	6.4	0	0	4.2	10.7	10.0
Histidine (H)	0	0	0	3.6	0	0	0	0	2.1	0	0	4.2	3.6	3.3
Isoleucine (I)	29.4	25.7	8.1	7.1	5.4	5.4	2.8	8.6	8.5	7.5	6.9	12.5	3.6	6.7
Leucine (L)	2.9	0	5.4	25.0	5.4	5.4	5.6	2.9	25.5	7.5	0	25.0	17.9	16.7
Lysine (K)	5.9	5.7	2.7	14.3	2.7	2.7	2.8	2.9	17.0	5.0	3.4	8.3	0.0	0
Methionine (M)	0	2.9	2.7	3.6	2.7	2.7	0	0	0	0	0	8.3	3.6	3.3
Phenylalanine (F)	8.8	11.4	16.2	10.7	16.2	16.2	22.2	8.6	12.8	20.0	17.2	16.7	10.7	10.0
Proline (P)	0	0	5.4	0	8.1	8.1	5.6	2.9	0	0	0	0	7.1	6.7
Serine (S)	14.7	14.3	16.2	0	21.6	21.6	19.4	14.3	0	15.0	27.6	0	3.6	3.3
Threonine (T)	2.9	5.7	5.4	0	0	0	2.8	5.7	0	10.0	10.3	0	7.1	10.0
Tryptophan (W)	2.9	2.9	2.7	0	2.7	2.7	2.8	5.7	0	5.0	0	0	0.0	0
Tyrosine (Y)	11.8	11.4	10.8	7.1	8.1	8.1	8.3	20.0	6.4	12.5	17.2	4.2	3.6	3.3
Valine (V)	0	0	0	1.8	0	0	0	0	2.1	0	0	4.2	0	0
Pyrrrolysine (O)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Selenocysteine (U)	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4: The Estimates of Evolutionary Divergence among fourteen species of silkworms

Species 1	Species 2	Distance
<i>Antheraeaassama</i>	<i>Antheraeafriithi</i>	0.09
<i>Antheraeaassama</i>	<i>Antheraeamylitta</i>	0.09
<i>Antheraeafriithi</i>	<i>Antheraeamylitta</i>	0.00
<i>Antheraeaassama</i>	<i>Antheraeapernyi</i>	0.11
<i>Antheraeafriithi</i>	<i>Antheraeapernyi</i>	0.09
<i>Antheraeamylitta</i>	<i>Antheraeapernyi</i>	0.09
<i>Antheraeaassama</i>	<i>Antheraeapolyphemus</i>	0.12
<i>Antheraeafriithi</i>	<i>Antheraeapolyphemus</i>	0.11
<i>Antheraeamylitta</i>	<i>Antheraeapolyphemus</i>	0.11
<i>Antheraeapernyi</i>	<i>Antheraeapolyphemus</i>	0.11
<i>Antheraeaassama</i>	<i>Antheraeaproylei</i>	0.11
<i>Antheraeafriithi</i>	<i>Antheraeaproylei</i>	0.09
<i>Antheraeamylitta</i>	<i>Antheraeaproylei</i>	0.09

<i>Antheraeapernyi</i>	<i>Antheraeaproylei</i>	0.00
<i>Antheraeapolyphemus</i>	<i>Antheraeaproylei</i>	0.11
<i>Antheraeaassama</i>	<i>Antheraearoylei</i>	0.12
<i>Antheraeafriithi</i>	<i>Antheraearoylei</i>	0.09
<i>Antheraeamylitta</i>	<i>Antheraearoylei</i>	0.09
<i>Antheraeapernyi</i>	<i>Antheraearoylei</i>	0.02
<i>Antheraeapolyphemus</i>	<i>Antheraearoylei</i>	0.12
<i>Antheraeaproylei</i>	<i>Antheraearoylei</i>	0.02
<i>Antheraeaassama</i>	<i>Antheraeayamamai</i>	0.09
<i>Antheraeafriithi</i>	<i>Antheraeayamamai</i>	0.10
<i>Antheraeamylitta</i>	<i>Antheraeayamamai</i>	0.10
<i>Antheraeapernyi</i>	<i>Antheraeayamamai</i>	0.08
<i>Antheraeapolyphemus</i>	<i>Antheraeayamamai</i>	0.12
<i>Antheraeaproylei</i>	<i>Antheraeayamamai</i>	0.08
<i>Antheraearoylei</i>	<i>Antheraeayamamai</i>	0.08
<i>Antheraeaassama</i>	<i>Attacus atlas</i>	0.18
<i>Antheraeafriithi</i>	<i>Attacus atlas</i>	0.19
<i>Antheraeamylitta</i>	<i>Attacus atlas</i>	0.19
<i>Antheraeapernyi</i>	<i>Attacus atlas</i>	0.18
<i>Antheraeapolyphemus</i>	<i>Attacus atlas</i>	0.19
<i>Antheraeaproylei</i>	<i>Attacus atlas</i>	0.19
<i>Antheraearoylei</i>	<i>Attacus atlas</i>	0.19
<i>Antheraeayamamai</i>	<i>Attacus atlas</i>	0.18
<i>Antheraeaassama</i>	<i>Bombyxmandarina</i>	0.19
<i>Antheraeafriithi</i>	<i>Bombyxmandarina</i>	0.18
<i>Antheraeamylitta</i>	<i>Bombyxmandarina</i>	0.18
<i>Antheraeapernyi</i>	<i>Bombyxmandarina</i>	0.16
<i>Antheraeapolyphemus</i>	<i>Bombyxmandarina</i>	0.21
<i>Antheraeaproylei</i>	<i>Bombyxmandarina</i>	0.16
<i>Antheraearoylei</i>	<i>Bombyxmandarina</i>	0.16
<i>Antheraeayamamai</i>	<i>Bombyxmandarina</i>	0.16
<i>Attacus atlas</i>	<i>Bombyxmandarina</i>	0.21
<i>Antheraeaassama</i>	<i>Bombyxmori</i>	0.20
<i>Antheraeafriithi</i>	<i>Bombyxmori</i>	0.18
<i>Antheraeamylitta</i>	<i>Bombyxmori</i>	0.18
<i>Antheraeapernyi</i>	<i>Bombyxmori</i>	0.16
<i>Antheraeapolyphemus</i>	<i>Bombyxmori</i>	0.20
<i>Antheraeaproylei</i>	<i>Bombyxmori</i>	0.16
<i>Antheraearoylei</i>	<i>Bombyxmori</i>	0.16
<i>Antheraeayamamai</i>	<i>Bombyxmori</i>	0.17
<i>Attacus atlas</i>	<i>Bombyxmori</i>	0.20
<i>Bombyxmandarina</i>	<i>Bombyxmori</i>	0.04
<i>Antheraeaassama</i>	<i>Gonometapostica</i>	0.18
<i>Antheraeafriithi</i>	<i>Gonometapostica</i>	0.18
<i>Antheraeamylitta</i>	<i>Gonometapostica</i>	0.17
<i>Antheraeapernyi</i>	<i>Gonometapostica</i>	0.18
<i>Antheraeapolyphemus</i>	<i>Gonometapostica</i>	0.18
<i>Antheraeaproylei</i>	<i>Gonometapostica</i>	0.18
<i>Antheraearoylei</i>	<i>Gonometapostica</i>	0.18
<i>Antheraeayamamai</i>	<i>Gonometapostica</i>	0.17
<i>Attacus atlas</i>	<i>Gonometapostica</i>	0.24
<i>Bombyxmandarina</i>	<i>Gonometapostica</i>	0.18
<i>Bombyxmori</i>	<i>Gonometapostica</i>	0.17
<i>Antheraeaassama</i>	<i>Hyalophoracecropia</i>	0.17
<i>Antheraeafriithi</i>	<i>Hyalophoracecropia</i>	0.17
<i>Antheraeamylitta</i>	<i>Hyalophoracecropia</i>	0.16
<i>Antheraeapernyi</i>	<i>Hyalophoracecropia</i>	0.17
<i>Antheraeapolyphemus</i>	<i>Hyalophoracecropia</i>	0.19
<i>Antheraeaproylei</i>	<i>Hyalophoracecropia</i>	0.17
<i>Antheraearoylei</i>	<i>Hyalophoracecropia</i>	0.18
<i>Antheraeayamamai</i>	<i>Hyalophoracecropia</i>	0.17
<i>Attacus atlas</i>	<i>Hyalophoracecropia</i>	0.17
<i>Bombyxmandarina</i>	<i>Hyalophoracecropia</i>	0.20
<i>Bombyxmori</i>	<i>Hyalophoracecropia</i>	0.21
<i>Gonometapostica</i>	<i>Hyalophoracecropia</i>	0.21
<i>Antheraeaassama</i>	<i>Samiaricini</i>	0.15
<i>Antheraeafriithi</i>	<i>Samiaricini</i>	0.14
<i>Antheraeamylitta</i>	<i>Samiaricini</i>	0.14
<i>Antheraeapernyi</i>	<i>Samiaricini</i>	0.16
<i>Antheraeapolyphemus</i>	<i>Samiaricini</i>	0.16

<i>Antheraeaaproylei</i>	<i>Samiaricini</i>	0.16
<i>Antheraearoylei</i>	<i>Samiaricini</i>	0.16
<i>Antheraeayamamai</i>	<i>Samiaricini</i>	0.17
<i>Attacus atlas</i>	<i>Samiaricini</i>	0.16
<i>Bombyxmandarina</i>	<i>Samiaricini</i>	0.20
<i>Bombyxmori</i>	<i>Samiaricini</i>	0.20
<i>Gonometapostica</i>	<i>Samiaricini</i>	0.22
<i>Hyalophoracecropia</i>	<i>Samiaricini</i>	0.08

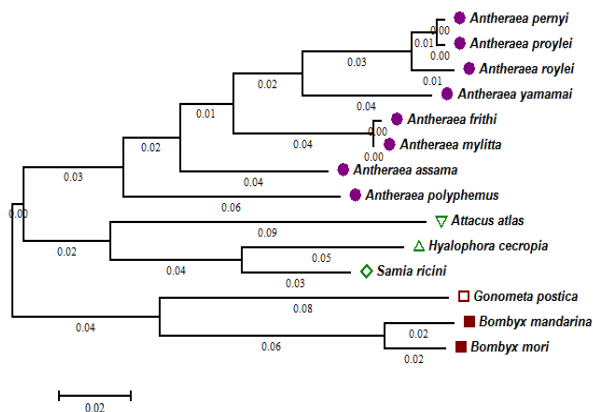


Fig 1: Maximum Likelihood tree based on Mitochondrial COI gene showing the relationships of fourteen silkworms

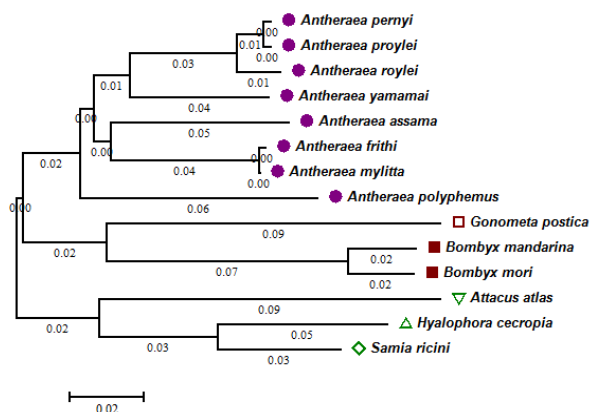


Fig 2: Neighbor joining tree based on Mitochondrial COI gene showing the relationships of fourteen silkworms

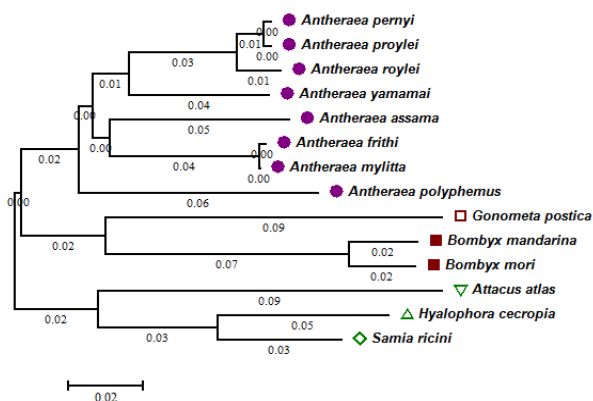


Fig 3. Minimum evolution tree based on Mitochondrial COI gene showing the relationships of fourteen silkworms

3. Results and Discussion

In the present study, the Mitochondrial COI genes have been selected and analysed to find out the variations and generic

similarity among wild and domesticated silk worms. The phylogenetic methods of Maximum Likelihood, Neighbour Joining and Minimum Evolution were employed to construct phylograms. In the earlier study, mitochondrial genes have been used to study the generic variations among wild and domesticated silk worms (Hwang *et al.*, 1999b; Yukuhiro, *et al* 2002; Mahendran *et al* 2002) [6, 15, 10] through phylogenetic analysis but here the results obtained from dendrogram analysis including three phylograms were also compared with Amino acid analysis, AT, GC composition and Evolutionary analyses. The three topologies i.e., Maximum Likelihood, Neighbour Joining and Minimum Evolution showed the monophyly uniformly among Bombycidae and Saturniidae as analyzed by Mahendran *et al* (2002) [10]. *B. mori* and *B. mandarina* are morphologically similar species but differ in biology and ecological behavior. *B. mori* and *B. mandarina* revealed their generic relationships by morphological, cytological (Jolly, 1985) [8], chromosomal studies (Astaurov *et al.*1959) [3], molecular analysis (Hwang *et al.*, 1999 a,b; Mahendran *et al* 2002; Prasad *et al.*, 2005;Arunkumar *et al.*, 2006) [7, 6, 10, 12, 2]. Yukuhiro *et al.*, (2002 [15] had suggested that the *B. mandarina* is believed to be the ancestor of *B.mori*. The findings from this analysis supported the generic affinity between *B.mori* and *B. mandarina* not only from the phylogenetic analysis but also by the Amino acid analyses in which the higher content of Isoleucine and the absence of Proline were observed in both the *Bombyx* species (Table 3) and the A, T, G and C composition and evolutionary analyses are further supporting the generic affinity between two *Bombyx* species (Table 4). Moreover, the *Bombyx* species shared a common node with *Gonomota postica* which reflects their inter-generic affinity. Monophyly was observed in all the *Antheraea* species which reflects their intra generic affinity. Moreover, species closeness was observed between geographically isolated Chinese (*A. pernyi*) and Indian species (*A. proylei*).

The intra generic affinity of the recently derived hybrid species, *A. proylei* originated from *A. pernyi* and *A. roylei* was revealed not only by phylogeny but also through amino acid analysis, ATCG analysis and Evolutionary estimation analysis (Table 2-4). The uniformity in the AT content of all the species and the absence of aminoacids such as Alanine, Pyrrolysine and Selenocystein in all the *Anthraea* species have been observed (Table 1-4). These results support the molecular phylogeny analyses carried out by Mahendran *et al* (2002) [10] and (2006) [11].

4. Conclusion

This study was intended to provide the geographic and generic context for understanding the relationships among wild and domestic silkworms. The results further revealed the utility of Cytochrome Oxidase subunit I DNA sequences in phylogenetic analysis along with Amino Acid analysis, A, T, G, C content, and Evolutionary analyses. Further studies in this direction could lead to meaningful generic analysis of silkworms.

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