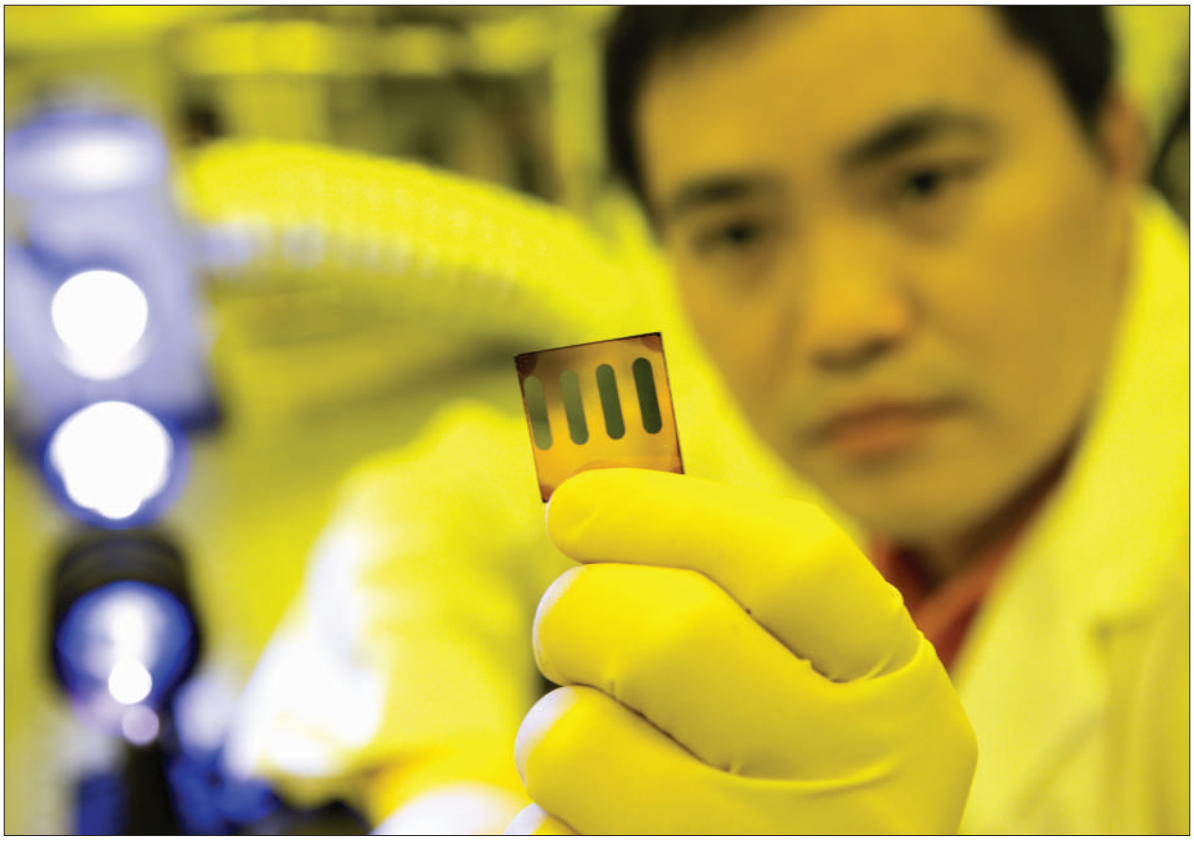

Singapore Journal of

Scientific Research

ISSN: 2010-006x

science alert

http://scialert.net/sjsr

OPEN ACCESS Singapore Journal of Scientific Research

ISSN 2010-006x
DOI: 10.3923/sjsres.2020.28.34

Research Article
Explorative Survey of Wild Mulberry Silkworm Genetic Resources

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Abstract

Background and Objective: India harbours a wide range of seri-biodiversity and a rich source of many sericigenous insect species in North-Eastern India and Andaman and Nicobar Islands and is enlisted as hotspots of biodiversity of invaluable economically important insect species. The diversity of wild sericigenous insects in these regions has not yet been fully understood. Therefore the main objective of the study was the explorative survey to collect the wild mulberry silkworm for further characterization, conservation and sustainable utilization of wild genetic resources. **Materials and Methods:** The wild *Bombyx* relative was collected through explorative survey at Ziro valley, Arunachal Pradesh, India. The larvae and cocoons of wild mulberry silkworm was collected from mulberry trees, moths were collected through light trap method. The silkworm samples were carried for further characterization and evaluation conducted at Central Sericultural Germplasm Resources Centre (CSGRC), Hosur. **Results:** The Silkworm specimens such as egg, larva, cocoon and adult (moth) was characterized as per the descriptor and identified that the silkworm belongs to wild *Bombyx* relative and it was *Bombyx huttoni* W. **Conclusion:** The study throw a light on the availability of wild silkworm (Bombycid relatives) in the areas surveyed, emphasizing the need to collect adequate samples so as to standardize the protocols for their conservation. The report opens up untapped treasure of wild insect genetic resources in this region of North-Eastern India to interested entomologists and ecologists.

Key words: Explorative survey, *ex situ* conservation, germplasm maintenance, wild mulberry silkworm, genetic resources, biodiversity, sustainable

Citation: G. Thanavendan, G. Lokesh and Geetha N. Murthy, 2020. Explorative survey of wild mulberry silkworm genetic resources. Singapore J. Sci. Res., 10: 28-34.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sericigenous fauna represent wider genetic variability and produce different kinds of silk of great economic value. The various anthropogenic interference and widespread climatic changes are leading the wild relatives of mulberry silkworm genetic resources to the verge of extinction in their natural niches. In this scenario of great concern, preventing deterioration of these invaluable genetic resources is highly pertinent which would be possible only through development of meticulous strategies for their *ex-situ* conservation. Conservation of such precious resource would aid in exploring of their commercially/economically desirable properties for silk productivity improvement and sustainable utilization. Recent survey provides adequate evidence for availability of wild bombycid species in India one among the 34 hotspots biodiversity rich centres of the world. Insects that produce silk of economic value are termed as Sericigenous insects. The natural silk producing insects are broadly classified as mulberry and wild or non-mulberry. At the present time many important genetic resources of silk moths are facing threats due to indiscriminate destruction of forests[1]. Therefore, considering the importance of sericigenous insects, national level surveys were carried out to explore and collect the sericigenous insects. The information obtained on the wild *Bombyx* relatives thus collected after proper quarantine and standardising protocols for conservation would help in development of breeds for different biotic and abiotic stress conditions.

The North-East Indian regions have unique distinction of being the homeland of four varieties of silk viz., mulberry, eri, muga and tasar along with a good number of wild sericigenous insects[2]. Although India is a mega diverse country with phytogeographical regions of immense biodiversity, the sericigenous insect wealth of the country is insufficiently explored. Among the wild bombycids a single genus *Ocinara* sp. has been reported from South Andaman and the bombycid moth species was collected through light trap from Mount Harriet of South Andaman[3-5]. Many reports on explorative surveys in Northern Western Ghats, Maharashtra recorded and identified 418 moth taxa (303 species and 116 genus level) from 28 moth families belonging to 15 super families of Bombycoidea of these, 3 Bombycoidae and 4 Saturniidae species viz., *Ocinara* sp., *Trilocha* c.f. *varians*, *Trilocha* c.f. *friedeli* were distributed in Sanjay Gandhi National Park (SGNP)[6,7], *Ocinara* sp., *Gunda javanica* Moore, *Trilocha varians* Walker in Northern Maharashtra[8]. The family Bombycidae belongs to the Lepidopteran superfamily, Bombycoidea and contains about 40 genera and 350 species[9]. The best known species is the silkworm, *Bombyx mori* (Linnaeus) which has been domesticated several years ago. Another well-known species is the wild silkworm, *B. mandarina*, which is also native to Asia. Several other Bombycid species are economically important pests in the agriculture, forestry, sericulture and horticulture industries. Bombycid moths are widely distributed around the world, of which 20% are recorded from China, which includes two of the 34 biodiversity hotspots in the world[10]. According to the Zoological Survey of India, 1500-2000 species of insects have been discovered on Andaman Nicobar Islands. These islands harbour a wide range of insect fauna which includes (Lepidoptera 45%) Bombycidae recorded 6 species of wild silk moth[11]. CSGRC Hosur during exploration survey in Andaman Islands reported the availability of different life stages of *Bombycidae* insect species *Ocinara* spp. on *Artocarpus* and *Ficus* spp. which are the main host plants for wild bombycids[12]. In the present paper, the explorative survey conducted by CSGRC at Ziro valley, Arunachal Pradesh for collection of wild bombycidae sericigenous insect resources for characterization, *ex situ* conservation at Sericultural Germplasm Centre, Hosur and for further utilization.

MATERIALS AND METHODS

Study area: The present work was undertaken as a part of mandate of Central Sericultural Germplasm Resources Centre (CSGRC), Central Silk Board, Hosur, Tamil Nadu. The survey was conducted at Ziro valley region, Arunachal Pradesh, India during July and August, 2016. The silkworm specimens collected were examined and data recording was made at CSGRC, Hosur.

Data collection: The North East regions of India are considered the floral and faunal gateway for main Asian land to Indian peninsula and have dense spectrum of species making it a rich reservoir of natural resources. In order to take up the survey, information and permission were sought from various authorities depending on the area surveyed as shown in Fig. 1. In present study, an explorative survey was conducted in Ziro valley region, Arunachal Pradesh of N-E India (Fig. 2).

Research procedure: Stratified random sampling framework was employed by taking random samples from a gradient/stratum ranging from lowest to highest altitude zones to record patterns of moth community assemblages in

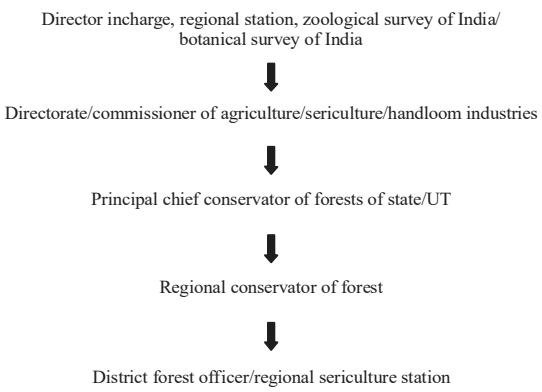

Fig. 1: Flow chart of survey
Source: Thangavelu and Bhagowati[2] Wild silkmoth

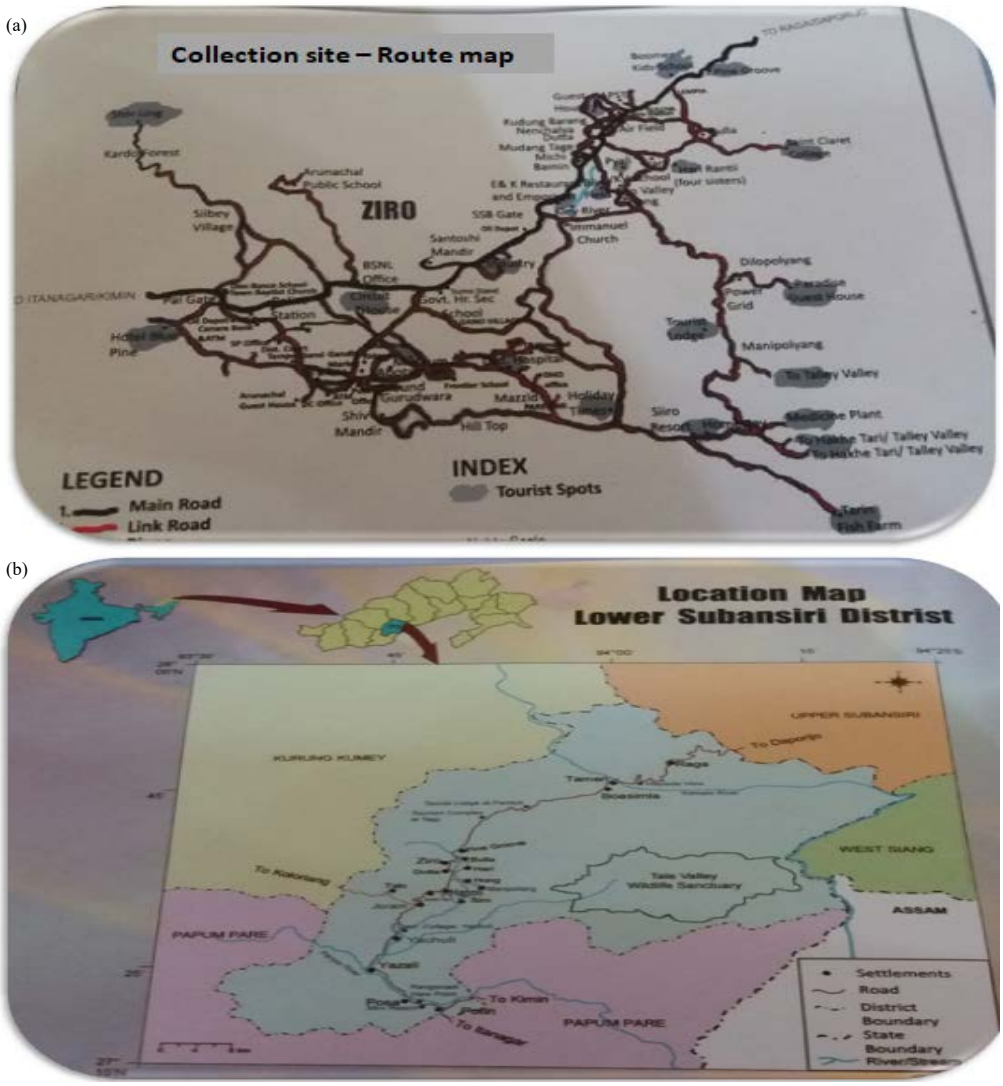

Fig. 2(a-b): Survey of wild silkworm and pupae collection at forest (Talley valley) area of Hakhe tari, (a) Ziro valley Tehsil map and (b) Lower Subansiri District map
Source: Google political district maps

both dominant and characteristic vegetation zones (Mulberry, Jack, *Artocarpus* and *Ficus* spp.) through trekking inside forest areas and battery operated light (Mercury lamp) trap, field survey and manual collections. The collected samples of the moths were identified through available literature, identification through taxonomic keys[13], standard reference books and taxonomist. The adults of Lepidoptera were collected during night with the help of light traps (200 watt mercury vapour light) with some collections made by hanging a makeshift source of light (200 watt mercury vapour light) on a white sheet/white washed wall. The survey team camped in resorts with close proximity to forests; the light traps were operated overnight from 18:00-05:00 h. The light trap comprised a 4×3 m white cloth stretched between two posts or trees, in front of which was hung a mercury vapour light bulb (Philips, B/73, ML160 W)[14]. The collected insects were processed using tetra benzene[15,16].

RESULTS AND DISCUSSION

During the survey at Ziro valley, a holometabolous insect, bombycid moth was found on the wild mulberry tree *Morus serrata* and *M. indica*. Different life stages of the insect viz., egg, larva, pupa and adult were observed (Fig. 3a-g) and collected carefully with the earlier scientific experience[17-19]. The morphological characters of egg, larva, cocoon and adult were observed and recorded the data presented in Table 1.

Egg: The colour of the egg is a pale straw-yellow and is of round shape, considerably larger than those of *B. mori*, but unlike the eggs of the Chinese races, the shape is retained till hatching. The eggs are firmly attached to the bark/trunk of the tree, more generally on the underside of the branches, where they remain spread out in clusters (150-300 nos.). They are exposed to the action of the frost all through the winter, but are at the same time protected from the rain and snow so as to run no risk of being washed off by the dissolving of their agglutinating gum.

Larvae: The larva moves through 5 stages of development viz. 1st, 2nd, 3rd, 4th and 5th instars. In this study, the 5th instars mature larvae were taken for morphology study. The head capsule was observed as brown, hard and roundish in all the populations. The body colours ranged from dark brown to pink with white spot and brown with black marking irrespective of population. The second and third segments in the larvae were swollen into a large globose mass; the anterior segment was creamy-yellow to yellow blending like tortoise-shell extending backwards on either side as a broad band through the 6th segment. The only method of rearing the larvae was to leave it at full liberty on the trees, where it remained perfectly quiet and contented. However, it has to contend with enemies in the form of birds, flies, bugs and wasps etc. to render a crop of silk very precarious and almost unattainable, without constant watching and expense.

Cocoon: The cocoon is spun on the leaf from the beginning of June-July to the end of the monsoon season. At the time of emergence, male moth emerged first, while, female emerged 3 days later. The cocoons were preserved at 25±1°C with relative humidity of 75±5%. The parameters viz., average single cocoon weights for male and female is 1.078 and 0.102 g, shell weights 2.320 g for male and 0.191 g for female and shell ratio is 9.461 and 8.232 of male and female, respectively.

Table 1: Description of collection of wild mulberry silk moth at Ziro valley

Particulars	Description of location
Name of collectors	Thanavendan and co-workers
Month of collection of original sample	July, 2016
Country/Province/State of collection	Arunachal Pradesh
Location of collection site/District	Ziro valley, Lower Subansiri District
Latitude of collection site	26.28° North
Longitude of collection site	97.30° East
Altitude of collection site from MASL	1564 m
Collection source	Wild and mulberry farm nearer to forest area through manual collections
Local/Vernacular name	Nil
Scientific name	*Bombyx huttoni* W.
Specimens sampled	15 larvae and 10 cocoons
Photograph	Enclosed
Museum specimen	Nil
Disease status	NA
End user	Research scholar, Insect breeding scientist, private stakeholder and cosmetic or pharmacological industry

MASL: Mean above sea level, NA: Not applicable

Fig. 3(a-g): Different bio stages of wild silkworm collected during exploratory survey in Ziro valley (Talley valley) of Himalayan regions, (a) Wild silk moth eggs laid on bark and stem of wild mulberry, (b) Wild silkworm cocoons collected from wild mulberry tree, (c) On-site observation of wild silkworm on the wild mulberry tree, (d) Observation of wild silk cocoons and collection from wild mulberry tree, (e) Observation of male and female pupae and cocoon of wild silkworm, (f) Male moth of *Bombyx huttoni* westwood and (g) Female moth of *Bombyx huttoni* westwood

Adults (moth): The Adults are pale brown in colour with distinct wing venation, male moths smaller in size compared to females. In the female moths, the wings (4-6 cm) had a distinctly dark crescent colour and acute apex, the outer margin was serrate in nature. In case of male moths, the wing size was 3-4 cm. In the thoracic region the male and female moths have dark band in rectangular shape. The abdomen shape is cylindrical in female moths and sharp in males. Based on the observations, the insect species was identified as *Bombyx huttani* is one of the insect belongs to the family Bombycidae. During the past two decades, much scientific progress has been made in the classification and phylogenetic analysis of the family Bombycidae, with great advances being made based on analysis of morphological characters[20] and molecular data[21-23]. The members of the Oriental Bombycidae to comprise two lineages based on characters of wing venation and male genitalia descriptions[24]. The observations made on phenotypic characters of various life stages of various wild bombycid populations.

Earlier, with an explorative survey in Andaman Islands, found different life stages of Bombycidae insect species *Ocinara* spp. on *Artocarpus* and *Ficus* spp.[12]. But they could not able to collect theses materials. Some eggs and cocoons of a wild silkworm belonging to Bombycidae were collected from wild mulberry tree *Morus serrata* and *M. indica* near Rudraprayag District of Uttarakhand at an altitude of 1600 m.a.s.l. The eggs were incubated and rearing was conducted on mulberry plants with nylon net covering at CSGRC, Hosur. It is a potential and interesting genetic material with several unique characters and utilising such wild relatives of *Bombyx* is quite possible to create additional Seri biodiversity and widen the genetic base.

CONCLUSION

Based on the earlier experiences of exploratory surveys conducted at Uttarakhand and Andaman Islands, the present survey was planned to collect wild silkmoth genetic resources at Ziro Valley, Arunachal Pradesh. One of the important wild silkworm and less known *Bombyx* spp. *Bombyx huttani* was collected from mulberry bushes and other Moraceae plants. The specimens collected were segregated; quarantined and preserved at CSGRC Hosur. However, the generations could not be perpetuated due to want of sufficient number of cocoons and non-synchronization of the moth emergence at CSGRC, Hosur. The study throw a light on the availability of wild silkworm (*Bombycid* relatives) in the areas surveyed emphasizing the need to conduct more number of surveys for collection of adequate samples so as to standardize the protocols for their conservation at local, regional and national levels.

SIGNIFICANCE STATEMENT

This study enumerates there is earnest efforts are needed from Government agencies as well as from public for protection of these precious sericigenous genetic resources from extinction and for sustainable utilization in posterity. Also the report open up possibilities of unravelling untapped treasure of wild insect genetic resources in North-Eastern region of India to interested entomologists and ecologists.

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