Dedicated
to
All my Teachers

DECLARATION

I declare that the thesis entitled **"Genetic Characterization of Nucleopolyhedrovirus Isolated from *Hyposidra talaca* Walker (Lepidoptera: Geometridae), a Tea Pest in Terai Region of Darjeeling Foothills, India."** has been prepared by me under the guidance of Professor Min Bahadur, Department of Zoology, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

We certify that Mr. Bappaditya Ghosh has prepared the thesis entitled **"Genetic Characterization of Nucleopolyhedrovirus Isolated from *Hyposidra talaca* Walker (Lepidoptera: Geometridae), a Tea Pest in Terai Region of Darjeeling Foothills, India."** for the award of Ph.D. degree of the University of North Bengal, under our guidance. He has carried out the work at the Department of Zoology, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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5 Chapter 1: Introduction, Review of Literature, Materials and Methods
6 Section 2: Introduction Tea cultivation in India Tea, Camellia sinensis (L.) O Kuntze, is the most common and widely consumed refreshing drink throughout the world. It is a resolutely and skillfully managed monoculture crop cultivated on large as well as small-scale between latitudes 41°N and 16°S with an annual precipitation of 1000-5000 mm and temperature of 8-35.0 C (Hazarika et al., 2008). As per 64th annual report (2017-18) of "Tea Board India", India is the World's second largest producer of tea sharing 23% (1325.05 Million Kg) of global tea production, while China is the first producing 45%. However, India exported 14% of world tea which comes after Kenya (23%), China (20%) and Srilanka (16%). India is also one of the largest tea consumers in the world accounting 19% of global tea consumption. Almost 76% of produced tea in India is consumed within the Country itself. During 2017-18, 256.57 Million Kg of tea was exported from India with Cost, Insurance, and Freight (CIF) value of Rs. 5064.88 Crs, while 20.59 Million Kg with CIF value of Rs. 288.56 Crs was imported into India (Anonymous, 2018). Tea is the chief foliar crop in northern part of West Bengal, Assam, Sikkim, Tripura, Nilgiri of Tamil Nadu and also grown in small scale in Himachal Pradesh, Kerala, Karnataka and Orissa (Fig 1). The Assam and Darjeeling tea are very popular in India and exclusively cultivated in large number of tea plantations in the Terai-Dooars region of West Bengal and Assam. In India tea is cultivated over an area of 6,36,557.07 hectare, of which 3,37,690.35 hectare (53%) is situated in Assam and 1,48,521.74 hectare (23%) in West Bengal including both big and small growers. The economy of both of these states largely depends on the production of tea. In the northern part of West Bengal, concerning the Himalayan foothills and plains, the Terai plantation is on the Western flank of the mighty Teesta river and the Dooars plantation stretches on the eastern terrain of the river continuing upto the state of Assam. Looper pests in the Tea plantations of Terai-Dooars region of India Tea plantations of the Terai and Dooars region of West Bengal and Assam are experiencing severe attacks of different leaf eating lepidopteran pests, among which, Biston (Buzura)
7 suppressaria Guen (Lepidoptera: Geometridae) was reported as a major tea pest for several decades from Assam and Tera-Dooars region of North Bengal (Das, 1965). Recent studies reveal that a few other species of geometrid pests are attacking tea plantations among which the pest species, Hypoxydra talaca Walker (Lepidoptera: Geometridae), early caterpillar of which are commonly known as 'Black Inch Looper' (Fig 2), has taken over as the major defoliating pest in tea plantations (Das et al., 2010a). Loopers of this species that primarily feed on a number of forest plants and weeds in India, Malayasia and Thiland (Das and Mukhopadhyay, 2009; Mathew et al., 2005; Winotai et al., 2005) have turned to tea as an active defoliating insect pest in plantations of Assam and Darjeeling Terai-Dooars of North Bengal (Basu Majumdar, 2004; Das et al., 2010). A substantial loss in tea production due to this defoliating lepidopteran pest has been reported from these regions (Guruubramanian et al., 2008; Hazarika et al., 2008), that severely has affected the economy of the country (Roy and Muraleedharan, 2014). Management of Tea pest population To manage pest infestations, there are two main methods of pest control: chemical and biological. The looper pests have so far been controlled by regular application of synthetic insecticides especially (Fig 3), organophosphates and pyrethroids, but, the pests are gradually becoming less susceptible, and have developed some extent of resistance to the pesticides (Das et al., 2010b; Das and Mukhopadhyay, 2008) often resulting in their control failures (Sannigrahi and Talukdar, 2003). Moreover, the application of these synthetic chemical insecticides is one of the major source of pollution of soil and water (Saravanan et al., 2009; Singh et al., 2017) and reported to be hazardous to the non-target organisms including human (Azmi et al., 2006; Mobed et al., 1992; Saravanan et al., 2009; Velmurugan et al., 2006). Because of these harmful effects, application of a number of these insecticides has been banned by the EPA of the USA and even by the Tea Research Organizations of India. The registration of many other insecticides have been reviewed (Chattopadhyay et al., 2008) and recommended under the Plant Protection Codes (PPC). Therefore, concerning the fact of development of pesticide resistance in insect pests and hazardous effects of the chemical pesticides, the organic tea has become more acceptable especially for export and health conscious tea consumers than the chemically managed conventional tea. In view of this, the

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Preface

The application of synthetic chemical pesticides to control different agricultural pests is a global concern because of their adverse effects on the environment and non-target organisms including humans. Moreover, due to the irrational use of chemical pesticides, insect pests may gradually develop resistance to synthetic chemical pesticides leading to control failure. Hence, the development of a pest management strategy that is non-polluting, eco-friendly, target-specific, and sustainable will be highly acceptable. In this regard, microorganisms as biopesticides/ bioinsecticides may be an alternative to synthetic chemical pesticides.

Among several microorganisms, baculoviruses, particularly nucleopolyhedroviruses (NPVs) are well known as bioinsecticides in the integrated pest management (IPM) strategies because of their host specificity and proved to be safe to the environment, humans, other plants, and natural enemies of pests. However, as the mode of action of NPVs is slower than chemical pesticides, genetic modifications to enhance the killing efficiency of NPVs are highly required for better pest control and management. Therefore, the studies to explore the genome of pest-specific NPVs will help design an effective biopesticide.

The present study has been contemplated to **characterize the genome of NPVs isolated from *Hyposidra talaca*, a major lepidopteran tea pest in the Terai-Dooars region of the northern part of West Bengal**. As the tea plantations of the Terai-Dooars regions are facing immense defoliation by the *Hyposidra talaca* larvae resulting in huge economic loss, several synthetic chemical pesticides are regularly applied in the tea plantations of the Terai-Dooars regions to manage the pest problem. Therefore, the development of ***Hyposidra talaca* nucleopolyhedrovirus** (HytaNPV) into a potential biopesticide will be beneficial to minimize the use of chemical pesticides. In this context, the characterization of the genome and evolutionary study of HytaNPV will be very helpful in designing a virus-based pesticide.

This thesis has been divided into several chapters. A brief **introduction**, **review of literature**, **objectives,** and **materials and methods** were provided in **Chapter 1**. **Chapter 2** comprises the **results** of the survey and sampling, restriction endonuclease fragment analyses and partial restriction map of HytaNPV genome, characterization of selected genes and phylogenetic analyses and **Chapter 3** contains the detailed **discussion** of the findings of the present study, **conclusion and summary**. **Chapter 4** comprises the list of research articles, and the papers presented at different conferences under the **Appendix**, along with the **Bibliography** and **Index**.

Abbreviations

$\%id_N$	:	percentage of identical sites for nucleotide
$\%id_A$	:	percentage of identical sites for amino acid
acc. no.	:	accession number (NCBI)
°N	:	degree North
°S	:	degree South
µg	:	microgram
µl	:	micro liter
^0C	:	degree centigrade
AcNPV	:	*Autographa californica* NPV
AdhoNPV	:	*Adoxophyes honmai* NPV
AdorGV	:	*Adoxophyes orana* GV
AdorNPV	:	*Adoxophyes orana* NPV
AgipNPV	:	*Agrotis ipsilon* NPV
AgseGV	:	*Agrotis segetum* GV
AgseNPV	:	*Agrotis segetum* NPV
alk-exo	:	*alkaline exonuclease*
AM	:	anti meridian
AngeNPV	:	*Anticarsia gemmatalis* NPV
AnpeNPV	:	*Antheraea pernyi* NPV
ApciNPV	:	*Apocheima cinerarium* NPV
BLAST	:	Basic local alignment search tool
BLASTN	:	nucleotide BLAST
BLASTP	:	protein BLAST
BmNPV	:	*Bombyx mori* NPV
BomaNPV	:	*Bombyx mandarina* NPV
bp	:	base pair
BSA	:	bovine serum albumin
Bt	:	*Bacillus thuringiensis*
BusuNPV	:	*Buzura suppressaria* NPV
BVs	:	budded virus
CapoNPV	:	*Catopsilia pomona* NPV
cds	:	coding sequence

CfGV	:	*Choristoneura fumiferana* GV
CfNPV	:	*Choristoneura fumiferana* NPV
ChchNPV	:	*Chrysodeixis chalcites* NPV
ChmuNPV	:	*Choristoneura murinana* NPV
ChocNPV	:	*Choristoneura occidentalis* NPV
ChroNPV	:	*Choristoneura rosaceana* NPV
CIF	:	Cost, Insurance, and Freight
ClanGV-H	:	*Clostera anachoreta* GV
ClanGV-HB	:	*Clostera anastomosis* GV
ClbiNPV	:	*Clanis bilineata* NPV
c_N	:	number of conserved sites for nucleotides
CnmeGV	:	*Cnaphalocrocis medinalis* GV
CoveNPV	:	*Condylorrhiza vestigialis* NPV
CrluGV	:	*Cryptophlebia leucotreta* GV
Crs	:	Crores
CuniNPV	:	*Culex nigripalpus* NPV
CypoGV	:	*Cydia pomonella* GV
DapuNPV	:	*Dasychira pudibunda* NPV
DekiNPV	:	*Dendrolimus kikuchii Matsumura* NPV
DisaGV	:	*Diatraea saccharalis* GV
d_N	:	number of variable sites for nucleotides
d_A	:	number of variable sites for amino acids
DNA	:	deoxyribonucleic acid
dNTPs	:	deoxyribonucleotide tri-phosphates
EcobNPV	:	*Ectropis Obliqua* NPV
EDTA	:	Ethylenediaminetetraacetic acid
egt	:	ecdysteroid glucosyltransferase
EPA	:	Environmental Protection Agency
EpapGV	:	*Epinotia aporema* GV
EppoNPV	:	*Epiphyas postvittana* NPV
ErelGV	:	*Erinnyis ello* GV
et al	:	and others
EuprNPV	:	*Euproctis (=Arna) pseudoconspersa* NPV
g	:	gravity (for centrifugation)

GM	:	genetically modified
gm	:	gram
GTR	:	General time reversible (model)
GV(s)	:	granulovirus(es)
HearGV	:	*Helicoverpa armigera* GV
HearNPV	:	*Helicoverpa armigera* NPV
HycuNPV	:	*Hyphantria cunea* NPV
HytaNPV	:	*Hyposidra talaca* nucleopolyhedrovirus
HytaNPV-ID1	:	*Hyposidra talaca* nucleopolyhedrovirus Dooars isolate
HytaNPV-ITK1	:	*Hyposidra talaca* nucleopolyhedrovirus Terai isolate
HytaNPV-R	:	*Hyposidra talaca* NPV as reference
HzNPV	:	*Helicoverpa zea* NPV
in silico	:	in or on a computer: done or produced by using computer software or simulation
in vitro	:	outside the living body and in an artificial environment
IPM	:	integrated pest management
kb	:	kilo base
kDa	:	kilo dalton
Kg	:	kilogram
LafiNPV	:	*Lambdina fiscellaria* NPV
LC_{50}	:	median lethal concentration
LD_{50}	:	median lethal dose
LdNPV	:	*Lymantria dispar* NPV
Lef	:	late expression factor
LeseNPV	:	*Leucania separata* NPV
LG	:	Le Gascuel
LoobNPV	:	*Lonomia obliqua* NPV
LT_{50}	:	median lethal time
LyxyNPV	:	*Lymantria xylina* NPV
MabrNPV	:	*Mamestra brassicae* NPV
MacoNPV-A	:	*Mamestra configurata* NPV-A
MacoNPV-B	:	*Mamestra configurata* NPV-B
MaviNPV	:	*Maruca vitrata* NPV
MEGA	:	Molecular Evolutionary Genetics Analysis

$MgCl_2$	:	magnesium chloride
ml	:	milliliter
mm	:	millimetre
mM	:	millimolar
MNPV	:	multiple-nucleopolyhedrovirus
MolaGV	:	*Mocis latipes* GV
Na_2CO_3	:	Sodium carbonate
NaCl	:	Sodium chloride
NCBI	:	National Centre for Biotechnology Information
nd	:	non-synonymous mutation
NeabNPV	:	*Neodiprion abietis* NPV
NeleNPV	:	*Neodiprion lecontei* NPV
NeseNPV	:	*Neodiprion sertifer* NPV
ng	:	nanogram
NPV(s)	:	nucleopolyhedrovirus(es)
OB	:	Occlusion body
ODVs	:	Occlusion-derived virus
OpNPV	:	*Orgyia pseudotsugata* NPV
ORF	:	Open reading frame
OrleNPV	:	*Orgyia leucostigma* NPV
PCR	:	Polymerase Chain Reaction
PeluNPV	:	*Perigonia lusca* NPV
PespNPV	:	*Peridroma* sp NPV
pH	:	potential of Hydrogen
PhcyNPV	:	*Philosamia cynthia ricini* NPV
Pif	:	*per os* infectivity factor
PiraGV	:	*Pieris rapae* GV
PlxyGV	:	*Plutella xylostella* GV
PlxyNPV	:	*Plutella xylostella* NPV
PM	:	post meridian
POBs	:	polyhedral occlusion bodies
PPC	:	Plant Protection Codes
PsinNPV	:	*Chrysodeixis (=Pseudoplusia) includens* NPV
PsunGV	:	*Pseudaletia unipuncta* GV

REN	:	Restriction endonuclease
RFLP	:	Restriction fragment length polymorphism
RoNPV	:	*Rachiplusia ou* NPV
s/v	:	transition over transversion
sd	:	synonymous mutations
SDS	:	sodium dodecyl sulphate
SeNPV	:	*Spodoptera exigua* NPV
SfGV	:	*Spodoptera frugiperda* GV
SfNPV	:	*Spodoptera frugiperda* NPV
SNPV	:	single nucleopolyhedrovirus
SpliNPV-A	:	*Spodoptera litura* NPV
SpliNPV-B	:	*Spodoptera littoralis* NPV
SujuNPV	:	*Sucra jujuba* NPV
TAE	:	Tris-acetate EDTA
ThorNPV	:	*Thysanoplusia orichalcea* NPV
TnGV	:	*Trichoplusia ni* GV
TnNPV	:	*Trichoplusia ni* NPV
UrprNPV	:	*Urbanus proteus* NPV
USA	:	United States of America
UV	:	ultra violet
VLF	:	very late factor
w/v	:	weight per volume
λ DNA	:	lambda DNA

Single letter code for nucleotide

A	adenine nucleotide
G	guanine nucleotide
C	cytosine nucleotide
T	thymine nucleotide
S	guanine nucleotide/cytosine nucleotide
W	adenine nucleotide/ thymine nucleotide
Y	pyrimidine nucleotide
R	purine nucleotide
H	adenine nucleotide/ cytosine nucleotide / thymine nucleotide
M	adenine nucleotide/ cytosine nucleotide (with nitrogen base having amino group)

Single letter code for amino acid

G	Glycine	**W**	Tryptophan
P	Proline	**H**	Histidine
A	Alanine	**K**	Lysine
V	Valine	**R**	Arginine
L	Leucine	**Q**	Glutamine
I	Isoleucine	**N**	Asparagine
M	Methionine	**E**	Glutamic Acid
C	Cysteine	**D**	Aspartic Acid
F	Phenylalanine	**S**	Serine
Y	Tyrosine	**T**	Threonine

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