I. INTRODUCTION
**Butterflies** are one of the most beautiful and colourful creatures on the earth. Along with moths, they are under Order Lepidoptera having two pairs of wings that are partly or wholly covered with tiny overlapping scales and have complete series of transformations (life cycle). Lepidoptera contains more than 180000 species in 128 families and 47 superfamilies. Estimates of species suggest that the order may have more species and is among the four largest, successful orders along with Hymenoptera, Diptera, and Coleoptera (Resh and Carde, 2003). Lepidoptera are found in variety of habitats, but almost always associated with higher plants, especially angiosperms (Gullan and Cranston, 2004).

Butterflies are taxonomically well studied group, which have received a reasonable amount of attention throughout the world (Ghazoul, 2002). Butterflies are classified into two superfamilies; Hesperioidea, consisting of a single family Hesperiidae (Skippers) and Papilionoidea, having four families: Papilionidae (Swallowtails), Pieridae (Whites and Yellows), Nymphalidae (Brush-footed butterflies) and Lycaenidae (Blues) (Kehimkar, 2008). Butterflies are important as pollinators for many species of flowering plants although in general they do not carry as much pollen load as Hymenoptera. They are however, capable of moving pollen over greater distances (Herrera, 1987). Adult butterflies are considered opportunistic foragers that visit a wide variety of available flowers (Courtney, 1986). Butterflies are one of the important food chain components of the birds, reptiles, amphibians, spiders and predatory insects. Butterflies are also good indicator of environmental changes as they are sensitive and directly affected by changes in the habitat (Haribal, 1992). Butterflies are sensitive biota which gets severely affected by the environmental variations and changes in the forest structure and composition as they are closely dependent on plants (Pollard and Yates, 1993). Being good indicators of climatic conditions as well as seasonal and ecological changes, they can serve in formulating strategies for conservation (Kunte, 1997). Many butterfly species are strictly seasonal and prefer only a particular set of habitat (Kunte, 1997).
and they are good indicators in terms of anthropogenic disturbance and habitat quality (Kocher and Williams, 2000). The diversity of butterfly species is high in natural habitats than the modified ones (Nayak et al., 2004). Variation in size, shape, colour and behavior within species of butterflies is quite common. Environmental factors such as geography, climate and season can also have their impact by influencing the movement, distribution and life cycle of butterflies, thus resulting in different forms. The four major types of variation found among butterflies are individual variability, sexual dimorphism, seasonal and geographical variability (Ackery and Vane-Wright, 1984; Kehimkar, 2008).

The **subfamily Heliconiinae** is an assorted group and includes the Costers, Lacewings, Fritillaries, Leopards, Vagrants, Yeomans and Rustic. The eggs of these groups are pale blue or yellow and are laid singly or in clusters. The larvae in general appearance are dark or conspicuous banded with numerous spines and may have a pair of dark horns on the head. Adults fly slowly to display bright warning colours (Beltran et al., 2008). They are commonly called heliconians or longwings as the fore wings are always elongated tipwards. Eyes are large, antenna long and typically brightly pigmented and upper wings usually tawny in colour with transverse lines of black spots. All members of the subfamily possess yellowish abdominal scent glands from which the butterflies evert and emit acrid odour when disturbed (Ross, 1976). They are usually unpalatable to predators as larvae feed on distasteful plants characteristically Passifloraceae vines and Urticaceae.

Heliconiinae is one subfamily of Nymphalidae, 21 species have been described so far in India (Kehimkar, 2008). These butterflies have played a key role in understanding evolutionary biology and ecology and it would be difficult to point a group of Neotropical butterflies that have contributed more to our knowledge of the biological processes in the tropics. The derived members of the tribe have undergone rapid speciation and divergence, while also exhibiting impressive mimetic convergence in wing patterns. The subfamily
Heliconiinae has really only been delimited as it is now since 1991, when Harvey (1991) placed the argynnines and acraeines with the heliconiines. A recent phylogenetic study of the subfamily by Penz and Peggie (2003) suggests that the subfamily should be divided into 4 tribes. The phylogenetic relationships of various groups in Heliconiinae have been extensively studied, especially in the tribe Heliconini by NSG (2009).

The vast amount of information gathered on this group spans a variety of topics in ecology, evolutionary biology and conservation biology (Muller and Beheregaray, 2010). They tend to show highly localized endemism in the region and high species diversity. However, the higher phylogenetic relationships of major groups of butterflies remain poorly hypothesised. This lack of knowledge is critical, since several disciplines in comparative biology (evolution of host plant preferences, mimicry and behavior) depend on robust phylogenetic hypotheses to provide a framework for interpreting the evolution of putatively adaptive character systems. Despite several recent important efforts to elucidate the higher level relationships of butterflies (Silva et al., 2010), there is still only fragmentary knowledge about patterns of relationships among lineages within the subfamily, one of the most diverse groups of butterflies and has been the focus of several recent phylogenetic studies (Kodandaramaiah et al., 2010; Muller and Beheregaray, 2010). Although many studies on morphological, ecological and molecular attributes of several species of Heliconiinae are available, very little is known about the species of the Oriental Zoogeographic region especially from the north-east India.

The Northeast India lies between 22°N and 29°5’N latitude and 88°00’E and 97°30’E longitudes, covering nearly 262379 sq. km. area and shares international border with Bhutan, China, Myanmar and Bangladesh (Chatterjee et al., 2006). Average rainfall in this region often exceeds 2000 mm, hosting more than 50% of the total Indian butterfly species (Kehimkar, 2008). The region has been divided into two biogeographic zones - Eastern
Himalaya and Northeast India (Rodgers and Panwar, 1988). The Eastern Himalaya comprising of Arunachal Pradesh and Sikkim is more mesic due to high degree of precipitation resulting from direct confrontation of monsoon laid wind blowing from Bay of Bengal by abruptly raising hills. The Northeast India biogeographic zone (Assam, Nagaland, Manipur, Meghalaya, Mizoram and Tripura) is most significant one and represents the transition zone between the Indian, Indo-Malayan, Indo-Chinese biogeographic regions as well as a meeting place of Himalayan mountains with that of Peninsular India (Rao, 1994). In the tropical zone, besides several tree species like the towering Hollong and Cinnamon, several evergreen shrubs and woody climbers, tree ferns, screw pine, wild banana, giant bamboos and ferns are conspicuous in the hill forests. Pitcher plants, several orchids and rhododendrons are endemic to the regions (Kehimkar, 2008). The region is geographical ‘gateway’ for much of India’s flora and fauna and as a result the region is one of the richest in biological values with vegetation types ranging from tropical rain-forest in the foothills to alpine meadows and cold deserts. The Northeast India contains more than one-third of the country’s total biodiversity. The region represents important part of Indo-Myanmar biodiversity hotspot, one of the global biodiversity hotspots (www.biodiversityhotspots.org).

This region needs imperative awareness for conservation because of high degree of host plant and butterfly endemism and the grave threats it faces. Global climate is changing rapidly with unpredicted consequences for the reason that elements of biodiversity responds closely to climate (IPCC, 2001). In this area, the shifting cultivation known as ”Jhum” is the major threat to biodiversity of insects especially to butterflies. To salvage the issue of extinction crisis, we need to prioritize and target conservation strategies in relation to systematics (conventional and molecular) and biodiversity of butterflies. This approach is a realistic platform to build a much more flexible system, with the potential for the rapid and accurate identification of butterflies using modern tools.
A **phylogeny** is an evolutionary tree that shows how different species are related to each other (Baldauf, 2003; Harrison and Langdale, 2006). One of the fundamental applications of phylogeny is in classification. Phylogeny help to systematically classify organisms in an evolutionary framework and less prone to errors and individual biases (Sidow and Thomas *et al.*, 1994, Zhang *et al.*, 2012). Morphology based classification can be compared and contrasted with molecular systematic with phylogeny (Lewis 2001). Therefore, phylogenies can be built using molecular data or morphological data, but in recent times molecular data are increasingly being used.

**RAPD-PCR** (Random Amplified Polymorphic DNA-polymerase chain reaction) uses short synthetic oligonucleotides (10-12 bases long) of random sequences as primers to amplify nanogram amount of total genomic DNA under low annealing temperature by PCR where a single oligonucleotide of random sequence is employed and no prior knowledge of the subjected genome is required and can be used for detecting polymorphisms at many loci between species and populations (Williams *et al.*, 1990). This technique amplifies anonymous fragments of DNA from any genome. The profile of amplified DNA primarily depends on nucleotide sequence homology between the template and oligonucleotide primer at the end of each amplified product. Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites. The size distribution of amplified fragments varies among species. Closely related taxa have similar fragment distributions, while distantly related ones are more divergent (Bardakci, 1999). Thus, RAPD-PCR and has been widely used in the determination of population structure without prior knowledge of DNA sequences while giving a good resolution of genetic differences, genetic polymorphisms and genetic diversity in natural populations between species.
DNA Barcoding is a relatively new concept that has been developed for providing a rapid and accurate species identification using standardized DNA sequences as tags. In fact, it started with the seminal work of Hebert et al. (2003). In DNA barcoding, the unique nucleotide sequence patterns of small DNA fragments (400-800 bp) are used as specific reference collections to identify specimens and to discover overlooked species. Any barcoding system should aim to acquire data for at least a nuclear and an organellar gene from single specimen for study of evolutionary pattern and taxonomic and systematic studies (Blaxter, 2004). In barcoding technology, the major point of focus is the DNA macromolecule and every sample which has to be characterized based on DNA barcodes access to its mitochondrial DNA (Ghosh, 2012). Barcode sequence data provide a shared genomic corner stone for the variable repertoire of genes that are used to build the phylogenetic tree. It can be used as a link between the deeper branches of the tree to its shallow, species-level branches (Hajibabaei et al., 2007).

The mitochondrial DNA (mtDNA) is a good choice for DNA barcoding because of its fast mutational rate which gives a significant variation between species, lack of introns, limited exposure to recombination and its haploid mode of inheritance (Lin and Danforth, 2004). These genes are generally easier to amplify than nuclear genes and conserved mitochondrial markers are widely available (Simon et al., 1994). Mitochondrial genes have been for many years the most commonly used source of data for studies of insect molecular phylogeny, biogeography and phylogeography (Morgan et al., 2009; Simon et al., 1994). Hebert et al., (2003) established that the mitochondrial gene Cytochrome c Oxidase 1 (CO1) can serve as the core of a global bio-identification system for all animal phyla. Rach et al., (2008) have shown that mitochondrial gene NADH Dehydrogenase Oxidase Subunit 1 (ND1) is well suited as an alternative or complement to CO1, since ND1 sequences have been shown to be highly informative at different taxonomic levels in dragonflies.
Although many studies on morphological, ecological and molecular attributes of several species of butterflies from world over are available, very little is known about the Indian species especially in northeast India. This region also needs imperative awareness for conservation because of high degree of host plant and butterfly endemism and the grave threats it faces. The present study, targeting the systematic and biodiversity of the subfamily may contribute towards the knowledge about Heliconiinae butterfly diversity, distribution and its evolutionary relationships in the region.