

Introduction

Black flies locally known as bhusana or peepsah are an integral part of the natural environment with the flowing waters which make their breeding habitat. Attention of the Government and the researchers have been drawn by the black flies due to their distribution, abundance, nuisance habits, economic impact on the human and livestock health. They invade both aquatic and the terrestrial environments at different stages of its life cycle. In aquatic environment, they are part of aquatic food web; in terrestrial environments, most of them are considered as pests.

The family Simuliidae is a small family of the world wide order Diptera, the family Simuliidae ranks about 30th in number of species among the approximately 130 Dipteran families. The family Simuliidae consists of 2,204 nominal species of which 22% are distributed among 24 species group in the subgenus *Simulium* Laterille (Adler and Crosskey 2016).

More than 20 nominal species and more than 50 cytoforms have been described on the basis of the polytene chromosomes. The cytologically described species may likely represent the valid

species (Adler and Crosskey 2011). For about one-quarter of the world's nominal species have been documented through the cytotaxonomical study confirmed by about 600 papers dealing with the chromosomes (Adler et al., 2010). The taxonomic structure of the family is constructed over the morphological work of more than 250 years and over 50 years of chromosomal study. Other studies on the systematics of the family Simuliidae will have to be made on this framework platform.

Black flies are small organisms about 1.5 to 6 mm in length and are humpbacked, with short legs, stout antennae, and strong anterior wing veins. Adult black flies are dark coloured, with variable shades of gray, orange, or yellow. Eyes of the males of most species are larger than the females. The eyes of males meet along the midline whereas in females they are separated. The extent of variation within the family is striking particularly in the colour pattern of larvae, gill configurations of pupae, and terminalia of adults. Black flies can be easily identified upto the family level due to the possession of fundamental character of the family. Eggs are not much of taxonomical importance; the shape and the texture of eggs are often variable.

Depending on the species the females either lay eggs over the water while flying very low near to the water surface or deposit them in masses at different levels of strata beneath the flowing water. Hatching of the larvae from the eggs and pupation of the larvae takes

place within a period of a few days to several months depending on the temperature and other environmental factors. Temperature plays the most important role in the life cycle of black flies at its different developmental stages. Larvae of black flies have an elongate, sausage shaped body, more expanded posteriorly with a well defined head capsule bearing a pair of labral. The size of the black fly larvae are variable ranging from 5 to 9 mm in length attached to various substrates in running waters, such as trailing grasses, aquatic plants, twigs, fallen tree leaves, stones, rocks, and plastic bottles and poly ethylene bags. All pupae are characterized by a pair of spiracular gills (=respiratory histoblast) on both lateral sides of thorax and a silk cocoon of varying complexity.

Black flies are among the top three or four most important groups of arthropod vectors only next mosquitoes that have received considerable attention after the discovery of the fact that they transmit filarial worms responsible for onchocerciasis (Blacklock 1926a, b). Out of 2,204, almost 98% of the females of black flies feed on avian and mammalian blood. Most of these flies transmit blood borne pathogens (Adler 2009, Adler and Crosskey 2016).

In West Bengal 27 nominal species were described with seven unnamed taxa (Datta 1992). Darjeeling, the area of this study, is situated in northern West Bengal, India, at an altitude of 2354m in the foothills of the Eastern Sub-Himalayan region. More than 19 nominal species of black flies have been reported from, Darjeeling,

West Bengal, on the basis of morphological characters (Datta 1973, 1974a, b, 1992, Datta and Pal 1975, Adler & Crosskey 2016). In recent years three more new species were described morphologically from Darjeeling area viz, *Simulium (Gomphostilbia) williei* (Takaoka et al., 2010), *Simulium (Gomphostilbia) sachini* (Takaoka et al., 2010), and *Simulium (Nevermannia) subratai* (Takaoka et al., 2011) along with the description of male *Simulium (Montisimulium) dasguptai* (Takaoka et al., 2009) that was not described earlier. Besides, two more species *Simulium (Nevermannia) praelargum* “IIL-1.2” and *Simulium (Nevermannia)* “IL” was described cytologically (Thapa et al., 2014, 2017). In spite of the high medical and socio-economic importance of black flies, only a few cytological studies of the polytene chromosomes of black flies from this region have been published so far (Dey et al., 1993, Henry et al., 2009, 2010, Thapa et al., 2014, 2017).

Black flies would have been most desired specimens for the genetic studies if mating and rearing in the laboratory were possible as in case of *Drosophila*. The larval polytene chromosomes of black flies are more explicit than that of Drosophilids. The alternating light and dark transverse bands of polytene chromosomes could be instrumental in inferring reproductive isolation (Rothfels 1956). The sibling species which otherwise is very difficult to identify morphologically can be visualized only through the polytene chromosomes information. The population structure, evolutionary

relationships and effects of toxicants can also be had from the information provided by the polytene chromosomes.

1.1 Medical and economic importance

Black flies, due to the blood sucking habits of the adult females of many species make them target of study as they are economically and medically important. Black flies bites can be detrimental to animal production due to weight loss, and reproductive dysfunction (Freeden 1977), decrease in milk and egg production (Jamnback 1973, Steelman 1976, Watts 1976), dermatitis and skin lesions (Gräfner 1981); death due to toxemia and systemic shock (Watts 1976, Steelman 1976, Freeden 1977); bovine onchocerciasis (Watts 1976, Steelman 1976) and avian leucocytozoonosis (Watts 1976, Snoddy and Noblet 1976, Fallis 1980). Black flies also affect the human health causing dermatitis, systemic reactions to bite (Jamnback 1973, Watts 1976, Newson 1977) and human onchocerciasis(Watts 1976) and to recreation and agricultural use namely, nuisance and loss of tourist revenue (Newson 1977, Merritt and Newson 1978), decrease in work efficiency in fields and forest (Jamnback 1973, Watts 1976).

It has also been observed that the black fly species of Darjeeling and adjoining hill areas are serious nuisance to local population. Their biting habits results in ulceration and occasional fever (black fly fever). Similar reports have also been received from Arunachal Pradesh and Assam regions of North-East India. Of all the species

involved, *Simulium (Simulium) himalayense* is especially responsible for causing annoyance to humans and cattle populations (Das et al., 1985).

Simuliids are the main causative agents of onchocerciasis and mansonellosis. Onchocerciasis commonly called as river blindness is the second serious cause of blindness in the world. Forty million people have been made blind due to onchocerciasis. As per the World Health Organization 1995 records (Basáñez et al., 2006, Remme et al., 2006), one million Disability Adjusted Life Year is lost due to onchocerciasis. It is reported that more than 25 species of Simuliids are the responsible for the transmission of *Onchocerca volvulus* causing river blindness in 6 Latin American countries, Yemen and 27 African countries. This accounts for 99 % of cases in Africa south of Sahara. A bacterium, *Wolbachia* has been discovered in the filarial worms that has proinflammatory role that establishes an obligate parasite-fly-human relationship.

In comparison to onchocerciasis, mansonellosis is a mild disease (Klion and Nutman 1999). Five Simuliid vectors along with Culicoides have been found to be responsible for this disease; mansonellosis is not widely spread and is limited to the New World tropics (Shelley and Coscarón 2001). As in case of all Dipterans, black flies have large salivary glands (Fig. 5). The polytene chromosomes from the larval salivary glands have the optimal working condition with maximum clarity.

OBJECTIVES:

Chromosomal characterization of few commonly occurring species of the genus *Simulium* found in the Darjeeling Hill:-

- a) Based on Polytene chromosome map
- b) Based on mitotic chromosomes

The literature review shows that the informations in the black flies of Darjeeling are very scanty. Darjeeling hill area with abundant streams and trickles support the existence of black flies. Only a very few species have been reported from this locality. Of the reported species, many of them have been reported incompletely by the morphotaxonomists. The existence of sibling species or the cytoforms have further made the identification of the species more complex.

In order to support the morphotaxonomical aspects of the species described is not sufficient enough to justify the taxonomic status of a species. The supporting study from other aspects of taxonomy is demanding. Therefore, the cytotaxonomic work through the study of chromosomes, both at the mitotic and the polytene chromosome level is of utmost importance. Out of 19 nominal species from this region, only six species have been described mitotically and seven at the polytene chromosome level.