Vector Biology

Anopheline Surveys and their Identification

The discovery by Sir Ronald Ross in 1897 implicating mosquitoes in malaria transmission created interest among entomologists for faunistic studies. Industrialization, water management projects, urbanization, deforestation, *etc*. have been the important developmental activities in post-independent era in India. Realizing the influence of environmental changes on species prevalence, the Institute carried out faunistic surveys to update the information on anopheline fauna and on other mosquitoes in different parts of the country (Fig. 1) (Nagpal and Sharma 1983, 1985, 1987; Nagpal *et al* 1983; Singh and Nagpal 1985; Singh *et al* 1985; Uprety *et al* 1983; Yadav *et al* 1989; Sharma *et al* 1985, 1999; Das *et al* 1998).

In addition, NIMR also took lead in publishing and authoring books describing anopheline species prevalent in India. The details of same are given in chapter–"Publications" of NIMR.

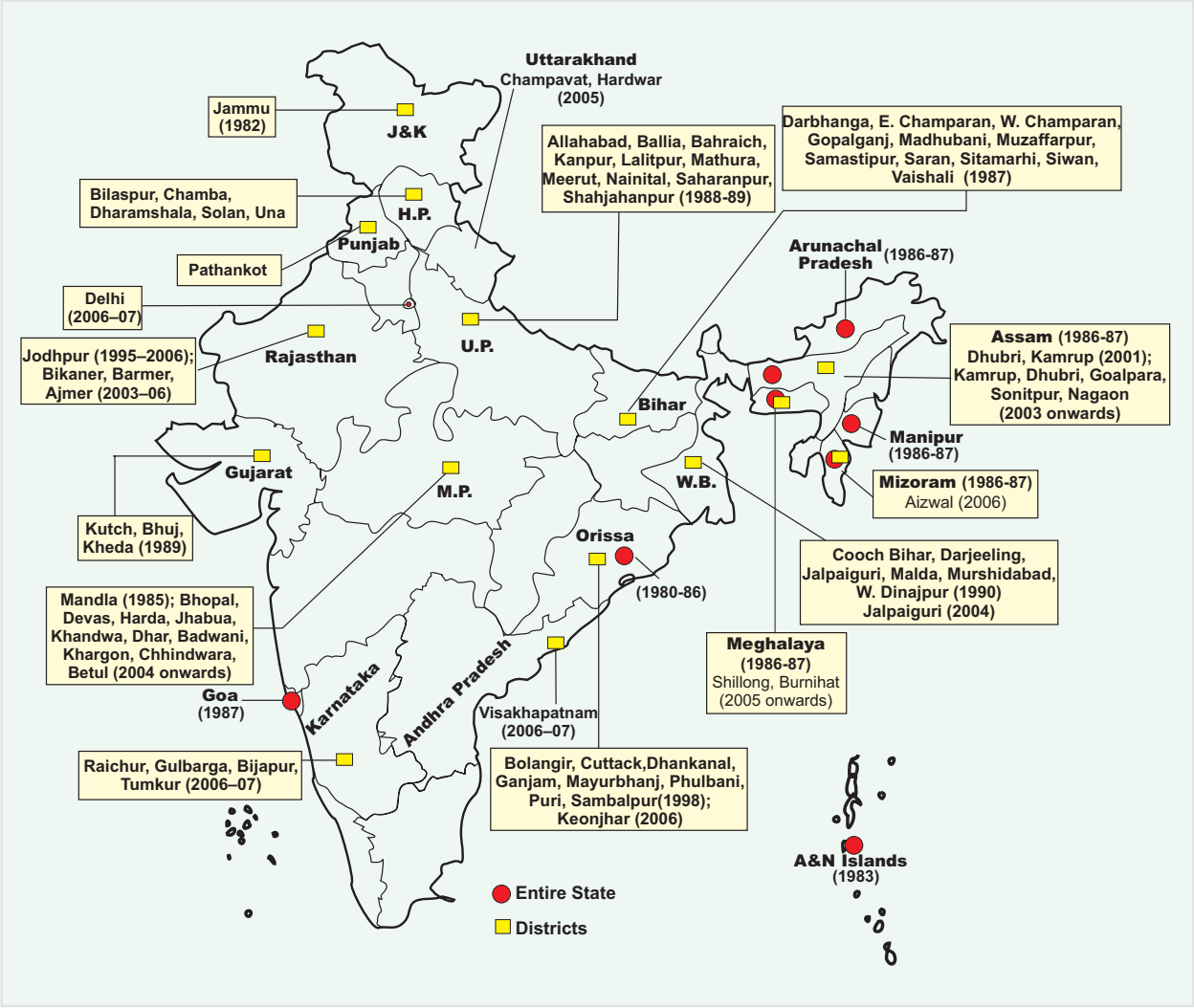

Fig. 1: Anopheline fauna surveys conducted by NIMR during 1980–2008

The references cited in the text are listed in the section "Research Articles Published by NIMR Scientists"

Highlights of Faunistic Surveys Carried out by NIMR

(i) Disappearance of *An. sundaicus* from coastal Orissa state and reappearance of *An. minimus* in northeastern states and Banbasa area of Champavat (Nainital district in erstwhile Uttar Pradesh state and now in Uttarakhand state)

(ii) Identification of *An. nivipes* (by morphological and cytotaxonomic methods) for the first time in India from northeastern states (Nagpal and Sharma 1987; Subbarao *et al* 2000)

(iii) A new focus of *An. sundaicus* in western coast (Kutchch, Bhuj) of India (Singh *et al* 1985); and

(iv) A number of morphological variations were recorded in more than 20 species in these surveys (Nagpal 1990; Nagpal and Sharma 1983).

Computer-aided Identification Tools for Indian Anopheline Species

Electronic Key for the Identification of Adult Anopheline Species

A computer-based identification key for all 58 female Indian anophelines belonging to subgenera *Anopheles* and *Cellia* has been developed. Besides being fast and easily upgradable, the added advantage of this electronic key over the earlier couplet keys is that it can identify the variant species. The software has been developed in Turbo Pascal ver. 6.0 and is menu driven (Nagpal *et al* 1995). The software is supported by computerized drawings (Fig. 2). The knowledge-base has been developed using the book by Nagpal and Sharma (1995). Mosquitoes are divided into two subgenera, namely *Anopheles* and *Cellia* depending on the number of pale areas on costa, subcosta and vein 1. The software of each subgenus consists of four modules. The module 1 is for beginners. Using a microscope or hand lens, the user enters his observations as prompted by computer menus, and species is identified. The module 2 is for experienced users who can recall the identification characters just by entering the species code assigned, these are displayed on the monitor. Characters for quick identification are also highlighted. The module 3 is for distant users in remote areas. The user completes a specially designed proforma and makes a string of coded numbers. As soon as the codes are entered, computer matches the string with the species code string stored. To identify the species, this task of computer can also be done manually using the species code given at the end of the proforma (Srivastava *et al* 1992). In module 4, based on successive characters, each subgenera, namely *Anopheles* and *Cellia* is divided in subgroups forming a nested sequence. At the end, the last subgroup contains only a few species with minor morphological variations leading to an approximate identification of the species.

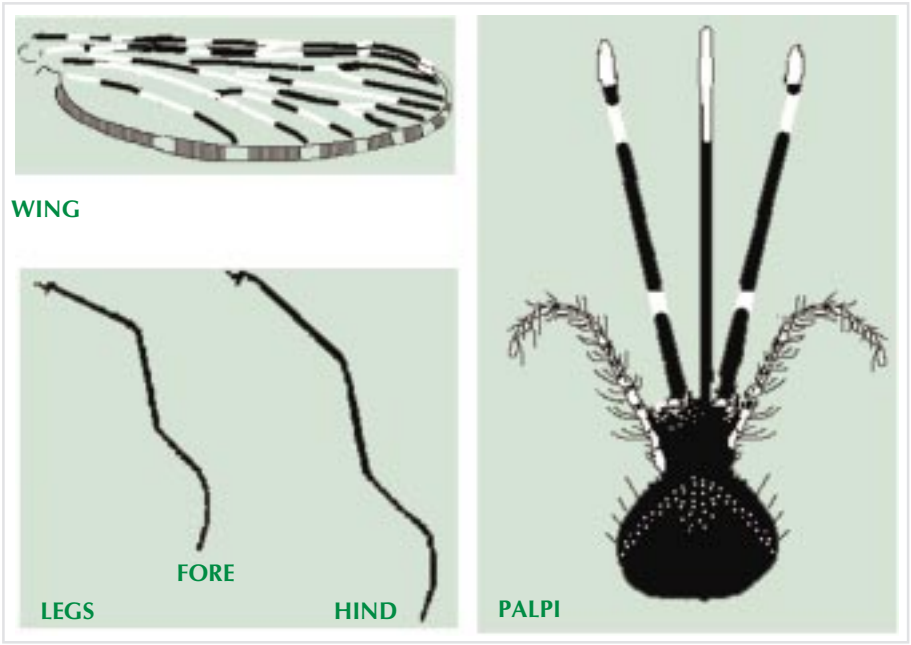

Fig. 2: Computerized drawings showing different characters for the identification of anopheline species through electronic key for identification

The software is very useful for beginners for quick and correct identification. It familiarizes the user with all required morphological characters. The software has proved to be an excellent training tool for entomologists, malariologists, researchers and public health workers.

Electronic Key for the Identification of Anopheline Larvae

A computer software for the identification of the IV instar larvae of 58 anopheline species has been developed. Knowledge-base consists of all the corresponding larval features of a species. The software has been divided into two modules. In the first module, user can enter the characters as seen under microscope through computerized menus and the larval species are identified. The second module is for an experienced user in which each species has got a unique code and by inputting the same, characters are displayed. One can cross-check the characters in the microscope and identify the species. Computerized drawings have also been linked to the modules. The software is user friendly and has been written in Foxpro 2.6. It has been field-tested and works accurately. The knowledge-base can be expanded and updated if new species are recorded. This software is a very useful tool for entomologists working in the field of malaria control in urban, foothill and forested areas of the country.

A Computerized Information System

An information system using updated knowledge-base on 58 species of Indian anophelines has been developed. The software is developed in dbase III plus. Species distribution, derivation of name, biology, biting habit, resting posture, preferred breeding sites, global occurrence, important references, *etc*. have been included in the database. The user can get any information about the Indian anophelines instantly. Also one can access state-wise information and species distribution in India. The software is easily upgradable to include new species. This information system is very useful for entomologists working in national and state health programmes.

Anopheline Identification Album

A CD has been prepared consisting of drawings of adult female and important identification characters, bio-ecology, vector status, Indian and global distribution, *etc*. for each Indian anopheline species (Fig. 3). To generate a tailor-made hard/soft copy album, a software in Visual Basic has been written, user can select the species codes of interest, the data would be processed and user can get album consisting of information on desired species. This album is extremely useful to the entomologists and field workers.

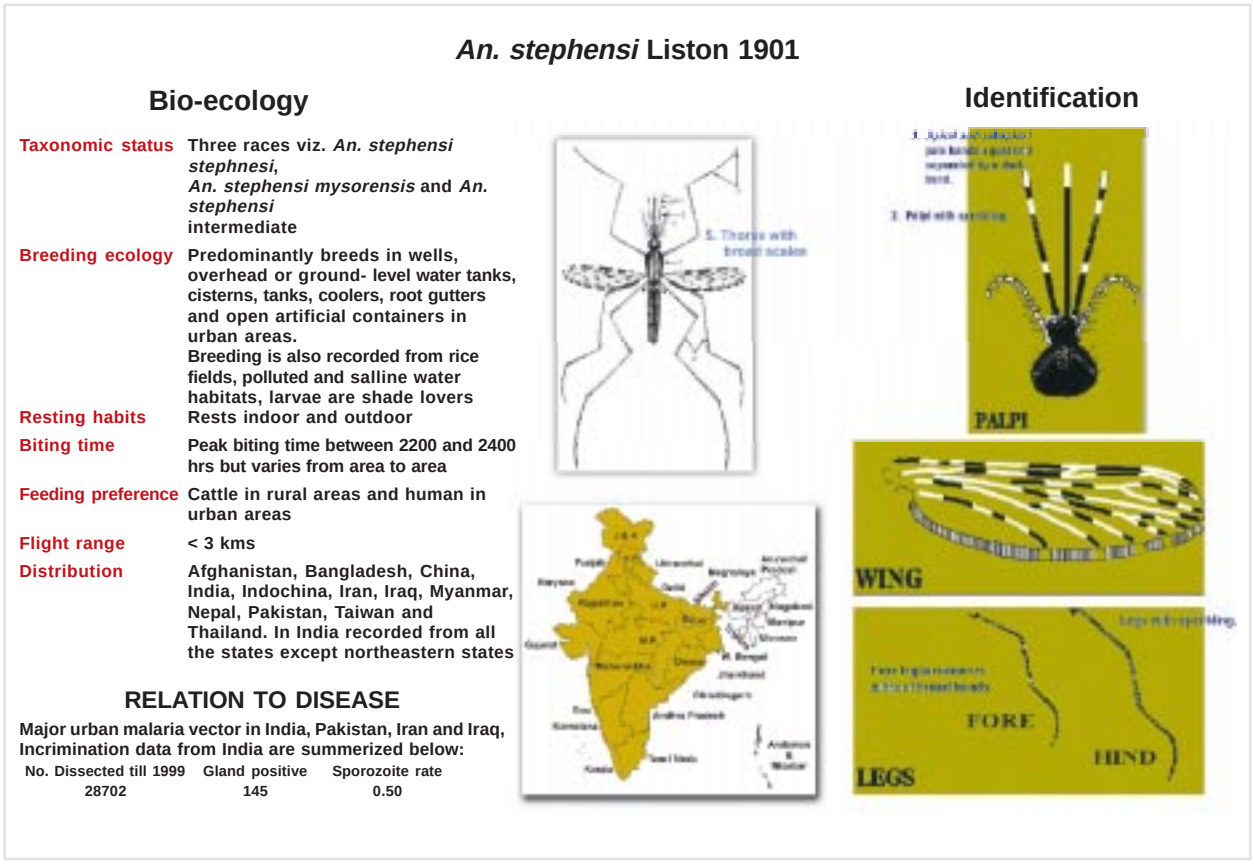

Fig. 3: A page of the album showing the identification characters, bio-ecology, distribution and relation to disease of *An. stephensi*

Pictorial Identification Key for Indian Anophelines

The pictorial identification key for 58 species of Indian anophelines has been published. The English version was released by the then Director General, ICMR (Fig. 4a) and Hindi version was released by the Director, NVBDCP, Delhi (Fig. 4b). The key was prepared on the request of Defence Research Laboratory, Tejpur, Assam and is meant for researchers, field workers and technicians. The pictorial key comprises of an introduction, checklist of Indian anophelines, morphological characters (pictures only) used for the identification and guidelines for using the key and pictorial identification. The breeding ecology of each species in brief is also given in the key.

Fig. 4a: Prof. NK Ganguly, the then Director General, ICMR releasing the pictorial identification key for Indian anophelines (English version)

Fig. 4b: Dr GPS Dhillon, Director, National Vector Borne Disease Control Programme releasing the pictorial identification key for Indian anophelines (Hindi version)

Morphometrics of *Anopheles stephensi*

Ecological Variants Based on Number of Ridges on Egg-float

Two variants which differ in egg-float ridge number and in egg length and width were reported earlier. These are referred as type form and var. *mysorensis*. To resolve the taxonomic status of these two forms, extensive surveys were carried in and around Delhi to collect *An. stephensi* and examine the egg-float ridge number. Several strains available in the insectary of NIMR were also examined (Subbarao *et al* 1987). The study grouped the *An. stephensi* into three categories—14–22, 12–17 and 9–15 ridges on the egg-floats respectively. The category with highest egg-float ridge number corresponded with the type form and the lowest with the var. *mysorensis* reported earlier, and the new egg-float category was designated as 'intermediate'. All the three forms were observed in semi-urban areas while only intermediate and var. *mysorensis* in rural areas. In this study typical urban localities were not surveyed. As genetic studies did not indicate any mating barrier between these forms, the three forms with different ridge number are best considered as ecological variants.

Spiracular Index to Identify Two Ecological Variants

To determine applicability of length of thoracic spiracle and its index as a taxonomic tool to identify two ecological variants of *An. stephensi* at adult stage, hand catches were made indoors and outdoors of *An. stephensi* populations during three seasons—summer, monsoon and post-monsoon. Gravid/semi-gravid females were allowed to lay eggs individually and batches of eggs-based on number of ridges on egg-float. *An. stephensi* type form with ≥15 and *mysorensis*≤14 ridges were separated. Corresponding females were subjected to measurement of thoracic spiracular indices to correlate with ridge counts of both variants. Studies clearly established statistically significant correlation between the ridge count with both thoracic spiracle length and spiracular index. In *An. stephensi* type form, the spiracle length ranged from 0.10 to 0.14 mm and spiracular index from 7–10 while in *mysorensis* these were 0.07 to 0.10 mm and 6–9 respectively. These parameters showed consistent variations in population of mosquitoes emerged during monsoon and summer seasons. The study established that the spiracle length and its index can be used to identify the two ecological variants of *An. stephensi* at the adult stage dispensing with the need of examining eggs.