

# **Faunistic studies on the diversity and distribution of mosquitoes of the high altitude Himalayan Region - Jammu & Kashmir**



## **THESIS**

Submitted to the Pondicherry University for the degree of  
**Doctor of Philosophy**  
in  
**ZOOLOGY**

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**February 2018**

## CERTIFICATE

This is to certify that the thesis entitled “**Faunistic studies on the diversity and distribution of mosquitoes of the high altitude Himalayan Region – Jammu and Kashmir**” submitted to Pondicherry University for the degree of Doctor of Philosophy by **Mr. Talib Hussain Dar**, is a bonafide record of original research work carried out by the candidate in ICMR - Vector Control Research Centre, Puducherry, India, during the period from 2011 to 2017 (Full-Time), under my supervision. This study has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles of this university or any other university and other institution of higher learning.

Place: Puducherry

Date:

**Dr. P. Jambulingam**

Scientist ‘G’ & Director  
(Guide of the Candidate)

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Place: Puducherry

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**Dr. S. Jayakumar**

Associate Professor

(Co-supervisor)

## ***Declaration***

I hereby declare that the thesis entitled “**Faunistic studies on the diversity and distribution of mosquitoes of the high altitude Himalayan Region – Jammu and Kashmir**” submitted to the Pondicherry University for the degree of Doctor of Philosophy is the original record of work carried out by me during the period from 2011 to 2017 (Full-Time), under the supervision of Dr. P. Jambulingam, Scientist ‘G’ & Director, ICMR - Vector Control Research Centre, Puducherry, India and under co-supervision of Dr. S. Jayakumar, Associate Professor, Department of Ecology and Environmental Sciences, Pondicherry University, Puducherry, has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles of this University or any other University and other similar Institution of higher learning.

Place: Puducherry

Signature of the Candidate

Date:

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**{O my Lord! Have mercy on them both even as out of compassion  
they did look after me, when I was (a) little (child)}**

**MY SIBLINGS**

**&**

**DR. R. NATARAJAN**

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# ***1. Introduction***

Mosquitoes belonging to family Culicidae and order Diptera are a large group of insects present throughout the temperate and tropical regions and even beyond the Arctic Circle of the world (Harbach 2007). They are the vectors of many diseases like malaria, dengue, Japanese encephalitis, etc. affecting human health. In addition, mosquitoes can be abundant nuisance biters that can further affect human health. At present a total of 3557 recognized mosquito species divided between two subfamilies and 112 genera are recorded in the world (Harbach 2017). The Oriental Region which includes India is regarded as one of the richest biogeographic regions for mosquitoes of the world, along with the Neotropics (Gaston & Hudson 1994). India is ranked fifth in terms of mosquito biodiversity after Brazil, Indonesia, Malaysia and Thailand (Foley *et al.* 2007). Mosquito faunistic studies in Indian subcontinent started from 1900 when Giles entitles his book *A Handbook of the Gnats or Mosquitoes* and himself added some seventeen new species. There can be no doubt, however, that it was Theobald who, in his gigantic task of grappling with the Culicidae of the world, in the five volumes of his monograph published over the years 1901-10 opened up the study of mosquitoes to workers all over the world. Later, two monographs by Christophers on anophelines and Barraud on culicines, marked as a land-mark in the history of mosquito studies in the Indian subcontinent were published in 1933 and 1934 respectively. The publications were the result of all the taxonomic studies in the subcontinent made by the earlier workers and the authors themselves. Noteworthy among the earlier works are James and Liston's (1904) volume on Anophelines of India; the Annotated Catalogue of Culicidae and the Critical Review of the Genera in Culicidae by Brunetti (1907-1920), and Larvae of Anopheline Mosquitoes by Puri (1931). After their monumental work, not many comprehensive biosystematics studies of Indian Culicidae have been undertaken and the year 1934 marks the end of an era of very active taxonomic research on Culicidae by virtue of which mosquitoes became one of the best known groups of insects in the area. During the period a total of 288 mosquito species (anophelines - 43, culicines - 245) were described from the Indian subcontinent. Post 1934, most of the taxonomic studies, if done, dealt exclusively with Anopheles mosquitoes possibly due to an unprecedented high significance tagged with malaria in the country. Due to a fervent nationwide anti-malaria campaign between 1950 and 1980, hardly any attention was given to the non-anopheline mosquitoes, and the

biomedical significance of culicine mosquitoes was almost completely overshadowed in terms of their taxonomic biodiversity, albeit many of the culicine mosquitoes playing key roles in transmitting several deadly and/or debilitating diseases. However, a faunistic study regarding culicines (anophelines also) was carried out by Rao and his colleagues from 1967 to 1969 in the Himalayan region and was published in subsequent years (Rao *et al.* 1973, Bhat and Kulkarni 1983, and Rao 1984). Since 1980s some faunistic surveys were carried out in various parts of India although fewer in numbers (Bhattacharyya *et al.* 2014). In this course of mosquito biodiversity studies, extensive collections made in different ecosystems and geographical regions detected many species which are new additions to the mosquito fauna of India. Attempts were made to comprehend distributional patterns of mosquitoes on regional basis such as those in Southern India including Western Ghats and Eastern Ghats as well as Andaman & Nicobar Islands (Reuben *et al.* 1993, 1994; Rajavel *et al.* 2005a, 2005b, 2005c), Northeastern states of seven sisters (Dutta *et al.* 2003), the Thar Desert in western Rajasthan and the Great Runn of Kutch in Gujarat (Tyagi *et al.* 1991), various districts of Punjab and Himachal Pradesh (Sagandeep *et al.* 1994) and Malwa region of Punjab (Jagbir & Jagdish 1999). The recent review of the published studies showed that the Indian mosquito fauna comprises 393 species (anophelines - 61, culicines - 332) in 49 genera and 41 subgenera with latest taxonomic situation (Bhattacharyya *et al.* 2014). Although mosquito faunal studies were carried out in many regions and states of India, since 1973 such studies were not carried out in Jammu and Kashmir State comprising the upper parts of Western Himalayas.

The earlier studies on mosquito fauna of Jammu & Kashmir were mainly made by Christophers (1933), Barraud (1934), Puri (1936 & 1948), Jacob (1950), Nair (1973), and Rao *et al.* (1973). Some other workers like James and Liston (1904, 1911), Adie (1913), Gill (1920), etc. have also carried out mosquito studies in Jammu and Kashmir but that was later compiled by Christophers (1933) regarding anophelines and Barraud (1934) regarding culicines in their two famous monographs. All the studies except Barraud (1934) and Rao *et al.* (1973) were made about anopheline fauna only due to malaria prevalence in Jammu and Kashmir State and other parts of Indian subcontinent. Barraud (1934) made study on culicine fauna of mosquitoes while as mentioned above Rao and his colleagues made faunistic studies on both anophelines and culicines and published their work in

subsequent years, Rao *et al.* (1973), Bhat and Kulkarni (1983), and Rao (1984). They reported 29 mosquito species (anophelines - 9, culicines - 20) and this was the last faunistic study made on Jammu and Kashmir mosquitoes. Later on some studies have been made but confined to vector species and vector borne diseases only (Mathew *et al.* 1977; Kaul *et al.* 1995; Sharma & Srivastava, 1995; Padbidri *et al.* 1996). Overall, from these studies 43 mosquito species (anophelines - 17, culicines - 26) were reported from Jammu and Kashmir State. But from about four decades not any faunistic study regarding mosquitoes was made in the State (see also last paragraph of *Review of Literature* part). Since then, the State has gone through much environmental changes particularly in the context of developmental activities and global warming (Talib 2007).

Furthermore, mosquito borne diseases, malaria and dengue are found in Jammu and Kashmir State. Mosquitoes may transmit four types of animal and human pathogens, i.e. the Plasmodium species that causes malaria in man (*Plasmodium falciparum* and *P. vivax*), filarial worms of genus *Wuchereria* and *Brugia* (Nematoda) causing lymphatic filariasis in man, arboviruses causing yellow fever and dengue, and, finally, bacteria causing tularaemia (Harwood & James 1979). In J&K State, as per District Malaria Office, Jammu report, malaria is found in Jammu division and some border areas of Kashmir division while as dengue is found in Jammu division only.

J&K State falls in Himalayan geographical zone. There are nine geographical zones of India based on physiography, climate and hydrology as followed by Rao (1984). The Himalayan zone includes partly alpine and partly subalpine mountain regions of Himalaya; generally cool weather in the summer and very cold snowy weather in winter. Indian Himalaya extends from Kashmir through Himachal Pradesh, parts of Uttar Pradesh, and West Bengal, Sikkim, Bhutan, Arunachal Pradesh and Nagaland. Himalaya is a biodiversity hotspot (Conservation International 2017). Of the estimated 10,000 species of plants in the Himalaya hotspot, about 3,160 species (71 genera) are endemic (Phoenix *et al.* 2006).

The State is situated in the northern part of India in the western Himalayan mountain ranges. The State has almost every description of climate in the world ranging from the tropical to the arctic (Jacob 1950). The State is divided into three geographically

and climatically different divisions, viz. Ladakh (cold desert), Kashmir (temperate) and Jammu (sub-tropical). So, faunistic studies of mosquitoes of the State are of paramount zoo-geographical importance and have to be updated in the light of latest taxonomic changes and environmental conditions.

Keeping these in view, mosquito surveys were made in Jammu and Kashmir State from 2011 to 2016. DNA bar-coding was incorporated as one of the objectives as this could be a new and additional tool for identification (molecular identification) of species and can be used effectively particularly when the material for morphological identification is not sufficient and when one species is difficult to distinguish from other species morphologically (viz., sibling species).

### **1.1. Objectives:**

- 1) To document mosquito species from the three divisions of Jammu and Kashmir State.
- 2) To study the diversity of the mosquitoes in the divisions and larval habitats.
- 3) To study the altitudinal distribution of the mosquitoes in the State.
- 4) To DNA barcode the mosquitoes of the State.



## *2. Review of Literature*

Mosquitoes are probably the most notoriously undesirable arthropods with an almost worldwide distribution. They are being found throughout the tropics and temperate regions but absent only from a few islands and Antarctica. (Rueda 2008)

The word 'mosquito' is of Spanish or Portuguese origin and it is probably correct to say that it must have come originally from Spanish or Portuguese America. But its modern use more probably has come from North America. The French name 'moustique' according to the Oxford English Dictionary is a metathetic equivalent of the Spanish 'mosquito'. Mosquito is now the name in common use in English for biting flies of the family Culicidae, suborder Nematocera, order Diptera. Formerly the name for the mosquito was 'gnat' or '*Culex*'. The change only took place about 1900, when as a result of Ross's discovery of the malaria cycle in mosquito the importance of these insects to man became realised for the first time. Thus, Kirby and Spence (1870) in their People's Edition are not at all sure that the foreign 'mosquito' is the same genus as English *Culex pipiens*. Hurst (1890) uses for his paper the title *The Life Development and History of a Gnat (Culex)* and Giles (1900) entitles his book *A Handbook of the Gnats or Mosquitoes*. But Theobald (1901) and practically all English writers since use simply the name mosquito.

*Burmaculex antiquus* (new genus and species) is the oldest known fossil mosquito described from a single partially preserved adult female in Burmese amber; the age of which was estimated between Upper Albian and Turonian, 100-90 million years ago but the origins of the Culicidae are notably older (Borkent & Grimaldi 2004).

The earliest references in literature are some passages in Aristotle (384-322 B.C.) relating to 'empis', a name which is generally accepted as signifying the mosquito. In Aristotle's *Historia Animalium* (some books translated into English by Peck (1943)) it is mentioned that some animals live at first in water and subsequently change their form as is the case with 'empis'. Approximately 350 years later are the writings of C. Plinius Secundus (A.D. 23-79), a Roman author. There are some dozen or so references to 'culices' in the thirty-seven books of his great natural history. From A.D. 200 to A.D. 1200 has been described as the dark ages for biology, and following on Plinius Secundus it is not until the seventeenth century that naturalists again begin to write about the

mosquito. Though from now till Ross's discovery the mosquito was never considered of any importance it still had features which gave it a special interest to the early naturalists. These centered especially about the origin of the fly from the worm-like aquatic stage, its complicated organ of puncture, the proboscis, and a character for which the insect has always been notorious, its hum.

Linnaeus was the first who in 10th edition of his *Systema Naturae*, published in 1758 started naming of mosquitoes on the binomial system, as does that for all forms of animal life. He gave the scientific name to mosquitoes by erecting the genus *Culex* to include what he considered as mosquitoes. Of these only two are known today as true mosquitoes and have valid names, viz., *Culex pipiens* L. 1758 and *Aedes aegypti* (L.) 1762. The latter had actually been named *Culex aegypti* by Linnaeus. Fabricius (1805) in his revision of the Diptera, presented a list of described species of mosquitoes classified on the basis of their mouth-parts. They number fifteen, including one or two that were possibly not mosquitoes. Meigen (1818) described thirteen more species and erected two more genera, *Anopheles* and *Aedes* in addition to the original genus *Culex* of Linnaeus. By 1889 about fifty-six species of mosquitoes had been described from Europe, North Africa and Egypt. And little was done on the taxonomy of mosquitoes until the classical discoveries during the last two decades of the 19<sup>th</sup> century of Manson (1879), Ross (1899) and Reed and Carroll (1901) that established their involvement in the transmission of filaria, malaria and yellow fever, respectively and initiated a drive to collect, name and classify these insects. Collections began to pour in from different tropical countries. Medical men and others interested in the new developments not only collected but intensively studied the many species in their natural surroundings. For the first time careful detailed descriptions were given and structural characters studied and made use of identification and classification which resulted in the publication of several monographs and catalogues. The family Culicidae today includes 3,557 species which are divided among two subfamilies (Anophelini and Culicini) and 112 genera (Harbach 2017).

## 2.1. Mosquito Studies in World

The first to attempt to correlate the mass of published descriptions in a treatise on world species was Giles, who in 1900 published his "Handbook of the Gnats or

Mosquitoes” and himself added some seventeen new species mostly from India. There can be no doubt, however, that it was Fred. V. Theobald, appointed in 1899 in the British Museum (Natural History), who in the five volumes of his monograph published over the years 1901-10 opened up the study of mosquitoes to workers all over the world. Brunetti (1907) compiled an annotated catalogue to provide a systematic list of the mosquitoes recorded from the Oriental Region, which included works spanning from that of James (1899) to Banks (1906). This catalogue also includes the work on the “*Anopheles Mosquitoes of India*” by James and Liston (1904) that deals with the distribution and classification of Indian species of *Anopheles*, besides providing notes on the eggs, larvae, habitats, collecting, mounting and preserving of specimens. Contemporary to this catalogue is the monograph of Culicidae of Malaya published by Leicester in 1908.

Fred. V. Theobald in his monograph *A Monograph of the Culicidae or Mosquitoes* of 5 volumes introduced many new generic names in an effort to classify numerous new species into seemingly natural groups. This monograph provides a lengthy list of works and essays, extensive information on life history, food, habitat, natural enemies and geographical distribution of the mosquitoes of the world. Nevertheless, the splitting of mosquitoes into many genera by Theobald who placed the 1,050 species known to him in 149 genera rendered his classification neither practical nor natural.

Significant changes were made toward a much more conservative system of classification during the following two decades, remarkable among them being the works of Edwards who published a series of papers from 1911 to 1932. The compendium of all his works was published as the “*Genera Insectorum. Family Culicidae*” (Edwards 1932). In this master treatment Edwards included the study of immature stages as well, along with the biological characteristics of the species that made it possible for him to develop a conservative system of classification of Culicidae, thus reducing the enormous number of genera from 149 to 30. He placed all the vectors of human malaria in the genus *Anopheles* rather than in many genera recognized by Theobald.

The current subgeneric classification of genus *Anopheles* is based primarily on the number and positions of specialized setae on the gonocoxites of the male genitalia, and this basis of classification has been accepted since it was introduced by Sir (Samuel)

Rickard Christophers in 1915 who worked on anopheline mosquitoes of British India. Christophers proposed three generic subdivisions, which Edwards (1921) and Root (1923) formally recognized as subgenera *Anopheles*, *Myzomyia* (= *Cellia*) and *Nyssorhynchus*. Edwards adopted this system and added subgenus *Stethomyia* in his classical treatise on family Culicidae published in 1932. This system recognized *Kerteszia* as an informal group within subgenus *Nyssorhynchus*. *Kerteszia* was elevated to subgeneric status by Komp (1937) and subgenus *Lophopodomyia* was proposed by Antunes (1937). In 2005, subgenus *Baimaia* was introduced by Harbach and his colleagues and very recently Harbach & Kitching (2016) resurrected subgenus *Christya* from synonymy with *Anopheles* Meigen for *An. implexus* (Theobald) and *An. okuensis* Brunhes, Le Goff & Geoffroy.

Culicine mosquitoes of the Oriental region, particularly that of the British India, were investigated extensively by Barraud who brought out “A Revision of the Culicine Mosquitoes of India” in 26 parts through publications spanning from 1923 to 1929. In these revisions many new species were described, besides descriptions made of larvae for many of the species. He compiled his work and including the treatment of Culicidae by Edwards (1932) and Senior-White (1923), published in 1934 in his classical volume “Fauna of British India including Ceylon and Burma”. It was during this period that biological characteristics became an important element in the classification giving rise to the concept of the biological species in mosquito classification.

The final number of species as given by Smart (1940) for Theobald’s revision (1910) was 1050, and that in Edwards’s ‘Genera Insectorum’ (1932) 1400. This big boost in the number of known species is especially apparent in the case of certain countries. The number of species described from Africa and its islands before 1900 was five. In 1941 the number listed in Edwards’s monograph of Ethiopian mosquitoes was 405 (*Anopheles*, 86; *Aedes*, 132; *Culex*, 99; other genera, 88). The number known from India prior to 1900 (that is before Giles’s 1st edition) seems to have been about three or four. In Barraud’s revision (1934) it was 245 (sixty-eight being new species under that author’s name).

The comprehensive works of Howard, Dyar and Knab (1912-1917), Dyar (1928) and Matheson (1944) contributed to the knowledge of mosquitoes of North America, but

it was Carpenter and LaCasse (1955) who brought together this information in the form of a monograph and closely followed the classification of Edwards (1932).

Belkin organized a project on the “Mosquitoes of Middle America” that began in 1962 and continued till 1980 and through which many papers on Neotropical mosquitoes were published. Belkin’s contribution to the classification of Culicidae was, however, made much earlier when he published the monograph on *Mosquitoes of the South Pacific* (Belkin 1962). This work is considered as the best example of a modern taxonomic study of an entire mosquito fauna as it includes all three levels of taxonomic study i.e., alpha, beta and gamma taxonomy. Belkin believed that the 155 named zoological species of true mosquitoes that he treated in the *Mosquitoes of the South Pacific* represented less than half the fauna, because practically no collection had been done in the interior of any of the islands. Because Belkin described as new or resurrected from synonymy 77 or 50% of these 155 species, the number of species known from the region before his studies may have been as little as 25% of the actual fauna. It would be estimated, then, that somewhere between 25 and 50% of mosquito species were known (Zavortink 1990). It was the first major work on Culicidae in which the pupae were treated as extensively as the larvae.

Edwards made three subfamilies of Culicidae in which Dixidae and Chaoboridae (midges) were two subfamilies and “true mosquitoes” as third subfamily, Culicinae. He divided subfamily Culicinae into three tribes, Anophelini, Toxorhynchitini (as Megarhinini) and Culicini and divided the tribe Culicini into five groups, *Sabethes*, *Uranotaenia*, *Theobaldia*, *Aedes* and *Culex*. Stone (1957) removed Dixidae and Chaoboridae from Culicidae and confined family Culicidae to the Culicinae of Edwards (1932). This brought about changes in subfamily and tribal designations that were adopted by Stone *et al.* (1959) in their world catalogue of mosquitoes and recognized three subfamilies, Anophelinae, Culicinae and Toxorhynchitinae and two tribes, Culicini and Sabethini of subfamily Culicinae. However, Belkin (1962) disagreed with Stone *et al.* (1959) and retained Anophelini and Toxorhynchitini but made 10 tribes in place of Culicini of Edwards and continued to treat Dixidae and Chaoboridae as subfamilies of Culicidae until Knight and Stone (1977) once again removed them from the family. This resulted in the division of family Culicidae into three subfamilies, Anophelinae, Culicinae

and Toxorhynchitinae, and subfamily Culicinae into the 10 tribes recognized by Belkin (1962). But Mattingly (1969, 1971, 1981) followed Stone *et al.* (1959) by accepting the division of Culicinae into 2 tribes only, i.e. Culicini and Sabethini and Service (1993) utilized Mattingly's tribal divisions as a matter of convenience. However, for the most part mosquito taxonomists accepted all of the tribal groups introduced by Belkin, including Toxorhynchitini (Harbach & Kitching 1998; Mitchell *et al.* 2002).

If leaving aside the proposals of Reinert *et al.* (2004, 2006, 2008, 2009) who divided tribe Aedini into 81 genera instead of 12, surprisingly few changes have been made in the recognition of mosquito genera since Edwards (1932) while as the number of formally recognized species has increased more than doubled, from 1400 to 3557.

But in the analysis of Reinert *et al.*, taxa that were widely accepted as genera (i.e. *Armigeres*, *Eretmapodites*, *Haemagogus*, *Heizmannia*, *Opifex*, *Psorophora*, *Udaya* and *Zeugomyia*) were taken as the reference clades for equivalence, monophyletic clades of equivalent rank to those taxa were afforded generic rank and monophyletic groups within those clades were afforded subgeneric rank. On this basis, and because most of phylogenetic systematists do not accept paraphyletic or polyphyletic classifications, Reinert *et al.* raised many of the subgenera and species groups of tribe Aedini to generic level. Based on the series of morphological and phylogenetic studies of Reinert and his colleagues (Reinert 1999, 2000a, 2000b; Reinert *et al.* 2004, 2006, 2008, 2009) and the addition of genera *Borichinda* Harbach & Rattanarithikul (Harbach *et al.* 2007), *Nyctomyia* (Harbach 2013) (replacement name for *Nyx* Harbach & Linton (Harbach *et al.* 2013)), the internal classification of the tribe Aedini was radically reorganized and now formally comprises 81 genera and 48 subgenera compared to 12 genera and 56 subgenera that were recognized prior to these studies and by this total genera of the family Culicidae have gone up to 112.

Parallel to the Mosquitoes of the Middle America Project was the Southeast Asia Mosquito Project (SEAMP). Beginning in 1961, a major study of the mosquito fauna of Thailand was undertaken by the United States Army Medical Component – Southeast Asia Treaty Organization (SEATO), Bangkok in connection with studies on a number of mosquito-borne diseases. It rapidly became abundantly clear that a major revision of the

mosquito fauna of the entire area would be needed. The SEATO collections became the nucleus for the Southeast Asia Mosquito Project. The Project was formally organized at the United States National Museum in October 1964 (De Meillon 1969).

This project was instrumental in documenting valuable information of mosquitoes, many of them medically important, besides description of many of the unknown life stages of different species, and the distribution and ecology of many mosquito species. Such information together with the vast mosquito material accumulated for the study made it possible to bring out state or regional level revisions of mosquitoes like those of Bram (1967), Delfinado (1967, 1968), Reinert (1970), Sirivanakarn (1972), and Huang (1972). Tanaka *et al.* (1979) made a revision of the mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Island) and Korea. Besides these, the mosquito fauna of the Philippines have been extensively covered in a monograph by Basio (1971). State and regional level revisions of mosquitoes are valuable and extremely useful to studies in mosquito biology and control in the particular areas involved and each can contribute valuable information to the overall picture of mosquito biology and distribution (Nielsen 1980).

Some of the group and subgroups of mosquitoes which are medically important have also been extensively studied in this regard. The subgroup vishnui in the subgenus *Culex* is comprised of important vectors of Japanese encephalitis (JE) was studied by Reuben *et al.* (1994) and prepared a key to identify the species of this complicated subgroup. Harrison (1980) has studied the important minimus group of *Myzomyia* series of subgenus *Cellia* and resolved the taxonomic status of the different species, together with the distribution and bionomics in Southeast Asia. Chen *et al.* (2002) carried out molecular and morphological studies on the minimus group in southern China and have elucidated its taxonomic, distributional and vectorial status. Another important group that comprises malaria vectors in Southeast Asia is the leucosphyrus group, a taxonomic revision of which has been made by Sallum *et al.* (2005). Rattanakul & Green (1986) studied *An. maculatus* group and prepared a key to adult females of the group while as Walton *et al.* (2007) studied genetic diversity and made molecular identification of the



species using the ITS2 region of rDNA. Trung *et al.* (2004) and Manguin *et al.* (2008) published about the bionomics and distribution of malaria vectors of Southeast Asia.

**In India**, G. M. Giles, was the first who in 1900 published his *Handbook of the Gnats or Mosquitoes* and himself added some seventeen new species mostly from India.

The Indo-Pakistan subcontinent, birth place as it was of Ross's discovery (i.e., discovery of malaria vector), started very late, so much so that at the time when Theobald published his Monograph of World Culicidae which contained a whole treasure of information on mosquitoes from other parts of the world, only very few species were known from India. Mosquito Taxonomy in India remained in slack waters for a long time. Even Giles's plea for collective investigation of Indian Culicidae, which appeared in 1901 the year that saw the publication of Theobald's Monograph of World Culicidae, did not have the desired effect. But fortunately this state of affairs did not last very long and soon workers in India also engaged themselves in a similar pursuit. (Qutubuddin 1960)

Malaria, the human malady that took a very heavy toll of life and cost India a great deal in terms of annual revenue was given top priority among the insect-borne diseases and consequently work started on mosquitoes of the genus *Anopheles*. A full-fledged research institute started functioning under the name of the Malaria Survey of India (later redesignated as the Malaria Institute of India) first at Kassauli and then at New Delhi. The main function of the institute was to train doctors regarding malaria and the identification of important mosquito vectors and their control. Basic research such as the taxonomy of anopheline adults and larvae was also carried out in the institute. Before Christophers's (1933) monographic work, James and Liston's (1904) volume on Anophelines of India was largely used as the guide to the identification of species of *Anopheles*.

Two monographs by Christophers on anophelines and Barraud on culicines, of the Indian subcontinent published in 1933 and 1934 respectively were the result of all the taxonomic studies in the subcontinent made by the earlier workers and the authors themselves. Noteworthy among the earlier works before these two monographs on culicidae are James and Liston's (1904, 1911) volumes on Anophelines of India; the Annotated Catalogue of Culicidae and the Critical Review of the Genera in Culicidae by

Brunetti (1907-1920), and Larvae of Anopheline Mosquitoes with Full Description of those of the Indian Species by Puri (1931). Some more works worth to mention are of Gill (1917, 1920), Senior-White (1923), and Covell (1927).

Christophers worked on anophelines of India from 1911 to 1931, published a bunch of papers and described many species. Christophers (1922) studied development and structure of the terminal abdominal segments and hypopygium of the adult mosquito to establish homologies with the terminal segments of the larva. As mentioned above, the current subgeneric classification of genus *Anopheles* based primarily on the number and positions of specialized setae on the gonocoxites of the male genitalia, has been accepted since it was introduced by Sir (Samuel) Rickard Christophers in 1915. Among other works, Christophers's (1906) paper on the importance of larval characters in the classification of mosquitoes, that of Sinton and Covell (1927) on the relationship of morphology of buccal cavity to the classification of anopheline mosquitoes, and Christophers and Barraud's (1931) description of the eggs of Indian Anophelines are worth mentioning.

About two years before *Edwards' Synopsis of Adult Oriental Culicine Mosquitoes* (1922) was published in two parts, Capt. P. J. Barraud had received a Commission under the Indian Research Fund Association to make a general survey of the mosquitoes of India. This investigation unearthed such a large number of culicine mosquitoes new to science that Barraud started publishing a series of papers entitled "A Revision of the Culicine Mosquitoes of India" in the Indian Journal of Medical Research and continued to do so for over a decade from 1923 onwards until in 1934 appeared his Fauna of British India (including Ceylon and Burma) devoted to the two tribes Megarhinini and Culicini. Christophers (1933) also came with a monograph on anophelines of British India and both the publications marked a land-mark in the history of mosquito studies in the sub-continent.

Barraud (1934) stated in his monograph - it is interesting to look back to 1900, when Colonel G. M. Giles, I.M.S., in reading a paper before the Bombay Natural History Society, stated that "Two years ago, when I took up the task of collecting the literature of the Culicidae, it is an actual fact that no more than four species were recorded as having

been found in all India. There was, in fact, hardly any other known country with such scanty records of the subject. The subjoined list includes 32 species, and I have little doubt the final total of species will be found to be not far off a hundred, as new species are constantly turning up.”

But the number (including 43 Anophelini) has now reached a total of 288, of the three tribes included in the subfamily Culicinae, not counting named varieties, some of which may eventually be shown to be distinct species (Barraud 1934).

The year 1934 marks the end of an era of very active taxonomic research on Culicidae by virtue of which mosquitoes became one of the best known groups of insects in the area. After 1934, not many comprehensive biosystematics studies of Indian Culicidae have been undertaken. Most of the taxonomic studies, if done, dealt exclusively with Anopheles mosquitoes possibly due to an unprecedented high significance tagged with malaria in the country!

From 1934-1959 following species were added as mentioned by Qutubuddin (1960):

1) *Anopheles habibi* Mulligan and Puri 1936. 2) *An. stephensi mysorensis* Sweet and Rao 1937. 3) *Culex (Culex) parainfantulus* Menon 1944. (since sunk as a synonym of *infantulus* Edwards by Mattingly 1959). 4) *Uranotaenia husaini* Qutubuddin 1946. 5) *Cx. (Culiciomyia) stylifurcatus* Carter and Wijesundara 1948 (synonymised by Mattingly (1955a) with *spathifurca* Edw.). 6) *Cx. (Mochthogenes) campilunati* C. and W. 1948. 7) *Aedes (Aedes) seculatus* Menon 1950. 8) *Uranotaenia mattinglyi* Qutubuddin 1951. 9) *Aedes (Aedes) petroelephantus* Wijesundara 1951. 10) *Aedes (Aedes) spermathecus* Wijesundara 1951. 11) *Aedes (Aedes) carteri* Wijesundara 1951 (sunk as synonym of *seculatus* Menon by Stone, 1956 (1957). 12) *Aedes (Stegomyia) patricease* Mattingly 1955. 13) *Culex (Neoculex) quettensis* Mattingly 1955. 14) *Cx. (Culex) afromidi* Qutubuddin 1956. 15) *Heizmannia reidi* Mattingly 1957. 16) *Aedes (Paraedes) menoni* Mattingly 1958.

Fifteen species and one variety, *An. stephensi mysorensis* were described new but later 4 species, *An. habibi* Mulligan and Puri 1936, *Cx. (Cux.) parainfantulus* Menon 1944,

*Cx. (Cui.) stylifurcatus* Carter and Wijesundara 1948, and *Cx. (Cux.) afromidi* Qutubuddin 1956 were found synonyms of *An. (Anopheles) claviger* (Meigen 1804), *Cx. (Lophoceraomyia) infantulus* Edwards 1922, *Cx. (Cui.) spathifurca* (Edwards 1915), and *Cx. (Oculeomyia) infula* Theobald 1901 species respectively.

Due to a fervent nationwide anti-malaria campaign between 1950 and 1980, hardly any attention was given to the non-anopheline mosquitoes, and the medical importance of culicine mosquitoes was almost completely overshadowed in terms of their taxonomic biodiversity, even though many of the culicine mosquitoes playing key roles in transmitting several deadly and/or debilitating diseases such as dengue, Japanese encephalitis, and lymphatic filariasis. Berlin (1972) rightly proposed for a systematic study of Indian Culicidae because of increasing mosquito-borne diseases, changing ecology and advances in mosquito systematics.

In terms of mosquito biodiversity, India is ranked fifth after Brazil, Indonesia, Malaysia and Thailand (Foley *et al.* 2007). Since 1980s some faunistic surveys were carried out in various parts of India although fewer in numbers, detected many new species and new country records which are new additions to the mosquito fauna of India (Bhattacharyya *et al.* 2014). Research workers were mainly concentrated on a region wise survey of mosquitoes. Hussainy (1981) presented distribution records of 14 culicine species from Bastar district, Madhya Pradesh although the survey was made between October 1968 and September 1974. The important surveys/collections/descriptions of mosquito species done in India since 1980 are: Nagpal and Sharma 1987; Rajput and Singh 1987a, 1987b, 1987c, 1989, 1990; Rajput and Kulkarni 1990, 1991a, 1991b; Tyagi *et al.* 1991; Tewari and Hiriyani 1991, 1992, 1995; Reuben *et al.* 1993; Sagandeep *et al.* 1994; Khan *et al.* 1998; Jagbir *et al.* 1999; Bhattacharyya *et al.* 2000, 2002, 2003, 2004a, 2004b, 2005, 2007, 2008, 2009; Dutta *et al.* 2003; Rajavel *et al.* 1998, 2004, 2005a, 2005b, 2005c, 2005d, 2011; Rajavel & Natarajan 2006, 2011. The recent review of the published studies showed that the Indian mosquito fauna comprises 393 species (anophelines - 61, culicines - 332) in 49 genera and 41 subgenera with latest taxonomic situation (Bhattacharyya *et al.* 2014).

Some of the medically important group and subgroups or species complexes of mosquitoes have also been extensively studied in India. The subgroup vishnui in the subgenus *Culex* is significant vectors of Japanese encephalitis and Chandipura virus diseases. The adult morphological identification of these species had been very much difficult or impossible although they were well differentiated in larval stages. Reuben *et al.* (1994) described adult morphological characteristics, towards their species identification of this complicated subgroup. Spatial distribution and molecular characterization of Maculatus Complex has been investigated in depth for possible role in malaria transmission specific to northeast India. Singh *et al.* (2012) recorded six species in this region, namely, *An. maculatus* (most abundant), *An. willmori*, *An. sawadwongporni*, *An. rampae*, *An. dravidicus* (restricted distribution) and *An. pseudowillmori* of the nine species of this complex known in the world. *An. culicifacies* *s.l.* has been widely studied in India regarding its biology and genetics (Sharma 2006; Barik *et al.* 2009), and presently is considered a species complex with five informally designated species A, B, C, D and E (Goswami *et al.* 2005, Manonmani *et al.* 2007). *Anopheles fluviatilis* *s.l.* also extensively studied and also considered a species complex comprising four sibling species, i.e., S, T, U, and V (Singh *et al.* 2004, Manonmani *et al.* 2003, Sharma *et al.* 1995). But Kumar *et al.* (2013) have found the members of the *Anopheles fluviatilis* species complex conspecific (belong to a single species) through DNA barcodes. *Anopheles minimus* *s.l.* known to be the predominant malaria vector in the Oriental Region (Garros *et al.* 2006) was extensively studied in Assam and Bengal for its bionomics and control (Muirhead Thomson 1941; Senior-White *et al.* 1945; Gilroy 1958). *An. fluviatilis* as known to be primary vector of malaria in the north-east India (Mohapatra *et al.* 1998) is now considered a seasonal variant of *An. minimus* (Singh *et al.* 2010). *Anopheles dirus* *s.l.* is a species complex of eight sibling species in which only *An. baimaii* and *An. elegans* are recognized in India (Subbarao 1998). In earlier records what was initially described as *An. balabacensis balabacensis* and later *An. dirus* (species D) in India are now referred as *An. baimaii* for all purposes. *Anopheles sundaicus* *s.l.*, an important vector of malaria is currently a complex of four species, i.e., *An. sundaicus* *s.s.*, *An. epiroticus* Linton & Harbach (formerly species A), *An. sundaicus* species D and *An. sundaicus* species E (Sinka *et al.* 2011) but in India only one species is prevalent in Kutch area of Gujarat, and

Andaman and Nicobar islands characterized to be cytotype species D (Alam *et al.* 2006; Dev & Sharma 2013). It is largely a brackish water species but breeding in fresh water collections has also been recorded. *Anopheles stephensi*, an important urban malaria vector is not a species complex but comprises three ecological variants, ‘type form’, ‘intermediate form’ and variety ‘mysorensis’ characterized by egg morphometrics (Rao *et al.* 1938; Dev & Sharma 2013). Only the ‘type form’ is known as an efficient vector of malaria.

Thirty two species known in India can transmit various pathogens to humans shown in Table 1 (Bhattacharyya *et al.* 2014; Kumar *et al.* 2016). Six mosquito-borne diseases, malaria, Japanese encephalitis, dengue, chikungunya, West Nile, and filariasis have been recorded in India. Malaria is transmitted by *Anopheles* species belonging to subgenus *Cellia* only. Thirteen species of *Anopheles* have been found vectors for malaria. Dengue and Chikungunya is transmitted only by the two species of genus *Stegomyia*. Japanese encephalitis is transmitted by 16 species, West Nile by 2 species and filariasis by 4 species. Mosquito taxonomy provides essential inputs for vector control.

**Table 1: Vectors of mosquito-borne diseases in India**

<b>Malaria:</b>		
<i>Anopheles (Cellia) culicifacies s.l.</i>	<i>An. (Cel.) sundaicus s.l.</i>	<i>An. (Cel.) varuna</i>
<i>An. (Cel.) baimaii</i>	<i>An. (Cel.) annularis s.l.</i>	<i>An. (Cel.) maculatus s.l.</i>
<i>An. (Cel.) fluviatilis s.l.</i>	<i>An. (Cel.) jeyporiensis</i>	<i>An. (Cel.) subpictus</i>
<i>An. (Cel.) minimus s.l.</i>	<i>An. (Cel.) philippinensis</i>	
<i>An. (Cel.) stephensi</i>	<i>An. (Cel.) nivipes</i>	
<b>Japanese encephalitis:</b>		
<i>Culex (Culex) vishnui</i>	<i>Cx. (Cux.) whitmorei</i>	<i>An. (Cel.) subpictus s.l.</i>
<i>Cx. (Cux.) pseudovishnui</i>	<i>Cx. (Oculeomyia) bitaeniorhynchus</i>	<i>Mansonia (Mansonioides) annulifera</i>
<i>Cx. (Cux.) tritaeniorhynchus</i>	<i>Cx. (Ocu.) infula</i>	<i>Ma. (Mnd.) indiana</i>
<i>Cx. (Cux.) fuscocephala</i>	<i>Cx. (Ocu.) epidesmus</i>	<i>Ma. (Mnd.) uniformis</i>
<i>Cx. (Cux.) quinquefasciatus</i>	<i>An. (Anopheles) barbirostris s.l.</i>	
<i>Cx. (Cux.) gelidus</i>	<i>An. (Ano.) peditaeniatatus</i>	
<b>Dengue:</b>		
<i>Stegomyia (Stegomyia) aegypti</i>	<i>St. albopicta</i>	
<b>Chikungunya:</b>		
<i>St. (Stg.) aegypti</i>	<i>St. albopicta</i>	
<b>West Nile:</b>		
<i>Cx. (Cux.) vishnui</i>	<i>Cx. (Cux.) quinquefasciatus</i>	
<b>Filariasis:</b>		
<i>Cx. (Cux.) quinquefasciatus</i>	<i>Ma. (Mnd.) uniformis</i>	
<i>Ma. (Mnd.) annulifera</i>	<i>Downsiomyia nivea</i>	

Source: Bhattacharyya *et al.* 2014; Kumar *et al.* 2016

## 2.2. Mosquito Studies in Jammu and Kashmir

As mentioned before, Giles (1900) was the first who carried out mosquito studies in India but regarding Jammu and Kashmir State he had not mentioned any mosquito species. James & Liston (1904, 1911) were the first who reported two anopheline species *An. willmori* and *An. turkhudi* from Kashmir in their monograph on anopheline mosquitoes of India. After James & Liston, other workers who reported mosquito species from Jammu and Kashmir State were Adies (1913), Gill (1920), Covell (1927), Senior-White (1923), etc. till Christophers (1933) came with a monograph on anophelines. Adies (1913) made an exhaustive search extending over a period of six months – from 21<sup>st</sup> April to 26<sup>th</sup> October, 1912 throughout the Valley reported two anopheline species, *An. barianensis* and *An. willmori*. However, Gill (1920) studied malaria in Kashmir region and reported six species from the region, namely, *An. gigas*, *An. splendidus*, *An. stephensi*, *An. willmori*, *An. fluviatilis* and *An. lindesayi*. Ronald Senior-White (1923) mentioned *An. fluviatilis*, *An. splendidus*, *An. lindesayi*, *An. stephensi*, *An. willmori*, *An. turkhudi* in his *Catalogue of Indian Insects*. Christophers by himself reported 3 anopheline species (*An. turkhudi*, *An. barianensis*, and *An. willmori*) from Kashmir Valley and later compiled all the work carried out by him and other researchers regarding anophelines, published in 1931 in a research article and later in his classical volume “Fauna of British India including Ceylon and Burma”. As mentioned before, most of the work was focused on anophelines due to importance of malaria. Culicine mosquitoes from Jammu and Kashmir were apparently first reported by Barraud in his research articles then in his monograph which appeared in 1934 although before him some researchers like Sinton, etc. collected some species from Kashmir Valley.

After Christophers (1933) and Barraud (1934), the studies on mosquito fauna of Jammu & Kashmir were made by Puri (1936 & 1948), Jacob (1950), Nair (1973), and Rao *et al.* (1973). All the studies except Barraud (1934) and Rao *et al.* (1973) were made about anopheline fauna only due to high malaria prevalence in Jammu and Kashmir State and other parts of Indian subcontinent. Barraud (1934) made study on culicine fauna of mosquitoes while as Rao *et al.* (1973) made faunistic studies on both anophelines and culicines. Rao *et al.* carried out surveys from 1967 to 1969 regarding haematophagous



arthropods in western Himalayas, Sikkim and Hill districts of West Bengal and published their work in subsequent years (Rao *et al.* (1973), Bhat and Kulkarni (1983), and Rao (1984)). The mosquito species reported by these earlier workers is given in Table 2.

Major V.P. Jacob (1950) surveyed whole Jammu and Kashmir State between August 1948 and October 1949 and did a good study about malaria and anopheline mosquitoes. Jacob has not done any incrimination of a vector but has pointed out that a malaria survey made by the Epidemiology Department, Jammu which was far from complete and confined to Jammu division have incriminated *An. (Cel.) culicifacies* as a vector. Of the 1,091 specimens dissected in 1946, two showed gland infections and 3 oocysts in the gut. *An. (Cel.) culicifacies* was common in the Jammu region and was noted as a vector but it was not found at locations above 4000 feet (1212 metres) msl. The only species found in any appreciable density above 4000 feet (1212 metres) (in a malarial area) was *An. (Cel.) fluviatilis*, so was regarded as a vector at the high altitudes. Later, C. P. Nair (1973) incriminated *An. fluviatilis* as a vector in Karnah Tehsil – Kashmir division. One *An. fluviatilis* out of 56 dissected showed sporozoites, the location being around at an elevation of 1829 m. Previously, Jacob (1950) found malaria transmission up to the elevation of 1829-1981 m (in Kamalkote village located at 1829-1981 m). Nair concluded that unstable endemic malaria existed in the region up to 2134 m altitude with low incidence at elevations higher than 1829 m.

Rao *et al.* (1973) carried out the mosquito collection in Jammu and Kashmir State from July 1967 to December 1969 under the title *A survey of haematophagous arthropods in Western Himalayas, Sikkim and Hill districts of West Bengal*. Collection was carried out in seven districts, namely, Anantnag, Srinagar, Baramullah, Ladakh, Doda, Rajouri, and Udhampur of all the three divisions. Two research articles (Rao *et al.* 1973, Bhat and Kulkarni 1983) were published on the mosquito fauna of the State. Anophelines collected were also mentioned by T. R. Rao (1984) in his book “Anophelines of India”. In first paper (Rao *et al.* 1973), 29 mosquito species (8 anophelines, 21 culicines) were reported. In both anophelines and culicines one species was mentioned ‘undetermined’ and *Cx. pseudovishnui* was also reported. In second research paper, authored by Bhat and Kulkarni (1983), only 27 species (7 anophelines, 20 culicines) were reported. *Cx.*

*pseudovishnui* was not mentioned but one species *Cx. sp.* (undetermined) was mentioned. T. R. Rao (1984) mentioned 9 species of anophelines instead of 7 species reported by Bhat and Kulkarni (1983) or 8 species reported by him earlier in his own research paper, Rao *et al.* (1973). The additional two species were *An. turkhudi* and *An. splendidus*. So, conclusively total 28 species were reported by Rao and his colleagues given in Table 2.

**Other mosquito studies carried out in Jammu and Kashmir:** Some studies confined to vector species and vector borne diseases have been made by Verma & Magotra (1976), Mathew *et al.* (1977), Dwivedi *et al.* (1979), Kaul *et al.* (1995), Sharma and Srivastava (1995), Padbidri *et al.* (1996), Sidhu *et al.* (2015), and Gupta *et al.* (2016). Among these, Mathew *et al.* (1977) and Padbidri *et al.* (1996) studied dengue outbreaks in Jammu division occurred in 1974 and 1993 respectively. Mosquito species, *Stegomyia aegypti* were collected by both for dengue antigen detection but found negative. And Sharma and Srivastava (1995) studied seasonal trends in population density of malaria vectors, *Anopheles culicifacies* and *Anopheles fluviatilis* in rural areas of Kathua district of Jammu division during 1989 and 1990 and observed the highest density for *Anopheles culicifacies* in the month of August while as for *Anopheles fluviatilis* in the month of February.

It is mentioned in paragraph 2 of *Introduction* part that from about four decades (from 1973) not any faunistic study regarding mosquitoes was made in the State and the faunistic studies made by Rao *et al.* (1973) was the last made on Jammu and Kashmir mosquitoes. However, recently a study on tree hole mosquitoes of Kashmir was done by Jebanesan *et al.* (2012) who reported *Aedes aegypti*, *Aedes albopictus*, *Anopheles barberi*, *Anopheles stephensi*, *Culex taeniorhynchus* and *Culex fatigans* species. This was not considered as **last** collection due to two main reasons: i) it was a small study (only three places were visited and six species were collected), and ii) two mosquito species, *An. barberi* and *Cx. taeniorhynchus* were reported wrongly. *An. barberi* is found in North America not in Kashmir or J&K, and *Cx. taeniorhynchus* is not a species at all. As per our guesswork, *An. barberi* might be *An. bariensis* and *Cx. taeniorhynchus* might be some other species.

**Table 2: Total number of mosquito species reported in previous studies**

S. No.	Taxa	Christophers (1933)	Barraud (1934)	Puri (1936, 48)	Jacob (1950)	Nair (1973)	Rao <i>et al.</i> (1973, 83, 84)	Jebanesan <i>et al.</i> 2012
1	<i>Aedimorphus vexans</i> (Meigen 1830)						L	
2	<i>Anopheles</i> ( <i>Anopheles</i> ) <i>barianensis</i> James 1911	K						
3	<i>An.</i> ( <i>Ano.</i> ) <i>gigas</i> ssp. <i>simlensis</i> James 1911	K			K		K	
4	<i>An.</i> ( <i>Ano.</i> ) <i>hyrcanus</i> (Pallas 1771)					K		
5	<i>An.</i> ( <i>Ano.</i> ) <i>lindesayi</i> Giles 1900	K		J	JK		J	
6	<i>An.</i> ( <i>Cellia</i> ) <i>annularis</i> van der Wulp 1884	K		J	JK	K	JK	
7	<i>An.</i> ( <i>Cel.</i> ) <i>culicifacies</i> Giles 1901			J	JK	K	J	
8	<i>An.</i> ( <i>Cel.</i> ) <i>dthali</i> Patton 1905				JK			
9	<i>An.</i> ( <i>Cel.</i> ) <i>fluviatilis</i> James 1902	K		J	JK	K	J	
10	<i>An.</i> ( <i>Cel.</i> ) <i>leucosphyrus</i> Donitz 1901					K		
11	<i>An.</i> ( <i>Cel.</i> ) <i>maculatus</i> Theobald 1901			JK	JK			
12	<i>An.</i> ( <i>Cel.</i> ) <i>moghulensis</i> Christophers 1924				K			
13	<i>An.</i> ( <i>Cel.</i> ) <i>splendidus</i> Koidzumi 1920	K		J	JK	K	√	
14	<i>An.</i> ( <i>Cel.</i> ) <i>stephensi</i> Liston 1901			J	JK			K
15	<i>An.</i> ( <i>Cel.</i> ) <i>subpictus</i> Grassi 1899			J	JK		J	
16	<i>An.</i> ( <i>Cel.</i> ) <i>turkhudi</i> Liston 1901	K			JK		√	
17	<i>An.</i> ( <i>Cel.</i> ) <i>willmori</i> (James 1903)	K				K	JK	
18	<i>Bruceharrisonius christophersi</i> Edwards 1922		K					
19	<i>Collessius</i> ( <i>Collessius</i> ) <i>elsiae</i> (Barraud 1923)						J	
20	<i>Co.</i> ( <i>Col.</i> ) <i>shorti</i> (Barraud 1923)		K					
21	<i>Culex</i> ( <i>Barraudius</i> ) <i>modestus</i> Ficalbi 1890		K					

22	<i>Cx. (Culex) barraudi</i> Edwards 1922						J	
23	<i>Cx. (Cux.) fuscocephala</i> Theobald 1907						J	
24	<i>Cx. (Cux.) mimeticus</i> Noe 1899		K				JK	
25	<i>Cx. (Cux.) quinquefasciatus</i> Say 1823						JK	K
26	<i>Cx. (Cux.) theileri</i> Theobald 1903		K				L	
27	<i>Cx. (Cux.) vagans</i> Wiedemann 1828		K				KL	
28	<i>Cx. (Cux.) vishnui</i> Theobald 1901						J	
29	<i>Cx. (Culiciomyia) pallidothorax</i> Theobald 1905						J	
30	<i>Cx. (Cui.) viridiventer</i> Giles 1901		K				JK	
31	<i>Cx. (Maillotia) hortensis</i> Ficalbi 1889						L	
32	<i>Cx. (Oculeomyia) bitaeniorhynchus</i> Giles 1901						J	
33	<i>Culiseta (Allotheobaldia) longiareolata</i> (Macquart 1838)						L	
34	<i>Fredwardsius vittatus</i> (Bigot 1861)						J	
35	<i>Gilesius pulchriventer</i> (Giles 1901)		K					
36	<i>Ochlerotatus (Finlaya) oreophilus</i> Edwards 1916						L	
37	<i>Oc. (Fin.) sintoni</i> (Barraud 1924)		K				L	
38	<i>Oc. (Fin.) versicolor</i> (Barraud 1924)		K					
39	<i>Oc. (subgenus uncertain) pullatus</i> (Coquillett 1904)		K					
40	<i>Stegomyia (Stegomyia) aegypti</i> (Linnaeus 1762)*							K
41	<i>St. (subgenus uncertain) albopicta</i> (Skuse 1895)						J	K
42	<i>St. (subgenus uncertain) w-albus</i> (Theobald 1905)						J	
43	<i>Uranotaenia (Pseudoficalbia) unguiculata</i> Edwards 1913		K					
	<b>Genera - 12; Subgenera - 13</b>	<b>8</b>	<b>12</b>	<b>8</b>	<b>12</b>	<b>7</b>	<b>28</b>	<b>4</b>

√ Species mentioned by Rao (1984) in his book 'Anophelines of India' but collection sites not mentioned.

\*Also reported by Mathew *et al.* (1977) and Padbidri *et al.* (1996) but from Jammu division who worked on dengue outbreaks in the division.

### ***3. Materials and Methods***

### **3.1. Geography and Climate of India – General account**

**3.1.1: Country profile:** India, the 7<sup>th</sup> largest country in the world occurs completely in the northern hemisphere (8° 4' and 37° 6' N, 68° 7' and 97° 25' E) has an area of 3,287,263 km<sup>2</sup>. It forms a different geographical entity marked off by the mountains and the sea. In the north it is bounded by the Great Himalayas and while stretching southwards tapers off at the tropic of cancer into the Indian Ocean between the Bay of Bengal on the east and the Arabian Sea on the west. It measures about 3,214 km from north to south and about 2,933 km from east to west. The land frontier and coastline of India is about 15,200 km 7,516.6 km respectively. Of the coastline distance, peninsular India contributes 5,423 km and Andaman, Nicobar, and Lakshadweep islands contribute 2,094 km.

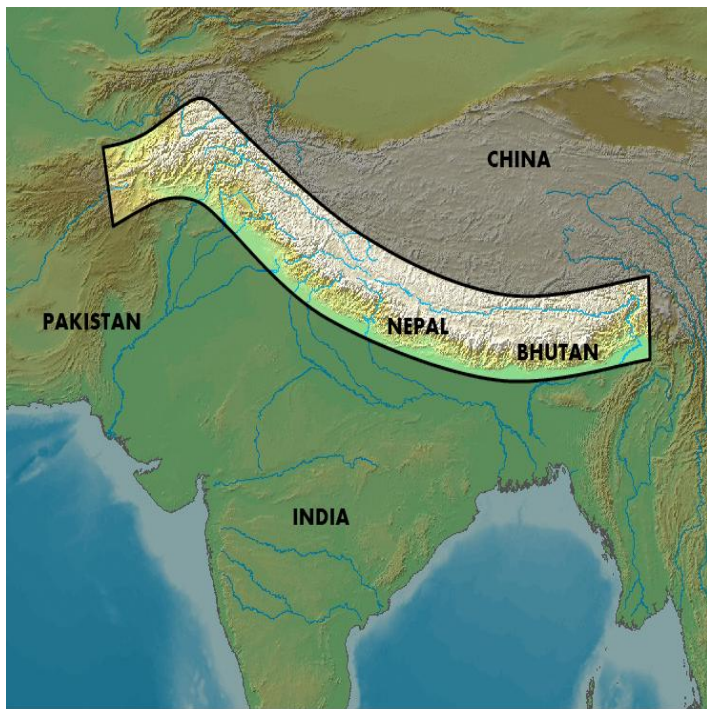
**3.1.2: Geographical Zones:** There are nine geographical zones of India based on physiography, climate and hydrology as followed by Rao (1984): 1. North Western Zone 2. Western Zone 3. Deccan Plateau 4. Gangetic Valley 5. East Central India 6. South Eastern Coastal Areas 7. Brahmaputra Valley 8. Himalayan Zone 9. Outlying Areas

**3.1.3: Physical Features:** The mainland comprises four regions: 1. The Great Mountain Zone (Himalayan Zone) 2. Plains of the Ganga and the Indus 3. The Desert Region 4. The Southern Peninsula.

**3.1.4: Climate:** In broad sense, the climate of India is tropical monsoon with four seasons (winter (January-February), summer (March-May), rainy (June-September), and post-monsoon (October-December)). India's climate is affected by two seasonal winds - the north-east monsoon and the south-west monsoon. The north-east monsoon, commonly known as winter monsoon blows from land to sea, whereas south-west monsoon, known as summer monsoon blows from sea to land after crossing the Indian Ocean, the Arabian Sea, and the Bay of Bengal. Most of the rainfall is brought by south-west monsoon.

### 3.2. Himalayan Zone (Fig. 1)

This zone is discussed here only because the state, Jammu and Kashmir where the present mosquito collection was carried out comes under it. The Himalayas comprise three almost parallel ranges interspersed with large plateaus and valleys, some of which, such as Kashmir and Kullu valleys, are fertile, extensive and of great scenic beauty. Some of the highest peaks in the world are found in these ranges. The mountain wall extends over a distance of about 2,400 km with a varying depth of 240 to 320 km. In the east, between India and Myanmar and India and Bangladesh, hill ranges are much lower. Garo, Khasi, Jaintia and Naga Hills, running almost east-west, join the chain to Mizo and Rkhine Hills running north-south.



**Fig. 1: The Himalaya** (Source: <https://en.wikipedia.org/wiki/Himalayas>)

The Himalayan zone includes partly alpine and partly subalpine mountain regions; generally cool weather in the summer and very cold snowy weather in winter. The Himalayas are the highest and also the youngest mountain ranges on Earth. It is characterized by extremes of elevation, slope and climate. With the largest snow and ice cover in the world outside the polar regions, the Himalayan region is one of the most

important mountain systems in the world and is referred to as the “third pole” (Schild 2008). The Himalaya also referred as the “water tower of Asia” is the largest natural freshwater reservoir in the world, and its melt-waters are a significant component of flow of several major rivers, including the Indus, Ganges, Brahmaputra, Mekong, Yangtze, and Yellow river. (Xu *et al.* 2009; Barnett *et al.* 2005).

Himalaya is a biodiversity hotspot (CI 2017). It is commonly said that most plants growing on the earth are found in the Himalayas. The abrupt rise of the Himalayan mountains from less than 500 m to more than 8,000 m results in a diversity of ecosystems that range, in only a couple of hundred kilometers, from alluvial grasslands (among the tallest in the world) and subtropical broadleaf forests along the foothills to temperate broadleaf forests in the mid hills, mixed conifer and conifer forests in the higher hills, and alpine meadows above the tree line. Of the estimated 10,000 species of plants in the Himalaya hotspot, about 3,160 species (71 genera) are endemic (Phoenix *et al.* 2006). Biogeographically, the Himalayan Mountain Range is a transition zone between the Palaearctic and Indo-Malayan realms. Species from both realms are represented in the Himalayas. Indian Himalayas (area - 236300 km<sup>2</sup>) extends from Kashmir through Himachal Pradesh, parts of Uttar Pradesh, and West Bengal, Sikkim, Arunachal Pradesh and Nagaland.

Jammu and Kashmir State, India comprises of three divisions, Jammu, Kashmir, and Ladakh falls in Western Himalayan region. Six Himalayan mountain ranges occur in J&K viz., Cis Himalayas - 1. Shiwalik Hills, 2. Lower or Middle Himalayas (Pir Panjal Range), 3. Greater Himalayas; Trans Himalayas - 4. Zaskar Range, 5. Ladakh Range, 6. Karakoram Range (Fig. 2). There is also an outer plain area which is a part of the Great Plains of India and stretches in the southern parts of Akhnoor, RS Pura, Samba and Kathua. Dhauladhar Range is the continuation of Pir Panjal Range which runs in the Himachal Pradesh State.

In present study, mosquito collections were made from Jammu and Kashmir State, constituting three divisions which are the three different climatic regions also (Jammu – sub-tropical, Kashmir – temperate, Ladakh – cold arid) (Fig. 3).





**Fig. 2: Himalayan ranges in Jammu and Kashmir (J&K) (Source: [knowledgeofindia.com/Himalaya-mountain](http://knowledgeofindia.com/Himalaya-mountain))**

### 3.3. Study Area

Jammu and Kashmir State, India lies in the Western Himalaya between  $32^{\circ}17'$  to  $36^{\circ}58'$  north latitudes and  $73^{\circ}26'$  to  $80^{\circ}30'$  east longitudes. It shares borders with the states of Himachal Pradesh and Punjab to the south. It has international border with China in the north and east, and the Line of Control separates it from Pakistani-administered territories of Azad Kashmir and Gilgit-Baltistan in the west and northwest respectively. The State has an area of  $101387 \text{ km}^2$  comprising of three distinct geographic and climatic regions/divisions, Jammu, Kashmir, and Ladakh with  $26293 \text{ km}^2$ ,  $15948 \text{ km}^2$ , and  $59146 \text{ km}^2$  areas respectively. Ladakh is a high altitudinal snow covered deserted area and accounts for nearly two-third of the State's area. It is one of the highest areas on earth which has an average altitude being above 3650 m (India Meteorological Department 2014). Arctic cold desert areas of Ladakh, temperate Kashmir Valley, and subtropical region of Jammu have 2617 – 7135 m, 1046 – 5420 m, and 261 – 2100 m altitude ranges respectively. A comparison of some parameters among these three divisions is given in Table 3.

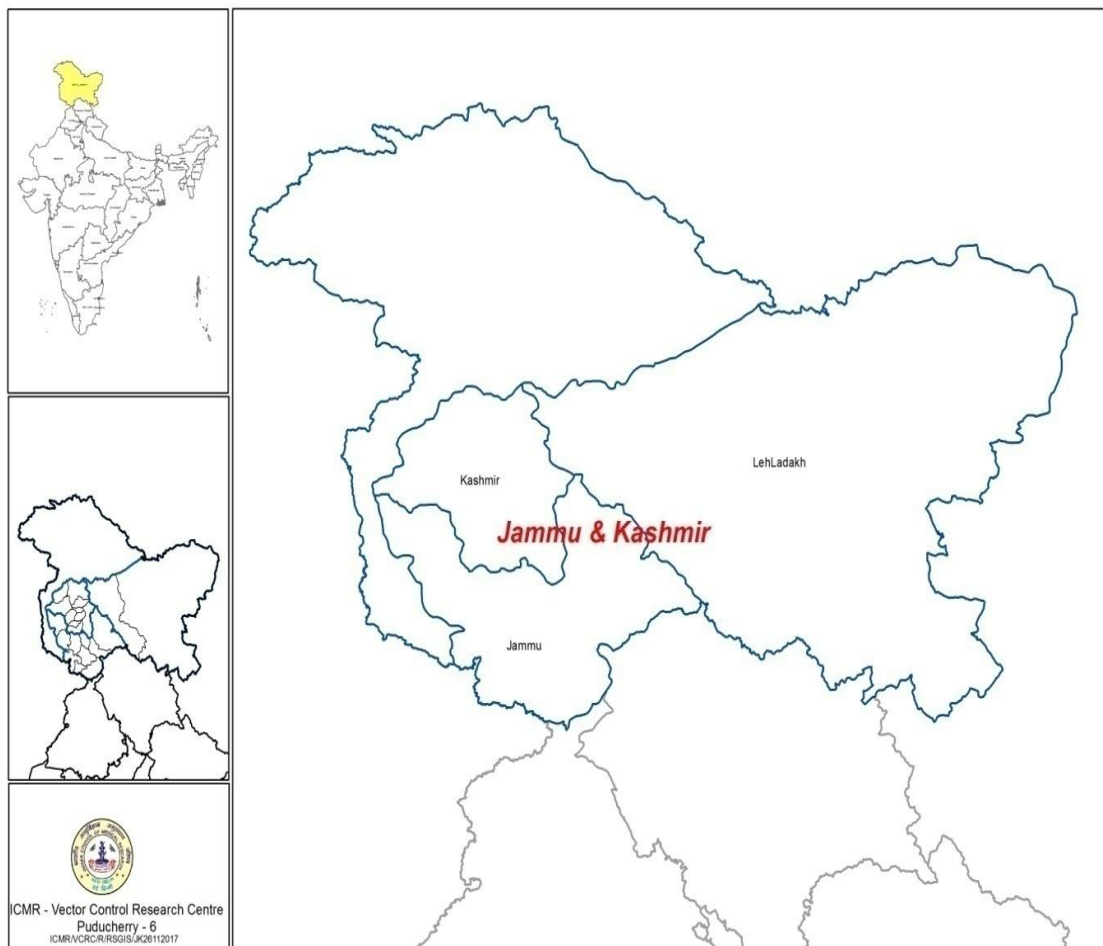
Jammu division is characterized by a rapid transition from sub-tropical to alpine ecoclimatic conditions as one moves from south (Kathua) to north (Kishtwar). It has a

geographical area of 26293 km<sup>2</sup> (25.93% of State area), borders Kashmir to the north, Ladakh to the east, and Punjab and Himachal Pradesh to the south. In the west, the Line of Control separates Jammu from Pakistani-administered Jammu and Kashmir. Most of the land is hilly or mountainous, including the Pir Panjal range which separates it from the Kashmir Valley. Jammu region has **two** different climatic zones (subtropical, and intermediate) depending primarily on altitude. In and around the Jammu district (lower hills and plains), the climate is similar to the nearby Punjab region (subtropical) with hot summers, rainy monsoon and mildly cold winters. The higher hills and mountains (intermediate) are snow-capped during the winter. In the high reaches of Chenab Valley, winters are severe and varied quantity of snow is received.

Kashmir, commonly called the Kashmir Valley, the ‘paradise on earth’, is situated between 330.20' - 340.54' N latitudes and 730.55' - 750.35' E longitudes, covering an area of 15,948 km<sup>2</sup> (15.73% of State area). Topographically, it is a deep elliptical bowl-shaped Valley bounded by lofty mountains of the Pir Panjal in the south and southwest and by the Great Himalayan range in the north and east, with 64% of the total area being mountainous. The Valley is asymmetrical, with 187 km diagonal length (from southeast to northwest corner), and considerably varying breadth, being 115.6 km along the latitude of Srinagar (Kaul 1977). The altitude of the Valley basin at Srinagar is 1,600 m and rises to 5,420 m at Kolahoi or “Gwashibror”, the highest peak among its surrounding mountains. Traversing the Valley is the river Jhelum and its tributaries, which feed many lakes for which Kashmir is famous. (Dar 2008)

Ladakh, ‘land of high passes’ lies between the Kunlun mountain range in the north and the main Great Himalayas to the south, having an area of 59146 km<sup>2</sup> (58.33% of State area) inhabited by people of Indo-Aryan and Tibetan descent. It has much in common with those of Central Asia generally and especially those of the Tibetan Plateau. It is the largest division (in area) but most sparsely populated region in Jammu and Kashmir State. It is a land of subalpine and alpine areas. Climate is cold and dry and most of the land is barren. The region has an enormous variation in altitude (2600 to >6500 m MSL). Snowline in the Trans-Himalaya is located at much higher altitude (5800 – 6000 m) as compared to Greater Himalaya where it is usually around 5500 m. This provides adequate area for the

plants of high alpine and sub-nival zone. The boundary between the moist alpine zone of Greater Himalaya and the cold arid region is generally sharp. Mean annual precipitation varies from 50 to 150 mm and it increases at higher altitudes, while deep river valleys such as lower Nubra and Zaskar are particularly dry. The dry landscape is interspersed with glacial streams, rivers and lake basins which form crucial Life-Support System for all living forms and provide ecosystem services (Rawat 2008). As Ladakh region is usually called ‘cold arid’ or ‘cold desert’ but Raj & Sharma (2013) did not agree fully with the term ‘desert’ because of its vegetation from alpine meadows to diverse woody plant species and increase of precipitation with elevation. They called it a typical semi-desert cold region.



**Fig. 3: Map of J&K showing divisions**

**Table 3: Comparison of geographical features and population among the three divisions of the State**

Parameters	Jammu	Kashmir	Ladakh
Avg. Altitude with Range (in meters)	550 (261 – 2100)	1650 (1046 – 5420)	3650 (2617 – 7135)
Mean Maximum and Mean Minimum Temperature	Summer : 29°C Winter : 16°C	Summer : 21°C Winter : 7°C	Summer : 20°C Winter : -10°C
Avg. Relative Humidity (%)	61	68.7	35
Annual Rainfall	42 inches or 107 cm	26 inches or 66 cm	8 inches or 20 cm
Climate	Subtropical & Intermediate	Temperate	Cold arid
Seasons	Four (Summer, Rainy, Autumn, Winter)	Four or Six (Spring, Summer, Autumn, Winter)	Four (Winter, Summer; Spring and Autumn are less)
Geographical Area	26293 km <sup>2</sup>	15948 km <sup>2</sup>	59146 km <sup>2</sup>
% of division area to State area (101387 km <sup>2</sup> )	25.93 %	15.73 %	58.33 %
Forest Area (% of forest area to division area)	12066 km <sup>2</sup> (45.89 %)	8128 km <sup>2</sup> (50.97%)	36 km <sup>2</sup> (0.06 %)
Population (12,54,130)	53,78,538	68,88,475	2,74,289
Population density (persons/km <sup>2</sup> )	204.56	431.93	4.63

References: India Meteorological Department 2014; Jammu and Kashmir Forest Department 2011; Census of India 2011

### 3.4. Mosquito Collection

Mosquito collections in all the three divisions (Jammu, Kashmir, and Ladakh) were made between 2011 and 2016. Ten surveys were carried out in 161 locations/study sites from which immature stages or adults were collected. Locations with habitats devoid of larvae or pupae of Culicidae were not considered for analysis. Some locations were surveyed more than once. In Jammu division, almost all the collections were carried out in subtropical zone only (Jammu, Udhampur, Samba, and Kathua districts). Sixty-four locations in Jammu (4 out of 10 districts), 48 locations in Kashmir (6 out of 10 districts),

and 49 locations in Ladakh (2 out of 2 districts) were surveyed (Table 4). Except from two locations geocoordinates (latitude, longitude, and altitude) were recorded from all the locations using mobile phone, or GPS device. All these geocoordinates and locations were checked on internet online software, GPS Geoplaner online (<http://www.geoplaner.com>) and the readings from this software were taken as final so as to keep uniformity. Fig. 4 shows the locations or sites of all the three divisions on map surveyed. Locations with lowest and highest altitudes from where mosquitoes (larvae) were collected were Satryan (RS Pura) – Jammu with altitude, 262 m and Lalung – Ladakh with altitude, 3879 m respectively (Table 5). Except in winter the surveys were carried out in all seasons (spring, summer, autumn, rainy) (Table 6). The fieldwork emphasized both immature and adult collection, and individual rearing of adults from the immature stages. Immatures were collected from a variety of larval habitats using a dipper, pipette, siphon, etc., whichever was appropriate for that habitat. Adults collected were those resting in indoor and outdoor habitats, using an oral aspirator/mechanical aspirator. Adults landing on the investigator during the field survey were also collected. Light traps and sweep nets were also used for adult collection.

**Table 4: Study sites with geo-coordinates**

S. No.	Division	District	Locality	Latitude (°N)	Longitude (°E)	Altitude (m)
1	Jammu	Jammu	Adhuvana Mori (Sohanjana)	32.697192	74.799203	275
2	Jammu	Jammu	Athem - View Point	32.777669	74.991186	522
3	Jammu	Jammu	Badsoo (Baghe)	32.873	74.9947	495
4	Jammu	Jammu	Bagh-e-Bahu	32.72522	74.87944	379
5	Jammu	Jammu	Baljata	32.75869	74.96325	402
6	Jammu	Jammu	Bantalab	32.8441	74.9433	509
7	Jammu	Jammu	Bantalab	32.78773	74.82137	373
8	Jammu	Jammu	Burn upper	32.84478	74.78069	336
9	Jammu	Jammu	Burn Morh (Kot Bhalwal)	32.848	74.811	455
10	Jammu	Jammu	Dansal	32.865	74.9963	500
11	Jammu	Jammu	Daskal	32.9109	74.7436	350
12	Jammu	Jammu	Deran	32.8194	74.76519	305
13	Jammu	Jammu	Dogra Hall	32.7393	74.8613	354
14	Jammu	Jammu	Ganderwan	32.95415	74.71553	509
15	Jammu	Jammu	Jaithly	32.7582	75.061	577
16	Jammu	Jammu	Jakli	Not known		

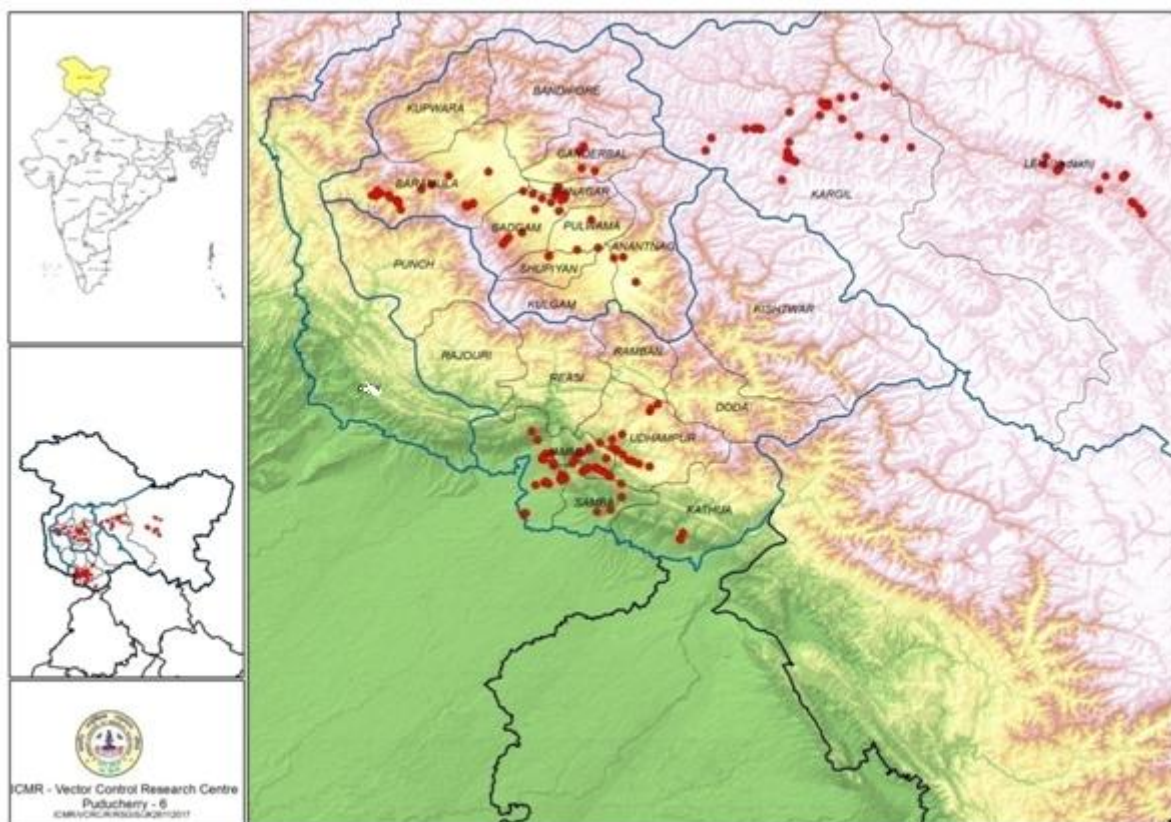
17	Jammu	Jammu	Jewel Chowk	32.721189	74.856847	299
18	Jammu	Jammu	Jindrah Challad	32.8224	75.0776	500
19	Jammu	Jammu	Kachi Chhawani	32.73736	74.87208	392
20	Jammu	Jammu	Kangrail	32.82578	74.76439	304
21	Jammu	Jammu	Kot Bhalwal	32.79668	74.81877	393
22	Jammu	Jammu	Kumbi Morh	32.733019	75.0985	639
23	Jammu	Jammu	MA Stadium	32.7228257	74.8536321	298
24	Jammu	Jammu	Mansar	32.69927	75.15033	660
25	Jammu	Jammu	Mishriwala	32.8156	74.7766	315
26	Jammu	Jammu	Nagrota - Balani Bridge	32.816967	74.926008	357
27	Jammu	Jammu	Nagrota - Chirwa Bridge	32.7975	74.91239	345
28	Jammu	Jammu	Nai Basti (Sohanjana)	32.6919	74.7334	262
29	Jammu	Jammu	Nandni	32.850333	74.9535	569
30	Jammu	Jammu	Panjoda	32.7566	74.9623	406
31	Jammu	Jammu	Raipur	32.80308	74.8177	390
32	Jammu	Jammu	Sagoon	32.7409	75.0889	644
33	Jammu	Jammu	Sagoon	32.74998	75.07555	619
34	Jammu	Jammu	Satrayan - RS Pura	32.55078	74.67415	262
35	Jammu	Jammu	Sidra - RS Pura	32.55552	74.68719	264
36	Jammu	Jammu	SRTC Yard	32.714069	74.859356	307
37	Jammu	Jammu	Surinsar	32.7727	75.0351	631
38	Jammu	Jammu	Surinsar - Urkhal	32.7768	75.0266	625
39	Jammu	Jammu	Surinsar (opp. to lake)	32.7674	75.0424	607
40	Jammu	Jammu	Thalwal	32.7031	74.777	274
41	Jammu	Jammu	Thalwal	32.70895	74.78365	275
42	Jammu	Jammu	Tootan di Khui	32.76687	74.98057	363
43	Jammu	Jammu	Tootan di Khui	32.77755	74.99167	523
44	Jammu	Kathua	Mearth	32.46012	75.45073	446
45	Jammu	Kathua	Barnoti	32.42988	75.43614	351
46	Jammu	Kathua	Budhi	32.4255	75.44155	349
47	Jammu	Kathua	Near National Highway	Not known		
48	Jammu	Samba	Sadyali Nud	32.63358	75.15267	391
49	Jammu	Samba	Near Govt. Degree College	32.57065	75.10047	345
50	Jammu	Samba	Vijaypur	32.5616	75.03232	338
51	Jammu	Udhampur	Battal Ballian	32.8619	75.1265	624
52	Jammu	Udhampur	Battal Ballian	32.8607	75.1283	627
53	Jammu	Udhampur	Battal Ballian (near bypass bridge)	32.8734	75.1084	608
54	Jammu	Udhampur	Birma	32.915786	75.105781	660
55	Jammu	Udhampur	Dabra	33.052186	75.287906	1407
56	Jammu	Udhampur	Domail - Jakheni Park	32.9389	75.1541	790
57	Jammu	Udhampur	Jallow	32.7957	75.2365	647

58	Jammu	Udhampur	Kaghote	32.8073	75.2084	603
59	Jammu	Udhampur	Kirmoo	32.7819	75.2881	760
60	Jammu	Udhampur	Kud	33.0657	75.2961	1483
61	Jammu	Udhampur	Patnitop	33.0864	75.328281	2017
62	Jammu	Udhampur	Phalata	32.8978	75.0459	677
63	Jammu	Udhampur	Ritti (to Ram nagar)	32.8413	75.157511	562
64	Jammu	Udhampur	Trilla	32.8123	75.1844	573
65	Kashmir	Anantnag	Achhabal	33.68206	75.2211	1666
66	Kashmir	Anantnag	Banderpora	33.804594	75.160094	1660
67	Kashmir	Anantnag	Bijbehara	33.7984	75.1148	1595
68	Kashmir	Baramulla	Boniyar	34.133539	74.178317	1510
69	Kashmir	Baramulla	Dachi	34.10646	74.01934	1307
70	Kashmir	Baramulla	Gulmarg	34.04962	74.40249	2545
71	Kashmir	Baramulla	Gulmarg	34.0586	74.393967	2652
72	Kashmir	Baramulla	Kamalkote	34.12807	73.96275	2006
73	Kashmir	Baramulla	Lagama	34.0816	74.059	1380
74	Kashmir	Baramulla	Mirgund	34.12508	74.67278	1581
75	Kashmir	Baramulla	Nowshera	34.1577	74.2293	1587
76	Kashmir	Baramulla	Rengee	34.2204	74.5048	1582
77	Kashmir	Baramulla	Saidpora	34.07054	74.06836	1389
78	Kashmir	Baramulla	Sultandaki	34.11519	73.9816	1423
79	Kashmir	Baramulla	Tangmarg	34.06402	74.42866	2131
80	Kashmir	Baramulla	Tangmarg	34.067656	74.428642	2115
81	Kashmir	Baramulla	Uri market	34.0789	74.0564	1377
82	Kashmir	Baramulla	Uri – Red-bridge	34.101	73.9577	1186
83	Kashmir	Baramulla	Uri - Salamabad	34.092694	74.026264	1279
84	Kashmir	Baramulla	Uri - Silikot	34.0344	74.0773	1578
85	Kashmir	Baramulla	Uri - Thajal road	34.0688	74.063556	1453
86	Kashmir	Baramulla	Uri - Uroosa	34.1053	73.9352	1206
87	Kashmir	Baramulla	Veerwan	34.1999	74.3112	1579
88	Kashmir	Budgam	Doodhpathri	33.8678	74.5746	2592
89	Kashmir	Budgam	Doodhpathri	33.8836	74.5879	2494
90	Kashmir	Budgam	Kaich	33.92239	74.6675	1977
91	Kashmir	Budgam	Railway station	34.03664	74.73158	1589
92	Kashmir	Budgam	Raiyar	33.9019	74.6037	2251
93	Kashmir	Ganderbal	Ganiwan	34.2244	75.0226	1988
94	Kashmir	Ganderbal	Naranag	34.3451	74.9669	2252
95	Kashmir	Ganderbal	Vaibagh - Mammar	34.2378	74.957506	1872
96	Kashmir	Ganderbal	Wangath	34.3188	74.94508	2021
97	Kashmir	Pulwama	CB Nath	33.810364	74.799239	1950
98	Kashmir	Pulwama	Dogripora	33.8493	75.0379	1591

99	Kashmir	Pulwama	Ladhu	33.987014	75.004797	1752
100	Kashmir	Pulwama	Qusbayar	33.8068	74.797	1976
101	Kashmir	Pulwama	Tahab	33.83994	74.93637	1649
102	Kashmir	Srinagar	Bemina	34.08939	74.76528	1580
103	Kashmir	Srinagar	Botanical Garden	34.092225	74.877817	1592
104	Kashmir	Srinagar	Golf-course	34.0835	74.863	1591
105	Kashmir	Srinagar	Habak crossing	34.1462	74.8436	1584
106	Kashmir	Srinagar	Hokersar	34.11129	74.71984	1584
107	Kashmir	Srinagar	Laam (Nishat)	34.111278	74.876	1581
108	Kashmir	Srinagar	Lal Chowk	34.07017	74.80895	1590
109	Kashmir	Srinagar	Naseem-bagh	34.138	74.8397	1597
110	Kashmir	Srinagar	Dal Lake (opp. Nigeen park)	34.117	74.8359	1586
111	Kashmir	Srinagar	Nowgam (Railway Station)	34.0275	74.8473	1584
112	Kashmir	Srinagar	Saida Kadal	34.1076	74.8341	1586
113	Ladakh	Kargil	Akchamal	34.55543	76.15725	2756
114	Ladakh	Kargil	Barchey	34.5798	76.2181	3490
115	Ladakh	Kargil	Bhimbat	34.43075	75.82125	3124
116	Ladakh	Kargil	Budhkhharbu	34.3401	76.5618	3500
117	Ladakh	Kargil	Bulbul-bagh	34.54649	76.14175	2693
118	Ladakh	Kargil	Damsna	34.18025	75.93263	3173
119	Ladakh	Kargil	Drass	34.43027	75.75668	3091
120	Ladakh	Kargil	Drass	34.42969	75.79708	3068
121	Ladakh	Kargil	Drass	34.43267	75.80473	3102
122	Ladakh	Kargil	Garkone	34.6347971	76.4338929	2716
123	Ladakh	Kargil	Gindial	34.42717	75.83005	3094
124	Ladakh	Kargil	Khachan	34.3644	75.9682	2865
125	Ladakh	Kargil	Kharchay Khar	34.26935	75.99955	3041
126	Ladakh	Kargil	Khumani Chowk	34.55858	76.12703	2677
127	Ladakh	Kargil	Khurbathang	34.5413	76.156722	2905
128	Ladakh	Kargil	Lalung	34.5868	76.2873	3879
129	Ladakh	Kargil	Lankerchey	34.3196	75.9588	2925
130	Ladakh	Kargil	Lankercheythang	34.3132	75.9626	2963
131	Ladakh	Kargil	Matayan	34.38385	75.58943	3223
132	Ladakh	Kargil	Minji-chutuk	34.49493	76.11587	2731
133	Ladakh	Kargil	Minamarg	34.32473	75.5606	3287
134	Ladakh	Kargil	Namikala	34.3834	76.434389	3657
135	Ladakh	Kargil	Sankoo	34.2875	75.9593	2987
136	Ladakh	Kargil	Shargol	34.3938	76.3095	3183
137	Ladakh	Kargil	Sher-bagh	34.540139	76.143014	2694
138	Ladakh	Kargil	Shimsa	34.51228	75.96885	2855
139	Ladakh	Kargil	Stakpa	34.2893	75.95565	2993



140	Ladakh	Kargil	Thasgam	34.28748	75.97267	2975
141	Ladakh	Kargil	Thasgam	34.27547	75.98587	3000
142	Ladakh	Kargil	Umbolung	34.4785	76.2251	2924
143	Ladakh	Leh	Basgo	34.225117	77.272808	3238
144	Ladakh	Leh	Diskit	34.54478	77.56957	3108
145	Ladakh	Leh	Ganglass	34.20628	77.60518	3857
146	Ladakh	Leh	Ganglass	34.19613	77.5943	3744
147	Ladakh	Leh	Horje	34.197	77.5957	3757
148	Ladakh	Leh	Horje Gompa	34.1913	77.5924	3704
149	Ladakh	Leh	Hundar	34.5725	77.49433	3081
150	Ladakh	Leh	Hundar	34.55247	77.52803	3099
151	Ladakh	Leh	Khalsar	34.49393	77.71703	3181
152	Ladakh	Leh	Likir	34.29395	77.21887	3687
153	Ladakh	Leh	Ney	34.25412	77.28837	3436
154	Ladakh	Leh	Ney	34.23913	77.2855	3375
155	Ladakh	Leh	Phey	34.1315	77.4764	3175
156	Ladakh	Leh	Phyang	34.199594	77.5082	3693
157	Ladakh	Leh	Shey	34.0643	77.6459	3242
158	Ladakh	Leh	Shey (near palace)	34.0728	77.6356	3241
159	Ladakh	Leh	Suspol	34.25527	77.2086	3383
160	Ladakh	Leh	Thiksey	34.04028	77.6727	3260
161	Ladakh	Leh	Thiksey (Rumbirpur)	34.014206	77.6873	3280



**Fig. 4: Map of J&K State showing study sites**

**Table 5: Number of districts and locations surveyed in each division and the altitudinal range**

Division	Total districts	Districts surveyed	No. of locations	Altitudinal range
Jammu	10	4 (Jammu, Udhampur, Kathua, Samba)	64	262 m (Satryian (RS Pura)) – 2017 m (Patnitop).
Kashmir	10	6 (Srinagar, Anantnag, Pulwama, Budgam, Baramulla, Ganderbal)	48	1186 m (Redbridge - Uri) – 2652 m (Gulmarg)
Ladakh	2	2 (Kargil, Leh)	49	2691 m (Bulbulbagh) – 3879 m (Lalung)

**Table 6: Months and seasons in which surveys were carried out**

Collection	Month	Jammu	Kashmir	Ladakh
1	July- August, 2011	-	Summer	-
2	November, 2012	Autumn	-	-
3	May-July, 2013	Rainy	Spr, Sum	Summer
4	August-November, 2013	Autumn	Autumn	Summer
5	April-May, 2014	Summer	Spring	-
6	July- August, 2014	Rainy	Summer	Summer
7	November, 2014	Autumn	-	-
8	May-June, 2015	Summer	Spring	-
9	August-September, 2015	Autumn	Autumn	Sum, Aut
10	July- August, 2016	Rainy	-	Summer

**3.4.1. Larval Sampling (Fig. 5):** Larval samples from each of the larval habitat of a particular collection site were maintained separately with a code number for the collection site and habitat. For example, in a day's collection, the first site of collection was assigned the number 1. If in this site larvae were collected from four different habitats such as a rock pool, ground pool, leaf axil, and bamboo, these were assigned serial numbers 1 to 4. Accordingly samples collected were assigned the code 1.1, 1.2, 1.3, and 1.4, where the first numeral denoted the collection site and the second numeral denoted the habitat. On the same day, when collection was made in a second site, it was assigned the number 2 and if larvae were collected from six different habitats in this site, they were coded 2.1, 2.2, 2.3, 2.4, 2.5 and 2.6 respectively. The collection sites were continued to be serially coded for the entire collection trip to a region or locality each of which ran through several days. Each collection trip to a region or locality or regions had one separate field

note book in which the larval samples collected were entered with the data on collection date, collection site, name of the habitat for each of the coded sample, along with the geo-coordinates of each collection site and information on the nature of the habitat such as stagnant, flowing, with algae, in shadow, etc., Larval samples were maintained in the field laboratory throughout the course of collection in each locality or region. They were checked daily for pupation and pupae were removed to a separate holding tube assigning the code of the sample until adults emerged. This process was considered as mass rearing of the larval samples. From the larval sample, a few fourth-instar larvae were removed and preserved in 70% alcohol with the respective code for later preparation of slide mounts. On completion of the collection trip, the larval samples in their original coded containers were brought to the laboratory at VCRC and the process of rearing continued till larvae in all the samples emerged to adults. Individual rearing of larva for obtaining associated material is essential for certain species that requires larval characters to make species identification. To facilitate this, fourth-instar larvae from the different larval samples were isolated individually in vials. In such cases the coding was taken one step further. For example, when 3 larvae were isolated individually from larval sample that was coded as 1.1, they were assigned the code 1.1.1, 1.1.2 and 1.1.3 respectively. On pupation, larval skin was carefully lifted and transferred to distilled water for few minutes and then preserved in 70% alcohol. The pupa was maintained until the adult emerged and the pupal skin was similarly preserved. The code assigned to each of the individual larva was carefully maintained throughout this process. Larval and pupal skins were mounted on slides along with their respective codes and the adult emerged was pinned with the same code.



**Fig. 5: Larval/Immature collection and rearing** a. Searching for immatures in a tree hole (Apple tree) b. Immature collection from a borrow pit c. Searching for immatures in steam pools at very high elevation (~4976 m) d. Immature rearing in laboratory

**3.4.2. Adult Preservation and Mounting (Fig. 6):** Adults collected in the field were transported to field laboratory and killed by using ethyl acetate. Each adult was mounted on a minuten pin under a binocular stereo microscope. The minuten was inserted to the lateral side of the mosquito keeping the proboscis towards the left side. After pinning, the other end of the minuten was held with a pair of forceps and inserted to the cork. Care was taken about the arrangement of the wings and legs so as to make all characters visible for



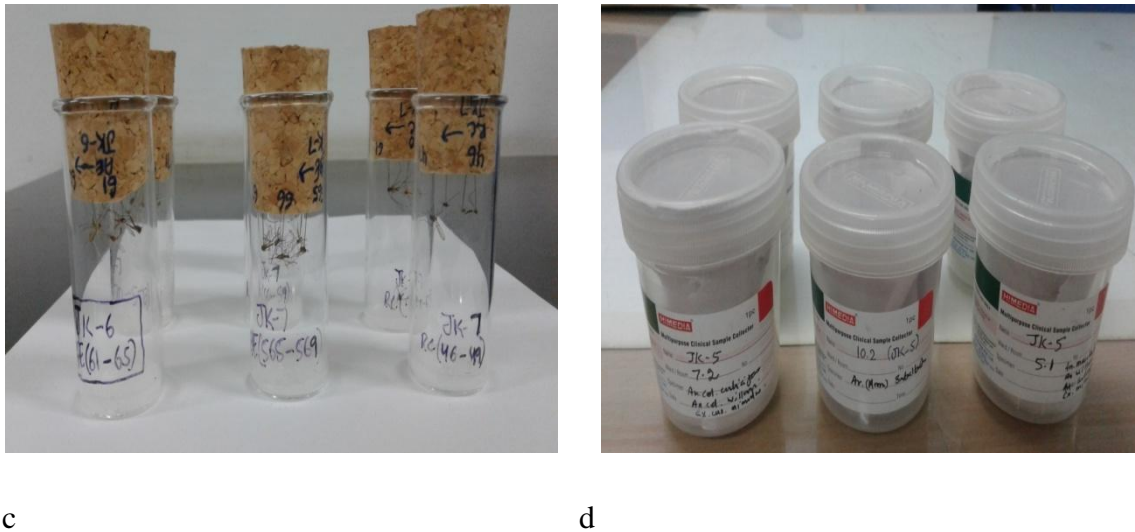
study. Adults emerged from the larval samples as well as the adults obtained through individual rearing were also mounted on minuten following the same procedure. Pinned adults inserted to the cork were preserved in a glass vial by inserting the cork onto/into the glass vial. Paradichlorobenzene (1, 4 dichlorobenzene) was used as a preservative to safeguard the specimen from fungus and other insects. The procedure followed for coding of the adults was as follows: Adults collected in the field were assigned the code RC (Resting Collection) and serially numbered on pinning as RC1, RC2, RC3, etc., with the date of collection and collection site and habit or habitat. Adults obtained from rearing of larval samples were assigned the code AE and serially numbered on pinning as AE1, AE2, AE3, etc., with the larval sample code 1.1 or 1.2 or 1.3 in the case of adults from mass rearing and with the larval sample code 1.1.1 or 1.1.2 or 1.1.3 in the case of adults from individual rearing.



a



b



**Fig. 6: Adult collection and preservation a. Adult collection from bushes (outdoor resting collection) b. Adult collection from a cowshed (indoor resting collection) c. Adults pinned on corks in glass vials d. Adults (in bulk) in stock vials or plastic vials**

### 3.5. Slide Mounts (Fig. 7)

Many of the characters of both adults and immature stages are most easily studied in slide mounts. The following procedures were adopted for preparing slide mounts of larva, larval and pupal skins, and male genitalia.

**3.5.1. Mounting Medium:** The mounting medium used was Hoyer's medium, the one considered to be most satisfactory for mosquito material by Belkin (1962). It is a modification of Berlese's medium and is prepared as follows: (1) dissolve 30 grams of clear gum arabic in 50 ml of distilled water by adding the gum gradually and stirring thoroughly in a large beaker; (2) let stand overnight and then filter through four to five fold muslin cloth; this may take longer time since the material is viscous; (3) add 200 gm. of chloral hydrate gradually, stirring thoroughly; and (4) add 20 ml of glycerine.

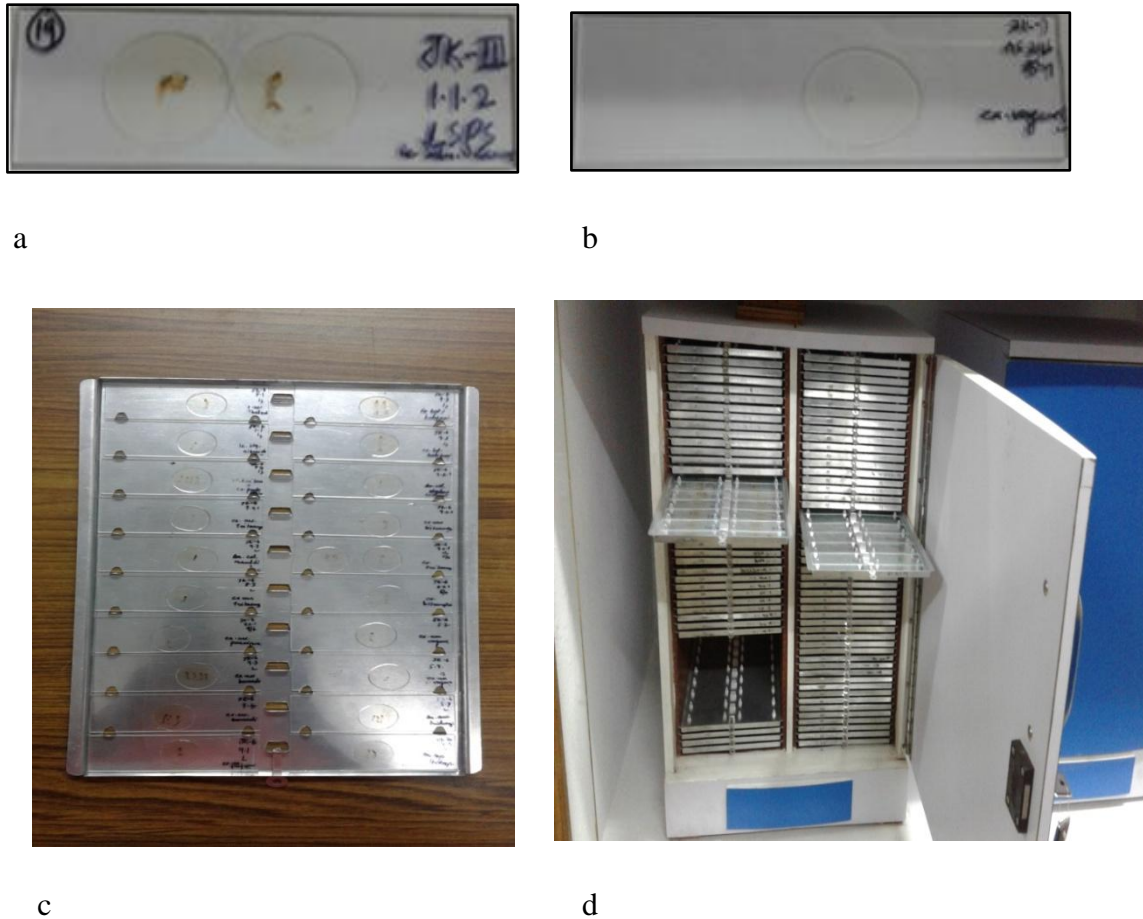
**3.5.2. Larval and Pupal Skins:** Larval and pupal skins particularly of individually reared larvae, the associated larval and pupal skins were mounted on the same slide and preferably under the same coverslip. When working with larval skin, the terminal abdominal segments was stretched or pulled by inserting one dissection needle in the thorax and the other at the base of the siphon or spiracular apparatus; the membrane

between abdominal segments VI and VII was partially torn to allow a lateral orientation of the remainder of the body. The pupal skin was dissected to separate the cephalothorax from the metanotum and abdomen, and then both cephalothorax and the abdomen was mounted using Hoyer's medium.

**3.5.3. Whole Larvae and Pupae:** Whole larvae and pupae were usually mounted without maceration directly into Hoyer's medium. In some cases of whole larvae, the specimens were kept in a clearing agent (Gater's fluid: 80 gms of chloral hydrate, 20 ml of 30% acetic acid) for one day after puncturing one or two safe places in the side of the thorax and abdomen. The specimen was then transferred on to a slide with the clearing agent and an incision was made carefully in the VII segment using a dissection needle without damaging the hairs. The terminal segment was mounted with the left side up and the main part of the body with the dorsal side up. Whole pupae normally do not make satisfactory or useful mounts, however in a few cases such mounts were made following the same procedure adopted for pupal skins.

**3.5.4. Male Genitalia:** More reliance for specific determination of mosquitoes is placed on male genitalia than any other structure in the adult, and, furthermore, the male genitalia show very excellent group characters. Therefore, slide mounting of male genitalia were made using Hoyer's medium for most of the specimens. The following procedure was followed: (1) Adults were relaxed in a moist test tube for at least 1 to 2 hours individually, 2) since individual specimens must always be associated with the corresponding genitalia, for a specimen bearing an individual number, the same adult number was used for the genitalia, 3) the tip of the abdomen was cut at about the middle of segment VII with the pair of fine scissors under binocular stereoscopic microscope, 4) the genitalia was transferred to diluted soap solution for 12 hours, 5) the specimen was washed in distilled water and transferred to clearing agent for 12 hours, 6) the specimen was transferred to a slide in a drop of clearing agent and the VIII segment was torn off gently with a needle to separate the sternite and tergite, 7) the genitalia was mounted in Hoyer's medium with the ventral side on top, 8) in some cases the genitalia was dissected to separate the basimere, distimere, phallosome and proctiger of one side and mounted laterally for more clear observation.





**Fig. 7: Larval, pupal, and genital mounting a. Larval and pupal skins on a SLIDE, b. Genitalia on a SLIDE, c. TRAY containing slides, and d. CABIN containing trays with slides**

### 3.6. DNA Bar-coding

DNA bar-coding of samples was carried out by using cytochrome c oxidase 1 (*COI*) gene. Extraction of DNA from mosquito legs was performed using GenElute™ Mammalian Genomic DNA Miniprep Kit. *COI* gene fragment (~700 bp) was amplified in a thermo-cycler (Eppendorf Mastercycler ep Gradient S or Bio-Rad T100™) using DNA primers described for Culicidae (Forward primer 5'-GGATTGGAATTGATTAGTTCCTT-3', Reverse primer 5'-AAAAATTTTAATTCCAGTTGGAACAGC-3') in India by Kumar *et al.* 2007. The parameters for PCR cycling were as follows: one cycle of 95°C for 5 min; 5 cycles of 94°C for 50 s, 45°C for 1 min and 72°C for 1 min; 35 cycles of 94°C for 50 s, 51°C for 1

min, 72°C for 1 min, followed by a terminal extension of 72°C for 10 min and a final ramping to 4°C. The amplified fragments were visualized by agarose gel electrophoresis. Positive samples were purified using QIAquick® PCR Purification Kit or/and QIAquick® Gel Extraction Kit. After purification samples were lyophilized and sent for custom sequencing. The generated sequences were analyzed using Chromas, MEGA7 and DNASP software. Both forward and reverse sequences were aligned and the consensus sequences were taken into consideration.

Species discrimination by DNA barcode methodology and genetic divergence among sequences was performed using the softwares such as MEGA7 and DNASP. Representative sequences available as DNA Barcodes for different species were retrieved from NCBI GenBank and analyzed along with the sequences generated in the present study to determine their phylogenetic relationships. The nucleotide composition, AT bias, genetic distances, genetic differentiation, etc., was calculated using DnaSP v5.1. The intra-specific divergence was computed using the Kimura-2-parameter genetic distances (MEGA7). Moreover, the average genetic divergence, intra-generic and inter-generic nucleotide divergences were estimated.

Two phylogenetic analysis tools, Neighbor-Joining (NJ) and Bayesian analysis (BA) were employed to test the reciprocal monophyletic criteria for species delimitation. Neighbor-Joining analysis was done by performing statistical analysis of the reliability of the tree generated by carrying out bootstrap analysis considering 1000 simulations. Species clade association was determined by Interior Branch tests (Nei and Kumar 2000). Beast software was used for Bayesian analysis of the sequences. The Bayesian analysis with the metropolis-coupled Markov Chain Monte Carlo (MCMC) was run for 10,000,000 generations with trees saving at every 100<sup>th</sup> generation (the first 25% of samples were discarded as burn-in). Bayesian posterior probabilities were calculated from the sample points once the MCMC algorithm began to converge. The trees generated through this process were visualized using FigTree v1.4.3.

### 3.7. Identification, Data analysis and Presentation

**3.7.1. Identification:** Taxonomic identifications of adults, larvae and genitalia were carried out under microscopes – Zeiss Stemi 2000 (Stereo-microscope), and Olympus CH-2 (Compound microscope). Identification was based on morphological characters, both for larvae and adults and keys followed were Christophers (1933), Barraud (1934), Bram (1967), Reid (1968), Yiau-Min Huang (1972), Reinert (1973), Sirivanakaran (1972, 1976, 1977), Rattanaarithikul and Green (1986), Reuben *et al.* (1994), Nagpal *et al.* (2005), and Becker *et al.* (2010). Classification of Culicidae was based on WRBU (2013) or Harbach (2017). Generic and sub generic abbreviations were used according to Reinert (2009). The specimens preserved were deposited in the Mosquito Museum of ICMR - Vector Control Research Centre, Puducherry (Fig. 8).



**Fig. 8: Mosquito Museum of ICMR – Vector Control Research Centre, Puducherry, India**

Species identification was done mainly based on adult male/female characters although immature stages were also used. The diagnostic characters of larvae were used for all the specimens that had associated material. Based on the observation of male genitalia also, species were identified.

**Adults:** The adults studied were mostly pinned dry specimens. Adults preserved in plastic vials were also studied. The usual method of presentation is to describe the female

first followed by the male. Diagnostic characters were checked on additional specimens, usually the entire available series.

**Immature stages:** Identification of the immature stages was largely done by observing diagnostic characters on their chaetotaxy. Diagnostic characters were also checked on additional specimens. Both whole larvae and larval skins were observed. Associated larval and pupal skins provided additional support for identification and confirmation of the species.

**Male genitalia:** Whole or dissected genitalia were examined particularly in case of culicines for the confirmation of species. In some instances all the genitalia were dissected and checked for the diagnostic characters of the genitalia.

**Recognition of new species:** Adults that exhibited striking differences in a combination of 2 or more characters on body, genitalia were provisionally regarded as different species. All reared specimens which appeared to be consistently different in at least 2 different stages were also considered as possible new forms. The final judgment in recognizing a new species was based on the constancy in correlated characters observed in a number of specimens. DNA barcoding (*COI* sequencing) showing intraspecific K2P genetic distance of more than 3% between the specimens were considered a separate (probably new also) species but needs further studies for confirmation.

**3.7.2. Data analysis and Presentation:** Specimens collected in the 10 surveys were analysed for number of species, new state records, and new country records. Division wise number of species, new species for the division was also analysed. Habitats, habitat types, and species found in habitats were also analysed.

Diversity indices (Shannon-Weiner and Gini-Simpson) were calculated for species for each division and habitat type. But the concept of true diversities (diversity indices are changed into effective numbers) given by Jost *et al.* (2006) was used for calculating the diversities. Exponential of Shannon-Weiner index (*i.e.*,  $\exp(H)$ ,  $H = -\sum p_i \ln p_i$ ), and subtracting Gini-Simpson index from unity and inverting (*i.e.*,  $1/1 - D$ ,  $D = 1 - \sum p_i^2$ ) give the measurements of effective number of species or effective or true diversities.

Shannon-Weiner index,  $H = -\sum p_i \ln p_i$

Gini-Simpson index,  $D = 1 - \sum p_i^2$

$p = n/N$  ( $n$  = no. of individuals of a species,  $N$  = total no. of individuals)

$\ln$  = natural log

Sorenson's Coefficient (CC) to find species similarity between the divisions was also calculated. Sorenson's Coefficient,  $CC = 2C/S1+S2$

$C$  = number of species the two communities have in common

$S1$  = total number of species found in community 1

$S2$  = total number of species found in community 2

Altitudinal distribution of the mosquito species with geographical distribution and climate of the divisions was also analysed and calculated.

Mosquito species barcoded were analysed by comparing the sequences of the specimens with each other and with the sequences available on GenBank and by constructing Neighbor-Joining and Bayesian trees. Intraspecific, intrageneric, and intergeneric distances were calculated and analysed.

Ecological notes consisting of *Material Examined*, *Identification* (for new country records only), *Present Record* and then general *Distribution*, *Habitat*, *Medical Importance*, and *Remarks* if any, were prepared for the species new for the State, and the important to mention. The symbols ♀, ♂, L, Le, Pe, ♂G and ♀G were used to represent female(s), male(s), fourth-instar larvae, larval exuviae, pupal exuviae, male genitalia and female genitalia respectively. *Material Examined*, and *Present Record* of the species was taken out from present study while as *Distribution*, *Habitat*, *Medical Importance* was searched out from the research articles and the other available literature. WRBU catalog was mostly referred for *Distribution* of the species.

## ***4. Results***

#### 4.1. Mosquito Species in J&K

In total, 7,094 specimens in 10 surveys were studied. Among the specimens, there were 1,172 whole larvae, 365 larval exuviae, 33 pupal exuviae; 2,413 adult♂, 3,111 adult♀; and 414 dissected male, 17 dissected female genitalia. In this study, 69 species were collected of which 3 tribes (Mansoniini, Sabethini, and Toxorhynchitini); 10 genera (*Armigeres*, *Coquillettidia*, *Hulecoeteomyia*, *Lutzia*, *Malaya*, *Mucidus*, *Neomelaniconion*, *Phagomyia*, *Toxorhynchites*, and *Verrallina*); 10 subgenera (*Alloeomyia*, *Armigeres*, *Coquillettidia*, *Culiseta*, *Eumelanomyia*, *Harbachius*, *Lophoceraomyia*, *Metalutzia*, *Mucidus*, and *Toxorhynchites*), and 36 species were reported for the first time (new occurrence records) in Jammu and Kashmir. The remaining 33 species (of 69 species) were the same as reported by the earlier workers. Ten species from the earlier reported species were not collected in the present surveys. Together with the ten species the total list of the mosquito species of J&K State increased up to 79. So, the mosquito fauna of J&K State is now composed of the two Culicidae subfamilies - Anophelinae and Culicinae, 8 tribes, 21 genera, 22 subgenera, and 79 species (Table 7). Species collected from the State in previous and present studies are shown in Table 8 and a summary is given in Table 9.

Five species, *Culex (Culex) pipiens* Linnaeus 1758, *Culex (Barraudius) pusillus* Macquart 1850, *Ochlerotatus* (subgenus uncertain) *caspius* Pallas 1771, *Ochlerotatus* (subgenus uncertain) *pulcritarsis* ssp. *asiaticus* Edwards 1926, *Coquillettidia (Coquillettidia) richiardii* Ficalbi 1889 were new country records. Three species (*Culex (Culex) mimeticus*, *Culex (Culex) vagans*, and *Culex (Culex) theileri* common to all the three divisions, and anophelines (*Anopheles (Anopheles) gigas* ssp. *simlensis* and *Anopheles (Anopheles) barianensis*) from Ladakh division were reported for the first time.

In the present study, from Jammu, Kashmir, and Ladakh divisions 49, 26, and 15 species were reported respectively out of which 30, 10, and 9 species were newly reported for Jammu, Kashmir, and Ladakh divisions respectively (Table 10). From Jammu division, 11 anophelines (3 new division records) and 38 culicines (27 new division records) were reported. *An. (Cel.) willmori*, and *Cx. (Cux.) quinquefasciatus* were found predominant. From Kashmir division, 5 anophelines and 21 culicines (10 new division records) were

reported. *Cx. (Cux.) vagans*, *Am. vexans*, *Cx. (Cux.) pseudovishnui*, and *Cx. (Cux.) quinquefasciatus* were found predominant. From Ladakh division, 2 anophelines (both new division records) and 13 culicines (7 new division records) were reported. *Cx. (Cux.) vagans*, *Cx. (Mai.) hortensis*, *Am. vexans*, and *Oc. (Fin.) sintoni* in Ladakh were found predominant.



Table 7: List of the total number of mosquito species reported from Jammu and Kashmir

Subfamily	Tribe	Genus	Subgenus	Species
Anophelinae	Anophelini	<i>Anopheles</i>	<i>Anopheles</i>	<i>barianensis</i> , <i>gigas</i> ssp. <i>simlensis</i> , <i>hyrcanus</i> *, <i>peditaeniatus</i> , <i>lindesayi</i>
			<i>Cellia</i>	<i>culicifacies</i> , <i>fluviatilis</i> , <i>stephensi</i> , <i>subpictus</i> *, <i>annularis</i> , <i>maculatus</i> , <i>willmori</i> , <i>pseudowillmori</i> , <i>dravidicus</i> , <i>splendidus</i> , <i>dthali</i> *, <i>turkhudi</i> *, <i>moghulensis</i> *, <i>leucosphyrus</i> *
Culicinae	Aedini	<i>Aedimorphus</i>		<i>vexans</i> , <i>pipersalatus</i> , <i>culicinus</i>
		<i>Armigeres</i>	<i>Armigeres</i>	<i>subalbatus</i>
		<i>Collessius</i>	<i>Alloeomyia</i>	<i>pseudotaeniatus</i>
			<i>Collessius</i>	<i>elsiae</i> *, <i>shortti</i> *
		<i>Bruceharrisonius</i>		<i>christophersi</i> *
		<i>Fredwardsius</i>		<i>vittatus</i>
		<i>Gilesius</i>		<i>pulchriventer</i>
		<i>Hulecoeteomyia</i>		<i>chrysolineata</i>
		<i>Mucidus</i>	<i>Mucidus</i>	<i>scatophagoides</i>
		<i>Neomelaniconion</i>		<i>lineatopenne</i>
		<i>Ochlerotatus</i>	<i>Finlaya</i>	<i>oreophilus</i> , <i>sintoni</i>
				<i>pullatus</i> , <i>pulcritarsis</i> ssp. <i>asiaticus</i> , <i>caspius</i> , <i>versicolor</i> *
		<i>Phagomyia</i>		<i>gubernatoris</i>
		<i>Stegomyia</i>	<i>Stegomyia</i>	<i>aegypti</i>
				<i>albopicta</i> , <i>w-alba</i> , <i>patriciae</i> , <i>unilineata</i>
		<i>Verrallina</i>	<i>Harbachius</i>	<i>yusafi</i>
	Culicini	<i>Culex</i>	<i>Culex</i>	<i>pipiens</i> , <i>quinquefasciatus</i> , <i>theileri</i> , <i>vagans</i> , <i>perexiguus</i> , <i>pseudovishnui</i> , <i>vishnui</i> , <i>barraudi</i> , <i>fuscocephala</i> , <i>tritaeniorhynchus</i> , <i>mimeticus</i> , <i>mimulus</i> , <i>murrelli</i> , <i>perplexus</i>
			<i>Oculeomyia</i>	<i>bitaeniorhynchus</i> , <i>infula</i> , <i>sinensis</i>

			<i>Eumelanomyia</i>	<i>brevipalpis, malayi</i>
			<i>Culiciomyia</i>	<i>pallidothorax, viridiventer</i>
			<i>Lophoceraomyia</i>	<i>minutissimus</i>
			<i>Maillotia</i>	<i>hortensis</i>
			<i>Barraudius</i>	<i>modestus, pusillus</i>
		<i>Lutzia</i>	<i>Metalutzia</i>	<i>fuscana, halifaxii</i>
	Mansoniini	<i>Coquillettidia</i>	<i>Coquillettidia</i>	<i>richiardii</i>
	Culisetini	<i>Culiseta</i>	<i>Culiseta</i>	<i>alaskaensis</i> ssp. <i>indica, niveitaeniata</i>
			<i>Allotheobaldia</i>	<i>longiareolata</i>
	Sabethini	<i>Malaya</i>		<i>genurostris</i>
	Uranotaeniini	<i>Uranotaenia</i>	<i>Pseudoficalbia</i>	<i>unguiculata</i>
	Toxorhynchitini	<i>Toxorhynchites</i>	<i>Toxorhynchites</i>	<i>splendens</i>
<b>2</b>	<b>8</b>	<b>21</b>	<b>22</b>	<b>79</b>

\*species not collected in the present surveys

**Table 8: List of the mosquito species reported from Jammu and Kashmir previously and presently**

S. No.	Taxa	Previously reported	Presently reported	Remarks
1	<i>Aedimorphus culicinus</i> (Edwards 1922)		J	New record
2	<i>Am. pipersalatus</i> (Giles 1902)		J	New record
3	<i>Am. vexans</i> (Meigen 1830)	L - R	KL	
4	<i>Anopheles (Anopheles) barianensis</i> James 1911	K	KL	
5	<i>An. (Ano.) gigas</i> ssp. <i>simlensis</i> Giles 1901	K - CPJaR	KL	
6	<i>An. (Ano.) hyrcanus</i> (Pallas 1771)	K - N		
7	<i>An. (Ano.) lindesayi</i> Giles 1900	JK - CPJaR	JK	
8	<i>An. (Ano.) peditaeniatus</i> (Leicester 1908)		J	New record
9	<i>An. (Cellia) annularis</i> van der Wulp 1884	JK - CPJaNR	J	
10	<i>An. (Cel.) culicifacies</i> Giles 1901	JK - PJaNR	J	
11	<i>An. (Cel.) dthali</i> Patton 1905	JK - Ja		
12	<i>An. (Cel.) dravidicus</i> Christophers 1924		J	New record
13	<i>An. (Cel.) fluviatilis</i> James 1902	JK - PJaNR	J	
14	<i>An. (Cel.) leucosphyrus</i> Donitz 1901	K - N		
15	<i>An. (Cel.) maculatus</i> Theobald 1901	JK - PJaN	J	
16	<i>An. (Cel.) moghulensis</i> Christophers 1924	K - Ja		
17	<i>An. (Cel.) pseudowillmori</i> (Theobald 1910)		J	New record
18	<i>An. (Cel.) splendidus</i> Koidzumi 1920	JK - CPJaNR	J	
19	<i>An. (Cel.) stephensi</i> Liston 1901	JK - PJaJe	JK	
20	<i>An. (Cel.) subpictus</i> Grassi 1899	JK - PJaR		
21	<i>An. (Cel.) turkhudi</i> Liston 1901	JK - CJaR		
22	<i>An. (Cel.) willmori</i> (James 1903)	JK - CPJaNR	JK	
23	<i>Armigeres (Armigeres) subalbatus</i> (Coquillett 1898)		JK	New record
24	<i>Bruceharrisonius christophersi</i> Edwards 1922	K - B		
25	<i>Collessius (Alloeomyia) pseudotaeniatus</i> (Giles 1901)		J	New record
26	<i>Co. (Collessius) elsiae</i> (Barraud 1923)	J - R		

27	<i>Co. (Col.) shortti</i> (Barraud 1923)	K - B		
28	<i>Coquillettidia (Coquillettidia) richiardi</i> (Ficalbi 1889)		K	New record
29	<i>Culex (Barraudius) modestus</i> Ficalbi 1890	K - B	L	
30	<i>Cx. (Bar.) pusillus</i> Macquart 1850		L	New record
31	<i>Cx. (Culex) barraudi</i> Edwards 1922	J - R	J	
32	<i>Cx. (Cux.) fuscocephala</i> Theobald 1907	J - R	J	
33	<i>Cx. (Cux.) mimeticus</i> Noe 1899	JK - BR	JKL	
34	<i>Cx. (Cux.) mimulus</i> Edwards 1915		J	New record
35	<i>Cx. (Cux.) murrelli</i> Lien 1968		J	New record
36	<i>Cx. (Cux.) perexiguus</i> Theobald 1903		J	New record
37	<i>Cx. (Cux.) perplexus</i> Leicester 1908		J	New record
38	<i>Cx. (Cux.) pipiens</i> Linnaeus 1758		L	New record
39	<i>Cx. (Cux.) pseudovishnui</i> Colless 1957		JK	New record
40	<i>Cx. (Cux.) quinquefasciatus</i> Say 1823	JK - RJe	JK	
41	<i>Cx. (Cux.) theileri</i> Theobald 1903	KL - BR	JKL	
42	<i>Cx. (Cux.) tritaeniorhynchus</i> Giles 1901		J	New record
43	<i>Cx. (Cux.) vagans</i> Wiedemann 1828	KL - BR	JKL	
44	<i>Cx. (Cux.) vishnui</i> Theobald 1901	J - R	J	
45	<i>Cx. (Culiciomyia) pallidothorax</i> Theobald 1905	J - R	JK	
46	<i>Cx. (Cui.) viridiventer</i> Giles 1901	JK - BR	K	
47	<i>Cx. (Eumelanomyia) brevipalpis</i> (Giles 1902)		J	New record
48	<i>Cx. (Eum.) malayi</i> (Leicester 1908)		J	New record
49	<i>Cx. (Lophoceraomyia) minutissimus</i> (Theobald 1907)		J	New record
50	<i>Cx. (Maillotia) hortensis</i> Ficalbi 1889	L - R	L	
51	<i>Cx. (Oculeomyia) bitaeniorhynchus</i> Giles 1901	J - R	JK	
52	<i>Cx. (Ocu.) infula</i> Theobald 1901		J	New record
53	<i>Cx. (Ocu.) sinensis</i> Theobald 1903		J	New record
54	<i>Culiseta (Allotheobaldia) longiareolata</i> (Macquart 1838)	L - R	L	
55	<i>Cs. (Culiseta) alaskaensis</i> ssp. <i>indica</i> (Edwards 1920)		L	New record
56	<i>Cs. (Cus.) niveitaeniata</i> (Theobald 1907)		K	New record

57	<i>Fredwardsius vittatus</i> (Bigot 1861)	J - R	J	
58	<i>Gilesius pulchriventer</i> (Giles 1901)	K - B	K	
59	<i>Hulecoeteomyia chrysolineata</i> (Theobald 1907)		J	New record
60	<i>Lutzia (Metalutzia) fuscana</i> (Wiedemann 1820)		J	New record
61	<i>Lt. (Mtl.) halifaxii</i> (Theobald 1903)		JK	New record
62	<i>Malaya genurostris</i> Leicester 1908		J	New record
63	<i>Mucidus (Mucidus) scataphagooides</i> Theobald 1901		J	New record
64	<i>Neomelaniconion lineatopenne</i> (Ludlow 1905)		J	New record
65	<i>Ochlerotatus (Finlaya) oreophilus</i> Edwards 1916	L - R	K	
66	<i>Oc. (Fin.) sintoni</i> (Barraud 1924)	KL - BR	KL	
67	<i>Oc. (Fin.) versicolor</i> (Barraud 1924)	K - B		
68	<i>Oc. (subgenus uncertain) caspius</i> (Pallas 1771)		L	New record
69	<i>Oc. (subgenus uncertain) pulcritarsis ssp. asiaticus</i> Edwards 1926		L	New record
70	<i>Oc. (subgenus uncertain) pullatus</i> Coquillett 1904	K - B	K	
71	<i>Phagomyia gubernatoris</i> (Giles 1901)		J	New record
72	<i>Stegomyia (Stegomyia) aegypti</i> (Linnaeus 1762)	JK - Je	JK	*
73	<i>St. (subgenus uncertain) albopicta</i> (Skuse 1895)	J - RJe	JK	
74	<i>St. (subgenus uncertain) patriciae</i> (Mattingly 1954)		K	New record
75	<i>St. (subgenus uncertain) unilineata</i> (Theobald 1906)		J	New record
76	<i>St. (subgenus uncertain) w-albus</i> (Theobald 1905)	J - R	J	
77	<i>Toxorhynchites (Toxorhynchites) splendens</i> (Wiedemann 1819)		J	New record
78	<i>Uranotaenia (Pseudoficalbia) unguiculata</i> Edwards 1913	K - B	K	
79	<i>Verrallina (Harbachius) yusafi</i> (Barraud 1931)		J	New record
	<b>Genera – 21, Subgenera - 22</b>	<b>43 species</b>	<b>69 species</b>	<b>36 species</b>

J = Jammu, K = Kashmir, L = Ladakh; B = Barraud (1934), C = Christophers (1933), Ja = Jacob (1950), Nair = Nair (1973), P = Puri (1936, 48), R = Rao *et al.* (1973, 83, 84), Je = Jebanesan *et al.* 2012

\*Reported from Jammu division by Mathew *et al.* (1977) and Padbidri *et al.* (1996) who worked on dengue outbreaks in the division, and from Kashmir division by Jebanesan *et al.* (2012).

**Table 9: Comparison between previous and present records on the number of mosquito species in the Himalayan Region**

	Previously reported	Presently reported
Total number of mosquito species	43	69
No. of genera	12	20
No. of subgenera	13	21
Species common to all the three divisions	Nil	03
Anophelines reported from Ladakh	Nil	02

**Table 10: Checklist of mosquito species collected in the present study with new records for the division (new records for the divisions are in bold)**

S. No.	JAMMU	KASHMIR	LADAKH
1	<i>Aedimorphus culicinus</i>	<i>Aedimorphus vexans</i>	<i>Aedimorphus vexans</i>
2	<i>Am. pipersalatus</i>	<i>Anopheles (Anopheles) barianensis</i>	<i>Anopheles (Anopheles) barianensis</i>
3	<i>Anopheles (Anopheles) lindesayi</i>	<i>An. (Ano.) gigas ssp. simlensis</i>	<i>An. (Ano.) gigas ssp. simlensis</i>
4	<i>An. (Ano.) peditaeniatus</i>	<i>An. (Ano.) lindesayi</i>	<i>Culex (Barraudius) modestus</i>
5	<i>Anopheles (Cellia) annularis</i>	<i>Anopheles (Cellia) stephensi</i>	<i>Cx. (Bar.) pusillus</i>
6	<i>An. (Cel.) culicifacies</i>	<i>An. (Cel.) willmori</i>	<i>Cx. (Culex) mimeticus</i>
7	<i>An. (Cel.) dravidicus</i>	<i>Armigeres (Armigeres) subalbatus</i>	<i>Cx. (Cux.) pipiens</i>
8	<i>An. (Cel.) fluvialtilis</i>	<i>Coquillettidia (Coquillettidia) richiardi</i>	<i>Cx. (Cux.) theileri</i>
9	<i>An. (Cel.) maculatus</i>	<i>Culex (Culex) mimeticus</i>	<i>Cx. (Cux.) vagans</i>
10	<i>An. (Cel.) pseudowillmori</i>	<i>Cx. (Cux.) pseudovishnui</i>	<i>Cx. (Maillotia) hortensis</i>

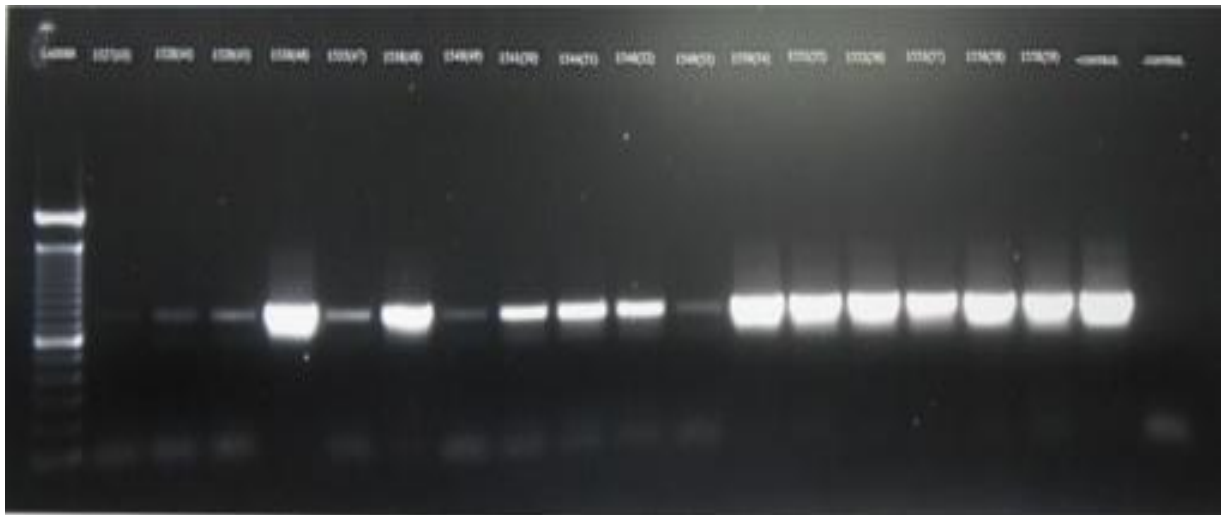
11	<i>An. (Cel.) splendidus</i>	<i>Cx. (Cux.) quinquefasciatus</i>	<i>Culiseta (Allotheobaldia) longiareolata</i>
12	<i>An. (Cel.) stephensi</i>	<i>Cx. (Cux.) theileri</i>	<i>Cs. (Culiseta) alaskaensis ssp. indica</i>
13	<i>An. (Cel.) willmori</i>	<i>Cx. (Cux.) vagans</i>	<i>Ochlerotatus (subgenus uncertain) caspius</i>
14	<i>Armigeres (Armigeres) subalbatus</i>	<i>Cx. (Culiciomyia) pallidothorax</i>	<i>Oc. (Finlaya) sintoni</i>
15	<i>Collessius (Alloeomyia) pseudotaeniatus</i>	<i>Cx. (Cui.) viridiventer</i>	<i>Oc. (subgenus uncertain) pulcritarsis ssp. asiaticus</i>
16	<i>Culex (Culex) barraudi</i>	<i>Cx. (Oculeomyia) bitaeniorhynchus</i>	
17	<i>Cx. (Cux.) fuscocephala</i>	<i>Culiseta (Culiseta) niveitaeniata</i>	
18	<i>Cx. (Cux.) mimeticus</i>	<i>Gilesius pulchriventer</i>	
19	<i>Cx. (Cux.) mimulus</i>	<i>Lutzia (Metalutzia) halifaxii</i>	
20	<i>Cx. (Cux.) murrelli</i>	<i>Ochlerotatus (Finlaya) oreophilus</i>	
21	<i>Cx. (Cux.) perexiguus</i>	<i>Oc. (Fin.) sintoni</i>	
22	<i>Cx. (Cux.) perplexus</i>	<i>Oc. (subgenus uncertain) pullatus</i>	
23	<i>Cx. (Cux.) pseudovishnui</i>	<i>St. (Stegomyia) aegypti</i>	
24	<i>Cx. (Cux.) quinquefasciatus</i>	<i>Stegomyia (subgenus uncertain) albopicta</i>	
25	<i>Cx. (Cux.) theileri</i>	<i>St. (subgenus uncertain) patriciae</i>	
26	<i>Cx. (Cux.) tritaeniorhynchus</i>	<i>Uranotaenia (Pseudoficalbia) unguiculata</i>	
27	<i>Cx. (Cux.) vagans</i>		
28	<i>Cx. (Cux.) vishnui</i>		
29	<i>Cx. (Culiciomyia) pallidothorax</i>		
30	<i>Cx. (Eumelanomyia) brevipalpis</i>		
31	<i>Cx. (Eum.) malayi</i>		
32	<i>Cx. (Lophoceraomyia) minutissimus</i>		
33	<i>Cx. (Oculeomyia) bitaeniorhynchus</i>		
34	<i>Cx. (Ocu.) infula</i>		
35	<i>Cx. (Ocu.) sinensis</i>		
36	<i>Fredwardsius vittatus</i>		
37	<i>Hulecoeteomyia chrysolineata</i>		
38	<i>Lutzia (Metalutzia) fuscana</i>		
39	<i>Lt. (Mtl.) halifaxii</i>		
40	<i>Malaya genurostris</i>		

41	<i>Mucidus (Mucidus) scataphagoides</i>		
42	<i>Neomelaniconion lineatopenne</i>		
43	<i>Phagomyia gubernatoris</i>		
44	<i>Stegomyia (Stegomyia) aegypti</i>		
45	<i>St.</i> (subgenus uncertain) <i>albopicta</i>		
46	<i>St.</i> (subgenus uncertain) <i>unilineata</i>		
47	<i>St.</i> (subgenus uncertain) <i>w-albus</i>		
48	<i>Verrallina (Harbachius) yusafi</i>		
49	<i>Toxorhynchites (Toxorhynchites) splendens</i>		
	<b>Genera - 15, Subgenera - 14</b> <b>New records for the division - 30</b>	<b>Genera - 11, Subgenera – 12</b> <b>New records for the division - 10</b>	<b>Genera - 5, Subgenera – 7</b> <b>New records for the division - 9</b>



## 4.2. DNA Barcoding

*COI* sequencing or DNA barcoding was carried out for many important species in which a total of 32 species (105 specimens) got barcoded. Barcoding has proven very helpful for the identification of species particularly the species which were difficult to identify morphologically like vishnui subgroup and sibling species of a species complex. *COI* sequences of around 700 base pairs (bp) can be obtained using BC-Kumar primers. In this study, *COI* sequences between 600 and 700 bp were generated using the same primers. For NJ (Neighbor-Joining) and Bayesian analysis 558 bp of consensus sequences were compared. The image of a gel showing barcode DNA or *COI* DNA bands and the list of the number of specimens taken from different divisions of the species barcoded in the present study with average and maximum observed intraspecific Kimura two-parameter (K2P) distances are shown in Fig. 9 and Table 11 respectively.



**Fig. 9:** Image of a gel showing bands of *COI* DNA isolated from mosquito specimens used in the present study

**Table 11: List of mosquito species, collection sites (here divisions) and the number of *COI* sequences obtained for each species along with average and maximum observed intraspecific Kimura two-parameter (K2P) distances**

S. No.	Taxa	Specimens from Jammu	Specimens from Kashmir	Specimens from Ladakh	Total no. of specimens	Average K2P distance (%)	Maximum K2P distance (%)
1	<i>Aedimorphus vexans</i>	-	5	5	10	1.4	3.6
2	<i>Anopheles (Anopheles) barianensis</i>	-	2	-	2	0.2	0.2
3	<i>Anopheles (Anopheles) gigas ssp. simlensis</i>	-	8	1	9	0.4	0.7
4	<i>Anopheles (Anopheles) lindesayi</i>	-	2	-	2	0.3	0.3
5	<i>Anopheles (Cellia) maculatus</i>	3	-	-	3	0.0	0.0
6	<i>Anopheles (Anopheles) peditaeniatus</i>	2	-	-	2	0.5	0.5
7	<i>Anopheles (Cellia) stephensi</i>	1	-	-	1	-	-
8	<i>Anopheles (Cellia) willmori</i>	2	-	-	2	0.3	0.3
9	<i>Coquillettidia (Coquillettidia) richiardi</i>	-	4	-	4	0.2	0.5
10	<i>Culex (Oculeomyia) bitaeniorhynchus</i>	-	1	-	1	-	-
11	<i>Culex (Maillotia) hortensis</i>	-	-	4	4	0.0	0.0
12	<i>Culex (Culex) malayi</i>	1	-	-	1	-	-
13	<i>Culex (Culex) mimeticus</i>	-	3	-	3	0.0	0.0
14	<i>Culex (Barraudius) modestus</i>	-	-	2	2	0.0	0.0
15	<i>Culex (Culex) perexiguus</i>	4	-	-	4	0.0	0.0
16	<i>Culex (Culex) pipiens</i>	-	-	5	5	0.1	0.3
17	<i>Culex (Culex) pseudovishnui</i>	1	2	-	3	0.8	1.0
18	<i>Culex (Culex) quinquefasciatus</i>	-	2	-	2	0.0	0.0
19	<i>Culex (Culex) theileri</i>	1	3	7	11	2.0	3.7
20	<i>Culex (Culex) vagans</i>	1	-	4	5	0.4	0.7
21	<i>Culex (Culiciomyia) viridiventer</i>	-	6	-	6	1.4	2.0
22	<i>Culiseta (Culiseta) alaskaensis ssp. indica</i>	-	-	3	3	0.0	0.0
23	<i>Culiseta (Allotheobaldia) longiareolata</i>	-	-	1	1	-	-

24	<i>Culiseta (Culiseta) niveitaeniata</i>	-	2	-	2	0.0	0.0
25	<i>Lutzia (Metalutzia) halifaxii</i>	-	2	-	2	0.0	0.0
26	<i>Ochlerotatus</i> (subgenus uncertain) <i>caspius</i>	-	-	4	4	0.4	0.5
27	<i>Ochlerotatus (Finlaya) oreophilus</i>	-	1	-	1	-	-
28	<i>Gilesius pulchriventer</i>	-	2	-	2	0.2	0.2
29	<i>Ochlerotatus</i> (subgenus uncertain) <i>pullatus</i>	-	3	-	3	0.2	0.3
30	<i>Ochlerotatus (Finlaya) sintoni</i>	-	-	2	2	0.2	0.2
31	<i>Stegomyia (Stegomyia) aegypti</i>	-	2	-	2	0.0	0.0
32	<i>Uranotaenia (Pseudoficalbia) unguiculata</i>	-	1	-	1	-	-
	<b>Genera - 10, Subgenera - 14</b>	<b>105</b>	<b>16</b>	<b>51</b>	<b>38</b>		<b>3.7</b>

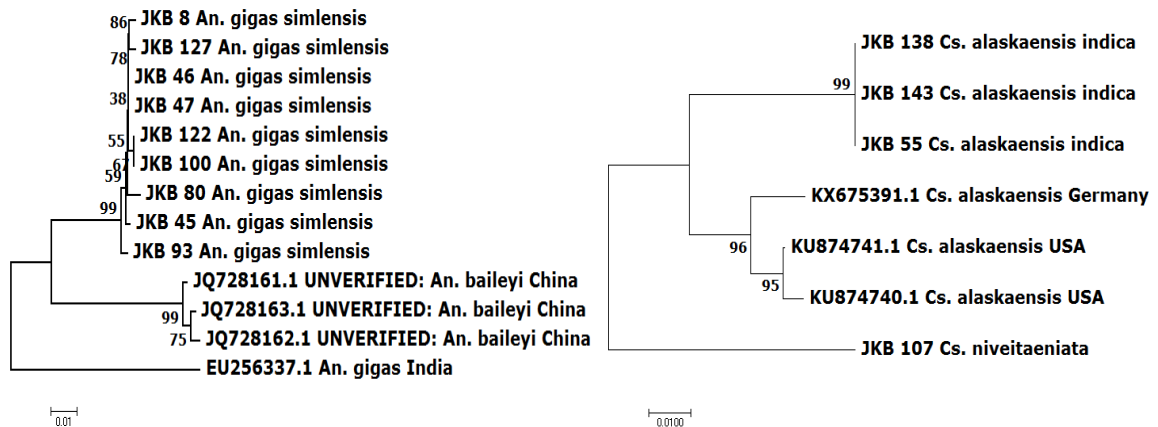
**Table 12: K2P sequence divergence at the *COI* barcode region within the same mosquito species with at least two specimens, within the same genera with at least two species, and between different genera**

Distance class	No. of specimens	Taxa	Min (%)	Mean (%)	Max (%)
Intraspecific	99	26	0.0	0.99	3.7
Intrageneric	84	4	1.3	10.37	13.8
Intergeneric	105	10	9	14.46	20

The mean genetic distance (K2P) computed for the different species of Culicidae belonging to 10 genera studied was found to be 0.124 or 12.4%. The intraspecific divergences ranged from 0.0–3.7% with a mean of 0.99% and the maximum intra-specific K2P value, 3.7% or 0.037 was found for *Culex (Culex) theileri*. The intrageneric K2P values ranged from 1.3% (*Culex (Culex) pipiens* and *Culex (Culex) quinquefasciatus*) to 13.8% (*Anopheles (Anopheles) barianensis* and *Anopheles (Cellia) willmori*) (Table 12). An overlap between the intraspecific and intrageneric K2P divergences was observed. The overlap was due to *Culex (Culex) quinquefasciatus* and *Culex (Culex) pipiens* which have shown 1.3% for intrageneric K2P genetic distance. These species cannot get distinguished by barcoding observed by other workers also (see ‘DNA Barcoding’ of *Discussion* part). Intraspecific distances of six species (*An. stephensi*, *Cx. bitaeniorhynchus*, *Cx. malayi*, *Cs. longiareolata*, *Oc. oreophilus*, *Ur. unguiculata*) could not be determined due to single representatives, but all of their minimum genetic distance to the nearest-neighbour (NN) was found higher than 4%. Sequence divergence increased with taxonomic rank with little overlap between intraspecific and intrageneric distances (Table 12).

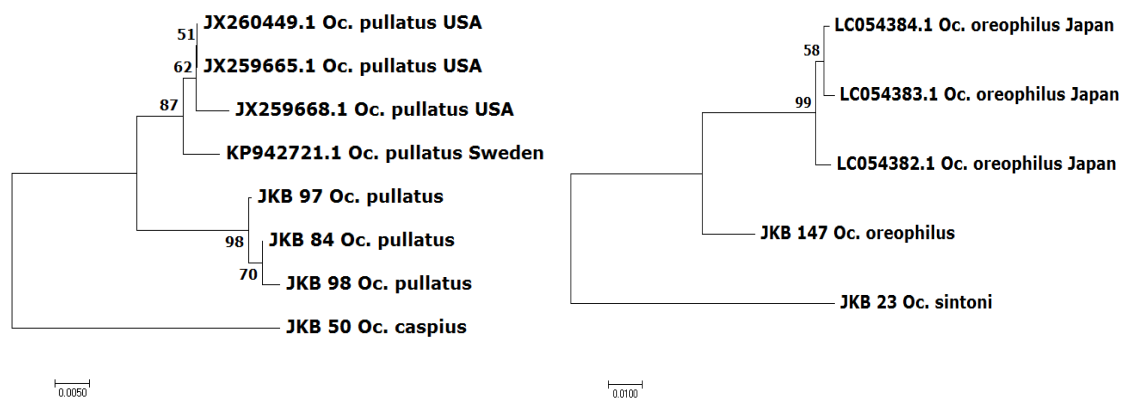
The average intraspecific distance (except two species, *Am. vexans*, and *Cx. (Cux.) theileri*) was found 0.4% ranging from 0 to 2% (Table 11). Hence, this study denoted that the K2P genetic distances were >0.02 between different species studied for Culicidae. Similar values have been used as a measure of intraspecific variation in other mosquito studies, for example, 0.00–2.58% for the *Anopheles strodei* subgroup of subgenus *Nyssorhynchus* (Bourke *et al.* 2013), 0.00–2.40% for 24 species in Pakistan (Ashfaq *et al.* 2014), 0.00–2.52% for 24 species in Belgium (Versteirt *et al.* 2015), 0.00–2.30% for 13 *Culex* species in Turkey (Gunay *et al.* 2015), and 0.00 to 2.67% for the *Cx. coronator* complex (Laurito *et al.* 2017). High intraspecific sequence divergence among *Am. vexans*, and *Cx. (Cux.) theileri* suggested the existence of cryptic species. In case of *Am. vexans*, only one specimen has shown the high divergence from 2.2 to 3.6% when compared with other 9 specimens. In case of *Cx. theileri*, 5 specimens (out of 11 specimens) have shown high divergence from 3.4 to 3.7% and some morphological differences regarding the arrangement of scales on thoracic region were also observed (see ‘DNA Barcoding’ of *Discussion* part).

In this study, the sequences of the species were also compared with their respective sequences available on GenBank which revealed some interesting results of some species (Fig. 10). The inference derived from the comparisons is discussed in ‘DNA Barcoding’ of *Discussion* part.



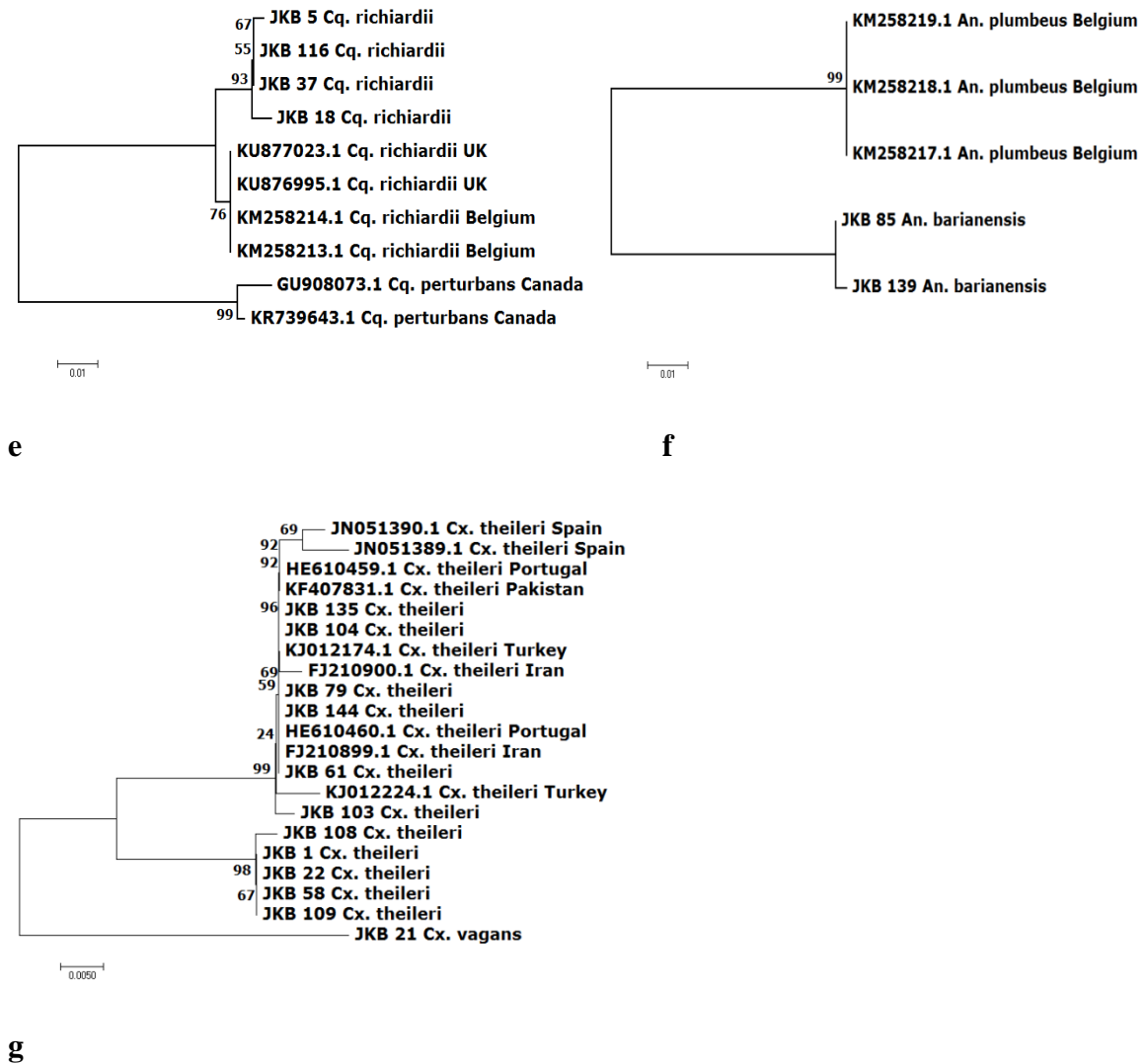
a

b



c

d

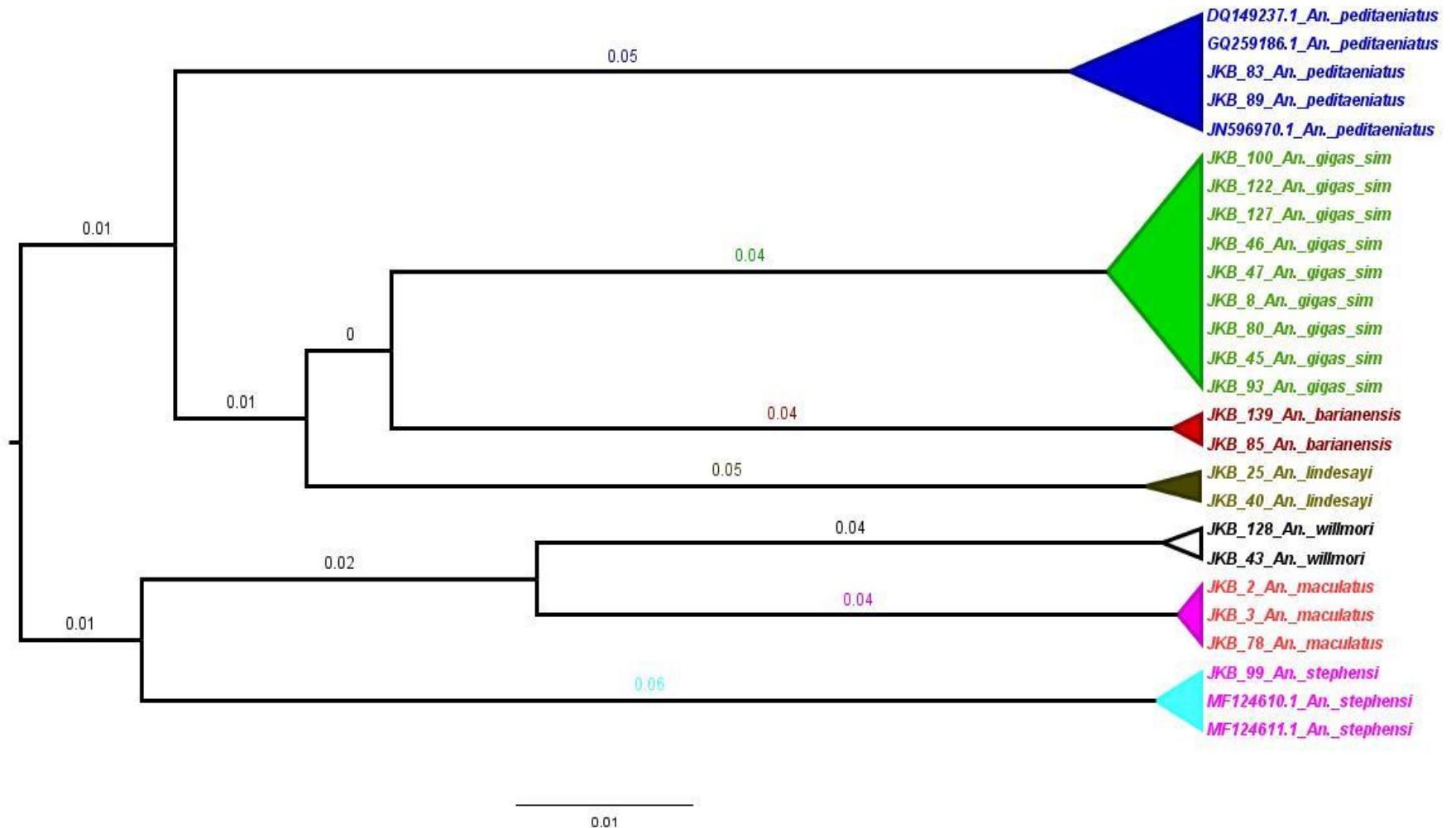


**Fig. 10: Neighbor-Joining (NJ) trees of the some species on comparing their *COI* sequences with reference/other sequences available on GenBank. Our sequences are named as JKB (Jammu Kashmir Barcode); other than these are the sequences taken from GenBank. a. *An. gigas simlensis*, b. *Cs. alaskaensis indica*, c. *Oc. pullatus*, d. *Oc. oreophilus*, e. *Cq. richiardii*, f. *An. barianensis*, and g. *Cx. theileri***

Comparing Bayesian and NJ trees (Fig. 11a,b and 12a,b) it is apparent that they are not same. *An. (Ano.) peditaeniatus* and *An. (Ano.) lindesayi*'s most recent common ancestor is more ancient than *An. (Ano.) gigas simlensis* and *An. (Ano.) barianensis* in Bayesian tree while as it is reverse in NJ tree. Subgenus *Cellia* and subgenus *Anopheles* are quite distinct or separated in Bayesian tree and share one ancestor only while as in NJ

tree these subgenera are not as separated and *An. stephensi* of subgenus *Cellia* and subgenus *Anopheles* share two ancestors. In culicines, both trees show that *Cq. richiardii* directly comes from the most recent common ancestor (MRCA) but they differ in other species. *Ur. (Pfc.) unguiculata* directly comes from second common ancestor in Bayesian tree while as it comes from fourth ancestor in NJ tree. In both trees *Cx. (Mai.) hortensis* goes with aedine species but in Bayesian tree all aedine species (not including other species) arise from sixth ancestor while as in NJ tree they arise from fourth and fifth ancestor. Moreover, Bayesian tree is more appropriate than NJ tree when compared with the morphological characteristic relationships.

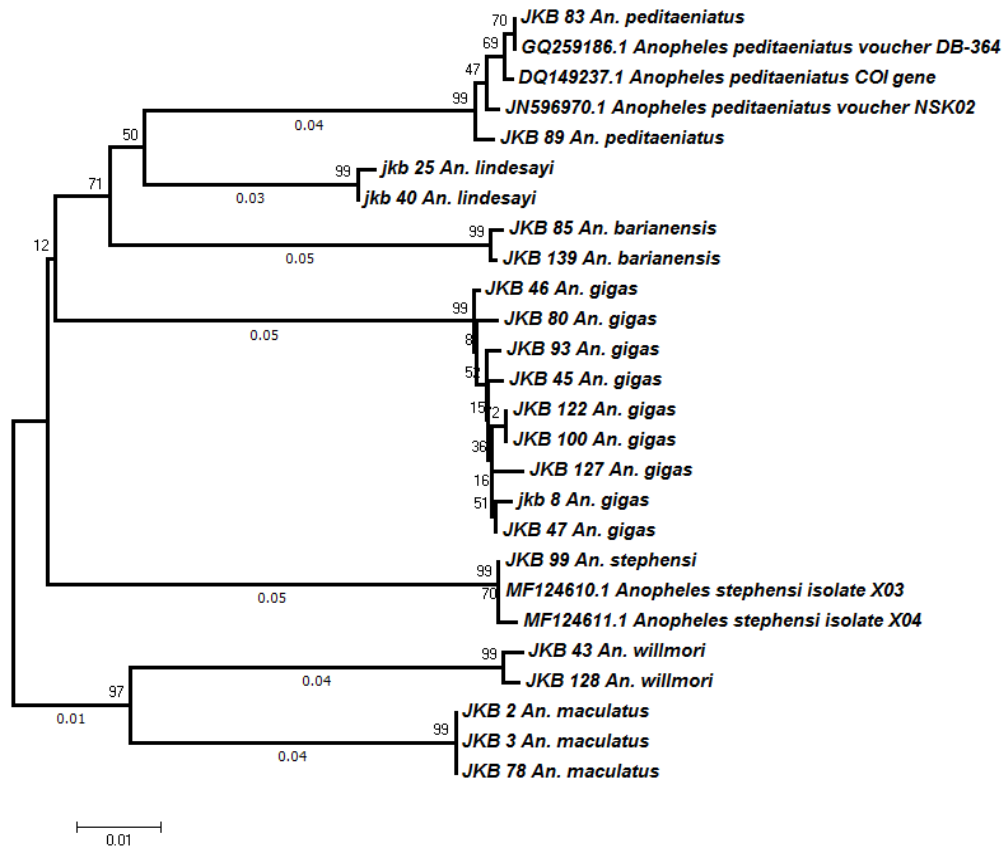
Of our *COI* sequences, sequences of 7 species (5 species and 2 subspecies which can be new species) *Oc. (Fin.) sintoni*, *Gi. pulchriventer*, *Cs. (Cus.) niveitaeniata*, *An. (Ano.) barianensis*, *An. (Cel.) willmori*, *Cs. (Cus.) alaskaensis* ssp. *indica*, *An. (Ano.) gigas* ssp. *simlensis* were not found on GenBank.



**Fig. 11a: Bayesian tree of Anophelines.** Our sequences are named as JKB (Jammu Kashmir Barcode); other than these are the sequences taken from GenBank.







**Figure 12:** NJ analysis of mosquitoes collected from Jammu, Kashmir, and Ladakh divisions of J&K. Bootstrap values (1000 replicates) are shown above the branches. The scale bar shows K2P distances. Analyses were conducted in MEGA7. Our sequences are named as JKB (Jammu Kashmir Barcode); other than these are the sequences taken from GenBank. a. *Anophelines* b. *Culicines* (below)

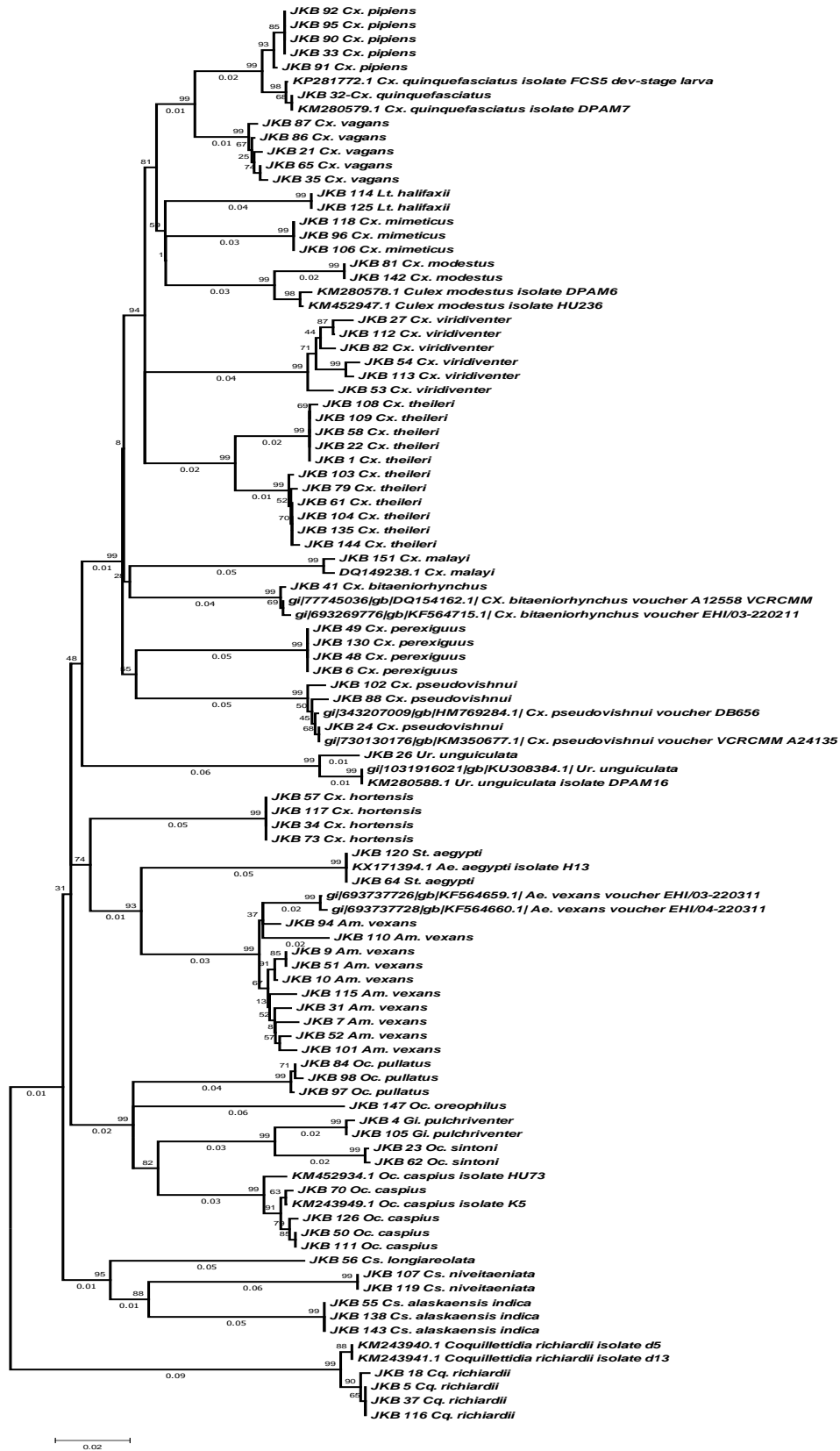


Fig. 4.4b

## 4.2. Ecological Notes

Notes for the 36 species newly reported from Jammu and Kashmir State in this study and for five additional species, *Ochlerotatus (Finlaya) sintoni*, *Aedimorphus vexans*, *Anopheles (Anopheles) barianensis*, *Ochlerotatus* (subgenus uncertain) *pullatus*, and *Anopheles (Anopheles) gigas* ssp. *simlensis* whose occurrence in the State and identification merits mention, are provided.

### 1. *Aedimorphus culicinus* (Edwards 1922):

**Materials examined:** 2♀. **Present record:** Collected from Jammu division. 2 ♀ reared from larvae collected from canal with grass margin near Govt. Degree College, Samba.

**Distribution:** India, Cambodia, Pakistan, South Vietnam, Thailand, Indonesia.

**Habitat:** Ground pools with grassy margins and decomposing algae on bottom. Adults prefer feeding on cattle to man at a ratio of 3:1 during night time. Bite in daytime also.

(References: Reinert 1973; Barraud 1934; WRBU catalog)

### 2. *Aedimorphus pipersalatus* (Giles 1902):

**Materials examined:** 3♂ 4♀ 3Le. **Present record:** Collected from Jammu division. 2♂ 2♀ reared from immature collected from ground pool with grass margin, Mansar, and 2♀ 1♂ collected as resting, Vijaypur in Jammu district.

**Distribution:** India, Cambodia, Sri Lanka, Pakistan, Bangladesh, Myanmar, Nepal, Laos, Thailand. In India, common from north-west as far east as Bengal, and through peninsular India to Sri Lanka. **Habitat:** Ground pools, water-filled ditches, paddy field. Adults were collected in indoor shelters and biting outdoors.

(References: Reinert 1973; Barraud 1934; WRBU catalog)

### 3. *Anopheles (Anopheles) peditaeniatus* (Leicester 1908):

**Materials Examined:** 4♂ 6♀ 5L 6Le. **Present record:** Collected from Jammu division. 4 larvae and 4♂ 6♀ reared from immature collected from a pond with algae,

Jindrah Challad, and 1 larva collected from irrigation canal with grass and algae, Nai Basti (Sohanjana) in Jammu district.

**Distribution:** India, Pakistan, Iran, Afghanistan, Sri Lanka, Bangladesh, Bhutan, Cambodia, Nepal, Myanmar, Indonesia, Malaysia, Thailand, Vietnam, southerly parts of China, Borneo, Philippines, and Celebes. **Habitat:** Zoophilous species, often abundant at cattle sheds. Larvae are found in grassy ponds and swamps and may be in rice-fields also. **Medical importance:** Transmits filarial infections of cattle. JE vector in India.

(References: Reid 1968; Bhattacharyya *et al.* 2014; WRBU catalog)

#### 4. *Anopheles (Cellia) dravidicus* Christophers 1924:

**Materials Examined:** 1♀. **Present record:** Collected from Jammu division. 1♀ reared from immature collected from stream margin with algae, Phalata, Udhampur district.

**Distribution:** India (Nilgiri Hills, Assam, Manipur, Sikkim); Myanmar; China; Laos; Nepal. **Habitat:** Could not find out the larval habitat of *dravidicus* from the available literature. So according to the present collection one of its larval habitat is stream margin. Adult female is found inside houses and bites man as Singh *et al.* (2012) collection method is concerned.

(References: WHO 2007; Rattanaarithikul & Green 1986; Singh *et al.* 2012; WRBU catalog)

#### 5. *Anopheles (Cellia) pseudowillmori* (Theobald 1910):

**Materials Examined:** 1♂ 2♀. **Present record:** Collected from Jammu division. 1♂ and 1♀ reared from immature collected from riverside pool, Nagrota – Balani bridge, Jammu district and stream margin with algae, Phalata, Udhampur district respectively; 1♀ collected as resting indoors, Sadyali Nud, Samba district.

**Distribution:** India (Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tripura, Punjab, Himachal Pradesh); Nepal; China; Thailand; Vietnam; Bhutan; Laos. **Habitat:** Larvae collected primarily from rice fields, stream margins,

ponds, wells, and pits. **Medical importance:** Malaria vector in Bhutan, Tibet, and Thailand

(References: Rattanaarithikul *et al.* 1995; Singh *et al.* 2012)

#### 6. *Armigeres (Armigeres) subalbatus* (Coquillett 1898):

**Materials Examined:** 106♂ 146♀ 17L 8Le 5Pe 3♂G. **Present record:** Collected from Jammu division. 66♂ 104♀ collected as resting and biting indoors and outdoors from localities: (Achabal, Banderpora) Anantnag district, (Dogripora, Tahab) Pulwama district, (Hokersar, Nowgam, Ishber, Naseem-bagh) Srinagar district, (Railway Station) Budgam district, (Uri – Thajal road, Uri - Uroosa) Baramulla district, (Gandharwan, Nagrota, Raipur) Jammu district, (Mearth, Barnoti) Kathua district; 17 larvae, 40♂ 42♀ reared from immature collected from bucket in Burn Morh and arum plant in Mansar, Jammu district, cactus in Mearth, Kathua district, and canal (probably mix of drain and canal water) in Uri – Thajal road, Baramulla district.

**Distribution:** India, Sri Lanka, Bangladesh, Myanmar, Nepal, Pakistan, Cambodia, China, Indonesia, **Japan**, Korea, South, Laos, Malaysia, Philippines, Taiwan, Thailand, Vietnam. **Habitat:** Larvae occur in various types of containers with strongly polluted water such as tree-holes, bamboos, and domestic collections of water. Adult females bite man throughout the day, being most active at dawn and dusk. **Medical importance:** Vector of *W. bancrofti*. Vector of dog heartworm *Dirofilaria immitis* in Peninsular Malaysia and zoonotic *Brugia pahangi* filariasis in suburban Kuala Lumpur, Peninsular Malaysia.

(References: Barraud 1934; Tanaka 1979; WRBU catalog; Muslim *et al.* 2013)

#### 7. *Collessius (Alloeomyia) pseudotaeniatus* (Giles 1901):

**Materials Examined:** 8♂ 17♀ 3L 6Le 2♂G. **Present record:** Collected from Jammu division. 2♂, 5♂ 9♀ and 1♂ 8♀ reared from larvae collected from a tyre in SRTC Yard, Jammu district, cement tank in Jakli, Jammu and canal with algae near Govt. Degree College, Samba district respectively. 3 larvae collected from a rock hole, Kaghote, Udhampur district.

**Distribution:** One of the commonest species both in the hills and plains and extending from the North-West Frontier (Khyber Pakhtunkhwa) and Baltistan to Nepal, Assam, Bangladesh, and Myanmar and throughout the Indian region to Sri Lanka. Also recorded from Cambodia and Thailand. **Habitat:** Tree-holes, rock-pools, cement sinks, iron cisterns, drains.

(References: Barraud 1934; WRBU catalog)

#### 8. *Culiseta (Culiseta) alaskaensis ssp. indica* (Edwards 1920):

**Materials Examined:** 17♂ 7♀ 13L 4Le 1Pe 2♂G. **Present record:** Collected from Ladakh division. 3 larvae and 9♂ 2♀ reared from immature collected from seepage, 3 larvae and 1♂ reared from immature collected from ground pool with aquatic plants and 1♀ reared from immature collected from pit with grass (polluted), Basgo, Leh district; 1 larva and 3♂ reared from immature collected from swamp and 6 larvae and 1♀ reared from immature collected from burrow pit, Shey, Leh district; 1♂ and 3♂ 2♀ reared from immature collected from riverside pool with grass margin, Khalsar and swamp, Shey (near palace), Leh district respectively.

**Distribution:** India, Pakistan, Russia, Tajikistan, Turkmenistan, Ukraine, Uzbekistan, Kazakhstan, Kyrgyzstan, Azerbaijan, Armenia, Georgia. **Habitat:** Larvae mostly confined to small pools, sometimes saline; prefer moderate hypothermal conditions and avoid shaded areas. It has been reported in forest free regions. Adults found rarely in human habitations and attack man more rarely but attack domestic animals and large mammals frequently. Polycyclic with female diapause.

(References: Maslov 1989; Barraud 1934; WRBU catalog)

**Remarks:** *indica* is a subspecies of *alaskaensis* as per literature (see Becker *et al.* 2010, page number, 300; Harbach 2017). But our present studies indicate *indica* is a separate species – see ‘DNA Barcoding’ of *Discussion* part.

### 9. *Culiseta (Culiseta) niveitaeniata* (Theobald 1907):

**Materials Examined:** 1♂ 1♀ 31L 1♂G. **Present record:** Collected from Kashmir division. 6 larvae and 1♂ 1♀ reared from immature collected from stream pool, and 25 larvae collected from hoof marks, Doodhpathri, Budgam district.

**Distribution:** Western Himalayas: Kasauli, Murree, Naini Tal, Muktesar, Dehra Dun; Eastern Himalayas: north of Yatung, Nepal, Tibet, Taiwan, China (North East, Central and South). **Habitat:** Larvae are found in mountainous areas at an elevation of 1600 m to 3658 m, live in clear or polluted water in a wide variety of habitats such as ground and rock pools, pits, ditches, seepages, shallow wells, artificial containers, and pools in beds of hill-streams. Larvae can survive in frozen pools. In China, adult bites cattle, water buffalo, and man.

(References: Barraud 1934; Dobrotworsky 1971; Darsie *et al.* 1996)

### 10. *Culex (Culex) mimulus* Edwards 1915:

**Materials Examined:** 8♂ 8♀ 4L. **Present record:** Collected from Jammu division. 1 larva and 1♂ 1♀ reared from immature collected from a pond with algae and grass, Jindrah Challad, Jammu district; 6♂ 7♀ reared from immature collected from a rock pool, Nandni, Jammu district; 1♂ reared from immature collected from a stream with algae, Kirmoo, Udhampur district; 2 larvae and 1 larva collected from a stream pool with grass, Phalata, Udhampur district and a pond with algae, Daskal, Jammu district respectively.

**Distribution:** Widespread throughout Southeast Asia, extending west in India and Sri Lanka; north in China, east and south in Papua-New Guinea and northern Australia. Also found in Pakistan. **Habitat:** Breed in ground pools, ponds, wells, ditches, stream pools and marshy depressions usually with green algae, mosses, grasses and other aquatic vegetation but not in rock pools. Also have been found in artificial containers such as concrete, stone or cement tanks, coconut shells, and snail shells.

(Reference: Sirivanakarn 1976; WRBU catalog)



**11. Culex (Culex) murrelli Lien 1968:**

**Materials Examined:** 2♀ 2L. **Present record:** Collected from Jammu division. 2♀ reared from immature collected from a rock pool, Nandni, Jammu district; one one larva collected from a rock pool with decayed leaves, Kirmoo, Udhampur district and an irrigation canal with grass and algae, Nai Basti (Sohanjana), Jammu district.

**Distribution:** Probably widespread in Southeast Asia and adjacent areas. **Habitat:** Ground pools with algae and rock pools with decayed leaves

(Reference: Sirivanakarn 1976)

**12. Culex (Culex) perexiguus Theobald 1903:**

**Materials Examined:** 5♂ 4♀ 5L 8Le 3♂G. **Present record:** Collected from Jammu division. 5 larvae and 5♂ 4♀ reared from immature collected from seepage, Surinsar – Urkhal, Jammu

**Distribution:** Widely distributed in northern Africa and southwestern Asia, and extends eastward into India. **Habitat:** Swamps, springs, stream pools, ponds, and wells usually containing quantities of emergent vegetation. Generally breeds away from dwellings. Adult probably feeds mainly on birds it apparently occasionally enters houses and bites man. **Medical importance:** West Nile virus has been isolated from this species in Israel and Egypt. Isolations of Sindbis virus have been made in Israel, Egypt, and Saudi Arabia

(Reference: Harbach 1988)

**13. Culex (Culex) perplexus Leicester 1908:**

**Materials Examined:** 1♂ 1♀ 6L 4Le. **Present record:** Collected from Jammu division. 5 larvae and 1♂ 1♀ reared from immature collected from a pond with algae and grass, Jindrah Challad, and 1 larva collected from a stream pool with algae, Bantalab in Jammu district.

**Distribution:** India, Thailand, Malaysia, Singapore, and Indonesia. **Habitat:** Ground pools, stream margins, swamps, and ponds. All pools usually contain numerous decayed leaves and swamps and ponds usually contain some green algae.

(Reference: Sirivanakarn 1976)

#### 14. *Culex (Culex) pseudovishnui* Colless 1957:

**Materials Examined:** 69♂ 105♀ 66L 9Le 19♂G. **Present record:** Collected from Jammu, and Kashmir divisions. 26♂ 69♀ collected as resting and biting from bushes, houses, and cattle-shed in districts, Jammu (Raipur), Srinagar (Bemina, Ishber, Nowgam, near nigeen park), Baramulla (Mirgund, Uri, Uri - Uroosa), and Pulwama (Dogripora); 66 larvae and 43♂ 36♀ reared from immature collected from canal, pond, ground pool, irrigation canal, paddy field, cement tank, spring pool, stream pool, stream margin, lake, seepage with grass, algae, water plants, and leaves in districts, Jammu (Jindrah Challad, Panjoda, Kumbi Morh, Bantalab, Daskal, Sagoon), Udhampur (Jakheni Park, Phalata), Srinagar (Habak crossing, Naseem-bagh, Nowgam, Saida Kadal), Baramulla (Dachi, Uri – Redbridge, Veerwan), and Pulwama (Dogripora).

**Distribution:** Widely distributed in Oriental region and occurs in China, Korea, Japan, Pakistan, and Iran. **Habitat:** Larvae are commonly found in rice fields, ground pools, ditches, stream margins, marshes, and ponds. Females are attracted to birds and domestic animals and occasionally bite man. **Medical importance:** It is a potential vector of Japanese encephalitis virus and has been associated with Kunjin and Tembusu viruses in Southeast Asia

(References: Harbach 1988; Sirivanakarn 1976)

#### 15. *Culex (Culex) tritaeniorhynchus* Giles 1901:

**Materials Examined:** 69♂ 35♀ 26L 26Le 7♂G. **Present record:** Collected from Jammu, and Kashmir divisions. 26♂ 15♀ collected as resting and biting from bushes, and cattle-shed in districts, Jammu (Nai Basti (Sohanjna), Thalwal), Samba (Sadyali Nud), Kathua (Barnoti), and Srinagar (Nowgam); 26 larvae and 43♂ 20♀ reared from immature collected in Jammu district from ground pool and paddy field (Bantalab), river margin

(Jewel Chowk), cement tank with leaves (Kumbi Morh), ground pool with grass margin (Mansar), irrigation canal with grass and algae (Nai Basti (Sohanjana)), seepage (Surinsar – Urkhal), riverside pool with algae (Thalwal), stream with grass margin (Battal Ballian), pond with algae and grass (Jindrah Challad), irrigation canal with grass margin (Adhuvana Mori (Sohanjana)), Udhampur district from cement tank with garbage (Jakheni park), rock pools and stream pool with algae (Phalata), spring pool with vegetation (Birma), and Kathua district from paddy field (Barnoti).

**Distribution:** Occurs in eastern Asia, the Oriental region, southwestern Asia, and Africa. **Habitat:** Larvae are found in rice fields, flood waters, and marshy areas with floating or emergent vegetation. Also found in ponds, swamps, streams, springs, irrigation ditches, grassy pools, seepages, and animal hoof prints. Females are known to enter houses and bite man during any time at night. **Medical importance:** Vector of Japanese encephalitis virus. Also has been associated with various viruses and filarial pathogens in many areas of eastern and southeastern Asia.

(References: Harbach 1988; Sirivanakarn 1976)

#### 16. *Culex (Eumelanomyia) brevipalpis* (Giles 1902):

**Materials Examined:** 7♂ 6♀ 5L 14Le 1♂G. **Present record:** Collected from Jammu division. 5 larvae and 7♂ 6♀ reared from immature collected from a tire with dead leaves, SRTC Yard, Jammu district.

**Distribution:** India, Sri Lanka, Myanmar, China, Taiwan, Ryukyu Islands, Thailand, Cambodia, South Vietnam, Malaysia, Indonesia, Philippines, Bismarck Archipelago. **Habitat:** The most frequent breeding sites of *brevipalpis* are treeholes and bamboos. On certain occasions, have also been collected from other containers such as coconut shells, water jugs, discarded barrels and stone basins. Females have never been collected biting man or other domestic animals.

(Reference: Sirivanakarn 1972)

**17. Culex (Eumelanomyia) malayi (Leicester 1908):**

**Materials Examined:** 2♂ 2♀ 5L 2♂G. **Present record:** Collected from Jammu division. 2 larvae and 1♀ reared from immature collected from a pond with algae and grass, Jindrah Challad, 2 larvae and 1♀ reared from immature collected from a stream pool with algae, Bantalab, and 1 larva and 2♂ reared from immature collected from a pond with algae, Daskal in Jammu district.

**Distribution:** India, Bangladesh, Nepal, Pakistan, Sri Lanka, Cambodia, China, Indonesia, Malaysia, Maldives, Myanmar, Taiwan, Thailand, Timor, Vietnam. **Habitat:** It is a common forest species utilize ground pools as the principal breeding habitat. Larval habitats also include ponds, ditches, rock pools, and crab holes.

(Reference: Sirivanakarn 1972; WRBU catalog)

**18. Culex (Lophoceraomyia) minutissimus (Theobald 1907):**

**Materials Examined:** 1♂ 2L 2♂G. **Present record:** Collected from Jammu division. 1♂ collected as resting indoors, Sadyali Nud, Samba district; 2 larvae collected from a stream pool with grass, Phalata, Udhampur district.

**Distribution:** Known from India, Bangladesh, Pakistan, Sri Lanka, Maldives, Indonesia, Malaysia, and Thailand. **Habitat:** It is a typical ground pool breeder. Reported from rock-springs, pools in ravines and river-beds, shallow wells, stagnant water in shaded culverts, coconut shells, flooded pools in mountain areas, etc. Adults were collected resting on stream banks, pit shelters, rock crevices, crab holes, and banana plantations.

(References: Barraud 1934; Sirivanakarn 1977; WRBU catalog)

**19. Culex (Oculeomyia) infula Theobald 1901:**

**Materials Examined:** 1♂ 1♂G. **Present record:** Collected from Jammu division. 1♂ collected as biting outdoors, Barnoti, Kathua district.

**Distribution:** India, Sri Lanka, Bangladesh, Nepal, Myanmar, Thailand, Malaysia, Vietnam, Indonesia, Singapore, Philippines, and Micronesia. **Habitat:** Restricted to breeding in large, algae filled ground pools. Adults have been caught in light traps and biting man or other animals. **Medical importance:** JE vector in India.

(References: Sirivanakarn 1976; Bhattacharyya *et al.* 2014; WRBU catalog)

#### 20. *Culex (Oculeomyia) sinensis* Theobald 1903:

**Materials Examined:** 4♂ 13♀. **Present record:** Collected from Jammu division. 1♀ resting indoors and 4♂ 12♀ resting outdoors collected in Mearth and Barnoti respectively, Kathua district.

**Distribution:** Apparently an uncommon species but widespread throughout the Oriental region with the range extending north and northeast into the Palaearctic region (China, USSR, Japan, and Korea). **Habitat:** Fresh water ground pools such as puddles, ponds and ditches in rice fields, and stream pools containing abundant vegetation, including green algae. Adult females were found biting man indoors and outdoors. Appear semi- or peri-domestic species.

(Reference: Sirivanakarn 1976)

#### 21. *Hulecoeteomyia chrysolineata* (Theobald 1907):

**Materials Examined:** 1L. **Present record:** Collected from Jammu division. 1 larva collected from a cement tank, Jakli, Jammu district.

**Distribution:** India, Sri Lanka, Bangladesh, Nepal, Cambodia, Indonesia, Laos, Malaysia, Philippines, Thailand, Vietnam. **Habitat:** Larvae reported from tree-holes, *Colocasia* leaf axils, banana tree leaf axil, fallen leaf, bamboos, clay pot, rubber cup, rock-pools, roof-gutter, and broken chatti. Adult recorded as being a sylvan biter of man.

(Reference: Barraud 1934; Knight 1968; WRBU catalog)

## 22. *Lutzia (Metalutzia) fuscana* (Wiedemann 1820):

**Materials Examined:** 1♂ 3♀ 2Le. **Present record:** Collected from Jammu division. 2♀ reared from immature collected from mix of drain and irrigation canal water, Satrayan - RS Pura, 1♂ reared from immature collected from a cement tank, Jakli, 1♀ reared from immature collected from a cement tank, Sagoon, and immature (2Le on slide) were collected from a Paddy field, Bantalab in Jammu district.

**Distribution:** India, Sri Lanka, Bangladesh, Pakistan, Nepal, Cambodia, Myanmar, Indonesia, Macau, Malaysia, Thailand, Timor, Vietnam, Malaya, Sumatra, Java, Borneo, Philippines, China, Taiwan, Russia, Korea, Japan, Singapore, Caroline Islands, Palau Islands, Mariana Islands, and Wake Island. **Habitat:** Natural pools, shallow wells, domestic collections of water, etc. Larvae are predatory in nature and potent destroyers of *quinquefasciatus* larvae under field conditions, but the predator persists at such a low level that no significant controlling effect is registered on the *quinquefasciatus* population. Adult females apparently feed primarily on avian hosts and could not be induced to feed on man.

(References: Barraud 1934; Bram 1967; WRBU catalog)

## 23. *Lutzia (Metalutzia) halifaxii* (Theobald 1903):

**Materials Examined:** 20♂ 12♀ 4L 6Le 1Pe 9♂G. **Present record:** Collected from Jammu and Kashmir divisions. 1♂ from Uri – Thajal Road, Baramulla district, 1♂ from Uri – Uroosa, Baramulla district, and 1♀ from Raipur, Jammu district collected as resting in bushes. 1♂ reared from immature collected from a canal with algae near Govt. Degree College, Samba district; 2♂ 2♀ and 9♂ 6♀ reared from immature collected from a cement tank, Sagoon and a cement tank with leaves, Kumbi Morh, Jammu district respectively; 4♂ and 1♂ 1♀ reared from immature collected from a rock pool, phalata and seepage, Ritti, Udhampur district respectively; 1 larva and 1♂ 2♀ reared from immature collected from a tyre, Surinsar, Jammu district; 1 larva from a spring pool, Sagoon, Jammu district, 1 larva from stream margin with algae, Panjoda, Jammu district, and 1 larva from stream with algae, Kirmoo, Udhampur were collected.

**Distribution:** India, Sri Lanka, Nepal, Bangladesh, Cambodia, Myanmar, Thailand, Malaya, Philippines, China, Taiwan, Russia, Korea, Japan, Singapore, Timor, Vietnam, Bismarck Archipelago, Ogasawara, Gunto, Ryukyu-Retto, Solomon Islands, Mariana Islands, New Guinea, Australia. **Habitat:** Larvae occur in artificial containers, rock pools, stream margins, tree holes, and various kinds of temporary and semi-permanent ground water habitats (rice fields, small ponds, road-side ditches, jungle-pools). Larvae are predacious on various mosquito species as well as other arthropods of appropriate size. Man has been reported as an occasional host of an adult female.

(References: Barraud 1934; Bram 1967; WRBU catalog)

#### **24. *Malaya genurostris* Leicester 1908:**

**Materials Examined:** 7♀ 4L 1Le. **Present record:** Collected from Jammu division. 4 larvae and 2♀ reared from immature collected from leaf axils of Arum plants (*Colocasia* sp.), Mansar, Jammu district; 5♀ reared from immature collected from leaf axils of Arum plants (*Colocasia* sp.) from an unknown locality near a National Highway (name and coordinates unavailable) in Kathua district.

**Distribution:** India, Bangladesh, Sri Lanka, Cambodia, China, Indonesia, Japan, Malaysia, Maldives, Myanmar, Nepal, Australia, New Guinea (Island); Papua New Guinea, Philippines, Singapore, Taiwan, Thailand. **Habitat:** Water in leaf-bases of a large Arum

(Reference: Barraud 1934; WRBU catalog)

#### **25. *Mucidus (Mucidus) scataphagoides* Theobald 1901:**

**Materials Examined:** 1♂. **Present record:** Collected from Jammu division. 1♂ reared from immature collected from a canal with algae near Govt. Degree College, Samba district.

**Distribution:** India, Sri Lanka, Bangladesh, China, Myanmar, Nepal, Pakistan, Gabon, Ghana, Madagascar (includes Glorioso & Juan De NovaIs), Mali, Mauritania,

Mozambique, Senegal, Thailand, Vietnam. **Habitat:** Open natural pools, ditch, rice paddy, swamp

(References: Barraud 1934; Tyson 1970; WRBU catalog)

## 26. *Neomelaniconion lineatopenne* (Ludlow 1905):

**Materials Examined:** 1♂ 3♀ 1Le 1Pe. **Present record:** Collected from Jammu division. 1♂ 1♀ reared from immature collected from an irrigation canal with grass and algae, Nai Basti (Sohanjana), Jammu district. 2♀ reared from immature, one collected from a canal with grass margin and another collected from a canal with algae near Govt. Degree College, Samba district.

**Distribution:** India, Sri Lanka, Indonesia, Bangladesh, Pakistan, Nepal, Malaysia, Benin, Borneo (island), Burkina Faso, Cambodia, China, Congo, Gabon, Ghana, Kenya, South Korea, Laos, Namibia, Nigeria, Philippines, Russia, South Africa, Thailand, Timor, Togo, Vietnam, Australia. **Habitat:** Open natural pools. **Medical importance:** A potential vector of West Nile (WN) virus in Pakistan, epizootic Rift Valley fever (RVF) virus in Kenya, a suspected vector of Japanese encephalitis (JE) virus in the Malaysian peninsular, and an efficient laboratory vector of the dog heartworm, *Dirofilaria immitis* in Thailand.

(References: Barraud 1934; Akhter *et al.* 1982; Linthicum *et al.* 1985; Vythilingam *et al.* 1997; Tippawangkosol *et al.* 1998; WRBU catalog)

## 27. *Phagomyia gubernatoris* (Giles 1901):

**Materials Examined:** 3♀ 1Le. **Present record:** Collected from Jammu division. 3♀ reared from immature collected from cactus hole, Mearth, Kathua district.

**Distribution:** India, Bangladesh, China, Nepal, Sri Lanka, Thailand. **Habitat:** Tree-holes, bamboo stump. Adult females more active at dusk and bite man.

(References: Barraud 1934; Krishnamoorthi & Livingstone 1980; Munirathinam *et al.* 2014; WRBU catalog)



**28. St. (subgenus uncertain) patriciae (Mattingly 1954):**

**Materials Examined:** 1♂1Le 1Pe 1♂G. **Present record:** Collected from Kashmir division. 1♂ reared from mud taken from a tree base, Uri – Silikot, Baramulla district.

**Distribution:** India, China, Malaysia, Pakistan, Thailand, Vietnam. **Habitat:** Tree-holes

(References: Huang 1972; WRBU catalog)

**29. St. (subgenus uncertain) unilineata (Theobald 1906):**

**Materials Examined:** 8♂ 20♀. **Present record:** Collected from Jammu division. 8♂ 20♀ reared from immature collected from cactus hole, Mearth, Kathua district.

**Distribution:** India, Nepal, Pakistan, Angola, Burkina Faso, Central African Republic, Cote d'Ivoire, Gabon, Ghana, Malawi, Nigeria, Saudi Arabia, Senegal, South Africa, Sudan and South Sudan, Zambia. **Habitat:** Tree-holes, hole in a pawpaw tree (*Carica papaya*), rock holes. Adult females bite man.

(References: Barraud 1934; Hopkins 1952; Ribiero & Ramos 1973; Huang 2004; WRBU catalog)

**30. Toxorhynchites (Toxorhynchites) splendens (Wiedemann 1819):**

**Materials Examined:** 2♂ 1♀ 3Le. **Present record:** Collected from Jammu division. 2♂ 1♀ reared from immature collected from a tire with decaying leaves, SRTC Yard, Jammu district. *Cx. brevialpilis* were found associated breeder.

**Distribution:** India, Bangladesh, Sri Lanka, Cambodia, China, Fiji, Indonesia, Malaysia, Myanmar, Nepal, Australia, New Guinea (Island); Papua New Guinea, Philippines, Thailand, Vietnam. **Habitat:** Tree-holes, bamboos, and sometimes domestic collections of water such as water-butts, jars, etc. Larvae prey upon and devour the larvae of other mosquitoes living in the same habitat. Adults do not bite and fly by day and visit flowers for the purpose of sucking honey.

(References: Barraud 1934; WRBU catalog)

### 31. Verrallina (Harbachius) yusafi (Barraud 1931):

**Materials Examined:** 1♀ 1♀G. **Present record:** Collected from Jammu division. 1♀ collected as biting, Gandharwan, Jammu district.

**Distribution:** India, Sri Lanka, Thailand. **Habitat:** In Thailand, numerous females were collected biting man. The adults presumably had emerged from the ground pool but no immatures could be found.

(References: Reinert 1974; WRBU catalog)

### 32. Culex (Culex) pipiens Linnaeus 1758:

**Materials Examined:** 40♂ 30♀ 6L 9♂G. **Identification:** It can be distinguished from its closest species *Cx. quinquefasciatus* on the basis of genitalia only (larva and adult are very similar to larva and adult of *Cx. quinquefasciatus*). In male genitalia of *Cx. pipiens* the ventral arm of aedeagus is slender and sickle shaped while as in *Cx. quinquefasciatus* the ventral arm of aedeagus is broad and leaf like. In the present collection, we found the adult of *Cx. pipiens* more yellow than the adult of *Cx. quinquefasciatus*. **Present record:** Collected from Ladakh division. 1♂ collected inside a hotel room, Khumani Chowk, Kargil district; 4♂ 3♀ and 2♂ collected as resting outdoors in Shey and Ganglass, Leh district respectively. 4♂ 3♀ reared from immature collected from ground pool with water plant and algae, Basgo; 3♂ 3♀ reared from immature collected from pond, Ganglass; 4♂ reared from immature collected from riverbed pool with grass margin, Khalsar; 1♂ reared from immature collected from ground pool with grass margin, Ney; 4♂ 3♀ reared from immature collected from swamp, Phey; 6 larvae and 6♂ 2♀ reared from immature collected from borrow pit, and 8♂ 3♀ reared from immature collected from swamp, Shey; 2♀ each reared from immature collected from pond with aquatic plants and swamp, Shey (near palace); 1♂ 1♀ reared from immature collected from seepage, suspol in Leh district. 2♂ 6♀ reared from immature collected from riverside pool, Sher-bagh; 1♀ reared from immature collected from ground pool with shade, Thasgam; 1♀ reared from immature collected from swamp with trees with Gindial in Kargil district.

**Distribution:** Widespread in the Holarctic region. Inhabits the temperate regions of Europe, Asia, Africa, Australia, and North and South America. In the southeasternmost areas of the northern hemisphere *Cx. pipiens* occurs sympatrically with *Cx. quinquefasciatus*. **Habitat:** Larvae are found in numerous and variable breeding places ranging from highly polluted cesspits to clear water pools and containers. Larvae can be found in rice field, larger pools with vegetation, barrels, tin cans, metal tanks, ornamental ponds; occasionally in tree-holes. They can tolerate a small amount of salinity and can occur in rock pools. Females are anautogenous, eurygamous, diapausing in wintertime, and ornithophilic although they have been observed to feed on wild mammals or on mice in the laboratory. **Medical importance:** WNV but plays a minor role as an arbovirus vector.

(References: Becker *et al.* 2010; Harbach 1988)

### **33. *Culex (Barraudius) pusillus* Macquart 1850:**

**Materials Examined:** 2♂ 4♀ 1Le 2♂G. **Identification:** It can be distinguished from its closely related species, *Cx. modestus* by darker coloration and the separated basolateral pure white patches of scales on the abdominal terga which do not form a continuous longitudinal stripe as usually found in *Cx. modestus*. Larva has siphon short and siphonal index at most 3.0. In male genitalia, gonostylus is distinctly shorter than half the length of gonocoxite and ventral arm of aedeagus is long extending beyond apex of paraproct. **Present record:** Collected from Ladakh division. 2♂ collected as resting outdoors and 4♀ reared from immature collected from swamp, Shey, and 2♀ reared from immature collected from swamp, Shey (near palace) in Leh district.

**Distribution:** Afghanistan, Algeria, Egypt, Greece, Iran, Iraq, Israel (and Gaza Strip & West Bank), Libya, Qatar, Saudi Arabia, Sudan and South Sudan, Syria, Tajikistan, Tunisia, Turkey, Turkmenistan, United Arab Emirates, Uzbekistan. **Habitat:** Apparently a halophilic species. Mainly found in coastal breeding places like saline marshes and swamps, lagoon or stagnant pools with or without vegetation and rarely in fresh water. Females probably do not feed on humans and have never been observed entering dwellings or stables.

(References: Becker *et al.* 2010; WRBU catalog)

**Remarks:** As this species is a new country record but due to its morphological similarities with *Cx. (Bar.) modestus* it is difficult to confirm fully its identification – see *Discussion* part.

#### **34. *Coquillettidia (Coquillettidia) richiardii* (Ficalbi 1889):**

**Materials Examined:** 13♂ 12♀ 7♂G. **Identification:** It can be identified with broad dark and pale scales intermixed on wing veins, numerous pale scales on proboscis and palpi, and pale rings on tarsomeres. In male genitalia, base of gonostylus slightly constricted below expanded portion and then sharply narrowed in the middle part. **Present record:** Collected from Kashmir division. 4♀ collected inside house, Bemina; 2♀ collected as biting and 12♂ as resting in vegetation (cattail), Hokersar; 1♂ 2♀ collected from vegetation, Ishber; 1♀ collected from vegetation, Naseem-bagh; 2♀ collected from vegetation, Nowgam in Srinagar district. 1♀ collected from vegetation (Apple orchard), Mirgund in Baramulla district.

**Distribution:** Europe and western Palearctic region - Afghanistan, Austria, Azerbaijan, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Iran, Ireland, Italy, Kazakhstan, Latvia, Lithuania, Luxembourg, Macedonia, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Syria, Tajikistan, Turkey, Ukraine, United Kingdom (includes Guernsey; Jersey; Isle of Man), Uzbekistan, Yugoslavia. **Habitat:** Larvae and pupae live submerged and obtain oxygen from aerenchyma of aquatic plants. Females can be very numerous and a severe nuisance to humans and domestic animals, in the surroundings of fresh water or slightly saline marshes, lakes, old river beds, and estuaries. They can bite indoor as well. Prefer to feed on mammals but can also feed on birds and amphibians. Biting activity is between 9 and 26°C temperature and 30 and 92 % relative humidity. Autogeny found but some do not show. One generation per year in the north and 2-3 generations in the south. **Medical importance:** Females infected with WNV, and OHF (Omsk hemorrhagic fever virus) were found in wild populations.

(References: Becker *et al.* 2010; WRBU catalog)

**35. Ochlerotatus (subgenus uncertain) caspius (Pallas 1771):**

**Materials Examined:** 80♂ 102♀ 2L 12Le 8♂G. **Identification:** Scutum with two narrow dorsocentral white stripes reaching to the posterior margin. Wing veins with dark and pale scales more or less evenly mixed. Siphonal tuft (1-S) of larva situated beyond the middle of siphon and antennal hair with 4 or more branches. Male genitalia with basal lobe gradually arising from gonocoxite, spines situated close together, and larger spine strongly curved apically. **Present record:** Collected from Ladakh division. 21♂ 25♀ collected as resting outdoors in vegetation, etc. from Ganglass, Horje Gumpa, Phey, Shey of Leh district; 31♂ 46♀ reared from immature collected from pond (Ganglass), ground pool, pit, seepage (Hunder), borrow pit (Phey), borrow pit, pit with grass, swamp (Shey), pond with aquatic plants, swamp (Shey (near palace)), and 2 larvae collected from ground pool (Ney), swamp (Shey) in Leh district.

**Distribution:** It is a palaearctic species found in Europe, China, north and north-eastern Africa, west and middle Asia. **Habitat:** Coastal areas, saline lakes and pools, marshes, rice fields. Halophilic and polycyclic but sometimes only one generation per year is produced due to nature of the breeding site. Adult females have zoophilic feeding habit and crepuscular-nocturnal activity. Females readily bite humans and animals both in rural and urban areas. Biting activity from 11.5 to 36 °C temperature and 47 to 92% relative humidity. Autogeny found. Long distance mosquito (10 km). Overwinters in egg stage (egg diapause). **Medical importance:** WNV (West Nile virus), Tahyna virus, RVF (Rift Valley Fever virus), Tularema, rabbit myxoma viruses

(References: Becker *et al.* 2010; Milankov *et al.* 2009)

**36. Ochlerotatus (subgenus uncertain) pulcritarsis ssp. asiaticus Edwards 1926:**

**Materials Examined:** 3♀ 1Le. **Identification:** Scutum mainly dark brown with a double median stripe of white scales on anterior half, narrowly bordered externally with yellow scales; on each side of the posterior end of this stripe, patch of whitish scales. Legs with speckling; hind femur (except for this speckling) dark on nearly the whole outer

surface. **Present record:** Collected from Ladakh division. 3♀ reared from immature collected from tree hole, Akchamal, Kargil district.

**Distribution:** Uzbekistan, Turkmenia, Pakistan, Tajikistan. **Habitat:** Tree-holes

(References: Barraud 1934; WRBU catalog)

**Remarks:** *Oc. pulcritarsis asiaticus* is a tentative name we gave to our specimens as speckling was found on legs (*asiaticus* character) but tip of hind femur was found white (*pulcritarsis* character); so mix of both characters which needs further taxonomic studies.

### 37. *Ochlerotatus (Finlaya) sintoni* (Barraud 1924):

**Materials Examined:** 102♂ 150♀ 23L 29Le 1Pe 37♂G. **Present record:** Collected from Kashmir and Ladakh divisions. 12♂ 45♀ collected as resting and 2♀ collected as biting outdoors in districts, Kargil (Bhimbat, Bulbul-bagh, Sher-bagh, Thasgam, Minji-chutuk), Leh (Ganglass), Budgam (Doodhpathri); 23 larvae and 90♂ 130♀ reared from immature collected in Kargil district from open stream pool (Barchey), ground pool (Budhkhharbu), ornamental fountain – unused (Bulbul-bagh), riverside pool (Sher-bagh), ground pool (Garkone), ground pool with shade (Lankerchey), open ground pool (Lankercheythang), Leh district from pit with grass and ground pool (Basgo), and Pulwama district from cement tank (Ladhu).

**Distribution:** India, Pakistan. **Habitat:** Larvae reported from a seepage pool with scrub vegetation, Dumgal near Batalik, Kargil – Jammu and Kashmir.

(References: WRBU catalog; Bhat and Kulkarni 1983; Barraud 1934)

**Remarks:** This species was originally described from Tangmarg in Kashmir by Barraud (1924) and appears endemic to J&K State – see *Discussion* part

### 38. *Aedimorphus vexans* (Meigen 1830):

**Materials Examined:** 148♂ 537♀ 6L 13Le 24♂G 11♀G. **Present record:** Collected from Kashmir and Ladakh divisions. 16♂ 414♀ collected as resting and biting, indoors and outdoors in districts, Anantnag (Banderpora), Pulwama (Dogripora), Srinagar (Ishber,

Naseem-bagh, Nowgam), Budgam (Railway Station), Baramulla (Mirgund, Tangmarg), Kargil (Bhimbat, Bulbul-bagh, Khumani Chowk, Khachan, Lankercheythang, Sher-bagh, Thasgam); 6 larvae and 130♂ 123♀ reared from immature collected in Pulwama district from paddy nursery, irrigation canal, ground pool, fallow field (Dogripora), Srinagar district from canal and ground pool (Hokersar), Baramulla district from cement tank (Tangmarg), and Kargil district from canal – cemented (Bulbul-bagh), swamp (Damsna), pond with algae and grass (Drass), swamp with trees (Gindial), riverside pool (Sher-bagh), ground pool with grass margin (Sankoo), ground pools with shade and without shade (Thasgam).

**Distribution:** India, Sri Lanka, Bangladesh, Pakistan, Indonesia, Iran, Iraq, Afghanistan, Myanmar, Nepal, Algeria, Australia, Austria, Azerbaijan, Belgium, Belize, Bosnia and Herzegovina, Bulgaria, Cambodia, Canada, China, Croatia, Czech Republic, Denmark, Estonia, Fiji, Finland, France, Georgia, Germany, Greece, Guam, Guatemala, Honduras, Hungary, Italy, Japan, Kiribati, South Korea, Laos, Latvia, Liberia, Libya, Lithuania, Macedonia, Malaysia, Mauritania, Mexico, Micronesia, Moldova, Mongolia, Morocco, Netherlands, New Caledonia, Papua New Guinea, Norway, Philippines, Poland, Portugal, Romania, Russia, Samoa, Saudi Arabia, Slovakia, Slovenia, Solomon Islands, South Africa, Spain, Sweden, Switzerland, Taiwan, Tajikistan, Thailand, Tonga, Turkey, Tuvalu, Ukraine, United Kingdom (includes Guernsey; Jersey; Isle of Man), United States, Vanuatu, Vietnam, Yemen, Yugoslavia. **Habitat:** Immatures prefer unshaded, fresh water flood pools in secondary scrub, open plains and rice fields but have also been collected from ditches, swamps, ground pools, foxhole, marshy depression, seepage spring, temporary puddle, grassy pool, fish pond, sunlit ground pool, and elephant foot prints. Habitats usually have little aquatic vegetation or algae. Adults were taken biting man, cattle and horses, in light traps, resting in houses and bamboo forests in Thailand. **Medical importance:** Natural infections with eastern equine encephalitis virus (EEE), western equine encephalitis virus (WEE), and California encephalitis (CE) group viruses have been reported from North America. Also involved in the transmission of *Tahyna* virus in Europe. It is also a vector of dog heartworm.

(References: Reinert 1973; Becker *et al.* 2010; WRBU catalog)

**Remarks:** It appears that two subspecies, *Aedimorphus vexans vexans*, and *Aedimorphus vexans nipponi* occur in J&K State – see ‘DNA Barcoding’ of *Discussion* part.

**39. Anopheles (Anopheles) barianensis James 1911, in James & Liston 1911:**

**Materials Examined:** 25♂ 54♀ 10L 1Le. **Present record:** Collected from Kashmir and Ladakh divisions. 9♀ collected as resting and biting in and around Chinar tree hollows (*Platanus orientalis*), Naseem-bagh, Srinagar district; 4♀ collected as resting outdoors in vegetation with trees, and 10 larvae and 25♂ 41♀ reared from immature collected from tree hole, Akchamal, Kargil district.

**Distribution:** India, Afghanistan, Pakistan, Tajikistan. **Habitat:** Habitat as described by Christophers (1933): Larvae are found in tree-holes, the water usually with deep brown coloration. At Simla such breeding places were chiefly in oaks. In parts of Simla, where this species is common, adult freely attacks man in the evenings in verandahs, houses, etc., but is chiefly found in the day resting inside hollow trees in the neighborhood, many in such situations being gorged with blood, probably, to a large extent, of human origin.

(References: WRBU catalog; Christophers 1933)

**Remarks:** Becker *et al.* (2010) in their book referenced Gutsevich *et al.* (1974) that there are no distinct differences between *An. barianensis* and *An. plumbeus*. But the present study shows *An. barianensis* a distinct species – see ‘DNA Barcoding’ of *Discussion* part.

**40. Ochlerotatus (subgenus uncertain) pullatus Coquillett 1904:**

**Materials Examined:** 11♀. **Present record:** Collected from Kashmir division. 11♀ collected as biting outdoors in forest vegetation, Gulmarg, Baramulla district.

**Distribution:** India (Gulmarg - Kashmir), Pakistan, Austria, Bosnia and Herzegovina, Bulgaria, Canada, Czech Republic, Finland, France, Germany, Greece, Hungary, Italy, Lithuania, Mongolia, Norway, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, United States, Yugoslavia. **Habitat:** Larvae can be found in tundra



mostly in small clear snow-melt pools and in mountainous regions in a variety of habitats like puddles and pools, without vegetation created by overflow of mountain streams or after heavy rainfall, small clear lakes with a rocky bottom. Females readily attack their hosts during any time of the day in forested areas. Adults are generally far remote from human habitations. Monocyclic species with egg diapause.

(References: Barraud 1934; Becker *et al.* 2010; WRBU catalog)

**Remarks:** Barraud (1934) pointed out about this species as: ‘This Kashmir specimen has rather broad and flat, scales on apn, ppn, and mid-lobe of scutellum, these being normally quite narrow, but the hypopygial structure is almost or quite identical with North American *pullatus*. Until more material is forthcoming it, is impossible to determine whether the Kashmir form is a distinct local variety.’ In the present study also some morphological differences were observed – see ‘DNA Barcoding’ of *Discussion* part.

#### **41. *Anopheles (Anopheles) gigas ssp. simlensis* Giles 1901:**

**Materials Examined:** 23♂ 18♀ 41L 15Le 10♂G. **Present record:** Collected from Kashmir, and Ladakh divisions. 7 larvae and 1♂ 2♀ reared from immature collected from ground pool, Boniyar; 1 larva and 1 larva, 1♂ reared from immature collected from stream margin with grass and spring pool with grass, Gulmarg respectively; 1 larva and 2 larvae, 1♂ 1♀ reared from immature collected from seepage pool and canal – cemented, Tangmarg respectively; 1 larva and 1♀ reared from immature collected from ground pools with grass, Uri in Baramulla district. 2♀ reared from immature collected from cement tank, 1 larva and 1♀ reared from immature collected from seepage pool with algae, 17 larvae and 1♂ 1♀ reared from immature collected from stream pool with algae, and 1 larva collected from borrow pit, Doodhpathri; and 2 larvae collected from ground pool with algae, Raiyar in Budgam district. 2 larvae and 10♂ 14♀ reared from immature collected from seepage, Naranag in Ganderbal district. 2♂ 2♀ reared from immature collected from ground pool, Bhimbat; 7♂ 3♀ reared from immature collected from ground pool with algae, Minamarg in Kargil district.

**Distribution:** India (Western Himalayas), Bangladesh, Sri Lanka, Nepal, China, Pakistan. **Habitat:** Small ponds, permanent pools, and suitable pools by the sides of

streams usually under an overhanging rock. In Kashmir it was found breeding in peaty water on uplands. It is a hilly and high elevation species usually found above 1000 m.

(References: Christophers 1933; Harbach *et al.* 2017; WRBU catalog)

**Remarks:** Nine specimens were *COI* sequenced which are not matching with the *Anopheles (Anopheles) gigas* reference sequence available on GenBank, so appears a separate species not subspecies – see ‘DNA Barcoding’ of *Discussion* part also.

### 4.3. Diversity

#### Species diversity:

Species diversity has two separate components: 1. the number of species present (species richness), and 2. their relative abundances (species dominance or species evenness). Diversity of a community usually can be calculated using Shannon-Weiner (or Shannon) and Gini-Simpson (or Simpson) indices. However, Shannon index is equally sensitive to both of the species diversity components while as Simpson index calculates mainly species dominance and is much less sensitive to species richness. Mosquito diversity in Jammu and Kashmir was studied using both these indices. But, here the concept of true diversities given by Jost *et al.* (2006) was used for calculating the diversities of mosquito species in divisions and in habitats. The true diversity method changes these indices into effective number of species.

**4.3.1. Species Diversity in the Divisions:** Both Shannon & Gini-Simpson indices with their true diversities were calculated in all the three divisions, namely, Jammu, Kashmir, and Ladakh with 49, 36, and 15 species respectively.

**Table 13: Diversity of mosquito species in divisions**

Division	Total species	Shannon index ( $H'$ )	True diversity ( $\exp(H')$ )	Gini-Simpson index ( $D$ )	True diversity ( $1/1-D$ )
Jammu	49	3.130	22.837	0.953	21.276
Kashmir	26	2.328	10.263	0.854	6.849
Ladakh	15	2.139	8.492	0.849	6.622

$\exp(H')$  (Shannon effective number of species) indicates that Jammu has the same diversity as a community with 22.837 equally common species, Kashmir has the same diversity as a community with 10.263 equally common species, and Ladakh has the same diversity as a community with 8.492 equally common species.

1/1-D (Gini-Simpson effective number of species) for Jammu (49 species) is 21.276; Kashmir (27 species) is 6.849; and Ladakh (15 species) is 6.622. Here, species richness is 49 for Jammu, 26 for Kashmir, and 15 for Ladakh. However, their Shannon effective number of species is less than the species richness and Gini-Simpson effective number of species is even less than Shannon effective number of species which indicates the degree of unevenness or dominance in the mosquito communities of all the divisions. When there is a degree of dominance, the Shannon effective number of species will be less than the species richness, and the Gini-Simpson effective number of species will be less than the Shannon effective number of species. The greater the dominance in the community, the greater the differences between these three numbers.

**4.3.2. Species Diversity in Larval Habitats:** Mosquito larval collection was carried out from 35 habitat types in the State. True diversities of Shannon and Gini-Simpson indices were calculated in these habitat types with atleast two species. (Table 14)

Table 14: Diversity of mosquito species in larval habitats

S. No.	Habitat	D	N	True diversity (exp(H'))	True diversity (1/1-D)	Taxa (with number of specimens) collected
1	Irrigation canal	JK	14	7.313	5.093	<i>Cx. fuscocephala</i> (6), <i>Ne. lineatopenne</i> (2), <i>Cx. pseudovishnui</i> (37), <i>Cx. quinquefasciatus</i> (7), <i>Cx. theileri</i> (2), <i>Cx. tritaeniorhynchus</i> (24), <i>Cx. vagans</i> (14), <i>Am. vexans</i> (6), <i>Fr. vittatus</i> (1), <i>Cx. barraudi</i> (3), <i>Cx. mimeticus</i> (2), <i>Cx. murrelli</i> (1), <i>An. peditaeniatus</i> (1), <i>Lt. fuscana</i> (2)
2	Canal	JKL	21	7.229	3.802	<i>An. annularis</i> (1), <i>Cx. bitaeniorhynchus</i> (13), <i>An. culicifacies</i> (3), <i>Am. culicinus</i> (2), <i>Cx. fuscocephala</i> (25), <i>An. gigas simlensis</i> (3), <i>Lt. halifaxii</i> (1), <i>Cx. hortensis</i> (15), <i>Ne. lineatopenne</i> (2), <i>Cx. tritaeniorhynchus</i> (3), <i>Cs. longiareolata</i> (4), <i>Co. pseudotaeniatus</i> (9), <i>Cx. pseudovishnui</i> (13), <i>Cx. quinquefasciatus</i> (4), <i>Mu. scatophagoides</i> (1), <i>Ar. subalbatus</i> (1), <i>Cx. theileri</i> (6), <i>Cx. vagans</i> (106), <i>Cx. viridiventer</i> (1), <i>Am. vexans</i> (3), <i>Ur. unguiculata</i> (3)
3	Drainage canal	JK	1	-	-	<i>Cx. quinquefasciatus</i> (108)
4	Stream margin	JKL	20	8.702	6.493	<i>An. annularis</i> (2), <i>Cx. barraudi</i> (29), <i>Cx. bitaeniorhynchus</i> (43), <i>An. culicifacies</i> (34), <i>An. dravidicus</i> (1), <i>An. fluviatilis</i> (15), <i>Cx. fuscocephala</i> (3), <i>An. lindesayi</i> (21), <i>An. maculatus</i> (54), <i>Lt. halifaxii</i> (2), <i>Cx. mimeticus</i> (34), <i>Cx. mimulus</i> (1), <i>Cx. pseudovishnui</i> (1), <i>An. pseudowillmori</i> (1), <i>An. willmori</i> (104), <i>An. stephensi</i> (2), <i>Cx. tritaeniorhynchus</i> (4), <i>Cx. hortensis</i> (1), <i>Cx. theileri</i> (2), <i>An. gigas simlensis</i> (1)
5	River margin	J	3	2.378	2.00	<i>An. culicifacies</i> (6), <i>Cx. quinquefasciatus</i> (22), <i>Cx. tritaeniorhynchus</i> (5)
6	Ground pool	JKL	21	9.821	7.142	<i>Oc. caspius</i> (32), <i>An. culicifacies</i> (4), <i>Cx. fuscocephala</i> (1), <i>An. gigas simlensis</i> (22), <i>Cx. hortensis</i> (83), <i>Cs. indica</i> (5), <i>An. lindesayi</i> (6), <i>Cx. mimeticus</i> (8), <i>Cx. modestus</i> (1), <i>Am. pipersalatus</i> (7), <i>Cx. pipiens</i> (5), <i>Cx. pseudovishnui</i> (3), <i>Cx. quinquefasciatus</i> (34), <i>Oc. sintoni</i> (184), <i>Am. vexans</i> (214), <i>An. stephensi</i> (2), <i>Cx. theileri</i> (47), <i>Cx. tritaeniorhynchus</i> (13), <i>Cx. vagans</i> (105), <i>Cx. viridiventer</i> (48), <i>Fr. vittatus</i> (54)
7	Stream pool	JKL	25	14.305	10.638	<i>An. annularis</i> (2), <i>Cx. bitaeniorhynchus</i> (1), <i>An. culicifacies</i> (2), <i>An. gigas simlensis</i> (23), <i>Cx. hortensis</i> (30), <i>Cs. longiareolata</i> (19), <i>Cx. malayi</i> (3), <i>Cx. mimeticus</i> (29), <i>Cs. niveitaeniata</i> (8), <i>Cx. quinquefasciatus</i> (6), <i>Oc. sintoni</i> (2), <i>Cx. theileri</i> (14), <i>Cx. vagans</i> (46), <i>Cx. viridiventer</i> (10), <i>An. willmori</i> (5), <i>Cx. tritaeniorhynchus</i> (1), <i>Cx. barraudi</i> (6), <i>An. fluviatilis</i> (1), <i>An. lindesayi</i> (14), <i>An. maculatus</i> (7), <i>Cx. mimulus</i> (2), <i>Cx. minutissimus</i> (2), <i>Cx. perplexus</i> (1), <i>Cx. pseudovishnui</i> (7), <i>An. stephensi</i> (1)
8	Spring pool	JKL	17	6.858	4.048	<i>An. annularis</i> (1), <i>Cx. bitaeniorhynchus</i> (3), <i>An. gigas simlensis</i> (1), <i>An. fluviatilis</i>

						(5), <i>Cx. hortensis</i> (49), <i>An. maculatus</i> (4), <i>Cs. longiareolata</i> (7), <i>Cx. mimeticus</i> (8), <i>Cx. pseudovishnui</i> (1), <i>Cx. quinquefasciatus</i> (2), <i>Cx. theileri</i> (5), <i>Cx. vagans</i> (15), <i>An. willmori</i> (2), <i>Cx. fuscocephala</i> (1), <i>Lt. halifaxii</i> (1), <i>An. lindesayi</i> (1), <i>Cx. tritaeniorhynchus</i> (1)
9	Riverside pool	JL	16	10.317	8.196	<i>An. culicifacies</i> (14), <i>Cx. hortensis</i> (39), <i>Cs. alaskaensis indica</i> (1), <i>An. maculatus</i> (12), <i>Cx. mimeticus</i> (12), <i>Cx. modestus</i> (1), <i>Cx. pipiens</i> (12), <i>An. pseudowillmori</i> (1), <i>Oc. sintoni</i> (3), <i>An. stephensi</i> (13), <i>Cx. theileri</i> (2), <i>Cx. tritaeniorhynchus</i> (7), <i>Cx. vagans</i> (40), <i>Am. vexans</i> (3), <i>An. willmori</i> (13), <i>Cs. longiareolata</i> (22)
10	Pond	JKL	19	10.138	6.746	<i>An. annularis</i> (1), <i>Cx. barraudi</i> (1), <i>Cx. bitaeniorhynchus</i> (1), <i>Oc. caspius</i> (8), <i>An. culicifacies</i> (1), <i>Cx. hortensis</i> (2), <i>Cs. longiareolata</i> (3), <i>Cx. malayi</i> (6), <i>Cx. mimeticus</i> (13), <i>Cx. mimulus</i> (4), <i>An. peditaeniatus</i> (20), <i>Cx. perplexus</i> (11), <i>Cx. pipiens</i> (2), <i>Cx. pseudovishnui</i> (17), <i>Cx. theileri</i> (55), <i>Ur. unguiculata</i> (16), <i>Cx. vagans</i> (9), <i>Am. vexans</i> (1), <i>Cx. tritaeniorhynchus</i> (3)
11	Rock pool	JK	16	9.323	4.784	<i>St. aegypti</i> (4), <i>Cx. barraudi</i> (3), <i>Cx. bitaeniorhynchus</i> (1), <i>An. culicifacies</i> (24), <i>An. fluviatilis</i> (7), <i>Lt. halifaxii</i> (5), <i>An. maculatus</i> (33), <i>Cx. mimeticus</i> (3), <i>Cx. mimulus</i> (13), <i>Cx. murrelli</i> (3), <i>Cx. quinquefasciatus</i> (12), <i>Cx. vagans</i> (2), <i>An. stephensi</i> (14), <i>Cx. tritaeniorhynchus</i> (11), <i>Fr. vittatus</i> (19), <i>An. willmori</i> (72)
12	Unused well	K	1	-	-	<i>Cx. vagans</i> (4)
13	Seepage	JKL	26	10.425	6.289	<i>Cx. barraudi</i> (34), <i>Cx. bitaeniorhynchus</i> (6), <i>An. culicifacies</i> (1), <i>An. gigas simlensis</i> (31), <i>Oc. caspius</i> (21), <i>An. fluviatilis</i> (6), <i>Lt. halifaxii</i> (2), <i>Cx. hortensis</i> (128), <i>Cs. indica</i> (16), <i>Cs. longiareolata</i> (2), <i>An. maculatus</i> (19), <i>Cx. mimeticus</i> (23), <i>Cx. modestus</i> (35), <i>Cx. perexiguus</i> (21), <i>Cx. pipiens</i> (16), <i>Cx. pusillus</i> (5), <i>Cx. quinquefasciatus</i> (1), <i>Oc. sintoni</i> (5), <i>Cx. theileri</i> (31), <i>Cx. vagans</i> (266), <i>An. willmori</i> (123), <i>Cx. tritaeniorhynchus</i> (10), <i>An. lindesayi</i> (4), <i>Cx. pseudovishnui</i> (22), <i>An. splendidus</i> (2), <i>An. stephensi</i> (1)
14	Swamp	JKL	11	6.587	4.784	<i>Oc. caspius</i> (2), <i>Cx. hortensis</i> (69), <i>Cs. alaskaensis ssp. indica</i> (9), <i>Cx. modestus</i> (17), <i>Cx. pipiens</i> (11), <i>Cx. pseudovishnui</i> (22), <i>Cx. theileri</i> (26), <i>Ur. unguiculata</i> (8), <i>Cx. vagans</i> (109), <i>Am. vexans</i> (24), <i>Fr. vittatus</i> (4)
15	Fallow field	K	4	2.426	2.036	<i>An. gigas simlensis</i> (1), <i>Cx. mimeticus</i> (2), <i>Cx. vagans</i> (26), <i>Am. vexans</i> (12)
16	Wheat/Grass field	K	4	2.556	2.049	<i>An. gigas simlensis</i> (6), <i>Cx. mimeticus</i> (2), <i>Cx. vagans</i> (17), <i>Cx. theileri</i> (1)
17	Paddy field	JK	9	3.899	3.067	<i>An. culicifacies</i> (1), <i>Cx. fuscocephala</i> (1), <i>Cx. pseudovishnui</i> (13), <i>Cx. theileri</i> (22), <i>Cx. tritaeniorhynchus</i> (12), <i>Cx. vagans</i> (56), <i>Am. vexans</i> (1), <i>Cx. mimeticus</i> (2), <i>Lt. fuscana</i> (2)
18	Borrow pit	KL	6	5.196	5	<i>Oc. caspius</i> (12), <i>Cx. hortensis</i> (13), <i>Cs. alaskaensis ssp. indica</i> (10), <i>Cx. pipiens</i> (14), <i>Cx. vagans</i> (17), <i>An. gigas ssp. simlensis</i> (1)
19	Roadside pool	JKL	9	5.419	3.839	<i>Cx. hortensis</i> (9), <i>Cx. mimeticus</i> (30), <i>Cx. quinquefasciatus</i> (16), <i>Cx. vagans</i> (72), <i>An. lindesayi</i> (2), <i>Fr. vittatus</i> (15), <i>Oc. caspius</i> (7), <i>Cx. pipiens</i> (6), <i>Cx. theileri</i> (4)

20	Hoof mark	K	2	1.302	1.158	<i>Cs. niveitaeniata</i> (25), <i>Cx. vagans</i> (2)
21	Lake margin	K	3	1.444	1.203	<i>Cx. pseudovishnui</i> (1), <i>Cx. quinquefasciatus</i> (1), <i>Ur. unguiculata</i> (20)
22	Fountain (Ornamental)	JL	7	3.861	2.958	<i>Cx. hortensis</i> (85), <i>Cs. longiareolata</i> (4), <i>Cx. mimeticus</i> (1), <i>Oc. sintoni</i> (40), <i>Cx. theileri</i> (11), <i>Cx. vagans</i> (21), <i>An. stephensi</i> (5)
23	Bucket	J	1	-	-	<i>Ar. subalbatus</i> (85)
24	Sintex tank	JK	3	2.217	1.814	<i>St. aegypti</i> (1), <i>Cx. vagans</i> (5), <i>Fr. vittatus</i> (1)
25	Fridge defrost tray	J	1			<i>St. albopicta</i> (1)
26	Cement tank	JK	26	13.013	8.264	<i>St. albopicta</i> (9), <i>An. annularis</i> (22), <i>Cx. barraudi</i> (84), <i>An. culicifacies</i> (5), <i>An. fluviatilis</i> (2), <i>Lt. fuscana</i> (2), <i>Cx. fuscocephala</i> (1), <i>An. gigas simlensis</i> (2), <i>Lt. halifaxii</i> (23), <i>An. lindesayi</i> (5), <i>An. maculatus</i> (7), <i>Cx. mimeticus</i> (2), <i>Cx. pallidothorax</i> (9), <i>Co. pseudotaeniatus</i> (19), <i>Cx. pseudovishnui</i> (13), <i>Gi. pulchriventer</i> (10), <i>Cx. quinquefasciatus</i> (12), <i>Oc. sintoni</i> (1), <i>An. stephensi</i> (1), <i>Cx. theileri</i> (2), <i>Cx. tritaeniorhynchus</i> (7), <i>Cx. vagans</i> (50), <i>Am. vexans</i> (1), <i>An. willmori</i> (11), <i>Fr. vittatus</i> (13), <i>Hl. chrysolineata</i> (1)
27	Barrel	K	1	-	-	<i>Gi. pulchriventer</i> (1)
28	Plastic pipe	J	2	2	2	<i>St. albopicta</i> (1), <i>Fr. vittatus</i> (1)
29	Tin	JK	4	3.053	2.512	<i>St. albopicta</i> (1), <i>Cx. fuscocephala</i> (2), <i>Cx. quinquefasciatus</i> (3), <i>Gi. pulchriventer</i> (8)
30	Tyre	JK	10	4.575	4.184	<i>St. albopicta</i> (27), <i>Cx. brevipalpis</i> (32), <i>Lt. halifaxii</i> (5), <i>An. maculatus</i> (1), <i>Cx. pallidothorax</i> (21), <i>Co. pseudotaeniatus</i> (2), <i>Cx. quinquefasciatus</i> (2), <i>Tx. splendens</i> (3), <i>Fr. vittatus</i> (2), <i>An. willmori</i> (2)
31	Tree hole	KL	3	1.251	1.104	<i>Oc. pulcritarsis asiaticus</i> (3), <i>An. bariensis</i> (77), <i>St. patriciae</i> (1)
32	Bamboo	J	1	-	-	<i>St. albopicta</i> (27)
33	Arum plant ( <i>Colocasia</i> sp.)	J	3	2.779	2.631	<i>Ml. genurostris</i> (11), <i>Ar. subalbatus</i> (14), <i>St. albopicta</i> (5)
34	Cactus hole	J	5	2.436	1.765	<i>St. albopicta</i> (4), <i>Mu. gubernatoris</i> (4), <i>Ar. subalbatus</i> (1), <i>St. unilineata</i> (28), <i>St. w-albus</i> (1)
35	Rock hole	J	1	-	-	<i>Co. pseudotaeniatus</i> (3)

D=Division, N=No. of species, J=Jammu, K=Kashmir, L=Ladakh

Larval collection was made in Ladakh from 13, in Kashmir from 25, and in Jammu from 26 habitat types. Most of the habitats in the State are ground habitats. The highest number of species were collected from seepage and cement tank (26 species each). Drainage canal, unused well, and container habitats – bamboo, barrel, bucket, fridge defrost tray, rock hole were found with least number of species (one species each). Bamboo and arum plant (natural container habitats) were found only in Jammu division. *Cx. vagans* was found in most of habitat types (20) followed by *Cx. theileri* (15), *Cx. mimeticus* (15), *Cx. quinquefasciatus* (14), *Cx. tritaeniorhynchus* (13), and *Cx. pseudovishnui* (12). First three species (*vagans*, *theileri*, and *mimeticus*) were collected from all the three divisions of the State. Species which were collected from only one habitat type were *An. barianensis* (tree hole), *St. patriciae* (tree hole), *St. unilineata* (cactus hole), *Hl. chrysolineata* (cement tank), and *Cx. perexiguus* (seepage).

Regarding the analysis of diversity in the habitat types, most of the species are found in seepage and cement tank (26 species each) but true diversity is more in cement tank (13.013) than seepage (10.425). This is because the unevenness in the number of specimens of the species collected from seepage is more than the number of specimens of species collected from cement tank although from seepage more number of the specimens was collected (831 specimens). Another habitat type, stream pool is next to these two habitat types in species richness (25 species) but is showing true diversity (14.305) more than these two habitats. This is again due to unevenness in number of specimens of species which is lesser in stream pool than seepage and cement tank although only 244 specimens were collected from the stream pool. From the true diversities calculated for the habitats it can be concluded that there is dominance in the habitats or uneven number of specimens by virtue of which the true diversities (Shannon effective number of species and Gini-Simpson effective number of species) are lesser than the species richness (number of species). Greater the difference between the species richness and true diversities means greater the dominance or unevenness in habitats.

**4.3.2. Community Similarity:** Sorenson's Coefficient (CC) was used to find species similarity between the divisions given in Table 15.

**Table 15: Mosquito species similarity between the divisions**

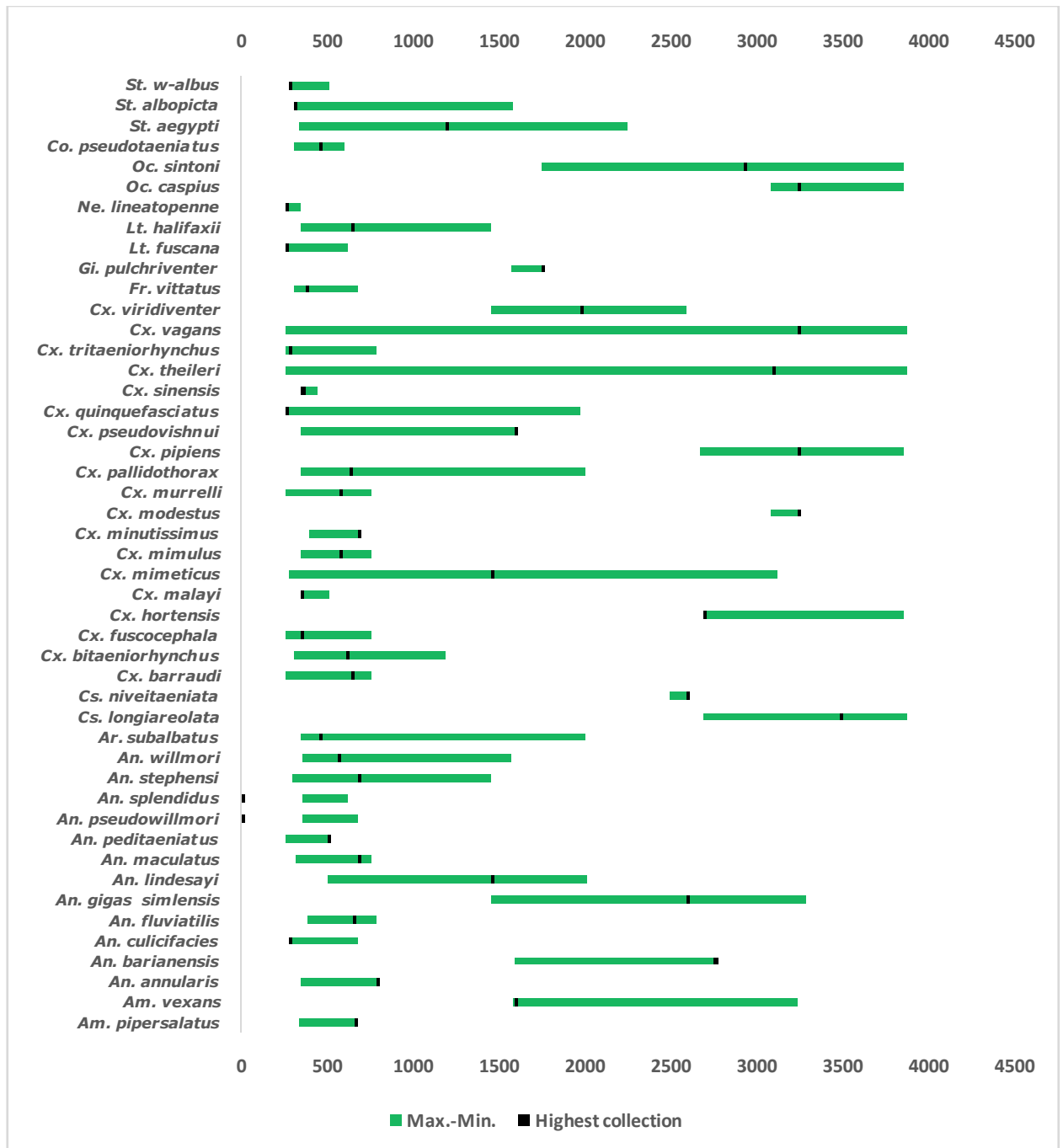
Division	Total species	C	CC
Jammu & Kashmir	49, 26	14	0.37
Kashmir & Ladakh	26, 15	7	0.34
Jammu & Ladakh	49, 15	3	0.09

It shows that Kashmir division has almost equal species similarity with Jammu (0.37) and Ladakh (0.34) divisions but Jammu division is quite different from Ladakh division (0.09) in species similarity. So, Kashmir division is a transition between Jammu, and Ladakh divisions in which species of both the divisions are found in almost equal ratios or numbers.

#### 4.4. Altitudinal distribution

Considering altitudinal distribution, mosquito surveys carried out between 2011 and 2016 from all the three divisions (with different altitudes and climates) of J&K State reported 69 mosquito species between the altitudes, 262 m (Jammu division) and 3879 m (Ladakh division). Fifteen species were collected from only one localities so with only one altitude readings as *An. dravidicus* (677 m), *Am. culicinus* (345 m), *Cx. brevipalpis* (307 m), *Cx. infula* (351 m), *Cx. perexiguus* (625 m), *Cx. vishnui* (351 m), *Oc. oreophilus* (1578 m), *Oc. pullatus* (2545 m), *Oc. pulcritarsis asiaticus* (2756 m), *St. patriciae* (1578 m), *St. unilineata* (446 m), *Tx. splendens* (307 m), *Mu. scatophagoides* (345 m), *Ph. gubernatoris* (446 m), and *Ve. yusafi* (509 m). *Ml. genurostris* was collected from two localities but altitude of one locality is not known, so also with one altitude reading only (660 m). And *Hl. chrysolineata* was collected from a locality with altitude not known. Remaining 52 species were collected from more than one locality and are with range of altitudes. Among them, 47 species are only shown in Fig. 13; remaining 5 species were found with very less range of altitudes as *Ur. unguiculata* (1581-1586 m), *Cs. alaskaensis indica* (3181-3242 m), *Cq. richiardi* (1580-1597), *Cx. pusillus* (3241-3242), and *Cx. perplexus* (500-509), so not shown in figure.





**Fig. 13: Altitudinal distribution of mosquitoes in J&K State**

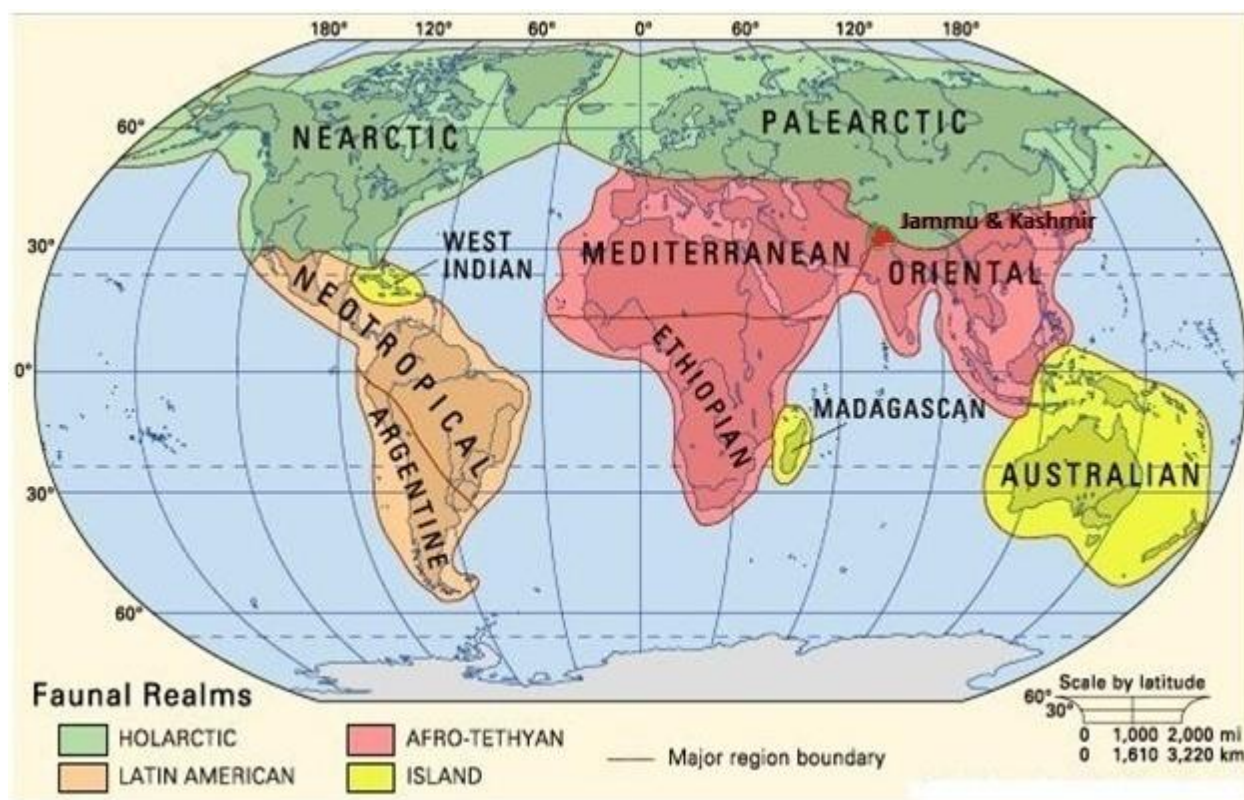
The high range of altitude of the State affects the diversity and distribution of mosquitoes. *Cx. pipiens*, *Cx. hortensis*, *Cx. modestus*, *Cx. pusillus*, *Oc. pullatus*, *Oc. caspius*, *Oc. pulcritarsis* ssp. *asiaticus*, *Cs. longiareolata*, *Cs. niveitaeniata*, *Cs. alaskaensis* ssp. *indica* species were collected above 2438 m altitude i.e. at high altitudes only (2438-5658 m (8000-12000 ft)). Species of genera *Ochlerotatus* (*caspius*,

*pulcritarsis asiaticus*, *pullatus*, *sintoni*), and *Culiseta* (*alaskaensis indica*, *longiareolata*, *niveitaeniata*) are usually confined to high altitudes. Some species of *Anopheles* (*barianensis* and *gigas simlensis*) and *Culex* (*hortensis*, *modestus*, *pusillus*, *vagans*, *theileri*) were also found at high altitudes. Thirty-five species of Jammu division were found up to 800 m altitude (altitude of Jammu division) only. Species, *Oc. sintoni*, *St. aegypti*, *An. gigas simlensis*, *Cx. quinquefasciatus*, *Am. vexans*, *Cx. pallidothorax*, *Ar. subalbatus*, *An. lindesayi*, *St. albopicta*, *Cx. pseudovishnui*, *An. willmori*, *An. barianensis*, *An. stephensi*, *Lt. halifaxii*, and *Cx. bitaeniorhynchus* were found in at least two divisions, so with wide altitudinal range. Some other species, *Cx. hortensis*, *Cx. pipiens*, *Cx. viridiventer*, and *Cs. logiareolata*, were also found with wide altitudinal range but were collected from one division only. Only three species, *Cx. vagans* (262 m – 3879 m), *Cx. theileri* (262 m – 3879 m), and *Cx. mimeticus* (255 m - 3124 m) were found in all the divisions showing their further wide range of altitudinal distribution and existence in different climates.

If we see the geographic location of J&K State, it is situated at the junction of three geographic realms viz. Palaearctic, Mediterranean and Oriental (Fig. 14). The State is surrounded by Palaearctic Region in the north-east, Mediterranean Region in the west, and Oriental Region in the south-east. The mosquitoes collected from the State show the affinity towards all these three regions. They show the affinity as Jammu mosquitoes (subtropical region) Oriental except *Cx. vagans* and *Cx. theileri*, Kashmir mosquitoes Oriental, Palaearctic or Mediterranean, and Ladakh mosquitoes Palaearctic or Mediterranean.

Considering mosquito collections, climate, and altitude of the State, the list of mosquito species recorded were categorized into five groups as shown in Table 16. All the mosquitoes of Jammu division were put in a single group – Plains and Lower Hills region (subtropical) from 262 to 1000 m altitude. Collection in this division was chiefly carried out in subtropical region (Jammu, Samba, Kathua, and Udhampur districts) upto around 800 m altitude. Beyond 800 m collection was carried out upto the elevation of 2017 m in Udhampur district but from few places. Mosquitoes of Kashmir division were made in three groups viz., Jhelum Valley from 1001 to 1600 m altitude, Kashmir Valley from

1550 to 1750 m altitude, and Montane region from 1751 to 2660 m altitude. One area (Kamalkote) of Jhelum Valley goes upto ~ 2100 m elevation but most of the area of the Valley falls in between 1001 and 1600 m elevation. All the three groups fall in temperate zone but Jhelum Valley is hotter than Kashmir Valley and Kashmir Valley is hotter than Montane region. Mosquitoes of Ladakh division were put in a single and fifth group – Subalpine and Alpine region (cold-arid) from 2661 to 3900 m altitude. Keeping in view Fig. 14 and Table 16, the general distribution of the mosquitoes of the State is discussed in ‘Distribution’ of *Discussion* part.



**Fig. 14: Geographical realms map showing J&K State bounded by three geographical realms (modified) (Source: <https://www.britannica.com/science/Oriental-region-faunal-region>)**

Table 16: Distribution of mosquitoes in different altitudinal ranges

S. No.	Sub-tropical (Jammu division)		Temperate (Kashmir division)			Cold-arid (Ladakh division)
	Plains and Lower hills region		Jhelum Valley	Kashmir Valley	Montane region	Subalpine and Alpine region
	262-1000 m alt.		1001-1600 m alt.	1550-1750 m alt.	1751-2660 m alt.	2661-3900 m alt.
1	<i>Am. culicinus</i>	<i>Cx. pallidothorax</i>	<i>An. gigas simlensis</i>	<i>Am. vexans</i>	<i>Am. vexans</i>	<i>Am. vexans</i>
2	<i>Am. pipersalatus</i>	<i>Cx. perexiguus</i>	<i>An. lindesayi</i>	<i>An. barianensis</i>	<i>An. gigas simlensis</i>	<i>An. barianensis</i>
3	<i>An. annularis</i>	<i>Cx. perplexus</i>	<i>An. stephensi</i>	<i>Ar. subalbatus</i>	<i>An. lindesayi</i>	<i>An. gigas simlensis</i>
4	<i>An. culicifacies</i>	<i>Cx. pseudovishnui</i>	<i>An. willmori</i>	<i>Cq. richiardi</i>	<i>Ar. subalbatus</i>	<i>Cs. alaskaensis indica</i>
5	<i>An. dravidicus</i>	<i>Cx. quinquefasciatus</i>	<i>Ar. subalbatus</i>	<i>Cx. mimeticus</i>	<i>Cs. niveitaeniata</i>	<i>Cs. longiareolata</i>
6	<i>An. fluviatilis</i>	<i>Cx. sinensis</i>	<i>Cx. bitaeniorhynchus</i>	<i>Cx. pseudovishnui</i>	<i>Cx. mimeticus</i>	<i>Cx. hortensis</i>
7	<i>An. lindesayi</i>	<i>Cx. theileri</i>	<i>Cx. mimeticus</i>	<i>Cx. quinquefasciatus</i>	<i>Cx. pallidothorax</i>	<i>Cx. mimeticus</i>
8	<i>An. maculatus</i>	<i>Cx. tritaeniorhynchus</i>	<i>Cx. pallidothorax</i>	<i>Cx. theileri</i>	<i>Cx. quinquefasciatus</i>	<i>Cx. modestus</i>
9	<i>An. peditaeniatus</i>	<i>Cx. vagans</i>	<i>Cx. pseudovishnui</i>	<i>Cx. vagans</i>	<i>Cx. theileri</i>	<i>Cx. pipiens</i>
10	<i>An. pseudowillmori</i>	<i>Cx. vishnui</i>	<i>Cx. quinquefasciatus</i>	<i>Oc. pulchriventer</i>	<i>Cx. vagans</i>	<i>Cx. pusillus</i>
11	<i>An. splendidus</i>	<i>Fr. vittatus</i>	<i>Cx. theileri</i>	<i>St. albopicta</i>	<i>Cx. viridiventer</i>	<i>Cx. theileri</i>
12	<i>An. stephensi</i>	<i>Hl. chrysolineata</i>	<i>Cx. vagans</i>	<i>Ur. unguiculata</i>	<i>Oc. pullatus</i>	<i>Cx. vagans</i>
13	<i>An. willmori</i>	<i>Lt. fuscana</i>	<i>Cx. viridiventer</i>		<i>Oc. sintoni</i>	<i>Oc. caspius</i>
14	<i>Ar. subalbatus</i>	<i>Lt. halifaxii</i>	<i>Lt. halifaxii</i>		<i>St. aegypti</i>	<i>Oc. pulcritarsis asiaticus</i>
15	<i>Co. pseudotaeniatus</i>	<i>Ml. genurostris</i>	<i>Oc. oreophilus</i>			<i>Oc. sintoni</i>
16	<i>Cx. barraudi</i>	<i>Mu. scatophagoides</i>	<i>St. aegypti</i>			
17	<i>Cx. bitaeniorhynchus</i>	<i>Ne. lineatopenne</i>	<i>St. albopicta</i>			
18	<i>Cx. brevipalpis</i>	<i>Ph. gubernatoris</i>	<i>St. patriciae</i>			
19	<i>Cx. fuscocephala</i>	<i>St. aegypti</i>				
20	<i>Cx. infula</i>	<i>St. albopicta</i>				
21	<i>Cx. malayi</i>	<i>St. unilineata</i>				
22	<i>Cx. mimeticus</i>	<i>St. w-albus</i>				
23	<i>Cx. mimulus</i>	<i>Tx. splendens</i>				
24	<i>Cx. minutissimus</i>	<i>Ve. yusafi</i>				
25	<i>Cx. murrelli</i>					

## ***5. Discussion***

### 5.1. Mosquito Fauna

Earlier studies made by Christophers (1933), Barraud (1934), Puri (1936, 48), Jacob (1950), Nair (1973), and Rao *et al.* (1973, 83, 84) reported 43 mosquito species from J&K State. In the present surveys, 69 species have been recorded; 33 species were the same as reported by the earlier workers and the remaining 36 species were reported for the first time from the State including five new country records. Ten species from the earlier reported species were not collected in the present surveys.

*Cx. (Bar.) modestus* was collected from Ladakh division in the present study while Barraud (1934) reported it from Kashmir division. Genus *Culiseta* in India is represented by three species, *Cs. (All.) longiareolata*, *Cs. (Cus.) alaskaensis ssp. indica*, and *Cs. (Cus.) niveitaeniata*. All these species were collected from J&K State during the present study. Maculatus group in India is represented by 6 species, *An. maculatus*, *An. willmori*, *An. pseudowillmori*, *An. dravidicus*, *An. rampae*, and *An. sawadwongporni*. Excluding *An. rampae*, and *An. sawadwongporni*, the remaining 4 species were collected from the State.

Regarding the literature about *An. (Cel.) maculatus* and *An. (Cel.) willmori* from Jammu and Kashmir is discussed here. *An. willmori* was first reported by James (1904) from Kashmir. He considered *An. willmori* and *An. maculatus* two species. Later, Adies (1913) and Gill (1920) also reported *willmori* from Kashmir division as a species. However, it was Christophers (1933) who treated *An. (Cel.) willmori* as a variety of *An. (Cel.) maculatus* as no morphological difference was found between the larvae of *maculatus* and *willmori* by Puri (1931) although they differ in adult stages from each other. Afterwards, Puri (1948) and Jacob (1950) reported *An. (Cel.) maculatus* from the two divisions, Kashmir, and Jammu of the State. Probably, they had collected both *willmori* and *maculatus* species from the State but as *willmori* was treated as a variety of *maculatus*, so they considered them only one species as *An. (Cel.) maculatus*. Later, Nair (1973) reported *An. (Cel.) maculatus* from Kashmir division but it appears the species would have been *An. (Cel.) willmori* (because till now, including the present collection, no one has reported *An. (Cel.) maculatus* from Kashmir division) and due to being a variety of *maculatus*, he considered it as *An. (Cel.) maculatus*. Rao *et al.* (1973) reported as *An. (Cel.) maculatus willmori* from Kashmir, and Jammu divisions. Subsequent to the

recognition of *An. willmori* as a distinct species by Rattanakul & Green (1986), we treated all the previous records of *An. maculatus* variety *willmori* from the State as distribution records of *An. willmori*, and of *An. maculatus* as distribution records of *An. maculatus* (except reported by Nair (1973)) (see Table 2).

Rao *et al.* (1973) collected mosquito species from all the three divisions but have not reported any species common to all the divisions. In the present collection, three species, *Cx. (Cux.) mimeticus*, *Cx. (Cux.) vagans*, and *Cx. (Cux.) theileri* were reported common in all the divisions. Earlier anophelines were reported only from Jammu, and Kashmir divisions but in present collection two anopheline species, *An. gigas simlensis*, and *An. bariensis* were collected from Ladakh division.

In this study, *Ar. subalbatus* was collected both as adult and larva in Jammu and Kashmir divisions. Adults were found in houses, therefore, appears to be a common species in the State. Rao *et al.* (1973) or Bhat and Kulkarni (1983) did not report this species. Probably, the species would have got introduced later or proliferated later in the division due to increase in drainage system. Further, three types of larvae (based on colour) and some variations on abdominal scales of adults were observed. It appears that the species has either subspecies or is composed of more than one species. DNA barcoding was tried for this species but sequences were not obtained and the species is left as such for further studies.

*Oc. (Fin.) sintoni* seems to be endemic to J&K State. It is found in India (J&K), Pakistan and probably in China. The species was originally described from Tangmarg - Kashmir by Barraud and subsequently recorded from Murree. Apparently this species is restricted to the Western Himalayas and probably is not found elsewhere in world. In the present study, numerous adult and immatures of the species were collected from Kashmir, and Ladakh divisions and the main larval habitat of the species was ground pools. Adults were collected during daytime resting and biting and also reared from immatures. Immatures were found very robust; hardly any death of larva or pupa was noticed while carrying them from one place to another place and even from one climatic condition to another climatic condition. The species was found breeding predominantly with other species in Ladakh - the cold-arid region while as it was restricted to forest areas in

Kashmir – the temperate region. Two specimens were DNA barcoded. The literature available regarding this species is only of Barraud (1924, 1934) who described the adult (♂ and ♀) and of Dong & Wang (1985) (in Chinese) who described the larva and the pupa. That is not sufficient for the complete description of the species. During the identification, some additional morphological characters of adult and immature stages (larva and pupa) were observed. It is perceived that the species should be completely revised and updated with more information and keys although some information on its bionomics and distribution here and in *Results* part has been provided.

In case of *Lutzia*, the predatory mosquitoes, two species, *Lt. halifaxii*, and *Lt. fuscana* were collected in the present study. *Lt. halifaxii* was found predominant when compared to *Lt. fuscana*. *Lt. halifaxii* was collected from two divisions, Jammu, and Kashmir while as *Lt. fuscana* was collected from one division only, Jammu. *Lt. halifaxii* was collected as adult (resting), immature and adult reared from immatures, from many places breeding in association with many anophelines (*An. culicifacies*, *An. fluviatilis*, *An. lindesayi*, *An. maculatus*, *An. stephensi*, and *An. willmori*) and culicines (*Cx. barraudi*, *Cx. bitaeniorhynchus*, *Cx. fuscocephala*, *Cx. mimeticus*, *Cx. mimulus*, *Cx. pallidothorax*, *Cx. pseudovishnui*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, and *Lt. fuscana*). *Lt. fuscana* was collected as immature and adult reared from immatures, from four places breeding in association with *An. maculatus*, *An. willmori*, *Cx. fuscocephala*, *Cx. pallidothorax*, *Lt. halifaxii*, *Co. pseudotaeniatatus*, and *Hl. chrysolineata*. In one habitat (cement tank) in Jammu division both the species were found breeding together.

*Ml. genurostris* was collected as larvae from the leaf axils of arum plants (*Colocasia* sp.) in Jammu division breeding in association with *Ar. subalbatus* and *St. albopicta*. Seven adult females were reared from the larvae.

Two species (*An. barianensis*, and *Ochlerotatus pulcritarsis* ssp. *asiaticus*) from tree holes as immatures were collected only from Ladakh division. From Kashmir division one adult (*St. patriciae*) was reared from mud taken from a tree stump. However, tree hole species can be more expected from Jammu, and Kashmir divisions than Ladakh division as tree holes are found in Jammu, and Kashmir divisions and rainfall in these divisions is higher as compared to Ladakh division. Further, Jammu, and Kashmir divisions contribute



more than 99% of forest area to the State and adults of the tree hole breeding species were collected from these divisions. So, their immatures are expected from the tree holes with additional species.

*Cx. (Bar.) pusillus* is a new country record in the present collection. It was collected along with *Cx. (Bar.) modestus* from Ladakh division. Key given by Becker *et al.* (2010) was used for the identification of the species. Adult male, female and larval exuvae were identified as *Cx. (Bar.) pusillus*. But it was noticed that the key characters given by Becker *et al.* (2010) are close to the key characters of *Cx. (Bar.) modestus* (see 'Ecological Notes' of *Results* part). In our specimens it was observed that the lateral scales on terga on males and females were not the same so it became difficult to distinguish these species from each other. Although the key was followed carefully and the species were observed deeply, it was felt that the species should be DNA barcoded. However, till now *modestus* only got barcoded and so *pusillus* remained still to be fully confirmed. Conclusively, *Cx. (Bar.) pusillus* was retained on the basis of morphological identification but DNA barcoding needs to be carried out to confirm its presence fully.

## 5.2. DNA Barcoding

DNA bar-coding of 105 specimens belonging to 32 species (10 genera) was carried out in the present study. The results were compared with morphological identification and many interesting findings were recorded.

While identifying *Cx. (Cux.) theileri*, the pattern of scaling on thorax of some specimens was found different. Then, eleven specimens of *Cx. (Cux.) theileri* were *COI* sequenced in which only 6 specimens clustered with the reference sequences of *theileri* available on GenBank and the remaining 5 specimens clustered separately (Fig. 10g, 11b, 12b). The average K2P genetic distance with the 6 specimens was found 3.5% (3.4-3.7%) and with the reference specimens, *theileri* was found 3.7% (3.5-4.3%). These 5 specimens found different will be possibly a new species. They were found in the three divisions of J&K (Jammu – 1 specimen, Kashmir – 3 specimens, Ladakh – 1 specimen), other 6 specimens clustered with *theileri* were all from Ladakh division only. Further studies are needed for confirmation of the new species.

Members of the Gigas Complex occur in mountainous areas in the Oriental Region, the Manchurian Subregion of the Palaearctic Region and the Austro-Malayan Subregion of the Australasian Region. All forms of the complex are recorded from altitudes at or usually above 1000 m (Harbach *et al.* 2017). *An. baileyi*, *An. gigas* and *An. prachongae* are currently recognised species of the Gigas Complex. *An. prachongae* was recently upgraded as a species of the Gigas Complex from subspecies *sumatrana* of *An. gigas* (Harbach *et al.* 2017). Now *An. gigas* has still eight subspecies in which *simlensis* is one (Harbach 2017). In India, *An. gigas* ssp. *simlensis* is reported from the Western Himalayas. As like other forms of the Gigas Complex it is an alpine form rarely occurring below 6,000 feet (~ 1818 m) altitude, but found sometimes at lower altitudes near the hills (Christophers 1933). In the present study, *An. gigas* ssp. *simlensis* (as per Christophers (1933) key) was collected from Kashmir, and Ladakh divisions of the J&K State. Immature stages were collected from ground pool, seepage, cement tank, spring pool with grass, algae, etc. Nine specimens of this subspecies (8 of Kashmir, 1 of Ladakh) were DNA barcoded and the sequences were compared with reference sequence of *An. gigas* available on GenBank. The sequences exhibited 11.9% K2P genetic distance with reference sequence of *An. gigas* indicating that the species is a distinct species (Fig. 10a). So, the species, *An. gigas simlensis* collected during this study appears to be not a subspecies. However, further taxonomic studies are needed to confirm its status or if it is to be upgraded to species.

*Culiseta (Culiseta) alaskaensis* ssp. *indica* (identified following Barraud (1934) key) was collected from Ladakh division. Three specimens were sequenced which showed average K2P genetic distance of 6.5% (6-6.9%) from the reference sequences of *Cs. (Cus.) alaskaensis* available on GenBank (Fig. 10b). This indicates that *indica* is no longer a subspecies but a distinct species. However, further studies are necessary to confirm its status.

*Ochlerotatus pullatus* was collected while biting in forest vegetated area, Gulmarg – Baramulla (Kashmir) in the present study. Eleven females were collected but no male or immature was collected. Barraud (1934) has noticed that the Kashmir specimen has rather broad and flat, scales on apn, ppn, and mid-lobe of scutellum, these being normally quite

narrow in North American *pullatus*. The same characters were observed in the present collection. As no male and immature was collected, it was not possible to observe genitalial and larval characters. However, 3 specimens sequenced (*COI*) were compared with the reference sequences of *Oc. pullatus* available on GenBank (Fig. 10c). Difference in K2P genetic distance (average 2.9% (2.5-3.5%)) was also found and possibly be a distinct species but before reaching to any conclusion some more taxonomic studies are needed.

Only one adult female of *Ochlerotatus (Finlaya) oreophilus* (identified following Barraud (1934) key) was collected from Uri – Baramulla (Kashmir). *COI* sequence of this species showed difference with the reference sequences of *oreophilus* available on GenBank (K2P genetic distance, 5.4%) (Fig. 10d). Further studies with more number of specimens are needed but presently only one female is available with only one leg remaining (5 legs were given for barcoding).

As usual before going for DNA barcoding, first morphological identification is carried out, so was done in case of *Am. vexans*, and *Cq. richiardi* and some interesting results were found. *Am. vexans*, and *Am. stenoetrus* were morphologically identified following Barraud (1934) key. But, DNA barcoding confirmed only one species viz. *Am. vexans* as all *COI* barcode sequences of these specimens clustered together in the Neighbor-Joining tree (Fig. 11b, 12b). Later it was found that Barraud (1934) key is not sufficient for the identification of *Am. stenoetrus*. This was solved by a book on *Aedimorphus* by Reinert (1973) in which the key character given by Barraud (1934) for *Am. stenoetrus* (hind tarsal claws are toothed) is shown also present on *Am. vexans nipponi* (a subspecies of *vexans*). Reinert (1973) has given additional key characters for *Am. stenoetrus* and also removed *Am. stenoetrus* from Southeast Asia species list along with *Am. taeniorhynchoides*. Our morphological identification has observed three types of *Am. vexans*, first in which both hind tarsal claws were untoothed, second in which both hind tarsal claws were toothed, and third in which one hind tarsal claw was toothed while as other was untoothed. So, from this observation and DNA barcoding it was concluded that probably *Am. vexans* of J&K consists of subspecies (not separate species) as, *Am. vexans vexans* (untoothed claws), *Am. vexans nipponi* (toothed claws), and intermediate

form (one claw toothed and other claw untoothed). In case of *Cq. (Coq.) richiardi*, adults were first identified as *Cq. (Coq.) perturbans*. The species is similar to *perturbans* in morphological and biological characteristics and some authors consider *Cq. (Coq.) perturbans* as a geographic race of *Cq. (Coq.) richiardi* (see Becker *et al.* 2010, page number, 310). *Cq. (Coq.) richiardi* was collected as adult (both ♂ and ♀) only, from Kashmir division and is a new country record. The morphological observations were compared with DNA barcoding. The *COI* sequences (4 specimens sequenced) clustered with the reference sequences of *Cq. (Coq.) richiardi* available on GenBank indicating it to be *Cq. richiardi* (Fig. 10e). Also, average K2P genetic distance between our specimen sequences and reference sequences of *Cq. (Coq.) perturbans* was found 11.85% (11.4-12.6%) i.e. very distinct from *Cq. perturbans*. Further, for being a *Cq. (Coq.) richiardi* species, it was supported by the fact that *Cq. (Coq.) perturbans* is a North American species while as *Cq. (Coq.) richiardi* occurs in Southwest Asia which is adjacent to J&K State. Moreover, *COI* sequences of our specimens and *COI* sequences of *Cq. (Coq.) richiardi* and *Cq. (Coq.) perturbans* available on GenBank confirm them as two different species.

Christophers (1933) mentioned about *An. (Ano.) barianensis* (first described by James and Liston (1911)) that the species was first given as synonymous with *An. (Ano.) plumbeus*, but shown later by Edwards (1921), to differ in some respects. It was known in India for some time as *An. plumbeus* var. *barianensis*, but it was cleared by Puri as a distinct species from the larval characters. In the Indian area, it is apparently restricted to the North-West Himalayas at altitudes of 5,000-8,000 feet (~ 1500-2500 m) and was recorded from Barian, Murree, Simla, Kasauli, Kangra Dist. (Naggar and Sil Madhani), and Kashmir (Dal Lake, Srinagar) (Christophers 1933). It was reported outside Indian subcontinent also, in Afghanistan and Tajikistan. However, later Gutsevich *et al.* (1974) pointed out that there are no distinct differences between *An. barianensis* and *An. plumbeus*, referenced by Becker *et al.* (2010) in their book at page number, 180. But in the present study, *COI* sequencing of two specimens was carried out and the sequences were compared with the reference sequences of *An. plumbeus* available on GenBank which clearly indicate that *An. barianensis* is distinct from *An. plumbeus* (average K2P genetic distance, 11.45% (11.3-11.6%)) (Fig. 10b). Furthermore, in the present collection,

the species, *barianensis* was collected at an altitude of 2756 m from tree hole in Akchamal – Kargil, Ladakh which is probably the highest altitudinal record for the species.

*An. (Cel.) maculatus* and *An. (Cel.) willmori* collected from Jammu, and Kashmir divisions got DNA barcoded (*maculatus* – 3 specimens, *willmori* – 2 specimens) and 8.9% K2P genetic distance (group mean distance) was found between them. Other two species, *An. (Cel.) pseudowillmori* and *An. (Cel.) dravidicus* of the Maculatus Group collected from the State were also tried but not successfully barcoded. However, it is important that the species, *An. (Cel.) maculatus* and *An. (Cel.) willmori* got distinguished by DNA barcoding also. But reference sequences of *willmori* are not available on GenBank and reference sequences of *maculatus* are not exact or correct as they have shown difference from each other when compared.

*Cx. (Cux.) pipiens* (a new country record) when collected from Ladakh in the present study, the adult was first identified as *Cx. (Cux.) quinquefasciatus* as no distinction in morphology was observed but only it was more yellow in colour when compared to *Cx. quinquefasciatus* from Jammu, and Kashmir divisions and then its genitalia (male) was dissected and the species was identified. DNA (*COI*) sequencing carried out for both the species (*pipiens* – 5 specimens, *quinquefasciatus* – 2 specimens) was not helpful to distinguish them. All the sequences of *pipiens* and *quinquefasciatus* clustered together and also with the reference sequences of *pipiens* and *quinquefasciatus* available on GenBank (Fig. 12b). Average pairwise K2P distance between *pipiens* (5 specimens) and *quinquefasciatus* (2 specimens) was found 1.2% (0.9-1.3%). Laurito and her colleagues (2013) also could not distinguish *pipiens* and *quinquefasciatus* on the basis of *COI* sequences, found the average K2P distance of 1.6% (0-3%) between these species. So, it was concluded that male genitalia is the only reliable material on which the species of Pipiens Complex, *pipiens* and *quinquefasciatus* can be distinguished (Dehghan *et al.* 2013; Harbach 2012).

In conclusion, our findings suggested that *COI*-based DNA barcode can effectively be used when morphological characters of certain species do not clearly distinguish one species from another. It can be used to distinguish the sibling species and

also to check the status of a subspecies. DNA barcodes also allow taxonomists to re-confirm the reference voucher specimens. However, empirical evidence has shown that *COI*-based barcoding is not successful all the time (Laurito *et al.* 2013; Kumar *et al.* 2007; Cywinska *et al.* 2006) and it was seen above between *Cx. quinquefasciatus* and *Cx. pipiens*. The limited availability of sequences to be used as references for comparison has restricted its usage on species identification. And the cut-off limit of barcode gap for species differentiation still remains controversial. The barcode gap proposed to differentiate vertebrate species is 2% and invertebrate species is 3% based on *COI* sequences (Rubinoff 2006) which cannot be always strictly followed. Therefore, *COI*-based DNA barcoding may not always be useful on its own, but would rather be an alternative tool to complement morphological identification. The phylogeny-assisted DNA barcode analyses (as, Bayesian analysis) can help us to refine the taxonomic identification and further understand the genetics and evolution of mosquito species in endemic habitats. Now advances in sequencing technology have made *de novo* sequencing readily accessible and complete mitochondrial genomes have been utilized as diagnostic markers across a wide range of taxa including mosquitoes (Coissac *et al.* 2016; Laurito *et al.* 2017).

### 5.3. Diversity

Considering richness of mosquito species, Jammu division has more richness of mosquito species followed by Kashmir and Ladakh divisions. Also, more number of species can be expected from the divisions in the order of Jammu>Kashmir>Ladakh. Although Ladakh division has the largest area in the State, the conditions prevailing in the area (higher altitude, less vegetation (forest area – 0.06%), less population, harsh climate (cold arid) with less summer days) are not favorable for more diversity of the mosquito species. On the other hand, Jammu, and Kashmir divisions bear good vegetation (forest area: Jammu division – 45.89%, Kashmir division – 50.97%), more population, more larval habitat types, suitable climate (Jammu – subtropical, Kashmir - temperate) which favor more number of mosquito species.

True diversities of species (Shannon and Gini-Simpson effective numbers of species) are found less than species richness in the divisions and larval habitats. This is

due to the dominance of species or unevenness in number of specimens of species collected. The unevenness in the number of species can be due to many factors such as seasonality, rare species, domestic and forest species, and collector. In Kashmir and Ladakh divisions usually mosquito species have to go for overwintering. Some species overwinter early, some overwinter late and some come out from winter diapause early, some come out late. Therefore, the species will have their own peak seasons and their densities will vary as per the season or month. *Ochlerotatus* species will be the first species of spring month and also the first species to go for overwintering. In the present collections in Kashmir division, *Am. vexans* was collected as larvae in March (early spring) when snow was melting and was found early species to enter the houses while as *Cx. vagans* probably be the last species to enter diapause as some males were collected inside the house in November (early winter). This seasonality of species can affect the number and density of species. Species like *An. bariensis*, *Ur. unguiculata*, *Cq. richiardi* were found at few localities only while species like *Cx. vagans*, *Cx. quinquefasciatus*, *Cx. pseudovishnui*, *Cx. theileri*, *Am. vexans* were found at most of the places and abundant which can affect the even collection of species. Some species like *Oc. pullatus* were found restricted to forest areas and some species like *Cx. vagans*, *Cx. quinquefasciatus*, *Cx. pseudovishnui* were found in domestic areas. Domestic mosquitoes are easily accessible while collection of forest mosquitoes needs extra effort. Collection of the number of specimens of a species also depends upon the collector i.e. how much a collector is cautious while collecting adult mosquitoes and searching for the larval habitats. Other factors like uneven rainfall, climatic conditions, etc. can also affect the evenness of collection.

As mosquito larval collection was carried out from 35 habitat types, most of the habitats in the State are ground habitats because of springs, rivers, streams and lakes. Of the ground habitats, one important characteristic of the Kashmir division is the presence of many lakes and extensive swamps which are found in other two divisions also but lesser than Kashmir division. It is quite surprising that mosquitoes (two species) were collected from tree holes in Ladakh division where precipitation or rainfall is very less (20 cm annual rainfall). Collection was carried out from 13 habitat types in Ladakh while in Jammu division it was carried out from 26 habitat types, and in Kashmir division from 25

habitat types. Although habitat types were less in Ladakh division, all its habitat types were almost found in Kashmir and Jammu divisions also. However, some habitat types like paddy field (found in Jammu, and Kashmir divisions), arum plant, and bamboo (found in Jammu division only) could not be found in Ladakh division. Container habitats (artificial or natural) other than tree hole were not also encountered in Ladakh division. The similarity of habitat types is due to streams, rivers, canals, swamps found in every division.

#### 5.4. Distribution

The mosquito species of Jammu division are Oriental (except *Cx. vagans* and *Cx. theileri*) and all the mosquito species of Ladakh division are Palearctic and Mediterranean while the mosquito species of Kashmir division are Oriental, Palearctic and Mediterranean. This indicates that Kashmir division is a transition between Oriental, Palearctic and Mediterranean Regions.

Now considering Fig. 14 and Table 16, general distribution of mosquito species is based on present collection, altitude, climate, geographic affinity, and past collections (collections by earlier workers). Thirty-five species (out of 49 species) were reported from Jammu division only, 8 species (out of 26 species) were reported from Kashmir division only, and 8 species (out of 15 species) were reported from Ladakh division only. These exclusive species of the divisions (species not common between the divisions) also show the same geographic affinity as the overall species of the divisions i.e. Jammu species Oriental, Kashmir species Mediterranean, Palearctic, and Oriental and Ladakh species Mediterranean, and Palearctic. Most of the anophelines were found in Jammu region (subtropical) then in Jhelum Valley of Kashmir division (temperate). Only two species of anophelines, *An. barianensis*, and *An. gigas simlensis* were found in Kashmir Valley, Montane region of Kashmir division, and Ladakh division (cold-arid). *An. barianensis* was collected at 1597 m and 2756 m elevation and *An. gigas simlensis* was collected from 1453 m to 3287 m elevation. Both these species appear restricted to Western Himalayas. However, earlier workers reported some more anophelines (*willmori*, *annularis*, *stephensi*, *splendidus*, *fluviatilis*, *subpictus*, *lindesayi*) from Kashmir Valley and Montane region of Kashmir division which were not encountered in the present study. *An. lindesayi*, a high



altitude mosquito was collected from 500 to 2017 m elevation in Jammu region and Jhelum Valley of Kashmir division. Anophelines which are malaria vectors were present in Jammu region and Jhelum Valley of Jammu division. The altitudinal range for anophelines in the State was from 262 to 3287 m. Culicine mosquitoes were collected from 262 to 3879 m altitude. Among these, three species, *Cx. gigas*, *Cx. theileri*, and *Cx. mimeticus* were found common in all the three divisions and two species, *Cx. gigas*, *Cx. theileri* were collected from lowest to highest altitude (i.e. 262-3879 m) of the present study. Barraud (1934) has reported *Cx. modestus* from Jhelum Valley and has mentioned that the species is common in Jhelum Valley but in the present study not even a single larva was collected. However, it was collected from Leh district of Ladakh division during the present study. *An. barianensis*, *An. gigas simlensis*, *Oc. pullatus*, and *Ur. unguiculata* were collected almost from the same places from where Christophers (1933) and Barraud (1934) have reported. However, *An. barianensis*, *An. gigas simlensis* were collected from Ladakh division also. *Ochlerotatus* species mostly belong to Holarctic Region were found in Kashmir and Ladakh division only and the minimum altitude at which *Ochlerotatus* species were found was 1578 m (*Oc. oreophilus*). *Oc. sintoni* was found restricted to forest areas in Kashmir division while it was found predominantly breeding with other species in Ladakh division. The species was reported between 1752 m and 3857 m elevation. *Oc. pullatus* collected from Gulmarg – Kashmir at 2545 m was found restricted to forest areas. *Culiseta* species usually considered as Holarctic species were also found in Kashmir and Ladakh division only and the minimum altitude at which *Culiseta* species were found was 2494 m (*Cs. niveitaeniata*). If the three groups or areas of Kashmir division (temperate) i.e. Jhelum Valley, Kashmir Valley, and Montane region are compared, they differ from each other in species composition. Also each area has some species that were not found in any other group like *Oc. oreophilus*, *St. patriciae* from Jhelum Valley, *Cq. richiardi*, *Gi. pulchriventer*, and *Ur. unguiculata* from Kashmir Valley, and *Cs. niveitaeniata*, and *Oc. pullatus* from Montane region. Species collected from Kargil and Leh districts of Ladakh division (cold-arid) are not showing much difference in their composition. From Jammu division collection was made above 1000 m in Udhampur district which had somewhat different climatic conditions but the collections were made few and only two species *An. lindesayi* and *Cx. mimeticus* were collected

which were collected up to 1000 m also. The most number of species were collected from Jammu division and the least number of species were collected from Ladakh division. This was mainly due to altitude and climate prevailing in the divisions although Ladakh division contributes 58.33% of State area. From the distribution of the mosquito species collected in the present study, impact of climate change or global warming was hardly perceived. Species reported by earlier workers were collected this time also and almost from the same places reported by them. Only *Cx. modestus* was not found in Jhelum Valley. However, developmental changes have occurred due to which *Ar. subalbatus* was found breeding in domestic areas that earlier workers have not reported. And due to developmental changes habitat displacement, loss, or confinement of habitats to some small places or pockets might have occurred, as a result of which some species reported by earlier workers were not collected in the present study and for which further collections are proposed.

Although dengue occurs in Jammu division only, the dengue vectors, *St. (Stg.) aegypti* and *St. albopicta* were collected from two divisions, Kashmir, and Jammu divisions of the State. Four *aegypti* adults were collected (reared from mud) from Kashmir division (temperate region) and one of them was collected at an altitude of around 2252 meters (Naranag – Ganderbal) which is now the highest elevation record for this species. Previous maximum elevation record for *aegypti* was around 2150 m in Puebla City in central Mexico (Lozano-Fuentes *et al.* 2012). In central Mexico, *aegypti* was found abundant at elevations up to 1,300 m, moderately abundant from 1,300 to 1,700 m, and still present but rare from 1700 to 2,150 m (Lozano-Fuentes *et al.* 2012). *St. aegypti* eggs show quiescence but lack diapause and the species that lack diapause are restricted to subtropical and tropical habitats (Denlinger & Armbruster 2014). The survivorship of overwintering *St. aegypti* eggs is minimal at such (maximum) elevation in Puebla City and that presence of the mosquito in the summer results largely from annual introductions of eggs or immatures through human transport of infested containers (Lozano-Fuentes *et al.* 2012). In the present study, it is interesting that *St. aegypti* was collected at an elevation of around 2252 meters from a rock pool (one adult reared from the mud taken from rock pool), Naranag – Ganderbal, Kashmir which is a natural container habitat

where introduction of the species through human transport can be doubtful but may be possible also as the place is a tourist spot. This needs further investigation.

The Vishnui group is almost wholly Oriental, but some members also occur in adjacent areas. Harbach (1988) pointed out that *Cx. tritaeniorhynchus* and *pseudovishnui* are the only members of the group which occur in southwestern Asia. *Cx. pseudovishnui* has been confused in the past with *Culex vishnui* Theobald, 1901, which is mainly restricted to the Oriental Region and occurs no farther west than eastern India. Although *pseudovishnui* closely resembles *vishnui*, it is definitely a distinct species which is well differentiated in the larval stage. In the present study, *Cx. vishnui* was collected from Jammu division only i.e., from the subtropical (or Oriental) region of the State while as *Cx. pseudovishnui* was collected from two divisions, Jammu, and Kashmir. So it appears that *Cx. vishnui* is distributed up to Jammu division only i.e. a fully Oriental species.

Species of the Maculatus group are Oriental but *An. (Cel.) maculatus* and *An. (Cel.) willmori* are the species of the Maculatus Group which penetrate the adjacent areas of the Oriental region. *An. (Cel.) maculatus* is found up to Pakistan while as *An. (Cel.) willmori* leaves behind Pakistan and goes up to Afghanistan (WRBU catalog; Morgan *et al.* 2013). *An. willmori* is a high altitude species occurring at 990–3170 m height MSL in northern Thailand (Rattanaarithikul *et al.* 1995). *An. (Cel.) willmori* was first reported in Kashmir by James in 1904. It breeds in the stream-beds of mountain-streams and torrents in the Himalayan area and adults were taken in houses in the villages in Gulmarg – Kashmir (Gill 1920; Christophers 1933). In the present study, *An. (Cel.) willmori* was collected from Kashmir, and Jammu division while as *An. (Cel.) maculatus* was collected from Jammu division only. It appears that *An. (Cel.) maculatus* is a subtropical or tropical species while as *An. (Cel.) willmori* along with being subtropical or tropical is a temperate species also.

### 5.5. Mosquito-borne diseases

Mosquito borne diseases, malaria and dengue are found in the State. Among J&K mosquito species collected in the present study, 15 are medically important species in India and 20 are medically important species in world.

J&K State is prone to two mosquito borne diseases - malaria and dengue (National Vector Borne Disease Control Programme 2017a,b). Jacob (1950) stated that on the occasion of the first health conference of Jammu and Kashmir in 1949, the Chief Medical Officer of the State pointed out that epidemics of cholera, smallpox, typhus and malaria were a great source of trouble in the State, and the last two diseases were more or less endemic. Currently, of these diseases, small pox (eradicated in 1980 worldwide) and typhus are no longer a source of trouble but cholera and malaria still persist in the State (National Family Health Survey 1999; National Rural Health Mission 2007). Malaria is found in Jammu division and some border areas of Kashmir division. Every year malaria cases are reported from different districts of Jammu division (District Malaria Office, Jammu). Thirteen mosquito species are malaria vectors in India (Bhattacharyya *et al.* 2014; Kumar *et al.* 2016) (Table 1). In the present study, the malaria vector species collected from the State were *An. annularis*, *An. culicifacies*, *An. fluviatilis*, *An. maculatus* and *An. stephensi*. Among these, *An. maculatus* was found at majority of the locations followed by *An. culicifacies*, *An. fluviatilis*, *An. annularis* and *An. stephensi*. Jacob (1950) pointed out that in 1946, the Epidemiology Department, Jammu who carried out studies in the division on malaria incriminated *An. culicifacies* as a vector. Of the 1091 *An. culicifacies* dissected, two showed gland infections and 3 oocysts in the gut. In Kashmir division, Nair (1973) incriminated *An. fluviatilis* as a vector. One *An. fluviatilis* out of 56 dissected showed sporozoites, the location being around at an elevation of 1829 m. Previously, Jacob (1950) found malaria transmission up to the elevation of 1829-1981 m (in Kamalkote village located at 1829-1981 m). Nair concluded that unstable endemic malaria existed in the region up to 2134 m altitude with low incidence at elevations higher than 1829 m.

Considering dengue, it is also a threat in the State and occurs only in Jammu division. Sidhu *et al.* (2015) have reported that the recent dengue outbreak occurred in 2013 was the first ever dengue outbreak. However, three outbreaks have been reported so far and now-a-days cases are reported every year. First two outbreaks occurred in 1974 and 1993. *St. aegypti* were collected for dengue antigen detection but found negative (Mathew *et al.* 1974; Padbidri *et al.* 1996). The recent outbreak occurred in 2013 was the third outbreak with 1837 cases and three deaths (NVBDCP 2017b). Mosquito species, *St.*

*aegypti* and *St. albopicta* are dengue vectors in India (Bhattacharyya *et al.* 2014). In present surveys, both the vector species were collected from the division. These species were collected from Kashmir division also but dengue is not found there.

Mosquito taxonomy plays essential role for vector control. *An. varuna*, a non-vector species targeted as a vector while misidentified as *An. minimus* in Vietnam (Bortel *et al.* 2001), and *An. fluviatilis*, a malaria vector now identified as a seasonal variant of *An. minimus* in north-east India (Singh *et al.* 2010) illustrate the role of mosquito fauna study. Also, malaria vector control in a region is dependant on the complexity and numbers of primary and secondary vectors which needs the study of whole anopheline fauna to devise proper and successful control strategy (Manguin *et al.* 2008).

Finally, taking this opportunity to sum up the total mosquito fauna of India, the result is: 393 species from the published data reported till 2014 (anophelines - 61, culicines - 332) (Bhattacharyya *et al.* 2014); four species *Aedes kolhapuriensis* Sathe & Girhe 2001, *Aedes panchgangee* Sathe & Girhe 2001, *Aedes sangitee* Sathe & Girhe 2001, *Aedes sangiti* Girhe & Sathe 2001 were removed due to *nomina nuda* (see *Mosquito Taxonomic Inventory*, What's New?, 12 November 2016, <http://mosquito-taxonomic-inventory.info/whats-new>); two species, *Cx. (Bar.) modestus*, and *Ur. (Pfc.) unguiculata* not included by Bhattacharyya *et al.* (2014) but collected in the present surveys and also reported by earlier workers; five new country records *Cx. (Cux.) pipiens*, *Cx. (Bar.) pusillus*, *Oc. (subgenus uncertain) caspius*, *Oc. (subgenus uncertain) pulcritarsis* ssp. *asiaticus*, and *Cq. (Coq.) richiardii* recorded from J&K in the present surveys; two new country records, *Ve. (Har.) srilankensis*, and *Cx. (Cui.) thurmanorum* collected by Natarajan – ICMR - Vector Control Research Centre – Puducherry, from Kerala (unpublished); *Hl. cherrapunjiensis* from Meghalaya (Natarajan *et al.* 2016); *Ur. maikalensis*, *Ur. dandakaranyensis*, and *Ur. satpuraensis*, three species of subgenus *Pseudoficalbia* from eastern hill ranges (Natarajan *et al.* 2017); *Tx. (Tox.) darjeelingensis* from West Bengal (Tyagi *et al.* 2015); and *Ur. (Pfc.) pseudostricklandi* from Kerala (Natarajan *et al.* (in submission process)). This brings the total number of species known to occur in India to 404 (anophelines - 61, culicines - 343).

### 5.6. Limitations of the Study

The main limitation of this study is the climate and political situation in the State. The climate of Ladakh division is extreme and the winter is so long that the roads remain open from May to October only and rest of the months remain blocked due to heavy snowfall and the division stays cut off from the State. The political situation in Kashmir Valley is also disturbing. Most of the time the Valley remains shut and curfewed and to go to forest areas for mosquito collection is risky. The situation makes many places inaccessible for mosquito collection.

COI sequencing or DNA barcoding was carried out for many important species in which a total of 32 species got barcoded and revealed many interesting points. However, some other important species showing morphological variations were not barcoded.

### 5.7. Future Perspectives

Mosquito-borne diseases, malaria, and dengue occur in J&K. Last vector incrimination for malaria was done in 1973 in Kashmir division (Baramulla district) by Nair and in 1946 in Jammu division by the Epidemiology Department, Jammu (Jacob 1950) after which lot of changes particularly developmental changes are taking place in the State. Vector incrimination should be carried out so as to control the diseases properly and effectively.

Many species such as *Am. vexans*, *Cq. richiardii*, *Cx. pipiens*, *Cx. modestus* etc. have been detected vectors and *Am. vexans* has been found a vector of animal diseases like EEE, WEE also in other countries but no such work has been carried out on these species in India.

Demirci *et al.* (2012) studied the altitudinal variation (between 808-2130 m) among populations of *Culex theileri* and have found a positive correlation between mosquito wing (body) size/shape and altitude in northeastern Turkey. J&K State has a quite wide altitudinal range (~261 m – 7000 m) which can be rarely found in any state in the world. In the present study, mosquitoes were collected from 262 m to 3879 m and three species were collected with a wide altitudinal range, *Cx. theileri* (262 m – 3879 m), *Cx. vagans* (262 m – 3879 m), and *Cx. mimeticus* (275 m – 3124 m). Such studies as done by Demirci *et al.* (2012) can be carried out in J&K State.

Study of winter survival of mosquitoes in J&K State can be a good research. In the State, Kashmir and Ladakh divisions bear very cold winter season, so mosquitoes have to go for diapause to cope the unfavorable conditions which is a neurohormonally mediated, dynamic state of low activity. To study diapause in these mosquitoes particularly in *An. gigas simlensis*, *Oc. pullatus*, *Oc. sintoni* will be quite interesting. Also, it will be the first study in India on overwintering of mosquitoes. *St. aegypti* was collected at 2252 m elevation which is quite surprising as the mosquito lacks diapause and to uncover this will be a good finding.

A number of other species found in adjacent countries of the J&K State, some may occur in the State also. Further surveys are needed to document their distribution in the State.

## ***6. Summary and Conclusion***



Ten mosquito surveys in 161 locations were carried out in different seasons (spring, summer, autumn, rainy) except in winter in all the three divisions of J&K State (Jammu, Kashmir, and Ladakh) between 2011 and 2016. Locations with lowest and highest altitudes from where mosquitoes (larvae) were collected were Satryian (RS Pura) – Jammu with altitude, 262 m and Lalung – Ladakh with altitude, 3879 m respectively.

A total of 7,094 specimens were studied from the 10 surveys. They were 1,172 whole larvae, 365 larval exuviae, 33 pupal exuviae; 2,413 adult♂, 3,111 adult♀; and 414 dissected male, 17 dissected female genitalia.

The study updated the mosquito fauna of J&K State which is composed of the two Culicidae subfamilies Anophelinae and Culicinae, 8 tribes, 21 genera, 22 subgenera, and 79 species.

Sixty-nine species were collected in the present study of which 3 tribes (Mansoniini, Sabethini, and Toxorhynchitini); 10 genera (*Armigeres*, *Coquillettidia*, *Hulecoeteomyia*, *Lutzia*, *Malaya*, *Mucidus*, *Neomelaniconion*, *Phagomyia*, *Toxorhynchites*, and *Verrallina*); 10 subgenera (*Alloeomyia*, *Armigeres*, *Coquillettidia*, *Culiseta*, *Eumelanomyia*, *Harbachius*, *Lophoceraomyia*, *Metalutzia*, *Mucidus*, and *Toxorhynchites*) and 36 species were reported for the first time in Jammu and Kashmir and represent new occurrence records for the State. Ten species from the earlier reported species were not collected in the present study.

Five species, *Culex (Culex) pipiens* Linnaeus 1758, *Culex (Barraudius) pusillus* Macquart 1850, *Ochlerotatus* (subgenus uncertain) *caspius* Pallas 1771, *Ochlerotatus* (subgenus uncertain) *pulchritarsis* ssp. *asiaticus* Edwards 1926, *Coquillettidia (Coquillettidia) richiardi* Ficalbi 1889 were reported as new country records.

Three species (*Culex (Culex) mimeticus*, *Culex (Culex) vagans*, and *Culex (Culex) theileri*) common to all the three divisions, and anophelines (*Anopheles (Anopheles) gigas* ssp. *simlensis* and *Anopheles (Anopheles) barianensis*) from Ladakh were reported for the first time.

Fifty, twenty-seven, and fifteen species were reported from Jammu, Kashmir, and Ladakh divisions respectively of which 31, 11, and 9 species were newly reported for Jammu, Kashmir, and Ladakh divisions respectively in the present study.

*COI* sequencing or DNA barcoding of 105 specimens was carried out belonging to 32 species (10 genera) due to which many interesting points got revealed.

*Am. vexans* was found to consist of subspecies, *vexans vexans*, and *vexans nipponi* revealed by barcoding and morphology.

*Oc. pullatus* collected from Kashmir division was found with the same morphological variation as described by Barraud (1934) and *COI* sequences have also shown some difference when they were compared with the reference sequences of *pullatus* available on GenBank (average K2P genetic distance was 3.1% (2.7-3.5%)). So can be a separate species but needs further taxonomical work.

When *COI* sequences of *Cs. (Cus.) alaskaensis* ssp. *indica*, and *An. (Ano.) gigas* ssp. *simlensis* were compared with the reference sequences of *Cs. (Cus.) alaskaensis*, and *An. (Ano.) gigas* respectively available on GenBank have shown quite difference from *COI* sequences of their respective species which indicated that these can be no longer subspecies but can be new species (K2P genetic distance between *Cs. alaskaensis* ssp. *indica* and *Cs. alaskaensis*, 6.5%; K2P genetic distance between *An. gigas* ssp. *simlensis* and *An. gigas*, 11.9%).

*An. barianensis* was found quite different from *An. plumbeus* when *COI* sequences of *barianensis* collected in present study were compared with the *COI* sequences of *plumbeus* available on GenBank as Gutsevich *et al.* (1974) have reported them similar species (K2P genetic distance, 11.6%).

*COI* sequence of *Oc. oreophilus* when compared with its reference sequences available on GenBank was not found similar (K2P genetic distance, 5.4%) which needs further studies with more number of specimens but only a single female specimen is available with only one leg remaining (5 legs were given for barcoding), so difficult to reexamine.

Larval collection was carried out in Ladakh from 13, in Kashmir from 25, and in Jammu from 26 habitat types. Most of the habitats in the State are ground habitats.

The highest number of species were collected from seepage and cement tank (26 species each). Drainage canal, unused well, and container habitats – bamboo, barrel, bucket, fridge defrost tray, rock hole were found with least number of species (one species each).

*Cx. vagans* was found in most of the habitat types (20). Species which were collected from only one habitat type are *An. barianensis* (tree hole), *St. patriciae* (tree hole), *St. unilineata* (cactus hole), *Hl. chrysolineata* (cement tank), and *Cx. perexiguus* (seepage).

True diversities (Shannon and Simpson effective number of species) were found less than species richness, both in divisions and larval habitats due to unevenness in the number of specimens of species collected.

*St. aegypti* was collected at an elevation of around 2252 meters from a natural container, rock pool (one adult reared from the mud taken from rock pool), Naranag – Ganderbal, Kashmir which is now the highest elevation record for this species. The presence of this species which lacks diapause at such an elevation is surprising and can be possible due to introduction of the species through human transport as the place is a tourist spot. But, as it was collected from a rock pool, a natural container, its introduction through human transport is doubtful, so needs further study.

The Vishnui group is almost wholly Oriental, but two members, *Cx. tritaeniorhynchus* and *pseudovishnui* occur in adjacent areas also. In the present study, *Cx. vishnui* was collected from Jammu division only i.e., from the subtropical (or Oriental) region of the State while as *Cx. pseudovishnui* was collected from two divisions, Jammu, and Kashmir. So it appears that *Cx. vishnui* is distributed up to Jammu division only i.e. a fully Oriental species.

*Cx. (Bar.) modestus* was collected from Ladakh division in the present study but previous worker(s) (Barraud) reported it from Kashmir division. Genus *Culiseta* in India

is represented by three species, *Cs. (All.) longiareolata*, *Cs. (Cus.) alaskaensis ssp. indica*, and *Cs. (Cus.) niveitaeniata* and all these species were collected from J&K State during the present study. And Maculatus group in India is represented by 6 species, *An. maculatus*, *An. willmori*, *An. pseudowillmori*, *An. dravidicus*, *An. rampae*, and *An. sawadwongporni*; excluding *An. rampae*, and *An. sawadwongporni* remaining 4 species were collected from the State. Species of the Maculatus group are Oriental but *An. (Cel.) maculatus* and *An. (Cel.) willmori* have penetrated to the adjacent areas of the Oriental region.

*Ar. subalbatus* was collected both as adult and larva in the present study from Jammu, and Kashmir divisions. Adults were found in houses, therefore, appears to be a common species in the State. Rao *et al.* (1973) or Bhat and Kulkarni (1983) have not reported this species. Probably, the species would have got introduced later or proliferated later in the division due to increase in drainage system.

From the distribution of the mosquito species collected in the present study, impact of climate change or global warming was hardly perceived. Species reported by earlier workers were collected this time also and almost from the same places reported by them. Only *Cx. modestus* was not found in Jehlum Valley. However, developmental changes have occurred due to which *Ar. subalbatus* was found breeding in domestic areas that earlier workers have not reported. And due to developmental changes habitat displacement, loss, or confinement of habitats to some small places or pockets might have occurred, as a result of which some species reported by earlier workers were not collected in the present study and for which further collections are proposed.

In conclusion, 69 species including 36 new state records with 5 new country records were collected from the State in the present study. And 4 species, *An. (Ano.) gigas simlensis*, *Cs. (Cus.) alaskaensis indica*, *Cx. (Cux.) theileri*, and *Oc. pullatus* collected in the present study may be new species (further taxonomical studies are needed). The climatic difference has shown the effect on the number of species and the habitat types. Both diversity and distribution of mosquitoes was found different in different divisions. Mosquito species diversity found in the divisions is in the order: Jammu>Kashmir>Ladakh, due to their climatic conditions (Jammu – sub-tropical,

Kashmir – temperate, Ladakh – cold arid). The number of species may also increase with further surveys or collections.

DNA barcoding though may not always be useful on its own was used as an additional tool to complement morphological identification in the study. In our analysis, *COI* barcode has differentiated mosquito species that were difficult to distinguish morphologically, cleared many doubts, and checked the status of subspecies.

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## *Annexure*

### **Publication:**

Dar TH, Natarajan R, Jayakumar S, Jambulingam P. 2016. Mosquitoes (Diptera: Culicidae) of Jammu division of Jammu and Kashmir State, India, with new records. *Journal of Entomology and Zoology Studies* 4(1): 388-393.

### **Presentation:**

Faunistic studies on the diversity and distribution of mosquitoes of the high altitude Himalayan Region – Jammu and Kashmir was presented in the 5<sup>th</sup> *International Conference on Climate Change and Sustainable Management of Natural Resources* held on from 9<sup>th</sup> to 11<sup>th</sup> February, 2015 at ITM University, Gwalior, MP - India.