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Comparative cytology of four families of spiders from two districts of Manipur, India

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Abstract
The cytological studies of spiders in Manipur is very limited and studies on this aspect is much needed for species exploration. The study was carried from four families from two districts of Manipur. The males were dissected in hypotonic solution and fixed on the slides and stained in 4% Giemsa stain by keeping the slides in solution in a couplin jar. Identity of family Salticidae was *Plexipus paykuhlii* from the morphological aspects while the other three could identified upto generic level but not be identified upto the species level due to complexities and much variation in morphological characteristics so they were put up to the generic level. The diploid count of the four families were same with 28. The chromosomes number in pachytene/diplotene stage was confirmed to be 28 and sex determination mechanism in four families was X_1 X_2 0 type while it seems to X0 in *Oxyopes*. There were not many variations in the stages of the stages of the meiosis among the four families. The sex gametes were like flattened top: *Plexipus* and *Pardosa* had pointed end (*Pardosa* had much pointed ends) while the *Oxyopes* and *Philodromus* had blunt ends. These differences might be differences in collection stages of the division. There was little variation in the Meiotic stages in four families of the spiders. The main differences were on the deposition of the heterochromatins. The heterochromatins were much in each chromosome starting from *Oxyopes*, *Pardosa* and *Philodromus* sp. than the *Plexippus*. The significance difference was most prominent in the zygotene stages, the chromomeres were darkly stains in *Oxyopes*, *Pardosa* and *Philodromus* while the *Plexipus* was less darkly stained. Again, the separation of X chromosomes was also significant observation, they were positively heteropycnotic behavior and seem to be separated at zygotene only in *Philodromus* while remain associated in *Plexipus*, *Pardosa and Oxyopes*. On the basis of identical chromosome diploid count of 28 (except 21 in *Oxyopes* sp with single X): 26 acrocentric somatic chromosomes, X_1X_2 allosomes and absence of distinct chromosomal behavior during the meiotic cycle, only evolutionary significant point to focus here is the role of the heterochromatin. On the basis of the heterochromatin deposition Salticidae is the primitive family then Philodromus, Lycosidae as the advance family. In future, this minimum karyological diversity in the families, their cytological studies are seeming to best resolved using molecular cytogenetic studies as well as molecular methods involving the DNA sequencing and species identification and delineation of the positive species identification and importance of the cytological data on speciation in the four families.

Keywords: Manipur, four families, cytological, minimum karyotype diversity

Introduction
Spiders are wonder Arthropods inhabiting every possible habitat in and around human inhabitant areas as commensal or free living in three habitats viz., land as jumping, aquatic as surface dwellers and arboreal in trees. They may be broadly of two types: harmless and poisonous that lethal to human beings. They are aspiration to human in many ways. One of the most important aspects is their importance in the food chains. They controlled many insect populations mainly house flies and mosquito. Till now there is no reports of carrier of human diseases. So there are many reasons for their systematic studies. Spiders are carnivorous creatures, feeding mainly on insects and small arachnids. Diving spiders like *Dolomedes* can even catch little fishes [1]. The wandering spiders are alerted to the presence of a prey by its movement. In case of web making species, the prey is trapped in the snare. The trap door spiders and wandering spiders just catch the prey and readily feed on it. Spiders ingest food in two different ways: Spiders having weak jaws inject digestive juice and the liquefied tissues is sucked to the left empty shell of the preys. The tarantulas, wolf spiders, large orb-weavers, and other having strong jaws, chewed their preys to pulp between the jaws, as the digestive fluid is

regurgitated over it [2].

Manipur is one of the regions included in Indo Burma hotspots [3] occupying the 6th hottest hot spots of the world. The main features of the divisions are endemism and destruction through human activities. The spider fauna of Manipur is virgin in sense that they are not thoroughly studied and these organisms are also not including in the criteria for hotspots.

Spiders belong to the class Arachnida, order: Araneae of the Phylum: Arthropoda. They are recognized by the possession of Cephalothorax and abdomen, four pairs of jointed legs, six to eight simple eyes and four to six spinnerets. They are one of the most diverse animals' group in the world. The spiders rank seventh in the total species diversity among all other groups of organisms.

Another division of spiders on the basis of owing a web: web-builders and hunting spiders. A few species of the genus *Dolomedes* are aquatic, can even remain under water for some time (Kumar, *et al*. 1999). According to the Platnick [4], 44032 species of spiders belonging to 3905 genera under 112 families. In India, 1685 species from 438 genera and 60 families (Keswani, *et al.* 2012) are reported so far. But according to World Spider Catalog [5] there are 4149 genera, 48304 species are available as valid species under 120 families with annual identification is more than 1, 000 in the year 2000. The family Lycosidae had 125 genera with 2438 species; Oxyopidae 9 genera, 453 species; Philodromidae with 31, 538 species and Salticidae 644 genera with 6162 species [5]. Despite the huge number of the spiders the least cytogenetic of 74 families, 320 genera and 867 species were cytologically analyzed.

According to Sharma and Sharma [6] had reported 325 cytogenetic records till date in India. Of these 232 species (71.38%) have sex chromosome system of the X_1X_20 type; 48 species (12.92%) have an X0 system; 39 species (12%) have an $X_1X_2X_30$ system; 1 species (0.3%) have sex chromosome system of the X_1X_2Y type; 4 species (1.23%) have an $X_1X_2X_3X_40$ system; 1 species (0.3%) has an SCS of the X_1X_2Y type. The cytology of the species of *Plexippus paykuhli* had reported only X_1X_2 but not the chromosome compliments.

The cytogenetic studies on the family Salticidae, Lycosidae, Oxyopidae and Philodromidae and their genera from Manipur is in nascent stage and the study had been taken up in the present paper.

Materials and Methods

The adult males and females of *Plexippus* (Salticidae), *Pardosa* (Lycosidae), *Oxyopes* (*Oxyopidae*) and *Philodromus* (Philodromidae) were collected from different locations of Imphal West-15 sites and Ukhrul district-5 study sites (Table 1, Fig. 1.) and properly photographed and kept specimens in the entomological deposits at D.M. College Imphal. Some of the males of each family were killed by keeping in the refrigerator (-10 ^{0}C) for 10 minutes and the testes were dissected in the hypotonic solution (0.075 M KCl) and kept for five minutes and crushed with tips of the dissecting needles in the above solution and fixed with fixative (1:3 Glacial acetic acid and Methanol) and air dry. The slide was stained in 4% Giemsa stain. The slides were observed under Opt-scopes compound microscope and photographs were taken with the camera attached to it with oil immersion. The species was identified according to appropriate keys. The respective cell stages were photographed and analyzed in the four families in the same stage.

Table 1: Details of study sites, GPS reading, and diploid count from Ukhrul and Imphal West district of Manipur.

SL. No.	Location	Districts	GPS reading	Date of collection	Family/Diploid (2n)
1.	Keishamthong	Imphal West	24^{0}47'80.0" 93^{0}55'93.3"	10/04/2015	Salticidae/28
2.	Keishampat	Imphal West	24^{0}79'76.1" 93^{0}93'38.3"	08/08/2015	Salticidae/28
3.	Keishamthong Laishom Leirak	Imphal West	24^{0}79'82.4" 93^{0}93'44.3"	12/04/2016	Salticidae/28
4.	Kwakeithel	Imphal West	24^{0}78'52.5" 93^{0}93'44.3"	23/04/2016	Philodromidae/28
5.	Keishamthong	Imphal West	24^{0}47'80.0" 93^{0}55'93.3"	06/04/2016	Salticidae/28
6.	Iroisemba	Imphal west	24^{0}80'96.9" 93^{0}89'25.4"	26/07/2016	Salticidae/28
7.	Naoremthong	Imphal west	24^{0}80'98.0" 93^{0}91'35.7"	26/07/2016	Salticidae/28
8.	CAU	Imphal west	24^{0}81'39.7" 93^{08}9'00.9"	26/07/2017	Salticidae/28
9.	CAU gate	Imphal west	24^{0}81'14.6" 93^0 88'94.6"	26/08/2017	Salticidae/28
10.	D M College	Imphal west	24^{0}79'82.4" 93^{0}93'44.3"	26/08/2018	Oxyopidae/21+ Salticidae/28
11.	D M College	Imphal west	24^{0}82'07.8" 93^{0}94'14.7"	26/08/2018	Oxyopidae/21+ Salticidae/28
12.	D M College	Imphal west	24^{0}82'08.2" 93^{0}94'28.9"	26/08/2018	Oxyopidae/21+ Salticidae/28
13.	Iroisemba	Imphal west	24^{0}80'96.9" 93^{0}89'25.4"	26/07/2018	Oxyopidae/28
14.	Naoremthong	Imphal west	24^{0}80'98.0" 93^{0}91'35.7"	26/07/2018	Oxyopidae/21
15.	Heirangoithoi	Imphal west	24^{0}77'47.9" 92^{0}01'02.2"	26/08/2018	Oxyopidae/21
16.	Jalembung	Ukhrul	24^{0}95'24.4" 94^{0}18'23.7"	27/02/2019	Salticidae/28
17.	Ukhrul Bazar	Ukhrul	24^{0}79'25.4" 92^{0}01'02.2"	24/04/2019	Salticidae/28
18.	Litan Ukhrul	Ukhrul	24^{0}57'10.0" 92^{0}12'90.0"	27/04/2019	Philodromidae/28
19.	Thoyee	Ukhrul	24^{0}93'76.5" 94^{0}15'13.1"	27/06/2019	Philodromidae/28
20.	Rambha	Ukhrul	25^{0}01'35.8" 94^{0}28'28.5"	28/07/2019	Oxyopidae/21

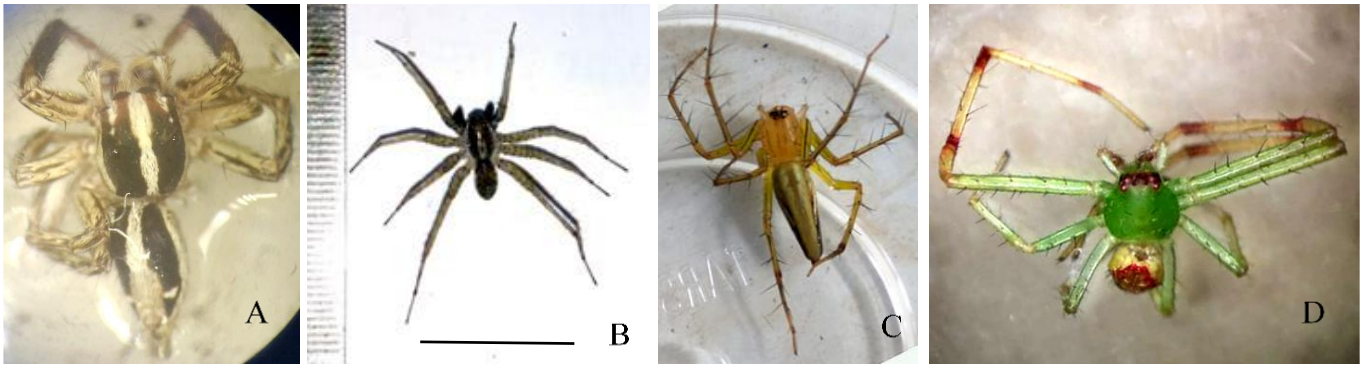

Fig 1: The ventral view of A-*Plexippus paykulli* (Salticidae), B-*Pardosa* sp. (Lycosidae), C-*Oxyopes* (Oxyopidae) and D-*Philodromus* sp. (Philodromidae). Bar represents 1.0 cm.

Taxonomic background

Salticidae blackwall, 1841 (Jumping spiders)

Salticidae are generally medium-sized among the spiders and the size ranges from (3-20) mm in length, ocular quadrangle on the cephalothorax delimited by eight eyes arranged in three or four rows. Front row comprises forwardly directed four eyes, of which two anterior median eyes are very large and prominent. Two anterior laterals form the next row and two posterior laterals almost same as anterior laterals, constitute the last. Thoracic region is in continuation with the cephalic part and in some separated by a constriction, sternum variable in size and shape, labium usually a triangular plate with blunt anterior edge bearing scopulae. Maxillary palps simple in female, complex in males and act as copulatory organs. Chelicerae with teeth and a fang on outer and inner margin in variable numbers. Legs segmented, long and stout with hairy tuft and two claws. Cephalothorax connected to the abdomen by a pidicel not visible in most. In some clearly visible, segmented in some, elongated, oval etc., covered with hairs. Spinnerets at the posterior end of the abdomen, epigynae variable and complex on structure. This family is the most dominant diverse family in India. A total of 207 species for 73 genera (Keswani, *et al.* 2012) are reported from India so far. Manipur recorded 20 genera with 28 species so far.

Genus: *Plexipus* C.L. Koch, 1846

Medium-sized less than 10mm long, cephalothorax long, slightly convex above, thoracic region broad, ovate, a little dilated behind dorsal eyes, with sides nearly vertical and parallel in front, rounded behind. The ocular quadrangle occupies approximately one third length of the cephalothorax, wider than long, a little broad in front than behind. Anterior eyes in a curved row, second row little nearer to the anterolaterals, posterior row of eyes slightly closer. Chelicerae with 2 teeth on inner and 1 on outer margin. Labium a little longer than wide. Sternum rather, rounded behind, with few spines. Leg III not longer than I, tibia I with 3 pairs of ventral spines, legs I and II similar. Abdomen oval, or elongate oval.

***Plexipus paykuhlii* (Audouin, 1825)**

Lycosidae Sindervall 1833 (Wolf Spiders)

Ground living, small to large spiders, ecribellate, entelegyne spiders. Eye 8 in three rows of 4, 2, 2 dark in colour, posterior row of eyes recurved strongly; anterior eyes small, other eyes large. Chelicerae long, robust, often hairy with 3 prolateral and 2 to 4 retrolateral marginal teeth. Maxilla longer than wide, anteriorly scopulate, labium free, quite short, sternum nearly heart shaped, pointed posteriorly. Legs long, slender, strong and spiny, tarsi bearing 3 claws, IV leg longest. Six-spinneretes present; colulus absent. Male palp without retrolateral tibial apophysis. Epigyne complex, with well sclerotised median septum, females carry eggsac attached to spinnerets. Abdomen oval covered with dense sitae.

A total of 126 species of 17 genera are reported from India so far. Among these *Hippasa* and *Lycosa* belong to the sub family: Lycosinae and *Pardosa* belongs to the sub family Pardosinae. Manipur recorded 5 genera with 22 species. (Sebastian and Peter 2009).

Distribution: Cosmopolitan.

Cephalic region raised and almost entirely occupied by 2 posterior rows of eye. Clypeus vertical, chelicerae much smaller than in most other Lycosids so that their height is less than height of the head. Anterior row of eyes shorter than the 2nd row. Labium usually wider than long with basal notch. Legs long, slender with long spines, metatarsus IV usually longer than or at least as long as Patella and Tibia together; tibia I with ventral spines in pairs. Abdomen oval, pubescent; dorsum decorated. A total of 44 species are reported from India so far. Manipur recorded 9 species only

***Oxyopidae* Thorell 1870 (Lynx spiders)**

1870. *Oxyopidae* Thorell, Nova. Acta. Reg. Soc. Sci., 793: 188

1970. *Oxyopidae*: Tikader Rec. Zool. Surv. India, 64 (1-4): 70

2004. *Oxylpidae:* Gajbe, P. Rec. Zool. Surv. India, 20(2): 2

2005. *Oxyopidae*: Siliwal, et al. Zoo's Print Journal, 20(11): 2028

Small to large sized, cribellate, entelegyne spiders. Cephalothorax longer than wide, high and convex anteriorly, sloping posteriorly. Clypeus is very high, vertical usually with conspicuous stripes and spots. Integuments clothed in thin setae and sometimes in iridescent scales. Eight-eyes in two rows, arranged in a hexagonal shape, anterior row strongly recurved and posterior row procurved. Anterior median eyes smallest, much smaller than the anterior lateral eyes. Chelicerae long and tapering at distal end with short fangs, cheliceral margin short and armed with one tooth on each side or without tooth, boss on the anterior lateral face of the chelicerae not so prominent. Sternum scutiform, tapers between coxa IV. Labium longer than wide, maxillae long and converging. Legs long, slender with long spines, 3-clawed, trochanters shallowly notched, without scapulae. Abdomen tapers posterioly with patches or bands. Spinnerets short, sub-equal, colulus present, epigyne complex, well sclerotised, male palp usually with tibial apophysis and paracymbium. Oxyopids are represented by genera, 430 species occurring worldwide (Platnick, 2013) while in India,

69 species from 4 genera are reported so far (Sebastian and Peter, 2009). In Manipur, 2 genera with 8 species are recorded.

***Oxyopes* Latreille 1804.**

Cephalothorax high and rounded with the anterior part vertical and it then continues almost level for most of its length to a steep thoracic part. Eye pattern 2,2,2,2 with very small anterior medians being over half way upto the front of the face and the posterior row of eyes strongly procurved and equidistant from each other. Ocular quadrangle longer than wide and limited by the posterior median eyes and the anterior lateral eyes. A thin black straight line starts from each of the anterior medians down the vertical face and continues down the centre of the long pale chelicerae to the tip. Abdomen long thin rounded and widest at the front and tapering all the way to spinnerets. A total of 48 species are reported from India so far. 8 species are recorded from Manipur.

The species from Imphal west district are *Oxyopes pankaji* Gajbe and Gajbe 2000, *Oxyopes shweta* Tikader 1970, *Oxyopes javanus* Thorell, 1877 and *Oxyopes birmanicus* Thorell, 1887.

Philodromidae Thorell, 1870 (Elongated crab spider)

Small to medium sized, eight recurved or sometimes posterior row strongly recurved eyed spiders with two clawed slender laterigrade legs having claw-tuft and scopulae. Cephalothorax slightly flattened, elongated, and smoothly convex on lateral sides, clothed with soft recumbent setae, white to pale cream and reddish-brown or greyish-brown in colour, frequently mottled with longitudinal bands or chevrons. Apex of sternum with an obtuse point between coxae IV, labium slightly longer than wide. Chelicerae without teeth. Legs I, III, IV almost equal in length, leg II usually longer, sometimes much longer, tarsi I and II with scopulae and claw tufts. Abdomen oval or elongated covered with soft recumbent setae. Colulus absent spinnerets simple, epigyne complex usually with median septum, sometimes with folds and copulatory openings on the laterals, spermethecae kidney-shaped. Male palp with small apophysis on tibia, embolus long to short, slender and arched at the distal end of tegulum.

A total of 48 species of 8 genera are reported from India so far (Keswani, *et al.* 2012). Only one genus is recorded from Manipur.

***Philodromus* Walckenaer, 1826.**

Cephalothorax broader than long, narrow in from, carapace flattened, smoothly convex at lateral sides. Eyes small and uniform in size and crescent shaped group, anterior row shortest, posterior row strongly recurved, medians farther from each other than from the lateral. Legs long, slender and laterigrade, leg II generally longer than leg I, Legs III and IV, a little shorter than I and II. Abdomen oval, angulated laterally, moderately flat and bears dorsally heart-\-shaped markings and chevrons.

Only one unidentified species is recorded from Manipur.

Results

Giemsa stained the spermatids as well as the spermatozoa. The head region of the spermatids was identical in the four families with little variation. The only difference was in pointed ends in *Plexipus* and *Oxyopes* while blunt in *Pardosa* and *Philodromus.* The only difference in four families regarding testes was the length their length (Fig. 2).

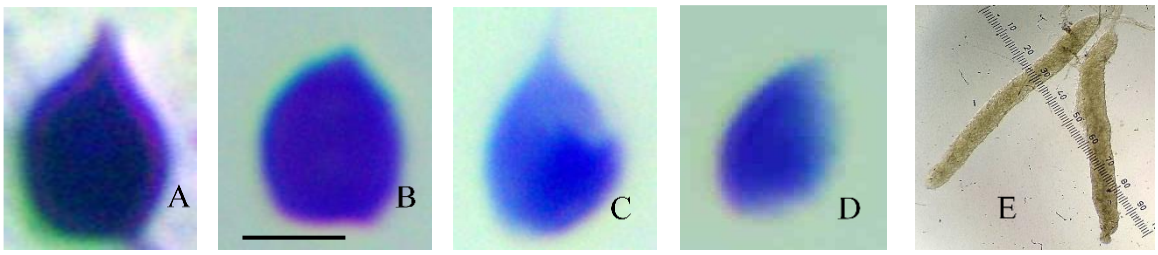

Fig 2: The reproductive gametes of A-*Plexippus paykulli* (Salticidae), B-*Pardosa* sp. (Lycosidae), C-*Oxyopes* (Oxyopidae) and D-*Philodromus* sp. (Philodromidae), testes of *Oxyopes*. Bar represents 10 µm.

Salticidae

The specimens of the family Salticidae from two districts were identified as *Plexipus paykulli* (Audouin, 1825). The species showed distinct cytological characteristics. There were no cytological differences in the specimens from the two districts.

The different stages of Meiosis I in the spermatogenesis in adult *Plexippus paykulli* (Audouin 1825), were studied after Giemsa staining. The diploid count of the species is 28 as other members of the Salticidae were in accordance with Sharma and Sharma (2014). The distribution of the 13 or 15 chromosomes in the diakinesis or metaphase I showed that the sex determination mechanism in this species is X_1X_20. Hence the karyotypic formula of the species could be 26A+ X_1X_20. The observations were made from the nuclei having the sufficient staining and started from the interphase stage. Early interphase showed densely stained dots like structures representing the two X chromosomes (Fig. 3. A, arrowed) were distinctly visible. Later those dots were become larger in size (Fig. 3. B, C arrowed) became almost rectangular blocks which could not be recognized as two Xs. In the early leptotene stage the two heteropycnotic blocks became separated out (Fig. 3.D arrowed) and fairly separate out in the late leptotene stage (Fig. 3. E, F arrowed), the separation could be seen in the late zygotene stage (Fig. 3. G arrowed), the connection of the two persisted again till late pachytene (Fig. 3. H, I arrowed), late pachytene showed distantly located X chromosomes (Fig. 3. J, K white arrowed), during diplotene, diakinesis stage they lie side by side (Fig. 3. L white arrowed) and persisted till metaphase I (Fig. 3. M white arrowed). The late stages of Meiosis might show some interesting features but present study focused on the Meiosis I.

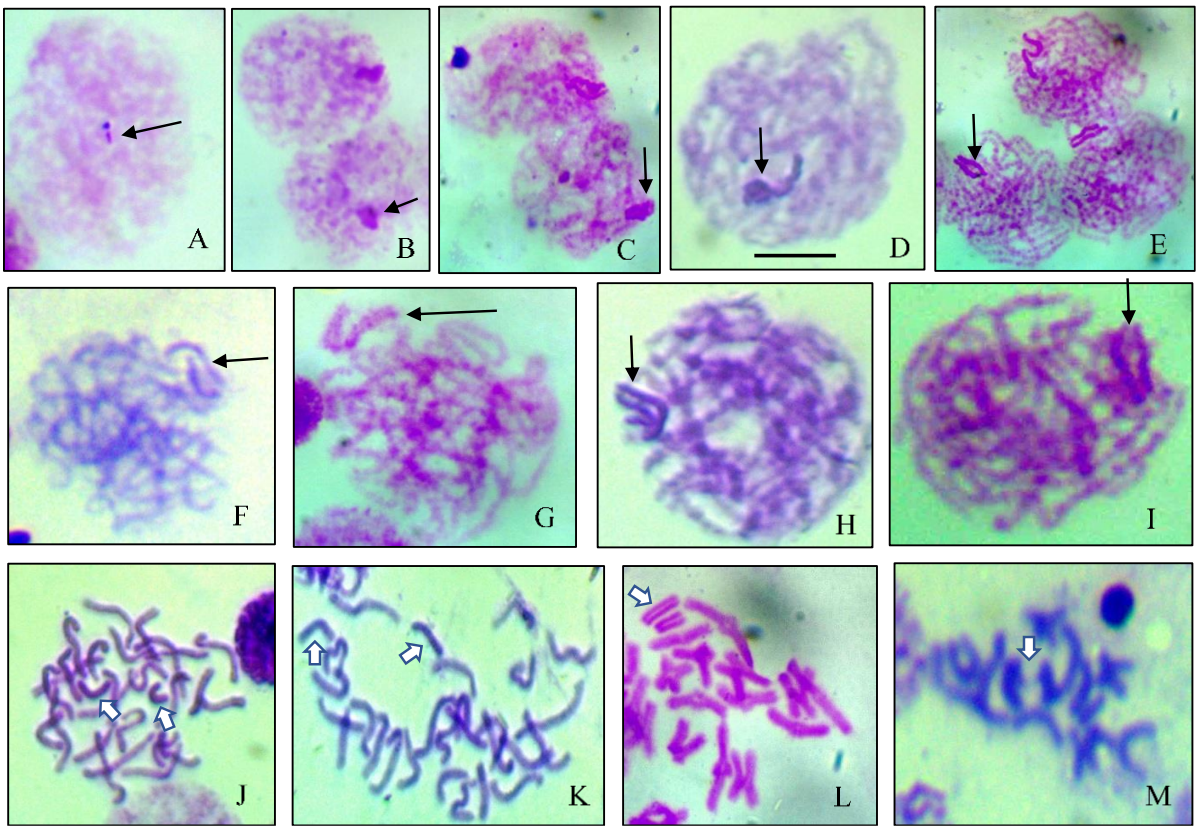

Fig 3: The different stages of Meiosis stages in the spermatogenesis in adult *Plexippus paykuhli*. Early interphase dots structures representing the two X chromosomes (A, arrowed), were become larger in size (B, C arrowed), in the late leptotene the two heteropycnotic blocks became separate (D arrowed) and fairly separate out in the late leptotene stage (E, F arrowed), the two chromosomes were fairly separate out in the zygotene stage (G arrowed), the joining of the two persist again till late pachytene (H, I arrowed), late pachytene showed distantly located X chromosomes (J, K white arrowed), during diplotene, diakinesis stage they lie side to side (L white arrowed) and persisted till metaphase I (M white arrowed). Bar represents 10 µm.

Oxyopidae

The different stages of Meiotic stages in the spermatogenesis in adult *Oxyopes* sp. were not upto the mark as expected but number of the chromosomes corresponded to 2n=21 with X chromosome. The conclusion came from the observation of 11 and 10 diplotene and metaphase respectively. The X chromosome was not much distinct from the somatic chromosomes. Late interphase showed densely stained dots structures whether X chromosomes or chromocenters could not be confirmed (Fig. 4. A), and also in leptotene (Fig. 4. B) with dense clump heteropycnotic regions, in the late leptotene stage the 4 or more heteropycnotic blocks located in regions (Fig. 4. C), the zygotene stage the pairing of the homologous chromosomes did show (Fig. 4. D), in early pachytene with roughly 21 chromosomes could be seen (Fig. 4. E), the diplotene-diakinesis stages with 11 chromosomes possessing one dark banded (white arrowed) seem to be X chromosome (Fig. 4. F), in metaphase I with 10 banded chromosomes (Fig. 4.G) and the spermatogonial pro-metaphase was having roughly 21 chromosomes were seen (Fig. 4.H).

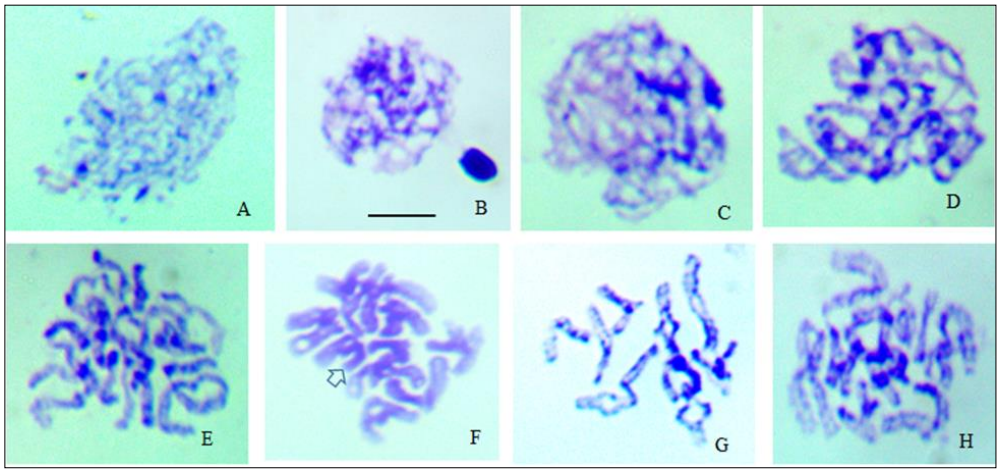

Fig 4: The different stages of Meiotic stages in the spermatogenesis in adult *Oxyopes* sp. Late interphase (A), leptotene (B) with dense clump heteropycnotic regions, in the late leptotene stage the 4 or more heteropycnotic blocks located in regions (C), the zygotene stage the pairing of the homologous chromosomes (D), in early Pachytene with 21 chromosomes (E), the diplotene-diakinesis stages with 11 chromosomes possessing one dark banded (white arrowed) seem to be X chromosome (F), in metaphase I with 10 banded chromosomes (G) and the spermatogonial pro-metaphase was having 21 chromosomes (H). Bar represents 10 µm.

Lycosidae

The genus of *Pardosa* sp. showed the characteristic meiotic stages from the testes and adult was perfect for the purpose. The diploid count of the species is 28. The distribution of the 13 or 15 chromosomes in the diakinesis or metaphase I showed that the sex determination mechanism in this genera was X_1X_2O. Hence the karyotypic formula of the species could be 26A+ X_1X_2O. Early interphase showed densely stained blocks without any distinction between the X chromosomes (Fig. A), were become segregated in leptotene stage (Fig. B), zygotene stage were showing distinct chromomeric pairing and two parallel X chromosomes (Fig. C), the pachytene stage was characterized by appearance of tetravalent chromosomes roughly 28 in numbers without any differences between X and somatic chromosomes (Fig. D), the diplotene stage was characterized by diplotene loop and much condensed chromosomes; the two X chromosomes were darkly stained and easily distinguished (Fig. E), diakinesis stage characterized by rod shaped X chromosomes and highly separating bivalents were seen clearly (Fig. F), the metaphase I characterized by condensed chromosomes arranged at equatorial plates as usual (Fig. G), the anaphase I showed 13 or 15 chromosomes configuration (Fig. H with premature condensed chromosomes), the metaphase II showed uncountable chromosomes (Fig. I), and anaphase II showed usual hollow radial configuration (Fig. J).

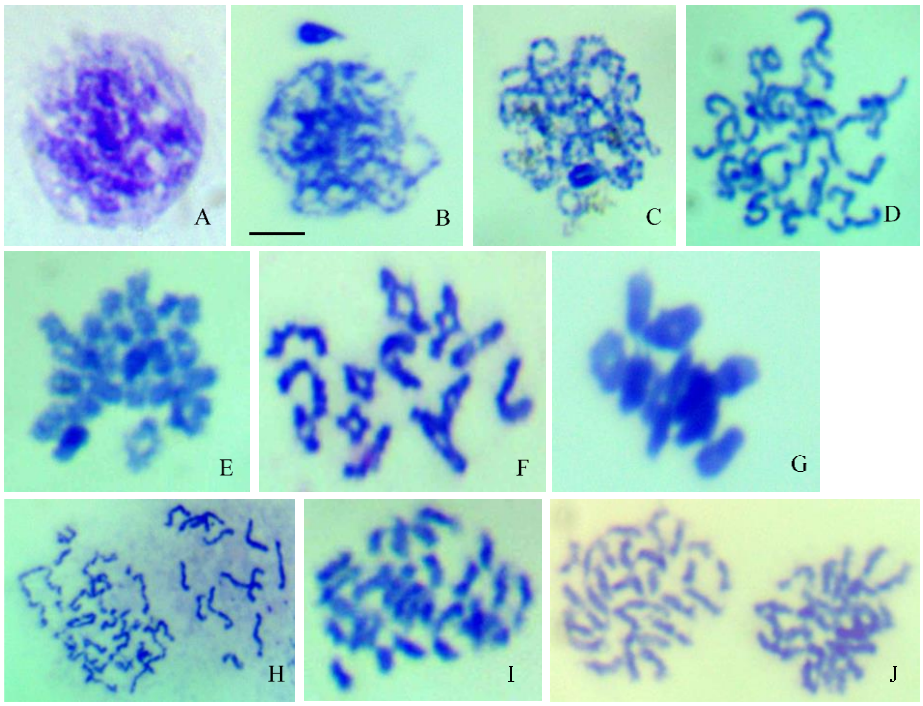

Fig 4.A: The different stages of Meiotic stages in the spermatogenesis in adult *Pardosa* sp. Early interphase (A), leptotene stage (B), zygotene stage (C), the pachytene stage (D), the early diplotene stage (E), diakinesis stage (F), the metaphase I (G), the anaphase I showed 13 or 15 chromosomes configuration (H with premature condensed chromosomes), the metaphase II showed uncountable chromosomes (I), and anaphase II showed radial cylindrical configuration (J). Bar represents 10 µm

Philodromidae

The different stages of Meiosis in the spermatogenesis in adult *Philodromus* sp. were quite beauty regarding the fine and distinct stages of division. Late leptotene showed densely stained peripherally located blocks having a loop at the middle, X chromosomes (A), were become segregated in zygotene stage with distinct chromomeric pairing and two X chromosomes (B), the pachytene stage was characterized by appearance of tetravalent chromosomes roughly 28 in numbers, two X chromosomes were fairly condensed and darkly stained than somatic chromosomes (D), the diplotene stage was characterized by diplotene loop and much condensed chromosomes; the two X chromosomes were darkly stained and easily distinguished (D), diakinesis stage characterized by rod shaped X chromosomes and highly separating bivalents (E), the metaphase I characterized by condensed chromosomes arranged at equatorial plates as usual (G), the anaphase I showed 13 chromosomes configuration (G), in prophase II the separation of the two cells could be seen (H) the metaphase II showed uncountable chromosomes (I), the anaphase II showed the floret features (I), and anaphase II showed usual radial cylindrical configuration (J). The spermatogonial metaphase chromosome plate showed 28 chromosomes (K) and premature prophase could not ascertain the chromosome numbers (L).

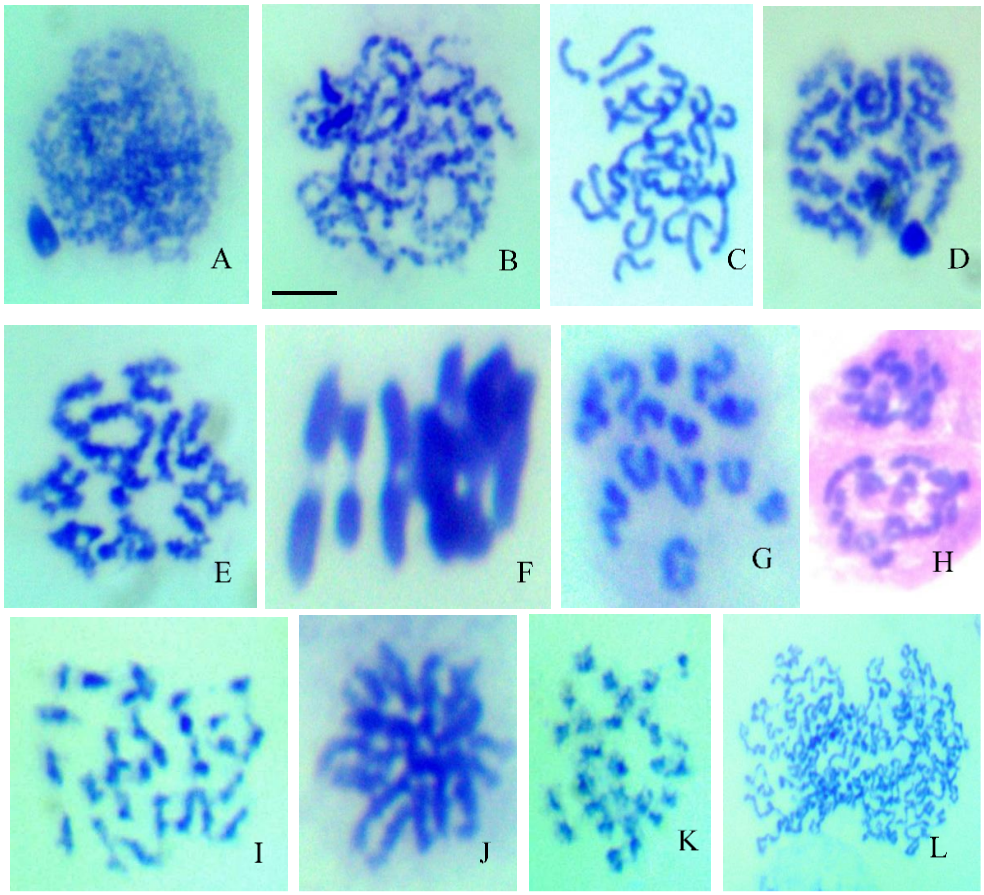

Fig 5: The different stages of Meiosis in the spermatogenesis in adult *Philodromus* sp. Late leptotene (A), leptotene (B), pachytene stage (D), the diplotene stage (E), the metaphase I (G), the anaphase I showing 13 chromosomes configuration (G), prophase II (I), the anaphase II (I), and anaphase II showed usual radial cylindrical configuration (J). The spermatogonial metaphase chromosome plate showed 28 chromosomes (K) and premature prophase could not ascertain the chromosome numbers (L). Bar represents 10 µm.

Discussion

According to the Platnick [4] there are 44, 032 species of spiders belonging to 3, 905 genera under 112 families. In India, 1685 species from 438 genera and 60 families [7] are reported so far. But according to World Spider Catalog [5] there are 4149 genera, 48304 species are available as valid species under 120 families with annual identification is more than 1, 000 in the year 2000. The family Lycosidae had 125 genera with 2438 species; Oxyopidae 9 genera, 453 species; Philodromidae with 31, 538 species and Salticidae 644 genera with 6162 species [5]. Despite the huge number of the spiders the least cytogenetic of 74 families, 320 genera and 867 species were cytologically analyzed [8]. So, the cytotaxonomy is lagging far behind as compare to the diversity of spiders. The present study is relevant to the scarcity of data on the cytological studies.

The family Salticidae, being the largest family in the present study, collected the genus *Plexippus paykulli* with 2n=28 from Imphal west and Ukhrul district, having karyological formula 26A+ X_1X_2O is in accordance with the available literatures [9, 10, 11, 12]. From the present data the amount of heterochromatin in the genome is less in all stages of meiotic cell division. Each gamete received 15 or 13 chromosomes and distribution was seem to be disturbed in latter stage of the Meiosis. The heteropycnotic X chromosomes that were always peripherally located, as reference point, the spatial positioning of the two sex chromosomes during the different stages of the interphase and Meiosis I were studied. During early stages of interphase, the two X chromosomes started as two dots and after S phase they became as larger irregular heteropycnotic body inside the nucleus. The dots were nearly jointed together and could be seen jointed till early pachytene probably at the centromeric regions. From the pachytene stage onwards close association could be seen till the metaphase I stage.

The species from Imphal west district are *Oxyopes pankaji* Gajbe and Gajbe 2000, *Oxyopes shweta* Tikader 1970, *Oxyopes javanus* Thorell, 1877 and *Oxyopes birmanicus* Thorell, 1887 but till date there is no report of the species from Ukhrul district. One small adult male was captured from the district but unable to get the meiotic division. Yet one thing is comnfirmed that the genera do occur in the district. The morphological study seems to be *Oxyopes javanus* Thorell, 1877.

The present cytological data reveal that species of the *Oxyopes* sp. from Imphal west with 2n=21: 20A+X chromosomes will be first report as it was identified as *Oxyopes sushilae* Tikader [13]. Other authors also reported 2n=21 [14]. The reports of 2n=22 are also coming up in viz., [15, 16] with 2 X chromosomes from India. From Japan [17] 2n=21 was described as *Oxyopes serratus* (L. Koch). *Oxyopes macilentus* with 2n=21 having 20A + X by Chen [17] has been the latest at the hand. All these available at hand are not up to date so intensive recent more data should be generated to fill the lacunae and update globally in future.

From Manipur three species had been identified so far are *Pardosa atropalpis* Gravely (Ukhrul and Imphal west), *Pardosa sumatrana* Thorell (Ukhrul) and *Pardosa bimanica* Simon (Imphal). The cytological data of *Pardosa atropalpis* is 2n=28: 26A+X_1X_2 A which are in accordance with Datta

and Chatterjee [19, 20], Parida and Sharma [21, 22], Sharma and Parida [23] from India in the name of *Pardosa birmanica*, Simon 1884 and *P. bifasciata* (C.L. Koch, 1834) by Kumbicak, *et al.* [24]. So, to ascertain the validity of the cytological with the morphological data we need to investigate more species and varied locations all over the India and if possible, globally covered.

Philodromus sp. of the family Philodromidae are collected from two study sites of Ukhrul district viz., Thoyee and Litan while from Imphal west district it was captured from Kwakeithel only. The male 2n=28: 26A+ X_1X_20 acrocetric chromosomes. The present data is in accordance with Datta and Chatterjee [25] from India. *Philodromus lividus* (2n = 28, X_1X_2 telocentric) revealed the same diploid number, chromosome morphology and sex chromosome system as in other members the family Philodromidae, which are characterized mostly by 2n = 28, monoarmed chromosomes and the X_1X_20 sex chromosome system, with the exceptions of *P. auricomus* L. Koch, 1878 (27, XO) [17] and *P. subaureolus* Bosenberg and Strand, 1906 (27, XO) [17]. As the data supports our observations but due to old cytological data it is hard to come to a concrete affirmation. Future works mainly the molecular will give much needed confirmation as the morphology as well as the cytological data could not arrive to a conclusive point.

The X_1X_20 reported for the first time by Revell [26], has been regarded as the ancestral mechanism of the sex determination mechanism in the spiders as this was found in the phylogenetically basal family Liphistiidae (Mesothelae) [27, 28] as reported in the present. White [29] suggested that duplication of the X chromosome from an X0 system gave rise to the multiple X chromosome systems. The present study with dissimilar X chromosomes of 7:5 in length could be explained as by Revell [26] suggested that these X chromosomes had undergone evolutionary differentiation after originating from an X0 system. Patau [30] suggested that the smaller X_1 and X_2 chromosomes had originated from the X0 system not only by simple centric fission, but through additional rearrangements such as 1) centric fragmentation and fission in the long arm terminal region followed by inversion of the long chromosome segment, resulting in a dicentric chromosome; 2) fission in the middle region of the dicentric chromosome, forming two acrocentric X_1 and X_2 chromosomes of similar size. Many explanations had been forwarded but need more studies on many families and genera to accept or discard the prepositions in future. The X chromosomes were seen to be connected at the centromeric region that could be the results of the homology and mechanism could be explained by nondisjunction of the X chromosomes during anaphase as Postiglioni and Brum-Zorrilla (1981) hypothesized that non-disjunction of one X chromosome of the X_1X_20 system, followed by loss of homology between the X chromosomes had occurred. The two X chromosomes were heteropycnotic throughout the Meiosis I and late replicating. They were fairly joint together at the centromeric region till the early Pachytene stage but seem to be separate out during the later stages. Future works on various families and genera will give insight into the mechanism of such fascinating phenomenon.

In future, this minimum karyological diversity in the families, their cytological studies are seeming to best resolved using molecular cytogenetic studies as well as molecular methods involving the DNA sequencing and species identification and delineation of the positive species identification and importance of the cytological data on speciation in the four families.

Conclusion

There was little variation in the Meiotic stages in four families of the spiders. The main differences were on the deposition of the heterochromatins. The heterochromatins were much in each chromosome starting from *Oxyopes*, *Pardosa and Philodromus* sp. than the *Plexippus*. The significance difference was most prominent in the zygotene stages, the chromomeres are darkly stains in *Oxyopes*, *Pardosa* and *Philodromus* while the *Plexipus* was less darkly stained. Again, the separation of X chromosomes was also significant observation, they are positively heteropycnotic and seem to be separated at zygotene only in *Philodromus* while remain associated in *Plexipus*, *Pardosa* and *Oxyopes*. On the basis of identical chromosome diploid count of 28 (except 21 in *Oxyopes* sp with single X): 26 acrocentric somatic chromosomes, X_1X_2 allosomes and absence of distinct chromosomal behavior during the meiotic cycle, only evolutionary significant point to focus here is the role of the heterochromatin. On the basis of the heterochromatin deposition Salticidae is the primitive family while Philodromus, Lycosidae are the advance families.

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