MATERIAL AND METHODS

The present investigations have been carried out in the Insect Biosystematic Laboratory, Network Project on Insect Biosystematics, Division of Entomology, Indian Agricultural Research Institute, New Delhi. The specimens were obtained from National Pusa Collection (NPC), Division of Entomology, Indian Agricultural Research Institute, New Delhi; Division of Entomology, Forest Research Institute (FRI), Dehradun, Uttarakhand and Entomology Section, Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh (DZAMU). In addition, specimens were collected from crops, vegetations and orchards from different agroecosystems under the auspices of the Network Project on Insect Biosystematics (NPIB). The bugs were collected from various states of India viz., Arunachal Pradesh, Delhi, Himachal Pradesh, Meghalaya, Uttarakhand, Uttar Pradesh and West Bengal and these deposited in the National Pusa Collection (NPC), Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi.

3.1 Collection, killing, drying, pinning and preservation

Collection of specimens from different agroecosystems was done by general sweeping with the help of insect net, hand picking method as well as light traps. Freshly collected insects were killed with the benzene or ethyl acetate and kept in butter paper envelopes, especially during the surveys. For pinning and stretching, these were relaxed in small air tight relaxing chambers with layers of wet cotton and covered with filter paper for 24 hrs. During stretching care was taken to expose the antennae and legs so as to reveal their characters of taxonomic importance. Adults were pinned on the right side of scutellum and kept in oven for 24 hrs at 60ºC. The specimens were labeled with data of locality, host, date and name of the collector. The juvenile stages of the bugs were preserved in small air tight vials, having 70% alcohol with few drops of glycerol. The procedure to study the genitalia, includes treatment of abdomen in 10% KOH for 30 min to soften it, thereafter it was washed and dissected in distilled water opening the same with fine needle on the lateral sides, extracting the genitalic structure, and boiling in 10% KOH for 5 min at 100ºC. The
genitalia were then transferred to glacial acetic acid for 15 min for dehydration, and studied by placing them on cavity slides. Mounting of genitalia on slides was avoided to prevent distortion of the structures. A cavity slide with a drop of glycerol was found most suitable medium for the study of genitalia from dorsal, ventral and lateral angles. These processed structures were studied and illustrated under Nikon MZ10 and Leica MZ16A stereozoom microscopes then stored in genitalia microvials containing few drops of glycerol, and pinned on to the respective specimens.

3.2 Taxonomic studies

The essential diagnostic characters of were examined under Nikon MZ10 fitted with a drawing tube and ocular micrometer and Leica MZ16A stereozoom microscopes; the former was used to make line diagrams of taxonomically important characters; and Leica’s EZ4 dissecting microscope was used for the dissection and study of genitalia. Photography was done under Leica MZ16A stereozoom microscope attached with Leica DFC425A, and Sony DSCH50 digital cameras.

3.3 Morphometric studies

Measurements of specimens as a whole and their body parts viz., head length and breadth (across eyes), pronotum length (medial) and breadth (between anterior and lateral pronotal angles), scutellum length (medially) and breadth (at base), abdominal length and breadth (at base) ventrally and their appendages like antennae, labium, legs were undertaken with the help of ocular and stage micrometers. Care was taken to ensure that the specimens were always in a level plane and set uniformly every time. For standardization, a trial run was made with five specimens for each species for every measurement chosen at a particular plane and angle to ensure uniformity and concordance of values. After standardizing the measurement procedure all selected characters were recorded. The scale shown in figures is 1 mm (Fig. 1b).

3.4 Scanning Electron Microscope studies

To study the structure of external thoracic scent efferent system of metathoracic scent glands, scanning electron microscope (SEM) studies were undertaken. Dried specimens were selected and washed with 0.1M phosphate buffer, repeated thrice, each for 15 min. After washing, dehydrated in series of 30%, 40%,
50%, 60%, 70%, 80%, 90%, 95% and 100% alcohol for 15 min. These were then ultrasonically cleaned and mounted on SEM stubs using double sided aluminium tapes. Palladium coatings were done of 15-18 nm of thickness in SC7620 Sputter coater at vacuum (1.19e-003 to 8.9e-004 Pa) and images taken with Zeiss EVO MA10 SEM under pressure of 10mbar/Pa and 15-20 kV.

3.5 Biology of Scutellera perplexa

To study the biology of *Scutellera perplexa*, adult males, females as well as nymphs were collected from jatropha plants grown in farm area of IARI, New Delhi. The collected individuals were kept in plastic jars (13.5× 10.5 cm), covered with muslin cloth for mass rearing, on tender shoots and unripe fruits. Freshly laid egg batches were shifted on to filter paper (5.0 cm dia) placed over wet cotton pads in petridishes (1× 5.5 cm) for recording different observations on eggs. Five neonates were shifted onto tender leaves placed in small jars (8.5× 8.5 cm) in ten replications and durations of instars were recorded. Newly emerged adults were separated in pairs (male and female) and placed in similar jars for recording the mating and ovipositional behaviour and durations. These studies were carried out at 24±2°C and 65±5% RH.

The following biological parameters were observed:

a) Fecundity, per-cent hatchability, incubation period and ovipositional pattern.

b) Nymphal instars and durations.

c) Adults, mating behavior, longevity and sex ratio.

Illustration of juvenile and adult stages was accomplished and preservation of juvenile stages was done in small vials in 70% alcohol.

Males and females were sorted out carefully based on the external male genitalia. The terminology used is after Pendergrast (1957), Scudder (1959) and McDonald (1966). Breadth of head calculated across the eyes and the breadth of scutellum taken at the base. All morphometrics are in mm, and the scales shown in illustrations equivalent to 1.0 mm.

3.6 List of abbreviations:

The abbreviations used in different plates/ illustrations for body parts, appendages and genitalia are as follows:
Abd: abdomen; Ans: antennal segment; Anv: anal vein; Apa: anterior pronotal angle; Bl: blade; Cja: conjunctival appendage; Cl: claw; Clf: claval fracture; Clv: clavus; Co: corium; Cu: cubitus; Df: distal flange; Dsd: distal spermathecal duct; Emb: embolium; Ev: evaporatorium; Ey: eye; Fm: femur; Frl: fore leg; Gnx: gonocoxa; Gp: gonopore; Hl: hind leg; Iv: Intervenal vein; Ju: Jugum vein; Jul: jugal lobe; Lbs: Labial segment; Lpa: lateral pronotal angle; M: median vein; Mem: membrane; Ml: middle leg; Mt: metasternum; Oc: ocellus; Ost: ostiole; P: pump; Pct: proctiger Pf: proximal flange; Pr: paramere; Prn: pronotum; Prt: peritreme; Ps: Peritremal surface; Psd: proximal spermathecal duct; Ptg: paratergite; R+M: radio-median vein; Sb: spermathecal bulb; Scu: scutellum; Sd: spermathecal dilation; Se: setae; Spr: spiracle; St: sternite; Str: strigil; Tb: tibia; Th: theca; Trb: trichobothria; Trc: trochanter; Trs: tarsomeres; Ty: tylus; Ve: vesica; Vn: veins; Vt: Vittae and Wg: wing.

The abbreviations for the depositories of specimens examined are as follows:
**DZAMU**: Department of Zoology Aligarh Muslim University, Aligarh; **FRI**: Forest Research Institute, Dehradun; **IARI**: Indian Agricultural Research Institute, New Delhi; **NPC**: National Pusa Collection, Division of Entomology, Indian Agricultural Research Institute, New Delhi and **NPIB**: Network Project on Insect Biosystematics, Division of Entomology, New Delhi.