Material & Methods
3. MATERIAL AND METHODS

Material

The present work is based on the study of a large number of specimens collected during 2007–2016 from several Indian States. Types and determined materials of some species housed in the ZDAMU were also studied.

Methodology

Techniques for collection and preservation summarized below are largely adapted from Noyes (1982).

1. Collection and preservation

The specimens were collected by a Sweep Net, Yellow Pan Traps and Malaise Traps which were then killed in ethyl acetate fumes. The collected specimens were transferred to 80% ethanol.

a) Card mounting

This procedure mainly consists of attaching the specimen with its meososma on rectangular card (14 × 5 mm) using water soluble glue. The cards were then arranged in a series with using micro-pins in rectangular cardboard boxes.

The card mounted specimens were used for the description of body colour and for measuring the total length of the body.

b) Preparation of slide mounts

The method of making permanent slides given by Noyes (1982) is followed as under:

i. Remove the wings from a card mounted specimen with the help of a fine needle and place these in a small drop of Canada balsam on the slide. Both the right wings were placed on top of the left hand side and vice versa, fore wings being placed on the top of the hind wing.
ii. Transfer the specimen to 10% KOH in a cavity block and leave it for several hours (24–48 hours). The highly sclerotized specimens were put for the maximum duration.

iii. Remove the KOH and add glacial acetic acid for 10 minutes.

iv. Remove the glacial acetic acid and add distilled water for 10 minutes.

v. Then dehydrate the specimen in ascending grades of ethanol. Starting with an equal mixture of distilled water and 80% ethanol and then to 80%, 90%, 96% and absolute. Each step required 10 minutes.

vi. Prior to removal of specimen from absolute alcohol, a few drops of clove oil were added in the cavity block for 10 minutes.

d) Slide mounting

Finally specimen was put on the slide with small drop of Canada balsam. And, dissected body parts were placed on slide as shown in the fig. I.

e) Drying and placing coverslips

The slides were put in incubator for about two weeks at 40°C ± 2°C and then coverslips were placed and the slides once again were put in the incubator for 6–8 weeks for further drying.

2. Measurements

The slide mounted specimens were used for the detailed study of setations, sculpture and internal structures like ovipositor. They were also used for measuring the dimensions of various sclerites.

Depending on the number of specimens available, one to several slides were prepared for each species. However, when only a single specimen of a species was available, this was partly dissected and mounted on a slide after the body colour and other details had been recorded.

Under the “Material examined” sections, the specimens, unless otherwise noted, are on cards.
3. Photography

For habitus view, insects as a whole were photographed under the stereo zoom binocular Nikon SMZ25. For slide mounted specimens the various parts were photographed under Leica DM2500 microscope attached with camera (Leica DFG295) scanning electron microscope. The photographs were taken either at 100× or 400× depending upon the size of body parts. These photographs were further retouched with Adobe Photoshop® CS3 and the photographic plates were made using Adobe Photoshop® 7.

4. SEM

For scanning electron microscopy the specimens were dehydrated in ascending grades of alcohol and then subjected to critical point drying. These were then glued on stubs with double sided sticky tape. Scanning electron micrographs were taken with JEOL-JSM-6510LV. These photographs taken were then retouched with Adobe Photoshop® CS3 and the photographic plates were made using Adobe Photoshop® 7.