



AN ILLUSTRATED DIAGNOSTICS OF LAC INSECT *KERRIA LACCA* (KERR) AS EXEMPLIFIED THROUGH ADVANCED STAINING AND MICROSCOPY TECHNIQUES

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ABSTRACT

A new and improved differential staining technique has been explored for the permanent mount preparation of the females of lac insect *Kerria lacca* (Kerr). The salient taxonomic characters had been illustrated using this technique, supplemented with the Scanning Electron Microscopy (SEM) studies. These have revealed a definite pattern of the dorsal duct cluster, stigmatic spinose setae on the brachial plate, pre-anal plate, sclerotized collar of the nuclear ducts of marginal duct cluster and six sectorial anal ring not recorded earlier. Post-oral lobes, rudimentary legs and traces of segmentation observed on the ventral aspect adds to the knowledge on its morphology. All these are likely to have implications on its classification in subgenera and species.

Key words: *Kerria lacca*, morphology, staining technique, SEM, taxonomic characters.

Lac insects are an isolated group of degenerated coccids, belonging to Tachardiidae of suborder Homoptera (Varshney, 1976), and these are known for their lac dye, resin and wax. Among the scale insects, the lac insects possess unusual and distinct morphological characters, and the family is easiest to be defined due to its unique anal tubercle, dorsal spine, brachium, brachial plate and post oral lobes. For deciphering these taxonomic characters, currently acid fuchsin is used as a stain and it requires a longer period of 12-72hr for optimum staining and visualization of characters. Also, after period of storage the stain fades resulting in difficulty in interpretation of the taxonomic characters. Hence, to resolve these problems and for improved understanding of the morphology, a new polychromatic stain with differential staining was explored for deriving definite advantage over the commonly used acid fuchsin and eosin. This new stain with phosphomolybedic acid, orange G, aniline blue and acid fuchsin renders a bluish colour to the membrane aspects and an orange tinge to the internal structures. Thus a differential staining is achieved which results in improved visualization of the cuticular features. In addition, the morphological characters of the female lac *Kerria lacca* (Kerr) explored with the polychromatic stain and thereby with improved slide mounts are enabled. Better interpretations of taxonomic characters used at all taxonomic levels achieved are

explained, and supplemented with Scanning Electron Microscope (SEM) studies.

MATERIALS AND METHODS

The ethyl alcohol preserved specimens were treated in 10% KOH, rinsed in 5-8 changes of distilled water, and internal contents cleaned through a small lateral incision in the integument in 1% glacial acetic acid (Varshney, 1976; with slight modifications). Cleared specimens were stained in polychromatic stain prepared by dissolving phosphomolybedic acid, orange G, aniline blue (WS) and acid fuchsin in distilled water in the sequence as mentioned, for a few minutes, then passed through grades of ethyl alcohol gradually dehydrating them, and then through grades of xylene, before mounting in DPX and finally dried on hot plate at 45 - 60°C. Illustrations were made after observing several specimens with a Canon Power Shot S50 attached to Leica DM 1000 phase contrast microscope and Leica EZ4 stereozoom microscope.

The specimens processed as before until staining were passed through xylene, and glued to the metal stub with the double sided silver tape for SEM study. An 18 nm gold palladium coating under vacuum pressure of 10 mbar/Pa was done before viewing under Zeiss EVO MA10 SEM at 15.00 kV and magnifications ranging from 70 x-5.97 k x.

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RESULTS AND DISCUSSION

The result on the morphology of the lac insect *Kerria lacca* (Kerr) obtained now are interpreted with earlier information and presented below:

Body

Both globose and pyriform body forms with a definite dorsal and ventral hemisphere, and ventral aspect with traces of segmentations near the posterior spiracles observed now adds to the morphology of the species. (Fig. 1, 2 & 19)

Dorsal aspect

Brachia elevated and highly sclerotized (Fig. 3 & 20), bearing the brachial plate brachial plates flat, subcircular with a shallow depression called the 'crater' which in turn bears smaller depressions called dimples having a central nuclear duct (Fig. 21). The stain enables the visualization of the numerous quinquelocular pores perforating the crater besides the dimples (Fig. 4). SEM study shows the presence of remnants of stigmatic spinose setae on the brachial plate (Fig. 22).

Dorsal spine is highly sclerotized and differentiate into pedicle or base and spine. The pedicle is long slender and highly sclerotized and with the spine on the apex. The spine is pointed with bifurcated tip. The base of the spine bears some spinosities (Fig. 5 & 23). The spine ducts are dendritic, with small nodes at the tips of the branches. These ducts can easily be visualized by the polychromatic stain (Fig. 6). SEM studies show the nodular tips of these ducts (Fig. 24).

Anal tubercle is the posterior prolongation of the body with a membranous pre-anal plate and there exist a highly sclerotized supra-anal plate, anal fringes and the anal ring with setae and pores. The pre-anal plate was observed in *Kerria lacca* (Kerr) as smooth and weakly sclerotized tube anterior to supra-anal plate. The latter smooth and highly sclerotized bearing setae with fringes at the apex getting clearly visualized by the polychromatic stain (Fig. 7 & 25). Anal fringes are variously digitate or fimbriate extensions of major length; and fringes of short type longer than the fringes (Fig. 8). Anal ring is projected bearing 10 long anal setae and dorsal, with two setae each or each bear single setae, and spiracles in each sector of the

Ventral

Mouth parts consists of two segmented labium, labial setae and two pairs of membranous, slightly sclerotized lobes (Fig. 8 & 28). The lobes anterior to the mouth pre-oral lobes and the pair posterior post-oral lobes, post-oral lobes membranous, oblong-ovate lobes, gets easily visualized using the polychromatic stain, and SEM study reveals the presence of fine scale-like sculpturing on the surface (Fig. 29). A pair of vestigial antennae lies anterior to the mouth, these very small with inconspicuous segmentation, and easily deciphered by the polychromatic stain, and number of segments varies from 3-4. Each antenna with 2 fleshy and 2 hairy setae at the apex (Fig. 9 & 30). Two pairs of thoracic spiracles present and the anterior one always larger than posterior, anterior pair always associated with brachia (Fig.10 & 31), posterior smaller, borne on a sclerotized ovate plate and bears spiracular pore on one side (Fig.11 & 32).

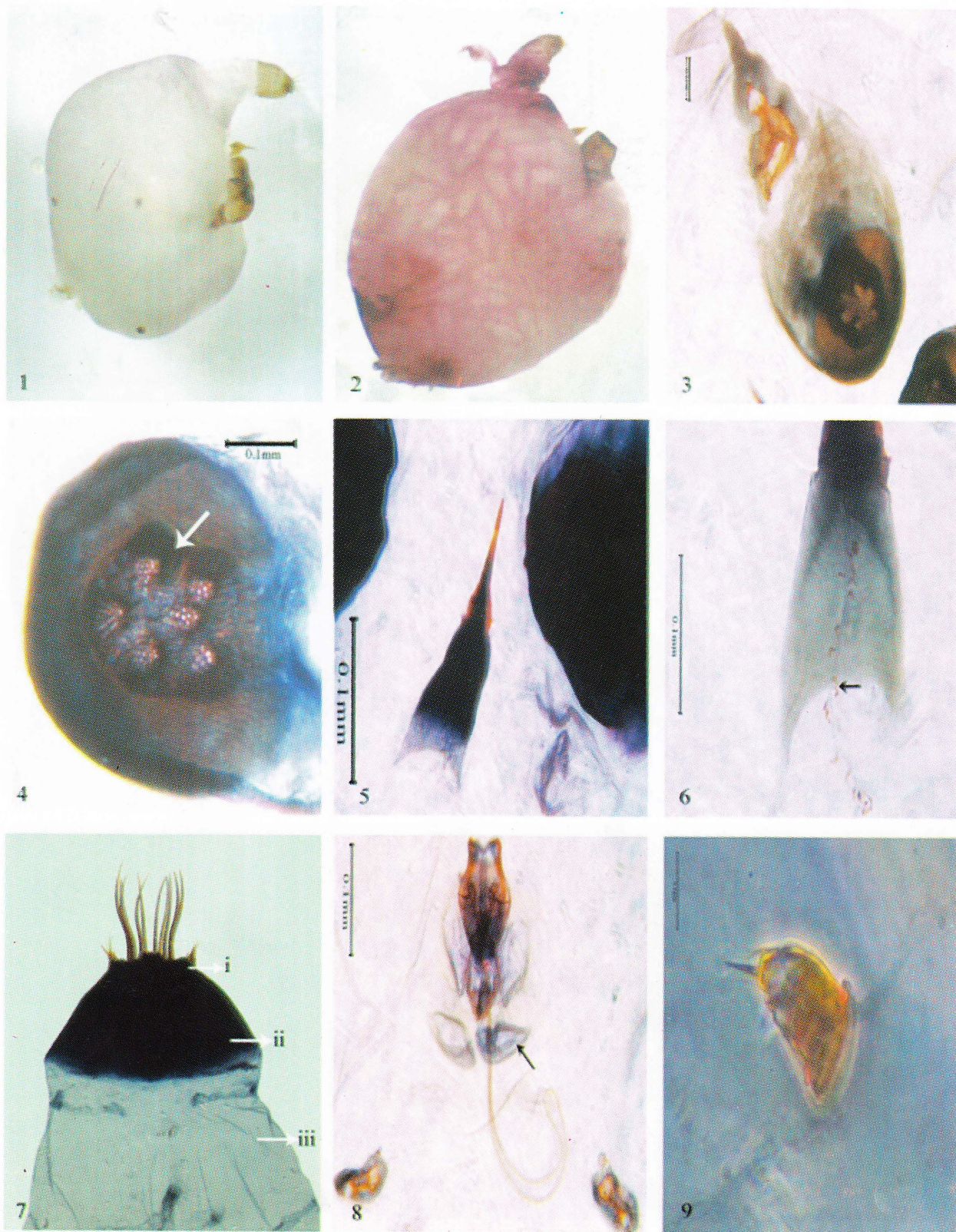
Pore and pore clusters

Perivulvar pore clusters are observed surrounding the vulva, their number more than 9 (Fig. 12 & 33), cluster comprises of many circular pores, each circular pore multilocular with 5-10 loculi (Fig.13 & 34). The multilocular nature gets easily deciphered only with polychromatic stain.

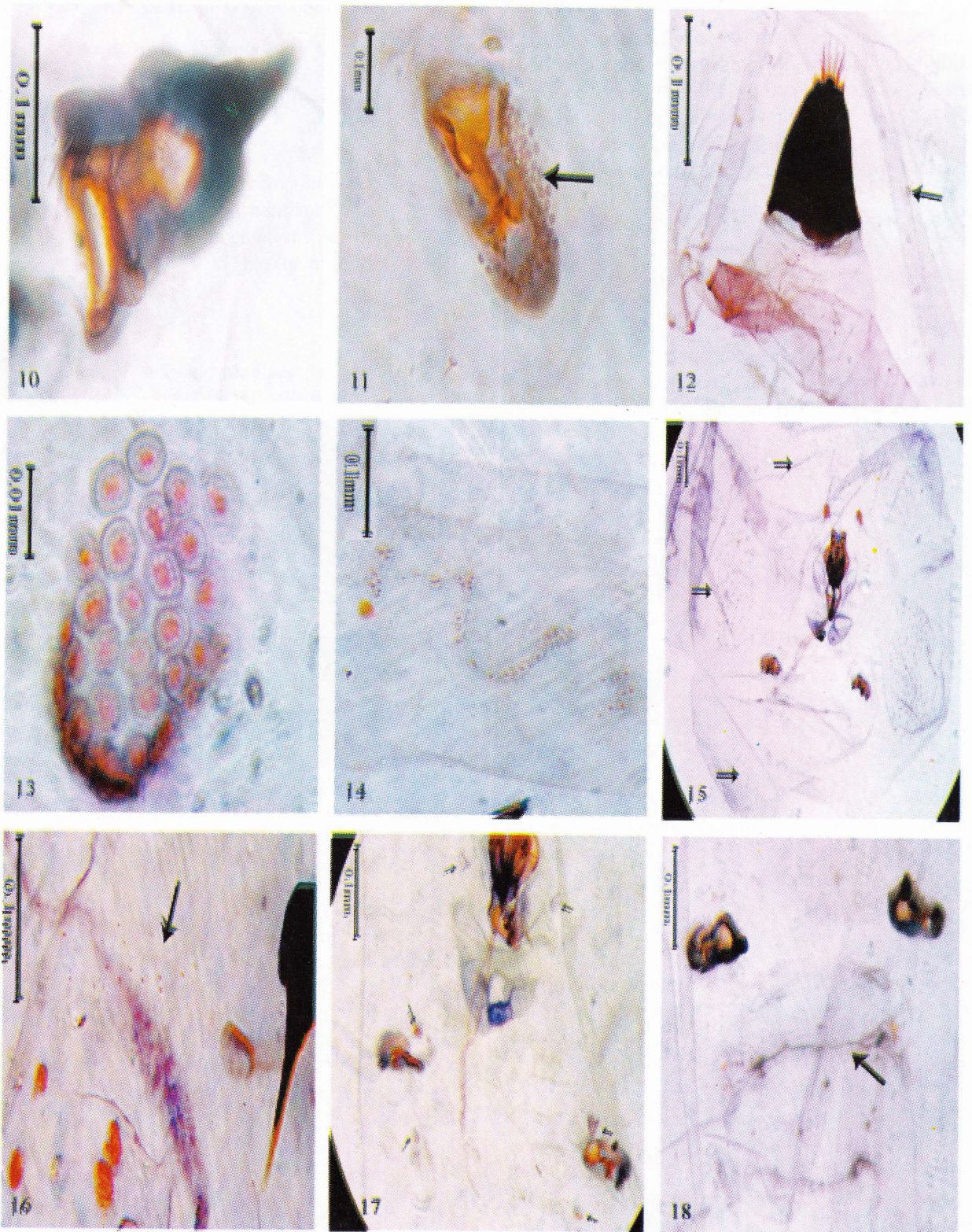
Spiracular pores observed distinct in a line associated with anterior and the posterior spiracles, small ceriferous pores observed by the side of the anterior spiracles on its ventral aspect and on one side of the posterior spiracles, and these usually quinquelocular but SEM study reveals 7 and 8-loculed pores too (Fig. 35). Brachial pores are the quinquelocular pores observed on the crater besides the dimple and visualized with polychromatic stain, SEM study reveals that majority of these are 5-loculed but 4- and 7-loculed pores too observed within the same (Fig. 36).

Duct and duct clusters

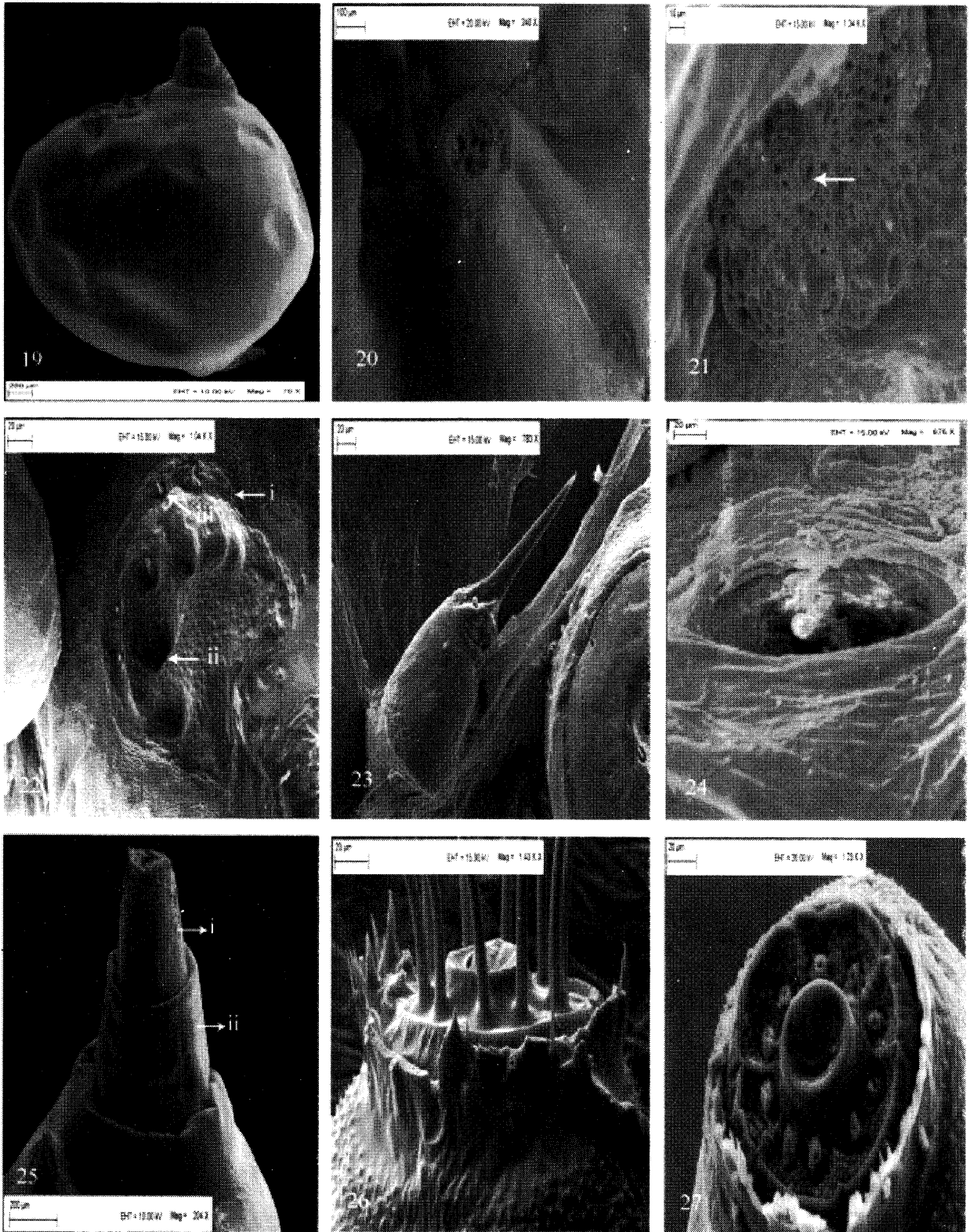
Marginal duct cluster occurs on the margins arranged in a wavy line (Fig. 14 & 37), and these are simplex type with elongated tubular ducts, with a median subcircular area surrounded by a narrow rim or collar with a small loculus. From the loculus arises a filamentous prolongation of the duct, this prolongation has a globular enlargement just after leaving the distal portion of the duct (Fig. 38). SEM studies reveal that the collar is raised in the ducts of marginal cluster. On



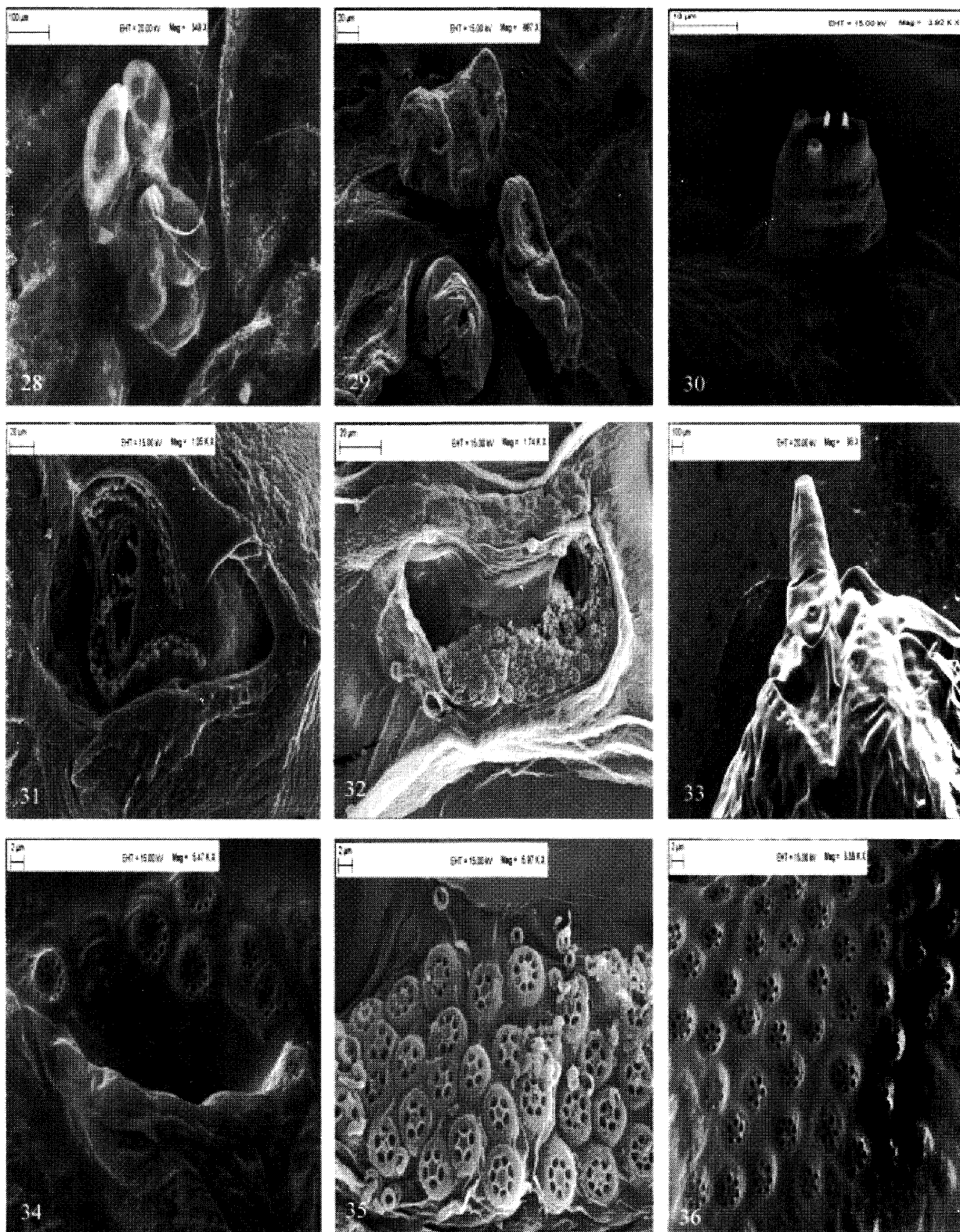
Figs 1-9. *Kerria lacca* (Kerr). (1) Globose body. (2) Pyriform body. (3) Brachia. (4) Brachial Plate (arrow showing dimple). (5) Dorsal spine. (6) Dendritic duct of spine. (7) Anal tubercle i- anal fringes, ii- supra-anal plate, iii- pre-anal plate. (8) Mouth parts (arrow indicating post-oral lobes). (9) Antennae.



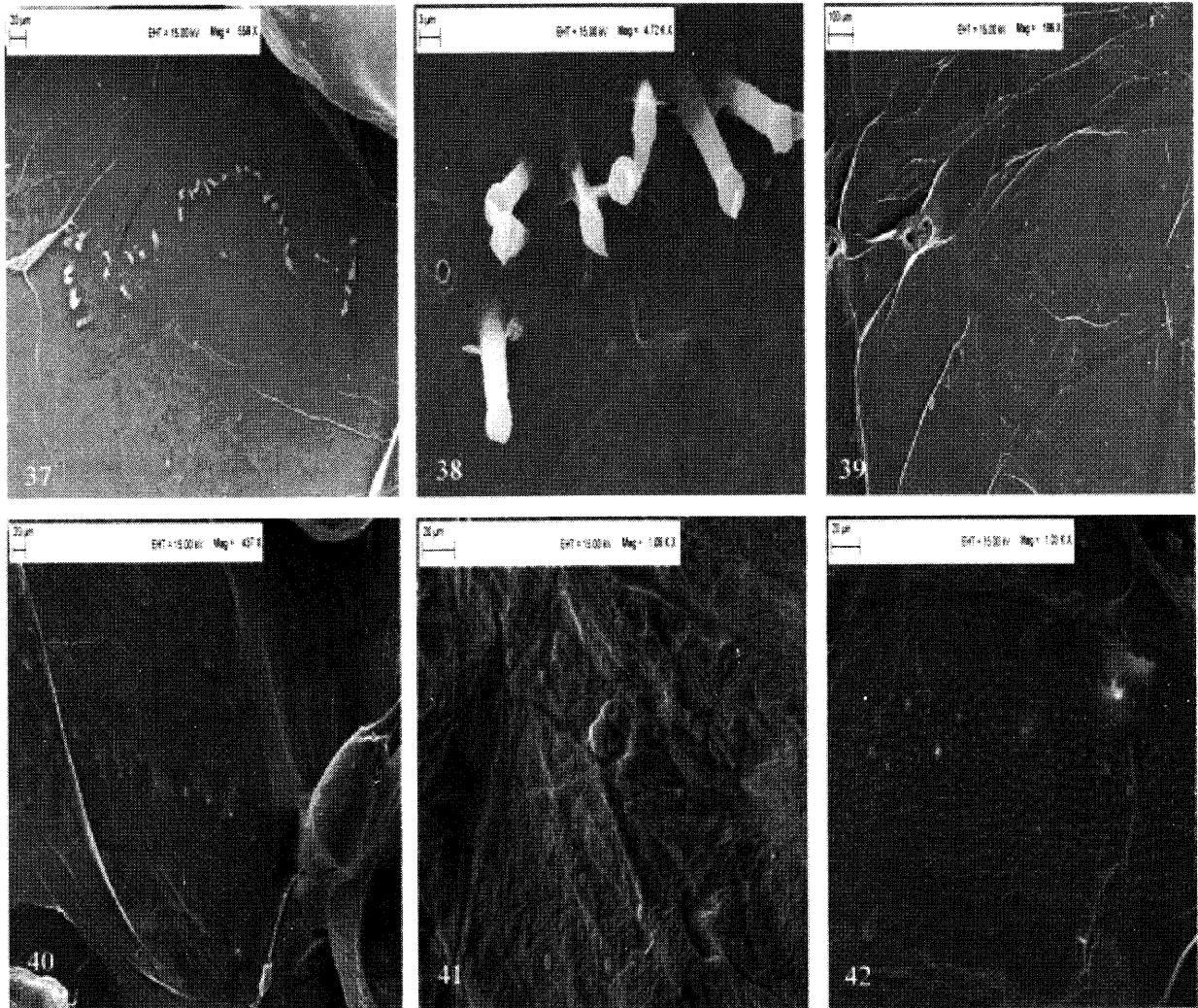
Figs 10-18. *Kerria lacca* (Kerr). (10) Anterior spiracle. (11) Posterior spiracle (arrow indicating spiracular pores). (12) Perivulvar pore cluster (indicated by arrow). (13) Single pore of perivulvar pore cluster. (14) Marginal duct cluster. (15) Ventral duct cluster (arrows indicating 3 pairs of duct cluster). (16) Dorsal duct cluster (arrow showing the ducts). (17) Rudimentary legs (arrows indicating 3 pairs of legs). (18) Segmentation on ventral side (indicated by arrow).



Figs 19-27. *Kerria lacca* (Kerr). (19) Body. (20) Brachia. (21) Dimple nuclear ducts (indicated by arrow). (22) Brachial plate-i-crater, ii-dimples, iii-stigmatic spinose setae. (23) Dorsal spine. (24) Dendritic duct. (25) Anal tubercle- i-supra-anal plate, ii-pre-anal plate. (26) Anal fringes (27) 6 sectored anal ring.



Figs 28-36. *Kerria lacca* (Kerr). (28) Mouth parts. (29) Post-oral lobes. (30) Antennae. (31) Anterior spiracle. (32) Posterior spiracle. (33) Perivulvar pore cluster. (34) Single opening of perivulvar pore cluster with multilocular pores (35) Spiracular pores. (36) Brachial pores.



Figs 37-42. *Kerria lacca* (Kerr). (37) Marginal duct cluster. (38) Magnified ducts of marginal cluster. (39) Ventral duct cluster. (40) Dorsal duct cluster. (41) Rudimentary leg. (42) Traces of segmentation on ventral side

the ventral surface there are three pairs of duct cluster, one anterior to the mouth parts, second being antero-lateral to it and the third posterior to the mouth just above the posterior spiracle and called the ventral duct cluster (Fig. 15 & 39). These consists of tubular ducts with a filamentous prolongation. Besides the marginal and the ventral duct cluster there is a row of tubular ducts between the anal tubercle and the dorsal spine region on the dorsal surface. (Fig. 16 & 40), and these had not been reported earlier in *Kerria*. Three pairs of rudimentary legs one adjacent to the mouth parts, second near the posterior spiracle and the third above the posterior spiracle are observed and these associated with some multilocular pores (Fig. 17 & 41). Traces of segmentation on the ventral surface above the posterior spiracle could easily be made out with

polychromatic stain and SEM (Fig. 18 & 42).

Further, SEM studies reveal the presence of stigmatic spinose setae on the brachial plate of the *Kerria* which corresponds to the brachia with the stigmatic plates or grooves of the Coccidae (Lit, 2002a). Presence of the membranous pre-anal plate revealed with polychromatic stain as well as the SEM studies are new observations not reported by Lit and Gullan (2001). The anal ring was so far thought to be 4-sectored (Lit and Gullan, 2001), whereas the present SEM study revealed a 6-sectored anal ring. The dendritic ducts of the dorsal spine were shown to possess nodular ends with the polychromatic stain and SEM which differs from the ducts of *Austrotachardia* sp. ex *Cassinia* (Lit, 2002a). Postoral lobes can easily

be visualized as oblong lobes by polychromatic stain and the SEM study indicates the absence of circular depression on the apex, which is contrary to the earlier observations (Lit, 2002a). The nuclear ducts in the marginal duct cluster were stated to have no raised collar in *Kerria* (Lit, 2002b), but the present study with polychromatic stain reveals a sclerotized collar and SEM study confirms these. According to Chamberlin (1923), the dorsal duct clusters are more or less distinct band of ducts which forms a large circle surrounding the area immediately dorsal to the mouth parts and present in *Tacharidella cornuta* (Cockerell), *Austrotachardiella gemmifera* (Cockerell) and *Austrotachardia angulata* (Froggatt); a distinct pattern of duct observed now on the dorsal surface between the anal tubercle and the dorsal spine region in *Kerria* is an additional observation. The rudimentary legs were reported earlier in most *Tachardiella* and one species each of *Tachardia* and *Austrotachardia* (Chamberlin, 1923); present study establishes the presence of these in the *Kerria* too. Traces of segmentation on the ventral side revealed now had not been mentioned earlier.

The present study concludes that the polychromatic stain used now for the light microscopy differentially stains the insect enabling better interpretation of taxonomically important characters. The SEM study and the results explained herein supplement these light microscopic observations enabling better understanding of the morphological characters. Present study is unique as it used both the whole and dissected permanent mounts of *Kerria lacca* for exploring the morphological characters and with improved staining

methodology. The concept that the characters like post-oral lobes, and number of ducts in ventral and dorsal duct cluster will be taxonomically important in differentiating the subgenera and species has been brought to the fore through the present study. However, there is need to study the variations in these taxonomic characters due to the season and geographical locations and to statistically validate them, for an improved understanding of the lac insect and its populations from India.

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