Distinction of Indian Commercial Lac Insect Lines of *Kerria* spp. (Homoptera: Coccoidea) Based on Their Morphometrics

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ABSTRACT. The lac insects belong to the genus *Kerria* (Hemiptera: Coccoidea: Kerriidae) and are commercially exploited worldwide for the production of lac, which comes from their waxy test and has diverse industrial applications. The insects are maintained by the Indian Institute of Natural Resins and Gums as distinctive lines that are cultivated and commercialized in the lac producing areas of India. The lines are all considered to belong to the genus *Kerria* but without validation of their taxonomic characters, and their identity to species has not been ascertained. This study used single-factor analysis of variance and several multivariate analyses, such as principal component analysis, discriminant function analysis, and canonical discriminant analysis to explore the morphometrics of some of the adult female lac insect lines. The results have enabled the identification of some taxonomically significant characters in adult females, which has grouped the 32 lac insect lines studied into 15 species along with validation of the most significant characters. Distinctive grouping patterns for the species of *Kerria* have been brought out using morphometrics.

Key Words: canonical discriminant analysis, discriminant function analysis, lac insect, new species, principal component analysis

Scale insects (Stenorrhyncha: Coccoidea) are phytophagous insects found in all terrestrial zoogeographical regions except Antarctica, with ~7,500 species in ~30 families (Ben-Dov et al. 2014). These are generally divided into two informal groups, the archaeococcoids and the neococcoids, based on the presence or absence of abdominal spiracles in the adult female. The neococcoids form a monophyletic group with 17 families and the Coccidae and the Tachardiidae form sister groups (Cook et al. 2002). The family Tachardiidae (=Kerriidae), which includes lac insects, consists of nine genera and 100 species (Ben-Dov et al. 2014). Lac insects (Hemiptera: Coccoidea: Tachardiidae) are morphologically distinctive scale insects that produce a gum-like or resinous secretion that forms a hard cover over the body (Chamberlin 1923, Varshney 1976). The word “lac” is derived from a Sanskrit word which mean “hundred thousand,” indicating the gregarious habit of this insect (Krishnaswami 1962). These insects belong to the genus *Kerria* and the most commonly cultivated species is *Kerria lacca* (Kerr). The species of *Kerria* are distributed throughout India but occur as isolated patches in a variety of habitats (Varshney 1976, Ramani et al. 2007).

Lac insects yield three commercially important products: resin, dye, and wax, which have major applications in a wide range of industries (Varshney 1976, Ramani et al. 2007). These lac products are preferred over other products due to their unique properties along with their environmental safety (Saha et al. 2011).

The commercially exploited species of lac insect belong to many distinctive genetic lines and these are maintained and cultivated by the Indian Institute of Natural Resins and Gums (INRG). These genetic lines are commercially exploited for lac production in different parts of India. Taxonomy of the lac insect is based on the monograph and its supplement by Chamberlin (1923, 1925) as well as subsequent works by Kapur (1958), Varshney (1976), and Kondo and Gullan (2007). All these commercially cultivated lines have been placed in the genus *Kerria* but without validation of their taxonomic characters and thus each line is commercially cultivated without a proper identification. The diversity and cultivation complexities of these lines require a critical analysis through a study of their morphology and morphometrics.

As there is much variability in their morphology, with significant overlapping of characters, these need to be analyzed and the most important characters clarified. Females are highly degenerative and undergo considerable changes in size and shape during sexual maturition, posing a challenge in their identification and so this intraspecific variation needs to be critically analyzed.

Hence, this study used single-factor analysis of variance (ANOVA) and multivariate analyses such as principal component analysis (PCA), discriminant function analysis (DFA), and canonical discriminant analysis (CDA) to explore the morphometrics of the commercial lac insect lines in India.

Materials and Methods

Collection and Preparation of Specimens. Thirty-two female lac insect lines were studied (Table 1). These included species of *Kerria* from the principal lac growing states, geographical races, some inbred lines, and the infra-subspecific forms kusumi and rangeeni. Kusumi and rangeeni are two distinct forms of lac insects, the latter thriving on *Butea monosperma* (Fabaceae) but not on *Schleichera oleosa* (Sapindaceae), which is a preferred host of kusumi. The samples were obtained from the cultures maintained on potted *Flemingia macrophylla* (Fabaceae), a lac host plant kept under culture conditions at the Lac Insect Field Gene Bank of National Lac Insect Germplasm Center, INRG campus, Ranchi (23° 19’51” N and 85° 22’18” E; elevation of 2,080 ft). These cultures are enclosed in synthetic mesh sleeves to exclude parasitoids and predators and are regularly sprayed with fungicide carbendazim (0.01%). In order to prepare the specimens for morphological studies, mature females were scraped from the twigs and placed in 100% ethyl alcohol for 48 hr to dissolve their resinous covering. The specimens were then cleaned carefully under a stereozoom microscope with a brush to remove any excess wax. These cleaned insects were preserved in 90% ethyl alcohol in a 1.5 ml epipendorf tube for further studies.

These alcohol-preserved specimens were slide mounted following the technique of Jena et al. (2011). Briefly, the specimens were placed in
Table 1. Lines of *Kerria* spp. studied and their species groups with locality and host

| Sl. no. | Species groups | Line | Locality of collection | Host                |
|--------|----------------|------|------------------------|---------------------|
| 1      | *Kerria brancheata* (Varshney) group | LIK0014 | Jammu, Jammu & Kashmir | *Ziziphus mauritiana* |
|        |                | LIK0027 | Silli, Jharkhand        | *Schleichera oleosa* |
|        |                | LIK0045 | Experimental           | *Flemingia macrophylla* |
|        |                | LIK0064 | Varanasi, Uttar Pradesh | *Ficus religiosa*    |
| 2      | *Kerria chamberlini* (Varshney) group | LIK0015 | Ambaji, Banaskantha, Gujarat | *F. religiosa*         |
|        |                | LIK0000 | Purulia, West Bengal    | *Butea monosperma*   |
| 3      | *Kerria chinensis* (Mahdihassan) group | LIK0023 | Thailand               | *Albizia saman*       |
| 4      | *Kerria dubeyi* (Ahmad & Ramamurthy) | LIK0008 | Bangalore, Karnataka   | *Ficus bengalensis*   |
| 5      | *Kerria ebrachiata* (Chamberlin) group | LIK0004 | Palamau, Jharkhand     | *B. monosperma*       |
|        |                | LIK0005 | Bokaro, Jharkhand      | *B. monosperma*       |
| 6      | *Kerria fici* (Green) group | LIK0013 | Ludhiana, Punjab       | *Z. mauritiana*       |
| 7      | *Kerria indicola* (Kapur) group | LIK0020 | Echoda, Andhra Pradesh | *Peltophorum ferrugineum* |
|        |                | LIK0029 | Korba, Chhattisgarh    | *B. monosperma*       |
|        |                | LIK0048 | Experimental           | *F. religiosa*        |
| 8      | *Kerria lacca* (Kerr) group | LIK0011 | Udaipur, Rajasthan     | *F. religiosa*        |
|        |                | LIK0012 | Jhalod, Rajasthan      | *F. religiosa*        |
|        |                | LIK0017 | Ahmednagar, Maharashtra| *Z. mauritana*        |
| 9      | *Kerria maduraiensis* (Ahmad & Ramamurthy) | LIK0003 | Sundergarh, Orissa    | *Malvaviscus penduliflorus* |
|        |                | LIK0001 | Korba, Chhattisgarh    | *S. oleosa*           |
| 10     | *Kerria manipurensis* (Ahmad & Ramamurthy) | LIK0039 | Selection               | *S. oleosa*           |
|        |                | LIK0040 | Selection               | *S. oleosa*           |
| 11     | *Kerria penneae* (Ahmad & Ramamurthy) | LIK0007 | Bankhedi, Madhya Pradesh | Original host not known |
| 12     | *Kerria pusana* (Misra) group | LIK0010 | Thrissur, Kerala       | *Amherita nobilis*    |
| 13     | *Kerria sharda* (Mishra & Sushil) group | LIK0007 | Sarat, Mayurbajaj, Orissa | *S. oleosa*           |
| 14     | *Kerria thrissurensis* (Ahmad & Ramamurthy) | LIK0063 | Patiala, Punjab        | *Z. mauritana*        |
| 15     | *Kerria varshneyi* (Ahmad & Ramamurthy) | LIK0001 | Korba, Chhattisgarh    | *Z. mauritana*        |

![Fig. 1. Hierarchical flow diagram for the classification of 32 lines studied based on the characters of anal tubercle and brachia with distinct character for each species.](image-url)
Table 2. Statistically significant characters ($P \leq 0.01$) for the morphometrics of 30 lines of species of *Kerria*

| Sl. no. | Characters | Acronym |
|---------|------------|---------|
| 1       | Length of canellar band | CL |
| 2       | Distance of anterior spiracle from crater rim | DCR |
| 3       | Length of brachia | BrL |
| 4       | Number of ducts in MDC III | MDCIII |
| 5       | Number of ducts in MDC II | MDCII |
| 6       | Number of dilome on brachia II | DII |
| 7       | Number of dilome on brachia I | DI |
| 8       | Number of ducts in MDC V | MDCV |
| 9       | Number of ducts in MDC I | MDCI |
| 10      | Length of pedicle | PL |
| 11      | Total length of dorsal spine | TDSL |
| 12      | Number of ducts in MDC IV | MDCIV |
| 13      | Number of ducts in MDC VI | MDCVI |
| 14      | Diameter of brachial plate | BPD |
| 15      | Width of crater | CW |
| 16      | Width of anterior spiral | ASW |
| 17      | Width of supra-anal plate | SPW |
| 18      | Length of antennae | AL |
| 19      | Length of anal tubercle | ATL |
| 20      | Length of supra-anal plate | SPL |
| 21      | Number of spiral pores | NSP |
| 22      | Perivulvar pore cluster II | PVCII |
| 23      | Width of body at middle | BWM |
| 24      | Number of antennal segments | NAS |

(Continued)

Table 2. (continued)

| Sl. no. | Characters | Acronym |
|---------|------------|---------|
| 25      | Width of pre-anal plate | PAW |
| 26      | Pedicle width at apex | PeWA |
| 27      | Number of antennal setae | NASe |
| 28      | Length of pre-anal plate | PAL |
| 29      | Perivulvar pore cluster I | PVCI |
| 30      | Length of antennal segment III | ALIII |
| 31      | Body length | BL |
| 32      | Length of spine | SL |
| 33      | Pedicle width at base | PeWB |
| 34      | Length of posterior spiracle | PSL |
| 35      | Length of anterior spiracle | ASL |
| 36      | Number of star pores | NSPo |
| 37      | Width of body at base | BWB |
| 38      | Width of body at apex | BWA |
| 39      | Length of oral lobe | OLL |
| 40      | Length of antennal segment IV | ALIV |
| 41      | Length of anal fringe | FL |
| 42      | Length of antennal segment II | ALII |
| 43      | Width of clypeolabral shield | WCS |
| 44      | Length of clypeolabral shield | LCS |
| 45      | Perivulvar pore cluster opening V | PoCV |
| 46      | Perivulvar pore cluster opening III | PoCIII |
| 47      | Perivulvar pore cluster opening II | PoCII |
| 48      | Perivulvar pore cluster opening I | PoCI |
| 49      | Length of antennal segment I | ALI |
| 50      | Perivulvar pore cluster opening IV | PoCIV |

(Continued)

Table 3. Proportion of variation and variable coefficients of the first five PCs for PCA and total sample standardized canonical coefficients of CDA for the 30 lines of *Kerria* spp.

| Sl. no. | Characters | Acronym |
|---------|------------|---------|
| 1 | Length of canellar band | CL |
| 2 | Distance of anterior spiracle from crater rim | DCR |
| 3 | Length of brachia | BrL |
| 4 | Number of ducts in MDC III | MDCIII |
| 5 | Number of ducts in MDC II | MDCII |
| 6 | Number of dilome on brachia II | DII |
| 7 | Number of dilome on brachia I | DI |
| 8 | Number of ducts in MDC V | MDCV |
| 9 | Number of ducts in MDC I | MDCI |
| 10 | Length of pedicle | PL |
| 11 | Total length of dorsal spine | TDSL |
| 12 | Number of ducts in MDC IV | MDCIV |
| 13 | Number of ducts in MDC VI | MDCVI |
| 14 | Diameter of brachial plate | BPD |
| 15 | Width of crater | CW |
| 16 | Width of anterior spiral | ASW |
| 17 | Width of supra-anal plate | SPW |
| 18 | Length of antennae | AL |
| 19 | Length of anal tubercle | ATL |
| 20 | Length of supra-anal plate | SPL |
| 21 | Number of spiral pores | NSP |
| 22 | Perivulvar pore cluster II | PVCII |
| 23 | Width of body at middle | BWM |
| 24 | Number of antennal segments | NAS |

(Continued)
10% potassium hydroxide overnight to soften the internal tissue. They were then washed thoroughly in distilled water with 8–10 changes and then placed in 1% glacial acetic acid where a small incision was made on the lateral aspect of the body using a scalpel in order to remove the internal contents. The specimens were then cleaned thoroughly with fine needles and a brush and placed in polychromatic stain for about 20 min. They were then dehydrated through grades of ethyl alcohol of 70%, 90%, and 100% followed by clearing in 30%, 50%, and 80% xylene before preparing a permanent mount in Distrene, Plasticiser, Xylene (DPX). Finally, the slide mounts were dried on a hot plate at 45–60°C. These permanent microslides were made using Leica EZ4 stereozoom microscope.

Table 3. (continued)

| ALIV  | 0.025 | −0.026 | 0.100 | 0.036 | 0.057 | 0.009 | 0.168 |
| FL    | −0.032 | 0.057 | −0.035 | −0.085 | −0.020 | 0.136 | 0.048 |
| ALII  | 0.052 | −0.033 | −0.039 | 0.113 | −0.094 | 0.181 | 0.031 |
| WCS   | 0.006 | −0.003 | 0.032 | −0.025 | −0.022 | 0.027 | −0.034 |
| LCS   | −0.011 | 0.023 | 0.187 | 0.000 | −0.324 | 0.002 | −0.091 |
| PoCV  | 0.006 | 0.003 | −0.124 | 0.028 | 0.466 | −0.024 | −0.190 |
| PoCII | 0.022 | 0.056 | −0.062 | −0.004 | −0.049 | 0.107 | −0.021 |
| PoCl  | 0.043 | 0.064 | −0.066 | 0.066 | −0.037 | 0.003 | 0.001 |
| AL  | −0.011 | −0.014 | 0.063 | 0.050 | −0.562 | 0.018 | −0.156 |
| PoCIV | 0.050 | 0.030 | −0.073 | −0.012 | 0.519 | 0.077 | −0.005 |

| Proportion of variation | 15.3% | 10.3% | 8.5% | 6.7% | 4.8% |

Fig. 2. Scatter plot of PCs 1 and 2 showing the grouping of 30 lines of Kerria spp. Encircled regions showing the compact clustering for Kerria chinensis (LIK0023), Kerria pusana group (LIK0001, LIK0039, LIK0040, and LIK0065), Kerria pennyae (LIK0003), and Kerria dubeyi (LIK0008) in the first, second, and third quadrants, respectively.

Table 4. Multivariate statistics and $F$ approximations for the 30 lines of Kerria spp.

| Statistic                          | Value       | $F$ value | Num DF | Den DF | Pr > $F$ |
|------------------------------------|-------------|-----------|--------|--------|----------|
| Wilks’ $\lambda$                   | 2.80 × 10$^{-7}$ | 11.83 | 1,450 | 20,841 | <0.0001  |
| Pillai’s trace                     | 9.2648      | 7.97 | 1,450 | 24,621 | <0.0001  |
| Hotelling–Lawley trace             | 33.7649     | 19.07 | 1,450 | 14,529 | <0.0001  |
| Roy’s greatest root                | 8.8518      | 150.3 | 50 | 849 | <0.0001  |
Selection and Measurement of Characters. Using the morphological characters of adult female *K. lacca* taken from Chamberlin (1923, 1925), Kapur (1958, 1962), Varshney (1976, 1985), Zhang (1993), Mishra and Sushil (2000), Lit and Gullan (2001), Lit (2002a,b), Kondo and Gullan (2007), a total of 65 characters were identified for morphometric analyses. A standardization experiment using 30 specimens of each line, in all at a time just before these reach maturity, was undertaken to identify the characters which were most stable and consistent. These resulted in the selection of 50 characters, which had been supported by the single-factor ANOVA. These selected characters were measured and their morphology observed at magnifications between $100 \times$ and $1,000 \times$ using a Leica DM1000 phase contrast microscope with a micrometer eyepiece. The measurements are as in the slide-mounted specimens. The measurements of width used in the study are as follows: 1) width at apex—width taken at clypeolabral shield position, i.e., middle of tentorium; 2) width at middle—width taken where it is maximum, generally taken at the middle of the body; and 3) width at base—width taken at the position of base of anal tubercle.

Statistical Analysis. Univariate one-way single-factor ANOVA was performed individually for all the characters to select those that were significant as a prelude to identifying the potential characters (Kalaisekar et al. 2012). These morphometrics were then analyzed using multivariate statistical approaches (Tabachnick and Fidell 2006) as follows: PCA (SAS procedure, PRINCOMP, SAS version 9.1.3, SAS Institute Inc., Cary, NC), without any prior assumption of groupings, assesses the components for total variation among the specimens by calculating linear combinations of variables that explain the maximum of total variation. PCA was also used as a dimension-reducing technique. CDA (SAS procedure, CANDISC) calculates linear combinations of variables that maximize the separation of means of previously defined classes. Contribution of the variables best summarizing

### Table 5. Canonical correlation analysis for the 30 lines of *Kerria* spp.

|        | Canonical correlation | Adjusted canonical correlation | Approximate standard error | Squared canonical correlation |
|--------|-----------------------|-------------------------------|-----------------------------|------------------------------|
| 1      | 0.948                 | 0.003                         | 0.898                       |
| 2      | 0.943                 | 0.004                         | 0.889                       |
| 3      | 0.881                 | 0.007                         | 0.777                       |
| 4      | 0.849                 | 0.009                         | 0.721                       |
| 5      | 0.793                 | 0.012                         | 0.629                       |
| 6      | 0.777                 | 0.013                         | 0.603                       |
| 7      | 0.749                 | 0.015                         | 0.561                       |
| 8      | 0.711                 | 0.017                         | 0.505                       |
| 9      | 0.669                 | 0.018                         | 0.447                       |
| 10     | 0.646                 | 0.019                         | 0.417                       |
| 11     | 0.615                 | 0.021                         | 0.379                       |
| 12     | 0.585                 | 0.022                         | 0.342                       |
| 13     | 0.565                 | 0.023                         | 0.319                       |
| 14     | 0.537                 | 0.024                         | 0.288                       |
| 15     | 0.473                 | 0.026                         | 0.224                       |
| 16     | 0.465                 | 0.026                         | 0.216                       |
| 17     | 0.416                 | 0.028                         | 0.173                       |
| 18     | 0.404                 | 0.028                         | 0.163                       |
| 19     | 0.355                 | 0.029                         | 0.126                       |
| 20     | 0.334                 | 0.030                         | 0.112                       |
| 21     | 0.313                 | 0.030                         | 0.098                       |
| 22     | 0.303                 | 0.030                         | 0.092                       |
| 23     | 0.281                 | 0.031                         | 0.079                       |
| 24     | 0.242                 | 0.031                         | 0.059                       |
| 25     | 0.206                 | 0.032                         | 0.042                       |
| 26     | 0.184                 | 0.032                         | 0.034                       |
| 27     | 0.183                 | 0.032                         | 0.033                       |
| 28     | 0.141                 | 0.033                         | 0.020                       |
| 29     | 0.128                 | 0.033                         | 0.016                       |

![Fig. 3. Scatter plot of the results of CDA for the 30 lines of *Kerria* spp. showing a similar clustering for lines as in PCA.](image-url)
the differences between classes is revealed by this technique. Since DFA (SAS procedure, DISCRIM) maximizes the variation among groups, it was used to separate groups. DFA also determines the potential misclassification of specimens and assesses the utility of characters used.

These analyses were carried out in two batches: one with each of the 30 lines and in the other with seven species of Kerria, namely Kerria chinensis, Kerria manipurensis, Kerria maduraeensis, Kerria thirssuresensis, Kerria pennuyae, Kerria dubeyi, and Kerria varshneyi (Ahmad et al. 2013a,b), to validate the species described. The sample size for each of the 30 lines was 30 and that for each of the seven species was 10.

### Results and Discussion

**Morphometrics and Species Distinctions.** The 32 lac insect lines fell into two broad categories based on the structure of the anal tubercle, i.e., whether the tubercle is elongated or abbreviated. Both groups were then subdivided based on the shape and status of brachia into five groups: those with an elongated tubercle into three subgroups: 1) brachia elevated and cylindrical, 2) brachia elevated and club shaped, and 3) brachia sessile and club shaped; and those with an abbreviated tubercle into two subgroups: 1) brachia elevated and club shaped and 2) brachia sessile and club shaped, as shown in Fig. 1. Based on the key to the adult females of Kerria, the species groups were differentiated.

**Morphometrics and Taxonomic Characters.** The evaluation of some taxonomic characters using one-way ANOVA revealed that 50 were statistically significant (P ≤ 0.01) (Table 2). These characters were subjected to PCA analyses. The first five principal components (PCs) with an eigenvalue more than 1.0 accounted for 45.5% of the total variation (Table 3). The first two PCs, i.e., PC1 and PC2, together explained about 25.6% of the total variation, with PC1 explaining 15.3% and PC2 explaining about 10.3%, respectively. These had positive loading for seven original variables, including the number of ducts in each marginal duct cluster (MDC), length of anal tubercle, length of pre-anal plate, distance of anterior spiracle from crater rim, length of brachia, length of pedicel, and total length of dorsal spine. The other PCs, i.e., PC3, PC4, and PC5, explained 8.5%, 6.7%, and 4.8% of the total variation, respectively. As the first two PCs accounted for 25.6% of the variability, those characters with maximum loadings were considered to be the major sources of variation. The plot for the first two PCs, i.e., PC1 and PC2, are shown in Fig. 2, and the clusters emphasize the grouping of the lac insect lines. A compact clustering was observed for the lines LIK0023 (K. chinensis), LIK0010 (K. thirssuresensis), LIK0001, LIK0039, LIK0040, and LIK0065 (K. pusana group), LIK0008 (K. dubeyi), and LIK0003 (K. pennuyae) in the first, second, and third quadrants, respectively, with the rest of the lines mostly overlapping.

CDA was carried out with priori grouping and using the lines as classification variables. The statistics used to test differences between the lines, namely Wilks’ λ, Pillai’s trace, Hotelling-Lawley Trace, and Roy’s greatest root, were found to be significant at P < 0.0001. These statistics clearly show the significant contribution toward the model, with a lower Wilks’ λ (2.8 × 10⁻⁸), holding true for all other statistics (Table 4). The first two canonical correlations (89.8% and 88.9%) were very high, signifying their importance (Table 5). The projection of the lines onto the first two canonical discriminant axes is shown in Fig. 3. The analysis was able to extract differences between the lines LIK0001, LIK0039, LIK0040, and LIK0065 (K. pusana group), LIK0008 (K. dubeyi), and LIK0003 (K. pennuyae) from the rest, with the main contribution being from canellar band length, while the second canonical root was not particularly helpful in discriminating between any lines (Table 3). This clustering obtained from CDA confirmed the grouping brought out by PCA. A cross-validation of group membership was performed identifying the misclassification of specimens and assessing the utility of the selected measurements/observations used. Overall, 78% of the classifications were correctly attributed to species, with relatively few (22%)
misclassifications (Table 6). The results of cross-validation accurately identified 100% of specimens to the lines LIK0003 (K. pennye), LIK0008 (K. dubeyi), LIK0017 (K. lacca group), and LIK0023 (K. chinensis); >90% to the lines LIK0005 (Kerria ebrachiata group), LIK0007 (Kerria sharda), LIK0040 (K. pusana group), LIK0045 (Kerria brancheata group), LIK0047 (K. lacca group), and LIK0065 (K. pusana group); and >80% to the lines LIK0001 (K. pusana group), LIK0012 (K. lacca group), and LIK0063 (K. varshneyi), respectively. Thus, the DFA results helped to identify these 13 species groups based on the merit of each of the morphological characters used in the analyses.

Establishment of Species Classification of New Species Described.

The status of recently described six new species—K. manipurensis (Ahmad & Ramamurthy), K. thriissuresis (Ahmad & Ramamurthy), K. maduraiensis (Ahmad & Ramamurthy), K. pennye (Ahmad & Ramamurthy), K. dubeyi (Ahmad & Ramamurthy), and K. varshneyi (Ahmad & Ramamurthy) (Ahmad et al. 2013a,b)—and K. chinensis (Mahdihassan) were supported by the multivariate analyses (PCA, K. Ramamurthy), maduraiensis (Ahmad & Ramamurthy), K. varshneyi, K. lasiusa, and little overlaps between these species with compact clustering for all except K. chinensis. Fig. 4. Scatter plot of PCs 1 and 2 along the two axes for seven Kerria species with compact clustering for all except Kerria maduraiensis and little overlaps between K. dubeyi and K. pennye and Kerria varshneyi with those of K. maduraiensis and Kerria thriissuresis. Symbols indicate species.
The PCA indicated that the first 10 PCs with eigenvalues more than 1 accounted for 79.9% of the total variation. Contribution of variables to the first three PCs accounted for 51.3% of the total variation (Table 7). PC1 reflected a generalized increase in the values of five characters: distance of anterior spiracle from crater rim, number of dents in each marginal duct cluster, width of anterior spiracle, length of anal tube and pre-anal plate length with a decrease in only one character, and number of dents on brachial plate. The main contributions to PC2 were from five characters: brachial plate diameter, crater width, body width, width of anterior spiracle and width of supa-anal plate and to PC3 were from three characters: total length of dorsal spine, length of spine, and length of anal fringe. The other PCs, namely PC4–PC10, explained 6.7%, 5.6%, 4.6%, 3.8%, 3.1%, 2.6%, and 2.2% of the total variation.

The differences in distribution across the common component were from three characters: total length of dorsal spine, length of spine, and number of dimples on brachial plate. The other PCs, namely PC4–PC10, explained 6.7%, 5.6%, 4.6%, 3.8%, 3.1%, 2.6%, and 2.2% of the total variation respectively, and therefore made little contribution toward explaining the variation. The differences in distribution across the common component of variation for the seven species of Kerria are evident in Fig. 4. The results of PCA show the distinctiveness of the species studied except for a small overlapping between K. dubeyi and K. pennsae and for K. varshneyi and K. maduraiensis. A dispersed clustering was observed for both K. maduraiensis and K. varshneyi.

The CDA showed a highly significant Wilks’ λ value (1.0 × 10⁻⁸), Pillai’s trace, Hotelling–Lawley Trace, and Roy’s greatest root (P < 0.0001) (Table 8). The first two canonical correlations with squared canonical values 99.4% and 98.3% in canonical correlation analysis (Table 9) were high, indicating their importance. Table 10 shows that the mean canonical variables with canonical roots having higher values for their respective variables (species) in canonical root 1 was able to separate the seven species studied, whereas canonical root 2 particularly separated K. chinensis and K. thrissurensis. The character brachial plate diameter contributed maximum (−10.413) to canonical root 1, toward the separation of species (Table 7). The projection of species onto the first two canonical axes is shown in Fig. 5.

A validation analysis through DFA of group participation/composition was performed for the seven species under study and it was observed that 87% of the classification was correctly attributed (Table 11). Also, the result of the validation analysis (DFA) correctly identified 100% of specimens to K. pennsae; 90% to K. dubeyi, K. manipuresnis, K. thrissurensis, and K. chinensis; and 70–80% to K. varshneyi and K. maduraiensis, respectively.

### Taxonomic Characters and Their Validation

The results of these analyses revealed that there are 14 characters which are consistent, without significant intraspecific variations, and which helped to separate the lac insect lines into species and groups. Most of these characters were in agreement with the 11 major characters noted by earlier taxonomists (Table 12). In this study, many characters have been added such as body widths (apex, middle, and base), number of star pores near the mouthparts, width of anterior spiracle, length of pre-anal plate (membranous extension below the supra-anal plate), length and width of supra-anal plate, pedicel length, spine length, width of pedicel at base, pedicel width at apex, total length of dorsal spine, perivulvar pore cluster openings, and length of antennal segments. These additional characters were not used by earlier taxonomists but were found to be significant in species delineation in our studies, while other characters

#### Table 8. Multivariate statistics and F approximations for seven Kerria species

| Statistic              | Value     | F value | Num DF | Den DF | Pr > F  |
|------------------------|-----------|---------|--------|--------|---------|
| Wilks’ λ               | 1.0 × 10⁻⁸| 9.17    | 282    | 110.24 | <0.0001 |
| Pillai’s trace         | 5.6189    | 6.9     | 282    | 132    | <0.0001 |
| Hotelling–Lawley trace| 263.8778  | 14.54   | 282    | 57.996 | <0.0001 |
| Roy’s greatest root    | 158.1423  | 74.02   | 47     | 22     | <0.0001 |

#### Table 9. Canonical correlation analysis for seven Kerria species

| Canonical correlation | Adjusted canonical correlation | Approximate standard error | Squared canonical correlation |
|-----------------------|--------------------------------|-----------------------------|------------------------------|
| 1                     | 0.997                          | 0.001                       | 0.994                        |
| 2                     | 0.992                          | 0.002                       | 0.983                        |
| 3                     | 0.974                          | 0.955                       | 0.949                        |
| 4                     | 0.962                          | 0.936                       | 0.925                        |
| 5                     | 0.942                          | 0.014                       | 0.887                        |
| 6                     | 0.938                          | 0.014                       | 0.880                        |

#### Table 10. Mean canonical variables based on discriminant functions of the morphological characters for seven Kerria species

| Species    | Canonical root 1 | Canonical root 2 | Canonical root 3 | Canonical root 4 | Canonical root 5 | Canonical root 6 |
|------------|------------------|------------------|------------------|------------------|------------------|------------------|
| K. varshneyi| −12.482          | −2.908           | −4.425           | 5.109            | 1.998            | 2.117            |
| K. dubeyi  | −13.940          | 0.915            | 5.408            | 1.991            | −1.639           | −3.775           |
| K. pennsae | −9.432           | −2.876           | 2.539            | −5.887           | 3.062            | 1.616            |
| K. chinensis| 18.140           | −12.964          | 2.420            | 1.296            | −0.526           | 2.227            |
| K. manipuresnis| −2.519          | 2.262            | −2.574           | −2.138           | −5.451           | 2.362            |
| K. thrissurensis| 7.186           | 2.206            | −6.424           | −2.033           | 1.122            | −4.113           |

Fig. 5. Scatter plot for the results of CDA for seven Kerria species showing compact clustering of the species studied as in the PCA, with slightest of overlap between K. varshneyi and K. pennsae.
Table 11. Classification matrix of the DFA for seven Kerria species, where rows = observed classification and columns = predicted classification

| Percentage | K. manipurensis | K. maduraiensis | K. thrisssuresis | K. pennae | K. dubeyi | K. chinensis | K. varshneyi |
|------------|-----------------|----------------|-----------------|-----------|-----------|-------------|-------------|
| K. manipurensis | 90.0 | 9.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| K. maduraiensis | 80.0 | 0.0 | 8.0 | 0.0 | 2.0 | 0.0 | 0.0 |
| K. thrisssuresis | 90.0 | 0.0 | 1.0 | 9.0 | 0.0 | 0.0 | 0.0 |
| K. pennae | 100.0 | 0.0 | 0.0 | 0.0 | 10.0 | 0.0 | 0.0 |
| K. dubeyi | 90.0 | 0.0 | 1.0 | 0.0 | 0.0 | 9.0 | 0.0 |
| K. chinensis | 90.0 | 1.0 | 0.0 | 0.0 | 0.0 | 9.0 | 0.0 |
| K. varshneyi | 70.0 | 0.0 | 1.0 | 0.0 | 1.0 | 0.0 | 7.0 |
| Total | 87.1 | 10.0 | 12.0 | 9.0 | 13.0 | 10.0 | 9.0 |

Table 12. Taxonomic characters versus lac insect species delineations, new characters (*) with statistical significance

| Taxonomic characters hitherto used | Taxonomic characters used now | Additional characters evaluated |
|-----------------------------------|-----------------------------|---------------------------------|
| Body width | Body width at middle | Body width at middle* |
| Antennae | Length of antennal segments | Length of pre-anal plate* |
| Number of ducts in marginal duct cluster | Number of ducts in each marginal duct cluster | Width of supra-anal plate* |
| Length of anal tubercle | Length of anal tubercle | Width of anterior spiracle* |
| Length of brachia | Length of brachia | Length of antennal segments |
| Brachial plate width | Brachial plate diameter | Spine length |
| Crater width | Crater width | Number of star pores near mouthparts |
| Number of dimples on brachial plate | Number of dimples on brachial plate | Number of openings in perivulver pore clusters |
| Distance of anterior spiracle from crater rim | Distance of anterior spiracle from crater rim | |
| Pedicel length | Pedicel length | |
| | Spine length | |
| | Width of pedicel at base | |
| | Pedicel width at apex | |
| Total length of dorsal spine | Total length of dorsal spine | |
| Length of anterior spiracle | Width of anterior spiracle | |

This study also provides an insight into the validity of the taxonomic characters deployed in the genus Kerria for the species delineation.

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