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Morphometric analysis of whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) puparia from different agroclimatic zones of Karnataka, India

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Abstract
Being an polyphagous pest of many agricultural and horticultural crops, whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) place an important role as a vector for transmission of more than 111 plant viruses which affects the crop development. *B. tabaci* is having 36 morphologically indistinguishable genetic groups. In the present study detailed survey was undertaken to collect *B. tabaci* puparia samples from eleven districts of Karnataka which were distributed in seven agro climatic zones during the year 2016-17. The morphometrics of *B. tabaci* puparia was recorded for 12 different characters and has recorded significantly higher values for 9 out of 12 characters in Dharwad population. The combined cluster analysis of *B. tabaci* puparia revealed the existence of two major clusters-Cluster 1 and Cluster 2 on which populations from different locations were distributed. The principal component analysis (PCA) based on morphometrics of puparia disseminate that, the first three principal components (PCs) account for 98.417 per cent of total variation. The morphometrics reveal that there are distinct variations which could be explored for morphological delineation of genetic groups/ putative species of *B. tabaci.*

Keywords: *Bemisia tabaci*, genetic groups, agro-ecological zones, morphometry, principal component analysis

1. Introduction
Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a haplodiploid, inconspicuous insect, well known for causing damage to plants either by direct or indirect way. Directly by feeding on plant sap and indirectly by transmitting viral diseases. In addition, they excrete honey dew which deposits on the surface of plant leads to the formation of sooty mould (e.g., *Capnodium* spp.) on the top of the leaves which adversely affects the photosynthesis and respiration in the plant. It is a polyphagous pest, reported on more than 900 host's plants and reported to transfer more than 111 plant viruses as a vector [7]. It has become a serious pest in numerous agricultural, horticultural and other commodity crops [11, 23].
Being a cryptic species complex, *Bemisia tabaci* consisting of 24 morphologically indistinguishable species and which are placed under 11 well-defined high-level groups [4]. *B. tabaci* as a complex species and variation among the population exists in different tomato growing areas because of its polyphagous nature.
With reference to systematics or taxonomy, morphology is considered as the top-tier basis for species separation [6, 30]. The taxonomic identification of *B. tabaci* is based on the morphology of the fourth larval instar, 'puparia' [15]. But some studies had shown that, these might vary within the species [6, 17, 25]. These phenotypic variations are largely determined by the host leaf morphology [20] and other factors. Such phenotypic plasticity has led to confusion in recognition of its populations/genetic groups/putative species established through other studies. This has also led to redescriptions and synonymization [4, 19]. Thus the morphologically indistinguishable or indistinct populations had been designated as biotypes/genetic groups/putative species. Many studies which propose new genetic groups/putative species and distinction of these in the *B. tabaci* species complex had been largely through non morphological definitions. Thus there is an impending need to analyze their morphological variations and document them, so as to enable differentiating them and if possible provide a

taxonomic methodology based on morphometrics. Hence, this study focusing on the morphometrics of puparia of the *B. tabaci* species complex from different agro-ecological zones of Karnataka.

2. Materials and methods

2.1 Sample collection

Survey was conducted in different geographical areas covering 11 districts distributed in seven agroclimatic zones of Karnataka where tomato is majorly grown (Table 1) and collected puparia of whitefly from tomato plants during February, 2016 to April, 2016. Tomato leaves containing sufficient number of *B. tabaci* puparia were collected in polythene bags of 30 × 40 cm size with proper labels. The samples were handled carefully and brought to the laboratory. The puparia was separated from the host plant with the help of camel hair brush and transferred to the different glass vials containing 70 per cent alcohol as preservative. The samples were drawn at randome from each population and used for morphological studies according to the methods of earlier studies [24, 26].

Puparia were processed for mounting as recommended by earlier Hodges an Evans method [10] with fewer modifications. The mounted specimens were observed under trinocular stereo zoom HD microscope make MEIJI EMZ 13 TR with 6MP HDMI camera attachment for studying measurement of essential characters and the photography was made under trinocular stereo zoom microscope Olympus SZX7 with cam (5 MP) attachment and Q-Imaging software for studying essential characters (n=5).

Table 1: Details of locations selected for collection of *Bemisia tabaci* samples across agroclimatic zones of Karnataka and the abbreviations used.

Sl. No.	Agro climatic zones	District	Abbrevations
1	Central dry zone Southern transition zone	Davanagere	DVN
2	Northern transition zone	Haveri	HVR
3	Northern transition zone	Dharwad	DWD
4	Northern dry zone	Belagavi	BLV
5	Eastern dry zone	Chikkaballapura	CKBR
6	Eastern dry zone	Kolar	KLR
7	Eastern dry zone	Bengaluru rural	BR
8	Northern dry zone	Bagalkot	BGK
9	Southern dry zone	Chamarajanagar	CHA
10	Hilly zone	Chikkamagaluru	CKM
11	Southern transition zone	Shivamogga	SHI

Puparia characters studied include Puparia length (PL), Puparia breadth (PB), Distance of Vasiform orifice from anterior end (DVOAE), Distance of transverse moulting suture from anterior end (DTMSAE), Length of Vasiform orifice (LVO), Breadth of Vasiform orifice (BVO), Length of lingula (LL), Length of operculum (LO), Breadth of operculum (BO), Length of caudal furrow (LCF), Length of caudal setae (LCS), Distance between caudal setae (DCS).

2.2 Statistical analysis

Descriptive statistical parameters such as mean, range (minimum and maximum), standard error and sample variance for individual characters were calculated by using SPSS 16.0 statistical package (SPSS, 2004). Cluster analysis and principal compound analysis was performed using software NTSYS-PC, Version 2.11w and Past 3.x software package for all quantitative features [8]. The aim of the cluster analysis was to have some preliminary segregation of specimens and to generate hypothesis suggesting the existence of grouping of populations, using taxonomic units.

3. Results

For the study, randomly selected 140 puparia samples of whitefly were selected belonging to 11 districts of Karnataka. Among the 12 morphological characters analyzed, six characters were statistically non-significant across populations. Remaining six characters were highly significant (p<0.001) among different locations.

Length of the puparia was significantly highest in Dharwad population (5.43 mm) followed by Bengaluru rural (5.31 mm), Shivamogga (5.25 mm) population and significantly lowest length was recorded from Bagalkot population (4.58 mm) which was statistically on par with Chamarajanagara population (4.65 mm) followed by Chikkamagaluru population (4.77 mm). Puparia breadth was significantly highest in Dharwad population (4.07 mm) which was statistically on par with the populations of Bengaluru rural (3.99 mm), Shivamogga (3.94 mm) and Chikkaballapura (3.89 mm). Significantly lowest breadth was recorded from Bagalkot population (3.42 mm) which was statistically on par with Chikkamagaluru population (3.46 mm) followed by Kolar (3.63 mm) populations.

Distance of vasiform orifice from anterior end (DVOAE) was recorded highest in Dharwad population (4.46 mm) which was statistically on par with Bengaluru rural (4.37 mm) followed by Shivamogga population (4.35 mm) and significantly lowest DVOAE was recorded from Chikkamagaluru population (3.68 mm) which was statistically on par with Bagalkot (3.72 mm) and Chamarajanagara population (3.76 mm).

Table 2: Morphometrics of *Bemisia tabaci* puparia on tomato from different agro climatic zones of Karnataka

Puparia characters	Measurements from different districts (mm)											Mean± SE	C.D (0.05)	C.V (%)	Statistical significance
	DVN	HVR	DWD	BLV	CKBR	KLR	BR	BGK	CHA	CKM	SHI				
PL	4.90 (3.37-5.85)	4.96 (4.09-5.83)	5.43 (5.19-5.70)	5.08 (4.28-5.69)	5.18 (4.45-5.68)	4.92 (4.11-5.71)	5.31 (4.28-5.83)	4.58 (4.05-5.29)	4.65 (4.28-5.30)	4.77 (4.32-5.25)	5.25 (4.84-5.75)	5.00±0.04	0.11	1.78	< 0.001
PB	3.77 (3.12-4.53)	3.79 (2.79-4.65)	4.07 (3.72-4.49)	3.76 (2.64-4.25)	3.89 (2.98-4.40)	3.63 (3.00-4.35)	3.99 (2.88-4.35)	3.42 (2.90-4.15)	3.63 (2.87-5.46)	3.46 (2.56-4.13)	3.94 (3.31-4.49)	3.76±0.08	0.22	4.53	< 0.001
DVOAE	4.21 (3.66-4.87)	4.14 (3.50-4.89)	4.46 (4.28-4.69)	4.21 (3.51-4.79)	4.29 (3.66-4.68)	4.05 (3.44-4.66)	4.37 (3.55-4.87)	3.72 (3.01-4.38)	3.76 (3.16-4.79)	3.68 (2.21-4.87)	4.35 (3.98-4.73)	4.11±0.03	0.10	1.88	< 0.001
DTMSAE	2.12 (1.74-2.52)	2.14 (1.51-2.53)	2.43 (1.95-3.22)	2.19 (1.86-2.45)	2.24 (1.80-3.10)	2.07 (1.03-2.48)	2.21 (1.79-2.60)	1.87 (1.01-2.30)	1.95 (1.69-2.38)	2.29 (1.74-4.35)	2.22 (1.98-2.45)	2.16±0.03	0.08	2.96	< 0.001
LVO	0.51 (0.42-0.60)	0.50 (0.41-0.56)	0.56 (0.50-0.60)	0.52 (0.40-0.58)	0.52 (0.44-0.59)	0.47 (0.38-0.57)	0.52 (0.41-0.57)	0.48 (0.39-0.57)	0.46 (0.37-0.51)	0.50 (0.43-0.55)	0.53 (0.46-0.63)	0.51±0.03		13.64	NS
BVO	0.39 (0.34-0.42)	0.37 (0.32-0.43)	0.41 (0.38-0.43)	0.38 (0.32-0.42)	0.39 (0.33-0.43)	0.37 (0.31-0.42)	0.39 (0.32-0.43)	0.35 (0.32-0.39)	0.35 (0.31-0.40)	0.38 (0.31-0.42)	0.37 (0.31-0.40)	0.38±0.02		9.02	NS
LL	0.18 (0.13-0.23)	0.18 (0.14-0.22)	0.19 (0.16-0.23)	0.18 (0.13-0.27)	0.18 (0.11-0.22)	0.17 (0.14-0.23)	0.17 (0.11-0.22)	0.16 (0.11-0.20)	0.16 (0.11-0.21)	0.17 (0.11-0.21)	0.20 (0.16-0.24)	0.18±0.00	0.01	6.13	< 0.001
LO	0.23 (0.18-0.27)	0.23 (0.18-0.27)	0.24 (0.21-0.26)	0.23 (0.20-0.28)	0.22 (0.16-0.26)	0.22 (0.18-0.25)	0.24 (0.17-0.27)	0.21 (0.11-0.26)	0.21 (0.15-0.25)	0.22 (0.17-0.28)	0.23 (0.17-0.30)	0.23±0.01		10.40	NS
BO	0.31 (0.25-0.34)	0.31 (0.26-0.34)	0.32 (0.31-0.34)	0.30 (0.23-0.33)	0.31 (0.24-0.38)	0.28 (0.19-0.34)	0.31 (0.25-0.35)	0.28 (0.24-0.32)	0.28 (0.23-0.36)	0.29 (0.25-0.33)	0.31 (0.26-0.43)	0.30±0.02		12.82	NS
LCF	0.36 (0.25-0.46)	0.34 (0.21-0.42)	0.41 (0.38-0.45)	0.37 (0.30-0.45)	0.37 (0.32-0.43)	0.35 (0.26-0.46)	0.40 (0.32-0.46)	0.33 (0.24-0.42)	0.39 (0.30-0.46)	0.35 (0.27-0.41)	0.37 (0.28-0.44)	0.37±0.02	0.04	9.54	< 0.001
LCS	0.57 (0.40-0.74)	0.61 (0.47-0.69)	0.56 (0.45-0.72)	0.54 (0.42-0.63)	0.50 (0.43-0.63)	0.59 (0.48-0.79)	0.54 (0.42-0.70)	0.49 (0.36-0.68)	0.53 (0.44-0.66)	0.54 (0.43-0.67)	0.53 (0.42-0.67)	0.55±0.03		14.35	NS
DCS	0.28 (0.23-0.41)	0.26 (0.20-0.36)	0.26 (0.20-0.30)	0.25 (0.18-0.31)	0.26 (0.20-0.30)	0.25 (0.17-0.29)	0.25 (0.22-0.28)	0.24 (0.17-0.28)	0.24 (0.20-0.27)	0.24 (0.18-0.31)	0.27 (0.21-0.31)	0.25±0.01		12.72	NS

Figures in the parenthesis are range values NS p > 0.001

Distance of transverse moulting suture from anterior end (DTMSAE) was significantly highest in Dharwad population (2.43 mm) followed by Chikkamagaluru and Chikkaballapura populations (2.29 mm), respectively. Significantly lowest DTMSAE was recorded from Bagalkot population (1.87 mm) which was statistically on par with Chamarajanagara (1.95 mm) followed by Kolar population (2.07 mm).

Length of lingula was significantly highest in Shivamogga population (0.20 mm) which was statistically on par with Dharwad population (0.19 mm), followed by Davanagere population (0.18 mm) and significantly lowest length was recorded from Chamarajanagara population (0.16 mm) which was statistically on par with Bagalkot, Bengaluru rural, Kolar and Chikkamagaluru populations.

Length of caudal furrow was significantly highest in Dharwad population (0.41 mm) which was statistically on par with Bengaluru rural (0.40 mm) and Chamarajanagara (0.39 mm) populations. Significantly lowest length was recorded from Bagalkot population (0.33 mm) which was statistically on par with Haveri (0.34 mm), Kolar (0.35 mm) and Chikkamagaluru (0.35 mm) populations.

Length of vasiform orifice ranged from 0.46 mm (Chamarajanagara) to 0.56 mm (Dharwad) with the mean value of 0.51 mm. The breadth of vasiform orifice was ranged from 0.35 mm (Chamarajanagara and Bagalkot) to 0.41 mm (Dharwad) with the mean of 0.38 mm. Length of operculum ranged between 0.21 mm (Bagalkot and Chamarajanagara) and 0.24 mm (Bengaluru rural and Dharwad) with the mean value of 0.23 mm. The breadth of operculum ranged between 0.28 mm (Kolar, Chamarajanagara and Bagalkot) to 0.32 mm (Dharwad) with the mean of 0.30 mm. Length of caudal setae ranged from 0.49 mm (Bagalkot) to 0.61 mm (Haveri) with the mean value of 0.55 mm. Distance between caudal setae ranged from 0.24 mm (Bagalkot, Chikkamagaluru and Chamarajanagara) to 0.28 mm (Davanagere) with the mean of 0.25 mm. All these six characters were found non-significant among the different populations.

3.1 Cluster analysis for *B. tabaci* puparia population

Cluster analysis was performed using software using Past 3.x software package for all quantitative features based on different morphological characters of *B. tabaci* puparia collected from different agro climatic zones of Karnataka revealed the existence of two major clusters-Cluster 1 and Cluster 2 (Fig 1). Cluster 1 was further divided into two major sub clusters (Cl 1a and Cl 1b). Sub-cluster C1 1a consisted of populations from Kolar on one hand which was deviated from other populations. On the other hand, Cl 1a consist of population from Davanagere, Haveri and Belagavi. The sub cluster Cl 1b consists of populations from Chikkaballapura, Bengaluru rural and Shivamogga on one hand and Dharwad population on other hand. Cluster 2 also further divided into

two sub-clusters (Cl 2a and Cl 2b). Sub-cluster Cl 2a consisted of single population populations from Chikkamagaluru and sub-cluster Cl 2b consisted of population from Chamarajanagara and Bagalkot. Chikkamagaluru and sub-cluster Cl 2b consisted of population from Chamarajanagara and Bagalkot.

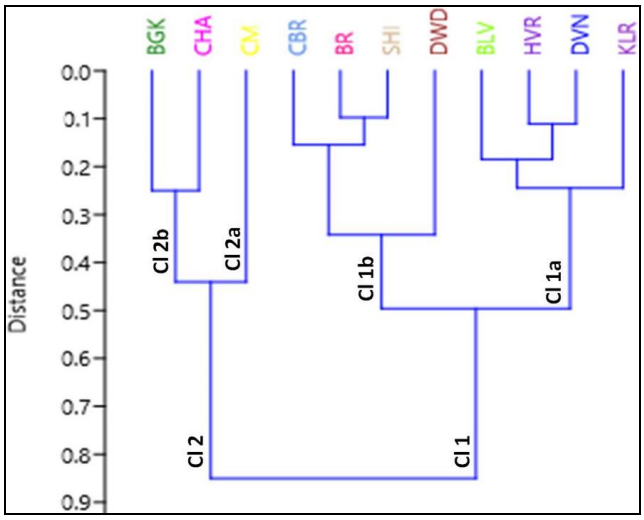

Fig 1: Cluster analysis for *Bemisia tabaci* puparia from different agro climatic zones of Karnataka based on 12 quantitative characters.

3.2 Principal component analysis (PCA) for the estimated variables of *Bemisia tabaci* puparia.

Principal component analysis (PCA) of different puparia characters were presented in Table 3. All the 12 morphological characters studied were divided into three major principal components. The first principal component (PC1) explains the maximum variability in the data regarding succeeding components.

There are total ten principle components present based on scree plot analysis (Fig. 2). The graph exhibits that, the first three Eigen values correspond to most of the variances in the dataset. Total 98.417 per cent of cumulative variance were observed from three major principal components extracted, in which, PC1 accounted for 90.781 per cent, PC2 for 6.337 per cent, PC3 for 1.299 per cent of the total variation. Among the three principal components analyzed, PC1 had shown highest positive contribution for puparia characters, which made the largest contribution of 90.781 per cent of the total variation for the characters *viz.*, puparia length (PL), puparia breadth (PB), distance of vasiform orifice from anterior end (DVOAE). Principle component 2, recorded highest positive contributions for the characters such as puparia length (PL) and distance of transverse moulting suture from anterior end (DTMSAE). Characters such as distance of vasiform orifice from anterior end (DVOAE) and length of caudal setae (LCS) has shown highest positive contribution in PC3 (Table 3).

The major share of variances contributed from first two principal components. In both the principal components all the characters under consideration contributing diversity positively and up to second principal component 97.118 per cent diversities were observed. So, other principal components were not considered as major contributing principal components or they were already considered and measured in first two principal components.

3.3 Score plot of principal components for *B. tabaci* puparia from different agro climatic zones of Karnataka

Scores for *B. tabaci* collected from different agro climatic zones of Karnataka, based on PC1 and PC2 are plotted in Fig. 3. The eleven districts were grouped into four major distinct clusters/ quarters. The distribution pattern revealed that, maximum number of entries (3) were included in quarter I, namely Bagalkot, Kolar, Chamarajanagara whereas, quarter II, included Chikkamagaluru. Quarter III shares one entry i.e. Dharwad whereas, quarter IV included entries *viz.*, Shivamogga and Bengaluru rural. Populations from Davanagere and Haveri shares their individuals in both quarter I and IV, whereas, Belagavi and Chikkaballapura population's shares between quarter III and IV (Fig. 3).

Table 3: Morphometrics of *Bemisia tabaci* puparia based on 12 quantitative characters- proportion of contribution and variable coefficients of the first three eigenvectors for principal component analysis

Eigen value	0.203	0.014	0.003
Proportion	90.781	6.337	1.299
Cumulative	90.781	97.118	98.417
Principal components			
Characters	PC 1	PC 2	PC 3
PL	0.601	0.236	-0.203
PB	0.451	-0.229	-0.631
DVOAE	0.599	-0.439	0.588
DTMSAE	0.262	0.831	0.194
LVO	0.055	0.069	0.040
BVO	0.031	0.057	0.068
LL	0.021	0.011	0.055
LO	0.020	0.008	0.029
BO	0.029	0.007	0.026
LCF	0.036	0.014	-0.273
LCS	0.015	-0.002	0.286
DCS	0.017	-0.031	0.104

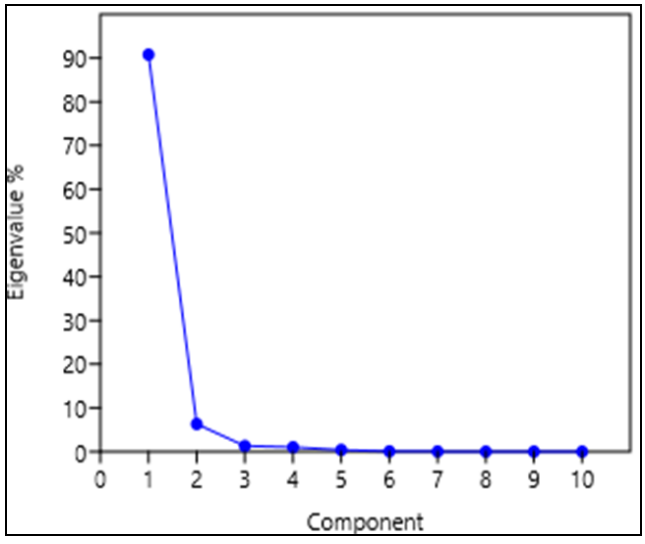

Fig 2: Scree plot analysis of the principal components for *Bemisia tabaci* puparia based on 12 quantitative characters

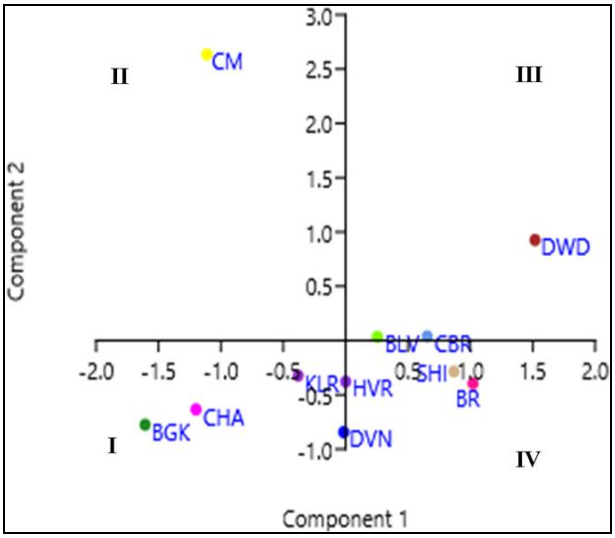

Fig 3: Score plot of principal components for *Bemisia tabaci* puparia from different agro climatic zones of Karnataka based on 12 quantitative characters.

4. Discussion

The puparia characters such as puparia length and breadth from Dharwad district recorded significant higher values of the puparia size, distance of vasiform orifice from anterior end (DVOAE), distance of transverse moulting suture from anterior end (DTMSAE), length of lingula (LL) and length of caudal furrow (LCF) and in addition, recorded comparatively higher values for the characters like length of vasiform orifice (LVO), breadth of vasiform orifice (BVO), length of operculum (LO), breadth of operculum (BO) and distance between caudal setae (DCS). Significantly lower values for PL, PB, DTMSAE, LL and LCF and numerically lowest values for LVO, BVO, LO, BO, DCS and Length of caudal setae (LCS) were recorded for the Bagalkot district population followed by the population from Chamarajanagara districts. Populations from remaining eight districts were on par with each other for the majority of the traits studied.

The results obtained from morphometric analysis of whitefly puparia revealed that, Dharwad population showed significant differences for majority of the characters studied. Significant variation in morphometry may be attributable to the presence of natural variation in geographically distinct populations. Host and geographical zones related to cropping pattern have known to influence on the traits that determine their immediate fitness. These results were similar to the earlier studies [18,30], reported that, the morphological variations of *B. tabaci* populations on different host plants and the variation in morphometrics corresponds to leaf surface features of host plants, environmental conditions and this was supported by Mound and Halsey; Jing-jing *et al.* [18,13]. Similar host associated variation in fruit borer *Helicoverpa armigera* (Hubner) has also been reported by previous author [22]. The host plant-associated morphological variations in *B. tabaci* resulted in very few stable morphological characters which can be used for taxonomy or identifications [14]. In fact, morphological variations across *B. tabaci* populations has confusions and resulted in more than 20 synonyms for this species [18]. The sex also can be reliably identified in puparia in *B. tabaci* species based on a combination of character such as the length and width of puparia, length of vasiform orifice (LVO) and the combination of some of their ratios [1].

Present results pertaining to morphometric analysis were similar to the study conducted by previous author [27], who reported significant differences in the length of egg, second, third and fourth in stars. Out of 62 measurements of the puparia analysed, 70 per cent of the head, 44 per cent of the thorax and 51 per cent of the abdomen showed significant differences between the cotton and leucaena populations. Positive correlation between body length and width of the nymphal instars and body sizes of males and females of *B. afer* singly and collectively recorded in earlier studies [16]. This was similar to the kind of growth pattern observed for *B. tabaci* nymphal instars on cassava [5, 29].

Cluster analysis for *B. tabaci* puparia population based on different morphological characters collected from different agro climatic zones of Karnataka revealed the existence of two major clusters-Cluster 1 and Cluster 2. Cluster 1 was further divided into two major sub clusters (Cl 1a and Cl 1b). Sub-cluster C1 1a consisted of populations from Kolar on one hand which was deviated from other populations. On the other hand, Cl 1a consist of population from Davanagere, Haveri and Belagavi. The sub cluster Cl 1b consists of populations from Chikkaballapura, Bengaluru rural and Shivamogga on one hand and Dharwad population on other hand. Cluster 2 also further divided into two sub-clusters (Cl 2a and Cl 2b). Sub-cluster Cl 2a consisted of single population populations from Chikkamagaluru and sub-cluster Cl 2b consisted of population from Chamarajanagara and Bagalkot. The results were similar to earlier reports [2] where, overlapped clustering was observed in six populations analyzed with 91.11 per cent of the classifications which were correctly attributable and confirm the distinctiveness of populations. Present results are in agreement with those of previous studies [12, 28]. Present conclusions are also corroborated with the previous conclusions that, the populations of *B. tabaci* morphologically and statistically vary to a significant extent in their taxonomic characters and they exhibited as phenotypically distinct populations [19, 3, 17].

The principal component analysis (PCA) based on 12 morphological characters of puparia divided these 12 traits into major three principal components. First two principal components contributed the major share of variances with 97.118 per cent diversity. The score plot analysis based on PC1 and PC2 also exhibited distribution pattern of eleven district populations into four major distinct clusters/ quarters. Similar results were observed by previous author [27] who stated that, principal component analysis separated whitefly population significantly according to host plants, with the first three principal components accounting for 66 per cent of the total variation. Whereas, 73.6 per cent of total variation was observed in earlier studies [2] where in, out of the 62 morphometrics characters, 30 characters vary significantly ($P<0.05$), which reinforce the complexity of *B. tabaci* species. Present results are also in agreement with those of previous studies [19, 3, 17, 12 & 28]. Similar results were also reported in earlier studies [9] which revealed the presence of different genetic groups among whitefly populations of agro-ecological zones of Kerala using morphometric variations, with fourteen different puparia characters. The principal component analysis revealed the presence of more variations in populations collected from Sulthan Bathery region compared to others.

5. Conclusion

Bemisia tabaci may consist of many cryptic species. Morphological variations in *B. tabaci*, may imply its genetic differentiations among populations or biotypes. The variations

might provide a support for distinguishing other populations as well. However, in the present study, existence of biotypes has not been tested, but, distinct clusters of different population give evidence for existence of biotypes based on morphological characters.

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