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Determination of sexual dimorphism in the puparia of four whitefly pest species from India (Hemiptera: Aleyrodidae)

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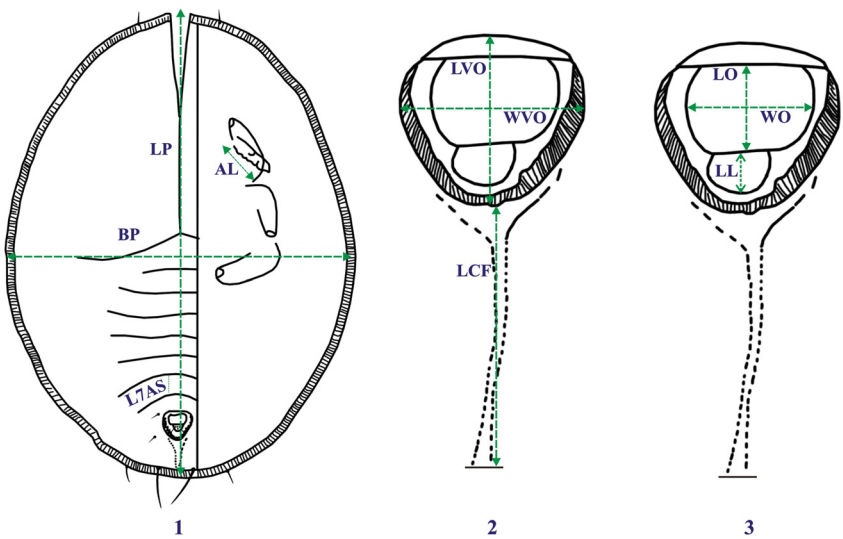
Abstract. We evaluated sexual dimorphism in puparia of four whiteflies, viz., *Aleyrodes* sp. on *Oxalis corniculata* (Oxalidaceae), *Bemisia tabaci* (Gennadius, 1889) on *Solanum melongena* (Solanaceae), *Dialeurodes delhiensis* David & Sundararaj, 1992 on *Ficus virens* (Moraceae), and *Trialeurodes vaporariorum* (Westwood, 1856) on *Phaseolus vulgaris* (Fabaceae), based on a set of ten morphometric characters analysed by univariate and multivariate statistics. Differences between males and females in means of the selected ten characters and in several character ratios were highly significant. The multivariate analyses including principal component analysis (PCA) and canonical discriminant analysis (CDA) revealed that sex can be reliably identified in puparia in these species based on a combination of characters such as the length and width of puparia irrespective of species; length of caudal furrow for *Aleyrodes* sp., *D. delhiensis* and *T. vaporariorum*; length of the seventh abdominal segment for *D. delhiensis* and *T. vaporariorum*; width of operculum alone for *Aleyrodes* sp.; and length of vasiform orifice (LVO) alone for *B. tabaci*; and the combination of some of their ratios. Identification of puparium sex is important for studies in taxonomy, biology and pest management of whiteflies. The results of this study show that morphometrics can provide a useful tool for its diagnosis and accurate identification.

Key words. Sternorrhyncha, Aleyrodomorpha, Aleyrodoidea, whiteflies, puparium, morphometrics, multivariate analyses, identification

Introduction

Whiteflies (Hemiptera: Sternorrhyncha: Aleyrodidae) are small sap-sucking insects, well known for causing damage to plants. They can harm plants directly by feeding and indirectly by transmitting viral diseases. Identification of whiteflies is based mainly on morphological characters of the fourth larval instar, "puparia", as opposed to the characteristics found in adults. A review of the literature showed that there have been very few detailed studies on sexual dimorphism in whitefly puparia (Fishpool et al. 1996, Dubey & Ko 2012). Only the puparial sizes (length and width) formed basis for the determination of dimorphism in many whiteflies but the differences between the sexes were found not so apparent in some species, e.g. *Aleuroinanis myrtacei* Martin, 1999 and *Vasdavidius cobarensis* (Martin, 1999) from Australia (Bellows et al. 1994, Martin 1999). Fishpool et al. (1996) recorded morphometric characters in the puparia of *Bemisia tabaci* (Gennadius, 1889) reared on *Manihot esculenta* (Euphorbiaceae) which showed two discrete size classes of which males were smaller. *Aleurocanthus* species are distinguished based on the number of spines on their dorsal disc, and these vary considerably between male and female puparia (Dubey & Ko 2012).

Since a better knowledge of sexual dimorphism in the puparia would be of significance in identification, and understanding the biology, reproductive potential and management of whiteflies, we explore the male and female forms in four whitefly species of quarantine importance in this paper, viz., *Aleyrodes* sp., *B. tabaci*, *Dialeurodes delhiensis* David & Sundararaj, 1992, and *Trialeurodes vaporariorum* (Westwood, 1856), the biology and morphology of which have recently been studied by Baig et al. (2015).



Figs 1–3. Morphometric measurements considered for sexual dimorphism in whitefly puparia. 1 – puparium, dorsal view; 2, 3 – enlarged view, vasiform orifice and caudal furrow. LP – puparium length; BP – puparium width; AL – antennal length; L7AS – seventh abdominal segment length, WVO – vasiform orifice width; LVO – vasiform orifice length; WO – operculum width; LO – operculum length; LL – lingula length; LCF – caudal furrow length.

Materials and methods

The puparia of *Aleyrodes* sp. (an unidentified species close to *A. proletella* (Linnaeus, 1758), studied also by Baig et al. 2015) were collected from *Oxalis corniculata* L. (Oxalidaceae), *B. tabaci* from *Solanum melongena* L. (Solanaceae), *D. delhiensis* from *Ficus virens* Ait. (Moraceae), and *T. vaporariorum* from *Phaseolus vulgaris* L. (Fabaceae). The former three whitefly species were collected at the Indian Agricultural Research Institute (IARI, 28°4′N, 77°09′E, 223 m a.s.l.), New Delhi, India and the latter species in Solan (30°55′N, 77°7′E, 1350 m a.s.l.), India. The *B. tabaci* population studied belongs to the Asia II-1 genetic group (Chaubey et al. 2015). The fourth instar specimens nearing moult were identified on a leaf surface and clipped individually with self-fabricated leaf cages (3 × 2.5 cm) as proposed by Gill & Rataul (1994). The sex of the emerged adults was confirmed by examination of the genitalia. The respective left over puparial materials after emergence were removed from the leaf, preserved in 90% ethylalcohol and later slide-mounted following the method suggested by Dubey & David (2012). For each sex, ~32 such samples were taken. Measurements of the following characters were made using a LEICA DM 500 research microscope equipped with an ocular eyepiece (Figs 1–3):

AL	antenna length;	LP	puparium length;
BP	puparium width;	LVO	vasiform orifice length;
LCF	caudal furrow length;	WO	operculum width;
LL	lingula length;	WVO	vasiform orifice width;
LO	operculum length;	L7AS	seventh abdominal segment length.

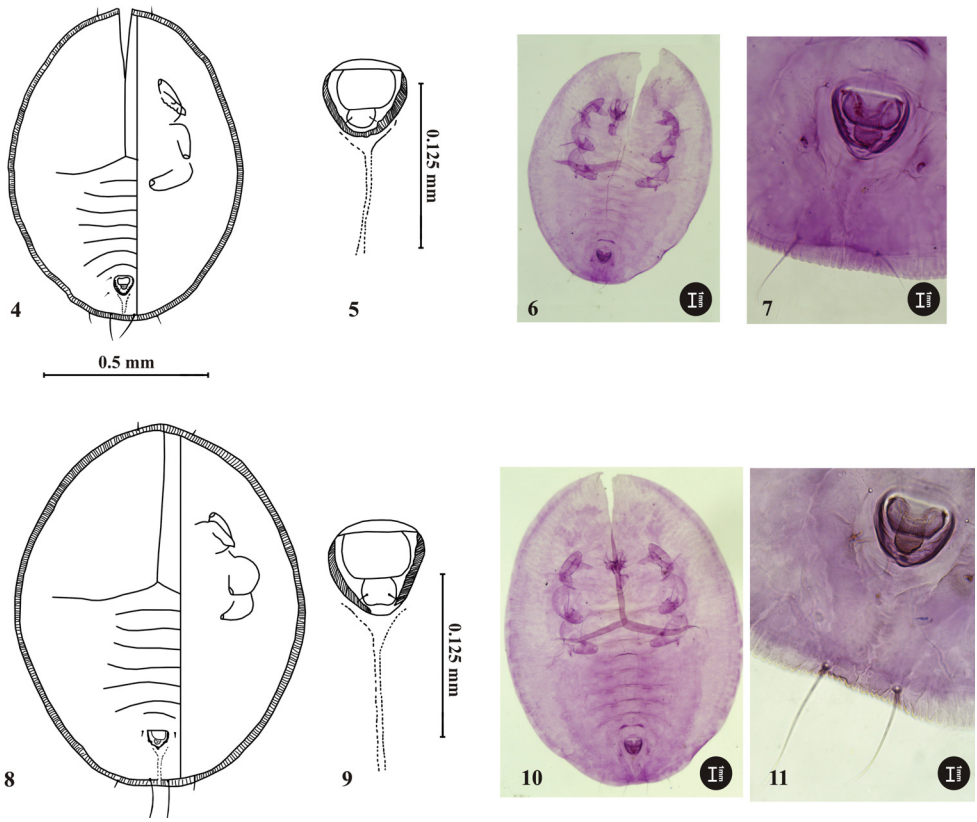
In puparia, the bases of the antennae are poorly defined and hence, the basal elevation was used as a boundary for measurement. The length of the seventh abdominal segment was omitted for *B. tabaci* as there was no significant distinction from the segment VI in this species. Ratios of the characters were also computed.

Means of the characters measured in each sex and species were compared by a Z-test at 5% and 1% probability levels, computed in MS Excel 2007. To further explore the sexual dimorphism, multivariate statistical analyses, viz., principle component analysis (PCA, based on correlation matrix), canonical discriminant analysis (CDA), and discriminant function analysis (DFA), were carried out using SAS 9.3.

The microslides of the puparia examined are deposited in the National Pusa Collection, Division of Entomology, IARI, New Delhi, India.

Results

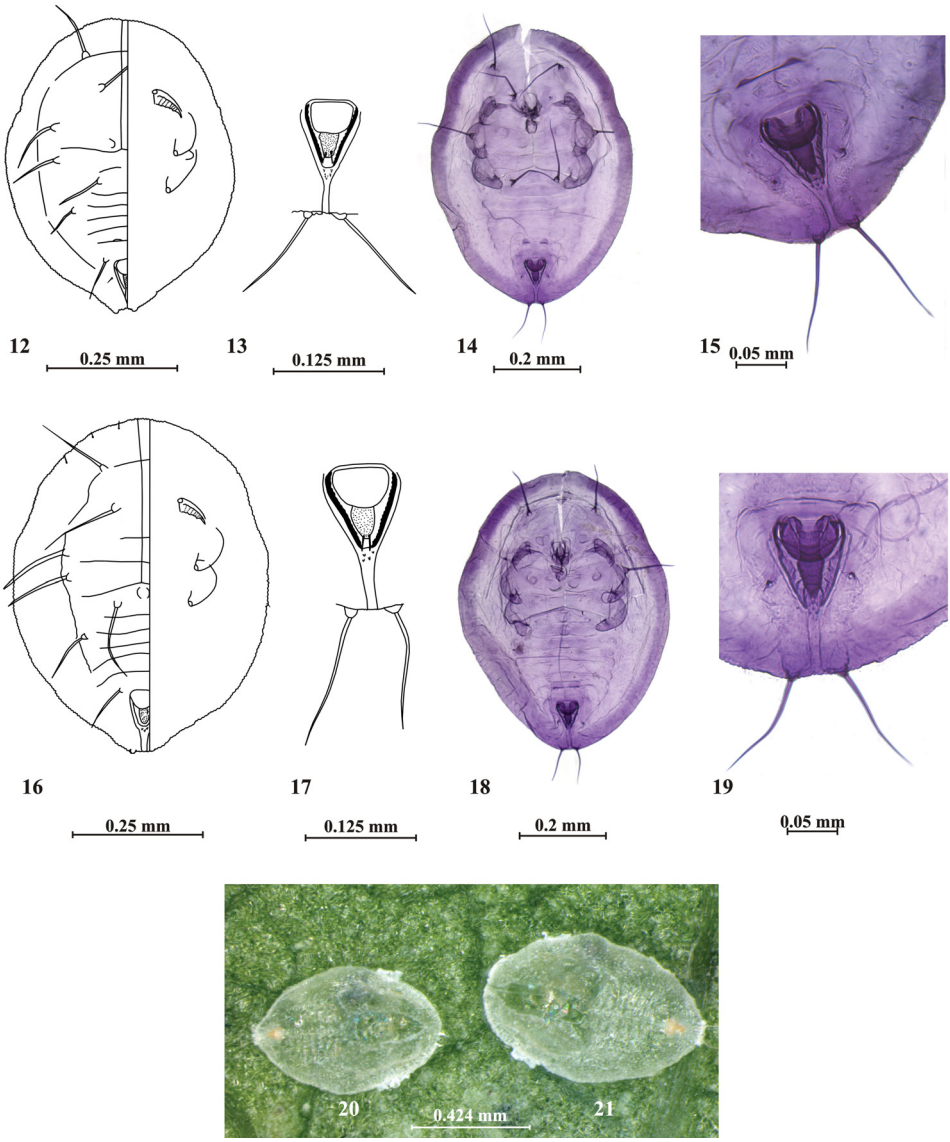
Observations of the four species revealed that the female puparia were generally larger than the male ones (Table 1; Figs 4–39); e.g., LP of females were 1.1–1.3-times larger than the same measurements in males in all four whitefly species. Though there were no other apparent morphological differences, differences in means were significant for all morphometric characters studied. Differences in character ratios, e.g., puparium length versus antennal length (LP:AL), were significant only in some species (Table 1).



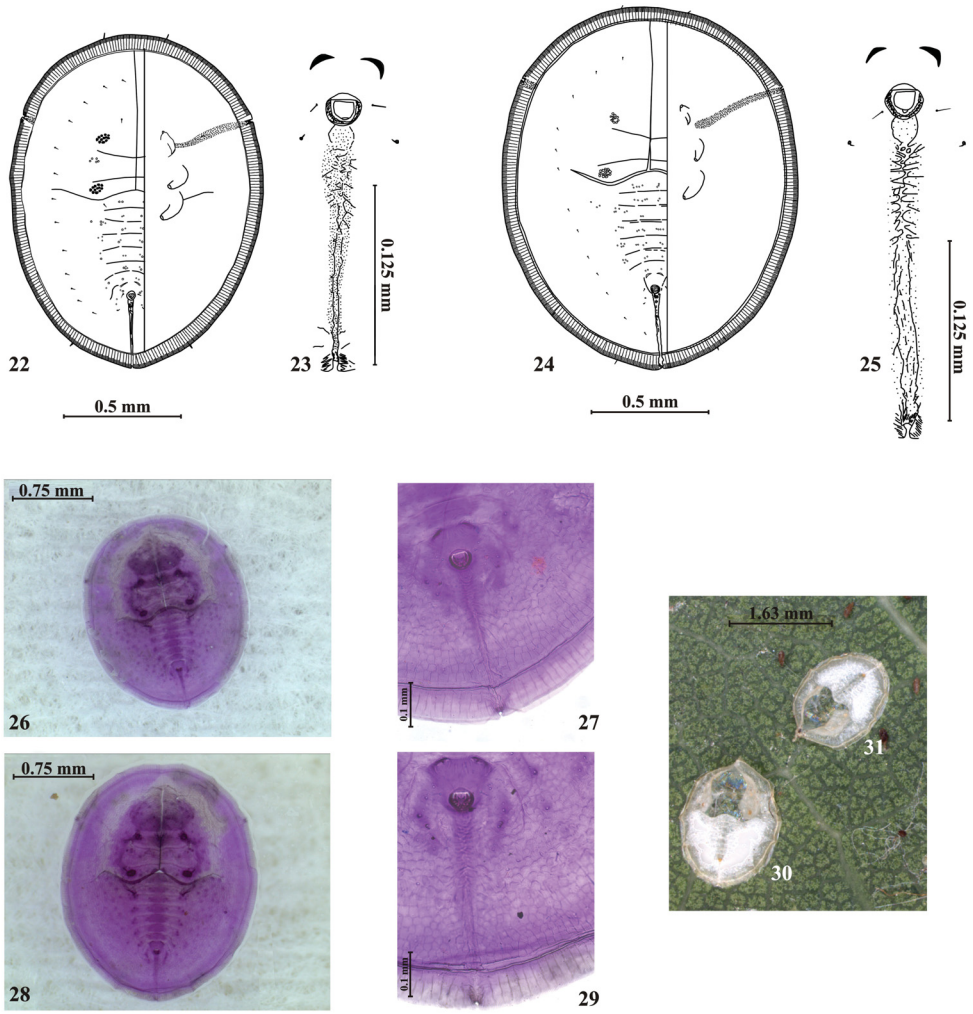
Figs 4–11. Sexual dimorphism in puparia of *Aleyrodes* sp. 4, 6 – males; 5, 7 – male vasiform orifice and caudal furrow; 8, 10 – females; 9, 11 – female vasiform orifice and caudal furrow.

PCA, CDA, and DFA were conducted separately for the characters selected and their ratios. The scatter plots generated through PCA (Figs 40–55) show that discrimination between sexes based on the combinations of the characters measured and their ratios is possible in the four species studied. This was confirmed also by the CDA (Wilk's lambda, Pillai's trace, Hotelling-Lawley trace, and Roy's greatest root were significant at $P \leq 0.0001$ in all four species), and the results of a cross-validation by DFA (100% of specimens were correctly identified to males and females).

Those characters and ratios with the highest loading values (positive or negative) in PCA (especially on the first two PCA axes) and the highest standardised canonical coefficients in CDA are considered to be the major sources of variation and can be used for discrimination

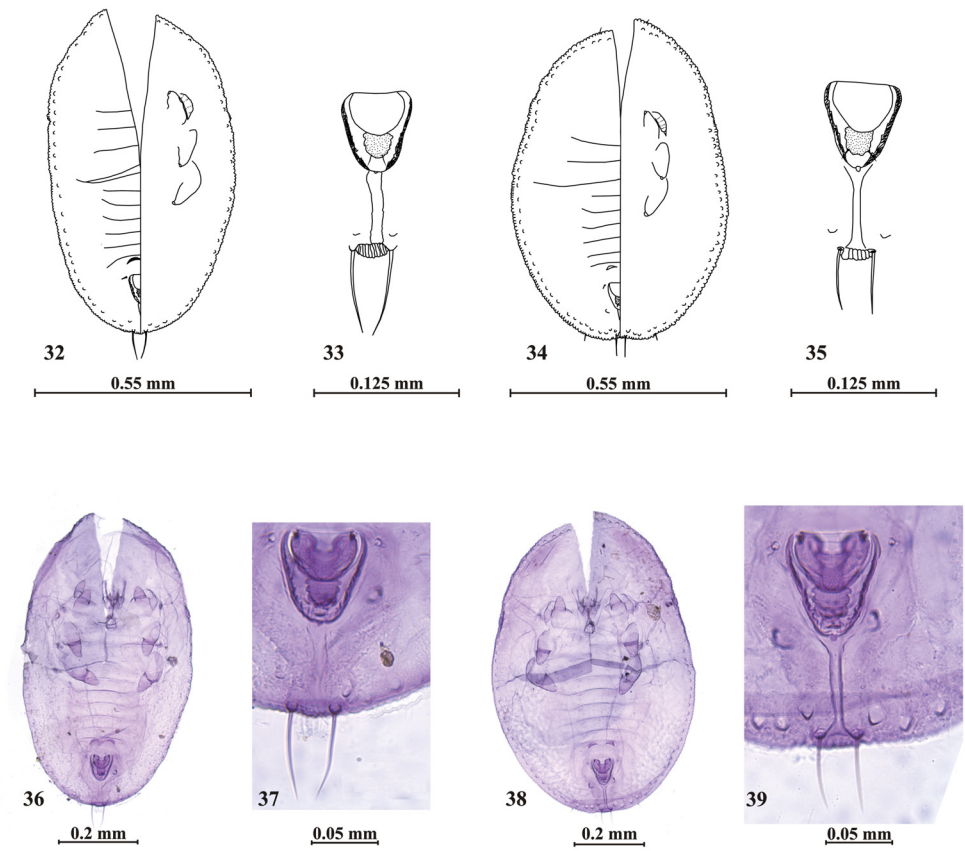


Figs 12–21. Sexual dimorphism in puparia of *Bemisia tabaci* (Gennadius, 1889). 12, 14, 20 – males; 13, 15 – male vasiform orifice and caudal furrow; 16, 18, 21 – females; 17, 19 – female vasiform orifice and caudal furrow.



Figs 22–31. Sexual dimorphism in puparia of *Dialeurodes delhiensis* David & Sundararaj, 1992. 22, 26, 31 – males; 23, 27 – male vasiform orifice and caudal furrow; 24, 28, 30 – females; 25, 29 – female vasiform orifice and caudal furrow.

between the males and females (Table 2). Thus, the males and females of *Aleyrodes* sp. can be best differentiated by LP, LCF, WO, LVO and their ratios, BP, AL, and LVO:LL; *B.tabaci* by LP, BP and their ratio, LL, LVO and their ratio, LP:LL, AL, etc.; *D. delhiensis* by LP, BP, LCF, AL, L7AS, LP:AL, LP:LCF, LVO:LL, etc.; *T. vaporariorum* by LP, L7AS and their ratio, AL, LCF, LVO:LL, and LP:LL (Table 2). LP and BP are of use in grouping males and females in all the species studied; LCF for *Aleyrodes* sp., *D. delhiensis* and *T. vaporariorum*; L7AS for *D. delhiensis* and *T. vaporariorum*; WO alone for *Aleyrodes* sp.; and LVO alone



Figs 32–39. Sexual dimorphism in puparia of *Trialeurodes vaporariorum* Westwood, 1856. 32, 36 – males; 33, 37 – male vasiform orifice and caudal furrow; 34, 38 – females; 35, 39 – female vasiform orifice and caudal furrow.

for *B. tabaci*; the ratios LVO:LL and LP:LL in all four species; LP:LVO for *Aleyrodes* sp., *B. tabaci*, and *T. vaporariorum*; LP:BP for *B. tabaci* and *D. delhiensis*; and LP:L7AS alone for *T. vaporariorum*.

Discussion

Our study supports the view that the sexual dimorphism in whiteflies could be gauged through variations in the puparium size. Earlier studies revealed that the length and width of puparia differ between males and females (Martin 1999, Fishpool et al. 1996). We could reveal that also other morphometric characters and their ratios vary significantly between sexes in all four species we studied. This was validated through both univariate and multivariate

Table 1. Morphometrics of whitefly (Hemiptera: Aleyrodidae) puparia– means, ratios, z test and its significance

Species	*Aleyrodes* **sp.**					*Bemisia tabaci*				
	Female (μ)		Male (μ)		z value	Female (μ)		Male (μ)		z value
Character	±SE	Range	±SE	Range		±SE	Range	±SE	Range	
LP	1157.2±2.6	1140–1200	976.6 ±2.5	950–1010	49.9**	795.4±3.2	772.2–821.7	672±2.4	653–693	30.6**
BP	871.2±4.5	820–920	727.8 ±2.8	700–780	26.9**	607.6±2.4	584–623.7	487±2.9	455–505	32.2**
L7AS	49.5±0.3	45–55	39.4 ±0.3	35–40	22.3**	–	–	–	–	–
AL	113.1±0.8	102–125	103.2 ±0.5	97.5–107.5	10.4**	62.7±0.1	62.5–65	58.8±0.4	55–62.5	9.5**
LVO	65.2±0.6	50–70	59.5 ±0.4	55–62.5	8.03**	80±0.1	77.5–82.5	68.3±0.4	65–70	29.6**
LO	27.7±0.3	25–35	22.6 ±0.2	20–25	13.3**	37±0.3	35–40	31.6±0.2	30–35	12.9**
LL	18.9±0.3	15–22.5	15.9 ±0.2	12.5–17.5	8.3**	31.9±0.2	30–32.5	25.9±0.2	25–27.5	22.1**
LCF	108.4±0.7	100–115	81.7 ±0.4	75–85	30.7**	60.4±0.5	57.5–65	47±0.5	45–52.5	19.7**
WVO	66.6±0.3	62.5–70	59.4 ±0.3	55–62.5	16.4**	63.2±0.5	60–67.5	52.7±0.3	50–55	17.1**
WO	50.6±0.3	47.5–52.5	41.8 ±0.3	40–45	21.6**	51.8±0.2	50–52.5	43.5±0.2	40–45	25.9**
LP:BP	1.32	1.3–1.4	1.34	1.27–1.4	-1.87	1.31	1.27–1.34	1.38	1.33–1.43	-9.11
LP:L7AS	23.40	21.1–26	24.85	23.75–28	-5.48	–	–	–	–	–
LP:AL	10.25	9.5–11.41	9.47	9.12–9.9	4.40**	12.68	11.88–13.15	11.44	10.89–12.06	12.70**
LP:LVO	17.80	16.6–23.2	16.44	15.68–17.8	6.30**	9.94	9.36–10.27	9.85	9.62–10.05	1.85
LP:LO	41.91	33.1–48	43.34	38.4–49.5	-2.27	21.53	19.8–22.63	21.32	19.23–22.44	0.95
LP:LL	61.34	53.3–76	61.70	54.86–76.8	-0.29	24.91	23.76–27.06	26.12	24.12–27.72	-4.45
LP:LCF	10.68	10–11.5	11.96	11.5–13	-13.30	13.19	11.88–13.77	14.32	12.82–14.96	-7.61
LVO:WVO	0.97	0.76–1.08	1.00	0.9–1.05	-1.73	1.27	1.19–1.33	1.30	1.27–1.33	-2.56
LVO:LL	3.45	2.9–4.2	3.75	3.43–4.4	0.69	2.51	2.46–2.67	2.65	2.45–2.80	-5.61
LO:WO	0.55	0.5–0.7	0.54	0.44–0.625	-4.55	0.72	0.67–0.80	0.73	0.67–0.81	-1.19

* significant (5%), ** highly significant (1%)

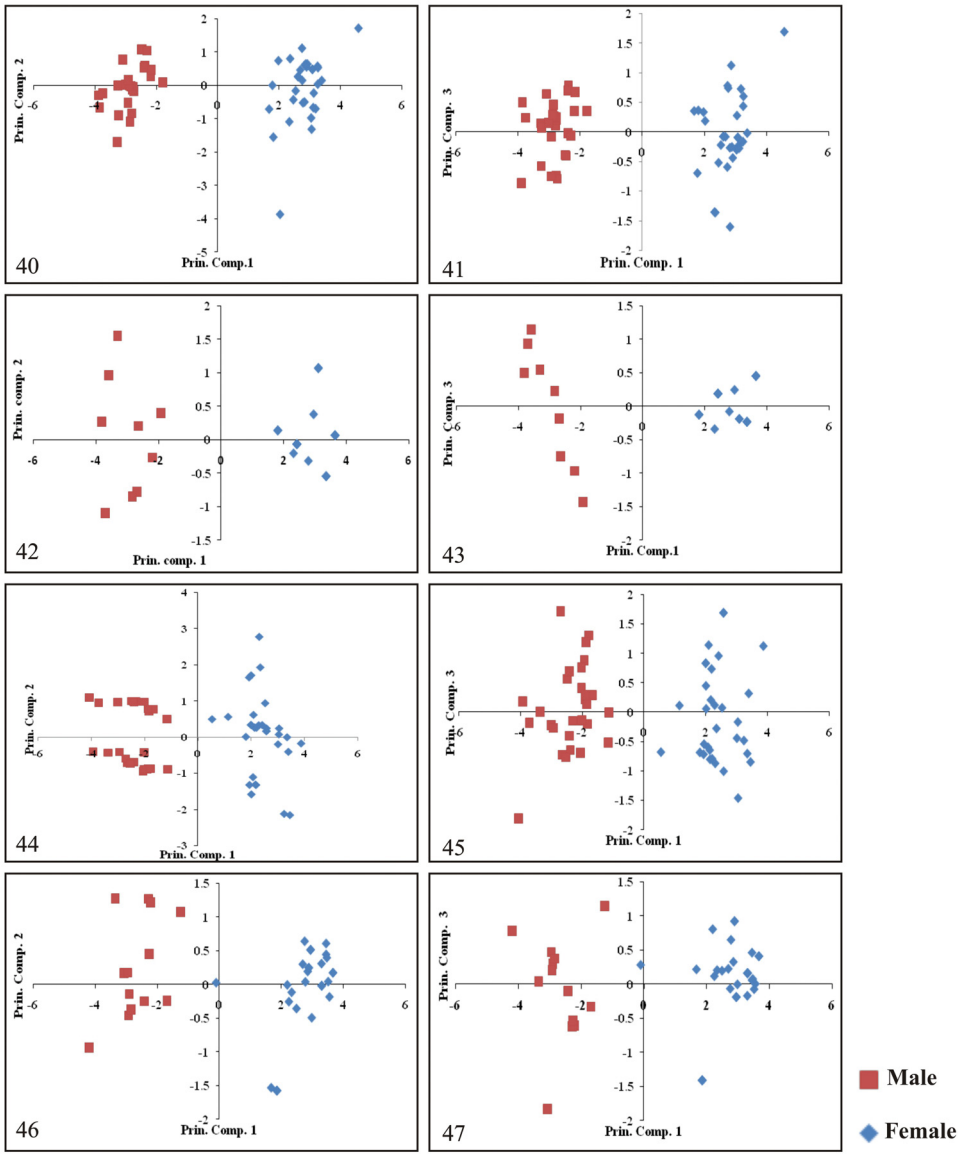
statistical analyses to overcome anomalies that might arise from host influences (Mohanty & Basu 1986; Martin 1999). Li et al. (2013) reported that the puparium size of exotic biotypes (B and Q) of *B. tabaci* was significantly larger than in the indigenous biotypes (ZH1–ZH3 and Cv) in China. However, intersexual variation may have partly obscured the results in Li's et al. (2013) study; a morphometric analysis involving puparia separated by sex needs to be undertaken to confirm any differences among the biotypes. We measured and analysed puparia only irrespective of biotypes in case of *B. tabaci*.

Species	*Dialeurodes delhiensis*					*Trialeurodes vaporariorum*				
	Female (µ)		Male (µ)		z value	Female (µ)		Male (µ)		z value
Character	±SE	Range	±SE	Range		±SE	Range	±SE	Range	
LP	2262.8±9.9	2160–2370	1921.5±7.9	1840–2000	26.9**	858.8±3.9	800–880	743.1±6.5	670–780	15.2**
BP	1845±13	1700–1950	1561.5±10.3	1430–1650	17.0**	557.8±3	500–590	479.4±4.8	430–510	13.7**
L7AS	80.9±0.6	75–85	65.6±0.6	55–70	18.1**	14.6±0.2	12.5–15	9.9±0.01	9.5–10	28.2**
AL	108.7±0.8	100–125	99.2±0.9	92.5–110	8.3**	71.8±0.7	62.5–77.5	64.1±0.7	57.5–70	7.4**
LVO	53.5±0.3	50–57.5	48.7±0.3	42.5–52.5	10.3**	78.5±0.4	72.5–82.5	65.4±0.5	60–70	21.4**
LO	22.4±0.3	20–27.5	21.1±0.2	20–22.5	3.7**	42.3±0.3	35–45	36.3±0.3	32–37	15.7**
LL	7.5	7.5	7.5	7.5	N.S	19.7±0.2	17.5–20	17.5±0.1	15–20	11.7**
LCF	432.6±2.2	405–450	350.6±2.5	325–370	24.6**	81±0.7	70–85	69.2±0.4	65–75	14.2**
WVO	55.3±0.5	50–62.5	51.3±0.3	50–55	7.0**	67.6±0.3	62–72	59.3±0.3	57–62	18.6**
WO	36.6±0.3	32.5–40	33.3±0.3	27.5–35	8.0**	51.6±0.3	47.5–55	44.4±0.4	40–50	14.6**
LP:BP	1.23	1.17–1.28	1.23	1.19–1.31	-0.51	1.54	1.43–1.64	1.55	1.42–1.74	-0.70
LP:L7AS	28.00	26–30.53	29.35	26.86–33.45	-3.97	59.06	53.33–70.4	74.44	67–80	-14.12
LP:AL	20.85	18.4–22.93	19.41	16.91–21.19	5.60**	12.00	10.8–13.76	11.61	10.71–13	2.28*
LP:LVO	42.31	39.64–45.4	39.48	36.8–44.24	7.35**	10.94	10.45–11.6	11.37	10.14–12	-5.14
LP:LO	101.52	81.09–115	91.74	81.78–100	5.41**	20.34	18.82–23.43	20.51	17.87–23.08	-0.50
LP:LL	301.71	288–316	256.21	245.33–266.67	26.95**	43.71	40–49.14	42.53	38–52	1.82
LP:LCF	5.23	4.97–5.61	5.49	5.22–6.09	-5.89	10.61	9.53–11.71	10.73	10.13–11.54	-0.99
LVO:W-VO	0.97	0.84–1.05	0.95	0.85–1	1.67	1.16	1.07–1.23	1.10	1.04–1.17	5.36**
LVO:LL	7.14	6.67–7.67	6.5	5.67–7	-1.33	4.00	3.63–4.57	3.74	3.25–4.5	4.35**
LO:WO	0.61	0.53–0.79	0.63	0.57–0.82	10.34**	0.82	0.74–0.9	0.82	0.72–0.94	-0.29

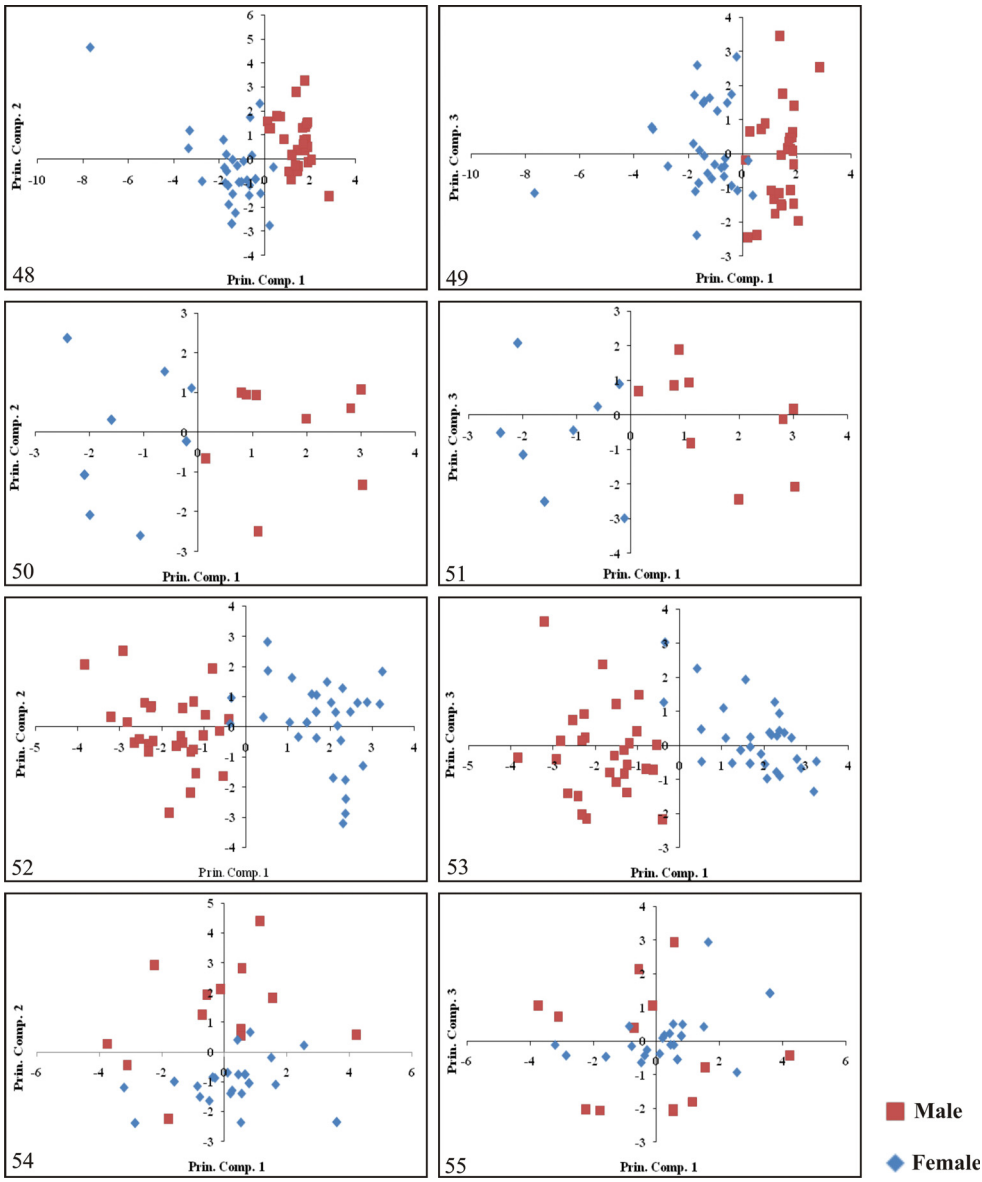
* significant (5%), ** highly significant (1%)

Our results indicate that sexual dimorphism in whitefly puparia is mainly size-correlated. However, morphometric variation is not the only source of intersexual differences in whiteflies. Dubey & Ko (2012) found that *Aleurocanthus* spp. can be identified mainly by counting the number of spines in the puparium stage; however, the number of spines observed varies between males and females. Such variations might result in multiple descriptions of new species, if dimorphic forms are not approached with caution.

Huldén (1986) observed a big overlap in the length of male and female puparia of



Figs 40–47. Scatter plots generated through PCA using puparium morphometric characters and differentiating sexes. 40, 41 – *Aleyrodes* sp.; 42, 43 – *Bemisia tabaci* (Gennadius, 1889); 44, 45 – *Dialeurodes delhiensis Dialeurodes delhiensis* David & Sundararaj, 1992; 46, 47 – *Trialeurodes vaporariorum* Westwood, 1856.



Figs 48–55. Scatter plots generated through PCA using ratios of puparium morphometric characters and differentiating sexes. 48, 49 – *Aleyrodes* sp.; 50, 51 – *Bemisia tabaci* (Gennadius, 1889); 52, 53 – *Dialeurodes delhiensis Dialeurodes delhiensis* David & Sundararaj, 1992; 54, 55 – *Trialeurodes vaporariorum* Westwood, 1856.

Table 2. Morphometrics of whitefly (Hemiptera: Aleyrodidae) puparia. Proportion of variation and variable coefficients of the first three eigenvectors for PCA and total sample standardised canonical coefficients for CDA with two groups (male and female).

Species	***Aleyrodes* sp.**				***Bemisia tabaci***			
	PC1	**PC2**	**PC3**	**CAN1**	**PC1**	**PC2**	**PC3**	**CAN1**
Proportion of total variation	81.11%	7.33%	3.54%		86.45%	5.37%	3.48%	
Character								
LP	0.34675	-0.0348	-0.1088	6.6402	0.33652	-0.0978	0.0468	2.7322
BP	0.34060	-0.0598	-0.1187	-1.7657	0.33166	-0.1101	0.06459	-0.5003
L7AS	0.33302	-0.0966	-0.2983	0.1039	–	–	–	–
AL	0.29402	-0.0869	0.8669	0.2241	0.2765	0.6026	-0.613	-0.5207
LVO	0.27125	0.65704	-0.1678	-0.0833	0.33005	-0.2044	-0.0449	5.3079
LO	0.29653	-0.4225	-0.0739	0.4419	0.30861	-0.2452	-0.1672	-0.1701
LL	0.27792	0.55439	0.14618	-0.2577	0.31818	-0.2013	0.3797	0.1131
LCF	0.34286	-0.0458	-0.1297	2.0252	0.32959	0.0734	-0.1906	0.8181
WVO	0.32247	-0.0843	0.16698	0.1808	0.32315	-0.2021	-0.0239	-1.9862
WO	0.32577	-0.227	-0.184	1.1759	0.33189	-0.0687	-0.0645	0.4464
Proportion of total variation	32.90%	20.46%	18.99%		34.99%	21.58%	19.79%	
Ratio								
LP:BP	0.12654	0.24916	-0.38284	-0.4022	0.4072	0.3040	-0.0915	1.6524
LP:L7AS	0.32893	0.21719	-0.17703	0.20620	–	–	–	–
LP:AL	-0.34339	-0.29662	0.09159	-0.4357	-0.4304	0.0805	-0.1344	-3.3599
LP:LVO	-0.46885	0.19884	0.14141	4.9363	-0.1837	0.5659	-0.0350	9.9731
LP:LO	0.35109	-0.36299	0.21139	-0.0211	-0.1364	0.1453	0.6534	0.0046
LP:LL	-0.00651	0.39854	0.56719	-9.3551	0.3890	0.4019	0.0739	-21.166
LP:LCF	0.39616	0.35726	-0.16563	1.7321	0.3060	-0.1397	0.1479	0.9338
LVO:WVO	0.29041	-0.39889	-0.18003	-0.7358	0.1340	-0.5538	0.2737	0.3016
LO:WO	-0.26607	0.34251	-0.41940	0.0708	0.1200	-0.1338	-0.6594	-0.0661
LVO:LL	0.32039	0.26182	0.43765	9.9720	0.4624	0.1492	0.0858	20.4703

LP – length of puparium, BP – breadth of puparium, L7AS – length of seventh abdominal segment, AL – antennal length, LVO – length of vasiform orifice, LO – length of operculum, LL – length of lingula, LCF – length of caudal furrow, WVO – width of vasiform orifice, WO – width of operculum. (–) – character not measured.

Aleyrodes sp., and his puparia from Finland were smaller (0.8–1.2 mm) than those of British origin (1.2–1.5 mm according to Mound 1966); our observations show that the puparia from India are in intermediate range (0.9–1.1 mm) between the British and Finnish puparia, which might be of taxonomic significance; further studies are needed to establish the identity of our *Aleyrodes* sp. close to *A. proletella* from India.

Species	*Dialeurodes delhiensis*				*Trialeurodes vaporariorum*			
	PC1	**PC2**	**PC3**	**CAN1**	**PC1**	**PC2**	**PC3**	**CAN1**
Proportion of total variation	69.30%	10.40%	6.70%		82.8%	5.33%	3.95%	
Character								
LP	0.38711	0.01493	–0.1494	2.76869	0.32075	0.04782	–0.3443	1.0924
BP	0.37854	–0.0326	–0.1145	–1.0389	0.31727	0.09245	–0.3591	0.4743
L7AS	0.37419	0.00473	–0.035	0.72594	0.32321	–0.2661	0.06391	3.7552
AL	0.31227	0.01183	0.58898	0.44783	0.28159	0.74938	–0.0265	0.1125
LVO	0.34133	–0.0124	–0.2164	–0.0334	0.33494	–0.0823	–0.3094	1.4221
LO	0.15986	0.92573	–0.0442	0.35398	0.30453	–0.5011	0.21224	1.4378
LL	0	0	0	0	0.29759	0.18662	0.691	1.5836
LCF	0.37711	0.02843	–0.1328	1.60574	0.32983	0.14659	0.23009	–2.1039
WVO	0.28875	–0.3254	–0.4438	0.33317	0.32961	–0.1702	0.13732	0.5719
WO	0.31887	–0.1863	0.59424	0.14795	0.31905	–0.1169	–0.2402	–0.2590
Proportion of total variation	39.50%	16.05%	12.82%		36.21%	26.21%	15.25%	
Ratio								
LP:BP	–0.0900	0.4233	0.0778	0.2992	0.2182	0.3228	0.2493	–0.1255
LP:L7AS	–0.2308	–0.0786	0.0862	–0.2856	0.0305	0.4781	–0.1304	2.4539
LP:AL	0.3640	0.1388	0.2421	–0.6104	0.2969	–0.0266	0.5696	–0.0234
LP:LVO	0.3338	–0.2362	0.5173	5.0625	0.0679	0.5701	0.1022	–1.8048
LP:LO	0.3957	–0.3236	–0.2422	–0.4100	0.3650	0.2518	–0.3777	–0.3563
LP:LL	0.4758	0.0772	0.1414	–3.7510	0.4733	–0.0737	0.0981	2.9547
LP:LCF	–0.3080	–0.1105	–0.1802	–0.4170	0.4126	0.1937	0.1032	0.1320
LVO:WVO	0.1229	0.5770	–0.3941	–0.2623	0.3570	–0.3243	–0.1364	–0.9696
LO:WO	–0.2066	0.4322	0.5836	–0.0662	–0.2274	–0.0140	0.6345	–0.2965
LVO:LL	0.3985	0.3110	–0.2304	6.3041	0.3896	–0.3599	0.0394	–3.385

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