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Host-race formation in *Chaetostomella cylindrica* (Diptera: Tephritidae): Morphological and morphometric evidence

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Abstract

Chaetostomella cylindrica is a highly oligophagous tephritid infesting the flower heads of six genera and 10 species of thistles in Lebanon, and is predominant on two hosts in sympatry, *Notobasis syriaca* and *Onopordum illyricum*. Adult flies emerging from *N. syriaca* fit more closely the description of the species with respect to the colour and pattern on the mesonotum. This study compares morphometrically and morphologically the host races associated with *N. syriaca* and *O. illyricum*. Immatures of both races were similar, but all stages of the *Onopordum*-associated race were significantly larger. Morphometric studies, based on two head and five wing measurements, using canonical discriminant analysis, allowed for the differentiation of the host races with more than 70% accuracy. The aculeus shape and length differed significantly between females of both races. The holotype of *Trypeta lurida* Loew 1844 was examined and appeared closer to the *Onopordum* host race. *Chaetostomella cylindrica* appears to be a complex of cryptic and reproductively isolated species.

Keywords: *Chaetostomella cylindrica*, fruit flies, host races, Lebanon, Tephritidae, thistles

Introduction

Host races are populations that are isolated from each other by preferred habitat or host (Diehl and Bush 1984). Rapid host-race formation and sympatric speciation are common among tephritid fruit flies. Phytophagous tephritid populations often show different host preferences but they are morphologically similar or even indistinguishable from one another (Zwölfer and Harris 1971). Host races sometimes represent unrecognized reproductively isolated sibling species that retain distinct host preferences in the absence of other barriers to gene flow (Bush 1975). Biological attributes such as mating on the host plant and positive correlation between host and mate selection, in addition to genetic control of host selection, allow the establishment and formation of new host races in areas of sympatry (Bush 1975).

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The genus *Chaetostomella* Hendel (Diptera: Tephritidae) is comprised of 15 described species (Norrbon et al. 1998; Basov 2000). In Lebanon (Eastern Mediterranean), where very little research has been done on fruit flies, the genus is represented by the generalist *Chaetostomella cylindrica* (Robineau-Desvoidy, 1830) and the specialist species referred to by Knio et al. (2002) as “*Chaetostomella* sp. possibly *lurida* (= *Trypeta lurida*) (Loew, 1844)”. While *C. cylindrica* is well-described and studied (White 1988; Freidberg and Kugler 1989), very little is known about *C. lurida*. The latter species was first described by Loew (1844) based on a holotype collected on the southern coast of Asia Minor, and deposited in the Museum für Naturkunde der Humboldt Universität, Berlin, Germany (ZMHU). However, according to Norrbom et al. (1998), *Trypeta lurida* is a synonym of *Chaetostomella cylindrica*.

In Lebanon, *Chaetostomella* spp. were reared from six genera and 10 species of thistles of the tribe Cardueae (Asteraceae: Lactucoideae). The flies were particularly common on two hosts: *Notobasis syriaca* (L.) Cass. and *Onopordum illyricum* L., which occur side by side in sympatry, in May to June. The flies emerging from these two hosts showed behavioural differences in oviposition (unpublished data) and, although not consistent, several differences in morphology (Knio et al. 2002). The *Notobasis*-associated flies matched more closely the description of *C. cylindrica* by having shiny black spots at the base of prescutellar setae and a black pattern on the mesonotum, compared with small faint brown spots or none at the base of prescutellar setae and grey or tan pattern on the mesonotum in the *Onopordum*-associated host race. Moreover, the aculeus of females from the *Onopordum* host race was slightly longer with a slightly blunter tip (Knio et al. 2002). Preliminary molecular studies also demonstrated that the population reared from *O. illyricum* in Lebanon is distinct from the one reared from *N. syriaca*, suggesting that they are probably distinct cryptic species.

Chaetostomella cylindrica seems to be a polymorphic complex of numerous populations associated with different host plants and having different levels of reproductive isolation in the Western Palaearctic (V. Korneyev, personal communication). Recently, Basov (2000) described a new species of *Chaetostomella*, *C. zhuravlevi*, from Russia associated with *Serratula coronata* L. The new species was similar to *C. cylindrica* and other members of the genus, but could be distinguished based on the aculeus apex and length as well as other morphometric measurements (Basov 2000). *Chaetostomella zhuravlevi* seems to be one of the reproductively isolated populations previously confused with *C. cylindrica*.

Because the taxonomic status of *C. lurida* is not clear in the literature and *C. cylindrica* seems to be a complex of species, there is a need to study these flies from all the recorded hosts from the Western Palearctic. In this study, we establish that *C. cylindrica* is actually a complex of host races by focusing on the differentiation of two host races of *C. cylindrica* morphologically and morphometrically. This paper will be followed by a comparative behavioural and genetic study of these two host races.

Materials and methods

Flower head collection and identification

Flower heads of the thistles *Notobasis syriaca* (L.) Cass. and *Onopordum illyricum* L., belonging to the Asteraceae, were collected from various locations in Lebanon, from sea level up to an altitude of 2000 m. Each sample consisted of 40–60 mature flower heads picked at random and identified using Post (1932) and Edgecombe (1970), and by comparison with voucher specimens from the Post Herbarium (BEI).

Rearing of adult flies from flower heads

Flower head samples were placed in glass-topped, sleeve insectary cages ($35 \times 35 \times 37$ cm) under fluorescent light (12h/12h cycle) and monitored for insect emergence (Goeden 1985). Adults of the two host races under study were reared from flower heads of *N. syriaca* and *O. illyricum*. The flies were identified using Korneyev (1986), White (1988), and Freidberg and Kugler (1989). Voucher specimens were deposited in the Natural History Museum, American University of Beirut.

Materials examined

Chaetostomella cylindrica adults were reared from flower heads of *N. syriaca* collected from various sites in Lebanon during 1995–2004: Mount Lebanon: 18 males, 14 females, Khaldeh, Baabda Co.; 10 males, 10 females, Aley Co.; 12 males, 12 females, Bhamdoun, Aley Co.; six males, four females, Saofar, Aley Co.; six males, eight females, Baabdat, Metn Co.; seven males, seven females, Jounieh Metn Co.; 13 males, 17 females, Barouk, Chouf Co.; six males, seven females, Meshref, Chouf Co.; seven males, seven females, Chehim, Chouf Co. Beqaa Valley: two females, Chtaura, Zahleh Co.; three males, one female, Kefraya, West Beqaa Co.; two males, five females, El Khraizat, West Beqaa Co. North Lebanon: four males, four females, Enfeh, Koura Co.; seven males, eight females, Kousba, Koura Co.; seven males, seven females, Chekka, Batroun Co.

Adults of the second host race, *Chaetostomella cylindrica* possibly *lurida*, were reared from flower heads of *Onopordum illyricum* collected during 1995–2005 from: Mount Lebanon: 40 males, 50 females, Bhamdoun, Aley Co. Beqaa Valley: 15 males, 14 females, Deir El Ahmar, Baalback Co.

The holotype specimen of *Chaetostomella lurida* was also examined: *Trypeta lurida* Loew, 1844 (Loew 1844, p 331). Holotype: Turkey, south coast of Asia Minor; ruins of Patara (ZMHU). The holotype specimen of *C. cylindrica* could not be examined as it has probably been destroyed (Norrbohm et al. 1998).

Comparative morphological studies of the immature stages

Immature stages, namely eggs, third instars, and pupae, of the *Onopordum*-associated host race were obtained from dissections of flower heads of *O. illyricum* (Mount Lebanon: Bhamdoun, Aley Co.) while those of the *Notobasis*-associated host race were obtained from heads of *N. syriaca* (Mount Lebanon: Khaldeh, Baabda Co. and Bhamdoun, Aley Co.; Beqaa Valley: Chtaura, Zahleh Co.; North Lebanon: Koura Co.) The length and width of the immatures were measured under a stereomicroscope (Leica, Zoom 2000) using a calibrated micrometer. The cephalopharyngeal skeletons, anterior spiracles, and posterior spiracles of the third instar larvae of both species were prepared following the method described by Phillips (1946).

Comparative morphological studies of adult flies

External morphology of adults. The following morphological characteristics of the *Notobasis*-associated host-race adults ($n=30$) and the *Onopordum*-associated host-race adults ($n=30$) were recorded: height, width, and colour of the head; length of the antennae (1st, 2nd, and 3rd segments except the arista); colour of the abdomen as well as the colour of the bristles on it; the colour of the thorax; colour of the spots at the base of the dorsocentral and

prescutellar setae; colour of the spot on the scutellum as well as the pattern on the mesonotum. For female flies, length and width at the base of the oviscapae were also measured.

Comparative morphometric studies of the adults. Five wing measurements (Figure 1), namely the width of the wing at the level of the stigma, the length of the discal medial (dm) cell, the length of the third radial (R_{4+5}) cell at the boundary with the medial and discal cells, and the length of the preapical crossband (as one entity and in parts), as well as the width and height of the head were determined for 30 randomly selected males and 30 selected females of the two races.

Multivariate analyses, namely principal component analysis and canonical discriminant analysis, were performed based on these measurements.

Morphology and morphometry of male terminalia. Dissection and slide preparation of the male terminalia of the *Notobasis*-associated host race ($n=13$) and the *Onopordum*-associated host race ($n=13$) were performed according to the method described by White (1988). Measurements of the length and width of the glans (sclerotized part and vesica) of the distiphallus were recorded.

Morphology and morphometry of the aculeus. Dissection of the ovipositors of the *Notobasis*-associated host-race ($n=58$) and the *Onopordum*-associated host-race ($n=41$) females was performed according to the method described by White (1988). The length of the oviscapae and the aculeus were measured under a stereo microscope and a compound microscope, respectively. A total of seven measurements of the aculeus were recorded: length and maximum width of the dissected aculeus; the location of the ventro-lateral groove on the aculeus tip as represented by two ratios (ratio 1 and ratio 2); the width of the aculeus tip at the level of the ventro-lateral groove (width 1 and width 2); the width of the pointed tip of the aculeus (Figure 2).

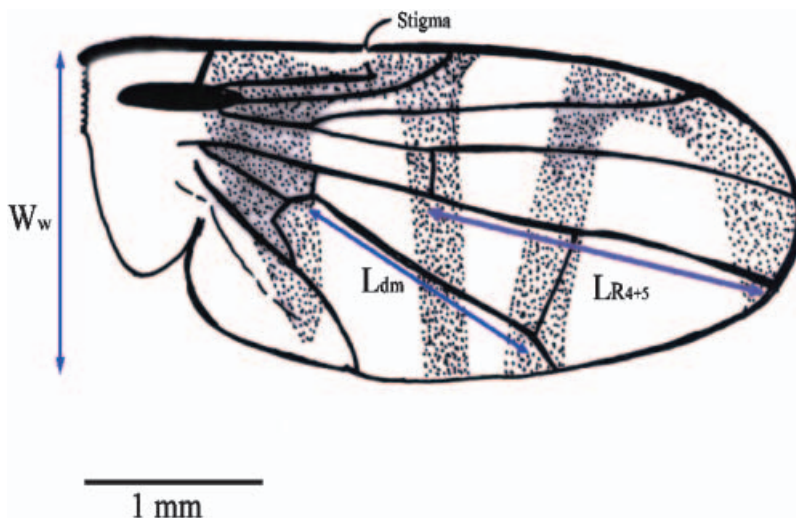


Figure 1. Tephritid wing showing the measurements taken for morphometric studies. WW, wing width at the level of the stigma; Ldm, length of the discal medial cell; LR_{4+5} , length of the third radial cell (R_{4+5}) at the boundary with the medial and discal cells.

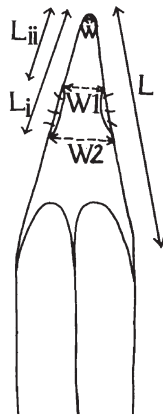


Figure 2. Tephritid ovipositor showing measurements made on the aculeus tip. W1 and W2, width 1 and width 2 of the aculeus tip below the distal and proximal ends of the ventro-lateral groove respectively; W, aculeus tip width measured 0.014 mm from the very tip of the aculeus; ratio 1 = L/L_{ii} ; ratio 2 = L/L_i .

A photograph of the aculeus tip was taken using a digital camera (Nikon E990) and a protractor was used to take two angle measurements of the aculeus tip. The first measurement is the measure of the angle formed by two imaginary lines joining the apex of the aculeus to the point where the aculeus starts to taper on each side. The second one is the measure of the aculeus apex angle, “the angle between two imaginary lines placed tangentially across each side of the tapering aculeus” (White and Marquardt 1989).

Statistical analysis

Statistical differences in morphometric characteristics between males and females within the same race, between males and females of the two races, and/or between the two races regardless of gender, were tested by performing two-tailed *t* tests for independent samples for variables that have a normal distribution, as well as Mann–Whitney tests for variables whose distribution is different from normal. The frequency distribution of measurements for each variable was tested for normality using one-sample Kolmogorov–Smirnov tests. All statistical tests were performed at 95% confidence level using SPSS (version 13.0).

To determine the dispersal of the 120 analysed specimens (considered as operational taxonomic units or OTUs), principal component analysis was performed using SPSS. A correlation matrix of the seven measured characters was generated, the values of the characters were standardized and the eigen values determined. Only the first two components were considered to determine the level of variation among the specimens. The same analysis was repeated for female specimens only ($n=60$ OTUs), and then for male specimens only ($n=60$ OTUs).

To determine whether the two races could be separated morphometrically, based on the two head and five wing measurements, canonical discriminant analysis (canonical variate analysis) was performed using SPSS. This analysis considers within- and between-group variations. A total of 120 specimens (30 females and 30 males reared from *O. illyricum*; 30 females and 30 males reared from *N. syriaca*) for which measurements had been taken were analysed; prior to the analysis, specimens were given codes to ensure that they belonged to either of the races.

Results and discussion

Systematics of the Chaetostomella cylindrica host races

Onopordum-associated host race: *Chaetostomella* sp. possibly *lurida* (Loew)

Trypeta lurida Loew 1844, p 331.

C. cylindrica: Norrbom et al. 1998, p 122 (in part), not Robineau-Desvoidy.

Holotype (female) examined. Turkey, south coast of Asia Minor, ruins of Patara (ZMHU). Head yellow, 1.1 mm in width and 1.33 mm in height. Antennae yellow, 0.48 mm in length. Thorax yellow and partly obscured by pin. No spots at the base of the prescutellar setae. Shiny black spots on scutellum. Abdomen yellow with black bristles and brown spots. Aculeus 1.73 mm long. Aculeus broken at very tip. Ratio 2 ca 4.2. Wing 4.8 mm long and 1.8 mm wide.

Males. Head yellow to yellow brown, 1 ± 0.02 mm in width ($n=30$), 1.28 ± 0.02 mm in height ($n=30$). Antennae yellow, yellow brown or yellow orange, 0.47 ± 0.00 mm ($n=6$) long. Thorax yellow with no spots at base of dorsocentral setae. Black spots at base of prescutellar setae, but sometimes faint brown or absent. Shiny black spots on scutellum. Usually faint brown, but sometimes brown or black pattern on mesonotum. Abdomen yellow with black bristles. Usually with two black or faint brown spots on each abdominal tergum.

Females. Head yellow, yellow brown or sometimes yellow orange, 1.1 ± 0.02 mm in width ($n=30$), 1.42 ± 0.03 mm in height ($n=30$). Antennae yellow or yellow orange, 0.49 ± 0.03 mm in length. Thorax yellow or yellow brown with no spots at the base of the dorsocentral setae. Black, sometimes very small faint brown or absent spots at the base of prescutellar setae. Shiny black spots on scutellum. Faint brown but sometimes dark brown, grey, or no pattern on mesonotum. Abdomen yellow to yellow brown with black bristles. In most cases, two black to faint brown spots on each abdominal tergum.

Puparia. Yellow brown or brown, barrel-shaped; 5.01 ± 0.07 mm long ($n=35$, range 4.2–6); 1.97 ± 0.03 mm wide ($n=35$, range 1.78–2.5).

Third instar larvae. Yellow white, elongated, and barrel-shaped; 5.56 ± 0.1 mm long ($n=58$, range 4.3–7.25); 1.91 ± 0.025 mm wide ($n=58$, range 1.4–2.3). Cephalopharyngeal skeleton sclerotized and red brown under light microscope, similar to that of *C. zhuravlevi* Basov (Basov 2000), 0.82–0.83 mm long (Figure 3A). Mouth hooks stout and strongly pigmented with a long apical tooth, a small median tooth, and a subapical tooth. Posterior spiracular plate, brown, with 14–16 thick internal trabeculae (Figure 3B). Posterior spiracles elongated, 0.058 mm long ($n=6$). Anterior spiracles with six rounded papillae (Figure 3C), unlike *C. undosa* and *C. zhuravlevi* with 11–13 and 9–10 papillae, respectively.

Eggs. White, smooth, cylindrical; 1.38 ± 0.026 mm long ($n=52$; range 1–1.8); 0.31 ± 0.006 mm wide ($n=52$; range 0.2–0.4).

A.

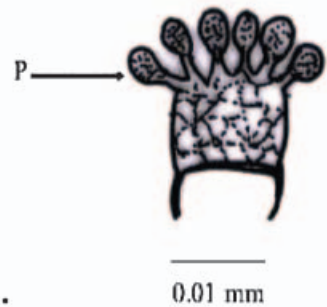
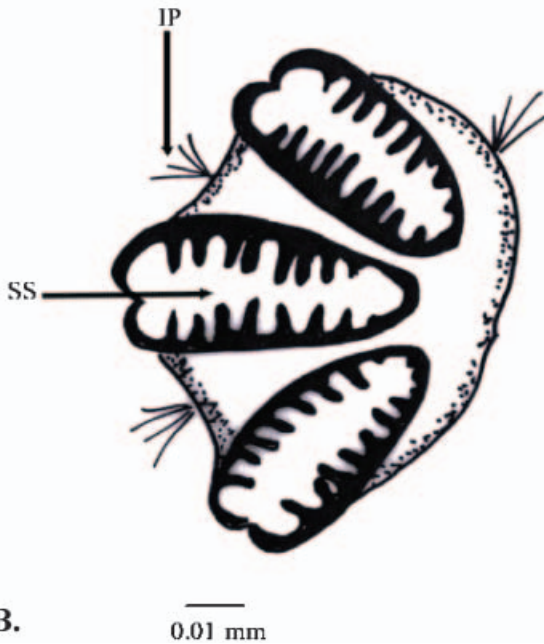
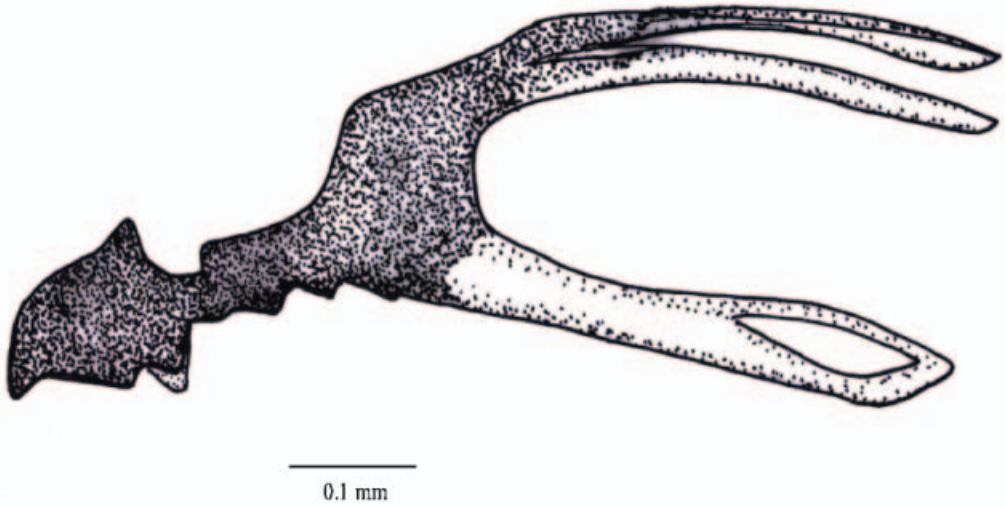


Figure 3. Third instar larvae of *Onopordum*-associated host race of *Chaetostomella cylindrica* (probably *C. lurida*). (A) Typical cephalopharyngeal skeleton; (B) posterior spiracle; (C) anterior spiracle. SS, spiracular slit; IP, interspiracular process; P, one of the six papillae.

Notobasis-associated host race of *C. cylindrica*

Males. Head yellow to yellow brown, sometimes yellow green, 0.89 ± 0.02 mm wide ($n=30$), 1.1 ± 0.02 mm long ($n=30$). Antennae yellow to yellow orange, 0.44 ± 0.01 mm ($n=11$) long. Thorax yellow or yellow brown; usually no spots or rarely very small faint

brown spots at the base of dorsocentral setae. Black spots at base of prescutellar setae, but sometimes brown or faint. Shiny black spots on scutellum. Grey black or black pattern on mesonotum. Abdomen similar to the *Onopordum*-associated host race.

Females. Head yellow to yellow green, 0.97 ± 0.02 mm wide ($n=30$), 1.23 ± 0.003 mm long ($n=30$). Antennae yellow, yellow orange, or sometimes orange, 0.46 ± 0.02 mm long. Thorax yellow, sometimes yellow brown or yellow green, with no spots at base of dorsocentral setae. Black or sometimes brown spots at base of prescutellar setae. Shiny black or dark brown spots on scutellum. Grey black or black pattern on mesonotum. Abdomen similar to the *Onopordum*-associated host race.

Puparia. Brown; 4.4 ± 0.1 mm long ($n=10$, range 4–4.8); 1.81 ± 0.06 mm wide ($n=10$, range 1.4–2.04).

Third instar larvae. Same appearance as the larvae from *Onopordum* but smaller; 4.35 ± 0.13 mm long ($n=17$; range 3.48–5.4); 1.54 ± 0.1 mm wide ($n=17$; range 1.1–2.29). Cephalopharyngeal skeleton similar in shape to that of the *Onopordum*-associated host race, sclerotized and reddish brown, 0.82 mm long (Figure 4A). Posterior spiracular plate and anterior spiracles similar to those of the *Onopordum*-associated host race (Figure 4B, C), but posterior spiracles slightly smaller, 0.048–0.05 mm long ($n=6$).

Eggs. White, smooth, cylindrical; 1.07 ± 0.029 mm long ($n=29$; range 0.85–1.26); 0.255 ± 0.006 mm wide ($n=29$; range 0.22–0.33).

Comparative morphometry of the immatures

The size of all the immature stages of the *Onopordum*-associated host race was significantly greater than those of the *Notobasis*-associated host race. The egg length and diameter of the two host races were statistically different ($t=7.4$, $df=79$, $P<0.001$ and $t=5.97$, $df=79$, $P<0.001$, respectively). The mean diameter and mean length of the third instar larvae were found to be significantly different between the two races ($t=5.5$, $df=73$, $P<0.001$ and $t=5.85$, $df=73$, $P=0.027$, respectively). The length and width of the puparia also showed significant differences between the two races ($t=4.19$, $df=43$, $P<0.001$ and $t=2.51$, $df=43$, $P=0.016$, respectively).

Comparative morphology and morphometry of the adults

Adult head and wings. Seven morphometric characteristics (five wing and two head measurements) were measured for a random sample of 60 flies of each race, 30 males and 30 females. Statistical analysis using two-tailed t test for independent samples showed that the means for the seven characters studied were significantly different ($P \leq 0.05$) for males and females within and between the two races, as well as between the 60 *Notobasis*-associated and 60 *Onopordum*-associated flies regardless of the gender (Table I). The variation within species reflects sexual dimorphism in the two races as males were usually smaller than females. Given the intraspecific variation, principal component analysis and canonical discriminant analysis were performed to determine whether the observed interspecific variation could be useful in differentiating between these two races.

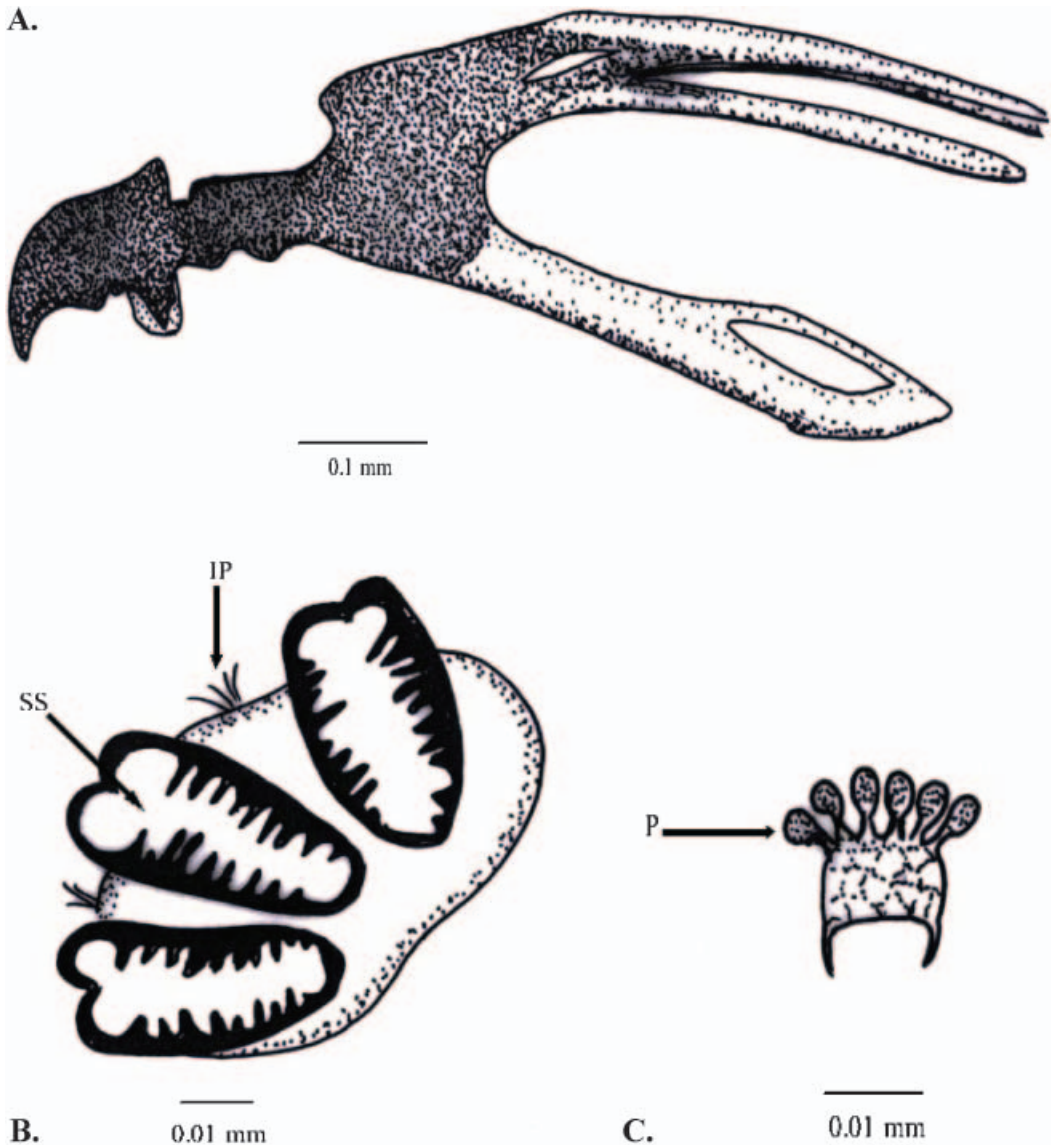


Figure 4. Third instar larvae of *Notobasis*-associated host race of *Chaetostomella cylindrica*. (A) Typical cephalopharyngeal skeleton; (B) posterior spiracle; (C) anterior spiracle. SS, spiracular slit; IP, interspiracular process; P, one of the six papillae.

Principal component analysis revealed that the first principal component which accounted for most of the observed variation between the specimens was the length of the preapical cross band measured as one entity (component loading (CL)=0.965), followed by the wing width (CL=0.947) (Table II). The first principal component also accounted almost equally for the variation due to any of the wing measurements. This suggests that the five wing measurements were highly correlated and including any one of them in any further analysis is sufficient. The second PC showed high loadings for the head width (CL=0.579) followed by the head length (CL=0.305).

Table I. Mean values of the seven morphometric characteristics measured for a sample of 30 adult females and 30 adult males, for each of the *Notobasis*-associated and the *Onopordum*-associated host races of *Chaetostomella cylindrica*.

Host race variable (mm)	<i>Notobasis</i> associated			<i>Onopordum</i> associated		
	Males	Females	Both	Males	Females	Both
HW	0.89 ± 0.02 (0.71–1.12)	0.97 ± 0.02 (0.71–1.12)	0.93 ± 0.02 (0.71–1.12)	1.01 ± 0.02 (0.83–1.18)	1.09 ± 0.02 (0.83–1.48)	1.05 ± 0.02 (0.83–1.48)
HH	1.1 ± 0.02 (0.89–1.36)	1.23 ± 0.03 (0.83–1.48)	1.17 ± 0.02 (0.83–1.48)	1.28 ± 0.02 (1.06–1.48)	1.46 ± 0.03 (1.12–1.77)	1.35 ± 0.02 (1.06–1.77)
WW	1.41 ± 0.03 (1.12–1.65)	1.63 ± 0.04 (1.06–2.01)	1.52 ± 0.03 (1.06–2.01)	1.58 ± 0.02 (1.24–2.01)	1.76 ± 0.03 (1.30–2.30)	1.67 ± 0.02 (1.24–2.30)
WLdm	1.36 ± 0.02 (1.12–1.59)	1.56 ± 0.04 (1.06–1.83)	1.46 ± 0.02 (1.06–1.83)	1.51 ± 0.02 (1.24–1.77)	1.69 ± 0.02 (1.48–2.12)	1.60 ± 0.02 (1.24–2.12)
WLR ₄₊₅	1.64 ± 0.03 (1.30–1.95)	1.85 ± 0.04 (1.36–2.18)	1.74 ± 0.03 (1.30–2.18)	1.76 ± 0.02 (1.36–2.01)	1.97 ± 0.02 (1.71–2.24)	1.87 ± 0.02 (1.36–2.24)
WLpband1	1.33 ± 0.02 (1.10–1.50)	1.53 ± 0.03 (1.06–1.83)	1.43 ± 0.02 (1.06–1.83)	1.47 ± 0.02 (1.12–1.71)	1.64 ± 0.02 (1.42–2.01)	1.55 ± 0.02 (1.12–2.01)
WLband2	1.33 ± 0.02 (1.12–1.59)	1.52 ± 0.04 (1.00–1.83)	1.42 ± 0.02 (1.00–1.83)	1.46 ± 0.02 (1.12–1.71)	1.64 ± 0.02 (1.42–1.95)	1.55 ± 0.02 (1.12–1.95)

Values are means ± SE with ranges in parentheses. HH, head height; HW, head width; WW, wing width; WLdm, wing length of the discal medial cell; WLR₄₊₅, wing length of R₄₊₅ cell; WLpband1, wing length of preapical cross band as one entity; WLpband2, wing length of preapical cross band in parts.

Similar results were obtained when PC analysis was done for only female specimens and for only male specimens. Plots of PC1 versus PC2 done for male specimens only and for female specimens only showed that there are two separate clusters reflecting the two races and that there is some overlap between the two groups (Figure 5). However, there was less overlap and better clustering for male specimens compared to female specimens.

To separate between the two races based on within- and between-group variations, two canonical discriminant analyses (CD) were performed, one using all the seven morphometric characters and the other using two characters, namely the head height and the wing width, as the canonical discriminant function based on these two variables

Table II. The component matrix of the first and second principal components (PC), extracted using principal component analysis, analysed for all specimens, and for female and male specimens of *Chaetostomella cylindrica* separately.

Variable	All specimens		Only females		Only males	
	PC1	PC2	PC1	PC2	PC1	PC2
HH	0.883	0.305	0.838	0.457	0.822	0.298
HW	0.784	0.579	0.794	0.548	0.730	0.606
WW	0.947	−0.154	0.936	−0.150	0.969	−0.037
WLdm	0.942	−0.148	0.918	−0.228	0.941	−0.175
WLR ₄₊₅	0.939	−0.139	0.928	−0.118	0.893	−0.252
WLpband1	0.965	−0.151	0.959	−0.207	0.961	−0.061
WLpband2	0.943	−0.171	0.925	−0.177	0.914	−0.223

HH, head height; HW, head width; WW, wing width; WLdm, wing length of the discal medial cell; WLR₄₊₅, wing length of R₄₊₅ cell; WLpband1, wing length of preapical cross band as one entity; WLpband2, wing length of preapical cross band in parts.

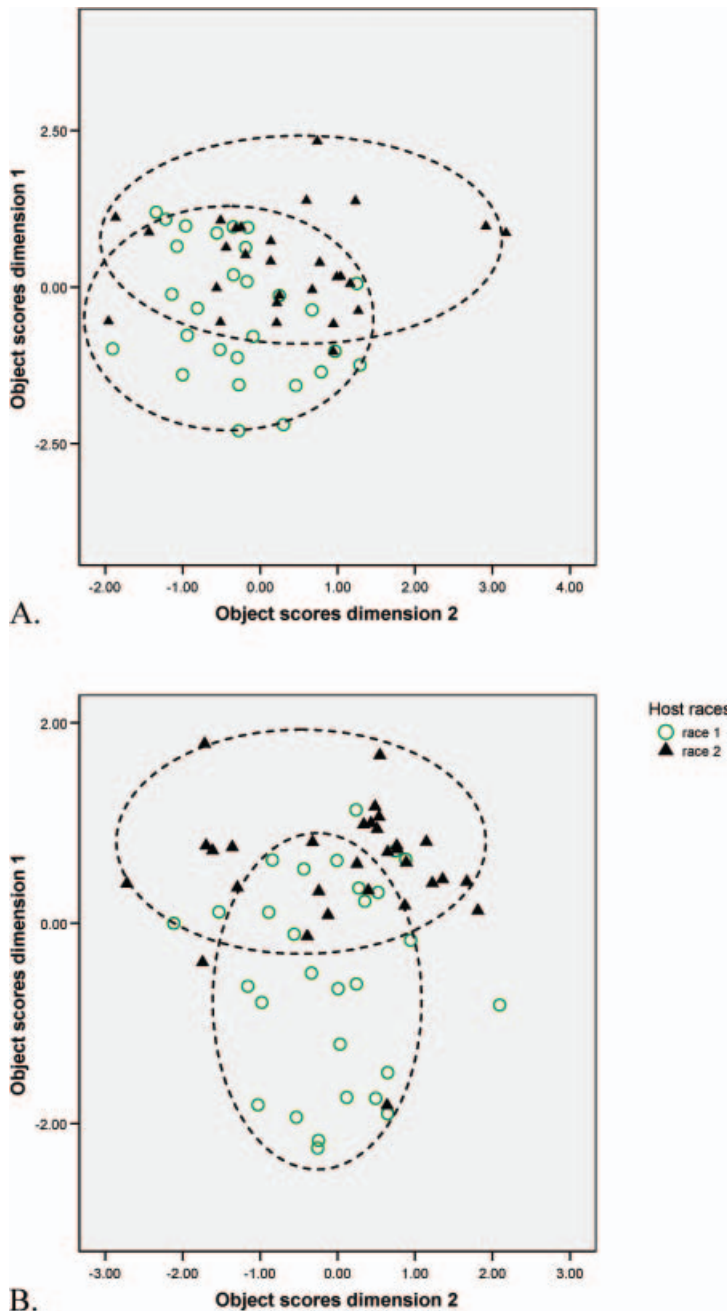


Figure 5. Plot of principal components 1 and 2 for (A) female specimens and (B) male specimens of *Chaetostomella cylindrica* from the *Notobasis*-associated race (race 1) and the *Onopordum*-associated race (race 2).

gave good separation between the two races. The characteristics of the two CD functions used are summarized in Table III.

Based on the two CD functions, the flies were classified into two groups, in such a way that each fly has an equal probability of belonging to any of the two groups. Based on the

Table III. Characteristics of the two canonical discriminant functions (CDFs) used in the two canonical discriminant analyses performed.

	Standardized CDF coefficients		Structure matrix ^a	
	CDF1	CDF2	CDF1	CDF2
HH	1.217	1.407	0.889	0.940
HW	0.182	N/A ^b	0.702	0.683 ^c
WW	-0.445	-0.578	0.529	0.560
WLdm	0.704	N/A	0.598	0.572 ^c
WLR ₄₊₅	-0.565	N/A	0.466	0.548 ^c
WLpband1	-0.706	N/A	0.532	0.589 ^c
WLpband2	0.440	N/A	0.554	0.566 ^c

HH, head height; HW, head width; WW, wing width; WLdm, wing length of the discal medial cell; WLR₄₊₅, wing length of R₄₊₅ cell; WLpband1, wing length of preapical cross band as one entity; WLpband2, wing length of preapical cross band in parts. CDF1: eigen value=0.474; variance=100%; canonical correlation=0.567; Wilks' λ =0.678; Chi square=44.461 (df=7; P =0.000). CDF2: eigen value=0.424; variance=100%; canonical correlation=0.546; Wilks' λ =0.702; Chi square=41.374 (df=2; P =0.000). ^aShows the pooled within-groups correlations between discriminating variables and the standardized CDF; ^bnot applicable (variable not used in the analysis); ^cvariable not used in the analysis.

first CD function, 79.2% of the flies were predicted to belong to the race they actually belonged to. However, 18.3% of the flies belonging to the *Notobasis*-associated host race were classified as *Onopordum*-associated and 23.3% of the flies belonging to the *Onopordum*-associated host race were classified as *Notobasis*-associated (Table IV). Based on the second CD function, in which only two variables were considered, 72.5% of the flies were predicted to belong to the race they originally belonged to. However, 26.7% of the flies belonging to the *Notobasis*-associated host race were classified as *Onopordum*-associated and 28.3% of the flies belonging to *Onopordum*-associated host race were classified as *Notobasis*-associated (Table IV). This shows that CD analysis can be useful in differentiating between these cryptic races with more than 70% accuracy (or 30% error).

Using the standardized CDF coefficients of the two CD functions (Table III), a score for each of the 120 flies analysed was calculated, and three scatter plots of these scores were constructed (Figure 6). In the first plot, the scores obtained for each fly using the second standardized CD function were plotted as a function of the scores obtained using the first CD function. The same was done in the second and third plots, except that only male scores were used in the second plot and only female scores were used in the third. Analysis of the plots shows that there is, to some extent, clustering of the flies into two groups. The presence of some flies of the first species in the vicinity of the second race can be attributed

Table IV. Classification results using first and second canonical discriminant functions (CDFs).

Count	Predicted group membership					
	CDF1			CDF2		
	<i>Notobasis</i> ^a	<i>Onopordum</i> ^b	Total	<i>Notobasis</i>	<i>Onopordum</i>	Total
<i>Notobasis</i>	49 (81.7%)	11 (18.3%)	60 (100%)	44 (73.3%)	16 (26.7%)	60 (100%)
<i>Onopordum</i>	14 (23.3%)	46 (76.7%)	60 (100%)	17 (28.3%)	43 (71.7%)	60 (100%)

^a*Notobasis*-associated host race of *Chaetostomella cylindrica*; ^b*Onopordum*-associated host race of *C. cylindrica*.

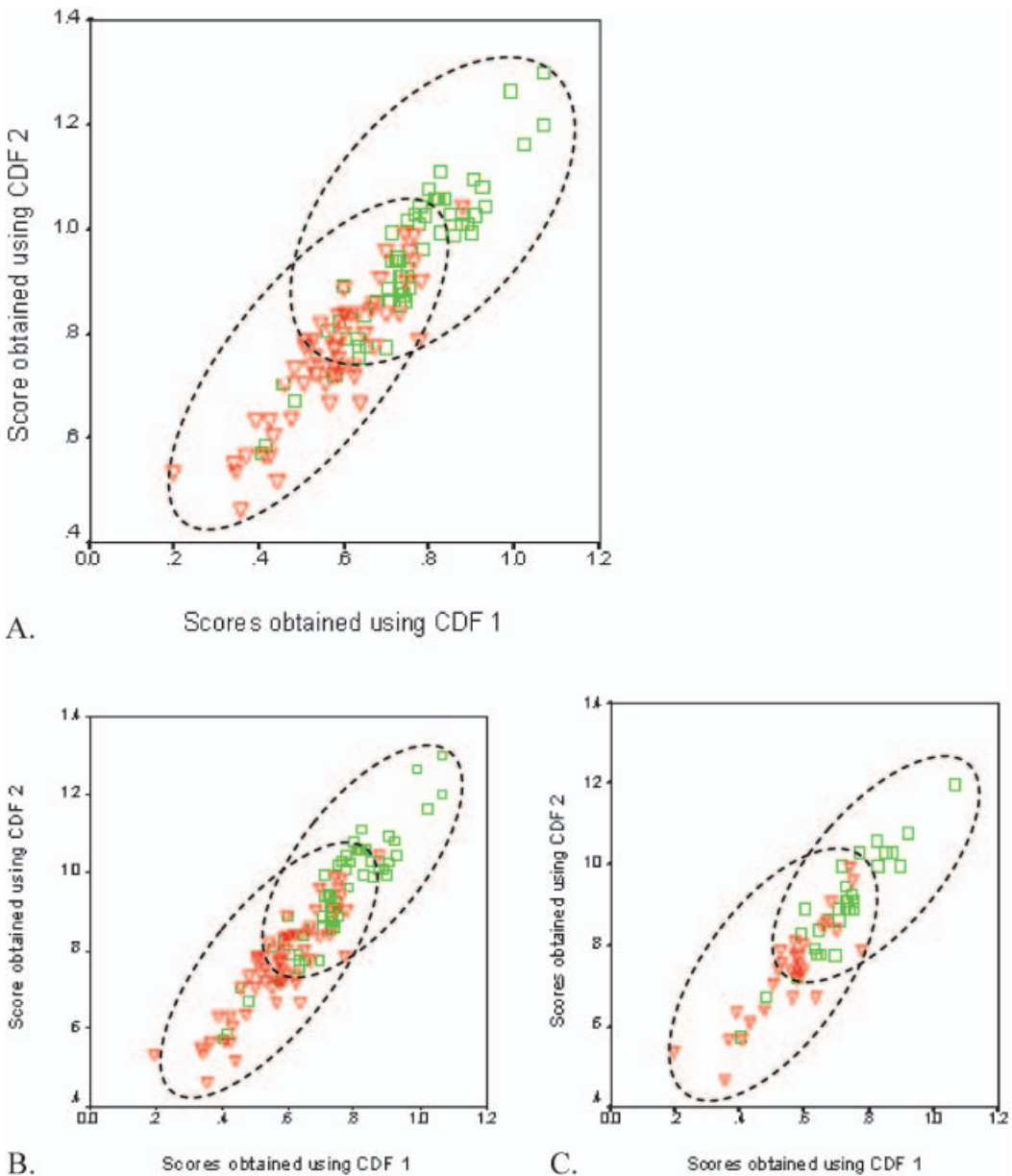


Figure 6. Scatter plots of: (A) the scores obtained for each fly using the second standardized CD function (CDF 2) as a function of the scores obtained using CDF 1; (B) for each male; and (C) for each female fly using CDF 2 as a function of the scores obtained using CDF 1. Squares represent *Chaetostomella cylindrica* from the *Onopordum* host race; triangles represent flies from the *Notobasis* host race.

to the fact that some small or less mature flower heads had been picked up and adult flies had been reared from these flower heads. These flies might have been smaller than normal due to the fact that their larvae did not have enough food and had to pupate ahead of time, before storing enough nutrients to be used in the pupal stages (Tsitsipis 1989).

Male terminalia. The morphological and morphometric comparisons undertaken proved that the terminalia of adult males belonging to the two races are similar, with the exception of fine differences in the glans, which was also longer in the *Onopordum* host race (Figure 7). The length of the epandrium was 0.48 ± 0.01 mm ($n=6$) and 0.5 ± 0.01 mm ($n=9$) while its width was 0.22 ± 0.02 mm ($n=6$) and 0.23 ± 0.01 mm ($n=9$), for the *Notobasis*- and *Onopordum*-associated host races, respectively. No significant differences were detected in the length ($t=0.99$, $df=13$) and width of the epandrium ($t=0.1$, $df=13$) between the two host races ($P>0.05$). On the other hand, the glans (the sclerotized part of the aedeagus together with the vesica) was significantly larger in the *Onopordum* host race. Its length was 0.58 ± 0.01 mm ($n=8$; range 0.54–0.62) and 0.64 ± 0.01 mm ($n=8$; range 0.57–0.68) ($t=3.23$, $df=14$, $P<0.001$) while its greatest width was 0.19 ± 0.004 mm ($n=9$; range 0.15–0.2) and 0.20 ± 0.006 mm ($n=9$; range 0.19–0.24) ($t=2.2$, $df=16$, $P<0.05$), for the *Notobasis*- and *Onopordum*-associated host races, respectively. The similarity in morphology of the male terminalia, however, does not necessarily mean that the two races can interbreed; taking into consideration that successful mating does not necessarily produce viable offspring.

Female terminalia. The oviscape of the *Onopordum*-associated host race was comparable to that of the *Notobasis*-associated host race. Its length was 1.26 ± 0.02 (1.18–1.36; $n=18$) in the *Onopordum*-associated host race and 1.25 ± 0.05 (0.89–1.48; $n=12$) in the *Notobasis*-

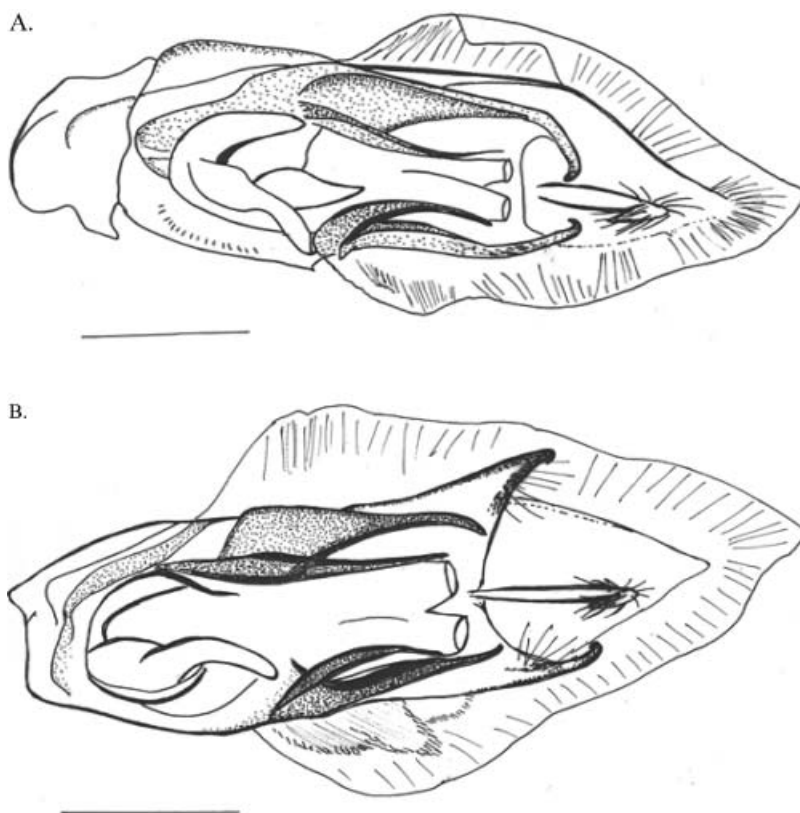


Figure 7. Typical glans of the male distiphallus of *Chaetostomella cylindrica* from the *Onopordum*-associated race (A) and the *Notobasis*-associated race (B). Scale bars: 0.1 mm.

associated host race. Its width was 0.92 ± 0.02 (0.71–1.06; $n=18$) in the *Onopordum*-associated host race and 0.85 ± 0.03 (0.65–1.06; $n=12$) in the *Notobasis*-associated host race. No significant differences in the means of the ov scape length ($t=0.13$, $df=28$) and width ($t=1.91$, $df=28$) between the two races were detected ($P>0.05$).

The size and shape of the aculeus of the two races differed greatly. Table V summarizes nine measurements taken on the aculeus of both races. Significant differences between the two races were detected in five of these measurements: mean length of the aculeus ($t=2.9$, $df=97$, $P=0.005$), tip width of the aculeus ($t=8.5$, $df=97$, $P<0.001$), ratio 1 ($t=4.7$, $df=97$, $P<0.001$), ratio 2 ($t=3.4$, $df=97$, $P=0.001$), and aculeus width 1 ($t=3.8$, $df=97$, $P<0.001$). No significant difference was detected in aculeus width 2 ($t=0.4$, $df=97$, $P=0.7$) (Figure 2). A marginally significant difference was found in the mean maximum width of the aculeus ($t=2.0$, $df=97$, $P=0.05$) of the two races (Table V).

The aculeus of the *Onopordum*-associated host race seems to be longer, a little wider and slightly blunter than the more pointed aculeus of the *Notobasis*-associated host race (Figures 8, 9), as is reflected by its mean length and width and the two angle measurements taken and as is cited in the literature (Knio et al. 2002). The aculeus apex angle differed by 1.2° between the two races; however, no statistical difference could be detected in this regard. The longer aculeus in the *Onopordum*-associated host race can be attributed to the difference in size between the hosts of the two races and can be considered a morphological adaptation of the females to their oviposition substrates. Since the flower heads of *Onopordum illyricum* are larger than flower heads of *Notobasis syriaca*, the *Onopordum*-associated females might need a longer aculeus to be able to penetrate deeper into the flower heads of their host and deposit their eggs between the bracts. Zwölfer (1972) suggested that a narrower and sharper aculeus was needed by a female fly in order to be able to deliver its eggs deeper into the larger flower heads it infests. However, the aculeus of the *Onopordum*-associated host race turned

Table V. Measurements of the aculeus of the *Notobasis*- and *Onopordum*-associated host races of *Chaetostomella cylindrica* collected from different sites in Lebanon.

Variable (mm)	Host race on	Number of samples measured	Range	Mean ^a	SE
Aculeus length	<i>Notobasis</i>	58	1.38–1.97	1.75 a	0.02
	<i>Onopordum</i>	41	1.52–2.00	1.82 b	0.02
Aculeus width	<i>Notobasis</i>	58	0.28–0.38	0.34 c	0.003
	<i>Onopordum</i>	41	0.27–0.41	0.35 c	0.005
Aculeus tip width	<i>Notobasis</i>	58	0.018–0.028	0.022 d	0.0004
	<i>Onopordum</i>	41	0.021–0.035	0.027 e	0.0005
Ratio 1	<i>Notobasis</i>	58	5.35–9.21	7.25 f	0.09
	<i>Onopordum</i>	41	6.17–10.44	8.02 g	0.15
Ratio 2	<i>Notobasis</i>	58	3.59–5.48	4.27 h	0.05
	<i>Onopordum</i>	41	3.95–9.34	4.69 i	0.13
Width 1	<i>Notobasis</i>	58	0.035–0.06	0.048 j	0.001
	<i>Onopordum</i>	41	0.035–0.074	0.053 k	0.001
Width 2	<i>Notobasis</i>	58	0.049–0.077	0.064 l	0.001
	<i>Onopordum</i>	41	0.042–0.084	0.065 l	0.001
Aculeus angle 1 (degrees)	<i>Notobasis</i>	16	24.5–29	27.0 m	0.4
	<i>Onopordum</i>	22	25–31	27.3 m	0.4
Aculeus angle 2 (degrees)	<i>Notobasis</i>	16	15–22	18.6 n	0.5
	<i>Onopordum</i>	22	15–29	19.8 n	0.6

^aMeans followed by different letters are significantly different ($P \leq 0.01$) using a two-tailed t test for independent samples.

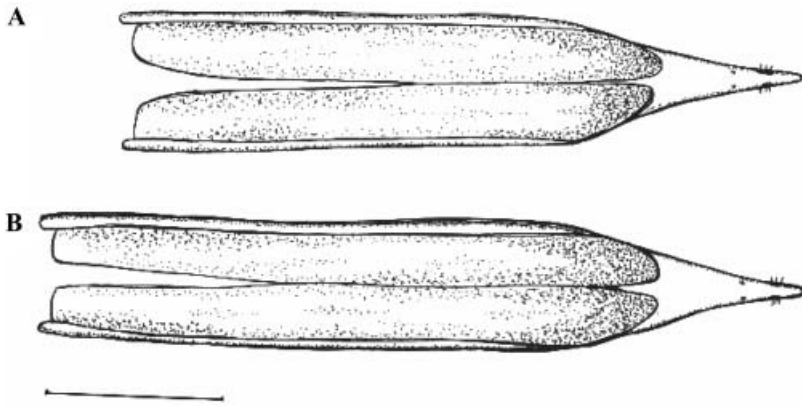


Figure 8. Typical aculeus of *Chaetostomella cylindrica* females from the *Notobasis*-associated host race (A) and the *Onopordum*-associated host race (probably *C. lurida*) (B). Scale bar: 0.5 mm.

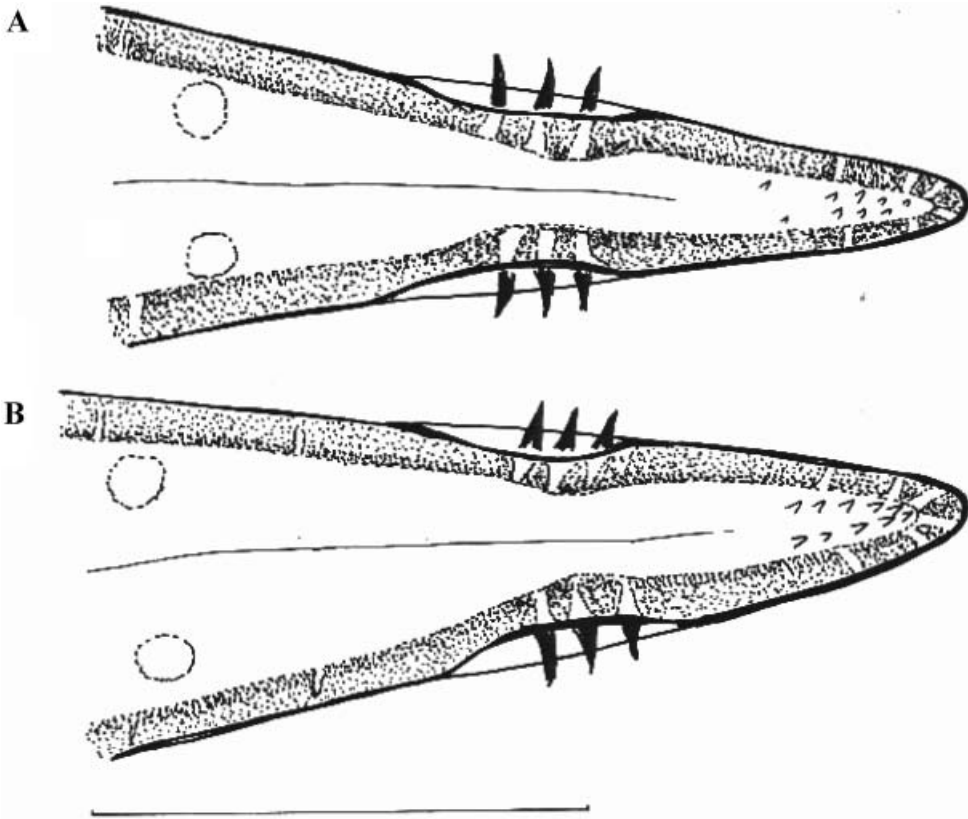


Figure 9. Aculeus tip showing the position of the sensory ventro-lateral groove in *Chaetostomella cylindrica* females from the *Notobasis*-associated host race (A) and the *Onopordum*-associated host race (B). Scale bar: 0.1 mm.

out to be a little wider and less pointed than that of the *Notobasis*-associated host races, although the flower heads of *Onopordum illyricum* are larger in size.

Another significant difference in the aculeus between the two races was in the location of the ventro-lateral grooves bearing three pairs of elongated sensilla as measured by ratio 1 and ratio 2 (Table V). In the *Onopordum*-associated females, the sensory ventro-lateral groove was located closer to the aculeus tip although the aculeus was longer (Figures 8, 9). This could also reflect the larger size of the host exploited by this race. The sensilla on the ventro-lateral grooves of the aculeus tip have been identified as mechano-chemosensilla and these are used by female tephritids to locate suitable hosts and to access the host quality and suitability for oviposition (Stoffolano 1989; Stoffolano and Yin 1987; Zacharuk et al. 1986).

Key to host races

This key allows the correct identification of at least 70% of specimens. The best way for identification is to check the plant host, examine sampled populations, and check the sample means for the diagnostic measurements mentioned in the following key.

Identifications (with 70% accuracy) could also be made by calculating the position of sampled specimens with respect to CV functions (or axes) I and II:

$$\text{CDF1} = 8.04 \text{ HH} + 1.51 \text{ HW} - 2.17 \text{ WW} + 4.12 \text{ WLdm} - 2.92 \text{ WLR}_{4+5} - 4.24 \text{ WLpband1} + 2.54 \text{ WLpband2} - 6.64.$$

$$\text{CDF2} = 9.3 \text{ HH} - 2.81 \text{ WW} - 7.21.$$

Key to adult females

- 1 Aculeus tip usually more pointed (Figures 8, 9) with tip width (90% of the cases) ≤ 0.025 mm (range 0.018–0.028; 70% of specimens < 0.025 mm). Aculeus length ranging from 1.3 to 1.97 mm; usually (70% of cases) ≤ 1.83 mm. Ventro-lateral grooves not very close to aculeus tip: ratio 1: 70% < 7.55 (range 5.4–9.2). Usually, faint brown or tan pattern on mesonotum. Head height ranging from 0.89 to 1.48 mm; usually 80% ≤ 1.36 mm. Head width ranging from 0.71 to 1.12 mm; 80% ≤ 1.06 mm. Length of discal medial (dm) cell ranging from 1.06 to 2.01 mm; ca 73% ≤ 1.65 mm *Notobasis*-associated host race
- Aculeus tip usually more blunt (Figures 8, 9) with tip width (90% of the cases) ≥ 0.025 mm (range 0.021–0.035; 70% of specimens > 0.025 mm). Aculeus length ranging from 1.52–2 mm; usually (70% of cases) ≥ 1.79 mm. Ventro-lateral grooves close to aculeus tip: ratio 1: 70% > 7.55 (range 6.2–10.4). Usually, grey black or black pattern on mesonotum. Head height ranging from 1.12 to 1.77 mm; ca 70% > 1.36 mm. Head width ranging from 0.83 to 1.48 mm; 70% ≥ 1.06 mm. Length of discal medial (dm) cell ranging from 1.48 to 2.12 mm; 70% ≥ 1.65 mm *Onopordum*-associated host race (possibly *C. lurida* (Loew))

Key to adult males

- 1 Grey black or black pattern on mesonotum. Head height ranging from 0.89 to 1.36 mm; usually 80% ≤ 1.18 mm. Head width ranging from 0.71 to 1.12 mm; 70%

of cases ≤ 0.94 mm. Wing width ranging from 1.12 to 1.65 mm; ca 70% ≤ 1.48 mm. Length of discal medial (dm) cell ranging from 1.12 to 1.59 mm; 70% ≤ 1.42 mm. Length of third radial (R_{4+5}) cell ranging from 1.3 to 1.95 mm; 73% ≤ 1.71 mm. Length of preapical crossband ranging from 1.12 to 1.53 mm; 80% ≤ 1.42 mm. Length of the glans ranging from 0.54 to 0.62 mm. Width of glans ranging from 0.15 to 0.2 mm . . . *Notobasis*-associated host race

- Usually faint brown, sometimes black, pattern on mesonotum. Head height ranging from 1.06 to 1.48 mm; ca 83% > 1.18 mm. Head width ranging from 0.83 to 1.18 mm; ca 70% of cases > 0.94 mm. Wing width ranging from 1.24 to 2.01 mm; 80% > 1.48 mm. Length of discal medial (dm) cell ranging from 1.24 to 1.77 mm; 77% > 1.42 mm. Length of third radial (R_{4+5}) cell ranging from 1.36 to 2.01 mm; 83% ≥ 1.71 mm. Length of preapical crossband ranging from 1.12 to 1.71 mm; 80% ≥ 1.42 mm. Length of the glans ranging from 0.57 to 0.68 mm. Width of the glans ranging from 0.19 to 0.24 mm *Onopordum*-associated host race (possibly *C. lurida* (Loew))

Conclusion

This study shows that *Chaetostomella cylindrica* consists of cryptic host races. *Chaetostomella* populations from *N. syriaca* were compared with sympatric populations from *O. illyricum*. Immatures of both host races were morphologically similar but could be differentiated morphometrically. All immature stages of the *Onopordum* host race, which could possibly be *C. lurida*, were significantly larger than those of the *Notobasis* host race. The adults of the two host races proved to be morphologically similar. However, they displayed slight differences in the pattern on the mesonotum: the pattern was usually darker in the *Notobasis*-associated host race, as compared to most literature descriptions of *C. cylindrica* (Knio et al. 2002). Morphometrically, adults of both races could be easily separated. The females were distinguished by the shape and length of the aculeus, which was significantly longer and slightly blunter in the *Onopordum*-associated population (possibly *C. lurida*), reflecting the larger size of flower heads exploited. Adults of both races also differed in two head and five wing measurements, and could be separated with 70% accuracy using canonical discriminant analysis, based on all or some of these measurements. The holotype of *Trypeta lurida* Loew 1844 was examined; however, it could not be conclusively identified as belonging to either of the biologically distinct host races recognized in this study, although it was morphometrically closer to the *Onopordum* host race. The holotype of *C. lurida* was collected from an unknown plant host in Patara, Turkey which also falls in the Eastern Mediterranean and has a similar vegetation to Lebanon. Future work will aim at genetically and behaviourally distinguishing these two host races.

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References

- Basov VM. 2000. A new species of the fruit fly genus *Chaetostomella* Hendel (Diptera, Tephritidae) from Russia. *Entomological Review* 80:569–573.
- Bush GL. 1975. Sympatric speciation in phytophagous parasitic insects. In: Price PW, editor. *Evolutionary strategies of parasitic insects and mites*. New York: Plenum Press. p 187–206.
- Diehl SR, Bush GL. 1984. An evolutionary and applied perspective of insect biotypes. *Annual Review of Entomology* 29:471–504.
- Edgcombe WS. 1970. *Weeds of Lebanon*. Beirut: American University of Beirut. 447 p.
- Freidberg A, Kugler J. 1989. *Fauna Palaestina, Insecta IV—Diptera: Tephritidae*. Jerusalem: Israel Academy of Sciences and Humanities. 212 p.
- Goeden RD. 1985. Host–plant relations of *Trupanea* spp. (Diptera: Tephritidae) in Southern California. *Proceedings of the Entomological Society of Washington* 87:564–571.
- Knio KM, Kalash SH, White IM. 2002. Flower head-infesting fruit flies (Diptera: Tephritidae) on thistles (Asteraceae) in Lebanon. *Journal of Natural History* 36:617–629.
- Korneyev VA. 1986. Fruit flies of the tribe Terelliini (Diptera: Tephritidae) in the USSR. *Entomological Review, Washington* 65:35–55.
- Loew H. 1844. Kritische untersuchung der europäischen Arten des Genus *Trypeta* Meig. *Zeitschrift für Entomologie (Germar)* 5:312–437.
- Norrbom AL, Carroll LE, Thompson FC, White IM, Freidberg A. 1998. Systematic database of names. In: Thompson FC, editor. *Fruit fly expert identification system and systematic information database*. Leiden: Backhuys Publishers. p 122–123.
- Phillips VT. 1946. The biology and identification of trypetid larvae (Diptera: Tephritidae). *Memoirs of the American Entomological Society* 12:1–161.
- Post GE. 1932. *Flora of Syria, Palestine and Sinai*. Volume 2. Beirut: American University of Beirut. 928 p.
- Robineau-Desvoidy AJB. 1830. *Essai sur les Myodaires. Mémoires Présentés par Divers Savans à l'Académie Royale des Sciences* 2(2):1–813.
- Stoffolano JG Jr. 1989. Structure and function of the ovipositor of the tephritids. In: Cavalloro R, editor. *Fruit flies of economic importance* 87. Rotterdam: A. A. Balkema. p 141–146.
- Stoffolano JG Jr, Yin LRS. 1987. Structure and function of the ovipositor and associated sensillae of the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). *International Journal of Insect Morphology and Embryology* 16:41–69.
- Tsitsipis JA. 1989. Nutrition requirements. In: Robinson AS, Hooper G, editors. *Fruit flies, their biology, natural enemies and control*. Volume 3A. Amsterdam: Elsevier Science. p 103–119.
- White IM. 1988. Tephritid flies (Diptera: Tephritidae): handbook for the identification of British insects. Volume 10, Part 5a. London: Royal Entomological Society of London. 134 p.
- White IM, Marquardt K. 1989. A revision of the genus *Chaetorellia* Hendel (Diptera: Tephritidae) including a new species associated with spotted knapweed, *Centaurea macutosa*, Lam. (Asteraceae). *Bulletin of Entomological Research* 79:453–487.
- Zacharuk RY, Lee RMKW, Berube DE. 1986. Fine structure of sensilla on the ovipositor of the tephritid fly *Urophora affinis*. *Canadian Journal of Zoology* 64:973–984.
- Zwölfer H. 1972. Investigations on *Chaetorellia* sp. associated with *C. solstitialis*. In: *Weed project for the University of California, Division of Biological Control*. Delémont, Switzerland: Commonwealth Institute of Biological Control. Report nr 7. 21 p.
- Zwölfer H, Harris P. 1971. Host specificity determination of insects for biological control of weeds. *Annual Review of Entomology* 16:156–178.