

Chaetotaxy of Fourth Instar Larvae of Four *Phlebotomus* (Diptera: Psychodidae) Sandflies Species from Jordan

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ABSTRACT. The morphological characteristics of the larval stages of four laboratory-reared *Phlebotomus* species, collected from Jordan, were studied. The length, shape and distribution of body hairs of the fourth larval instar (L₄) of *P. syriacus*, *P. mascittii*, *P. kazeruni* and *P. tobbi* formed a pattern that can distinguish one species from another. *P. tobbi* possessed a single pair of caudal bristles. Distinctive chaetotactic features of L₄ instars of the described four species were compared to those of *P. langeroni*.

The immature stages of few sandfly species have been described and larvae found in nature have often remained unidentified (Killick-Kendrick 1987). Sandfly larvae have characteristic brush-like hairs that vary in length, form, and distribution and thus can help in species identification (Lane and El-Sawaf 1986). Ward (1976) produced a numerical chaetotaxy for neotropical phlebotomine sandfly larvae. Much work is needed to determine whether Old World phlebotomine sandflies can also fit into this system. Trouillet (1979) provided a system in which the hairs of larvae of Old World phlebotomine sandflies are numbered radially from the mid dorsal line. This system was used by Lane and El-Sawaf (1986) to describe the chaetotaxy of the immature stages of *P. langeroni*. The latter workers also noted on interspecific differences in the chaetotaxy of larval and pupal stages of other species.

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Key words: *P. syriacus*, *P. mascittii*, *P. kazeruni*, *P. tobbi*, sandfly chaetotaxy.

The purpose of the present study is to describe the features and chaetotaxy of the fourth larval instars of *P. syriacus*, *P. mascittii*, *P. kazeruni*, and *P. tobbi* and to determine whether they could be used as a tool for species identification.

Materials and Methods

Sandflies were captured from their resting sites by aspirators. *P. syriacus*, *P. mascittii*, and *P. tobbi*, were collected from animal sheds in Um-Quais, Bani Kinana District, northern Jordan. *P. kazeruni* was collected from a cave in Ras el Naqb, southern Jordan. For location and biotope description of these locations see Kamhawi *et al.* (1995 a, b).

Establishment, rearing and maintenance of sandflies of laboratory-reared colonies of collected sandflies were carried out according to Endris *et al.* (1982), Modi and Tesh (1983), and Killick-Kendrick and Killick-Kendrick (1991). Colonies were maintained at a temperature of 27-31 °C and a relative humidity of 80-90%. The food for sandfly larvae was prepared according to the method described by Young *et al.* (1981).

Slide preparations of immature stages were made by mounting them in Berlese medium (Lewis 1973) following killing in hot water. *Camera Lucida* drawings of fourth-instar larvae of each species were made to show the distribution, morphology, and lengths of their hairs.

Results

Figures 1, 2, and 3 show the dorsal and ventral distribution of hairs on the head, thorax, and abdomen of L₄ of *P. syriacus*, *P. mascittii*, and *P. kazeruni*, respectively. Tables 1, 2 and 3 show the length of hairs distributed all over the body of L₄ of each species. Hairs were numbered following the system of Trouillet (1979) and Lane and El-Sawaf (1986). The head of L₄ of *P. mascittii* and *P. kazeruni* bore 9 major hairs numbered 1-9 (Figs. 2 and 3) of which hairs 1, 2, 4, 5, 6 were slender, while all others were brush-like. *P. syriacus* showed the same arrangement excluding hair 6 (Fig. 1). Hairs 2, 3, 4, 5, 6, 8 and 9 of this species were consistently longer than those of *P. mascittii* ($P < 0.005$). Moreover, all head hairs of L₄ *P. syriacus*, except for 1, 2 and 5, were longer than the corresponding ones in *P. kazeruni* ($P > 0.05$) (Tables 1-3). On the other hand, head hairs 2, 4, 5, 6 and 8 of L₄ *P. kazeruni* were longer than those of *P. mascittii* ($P < 0.001$), while hair 7 was longer in L₄ of *P. mascittii* than in that of *P. kazeruni* ($P < 0.05$).

Each of the thoracic segments of L₄ of the three species bore a maximum of 9 hairs (1-9), except for the anterior prothoracic segment, which bore only 6 hairs. Additional hairs termed a, b, c, d were also encountered on the thoracic segments of some species. Thoracic hairs of the three species showed similarities in terms of morphology and length, except for the dorsal anterior prothoracic hair designated b. L₄ of *P. kazeruni* had a brush-like b hair while those of *P. syriacus* and *P. mascittii* had spiny ones (Figs. 1-3). Moreover, in L₄ of *P. syriacus* and *P. mascittii* hair d was present on all of the thoracic segments, and hair a was found on the meso- and metathoracic segments. None of these hairs was seen on *P. kazeruni* L₄ specimens. Both thoracic and abdominal hairs of *P. mascittii* had club-shaped endings, a feature observed only on the dorsal thoracic and all the abdominal hairs of L₄ of *P. kazeruni*. The ventral thoracic hairs of *P. kazeruni* and all hairs on the thorax and abdomen of *P. syriacus* L₄ had pointed endings (Figs. 1-3).

Dorsally, the abdominal hairs 1 and 2 of *P. syriacus* L₄ (Fig. 1, Table 1) as well as hair 1 of abdominal segments VII and VIII of *P. mascittii* L₄ (Fig. 2, Table 2) were significantly shorter than those of *P. kazeruni* (Fig. 3, Table 3) ($P < 0.001$), and those of *P. mascittii* were longer than their corresponding ones in *P. syriacus* ($P < 0.001$). Moreover, the 1S hair found on abdominal segments I-VII of L₄ of the three species were longer in *P. kazeruni* than those of the other two species ($P < 0.001$). The shape of this hair varied among the three species. It was brush-like in *P. kazeruni* (Fig. 3), spatula-shaped in *P. syriacus* (Fig. 1) and fan-like in *P. mascittii* (Fig. 2). Also, hair 3 of the abdominal segment VIII of *P. kazeruni* L₄ was hairy while it was spiny in case of L₄ of the other two species. Ventrally, hair 6 of the abdominal segments I-VII of *P. mascittii* (Fig. 2) and *P. kazeruni* L₄ (Fig. 3) were hairy and brush-like while those of *P. syriacus* (Fig. 1) were spiny. Moreover, hairs 6 and A of *P. syriacus* and *P. kazeruni* L₄ were 2-3 times longer than their corresponding ones in *P. mascittii* L₄ ($P < 0.005$).

A preliminary description of the chaetotaxy of *P. tobbi* L₄ based on a single specimen is shown in Fig. 4 and Table 4. This larva appeared to bear long dorsal hairs with club-shaped endings, except for hair 1 in the abdominal segment VIII, which was shorter and devoid of a distinct ending, being similar to all other ventral hairs. The 1S hairs were fan-shaped. Only one pair of caudal bristles is present in all of the four larval instars of *P. tobbi* (Fig. 5a), a feature that is not shared by larvae of all other *Phlebotomus* species studied so far in which larval instars L₂-L₄ bear two pairs of caudal bristles (Fig. 5b).

Discussion

Using chaetotaxy, it was possible to distinguish among L₄ instars of *P. syriacus*, *P. mascittii*, *P. kazeruni* and *P. tobbi*. The specific features of L₄ hairs of each of the above mentioned species are given in Table 5. In the head region, the length of hairs and the presence or absence of hair 6 were the most important distinguishing features. As for hairs on the thorax and abdominal segments I-VIII, hair morphology as well as the length of abdominal hairs 1 and 2 formed a pattern that distinguished one species from another. Moreover, the number of caudal bristles on abdominal segment IX clearly distinguish *P. tobbi* from the other 3 species studied. The observation of having only one pair of caudal bristles in all four larval instars of *P. tobbi* confirmed an earlier observation made by Killick-Kendrick *et al.* (1989), who collected their specimens from the Greek Island. One pair of caudal bristles in all larval instars is also a constant feature in all species of the New World genus *Brumptomyia* (Killick-Kendrick *et al.* 1989). The features outlined in Table 5 also distinguished L₄ of the species studied presently from that of *P. langeroni* described by Lane and El-Sawaf (1986).

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Table 1. Hair length (in μm) of L₄ of *P. syriacus*. Data show means \pm standard errors for 5 specimens. For the locality of each hair, see Fig. 1. (The measurement of only 1 or 2 specimens is indicated by*).

Hair No.	Head	Prothorax anterior	Prothorax posterior	Mesothorax	Metathorax	Abdominal segments			
						I-VI	VII	VIII	IX
1	24.8 \pm 2.5	123.6 \pm 8.9	80.0 \pm 12.6	103.6 \pm 16.1	90.9 \pm 14.2	34.5 \pm 7.0	30.3 \pm 5.7	33.3 \pm 8.0	50.0 \pm 30.7
2	133.3 \pm 10.2	66.7 \pm 12.4	122.4 \pm 12.6	134.5 \pm 15.5	133.3 \pm 24.5	33.9 \pm 15.9	20.9 \pm 4.8	36.4 \pm 11.7	60.0 \pm 10.4
3	157.6 \pm 10.3	127.9 \pm 15.8	105.4 \pm 11.6	109.0 \pm 14.4	113.9 \pm 13.0	112.7 \pm 21.4	96.0 \pm 22.2	7.1 \pm 1.4	
4	133.9 \pm 7.2	123.6 \pm 13.6	63.6 \pm 14.2	110.9 \pm 19.2	120.6 \pm 22.4	138.8 \pm 22.3	136.4 \pm 19.4	154.5 \pm 11.9	
5	125.0 \pm 12.0	121.8 \pm 7.8	17.4 \pm 7.6	14.1 \pm 3.4	13.7 \pm 7.5	67.3 \pm 13.1	70.3 \pm 15.9	106.7 \pm 15.1	
6		120.6 \pm 12.2	104.7 \pm 18.3	103.0 \pm 20.8	113.3 \pm 18.8	39.1 \pm 6.3	39.1 \pm 10.8	51.1 \pm 3.7	
7	129.7 \pm 12.6		66.7 \pm 27.7	56.4 \pm 12.6	63.6 \pm 12.1			57.1 \pm 6.4	
8	126.7 \pm 19.3		16.6 \pm 3.1	18.9 \pm 3.5	18.3 \pm 2.1				
9	142.7 \pm 9.8		28.6 \pm 5.3	28.4 \pm 5.3	34.9 \pm 8.5				
IS						10.9 \pm 1.3	11.0 \pm 1.5		
a		6.1 \pm 1.5		7.4 \pm 1.4	7.4 \pm 1.6	12.4 \pm 7.5	13.1 \pm 5.3	9.6 \pm 1.7	
b		18.2 \pm 3.7		24.4 \pm 20.6	N.D.			17.5 \pm 5.1	
c			12.6 \pm 2.3	10.0 \pm 4.4	9.1 \pm 2.6				
d			4.6 \pm 1.3	7.7 \pm 1.6	8.7*				
A						123.2 \pm 15.7	123.3 \pm 18.4		98.8 \pm 4.6
B									321.1 \pm 27.4
C									92.7 \pm 8.7
D									63.0 \pm 9.2
E									43.6 \pm 4.1

Table 3. Hair length (in μm) of L_4 of *P. kazeruni*. Data show means \pm standard errors for 5 specimens except were indicated by (*) when 1-2 specimens were measured. For the locality of each hair, see Fig. 3.

Hair No.	Head	Prothorax anterior	Prothorax posterior	Mesothorax	Metathorax	Abdominal segments			
						I-VI	VII	VIII	IX
1	33.5 \pm 4.6	77.8 \pm 20.2	86.4*	121.2 \pm 15.2	130.3*	191.9 \pm 31.5	103.5 \pm 67.3	119.2 \pm 27.3	58.2*
2	142.4 \pm 10.9	74.7 \pm 12.2	110.1 \pm 14.3	141.4 \pm 21.5	140.9*	194.9*	181.8 \pm 15.2	146.5 \pm 7.6	65.7 \pm 12.6
3	87*	88.9 \pm 3.5	100.0 \pm 8.0	125.7*	141.4 \pm 9.3	180.8 \pm 28.8	175.7 \pm 21.8	14.8	
4	118.2 \pm 10.9	88.9 \pm 9.3	60.6*	90.9 \pm 12.1	10.0 \pm 16.9	144.4 \pm 20.6	141.7 \pm 19.4	130.3 \pm 13.2	
5	117.2 \pm 9.7	98.0 \pm 25.2	31.3 \pm 9.3	7.6*	12.0 \pm 6.2	121.2 \pm 18.9	94.7 \pm 20.2	83.8 \pm 20.2	
6	119.5*	91.9 \pm 22.7	89.4 \pm 15.0	77.8 \pm 3.5	84.8 \pm 8.0	47.5 \pm 15.3	39.4*	86.9 \pm 24.3	
7	78.8 \pm 3.0		75.8 \pm 13.2	72.7 \pm 12.1	76.8 \pm 7.6			91.9 \pm 16.7	
8	96.0 \pm 4.6		21.2 \pm 8.0	25.3 \pm 12.2	29.2 \pm 11.6				
9	88.9 \pm 9.3		46.5 \pm 16.7	46.5 \pm 16.7	45.5 \pm 13.2				
1S						53.0*	43.4 \pm 12.2		
a		15.2 \pm 3.0				51.5*	17.4*		
b		29.3 \pm 6.3		26.3 \pm 16.7	8.1*			7.0*	
c			7.5 \pm 4.3	5.4*				17.2 \pm 3.5	
d									
A						177.3*	161.3 \pm 18.9		118.2*
B									237.0 \pm 6.0
C									93.0 \pm 17.2
D									88.5 \pm 11.1
E									71.7 \pm 5.0

Table 5. A comparison of the specific features of L₄ hairs of *P. syriacus*, *P. mascittii*, *P. kazeruni*, and *P. tobbi* and those described for *P. langeroni* * by Lane and El-Sawaf (1986).

Body part	<i>P. syriacus</i>	<i>P. mascittii</i>	<i>P. kazeruni</i>	<i>P. tobbi</i>	<i>P. langeroni</i> *
Head	- Hair longest and thinnest; hair 6 absent.	- Hair 7 longer than in <i>P. kazeruni</i> ; hair 6 present.	- Hairs 2-5 & 8 longer than those of <i>P. mascittii</i> ; hair 6 present	- Not all hairs recorded.	- Lengths not recorded; hair 6 absent.
Thorax	- All hairs with pointed endings; hair b* spiny; hairs a-d present along all segments.	- Hairs 1-9 with smooth club-shaped endings; prothoracic hair b spiny; hairs a-d present on all segments.	- Dorsal hairs 1-3 with smooth club-shaped endings, ventral ones with pointed endings; prothoracic hair b brush like; hair c on thoracic segments and hair a on the meso and meta-thoracic segments absent.	- Dorsal hairs 1-3 with smooth club-shaped endings; ventral hairs with blunt endings covered by fine hairs; prothoracic hair b spiny; meso and metathoracic segments devoid of hairs a-d.	- Hairs 1-9 with blunt endings covered by fine hairs; hairs b,c, and d on all thoracic segments absent.
Abdominal segments I-VIII-dorsal surface.	- Hairs 1-2 on all segments and hair 1S on segments I-VII distinctly short; hair 1S spatula - shaped; hairs 1 & 2 had blunt ending covered with fine hairs and on segments I-VII with pointed ending have fine hairs; hair 3 on segment VIII minutes and spiny.	- Hair 1 on segments VII and VIII and hair 1s on segments I-VII shorter than those of <i>P. kazeruni</i> and <i>P. langeroni</i> ; 1S hair fan-shaped; hair 3 on segments VIII minute and spiny; other hairs with club-shaped endings.	- All hairs (except hair 3 on segment VIII) long will smooth club-shaped endings; 1S hair brush - like, hair 3 on segment VIII short and covered with smaller fine hairs.	- Hair 1 on segment VIII shorter than that of <i>P. kazeruni</i> * <i>P. langeroni</i> ; hair 1 on segments VII longer than that of <i>P. mascittii</i> ; 1S hairs fan-shaped; hair 3 on segment VIII minute and spiny; other hairs with smooth club-shaped endings.	- All hairs (except 3 on segment VIII) long with blunt endings covered with hairs; hair 1S brush-like; hair 3 on segment VIII slender with fine on hair tip.
Abdominal segments I-VIII-ventral surface.	- All hairs with pointed endings; hair 6 on segments I-VII spiny.	- Hairs 4, 5 and 7 and hair 6 on segment VIII with smooth club-shaped endings; hair 6 on segment I-VII short and brush-like.	- Most hairs with smooth club-shaped endings; hair 6 on segment I-VII brush like and longest.	- Hairs 4, 5 and 7 with blunt endings covered by hairs; hair 6 short and spiny in all segments except segment VIII (blunt brush ending).	- Hair 5, 6 and 7 with blunt endings covered with hairs; hair 6 short and brush-shaped.
Abdominal segment IX.	- Two pairs of caudal bristles, longer than in <i>P. kazeruni</i> and <i>P. mascittii</i> .	- Two pairs of caudal bristles.	- Two pairs of caudal bristles.	- One pair of caudal bristles.	- Two pairs of caudal bristles.

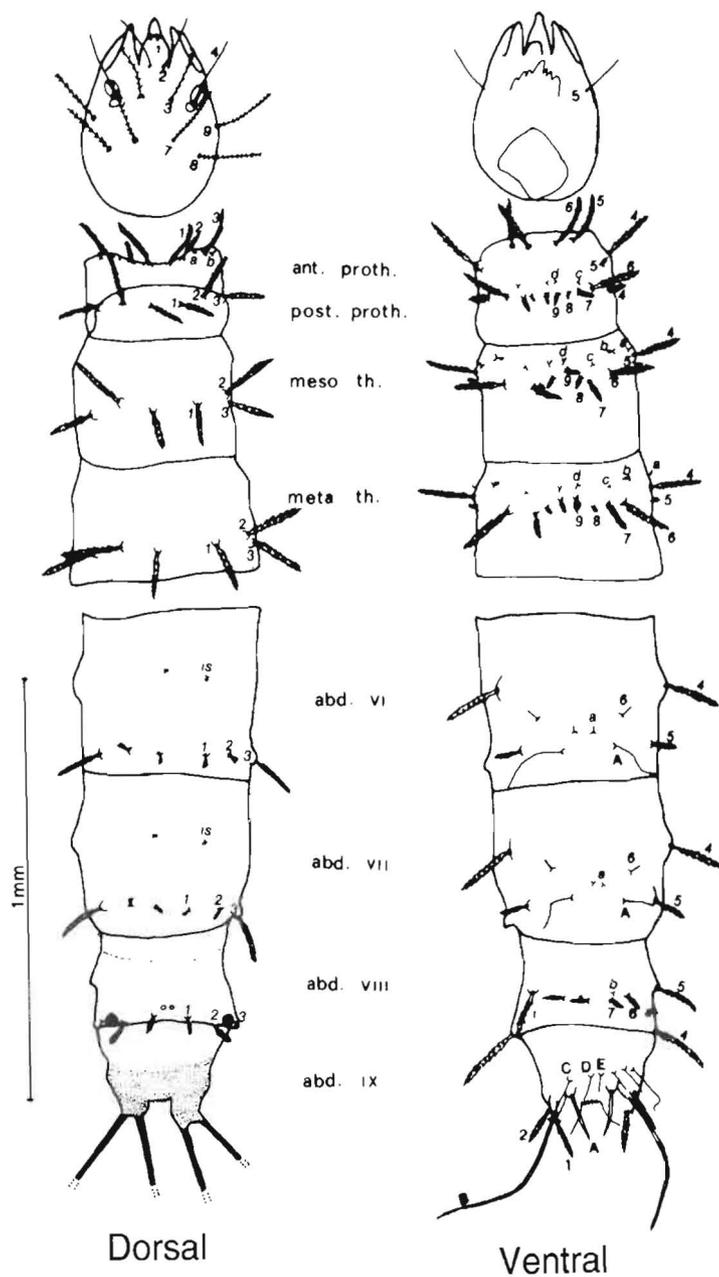


Fig. 1. Chaetotaxy of L₄ of *P. syriacus*.

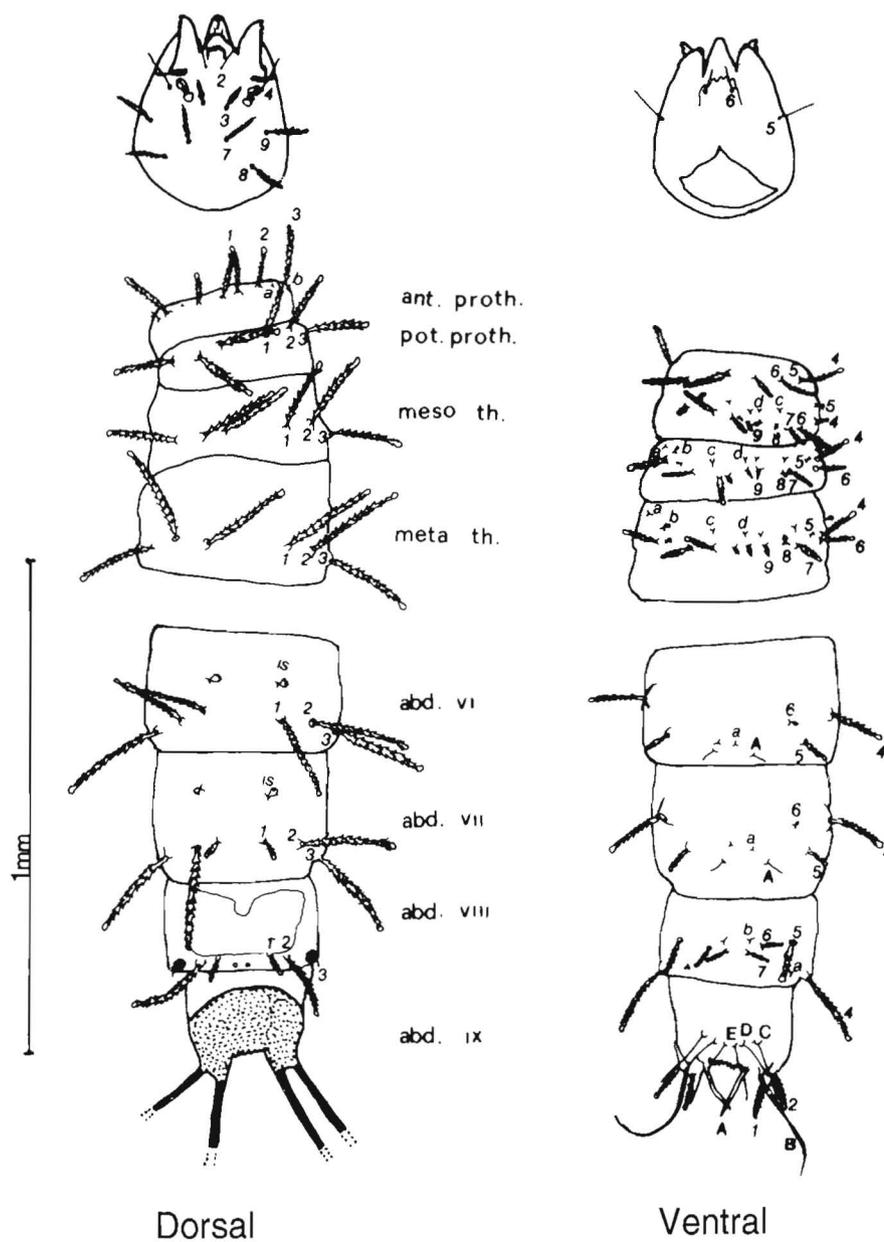


Fig. 2. Chaetotaxy of L₄ of *Pmascittii*.

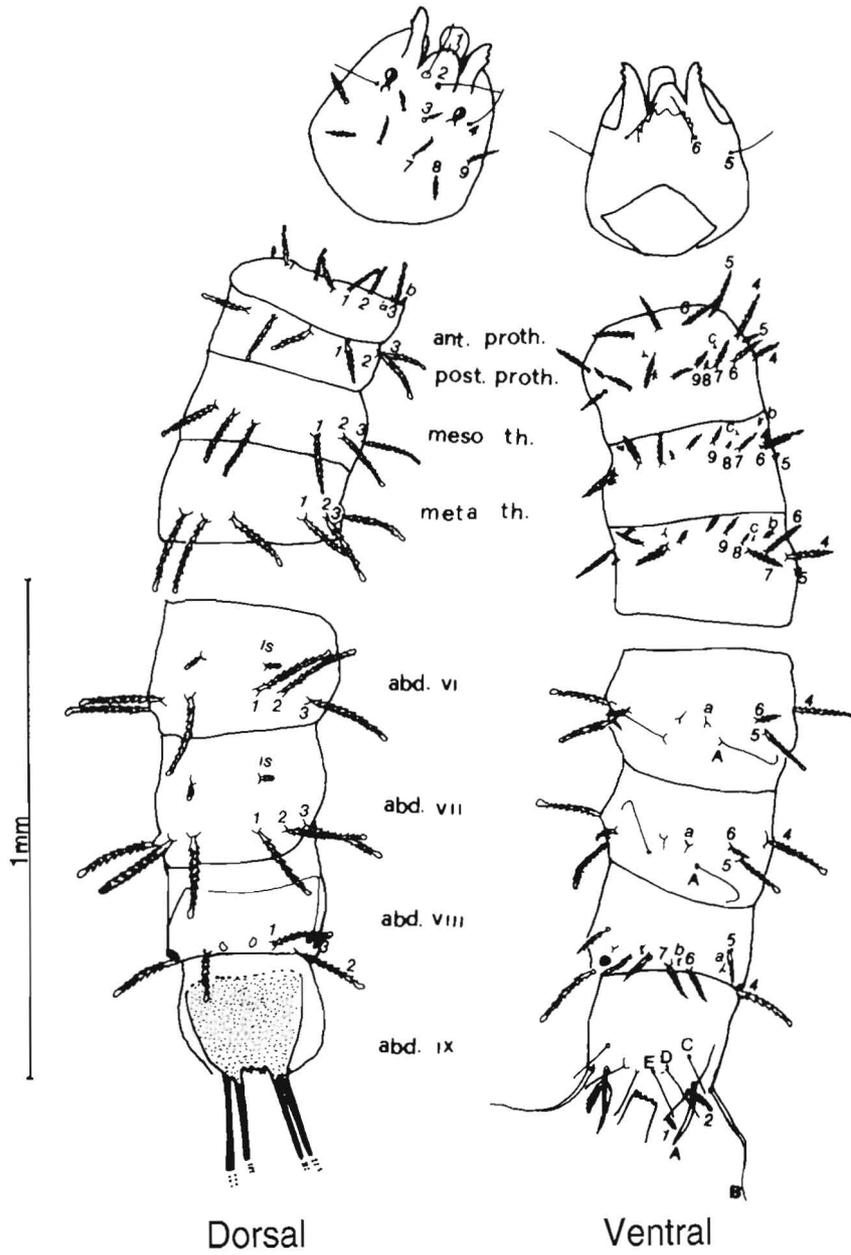


Fig. 3. Chaetotaxy of *L4* of *kazeruni*.

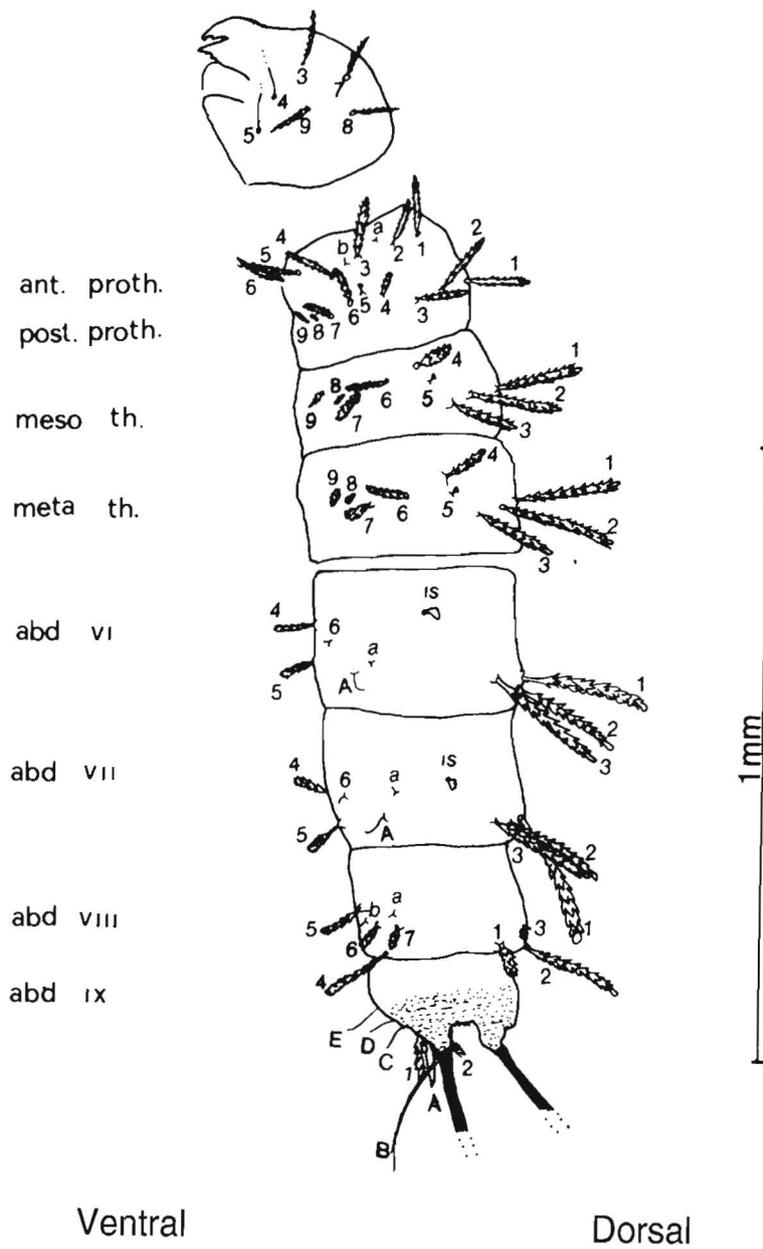


Fig. 4. Chaetotaxy of L₄ of *P. tobbi*.

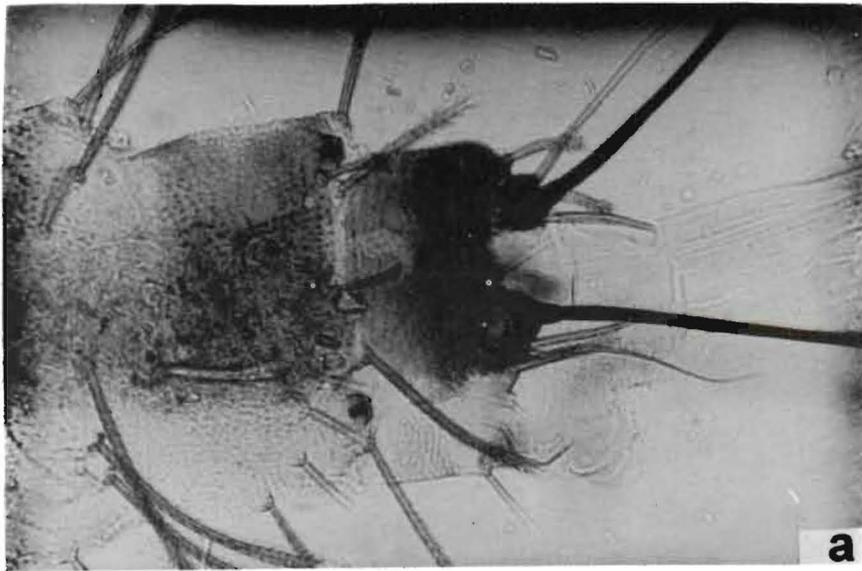


Fig. 5. a. Two caudal bristles in L_4 of *P. tobbi*.

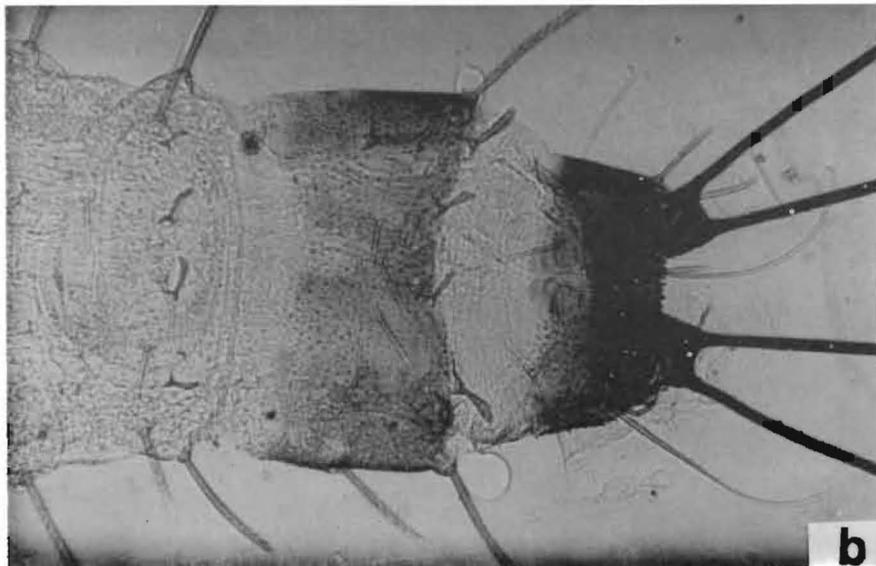


Fig. 5. b. Four caudal bristles in L_4 of *P. syriacus*.

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توزع الأشعار في يرقات الطور الرابع لأربعة أنواع من ذباب
الرمل من جنس *Phlebotomus* (Diptera, Psychodidae)
من الأردن

منى زكي قدومي و سامي خضر عبد الحافظ و شادن عبد السلام قمحاوي

قسم العلوم الحياتية - كلية العلوم - جامعة اليرموك - اربد - الأردن

تبين الدراسة الخصائص الشكلية لتوزع الأشعار في يرقات الطور الرابع لأربعة أنواع من ذباب الرمل من جنس *Phlebotomus* جمعت من الأردن وربيث في المختبر . لقد شكل توزع وأطوال وأشكال الأشعار الموجودة على أجسام يرقات الطور الرابع لأنواع *P.kazeruni*, *P.mascittii*, *P.syriacus* نظاماً يميز كل نوع عن الآخر وقد تميزت ذبابة النوع *P.tobbi* بشفع واحد من الأهلاب الذيلية . وقد قورن التوزع الشعري ليرقات الطور الرابع لهذه الأنواع الأربعة مع مثيله في ذبابة الرمل من النوع *P.langeroni* .