Culex (*Neoculex*) *judaicus* (Diptera: Culicidae), larval description and main characters distinguishing it from larvae of *Culex* (*Neoculex*) *martinii* in Israel

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Abstract: Adults of *Culex judaicus* were described by Edwards in 1926. However, the larva of this species has not yet been described and it was deemed useful to describe details of the larva and compare it with the larva of *Cx. martinii*, both species that occur in northern Israel. Some comparisons are made between the larva and pupa of *Cx. judaicus* and the larva and pupa of *Cx. europaeus*, which was described from Portugal in 2003. ***Journal of the European Mosquito Control Association*** **38: 11–16, 2020**

Keywords: *Neoculex*, *Culex judaicus*, *Culex martinii*, *Culex europaeus*, larval and pupal characteristics.

Introduction

Adult males and females of *Culex judaicus* Edwards were first collected in Palestine in May, 1923, by Buxton (1924), and identified as *Cx. apicalis* Adams from "Wadi Kabala, 10 miles west of Jerusalem" (En Hemed of today). They were later described by Edwards (1926) as *Cx. apicalis* var. *judaicus*. Subsequently, Mattingly (1953) considered these specimens to be sufficiently different from both *Cx. apicalis* and *Cx. territans* Walker to be considered as a distinct species, *Cx. judaicus*. It was later described in slightly more detail by Senevet and Andarelli (1959), also based on the original material collected in Palestine and deposited in the Natural History Museum, London.

There is some confusion in the older literature concerning the status of *Cx. apicalis*, now known to be confined to the southern United States, and *Cx. territans* which is distributed in Canada, the northern United States and possibly also across Europe, Caucasus, Middle East and North Africa (Becker *et al*., 2003; Robert *et al*., 2019). However, after the re-examination of the widespread *Cx. territans* from Portugal, a new species of the subgenus *Neoculex*, *Cx. europaeus* da Cunha Ramos, Ribeiro & Harrison, was described and named after its presumed area of distribution (da Cunha Ramos *et al*., 2003). *Culex europaeus* has also recently been reported from western Spain by Bravo-Barriga *et al*. (2017), thus it is possible that some of the "*Cx. territans*" recorded from across Europe will turn out to be *Cx. europaeus*.

Culex judaicus has so far only been recorded from Israel, Jordan and Lebanon. Another species of the subgenus *Neoculex*, *Cx. martinii* Medschid, first described from Turkey in 1930, has also been found in the northern half of Israel in the same region as *Cx. judaicus*. *Culex martinii* is also known from several central and southern European countries as well as Caucasus, middle Asia (Uzbekistan, Tajikistan and Kyrgyzstan), Egypt and Morocco (Robert *et al*., 2019).

A description of the *Cx. judaicus* larva is given here, based on material newly collected in the field and raised to adults, as well as preserved specimens. Since the larvae of *Cx. judaicus* and *Cx. martinii* are quite similar, a comparison of the main distinguishing features was made. In addition, pupal features described for *Cx. europaeus* by da Cunha Ramos *et al*. (2003) are compared with those of *Cx. judaicus*.

Materials & Methods

Collections of both adult and larval mosquitoes during ongoing surveys by the Ministry of Health (MoH), Jerusalem were examined from various sites all over Israel, as well as some larval specimens in the MoH collections. While adult *Neoculex*, particularly males, were rarely collected in standard CDC traps using dry ice as bait, the larvae of *Cx. judaicus* and *Cx. martinii* were found fairly frequently in northern Israel. Some of the few adults trapped were pinned, while others were kept frozen for later study. Field collected larvae, preserved in 70% ethanol and thought to be of the subgenus *Neoculex*, were normally mounted on slides in a drop of Hoyer's solution and examined microscopically. In addition, some pinned adults and slides of larval specimens were borrowed from both the Natural History Museum (NMH), London, and from the US National Museum of Natural History (USNM), Smithsonian Institution, Washington, D.C.

Adults of *Cx. judaicus* have been recorded from five sites: En Hemed, Judean Hills (the presumed type locality), Theodor's specimens, collected in April and May 1947 (Tel Aviv University collections) and Buxton's original specimens from "Wadi Kabala", collected in 1923 (NHM); Lotem, Lower Galilee, collected June 1984 (USNM); Elon, Upper Galilee, collected June 1984 (USNM); Nahal Dalyiot, Lower Golan, collected November 2006 (MoH); En Meholelim, northern Samaria, collected September 2008, June 2009, July 2011 (MoH).

An examination of larval specimens of *Cx. judaicus* on slides in the MoH collections, identified as "*Cx. apicalis*", from the 1970s and 1980s, as well as from more recent collections, allowed the identification of *Cx. judaicus* from about 30 sites in northern Israel: the Carmel area (En Alon, En Hod), Central Coastal Plain and Yizre'el Valley (En Ramat-Yishay), northern Samaria (En Meholelim, En HaShofet, En Tut), Lower Galilee (Lotem*, Nahal Zalmon, En Tovim, Enot Zippori), Upper Galilee (Hanita, Nahal Betzet, En Tamir, En Ziv, N. Keziv, Elon*, Kabri, En Merav, N. Ga'ton, Peki'in, En Avel*, N. Iyyon, Ghajar, Tel Dan seepage) and the Golan Heights (Nahal Dalyiot, N. Meshushim, N. Zavitan, Banias, Umm el Kanatir). Additional larvae were collected from En Meholelim and raised to adults in the laboratory.

Both larvae and adults of *Cx. martinii* were recorded from the springs of Tel Nagila* in the Northern Negev, south of

Kiryat Gat, in southern Israel (Margalit *et al*., 1987, 1988), collected in August 1983 and May 1984. Since the 1990s, both adults and larvae of *Cx. martinii* have been collected sporadically at various sites mainly in northern Israel, the southernmost sites being Nahal Gerar and N. Shiqma* in the Northern Negev and Enot Gibbeton in the Judean foothills. Larvae newly collected from Enot Gibbeton were raised to adults in the laboratory. Other collection areas include: Central Coastal Plain (Nahal Yarqon, Talmei Elazar); Yizre'el Valley (N. Midrakh); Bet She'an Valley (N. Amal, En Malkoah); the Galilee (HaZore'im, Ne'ot Mordekhay) and the New Hula Lake area. Some larval specimens on slides in the MoH collections, originally identified as "*Cx. apicalis*", were examined and re-identified as *Cx. martinii* from other sites in the same general area, including Akko, the northern entrance of the Jordan River into Lake Kinneret, Hulata, En Bedolah and En Nucheila* near Kibbutz Dan in the Upper Galilee. Where indicated with an asterix (*), specimens examined include material borrowed from NHM (En Avel) and from USNM (Lotem, Elon, Nahal Shiqma, Tel Nagila and En Nucheila).

Results and discussion

The distribution of *Cx. martinii* in Israel is wider than and overlaps that of *Cx. judaicus*, and spans the springs of the Western Negev, the Coastal Plain, the Yizre'el Valley and the Jordan Valley from the Bet She'an area northwards to the Hula Valley and Kibbutz Dan. However, the two species have not yet been found in the exact same habitats, see Figure 1.

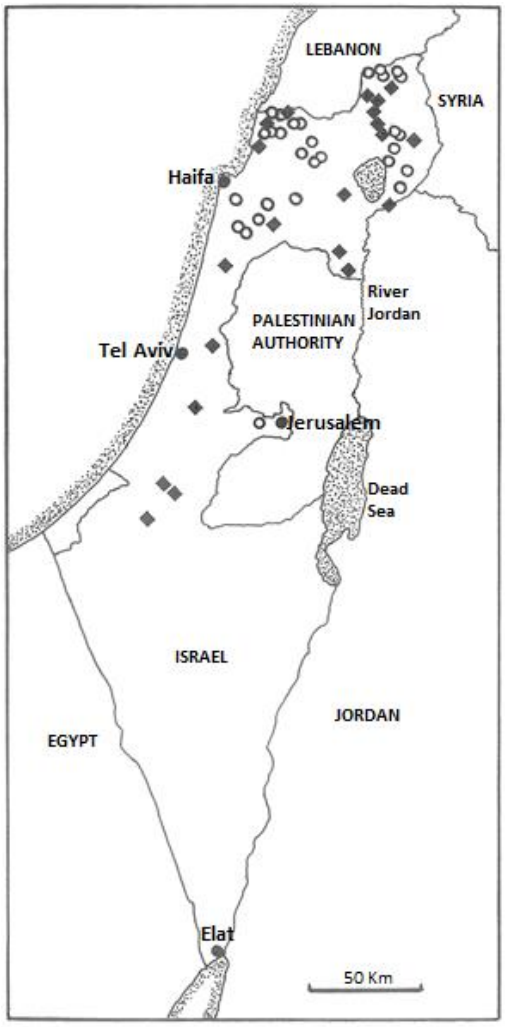

Figure 1: Sampling sites of *Cx. judaicus* (open circles) and *Cx. martinii* (closed diamonds) in Israel.

Adults of *Cx. judaicus* and *Cx. martinii* are fairly easily distinguished by differences in, for example, patterns of the abdominal terga and hindfemur, see Table 1.

Table 1: Main features used to separate adult female of *Cx. judaicus* and *Cx. martinii.*

	Cx. judaicus	*Cx. martinii*
Abdominal terga	Terga very dark, almost black, with narrow white apical bands, 1 to 2 scales deep, which may be interrupted in the middle. The apical bands are produced laterally into small pale triangles.	Terga dark-brown, without pale apical bands, but with pale apical triangles seen laterally on terga IV–VII, barely visible dorsally.
Femora	Dark anterior stripe extends about 7/8 of hindfemur length. Apical ends of femora with a few white scales forming "knee-spots".	Dark anterior stripe extends whole length of hindfemur. Knee-spots absent.
Prealar scales	Lower prealar scales present.	Prealar scales absent.

However, it is usually necessary to make slide preparations of *Neoculex* larvae, easily recognised by their distinctive long and narrow siphon, in order to examine details of head setae 5- and 6-C, the dorsomentum, the ventral brush (4-X) and the pecten spines. Although there is a great deal of overlap in many of the larval features of the two species, a combination of some or all of the six main characters shown in Table 2 is generally sufficient to assign a fourth-instar larva to one or other of the species. Characters 2 and 3 are perhaps the most useful and constant in distinguishing *Cx. judaicus* and *Cx. martinii* in Israel.

Concerning these six characters, there are no data for *Cx. europaeus* from Portugal (da Cunha Ramos *et al*., 2003) for characters 2, 3 and 5. Regarding character 1, *Cx. judaicus* and *Cx. europaeus* are similar with 6-C usually single and 5-C with 1 or 2 branches. Regarding character 4, *Cx. judaicus* is similar to *Cx. europaeus*, often with the pecten spines with a single denticle, although no specimens of *Cx. judaicus* have been found with *all* pecten spines with only a single denticle as was reported for *Cx. europaeus*. Regarding character 6, *Cx. judaicus* is quite similar to *Cx. europaeus*, where the dorsal anal papillae are as long as the saddle and the ventral papillae are slightly shorter. However, the usual condition for *Cx. judaicus* is with papillae 0.67 to 0.75 the length of the saddle.

Description of the fourth-instar larva of Culex (Neoculex) judaicus.

The description is based on material from En Meholelim, Lotem, N. Betzet, Enot Zippori, En Merav, Elon, N. Meshushim and N. Zavitan. See also Figure 2.

Head: Length 0.60–0.84 mm, often 0.68–0.74 mm, mean 0.72 mm; width 0.95–1.15 mm, usually 1.03–1.10 mm, mean 1.05 mm. Colour patterns on dorsal apotome (not always seen) consist of a small, round, darkish patch midway between setae 5-C and a larger, median, oval patch posterior to this; 2 lateral oval patches anterior to seta 8-C, forming large indistinct

triangles which may merge with the central oval patch or be divided into 3 distinct patches; most common formula of dorsomentum is 1-3-2-1 or 1-3-2-½ (for details, see Table 2); seta 1-C heavily sclerotized, stout and pointed, as thick at base as 6-C, length 75–125 µm, usually 98–110 µm; seta 4-C single, delicate, sometimes split in distal half; seta 5-C with 1 or 2 branches, usually 2, long and slightly aciculate; seta 6-C single or double, usually single, very long, and slightly aciculate, longer than 5-C; ratio length of 5-C to length of 6-C, 0.4–0.65 of 6-C, usually about 0.55; seta 7-C with 4–11 branches, usually 5–7; seta 8-C with 2–5 branches, usually 3 or 4; seta 9-C with 2–6 branches, usually 3–5; seta 10-C single, occasionally double, usually split into 2 or 3 branches distally. **Antenna:** Length about same as head length or slightly shorter, 0.59–0.69 mm, mean 0.65 mm; dark at base and from just before seta 1-A insertion to tip; proximal spines slender and transparent; spines around seta 1-A insertion and in distal part strongly sclerotized and shorter but stouter than proximal ones; seta 1-A insertion at 0.69–0.76 from antennal base, mean 0.72; seta 1-A with 23–37 branches, usually 28–35, mean 31. **Thorax**: Integument densely spiculate; seta 1,2-P always long, strong and single, slightly aciculate; seta 3-P delicate and much shorter than 1,2-P with 2–5 branches, usually 2 or 3, rarely 4 or 5. **Abdomen:** Integument lightly spiculate; seta 6-I,II almost always with 3 branches (rarely 2); seta 6-III–VII always with 2 branches; seta 2,4-VIII always single; seta 1-VIII with 2–7 branches, usually 3–5, commonly 4 or 5; seta 3-VIII with 6–11 branches, usually 7–9, most often 8 or 9; seta 5-VIII with 2–5 branches, usually 3 or 4, mode 3. Comb of segment VIII with 40–74 scales each with rounded apical fringe, usually 50–60 scales, average 53. **Siphon:** Darkened, long, slender and tapering, slightly but distinctly widened at apex; siphon index 6.5–10.1, usually 8 to 9, mean 8.4; siphon length/pecten length 3.0–6.0, usually 3.5–4.5, mean 4.1; pecten with 9–20 spines, usually 13–17, mode 15, distal spines sometimes more widely spaced; spines with 1–4 denticles, usually 1 or 2 (1–3 spines closest to siphon base may have 3 or 4 denticles, but 4 are rare); seta 1-S inserted distal to pecten, comprising 8–12 posterolateral setae, usually 9 or 10, imperfectly paired; seta 1-S with 1–4 branches, usually 2 or 3, mode 2; ratio of length of first pair of 1-S to siphon width at point of insertion 1.1–2.9, usually 1.4–1.9. **Segment X:** Saddle well sclerotized and clearly spiculate; strongest spicules on distal dorsal area near insertion of setae 2,3-X; seta 1-X/saddle length: 0.45–0.9, usually 0.55–0.65, average 0.6; seta 1-X with 1–3 branches, usually 2, occasionally 1 or 3; seta 2-X with 3 or 4 unequal branches, usually 4, occasionally 5; seta 3-X single; seta 4-X with 12–15 setae, usually 14, occasionally 13 or 15, rarely 12; mode 14; most setae borne on a grid (cratal setae) each with 4–7 branches; 1 or 2, rarely 3, short precratal setae, each with 1 or 2 branches. Dorsal anal papillae length/saddle length: 0.5–1.1, usually 0.65–0.75, mean 0.7; ventral pair slightly shorter.

Table 2: Features distinguishing fourth-instar larvae of *Cx. judaicus* and *Cx. martinii*.

Cx. judaicus	*Cx. martinii*
1. Head setae: 5-C single or double, usually double (mode 2); 6-C single or double, usually single.	1. Head setae: 5-C with 2–5 branches, usually 2 or 3 (mode 2 or 3, depending on population); 6-C single or double, usually double.
2. Dorsomentum: Most common formula is: 1-3-2-1 or 1-3-2-½ (smallest tooth only on one side). May also occasionally be 1-3-2 or 1-3-3, or rarely, 1-2-2, and once, 1-4-2-1. [1 = central tooth, 3 = 3 smaller teeth on either side of central tooth, 2 = 2 slightly larger teeth on either side and 1 = smaller, outermost tooth on either side.]	2 .Dorsomentum: Most *Cx. martinii* from Israel have the formula of 1-3-3-1, but may be 1-3-3-½ (smallest tooth only on one side), or 1-3-3. Occasionally also 1-3-2-1 or 1-2-3-1. [Medschid found 1-3-3-1, where 1 = central tooth, 3 = 3 smaller teeth on either side of central tooth, 3 = 3 slightly larger teeth on either side and 1 = smaller, outermost tooth on either side.]
3. Ventral brush, 4-X: setae range from 12 to 15 but most commonly 14, occasionally 13, and rarely 12 or 15 (mode 14). 1–3 short precratal setae, usually 2.	3. Ventral brush, 4-X: setae range from 10 to 13 but usually 12 (mode 12). 0 or 1 short precratal seta.
4. Denticles of pecten spines: range 1–4, usually 1 or 2, but spines close to base of siphon *may* have more; 4 are rare.	4. Denticles of pecten spines: range 1–7, usually 3 or 4, but may be 5 or 6 denticles on spines at base of siphon.
5. Siphon length/pecten length: range 3.0–6.0, usually 3.5–4.5, mean 4.1.	5. Siphon length/pecten length: range 4–6, usually 4.5–5.5. In type material from Turkey, pecten is 0.2 of siphon length (Medschid).
6. Dorsal anal papillae length/saddle length: range 0.5–1.1; usually 0.65–0.75, mean 0.7.	6. Dorsal anal papillae length/saddle length: range 0.3–1.0; usually 0.4–0.6. Papillae of type material are 0.5 of saddle length (Medschid).

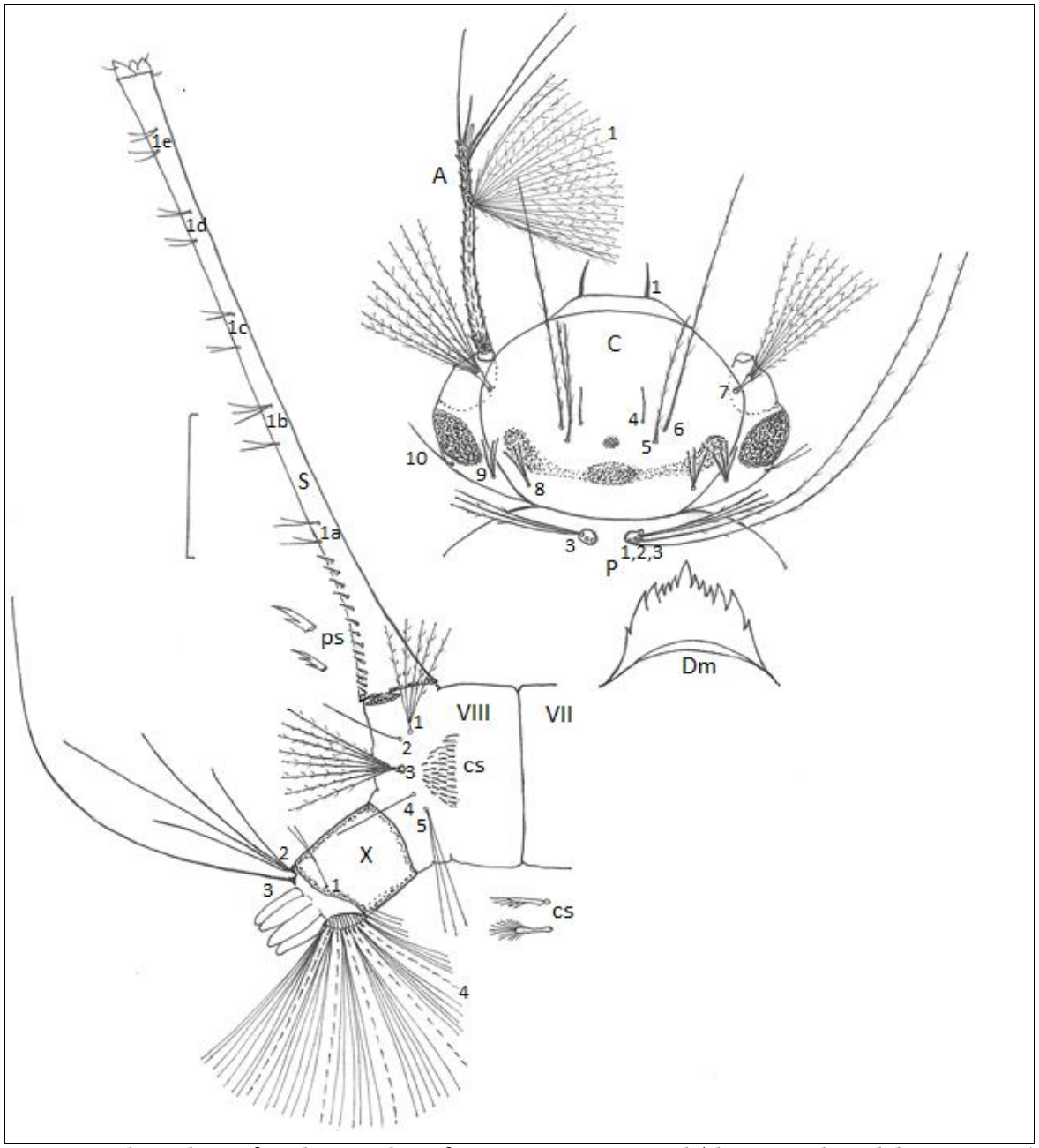

Figure 2: *Culex judaicus*, fourth-instar larva from Enot Zippori. Head (showing enlarged dorsomentum) and terminal abdominal segments (showing enlarged comb scales and pecten spines).
A = antenna; C = head; cs = comb scales; Dm = dorsomentum; P = prothorax; ps = pecten spines; S = siphon; VII, VIII, X = abdominal segments. Scale bar = 0.5 mm.

Notes on significant differences between pupae of Cx. europaeus *and* Cx. judaicus

In distinguishing *Cx. europaeus* from *Cx. territans*, da Cunha Ramos *et al.* (2003) noted that several of the larval characters of *Cx. territans* are highly variable, including the following: the number of branches of seta 5-C and 6-C; the length of 5-C in relation to 6-C; the siphon index; the number and length of seta 1-S on the siphon; the number of denticles of individual pecten spines; and the presence or absence of banding patterns on the abdominal terga. Thus, the authors did not try to separate *Cx. europaeus* from *Cx. territans* using larval features. However, these authors noted promising features of the pupa of *Cx. europaeus* that distinguish it from the pupa of *Cx. territans*. It was therefore decided to compare the pupal features recorded for *Cx. europaeus* with those of *Cx. judaicus* from Israel (Table 3).

It is clear from Table 3 that pupae of *Cx. judaicus* and *Cx. europaeus* are similar but with many overlapping differences in chaetotaxy. However, several of the characters listed in Table 3 point to more significant differences between the two species, in particular the pinna index, the branching of abdominal setae 1-III, 1-IV, 1-V, 5-IV, 9-VII, and 9-VIII, and the paddle index. The pinna index is greater in *Cx. judaicus*; the number of branches of abdominal setae 1-III–V, while overlapping, are generally more numerous in *Cx. judaicus* than in *Cx. europaeus*; there are differences in the branching and lengths of abdominal setae 5-IV, 9-VII, and 9-VIII; and the paddle index is generally smaller in *Cx. judaicus*. These eight characters are marked in bold in Table 3. Da Cunha Ramos *et al.* (2003) used the number of branches of abdominal seta 1-III–V, diagnostically in part, to separate *Cx. europaeus* and *Cx. territans*, which in the latter species, has fewer branches in each case, while the largest numbers were seen in *Cx. judaicus*.

No definite conclusions can be made at this time concerning the similarity between the larvae of *Cx. judaicus* and *Cx. europaeus*. However, it is clear that there are significant differences between the pupae of the two species. These differences are of the same order of magnitude as those used by da Cunha Ramos *et al.* (2003) to distinguish pupae of *Cx. europaeus* and *Cx. territans*.

Table 3: Comparison of pupae of *Cx. judaicus*, based on material mainly from En Meholelim, with pupae of *Cx. europaeus*, from description in da Cunha Ramos et al., 2003.

	Cx. europaeus	*Cx. judaicus*
Cephalothorax	Moderately dark	Moderately dark
Seta 8-CT	2–5 branches, usually 3 or 4	3–5 branches, usually 3 or 4, mode 4, (n=29)
Seta 9-CT	Always double	Usually double, sometimes triple (7/20, n=20)
Trumpet	Very dark, somewhat narrowed in middle	Very dark, slightly narrowed just before middle, near end of tracheoid area
Trumpet index	8–10, usually about 9	8–12.4, usually 10–12, mean 10.7 (n=46)
Tracheoid area	Up to 0.5 of trumpet length	0.43–0.5 of trumpet length, mean 0.46
Pinna index	**Short, ± 0.15 trumpet length**	**0.17–0.25 trumpet length, mean 0.21**
Metathorax		
Seta 10-M	5–8 branches	3–6 branches, usually 4 or 5 (n=28)
Seta 11-M	Double	Double (except one specimen with single but very thick seta) (n=36)
Seta 12-M	2–4 branches	3 or 4 branches, usually 3 (25/32, n=32)
Abdomen		
Seta 1-III	**8–13 branches**	**7–17 branches, usually 11 or 12 (n=40)**
Seta 1-IV	**8–10 branches**	**6–13 branches, usually 9 to 11 (n=34)**
Seta 1-V	**5–8 branches**	**4–10 branches, usually 6 to 8 (n=31)**
Seta 1-VI	4 or 5 branches	3–8 branches, usually 3–5 (n=21)
Seta 1-VII	2–4 branches (given as seta 1-VIII in da Cunha Ramos *et al.*)	2–6 branches, usually 3 or 4 (n=21)
Seta 4-I	4–6 branches	2–5 branches, usually 3 or 4 (n=32)
Seta 4-II	No data	3 or 4 branches, usually 3 (n=11)
Seta 4-III	No data	2–5 branches, usually 3 (n=12)
Seta 4-VIII	No data	1 or 2 branches, usually 2 (n=45)
Seta 5-I	No data	2 or 3 branches (n=9)
Seta 5-II	4–7 branches	3–6 branches, usually 4 (n=27)
Seta 5-III	5–7 branches	4–9 branches, usually 6 (n=28)
Seta 5-IV	**3 or 4, long branches reaching beyond end of segment V**	**4–6 branches, usually 5 (n=24), reaching to about 0.75 length of segment V**
Seta 5-V	2 long branches, reaching beyond middle of segment VII	2 branches, reaching to middle of or beyond segment VII
Seta 5-VI	2 long branches, reaching beyond middle of segment VIII	2 branches, reaching to middle of or beyond segment VIII
Seta 6-III	No data	2–6 branches, usually 4 (n=18)
Seta 6-IV	3–5 branches	3–6 branches, usually 4 or 5 (n=35)
Seta 6-V	No data	3–6 branches, usually 4 or 5 (n=32)
Seta 6-VI	4 or 5 branches	3–6 branches, usually 5 (n=36)
Seta 6-VII	2–4 branches	2–4 branches, usually 2 (n=40)
Seta 9-VII	**2 branches, small, ± 0.5 length of segment**	**2 or 3 branches, usually 2 (n=24), reaching ± 0.5 to 0.75 length of segment**
Seta 9-VIII	**3 branches ± 0.5 length of segment**	**2–4 branches, usually 3 (n=26), some 2-branched but split into 3 or 4 branches, ± 0.75 length of segment**
Paddle	Unpigmented, midrib not reaching apex; outer border minutely and sparsely spiculate on distal half	Unpigmented, midrib not reaching apex, no spicules observed on outer, distal border
Paddle index	**1.59–1.74, mean 1.68**	**1.33–1.64, mean 1.49 (n=24)**
Seta 1-Pa	Always present, very small	Always present, very small
Seta 2-Pa	Always present, minute	Always present, minute, ± 0.75 length of 1-Pa

Ecological notes

The larvae of *Cx. judaicus* were typically found in shaded areas of small, slow-flowing streams of spring-fed water with emergent vegetation. *Culex judaicus* larvae have been recorded in Israel between February and July as well as October and November. The larvae of *Cx. martinii* were found at Enot Gibbeton in quiet, heavily shaded water with dense stands of *Phragmites*. There are records of *Cx. martinii* larvae in Israel throughout the year except in February and October.

Culex judaicus has so far been recorded only from Israel, Jordan and Lebanon. In Lebanon, a total of 22 females and 12 males were collected from two sites in the area of Mt. Lebanon in the north and one site in the south (Knio *et al*., 2005). In Jordan, six larvae were collected from a single site of Ain Rahoob in 1996 (Amr *et al*., 1997; Al-Khalili *et al*., 2000), although in the second paper they were identified as *Cx. territans*, and it was noted that the existence of both species are to be expected in Jordan. In both Jordan and Lebanon, *Cx. judaicus* was considered rare and was the only species of the subgenus *Neoculex* to be recorded at that time. More recently, however, *Cx. martinii* was recorded in small numbers from Lebanon (Zakhia, 2017).

While records of feeding behaviour of species of the subgenus *Neoculex* are scarce, none are known to take a blood meal from humans (Becker *et al*., 2003). Edman (1974) analysed feeding patterns of some Florida mosquitoes and noted that *Cx. territans* feeds largely (75–90%) on amphibians, particularly frogs, but 10–25% had fed on birds, small mammals (rabbit), as well as reptiles (lizard and snake). More recently, Shepard *et al.* (2016) recorded that *Cx. territans* obtained blood meals from both avian and amphibian hosts in the USA. In addition, mixed blood meals originating from both avian and mammalian hosts were also identified from *Cx. territans* and the authors suggested that this species may contribute to the epizootic transmission of eastern equine encephalitis virus. It thus seems likely that both *Cx. judaicus* and *Cx. martinii* feed mainly on amphibians but also possibly on birds and small mammals like some other members of the subgenus.

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