

## CROSSMATING AND MORPHOLOGICAL STUDIES OF *CULEX NEAVEI* AND *CULEX PEREXIGUUS* (DIPTERA: CULICIDAE) TO ELUCIDATE THEIR TAXONOMIC STATUS<sup>1</sup>

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**ABSTRACT.** Comparative studies were conducted on *Culex perexiguus* and *Cx. neavei*, two members of the medically important *univittatus* complex, to determine if they represent the same or different species. Specimens of *neavei* from countries south of the Sahara were compared with specimens of *perexiguus* from countries in northern Africa and southwestern Asia. Slight and variable differences were observed in the ornamentation of adults, males differed constantly in a single feature of the genitalia, and several tenuous distinctions were noted in larvae. A high degree of unidirectional incompatibility occurred when *neavei* females from South Africa were mated with *perexiguus* males from Egypt. It is concluded that *perexiguus* and *neavei* represent different, largely allopatric species.

### INTRODUCTION

The *univittatus* complex of the subgenus *Culex* includes three nominal forms: *univittatus* Theobald, 1901 (type locality Salisbury, Zimbabwe), *perexiguus* Theobald, 1903 (type locality Sidon, Lebanon), and *neavei* Theobald, 1906 (type locality Lualas, Sudan). The nominotypical form appears to be restricted to the temperate highlands of southern and eastern Africa and the southwestern corner of the Arabian Peninsula. *Culex neavei* seems to occur throughout the subtropical and tropical lowlands south of the Sahara. *Culex perexiguus* inhabits the arid areas of northern and eastern Africa and southwestern Asia eastward into India.

*Culex neavei* was originally described as a variety of *univittatus*. It was elevated to specific status based on morphological discontinuity and reproductive isolation (Jupp 1971). *Culex perexiguus* was synonymized with *univittatus* by Edwards (1912), treated as a form of *univittatus* by Mattingly (1954), and recognized as a variety of this species by Mattingly and Knight (1956). Jupp (1972) performed a morphological comparison between *neavei* and specimens attributable to *perexiguus* and showed that the two forms were distinguishable by minor characteristics of the male genitalia. Two other differences between *neavei* from South Africa and *perexiguus* (as *univittatus*) from Egypt became evident during laboratory colonization and vector competency tests involving West Nile and Sindbis viruses. While *perexiguus* readily adapts to mating in 30-35 cm<sup>3</sup> cages under normal laboratory conditions

(Hurlbut and Weitz 1956, Gad et al. 1987), *neavei* does not and requires a simulation of crepuscular lighting similar to that required by *univittatus* in South Africa (Jupp and Brown 1967, Jupp 1971). *Culex perexiguus* in Egypt is a superior vector of both West Nile and Sindbis viruses (Hurlbut 1956, Taylor et al. 1955) as compared with *neavei* in South Africa (Jupp et al. 1986).

Jupp (1972) suggested that *perexiguus* and *neavei* may represent sibling species, but added that further morphological and crossing experiments were necessary before the taxonomic status of these forms could be settled. White (1975) apparently disregarded this statement when he recognized *perexiguus* as a valid species in his "Notes on a Catalog of Culicidae of the Ethiopian Region." White stated that "*perexiguus* is now reinstated to full species rank, on the evidence marshalled by Jupp (1971, 1972)." But since the evidence presented by Jupp was meager and inconclusive, the status of *perexiguus* and *neavei* remained uncertain. In this paper we describe the results of morphological studies and crossmating experiments which support the contention that these nominal forms represent different species.

### MATERIALS AND METHODS

**Morphology.** Morphological studies were conducted on specimens deposited in the National Museum of Natural History (Smithsonian Institution), the British Museum (Natural History), and the London School of Hygiene and Tropical Medicine. A total of 253 specimens of *neavei* (111 females, 52 males, 44 male genitalia, 14 pupal exuviae, 18 larval exuviae, and 14 fourth-instar larvae) were examined from 15 countries south of the Sahara. Some 2,696 specimens of *perexiguus* (735 females, 474 males, 92 male genitalia, 895 pupal exuviae, 446 larval exuviae, and 54 fourth-instar larvae) were examined from seven countries in southwestern Asia and nine in northern Africa (Harbach 1988). Ethiopia, Sudan, and Upper Volta were the only countries from which specimens of both forms were

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examined. The morphological terminology and the abbreviations used in the figures follow Harbach and Knight (1980).

**Crossing experiments.** Virgin mosquitoes drawn from laboratory colonies of *neavei* (F<sub>4</sub>) and *perexiguus* (F<sub>29</sub>) were used for the crosses. The *neavei* colony was started with specimens collected at Mtubatuba, northern coastal Natal, South Africa and the *perexiguus* colony was started with specimens collected at Kom Ombo, Egypt (collection number 055 of Harbach et al. 1989). Reciprocal crosses were done in duplicate in 35 cm<sup>3</sup> cages using adult mosquitoes not more than seven days old. From 221 to 269 mosquitoes of each sex were placed in each cage. The cages were maintained at 25-26° C and 75-80% RH. The daily light-cycle was provided by controlled artificial lighting and consisted of a day period of 12 hours, a night period of 10 hours, and twilight periods of 60 minutes each when the light intensity was either gradually increased (dawn) or decreased (dusk). Hamsters were exposed to the mosquitoes as a source of blood. Gravid mosquitoes were placed individually in tubes for oviposition 5-6 days after feeding.

Hybrid progeny resulting from the crosses were backcrossed with the parental strains. These crosses were performed under the same conditions as the primary crosses. The mosquitoes were 1-13 days old.

The larvae and adult males resulting from all of the crosses were examined for evidence of abnormal morphology. Larvae and male genitalia were mounted on slides and compared with specimens from the parental colonies.

Egg rafts were placed in cedar oil and examined under a stereomicroscope to determine the percentage of hatched, unhatched-infertile (unembryonated), and unhatched-embryonated eggs. For purposes of discussion, rafts containing eggs that hatched are termed "viable rafts" while those containing only unhatched eggs are termed "nonviable rafts." Spermathecae of a sample of females used in the crosses were examined for the presence of sperm.

## RESULTS

**Morphology.** *Culex neavei* and *perexiguus* are remarkably similar in all life stages. Slight and variable differences between the adults of these forms and *univittatus* were listed previously by Harbach (1988, Table 41). Differences observed between *neavei* and *perexiguus* are compared in Table 1. The most reliable distinction between females of these forms is the presence or absence of pale scales at the base of the costa. A short line of pale scales is present in *perexiguus* and apparently absent in *neavei*. Males of these forms appear to differ constantly in a single character of the genitalia, i.e., the length of the ventral arm of the lateral plate. The ventral arm (VA) is always long (projecting beyond distal margin of lateral plate, LP) in *neavei* (Fig. 1) and short (not projecting beyond distal margin of LP) in *perexiguus* (Fig. 3).

Too few larval and pupal specimens of *neavei* were available for detailed study. Most of the available specimens were from South Africa and no significant differences were found to distinguish them from larval and pupal specimens of *perexiguus* from southwestern Asia and northern Africa (primarily Egypt). The pupae of these forms appear to be indistinguishable (cf. Figs. 1 and 3) and only vague and variable differences were noted in the larvae (cf. Figs. 2 and 4). Equivocal morphological differences between the larvae of these forms include the following: (1) pecten spines (PS) with more denticles in *neavei*; (2) seta 1-S usually in 5.5 or 6.0 pairs in *neavei* (5.0-6.5) and 5.0 pairs in *perexiguus* (4.5-6.0); (3) seta 2-I frequently (frequency of nearly 50%) inserted directly anterior or anterior and slightly lateral to seta 3-I in *perexiguus* (as shown in Fig. 4) and anterior and normally well mesad of 3-I in *neavei*; (4) seta 5-IV double or triple in both forms but more often triple in *neavei* and double in *perexiguus*; and (5) seta 2-X often double and frequently triple in *neavei* (rarely with 4 branches) but almost always double in *perexiguus* (rarely single or triple).

**Crossing experiments.** Table 2 records the viability of egg rafts deposited by females used in the reciprocal crosses. The viabilities of egg rafts resulting from crossing *perexiguus* females with *neavei* males were 90.2 and 96.7%. These percentages were on the same order of magnitude as the viabilities for the parental colonies, i.e., 95.5% for *neavei* and 85.1% for *perexiguus*. Indices for egg rafts resulting from the reciprocal cross were only 5.7 and 12.9%, thus there was a measure of unidirectional incompatibility between the two forms.

The results of spermathecal examinations are given in Table 3. All of the *neavei* females crossed with *perexiguus* males which deposited viable rafts were inseminated, while both inseminated and uninseminated females deposited nonviable rafts. In contrast, judging from the samples examined, all nonviable rafts resulting from the cross between *perexiguus* females and *neavei* males were deposited by uninseminated mosquitoes. This further indicates that there was some incompatibility in the cross between *neavei* females and *perexiguus* males.

The classification of eggs comprising viable rafts obtained from the primary crosses and the parental colonies is compared in Table 4. This table contrasts the mean percentage of hatched, unhatched-embryonated, and infertile (unembryonated) eggs contained in all of the rafts examined in each case. Of interest is that *neavei* females crossed with *perexiguus* males produced rafts which contained few eggs that hatched and that a large proportion of embryonated eggs failed to hatch.

The mean percentages of unhatched-embryonated and infertile (unembryonated) eggs comprising nonviable rafts that resulted from the primary crosses are compared in Table 5. Nonviable rafts from *neavei* females crossed with *perexiguus* males contained a mixture of embryonated and

**Table 1.** Morphological differences observed between *Culex perexiguus* and *Cx. neavei*.

Character	<i>perexiguus</i>	<i>neavei</i>
1) Ventral surface of proboscis	pale except at base, weakly pale on distal 0.25	inconspicuously pale in middle
2) Postspiracular area	tendency for scales to cover more than dorsal 0.5	tendency for scales to occur in small patch near spiracle
3) Forefemur	usually with indistinct anterior pale stripe	no anterior pale stripe
4) Hindtibia	with distinct anterior and posterior pale stripes on proximal 0.8, partly separated on proximal 0.5 or less by weak ventral dark stripe; with distinct apical pale spot	with rather indistinct anterior and posterior pale stripes ending before base; with rather indistinct apical pale spot
5) Wing - costa	with short line of pale scales at base	without pale scales at base
6) Abdomen - pale bands of terga	normal	reduced or absent
7) Male genitalia - ventral arm of lateral plate	short	long

**Table 2.** Viability of egg rafts from primary crosses and parental stocks.

Cross/colony	Cage	Proportion of viable rafts	Percent viable
<i>neavei</i> ♀ x <i>perexiguus</i> ♂	1	7/122	5.7
	2	11/85	12.9
<i>perexiguus</i> ♀ x <i>neavei</i> ♂	1	111/123	90.2
	2	116/120	96.7
<i>neavei</i>	-	42/44	95.5
<i>perexiguus</i>	-	40/47	85.1

**Table 3.** Proportion of inseminated females which deposited viable and nonviable rafts.

Cross	Cage	Inseminated ♀s /viable rafts	Inseminated ♀s /nonviable rafts
<i>neavei</i> ♀ x <i>perexiguus</i> ♂	1	7/7	9/20
	2	5/5	15/20
<i>perexiguus</i> ♀ x <i>neavei</i> ♂	1	ND	0/11
	2	ND	0/2

ND = Not determined.

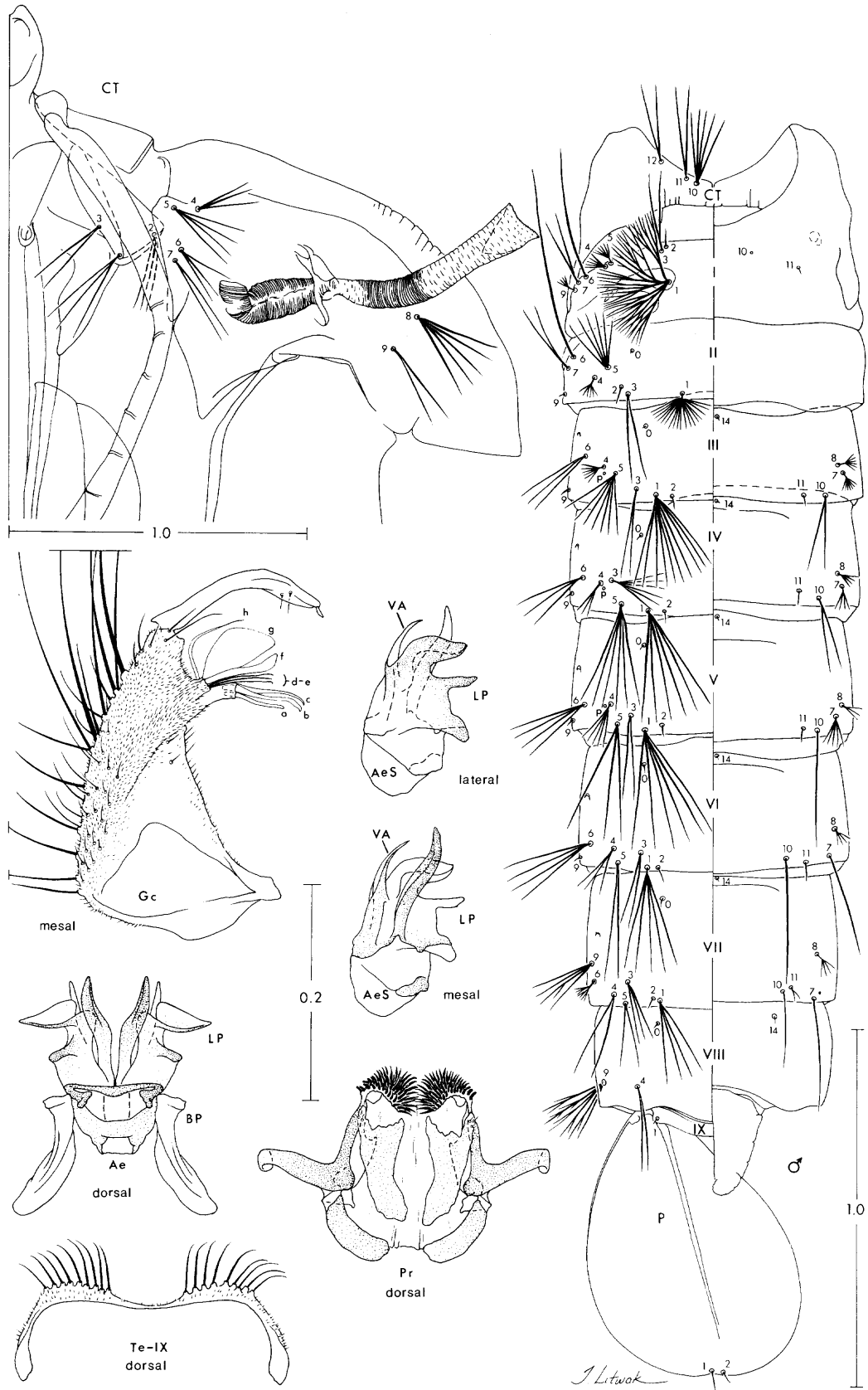


Fig. 1. *Culex (Culex) neavei*. Male genitalia and pupa, South Africa.



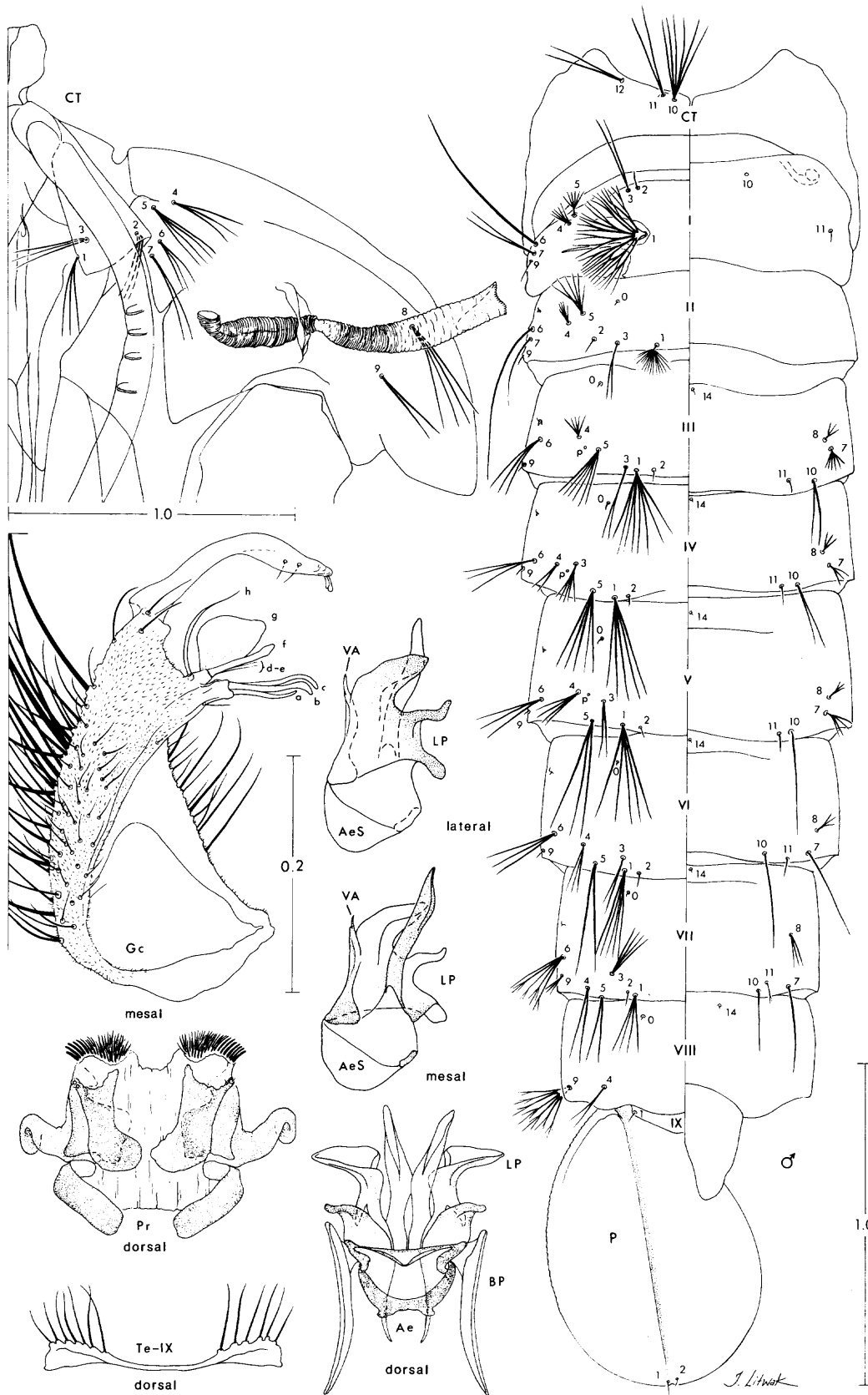


Fig. 3. *Culex (Culex) perexiguus*. Male genitalia and pupa, Egypt.

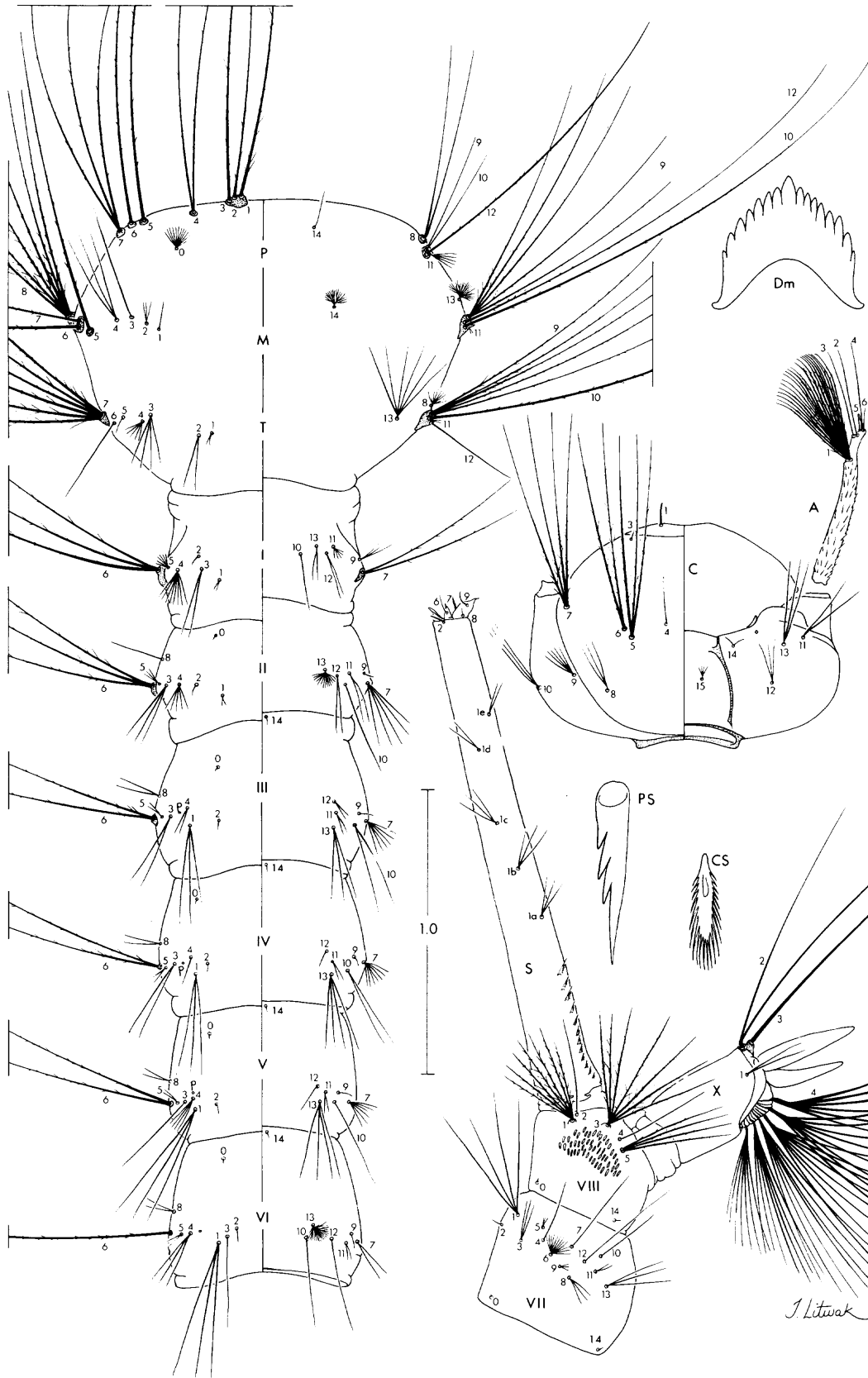


Fig. 4. *Culex (Culex) perexiguus*. Larva, Egypt.

**Table 4.** Classification of eggs in viable rafts from primary crosses and parental stocks.

Cross/colony	Cage	No. rafts examined	Mean % of eggs		
			Hatched	Unhatched-embryonated	Infertile (unembryonated)
<i>neavei</i> ♀ x <i>perexiguus</i> ♂	1	7	2	96	2
	2	11	3	95	2
<i>perexiguus</i> ♀ x <i>neavei</i> ♂	1	16	72	2	26
	2	22	89	1	10
<i>neavei</i>		23	95	3	2
	<i>perexiguus</i>	21	93	2	5

**Table 5.** Classification of eggs in nonviable rafts from primary crosses.

Cross	Cage	No. rafts	Mean % of eggs	
			Embryonated	Infertile (unembryonated)
<i>neavei</i> ♀ x <i>perexiguus</i> ♂	1	48	44	56
	2	49	50	50
<i>perexiguus</i> ♀ x <i>neavei</i> ♂	1	12	0	100
	2	4	4	96

infertile (unembryonated) eggs while unhatched eggs from the reciprocal cross were virtually all infertile (unembryonated).

The hybrids that resulted from the crosses had normal sex ratios and developed normally, without morphological abnormalities, to produce healthy, vigorous adults. However, only a limited number of F<sub>1</sub> adults resulted from the cross between *neavei* females and *perexiguus* males because of the limited number of viable rafts produced, each with a low egg hatch. In comparison, a large number of F<sub>1</sub> adults resulted from the reciprocal cross. These hybrids were backcrossed with the parental stocks to obtain the results shown in Tables 6 and 7. Table 6 shows that viabilities were high for the egg rafts resulting from each of the backcrosses. The ensuing progeny developed at a normal rate, no morphological abnormalities were observed, and healthy adults were produced which exhibited a normal sex ratio.

The classification of eggs comprising viable rafts deposited by females used in the backcrosses is shown in Table 7. The overall viability of the eggs that resulted from back-

crossing the hybrids was not always as high as it was for eggs in rafts obtained from the parental colonies. However, a high percentage of eggs hatched in some of the rafts that resulted from each of the backcrosses.

## DISCUSSION

The results of the crossing experiments show that there is a high degree of unidirectional incompatibility when *neavei* females are mated with *perexiguus* males. The high proportion of females which were inseminated in this cross (viz. 36/52, 69.2%), and which deposited eggs that developed embryos, indicates that there was little or no pre-mating isolation between the laboratory colonies of these forms, presuming, of course, that normal pre-mating isolating mechanisms were not disrupted in the laboratory. The effect of crossing *neavei* females with *perexiguus* males is certainly indicative of a measure of postmating isolation, but also could be due to the phenomenon of cytoplasmic incompatibility caused by a rickettsia-like symbiont similar to that found in different geographical populations of *Cx.*



**Table 6.** Viability of egg rafts from backcrosses between F<sub>1</sub> hybrids and parental forms.

Cross	Proportion of viable rafts
hybrid ♀ x <i>neavei</i> ♂	89/98 (90.8%)
hybrid ♀ x <i>perexiguus</i> ♂	107/111 (96.4%)
<i>neavei</i> ♀ x hybrid ♂	74/76 (97.4%)
<i>perexiguus</i> ♀ x hybrid ♂	62/92 (67.4%)

**Table 7.** Classification of eggs in viable rafts from backcrosses and parental stocks.

Cross/colony	No. rafts examined	Mean % of eggs		
		Hatched	Unhatched-embryonated	Infertile (unembryonated)
F <sub>1</sub> hybrid ♀ x <i>neavei</i> ♂	31	80	8	12
F <sub>1</sub> hybrid ♀ x <i>perexiguus</i> ♂	30	68	21	11
<i>neavei</i> ♀ x F <sub>1</sub> hybrid ♂	30	73	20	7
<i>perexiguus</i> ♀ x F <sub>1</sub> hybrid ♂	30	86	8	6
<i>neavei</i>	23	95	3	2
<i>perexiguus</i>	21	93	2	5

*pipiens* Linnaeus (Yen and Barr 1973, Irving-Bell 1974, Jupp 1987). However, if the evidence obtained from the crossing experiments is considered in combination with the morphological differences that exist between the two forms, then the conclusion is reached that *neavei* and *perexiguus* are separate, largely allopatric species.

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