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Morphological study and description of *Anopheles* (*Anopheles*) *persiensis*, a member of the Maculipennis Group (Diptera: Culicidae: Anophelinae) in Iran

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Abstract

The morphology of the adults, egg, larva and pupa of *Anopheles persiensis* Linton, Sedaghat & Harbach, an Iranian member of the Holarctic Maculipennis Group, was studied and compared with the morphology of the closely related and sympatric *A. maculipennis* Meigen. *Anopheles persiensis* was formally recognized based on DNA evidence and egg morphology. The fourth member of the group, *Anopheles persiensis*, is described in detail and the egg illustrated using scanning electron microscopy.
Key words: *Anopheles persiensis*, morphology, scanning electron microscopy, sibling species, Iran, taxonomy, mosquito, malarialogy, Culicidae

چکیده

شکل‌شناسی حشرات بالغ، تخم، لارو و شفیره‌ی *Anopheles persiensis* Linton, Sedaghat & Harbach، عضو ایرانی گروه Maculipennis منطقه‌ی هولارکتیک (Holarctic)، مورد مطالعه قرار گرفته و با گونه‌ی نزدیک و هم‌محل (sympatric) خود، *A. maculipennis* Meigen، مقایسه می‌شود. گونه‌ی *A. persiensis* در ابتدا بر اساس شواهد مولکولی و DNA و همچنین مشخصات تخم تشخیص داده شد. این گونه که چهارمین عضو این گروه است، با جزییات بیشتر توصیف و تخم‌های آن با استفاده از میکروسکوپ الکترونی نشان داده شده است.
واژگان کلیدی: *Anopheles persiensis*، شکل‌شناسی، میکروسکوپ الکترونی، گونه‌ی خواهر- برادر، ایران، تاکسونومی، پشه، مالاریاشناسی، Culicidae

Introduction

Anopheles maculipennis Meigen is the nominotypical member of the first mosquito species complex to be discovered (Falleroni, 1926; van Thiel, 1927). Interest in the group was initially in response to the phenomenon of "anophelism without malaria", this phenomenon define as the presence of anopheline mosquitoes in areas with no malaria transmission. Today, the Maculipennis Group remains the classic example of how knowledge of cryptic species impacts on malaria epidemiology and control. Current understanding of this group in the Palaearctic Region stems from White (1978), who recognized nine species: *An. atroparvus* van Thiel, *A. beklemishevi* Stegnii & Kabanova, *A. labranchiae* Falleroni, *A. maculipennis*, *A. martinius* Shingarev, *A. melanoon* Hackett (with its variety *A. subalpinus* Hackett & Lewis), *A. messeae* Falleroni, *A. sacharovi* Favre and *A. sicaulti* Roubaud. Field and laboratory investigations involving morphological, enzyme electrophoresis, crossing-mating and

chromosome studies later revealed that *A. sicaulti* was conspecific with *A. labranchiae* (de Zulueta *et al*., 1983). Ribeiro *et al*. (1988) treated *A. subalpinus* as a separate species, but it was recently shown to be conspecific with *A. melanoon* (Linton *et al*., 2002). More recently, Sedaghat *et al*. (2003b) described *A. persiensis* Linton, Sedaghat & Harbach in Iran, Nicolescu *et al*. (2004) distinguished *A. daciae* Linton, Nicolescu & Harbach in Romania and Gordeyev *et al*. (2005) recognized *A. artemievi* Gordeyev *et al*. in Kyrgyzstan. White (1978) suggested that *A. lewisi* Ludlow may be synonymous with *A. messeae* or *A. beklemishevi*, but its identity remains resolved. Consequently, 12 species of the *A. maculipennis* group (*sensu* Harbach, 2004) are currently recognized in the Palaearctic Region, namely *A. artemievi*, *A. atroparvus*, *A. daciae*, *A. beklemishevi*, *A. labranchiae*, *A. lewisi*, *A. maculipennis*, *A. martinius*, *A. melanoon*, *A. messeae*, *A. persiensis* and *A. sacharovi*. Six of these species (*A. atroparvus*, *A. labranchiae*, *A. maculipennis*, *A. melanoon*, *A. messeae* and *A. sacharovi*) have been identified as primary or secondary vectors of malaria in parts of Europe and the Middle East. Moreover, it seems likely that *A. daciae* and *A. persiensis* could be responsible for malaria transmission attributed to *A. messeae* in eastern Europe (Nicolescu *et al*., 2004) and *A. maculipennis* in northern Iran (Sedaghat *et al*., 2003a), respectively.

With the discovery of *A. persiensis*, 25 species of the genus are currently recognized in Iran (Sedaghat & Harbach, 2005; Azari-Hamidian *et al*., 2006). This species was formally described based on sequences for the internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA and egg morphology (Sedaghat *et al*., 2003b). The morphology of the adults, egg, larva and pupa of *A. persiensis* are described and compared with those of *A. maculipennis* in this paper.

Materials and methods

The progeny of three wild-caught females captured in Gilan and Mazandaran provinces near the Caspian Sea littoral in northern Iran were individually reared to provide adults with associated larval and pupal exuviae for morphological study. Eggs for morphological study were obtained from two additional females collected in the same areas. The five mothers were identified to species based on ITS2 sequence (see Sedaghat *et al*., 2003b). A total of 44 specimens were examined in detail, including 3 males, 18 females, 8 larval exuviae, 5 fourth-instar larvae, 10 pupal exuviae and 22 eggs. Observations of adults were made under simulated natural light. Larval and pupal chaetotaxy were studied using bright field and differential interference contrast microscopy. Measurements and counts were carried out on

10 pupae and 13 larvae. Unless indicated otherwise, numbers in parentheses represent the modes of the reported ranges. Eggs were held for 36 h to allow them to embryonate before transfer to vials containing Bouin's fixative. The eggs were rinsed twice in 20% ethanol to remove fixative, held in 20% ethanol overnight and dehydrated through a graded series of ethanol (5% increments) to 100%. They were held for 2-3 h in each concentration of ethanol. Fully dehydrated eggs were pipetted into millipore capsules (Agar Scientific Ltd, Stansted, England) and stored in vials containing a molecular sieve and cold 100% ethanol before being critical point dried. Eggs were "tapped" out of capsules on to SEM stubs (one brood per stub) covered with Sticky Tabs (Agar Scientific Ltd, Stansted, England). Individual eggs were re-positioned, if necessary, using a single-hair artist's brush. Specimens were then sputter-coated with palladium and examined and photographed in a Philips XL–30 scanning electron microscopic.

The morphological terminology of Harbach & Knight (1980, 1982) is used in the description below. All specimens are deposited in The Natural History Museum, London.

***Anopheles* (*Anopheles*) *persiensis* Linton, Sedaghat & Harbach, 2003**

Diagnosis – It is not possible to provide a morphological diagnosis of *A. persiensis* until all members of the Maculipennis Group in the Palaearctic Region have been studied comparatively. To date, the life stages of only *A. maculipennis*, *A. sacharovi* and *A. daciae* have been described in detail (Linton *et al*., 2003; Sedaghat *et al*., 2003a; Nicolescu *et al*. 2004, respectively). The adults of *A. persiensis* resemble other members of the Maculipennis Group in the Palaearctic Region that have distinct clusters of dark scales on the wings. They differ from those of *A. sacharovi* (but resemble the other species) in their overall darker habitus, and in having the dark clusters of scales on the wings more distinct. The eggs differ from those of *A. martinius* and *A. sacharovi* (but resemble the other species) in having floats; however, the eggs of these two species may develop rudimentary floats at low temperatures (Mer, 1937). The deck varies from more or less entirely dark to having a rather faint pattern consisting of irregular mottling and a transverse dark band at either end of the floats, similar to the eggs of *A. melanoon*.

Female – Head: vertex with pale erect scales behind frontal tuft, dark erect scales posterolaterally. Clypeus dark brown, bare. Antenna dark brown with dark setae. Proboscis length about 2.1 mm (generally shorter than *A. maculipennis* with length about 2.9 mm), approximately same length as forefemur and maxillary palpus, entirely dark-scaled, some

semi-erect scales proximally; labella dark. Thorax: integument brown, broad central area of scutum encompassing dorsocentral and prescutellar areas from anterior promontory to scutellum paler due to silvery pruinescence and dense lines of short golden piliform scales. Wing: length about 4.2 mm (generally shorter than *A. maculipennis* with length about 5.1 mm), apex with very faint fringe spot, sometimes unnoticeable; scales clustered to form darker spots at bases of R_s, R_{1+2}, R_3, R_{4+5}, M_{1+2}, M_2 and M_{3+4}. Halter: scabellum and pedicel pale, without scales; capitellum dark-scaled. Abdomen: integument brown to light brown, terga and sterna without scales, sterna and lateral margins of terga distinctly paler.

Male – Like female except for sexual characters. Head: proboscis length about 2.8 mm, generally longer than *A. sacharovi* (length about 2.2 mm), generally slightly shorter than *A. maculipennis* (length about 3.0 mm). Wing: length about 4.4 mm, generally longer than *A. sacharovi* (length about 3.5 mm), veins with fewer scales than female but dark clusters of scales generally prominent.

Pupa – Character and positions of setae as in *A. maculipennis* (see Linton *et al*., 2003); numbers of branches compared with those of *A. maculipennis* in table 1. Cephalothorax: lightly to moderately pigmented; mesothoracic wing usually without lattice pattern of darker markings. Trumpet: length 0.45-0.66 mm (mean = 0.55 mm), width 0.21-0.37mm (mean = 0.28mm), index 1.71-2.25 (mean = 1.95). Abdomen: lightly to moderately pigmented with few areas of darker pigmentation. Segments III-VI sometimes with an extra single seta near seta 4 and another near seta 9; setae 10-VI and 7-VII single, inserted on posterior edge of respective segment. Paddle: lightly pigmented; length 0.80-1.13 mm (mean = 0.93 mm), width 0.56-0.89 mm (mean = 0.70 mm), index 1.30-1.46 (mean = 1.34); seta 1-Pa rather stout, bent, with 1, 2(1) branches.

Larva, fourth-instar – Character and positions of setae as in *A. maculipennis* (see Linton *et al.*, 2003); numbers of branches compared with those of *A. maculipennis* in table 2. Head: slightly longer than width, length 0.72-0.97 mm (mean = 0.86 mm), width 0.66-0.81 mm (mean = 0.74 mm), integument brown with darker areas behind setae 5, 6-C, more or less triangular dark area in middle with 4-6 additional darker areas around it; collar strongly pigmented. Antenna: lightly pigmented, length 0.28-0.36 mm (mean = 0.32 mm); mesal surface with spicules; apex darkly tanned. Seta 1-A inserted 0.25-0.33 from base of antenna, short, about 1.5 times diameter of antenna at point of attachment, with 1-9 branches, 4-A with 3-7(4) branches. Thorax: integument hyaline, smooth. Setae 1-3-P without support plate(s). Abdomen: integument hyaline. Anterior tergal plates on segments I-VIII about 0.2 width of

segment; median accessory tergal plates on segments I-VII; seta 1-I,II palmate, with simple lanceolate leaflets; 1-III-VII fully palmate, with shoulders and filaments slightly more darkly pigmented than blades, shoulders broad with more-or-less squared notches rather than pointed teeth, filaments relatively long and acuminate.

Table 1. Range (mode) of branches for pupal setae of *A. persiensis* and *A. maculipennis*. Data for *A. maculipennis* are taken from Linton *et al*. (2003).

	Seta	Cephalothorax	Abdominal segments								Paddle
Anopheles **species**	**no.**	**CT**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**Pa**
persiensis	0	-	-	1,2(1)	1-4(1)	1-4(2)	1-3(3)	1-3(3)	1-4(2)	1,2(1)	-
maculipennis		-	-	1	1-3(2)	1-3(2)	1-3(1)	1-3(1)	1-3(1)	1,2(1)	-
persiensis	1	2-4(3)	34-83(38)	5-9(5)	5-10(7)	5-8(6)	1-3(2)	1-6(1)	1-4(1)	-	1,2(1)
maculipennis		2-4(3)	26-77(39)	4-9(7)	5-9(7)	4-11(6)	1-5(3)	1-3(1)	1-3(1)	-	1,2(1)
persiensis	2	2,3(2)	2-4(3)	4-7(6)	5-8(7)	3-5(4)	3-5(4)	2-4(3)	3-5(3)	-	1-3(1)
maculipennis		1-5(3)	1-4(3)	4-8(6)	4-8(5)	2-5(3)	2-4(3)	2-5(3)	1-5(3)	-	1-3(1)
persiensis	3	2-5(4)	3-7(5)	1,2(1)	2-4(3)	2-6(5)	1-3(?)	1	1-4(3)	-	-
maculipennis		2-4(3)	3-6(5)	1-4(1)	1-5(3)	3-6(5)	1-3(2)	1-3(1)	1-4(2)	-	-
persiensis	4	1-4(3)	4-8(6)	3-5(3)	2-4(3)	1-4(3)	2-4(4)	1,2(2)	1,2(1)	1-3(2)	-
maculipennis		1-4(2)	3-8(5)	1-6(4)	1-5(3)	1-4(1)	1-4(3)	1-3(1)	1-3(1)	1-3(2)	-
persiensis	5	3-6(5)	1-3(2)	2-4(3)	5-9(8)	5-8(7)	3-7(4)	3-6(3)	1-4(3)	-	-
maculipennis		1-6(3)	1-4(2)	2-5(4)	5-12(7)	4-8(5)	3-5(3)	2-6(3)	1-5(3)	-	-
persiensis	6	2,3(3)	1-3(2)	1-3(2)	2-4(2)	1-3(2)	1-3(2)	1	1-3(3)	-	-
maculipennis		1-3(1)	1-4(2)	1-5(2)	2-7(4)	1-3(3)	1-3(2)	1,2(1)	1-3(1)	-	-
persiensis	7	1	1-4(3)	2-4(3)	2-5(3)	1-4(2)	1-4(2)	1	1	-	-
maculipennis		1	2-5(3)	2-6(3)	1-5(2)	1-4(2)	1-4(3)	1-4(1)	1	-	-
persiensis	8	1-3(2)	-	-	1-4(2)	1-4(2)	1-3(1)	1-3(2)	3-5(3)	-	-
maculipennis		1-3(2)	-	-	1-4(2)	1-4(2)	1-3(1)	1-3(2)	2-5(3)	-	-
persiensis	9	1-4(2)	2,3(2)	1	1	1	1	1	1	11-17(14)	-
maculipennis		1-4(2)	1-4(2)	1	1	1	1	1	1	9-17(13)	-
persiensis	10	1-4(2)	-	-	1-3(2)	1-3(2)	1,2(2)	1	1,2(2)	-	-
maculipennis		1-5(3)	-	-	1,2(1)	1,2(1)	1,2(1)	1-3(1)	1-3(1)	-	-
persiensis	11	2-6(3)	-	-	1-3(3)	1-3(1)	1-3(1)	1-3(1)	1,2(1)	-	-
maculipennis		2-4(3)	-	-	1-3(1)	1,2(1)	1,2(1)	1	1,2(1)	-	-
persiensis	12	1-3(2)	-	-	-	-	-	-	-	-	-
maculipennis		1-3(2)	-	-	-	-	-	-	-	-	-
persiensis	14	-	-	-	1	1	1	1	1	1	-
maculipennis		-	-	-	1	1	1	1	1	1	-

Egg – (fig. 1, table 3). Length 569-660 μm (mean = 608 μm). Black, boat-shaped in dorsal and lateral views (fig. 1A). Floats present, well developed, with 17-22 (mean = 18) float ridges, grooves between ridges smooth (finely corrugated in *A. maculipennis* according to White (1978)). Deck more or less entirely dark or with faint pattern consisting of irregular mottling and a transverse dark band at either end of the floats (darker areas of the deck correspond to areas with smaller tubercles in figs. 1A, B; eggs of *A. maculipennis* generally have larger tubercles and a distinct band at either end of the floats). Ventral surface uniformly covered with well-defined pattern of hexagonal outer chorionic cells, cells longer than wide,

Table 2. Range (mode) of numbers of branches for fourth-instar larval setae of *A. persiensis* and *A. maculipennis*. Data for *A. maculipennis* are taken from Linton *et al*. (2003).

	Seta	Head	Thorax			Abdominal segments								
Anopheles species	no.	C	P	M	T	I	II	III	IV	V	VI	VII	VIII	X
persiensis	0	1	1	-	-	-	1,2(1)	1,2(2)	1-3(1)	1,2(2)	1,2(1)	1	1,2(1)	-
maculipennis		1	1	-	-	-	1	1-3(1)	1,2(1)	1,2(1)	1,2(2)	1,2(1)	1,2(1)	-
persiensis	1	1	1-3(1)	10-21(18)	1-3(1)	6-15(?)	4-19(12)	12-22(20)	14-24(19)	16-21(?)	14-20(19)	13-21(?)	1	1
maculipennis		1	1-6(1)	17-26(19)	1-4(2)	8-15(8)	10-17(12)	18-23(21)	16-27(23)	14-23(20)	17-23(20)	16-22(18)	1	1
persiensis	2	1-6(2)	4-11(8)	1-4(1)	1-3(1)	3-6(5)	4-8(6)	3-8(5)	2,3(2)	1-3(2)	2-4(3)	3-6(4)	5-8(6)	10-23(?)
maculipennis		1-6(2)	6-14(10)	1-3(1)	1	4-11(7)	6-13(9)	5-14(8)	3-6(3)	2-5(3)	5-9(5)	5-9(6)	4-9(7)	14-21(16)
persiensis	3	25-45(32)	1	1	8-14(11)	1	1	1	2-4(3)	1	1	1-4(3)	4-10(7)	10-24(11)
maculipennis		20-43(32)	1,2(1)	1	8-15(9)	1	1	1	2-4(2)	1,2(1)	1	1-4(2)	5-10(7)	7-12(11)
persiensis	4	2-7(4)	14-25(16)	2,3(3)	2-4(3)	3-6(4)	2-6(5)	3-7(4)	3-5(3)	2-5(3)	1	1	1	9,10(9)
maculipennis		2-5(3)	13-21(17)	2-4(3)	2-6(3)	4-8(5)	3-6(5)	3-6(5)	3-8(5)	3-5(3)	1	1	1	9-20
persiensis	5	12-17(13)	18-33(18)	1	17-32(?)	3-7(5)	6-12(9)	5-10(8)	3-6(5)	4-6(5)	3-6(5)	4-7(6)	4-6(5)	-
maculipennis		10-17(11)	19-34(26)	1,2(1)	22-27(24)	4-7(5)	7-11(9)	7-12(8)	4-8(6)	5-11(6)	6-9(6)	4-10(7)	4-7(5)	-
persiensis	6	12-19(?)	1	1-4(?)	1-3(1)	12-27(?)	19-35(?)	11-21(20)	3,4(3)	1-4(3)	1-4(3)	1-4(3)	-	-
maculipennis		10-18(13)	1,2(1)	3-5(3)	1-3(2)	15-25(18)	16-26(21)	14-22(17)	2-5(3)	3	3-6(3)	2-5(4)	-	-
persiensis	7	11-20(16)	14-25(21)	1-5(1)	22-33(?)	16-24(?)	15-27(20)	2-7(4)	2-5(3)	2-5(3)	2-6(2)	4-6(5)	1-S,	4-6(5)
maculipennis		12-24(14)	17-30(24)	1-4(2)	15-27(19)	14-22(19)	16-27(22)	3-5(3)	3-6(3)	2-5(3)	2-4(3)	4-7(6)		4-6(5)
persiensis	8	3-8(6)	14-34(?)	11-23(11)	17-30(26)	-	1-4(2)	1-3(2)	1-3(2)	2,3(2)	2-4(2)	2-5(3)	2-S,	2-5(?)
maculipennis		5-8(6)	20-29(23)	12-27(16)	16-29(22)	-	2-4(3)	2-4(3)	2-4(3)	2-4(3)	2-4(3)	3-6(3)		3-8(5)
persiensis	9	3-6(4)	2-4(3)	1,2(1)	1-3(1)	4-7(4)	4-11(6)	4-9(6)	4-8(7)	4-8(6)	4-8(7)	2-6(3)	6-S,	1-3(2)
maculipennis		4-7(5)	1-4(2)	1	1-3(1)	3-7(5)	5-11(7)	6-14(8)	7-11(7)	5-9(7)	6-9(7)	2-6(3)		1-4(2)
persiensis	10	1-3(2)	1,2(1)	1	1	1	1-4(?)	1	1,2(1)	1	1,2(2)	1-4(3)	7-S,	1,2(2
maculipennis		2-4(2)	1	1	1	1	2-4(3)	1-3(1)	1	1	1-3(2)	2-5(4)		1,2(2)
persiensis	11	16-42(25)	1	1	1	2-5(4)	1,2(1)	2-4(3)	2,3(2)	1-3(2)	1-3(2)	1-3(1)	8-S,	2-4(3)
maculipennis		21-43(25)	1	1	1	3-6(4)	1-4(1)	2-4(3)	2-4(3)	2,3(2)	1-3(3)	1-3(2)		2-5(5)
persiensis	12	1-3(3)	1	1	1,2(2)	1-4(?)	1,2(1)	1-3(2)	1-4(2)	2-4(2)	1-3(1)	1	9-S,	3-5(4)
maculipennis		2-4(3)	1	1,2(1)	1	2,3(3)	1	1-3(3)	2,3(3)	2-4(2)	1	1		3-7(4)
persiensis	13	2-6(4)	6-12(?)	2-8(4)	2,3(2)	4-8(5)	4-12(?)	3-6(4)	3	3-6(4)	5-13(7)	2,3(3)	-	-
maculipennis		3-6(4)	6-12(9)	3-8(5)	2-4(3)	4-8(5)	6-10(8)	4-8(6)	3,4(3)	3,4(3)	5-10(7)	2-4(3)	-	-
persiensis	14	1,2(1)	5-10(8)	4-11(9)	-	-	-	1	1	1	1,2(1)	1	1	-
maculipennis		4,5(5)	6-12(8)	5-13(8)	-	-	-	1	1	1	1	1	1	-
persiensis	15	3-6(4)	-	-	-	-	-	-	-	-	-	-	-	-
maculipennis		4-10(6)	-	-	-	-	-	-	-	-	-	-	-	-

longer sides more or less parallel longitudinal axis of egg (fig. 1C). Both ends similar but posterior end slightly narrower and generally with fewer lobed tubercles; lobed tubercles well developed, oval or round, number of convolutions indicated in table 3.

Table 3. Egg characteristics of *A. persiensis* (ranges of lengths and counts with means and modes in parentheses, respectively).

Character	Measurement
Egg length	569-660 µm (608 µm)
Float length	17-22 (18)
Anterior lobed tubercles	6-11 (8)
Number of convolutions	5-10 (9)
Posterior lobed tubercles	5-9 (6)
Number of convolutions	5-11 (8)

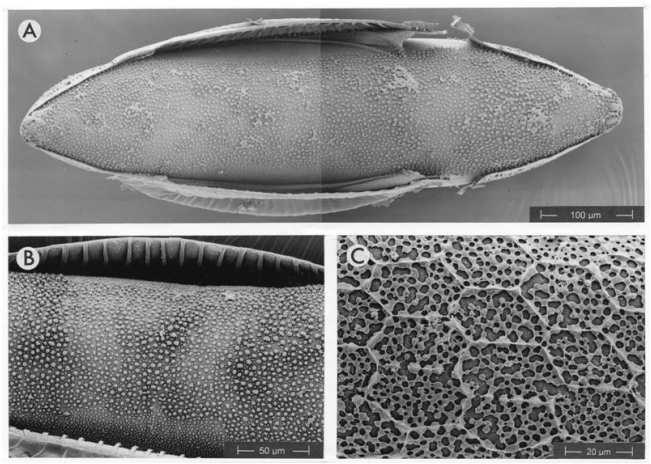

Figure 1. Egg of *A. persiensis.* A, entire egg, ventral (upper) surface, anterior to right; B, central area of deck; D, central area of dorsal (lower) surface.

Distribution and bionomics – *Anopheles persiensis* is currently only known to occur in areas of Gilan and Mazandaran provinces of Iran adjacent to the Caspian Sea (fig. 2), but it is likely to have a much wider distribution because it is very common in these provinces. Based on the identification of ITS2 amplification products, the majority of mosquitoes of the Maculipennis Group collected in Gilan and Mazandaran in 2001 and 2002 were *A. persiensis*

(63%). In comparison, *A. maculipennis* comprised 30% of specimens and *A. sacharovi* merely 7%.

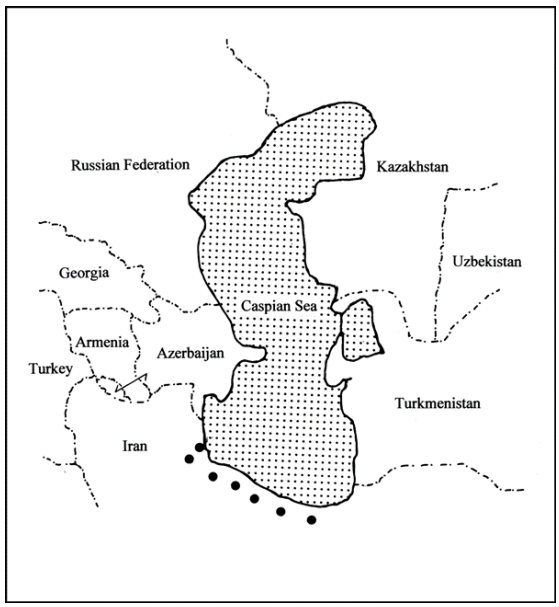

Figure 2. Map of the Caspian Sea region showing the localities (dots) in Gilan and Mazandaran provinces of Iran where *A. persiensis* was collected in Iran.

Specimens of *A. persiensis* were collected in mountainous areas, forest, near the coast of the Caspian Sea and in rural areas in both provinces. The coastal plain of the Caspian Sea in northern Iran has a subtropical climate and is humid throughout the year. Gilan province is located between latitudes 36° 36' and 38° 27' N and longitudes 48° 45' and 50° 34' E and has an average annual rainfall of more than 1900 mm. Mazandaran province is located between latitudes 35° 47' and 38° 5' N and longitudes 50° 34' and 56°14' E and receives an average of 650 mm of rainfall annually.

Anopheles maculipennis is widespread across Iran. *Anopheles persiensis* was found in sympatry with *A. maculipennis* in Gilan province, and with *A. maculipennis* or *A. sacharovi* in Mazandaran province. Since *A. persiensis* was found in cow and sheep resting places, it appears to be mainly exophilic and zoophilic. However, its involvement in malaria transmission in northern Iran, which has been attributed to *A. maculipennis*, cannot be

discounted because the latter species is predominantly zoophilic and is not regarded as an important vector of malaria elsewhere (Jetten & Takken, 1994). Rice fields and small groundwater pools that occur in areas where adults of *A. persiensis* were collected are considered to be habitats for the immature stages of this species.

Discussion

As currently recognized, the Maculipennis Group includes 12 species in the Palaearctic Region that bear identical or overlapping morphological characters. Egg morphology, despite seasonal and intraspecific variation in surface features (see Linton *et al*., 2003), is still used routinely by some entomologists for species identification. White (1978) and Jetten & Takken (1994) provided keys that distinguish the eggs of some Palaearctic species of the Maculipennis Group. These keys are based on the overt appearance of eggs observed with a light microscope. It seems likely that the ultrastructure of eggs revealed by scanning electron microscopy may provide more definite structural characters for distinguishing the species.

Hitherto, *A. persiensis* has been largely misidentified as *A. maculipennis* in northern Iran (Sedaghat *et al*., 2003b), and we failed to find any reliable morphological characters for separating these two species in the egg, larval, pupal and adult stages. However, these two species have different ITS2 sequences (Sedaghat *et al*., 2003b), which can be used to conduct comparative studies of the biology, ecology and behaviour of *A. persiensis* and *A. maculipennis*, to evaluate their specific roles in malaria transmission and refocusing vector control operations.

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