TAXONOMIC DIFFERENTIATION OF *ANOPHELES SACHAROVI* AND *AN. MACULIPENNIS S.L.* (DIPTERA: CULICIDAE) LARVAE BY SETA 2 (ANTEPALMATE HAIR)

S. Doosti[1], S. Azari-Hamidian[2], H. Vatandoost*[1], M. A. Oshaghi[1] and M. Hosseini[3]

1) Department of Medical Entomology and Vector Control, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran
2) School of Public Health, Guilan University of Medical Sciences, Rasht, Iran
3) Department of Biostatistics, School of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran

Abstract- Malaria has reemerged in northern borderline of Iran after the collapse of former Soviet Union. There have been several reports of malaria epidemics in Azerbaijan and Ardebil provinces of Iran. The *Anopheles maculipennis* complex is assumed to play an important role in malaria transmission in these regions. For the first time in Iran, a diagnostic character in 4th instar larvae, *i.e*. seta 2 (antepalmate hair) in the tergum of 4th and 5th segments of abdomen was used to differentiate *An. sacharovi* from *An. maculipennis s.l.* A total of 149 larval samples from 17 different locations of Iran were examined by light microscope. It was found that the mean number of seta 2 branches in *An. sacharovi* was 36.84 ± 1.94 whereas it was 16.52 ± 5.05 for *An. maculipennis s.l.* It seems that this character can be added to the national identification key of larval stage of Iranian anopheline mosquitoes.

Acta Medica Iranica, 44(1): 21-27; 2006

Key words: Antepalmate hair, *Anopheles maculipennis* complex, Iran, malaria, larvae, numerical taxonomy

INTRODUCTION

Some important mosquito-borne diseases, including malaria, West Nile encephalitis and dirofilariasis, have been reported in Iran (1-3). After collapsing of the former Soviet Union, several epidemics of malaria imported from beyond Iranian borderline have been reported in Azerbaijan and Ardebil provinces in northern Iran (4). According to the traditional classification, the family Culicidae includes three subfamilies (Anophelinae, Culicinae, and Toxorhynchitinae), 10 tribes, 39 genera, 135 subgenera and more than 3450 species and subspecies (5-10). The genus *Anopheles* includes six subgenera and 484 species (11). The *An. maculipennis* complex has been reported in most parts of Palaearctic regions except far eastern Asia and far northern Europe and Asia (12, 13). The last taxonomic changes of the Maculipennis Complex are the synonymy of *An. subalpinus* and *An. melanoon* and the description of two new species according to the molecular data in Iran and Romania (14-16). The complex includes at least nine species in Palaearctic region (11).

Based on different taxonomic methods, 22 to 28 anopheline species have been reported in Iran (15, 17-21), out of which 8 have been assumed as malaria vector (15, 22). The *An. maculipennis* complex species is present in almost all parts of Iran except southeastern parts (23). There is no comprehensive information on the distribution and medical

Received: 6 Feb. 2005, Revised: 5 Apr. 2005, Accepted: 13 Jun. 2005

*** Corresponding Author:**
H. Vatandoost, Department of Medical Entomology and Vector Control, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 66402095, Fax: +98 21 66462267
E-mail: hvatandoost@yahoo.com

importance of all species in Iran. The Maculipennis complex is the most important malaria vector in northern and western parts and central plateau of the country (15, 23, 24). Based on morphological characteristics, two species, *An. sacharovi* and *An. maculipennis s.l.*, were generally reported in Iran (18, 25-36). However, according to the egg pattern, adult morphology including wing scale index and DNA based methods, 8 species of *An. maculipennis* complex have been reported in Iran, including *An. atroparvus* (37, PCR), *An. labranchiae* (38, PCR), *An. maculipennis s.s.* (12, 17, 20, 23, 39-48, egg pattern; 15, 24, 37, 49, PCR), *An. martinius* (13, 19), *An. melanoon* (12, 17, 19, 40, 50, 51 as *An. subalpinus*; 20, 23, 47, 48, 52, egg pattern), *An. messeae* (46-48, 51, egg pattern; 53, wing index; 37 PCR), *An. sacharovi* (54 as *An. elutus*; 17, 19, 23, 39-43, 45, 46, 52, 55-60, egg pattern and adult morphology; 15, 24, 37, PCR) and *An. persiensis* (15, PCR). However, some reports need to be verified.

Biosystematics and ecology of the *An. maculipennis* complex need complete investigations in Iran. Previous literatures are cited as a historical review and for providing a faunal bibliography for future studies. Most common characters to identify the members of the *An. maculipennis* complex are egg pattern, polytene chromosome, isoenzymes and species-specific PCR (13, 15). In the larval stage, situation of seta 3C (outer clypeal hair) in comparison to lateral palatal brush (61) and seta 1II (62-64) were noted. The most reliable morphological character to differentiate in the larval stage is seta 2 (antepalmate hair) (62-65) (Fig.1).

Since sibling species show different biology, ecology, behavior, host preference and vectorial capacity, the identification of vector species is very important to malaria control programs. This study is the first one to differentiate *An. sacharovi* and *An. maculipennis s.l.* at larval stage in Iran.

MATERIALS AND METHODS

Totally, 149 larval slides of the *An. maculipennis* complex originated from different locations of Iran which were deposited in Medical Entomology Museum, School of Public Health, Tehran University of Medical Sciences were examined.

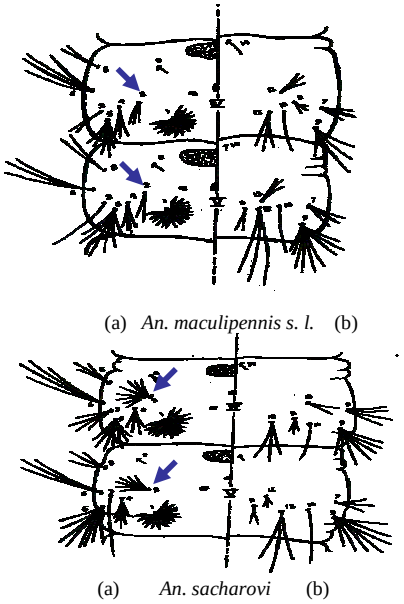

Fig. 1. Dorsal (a) and ventral (b) view the of the IV-V abdominal segments of larvae in *An. sacharovi* and *An. maculipennis s.l.* (seta 2 is shown by arrow).

The number of seta 2 (antepalmate) of the fourth and fifth abdominal segments of each larva was counted using light microscope. Mean and standard deviation (SD) of total branches of seta 2 IV-V were calculated for all specimens in every location by SPSS (version 11.5). The means were compared with the ones provided by Bates (63) and Romi *et al.* (65), using the same package. The origins of specimens are shown in figure 2.

RESULTS

Comparison of mean number of seta 2 branches of Iranian specimens with the ones available in literature (63, 65) showed that in four locations, samples have got the highest value and hence the species were identified as *An. sacharovi*. Those locations are in Qazvin, West Azerbaijan and Khuzestan provinces (Table 1, Figures 2 and 3). Average mean number of branches was 36.84 ± 1.94 (n = 45) for *An. sacharovi*. This character for *An. maculipennis s.l.* which were found in the 13 locations of other provinces was 16.52 ± 5.05 (n = 104). Those locations are in Fars, Isfahan, East Azerbaijan, West Azerbaijan, Khorassan, Kurdistan, Mazandaran and Guilan provinces (Table 1, Fig. 2 and 3).

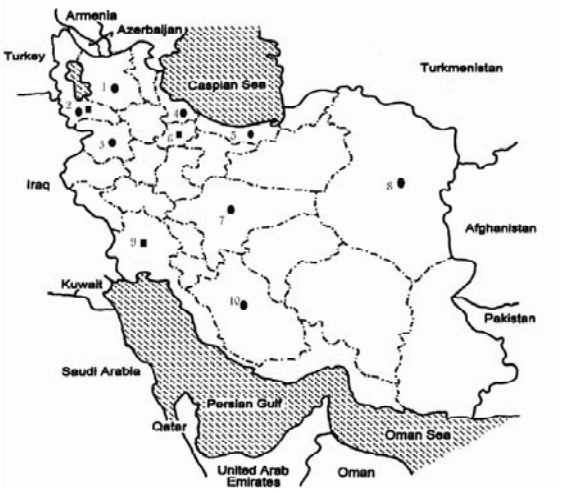

Fig. 2. Geographical distribution of *An. sacharovi* and *An. maculipennis s.l.* in Iran based on the mean number of seta 2 branches of available larval slides in the Medical Entomology Museum of School of Public Health, Tehran University of Medical Sciences, Iran. ■ *An. sacharovi*; ● *An. maculipennis s.l.*; 1. East Azerbaijan; 2. West Azerbaijan; 3. Kurdistan; 4. Guilan; 5. Mazandaran; 6. Qazvin; 7. Isfahan; 8. Khorassan; 9. Khuzestan; 10. Fars.

Location, mean, and SD of seta 2 branches and the number of the studied larval slides are shown in table 1. Original photos obviously show different number of branches in seta 2 IV and V in *An. Sacharovi* and *An. maculipennis s.l.* specimens (Fig. 3).

DISCUSSION

Results of this study showed that taxonomic differentiation of *An. sacharovi* from other members of the *An. maculipennis* complex at larval stage is relatively easy since seta 2 in the 4th and 5th abdominal segments of *An. sacharovi* specimens is highly branched. Bates (63), Missiroli (62), Darsie and Samanidou-Voyadjoglou (64) also used the mean number of IV and V seta 2 branches to separate different species of the *An. maculipennis* complex by numerical taxonomy. However, except for *An. sacharovi* the differentiation of other members of the complex sometimes is quite difficult because of their close mean number of seta 2 branches. In addition, different geographical population of each species may show different mean number of branches as seen in *An. messeae* from Albania and the Netherlands (66). This limitation in the differentiation of the *An. maculipennis* complex in the larval stage, particularly in Iran where there is no available local numeric standard, causes more difficulties in species identification. Therefore, results of this study showed that counting the seta 2 branches of IV and V abdominal segments could be recommended as an easy and definite way to

Table 1. Location, mean and standard deviation (SD) of seta 2 branches, and the number of larval slide of the studied Iranian *An. maculipennis* complex specimens.

Province	No. larvae	Location	Species	Mean of seta 2 branches ± SD
Fars	14	Dasht khezri	*An. maculipennis s.l.*	27.21 ± 4.28
Qazvin	13	Taherabad	*An. sacharovi*	36.92 ± 3.79
Qazvin	9	Nazarabad	*An. sacharovi*	34.55 ± 7.55
Isfahan	5	Esmailtarkhan	*An. maculipennis s.l*	14.40 ± 2.51
East Azerbaijan	13	Takhtolia	*An. maculipennis s.l*	14.84 ± 2.91
West Azerbaijan	7	Dehriz	*An. maculipennis s.l*	16.57 ± 4.57
West Azerbaijan	6	Band Rezaie	*An. maculipennis s.l*	16.16 ± 2.92
West Azerbaijan	6	A marsh near Jabal	*An. sacharovi*	36.66 ± 8.47
West Azerbaijan	7	Ghagharalu	*An. maculipennis s.l*	14.71 ± 1.70
West Azerbaijan	6	Dehjabal	*An. maculipennis s.l*	27.66 ± 7.22
Khorassan	17	Sarvelayat	*An. maculipennis s.l*	15.47 ± 4.001
Khorassan	6	Marsuk	*An. maculipennis s.l*	15.66 ± 2.80
Khuzestan	17	Kaldusakh	*An. sacharovi*	39.23 ± 6.15
Kurdistan	5	Gheybisur	*An. maculipennis s.l*	13.20 ± 1.30
East Azerbaijan	5	Sarik	*An. maculipennis s.l*	14.80 ± 3.42
Mazandaran	8	Abdangeh	*An. maculipennis s.l*	13.12 ± 1.80
Guilan	5	Fuman	*An. maculipennis s.l*	11 ± 1.58

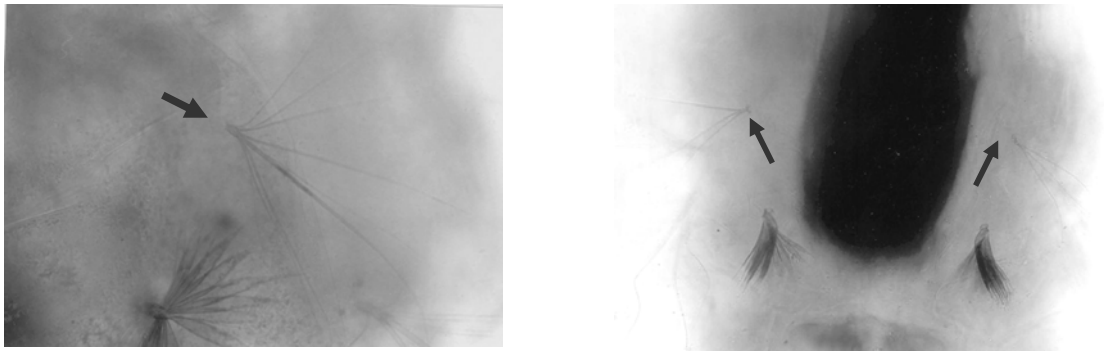

Fig. 3. Dorsal view of larval abdominal segment of IV-V in *An. sacharovi* (a) and *An. maculipennis s.l.* (b). Seta 2 is shown by arrow.

distinguish *An. sacharovi* specimens from other members of the complex in the larval stage in Iran. It is noteworthy that *An. sacharovi* was previously reported from the provinces where it has been found in this study (23, 25, 40). This method, like other morphological methods, is less expensive and complex than others such as cytological and molecular methods.

In Iran, most reports about the members of the Maculipennis complex have been mostly based on single characters like egg pattern or molecular data. Since each species identification method inherits some limitation or difficulties, higher confidence will be achieved if different methods could be used simultaneously. Hence, each specimen or population should be reared separately to prepare a progeny brood, then their egg pattern, larval and pupal chaetotaxy, adult morphology including hypopygia, and finally their molecular characteristics, particularly second internal transcribed spacer (ITS 2) of ribosomal DNA (rDNA) should be studied at the same time to reach a consensus identification. This process will help us to determine the correlation of different characters and to validate each character in species identification of the Iranian *An. maculipennis* complex.

Conflicts of Interests

We have no conflicts of interest.

Acknowledgements

The authors are grateful to Dr S. Tirgari, Mrs. M. Jedari and Mrs. F. Naini, Department of Medical Entomology and vector control, School of Public Health Tehran University of Medical Sciences, for providing the specimens and S. Poorassad, School of Public Health Tehran University of Medical Sciences for taking photos.

REFERENCES

1. Sadighian A. Helminth parasites of stray dogs and jackals in Shahsavar area, caspian region, Iran. J Parasitol. 1969 Apr; 55(2):372-374
2. Saidi S, Tesh R, Javadian E, Nadim A. The prevalence of human infection with West Nile in Iran. Iranian J Publ Health. 1974; 5: 8-14.
3. Siavashi MR, Massoud J. Human cutaneous dirofilariasis in Iran: a report of two cases. Iranian J Med Sci. 1995; 20(12): 85-86.
4. Edrissian GH. Malaria history and status in Iran. J Sch Pub Health and Ins Pub Health Res. 2002; 1(1):50-61 [Persian].
5. Knight KL, Stone A. A catalog of the mosquitoes of the world (Diptera: Culicidae). 2ed. Maryland, College Park: Entomological Society of America, 1977.
6. Knight KL. Supplement to catalog of the mosquitoes of the world (Diptera: Culicidae). Maryland, College Park: Entomological Society of America, 1978.
7. Ward RA. Second supplement to “A catalog of the mosquitoes of the world (Diptera: Culicidae)”. Mosq Sys. 1984; 16: 227- 270.
8. Gaffigan TV, Ward RA. Index to the second supplement to “A catalog of the mosquitoes of the world” with corrections and additions. Mosq Syst. 1985; 17: 52 -63.
9. Ward RA. Third supplement to “A catalog of the mosquitoes of the world (Diptera: Culicidae)”. Mosq Syst. 1992; 24: 177-230.

10. Reinert JE. Revised list of abbreviations for genera and subgenera of Culicidae (Diptera) and notes on generic and subgeneric changes. J Am Mosq Control Assoc. 2001 Mar; 17(1):51-55.
11. Harbach RE. The classification of genus Anopheles (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. Bull Entomol Res. 2004 Dec; 94(6):537-553.
12. Bates M, Beklemishev WN, Laface L. Anophelines of the Palearctic region. In: Boyd MF, Ed Malarialogy 1. Philadelphia: Saunders Co. 1949. p. 419- 442.
13. White GB. Systematic reappraisal of *Anopheles maculipennis* complex. Mosq Sys. 1978; 10: 13-44.
14. Linton YM, Smith L, Harbach RE. Observation on the taxonomic status of *Anopheles subalpinus* Hackett and Lewis and *An. melanoon* Hackett. Euro Mosq Bull. 2002; 13: 1-7.
15. Sedaghat MM, Linton YM, Oshaghi MA, Vatandoost H, Harbach RE. The Anopheles maculipennis complex (Diptera: Culicidae) in Iran: molecular characterization and recognition of a new species. Bull Entomol Res. 2003 Dec; 93(6):527-535.
16. Nicolescu G, Linton YM, Vladimirescu A, Howard TM, Harbach RE. Mosquitoes of the Anopheles maculipennis group (Diptera: Culicidae) in Romania, with the discovery and formal recognition of a new species based on molecular and morphological evidence. Bull Entomol Res. 2004 Dec; 94(6):525-535.
17. Dow RP. Notes on Iranian mosquitoes. Am J Trop Med Hyg. 1953 Jul; 2(4):683-695.
18. Shahgudian ER. A key to the anophelines of Iran**.** Acta Med Iran. 1960; 3(3): 38-48.
19. Glick JI. Illustrated key to the female *Anopheles* of southwestern Asia and Egypt (Diptera: Culicidae). Mosq Syst 1992; 24(2):125- 140.
20. Dinparast-Jadid N, Manouchehri AV. Study of the Anopheles *maculipennis* complex ecology in northern Iran. First Iranian Cong Med Parasitol, Rasht. 1990; p106 [Persian].
21. Oshaghi MA, Taghilo B, Moradi MT, Vatandoost H. Detecting the *Anopheles culicifacies* complex, species A and B in Baluchestan using mtRNA PCR RFLP assay, the first report of species B from Iran. Hakim Research Journal. 2004; 7(1): 35-42 [Persian].
22. Zaim M, Subbarao SK, Manouchehri AV, Cochrane AH. Role of Anopheles culicifacies s.l. and An. pulcherrimus in malaria transmission in Ghassreghand (Baluchistan), Iran. J Am Mosq Control Assoc. 1993 Mar; 9(1):23-26.
23. School of Public Health, Geographical Pathology of Iran. Tehran: Scientific Publication of School of Public Health and Public Health Research Institute, University of Tehran. 1970; 1802: 1-120.
24. Sedaghat MM, Linton YM, Nicolescu G, Smith L, Koliopoul G, Thanassius KZ, Oshaghi MA, Vatandoost H, Harbach RE. Morphological and molecular characterization *Anopheles (Anopheles) sacharovi* Favre, a primary vector of malaria in the Middle East**.** Syst Ent. 2003; 28(2):241-256.
25. Macan TT. The Anopheline mosquitoes of Iraq and north Persia. Lond Sch Hyg Trop Med Res Mem No 7, London: H.K & Lewis Co Ltd, 1950.
26. Monchadskii AS. The Larvae of blood–sucking mosquitoes of the U.S.S.R. and adjoining countries (Subfam-Culicinae). Opred. Faune SSR Moscow, Zool Inst Akad Nauk SSR 1951; 37: 1- 290[Russian].
27. Smart J. A Handbook for the identification of insects of medical importance. London: Trustees of the British Museum (Natural History), 1965.
28. Zaim M. Mosquito fauna of Iran. XVII Inter Cong Entomol, Hamburg, FG Germany. 1984; p29.
29. Zaim M. The mosquito fauna of Kashan, public health importance and control. Desert Scientific Research.1987; 17:1- 41[Persian].
30. Weiser J, Zaim M, Saebi E. *Coelomomyces irani* sp.n. infecting *Anopheles maculipennis* in Iran. J Inver Path.1991; 57:290-291.
31. Zaim M, Karami M, Eddalat H. Fauna, distribution and night biting activity of Culicidae in Amol district (Caspian Sea littoral), Iran (1994).IX SOVE European Meeting, Prague, Czech Republic.1995; p 51- 52.
32. Karimi M. Fauna, seasonal activity and adult behaviour characteristics of *Anopheles* species (endophilic) in Babol district (Caspian Sea littoral), Iran 1999. 2nd Iranian Cong Malaria, Tehran. 2000; p60 [Persian].
33. Mousakazemi S, Zaim M, Zahraii A. Fauna and ecology of Culicidae of the Zarrin-shahr and Mobarakeh area in Isfahan province. Armaghan Danesh, J Yasuj Univ Med Sci. 2000; 5(17-18):46-45 [Persian].
34. Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E. A study of mosquito (Diptera: Culicidae) fauna in Rasht county (IRAN). Modarress J Med Sci. 2001; 3(2):65-70 [Persian].

35. Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E. The distribution and larval habitat characteristics of mosquitoes (Diptera: Culicidae) in Rasht county (Guilan province, Iran). Modarress J Med Sci. 2002; 4(2):87-96[Persian].
36. Azari-Hamidian S, Joeafshani MA, Mosslem M, Rassaei AR. Adult mosquito habitats and resting-places in Guilan province (Diptera: Culicidae). Hakim, Research Journal. 2003; 6 (3):55-62 [Persian].
37. Dinparast-Jadid N, Kordiev M, Townson H. Molecular key to *Anopheles maculipennis* species complex and its application for malaria control in the costal area of Caspian Sea.3rd Iranian Cong Med Parasitol, Sari. 2001; p108 [Persian].
38. Gholizadeh S, Tafsiri E, Zakeri S, Dinparast-Jadid N. Molecular identification of a new species to Iranian anopheline fauna of *Anopheles maculipennis* complex (Diptera: Culicidae). The 13th Iranian Congress on Infectious Diseases and Tropical Medicine, Tehran. 2004; pp. 197 [Persian].
39. Gutsevich AV. On the mosquitoes of north Iran. Comptes Rendus Academy of sciences, USSR. 1943; 40(3):123- 125[Russian].
40. Zolotarev ER. *Anopheles maculipennis* of northern Iran. Med Parasit and Parasitic Dis, Moscow.1945; 14 (2):50- 57[Russian].
41. Etherington D, Sellick G. Notes on the bionomics of *Anopheles sacharovi* in Persia and Iraq. Bull Entomol Res .1946; 37:191- 195.
42. Kalandadze LP, Kaviladze OP. On the blood-sucking mosquitoes of the western part of the Iran Azerbaijan. Med Parasit and Parasitic Dis, Moscow. 1947; 16(1):57- 65[Russian].
43. Gutsevich AV. Mosquitoes and Malaria in Iran. In: Pavlovsky EN, Ed. Epidemio-parasitological expeditions to Iran and parasitological investigation. .Moscow: Academy of Science, USSR.1948; p 1941-1943[Russian].
44. Garrett-Jones C. An experiment in trapping and controlling *Anopheles maculipennis* in north Iran**.** Bull WHO. 1951; 4: 547- 562.
45. Foote RH, Cook DR. Mosquitoes of Medical Importance. Washington, D.C: Agriculture Handbook, No. 152, Agriculture Research Service, U.S. Department of Agriculture, 1959.
46. Momeni S, Manouchehri AV, Zaim M, Mottaghi M. The distinguish between the species of *Anopheles maculipennis* complex by using of egg characters in Gilan and Mazandaran provinces, northern areas of Iran 1990-91.Iranian Cong Malaria, Zahedan. 1992; p. 29 [Persian].
47. Azari-Hamidian S, Joeafshani MA, Mosslem M, Rassaei AR. Taxonomic survey of mosquitoes (Diptera: Culicidae) in Guilan province with reporting of a subgenus new to Iranian mosquito fauna.15th Iranian Plant Prot Cong, Kermanshah.2002; p319-320 [Persian].
48. Azari-Hamidian S, Joeafshani MA, Rassaei AR, Mosslem M, Mousavi-Eivanaki, E. Mosquito fauna of the Genus *Anopheles* (Diptera: Culicidae) in Guilan province. Modarres J Med Sci. 2003; 6(2): 11-22 [Persian].
49. Linton YM, Smith L, Koliopoulos G, Samanidou-Voyadjoglou A, Zounos AK, Harbach RE. Morphological and molecular charecterization of *Anopheles (Anopheles) maculipennis* Meigen, type species of the genus and nominotypical member of the Maculipennis Complex. Sys Entomol .2003; 28:39-55.
50. Horsfall WR. Mosquitoes. Their bionomics and the relation to disease. NewYork: Hafner Publishing, 1955.
51. Zulueta JD, Jolivet P, Thymakis K, Caprari P. Seasonal variation in susceptibility to DDT of *Anopheles maculipennis* in Iran. Bull WHO.1957; 16: 475- 479.
52. Dahl C, White GB. Culicidae. In: Illies, J (Ed) Limnofauna Europaea (2nd ed). Stuttgart: Gustav Ficher Verlag.1978; p 390- 395.
53. Minar J. Results of the Czechoslovak-Iranian entomological expendition to Iran 1970. Diptera: Culicidae. Acta Ent Musei Nat Pragae. 1974; 6:87- 89.
54. Edwards FW. A revision of the mosquitoes of the Palaearctic region. Bull Entomol Res. 1921; 12(3): 263- 351.
55. Beklemishev VN, Gontaeva AA. Anophelogenous landscapes of northwest Iran. Med Parasit and Parasitic Dis, Moscow.1943; 12 (5):17- 32[Russian].
56. Ross ES, Roberts HR. Mosquito atlas (Part II). Eighteen old world Anophelines important to malaria. Philadelphia: The American Entomological Society, the Academy of Natural Sciences, 1943.
57. Russell PF, Rozeboom LE, Stone A. Keys to the anopheline mosquitoes of the world with note on their identification, distribution, biology and relation to malaria. Philadelphia: The American Entomological Society, the Academy of Natural Sciences, 1943.

58. Zahar AR. Review of the ecology of malaria vectors in the WHO Eastern Mediterranean region. Bull WHO.1974; 50:427-440.
59. Yaghoobi-Ershadi MR, Namazi J, Piazak N. Bionomics of *Anopheles sacharovi* in Ardebil province, northwestern Iran during a larval control program. Acta Tropica. 2001; 78:207- 215.
60. Abul-Hab J. The differentiation of larval population of *An. sacharovi* and *An.maculipennis* Meigen in north Iraq**.** Bull End Dis. 1955; 1:265- 273.
61. De Buck A, Schoute E, Sewllengrebel NH. Racial differentiation of "*Anopheles maculipennis*" in Netherlands and its relation to malaria. Riv Malariol. 1930; 9: 97- 110.
62. Missiroli A. Varieties of *Anopheles maculipennis* and malaria in Italy. 7th Inter Cong Entomol, Berlin. 1938; p1619-1640.
63. Bates M. Variation in the antepalmate hairs of larvae of the *Anopheles maculipennis* complex. Riv Malariol. 1939; 18: 299-312
64. Darsie RE, Samanidou-Voyadjoglou A. Keys for the identification of the mosquitoes of Greece. J Am Mosq Cont Assoc.1997; 13 (3): 247- 254.
65. Romi R, Boccolini D, Horanesyan I, Grigoryan-G, Diluca M, Sabatinelli G. *Anopheles sacharovi* (Diptera: Culicidae): a reemerging malaria vector in the Ararat Valley of Armenia. J Med Entomol. 2002; 39:447-450.
66. Hackett LW, Lewis DJ. A new variety of *Anopheles maculipennis* in southern Europe. Riv Malariol. 1935; 14: 377-383.