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

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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.

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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...
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. martininii (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.

Bio-systematics 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.

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 comparison to lateral palatal brush (61) and 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).

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).

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.

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

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. messae 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
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.

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