

Original Article

Sand Flies of the Subgenus *Adlerius* (Diptera: Psychodidae) in an Endemic Focus of Visceral Leishmaniasis and Introduction of *Phlebotomus (Adlerius) comatus* as a New Record for Iran

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Abstract

Background: Sand flies of subgenus *Adlerius* has a wide geographical distribution in Iran and are mostly found in wild form in mountainous areas. They are always considered as probable vectors of visceral leishmaniasis. The objective of this study was to determine the *Adlerius* species and its composition in an endemic focus of zoonotic visceral leishmaniasis in northwest of the country.

Methods: Sand flies were collected from 6 different areas of Azarbaijan-e-Sharqi Province using sticky paper traps from August to September which is active season for sand flies in this area, in 2009. The flies were mounted and identified. The length of third antennal segments, ascoid, labrum, coxite, surstyle, style, aedeagus, genital filament, genital pump, width of style, and the end of aedeagus were measured and the number of costal hairs group was also counted as the morphological characters.

Results: A total of 30 adult sand flies, (26 males and 4 females) including *Phlebotomus halepensis* (46.8%), *P. longiductus* (13.3%), *P. balcanicus* (23.3%), *P. comatus* (3.3%), and *Adlerius* spp. (13.3%) belong to subgenus *Adlerius* were identified respectively in 6 counties. One *P. comatus* male was captured in front of a cave located in the hillside of a mountain covered with the vegetation in Varzeqan area.

Conclusion: The presence of at least 5 species of the subgenus *Adlerius* in Azarbaijan-e-Sharqi Province, an endemic focus of zoonotic visceral leishmaniasis in Iran, shows that the risk of parasite transmission among man and reservoir animals is high during the active season of sand flies. *P. comatus* is a new record for Iran and needs to be added to the list of Iranian phlebotomines of subgenus *Adlerius*.

Keywords: *Phlebotomus (Adlerius) comatus*, Phlebotomine sand flies, Visceral leishmaniasis, New species, Iran

Introduction

Visceral Leishmaniasis (VL) is a fatal protozoan disease caused by *Leishmania donovani* complex (Kinetoplastidae: Trypanosomatidae) transmitted by the bites of phlebotomine sand flies (Diptera: Psychodidae) of the genus *Phlebotomus* in The Old World and *Lutzomyia*

in The New World. It is a serious illness that gives rise to epidemics and causes high mortality if left untreated. Visceral leishmaniasis appears as an opportunistic disease associated with the HIV infection and in other immune suppressed patients (Desjeux and Alvar 2003).

Leishmania infantum Nicolle, 1908 can also cause cutaneous lesions (WHO 2010). There are at least 7 endemic foci of Zoonotic Visceral Leishmaniasis (ZVL) in Iran as follows:

In Iran, some parts of Fars Province in the south and Ardabil Province in the northwest are indicated as the highly endemic areas while some parts of Azarbaijan-e-Sharqi Province in the northwest, Saveh and Qom in the central, Khorramabad area of Lorestan Province in the west, some parts of Khuzestan Province in the southwest and Khorassan-e-Shomali area in the northeast are known as the low endemic areas. In all foci, kala-azar is caused by *L. infantum*. Dogs, foxes and Jackals are main reservoir hosts (Yaghoobi-Ershadi 2012). Kala-azar is endemic in four counties of Azarbaijan-e-Sharqi province including Ahar, Sarab, Kaleybar and Azarshahr.

Five species of sand flies including *P. kandelakii*, *P. perfiliewi*, *P. keshishiani*, *P. neglectus* and *P. alexandri* are considered as probable vector species of ZVL in Iran (Yaghoobi-Ershadi 2012).

The subgenus *Adlerius* Nitzulescu includes about 20 species which some are suspected or proven vectors of ZVL in the old world, as follows:

Phlebotomus balcanicus in Armenia and Georgia, *P. longiductus* in China, Kazakhstan, Kyrgyzstan, Ukraine and Uzbekistan, *P. halepensis* in Syrian Arabic Republic and Georgia (WHO 2010).

The main key to species identification of subgenus *Adlerius* of Iran was prepared by Seyedi-Rashti and Nadim (1992). They believed that females of the subgenus are unidentifiable. So the key is based on male morphology as follows:

- 1- Coxite with less than 29 hairs. Subterminal tubercle of aedeagus 19–28 μm from tip.
Aedeagus short and thick. *P. brevis*
- Coxite with more than 30 hairs. Aedeagus long. 2
- 2- Aedeagus with rectangular subterminal notch, coxite with 50 hairs or less (39–

40).

P. halepensis

- Subterminal tubercle of aedeagus not rectangular. Coxite with more than 50 hairs. 3
- 3- Coxite with 50–80 hairs. Genital filaments very long 8–15 times length of pump. *P. longiductus*
- Coxite with 69–105 hairs. Genital filament 8 times length of pump. *P. balcanicus*

In the last 3 decades ZVL has become an endemic disease in Azarbaijan-e-Sharqi Province, northwest of the country.

The objective of this study was to determine the *Adlerius* species and their population size in an endemic focus of ZVL in northwest of the country.

Materials and Methods

Study area

The Azarbaijan-e-Sharqi Province is located in the northwest of Iran (38.0766 ° N, 46.2800 ° E) bordering Armenia and the Republic of Azerbaijan countries and the provinces of Ardabil, Western Azarbaijan, and Zanjan (Fig. 1). It covers an area of approximately 47830 km², with altitude range of 1310 m in plain locale to 3722 m in highland locale. It has a cool and dry climate being in the main a mountainous region. Temperatures run between -17 °C in the winter and up to 40.6 °C in the summer. The annual precipitation is about 255 mm. It has a population of 3691270 people (SCI, 2006).

Sand flies collection

Sand flies were collected monthly from 6 different areas including Tabriz, Ahar, Sharabian-Esmailabad, Varzeqan, Kaleibar and Marand in Azarbaijan-e-Sharqi Province using 50 sticky paper traps (castor oil coated white papers 15×21 cm) from the beginning (August) to the end (September) of the active season in 2009. Traps were installed before sunset and collected before sunrise. Collected sand flies

were removed from sticky papers using needle dipped and stored in 75% ethanol. Females of sand flies belong to the subgenus *Adlerius* are more or less morphologically identical and there is no key for identification so only male specimens were selected for morphological study (Seyedi-Rashti and Nadim 1992).

Mounting

The head and genitalia of individual male sand flies were cut off within a fresh drop of sterile saline on a clean slide then they were transferred to a drop of puri's media between a slide and cover slip (Smart et al. 1965). Identification of specimens was carried out after 24 h using the keys of Theodor and Mesghali 1964, Artemiev 1973, Lewis 1982, Seyedi-Rashti and Nadim 1992.

Morphometric measurements

Morphometric measurements were done by Olympus microscope (CH-2) and photographs were taken by camera of Leica microscope (Model: DM 2000).

The specimen characters on the length of third antennal segments, ascoid, labrum, coxite, surstyle, style, aedeagus, genital filament, genital pump, and also the width of style were measured with different magnifications. In addition, for the *P. comatus* male, the end of aedeagus was measured. Number of costal hairs group, a very important morphological character, for all *Adlerius* specimens were counted. The entire given measurements are in micrometer (μm).

Results

Adult sand flies belong to genus *Phlebotomus* were collected and identified. Males were significantly larger numbers than females from the area sampled (30:4). Thirty four *Phlebotomus* sand flies belong to the subgenus *Adlerius* (30 males and 4 females) were identified. *Phlebotomus halepensis* (46.8%), *P. longiduc-*

tus (13.3%), *P. balcanicus* (23.3%), *P. comatus* (3.3%), and females of *Adlerius* spp. (13.3%) have been captured during this study in 6 areas of the Azarbaijan-e-Sharqi Province (Table 1).

Phlebotomus halepensis was present in the all areas. The relative abundance of this species was 46.8%, higher than of the other species in comparison. The most captured *Adlerius* specimens, including *P. halepensis*, *P. longiductus* and *P. balcanicus*, were related to Varzeqan area.

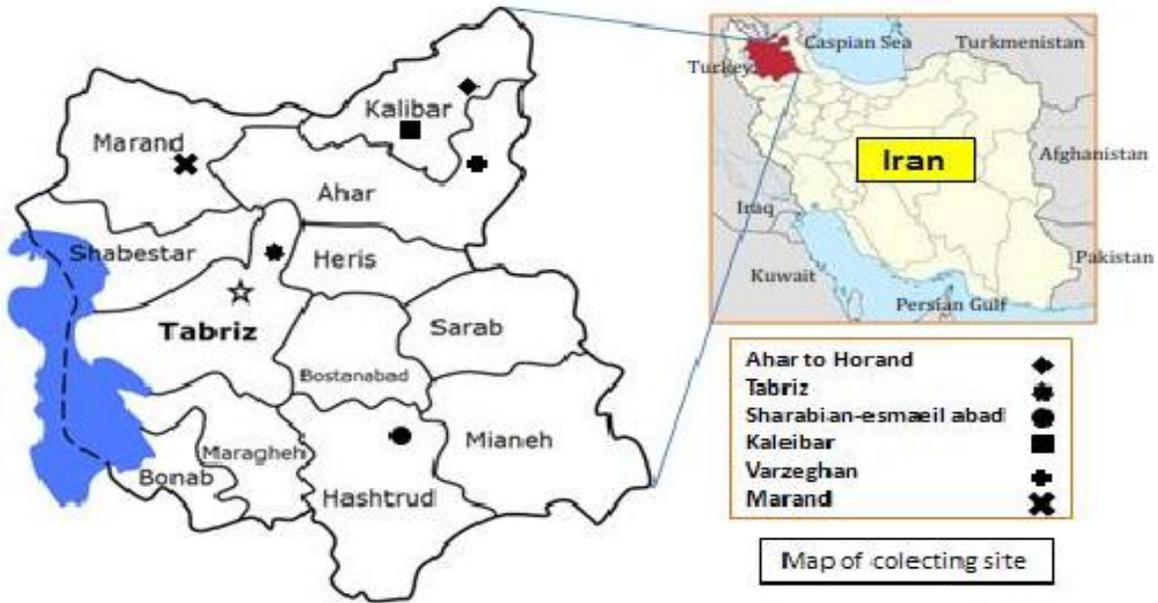
The altitudinal distribution and abundance of all *Adlerius* species collected from the study areas are shown in Table 1. *Phlebotomus halepensis* was found at an altitude range of 1200–1670 m, *P. longiductus* was captured from two different altitudes at 1200 and 1670 m, while *P. balcanicus* and *P. comatus* were only trapped at 1670 m. As the table shows different species of subgenus *Adlerius* have been collected at altitude of 1670 m in Varzeqan area. It seems that the highest altitudes are preferred for breeding places of these phlebotomine sand flies.

We found only one *Phlebotomus comatus* male from Varzeqan area, which was a new record for Iran. It was collected in front of a cave located in the hillside of a mountain covered with the vegetation in Varzeqan area. The humidity was measured as normal (60%). Table 2 shows the measurements of the species. In this specimen, the coxite is wide with dense group of about 176 hairs. The hairy spots are located almost the middle of the coxite. The paramere is pigmented at the end. Aedeagus with subterminal tooth at 23 μm from the end. The length of genital filament is 931 μm and F/P=6.5. Figure 2 shows photos of some important characters on the head eg, antennal segment 3, clypeus, epipharynx and palp and also the genitalian parts at the end of the abdomen eg, genital filament, genital pump, length and width of aedeagus, dense group of 176 hairs on coxite and pigmented paramere.

Table 1. Sand flies of subgenus *Adlerius* and their relative abundance (%) in 6 areas of Azarbaijan-e-Sharqi, an endemic province of visceral leishmaniasis in northwest of Iran

Row	Area	Altitude*	Species of subgenus <i>Adlerius</i> (male)										
			<i>P. halepensis</i>		<i>P. longiductus</i>		<i>P. balcanicus</i>		<i>P. comatus</i>		<i>Adlerius</i> sp. (Female)		Total
			No.	%	No.	%	No.	%	No.	%	No.	%	
1	Tabriz	1200 m	1	25	3	75	0	0	0	0	0	0	4
2	Ahar	1367 m	3	100	0	0	0	0	0	0	0	0	3
3	Mianeh	1300 m	1	25	0	0	0	0	0	0	3	75	4
4	Varzeqan	1670 m	6	40	1	6.7	6	40	1	6.7	1	6.6	15
5	Kaleibar	1240 m	2	100	0	0	0	0	0	0	0	0	2
6	Marand	1334 m	1	50	0	0	1	50	0	0	0	0	2
Total			14	46.8	4	13.3	7	23.3	1	3.3	4	13.3	30

*(Statistical Center of Iran, 2006)



Scale: 1/50000

- 20 Km of Tabriz -Ahar Road
- ◆ 10 Km of Horand -Ahar Road in front of Moradlou village, near of Cheno village
- Sharabeian-Esmaeil-Abad village
- ✕ Marand-Heidarlou village 5Km near of Sary tapah village
- ⊕ 12Km of Varzeghan-Ahar, near of Sardarkandy village
- Kaleibar (Horand Road- 10Km to Abesh-ahmad village)

Fig. 1. Map of Azarbaijan-e-Sharqi province, showing sand fly sampling areas

Table 2. The comparison of morphometric measurements (μm) on males of *Phlebotomus (Adlerius) comatus* from Iran and Afghanistan

Country	Iran Present study	Afghanistan (Seyedi- Rashti and Nadim) *
Number of Coxal setae	176	-
Ascoid 3 Length	49	-
Labrum Length	270	270–300
Clypeous Length	180	-
Cibarium teeth	Unarmed	Unarmed
Palp1 Length	60	-
Palp2 Length	175	-
Palp3 Length	190	-
Coxite Length	450	420
Surstyle Length	450	-
Style Length	220	200
Style width	50	-
Aedeagus Length	170	-
Aedeagus End	Subterminal tooth	-
A3 Length	399	310–370
Genital Filament	931	-
Genital Pump	144	-

*(Seyedi-Rashti and Nadim 1992)

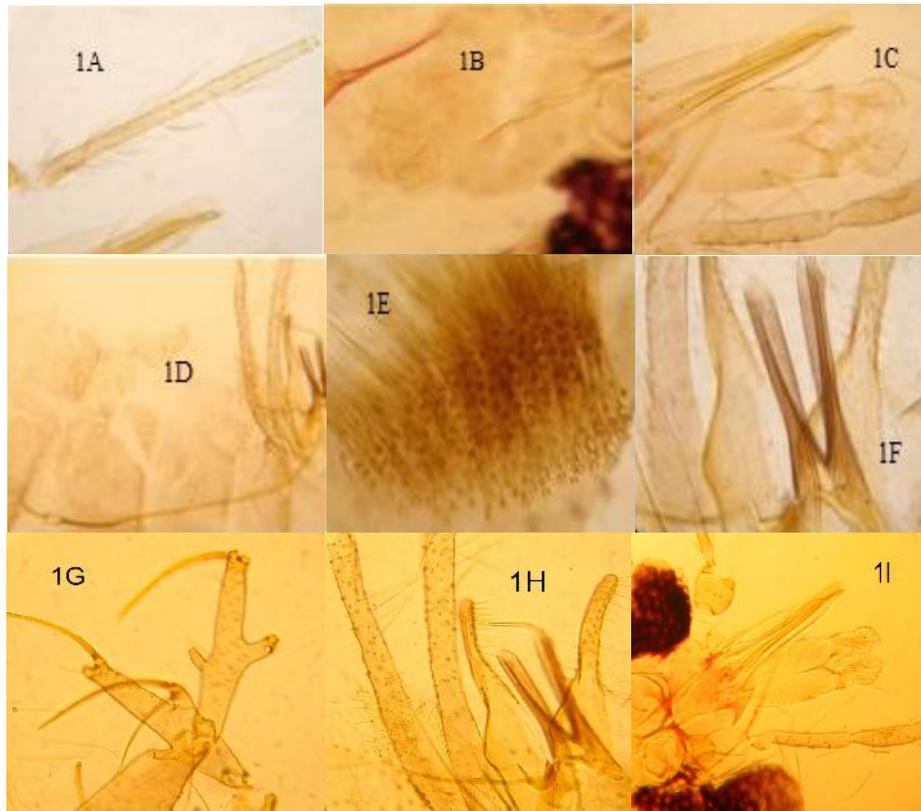


Fig. 2. *Phlebotomus (Adlerius) comatus* Artemiev male: **1A**, Antennal 3($\times 400$), **1B**, Pharynx and pharyngeal armature ($\times 400$), **1C**, Epipharynx (labellum, maxilla) and palp ($\times 400$), **1D**, Genital filament, genital pump, Surstyle ($\times 100$), **1E**, dense group of 176 hairs on coxite ($\times 1000$), **1F**, Length and width of aedeagus, paramere ($\times 630$), **1G**, Style and the seta($\times 400$), **1H**, Pigmented paramere ($\times 400$), **1I**, Clypeus($\times 400$)

Discussion

Most species of the subgenus *Adlerius* were considered as subspecies of *Phlebotomus chinensis* (Lewis 1978). Theodor and Mesghali (1964) supposed that some of the subspecies may have to be made into species. After the examination of numerous specimens from Afghanistan, central Asia and Caucasus it was concluded that the supposition of these scientists was right (Artemiev 1973).

Males of all described *Adlerius* species from Iran can be easily identified according to antennal formula, position of the coxal tuft and its number of setae and shape of aedeagus, but females are much more uniform. Prior to this study six species including *P. halepensis*, *P. brevis*, *P. longiductus*, *P. balcanicus*, *P. turanicus* and *P. salangensis* were recorded from Iran (Seyedi-Rashti and Javadian 2008, Akhundi et al. 2012). Whether *P. kabulensis* has also been recorded in the past (Kasiri et al. 2000) but it has not been confirmed by senior entomologists in the country yet. Some morphometric measurements have been processed to characterize another new species for the list of phlebotomine sand flies of Iran (Table 3). Artemiev revised the *Adlerius* of Afghanistan in 1978. He caught and identified 44 males of *P. comatus* from Northern and Central Afghanistan including Kala-i-Naw (Badghis), Khulm, Bamyan and Gorband valley. He mentioned that it is a species of rocky mountain (1000–2600 m) and it seems to be moderately thermophilic and hydrophilic. He described that male of *P. comatus* has antennal formula 2/3–5, 1/6–15, coxite very wide, with dense group of 165(126–203) hairs, distal border of the hairy spot at 0.47 (0.41–0.51) of coxite, paramere with wide pigmented end, aedeagus rather short, with subterminal tooth a 20 (16–24) μ m from the end, Genital filaments 1040 (800–1280) μ m long, F/P=8.2 (6.9–9.4).

Seyedi-Rashti and Nadim (1992) mentioned that in Afghan specimens of *P. comatus*, the

measurements are as follows: the length of antennal segment 3, 310–370 μ m, labrum 270–300 μ m, coxite 420 μ m and style 200 μ m (Table 3).

The Iranian male specimen of *P. comatus* has 176 numbers of coxal hairs. It is in the range record numbers of the Afghan specimen by Artemiev in 1978. Antenna 3 observed to be longer than flies recorded by Seyedi-Rashti and Nadim (1992) but labrum is in the range record of the latter authors. The length of coxite is larger and style length is also bigger than which was described by Seyedi-Rashti and Nadim in 1992.

According to the literature *P. halepensis* was recorded by Theodor and Mesghali from Tabriz county, Azarbaijan-e-Sharqi Province in 1964 and from Sarab county by Mesghali in 1961. During an entomological study of visceral leishmaniasis *P. halepensis*, *P. brevis*, *P. longiductus* and *P. balcanicus* were recorded in Kaleybar county at the same province in 2000 (Rassi et al. 2000). Our captures and identifications showed that *P. halepensis*, *P. longiductus* and *P. balcanicus* are common species in this province.

Phlebotomus brevis which had recorded from the area previously was not found in this study. *Phlebotomus halepensis* and *P. brevis* have wide distribution in Iran at altitudes between 800 and 1700 m above sea level mostly in mountainous regions. *Phlebotomus longiductus* and *P. balcanicus* have limited distribution in comparison of *P. halepensis* and *P. brevis* in the country. These four species are present in sympatry in the northwest of Iran (Ardehali et al. 1995).

We caught the Iranian specimen of *P. comatus* from Varzeqan area in elevation of 1670 m. Its coxite is also wide, paramere with wide pigmented ends. In comparison with other species aedeagus is short with subterminal tooth at 23 μ m from the end and it is in the range record numbers of Artemiev's

specimens. The ratio of F/P in Iranian specimen is also in the range of his record from Afghanistan.

It should be mentioned that Varzaqan is the mining center for base metal mining as well as gold and farming is the predominant occupation in the rural areas. Recently Spruce trees have also been planted as the start of a forestry industry at the same place.

More investigations are needed to reveal the distribution of *P. comatus* in Iran and its role in the transmission of *L. infantum* in the study area remains to be specified.

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