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Original Article

Morphometric Characterization and Distribution of the Subgenus *Adlerius* of Sand Flies (Diptera: Psychodidae, Phlebotominae) in Southeastern Iran: New Records from Sistan and Baluchistan Province

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Abstract

Background: Phlebotomine sand fly taxonomy has been widely studied, often due to their role as vectors of various pathogens, including arboviruses, *Leishmania* spp. and *Bartonella* spp., which cause infections in both animals and humans. The objective of this research was to determine the morphological characteristics of various populations of *Phlebotomus halepensis*, *Ph. kabulensis* and *Ph. salangensis* collected in the southeastern region of Iran, using morphometric analysis of male sand fly features.

Methods: Specimens were captured by sticky paper traps and preserved in 70% ethanol**.** They were mounted in Puri's medium on microscope slides. The sand flies were identified by examining them under a light microscope following valid morphological keys. The morphometric analysis of the specimens was done using a microscope aided by a micrometer. All measurements were recorded in millimeters (mm). Photographs and drawings of identifying characteristics of male specimens were taken.

Results: A total of 87 phlebotomine sand flies belonging to the subgenus *Adlerius* were caught in four counties of Sistan and Baluchistan Province, Iran. We report the presence of *Adlerius* in the counties: Iranshahr (*Ph. kabulensis*), Khash (*Ph. halepensis, Ph. kabulensis* and *Ph. salangensis*), Nikshahr (*Ph. halepensis* and *Ph. kabulensis*) and Zahedan (*Ph. halepensis*, *Ph. kabulensis* and *Ph. salangensis*). The morphometric characteristics of *Ph. halepensis*, *Ph. kabulensis* and *Ph. salangensis* are listed in detail in this article.

Conclusion: More intensive investigations are required to find sand fly infections, their relative abundance and distribution; moreover, subsequent DNA molecular comparisons are needed to control them in Iran.

Keywords: Phlebotomines; Taxonomy; Identification; Iran

Introduction

Phlebotomine sand flies are hematophagous insects feeding on various vertebrate hosts**.** In total, there are 1063 species of sand flies (549 in the New World and 514 in the Old World), distributed among 23 and 17 genera, respectively. Of these, approximately 10% of species and subspecies are implicated in the transmission of various diseases, including Chandipura virus encephalitis, Papataci fever, bartonellosis (Carrión disease), Isfahan virus (Rhabdoviridae: Vesiculovirus) and leishmaniasis, the latter being recognized as one of the major neglected tropical diseases. Notably, leishmaniasis alone accounts for nearly one million new cases annually (1–4).

The subgenus *Adlerius* (Chinensis group) was proposed by Nitzulescu. *Phlebotomus chinensis* Newstead is the type species of this subge-

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nus, the vector of *Leishmania infantum* in China (5). Artemiev (6) paid attention to all species of this subgenus as reliable species and characterized them. Morphologically, females have wrinkled spermathecae with disordered segments, a short neck and gently decreased width towards the end. Ducts of the spermatheca are separate, lengthy and expanded at their base. The pharyngeal armature of the females is specified by two types of teeth. The antennal formula is 2/III-XV. Males of medium size have a long parameral sheath (aedeagus) showing a subterminal tubercle, including a coxite with a group of setae on the internal surface and without a basal process, a simple paramere, five long spines on the style (two spines are on the dorsal surface, two spines at the end of the style and one spine on the ventral surface of the style). The genital filament is long, 5–12 times the length of the sperm pump. The antennal formulas differ from 2/III–XV to 2/III–V-1/VI–XV, with many intermediate combinations. The identification of the *Adlerius* species is based on male morphology, whereas females are considered unidentifiable. The location and number of setae on the coxite are the most important morphological characteristics in identifying and distinguishing *Adlerius* species from others. At times, it is important to identify the number and location of setae on the body of different species of sandflies to check how similar or different they are. According to morphological keys, the total number of coxite setae in *Ph. halepensis* is between 34 and 53, in *Ph. kabulensis* is 27–50 and in *Ph. salangensis* is 30–85. In both *Ph. kabulensis* and *Ph. salangensis*, the parameral sheath has a normal obtuse-angle subterminal notch, but in *Ph. halepensis* the parameral sheath has a rectangular subterminal notch (6–8). The females of this subgenus have been identified using sequence analysis of the cytochrome b gene (Cyt b) (9).

The subgenus *Adlerius* includes 21 species. They have been involved in the transmission of both visceral leishmaniasis and cutaneous leishmaniasis (3, 8, 10). In the present study, we report new records of the subgenus *Adlerius* from Sistan and Baluchistan Province, along with their morphometric analysis. This paper also provides fundamental taxonomic information on these sand flies and contributes to a broader understanding of the subgenus *Adlerius* in Iran.

Materials and Methods

This study was conducted on sand fly samples collected in 1997 from the Baluchistan region of Sistan and Baluchistan Province, in southeastern Iran. The region has an average elevation of 763 meters above sea level. Sistan and Baluchistan Province is the second largest of Iran's 31 provinces, covering an area of 180,726 km². Its capital is Zahedan, and the province shares borders with Afghanistan and Pakistan (Fig. 1).

Sand flies were collected with sticky paper traps (consisting of white sheets 15×21 cm, coated with castor oil) from indoors (human and animal places, such as living room, bathroom, warehouse, chicken coop and stable) and outdoors (rodent burrows, mountain and cave rifts, wall rifts, roach rifts) in both plain and mountainous areas. Traps were installed before sunset and were collected after sunrise. Specimens were collected from six counties: Chabahar (25°17′34″N, 60°38′59″E), Iranshahr (27°11′52″N, 60°41′01″E), Khash (28°13′12″N, 61°12′41″E), Nikshahr (26°13′31″N, 60°13′00″E), Saravan (27°22′15″N, 62°19′57″E) and Zahedan (29°30′09″N, 60°51′21″E)..

Sand flies were removed from sticky paper traps with a scientific tool, rinsed in acetone and then conserved in 70% ethanol. Sand flies were mounted on microscope slides. The specimens were mounted one by one on each slide, Puri’s medium was added, and the cover slip was slowly put on the specimen. The specimens were identified according to the valid morphological keys (11–13). The study

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and confirmation of the identification of these specimens were carried out in the Natural History Museum of London. The specimens are deposited in the personal collection of the corresponding author and are available on request. Morphometric analysis was performed on three specimens of *Ph. halepensis*, 25 of *Ph. kabulensis* and 19 of *Ph. salangensis*, with 16 characters measured per specimen (Table 1) to test for intraspecific variation. For morphometric analysis, an Olympus microscope was used for all measurements, and hand drawings were made to document key features. Measurements were recorded in millimeters (mm), and species identification followed the morphological terminology of Galati et al. (14).

Results

A total of 87 phlebotomine sand fly specimens belonging to the subgenus *Adlerius* were caught in four counties of Sistan and Baluchistan Province. No *Adlerius* specimens were caught in the counties of Chabahar and Saravan. The principal morphological features of the male subgenus *Adlerius* were that the style bears five long spines, the coxite has no tubercle and the paramere has no ventral lobe and is not truncated. The parameral sheath has a subterminal tiny notch. The morphological description of male *Ph. kabulensis* was: parameral sheath with an obtuse-angled subterminal tooth, genital filament/genital pump was 7.8–10.4, presence of one ascoid on antennal segments 9–15, antenna 3–8 with two ascoids, coxite is relatively wide, the border of the coxite brush from the base of the coxite is 0.56–0.64, and a group of 44–58 setae on the coxite (Table 2). The morphometric characteristics of *Ph. salangensis* and *Ph. halepensis* are listed in Table 3 and Table 4, respectively. Drawings of the Iranian male specimens are shown in Fig. 2. Pictures of male genitalia of three species and female pharyngeal armature of two species of sand flies mentioned above are in Figs. 3 and 4.

We report the presence of *Adlerius* in counties as follows: Iranshahr (*Ph. kabulensis*), Khash (*Ph. halepensis*, *Ph. kabulensis* and *Ph. salangensis*), Nikshahr (*Ph. halepensis* and *Ph. kabulensis*) and Zahedan (*Ph. halepensis*, *Ph. kabulensis* and *Ph. salangensis*). We captured the *Adlerius* species from different rural regions and mountainous habitats. The most captured specimens were related to the counties of Khash, Zahedan, Nikshahr and Iranshahr, respectively. *Phlebotomus kabulensis*, *Ph. salangensis* and *Ph. halepensis* accounted for approximately 0.3%, 0.06% and 0.03% of the caught *Phlebotomus* spp. from Sistan and Baluchistan Province.

The highest catch rate of *Ph. kabulensis* was in Khash County, accounting for 1.8% of the caught *Phlebotomus* spp. The catch rates of *Ph. kabulensis* in Iranshahr, Zahedan and Nikshahr Counties were 0.03%, 0.2% and 0.1% of the caught *Phlebotomus* spp., respectively. The catch rates of *Ph. salangensis* in Khash and Zahedan Counties were 0.4% and 0.06% of the caught *Phlebotomus* spp., respectively. The catch rates of *Ph. halepensis* in Khash, Zahedan and Nikshahr Counties were 0.1%, 0.1% and 0.03% of the caught *Phlebotomus* spp., respectively.

During the study months (from April to September), the highest number of *Ph. kabulensis* catches was in April (n=29). In May, June and July, 27, 2 and 7 specimens were caught, respectively. Accordingly, the highest number of *Ph. salangensis* catches was in May (n= 10), April (n=3) and August (n=2). In addition, the highest catch rates for *Ph. halepensis* were in the months of May (n=3), August (n= 2), April (n=1) and July (n=1).

A total of 65 *Ph. kabulensis* (62 males and 3 females), 15 *Ph. salangensis* (14 males and 1 female), and 7 *Ph. halepensis* (males) specimens were caught. All three species mentioned above were hunted from mountainous and foothill areas and were not found in plain regions.

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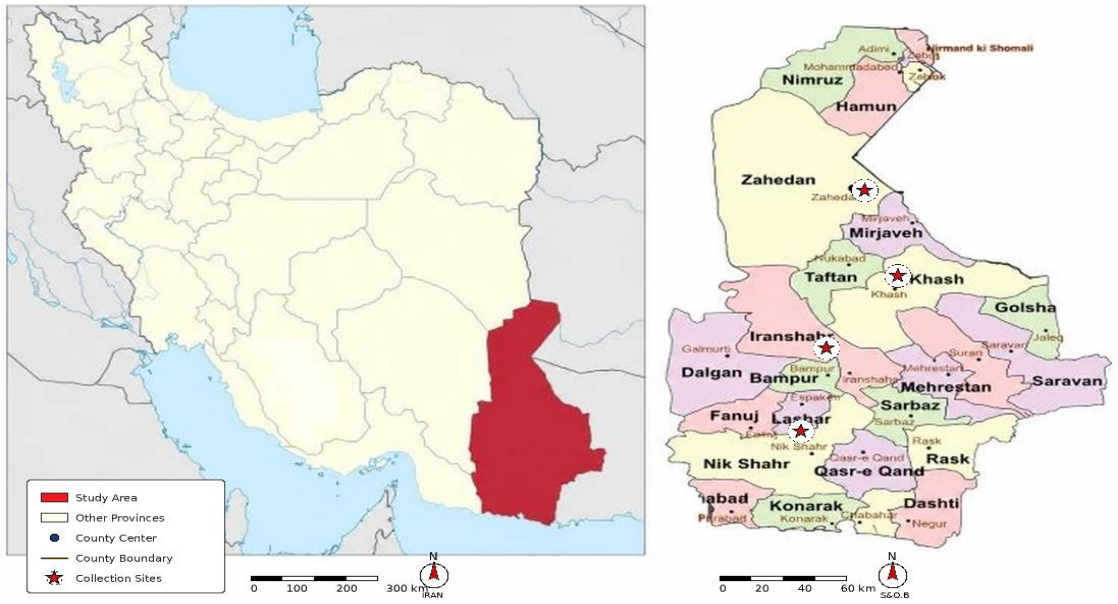


Fig. 1. Sampled regions for capturing the subgenus *Adlerius* species, Sistan and Baluchistan Province, southeastern Iran

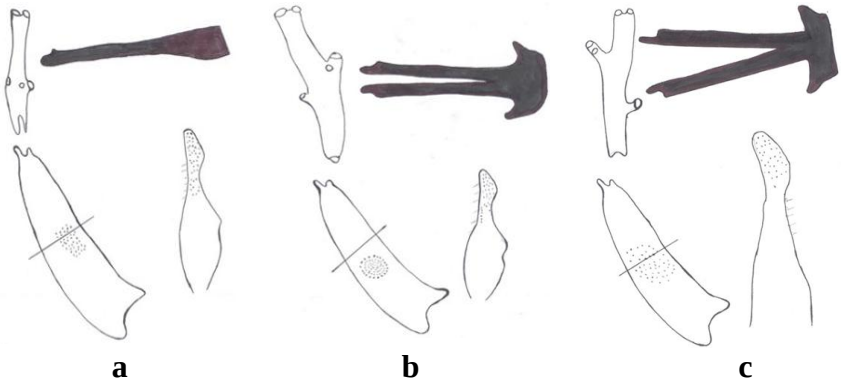


Fig. 2. Drawings of male genitalia in (a) *Phlebotomus kabulensis*, (b) *Ph. salangensis* and (c) *Ph. halepensis*, caught in Sistan and Baluchistan Province, southeastern Iran



Fig. 3. Photos of male terminalia in (a) *Phlebotomus kabulensis*, (b) *Ph. salangensis* and (c) *Ph. halepensis*, caught in Sistan and Baluchistan Province, southeastern Iran. Figures are from H. Kassiri's personal archive and require permission for reproduction

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Table 1. List of morphological characteristics and description of feature measurements conducted on *Phlebotomus halepensis*, *Ph. kabulensis* and *Ph. salangensis*

Characters	Descriptions	Characters	Descriptions	Characters	Descriptions
A	Length of style	F	$\frac{\text{Length of coxite}}{\text{Length of the parameral sheeth}}$	K	Length of genital filament
B	Length of coxite	G	Length of antenna 3	L	$\frac{\text{Length of genital filment}}{\text{Length of sperm pump}}$
C	Barb of the parameral sheeth from the tip	H	$\frac{\text{Length of antenna 3}}{\text{Length of epipharynx}}$	M	Border of the coxite brush from base of coxite
D	Number of hairs on the coxite brush	I	Length of ascoid an antenna 4	N	Antennal formula
E	Length of the parameral sheeth	J	$\frac{R_2}{R_{2+3}}$	O	Body length
				P	Wing length

Table 2. Morphometric measurements (in mm) of male *Phlebotomus kabulensis* sand flies collected from four counties (Iranshahr, Khash, Nikshahr and Zahedan) in Sistan and Baluchistan Province, southeastern Iran

No	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	0.17	0.35	0.021	45	0.18	1.94	0.4	1.67	0.048	1.80	1.1	10	0.60	2/3-8,1/9-15	2.87	2.43
2	0.16	0.33	0.018	44	0.17	1.94	0.34	1.48	0.049	1.75	1.02	7.8	0.60	2/3-8,1/9-15	2.73	2.56
3	0.17	0.4	0.018	47	0.18	2.22	0.46	1.84	0.054	1.88	1.3	9.3	0.64	2/3-8,1/9-15	2.33	2.27
4	0.16	0.33	0.015	49	0.17	1.94	0.31	1.29	0.040	1.84	0.97	8.08	0.61	2/3-8,1/9-15	2.87	2.01
5	0.19	0.37	0.024	54	0.19	1.95	0.43	1.65	0.048	1.37	1.15	10	0.61	2/3-8,1/9-15	2.48	2.42
6	0.17	0.36	0.018	49	0.18	2	0.39	1.5	0.054	1.59	1.05	9.5	0.64	2/3-8,1/9-15	2.49	2.43
7	0.17	0.38	0.024	44	0.19	2	0.45	1.73	0.048	1.74	1.25	10.4	0.63	2/3-8,1/9-15	2.16	2.42
8	0.16	0.34	0.018	47	0.18	1.89	0.45	1.73	0.054	1.84	1.16	8.9	0.64	2/3-8,1/9-15	2.20	2.56
9	0.16	0.31	0.024	49	0.18	1.89	0.37	1.32	0.078	1.87	1.13	8.07	0.63	2/3-8,1/9-15	2.9	2.38
10	0.16	0.34	0.018	47	0.18	1.89	0.45	1.73	0.054	1.67	1.16	8.9	0.64	2/3-8,1/9-15	2.78	2.22
11	0.18	0.37	0.018	48	0.19	1.94	0.43	1.59	0.054	1.68	1.11	9.25	0.63	2/3-8,1/9-15	2.83	2.43
12	0.16	0.33	0.018	47	0.18	1.83	0.37	1.32	0.054	1.75	1.1	10	0.60	2/3-8,1/9-15	2.78	2.56
13	0.17	0.34	0.021	50	0.18	1.89	0.36	1.5	0.054	1.74	1.13	9.4	0.62	2/3-8,1/9-15	2,48	2.27
14	0.19	0.38	0.018	52	0.19	2	0.47	1.68	0.061	1.75	1.25	8.92	0.61	2/3-8,1/9-15	2.73	2.34
15	0.19	0.34	0.018	58	0.18	1.89	0.45	1.73	0.054	1.39	1.14	8.44	0.56	2/3-8,1/9-15	2.22	2.42
16	0.17	0.38	0.021	47	0.19	2	0.43	1.65	0.048	1.67	1.25	8.93	0.61	2/3-8,1/9-15	2.79	2.56
17	0.17	0.34	0.018	46	0.17	1.94	0.37	1.61	0.054	1.75	1.05	8.08	0.63	2/3-8,1/9-15	2.85	2.45
18	0.17	0.36	0.018	49	0.18	1.89	0.39	1.5	0.054	1.56	1.05	9.5	0.6	2/3-8,1/9-15	2.79	2.27
19	0.174	0.35	0.021	45	0.18	1.94	0.4	1.67	0.048	1.84	1.1	10	0.6	2/3-8,1/9-15	2.45	2.42
20	0.17	0.38	0.024	44	0.19	2	0.45	1.73	0.048	1.88	1.25	10.42	0.63	2/3-8,1/9-15	2.9	2.58

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Table 2. Continued ...

21	0.18	0.37	0.018	48	0.19	2	0.43	1.59	0.054	1.68	1.11	9.25	0.64	2/3-8,1/9-15	2.33	2.49
22	0.16	0.33	0.018	47	0.18	1.9	0.37	1.68	0.054	1.67	1.1	10	0.60	2/3-8,1/9-15	2.48	2.37
23	0.17	0.4	0.018	47	0.19	2	0.46	1.84	0.054	1.88	1.3	9.3	0.64	2/3-8,1/9-15	2.87	2.42
24	0.18	0.34	0.015	51	0.17	1.93	0.31	1.29	0.048	1.82	0.97	8.08	0.63	2/3-8,1/9-15	2.22	2.52
25	0.19	0.37	0.024	54	0.19	1.95	0.43	1.65	0.048	1.47	1.15	10	0.61	2/3-8,1/9-15	2.49	2.46
Mean	0.171	0.35	0.194	48.3	0.182	1.95	0.41	1.58	0.052	1.71	1.13	9.22	0.62	----	2.41	2.6
SD	0.010	0.23	0.027	3.4	0.007	0.07	0.047	0.16	0.001	0.14	0.09	0.78	0.19	----	0.13	0.24
Max	0.19	0.40	0.024	58.0	0.19	2.22	0.47	1.84	0.078	1.88	1.3	10.42	0.64	----	2.58	2.90
Min	0.16	0.31	0.015	44.0	0.17	1-83	0.31	1.29	0.040	1.37	0.97	7.8	0.56	----	2.01	2.16

Table 3. Morphometric measurements (in mm) of male *Phlebotomus salangensis* sand flies collected from two counties (Khash and Zahedan) in Sistan and Baluchistan Province, southeastern Iran

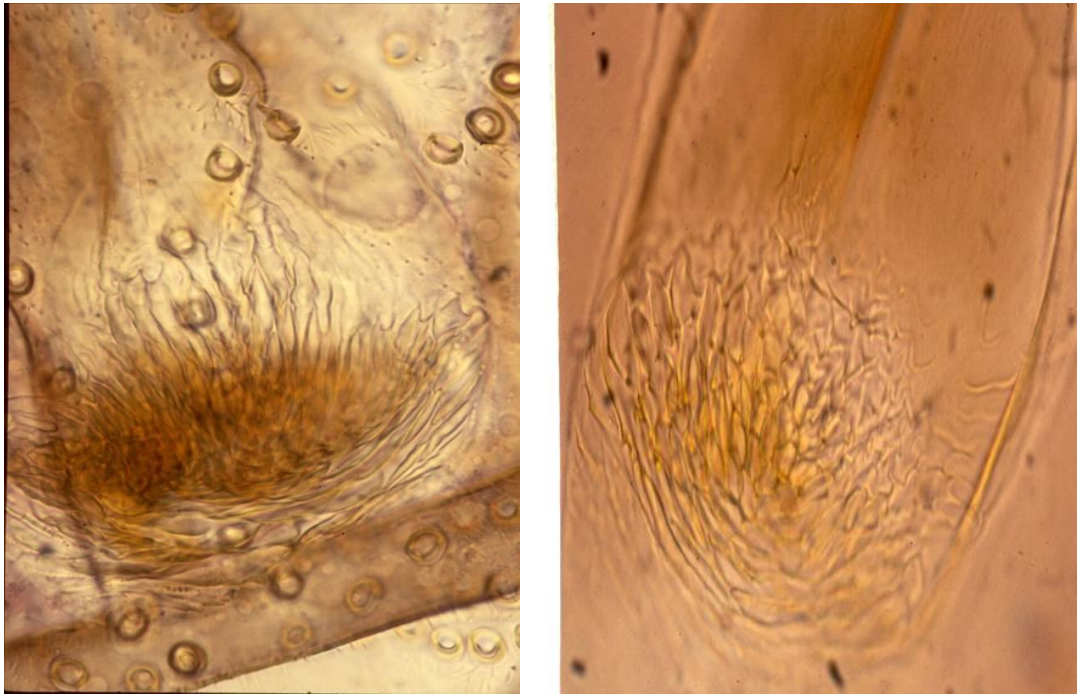
No	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	0.16	0.37	0.018	71	0.18	2.05	0.36	1.33	0.04	1.7	0.93	8.11	0.48	2/3-5,1/6-15	2.56	2.43
2	0.17	0.40	0.018	71	0.18	2.22	0.40	1.40	0.04	1.8	1.0	8.77	0.48	2/3-5,1/6-15	2.72	2.43
3	0.17	0.38	0.019	80	0.17	2.23	0.34	1.31	0.04	1.6	0.97	7.46	0.49	2/3-5,1/6-15	2.83	2.42
4	0.17	0.41	0.019	96	0.18	2.27	0.40	1.40	0.04	1.7	1.04	8.32	0.48	2/3-5,1/6-15	2.60	2.29
5	0.19	0.40	0.019	77	0.18	2.22	0.36	1.33	0.04	1.6	1.1	9.2	0.48	2/3-5,1/6-15	2.75	2.50
6	0.18	0.37	0.018	72	0.17	2.18	0.36	1.33	0.04	1.7	1.0	8.77	0.49	2/3-5,1/6-15	2.63	2.49
7	0.17	0.40	0.018	85	0.18	2.21	0.36	1.33	0.04	1.8	1.1	9.2	0.49	2/3-5,1/6-15	2.80	2.29
8	0.17	0.40	0.018	71	0.18	2.22	0.42	1.41	0.04	1.6	1.0	8.77	0.48	2/3-5,1/6-15	2.79	2.44
9	0.16	0.37	0.018	71	0.18	2.05	0.36	1.33	0.04	1.8	0.92	8.11	0.48	2/3-5,1/6-15	2.66	2.54
10	0.18	0.39	0.018	82	0.18	2.17	0.38	1.36	0.04	1.8	1.03	7.92	0.48	2/3-5,1/6-15	2.73	2.59
11	0.17	0.40	0.018	71	0.18	2.22	0.38	1.34	0.04	1.7	1.0	8.77	0.48	2/3-5,1/6-15	2.56	2.42
12	0.17	0.40	0.020	77	0.17	2.35	0.40	1.54	0.03	1.6	1.1	7.86	0.48	2/3-5,1/6-15	2.72	2.31
13	0.16	0.37	0.018	71	0.18	2.06	0.36	1.44	0.04	1.6	0.92	8.11	0.48	2/3-5,1/6-15	2.83	2.29
14	0.19	0.40	0.017	90	0.18	2.22	0.42	1.42	0.04	1.5	1.1	9.2	0.48	2/3-5,1/6-15	2.72	2.30
15	0.17	0.37	0.016	89	0.17	2.18	0.38	1.36	0.04	1.5	1.1	10	0.48	2/3-5,1/6-15	2.83	2.51
16	0.19	0.40	0.019	77	0.18	2.22	0.36	1.33	0.04	1.6	1.1	9.2	0.48	2/3-5,1/6-15	2.70	2.57
17	0.17	0.39	0.017	72	0.18	2.17	0.36	1.33	0.04	1.6	1.1	9.2	0.48	2/3-5,1/6-15	2.56	2.46
18	0.17	0.38	0.018	80	0.17	2.23	0.34	1.26	0.04	1.8	0.97	7.46	0.49	2/3-5,1/6-15	2.70	2.47
19	0.17	0.41	0.019	96	0.18	2.27	0.42	1.41	0.04	1.7	1.04	8.32	0.49	2/3-5,1/6-15	2.83	2.33
Mean	0.17	0.39	0.018	78.9	0.18	2.20	0.38	1.37	0.04	1.67	1.03	8.57	0.48	----	2.71	2.43
SD	0.01	0.014	0.01	7.78	0.05	0.76	0.02	0..06	0.02	0.10	0.69	0.69	0.04	----	0.93	0.98
Max	0.19	0.41	0..02	96	0.18	2.35	0.42	1.54	0.04	1.8	1.1	10	0.49	----	2.83	2.59
Min	0.16	0.37	0.016	71	0.17	2.05	0.34	1.26	0.03	1.5	0.92	7.46	0.48	----	2.56	2.29

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Table 4. Morphometric measurements (in mm) of male *Phlebotomus halepensis* sand flies collected from three counties (Khash, Nikshahr and Zahedan) in Sistan and Baluchistan Province, southeastern Iran

No	A	B	C	D	E	F	G	H	I	K	L	M	N
1	0.15	0.33	0.018	42	0.19	1.73	0.37	1.27	0.048	1.25	8.93	0.61	2/3-8,1/9-15
2	0.16	0.31	0.018	36	0.18	1.73	0.37	1.32	0.078	1.13	8.07	0.63	2/3-8,1/9-15
3	0.16	0.33	0.017	38	0.18	1.73	0.37	1.27	0.051	1.25	8.93	0.62	2/3-8,1/9-15
Mean	0.157	0.323	0.177	38.3	18.3	1.72	0.37	1.28	0.059	1.21	8.64	0.62	----
SD	0.057	0.116	0.006	3.5	0.06	0.06	0.0	0.03	0.016	0.07	0.496	0.010	----
Max	16	0.33	0.18	42	0.19	1.72	0.37	1.32	0.078	1.25	8.93	0.63	----
Min	0.15	0.31	0.17	38	0.18	1.73	037	1.27	0.048	1.13	8.07	0.61	----



a **b**

Fig. 4. Photos of female pharynx and pharyngeal armature in *Phlebotomus salangensis* (a) and *Ph. kabulensis* (b), caught in Sistan and Baluchistan Province, southeastern Iran. Figures are from H. Kassiri's personal archive and require permission for reproduction

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Discussion

We report the presence of *Adlerius* (*Ph. halepensis*, *Ph. kabulensis* and *Ph. salangensis*) in Sistan and Baluchistan Province, where this subgenus had never been recorded before 1997 (15). Male *Phlebotomus comatus* specimens were captured in East Azerbaijan Province, northwestern Iran (10). Meanwhile, *Ph. comatus* and *Ph. kabulensis* have been collected from East Azerbaijan and Lorestan Provinces, respectively (16). On the other hand, *Ph. turanicus* and *Ph. salangensis* have been caught from the north-east of Iran in provinces bordering Turkmenistan and Afghanistan (8). *Phlebotomus kabulensis* was collected from Esfahan, Lorestan and Ilam Provinces (17). *Phlebotomus halepensis* has been found in many provinces of Iran (8, 10, 16).

Morphometric study is a helpful method for the characterization of populations and classification into various species. Meanwhile, it is more economical than more costly conventional molecular identification assays. Comparing morphology and morphometrics of sand flies may be beneficial for the identification of similar or sibling species and changes in the population of sand flies in field collections. By the way, it may play a role in monitoring and assessing sand fly populations in connection with preventing and controlling different forms of leishmaniasis (18). Past investigations have shown that the flight length of adult sand flies is short and often limited to the region where larval development happens. Sand flies have weak flying capability, and their dispersal range does not exceed 100 meters (18, 19). So, it may be supposed that sand flies have limited populations within each limited region and that genetic diversity may occur not only between species but also between different populations of the same species of sand fly in various regions if no gene flow exists. Morphological and morphometric variations of sand flies may be because of the geography of the counties under study in various regions, differences in their biological and physical features, such as physical obstacles, the vertebrate host population abundance and distribution, precipitation, environmental conditions, temperature, humidity percent, latitude, altitude, the height above sea level, and access to habitat (20).

The genus *Phlebotomus* sand flies in the Old World (Eastern Hemisphere) are included: *Adlerius*, *Anaphlebotomus*, *Euphlebotomus*, *Kasaulius*, *Larroussius*, *Lewisius*, *Madaphlebotomus*, *Paraphlebotomus*, *Phlebotomus*, *Synphlebotomus* and *Transphlebotomus*. All of the subgenus *Adlerius* species of Iran are as follows: *Ph. balcanicus* Theodor, *Ph. brevis* Theodor and Mesghali, *Ph. comatus* Artemiev, *Ph. halepensis* Theodor, *Ph. kabulensis* Artemiev, *Ph. longiductus* Parrot, *Ph. salangensis* Artemiev, *Ph. simici* Nitzulescu, *Ph. turanicus* Artemiev and *Ph. zulfagarensis* Artemiev. *Phlebotomus simici* was recorded in Iran by Theodor (1958). Although the species has not been recorded in Iran after that, Galati et al. (3) mentioned its distribution in Iran. *Phlebotomus zulfagarensis* was described by Artemiev (1978) based on specimens from Afghanistan and Iran. So, one of its type localities is in Iran. *Phlebotomus kabulensis* is suspected to be a potential vector of visceral leishmaniasis (3, 16, 17, 21). Also, *Ph. halepensis* is a potential vector of cutaneous as well as visceral leishmaniasis (22, 23). The collection of *Ph. salangensis* in Baluchistan, southeastern Iran, adds to the growing evidence of this species' wide distribution across the region. Its presence in Iran, particularly in a province bordering Pakistan, is of considerable epidemiological interest. In neighboring Pakistan, *Ph. salangensis* has been reported as the most abundant sand fly species (42.2%) in Dir Districts, Khyber Pakhtunkhwa, with a male-to-female ratio of 3:1, surpassing even *Ph. sergenti* (36.6%) (24). Given that cutaneous leishmaniasis (CL) is endemic in various parts of Khyber Pakhtunkhwa, and that *L. tropica* has been identified as the predominant causative agent (89.2%), followed by *L. major* (6.8%) and *L. infantum* (4.1%) (25),

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the potential role of *Ph. salangensis* in *Leishmania* transmission cannot be overlooked. Although the vector competence of this species has not been definitively established in Iran, its high abundance in neighboring endemic foci and its sympatric occurrence with proven vectors such as *Ph. sergenti* raise concerns about its possible involvement in the transmission cycle. Furthermore, the movement of populations across the Iran–Pakistan border, combined with similar ecological and climatic conditions in Baluchistan, may facilitate the spread of both sand fly vectors and *Leishmania* parasites. Therefore, the presence of *Ph. salangensis* in Iran warrants further investigation, including molecular detection of Leishmania infection, blood-feeding behavior studies and vector competence assays, to better assess its role in the epidemiology of leishmaniasis in this understudied region.

From the morphological analysis of *Ph. kabulensis* sand flies collected in Sistan and Baluchestan Province, certain traits exhibited high standard deviation (SD), including the number of setae on the coxite brush, the ratio of genital filament length to sperm pump length, wing length, and coxite length. In contrast, traits such as the length of the ascoid on antenna 4 and the length of the parameral sheath showed low SD. These observed variations, both high and low, may reflect morphological adjustments within local populations, potentially driven by habitat-specific factors such as selective pressures, food availability and environmental conditions (26).

Conclusion

There are characteristic differences in the morphology of these species of sand flies at the population level which may be utilized for classification of sand flies in the future. It is possible that the populations take part in vector competence. These results emphasize the importance of sustained and systematic entomological surveillance to improve understanding of the diversity of sand flies and assess their potential role in disease transmission. The experimental or natural infection of *Ph. halepensis, Ph. kabulensis* and *Ph. salangensis* with *Leishmania* species appears interesting for future research.

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Ethical considerations

This study was approved and registered by the Ethics Committee of Research at Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Code: IR-AJUMS.EEC. 1405.043).

Conflicts of interest statement

The authors declare that there is no conflict of interest.

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