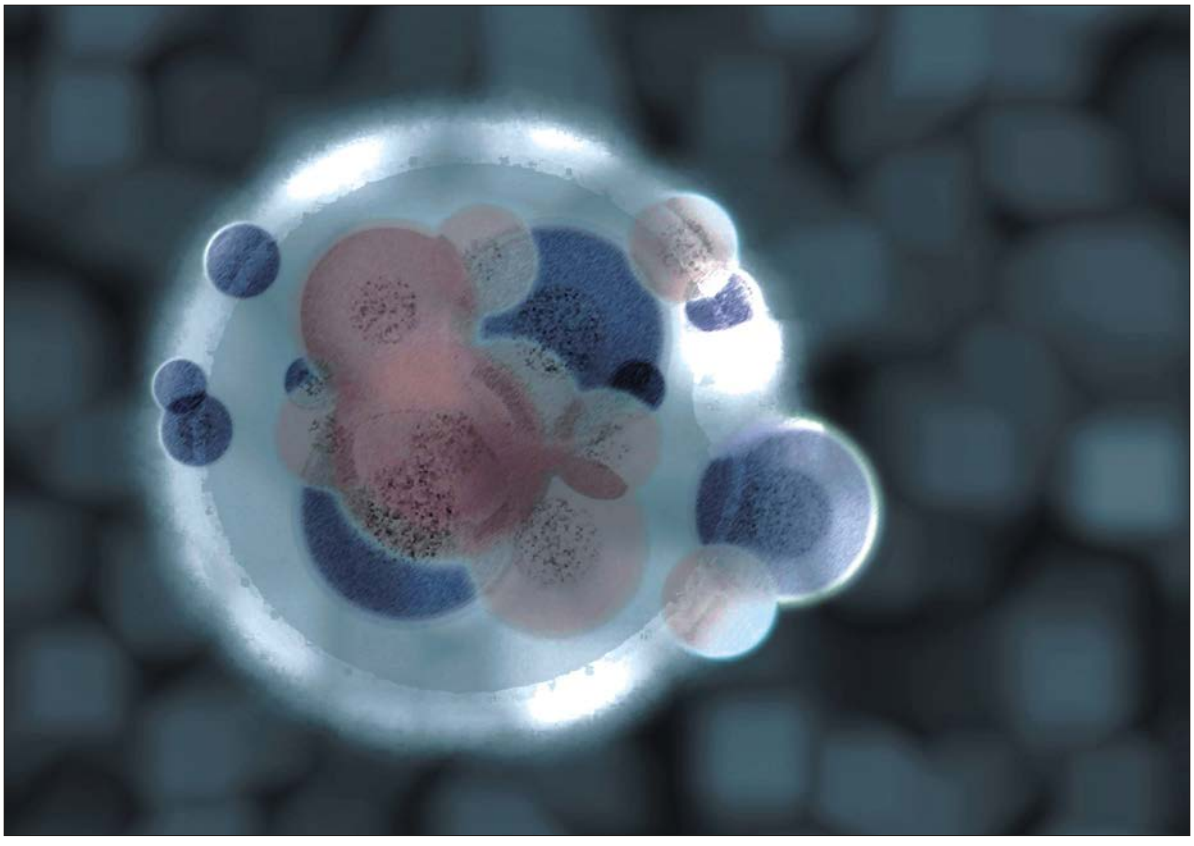

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A Comparative Analysis of Key Parameters of the Species of the Subgenus *Paraphlebotomus* (Diptera, Psychodidae)

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Abstract: During an extensive taxonomic study conducted in the whole of the Balochistan Province in 1996-2001, 47 key parameters of male and 38 of female of the four species of the subgenus *Paraphlebotomus* viz., *Phlebotomus sergenti, Ph. alexandri, Ph. andrejevi* and *Ph. nuri* so far recorded from Pakistan are investigated. Some extra facts like hypopharynx and pharynx length / breadth ratio are also found of taxonomic value in both the sexes. In addition, position of ascoid on antennal segments, presence and absence of striations in spermathecal ducts in female and alar index, filament / pump ratio and surstyle length in male were observed to be of taxonomic value. Mouth-parts, antenna 3 and spermathecae of female and male genitalia and antenna 3 of the male Pakistani specimens were compared with the published data of these species from other territories. It is suggested that these extra facts may also be taken in to consideration while identifying sandflies of the genus *Paraphlebotomus.*

Key words: Sandflies, *Paraphlebotomus, Ph. sergenti, Ph. alexandri, Ph. andrejevi, Ph. nuri*

INTRODUCTION

Sandflies (Diptera, Psychodidae, Phlebotominae) are best known for their bites and their role as vectors of leishmaniasis, a protozoan disease. In the last two decades with the arrival of Afghan and Iranian refugees into Pakistan in general and Balochistan in special and also due to the urbanization of the disease, the importance of leishmaniasis in Public Health has increased. Identification of the circulating species of phlebotomine in areas of the disease is of crucial importance in epidemiological studies of leishmaniasis, as certain *Phlebotomus* species may act as vectors. In Pakistan, the first cases of leishmaniasis were described from northern areas of Pakistan (Baltistan) in 1979[1]. Cutaneous leishmaniasis is known to prevalent in the Balochistan Province since a long time and in 1993, few cases of Visceral leishmaniasis were reported from Quetta by Nagi and Nasimullah[2]. The local fauna of phlebotomine was poorly known. Previously, no such attempt has been done in the identification of the sandflies species of Balochistan except Lewis[3] whose main focus was on the species from Punjab, Sindh and NWFP. The largest Province of the country containing most of the cases of cutaneous leishmaniasis (CL) was left un-surveyed. Lewis[3] reported *Ph. alexandri* from a single male collected from Northern Pakistan. The data of the female *Ph. nuri* and male of *Ph. andrejevi* were unknown from Pakistan in the literature prior to the present study.

Therefore, to fill this gap of knowledge, an extensive taxonomic study to identify the species of sandflies prevalent in the Balochistan Province was conducted by the author in 1996-2001 and 2013 sandflies were collected comprising of the genera *Phlebotomus, Sergentomyia* and *Grassomyia*[4]. During the revision of the genera, forty seven characters of male sandflies and 38 characters of female of the four species of the subgenus *Paraphlebotomus* viz., *Ph. sergenti* (N=140, from 11 localities), *Ph. alexandri* (N=82, from 5 localities), *Ph. andrejevi* (N= a single male) and *Ph. nuri* (N=09, from 2 localities) were investigated. In view of the insufficient description of Lewis (*loc. cit.*), diagnostic parameters for the identification of the species of the subgenus *Paraphlebotomus* are constructed and given in the present paper. Some extra facts of both the sexes are also observed to be of taxonomic value. Parameters of Pakistani species are compared with the published data of these species from other territories. The results add new supportive-extra facts and so facilitate the identification of these four species of the subgenus *Paraphlebotomus.*

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MATERIALS AND METHODS

For collection, preservation, dissection and examination of external and internal characters of sandflies, techniques adopted by Johnson *et al.*[5], Lewis[6], Killick-Kendrick[7] and Killick-Kendrick *et al.*[8] and Lawyer *et al.*[9] were followed. Species identification was done with the keys framed by Artemiev[10] and Lewis[3,11,12]. Measurements are in millimeter (mm). Specimens are housed with the Author's collection, Department of Zoology, University of Balochistan, Quetta.

RESULTS AND DISCUSSION

Key parameters studied: Taxonomic features taken into account in the present study are primarily those of earlier workers, but many are modified and others are new. Forty-seven key parameters of male and 38 of female were studied, compared and are presented in the given Table 1 and 2, respectively. Mouth parts, antenna 3 and spermathecae of the female specimens and male genitalia and antenna 3 of the male Pakistani specimens are compared with the published data of these species from other territories (Table 3).

Differential diagnosis: *Ph. sergenti* Parrot[13] Female antenna 3 long (0.263-0.30 mm), A3/labrum=0.989 female pharynx with uniform strong pharyngeal teeth directed obliquely down towards center, spermatheca with 4 or 5 segments, comparatively broader apical segments (0.014-0.016 mm) and spermathecal ducts are without striations are important characters of female specimens. Male can be recognized having shorter style with 2 apical spines, basal process with thin small asymmetrical head (0.016-0.02 mm broad) obliquely down and long genital punip (0.16-0.193 mm).

Table 1: Morphometrics of characteristics (mm) of the species of sub genus *Paraphlebotomus*

Key parameters	*Ph. sergenti* (N=140)	*Ph. alexandri* (N=82)	*Ph. andrejevi* (N=1)	*Ph. nuri* (N=9)
Male				
Head length	0.48-0.51	-	-	0.44
	0.24x wing length	-		
Breadth	0.48-0.52	0.384-0.4	0.464	
Eye length	0.224-0.256	0.16-0.2	-	0.176
	0.489x head length	-		
Breadth	0.16-0.19	0.144	0.144	
Distance between eyes	0.15-0.168	0.152-0.176	-	0.128
Wing length	1.842-2.172	1.36- 1.76	1.7	2.0
Breadth	0.511-0.649	0.36-0.48	0.52	0.56
α	0.303-0.427	0.176-0.28	0.32	0.32
β	0.288-0.397	0.16-0.2	0.304	0.24
δ	0.062-0.147	0.064-0.08	0.08	0.064
γ	0.32-0.456	-	0.32	0.32
Π	0.04	0.04-0.12	0.12	0.064
Alar index	1.052-1.075	1.1-1.4	1.33	
Palp length	0.85	0.66		0.84
Formula	1,2,4,3,5; 1,2-4,3,5; 1,4,2,3,5	1,2-4,3,5; 1,2,4,3,5		1,4,2,3,5
Relative length	1, 2.75, 3.51, 2.85, 6.36	1, 2.85, 3.42, 2.85, 7.71	1, 3.5, 4.5 3.0, 9.0	
Antenna 3	0.253-0.311 long	0.12-0.14 long	*Antennae missing*	0.27-0.31 long
	0.139x wing length	0.083x wing length		0.145x wing length
	1.239x labrum length	0.868x labmm length		1.348x labrum length
	1.096x A4+A5	0.822x A4+A5		1.115x A4+A5
Ascoid length	0.04-0.06	0.03		0.04-0.05
	0.175x length of segment	0.23x length of segment		0.155x length of segment
Antenna 4	0.12-0.14 long	0.07-0.09 long		0.11-0.15
Ascoid length	0.04-0.06	0.03		0.04-0.044
	0.384x length of segment	0.0375x length of segment -		0.323x length of segment
Antenna 5	0.12-0.14 long	0.07-0.086 long		0.11-0.15 long
Ascoid length	0.04-0.06	0.03		0.04-0.044
	0.384x length of segment	0.384x length of segment		0.323x length of segment
A single papilla and its position	On A3, 0.864; A4, 0.7; A5,0.666	On A3, 0.769; A4, 0.648; A5, 0.657	-	-
Two asoids and their position	On A3, 0.68; A4,0.2; A5, 0.25	On A3, 0.692; A4, 0.351; A5, 0.368		On A3, 0.79; A4, 0.33; A5, 0.346
Proboscis length	0.23-0.29, 0.129x wing length	0.16-0.2	0.26-0.28	
Labrum length	0.20-0.25	0.14-0.16, 0.026 broad,	0.17 long,	0.21-0.22 long,
	0.112x wing length			
	labrum with 2 stout apical sensilla and about 20 long narrow laterals	with 3-4 apical sensilla and 15 long laterals	with 2 long stouter apical sensilla and 1 short apical	with 3 long apical sensilla and laterals short

Table 1: Continue

Key parameters	*Ph. sergenti* (N=140)	*Ph. alexandri* (N=82)	*Ph. andrejevi* (N=1)	*Ph. nuri* (N=9)
Sensilla depth	0.044	0.036	0.058	0.052
Hypopharynx	about 14 teeth on each side	with 3 long apical and 8 lateral teeth	0.016 broad, with 4 apical pointed and 14 lateral teeth	0.022 broad, with 14 lateral teeth
Dental depth	0.028	0.024	0.04	0.032
Maxilla	sword like structure, without teeth	-	-	-
Cibarium breadth	0.039-0.057, chitinous dots and spicules weak	0.03-0.04, armature not seen	0.056, teeth absent	0.04-0.045 broad, armed with many long lateral spicules and scattered minute dentices
Pharynx length	0.19-0.22; 3.14-3.39x its breadth,	0.158-0.17; 3.15-4.93x its	0.18 long,	0.19, 2.53x its breadth
Fore breadth	0.045-0.05,			
Hind breadth	0.056-0.07			
	1.4 times as wide posteriorly as anteriorly,			1.7 times as wide posteriorly as anteriorly
	3.14-3.39 times long its breadth			
Armature height	0.05-0.06	0.03-0.04		0.05
	0.268x pharynx length, anterior edge of armature forms an almost straight line, proximally a series of transverse ridges with serration and more distally long and broad spicules pointing towards center.		0.22x pharynx length	0.263x pharynx length anterior edge of armature forms an almost straight line, antero-central part composed of long spicules directing obliquely down towards center, there are also some short and broad teeth
Coxite	0.22-0.29 long, 0.07-0.11broad	0.18-0.22 long, 0.08-0.12 broad, 0.025-0.04 long	0.024 long	0.2-0.23 long, 1.466x length of style
Basal process	0.044-0.06 long		0.064 long	
Head	0.016-0.02 broad, slightly elongated with tapering apex, with 13-15 thick hairs yellow pigmented and slightly curved ventrally	0.022-0.025 broad, almost rounded, with 15-18 thick brush like hairs directed obliquely down	very large, asymmetrical, 0.036 long, 0.034 broad, with almost straight hairs form dark dense tuft	0.024 broad, with long hairs, distal part of hairs characteristically curved downward at 90 ^{0} angle
Neck	0.014-0.017 broad,	0.014-0.015 broad	0.03 long, 0.026 broad,	0.02 broad
Style	0.09-0.1 long, 0.04-0.05 broad, style with 2 terminal spines (longest slightly curved situated on small tubercles of equal length and thickness) and 2 median spines (one of which at the middle of the body of style short and thin and almost straight 0.06-0.086 long, the other located closer to the base and lateral border of style, is the longest 0.12-0.13 and curved and thiuner than the terminal spines but relatively thicker than the shorter thin medium spine.	0.11-0.124 long, 0.04-0.046 broad bearing 4 spines (2 median, of unequal length, stand on two small tubercles) and 2 median spines are also of unequal length, stand at 0.58 x length of style	0.1 long, 0.044 broad, 2 terminal spines of unequal length, 2 median also of unequal length	Slender, 0.15 long, 0.04 broad, with 4 spatulate spines (1 apical,1 subapical and 2 submedian)
Paramere	0.091-0.152 long, flat elliptical surface with about 15 short spinules	0.114 long, flat elliptical surface with short hairs	0.08 long,	0.13-0.14 long,
Aedeagus	0.053-0.073 long, darkly pigmented, almost oval with slightly curved apex	0.074-0.076 long, pigmented, apex curved ventrally	0.06 long, slightly curved apex	0.07-0.08, apical ends almost straight,
Genital filament	0.18-0.2 long,	0.15-0.19 long	0.2 long,	0.23-0.24
Funnel	0.048 long, 0.024 broad	0.026 long, 0.016 broad	0.05 long, 0.02 broad,	0.056
Pump	0.16-0.193 long	0.11-0.12	0.18	0.17-0.18
Filament/ pump	1.036-1.125	1.36-1.58	1.11	1.33-1.35
Surstyle	0.24-0.27 long, about the length of coxite.	0.18-0.21 long, 1.0-0.954x length of coxite	0.26 long, 1.08x length of coxite	very long, 0.34, 1.545x length of coxite

Ph. alexandri Sinton[14] Female pharyngeal armature occupies only base of the pharynx and almost rectangular in shape, 7-9 segmented spermahtheca, apical segment not larger (0.12-0.14 mm), apical and basal segments are almost of same size, ducts are also without striations. Whereas in male basal process with small rounded head (0.022 mm) having brush like long hairs, genital pump short (0.11-0.12 mm) with small funnel (0.026 mm) are of significance.

Ph. andrejevi Shakirzyanova[15] Males of this species can easily be differentiated from other species of the subgenus *Paraphlebotomus* by having a very large but

Table 2: Morphometrics of characteristics (mm) of the species of sub genus *Paraphlebotomus*

Key parameters	*Ph. sergenti* (N=140)	*Ph. alexandri* (N=82)	*Ph. andrejevi* (N=1)	*Ph. nuri* (N=9)
Female	Not known from Pakistan			
Head length	0.48-0.576, 0.228x wing length,	-	-	
Breadth	0.512-0.6	0.464-0.496, 0.271x wing length		
Eye length	0.24-0.28, 0.5x length of head,	0.24-0.264, 0.533x length of head	-	-
distance				
between eyes	0.20-0.24	0.216-0.232	-	
Wing	2.0-2.42 long, 0.588-0.753 broad,	1.6-1.84 long, 0.48-0.60 broad	-	2.24-2.36 long, 0.64-0.68 broad
α	0.357-0.465	0.24-0.424		0.43-0.45
β	0.3-0.36	0.20-0.30		0.33-0.35
δ	0.02-0.16	0.04-0.08		0.011
γ	0.32-0.52	0.36-0.48		0.48
Π	0.08-0.12	0.056-0.12		0.08-0.12
Alar index	1.15-1.29	1.2-1.41		1.29
Palp length	0.70-0.81	0. 708	-	0.95
Formula	1,4,2,3,5; 1,2-4,3,5; 1,2,4,3,5	1,4,2,3,5		1,2-4,3,5
Relative length	1.35, 4.31, 3.2, 6.9	1.29, 3.9, 2.8, 6.58		3.55, 4.53, 3.55, 8.0
A3 length	0.263-0.3, 0.123x wing length,	0.11-0.14, 0.07x wing length	-	0.23-0.27, 0.108x wing length
	0.989x labrum length,	0.51x labrum length		1.02x labrum length
	1.137x A4+A5	0.925x A4+A5	-	1.063x A4+A5
Ascoid length	0.05-0.07	0.03	-	0.05
	0.212x length of segment	0.24x length of segment	-	0.2x length of segment
A4 length	0.112-0.134	0.066-0.07	-	0.12
Ascoid length	0.06-0.07	0.024-0.03		0.05
	0.532x length of segment	0.41x length of segment	-	0.416
A5 length	0.11-0.134	0.064-0.07	-	0.11-0.12
Ascoid length	0. 06-0.07	0.028-0.03	-	0.05
	0.532x length of segment in almost all female flies, ascoid reach the anterior end of segments	0.432x length of segment	-	0.434x length of segment
Two ascoids on each segments and their position				
	On A3,0.692; A4, 0.344,A5, 0.272	On A3,0.66;A4, 0.37;A5, 0.31	-	On A3, 0.678; A4, 0.416; A5, 0.466
A single papilla				
and its position	On A3, 0.807;A4, 0.724; A5,0.727	On A3, 0.83; A4, 0.68; A5, 0.62		On A3,0.869; A4, 0.75; A5, 0.8
Labrum	0.265-0.31 long, 0.125x wing	0.24-0.25 long, 0.138x wing	-	0.24-0.25 long, 0.106x wing length,
with				
	length, 0.038 broad, with 4 median terminal sensilla stout and longer, 15 adorals	length, 0.03 broad, with 3 thin apical sensilla, adorals small and narrow.		3 apical long stout sensilla and laterals short
Sensilla depth	0.056	0.048		
Hypopharynx	0.025 broad, with 3 apical pointed teeth and with 16 curved teeth on each side,	0.024 broad with 3 apical and 9 lateral teeth on each side	-	with about 15 teeth at each side
Dental depth	0.04	0.04	-	0.048
Maxilla	0.012 long with 7 lateral (2 large prominent, other 2 rounded and less prominent and remaining 3 very minute dot like) and 18 ventral teeth	with 5 lateral and nine ventral teeth	-	with 4 laterals and 21 ventral teeth,
Dental depth	0.088	0.056		0.096
Mandible	0.009 broad, with 35 teeth	narrow, 0.017 broad with 7-8 re-curved teeth per 0.01	-	narrow, 0.017 broad, with 7-8 small re-curved teeth per 0.01, dental depth 0.056
Dental depth	0.048, 3 curved teeth per 0.004			
Proboscis	0.265-0.31 long, 0.125x wing	0.26-0.27 long, 0.15x wing length	-	-
Length				
Cibarium	-			
Breadth	0.044-0.065, side walls bears 5-6 small spicules and 3-4 minute chitinous dots scattered at antero-central part, chitinous arch well developed, pigment patch and anterior process both absent	0.036-0.049, side walls bear 4-6 small spicules, 7-10 minute rounded denticles at antero-central part and posterior to these are 5-8 triangular denticles in a zigzag row		0.04 with 7-9 dot like denticles at antero-central part whereas 4-8 relatively long spicules at both the sides, pigment patch and anterior process both absent
Pharynx				
Length	0.22-0.256, 1.31-1.42 times as	0.16-0.21, 2.1-2.42 times as long	-	0.22-0.23, 2.3-2.75 times as long as
Broad,	long as broad, posterior part 3.87-3.9 times as wide as narrowest anterior part	as broad, posterior part 1.78-2.0 times as wide as the narrowest anterior portion		posterior portion is 1.5 times as wide as its anterior part

Table 2: Continue

Armature	Anterior edge of armature forms straight line, posterior part composed of broad and long leaves like spicules median armature consists of long and broad spicules directing obliquely towards the center, basal part consists of straight transverse ridges with minute teeth	Anterior edge of armature forms almost straight line, armature darkly pigmented and rectangular in shape armature occupies base of pharynx height of armature 0.03-0.05, 0.211x length of pharynx	-	Most of the broader part occupied by armature, anterior edge of armature forms a straight line, median and posterior part of armature was in blunt, long and wider teeth directing obliquely to the center, basal part of armature consists of fine curved or straight punctiform ridges
Spermatheca	4-5 segmented, global-apical segment larger and broader (0.011-0.032 long, 0.014-0.016 broad) remaining segments 0.007-0.008 long and 0.012-0.014 broad	7-9 segmented, anterior segments (0.012-0.014 broad) not expanded and not larger than the other segments	-	7-8 segmented, anterior segments 0.012 broad median segments 0.016 broad and basal segments 0.012 broad,
Ducts	0.024-0.026 long with separate openings	0.15-0.195	-	0.192 long with separate openings
Genital atrium	0.048 broad	0.048-0.054	-	0.052
Genital fork	0.11-0.12 long	0.096-0.1	-	0.08

Table 3: Comparison of key parameters (mm) of species of *Paraphlebotomus*

Key parameters	Balochistan (Present study)	Oriental region [11]	Afghanistan [10]	Countries of EMR+ [16]	Egypt [17]
♀					
Mouth parts					
Ph. sergenti	Hypopharynx with 3 apical and 16 re-curved teeth on each side and a dental depth of 0.04. Maxilla with 7 lateral and 18 ventral teeth, a dental depth of 0.088	Hypopharynx with 16 teeth on each side, Maxilla with 7 lateral 15 ventral teeth and a dental depth of 0.09	-	-	-
Ph. alexandri	Hypopharynx with 3 apical and 9 teeth on each side, a dental depth of 0.04. Maxilla with 5 lateral and 9 ventral teeth, a dental depth of 0.056	Hypopharynx with 16 teeth on each side, Maxilla with 4 lateral and 9 ventral teeth, a dental depth of 0.06	-	-	-
Ph. nuri	Hypopharynx with 15 teeth at each side, a dental depth of 0.048. Maxilla with 4 lateral and about 21 ventral teeth, dental depth of 0.096. Mandible narrow 0.017 broad, with 7-8 re-curved teeth per 0.01, dental depth of 0.056.				
Antenna 3 length					
Ph. sergenti	0.263-0.3, 0.967-0.992 times length of labrum	0.23-0.33,0.7-1.0 times length of labrum	240-300 µm	-	longer than labrum
Ph. alexandri	0.11-0.14, 0.51 times length of labrum	0.12-0.16,05-0.6 times length of labrum	A3 short	-	A3 much shorter than labrum
Ph. nuri	0.23-0.27, 1.02 times length of labrum	-	264-296 µm, 0.71-0.84 times length of labrum	-	-
Spermatheca					
Ph. sergenti	4-5 segmented, apical segment larger and broader	4 -5 segmented	4-5 segmented with global terminal segment larger	3-7 segments, terminal segments larger	with equal segments
Ph. alexandri	7-9 segmented, apical segments similar as of posterior ones	-	6-9 segmented, apical segment narrow	-	with 6-7 segments
Ph. nuri	7-8 segmented, anterior segments 0.012 broad, median segments 0.016 broad, basal segments 0.012 broad	-	7-8 segmented, narrow apical segment	-	-

Table 3: Continue

Key parameters	Balochistan (Present study)	Oriental region [11]	Afghanistan [10]	Countries of EMR+ [16]	Egypt [17]
♂					
Antenna 3 length					
Ph. sergenti	0.253-0.311, 1.239x length of labrum	0.25-0.34, 1.0-1.4x length of labrum	-	-	A3 slender, longer than labrum
Ph. alexandri	0.12-0.14, 0.866x length of labrum labrum	short, 0.12-0.16, 0.7-0.9, x length of labrum	A3 short,	-	A3, stout, very much shorter than
Ph. nuri	0.27-0.31, 1.348x length of labrum	0.31-0.37,1.1 times length of labrum[3]	-	-	
Ph. andrejevi					
Genitalia					
Ph. sergenti	Coxite 0.22-0.29 long, basal process is the characteristics of this fly, style 0.09-0.1 long, with 4 spines	Slender basal lobe on coxite, 4 spines on a short style	Terminalia short, basal process thin, style very short	Coxite two times as long as style, process slender	Coxite short and stout, basal process slender
	Genital pump long (0.16-0.193) with broad basal funnel (0.024)	Genital pump long (0.17-0.2) with broad basal fiiunel	-	-	-
Ph. alexandri	Coxite 0.18-0.22 long, style 0.11-0.12 long, Genital pump short (0.11-0.12) with smal basal fiiunel (0.016)	Basal lobe of coxite small and thin Genital pump short (0.12) with small basal funnel	Coxite with short basal process, style rather long	Style long, four times as long as thick	Terminalia very short basal process short and broad
Ph. nuri	Coxite 0.2-0.23 long, basal process broad, (0.024) hairs characteristically downward, style 0.15 long	Basal process of coxite very large and thick, with many hairs	Basal process of coxite long and thick, with long hairs, style long	-	-

+ East Mediterranean Region countries

symmetrical head of basal process (0.03 mm long, 0.036 mm broad) and broad neck (0.03 mm long, 0.026 mm broad), short hairs of basal process, short style (0.1 mm long) with 2 terminal spines are of equal length, long genital pump (0.18 mm).

Ph. nuri Lewis[3] Female pharyngeal armature occupy most of the broader part of the pharynx, very long slender style (0.15 mm), spermatheca with 7-8 segments, anterior segments 0.012 mm broader, median and basal segments are 0.016 and 0.012 mm broader respectively (anterior and basal segments are of sanie size). In male style is 3.75 times as long as wide, head of the basal process 0.024 mm broad (comparatively larger than of *Ph. sergenti, Ph. alexandri* and is the only Oriental species having relatively larger basal process[11], distal part of the hairs characteristically curved downward at 90 degree, long pump (0.17-0.18 mm), surstyle very long (0.34 mm) are important features for its identification.

In conclusion, it can be said that the aforementioned key paranieters indeed facilitate the correct identification of the species and is suggested that these should also be taken into consideration in taxonomic studies of the subgenus *Paraphlebotomus*.

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