INT. J. BIOL. BIOTECH., 3 (2): 257-270, 2006.

REVISION OF THE GENUS *LYCHUS* KOCH (ARACHINDA : BUTHIDAE) WITH THREE NEW SPECIES FROM PAKISTAN AND THEIR CHROMATOGRAPHY AND ELECTROPHORESIS OF VENOM

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ABSTRACT

Genus *Lychus* Koch is revised and keyed out to accommodating three new species and eleven already described species from Indo-Pakistan sub-continent. The chromatography and electrophorasis of the venom of new species is also performed. A cladogram is constructed by using their apomorphies and cladistic relationship is discussed.

Key word: *Lychus*, new species, Chromatography,electrophorasis, Pakistan.

INTRODUCTION

The genus *Lychus* first time described by Koch (1845), accommodating the type species from Oriental,Australion and Ethiopean region. Kraepelin (1891, 1899) later described the genus *Archisometrus* which have been synonymised under *Lychus*. Pocock (1900) and Stahnke (1972) described the genus *Lychus.* Tikader and Bastawadae (1983) in their extensive work on scorpion from the fauna of India described the genus *Lychus* Koch. They also formulated a key to the sub-genera and species and described the morphological characters in detail.

MATERIALS AND METHODS

The animals were collected from various localites of Sindh areas like Ghotki, Sehwan and Sanghar and were killed with the help of formalin and preserved in 70% alcohol. For the study of male genitalia the specimens were dissected out by removing the tergites of mesosoma. After dissection the aedeagus was mounted on slide then taken the photograph using photographic microscope. For the study of electrophorasis and chromatograpgy the technique generally followed by Amir *et al*. (1994 a, b, c and d and 2003 and 2004).

RESULT

Genus : *Lychus* Koch.
Lychus Koch 1843, *Archn. 12* : 3 ; Pocock 1900, *Fauna Porit.India Arch* : 35 ;Stahnke 1972, *Ent. News. 83* : 128 ; Tikader & Bastawadae 1983, *Fauna. Ind. Arcn. 3* : 40.
Archisometrus Kraepelin, 1891, *Jb. Hemb. Wiss, Anst. 8* : 217 ; 1899 ,*Tierr. Scor. etc* : 41.

Diagnostic features:

Carapace with weak posterior and lateral carinae, interocular area horizontal, mesosomal tergites with two or three carinae, cephalo – thoracic sternum triangular and longer than wide, metasomal segments carinated and vesicle with a conspicuous, strong triangular subaculeus spine below the vesicle, inferior surface of cheliceral fixed finger with one tooth, dentition on fingers of pedipalp non-imbricated, trichobothrial dorsal 1 dorsal 3,and dorsal 4 on femur from β angle.

Comparative note:

This genus is most closely related to genus *Hemibuthus* in having carapace with out posterior and lateral carinae, mesosomal tergites tricarinated and dentition on fingers of pedipalp non-imbricuted, but it can easily be separated from the same in having carapace with weak posterior median or lateral carinae, metasomal trgites with two or three carinae, dentition on fingers of pedipalp non-imbricuted and by the other characters as noted in the descriptions.

Type species : *Lychus scutilus* Koch.
Distribution: India, Burma, Malaya, Philippines, Thailand, China, Austraila, East, west and South Africa, Pakistan : Sindh.

Key to the species and sub-genera of the genus *Lychus* Koch.

1. Trichobothria of type db is proximal to et or est . - 2
…….Trichobothria of type db is distal to et .- - - - - - *Distotrichus* - 5

2. Trichobothria of type db is proximal to et only.- - - - - - *Alierotrichus* - - - - - - - - - - - - - - - - - 3
……Trichobothria of type db is proximal to est only. - - - - -*Endotrichus* - - - - - - - - - - - - - - - - 7

3. Body always more than 35mm in length, pectin more than 5X as wide and pectinal teeth more than 20-21 in number, immovable finger arched in male - - - - - - - -- - - *L.(A.) mucronatus* (F.).
…….Body not more than 30mm in length, pectin not more than 5X as wide and pectinal teeth never above 20 in number, immoveable finger never arched in male - 4

4. Body length about 25mm, pectin 2 3/4X as wide and pectinal teeth 12-13 in number -*L. (A.) rugosus* Pocock.
…….Body length more than 25mm, pectin 4X as wide and pectinal teeth 17-18 in number - *L. (A.) hendersoni* Pocock.

5. Pectin 3.5X as wide, pectinal teeth 12-13 in number. - - - - - - - - - - *L.(D.) gravelyi* Henderson. …….Pectin 4.25X as wide, pectinal teeth 16-28 in number. - 6

6. Body yellowish in colour, entire surface finely and granular, pectinal teeth 17 in number - - - - - - - -- *L.(D.) nigristornis* Pocock.
…….Body mustard yellow, entire surface weakly granular, pectinal teeth 28 in number.- -*L. (D.) zainii* (Sp.n.)

7. Mesosomal tergites II-IV with three carinae, exterior median on patella placed more distal but close to exterior sub-terminal and exterior terminal -8
…..…Mesosomal tergites II-IV with one carinae, exterior median on patella placed proximal and always from exterior sub-terminal and exterior terminal -10

8. Lateral carinae on mesosomal tergites not well developed and represented by a single large granule on posterior margin, exterior sub-basal 2 is proximal to exterior sub-basal "1" on patella -9
……. Lateral carinae on mesosomal tergites granular and well developed on posterior portion, exterior sub-basal 2 is slightly distal to exterior sub-basal 1 on patella - - - - - - *L.(E.) tricarinatus* Simon

9. Body colour yellow,entire surface coarsly and closely granular, pectinal teeth 22 in numbers. - *L. (E) laevifrons* Pocock
……..Body colour mustard yellow, entire surface weakly smooth, pectinal teeth 28 in numbers. - *L.(E) khalanderii* Sp.n.

10. Fingers of chela long, terminal and dorsal basal are always from each other, distance between exterior terminal and exterior sub-terminal also more, distal 5 and exterior 1 are on same plane to femur, exterior sub-basal 2 distal to exterior sub-basal 1 on patella - *L.(E.)scaber* Pocock
………Fingers of chela short dorsal terminal and dorsal basal not much away from each other, exterior terminal and exterior sub-terminal also very closer, exterior 1 proximal to dorsal on 5 femur, exterior sub-basal 1 and exterior sub-basal 2 on same plane or exterior sub-basal 1 slightly distal to exterior sub-basal - - - - - - - - - - - 11

11. Exterior terminal,exterior sub-terminal and exterior sub-basal placed very close to each other on menus, exterior sub-basal 1 and exterior sub-basal 2 always slightly proximal to exterior sub-basal on patella, manus not clear yellow -13

………Exterior terminal placed away from exterior sub-basal on manus, exterior sub-basal 1 and exterior sub-basal 2 on patella, manus clear yellowish colour - 12

12. Entire body obsoletly granular, Tarsomera II smooth, pectinal teeth 21 in number- -*L.(E.) albimanus* Henderson
………Entire body slightly granular, Tarsomera II weakly smooth, pectinal teeth 27 in number- *L. (E.) azraae* Sp.n.

13. Pectin 6X longer than wide, and pectinal teeth 24/24 in number, exterior sub-terminal placed more distal to dorsal basal on immovable finger - *L.(E) biharensis*
………Pectin less than 5X longer than wide and pectinal teeth 20/20 in number, exterior sub- terminal placed closer to dorsal basal on immovable finger - - - - - - - - - - - *L.(E.) kamshetensis*

Lychus (Endotrichus) azraae **Sp.n.**

(Figs.1 to 13)

Colouration:

Body generally yellow.

Prosoma (Fig. 3)**:**
Carapace: Entire surface of carapace smooth, all carinae weakly granular,ocular tubercles blackish brown, anterior margins weakly granular and provided with 24-26 small blackish setae, lateral margins not cranulated and smooth on anterior portion.

Pedipalp (Fig. 4 - 6)**:**
Manus stout and much lobed, inner portion longer than femur, shorter than carapace, almost all carinae weakly granular, outer and anterior side provided with a crenulated crest of 15-17 denticular tubercles. Patella longer than femur but always shorter than carapace. Inner or anterior surface provided almost weakly granular crest with 13 sub-denticular tubercles. Fixed finger almost as long as femur but movable finger longer than carapace. Dentition on the fingers consisting of three rows of non-imbricated teeth granular on the fixed and movable finger. Trichobothrial pattern of pedipalp “B” type.

Legs (Fig. 7)**:**
Femur weakly granular and patella smooth and carinae crenulated, tibiae with strong tibial spurs, on the legs III and IV, size 0.1 cm., pedal spurs spiny, tarsomere I laterally smooth furnished with few spines, tasomere II weakly smooth and lateral margin over lapping the base of the claw below which two rows of mid ventral reddish spines.

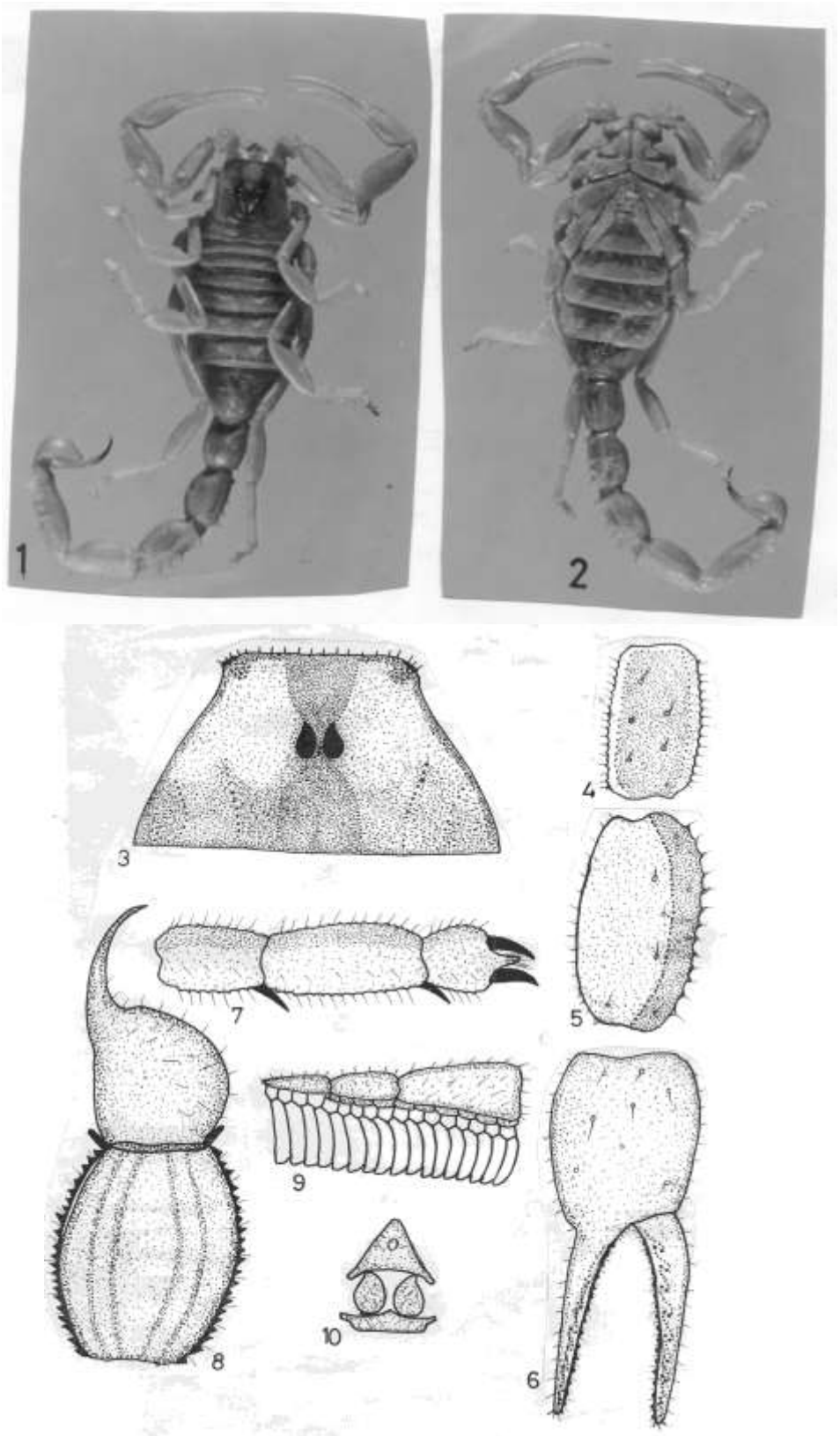
Pectin (Fig. 9)**:**
Pectin well developed and almost two times longer than wide, ten middle lamellae present, fulcra distinct triangular and more pointed, not covered with setae, pectin pale yellow with 27 teeth.

Gential operculum (Fig. 10)**:**
Genital operculum wider than longer and sclerites slightly divided on posterior portion from which small genital papillae produced in male, sternum small and triangular.

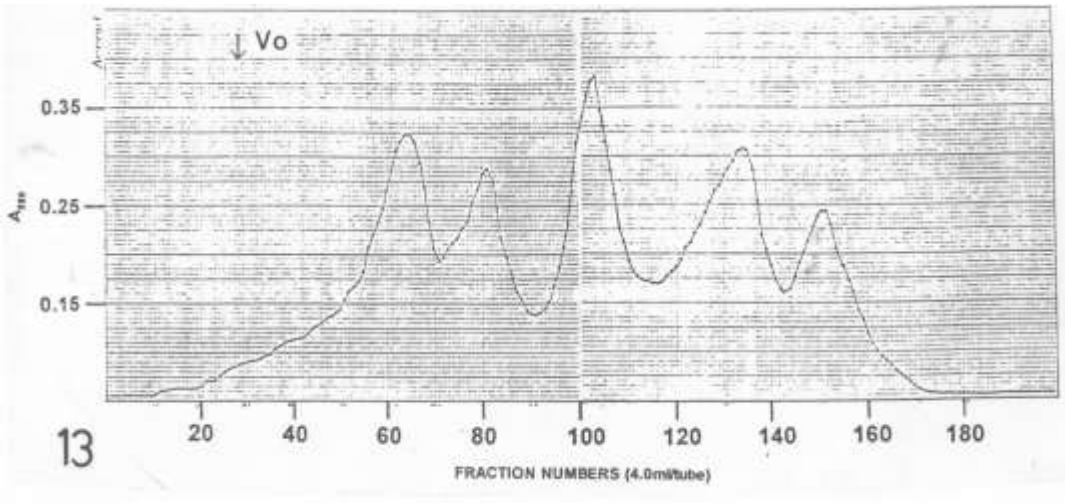
Mesosoma:
All tergites weakly granular but more granular on posterior portin of each tergite, sternites I-IV smooth and each provided with slite-like stigmata for book lungs.

Metasoma:
Cauda five times as long as carapace, first segment shorter than wide, segments I-IV with dorsal carinae crenulated, dentiform on posterior portion much more elevated on segment III and IV, dorsolateral carinae evenly crenulated, lateral carinae weakly developed only on posterior of segment III-IV.

Telson (Fig. 8)**:**
Telson with vesicle not as wide or deep as segment V, ventral surface densely smooth, ventral medium crest developed, sub-aculeus nodule present, aculeus weakly curved, as long as vesicle.



Figs. 1 - 13: *Lychus* (*Endofrichus) azraae*; 1. entire, dorsal view; 2. entire, ventral view; 3. prosoma, dorsal view; 4 - 6. pedipalpi, lateral view ; 7. leg, lateral view; 8. telson, lateral view; 9. pectin, ventral view ; 10. genital operculum, ventral view.



Figs. 11 - 13: *Lychus* (*Endofrichus) azraae*; 11. aedeagus, lateral view ; 12. electrophorasis of venom; 13. gel fieteration chromatography of venom.

Male genetalia (Fig. 11)**:**

Flagellum 0.7mm long, elastic and elongated, trunk 0.4mm long, 0.11mm wide, cylindrical, basally slightly dilated, pedicel very flat, 0.3mm long and 0.12mm wide, sperm spine blunt, sperm tube elongated, apex coiled.

Material examined:

Holotype: Male, Pakistan: (Sind), 12.6.93 leg. Azra Umar, lodged at MEMUK No.102.

Paratypes: 10 female other data same as holotype, lodged at ZMUK.

Comparative note:

This new species is most closely related to *Lychus (Endotrichus) albimanus* Henderson in having entire body obsoletely granular, 21 pectinal teeth are present but it can easily be separated from the same in having entire surface of carapace slightly granular, 27 pectinal teeth are present and by the other characters as noted in the key and description.

Table 1. ·Measurement in cm/mm meristic characters of the male holotype *Lychus (Endotrichus) azraae* sp.n.

Characters	Holotype Male
Total length	3.4 cm.
Carapace length	0.5 cm.
Mesosoma length	1.0 cm.
Metasoma length	1.9cm.
I segment length/width	0.3/0.22 cm.
II segment length/width	0.35/0.25 cm.
III segment length/width	0.4/0.3 cm.
IV segment length/width	0.45/0.32 cm.
V segment length/width	0.5/3.5 cm.
Telson length	5.5 cm.
Vesicle length/width	0.35/0.25cm.
Aculeus length	0.2 cm.
Pedipalp length	1.6 cm.
Femur length/width	0.35/0.2 cm.
Patella length/width	0.5/0.25 cm.
Chela length/width	0.85/0.3 cm.
Fixed finger length	0.4 cm.
Movable finger length	0.5 cm.
Chelicera Chela length/width	0.3/0.2 cm.
Fixed finger length	0.11 cm.
Movable finger length	0.12 cm.
Pectinal teeth count	27
Male genitalia	1.4 mm

Table 2. Variation in tarsomere counts in *Lychus (Endotrichus) azraae* sp.n. on each specimen,the spine of the left and right legs of each pair were counted.

Legs	Margin	4	5	6	7	8
I	Prolateral	5	3	5	6	4
	Retrolateral	4	5	4	5	5
II	Prolateral	4	5	5	8	5
	Retrolateral	3	3	6	7	5
III	Prolateral	6	5	5	7	4
	Retrolateral	5	7	5	7	4
IV	Prolateral	6	7	4	4	7
	Retrolateral	6	7	4	5	5

***Lychus (Endotrichus) khalanderii* sp.n.**
(Figs, 14 - 26)

Colouration:
Body generally mustard yellow.

Prosoma (Fig. 16)**:**
Carapace: Entire of carapace weakly smooth. All carinae granular. Ocular tubercles blackish brown and granular. Anterior margins smooth and provided with 26-28 small brownish setae, lateral margins crehnulated and more crenulated on anterior portion.

Pedipalp (Fig. 17 - 19)**:**
Manus globular, longer than femur, shorter than carapace, almost all carinae smooth and strong, outer and anterior side provided with a crenulated crest, 16-18 denticular tubercles. Patella longer than femur but always shorter than carapace. Inner or anterior surface provided almost granular crest with 16 sub-denticular tubercles. Manus or hand globular and length of underhand shorter than femur. Fixed finger asmost as femur but movable finger longer than carapace. Dentitin on the finger consisting of three rows of non-imbricated granular teeth on the fixed and movable fingers, Trichobothrial pattern of pedipalp "C" type.

Legs (Fig. 20)**:**
Femur patella smooth and cariae crenulated. Tibia without tibia spur on the legs III and IV. Tarsomere I laterally flat and provided with microscopic hairs on dorsal and ventral margins. Tarsomere II cylindrical.

Pectin (Fig. 22)**:**
Pcetines well developed and almost two and a half times longer than wide. Five middle lamellae present. Fulcra nearly triangular. Pectines pale yellow with 28 teeth.

Genital Operculum (Fig. 23)**:**
Genital operculum wider than long and sclerites slightly divided on posterior portion from which small genital papillae in male. Sternum small and triamgular.

Mesosoma:
All tergites smooth but more smooth on posterior portion of each tergite. Sternites I-IV smooth and each provided with slit-like stigmata for book lungs.

Metasoma:
Cauda four times as long as carapace, first segment shorter than wide. Segments I-IV with dorsal carinae crenulated, dentiform on posterior portion, much more elevated on segment III and IV, dorsolateral carinae evenly crenulated, lateral carinae very weakly developed only on posterion portion of segment III-IV.

Telson (Fig. 21)**:**
Telson with vesicle not wide or deep as segment V. Ventral surface densely smooth. Ventral-median crest developed, sub-aculeus nodule present. Aculeus weakly curved, as long as vesicle.

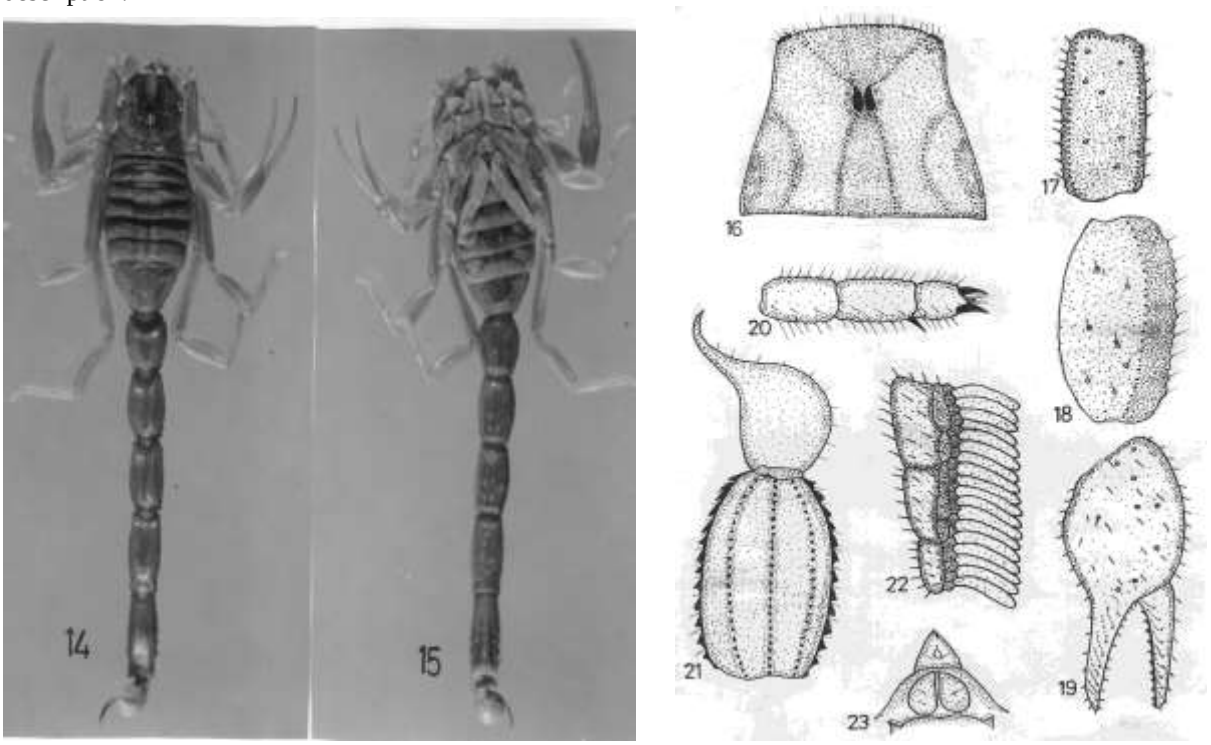
Male genitalia (Fig. 24)**:**
Flagellum 0.62mm long, flagella elongated, flagellar like. Trunk 0.6mm long, 0.12mm wide, Trunk cylindrical basally dilated, Pedicel 0.25mm long, 0.11mm wide. Seprm spine small thorn-like. Sperm tube elongated, apically curved.

Material examined:
Holotype: Male, Pakistan: Sehwan (Sind), 10.10.1994 leg Sattar, lodged in MEMUK No.68.

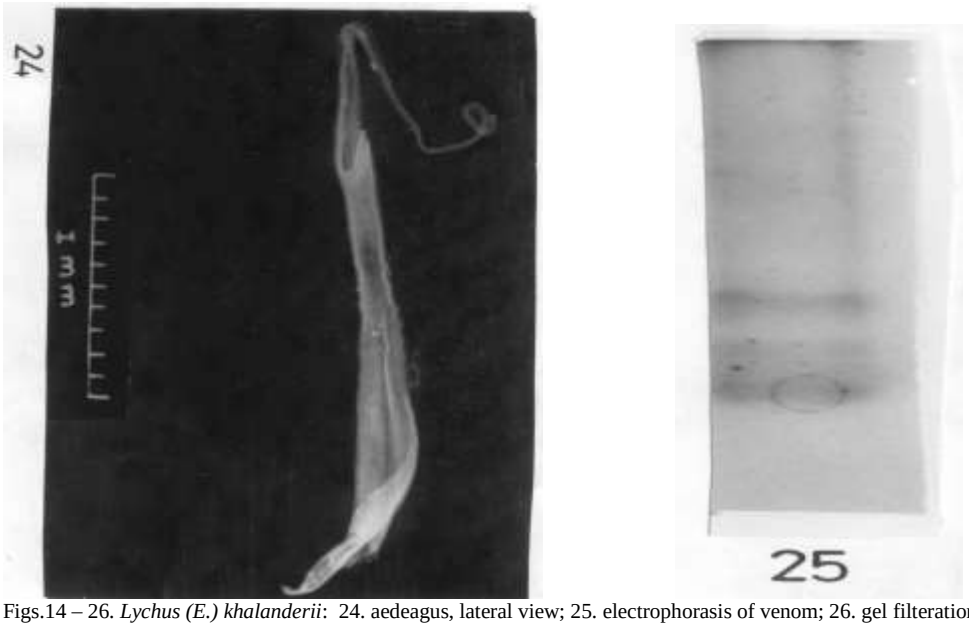
Paratypes: 11 females lodged at ZMUK, other data same as holotype, lodged at ZMUK.

Comparative note:
This new species is most closely related to *Lychus (Endotrichus)* leavifrons (Pocock) in having entire surface coarsely and closely granular, 22 Pectinal teeth are present, but it can easily be separated from the same in having

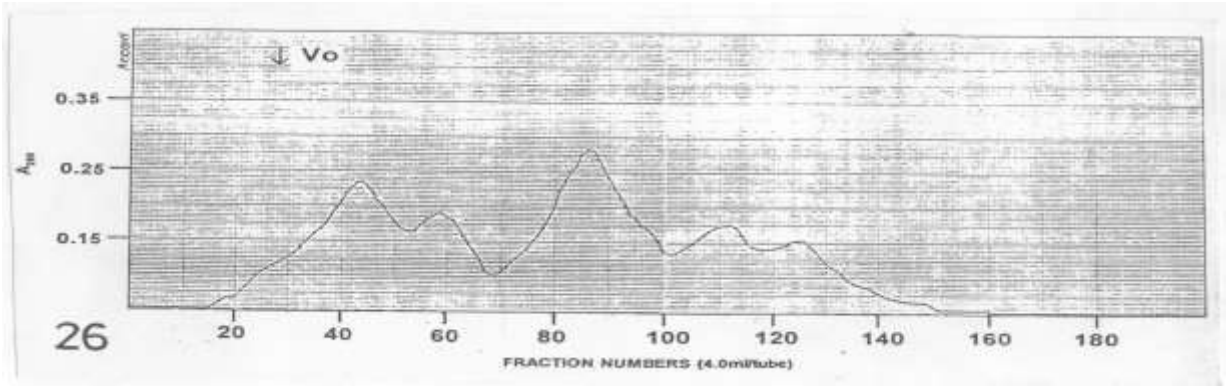
entire surface weakly smooth, 28 pectinal teeth are present and by the other characters as noted in the key and description.



Figs.14 - 26: *Lychus (E.) khalanderii*: 14. entire, dorsal view; 15. entire, ventral view; 16. prosoma, dorsal view; 17 -19. pedipalpi, lateral view; 20. leg, lateral view ; 21. telson, lateral view; 22. pectin, ventral view, 23. genital operculum, ventral view;



Figs.14 – 26. *Lychus (E.) khalanderii*: 24. aedeagus, lateral view; 25. electrophorasis of venom; 26. gel filteration chromatophoresis of venom.



Figs.26. *Lychus (E.) khalanderii*: gel filteration chromatophoresis of venom.

Table 3. Measurment in cm/mm meristic character of the male holotype *Lychus (Endotrichus) khalanderii* sp.n.

Characters	Holotype Male
Total length	3.5 cm.
Carapace length	0.5 cm.
Mesosoma length	1.0 cm.
Metasoma length	2.0 cm.
I segment length/width	0.3/0.25 cm.
II segment length/width	0.35/0.22 cm.
III segment length/width	0.4/0.20 cm.
IV segment length/width	0.45/0.18 cm.
V segment length/width	0.5/0.25 cm.
Telson length	0.4 cm.
Vesicle length/width	0.3/0.2 cm.
Aculeus length	0.1 cm.
Pedipalp length	1.7 cm.
Femur length/width	0.4/0.2 cm.
Patella length/width	0.6/0.3 cm.
Chela length/width	0.7/0.3 cm.
Fixed finger length	0.3 cm.
Movable finger length	0.31 cm.
Cheicera, Chela length/width	0.2/0.1 cm.
Fixed finger length	0.1 cm.
Movable finger length	0.11 cm.
Pectinal tooth count	28
Male Genitalia	1.47 mm.

Table 4. Variation in tarsomere II spine counts in *Lychus (Endotrichus) khalanderii* sp.n., on each specimen, the spine of the left and right legs of each pair were counted

Legs	Margin		4	5	6	7	8
I	Prolateral	4	3	5	5	4	
	Retrolaterla	3	3	3	5	3	
II	Prolateral	5	4	7	7	7	
	Retrolateral	6	5	4	4	5	
III	Prolateral	6	5	6	5	8	
	Retrolateral	5	4	5	6	7	
IV	Prolateral	5	4	5	4	4	
	Retrolateral	5	3	5	4	5	

***Lychus (Distotrichus) zainii* Sp.n.**
(Figs 27 – 39)
Colouration:
Body generally mustered colour.

Prosoma (Fig. 29)**:**
Carapace: Entire surface of carapace weakly granular. All carinae smooth. Oculartubercles brownish mustered colour. Anterior margins smooth and provided with 37-39 small brownish setae, lateral margins crenulated on anterior portion.

Padipalp (Fig. 30 - 32)**:**
Manus stout longer than femur and carapace separately. Almost all carinae granular, outer and anterior side provided with a crenulated crest of 14 denticular tubercles. Patella longer than femur but always shorter than carapace. Inner or anterior surface provided almost granular crest with 15 sub-denticular tubercles. Manus or hand stout and length of longer than femur. Fixed finger almost as long as femur but movable finger longer than carapace. Dentition on the fingers consisting of 1 row of imbricated teeth granular on the fixed and movable finger. Trichobothrial pattern of pedipalp "C" type.

Legs (Fig. 33)**:**
Femur granular, patella weakly granular and carinae crenulated, tibia with strong tibia spurs, on the legs III and IV, size 0.12 cm tarsmere I laterally flat6, tarsomere II cylindrical.

Pectin (Fig. 35)**:**
Pectin well developed and almost two times longer than wide. Nine middle lamellae present. Fulera nearly ventrally triangular. Pectinal teeth are pale yellow with 28 teeth.
Genital operculum (Fig. 36)**:**
Genital operculum wider than long and sclerites slightly divided on posterior portion from which small genital papillae produced in male. Sternum small and triangular.

Mesosoma:
All tergites granular on posterior portion of each tergite. Sternites I-IV granular, each provided with slit- like stigmata for book lungs.

Metasoma:
Cauda five times as long as carapace, First segment shorter than wide, Segments I-IV with dorsal carinae crenulated, dentiform on posterior portion much more elevated on segment III and IV, Dorsolateral carinae evenly crenulated, lateral carinae very weakly developed only on posterior portion of segment III-IV.

Telson (Fig. 34)**:**
Telson with vesicle not deep as segment V. Ventral surface densely granular, ventral median crest not developed, sub-aculeus nodule present. Aculeus not curved, as short as vesicle.

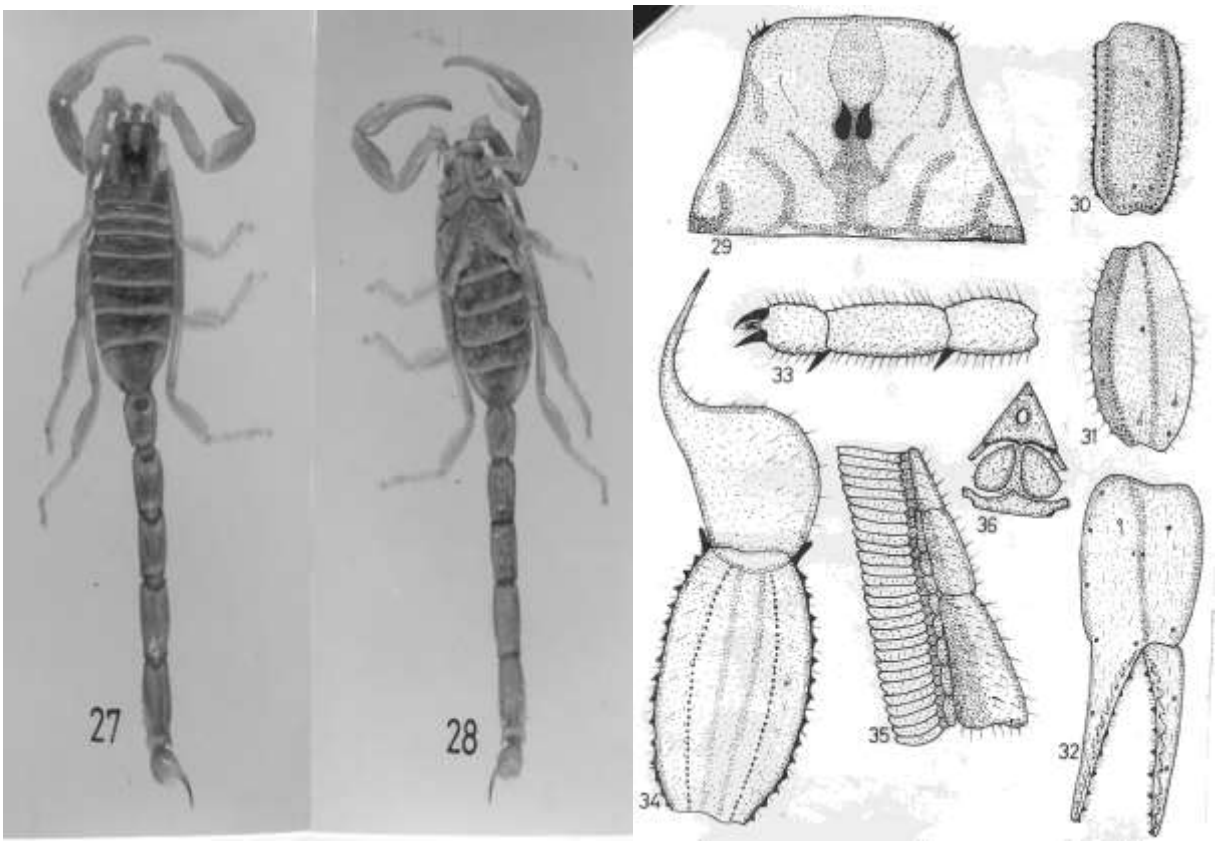
Male Genitalia (Fig. 37)**:**
Flagellum 0.6 mm long, flagellum elongated and elastic. Trunk 0.5 mm long and 0.16 wide, trunk cylindrical medially narrowed pedicel 0.25 mm long, 0.15 mm wide, pedicel very flat. Sperm spine blunt, sperm tube narrowed, apically curved.

Material examind:
Holotype, Male, Pakistan: Sanghar (Sind), 10.12.1993, leg. Rafat Amir, loading at MEMUK No.134.

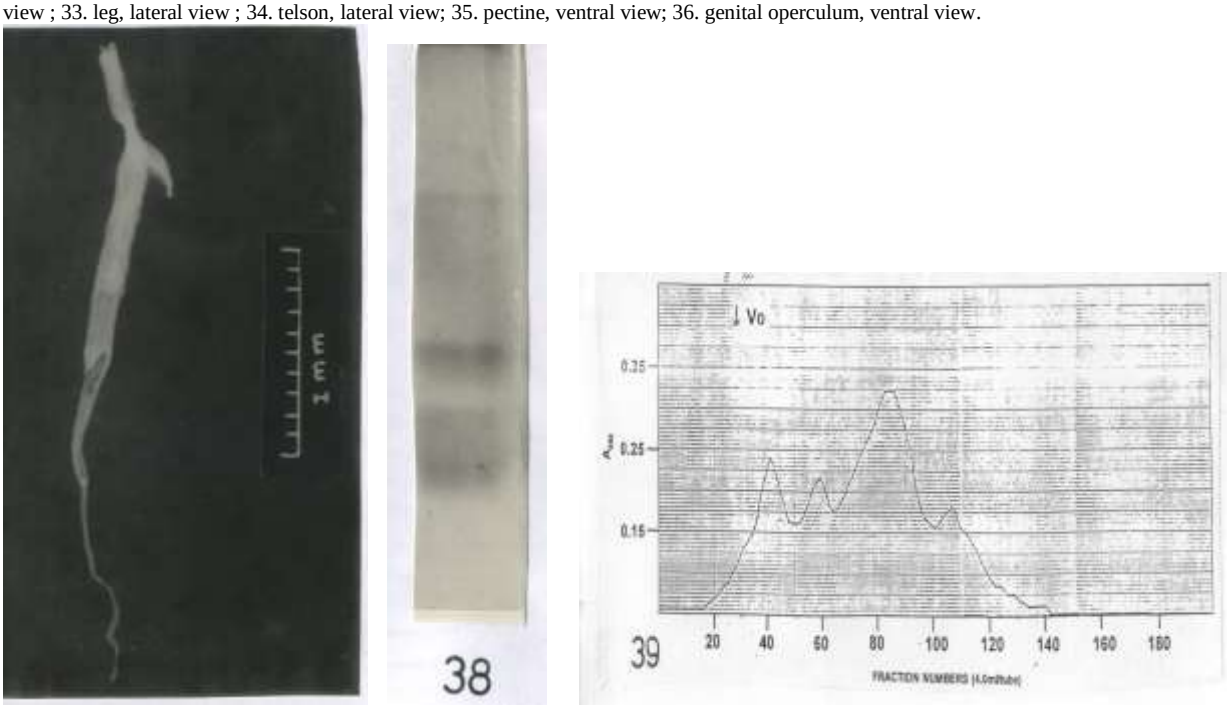
Paratypes: 3females,other data same as holotype, lodged at ZMUK.

Comparative notes:
This newspecies is most closely related to *Lychus* (*Distotrichus) nigristernis* Pocock (1899) in having entire surface finely and also granular but smooth on few spots, 17 pectinal teeth are present, but it can easily be separated

from the same in having entire surface of carcpace weakly granular, 28 Pectinal teeth are present and by the other characters as noted in the key and description.



Figs. 27 - 39.*lychus* (*Distotriclus*) *zaini*: 27. entire, dorsal view, 28. entire, ventral view; 29. prosoma, dorsal view ; 30----32. pedipalpi,lateral view ; 33. leg, lateral view ; 34. telson, lateral view; 35. pectine, ventral view; 36. genital operculum, ventral view.



Figs. 27 - 39.*lychus* (*Distotriclus*) *zaini*: 37. aedeagus, lateral view ; 38. electrophoresis of venom ; 39. gel filteration chromatography of venom.

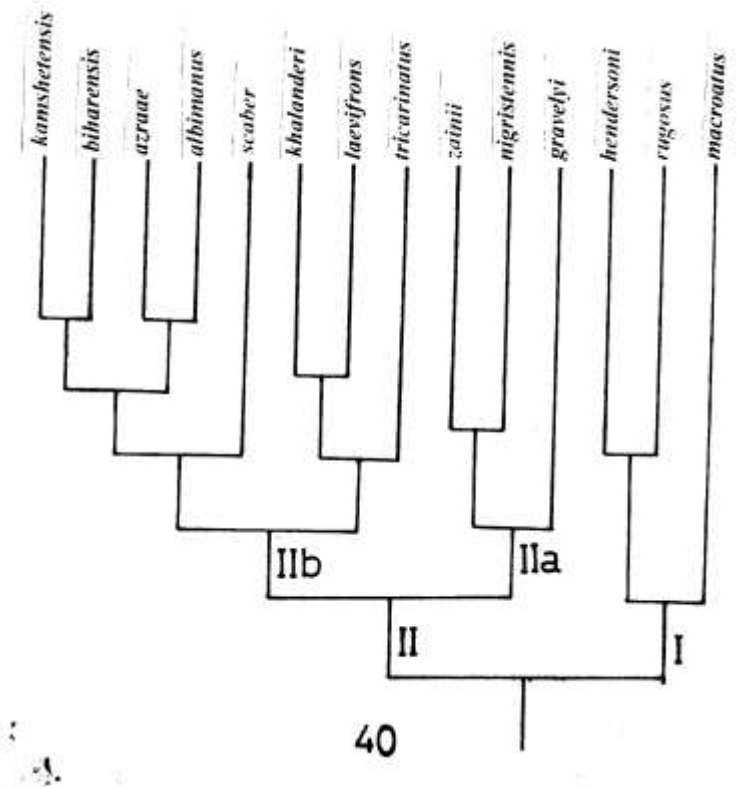


Fig. 40. Cladogram showing relationship of included taxa.

Table 5. Measurement in cm/mm meristic character of the male holotype *Lychus (Distotrchus) zainii* sp.n.

Characters	Holotype Male
Total length	5.0 cm.
Carapace length	0.55 cm.
Mesosoma length	1.5cm.
Metasoma length	2.95 cm.
I segment length/width	0.55/0.30 cm.
II segment length/width	0.55/0.35 cm.
III segment length/width	0.58/0.37 cm.
IV segment length/width	0.62/0.39 cm.
V segment length/width	0.65/0.39 cm.
Telson length	0.7 cm.
Vesicle length/width	0.35/0.3 cm.
Aculeus length	0.35 cm.
Pedipalp length	1.8cm.
Femur length/width	0.45/0.2 cm.
Patella length/width	0.5/0.25 cm.
Chela length/width	0.85/0.3 cm.
Fixed finger length	0.4 cm.
Movable finger length	0.45 cm.
Cheicera, Chela length/width	0.2/0.2 cm.
Fixed finger length	0.1 cm.
Movable finger length	0.12 cm.
Pectinal tooth count	28
Male Genitalia	1.35 mm.

Table 6. Variatin in tarsomere II spine counts in *Lychus (Distotrichus*) *zainii* sp.n. on each specimen, the spine of the left and right legs of each pair were counted.

Legs	Margin	4	5	6	7	8
I	Prolateral	5	7	4	7	4
	Retrolaterla	4	5	4	4	5
II	Prolateral	6	4	5	4	3
	Retrolateral	4	5	3	3	3
III	Prolateral	6	4	5	5	4
	Retrolateral	4	3	6	8	4
IV	Prolateral	7	5	4	7	6
	Retrolateral	5	5	5	7	6

Polyacrylamide Gel electrophorasis of Scorpion venom

Polyacrylamide gel electrophorasis of scorpion venom were performed under deutaturing condition using SDS (Sodium Dodecyl Sulphate) and B-Merespoethenol as deutaturing agents NW-SDS -70 L. Standard protein markers were used to prepare standard curue. The marker include, bovine serum albumin 66.0 KDa), egg albumin (45.0 KDa), glycraldehyde-3 Phosphate, dehydrogenises, trypsinogen (24.0 KDa), trypsin inhibitor (20.0 KDa) and lactalbumin (14.2 KDa).

Result (Fig. 12, 25 & 38)**:**

The *Lychus* venom on the contrary, showed very few hazy bands in the molecular weight range of 70.0 KDa to 14.0 KDa.

Gel filtration chromatography of genus *Lychus*.

1000 mg of *Lychus* (*Endotrichus*) *khalanderii* sp.n. *L. pistotrichus zainii* sp.n. *Lychus* (*Endotrichus) azraee* sp.n, venom was loaded on sephadex chulam -50 colum (2.5 x 9000cm) and eluted with 0.1M ammonium acetate buff (p4.69.0).

Result:

All three venoms was resolved into five peaks, (1toV), but exhibits different molecular mass and elution patterns. The venoms of *Lychus azraee* and *L.zainii* species possess components in the molecular weight range of hight to low where as *Lychus khalanderii* venom
contain components in the range of high to moderate. *L. khalanderii* venom resolved in to two
major and three minor peaks, *L. azraee* resolved into three major, one modrate and one minor peaks. *L. zainii* venom on the contrary, possess all five major peaks.

Discussion (Fig. 40)**:**

The genus *Lychus* Koch is distributed in desert and semideserts areas of Ethiopean, Oriental and Australian regions. The genus *Lychus* plays sister group relationships with *Hemibuthas* by their synapomorphy like immovable finger of chelicera furnished below with one minute tooth and isolated by its autapomorpheis like carapace with posterior median or lateral carinae and vesicle elongated and provided with strong triangular subaculear spine. Tikadar and Bastawadae (1983) erected three sub-genera on their apomorphies like trichobothrial patterns on immovable finger of pedipalp, specially the different position of et and est to dt and db on immovable finger.

The genus *Lychus* comprises fourteen species, which fall into two groups, the first group comprises *hendersoni, rugosus* and *maeroatus* and the second group comprises eleven species viz. *kamshetensis, biharensis, azraae,*

albimanus, scaber, khalanderi, laevifrons, tricarinatus, zainii, nigristennis and *gravelyi.* Among the first group *handrsoni* plays sister group relationship with *rugosus* and out group relationship with *macroatus.*

The second group also falls into two sub-groups, the first sub-group comprises three species viz. *zainii, nigristennis* and *gravelyi* where as second sub-group comprises rest of the eight species. Among first sub-group the *zainii* and *nigristennis* play sister group relationship to each other and out group relationship with *gravelyi.*

Among second sub-group the *kamshetensis* and *biharensis* play sister group relationship to each other and out group relationship with *azraae* and *albimanus,* which further play sister group relationship to each other. All these play sister group relationship to each other and out group relationship with *scaber*. The species *khalanderi* and *laevifrons* play sister group relationship to each other and out group relationship with *tricarinatus*.

Among the new species with reference to gel-filtration chromatography of the venom, the species *azraae* is very closely related to *zainii* by having molecular weight range of high to low as compare to molecular weight range of high to moderate as in *khalanderii*.

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(*Accepted for publication March 2006*)