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ON THE OCCURRENCE OF *Scorpiops longimanus* (POCOCK, 1893) (ARACHNIDA: SCORPIOPIDAE) IN MIZORAM, NORTHEAST INDIA

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Abstract
In this study, we confirm the occurrence of the tropical forest scorpion *Scorpiops longimanus* (Pocock, 1893) in Mizoram, India based on specimens collected from 13 different localities within the state. The total length of the animal was found to range from 42.22 mm up to 65.16 mm. The number of trichobothria on the ventral surface of the pedipalp patella and that of the external face alternates between 10–11 and 18–19, respectively. The pectines showed dissimilarities in the structure of the lamellae and in the number of teeth. We generated the first molecular data for the species and performed a maximum likelihood phylogenetic analysis using the partial mitochondrial 16S rRNA gene which showed the formation of a distinct clade of *S. longimanus*. The uncorrected k2p distance showed an intraspecific genetic variation of 2.9% among the species studies.

***Key words*:** Distribution, molecular phylogeny, Neoscorpii, new record, Orthosterni, Scorpiones

Introduction

Upon a recent major revision of Scorpiopidae, the genera: *Dasyscorpiops* Vachon, 1974; *Neoscorpiops* Vachon, 1980; *Alloscorpiops* Vachon, 1980; *Euscorpiops* Vachon, 1980; *Vietscorpiops* Lourenço & Pham, 2015; and *Plethoscorpiops* Lourenço, 2017 were synonymised with the genus *Scorpiops* Peters, 1861 under the family Scorpiopidae, which is currently made up of 99 species (Kovařík *et al*. 2020, Rein 2021). To our knowledge, 28 species of *Scorpiops* are present in India among which *S. asthenurus* (Pocock, 1900); *S. dastychi* Kovarik, 2000; *S. feti* Kovarik, 2000; *S. furai* Kovarik, 2000; *S. hardwickii* (Gervais, 1843); *S. kamengensis* (Bastawade, 2006); *S. leptochirus* Pocock, 1893; *S. longimanus* (Pocock, 1893); and *S. vonwicki* Birula, 1913 are found within northeast India (Kovařík *et al*. 2020).

Scorpiops longimanus is characterized by a total length of 47.2–50.0 mm, pectines provided with 8 teeth, carapace and pedipalps coarsely granular and the presence of 10 trichobothria on the ventral surface of the pedipalp patella (Pocock 1893). Their recorded distribution includes Assam, India (Tikader & Bastawade

1983), Bangladesh and Myanmar (Szubert 2021). This study investigates of the Scorpion fauna of Mizoram which has been poorly explored for its invertebrate fauna.

Material and Methods

Sampling and species identification. Surveys of scorpion fauna were conducted between November 2020 and September 2021 at multiple localities within the state of Mizoram, India (Fig. 1). All the specimens were observed at night using hand held torchlight coupled with Ultra-Violet torchlight, and were collected with the aid of forceps and long tweezers. GPS coordinates of the collection sites were recorded using Garmin Montana 650-GPS navigator. Photographs were taken using a Canon EOS 60D II digital camera. All the collected specimens were catalogued and deposited in the Departmental Museum of Zoology (MZMU), Mizoram University where they are preserved in 70% ethanol (Table 1).

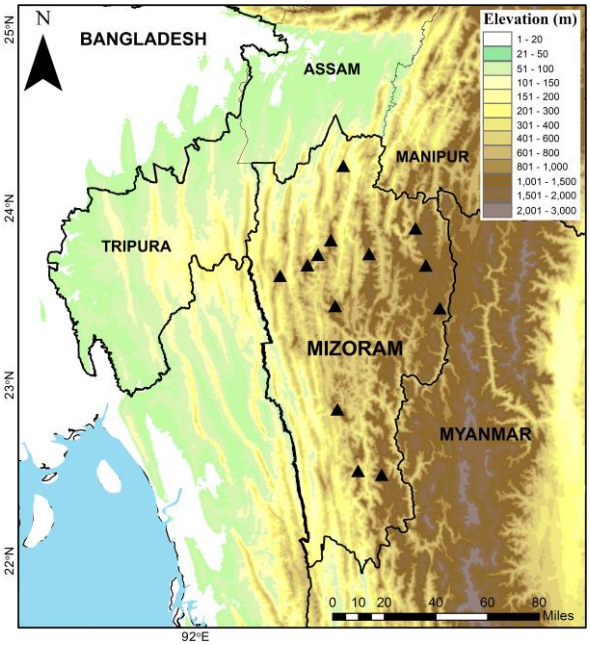

Figure 1. The current distribution of *Scorpiops longimanus* in Mizoram, India

Species identification is based on Kovařík *et al*. (2020). Morphological terminology and measurements follow Stanhke (1970) and Soleglad & Sissom (2001) with trichobothrial notations as given by Vachon (1974). Morphometric measurements (Table 2) were taken using MitutoyoTM (505-730) dial callipers with accuracy to the nearest 0.01 mm. Graphic illustrations were created using Wacom Pen Tablet CTL–472/K0-C and Adobe Photoshop 2020 software. The distribution map was prepared using ArcMap 10.3 software.

Phylogenetic analyses. Whole genomic DNA was extracted from ethanol-fixed soft tissue of seven specimens collected from Tamdil National Wetland, Saitual District (MZMU 2010, MZMU 2010A and MZMU 2010B), Sihphir, Aizawl District (MZMU 2042), Lallen, Mamit District (MZMU 2246), Theiriat, Lunglei District (MZMU 2174A) and Hmuifang Community Reserved Forests, Aizawl District (MZMU 2304A).

Genomic DNA extraction was performed using QIAamp DNA Mini Kit (Cat No.ID: 51306) following the manufacturer's protocols. Extracted gDNA was used as a template for the amplification of the partial 16S rRNA mitochondrial gene. PCR reaction was prepared for 20 µL reaction mixture containing 1X amplification buffer, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 1 µL genomic DNA, and 1 µL Taq DNA polymerase with a pair of partial 16S rRNA primers: forward (L02510 - CGC CTG TTT ATC AAA AAC AT) (Palumbi 1996) and reverse (H03063 - CTC CGG TTT GAA CTC AGA TC) (Rassmann 1997).

The PCR amplification was performed using ProFlex™ thermocycler (3x32-Well PCR System, Applied Biosystems) with the thermal regimes; 5 minutes at 95°C for initial denaturation, followed by 35 cycles, each of 1 minute at 95°C for second denaturation, 30 seconds for annealing at 50.3°C, elongation for 1 minute at 72°C, and a final elongation for 5 minutes at 72°C. PCR products were checked using 1.5% agarose gel electrophoresis containing Ethidium bromide.

Samples were then sequenced using Sanger's dideoxy method, and sequencing reactions were carried out in both directions on a sequencer (Barcode Bioscience, Bangalore, India). All the generated sequences were submitted to GenBank to obtain accession numbers. The phylogenetic analyses included 17 nucleotide sequences consisting of 372 bp for 16 ingroup taxa and one outgroup taxon. All the alignments were performed using MUSCLE (Edgar 2004) in MEGA X (Kumar *et al*. 2018) with default parameter settings and their uncorrected pairwise distances were calculated (Table 3). Phylogenetic relationships were estimated with Maximum Likelihood (ML) in RaxmlGUIv1.3 (Silvestro & Michalak 2012) using the GTR+gamma model with 1000 rapid bootstraps. Bayesian Inference phylogenies were

Plate 1

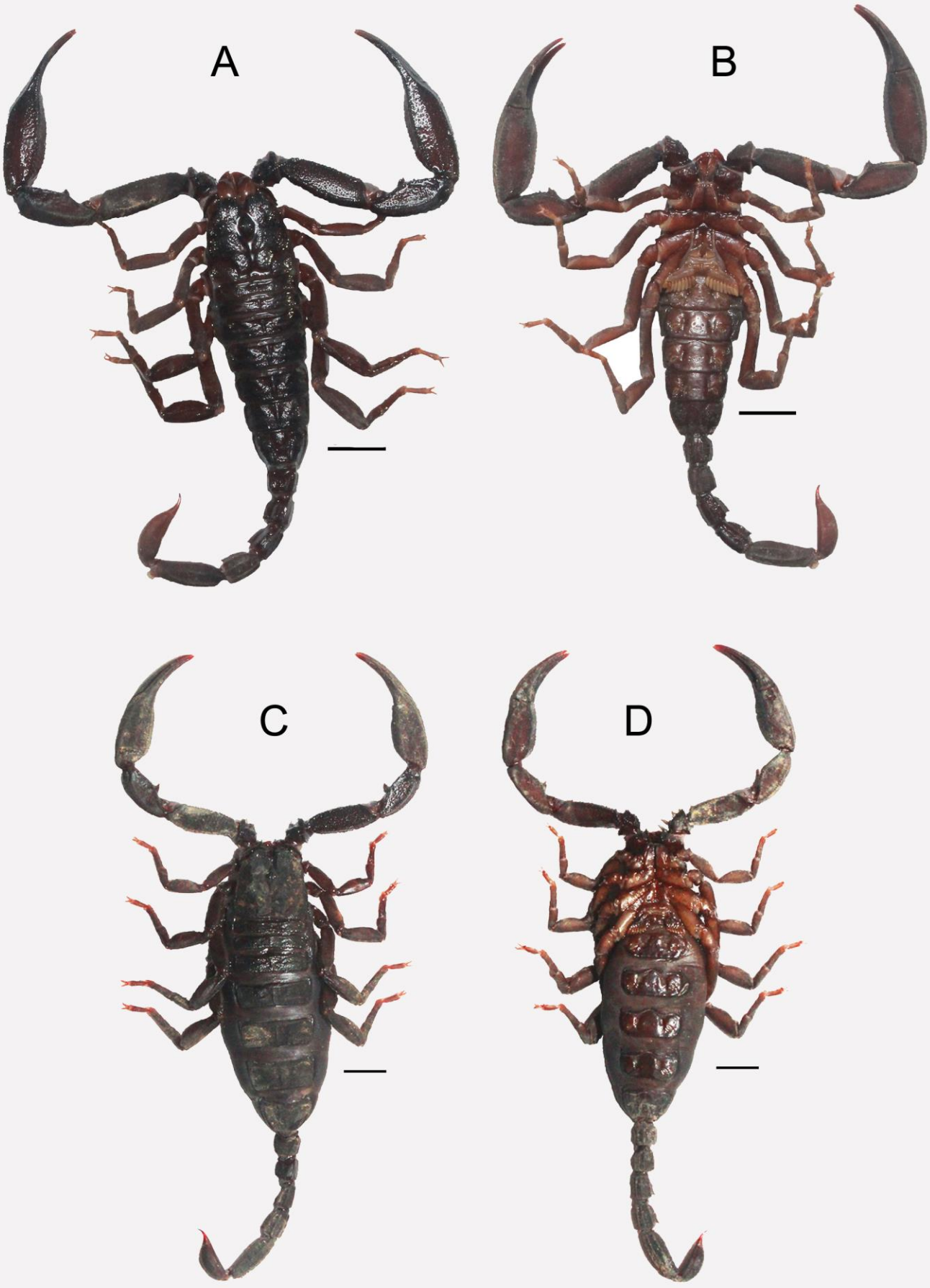

Figure 2. *Scorpiops longimanus* collected from Mizoram, India: **(A)** dorsal and **(B)** ventral views of a male (MZMU 2174C); **(C)** dorsal and **(D)** ventral views of a female (MZMU 2174A); scale: 5.0 mm.

Plate 2

Figure 3. Different pectine morphology and pectinal teeth counts of *Scorpiops longimanus*

inferred using MrBayes 3.2.6 (Ronquist *et al*. 2012) under GTR+I+G model (2 parallel runs, 10000000 generations), sampled every 500 generations in which the initial 25% of sampled data were discarded as burn-in. The phylogenetic tree was visualised and edited using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 1. List of *Scorpiops* specimens used in the phylogenetic studies; CRF, Community Reserved Forest

Species name	Location	GenBank No.	Source
S. longimanus 1	Tamdil National Wetland, Mizoram, India	MW665151	this study
S. longimanus 2	Tamdil National Wetland, Mizoram, India	MW665152	this study
S. longimanus 3	Tamdil National Wetland, Mizoram, India	MW665153	this study
S. longimanus 4	Sihphir, Mizoram, India	MZ057759	this study
S. longimanus 5	Hmuifang CRF, Mizoram, India	MZ057760	this study
S. longimanus 6	Lunglei, Mizoram, India	MZ057758	this study
S. problematicus	Thailand	MW269424	Šťáhlavský *et al.* 2021
S. orioni	Thailand	MW269411	Šťáhlavský *et al.* 2021
S. leptochirus	India	MW269410	Šťáhlavský *et al.* 2021
S. neradi	Thailand	MW269446	Šťáhlavský *et al.* 2021
S. wongpromi	Thailand	MW269418	Šťáhlavský *et al.* 2021
S. montanus	India	MW269419	Šťáhlavský *et al.* 2021
Scorpiops sp. 1	Thailand	MW269440	Šťáhlavský *et al.* 2021
S. grandjeani	Thailand	MW269443	Šťáhlavský *et al.* 2021
S. citadelle	Thailand	MW269433	Šťáhlavský *et al.* 2021
S. oligotrichus	Laos	MW269412	Šťáhlavský *et al.* 2021
Buthus draa	Tata, Morocco	KF825074	Pedroso *et al.* 2013

Results

A total of 54 specimens (34 males and 20 females) of *Scorpiops longimanus* were collected (Fig. 2) between November 2020 and September 2021 from 13 different localities: Theiriat Village (Lunglei District), Tanhril Village (Aizawl District), Hmuifang community reserved forest (Aizawl District), Reiek community reserved forest (Aizawl District), Sihphir (Aizawl District), Tamdil National Wetland (Aizawl District), Sihzau (Champhai District), Murlen National Park (Champhai District), Ngopa (Champhai District), Lallen (Mamit District), North Hlimen (Kolasib District), Lawngtlai District and Siaha District (Fig. 1). Based on the collection sites, the species is most likely present throughout the state.

The total length of the adult specimens was between 42.2–65.2 mm (Table 1). The pectinal morphology showed the highest degree of intraspecific variation among the observed characteristics with teeth number showing ten different variations (Left/Right); 5/4, 5/6, 6/6, 6/7, 7/6, 7/7, 8/7, 7/8, 9/9 and 8/9 (Fig. 3) coupled with a varying extent of the development of the marginal and middle lamellae. The number of the trichobothria on the dorsal and ventral faces of the chela manus did not vary. However, the number of trichobothria on the external surface of the pedipalp patella can either be 18 or 19 and that of the ventral surface alternates between 10 and 11 (Fig. 4). These observed characteristics differ from that of the original descriptions of Pocock (1893) where the total length of the animal was given as 50 mm and the pectinal teeth as 8 in number. Some individuals also showed the presence of a pair of accessory trichobothria on the carapace, anterolateral to the median eyes. Trichobothria Eb3 is at similar height to Dt in some individuals, which supported the independent positional variation of the Dt trichobothrium with respect to Eb3 (Kovařík *et al*. 2020). Difference in colouration was not observed.

The BI and ML analyses yielded a similar topology, which showed *S. longimanus* is forming a sister taxon with *S. problematicus* (MW269424) and *S. orioni* (MW269411) by a considerably high nodal support value (BI/BS=0.80/81). However, the *S. longimanus* can be distinguished from *S. problematicus* based on the number and pattern of external trichobothria on the patella along with the morphology of the fingers of the pedipalp (Kovařík 2000) and from *S. orioni* by the length to width ratio of the chela manus (Kovařík *et al.* 2015). *S. longimanus*, formed a monophyletic clade (Fig. 5). From the estimated pairwise distances (Table 3), we diagnosed that genetic

distance of *S. longimanus* (MW 665151) to be 4.9 % from *S. problematicus* (MW 269424), its closest sister taxon. Intraspecific nucleotide variation was detected among the studied species within the range of 0.0 to 2.9% (Table 3).

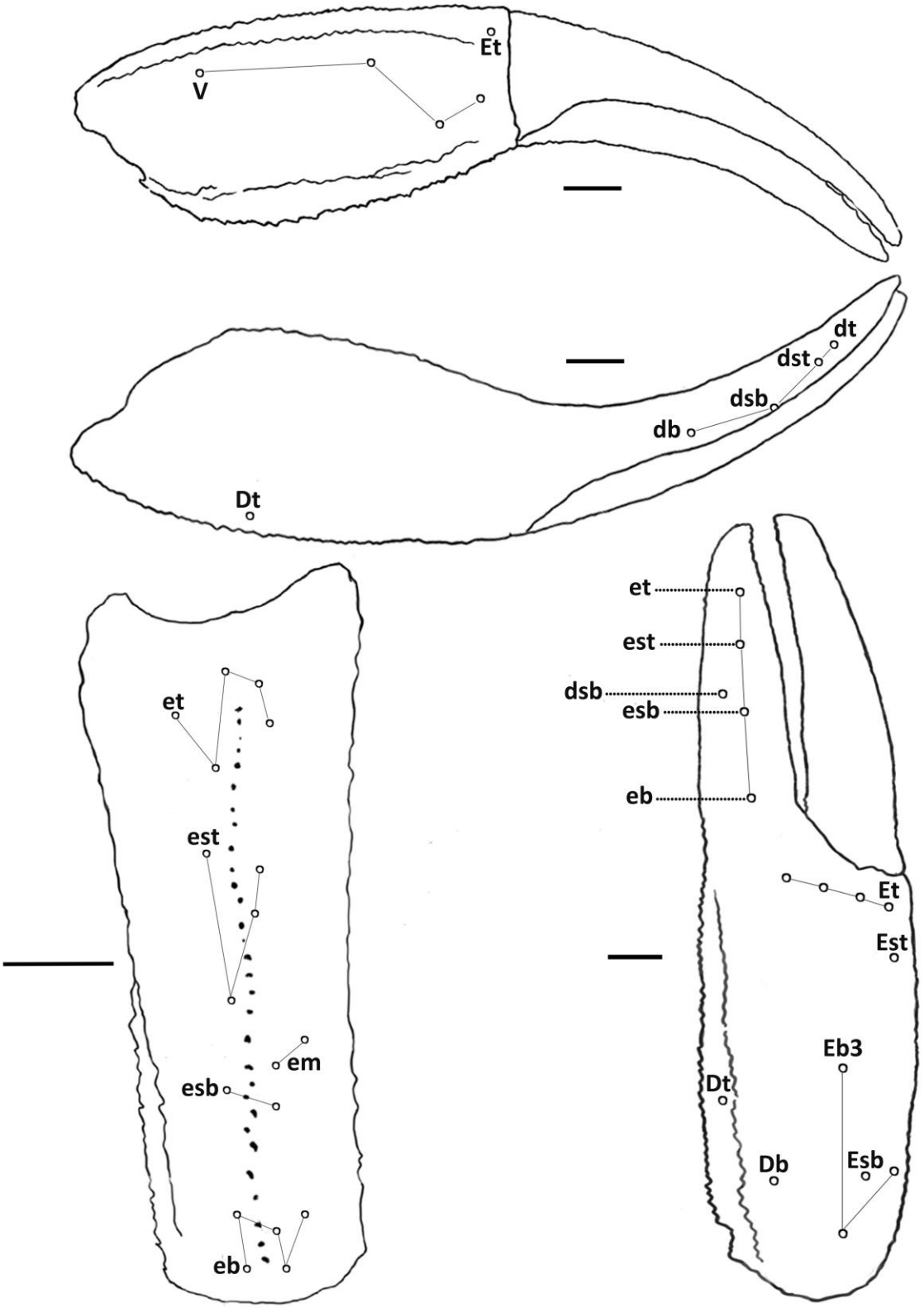

Figure 4. Diagrammatic representation of the trichobothriotaxy of *Scorpiops longimanus*: **(A)** ventral view of chela, **(B)** dorsal view of chela, **(C)** external view of patella, and **(D)** external view of chela; scale 1.0 mm.

Table 2. Morphometric data of *Scorpiops longimanus* (in mm) collected (14 specimens out of 54) from Mizoram, India

Character	Males										Females			
	MZMU 2174C	MZMU 2174D	MZMU 2174F	MZMU 2393A	MZMU 2393B	MZMU 2393C	MZMU 2393D	MZMU 2392A	MZMU 2392B	MZMU 2392D	MZMU 2174A	MZMU 2174B	MZMU 2174E	MZMU 2392C
Total Length	58.26	59.96	42.22	63.54	62.98	65.16	42.44	51.78	57.46	48.32	59.24	59.02	40.46	53.12
Carapace length	7.60	7.62	5.90	8.86	8.34	8.86	6.32	7.80	8.90	7.44	8.22	8.10	5.36	7.76
Carapace width (anterior)	4.48	5.00	1.54	3.54	2.44	3.48	1.58	3.10	3.20	2.38	4.00	4.54	1.42	2.70
Carapace width (Posterior)	6.66	6.64	4.60	7.74	7.46	7.82	6.12	7.12	7.96	6.76	8.50	6.82	4.38	7.70
Mesosomal Segmemt length	24.62	25.20	17.44	25.54	26.28	26.12	16.02	18.18	19.36	15.00	25.22	24.56	16.6	20.36
Mesosomal Segmemt 1 length	2.72	2.76	1.90	3.00	2.98	3.20	2.22	2.54	2.56	2.54	2.68	2.78	1.66	2.32
Mesosomal Segmemt 1 width	2.34	2.44	1.78	3.24	2.98	3.02	1.88	2.42	3.52	2.80	2.42	2.44	1.82	2.88
Mesosomal Segmemt 2 length	2.88	2.86	1.96	2.98	3.24	3.02	2.30	2.56	3.00	2.78	2.72	3.00	2.00	2.14
Mesosomal Segmemt 2 width	2.38	2.34	1.76	2.70	2.56	2.88	1.84	2.64	3.14	2.54	2.66	2.44	1.82	2.48
Mesosomal Segmemt 3 length	3.50	3.62	2.00	3.84	3.24	3.88	2.66	2.88	3.14	2.82	2.76	3.54	2.46	2.88
Mesosomal Segmemt 3 width	1.98	2.20	1.68	2.60	2.60	2.72	1.44	2.22	2.64	2.18	2.70	2.00	1.52	2.32
Mesosomal Segmemt 4 length	4.02	4.22	2.36	4.12	4.00	4.32	2.94	3.50	4.24	3.86	3.32	4.06	2.60	3.84
Mesosomal Segmemt 4 width	1.92	2.20	1.40	2.30	2.30	3.10	1.54	2.34	2.62	2.12	2.76	1.96	1.66	2.30
Mesosomal Segmemt 5 length	5.54	6.00	5.32	7.56	7.20	7.88	4.44	6.58	6.96	6.54	6.72	5.56	4.48	6.50
Mesosomal Segmemt 5 width	1.70	2.12	2.2S2	2.30	2.14	2.62	1.30	2.14	2.54	1.98	2.24	1.74	1.46	2.36
Telson length	4.88	5.22	3.48	5.44	5.16	5.52	3.66	5.48	6.50	5.24	5.20	4.90	3.40	5.26
Telson width	1.96	2.20	1.42	2.22	2.36	2.30	1.40	2.26	2.56	2.08	2.22	1.96	1.26	2.12
Aculeus length	2.50	2.46	1.86	2.20	2.54	2.36	1.88	2.26	2.80	2.10	2.40	2.52	1.90	2.06
Pedipalps: femur length	7.28	7.16	5.48	8.86	8.60	8.90	5.64	8.10	10.82	8.62	7.54	7.32	5.14	7.88
Pedipalps: femur width	2.48	2.44	1.84	3.24	3.06	3.34	1.92	3.02	4.50	2.78	3.26	2.58	1.64	2.88
Pedipalps: patella length	6.72	7.04	5.02	8.06	7.46	8.22	5.56	7.44	9.84	7.88	7.56	6.80	4.36	7.00
Pedipalps: patella width	3.00	3.22	2.44	3.42	3.28	3.48	2.36	3.30	4.62	3.14	4.34	3.02	1.84	4.10
Pedipalps: chela length	14.44	15.92	10.92	18.40	17.18	18.42	12.30	16.02	20.58	16.88	16.60	14.94	10.84	15.88
Pedipalps: chela width	3.82	3.94	2.36	4.86	4.36	4.84	3.04	4.02	5.16	4.28	4.82	4.00	3.22	4.20
Pedipalps: chela depth	2.76	3.02	2.22	3.64	3.06	3.66	2.20	3.18	4.34	3.04	3.42	2.82	2.00	3.2
Pedipalps: moveable finger length	8.92	8.96	6.04	9.74	9.14	9.82	6.62	7.90	9.30	8.14	8.24	9.32	6.28	8.08
Pedipalps: pectines	7-7	7-7	7-7	7-7	7-7	8-7	7-7	7-7	7-7	7-7	7-7	6-7	6-7	7-8

Table 3. Uncorrected pairwise genetic distance of *Scorpiops longimanus* and its congeners retrieved from the NCBI database

No	Species	*P*-distance																
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
1	*Scorpiops longimanus* 1																	
2	*S. longimanus* 2	0.000																
3	*S. longimanus* 3	0.006	0.006															
4	*S. longimanus* 4	0.023	0.023	0.029														
5	*S. longimanus* 5	0.023	0.023	0.029	0.000													
6	*S. longimanus* 6	0.029	0.029	0.035	0.026	0.026												
7	*Scorpiops problematicus*	0.049	0.049	0.047	0.055	0.055	0.047											
8	*Scorpiops orioni*	0.049	0.049	0.047	0.058	0.058	0.049	0.032										
9	*Scorpiops leptochirus*	0.096	0.096	0.093	0.096	0.096	0.105	0.102	0.110									
10	*Scorpiops neradi*	0.099	0.099	0.102	0.090	0.090	0.099	0.105	0.108	0.105								
11	*Scorpiops wongpromi*	0.102	0.102	0.105	0.093	0.093	0.102	0.110	0.113	0.122	0.110							
12	*Scorpiops montanus*	0.116	0.116	0.116	0.122	0.122	0.119	0.116	0.125	0.125	0.131	0.145						
13	*Scorpiops* sp. 1	0.119	0.119	0.122	0.116	0.116	0.113	0.122	0.119	0.119	0.108	0.125	0.142					
14	*Scorpiops grandjeani*	0.122	0.122	0.125	0.119	0.119	0.131	0.128	0.131	0.134	0.125	0.093	0.137	0.160				
15	*Scorpiops citadelle*	0.131	0.131	0.134	0.122	0.122	0.131	0.131	0.128	0.125	0.119	0.125	0.131	0.067	0.154			
16	*Scorpiops oligotrichus*	0.131	0.131	0.134	0.131	0.131	0.131	0.131	0.128	0.134	0.125	0.134	0.142	0.090	0.166	0.084		
17	*Buthus draa*	0.294	0.294	0.294	0.276	0.276	0.285	0.279	0.297	0.294	0.291	0.279	0.314	0.317	0.299	0.305	0.331	

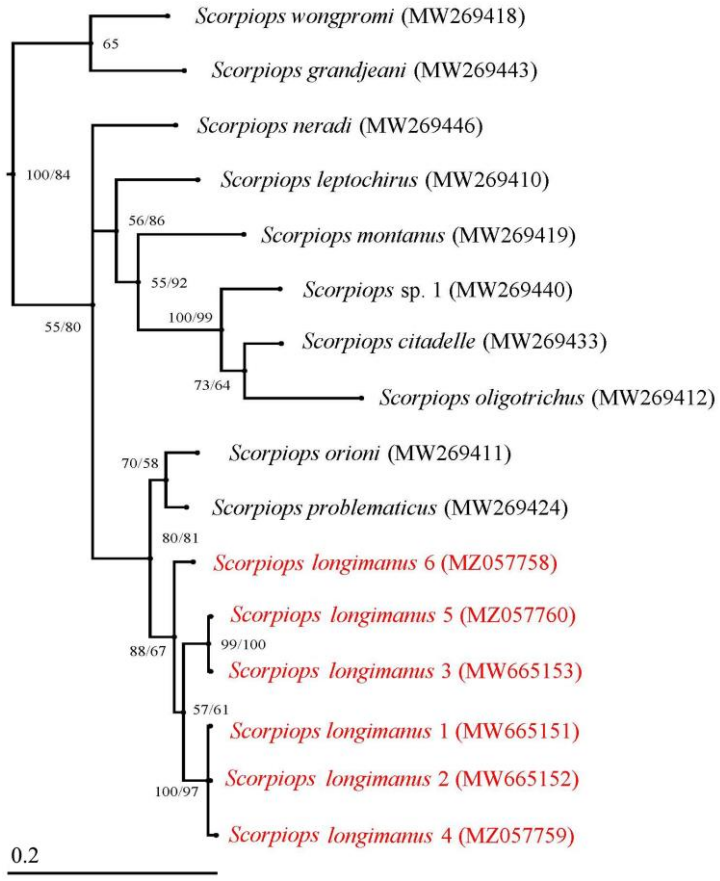

Figure 5. Maximum likelihood phylogenetic tree of *Scorpiops longimanus* constructed based on the partial 16S rRNA gene and the comparison with its congeners retrieved from the NCBI database; numbers at nodes indicate BI/BS support values; red coloured font denotes the studied species; outgroup taxon, *Buthus draa* is not shown.

Discussion

The present study on the scorpion fauna of Mizoram revealed that the species *Scorpiops longimanus* is the second most abundant and widespread species of scorpion in the central and eastern parts of the state after *Liocheles australasiae* (Fabricius, 1775), reported by Malsawmdawngliana *et al*. (2021). No other species of *Scorpiops* was encountered during the survey. Most of the studied specimens were collected from rock crevices, under and inside fallen trees and in the open, often lying still on rock surfaces and tree bark (Fig. 6). Based on the sites of collection, this species of scorpion prefers wet, cool and humid places, in areas devoid of direct sunlight. They were collected nearby streams and from the walls of riverbeds. The species was observed to exhibit a selective feeding behaviour in captivity. A single instance of predation on *Tettigonia* sp. was observed in the wild (Fig. 6). Their breeding season is from April to September based on the collection of gravid females. Juveniles were collected throughout all the months from September 2020 to April 2021. They are an extremely elusive animal reacting to every form of disturbance including lights, noises and vibrations. Practices of slash and burn cultivation, logging and construction are likely to threaten the species in this region. The molecular analysis based on the 16S nucleotide sequence showed a monophyletic clade, closest to *S. problematicus* and *S. orioni* with 4.9% genetic divergence (Table 3). The morphological differences between *S. longimanus*, *S. problematicus* and *S. orioni* are based on the characters of the pedipalp which have been shown to be taxonomically unstable (Kovařík *et al.* 2021) suggesting the need for further investigation. The monophyly of the family Scorpiopidae has been established using multi locus phylogenetic analysis (Šťáhlavský *et al*. 2021). However, *S. longimanus* has not been sequenced by anyone and this is the first molecular data reported along with their predicted phylogenetic position. The branching patterns from our BI topology mostly accorded with the cladogram in Šťáhlavský *et al*. (2021). The phylogenetic position of *S. longimanus* in our analysis may be affected by the insufficient molecular data for *Scorpiops* available in the

data depository. We assume that this new information will improve the limited molecular understanding of this species group in the future.

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Plate 3

Figure 6. *Scorpiops longimanus* from Mizoram, India: habitat in **(A)** Sihphir and **(B)** Tamdil National Wetland; **(C)** adult female with juveniles before the first ecdysis; and **(D)** predate on *Tettigonia* sp. in the wild