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A new record of *Liocheles australasiae* (Fabricius, 1775) (Scorpiones: Hormuridae) from the state of Mizoram, India

Fanai Malsawmdawngliana[1], Mathipi Vabeiryureilai[1], Tara Malsawmdawngzuali[2], Lal Biakzuala[1], Lalengzuala Tochhawng[1] and Hmar Tlawmte Lalremsanga[1*]

[1]*Developmental Biology and Herpetology Laboratory, Department of Zoology, Mizoram University, Aizawl 796004, Mizoram, India*

[2]*Department of Life Sciences, Pachhunga University College, Mizoram University, Aizawl 796001, Mizoram, India*

[*]*Corresponding author* ✉: *htlrsa@yahoo.co.in*

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Abstract

The occurrence of the hormurid scorpion *Liocheles australasiae* (Fabricius) is reported for the first time from the state of Mizoram, northeast India. The specimens were identified on the basis of morphological characters and molecular analysis using a fragment of the mitochondrial cytochrome C oxidase subunit I gene. The species is reported from multiple localities within the state, constituting at least seven different populations. The specimens were larger than those from previous records.

Key words: New state report, mitochondrial *COI* gene, north-eastern India

Introduction

Scorpions are an extremely conserved group of organisms and are often referred to as "living fossils" whose ancestral lineage can be traced back to the Silurian period (Dunlop and Penney, 2012). With 2580 species described worldwide (Rein, 2021), the order Scorpiones is one of the least diversified group of organisms. Though several attempts were made to resolve their taxonomy, the higher systematics of scorpions is under constant scrutiny and revisions (Lourenço et al., 2109; Kovařík et al., 2020; Prendini and Loria, 2020; Santibáñez-López et al., 2020; Tropea and Onnis, 2020; Šťáhlavský et al., 2021).

Previously, the family Hormuridae was placed under Hemiscorpiidae as a subfamily and was reinstated as a family by Monod and Prendini (2015) in their revision of several taxa. It has also been rendered paraphyletic based on phylogenomic analysis (Sharma et al., 2015). Currently, this family is represented by 93 species in 11 genera distributed across the South-eastern Asia and Australian zoogeographic realms (Rein, 2021). Three genera of Hormuridae have been recorded from India: *Chiromachetes* Pocock, *Iomachus* Pocock, and *Liocheles* Sundevall (Mirza, 2017), where *Liocheles* in India is represented by *Liocheles australasiae* (Fabricius), *L. nigripes* (Pocock) and *L. schalleri* Mirza (Pocock, 1900; Tikader and Bastawade, 1983; Mirza, 2017). To our knowledge, the distribution of *L. australasiae* in India includes the Andaman and Nicobar Islands (Tikader and Bastawade, 1983) and Malabar, Kerala on the southwestern Malabar Coast of India (Sureshan et al., 2007). Globally, the species is distributed over the whole of the Indo-pacific regions including Australia, Bangladesh, China, Fiji, Indonesia, Japan, Kiribati, Korea, Malaysia, Marshall Islands, Micronesia, Myanmar, New Caledonia, Palau, Papua New Guinea, Philippines, Samoa, Solomon Islands, Thailand, Tonga, Tuvalu, Vanuatu and Vietnam (Szubert, 2020). *Liocheles australasiae* has also been recently reported from Sri Lanka (Kovařík et al., 2018).

They are small-sized, brownish scorpions, characterized by a smooth, punctate carapace and having a dorso-ventrally compressed body with a weak and small aculeus. This species possesses Type C trichobothriotaxy and is one of the most common species found in its distributional range (Tikadar and Bastawade, 1983; Kovařík et al., 2018).

Material and Methods

Field work and collection sites

Survey of the scorpion fauna of Mizoram was conducted between March–November 2020 and yielded a total of 21 specimens of *L. australasiae* from five localities within Aizawl District, Mizoram State (Fig 1): nine specimens (MZMU 2136a, b, c d, e, f, g, h and MZMU 2137a) were collected between October and November from Durtlang North (23°47'30"N, 92°43'37"E, 1309 m a.s.l), two specimens (MZMU 2140a and MZMU 2140b) from Kanan Veng (23°44'8.26"N, 92°42'23.51"E, 968 m a.s.l), eight specimens (MZMU 2135a, b, c, d, e, f, g and MZMU 2138a) from Republic Veng (23°43'5.38"N, 92°43'22.67"E, 960 m a.s.l), one specimen (MZMU 2141a) from Tanhril Veng (23°44'34.69"N, 92°40'27.60"E, 895 m a.s.l), Aizawl District during the months of March to October and one specimen (MZMU 2142a) from Sailam Village (23°20'44.12"N, 92°48'21.88"E, 1258 m a.s.l), Aizawl District. Two specimens (MZMU 2143a and MZMU 2143b) were also collected during October from Theiriat Village (22°52'3.95"N, 92°46'27.80"E, 1180 m a.s.l) located within Lunglei District and 15 individuals were also identified based on non-catalogued specimens from Pangzawl Village (23° 5'5.83"N, 92°54'9.64"E, 700 m a.s.l), Hnahthial District, Mizoram State.

The collected specimens (Fig. 2) were dug out and collected with the help of tweezers from their microhabitats (Fig 3), then were transferred to 70% ethanol and catalogued into the Departmental Museum of Zoology (MZMU), Mizoram University. Locality coordinates were recorded using a portable GPS unit (Garmin Montana 650-GPS navigator). Photographs were taken using a Canon EOS 60D II digital camera. The distribution map was prepared using the open source QGIS 10.3.8 software. Morphometric data were taken using Mitutoyo™ (505-730) dial calliper with accuracy to 0.01 mm. After species identification was confirmed, morphometric measurements of four specimens (MZMU 2135a, MZMU 2136c, MZMU 2137a and MZMU 2138a) were taken and highlighted in Table 1.

Genomic DNA extraction:

Two specimens of *L. australasiae* collected from Republic Veng, Aizawl (MZMU 2135a) and from Durtlang North, Aizawl (MZMU 2136c) were selected for molecular analysis. DNA was extracted from the thoracic tissues. The extraction method followed a modified protocol by Sambrook et al. (1989). For DNA extraction, the thoracic tissue was washed with 1x PBS and macerated with the help of sharp scissors in 1.5 mL Eppendorf tubes. The tissue was homogenized with a pestle, and 300µL of extraction buffer (50 mM Tris-HCl, 25 mM NaCl, 25 mM EDTA, 1 % SDS) was added and mixed gently. Proteinase K (20 mg/mL, 20µL) was added and incubated at 55°C overnight. After incubation, 300 µL of phenol/chloroform/isoamyl alcohol was added, mixed gently and centrifuged at 13,000 rpm for 10 min. The supernatant was carefully taken out and collected in a new Eppendorf tube. 500 µL of ice-cold ethanol and 50 µL of sodium acetate (3M) were added to the supernatant and mixed gently by inverting the tube several times and were kept in -20°C for overnight. The tube was centrifuged at 13,000 rpm for 5 min at 4 °C. Ethanol was poured off without dislodging the pellet, 200 µL of 70% ethanol was then added, and flash spun at 6000 rpm for 1 min. The ethanol was poured off and the pellet was dried. Nuclease free water (70 µL) was then added to the tube; the pellet was re-suspended by gently flicking the tube and was stored at -20 °C for further use.

Amplification of COI gene using PCR

PCR reactions were prepared with universal primers - forward primer: LepF1 (5′-ATTCAACCAATCATAAAGATATTGG-3′) and reverse primer: LepR1 (5′-TAAACTTCTGGATGTCCAAAAAATCA-3′) (Hebert et al., 2004). The 20 µL reaction mixture contained 1x amplification buffer, 2.5 mM MgCl2, 0.25 mM dNTPs, 0.2 pM each forward and reverse primer,0.8 µL BSA, 2 µL genomic DNA, and 1 µL Taq DNA polymerase. The PCR thermal regime for amplification was 5 minutes at 95 °C for initial denaturation, followed by 35 cycles of 1 minute at 95 °C for denaturation, 40 seconds for annealing at 48–49 °C, elongation for 1 minute at 72 °C, and a final elongation for 5 minutes at 72 °C. PCR products were checked by gel electrophoresis on a 1.5% agarose gel containing ethidium bromide. Samples were sequenced using the Sanger's dideoxy method, and sequencing reactions were carried out in both directions on a sequencer (Agrigenome Labs Pvt Ltd., Kochin, India). All sequences were checked using BLAST (NCBI). All protein-coding sequences were translated into amino acids and their ORFs checked (ORF Finder, NCBI). Scorpion's *COI* sequences were submitted to GenBank under the accession numbers MW387531 (MZMU 2135a) and MW387532 (MZMU 2137a).

Phylogenetic analysis

The analysis involved 13 nucleotide sequences, with a total of 382 base pairs in the final dataset. The best partitioning model was estimated with MEGA7 (Kumar et al., 2016) based on the Bayesian Information criterion. A phylogenetic tree was inferred using Maximum Likelihood based on the General Time Reversible model (Nei and Kumar, 2000). The bootstrap consensus tree inferred from 1000 replicates was generated to represent the evolutionary history of the target group. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Phylogenetic analyses were conducted in MEGA7 (Kumar et al., 2016). Two species *Buthus draa* Lourenco and Slimani (JQ775941) and *Buthus mariefranceae* Lourenco (JQ775957.1) were used as an out-group in the phylogenetic analysis.

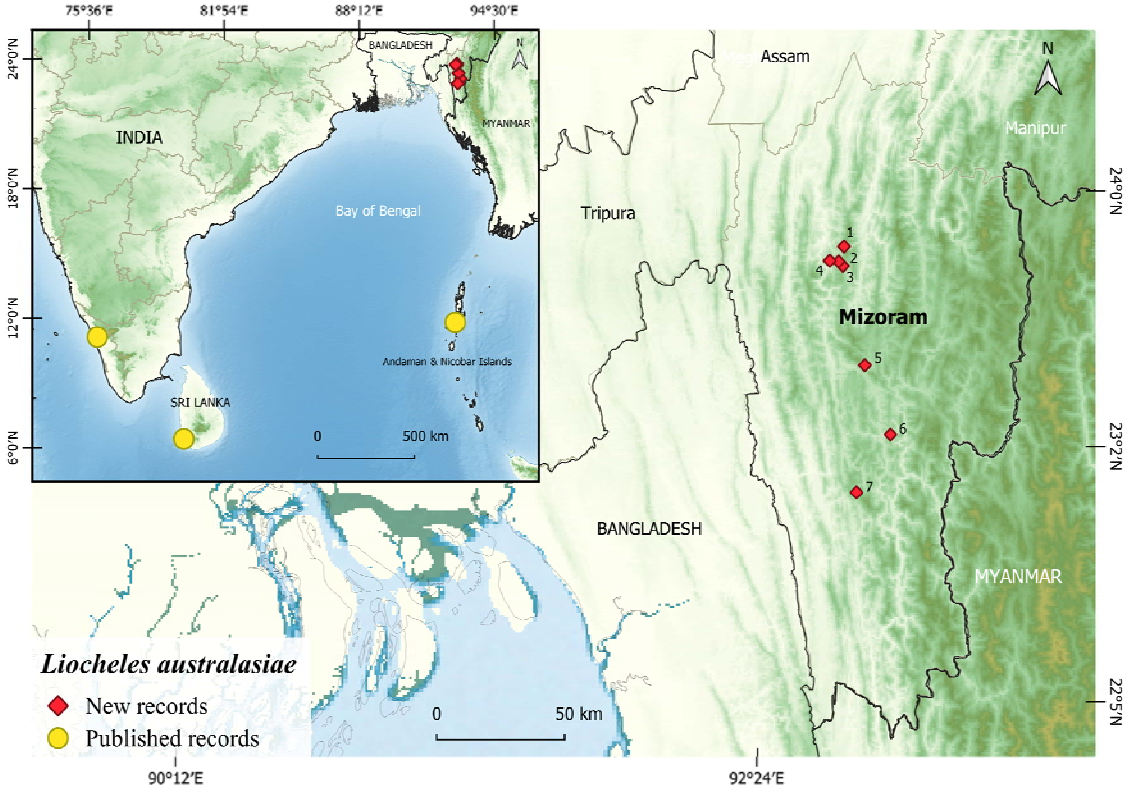

Figure 1: Distribution map of *Liocheles australasiae* within the Indian subcontinent, based on the previous (small yellow circles) and present records (small red polygons), respectively. 1 – Durtlang North; 2 – Kanan Veng; 3 – Republic Veng; 4 – Tanhril Veng; 5 – Sailam Village; 6 – Pangzawl Village; 7 – Theiriat Village.

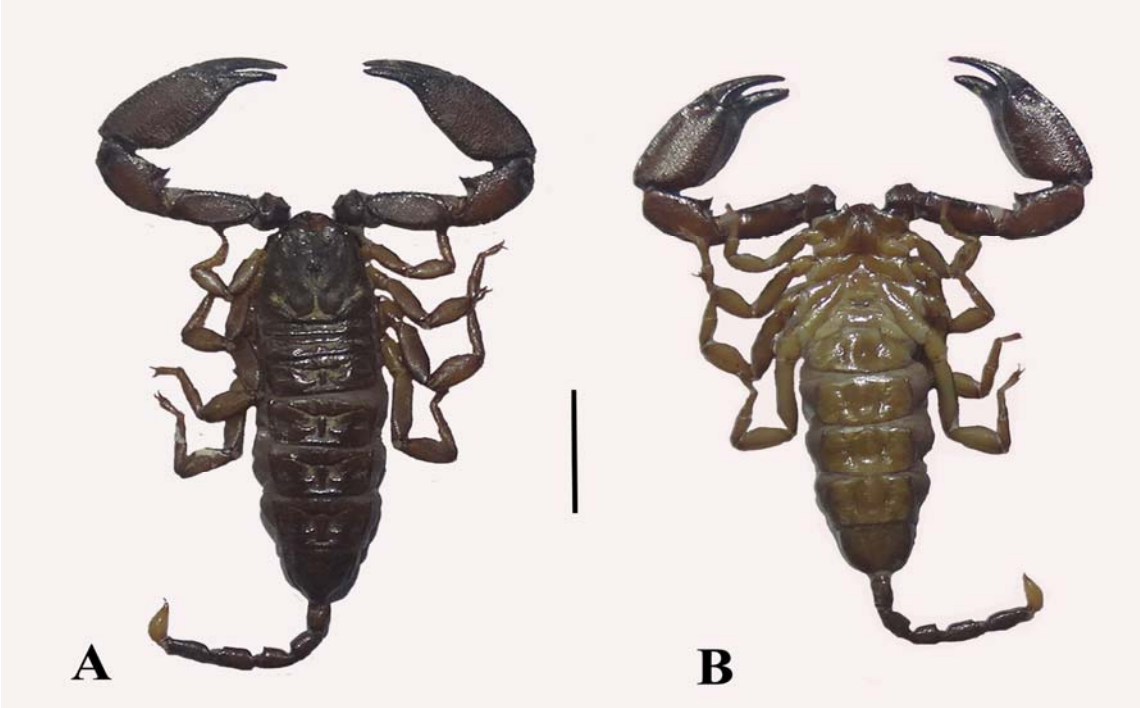

Figure 2: *Liocheles australasiae* (MZMU 2135a) from Mizoram, dorsal (A) and ventral (B) views. Scale bar = 10 mm.

Figure 3: Microhabitat and collection site of *Liocheles australasiae*, crevices and clefts of rock (A) and retaining wall (B) at Durtlang North, Aizawl, Mizoram, India.

Results and Discussion

Morphology and meristic data for the newly collected specimens (Table 1) are in good agreement with the description of *L. australasiae* by Tikadar and Bastawade (1983) and Kovařík et al. (2018): patella of pedipalp granulated and furnished with three ventral trichobothria with the trichobothrial pattern 5*eb*, 2*esb*, 2*em*, 1*est*, 3*et* on the external face of the pedipalp patella. Trichobothria *Db*, *Eb1*, *Eb2*, *Eb3* and *Esb* located at the proximal base of the chela manus. Ventral surface of the chela manus is provided with four trichobothria (Kovařík et al., 2018). The coloration of the body is yellowish brown with darker coloration on the chela and lighter coloration on the walking legs. All the specimens in the present study showed a dorsoventrally flattened body, larger size of chela in proportion to other body parts (Fig. 2), and reduction in size of metasoma owing to their mode of prey acquisition.

Table 1: Morphometric (in mm) and meristic characteristics of collected specimens of *Liocheles australasiae* from Durtlang North (MZMU 2136c, MZMU 2137a) and Republic Veng (MZMU 2135a, MZMU 2138a), Mizoram, India.

No.	Characters	MZMU 2135a	MZMU 2136c	MZMU 2137a	MZMU 2138a
1.	Total length	43.22	40.26	46.26	39.20
2.	Carapace				
	- Length	6.70	5.78	6.66	5.82
	- Anterior width	3.00	2.56	3.48	3.22
	- Posterior width	7.20	6.50	7.00	6.60
3.	Metasomal segment I				
	- Length	2.32	2.30	2.38	2.02
	- Width	1.70	1.60	1.50	1.46
4.	Metasomal segment II				
	- Length	2.22	2.34	2.28	2.12
	- Width	1.44	1.34	1.28	1.26
5.	Metasomal segment III				
	- Length	2.10	2.10	2.22	2.10
	- Width	1.38	1.28	1.28	1.26
6.	Metasomal segment IV				
	- Length	2.70	2.66	2.78	2.14
	- Width	1.40	1.66	1.28	1.20
7.	Metasomal segment V				
	- Length	3.02	3.00	3.22	3.12
	- Width	1.22	1.12	1.16	1.24
8.	Telson				
	- Length	4.43	2.66	2.76	2.38
	- Width	1.34	1.22	1.26	1.20
	- Aculeus length	1.20	1.06	1.06	1.00
9.	Pedipalps				
	- Femur length	6.04	5.20	6.10	5.12
	- Femur width	2.66	2.54	2.78	2.50
	- Patella length	6.28	5.92	6.34	5.54
	- Patella width	3.44	3.30	3.52	3.00
	- Chela length	12.78	11.70	12.94	11.60
	- Chela width	5.00	4.64	4.72	4.46
	- Chela depth	2.46	2.48	2.36	2.28
	- Movable finger length	6.14	5.00	5.70	5.32
10.	**Pectines**				
	- Teeth-count	6 – 6	7 – 7	7 – 7	7 – 7

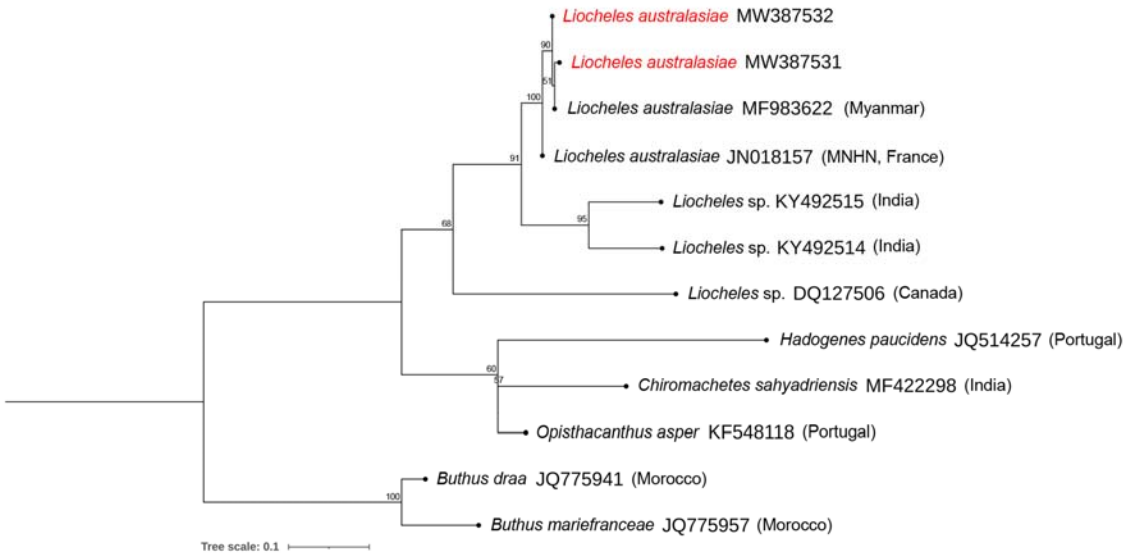

Figure 4: Phylogenetic tree inferred from mitochondrial *COI* gene sequence by using the Maximum Likelihood method based on the General Time Reversible model. Numbers above the lines/ besides the nodes are given as Bayesian posterior probabilities (BPP)/bootstrap support (BSP) for maximum likelihood analysis.

Morphometric data of the specimens (Table 1) showed that the collected specimens were larger in size (39.20–46.26 mm) compared to the size (22–36 mm) given by Kovařík (2000). However, the museum specimen from the British Museum of Natural History, London (voucher number not specified), examined by Tikadar and Bastawade (1983) was reported to be 39.25 mm in length. Based on our data, we observed the upper limit of the total length of the species to reach 46.26 mm (MZMU 2137a). No male individual was encountered in the present collection which is congruent with the observation of Yamazaki and Makioka (2005) on the potentially parthenogenetic nature of the species.

Inferring from the molecular analysis, *L. australasiae* specimens from this study are clustered with the conspecific sequences from GenBank with a significant bootstrap support in the present Maximum Likelihood phylogenetic tree inferred using *COI* gene (Fig. 4), and are genetically closest to *L. australasiae* (MF983622) from Myanmar with intra-species genetic distance of 4.8% with specimens MZMU 2135a (MW387531) and MZMU 2136c (MW387532) (Appendix).

The distribution map (Fig. 1) of *L. australasiae* within the state of Mizoram shows that the species is not uncommon, widespread and found mostly near human settlements. Several specimens were collected indoors in places such as crevices and clefts in rocks and retaining walls, under flower pots and basements of houses. They prefer dark, humid areas and present no real threat to humans due to their mild venom and weak aculeus which often fails to penetrate human skin (Rein, 2021). This report added a second locality of the species apart from Malabar, Kerala (Sureshan et al., 2007) from mainland India, that links the distribution range from the latter with Tanintharyi, Myanmar (Slapcinsky and Mulcahy, 2017) towards the east.

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Conflict of interest

The authors declare that there are no conflicting issues related to this research article.

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Appendix: Uncorrected K2P distance of *COI* gene for *Liocheles* species from Mizoram and sequence retrieved from NCBI database.

No.	Species	K2P distance											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	*Liocheles australasiae* (MW387531) This study												
2	*Liocheles australasiae* (MW387532) This study	0.048											
3	*Liocheles australasiae* (MF983622) Myanmar	0.048	0.032										
4	*Liocheles australasiae* (JN018157) France	0.053	0.026	0.016									
5	*Chiromachetes sahyadriensis* (MF422298) India	0.116	0.135	0.135	0.129								
6	*Hadogenes paucidens* (JQ514257) Portugal	0.153	0.154	0.160	0.167	0.140							
7	*Opisthacanthus asper* (KF548118.1) Portugal	0.129	0.111	0.111	0.105	0.105	0.160						
8	*Liocheles* sp. (KY492515) India	0.081	0.099	0.124	0.105	0.159	0.173	0.148					
9	*Liocheles* sp. (KY492514) India	0.111	0.124	0.124	0.117	0.179	0.185	0.147	0.093				
10	*Liocheles* sp. (DQ127506) Canada	0.165	0.152	0.165	0.159	0.178	0.219	0.159	0.159	0.193			
11	*Buthus draa* (JQ775941) Morocco	0.206	0.173	0.159	0.153	0.226	0.185	0.165	0.206	0.198	0.938		
12	*Buthus mariefranceae* (JQ775957) Morocco	0.165	0.159	0.159	0.166	0.205	0.140	0.179	0.206	0.212	0.827	0.089	

The NCBI accession numbers are provided within parenthesis after species name followed by locality in the table.