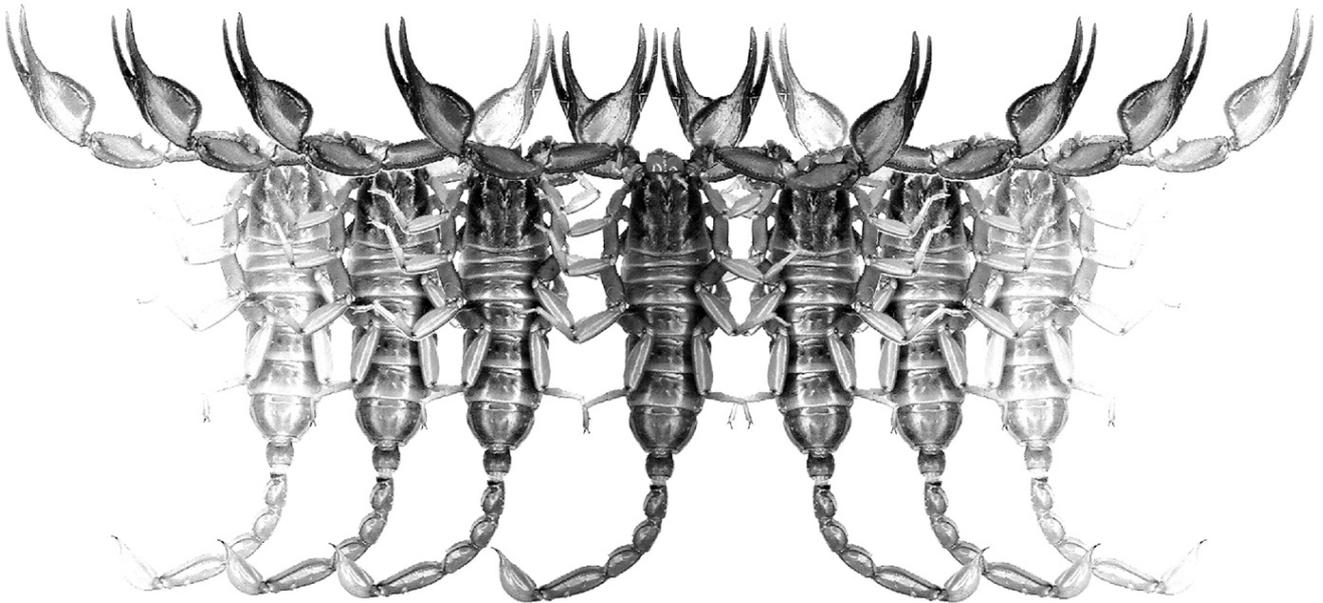


# *Euscorpius*

Occasional Publications in Scorpiology



**A new species of *Isometrus* Ehrenberg, 1828  
(Scorpiones: Buthidae) from southeastern  
plains of Tamil Nadu, India**

**Shubhankar Deshpande, Gaurang Gowande,  
Deshabhusan Bastawade & Shauri Sulakhe**

**June 2022 — No. 353**

# *Euscorpius*

## *Occasional Publications in Scorpiology*

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<http://zoobank.org/urn:lsid:zoobank.org:pub:8B9DDB23-F917-40BD-AEB5-EED78870A7DE>

# A new species of *Isometrus* Ehrenberg, 1828 (Scorpiones: Buthidae) from southeastern plains of Tamil Nadu, India

Shubhankar Deshpande<sup>1,3</sup>, Gaurang Gowande<sup>1,2,4</sup>, Deshabhushan Bastawade<sup>1,5</sup> & Shauri Sulakhe<sup>1,5</sup>

<sup>1</sup>InSearch Environmental Solutions, Flat no 1, Omkar Apartments, Near Shivaji Statue, Sant Gangaram Road, Pune: 411038, Maharashtra, India.

<sup>2</sup>Annasaheb Kulkarni Department of Biodiversity, Abasaheb Garware College, Karve 13 Road, Pune, Maharashtra – 411004, India.

<sup>3</sup>Department of Environmental Science, Fergusson College, Pune, Maharashtra – 411004, India.

<sup>4</sup>Department of Biotechnology, Fergusson College, Pune, Maharashtra – 411004, India.

<sup>5</sup>Institute of Natural history, Education and Research (INHER), B1–602, Kumar Parisar, Kothrud, Pune, Maharashtra – 411038, India.

Corresponding author: Shauri Sulakhe ([shaurisulakhe@gmail.com](mailto:shaurisulakhe@gmail.com))

<http://zoobank.org/urn:lsid:zoobank.org:pub:8B9DDB23-F917-40BD-AEB5-EED78870A7DE>

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## Summary

We here describe a new species of *Isometrus* Ehrenberg, 1828 from the southeastern plains of Tamil Nadu, India, which is known only from its type locality, Chengalpattu. The new species was recovered as sister to *Isometrus nakshatra* based on a molecular phylogeny inferred from COI and 16S mitochondrial genes. Sequence divergence between the new species and all the Indian congeners was 12.6–15.7 % for COI and 12.2–14.4 % for 16S. The new species can also be differentiated from all the Indian congeners based on the following set of characters: surface of carapace with mixed (coarse and fine) and dense granulation; telson vesicle length to depth ratio in males 3.5–3.7; chela length to width ratio in males 5.0–5.4; metasomal length to carapace length ratio in males 8.2–8.9; coarse granulation on mesosomal tergites V and VI along the margins; lateral supramedian and ventral lateral carinae on metasomal segments II–IV moderately to weakly granular; ventral median carina of telson vesicle weakly granular; spiniform granules of promedian carina of the pedipalp patella strongly developed. This is the ninth species of *Isometrus* described from India and the first species from southeastern peninsular India. This discovery highlights the need for more surveys from the eastern peninsular region.

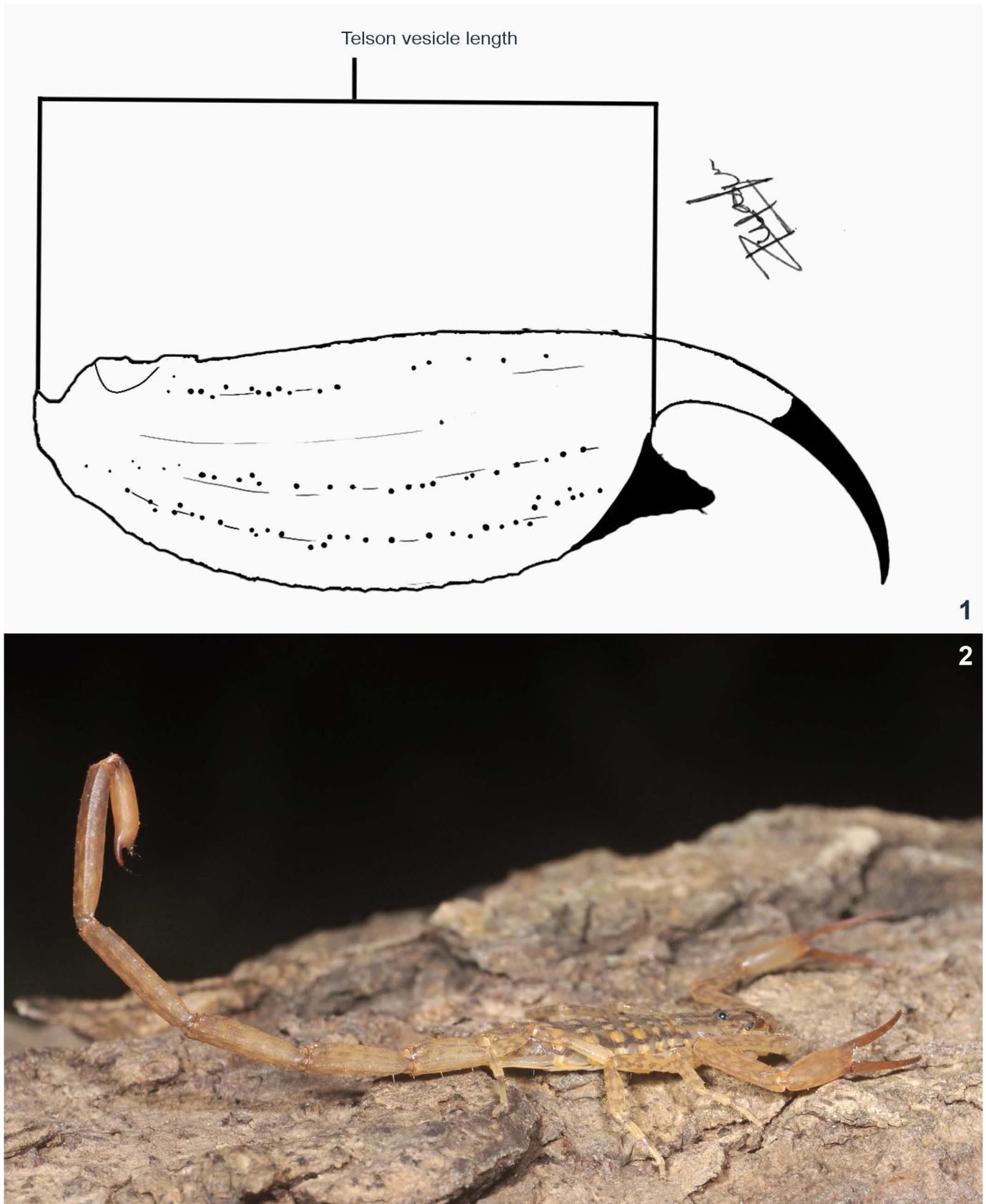
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## Introduction

The family Buthidae C. L. Koch, 1837 is the largest and most diverse scorpion family, currently comprising 96 genera and 1312 species across the world (Rein, 2022). The genus *Isometrus* Ehrenberg, 1828 was described based on the type species *Isometrus maculatus* (De Geer, 1778) from a vague type locality “Suriname and Pennsylvania” and is considered to be a cosmopolitan species with records from ca. 70 countries in the world (Fet & Lowe, 2000; Kovařík, 2003); although Veronika et al. (2013) and Kovařík et al. (2016) considered it to be endemic to Sri Lanka. Kovařík et al. (2016) and Sulakhe et al. (2020a) proposed the need to undertake molecular studies to assess the taxonomic status of this widely distributed species. The genus *Isometrus* comprises eight species in India including *I. maculatus*, which is known to occur in India based only on a few morphologically identified records (Kovařík, 2003). The remaining species include: *Isometrus*

*thurstoni* Pocock, 1983 from Shevaroy Hills (Tamil Nadu), *I. sankeriensis* Tikader & Bastawade, 1983 from Sunkeri, Karwar (Karnataka), *I. tamhini* Sulakhe et al., 2020 from Tamhini, Pune (Maharashtra), *I. amboli* Sulakhe et al., 2020 from Amboli, Sindhudurg (Maharashtra), *I. kovariki* Sulakhe et al., 2020 from Bengaluru Urban District (Karnataka), *I. nakshatra* Sulakhe et al., 2022 from Kadmane Tea Estate, Hassan District (Karnataka), and *I. wayanadensis* Sulakhe et al., 2022 from Wayanad District (Kerala).

*Isometrus sankeriensis* was considered a junior synonym of *I. thurstoni* by Kovařík (2003); however, recently Sulakhe et al. (2022) resurrected *I. sankeriensis* from the synonymy of *I. thurstoni* based on fresh collections from the type locality and also tested the validity of *I. tamhini*, *I. amboli* and all other species of *Isometrus* found in India based on geographical, morphological and molecular data. Establishing the validity of *I. tamhini* and *I. amboli* was all the more important given the morphological similarity and geographical proximity with



**Figures 1–2:** **Figure 1.** The telson of *I. wayanadensis* Sulakhe et al., 2022, male, holotype BNHS SC 190 (bracket showing the character ‘telson vesicle length’). Illustration by Ruta Karmarkar. **Figure 2.** *Isometrus longitelson* sp. n., male, paratype, BNHS SC 287 in vivo habitus.

*I. sankeriensis*. Designation of a neotype of *I. sankeriensis* stabilized the taxonomy and understanding of the cryptic diversity by delineating the species (Sulakhe et al., 2022).

As a part of our continuous field surveys of *Isometrus* populations in the peninsular India we came across a population, which was morphologically and genetically distinct from all the known species. In this communication, we describe this distinct lineage of *Isometrus* from the southeastern plains of Tamil Nadu, India based on the diagnostic character 'telson elongation' for the morphological separation of the new species from all the Indian congeners.

## Methods, Material & Abbreviations

**Sampling.** Sampling was carried out in south India. Scorpions were located with the help of ultraviolet light (uvBeast V3 385-395nm UV Torch) and collected. Photographs of holotype and paratypes were taken using Nikon D500, 105mm F2.8 micro lens and R1C1 flash kit. Specimens were euthanized and preserved in absolute ethanol, and later transferred to 70% ethyl alcohol in collection jars for long term preservation. Examination and morphological measurements were done using LEICA EZ4HD microscope with LEICA application suite. Morphometry was performed following Stahnke (1971); trichobothrial terminology follows Vachon (1974); metasoma carination follows Francke (1977); pedipalp carination, chela dentition and leg terminology follow González-Santillán & Prendini (2013); morphological terminology follows Hjelle (1990); lateral ocelli terminology follows Loria & Prendini (2014). The surface morphology was examined and photographed under UV light after Volschenk (2005). Measurements were taken (in mm) for 34 morphological characters (Tables 1, 2). Specimens collected and studied are deposited in the museum collection of Bombay Natural History Society (BNHS), Mumbai and InSearch Environmental Solutions (IES), Research laboratory, Pune, Maharashtra in India.

**Comparative material examined.** Data used for comparison and diagnosis of *I. maculatus*, *I. thurstoni*, *I. tamhini*, *I. amboli*, *I. kovariki*, *I. sankeriensis*, *I. nakshatra* and *I. wayanadensis* has been sourced from (Sulakhe et al., 2020a, 2020b, 2022).

**Molecular analysis. DNA extraction, amplification and sequencing.** DNA extraction, amplification and sequencing protocols as per Sulakhe et al. (2020a) were followed. Genomic DNA was isolated from preserved (ethanol 99.9%) muscle tissue (leg fragment) of *Isometrus* species (voucher numbers of specimens used for DNA analysis are mentioned in Figs. 31, 32) with the help of MACHEREY-NAGEL NucleoSpin® DNA Insect kit as per manufacturer's protocols. A 550–600 base pair (bp) fragment of the cytochrome c oxidase subunit I (COI) and a 450–500 base pair (bp) fragment of 16S rRNA (16S) mitochondrial genes were amplified by polymerase chain reaction (PCR) using the primers as per previous studies (Table 3). A 25 µl PCR reaction (TaKaRa Taq™ DNA Polymerase) was prepared containing 1 unit of Taq DNA polymerase (0.2µL), 2.5 µL of 10x buffer, 2

µl of dNTPs (2.5mM each), 2 µl (5mM) of each primer, 2 µl template DNA, and 14.3 µl of water, and reactions were carried out with an Miniamp Thermal Cycler. Thermal cycler profiles used for amplification of COI gene were as follows: 95°C for 30 seconds (initial denaturation temperature 95°C for 3 minutes, annealing temperature 55°C for 30 seconds, elongation temperature 72°C for 45 seconds and 3 minutes x 35 cycles. Thermal cycler profiles used for amplification of 16S gene was as follows: 95°C for 30 seconds (initial denaturation temperature 95°C for 3 minutes, annealing temperature 52°C for 30 seconds, elongation temperature 72°C for 45 seconds and 3 minutes x 35 cycles. PCR products were cleaned through column purification with Qiagen PCR Cleanup Kit and sequenced with a 3730 DNA Analyzer. Sequencing primers were the same as those used in PCRs. All newly generated sequences were deposited in the GenBank® nucleotide sequence database (<http://www.ncbi.nlm.nih.gov>) under accession numbers: COI (ON103619–IES 368, ON103620–IES 372); 16S (ON113165–IES 368, ON113166–IES 272).

**Sequence alignment.** Generated sequences were cleaned manually in MEGA v.7 (Kumar et al., 2016) using chromatograms visualised in Chromas v.2.6.5 (Technelysium PTY. Ltd.). Cleaned sequences were aligned using MUSCLE (Edgar 2004) implemented in MEGA v.7 with default parameters and were then added to the sequence matrix generated by Sulakhe et al., (2022). The final COI alignment contained 33 sequences each 525 bp in length, whereas the 16S alignment contained 14 sequences each of 500 bp length. The alignment included one sequence of *Lychas mucronatus* Simon, 1884 used as outgroup to root the phylogenetic tree. The COI and 16S datasets were concatenated and the resultant 1025 bp long alignment was used for molecular phylogenetic analyses.

**Genetic divergence (p-distance).** The *p-distances* were calculated separately for the mitochondrial COI and 16S loci in MEGA v.7. The substitution type was set as nucleotide, the model was kept as *p-distance* and the substitutions were included as d: Transitions + Transversions. Uniform rates were kept for the analysis. Missing data were partially deleted and the site Cutoff % was set as 95. At last, all three codon positions and noncoding sites were selected (the *p-distances* are mentioned in Tables 4, 5).

**Molecular phylogenetics analysis.** Maximum Likelihood (ML) and Bayesian Inference (BI) methods of phylogenetic analyses were implemented. The COI region was partitioned per codon position, whereas the non-coding 16S region was not partitioned and the best substitution model for BI phylogenetic analysis was determined using PartitionFinder V.1.1.1 (Lanfear et al., 2012). Model search was performed with a greedy search algorithm (Schwarz, 1978) and models were selected using the Akaike Information Criterion (AIC). Maximum Likelihood analysis was performed using the web implementation of IQ-tree (Nguyen et al., 2015) under the TVM+F+I+G4 models of sequence evolution for all the positions. The model of sequence evolution was determined

Dimensions (mm)		<i>Isometrus longitelson</i> sp. n.				
		♂ holotype BNHS SC 287	♂ paratype BNHS SC 288	♂ paratype BNHS SC 289	♂ paratype IES 368	♂ paratype IES 370
Carapace	L / W	4.27 / 4.60	3.95 / 3.64	4.16 / 3.49	4.23 / 3.60	4.32 / 4.11
Mesosoma	L	14.94	9.52	11.22	11.05	12.66
Tergite VII	L / W	3.95 / 3.42	2.79 / 2.56	3.13 / 2.87	3.14 / 3.05	3.35 / 2.87
Metasoma and telson	L	35.51	35.25	37.05	36.02	35.43
Segment I	L / W / D	1.72 / 1.92	4.15 / 1.62	4.46 / 1.70	4.24 / 1.63	4.72 / 1.52
Segment II	L / W / D	5.07 / 1.68	5.19 / 1.44	5.44 / 1.60	5.57 / 1.49	5.89 / 1.45
Segment III	L / W / D	5.84 / 1.51	5.47 / 1.31	6.20 / 1.45	5.79 / 1.22	5.33 / 1.37
Segment IV	L / W / D	7.73 / 1.29	6.74 / 1.19	6.94 / 1.32	6.84 / 1.15	6.23 / 1.28
Segment V	L / W / D	8.44 / 1.46	7.82 / 1.28	7.87 / 1.31	7.63 / 1.40	6.95 / 1.32
Telson	L / W / D	6.71 / 1.39 / 1.47	5.88 / 1.19 / 1.30	6.14 / 1.19 / 1.38	5.95 / 1.27 / 1.34	6.31 / 1.32 / 1.38
Pedipalp	L	23.51	19.3	18.84	19.94	22.24
Femur	L / W	6.99 / 1.49	5.23 / 0.94	4.93 / 0.99	5.30 / 1.15	6.10 / 1.11
Patella	L / W	7.39 / 1.86	5.51 / 1.44	5.24 / 1.35	5.56 / 1.56	6.30 / 1.77
Chela	L	9.13	8.56	8.67	9.08	9.84
Manus	W / D	1.71 / 1.86	1.72 / 1.36	1.63 / 1.62	1.69 / 0.95	2.05 / 1.47
Movable finger	L	5.56	5.39	5.54	5.48	6.06
Pectine	L / W	4.55 / 0.80	2.94 / 0.54	3.44 / 0.64	4.05 / 0.94	3.86 / 0.74
Genital Operculum	L / W	0.80 / 1.36	0.66 / 0.93	0.79 / 0.79	1.27 / 2.19	0.64 / 1.26
<b>Total</b>	<b>L</b>	<b>54.72</b>	<b>48.72</b>	<b>52.43</b>	<b>51.30</b>	<b>52.41</b>
Pectinal teeth count	PTC	17 / 18	17 / 17	16 / 17	15 / 16	17 / 17

Dimensions (mm)		<i>Isometrus longitelson</i> sp. n.			
		♀ paratype IES 372	♀ paratype BNHS SC 290	♀ paratype IES 366	♀ paratype IES 369
Carapace	L / W	3.99 / 3.42	3.68 / 3.40	3.86 / 3.51	4.46 / 3.71
Mesosoma	L	10.47	11.36	11.21	12.3
Tergite VII	L / W	2.47 / 3.34	2.63 / 3.69	2.48 / 3.60	3.17 / 3.82
Metasoma and telson	L	22.64	22.68	22.09	24.09
Segment I	L / W / D	2.50 / 1.92	2.26 / 1.92	2.54 / 1.96	2.83 / 2.11
Segment II	L / W / D	3.31 / 1.59	3.30 / 1.74	3.44 / 1.70	3.52 / 1.75
Segment III	L / W / D	3.38 / 1.57	3.70 / 1.57	3.52 / 1.61	3.58 / 1.73
Segment IV	L / W / D	4.15 / 1.38	3.96 / 1.53	4.17 / 1.47	4.16 / 1.51
Segment V	L / W / D	5.25 / 1.25	5.36 / 1.48	4.63 / 1.39	5.87 / 1.10
Telson	L / W / D	4.05 / 1.12 / 1.16	4.10 / 1.06 / 1.21	3.79 / 1.09 / 1.11	4.13 / 1.04 / 1.12
Pedipalp	L	15.21	14.32	14.81	14.92
Femur	L / W	3.66 / 1.04	3.45 / 1.10	3.32 / 1.11	3.58 / 1.02
Patella	L / W	4.44 / 1.50	4.18 / 1.59	4.22 / 1.52	4.34 / 1.38
Chela	L	7.11	6.69	7.27	7.00
Manus	L / W / D	1.41 / 1.10	1.25 / 1.27	1.38 / 1.22	1.34 / 1.26
Movable finger	L	4.8	4.77	4.7	4.64
Pectine	L / W	2.85 / 0.56	3.23 / 0.67	3.52 / 0.52	3.46 / 0.63
Genital Operculum	L / W	0.64 / 1.16	0.73 / 1.00	0.56 / 1.11	1.01 / 1.86
<b>Total</b>	<b>L</b>	<b>37.10</b>	<b>37.72</b>	<b>37.16</b>	<b>40.85</b>
Pectinal teeth count	PTC	15 / 16	17 / 17	17 / 17	16 / 16

**Table 1.** Morphometric data for *Isometrus longitelson* sp. n. Abbreviations: length (L), width (W, in carapace it corresponds to median width), depth (D).

Telson vesicle lengths of male specimens of <i>Isometrus</i> Ehrenberg, 1828 species from India.		
Species	Voucher Number	Telson Vesicle Length
<i>I. tamhini</i>	BNHS SC 155	4.82
<i>I. tamhini</i>	INHER 92	4.69
<i>I. tamhini</i>	INHER 73	4.49
<i>I. amboli</i>	BNHS SC 157	4.75
<i>I. amboli</i>	INHER 42	4.14
<i>I. amboli</i>	INHER 111	4.19
<i>I. amboli</i>	INHER 110	3.98
<i>I. amboli</i>	INHER 113	3.19
<i>I. amboli</i>	INHER 115	3.17
<i>I. amboli</i>	INHER 112	3.06
<i>I. thurstoni</i>	INHER 134	3.10
<i>I. thurstoni</i>	INHER 136	3.26
<i>I. thurstoni</i>	INHER 141	3.19
<i>I. thurstoni</i>	INHER 139	4.09
<i>I. kovariki</i>	BNHS SC 161	4.87
<i>I. kovariki</i>	BNHS SC 164	4.64
<i>I. kovariki</i>	BNHS SC 163	4.51
<i>I. kovariki</i>	INHER 151	3.39
<i>I. kovariki</i>	INHER 146	3.82
<i>I. kovariki</i>	INHER 152	3.84
<i>I. kovariki</i>	INHER 149	3.67
<i>I. nakshatra</i>	BNHS SC 195	3.92
<i>I. nakshatra</i>	INHER 275	3.33
<i>I. wayanadensis</i>	BNHS SC 190	4.11
<i>I. wayanadensis</i>	BNHS SC 193	3.93
<i>I. wayanadensis</i>	INHER 279	3.84
<i>I. sankeriensis</i>	BNHS SC 194	2.69
<i>I. sankeriensis</i>	INHER 288	3.03
<i>I. longitelson</i> sp. n.	BNHS SC 287	5.11
<i>I. longitelson</i> sp. n.	BNHS SC 288	4.80
<i>I. longitelson</i> sp. n.	BNHS SC 289	4.94
<i>I. longitelson</i> sp. n.	IES 368	5.02
<i>I. longitelson</i> sp. n.	IES 370	5.02

**Table 2.** Telson vesicle lengths of male specimens of Indian *Isometrus* species.

using ModelFinder (Kalyaanamoorthy et al., 2017) on the IQ-tree web platform, and branch support was tested using 1000 non-parametric rapid ultrafast bootstrap pseudo-replicates (Minh et al., 2020). Bayesian trees were generated using MrBayes v .3.2.6 (Ronquist et al., 2012). The models of sequence evolution were as follows: HKY for codon position 1, HKY+I for codon position 2, and HKY+I for codon position 3 and HKY for codon position 4. For the BI analysis, two simultaneous, independent analyses were run

starting from different random trees. Three heated and one cold chain was used in the analysis. Markov chains were sampled every 500 generations for 50 million generations. At the end of the run, we tested convergence of the two MCMC runs by checking the standard deviation of split frequencies, which was less than 0.001, and by checking the trace plots using Tracer v. 1.7 (Rambaut et al., 2018). The Effective Sample Size (ESS) values for all the parameters were above 200, further indicating convergence. A total of 25% trees were discarded as burn-in. The tree representing the best evolutionary hypothesis was selected using a 50% majority consensus rule.

## Systematics

**Buthidae** C. L. Koch, 1837

***Isometrus*** Ehrenberg, 1828

(Figures 1–33, Tables 1–5)

*Isometrus* (selected references): Pocock, 1893: 297; Pocock, 1900: 44 (in part); Vachon, 1972: 169; Tikader & Bastawade, 1983: 254 (in part); Kovařík, 1994: 189 (in part); Fet & Lowe, 2000: 146 (in part; complete reference list until 1998); Kovařík, 2003: 1 (in part); Kovařík & Ojanguren, 2013: 180 (in part).

TYPE SPECIES. *Scorpio maculatus* DeGeer, 1778

***Isometrus longitelson* sp. n.**

(Figures 1–33, Tables 1, 2)

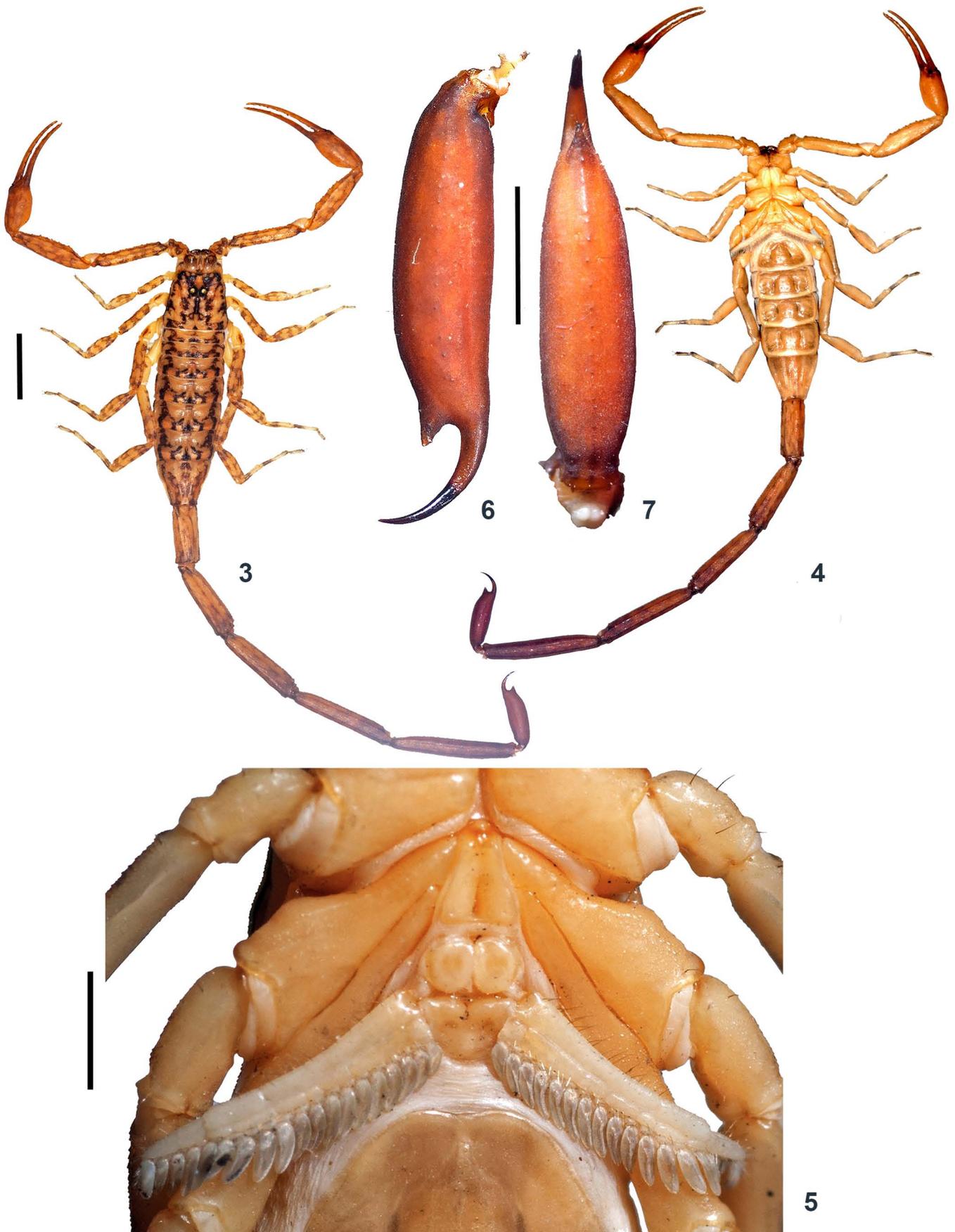
<http://zoobank.org/urn:lsid:zoobank.org:act:B6C82C3A-BF0E-4D96-BD65-782FC266DEDC>

TYPE LOCALITY AND TYPE REPOSITORY. India, Tamil Nadu State, Chengalpattu District, Keelavedu Village, Chengalpattu-Thiruporur Road, 12.68°N 80.01°E, 57 m a. s. l.; BNHS.

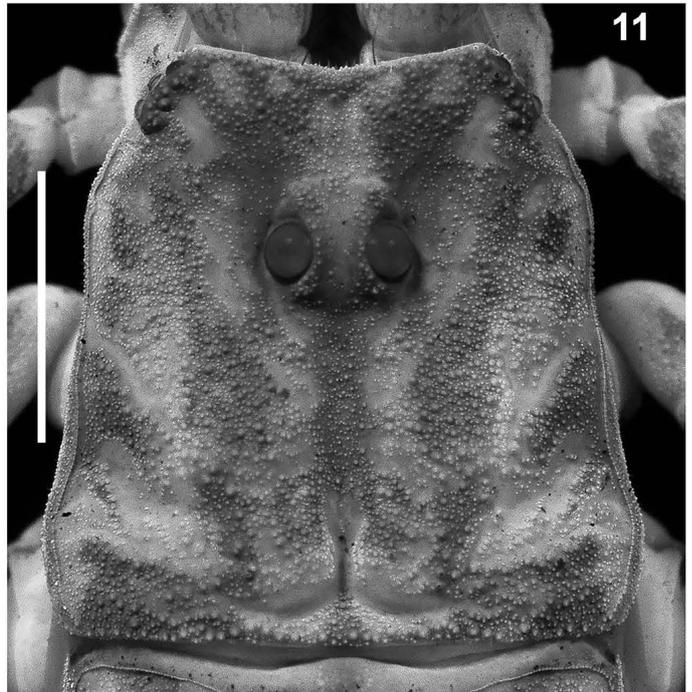
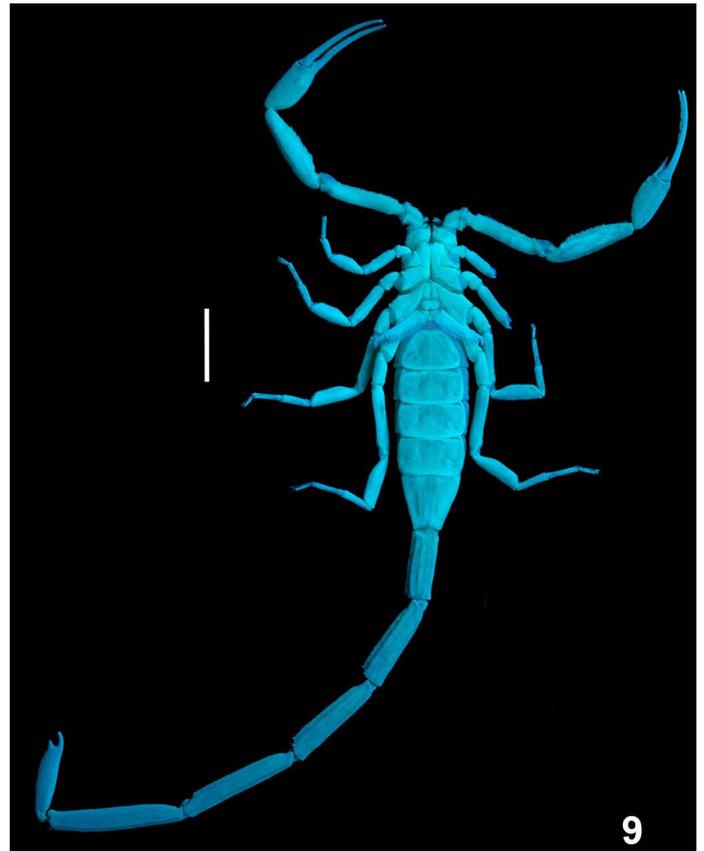
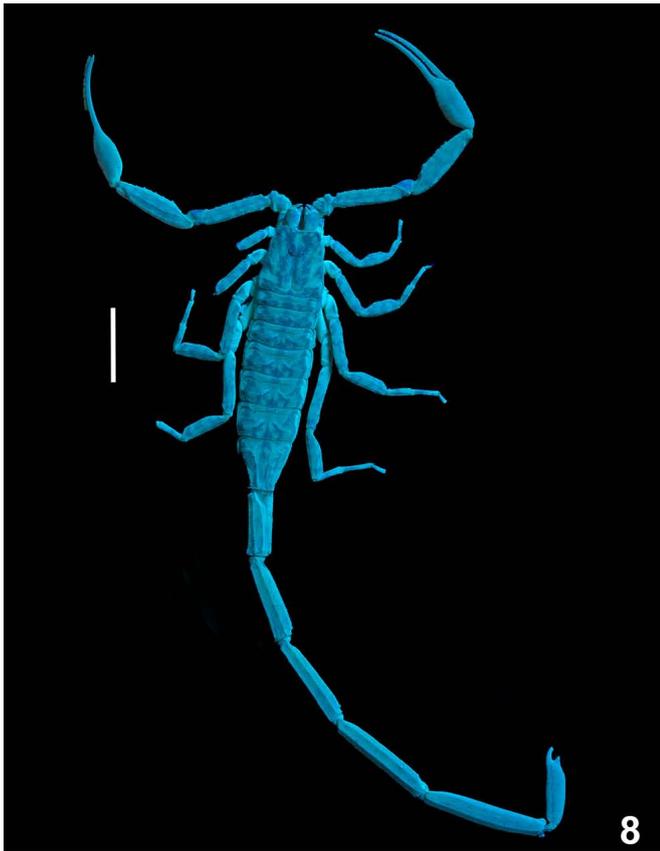
TYPE MATERIAL. India, Tamil Nadu State, Chengalpattu District, Keelavedu Village, Chengalpattu-Thiruporur Road, 12.68°N 80.01°E, 57 m a. s. l., leg. S. Datta, 27 September 2016, 1♂ (holotype, BNHS SC 287) 4♂ (paratypes, IES 368, 370, BNHS SC 288, 289) 4♀ (paratypes IES 366, 369, 372, BNHS SC 290).

ETYMOLOGY. The specific epithet is an adjective referring to the elongated telson in the males of the species

DIAGNOSIS. (♂♀) Total length 37.10–54.72 mm. Base colouration yellowish-brown and variegated with black-brown stripes and spots. Basal segments of chelicerae dorsally yellowish with blackish reticulation. Pectinal tooth number 15–18 in both sexes. Median supra-ocular area, finely granular. Median ocelli anteriorly situated in the ratio 1:2.2. Promedian carina on pedipalp patella strongly developed. Tergites I–VI finely granular with median carina more strongly developed on posterior side.



Figures 3–7. *Isometrus longitelson* sp. n., male, holotype, dorsal (3) and ventral (4) views, sternopectinal area (5), telson lateral (6) and ventral (7) views. Scale bars = 5 mm (3–4); 2 mm (5, 6–7).

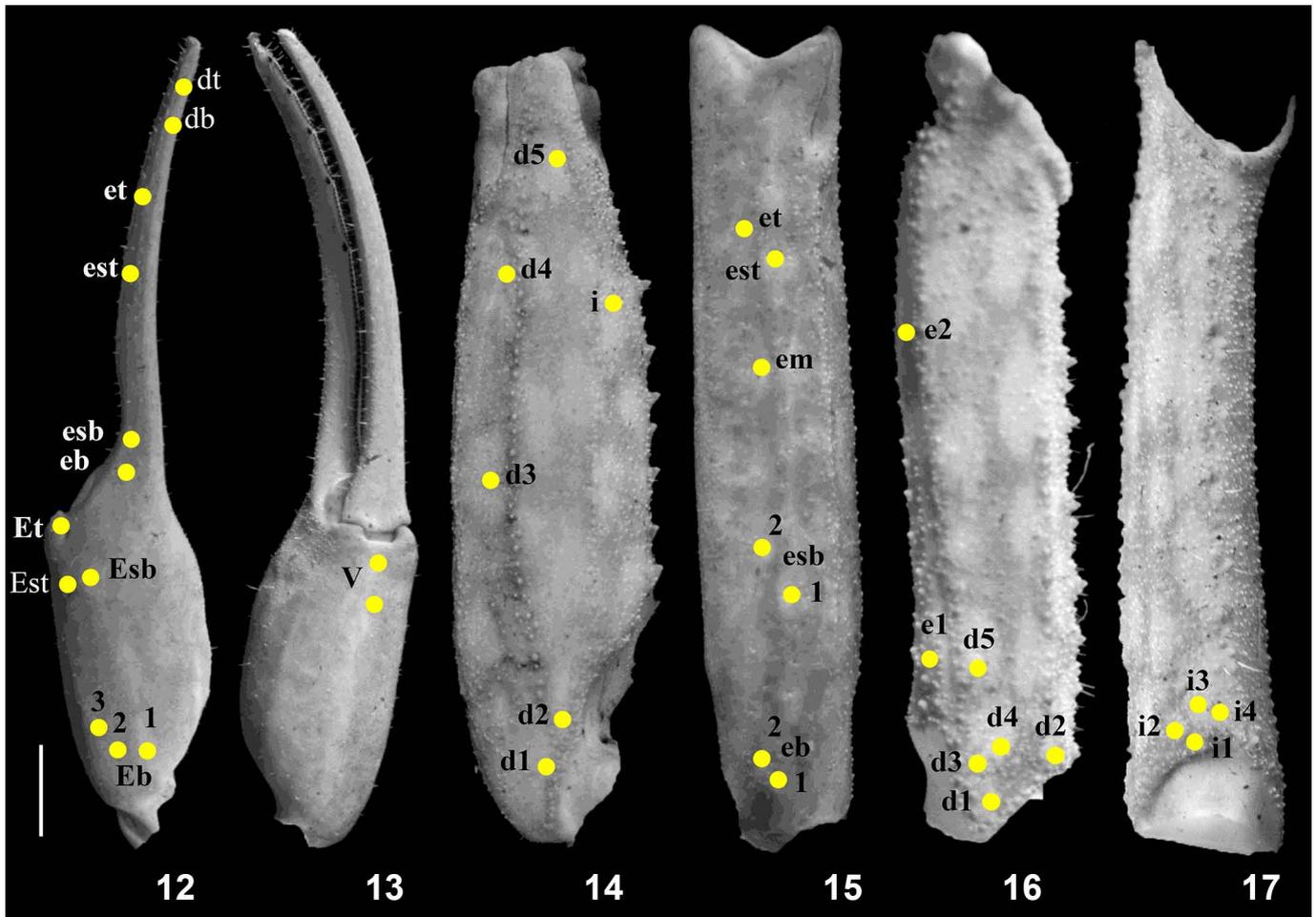


**Figures 8–11.** *Isometrus longitelson* sp. n., male, holotype, dorsal (8) and ventral (9) views under UV light, carapace under white light (10), carapace under UV light (11). Scale bars = 5 mm (8, 9); 2 mm (10, 11).

**DESCRIPTION** (♂ holotype; measurements in Table 1)

**Coloration** (Figs. 3–5, 10). Body yellowish brown and variegated with blackish brown stripes and spots; appendages yellowish with blackish brown stripes and spots; metasomal segment V dark brownish to blackish, darker on posterior portion; pedipalp

fingers yellowish with dark brown on one third proximal portion. Ventral portion uniformly yellow. Basal segments of chelicera yellow with blackish reticulation ending anteriorly into blackish transverse thin patch. Fingers of chelicera dark brown with tip of the fingers black. Telson dark brown in color.



**Figures 12–17.** *Isometrus longitelson* sp. n., male, holotype. Pedipalp chela dorsal (12) and ventral (13) views, patella dorsal (14) and external (15) views, femur dorsal (16) and internal (17) views. Trichobothrial pattern indicated by yellow circles. Scale bar = 1 mm.

**Carapace** (Figs. 10, 11). Surface densely granular with coarse and fine granules (mixed). Carapace without carinae. Median supra-ocular area, finely granular. Inter-ocular area with fine and dense granules. A pair of median eyes situated anteriorly in the ratio 1:2.2 (ratio of median eyes to anterior margin and median eyes to posterior margin). Antero-lateral ocular tubercle granular with type 5 lateral ocelli. Three pairs of large major ocelli and two small minor ocelli situated behind the major ocelli. Longitudinal furrow shallow anteriorly and deep posteriorly. Anterior margins finely granular. Lateral margins weakly crenulated below lateral ocelli. Posterior margin almost entirely smooth.

**Chelicerae.** Characteristic of the family Buthidae. Basal segments and movable fingers with tuft of short and firm setae on basal and ventral surfaces.

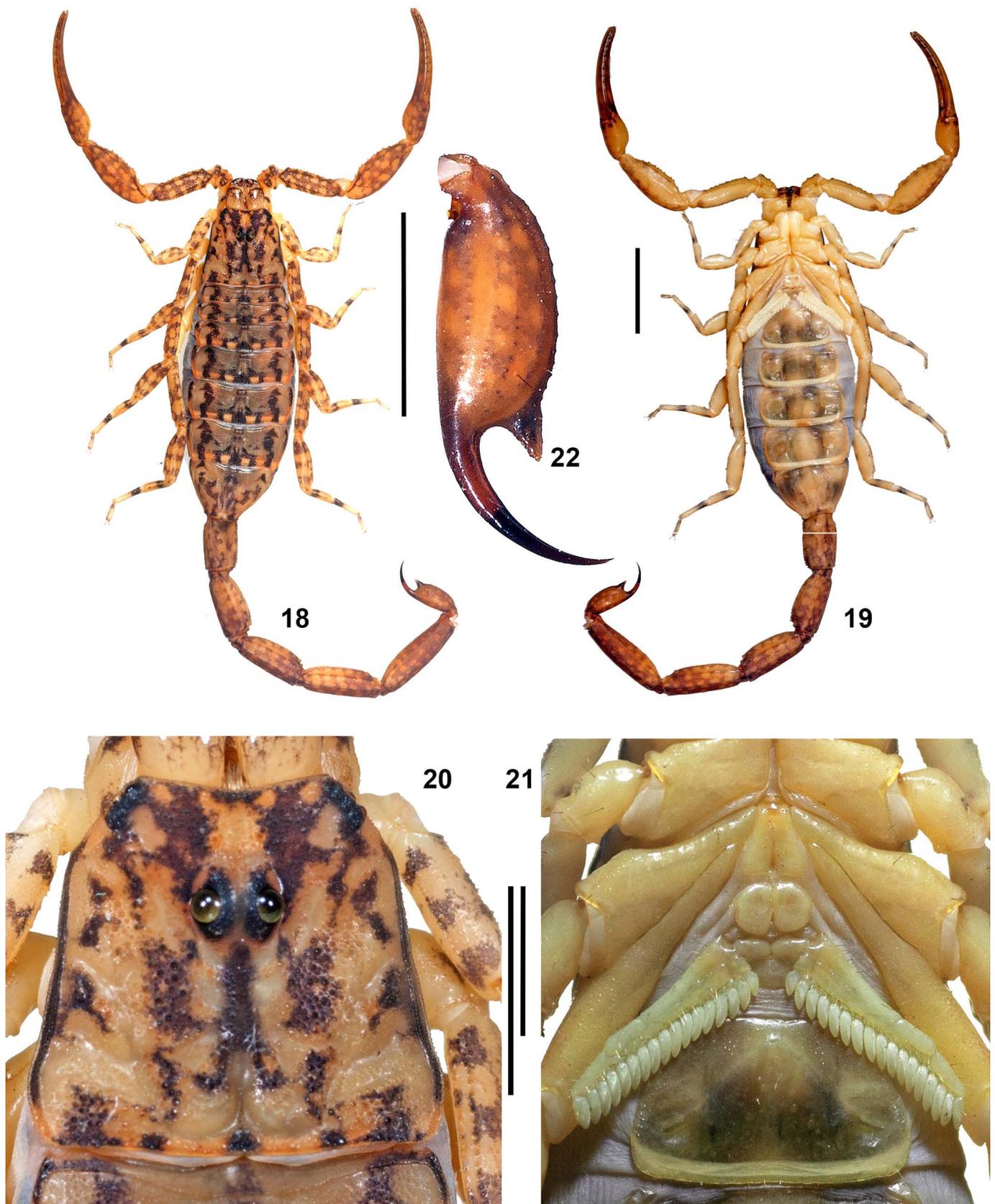
**Pedipalp** (Figs. 12–17). Femur with five carinae (prodorsal, retrodorsal, promedian, retromedian and proventral). All carinae crenulated. Intercarinal surfaces with mixed and sparse granulation except ventral surface smooth with few fine granules on proximal portions. Patella with seven distinct carinae (dorsomedian, prodorsal, retrodorsal, retromedian, retroventral, promedian and proventral). Intercarinal surfaces weakly granular on dorsal surface and smooth on

ventral surface. Manus with four carinae (dorsomedian and dorsoretrosulmedian accessory prominent; retromedian and retroventral obsolete). Fixed fingers with two smooth digital and inner secondary carinae. Movable and fixed fingers slightly scalloped with six rows of prolateral and retrolateral denticles in pairs and one additional single row of retrolateral denticles on proximal portion. Trichobothrial pattern of type A, typical for the genus (chela dorsal 12, chela ventral 2, patella dorsal 6, patella retrolateral 7, femur dorsal 7 and femur prolateral 4).

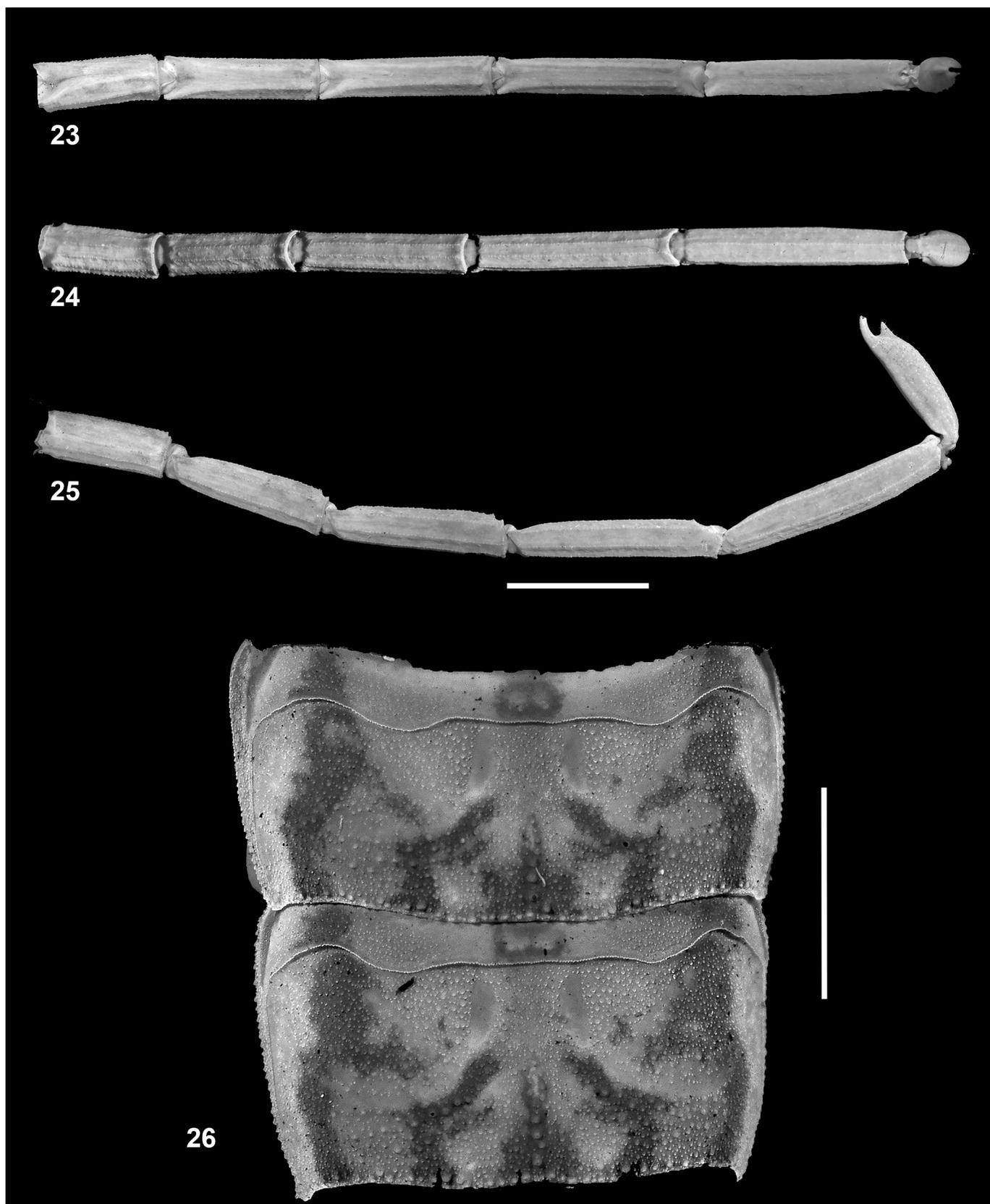
**Legs** (Figs. 3, 4, 8, 9). Femur and patellae carinated. All anterior carinae granular whereas all posterior carinae smooth. Tibia 3 and 4 without tibial spur. All legs with a pair of pedal spurs. Tarsomere covered with long delicate setae arranged in parallel rows on ventral side. Tarsomere I with a tuft of short, stout blackish setae on ventral side. Tarsomere II compressed laterally and ventrally with paired row of short, pointed, anteriorly directed, closely placed setae.

**Genital operculum** (Fig. 5). Wider than long, elliptical, separated with a pair of short male genital papillae.

**Pectines** (Fig. 5). Basal piece rectangular, notched on anterior median margin. Posterior margin of basal piece curved. Marginal lamella of 3/3 digits and median lamella of 6/7 digits, outer margin armed with a row of stout short red setae



Figures 18–22. *Isometrus longitelson* sp. n., female, paratype, BNHS SC 290, dorsal (18) and ventral (19) views carapace in dorsal view (20), sternoplectinal area (21), and telson in lateral view (22). Scale bars = 5 mm (18–19); 2 mm (20–22).



Figures 23–26. *Isometrus longitelson* sp. n., male, holotype, metasoma in dorsal (23), ventral (24) and lateral (25) views under UV light and mesosomal tergites V–VI of dorsal view under UV light (26). Scale bars = 5 mm (23–25); 2 mm (26).



Figures 27–29. The type locality of *Isometrus longitelson* sp. n., *Tamarindus* sp. on which specimens were observed (27), a hillock near the type locality (28), Chengalpattu-Thiruporur Road, Keelavedu village from where the type series was collected (29).

Primers- Cytochrome c Oxydase I	5' –3' Primer Sequence	Source
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
HCOoutout	GTAATATATGRTGDGCTC	Prendini et al. (2003)
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
Nancy	CCCGGTAAAATTAATAATAAACTTC	Simon et al. (1994)
Chelicerate F1	TACTCTACTAATCATAAAGACATTGG	Barrett & Hebert (2005)
Chelicerate R1	CCTCCTCTGAAGGGTCAAAAAATGA	Barrett & Hebert (2005)
Chelicerate R2	GGATGGCCAAAAATCAAATAAATG	Barrett & Hebert (2005)
Primers- 16S rRNA	5' –3' Primer Sequence	Source
16Sar	CGCCTGTTTATCAAAAACAT	Simon et al. (1994)
16Sbr	CTCCGGTTTGAAGTCAAGATCA	Giribet et al. (1996)

**Table 3.** Primers used for PCR amplification and sequencing of (COI and 16S) mitochondrial gene.

and few setae on surface. Fulcra 17/18, very small, roughly triangular, each armed with few short red setae, placed in between adjacent pectinal teeth. Teeth 17/18, strong and stout. **Mesosoma** (Figs. 3, 4, 8, 9, 26). Tergites I–VI finely granular with median carina more strongly developed on posterior side. Posterior and lateral margins granular. Coarse granulation on mesosomal tergites V and VI along the margins. Tergite VII granular, narrowed posteriorly, with two pairs of lateral granular carinae. Broad median carina present, more strongly developed on anterior portion. Sternites III–V almost entirely smooth, each with a pair of spiracles. Sternite VI finely granular on lateral portion. Sternite VII entirely granular, more closely granular on lateral portion; two pairs of granular carinae present on two third posterior portion.

**Metasoma** (Figs. 3, 4, 8, 9, 23–25). All segments longer than wide. Segment I with five pairs of granular carinae (dorsal lateral, lateral suprmedian, lateral infrmedian, ventral lateral and ventral submedian). Segments II–IV with four pairs of carinae (dorsal lateral, lateral suprmedian, ventral lateral and ventral submedian). Lateral suprmedian and ventral lateral carinae on segments II and III moderately granular. Intercarinal surfaces almost smooth. Dorsal lateral carina on segments I–IV ending in very weak tubercles. Segment V with five carinae (dorsal lateral, lateral suprmedian, ventral lateral pairs, ventral submedian and single ventral median). Dorsal lateral carina weakly developed. Intercarinal surfaces almost smooth. Anal rim weakly granular.

**Telson** (Figs. 2, 6–9, 25). Telson with slender and elongated vesicle, smooth on dorsal surface. Ventral median carina weakly granular, ending in triangular, subaculear, pointed nodule, armed with a pair of minute denticles on inner basal margin. Lateral and ventral intercarinal surfaces weakly granular. Aculeus elongated and moderately curved.

**DISTRIBUTION AND ECOLOGY.** *I. longitelson* **sp. n.** is only known from its type locality, Chengalpattu-Thiruporur Road, Keelavedu Village, Chengalpattu District, Tamil Nadu, India. All the specimens were collected from the tree bark of *Tamarindus indica* Linné, 1753 (Tamarind trees) adjacent to the road just after dusk at an elevation of 4–6 feet from

the ground. A population of the new species was observed on these trees along the entire road. Multiple individuals were observed on single trees. The new species was observed to be strictly arboreal living below the tree bark similar to all the other species of *Isometrus* found in India. The new species might be occurring in the forest on the hills surrounding the Chengalpattu-Thiruporur Road, however this needs to be confirmed. It is interesting to note that the new species was observed to be closely associated with the Tamarind trees present in the type locality. The precise origin of *Tamarindus indica* is still subject to controversy. It is claimed (Lefèvre 1971; Grollier et al., 1998; El-Siddig et al., 1999) that it originated in Africa and was only introduced into India; however, Wunderlin (1998) and Chauvin & Poupon (1983) assumed that its origin is in Asia, particularly in India, because of its appellation “Tamar hindi”, which means “Indian date”. This close association between the two species might support the later hypothesis; however this needs to be confirmed with biogeographic analysis (Figs. 27–29, 33).

**AFFINITIES.** *Isometrus longitelson* **sp. n.** differs from all other Indian species of *Isometrus* based on the following set of morphological characters:

1. Surface of carapace with mixed (coarse and fine) and dense granulation (Figs 10, 11) as opposed to coarse and dense granulation in *I. tamhini* and *I. wayanadensis*; coarse and sparse granulation with some areas without granules in *I. sankeriensis* and *I. thurstoni*; granular throughout with mixed granules, more densely granular in inter-ocular area and median posterior ocular area in *I. kovariki* and granular throughout but obsolete in *I. maculatus*.
2. Telson vesicle length to depth ratio in males 3.5–3.7 as opposed to 2.6–2.8 in *I. tamhini*; 2.3–2.6 in *I. amboli*; 2.4–2.5 in *I. sankeriensis*; 2.2–2.6 in *I. thurstoni*; 2.0–2.4 in *I. kovariki*; 2.1–2.2 in *I. nakshatra* and 2.3–2.4 in *I. wayanadensis* (Figs. 1, 6, 7–9).
3. Coarse granulation on mesosomal tergites V and VI along the margins (Fig. 26) as opposed to fine granulation along margins in *I. wayanadensis*, *I. nakshatra*, *I. sankeriensis*, *I. amboli*, *I. thurstoni* and *I. kovariki*.

Species	IM	IT	IA	IK	IS	ISP
<i>Isometrus maculatus</i> (IM)	(0)					
<i>Isometrus tamhini</i> (IT)	14.2–14.4	(0.0–0.6)				
<i>Isometrus amboli</i> (IA)	13.8–14.2	6.6–7.2	(0.0–0.8)			
<i>Isometrus kovariki</i> (IK)	16.5	9.7–9.9	10.1	(0)		
<i>Isometrus sankeriensis</i> (IS)	14.2–14.0	7.2–7.4	4.7–5.4	10.7–10.9	(0.0–0.4)	
<i>Isometrus</i> sp. (ISP)	13–14.0	6.8–7.4	3.8–5.4	10.6–12.2	5.1–6.2	(0.2–1.7)
<i>Isometrus thurstoni</i> (ITH)	13.8–14.2	11.1–12.0	12.6–13.2	10.1–10.5	13.6–14.0	12.3–13.4
<i>Isometrus wayanadensis</i> (IW)	13.8–14.0	10.9–11.3	11.1–11.8	11.8–12.0	10.5–10.9	9.1–10.3
<i>Isometrus nakshatra</i> (IN)	13.2–14.4	12.8–13.4	12.8–13.6	14.6–14.8	12.6–12.8	11.9–13.6
<i>Isometrus longitelson</i> sp. n. (IL)	13.8	13.2–13.4	14.0	15.7	14.6–14.8	14.3–14.8
<i>Lychas mucronatus</i> (LM)	14.4	16.7–17.1	16.3–17.1	18.4	16.9–17.1	16.3–16.5

Species	ITH	IW	IN	IL	LM
<i>Isometrus thurstoni</i> (ITH)	(0.2–0.4)				
<i>Isometrus wayanadensis</i> (IW)	12.0–12.4	(0.0–0.2)			
<i>Isometrus nakshatra</i> (IN)	14.2–14.8	13.2–14.2	(0–3.5)		
<i>Isometrus longitelson</i> sp. n. (IL)	14.8–15.1	13.8–14.0	12.6–13.6	(0)	
<i>Lychas mucronatus</i> (LM)	(17.5–17.9)	(14.6–14.8)	(15.7–16.5)	16.5	(0)

**Table 4.** Pairwise uncorrected raw distances (%) expressed as minimum-maximum based on COI gene sequences for Indian *Isometrus* species. Values in brackets are intra-clade distances.

Species	IM	IT	IA	IK	IS	ISP
<i>Isometrus maculatus</i> (IM)	(0.0)					
<i>Isometrus tamhini</i> (IT)	13.1	(0.0)				
<i>Isometrus amboli</i> (IA)	13.5	3.5	(0.0)			
<i>Isometrus kovariki</i> (IK)	13.3	10	10.2	(0.0)		
<i>Isometrus sankeriensis</i> (IS)	13.5	4.0	2.7	9.3	(0.0)	
<i>Isometrus</i> sp. (ISP)	13.9	5.3	3.1	10.2	4.2	(0.7)
<i>Isometrus thurstoni</i> (ITH)	11.9	8.8	9.1	7.1	10.2	10.6
<i>Isometrus wayanadensis</i> (IW)	13.1	10.0	9.5	8.0	10.4	10.0
<i>Isometrus nakshatra</i> (IN)	12.2–12.4	13.9	13.5	13.3	13.3	13.7–14.2
<i>Isometrus longitelson</i> sp. n. (IL)	14.2	13.7	14.4	13.1	13.3	14.4
<i>Lychas mucronatus</i> (LM)	21.5	21.5	21.5	19.7	20.6	21.0–21.2

Species	ITH	IW	IN	IL	LM
<i>Isometrus thurstoni</i> (ITH)	(0.0)				
<i>Isometrus wayanadensis</i> (IW)	6.9	(0.0)			
<i>Isometrus nakshatra</i> (IN)	12.2	12.8	(0.0)		
<i>Isometrus longitelson</i> sp. n. (IL)	13.7	14.4	12.2–12.8	(0.0)	
<i>Lychas mucronatus</i> (LM)	20.6	21.2	18.6	21.5	(0.0)

**Table 5.** Pairwise uncorrected raw distances (%) expressed as minimum-maximum based on 16S gene sequences for Indian *Isometrus* species. Values in brackets are intra-clade distances.

4. Chela length to width ratio in males 5.0–5.4 as opposed to 6.1–6.5 in *I. tamhini*, 5.7–5.8 in *I. sankeriensis* and 10.6 in *I. nakshatra* and in females 5.0–5.4 as opposed to 5.7 in *I. amboli*, 4.8 in *I. kovariki* and 6.1–6.2 in *I. nakshatra* (Table 1).  
 5. Metasomal length to carapace length ratio in males 8.2–8.9 as opposed to 5.9–6.1 in *I. sankeriensis*, 6.5–7.3 in *I. kovariki*, 5.3–5.8 in *I. nakshatra*, 6.8–7.2 in *I. wayanadensis* 9.6 in *I. maculatus* (Table 1).  
 6. Lateral suprmedian and ventral lateral carinae on metasomal segments II–IV moderately to weakly granular

(Fig. 23–25) as opposed to strongly granular in *I. tamhini* and *I. sankeriensis*.

7. Ventral median carina of telson vesicle weakly granular (Fig. 7) as opposed to moderately granular in *I. amboli*, *I. wayanadensis* and strongly granular in *I. tamhini*.

8. Spiniform granules of promedian carina of the pedipalp patella strongly developed (Fig. 14) as opposed to moderately developed in *I. tamhini*, *I. amboli*, *I. sankeriensis*, *I. kovariki* and weakly developed in *I. nakshatra*.

**Key to *Isometrus* species from India  
(excluding *I. maculatus*)**

1. Telson vesicle length to depth ratio in males 3.5–3.7 .....  
..... *I. longitelson* sp. n.
- Telson vesicle length to depth ratio in males 2.0–2.8 ..... 2
2. Ventral median carina on vesicle strongly granular .....  
..... *I. tamhini* Sulakhe et al., 2020
- Ventral median carina on vesicle moderately granular ... 3
- Ventral median carina on vesicle weakly granular ..... 4
3. Surface of carapace coarsely and densely granular .....  
..... *I. wayanadensis* Sulakhe et al., 2022
- Surface of carapace finely and densely granular .....  
..... *I. amboli* Sulakhe et al., 2020
4. Chela length to width ratio in males 10 .....  
..... *I. nakshatra* Sulakhe et al., 2022
- Chela length to width ratio in males 5.0–6.4 ..... 5
5. Lateral supramedian and ventral lateral carinae on  
metasomal segments II–IV strongly granular; metasomal  
length to carapace length ratio in males 5.9–6.1 .....  
..... *I. sankeriensis* Tikader & Bastawade, 1983
- Lateral supramedian and ventral lateral carinae on  
metasomal segments II–IV moderately to weakly granular;  
metasomal length to carapace length ratio in males 6.5–  
8.2 ..... 6
6. Surface of carapace coarsely and sparsely granular with  
some areas without granules; anterior margin of carapace  
sharply curved near lateral ocelli .....  
..... *I. thurstoni* Pocock, 1983
- Surface of carapace more closely granular in inter-ocular  
area and median posterior ocular area; anterior margin of  
carapace curved near lateral ocelli .....  
..... *I. kovariki* Sulakhe et al., 2020

**Molecular analysis**

*Molecular phylogenetic* (Figs. 31, 32) All known species of the genus *Isometrus* found in India are included in our phylogenetic analysis of 525 bp fragment of the COI and a 500 bp fragment of the 16S mitochondrial genes. Maximum Likelihood and Bayesian Inference analyses generated trees with different topologies, but the new species in both these analyses were recovered as monophyletic with high ultrafast bootstrap support in ML analysis (100) and high posterior probability values in the BI analysis (pp = 1). The new species *I. longitelson* sp. n. was recovered as sister to *I. nakshatra* in both ML and BI analyses. The phylogenetic position of *I. maculatus* from Sri Lanka is not completely understood as it recovered as sister to a clade comprising *I. nakshatra* and *I. longitelson* sp. n. in the ML analysis and was recovered as sister to a clade comprising all the Indian *Isometrus* in BI

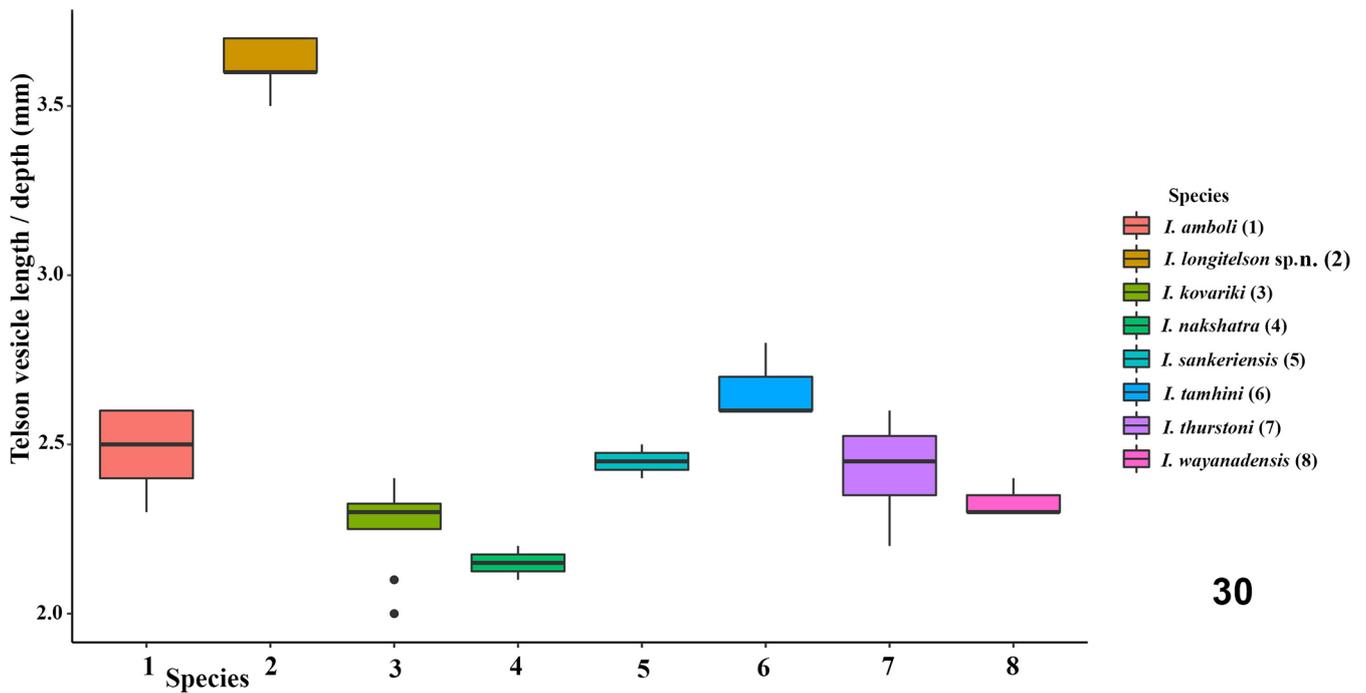
analysis. This might be due to incomplete taxon sampling and missing data. Nevertheless, incomplete taxon sampling or missing data may not affect the accuracy of the phylogenetic relationships in other complete taxa included in the analyses (Wiens. 2003).

*Genetic divergence (p-distance)* (Tables 4, 5) The new species *I. longitelson* sp. n. was well separated from all the congeners based on the 525 bp fragment of COI mitochondrial gene. It was recovered as nearest to *I. nakshatra* and separated with a minimum genetic divergence of 12.6%, but showed 13.2–15.7% divergence from all other Indian congeners. *I. longitelson* sp. n. also clearly separated from all the congeners based on 500 bp fragment of 16S mitochondrial gene. It was recovered as nearest to *I. nakshatra* and separated with a minimum genetic divergence of 12.2%, but showed 13.1–14.4% divergence from all other Indian congeners.

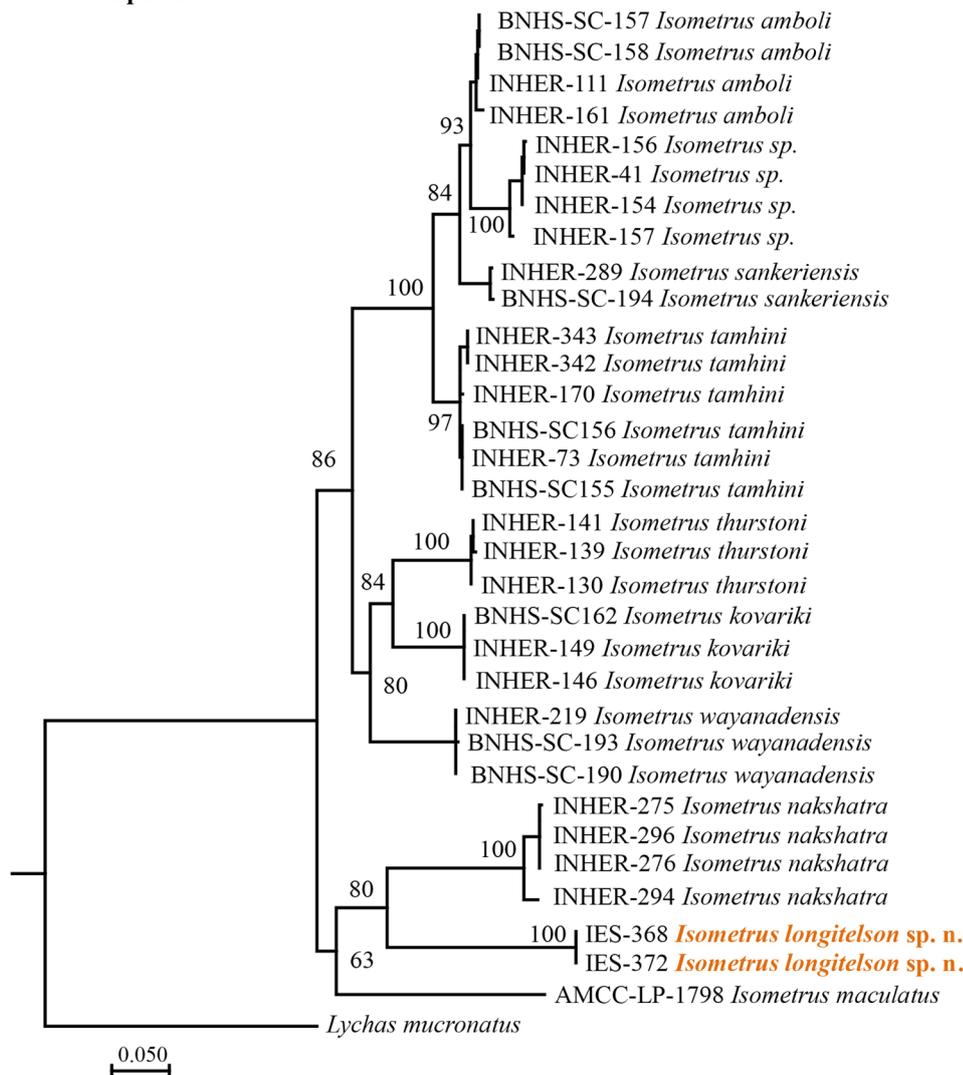
**Discussion**

The discovery of a new species of Indian *Isometrus* highlights the necessity for more extensive surveys across South Asia. Until early 2020, the genus *Isometrus* included only two species from India; however, there were 5 new species descriptions and one reinstatement within a span of two years (Sulakhe et al., 2020a, 2020b, 2022). Based on the multiple Indian localities of *I. maculatus* and *I. thurstoni* listed in Kovařík (2003), it was assumed that these lineages were widely distributed; however, based on the recent studies and new species descriptions where molecular data was integrated with morphological data, it is now evident that the diversity of Indian *Isometrus* was highly underestimated. It is most important to test the species boundaries of such species with wide distribution records so as to understand the ecology and evolutionary history of these lineages (Wiens, 2007). Robust delimitation tools with multilocus genetic data can greatly help in accurate estimation of the species diversity and thus identify conservation priorities (Welton et al., 2013).

After this new description the total number of *Isometrus* species in India now accounts to 9 and one novel lineage still awaits description from northern Karnataka. *Isometrus longitelson* sp. n. is the first *Isometrus* species from the southeastern plains of India and is distinct from all other *Isometrus* as it can be easily identified in the field due to its elongated telson vesicle. The presence of more of such morphologically distinct lineages in India cannot be denied. Southeastern peninsular India, especially across a longitudinal gradient from the eastern end of Western Ghats to the Coromandel Coast, is poorly studied in terms of biodiversity (Mani, 1974; Pitchairamu et al., 2008). There could be more novel species belonging to the clade of the new species from this region and should be investigated with extensive surveys. Such work will increase the species richness of this neglected region and will boost up the biodiversity value, which will finally lead to setting up priorities for conservation.



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Figures 30–31: **Figure 30.** Boxplots showing variations in ratios of telson length to depth in males for all *Isometrus* species from India except *I. maculatus* DeGeer, 1778. **Figure 31.** Maximum Likelihood phylogenetic tree (ML) for *Isometrus*. Values along the nodes are ultrafast bootstraps for 1000 iterations.

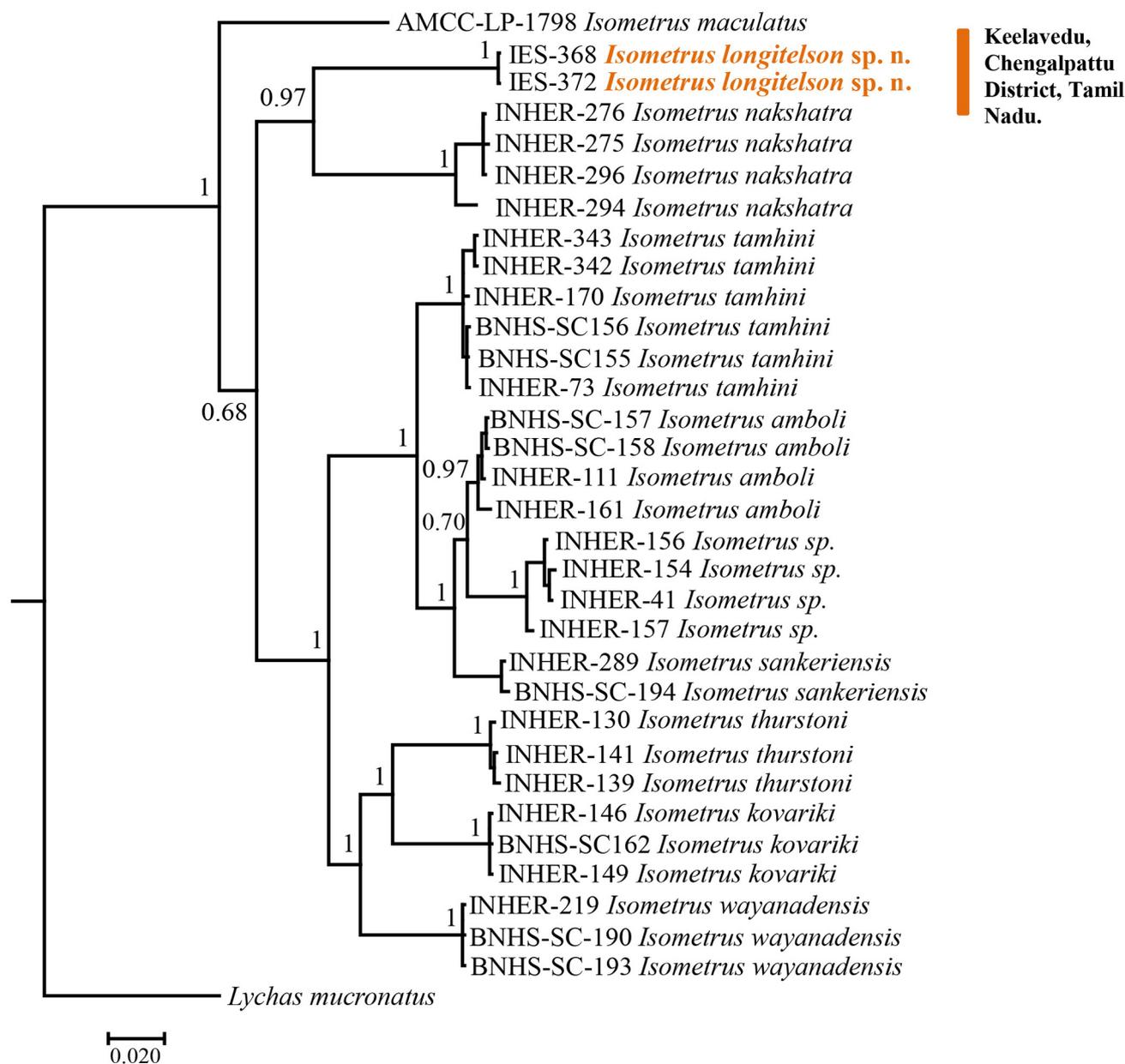


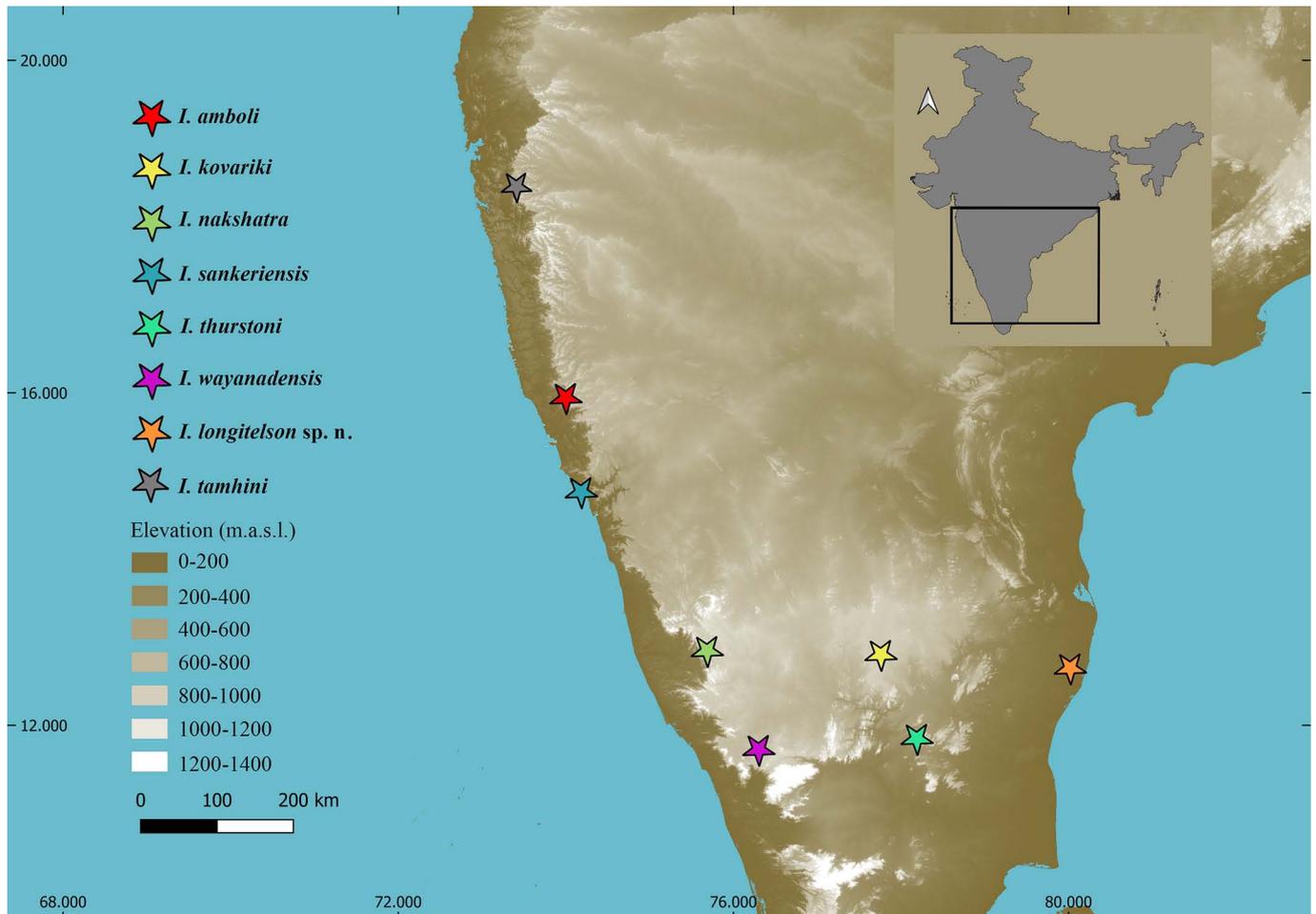
Figure 32. Bayesian phylogenetic tree for *Isometrus*. Values along the nodes are Bayesian posterior probabilities for Bayesian Inference.

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**Figure 33.** Distribution of Indian *Isometrus* species with elevation data. The stars represent the type localities.

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