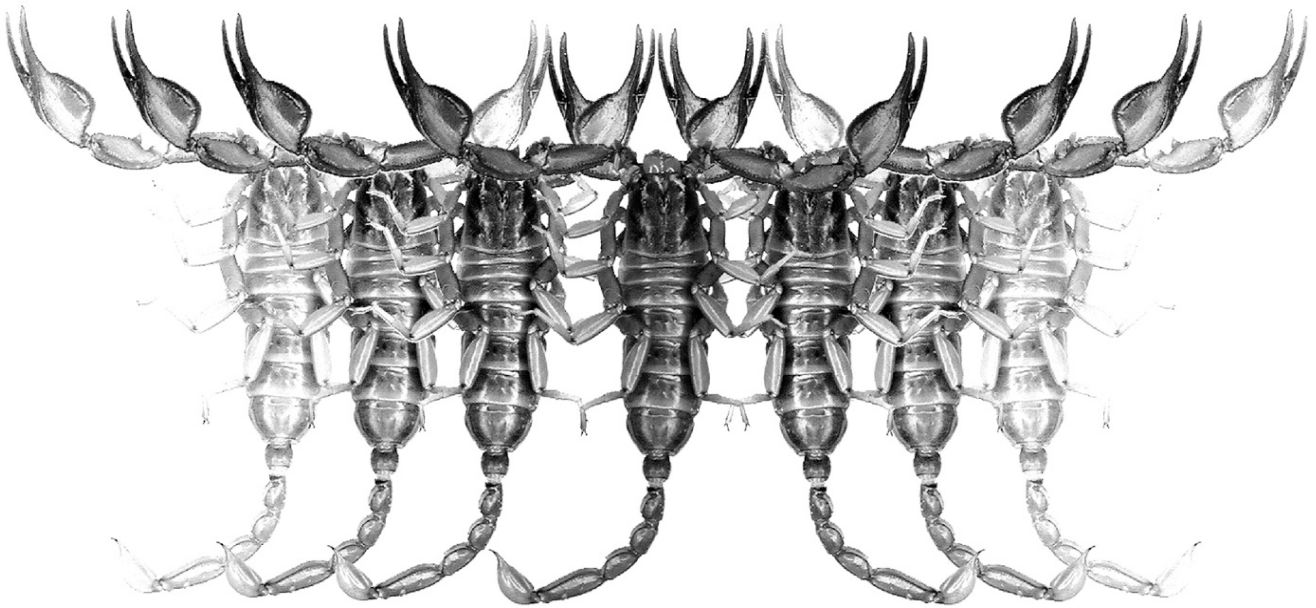


Euscorpius

Occasional Publications in Scorpiology



**Molecular and morphological confirmation of
Isometrus maculatus (DeGeer, 1778)
(Scorpiones: Buthidae)
from Northeast India and East Asia**

Deshpande, Joshi, Kawai, Deb, Lee, Bastawade, Gowande & Sulakhe

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Euscorpius

Occasional Publications in Scorpiology

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Molecular and morphological confirmation of *Isometrus maculatus* (DeGeer, 1778) (Scorpiones: Buthidae) from Northeast India and East Asia

Shubhankar Deshpande^{1,3}, Mihir Joshi^{1,4}, Kazusa Kawai⁵, Arnab Deb⁶, Jeng-Di Lee⁷,
Deshabhushan Bastawade¹, Gaurang Gowande^{1,2} & Shauri Sulakhe¹

¹ InSearch Environmental Solutions, Flat no 1, Omkar Apartments, Near Shivaji Statue, Sant Gangaram Road, Pune – 411038, Maharashtra, India.

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

³ Department of Environmental Science, Fergusson College, Pune, Maharashtra, 411004, India.

⁴ Department of Biotechnology, Modern College of Arts, Science and Commerce, Shivajinagar, Pune – 411005, Maharashtra, India.

⁵ The Japan Scorpion laboratory, 4-7, Motomachi, Gifu-shi, Gifu-ken, 500-8185, Japan.

⁶ Assam Down Town University, Panikhaiti, Guwahti – 781026, Assam, India.

⁷ No. 1, Jianye New Residential Quarter, Zuoying District, Kaohsiung City – 813003, Taiwan (R.O.C.)

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

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Summary

We present molecular data on *Isometrus maculatus* (DeGeer, 1778) for the first time from India, Japan, and Taiwan based on the COI gene and justify the cosmopolitan nature of this species. We also address the first population of *I. maculatus* found in Teliamura, Tripura, India and give its detailed description and comparison with the rest of the *Isometrus* species from India. Furthermore, we discuss the justification for considering Sri Lanka as the true home of *I. maculatus*.

Introduction

The genus *Isometrus* Ehrenberg, 1828 was described based on the type species *Isometrus maculatus* (DeGeer, 1778) from “Suriname and Pennsylvania”, which was later recorded from ca. 70 countries across the world. It was thereby considered as a cosmopolitan species (Fet & Lowe, 2000; Kovařík, 2003). Lourenco & Huber (2002) suggested that Sri Lanka was the original home of *I. maculatus* before becoming cosmopolitan due to human activities. Referring to the statement by Lourenco & Huber (2002), Veronika et al. (2013) cited the fact about *I. maculatus* being endemic to Sri Lanka. Kovařík et al. (2016) raised the possibility of *I. maculatus* being indigenous to India and Sri Lanka and its similarity to *Isometrus thurstoni* Pocock, 1893 and *I. thwaitesi* Pocock, 1897 based on morphology. However, Kovařík et al. (2016) warranted a need to undertake molecular studies to test the endemism of Sri Lankan *I. maculatus* and to assess the global distribution of the species.

The high species diversity of *Isometrus* in peninsular India was highlighted recently by our research group (Sulakhe et al. 2020a, 2020b; Deshpande et al., 2022; Sulakhe et al., 2022) using an integrated taxonomic approach. Yet, these studies did not confirm any population of *I. maculatus* in peninsular India. Recently, a few specimens were collected from Northeast India, Japan and Taiwan. In a continuous effort to understand the diversity and distribution of *Isometrus*, we assessed the distribution of *I. maculatus* based on molecular confirmation and detailed morphological comparison from various localities in Asia, contingent the newly collected material as well as available data. We also give the first morphological description of *I. maculatus* from Northeast India. Additionally, we compare *I. maculatus* populations from Northeast India, Japan and Taiwan with the rest of the *Isometrus* species from India and Sri Lanka. Morphological comparisons also included data on *I. maculatus* from Cuba published by Teruel (2009).



Figures 1–2. Figure 1. *Isometrus maculatus*, male, IES-522, from Teliamura, Tripura, India in vivo habitus. **Figure 2.** *Isometrus maculatus*, female, IES-525, from Teliamura, Tripura, India in vivo habitus with newborns. Photos taken by Arnab Deb.

Methods, Material and Abbreviations

Sampling

Scorpions were located with the help of ultraviolet light (uvBeast V3 385-395nm UV Torch) and collected. Photographs of specimens were taken using Nikon D500, 105mm F2.8 micro lens and RIC1 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 based on the methodologies followed by (Volschenk 2005). Measurements were taken (in mm) for 34 morphological characters (Tables 1, 2). Specimens collected and studied are deposited in InSearch Environmental Solutions (IES), Research Laboratory, Pune, Maharashtra, India.

Comparative material examined

Data used for comparison and diagnosis of *I. amboli*, *I. kovariki*, *I. longitelson*, *I. nakshatra*, *I. sankeriensis*, *I. thurstoni*, *I. tamhini*, and *I. wayanadensis* have been sourced from our previous publications (Sulakhe et al., 2020a, 2020b, 2022; Deshpande et al., 2022). The morphometric data of *I. maculatus* from Cuba have been sourced from Teruel (2009); however, morphometric data of *I. maculatus* from Sri Lanka were not included due to unavailability.

Molecular analysis

DNA extraction, amplification and sequencing

DNA extraction, amplification and sequencing protocols followed Sulakhe et al. (2020b). 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. 23, 24) 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) gene was 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 (0.2 µL), 2.5 µL of 10x buffer, 2 µl of dNTPs (2.5 mM each), 2 µl (5 mM) of each primer, 2 µl template DNA, and 14.3 µl of water, and reactions were carried out with a 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. 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 as per Table 4.

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 Deshpande et al. (2022). The final COI alignment contained 36 sequences each 525 bp in length, including a sequences of *I. maculatus* (Sri Lanka) from Esposito et al. (2018) and *I. thwaitesi* from Štundlová et al. (2022). Sequences of *Reddyanus basilicus* (Karsch, 1879) from Štundlová et al. (2022), and of *R. brachycentrus* (Pocock, 1899) generated in this study were used as outgroups to root the phylogenetic tree.

Genetic divergence (p-distance)

The *p-distances* were calculated for the mitochondrial COI 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 analysis. Missing data were partially deleted and the site cutoff was set as 95%. Finally, all three codon position sites were selected (the *p-distances* are mentioned in Appendix 1).

Molecular phylogenetics analysis

Maximum Likelihood (ML) and Bayesian Inference (BI) methods of phylogenetic analyses were implemented. The COI region was partitioned per codon position 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 TIM2+F+G4 models of sequence evolution for all the positions. The model of sequence evolution was determined 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+G for codon position 1, GTR+G for codon position 2, and F81+I for codon position 3. For the BI analysis, two simultaneous, independent analyses were run starting from different random trees. Three heated and one cold chain were used in the analysis. Markov chains were sampled every 200 generations for 10 million generations. At

Dimensions (mm)		<i>Isometrus maculatus</i>		
		♂ (Tripura, India) IES 521	♂ (Tripura, India) IES 522	♂ (Tripura, India) IES 523
Carapace	L / W	4.23 / 3.84	4.89 / 4.25	4.42 / 4.25
Mesosoma	L	11.77	13.24	12.02
Tergite VII	L / W	3.57 / 3.42	3.92 / 3.79	3.63 / 3.44
Metasoma and telson	L	31.75	37.44	32.81
Segment I	L / W	3.78 / 1.79	4.22 / 1.72	3.83 / 1.36
Segment II	L / W	4.76 / 1.51	6.02 / 1.52	4.81 / 1.25
Segment III	L / W	5.09 / 1.32	6.57 / 1.33	4.98 / 1.2
Segment IV	L / W	6.14 / 1.19	6.62 / 1.28	6.3 / 1.09
Segment V	L / W	6.86 / 1.04	8.2 / 1.28	7.69 / 1.01
Telson	L / W / D	5.12 / 1.14 / 1.4	5.81 / 1.45 / 1.49	5.21 / 1.2 / 1.42
Pedipalp	L	21.73	27.1	24.41
Femur	L / W	6.01 / 0.94	7.84 / 1.1	6.97 / 1.05
Patella	L / W	6.48 / 1.12	8.29 / 1.27	7.61 / 1.17
Chela	L	9.24	10.97	9.83
Manus	W / D	1.23 / 1.08	1.4 / 1.18	1.24 / 1.07
Movable finger	L	5.65	5.97	*
Pectine	L / W	3.41 / 0.73	3.83 / 0.83	3.4 / 0.7
Genital Operculum	L / W	0.61 / 1.03	0.71 / 1.23	0.67 / 1.22
Total	L	47.75	55.57	49.25
Pectinal teeth count	PTC	18 / 18	18 / 18	19 / 19

Dimensions (mm)		<i>Isometrus maculatus</i>		
		♀ (Tripura, India) IES 524	♀ (Tripura, India) IES 525	♀ (Tripura, India) IES 526
Carapace	L / W	4.89 / 4.76	5 / 4.9	4.66 / 4.41
Mesosoma	L	12.78	11.38	13.44
Tergite VII	L / W	3.63 / 4.85	3.06 / 5.1	3.35 / 4.7
Metasoma and telson	L	27.1	26.69	24.79
Segment I	L / W	3.17 / 2.2	3.24 / 2.15	2.94 / 1.93
Segment II	L / W	3.91 / 2.07	3.96 / 2.02	3.47 / 1.85
Segment III	L / W	4.29 / 1.82	4.09 / 1.89	3.9 / 1.72
Segment IV	L / W	5.19 / 1.65	4.91 / 1.78	4.15 / 1.53
Segment V	L / W	5.72 / 1.53	5.53 / 1.48	5.58 / 1.3
Telson	L / W / D	4.81 / 1.3 / 1.49	4.96 / 1.21 / 1.5	4.75 / 1.16 / 1.43
Pedipalp	L	19.95	20.45	18.77
Femur	L / W	5.31 / 1.29	5.38 / 1.31	4.99 / 1.27
Patella	L / W	5.94 / 1.7	5.99 / 1.77	5.55 / 1.58
Chela	L	8.7	9.08	8.23
Manus	W / D	1.56 / 1.4	1.66 / 1.43	1.37 / 1.4
Movable finger	L	5.44	5.07	5.69
Pectine	L / W	3.44 / 0.75	3.37 / 0.81	3.12 / 0.7
Genital Operculum	L / W	0.71 / 1.24	0.63 / 1.37	0.69 / 1.29
Total	L	44.77	43.07	42.89
Pectinal teeth count	PTC	17 / 17	18 / 17	17 / 18

Table 1. Morphometric data for *Isometrus maculatus* from Tripura, India. Abbreviations: length (L), width (W), in carapace it corresponds to median width, depth (D). * Movable finger length of IES-523 could not be recorded due to damaged specimen.

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 0.002, 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.

Results

Systematics

Family Buthidae C. L. Koch, 1837

Genus *Isometrus* Ehrenberg, 1828

(Figures 1–25, Tables 1–2)

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 maculatus (DeGeer, 1778)

(Figures 1–25, Tables 1–2)

<http://zoobank.org/urn:lsid:zoobank.org:act:897D4BDF-4DD1-47BC-A0B4-004EE28B1745>

MATERIAL EXAMINED. **India**, Tripura, Teliamura, 23.84°N 91.65°E, 70 m a. s. l., leg. Arnab Deb, 9 November 2022, 3♂ (IES 521, IES 522, IES 523) 3♀ (IES 524, IES 525, IES 526). **Japan**, Haterumajima Island, Okinawa Prefecture, 24.06°N 123.75°E, 14 m a. s. l., leg. Kazusa Kawai, 13 October 2019, 1♀ (IES 585); Shimojishima Island, Okinawa Prefecture, 24.83°N 125.14°E, 10 m a. s. l., leg. Kazusa Kawai, 30 March 2020, 3♀ (IES 582, IES 583, IES 584); Kuroshima Island, Okinawa Prefecture, 24.24°N 124.01°E, 13 m a. s. l., leg. Kazusa Kawai, 21 February 2021, 1♂ (IES 541) 1♀ (IES 542). **Taiwan**, Cijin Island, Kaohsiung City, 22.61°N 120.26°E, 6 m a. s. l., leg. Jeng-Di Lee, 3 March 2023, 2♀ (IES 580, IES 581).

DIAGNOSIS. (♂♀) Total length 29.68–55.57 mm. Base colouration yellowish brown and variegated with yellowish brown stripes and spots; appendages yellowish with blackish yellow stripes and spots. Basal segments of chelicera yellowish with black reticulation ending anteriorly into blackish transverse patch. Pectinal teeth number 17–20 in both sexes. Median supra-ocular area finely granular. Median ocelli anteriorly situated in the ratio 1:2.0. Promedian carina on pedipalp patella weakly developed. Tergites I–VI with fine granulation, with median carina stronger on posterior region.

Colouration (Figs. 3–4): Body yellowish brown and variegated with blackish brown stripes and spots; appendages yellowish

with blackish brown stripes and spots; dark brownish to blackish last metasomal segment, darker on posterior portion; pedipalp fingers dark blackish to brown with yellow on one third distal portion. Ventral portion yellow anteriorly and light brown posteriorly. Basal segments of chelicera yellowish with black reticulation ending anteriorly into blackish transverse patch. Fingers of chelicera brownish black with tip of fingers black. Telson yellowish brown in color.

Carapace (Fig. 10, 11): Surface of carapace with mixed (coarse and fine) and dense granulation. 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 1:2.0 (Ratio of median eyes to anterior margin and median eyes to posterior margin). Anterio-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 finely 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. 3–4, 8–9, 12–17): Femur with five carinae (prodorsal, retrodorsal, promedian, retromedian and proventral). All carinae crenulated. Intercarinal surfaces with coarse and sparse granulation except ventral surface smooth with few fine granules on proximal portions. Patella with seven distinct carinae (dorsomedian, prodorsal, retrodorsal, promedian, retromedian, proventral, retroventral). Promedian carina moderately developed. Intercarinal surfaces weakly and sparsely granular on dorsal surface and smooth on ventral surface. Chela smooth. Movable and fixed fingers with six rows of prolateral and retrolateral denticles in pairs and one additional single row of retrolateral denticles on proximal portion. Fingers slightly scalloped at the base. 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. 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 (basitarsus) with a tuft of short, stout blackish setae on ventral side and a small bulge situated laterally on the proximal portion. Tarsomere II (telotarsus) 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 7/6 digits, outer margin armed with a row of stout short red setae and few setae on surface. Fulcra 17/17, very small, roughly triangular, each armed with few short red setae, placed in between adjacent pectinal teeth. Teeth 18/18, strong and stout.

Dimensions (mm)		<i>Isometrus maculatus</i>			
		♂ (Kuroshima Island, Japan) IES 541	♀ (Kuroshima Island, Japan) IES 542	♀ (Cijin Island, Taiwan) IES 580	♀ (Cijin Island, Taiwan) IES 581
Carapace	L / W	5.23 / 4.38	4.83 / 3.93	4.08 / 3.69	4.76 / 3.99
Mesosoma	L	10.76	14.28	14.53	13.05
Tergite VII	L / W	3.42 / 3.73	2.66 / 4.54	3.02 / 3.82	3.11 / 1.15
Metasoma and telson	L	38.87	22.99	24.11	24.32
Segment I	L / W	4.57 / 2.18	3.17 / 2.29	2.45 / 1.96	2.84 / 2.09
Segment II	L / W	6.0 / 1.86	3.28 / 1.77	3.43 / 1.75	3.61 / 1.87
Segment III	L / W	6.17 / 1.33	3.59 / 1.68	3.51 / 1.63	3.85 / 1.62
Segment IV	L / W	7.23 / 1.28	4.18 / 1.48	4.57 / 1.26	4.41 / 1.56
Segment V	L / W	8.87 / 1.16	4.5 / 1.28	5.57 / 1.1	4.89 / 1.05
Telson	L / W / D	6.03 / 1.45 / 1.71	4.27 / 1.14 / 1.21	4.58 / 1.11 / 1.29	4.72 / 1.13 / 1.3
Pedipalp	L	29.2	19.28	17.98	19.19
Femur	L / W	8.7 / 1.23	5.14 / 1.21	5.08 / 1.08	5.22 / 1.19
Patella	L / W	8.97 / 1.52	5.66 / 1.76	5.21 / 1.48	5.58 / 1.64
Chela	L	11.53	8.48	7.69	8.39
Manus	W / D	1.22 / 1.33	1.19 / 1.34	1.4 / 1.3	1.39 / 1.44
Movable finger	L	5.41	5.21	5.63	5.94
Pectine	L / W	4.09 / 0.78	- / 0.51	3.03 / 0.6	3.24 / 0.68
Genital operculum	L / W	0.7 / 1.18	0.63 / 1.21	0.41 / 1.13	0.5 / 1.17
Total	L	54.77	42.1	42.72	42.13
Pectinal teeth count	PTC	17 / 18	-	17 / 17	18 / 18

Dimensions (mm)		<i>Isometrus maculatus</i>			
		♀ (Shimojishima Island, Japan) IES 582	♀ (Shimojishima Island, Japan) IES 583	♀ (Shimojishima Island, Japan) IES 584	♀ (Haterumajima Island, Japan) IES 585
Carapace	L / W	4.88 / 4.3	3.51 / 2.92	4.62 / 3.81	4.78 / 3.9
Mesosoma	L	11.16	6.85	10.53	10.74
Tergite VII	L / W	3.02 / 3.07	1.96 / 2.26	2.68 / 3.77	2.96 / 3.39
Metasoma and telson	L	24.23	19.32	22.6	23.8
Segment I	L / W	2.37 / 2.16	2.34 / 1.47	2.49 / 2.05	2.91 / 2.13
Segment II	L / W	3.8 / 1.73	2.94 / 1.32	3.35 / 1.83	3.47 / 1.91
Segment III	L / W	4.0 / 1.45	3.23 / 1.23	3.57 / 1.76	4.01 / 1.77
Segment IV	L / W	4.4 / 1.42	3.43 / 1.08	4.17 / 1.5	4.3 / 1.1
Segment V	L / W	5.01 / 1.4	3.72 / 0.98	4.35 / 1.4	4.71 / 0.97
Telson	L / W / D	4.65 / 1.27 / 1.46	3.36 / 0.93 / 1.02	4.67 / 1.16 / 1.35	4.4 / 1.36 / 1.39
Pedipalp	L	18.61	15.01	18.04	19.07
Femur	L / W	4.79 / 1.3	4.2 / 0.83	4.81 / 1.25	5.15 / 1.24
Patella	L / W	5.02 / 1.67	4.37 / 1.12	5.3 / 1.63	5.54 / 1.66
Chela	L	8.8	6.44	7.93	8.38
Manus	W / D	1.44 / 1.34	0.95 / 0.92	1.29 / 1.39	1.42 / 1.4
Movable finger	L	5.91	4.21	4.86	5.77
Pectine	L / W	3.27 / 0.75	2.53 / 0.57	2.99 / 0.71	3.37 / 0.7
Genital operculum	L / W	0.68 / 1.39	0.5 / 0.88	0.53 / 1.33	0.58 / 1.26
Total	L	40.27	29.68	37.75	39.32
Pectinal teeth count	PTC	- / 18	19 / 20	19 / 18	17 / 17

Table 2. Morphometric data for *Isometrus maculatus* from Japan and Taiwan. Abbreviations: length (L), width (W), in carapace it corresponds to median width, depth (D). *Note: First two segments of mesosoma broken in IES-580.

Primers- Cytochrome c Oxidase I	5' -3' Primer Sequence	Source
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
HCOoutout	GTAAATATATGRTGDGCTC	Prendini et al. (2003)
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
Nancy	CCCGGTAAAATTTAAAATATAAACTTC	Simon et al. (1994)
Chelicerate F1	TACTCTACTAATCATAAAGACATTGG	Barrett & Hebert (2005)
Chelicerate R1	CCTCCTCCTGAAGGGTCAAAAAATGA	Barrett & Hebert (2005)
Chelicerate R2	GGATGGCCAAAAAATCAAATAAATG	Barrett & Hebert (2005)

Table 3. Primers used for PCR amplification and sequencing of COI mitochondrial gene.

Mesosoma (Figs. 3–4, 8–9): Tergites I–VI with fine granulation. Median carina more strongly developed on posterior side. Posterior and lateral margins granular. Mesosomal tergites V and VI with mixed granulation along margins. Tergite VII granular, narrowed posteriorly, with two pairs of lateral granular carinae. Weak 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 granular on lateral portion; two pairs of granular carinae present on two third portion.

Metasoma (Figs. 3–4, 8–9, 18–20): All segments longer than wide. Segment I with five pairs of granular carinae (dorsal lateral, lateral suprmedian, lateral inframedian, ventral lateral and ventral submedian). Intercarinal surfaces on segments I–IV finely and sparsely granular. Segments II–IV with four pairs of carinae (dorsal lateral, lateral suprmedian, ventral lateral and ventral submedian). Lateral suprmedian carina and ventral lateral carinae strongly granular on segments II–IV. Dorsal lateral carina on segments I–IV ending in weak tubercles. Intercarinal surfaces almost smooth. Dorsal lateral carina on segments I–IV ending in weak tubercles. Segment V with five carinae (lateral suprmedian and ventral lateral pairs and single ventral median). Intercarinal surfaces coarsely to weakly granular; more granular on posterior one third portion. Anal rim weakly granular.

Telson (Figs. 6–9, 18–20): Telson with slender vesicle. 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.

AFFINITIES. *Isometrus maculatus* differs from all the Indian species of *Isometrus* based on the following set of morphological characters:

a. 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 interocular area and median posterior ocular area in *I. kovariki*.

b. Spiniform granules of promedian carina of pedipalp patella weakly developed (Fig. 14) as opposed to moderately developed in *I. tamhini*, *I. amboli*, *I. sankeriensis* and *I. kovariki*; strongly developed in *I. thurstoni*, *I. wayanadensis*, *I. longitelson*.

c. Mixed granulation on mesosomal tergites V and VI along the margins as opposed to coarse granulation in *I. tamhini*, and *I. longitelson*; fine granulation along margins in *I. wayanadensis*, *I. nakshatra*, *I. sankeriensis*, *I. amboli*, *I. thurstoni* and *I. kovariki*.

d. Ventral median carina of telson vesicle weakly granular (Fig. 6) as opposed to moderately granular in *I. amboli*, *I. wayanadensis*; strongly granular in *I. tamhini*.

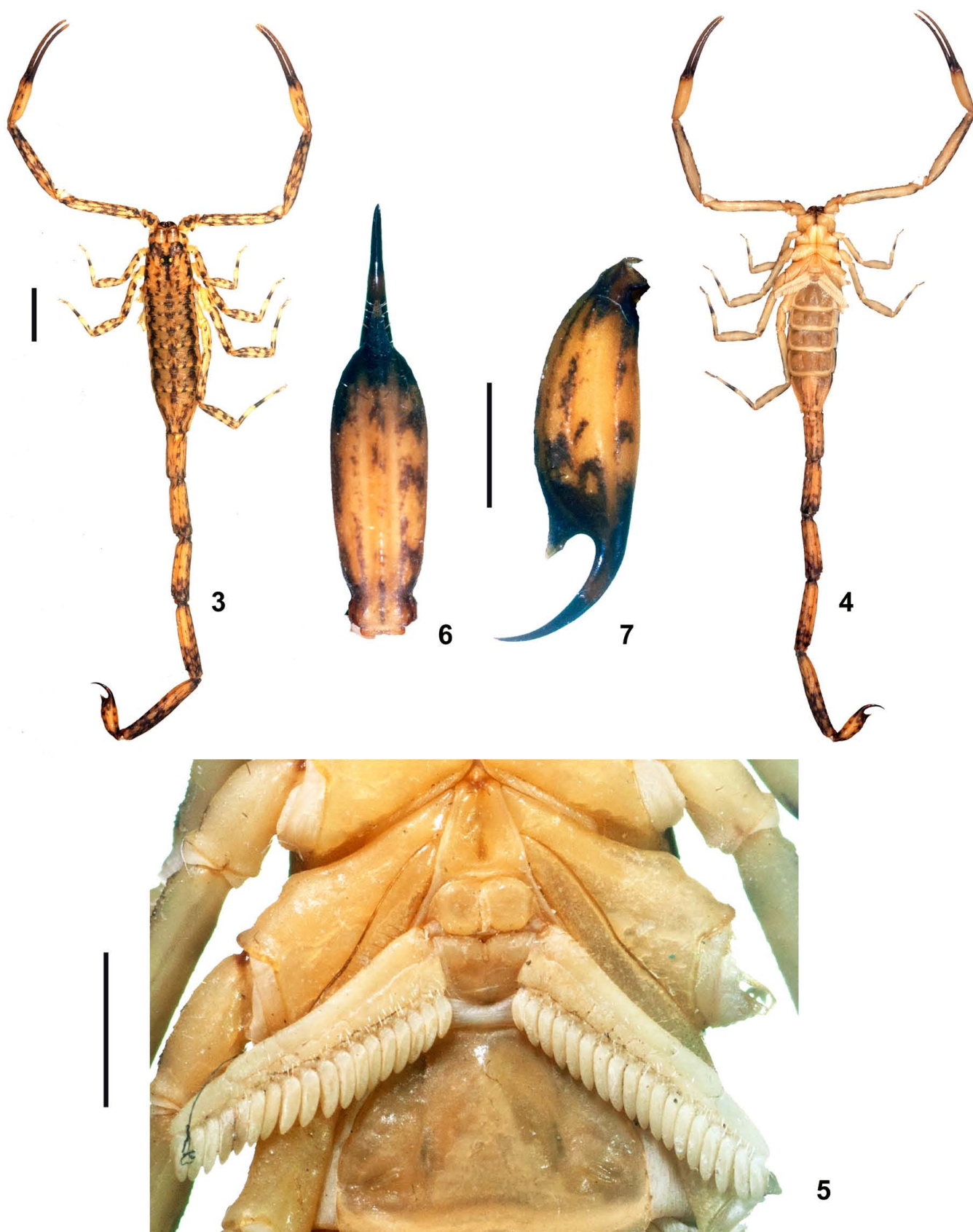
e. Chela length to width ratio in males 7.5–10.4 as opposed to 6.1–6.5 in *I. tamhini*, 5.3–5.9 in *I. amboli*; 5.7–5.8 in *I. sankeriensis*, 5.1–6.4 in *I. kovariki*, 5.0–5.2 in *I. thurstoni*, 5.0–5.3 in *I. wayanadensis*, 10.6 in *I. nakshatra* and 5.0–5.4 in *I. longitelson*; in females 5.6–6.3 as opposed to 4.8 in *I. kovariki*; 4.8–5.5 in *I. thurstoni*; 4.8–5.0 in *I. wayanadensis*; 5.0–5.4 in *I. longitelson* (Table 1, 2).

f. Metasomal length to carapace length ratio 7.4–8.2 in males as opposed to 8.8–9.1 in *I. tamhini*; 6.5–7.3 in *I. kovariki*, 5.3–5.8 in *I. nakshatra*, 6.8–7.2 in *I. wayanadensis* and 8.2–8.9 in *I. longitelson* (Table 1, 2).

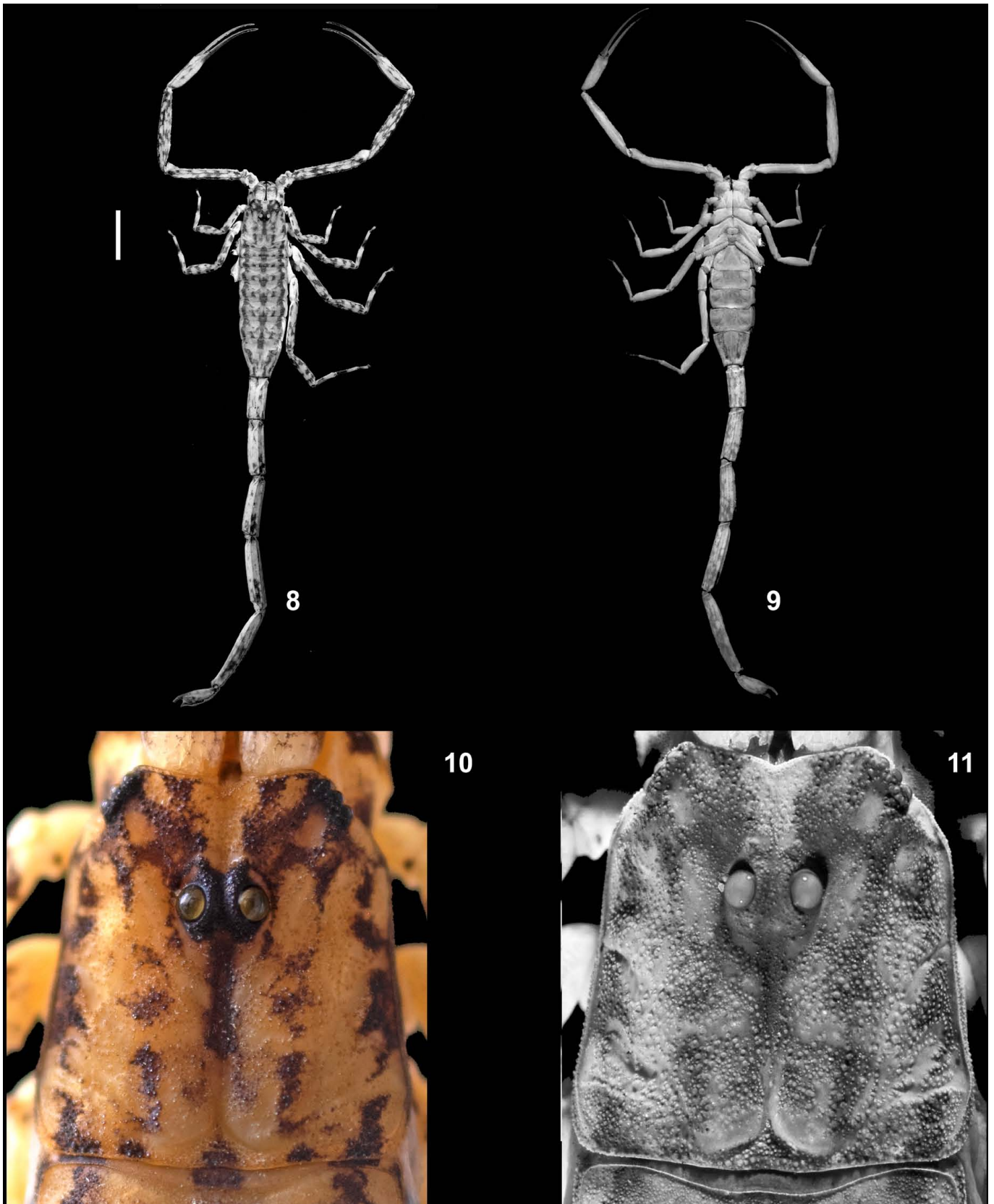
g. Telson vesicle length to depth ratio in males 2.1–2.8 (Figs. 6, 7, 18–20) as opposed to 2.0–2.4 in *I. kovariki*, 2.3–2.4 in *I. wayanadensis* and 3.5–3.7 in *I. longitelson* (Table 1, 2).

Molecular analysis

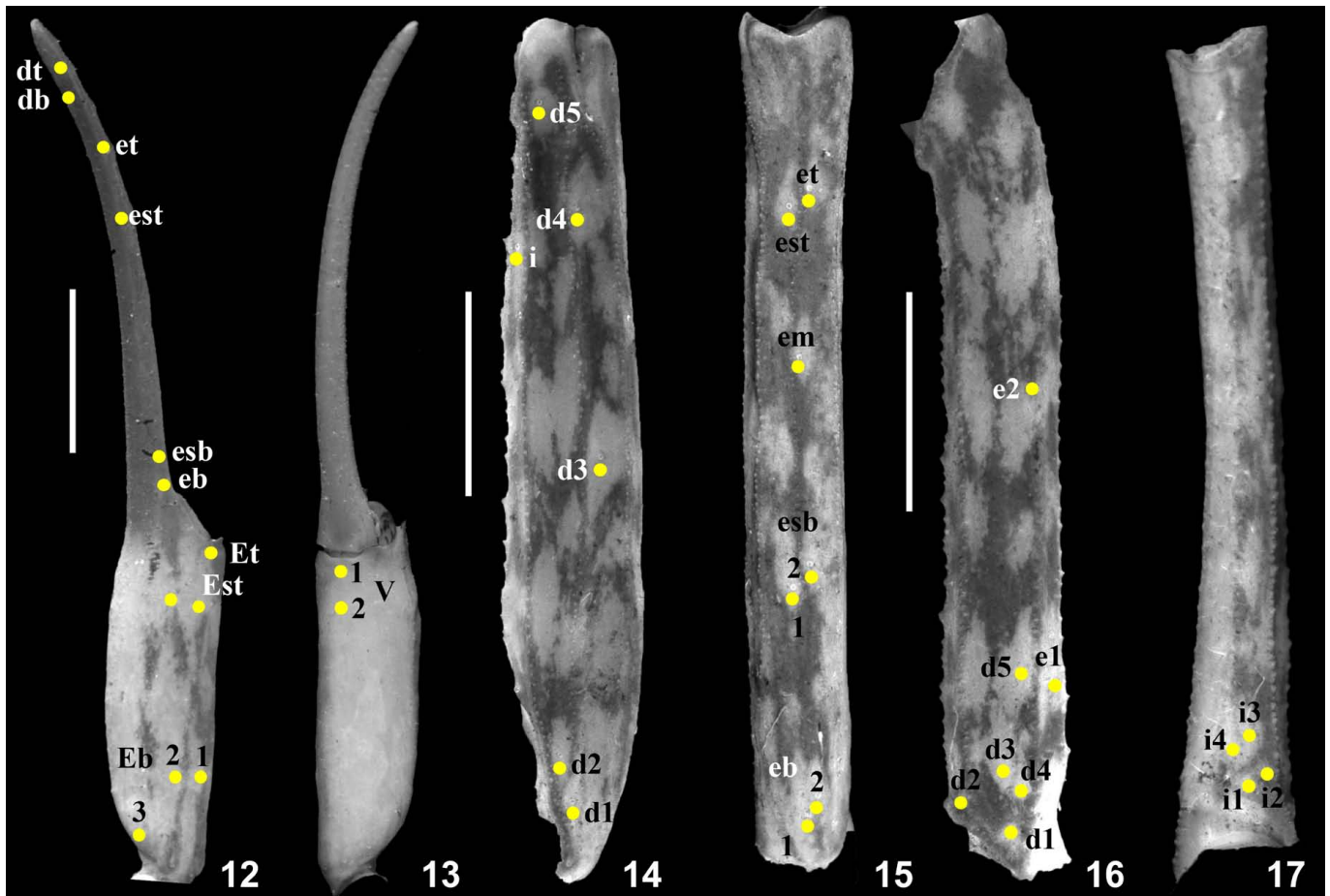
Molecular phylogenetics (Figs. 24, 25). All known species of the genus *Isometrus* found in India and Sri Lanka were included in our phylogenetic analyses along with *I. maculatus* from Northeast India, Japan and Taiwan of a 525 bp fragment of the COI gene. Maximum Likelihood and Bayesian Inference analyses generated trees with different topologies, but *I. maculatus* from Sri Lanka, India, Japan and Taiwan in both these analyses were recovered as monophyletic. *I. maculatus* was recovered as sister to *I. nakshatra* in ML analysis with ultrafast bootstrap support (bp) of 46 and was recovered as sister to *I. longitelson* in BI analysis with posterior probability (pp) values of 0.65. The low support values in ML and BI analyses could be attributed to the use of a single mitochondrial marker.



Figures 3–7. *Isometrus maculatus*, male, IES-522, from Teliamura, Tripura, India, dorsal (3) and ventral (4) views, sternopectinal area (5), telson lateral (6) and ventral (7) views. Scale bars = 5 mm (3–4); 2 mm (5–7).



Figures 8–11. *Isometrus maculatus*, male, IES-522, from Teliamura, Tripura, India, dorsal (8) and ventral (9) views under UV light, carapace under white light (10), carapace under UV light (11). Scale bar = 5 mm (8, 9).



Figures 12–17. *Isometrus maculatus*, male, IES-522, from Teliamura, Tripura, India, 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 bars = 1 mm.

Genetic distance (*p*-distance) (Appendix 1)

I. maculatus was found well separated from all congeners based on a 525 bp fragment of the COI mitochondrial gene. It was recovered as nearest to *I. wayanadensis* but can be separated with a minimum divergence of 12.6% and a maximum divergence of 13.7%. The maximum intra-specific divergence within *I. maculatus* was recovered as 3.6%.

Distribution and Ecology

I. maculatus from Teliamura, Tripura, India was only observed on the trees with money plants coiled around the bark (Fig. 22, 23). Intriguingly, such trees were only found near human settlements where the individuals were observed, photographed and collected. The rigorous surveys conducted in the forested areas in Tripura did not yield any different species of *Isometrus*. In Nansei Islands of Japan, *I. maculatus* was only found where the minimum temperature is higher than 10°C (Kawai, 2021).

Discussion

The presence of *I. maculatus* in Sri Lanka, India, Japan and Taiwan proves the cosmopolitan nature of the species not only

based on morphology but molecular data as well. Previously, Lourenço & Huber (2002) suggested that Sri Lanka was the original home of *I. maculatus*; however, Kovarik (2016) called this a pure speculation. By taking geography into account, Sri Lanka is quite distant from Northeast India, Japan and Taiwan, yet all individuals show an intraspecific divergence of 0.4%–3.6% for the COI gene and form a monophyletic cluster. During the Portuguese and Dutch occupation of maritime regions (1505–1796), Sri Lanka served as a major harbour for the exportation of timber (Wijesinghe, 2003) to the Netherlands and other European countries (Nanayakkara, 1981). Later, during the British colonial period, timber was mainly imported from the Baltics and North America. The natural forest cover of Sri Lanka also decreased from 85 to 75 percent of its land area during British rule which lasted from 1881 to 1900 (Zoysa, 2001). India also had frequent connections with Sri Lanka during the colonial period where Colombo, Sri Lanka as well as Kolkatta and Mumbai, India served as the main ports for regional trade (Tsubota et al., 2017). Concerning these scenarios, it is likely that multiple individuals from various populations of *I. maculatus* from Sri Lanka were introduced across the globe along with the timber, this being the reason for the 3.6% maximum interspecific divergence on the COI gene.



Figures 18–21. *Isometrus maculatus*, male, IES-522, from Teliamura, Tripura, India, metasoma in dorsal (18), lateral (19) and ventral (20), movable finger under UV light (21), Scale bars = 5 mm (18–20); 2 mm (21).



Figures 22–23. A tree on which individuals of *Isometrus maculatus* were observed in Teliamura, Tripura, India.

Sri Lanka is quite geographically distant from Northeast India, yet *I. maculatus* from both these localities shows a small divergence of 1.9%. Additionally, *I. maculatus* from Cijin Island, Taiwan is completely identical to *I. maculatus* from Northeast India. At the same time, *I. maculatus* from Cijin Island, Taiwan shows a divergence of 2.7% from Shimojishima Island, Japan despite their geographical closeness. These p-distance values are quite discordant in comparison with the geographical distances. Hence, it can be strong evidence for multiple anthropogenic introduction of the species. Later, individuals might have been able to establish themselves in their respective localities where timber was exported.

It is also quite interesting to note that the morphological characters of *I. maculatus* from Teliamura, Tripura, India match those of *I. maculatus* from Cuba reported by Teruel (2009) and Teruel & Kovařík (2012). The morphometric data of Cuban *I. maculatus* were included in the comparisons which

form a range that strongly differentiates it from the rest of the *Isometrus* species from the peninsular India. Teruel (2009) and Teruel & Kovařík (2012) also specifically mentioned presence of *I. maculatus* in human settlements where they were found in the cracks and spaces of wooden tables, walls and beams (see figs. 202–206 in Teruel & Kovařík, 2012: 90 and map in page 91). These statements justify the close association of this species with human settlements and anthropogenic activities.

Similar cases of a possible anthropogenic introduction in scorpions were discussed previously (Fet et al., 2005; Tropea et al., 2017). Fet et al. (2005) found that *Euscorpius italicus* (Herbst, 1800) from Switzerland, Italy, Slovenia, Greece and Turkey had little to negligible divergence of the 16S rDNA gene. Fet et al. (2005) hypothesized that the natural populations of the species underwent a bottleneck during glaciations and its introduction in multiple localities was a recent event that occurred due to anthropochory. Interestingly, *E. italicus* is

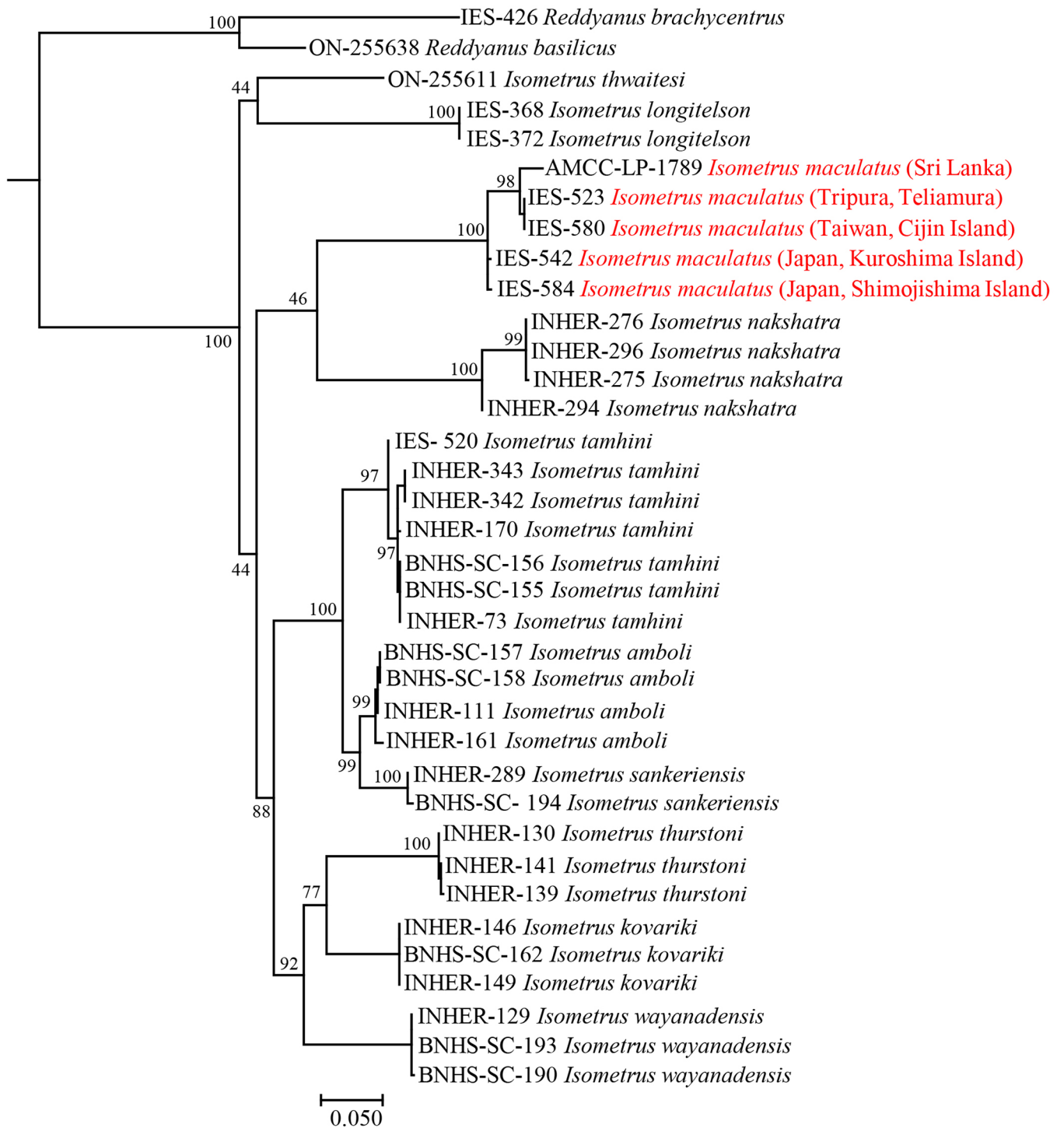


Figure 24. Maximum Likelihood phylogenetic tree (ML) for *Isometrus*. Values along the nodes are ultrafast bootstraps for 1000 iterations. The lineage in red indicates *Isometrus maculatus*.

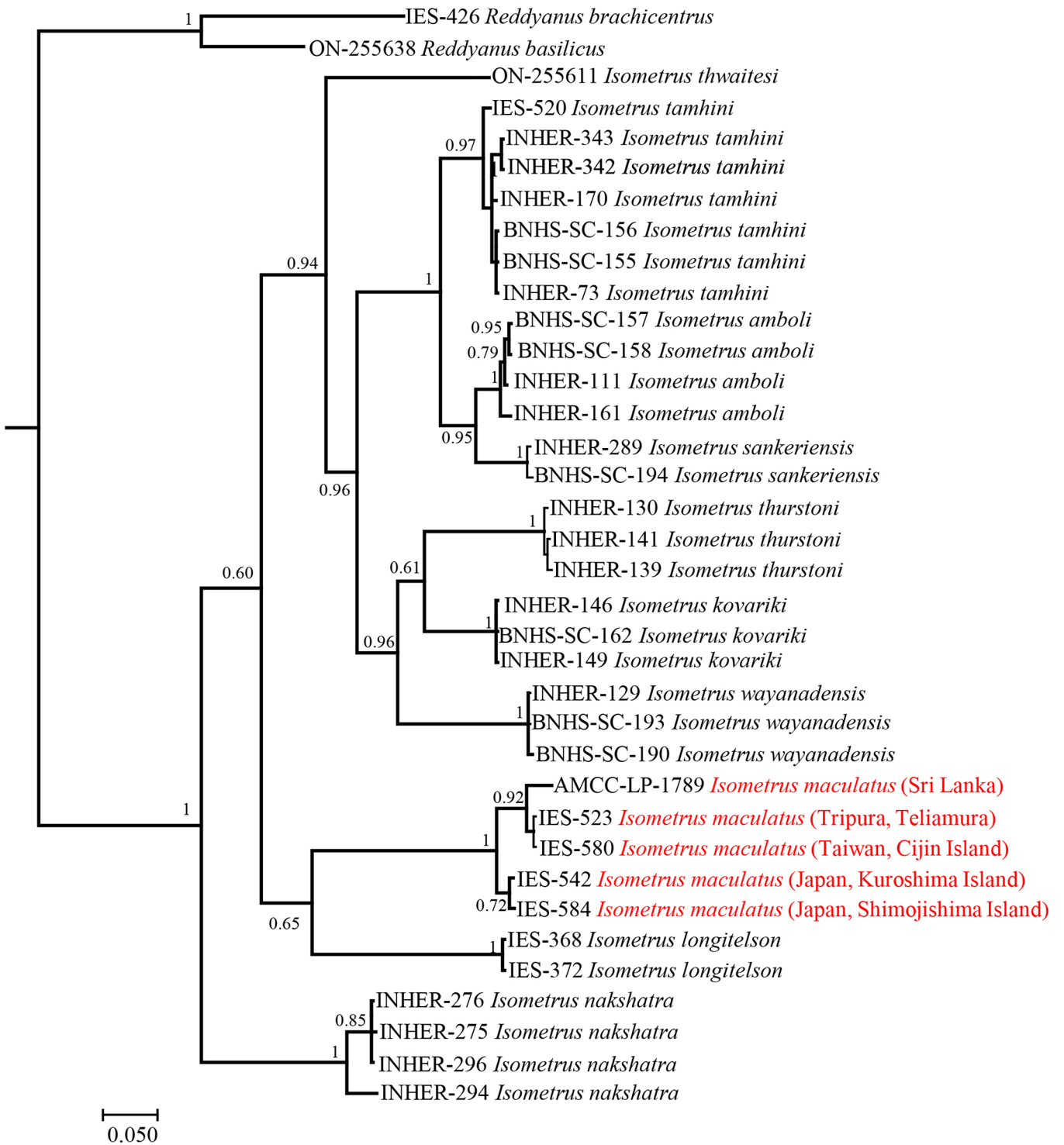


Figure 25. Bayesian phylogenetic tree for *Isometrus*. Values along the nodes are Bayesian posterior probabilities for Bayesian Inference. The lineage in red indicates *Isometrus maculatus* DeGeer, 1778.

Species	Location	Voucher	GenBank Accession
<i>Isometrus maculatus</i>	India, Tripura, Teliamura	IES-523	OR091376
<i>Isometrus maculatus</i>	Japan, Kuroshima Island, Okinawa Prefecture	IES-542	OR091378
<i>Isometrus maculatus</i>	Taiwan, Cijin Island, Kaohsiung City	IES-580	OR091377
<i>Isometrus maculatus</i>	Japan, Shimojishima Island, Okinawa Prefecture	IES-584	OR091379
<i>Isometrus tamhini</i>	India, Maharashtra, Varandha Ghat	IES-520	OR091380
<i>Reddyanus brachycentrus</i>	India, Karnataka, Dakshina Kannada District, E of Mangaluru, Charmadi Ghat	IES-427	OR134097

Table 4. Voucher numbers and GenBank accession numbers for the newly generated sequence data used for the phylogenetic analysis.

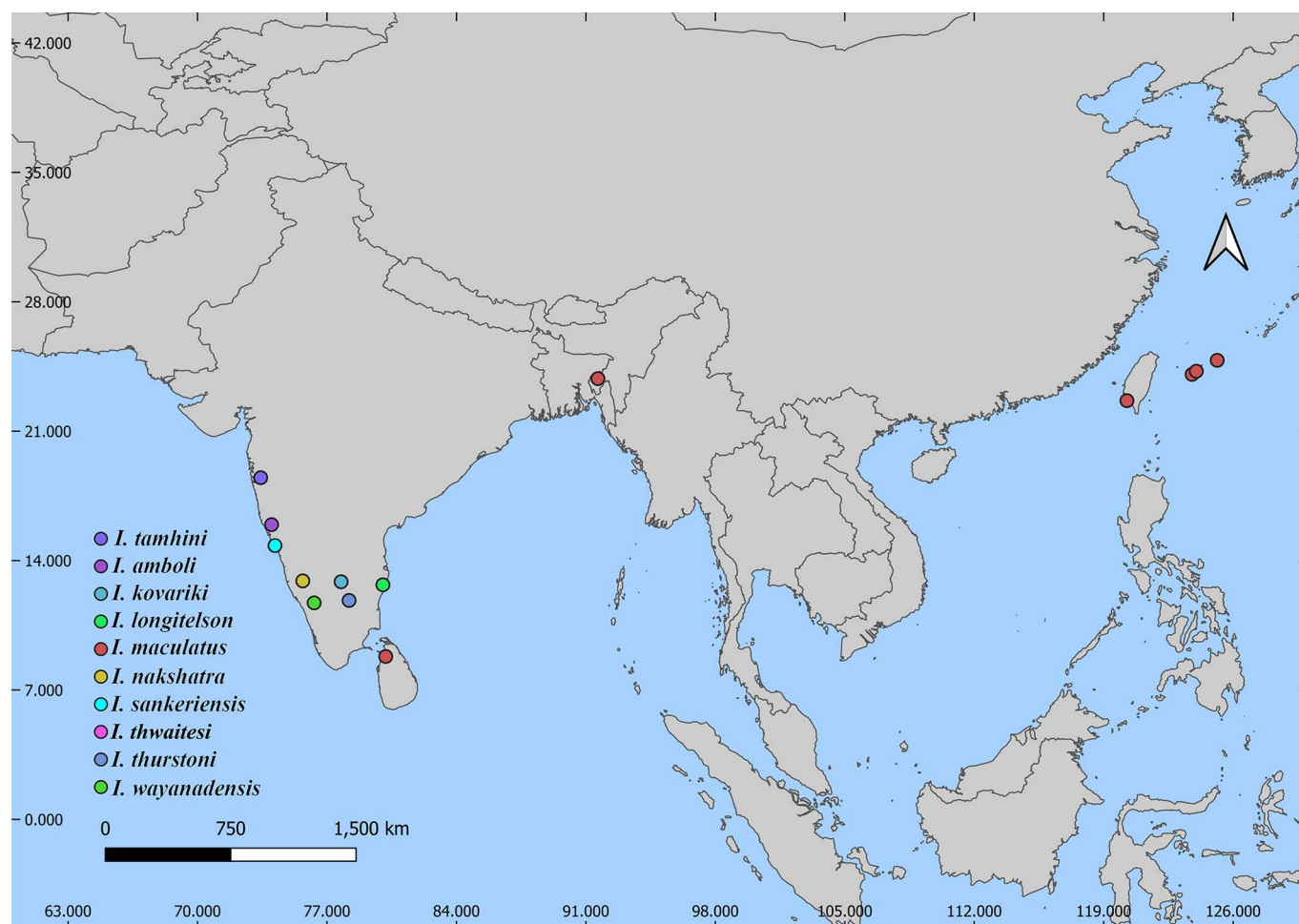


Figure 26. Distribution of *Isometrus* specimens analysed here (see Figs 24–25).

commonly found near human habitations, which is also a case in *I. maculatus*. *Euscorpius tauricus* (C. L. Koch, 1837) was described from the Crimea Peninsula in Ukraine, and further, Tropea et al. (2017) reported its presence in the Cyclades Islands (Greece) and northwest Anatolia (Turkey). Tropea et al. (2017) speculated that the species might have been introduced from the Cyclades by the Greeks who founded colonies in the Mediterranean.

Concerning the high species diversity of *Isometrus* in peninsular India (Sulakhe et al. 2020a, 2020b, 2022; Deshpande et al., 2022) and the presence of two species, *I.*

maculatus and *I. thwaitesi*, in Sri Lanka, it is plausible that the genus is originally endemic to both these countries as previously discussed by Kovařík et al. (2016). Kovařík et al. (2016) further questioned the validity of *Isometrus formosus* Pocock, 1984 from Indonesia and hypothesized that *I. formosus* might comprise a separate genus. The *I. maculatus* collected from Northeast India, Japan and Taiwan were only found near human settlements justifying that these populations might have been introduced to these localities. Hence, in accordance with the facts and available morphological and molecular data, we hereby propose to consider Sri Lanka as the true

home of *I. maculatus*. We furthermore encourage to sample localities across geographic regions from where *I. maculatus* has reported and to test their status based on morphological and molecular data.

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Appendix 1. Pairwise uncorrected raw distances (%) for COI gene sequences for *Isometrus* species.

No. Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1 INSC-244 <i>Reddyanus brachycentrus</i>																			
2 S880- <i>Reddyanus basilicus</i>	0,124																		
3 S809- <i>Isometrus thwaitesi</i>	0,204	0,175																	
4 AMCC-LP-1798 <i>I. maculatus</i> (Sri Lanka)	0,187	0,183	0,143																
5 IES-523 <i>I. maculatus</i> (Tripura, Teliamura)	0,185	0,181	0,147	0,019															
6 BNHS-SC-156 <i>I. maculatus</i> (Taiwan, Cijin Island)	0,185	0,181	0,147	0,019	0,000														
7 IES-542 <i>I. maculatus</i> (Japan, Kuroshima Island)	0,177	0,170	0,143	0,036	0,027	0,027													
8 IES-584 <i>I. maculatus</i> (Japan, Shimojishima Island)	0,175	0,168	0,143	0,036	0,027	0,027	0,004												
9 INHER-368 <i>Isometrus longitelson</i>	0,179	0,183	0,137	0,139	0,139	0,139	0,133	0,133											
10 INHER-372 <i>Isometrus longitelson</i>	0,179	0,183	0,137	0,139	0,139	0,139	0,133	0,133	0,000										
11 IES-520 <i>Isometrus tamhini</i>	0,190	0,171	0,124	0,147	0,139	0,149	0,147	0,147	0,139	0,139									
12 INHER-343 <i>Isometrus tamhini</i>	0,200	0,179	0,128	0,149	0,150	0,150	0,149	0,147	0,137	0,137	0,013								
13 INHER-342 <i>Isometrus tamhini</i>	0,200	0,179	0,128	0,149	0,150	0,150	0,149	0,149	0,137	0,137	0,013	0,000							
14 INHER-170 <i>Isometrus tamhini</i>	0,198	0,179	0,126	0,149	0,150	0,150	0,149	0,149	0,141	0,141	0,010	0,008	0,008						
15 BNHS-SC-156 <i>Isometrus tamhini</i>	0,198	0,175	0,126	0,149	0,150	0,150	0,149	0,149	0,139	0,139	0,010	0,008	0,008	0,004	0,000				
16 BNHS-SC-155 <i>Isometrus tamhini</i>	0,198	0,175	0,126	0,149	0,150	0,150	0,149	0,149	0,139	0,139	0,010	0,008	0,008	0,004	0,000				
17 INHER-73 <i>Isometrus tamhini</i>	0,198	0,175	0,126	0,149	0,150	0,150	0,149	0,149	0,139	0,139	0,010	0,008	0,008	0,004	0,000	0,000			
18 BNHS-SC-157 <i>Isometrus amboli</i>	0,194	0,187	0,124	0,147	0,150	0,150	0,139	0,139	0,147	0,147	0,057	0,069	0,069	0,065	0,065	0,065	0,065		
19 BNHS-SC-158 <i>Isometrus amboli</i>	0,194	0,187	0,124	0,147	0,150	0,150	0,139	0,139	0,147	0,147	0,057	0,069	0,069	0,065	0,065	0,065	0,065	0,000	
20 INHER-161 <i>Isometrus amboli</i>	0,194	0,185	0,122	0,149	0,152	0,152	0,141	0,141	0,145	0,145	0,059	0,070	0,070	0,067	0,067	0,067	0,067	0,010	
21 INHER-111 <i>Isometrus amboli</i>	0,196	0,185	0,122	0,145	0,149	0,149	0,137	0,137	0,145	0,145	0,055	0,067	0,067	0,063	0,063	0,063	0,063	0,002	
22 INHER-289 <i>Isometrus sankeriensis</i>	0,198	0,192	0,124	0,147	0,154	0,154	0,141	0,143	0,152	0,152	0,070	0,072	0,072	0,072	0,072	0,072	0,072	0,048	
23 BNHS-SC-194 <i>Isometrus sankeriensis</i>	0,196	0,194	0,122	0,145	0,152	0,152	0,143	0,145	0,150	0,150	0,070	0,072	0,072	0,072	0,072	0,072	0,072	0,050	
24 INHER-130 <i>Isometrus thurstoni</i>	0,204	0,173	0,137	0,141	0,137	0,137	0,135	0,135	0,150	0,150	0,124	0,126	0,126	0,122	0,120	0,120	0,120	0,131	
25 INHER-141 <i>Isometrus thurstoni</i>	0,202	0,171	0,135	0,139	0,135	0,135	0,133	0,133	0,152	0,152	0,122	0,124	0,124	0,120	0,118	0,118	0,118	0,130	
26 INHER-139 <i>Isometrus thurstoni</i>	0,200	0,170	0,137	0,137	0,133	0,133	0,131	0,131	0,152	0,152	0,120	0,122	0,122	0,118	0,116	0,116	0,116	0,131	
27 INHER-276 <i>Isometrus nakshatra</i>	0,177	0,171	0,141	0,139	0,141	0,141	0,147	0,145	0,135	0,135	0,128	0,133	0,133	0,133	0,133	0,133	0,133	0,139	
28 INHER-275 <i>Isometrus nakshatra</i>	0,179	0,173	0,143	0,141	0,143	0,143	0,149	0,147	0,135	0,135	0,130	0,135	0,135	0,135	0,135	0,135	0,135	0,141	
29 INHER-296 <i>Isometrus nakshatra</i>	0,177	0,171	0,141	0,139	0,141	0,141	0,147	0,145	0,135	0,135	0,128	0,133	0,133	0,133	0,133	0,133	0,133	0,139	
30 INHER-294 <i>Isometrus nakshatra</i>	0,173	0,168	0,135	0,130	0,131	0,131	0,137	0,135	0,126	0,126	0,131	0,137	0,137	0,137	0,137	0,137	0,137	0,135	
31 INHER-219 <i>Isometrus wayanadensis</i>	0,187	0,173	0,130	0,135	0,131	0,131	0,128	0,126	0,139	0,139	0,109	0,114	0,114	0,114	0,114	0,114	0,114	0,114	
32 BNHS-SC-190 <i>Isometrus wayanadensis</i>	0,187	0,173	0,130	0,135	0,131	0,131	0,128	0,126	0,139	0,139	0,109	0,114	0,114	0,114	0,114	0,114	0,114	0,114	
33 BNHS-SC-193 <i>Isometrus wayanadensis</i>	0,189	0,175	0,131	0,137	0,133	0,133	0,130	0,128	0,141	0,141	0,110	0,116	0,116	0,116	0,116	0,116	0,116	0,116	
34 INHER-146 <i>Isometrus kovariki</i>	0,208	0,185	0,118	0,164	0,156	0,156	0,147	0,147	0,156	0,156	0,103	0,105	0,105	0,101	0,101	0,101	0,101	0,105	
35 BNHS-SC-162 <i>Isometrus kovariki</i>	0,208	0,185	0,118	0,164	0,156	0,156	0,147	0,147	0,156	0,156	0,103	0,105	0,105	0,101	0,101	0,101	0,101	0,105	
36 INHER-149 <i>Isometrus kovariki</i>	0,208	0,185	0,118	0,164	0,156	0,156	0,147	0,147	0,156	0,156	0,103	0,105	0,105	0,101	0,101	0,101	0,101	0,105	

No. Species	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
1 INSC-244 <i>Reddyanus brachycentrus</i>																			
2 S880- <i>Reddyanus basilicus</i>																			
3 S809- <i>Isometrus thwaitesi</i>																			
4 AMCC-LP-1798 <i>I. maculatus</i> (Sri Lanka)																			
5 IES-523 <i>I. maculatus</i> (Tripura, Teliamura)																			
6 IES-580 <i>I. maculatus</i> (Taiwan, Cijin Island)																			
7 IES-542 <i>I. maculatus</i> (Japan, Kuroshima Island)																			
8 IES-584 <i>I. maculatus</i> (Japan, Shimojishima Island)																			
9 INHER-368 <i>Isometrus longitelson</i>																			
10 INHER-372 <i>Isometrus longitelson</i>																			
11 IES-520 <i>Isometrus tamhini</i>																			
12 INHER-343 <i>Isometrus tamhini</i>																			
13 INHER-342 <i>Isometrus tamhini</i>																			
14 INHER-170 <i>Isometrus tamhini</i>																			
15 BNHS-SC-156 <i>Isometrus tamhini</i>																			
16 BNHS-SC-155 <i>Isometrus tamhini</i>																			
17 INHER-73 <i>Isometrus tamhini</i>																			
18 BNHS-SC-157 <i>Isometrus amboli</i>																			
19 BNHS-SC-158 <i>Isometrus amboli</i>																			
20 INHER-161 <i>Isometrus amboli</i>	0,010																		
21 INHER-111 <i>Isometrus amboli</i>	0,002	0,008																	
22 INHER-289 <i>Isometrus sankeriensis</i>	0,048	0,050	0,046																
23 BNHS-SC-194 <i>Isometrus sankeriensis</i>	0,050	0,051	0,048	0,004															
24 INHER-130 <i>Isometrus thurstoni</i>	0,131	0,133	0,130	0,137	0,139														
25 INHER-141 <i>Isometrus thurstoni</i>	0,130	0,131	0,128	0,135	0,137	0,002													
26 INHER-139 <i>Isometrus thurstoni</i>	0,131	0,133	0,130	0,137	0,139	0,004	0,002												
27 INHER-276 <i>Isometrus nakshatra</i>	0,139	0,135	0,137	0,130	0,130	0,150	0,149	0,147											
28 INHER-275 <i>Isometrus nakshatra</i>	0,141	0,137	0,139	0,131	0,131	0,150	0,149	0,147	0,002										
29 INHER-296 <i>Isometrus nakshatra</i>	0,139	0,135	0,137	0,130	0,130	0,150	0,149	0,147	0,000	0,002									
30 INHER-294 <i>Isometrus nakshatra</i>	0,135	0,131	0,133	0,130	0,130	0,152	0,150	0,149	0,030	0,032	0,030								
31 INHER-219 <i>Isometrus wayanadensis</i>	0,114	0,116	0,112	0,107	0,105	0,114	0,112	0,114	0,141	0,143	0,141	0,135							
32 BNHS-SC-190 <i>Isometrus wayanadensis</i>	0,114	0,116	0,112	0,107	0,105	0,114	0,112	0,114	0,141	0,143	0,141	0,135	0,000						
33 BNHS-SC-193 <i>Isometrus wayanadensis</i>	0,116	0,118	0,114	0,109	0,107	0,116	0,114	0,116	0,143	0,145	0,143	0,137	0,002	0,002					
34 INHER-146 <i>Isometrus kovariki</i>	0,105	0,103	0,103	0,107	0,109	0,101	0,103	0,105	0,149	0,150	0,149	0,149	0,114	0,114	0,116				
35 BNHS-SC-162 <i>Isometrus kovariki</i>	0,105	0,103	0,103	0,107	0,109	0,101	0,103	0,105	0,149	0,150	0,149	0,149	0,114	0,114	0,116	0,000			
36 INHER-149 <i>Isometrus kovariki</i>	0,105	0,103	0,103	0,107	0,109	0,101	0,103	0,105	0,149	0,150	0,149	0,149	0,114	0,114	0,116	0,000	0,000		