Euscorpius

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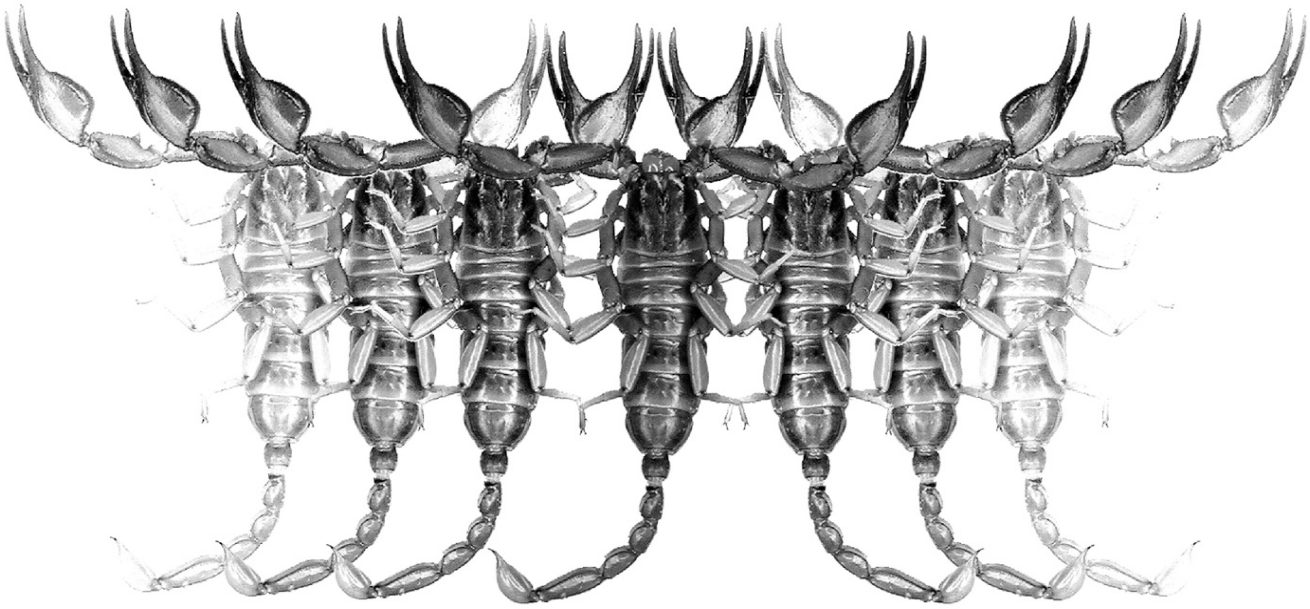

A new species of *Isometrus* (Scorpiones: Buthidae) from southern India

Shauri Sulakhe, Nikhil Dandekar, Shomen Mukherjee, Malay Pandey, Makarand Ketkar, Anand Padhye & Deshabhushan Bastawade

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Euscorpius — Occasional Publications in Scorpiology. 2020, No. 310

A new species of *Isometrus* (Scorpiones: Buthidae) from southern India

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Summary

A new species of *Isometrus* (Buthidae) is described from India using integrated taxonomic approach. *Isometrus kovariki* **sp**. **n**. is closely related to *I. thurstoni*, and differs in morphological features and raw genetic divergence of more than 9%.

Introduction

The Indian fauna of the scorpion genus *Isometrus* Ehrenberg, 1828, includes four species (Kovařík, 2003; Sulakhe et al., 2020). These are: the type species, *Isometrus maculatus* (DeGeer, 1778), *I. thurstoni* Pocock, 1893, and two recently described species from northern Western Ghats of India, *I. tamhini* Sulakhe et al., 2020 and *I. amboli* Sulakhe et al., 2020. *I. maculatus* is a cosmopolitan species found in more than 70 countries (Kovařík, 2003). Records in India show the presence of this species in the states of Maharashtra, Karnataka, Tamil Nadu, West Bengal and Meghalaya (Kovařík, 2003; Fet & Lowe, 2000). It has an ambiguous type locality with original listing from "Suriname and Pennsylvania"; however, this species is assumed to have originated from South Asia (Fet & Lowe, 2000). The remaining three species of this genus from India include *I. thurstoni* described from Shevaroy Hills (Tamil Nadu), *I. tamhini* from Tamhini, Pune (Maharashtra), and *I. amboli* from Amboli, Sindhudurg (Maharashtra). While *I. thurstoni* is distributed in the states of Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu (Pocock, 1893; Tikader & Bastawade, 1983; Fet & Lowe, 2000) and in Sri Lanka (Pocock 1900), *I. tamhini* and *I. amboli* are known only from their type localities. In view of the cosmopolitan distribution of *I. maculatus*, which might indicate cryptic speciation within this genus, it is essential to study this genus based on integrated taxonomy (Sulakhe et al. 2020). In this communication, we describe a new species of *Isometrus* from southern India, discovered during recent scorpion surveys.

Methods, Materials & Abbreviations

Sampling was carried out in Chikkadunnasandra (12.85°N 77.76°E, 893 m a. s. l.), Bangalore, Karnataka State, India. Specimens were located with the help of ultraviolet light (The Scorpionator, Model: Scorpion Master). A total of 9 specimens were collected (7 males and 2 females). Photographs of holotype and paratype were taken using Nikon D90, 105mm F2.8 micro lens and R1 flash kit. Specimens were euthanized and preserved in absolute ethanol, and later transferred to 70% ethanol 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); morphological terminology follows Hjelle (1990). Measurements were taken (in mm) for 38 morphological characters (Table 1). The trichobothrial terminology follows Vachon (1974).

Specimens are deposited in the museum collections of Bombay Natural History Society (BNHS), Mumbai and Institute of Natural History Education and Research (INHER), Research Laboratory, Pune, Maharashtra, India.

Comparative material examined:

Isometrus tamhini Sulakhe et al., 2020

Type material and 1♂ (INHER-170), India, Maharashtra State, Pune District, Saltar Khind, near Ambawne, 18.58°N 73.36°E, 743 m a. s. l., 21 July 2019, leg. S. Sulakhe, S. Deshpande.

Isometrus amboli Sulakhe et al., 2020

Type material and 1♂ (INHER-161, India, Maharashtra State, Sindhudurg District, Amboli, 15.94°N 74.00°E, 872 m a. s. l., 30 August 2019, leg. S. Sulakhe.

Figure 1. *Isometrus kovariki* **sp**. **n**., male holotype, in vivo habitus.

Morphometric data used for comparison and analysis of *I. thurstoni*, *I. maculatus*, *I. tamhini* and *I. amboli* has been sourced from Sulakhe et al. (2020).

Statistical Analysis

Multivariate normality of the data was checked following Doornik & Hansen (2008). A discriminant function analysis (DFA) using Principal Component Analysis (PCA) factors was conducted to assess the degree of morphological differentiation among the new species and their closest relatives. In order to nullify the influence of body size, PCA were performed using 25 size-corrected morphometric parameters taken from adults of both sexes. Sets of 25 predictor variables were generated from PCA and all PCA factor scores were used as input variables for DFA to determine the classification success of our samples (Sulakhe et al., 2020). PCA and DFA were performed using the statistical software PAST 3.25 (Hammer et al., 2001).

Molecular Analysis

For genetic analyses, protocol as per Sulakhe et al. (2020) was followed. Whole genomic DNA was extracted from preserved (ethanol 99.9%) muscle tissue (leg fragment) of *Isometrus kovariki* **sp**. **n**., *I. tamhini* and *I. amboli* (see voucher numbers of specimens used for DNA analysis in Table 4 and Fig. 37) 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*) mitochondrial gene was amplified by polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer et al., 1994). A 25 µl PCR reaction (TaKaRa Taq™ DNA Polymerase) was set containing 1 unit of Taq DNA polymerase (0.2µL), 2.5µL of 10x buffer, 2 µl of dNTPs (2.5mM each), 2 µl (5mM) of each primer, 2µl template DNA, and 14.3 µl of water, carried out with an Miniamp Thermal Cycler. Thermal cycler profiles used for amplification were as follows: 95° C for 3 min (denaturation temperature 95° C for 30 seconds, annealing temperature 50° C for 30 seconds, elongation temperature 72° C for 1 minute) x 35 cycles, 72° C for 7 minutes, hold at 4° C. PCR product was cleaned through column purification method with Barcode Biosciences PCR Cleanup Kit and sequenced with a 3730 DNA Analyzer. The sequencing primers were the same as those used in the PCRs. All sequences were deposited in the GenBank (http://www.ncbi.nlm.nih.gov) under accession numbers as per Table 4.

The sequences were also checked on the Blast tool to find the closest available sequences and the related ones were downloaded for analysis. Gene sequences were aligned using MUSCLE (Edgar, 2004). Molecular phylogenetic analysis was performed using MEGA 6 (Tamura et al., 2013). Pairwise raw phylogenetic distances were calculated in MEGA 6 (Tamura et al., 2013). The best fit model for nucleotide substitution was selected from 24 models using MEGA 6 (Tamura et al. 2013) based on the minimum Bayesian Information Criterion (BIC) value (Schwarz, 1978; Nei & Kumar, 2000). The best fit nucleotide substitution model was used for testing the phylogenetic hypothesis using maximum likelihood method. Phylogenetic tree was built using MEGA 6 (Tamura et al., 2013) and reliability of the tree was estimated using bootstrap values from 1000 replicates.

I. kovariki **sp. n.** **Dimensions (mm)**		♂ **HT** BNHS SC161	♂ **PT** BNHS SC163	♂ **PT** BNHS SC164	♂ **PT** INHER151	♂ **PT** INHER146
Carapace	L / W	5.4 / 4.6	5.3 / 4.4	5.0 / 4.6	4.4 / 3.8	4.6 / 4.0
Mesosoma	L	16.0	15.07	11.66	9.73	12.3
Tergite VII	L	3.8	3.9	3.2	2.7	3.1
Metasoma + telson	L	39.7	37.1	34.8	28.8	32.6
Segment I	L / W	4.3 / 2.4	4.0 / 2.3	3.3 / 2.1	3.0 / 1.9	3.5 / 2.0
Segment II	L / W	5.6 / 2.1	5.2 / 1.9	5.0 / 2.0	3.9 / 1.8	4.4 / 1.8
Segment III	L / W	6.1 / 2.0	5.7 / 1.8	5.4 / 2.0	4.3 / 1.7	4.9 / 1.7
Segment IV	L / W	6.8 / 2.1	6.4 / 2.0	6.1 / 1.8	5.1 / 1.6	5.7 / 1.6
Segment V	L / W	8.6 / 2.2	8.4 / 1.9	7.6 / 1.7	6.5 / 1.6	7.3 / 1.6
Telson	L / W / D	8.3 / 2.1 / 2.1	7.4 / 1.8 / 1.9	7.5 / 1.8 / 1.9	6.1 / 1.4 / 1.7	6.7 / 1.5 / 1.7
Telson aculeus	L	3.07	2.58	2.70	2.34	2.50
Pedipalp	L	28.67	26.66	26.27	20.85	23.84
Femur	L / W	8.0 / 1.5	7.4 / 1.4	7.4 / 1.3	5.5 / 1.2	6.6 / 1.2
Patella	L / W	8.3 / 1.7	7.7 / 1.7	7.4 / 1.7	5.8 / 1.5	6.9 / 1.5
Chela	L	12.4	11.6	11.51	9.55	10.35
Manus	W / D	2.2 / 1.8	2.0 / 1.7	1.9 / 1.5	1.7 / 1.2	1.6 / 1.5
Movable finger	L	7.86	6.98	7.23	6.04	6.37
Pectine	L / W	4.2 / 0.8	3.9 / 0.9	3.9 / 0.4	3.7 / 0.8	3.8 / 0.8
Total	**L**	**61**	**57.4**	**51.41**	**42.93**	**49.53**

I. kovariki **sp. n.** **Dimensions (mm)**		♂ **PT** INHER152	♂ **PT** INHER149	♀ **PT** BNHS SC162	♀ **PT** INHER148
Carapace	L / W	4.6 / 3.8	4.5 / 3.8	4.8 / 4.1	4.7 / 3.9
Mesosoma	L	12.03	13.41	13.57	12.51
Tergite VII	L	3.2	3.4	3.0	2.8
Metasoma + telson	L	31.3	31.1	25.2	23.3
Segment I	L / W	2.9 / 2.0	3.5 / 2.0	2.9 / 2.4	2.1 / 2.2
Segment II	L / W	4.3 / 1.6	4.3 / 1.9	3.5 / 2.1	3.3 / 2.0
Segment III	L / W	4.9 / 1.7	4.8 / 1.8	3.9 / 2.1	3.6 / 1.9
Segment IV	L / W	5.5 / 1.6	5.5 / 1.8	4.3 / 1.9	4.1 / 1.8
Segment V	L / W	7.0 / 1.7	6.6 / 1.7	5.5 / 1.9	5.2 / 1.8
Telson	L / W / D	6.7 / 1.6 / 1.8	6.5 / 1.5 / 1.6	5.2 / 1.5 / 1.6	5.5 / 1.4 / 1.5
Telson aculeus	L	2.48	2.49	1.97	2.34
Pedipalp	L	23.57	22.2	19.71	17.98
Femur	L / W	6.5 / 1.2	6.3 / 1.3	5.2 / 1.4	4.8 / 1.3
Patella	L / W	6.8 / 1.6	6.4 / 1.5	5.7 / 1.8	5.1 / 1.7
Chela	L	10.28	9.53	8.85	8.07
Manus	W / D	1.9 / 1.4	1.9 / 1.6	1.8 / 1.6	1.7 / 1.5
Movable finger	L	6.18	5.67	5.9	5.51
Pectine	L / W	3.9 / 0.9	3.5 / 0.8	3.4 / 0.7	3.2 / 0.8
Total	**L**	**47.91**	**49.05**	**43.68**	**40.98**

Table 1. Comparative measurements of adults of *Isometrus kovariki* **sp. n.** Abbreviations: length (L), width (W, in carapace it corresponds to posterior width), depth (D), holotype (HT), paratype (PT).

Systematics

Family Buthidae C. L. Koch, 1837
Genus ***Isometrus*** Ehrenberg, 1828
(Figures 1–37, Tables 1–4)

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

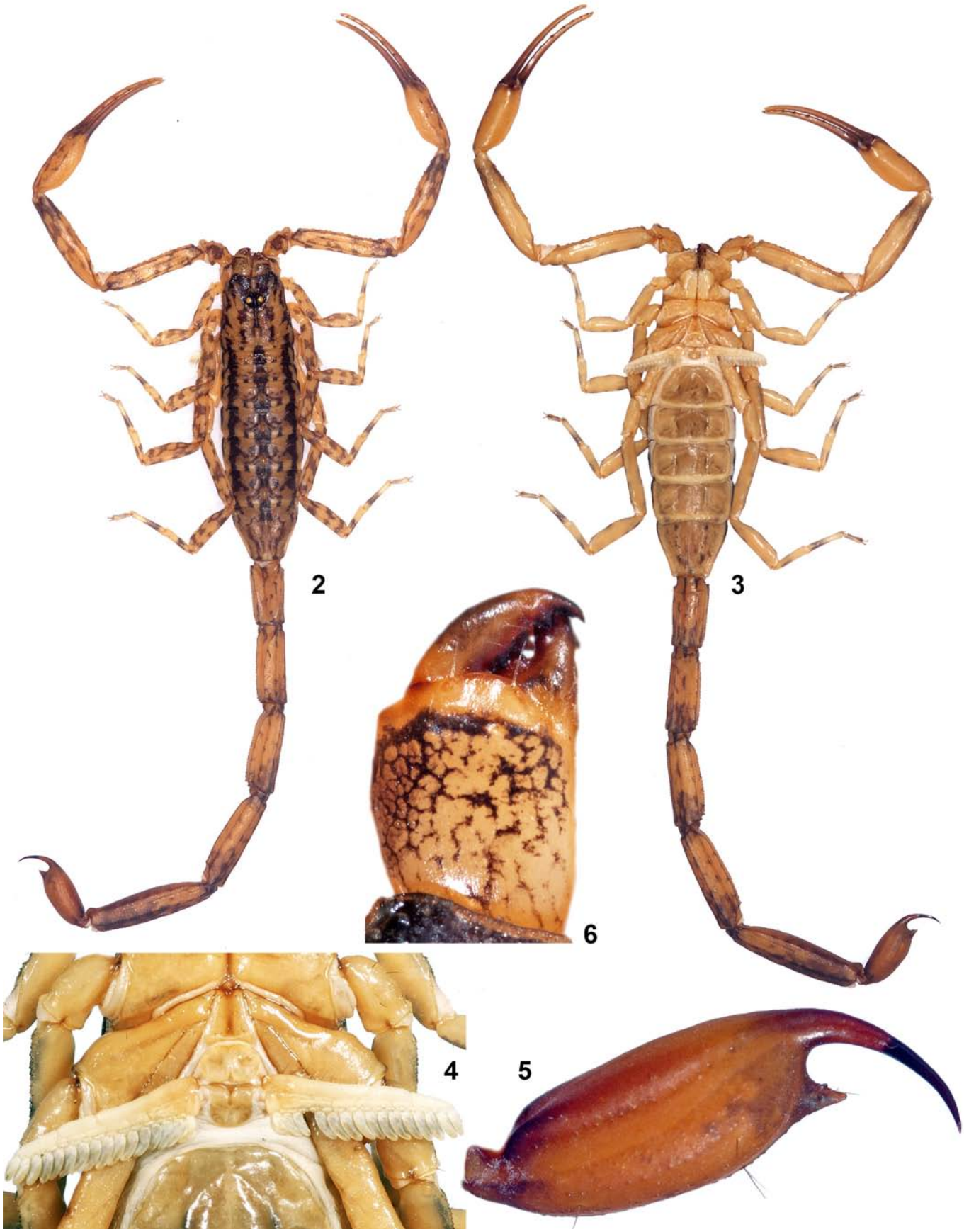
Isometrus kovariki sp. n.
(Figures 1–21, 24, 28, 32, Tables 1–4)
http://zoobank.org/urn:lsid:zoobank.act:225D3716-8AFB-4A08-A01D-FFCEBEE276CF

Type locality and type repository. India, Karnataka State, Bengaluru Urban District, Chikkadunnasandra, 12.85°N 77.76°E, 893 m a. s. l.; BNHS.

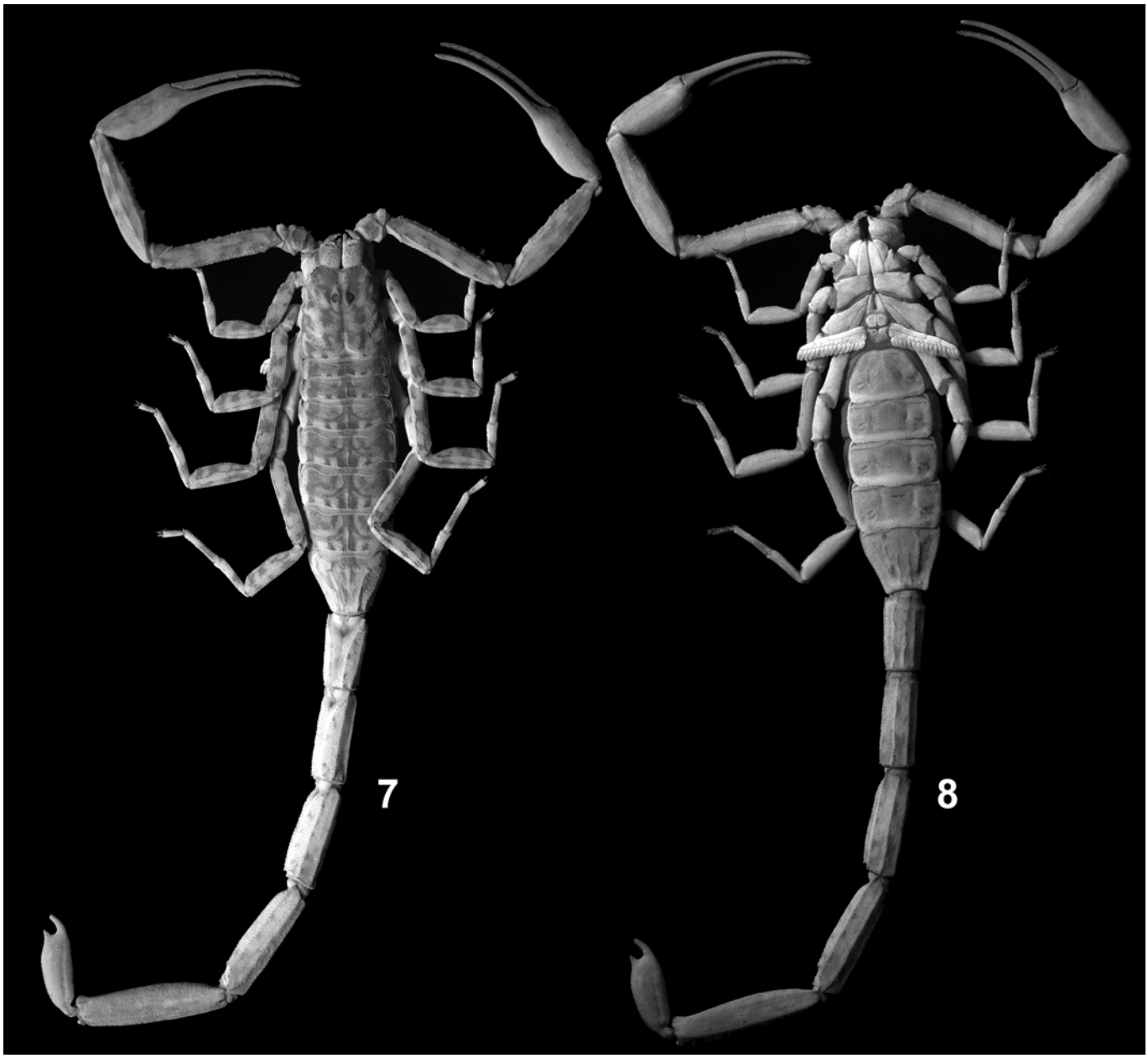
Type material. **India**, Karnataka State, Bengaluru Urban District, Chikkadunnasandra, 12.85°N 77.76°E, 893 m a. s. l., 1♂ (holotype, BNHS SC 161), 29 August 2019, 6♂ (paratypes, INHER- 146, 149, 151, 152, BNHS SC 163, 164), 2♀ (paratypes, INHER-148, BNHS SC 162), 29 August 2019. All specimens collected by S. Mukherjee, S. Sulakhe, M. Ketkar, S. Deshpande & M. Kulkarni.

Etymology. The species epithet is a patronym honoring František Kovařík for his remarkable contribution to the scorpion taxonomy of the world. Suggested common name: Kovařík's Tree Scorpion.

Description (♂ holotype).
Coloration (Figs. 2, 3, 9, 10). Body and appendages light yellowish brown and variegated with blackish brown stripes and spots; light brownish to yellowish last metasomal segment, more darker on posterior portion; pedipalp fingers dark brownish at the base. Ventral portion uniformly yellow and sternite V with few dark spots. Basal segments of chelicera dorsally yellowish with blackish reticulation ending anteriorly into blackish transverse patch; ventral portion of chelicera yellowish; fingers of chelicera dark brown with tip of the fingers black. Telson uniformly brown in color.
Carapace (Figs.18, 20). Surface granular throughout with mixed granules, more closely granular in inter-ocular area and median posterior ocular area. Carapace without carinae, median supra-ocular area, with mixed granulation. 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). Anteriolateral ocular tubercle granular, provided with 5 pairs of lateral ocelli. Three pairs of subcontiguous lateral ocelli and two micro-ocelli situated behind the lateral ocelli. Median longitudinal furrow visible only till the anterior portion of median eyes. Anterior margin smooth with deep emargination. Lateral margins finely crenulated below the lateral ocelli. Posterior margin almost entirely smooth.
Chelicerae (Fig. 6). Characteristic of the family Buthidae. Basal segments and movable fingers with short and firm setae on the basal and ventral surfaces. Dorsal surface of basal segment with two prominent tubercles on anterior portion.
Pedipalp (Figs. 12–17, 24). Femur with 5 carinae (dorsal exterior, dorsal interior, exterior median, interior median and exterior ventral). Exterior median carina with few granules more prominent and subtriangularly tuberculate. All remaining carinae are evenly crenulated. Intercarinal space very weakly granular except ventral surface with few closely set granules on proximal portions. Patella with 7 distinct carinae (Dorsal median, dorsal interior, dorsal exterior, exterior median, ventral exterior, interior median and ventral interior). Dorsal exterior, dorsal interior and dorsal median carinae granular. Interior median carina strongly tuberculated with few sub-denticulate granules. Exterior median carina weakly granular. Ventral interior carina evenly granular. Ventral exterior carina weakly granular on proximal portion and obsolete on distal portion. Intercarinal space almost entirely smooth. Manus almost smooth. Fixed fingers with 1 smooth and obsolete carina on dorsal exterior surface. Fixed and movable finger armed with 6 rows of linear denticles with one external denricle only on movable finger. Trichobothrial pattern typical for the genus.
Legs (Figs. 2, 3, 7, 8). Femur and patellae carinated. All carinae granular. Tibiae 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 provided with tuft of short, stout blackish setae on ventral side. Tarsomere II compressed laterally and ventrally provided with paired row of short, pointed, anteriorly directed, closely placed setae.
Genital operculum (Figs. 4, 11). Wider than long, elliptical, separated with a pair of short male genital papillae.
Pectines (Figs. 4, 11). Basal piece rectangular, deeply notched on anterior median margin. Posterior margin of basal piece curved; smooth on surface with a parallel wide sub-basal piece along the posterior margin. Pectin 5 times longer than its width, marginal lamella of 3 digits and median lamella of 6 digits, outer margin armed with a row of stout short red setae and few setae on surface. Fulcra 15, roughly triangular each armed with few short red setae, placed in between adjacent pectinal teeth. Teeth 16, strong and stout.
Mesosoma (Figs. 2, 3, 7–10). Tergites I – V sparsely and finely granular and provided with a short median carina. Posterior and lateral margins granular. Tergite VI with continuous median carina. Tergite VII narrowed posteriorly, granular, provided with 2 pairs of lateral granular carinae, present only on 2/3 posterior portion and ends abruptly. A broad median carina limited to anterior 1/3 of median portion. Sternites III–VI almost entirely smooth with a pair of spiracles. Sternite V exceptionally smooth. Sternite VII smooth on posterior



Figures 2–**6**. *Isometrus kovariki* **sp**. **n**., male holotype, dorsal (2) and ventral (3) views, sternopectinal area (4), telson in lateral view (5) and chelicera in dorsal view (6).



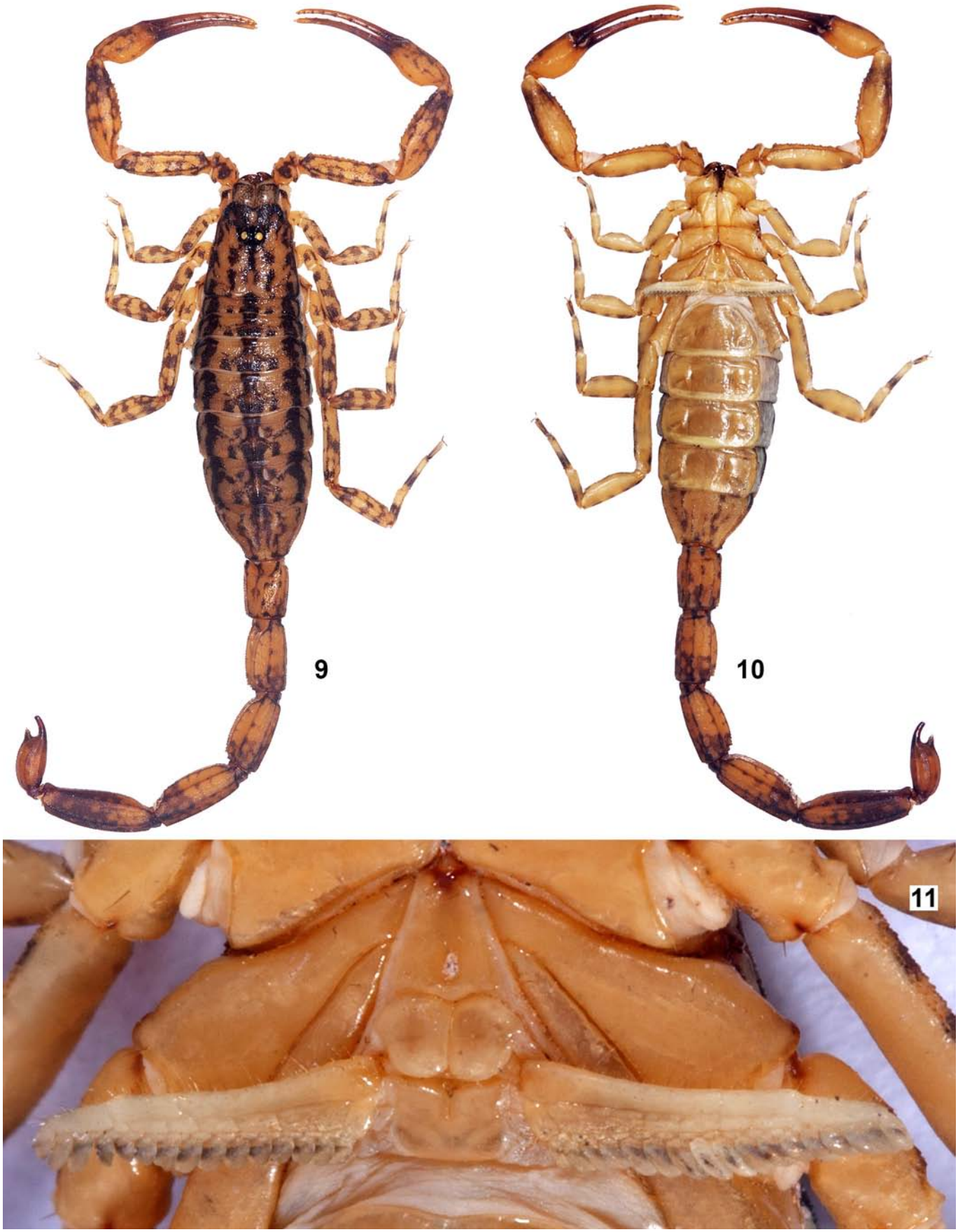
Figures 7–**8**. *Isometrus kovariki* **sp**. **n**., male holotype in dorsal (7) and ventral (8) views under UV fluorescence.

margin while finely crenulated to serrated on lateral margins; with 2 pairs of granular carinae; median carinae restricted to posterior 2/3 portion; lateral carinae present in the middle half region.

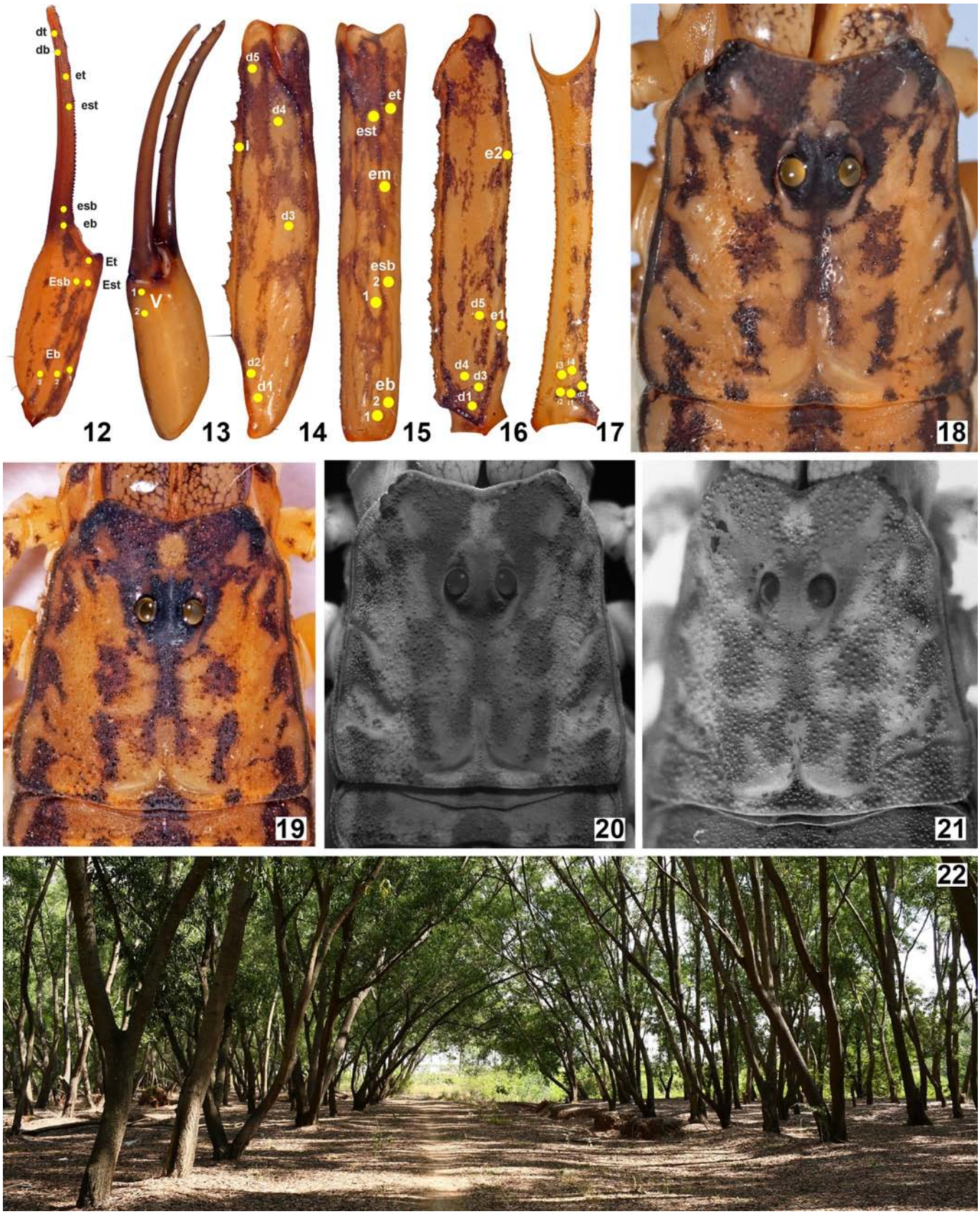
Metasoma (Figs. 2, 3, 7–10). All segments longer than wide; basal segment 1.8 times longer than wide. Segment I with 5 pairs of carinae (dorsal, dorso-lateral, lateral, ventrolateral and ventral) weakly granular. Intercarinal space weakly and finely granular, anterior margin smooth. Segments II and III provided with 4 pairs of carinae (dorsal, dorsolateral, ventrolateral and ventral). Intercarinal portion irregularly granular, dorso-lateral and dorsal carinae posteriorly ending into weak subtringular tubercles. Segment IV with 4 pairs of weakly granular carinae (dorsal, dorsolateral, ventrolateral and ventral). Dorsals ending into very weak subtringular tubercles. Intercarinal space weakly and irregularly granular. Segment V with 7 carinae (dorsal, dorsolateral and ventrolateral pairs and a single ventral); dorsal carinae weakly, sparsely granular. Dorsolateral carinae present throughout. Laterals totally absent. Ventrolateral carinae and single ventral median carina granular and ending posteriorly into a weakly granular anal rim. Intercarinal space irregularly and weakly granular than segments I–IV.

Telson (Figs. 5, 30). Telson with stout vesicle, bulbous on distal portion and smooth on dorsal surface. Lateral surface demarcated with weakly granular ridge. Ventral median carina very weakly granular ending into triangular, subacular, pointed nodule, armed with a pair of minute denticle on inner margin. Ventral portion with 2 pairs of sparsely and finely granular carinae. Intercarinal space weakly and finely granular. Aculeus elongated, sharp and moderately curved.

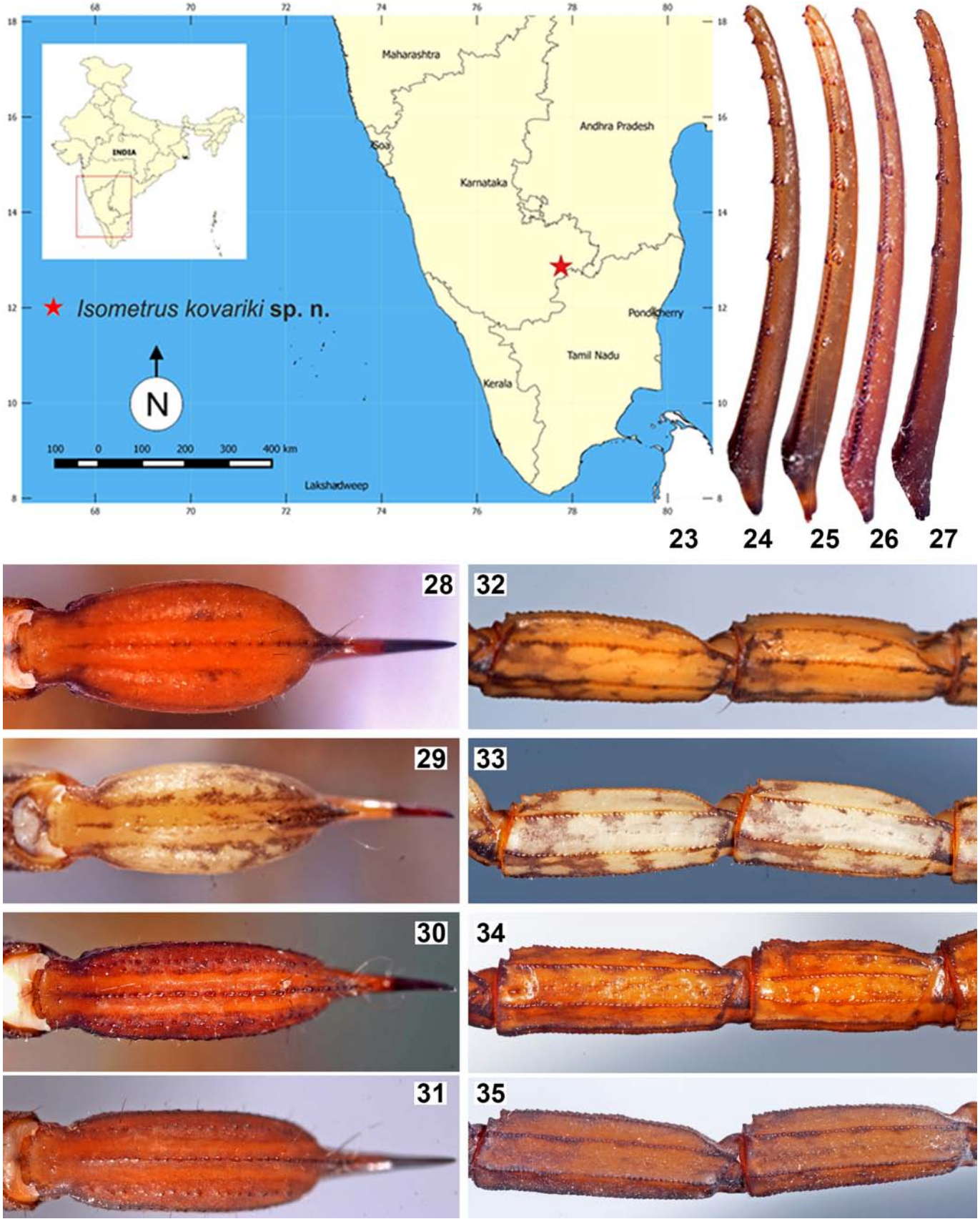
Measurements. See Table 1.



Figures 9–**11**. *Isometrus kovariki* **sp**. **n**., female, paratype, BNHS SC 162, in dorsal (9) and ventral (10) views, and sternopectinal area (11).



Figures 12–**22**: *Isometrus kovariki* **sp**. **n**. **Figures 12**–**18**, **20**. Male, holotype, pedipalp chela dorsoexternal (12) and ventral (13), patella dorsal (14) and external (15), and femur dorsal (16) and internal (17) views. Trichobothrial pattern indicated by yellow circles. Carapace under white light (18) and UV fluorescence (20). **Figures 19**, **21**. Female, paratype, BNHS SC 162, carapace under white light (19) and UV fluorescence (21). **Figure 22**. Type locality, a view of dense plantation with tall trees.



Figures 23–**35**: **Figure 23**. Distribution of *Isometrus kovariki* **sp**. **n**. **Figures 24–27**: Comparison of pedipalp movable finger dentition. **Figures 28–31**: Comparison of telson in ventral view. **Figures 32–35**: Comparison of metasoma II–III in lateral view. **Figures 24**, **28**, **32**. *I. kovariki* **sp**. **n**., male, holotype. **Figures 25**, **29**, **33**. *I. thurstoni*, male, INHER-SC-139. **Figures 26**, **30**, **34**. *I. tamhini*, male, holotype. **Figures 27**, **31**, **35**. *I. amboli*, male, holotype.

Ratios of males	*I. kovariki* sp. n. (7♂)	*I. amboli* (7♂)	*I. tamhini* (3♂)	*I. thurstoni* (5♂)	*I. maculatus* (1♂)
Pedipalp / Carapace (L)	4.7–5.3	4.5–5.0	5.1–5.4	3.5–5.0	4.5
Telson (L/W)	4.0–4.5	3.5–4.4	4.4–4.7	3.8–4.1	–
Metasoma / Carapace (L)	5.1–5.8	6.0–7.2	7.4–7.6	6.3–7.1	7.6
Total length	**42.9–61.0**	**38.4–57.7**	**51.0–58.4**	**35.8-53.8**	**62.6**

Table 2. Comparison among *Isometrus* species based upon selected morphometric ratios of adults. Abbreviations: length (L), width (W).

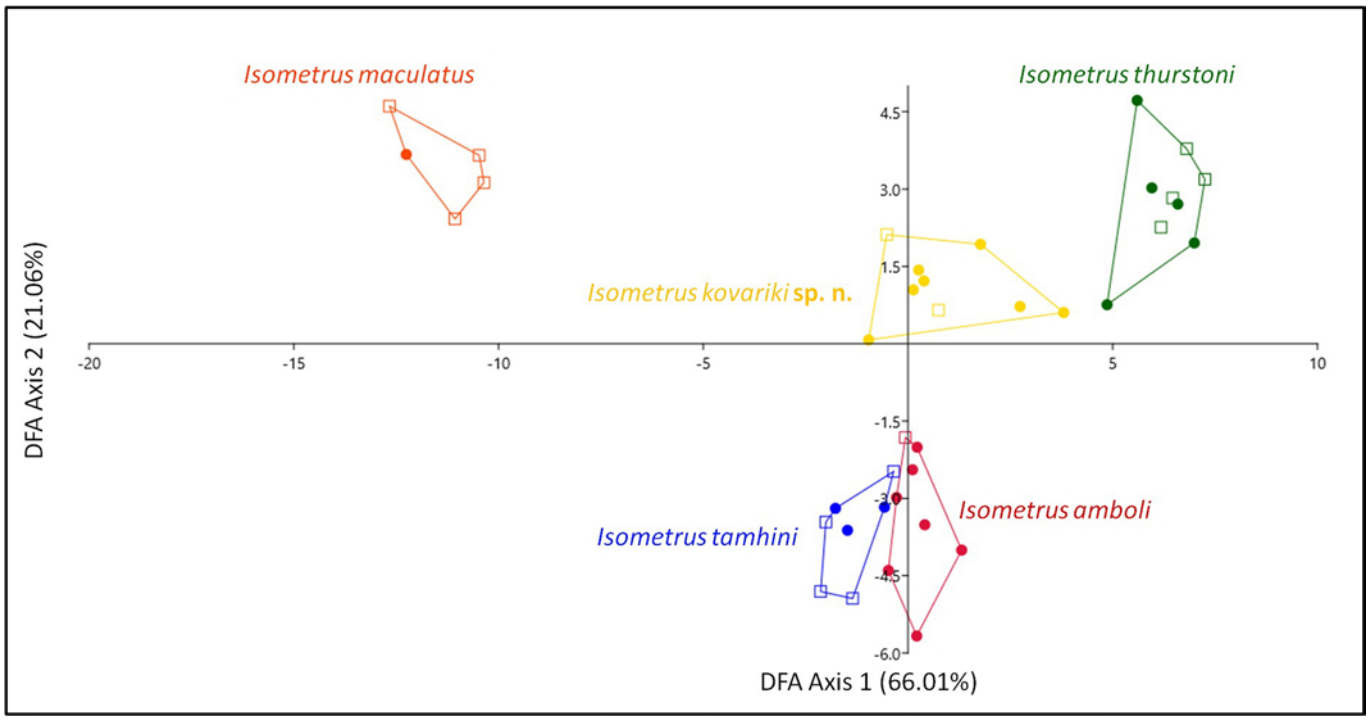


Figure 36. Discriminant function analyses projection on first two factor planes explaining 87.07% of variation among the five species. Abbreviations: Circle cells (Males), Square cells (Females).

Sexual Dimorphism. Male genital operculum partially exposed on posterior portion, from which a pair of small genital papillae is seen. In females the genital operculum is separated with a median suture covering the female genital orifice (Figs. 4, 11).

Affinities. *Isometrus kovariki* **sp. n.** differs from all other Indian species of *Isometrus* by a raw genetic distance of about 10 to 16 % (Table 3) (see below). It is distinguished from its congeners based on the following set of morphological characters:

1. Average total length larger in *Isometrus kovariki* **sp. n.** as opposed to *I. thurstoni*. Males (51.3 ± 6.1mm vs 43.0 ± 7.0mm), females (42.3 ± 1.9mm vs 39.6 ± 3.0mm) (Table 2).

2. Metasomal length to carapace length ratio in males 5.1–5.8 as against 6.3–7.1 in *I. thurstoni*, 7.4–7.6 in *I. tamhini*, 6.0–7.2 in *I. amboli* and 7.6 in *I. maculatus* (Table 2).

3. Carapace granular throughout with mixed granules, more closely granular in inter-ocular area and median posterior ocular area as opposed to sparsely granular with some areas without granules in *I. thurstoni* (Figs. 18–21).

4. Anterio-lateral margin of carapace curved near the lateral eyes as opposed to anterio-lateral margin of carapace sharply curved near the lateral eyes in *I. thurstoni* (Figs. 18–21).

5. Telson length greater than or equal to 4 times the telson width in males as opposed to less than or equal to 4 times in *I. thurstoni* (Table 2, Figs. 28–31).

6. Anterior margin of carapace with deep emargination in *Isometrus kovariki* **sp. n.** and *I. thurstoni* as opposed to anterior margin of carapace with shallow emargination in *I. tamhini* and *I. amboli* (Figs. 18–21).

7. Ventral median carina on vesicle weakly granular in *Isometrus kovariki* **sp. n.** and *I. thurstoni* as opposed to strongly granular in *I. tamhini* and *I. amboli* (Figs. 28–31).

All the diagnostic characters mentioned above are based on all the specimens collected and studied (including holotype and all paratypes).

Species	IM	ITH	ITM	IAM	IKV
Isometrus maculatus (IM)	0				
Isometrus thurstoni (ITH)	13.7-14.2	(0.2-0.5)			
Isometrus tamhini (ITM)	13.5-13.7	11.6-12.4	(0.2-1.2)		
Isometrus amboli (IAM)	13.4	13-13.2	6.6-7.4	(0-0.2)	
Isometrus kovariki **sp. n.** (IKV)	15.4-15.8	9.9-10.3	10.3-11.1	10.8-11	(0.0-0.9)

Table 3. Pairwise uncorrected raw distances (%) expressed as minimum–maximum based on COI gene sequence for *Isometrus* species. Values in brackets are intra-clade distances.

Species	Voucher	GeneBank Accession Number
Isometrus kovariki **sp. n**.	BNHSC SC 161	MT260062
Isometrus kovariki **sp. n**.	BNHS SC 162	MT260061
Isometrus kovariki **sp. n**.	INHER 146	MT260065
Isometrus kovariki **sp. n**.	INHER 149	MT260060
Isometrus amboli	INHER 161	MT260056
Isometrus tamhini	INHER 170	MT250512

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

Distribution, habitat and ecology. The new species is currently known only from the type locality in southern peninsular India. It is an approximately 10–15 year old *Acacia auriculiformis* plantation, on the outskirts of the Bengaluru city. In this area they were found to be most active during summer and used both the bark of the trees and leaf litter (in equal numbers) for ambushing their prey such as field cockroaches (*Blatella* sp.) and crickets. However during the rainy season they were largely found ambushing only on the trees. The scorpions are not active during winter (December, January); however, they start becoming active by the end of February. Live individuals brought and maintained in the lab of S.M. (at Azim Premji University) indicated that this species breeds in May (summer), and their clutch size ranges from 12–20 individuals (n=4). The juveniles dispersed after their first moult which occurred two days after birth. (Figs. 22, 23).

Statistical Analysis

Size corrected morphometric data was not significantly different from multivariate normal (Doornik and Hansen omnibus, within group Ep = 145.3, P <0.001). First four PCA factors with eigenvalues more that 1.0 explained 97.32% of variation among the species. The DFA using all the PCA factors as input resulted in 100% individuals being classified into their respective species. The three discriminant functions with eigenvalues greater than 1.0 explained 100% of variation among these species, all the species formed distinct clusters on the factor plane using the first two DFA axes (Fig. 36) (PCA data available from the authors) .

Genetic Analysis

Model selection suggested transition model with gamma distribution (TN93+G, ln*L* = –2360.8833, *df* = 53, *BIC* = 5230.40) as the best nucleotide substitution model. Maximum likelihood analysis indicated that *Isometrus kovariki* **sp**. **n**. is a monophyletic clade distinct from *I. maculatus*, *I. thurstoni*, *I. tamhini*, and *I. amboli* (Fig. 37). *I. kovariki* **sp**. **n**. differed from *I. maculatus* by a raw genetic distance of 15.4–15.8 %, from *I. thurstoni* by 9.9–10.3 %, from *I. tamhini* by 10.3–11.1 % and from *I. amboli* by 10.8–11 % (Table 3).

Discussion

Although the distribution records of *Isometrus maculatus* and *I. thurstoni*, based on old museum collections, are from multiple states in India (Kovařík, 2003), none of specimens from India collected by us in this study have matched these two species except for the collection of *I. thurstoni* from the type locality itself (Sulakhe et al., 2020). A wide distribution of a species with low dispersal ability has intrigued scientists for decades. It could be a result of human-mediated transport or it could be an evidence for cryptic species (Pérez-Portela et al., 2013). The two hypotheses are not mutually exclusive. Although some of the Indian fauna could have been distributed due to the extensive timber trade during British governance in India, it is also likely that a cosmopolitan species such as *I. maculatus* is actually a cryptic species, and the discovery of a new species (*I. kovariki* **sp**. **n**.) tends to suggest that. Future studies should focus on prevalence of *I. maculatus* and distribution of *I. thurstoni* in various other states in India with integrated taxonomic approach.

It is interesting to note that the population of *I. kovariki* **sp**. **n**. has been found only in a small area which has been modified by human activities. Such a restricted population in area of high disturbance is definitely threatened and thus needs immediate protection.

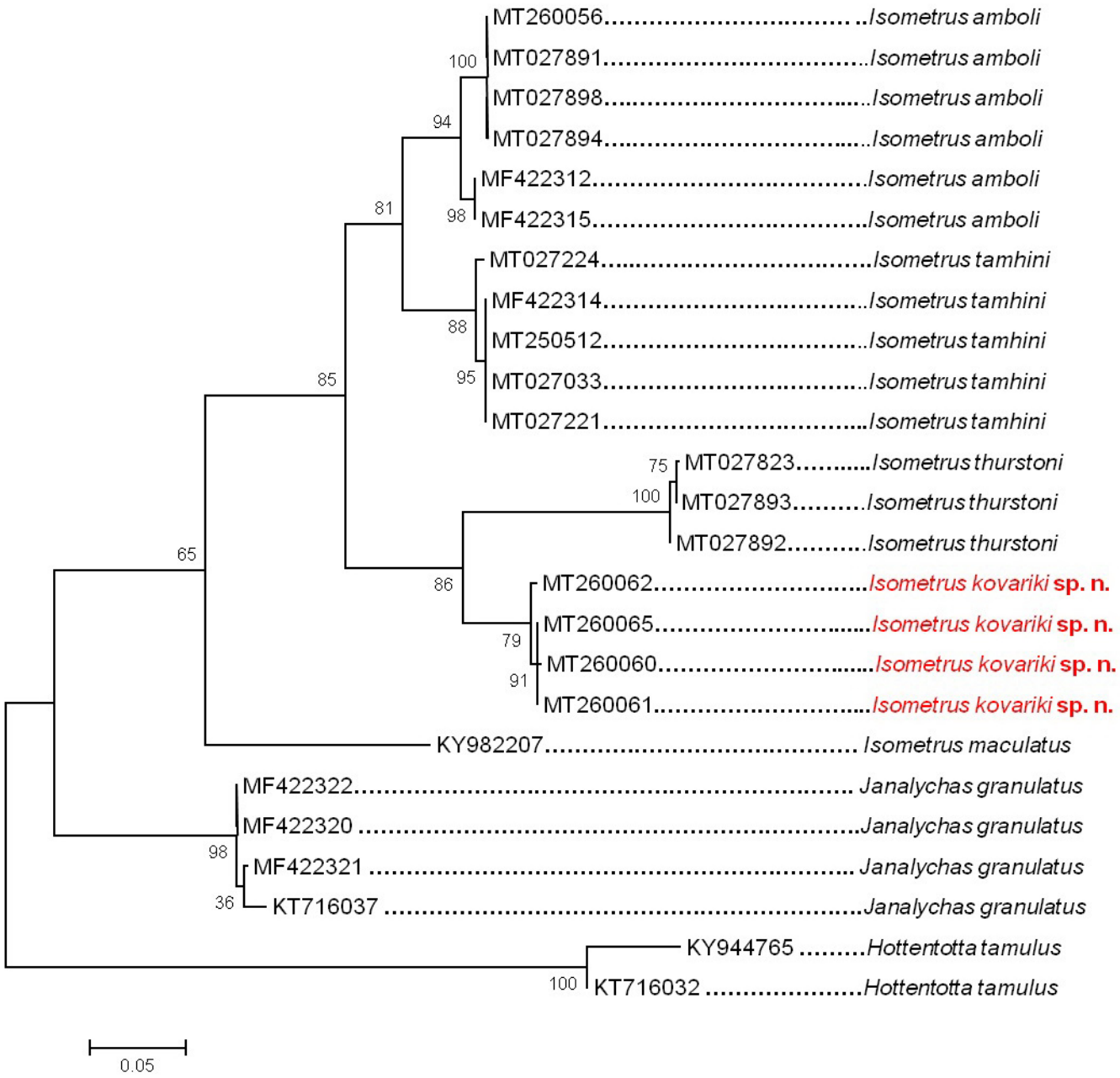


Figure 37. Maximum likelihood (ML) phylogeny of the Indian *Isometrus*. Values along the nodes are % bootstraps for 1000 iterations.

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References

DOORNIK, J. A. & H. HANSEN. 2008. An Omnibus Test for Univariate and Multivariate Normality. *Oxford Bulletin of Economics and Statistics*, 70: 927–939.

EDGAR, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32(5): 1792–1797.

FET, V. & G. LOWE. 2000. Family Buthidae C. L. Koch, 1837. Pp. 54–286 *in* Fet, V., W. D. Sissom, G. Lowe & M. E. Braunwalder. *Catalog of the Scorpions of the World (1758–1998)*. New York: New York Entomological Society, 690 pp.

FOLMER, O., M. BLACK, W. HOEH, R. LUTZ & R. VRIJENHOEK. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: 294–299.

HAMMER, Ø., D. A. T. HARPER & P. D. RYAN. 2001. PAST: Paleontological statistics software package for education and data analysis. *Paleontologia Electronica*, 4(1): 9 pp.

HJELLE, J. T. 1990. Anatomy and morphology. Pp. 9–63 *in* Polis, G. A. (ed.), *The Biology of Scorpions.* Stanford, California: Stanford University Press.

KOVAŘÍK, F. 1994. *Isometrus zideki* sp. n. from Malaysia and Indonesia, and a taxonomic position of *Isometrus formosus*, *I. thurstoni* and *I. sankariensis* (Arachnida: Scorpionida: Buthidae). *Acta Societatis Zoologicae Bohemicae*, 58: 195–203.

KOVAŘÍK, F. 2003. A review of the genus *Isometrus* Ehrenberg, 1828 (Scorpiones: Buthidae) with descriptions of four new species from Asia and Australia. *Euscorpius*, 10: 1–19.

KOVAŘÍK, F. & A. A. OJANGUREN AFFILASTRO. 2013. *Illustrated catalog of scorpions. Part II. Bothriuridae; Chaerilidae; Buthidae I. Genera* Compsobuthus, Hottentotta, Isometrus, Lychas, *and* Sassanidotus. Prague: Clairon Production, 400 pp.

NEI, M. & S. KUMAR. 2000. *Molecular Evolution and Phylogenetics.* New York, Oxford University Press.

PÉREZ-PORTELA, R., V. ARRANZ, M. RIUS & X. TURON. 2013. Cryptic speciation or global spread? The case of a cosmopolitan marine invertebrate with limited dispersal capabilities. *Scientific Reports*, 3: 3197.

POCOCK, R. I. 1893. Report upon a small collection of scorpions sent to the British Museum by Mr. Edgar Thurston, of The Government Central Museum, Madras. *Journal of the Bombay Branch of the Royal Asiatic Society*, 7(3): 297–312.

POCOCK, R. I. 1900. *Arachnida. The Fauna of British India*, *Including Ceylon and Burma*. Published under the authority of the Secretary of State for India in Council. London: W. T. Blandford, xii, 279 pp.

SCHWARZ, G. 1978. Estimating the dimension of a model. *Annals of Statistics*, 6: 461–464.

STAHNKE, H. L. 1971. Scorpion nomenclature and mensuration. *Entomological News*, 81: 297–316.

SULAKHE, S., N. DANDEKAR, A. PADHYE & D. BASTAWADE. 2020. Two new cryptic species of *Isometrus* (Scorpiones: Buthidae) from the northern Western Ghats, India. *Euscorpius*, 305: 1–24.

TAMURA, K., G. STECHER, D. PETERSON, A. FILIPSKI & S. KUMAR. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30: 2725–2729.

TIKADER, B. K. & D. B. BASTAWADE. 1983. Scorpions (Scorpionida: Arachnida). In *The Fauna of India*, *Vol. 3.* Calcutta: Zoological Survey of India, 671 pp.

VACHON, M. 1972. Remarques sur les scorpions appartenant au genre *Isometrus* H. et E. (Buthidae*)* à propos de l'espéce *Isometrus maculatus* (Geer) habitant l'île de Páques. *Cahiers Pacifique*, 16: 169–180.

VACHON, M. 1974. Etude des caractères utilisés pour classer les familles et les genres de Scorpions (Arachnides). 1. La trichobothriotaxie en arachnologie. Sigles trichobothriaux et types de trichobothriotaxie chez les Scorpions. *Bulletin du Muséum National d'Histoire Naturelle*, *Paris*, 140: 857–958.