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New Morphological and Molecular Data Reveal an Underestimation of Species Diversity of Mites of the Genus *Geckobia* (Acariformes: Pterygosomatidae) in India

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Abstract: Mites of the genus *Geckobia* (Acariformes: Pterygosomatidae) are permanent and highly specialised ectoparasites of geckos (Gekkota). We conducted a local study on *Geckobia* mites associated with the geckos of the family Gekkonidae found mainly in the territory of the Indian Institute of Science's campus (Bangalore, India). In total, we examined 208 lizards belonging to two genera: *Hemidactylus* and *Cnemaspis*. We assessed the prevalence of the mites and identified the preferred site for their infestation. We extended the standard morphological identification of the mite species by using DNA barcode markers, partial sequences of the mitochondrial cytochrome c oxidase subunit I (*COI*) gene and nuclear ribosomal gene sequences: 18S rRNA and hypervariable region D2 of nuclear 28S rRNA. We checked the suitability of *COI* and nuclear (D2 of 28S rRNA) markers for species delimitations and identification purposes of the genus. The distance- and phylogeny-based approaches were applied: (i) to test the presence of a barcoding gap, we used the automated barcoding gap discovery tool (ABGD) and investigated intra- and interspecific genetic distances, and (ii) to reconstruct evolutionary relationships within the species, we performed maximum likelihood (ML) and Bayesian inference with Markov-Chain Monte Carlo (BI) analyses. As a result, we described five new species—*Geckobia gigantea* sp. n., *G. treutleri* sp. n., *G. unica* sp. n. and *G. brevicephala* sp. n.—from four *Hemidactylus* species: *H. giganteus*, *H. treutleri*, *H. parvimaculatus* and *H. frenatus*, respectively, and *G. mysoriensis* sp. n. from *Cnemaspis mysoriensis*. Additionally, we found three already described species: *Geckobia indica* Hirst, 1917 on *H. treutleri* (new host), *Geckobia bataviensis* Vitzhum, 1926 on *H. parvimaculatus* (new host) and *H. frenatus* (new locality) and *Geckobia phillipinensis* Lawrence, 1953 on *H. frenatus* (new locality). The diagnoses of *G. indica* and *G. phillipinensis* were improved and supplemented by descriptions of the males and juveniles. Both topologies of the BI and ML phylogenetic trees, as well as genetic distances, supported the species boundaries in the mite population shown by the morphological data. *Hemidactylus frenatus* was the most infected gecko species (61% prevalence), with the highest number of mite species (three spp.). The scale-mite richness was higher than expected; therefore, further research is required to evaluate the true diversity of *Geckobia* mites.

Keywords: Acari; biodiversity; scale mites; *Hemidactylus*; species delimitation; barcoding; phylogeny; ABGD

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1. Introduction

Geckobia Mégnin, 1878 is the most species-rich genus belonging to the family Pterygosomatidae (Acariformes: Prostigmata). To date, there are over 80 valid species and subspecies associated with reptiles from all zoogeographical regions, except for Antarctica [1–4]. The range of their hosts comprises six lizard families (Gekkonidae, Phyllodactylidae, Diplodactylidae, Carphodactylidae, Eublepharidae and Liolamidae) and with one species, *Geckobia enigmatica* Bertrand and Pedrono, 1999, found on the *Astrochelys yuniphora* (Vaillant) (Testudinidae) [5].

All species of this genus are permanent and obligatory ectoparasites of reptiles and cannot survive in the off-host environment; therefore, they have evolved over diverse morphological specialisations that allow them to spend their entire life on the host's body (e.g., idiosoma, which is wider than it is longer and allows hiding beneath the scales; unequal legs directed forward and numerous idosomal setae, which play a role in fixing to the host's body). This strong dependency has led to the paradigm that scale mites are highly host-specific, being mono- or oligoxenous parasites, and co-evolved with their reptilian hosts [1]. However, this literature review [1] reveals the fragmentary examination of numerous host species (testing for mites in phylogenetically distant gecko species taken frequently from distant localities). Therefore, the meticulous checking for mites from closely related hosts may reveal that mites considered as oligoxenous, infesting different host species from distant localities, might be monoxenous (i.e., cryptic taxa) or stenoxenous species that infest closely related hosts.

Previous records of *Geckobia* mites include 23 species described from 13 species of Asian geckos, of which six species have been reported on three host species in India [1,6,7]. Recently, several new species of *Hemidactylus* (which are common hosts of *Geckobia* mites) have been described in India, and numerous species complexes have been identified, e.g., [8,9]. Because the distribution of *Geckobia* species on their hosts is highly host-specific, we suspect that the actual number of *Geckobia* species is much larger.

Despite their diversity and species richness, mites of the genus *Geckobia* are rarely targeted for biodiversity assessments because of serious taxonomic issues that have also been observed in other groups of mites [10]. The status of many species is uncertain due to synonymies [11–13], obscure morphological differences or vague species description, e.g., [14,15]. Moreover, immature life stages are excluded from many surveys as they are rarely collected or lack diagnostic morphological characteristics. The morphological identification of immature stages and males of closely related scale mites is a laborious task even for experienced taxonomists. In addition to all these challenges, there is a scarcity of taxonomic experts specialised in the morphological identification of pterygosomatid mites. Consequently, surveys have often been limited to new species' descriptions based on records from a single locality or host specimen, which prevents detailed assessments of the mite fauna, such as the examination of species turnover on the hosts in space or time.

Currently, *Geckobia* mites are arranged into five species groups based on the trochanter-tibia chaetotaxy of legs I–IV (i.e., *latasti*, *haplodactyli*, *ovambica*, *indica* and *simplex*) and into groups A and B based on differences in the tarsal chaetotaxy of leg I (see [3,6,16]). Nonetheless, approximately one-third of the species in this genus has not been assigned to any group because of their vague descriptions or unique morphological characteristics. So far, all species descriptions of *Geckobia* mites have been made solely based on external morphology. This was mostly dictated by the fact that the morphology-based investigations were carried out on pterygosomatids collected from museum host specimens caught at the turn of the 20th century and initially conserved in the industrial methylated spirit. In such materials, the DNA is often too highly degraded to undertake molecular-based investigation of the mites. Moreover, for most species, the gene sequence template for designing primers is not available; thus, they cannot be identified by molecular techniques. So far, more than 180 species of the family Pterygosomatidae have been identified in nature [1,17], whereas less than 10 species have molecular data available in GenBank.

Recently, the systematics of acariform mites have largely benefited from the ongoing development of molecular techniques. An integrative approach combining morphology and mitochondrial and nuclear sequences has been successfully used in delimiting species boundaries, e.g., [18], and discovering cryptic taxa, e.g., [19–21], but previous research has focused on phylogenetic studies of a few species or genera, e.g., [19,22]. Hence, the molecular data of *Geckobia* mites, which have not been included in any investigations of the mites before, hold much promise for documenting and understanding both the extent and patterns of species diversity by providing a transparent, consistent method for delineating species, which also allows the inclusion of all life stages and both sexes.

Therefore, this paper aims to (i) describe new species and conduct the first comprehensive assessment of diversity within the analysed population of *Geckobia* spp., using both morphological and molecular data; (ii) check the suitability of cytochrome c oxidase subunit I and nuclear markers for species delimitation and identification; (iii) assess the levels of genetic variability in the analysed population of *Geckobia* spp.; (iv) conduct preliminary analysis of the phylogenetic relationship of the analysed species; (v) assess the prevalence of mites and confirm or exclude the possibility of host switches between hosts living sympatrically in the Indian Institute of Science campus (IISc); and (vi) identify the preferred site of mite infestation.

2. Materials and Methods

Mite sampling. The mite material used in this study was obtained from the geckos collected from 13 September 2019 to 13 November 2019 in IISc from approximately 6.30 pm. to 11.30 p.m. The lizards (179 geckos) were kept in separate containers, and each specimen was identified to species following the key in [23] and the description presented by [24]. Additional lizards were checked for mites in the National Centre for Biological Sciences (NCBS) campus in Bangalore (Karnataka, India) on 29 November 2019 (20 geckos) and in Yerramaranahalli (Karnataka, India) on 12 November 2019 (9 geckos). All collected lizards were examined for mites, which were removed from the lizards under the stereo microscope LEICA M205 C. Mites infesting different regions of the host's body were counted to identify the preferred sites of their infestation and placed in small vials (2 mL) containing 96% ethyl alcohol. Then, the lizards were released in the place of their collection.

Morphological analysis. Some mite specimens were used a day after collection for DNA extraction, where the remaining specimens, before mounting in Hoyer's medium, were cleared and softened in Nesbitt's solution at +45 °C for 1–5 h. Then, all specimens (including exoskeletons left after DNA extraction) were mounted as vouchers, using Hoyer's medium on a glass slide using the standard method [25]. Fragments of the mites crushed during the extraction were also mounted for species identification. The mites were studied using a Leica DMD108 microscope. In the species descriptions, names of the leg and idiosomal setae followed [26,27] as described by [28], whereas those of the palpal setae followed [29]. Grandjean's nomenclatures [26,27,29] were applied to the family Pterygosomatidae by [30]. All measurements in the descriptions and values for scale bars in figures are presented in micrometres (μm), and data for the holotype are followed in brackets by the ranges for corresponding paratypes. The scientific names of lizards followed [24].

All specimens and vouchers were deposited in the mite collection of the Centre of Ecological Sciences (CES) in IISc, Bangalore, India.

DNA extraction and PCR amplification. Several mite specimens kept in 96% ethanol, before mounting on microscope slides, were subjected to DNA extraction using a DNeasy Blood and Tissue Kit (Qiagen), following a previously reported modified protocol described by [19].

The complete 18S rRNA (about 1.8 kb) was PCR-amplified in two overlapping fragments of approximately 950 and 1500 bp, each using primer pairs 18SF/rev960 and fw770/rev18d, respectively. The *COI* gene fragment (covering about 650 bp of the 5'-terminus of the *COI* gene) was amplified by PCR using the primers bcdF04 and bcdR04. The D2 region of the 28S rRNA gene (*c.* 900 bp) was amplified using the 28F0001 and 28R0990 primers (all primers are listed in Table 1).

PCR amplifications were conducted in 25 μL reaction volumes containing *Taq* reaction buffer B, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.25 μL of each primer, 1 U *Taq* polymerase (GeNei^{TM}, Bangalore, India) and 3 μL of DNA template using a thermocycling profile of 12 min at 95 °C followed by 35 cycles consisting of 95 °C for 15 s, 50 °C for 1 min and 72 °C for 1 min, with a final step of 7 min at 72 °C. After amplification, 2 μL of each PCR product was analysed by electrophoresis on a 1% agarose gel. Samples containing visible bands were purified with the QIAquick PCR & Gel Cleanup Kit (Qiagen). Purified PCR products were sent for sequencing to Barcode Biosciences, Bangalore.

DNA matrices and sequence alignments. Sequences of three gene (*COI*, 18S and 28S) fragments of *Geckobia* spp. representing eight morphospecies were blasted in GenBank and checked for possible contaminants. The sequence chromatograms were also checked for accuracy and edited using Chromas Lite 2.1.1 (Technelysium Pvt. Ltd., South Brisbane, QLD, Australia).

Alignments of the sequence data were prepared by the ClustalW algorithm in MEGA ver. 11.0.8 software with the default parameters [31]. The nucleotide sequences of *COI* were converted into amino acid sequences to check for sequencing errors and pseudogenes.

All sequences were deposited in GenBank under the accession number presented in Table 2.

Genetic distance, barcode gap discovery and species delimitation. Genetic distances were calculated for *COI*, 18S and 28S fragments using MEGA ver. 11.0.8 [31] under the Kimura two-parameter (K2P) model [32] for all codon positions. To calculate the genetic distances between populations of different genera, we used sequences of Pterygosomatidae previously deposited in GenBank (Table 2).

Additionally, the sequence data were analysed using Automatic Barcode Gap Discovery (ABGD) method to delimit the genetic clusters by detecting a significant gap in pairwise distance distribution [33]. We used the ABGB web server for performing the analysis (https://bioinfo.mnhn.fr/abi/public/abgd/, accessed on 30 January 2021), with the default settings and K2P distance model.

Phylogenetic analysis. Phylogenetic relationships among the studied taxa were estimated with two methods: maximum likelihood (ML) and Bayesian inference (BI). For the two likelihood-based methods, an appropriate model of DNA sequence evolution was determined using PartitionFinder v.1.1.1 [34]. As a result, for each codon position of *COI*, subsequent models were used: TrN + I, F81 + G and HKY. For nuclear data, K81uf + G was used as a sequence evolution model.

Table 1. Primers used in this study.

Primer	Sequence	Product	Source
bcdF04	TTTTCTACHAAYCAYAAAGATAT	COI	[19]
bcdR04	TATAAACYTCDGGATGNCCAAAAAA	COI	[35]
18Sfw	CTTGTCTCAAAGATTAAGCCATGCA	18S rDNA	[35]
rev960	GACGGTCCAAGAATTTCAC	18S rDNA	[35]
fw770	ACTTTGAAAAAATTAGAGTGC	18S rDNA	[35]
rev18S	TGATCCTTCCGCAGGTTCACCT	18S rDNA	[35]
28SF0001	ACCCVCYNAATTTAAGCATAT	28S rDNA	[21]
28SR0990	CCTTGGTCCGTGTTTCAAGAC	28S rDNA	[21]

Similar settings were used when analysing nuclear and mitochondrial data. ML was performed in raxmlGUI v.1.5 using the GRTGAMMA model, and a thorough bootstrap was carried out for 1000 reps with 10 ML searches [36]. The nodes supported by bootstrap values (BSP) $\geq$ 70% were considered strongly supported [37]. Bayesian analysis was performed in MrBayes v. 3.2.6. Each run of 5 million generations was sampled every 500 [38]. The value of the estimated sample sizes was checked in Tracer v. 1.6 to ensure appropriate chain length and to check for stationarity. The first 25% of the tress was discarded as "burn-in". Nodes supported by posterior probabilities (BPP) $\geq$ 95% were considered strongly supported [39]. Tree visualisations were prepared using FigTree ver. 1.4.3 [40].

Table 2. Mites and sequences used in this study.

Mite Species	Host Species	Sample ID			Accession No.			Reference
		COI	D2	18S	COI	D2	18S	
Geckobia gigantea sp.n.	*Hemidactylus giganteus*	–	88_28SF01	88_18SF	–	MZ824666	MZ666419	This study
		–	86_28SF01	86_18SF	–	MZ824665	MZ666420	
		–	87_28SF01	87_18SF	–	MZ824667	MZ666421	
		–	84_28SF01	84_18SF	–	MZ824668	MZ666422	
		–	1_28SF001	13_18SGF	–	MZ683418	MZ683429	
		–	2_28SF001	27_18SF	–	MZ683419	MZ683430	
		–	13_28SR0990	1_18SF	–	MZ683420	MZ683431	
Geckobia mysoriensis sp.n.	*Cnemaspis mysoriensis*	–	14_28SF001	2_18SF	–	MZ683421	MZ683432	This study
		15_bcdF04	15_28SF001	16_18SF	MZ682028	MZ683422	MZ683433	
		16_bcdF04	16_28SF001	14_18SF	AF142139	MZ683423	MZ683434	
		–	24_28SF001	24_18SF	–	MZ683424	MZ683435	
		–	47_28SF001	15_18SF	–	MZ683425	MZ683437	
		–	–	47_18SF	–	–	MZ683436	
Geckobia bataviensis Vitzhum, 1926	*Hemidactylus frenatus* *Hemidactylus parvimaculatus*	–	3_28SF001	3_18SF	–	MZ750859	MZ750870	This study
		–	9_28SF001	9_18SF	–	MZ750860	MZ750871	
		–	21_28SF001	21_18SF	–	MZ750861	MZ750872	
		–	23_28SF001	23_18SF	–	MZ750862	MZ750873	
		28_bcdF04	28_28SF0001	28_18SF	OK668315	MZ750863	MZ750874	
		–	40_28SF0001	40_18SF	–	MZ750864	MZ750875	
		–	42_28SF0001	42_18SF	–	MZ750865	MZ750876	
		–	57_28SF0001	57_18SF	–	MZ750866	MZ750877	
		58_bcdF04	58_28SF0001	59_18SF	OK647841	MZ750867	MZ750878	
		59_bcdF04	59_28SF0001	58_18SF	OK647840	MZ750868	MZ750879	
		–	78_28SF0001	78_18SREV	–	MZ750869	MZ750880	
		–	–	92_18SF	–	–	MZ750881	
Geckobia treutleri sp. n.	*Hemidactylus treutleri*	–	70_28SF001	70_18SF	–	OK256888	OK256880	This study
		–	73_28SF001	72_18SF	–	OK256887	OK256881	
Geckobia indica Hirst, 1917	*Hemidactylus treutleri*	71_bcdF04	71_28SF001	71_18SF	OK668260	OK256886	OK256878	This study
		79_bcdF04	79_28SF001	79_18SF	OK668316	OK256885	OK256879	
Geckobia phillipinensis Lawrence, 1953	*Hemidactylus frenatus*	–	60_28SR0990	51_18SF	–	OK360925	OK360926	This study
		–	100_28SF0001	60_18SF	–	OK360924	OK360928	
		–	–	99_18SF	–	–	OK360929	
		–	–	100_18SF	–	–	OK360927	
Geckobia unica sp. n.	*Hemidactylus parvimaculatus*	49_bcdF04	49_28S0001	49_18SF	OK626786	OK642373	OK642374	This study
Geckobia brevicephala sp. n.	*Hemidactylus frenatus*	–	4_28SF001	4_18SF	–	OL334759	OL334757	This study
		–	7_28SF001	7_18SF	–	OL334760	OL334758	
Pimeliaphilus hemidactyli Fajfer and Karanth, 2021		–	–	–	MT668542	MT669008		[41]
		–	–	–	MT668543	MT669007		
		–	–	–	MT668545	MT669009	MT669010	
		–	–	–	MT668541	MT669006		
		–	–	–	MT668544	MT669005		
Pterygosoma theobaldi Fajfer Melnikov and Dabert, 2016	*Phrynocephalus theobaldi*	–	–	–	KT962103	KT962106	–	[42]
Pterygosoma pallidum Fajfer Melnikov and Dabert, 2016	*Trapelus pallidus*	–	–	–	KT962104	KT962105	–	[42]
Pterygosoma parasiniatum Fajfer, Melnikov and Dabert, 2016	*Pseudotrapelus* cf. *sinaitus*	–	–	–	–	KT962107	–	[42]

3. Results

3.1. Species Composition

Eight species of the genus *Geckobia* were found on six gecko species based on morphological criteria. Five of the recorded mite species corresponded to new undescribed species: *Geckobia gigantea* sp. n., *Geckobia mysoriensis* sp. n., *Geckobia treutleri* sp. n., *Geckobia unica* sp. n. and *Geckobia brevicephala* sp. n. In addition, three already described species have been found: *Geckobia indica* Hirst, 1917, *Geckobia bataviensis* Vitzhum, 1926 and *Geckobia phillipinensis* Lawrence, 1953. The largest number of scale-mite species was hosted by

Hemidactylus frenatus (three spp.), whereas the remaining host species harboured their own scale-mite species (Table 3).

Table 3. Prevalence of *Geckobia* spp. in different species (specimens caught from September 2019 to November 2019).

Host Species:	No. of Checked Host Specimens:	No. of Infested Host Specimens	Prevalence (%)
		at IISc and NCBS, respectively:	
Hemidactylus frenatus Duméril and Bibron, 1836	72/12	44/7 [1]	61/58
H. parvimaculatus Deraniyagala, 1953	49/6	0/2 [2]	12/33
H. leschenaultii Duméril and Bibron, 1836	13/0	0/0 [2]	-
Cnemaspis mysoriensis (Jerdon, 1853)	45/2	21/0	47/0
		at Yerramaranahalli	
Hemidactylus treutleri Mahony, 2009	5	2 [3]	40
Hemidactylus giganteus Stoliczka, 1871	4	1	25

[1] *G. phillipinensis* was found on four host specimens together with *G. bataviensis* at IISc. [2] These species were infested also by *Pimeliaphilus* mites (see [41]). [3] *G. indica* and *G. treutleri* sp. n. were found on different host specimens.

3.2. Systematics

Family Pterygosomatidae Oudemans, 1910.
Genus *Geckobia* Mégnin, 1878.

3.2.1. Description

Geckobia gigantea sp. n. (Figures 1–5).

Female (holotype, range for nine paratypes). *Gnathosoma*. Chelicerae 85 (80–95) long. Swollen, proximal part of cheliceral base 45 (30–40) long and slender distal part 45 (45–55) long. Movable cheliceral digit three-pronged while fixed cheliceral digit spinous and approximately 5 (5–10) long. Palpal femur with thick plumose seta *dF* 15 (15–20) long; palpal genu with filiform smooth seta *dG*, 65 (50–60) long. Palpal tibia with three smooth setae (*dTi*, *l′Ti* and *l″Ti*) and slender curved claw. Palpal tarsi with four smooth setae. Subcapitular seta *n* filiform and smooth, about 45 (40–50) long. Each branch of peritremes with barely visible chambers 75 (70–85) long. Hypostome with ornamented apex (Figure 2b). *Idiosoma* 210 (155–260) long and 305 (295–385) wide. Dorsum (Figure 1). Propodonotal shield well outlined, 110 (85–115) long and 230 (220–270) wide, covered by minute punctations (Figure 2a). On propodonotal shield, small eyes situated on lateral margins present and 73 (73–81) stout and plumose setae, 10–20 long. These setae decrease in length from anterior to posterior part of propodonotal shield. Posterior to propodonotal shield, four rows of numerous stout and plumose setae (about 15) long present. These setae increase in length from anterior to posterior part of idiosoma (10–15 long) and resemble setae situated posteriorly on propodonotal shield. Postero-lateral and most posterior part of idiosoma with numerous flattened longer setae (20–50 long) that increase in length from anterior to posterior part of idiosoma; these setae are slightly serrate only at tip (as in Figure 2c). Venter (Figure 1b). Anterior part with numerous thick and plumose setae (10–15 long) and posterior part with about 60 pairs of thick and flattened setae (40–50 long). Genital region (Figure 1c). Genital setae represented by four pairs of slender blunt pointed setae *g1–g4*. Setae *g1* and *g2* about 20 long, *g3* about 10 long and *g4* about 30 long. Setae *g1–g3* situated medially on genital valves and setae *g4* situated laterally. Pseudanal series represented by 11 pairs of blunt-pointed smooth and flattened setae *ps1–ps11*, about 50 (40–55) long. *Legs*. Coxal setation: *1a*, *1b*, *2a*, *2b*, *3a*, *3b*, *4a*, *4b* and *4c* arranged in formula: 2–2–2–3. All coxal setae thick and plumose, except for filiform and smooth setae *1a* and *1b*. Two plumose setae present between coxal plates I and II. Leg chaetotaxy as follows: tibiae I–IV (5–5[4]–4–5), genua I–IV (1–0–0–1), femora I–IV (3–2–1–2) and trochanters I–IV (1–1–1–2). Setae *d′TiI–IV*, *d″TiI*, *d′TiIV*, *v′TiI–IV*, *v″TiI–IV*, *l′TiI–IV*, *lGI*, *lGIV*, *dlFI–IV* and *dFIV* filiform and smooth;

setae *d″FI*, *vFI–II*, *vTrI–IV* and *v″TrIV* filiform and serrate. Setation of tarsi I: 14 setae (*ft*, *tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *it′*, *it″*, *u′*, *u″*, *vs′*, *vs″* and *pl′*) and solenidion *ω1*; tarsi II: 10 setae (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*) and *ω1*; tarsi III and IV with 10 setae each (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*). Solenidion *ω1* (about 25 long) longer than seta *ft* (about 5 long). Setae *tc′*, *tc″*, *it′* and *it″* of leg I represented by euphatidia; *tc′* and *tc″* of legs II–IV, *u′*, *u″*, *vs′*, *vs″*, *a′*, *a″* and *pl′* of legs I–IV filiform.

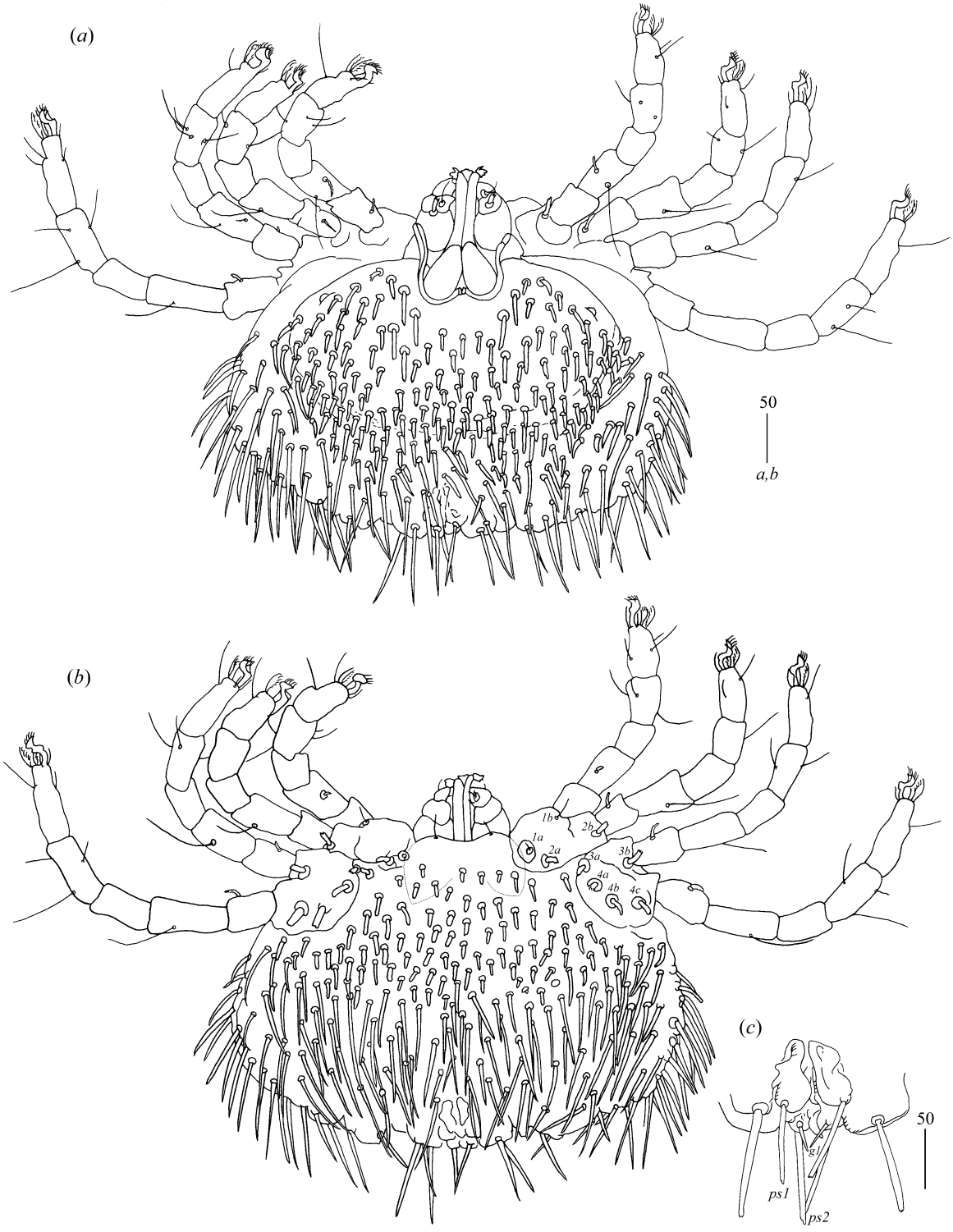

Figure 1. *Geckobia gigantea* sp. n. female: (**a**) in dorsal view; (**b**) in ventral view; (**c**) genital region, enlarged.

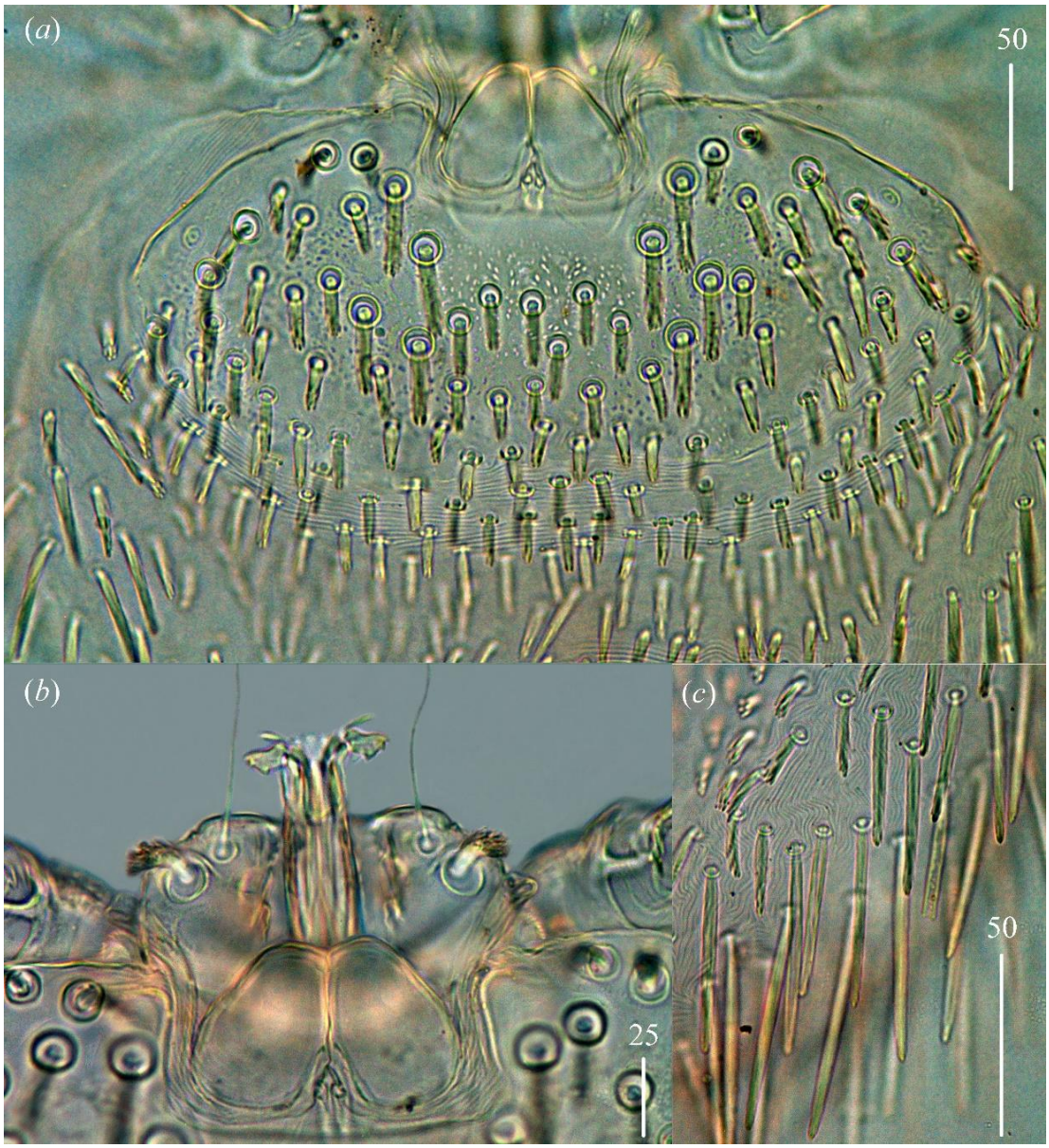

Figure 2. *Geckobia gigantea* sp. n., female, details: (**a**) propodonotal shield; (**b**), gnathosoma in dorsal view; (**c**) posterior dorsal setae.

MALE (range for two paratypes). *Gnathosoma* as in female. Chelicerae 80 long; swollen proximal part and slender distal part subequal in length, about 40 long. Fixed cheliceral digit about 5 long. Setae *dF* and *dG* 15 and 45 long, respectively. Subcapitular setae *n* 35 long. Each branch of peritremes 50 long. *Idiosoma* 150–155 long and 145–180 wide. Dorsum (Figure 3a). Propodonotal shield reniform, 45–55 long and 70 wide, with small eyes present on lateral margins and six plumose and thick setae: two pairs (15 long) present antero-laterally and four pairs (10–20 long) present postero-laterally. Posterior to propodonotal shield, 21–23 pairs of slightly plumose setae (10–40 long) present. These setae increase in length from anterior to posterior part of idiosoma. Venter (Figure 3b) with 18 short and plumose setae in antero-medial part, 5–10 long, and 29 thick and smooth setae in postero-lateral part, 20–40 long. Aedeagus 90 long, ended with spine-like structure. Most posteriorly situated one pair of spine-like setae about 10 long. Ano-genital opening covered with two curved spines, about 5 long. Laterally to aedeagus one pair of slightly serrate setae, 20 long, present. *Legs*. Coxal setation: *1a*, *1b*, *2a*, *2b*, *3a*, *3b* and *4a* arranged in formula: 2–2–2–1. All coxal setae thick and plumose, except for filiform and smooth setae *1a* and

1b. Leg chaetotaxy: tibiae I–IV (5–5–5[4]–5), genua I–IV (1–0–1–1), femora I–IV (3–1–1–2) and trochanters I–IV (1–1–1–1). Setae *d′TiI–IV*, *d″TiI–IV*, *lTiI–IV*, *v′TiI–IV*, *v″TiI*–IV, *l′GI*, *lGIII–IV*, *vFI*, *l′FI* and *vFIV* filiform and smooth; setae *l″FI* with barely discernible serration; setae *l″FII–FIV* slightly serrate and setae *lTrI–IV* filiform and smooth. Setation of tarsi I–IV as in female.

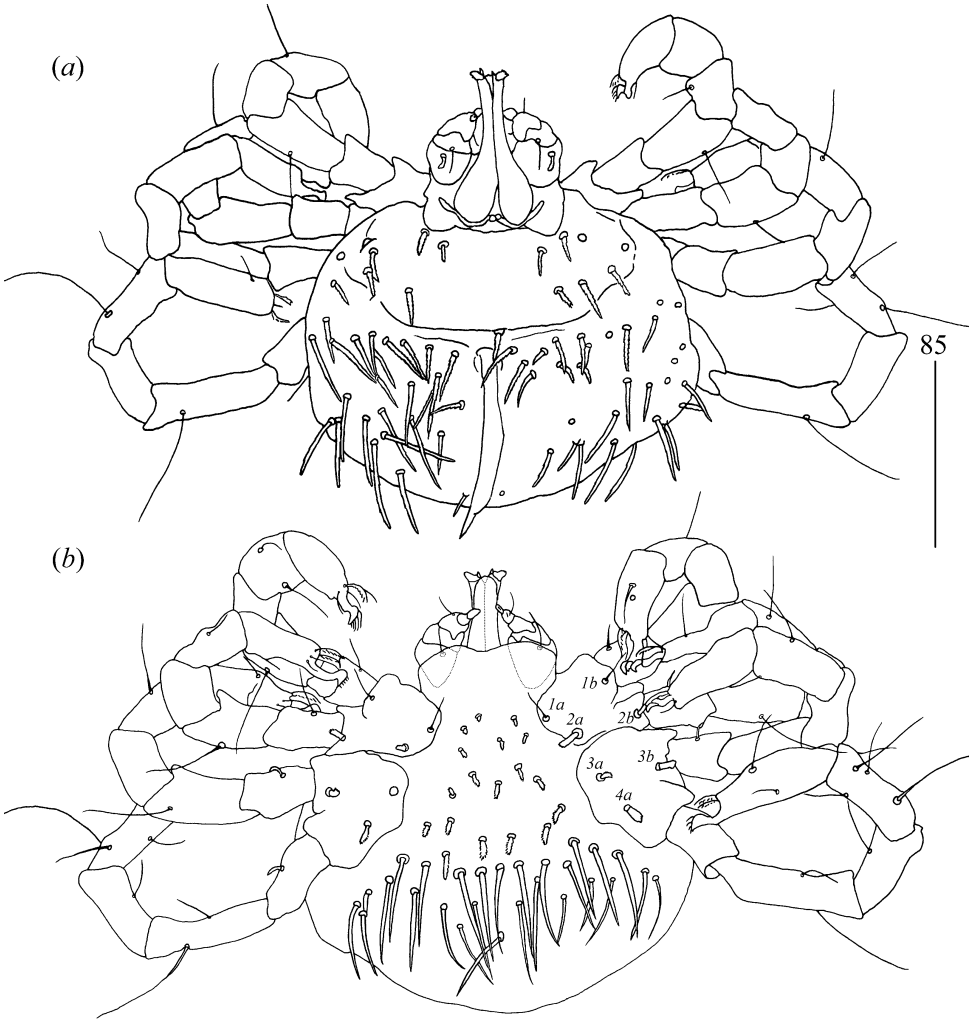

Figure 3. *Geckobia gigantea* sp. n., male: (**a**) in dorsal view; (**b**) in ventral view.

Deutonymph (one paratype). *Gnathosoma* as in female. Chelicerae about 45 long. Swollen proximal part of chelicerae 20 long, slender distal part 25 long. Fixed cheliceral digit spinous, 5 long. Palpal tibia and tarsi with smooth setae. Each branch of peritremes 50 long. *Idiosoma* 150 long and 200 wide. Dorsum. Propodonotal shield 150 wide and 40 long, punctuate, with 16 plumose setae, 15–20 long. Dorsum covered by setae resembling that of propodonotal shield, about 15 long. Posterior and posteo-lateral setae serrate and 35–40 long. Venter with 20 pairs of short (about 15 long) plumose setae situated in anterior half of idiosoma and 13 pairs of longer (about 40 long) slightly serrate setae situated in posterior half of idiosoma as in Figure 4. Genital region (Figure 5a). Genital setae slightly serrate, setae *g1* 20 long, *g2* and *g3* about 10 long each. Pseudanal setae *ps1–ps3* slightly serrate 30, 35 and 20 long, respectively. Additional unpaired seta *ps4* on one side of idiosoma present. *Legs*. Coxal setation: *1a*, *1b*, *2a*, *2b*, *3a*, *3b* and *4a* arranged in formula: 2–2–2–1. Coxal seta *1a* and *1b* filiform, *2a*, *2b*, *3a*, *3b* and *4a* spur-like and serrate. All coxae punctate. Chaetotaxy of trochanters-tibiae I–IV as in female, except for lack of setae *v″TrIV*. All setae of tibiae-trochanters I–IV filiform and smooth. Setation of tarsi I–IV as in female.

Figure 4. *Geckobia gigantea* sp. n. deutonymph in ventral view.

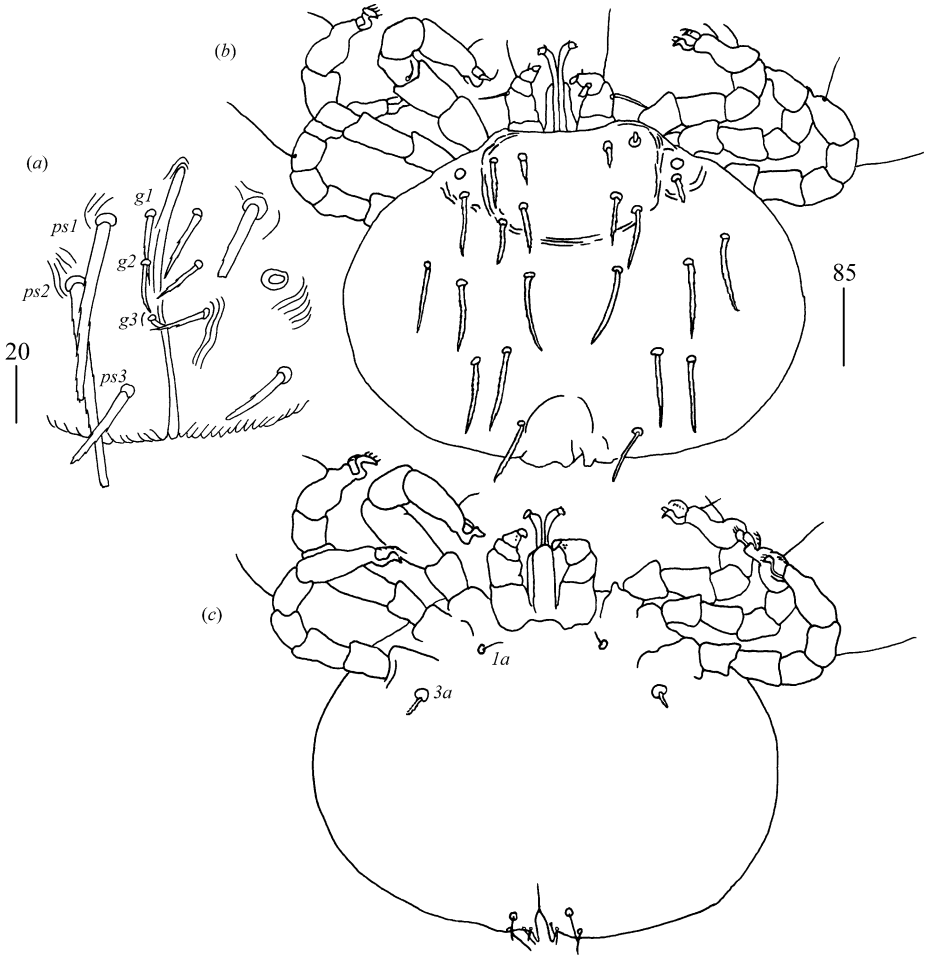

Figure 5. *Geckobia gigantea* sp. n.: (**a**) dorsal view of genital region of deutonymph; (**b**) larva in dorsal view; (**c**) larva in ventral view.

Larva (range for two paratypes). *Gnathosoma* as in female. Chelicerae 35 long. Swollen proximal part of chelicerae 15 long and slender distal part 20 long. Fixed cheliceral digit about 5 long. Setae *dF* slightly serrate, and 20 long and setae *dG* filiform and smooth, 25 long. Each branch of peritremes 30 long. Hypostome 30 long. *Idiosoma* 195–210 wide and 155–185 long. Dorsum with barely discernible punctate propodonotal shield 45 long and 70 wide and with 11 serrate setae (10–30 long) situated as in Figure 5b. Eyes present laterally to propodonotal shield. Venter devoid of any setation (Figure 5c). Genital region. Genital setal series represented by three pairs of filiform setae *g1–g3* 5–10 long; pseudanal setal series represented by two pairs of filiform pseudanal setae *ps1* and *ps2* with barely discernible serration. Setae *ps1* 10–15 long and setae *ps2* about 20 long. *Legs*. Coxal setation arranged in formulae: 2–0–1. Coxal setae *1a* filiform and smooth; *1b* filiform and slightly serrate; setae *3a* thick and serrate. Chaetotaxy of legs I–III as follows: tibiae I–III (5–4–4), genua I–III (1–0–0), femora I–III (3–2–1) and trochanters I–III (0–0–0). Setation of tarsi I–III as in female, except for lack of setae *p″* on tarsi I.

Type material.

Female holotype and paratypes: nine females, two males, one deutonymph and two larvae (CES19109) from *Hemidactylus giganteus* Stoliczka (Squamata: Gekkonidae) (no. CES19101), India, Karnataka, Yerramaranahalli, 13°32′55.4″ N, 77°39′18.5″ E, 12.11.2019, coll. P. Karanth.

Molecular data.

The D2 region of 28S rRNA of *G. gigantea* is 898 bp long and comprises four sequences represented by two haplotypes differing in terms of five nucleotide positions (0.04%, SD = 0.002, K2P). The 18S region of the rRNA is 1708 bp (two sequences) and 844 bp long (two sequences) and comprises four sequences represented by two haplotypes differing by two nucleotide positions (0.20%, SD = 0.001, K2P).

Etymology.

The species name is derived from the species name of the host.

Differential diagnosis.

This species is most similar to *Geckobia indica* Hirst, 1917 from *Hemidactylus gleadowi* Murray, India ("Upper Sind" according to original description of Hirst [43]) [10,40]. In both species, the propodonotal shield and eyes are present, the shape and arrangement of the dorsal and ventral setae are the same, palpal setae *dF* are thick and serrate and the setation of tarsi I–IV is the same. In *Geckobia gigantea* sp. n., the propodonotal shield has a rounded posterior part and 73–81 setae on the shield, which decrease in length from the anterior to posterior part; leg seta *dFIII* is absent, and setae *lGIV* and *v″TrIV* are present. In *G. indica*, the propodonotal shield is concave in its posterior part and possesses 34–46 setae, which increase in length from the anterior to posterior part of the shield; leg setae *dFIII* are present, and setae *lGIV* and *v″TrIV* are absent.

Geckobia mysoriensis sp. n. (Figures 6–11).

Female (holotype, range for eight paratypes). *Gnathosoma* (Figure 8a). Chelicerae 110 (105–110) long. Swollen proximal cheliceral part 40 (40–45) long and slender distal part 70 (70) long. Movable cheliceral digit three-pronged. Fixed cheliceral digit spinous, about 5 long. Palpal femur with serrate seta *dF*, 20 (20–25) long; palpal genu with smooth setae *dG*, 50 (50–55) long. Palpal tibia with three smooth setae: *l′Ti*, *l″Ti* and *vTi*. Palp tarsi with four filiform smooth setae. Supcapitular setae *n* serrate and 50 (50) long. Each peritremal branch about 80 long. *Idiosoma* 480 (395–490) long and 515 (445–520) wide. Dorsum covered by numerous serrate setae, 20–35 long, arranged as in Figure 6. These setae slightly increase from anterior to posterior part of idiosomal dorsum. Small eyes present. Venter (Figure 7) with numerous setae that cover all idiosoma except for most posterior part. These setae are less serrate than those on dorsum and 30–45 long. Genital series with one pair of filiform slightly serrate genital setae *g1*, about 20 long and 10–11 pairs of serrate pseudanal setae *ps* (in holotype, 10 setae *ps* present on left side and 11 pairs on right side of idiosoma), 35–55 long. *Legs*. Coxal setae as follows: 2–2–2–3. Setae *1a* and *1b* filiform and smooth *2a*, *2b*, *3a*, *3b*, *4a*, *4b* and *4c* thick and serrate apically. Setae of tibiae I–IV (5–5–5–5), genua I–IV

(1–0–0–1), femora I–IV (3–2–2–2) and trochanters I–IV (1–1–1–1). Setae *vTrI–IV* thick and serrate, *vFI–IV* short and serrate, *dFI* and *lFI–IV* filiform and slightly serrate, *lGI* and *lGIV* with barely discernible serration. Setae *v′TiI–IV*, *v″TiI–IV*, *d′TiI–IVd″TiI–IV* and *l′TiI–IV* smooth. Setation of tarsi I: 14 setae (*ft*, *tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *it′*, *it″*, *u′*, *u″*, *vs′*, *vs″* and *pl′*) and solenidion *ω1*; tarsi II: 10 setae (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*) and *ω1*; tarsi III and IV with 10 setae each (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*). Setae *it′*, *it″*, *tc′* and *tc″* of legs I in form of euphatidia. Setae *pl′* smooth, setae *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″* serrate. Setae *ft* smooth, about 5 long.

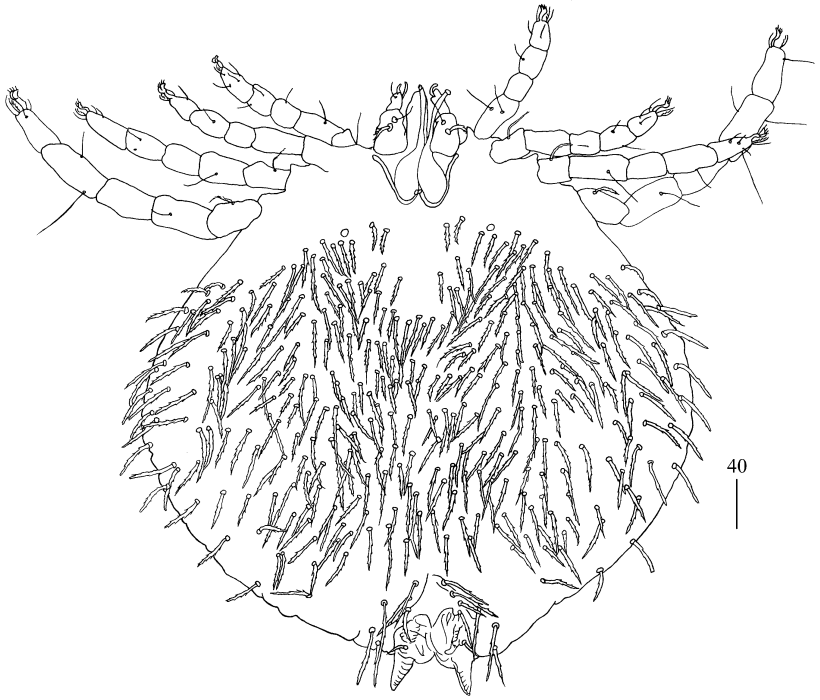

Figure 6. *Geckobia mysoriensis* sp. n. female in dorsal view.

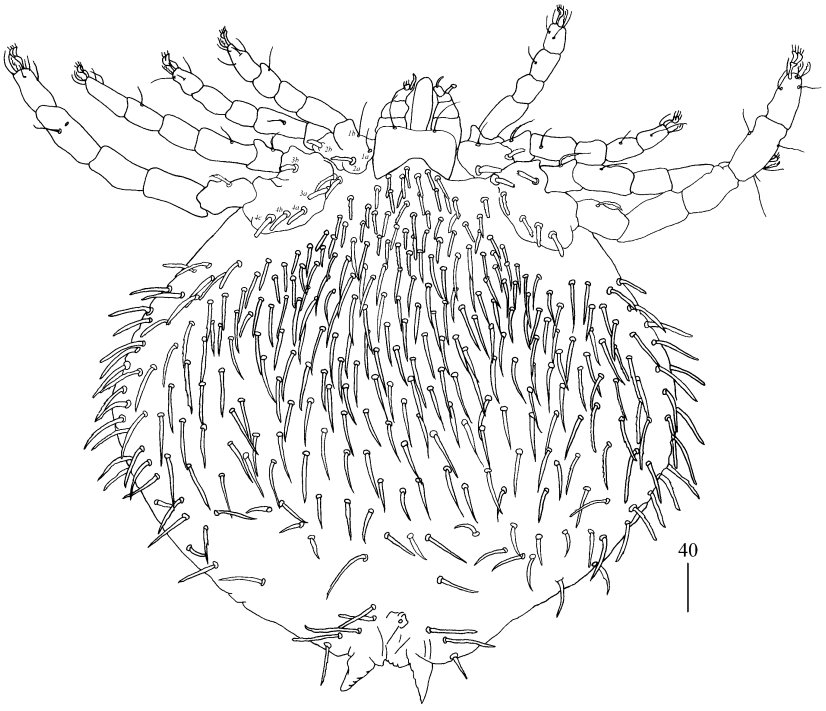

Figure 7. *Geckobia mysoriensis* sp. n. female in ventral view.

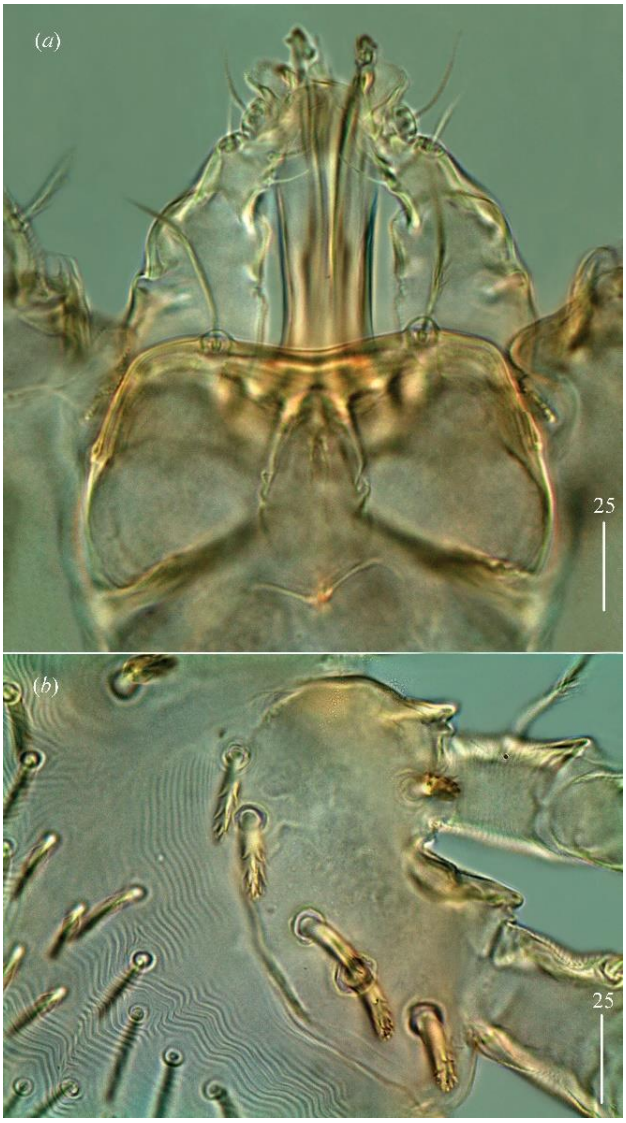

Figure 8. *Geckobia mysoriensis* sp. n. female, details: (**a**) gnathosoma in ventral view; (**b**) coxae III and IV.

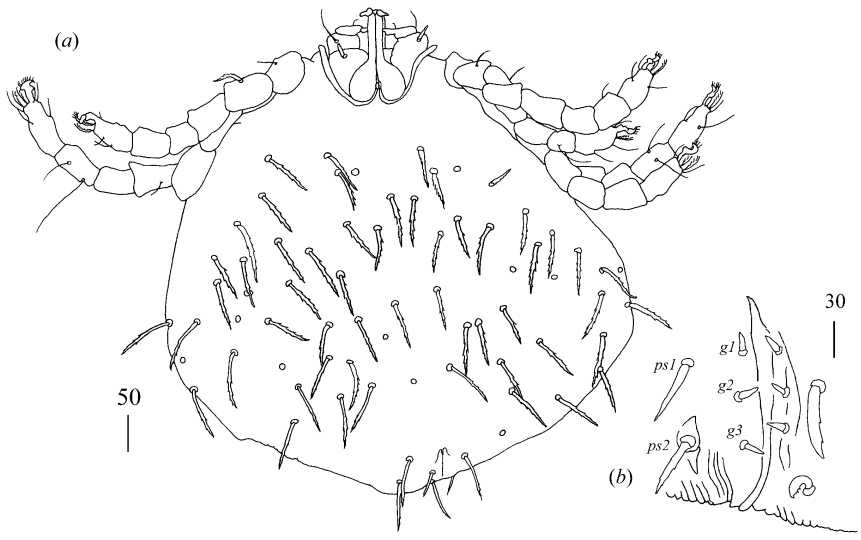

Figure 9. *Geckobia mysoriensis* sp. n.: (**a**) deutonymph in dorsal view; (**b**) genital region of larva.

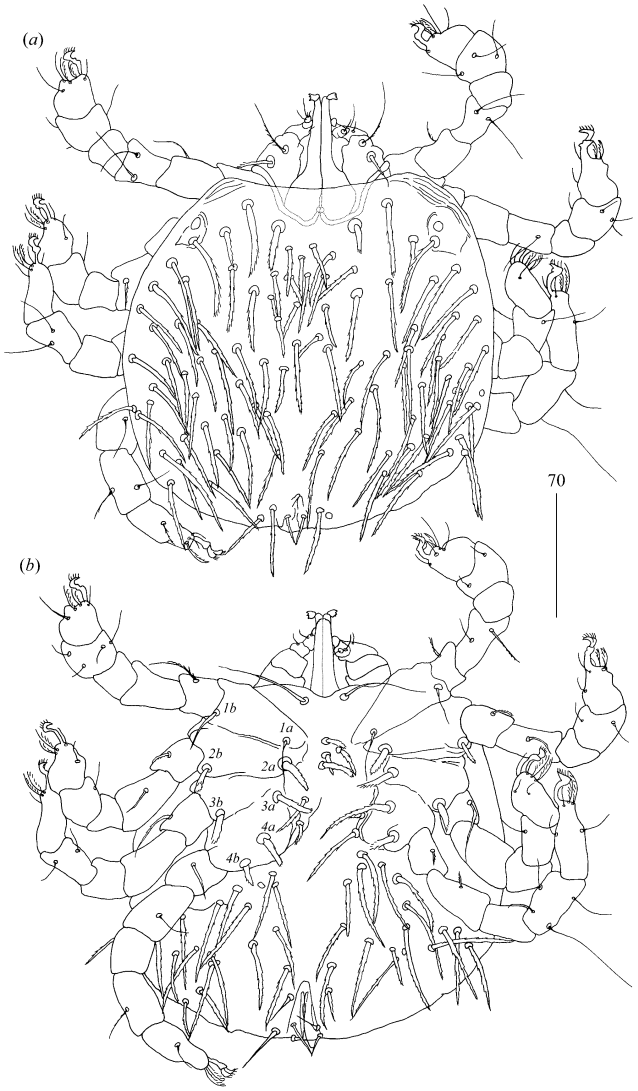

Figure 10. *Geckobia mysoriensis* sp. n. protonymph: (**a**) in dorsal view; (**b**) in ventral view.

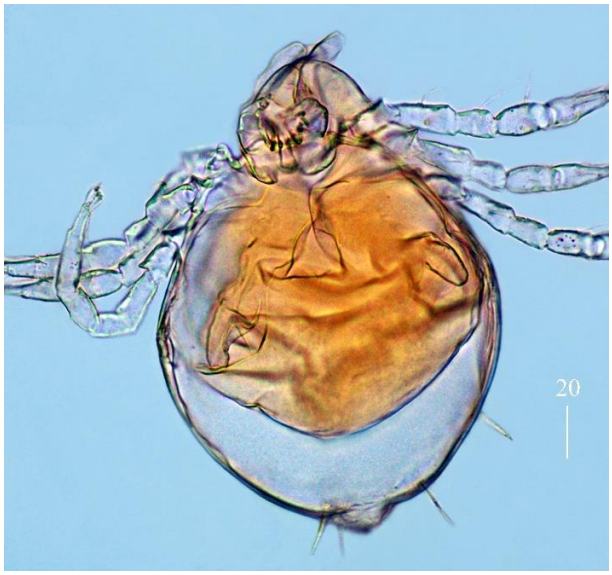

Figure 11. *Geckobia mysoriensis* sp. n. larva with forming chrysalis, dorsal view.

Deutonymph (one paratype). *Gnathosoma* as in female. Chelicerae 70 long. Swollen cheliceral part 20 long and slender cheliceral part 50 long. Setae *dF* thick and serrate, 25–30 long; setae *dG* filiform with barely discernible serration, about 30 long. Each peritremal branch about 45 long. Subcapitular setae *n* 20 long. *Idiosoma* 310–335 wide and 300–315 long. Dorsum with about 30 pairs of dorsal serrate setae (30–40 long) arranged as in Figure 9a. Venter with five–six short setae, 10–20 long, situated anteriorly and longer setae, about 35 long, situated on remaining part of idiosomal venter, except for most posterior part. Genital region (Figure 9b) with three genital setae *g1–g3* 10–15 long and three pseudanal setae *ps1–ps3*. Setae *ps1* 35 long, *ps2* 30 long and *ps3* 20 long. *Legs*. Coxal setae as follows: 2–2–2–2. Coxal setae *1a* and *1b* filiform with barely visible serration, *2a*, *2b*, *3a*, *3b*, *4a* and *4b* thick and serrate. Setae of tibiae-trochanters I–IV as in female except for lack of setae *lGI* and *lFIII*.

Protonymph (one paratype). *Gnathosoma* as in female. Chelicerae 70 long. Swollen proximal part of chelicerae 30 long and slender distal part about 40 long. Setae *dF* and *dG* filiform and serrate, about 35 long. Subcapitular setae *n* slightly serrate, about 45 long. Peritremes about 55 long. *Idiosoma* 165–195 wide and 185–190 long. Dorsum with numerous serrate setae 25–30 long situated as in Figure 10a. Venter (Figure 10b) with four short serrate setae situated anteriorly (10–15 long) and numerous longer setae situated in posterior half of idosomal venter, 25–30 long. Genital region with three setae *g1–g3*, about 15 long, and three pseudanal setae *ps1–ps3*. Setae *ps1* 15–20 long, *ps2* and *ps3* 20–35 long. *Legs*. Coxal setae as follows: 2–2–2–2. Setae *1a* and *1b* filiform and slightly serrate, setae *2a*, *2b*, *3a*, *3b*, *4a* and *4b* plumose, thick and spur-like. Setae of tibiae-trochanters I–IV as in female except for lack of setae *lGI*, *lGIV* and *lFII–III*.

Larva (range for five paratypes). *Gnathosoma* as in female. Chelicerae about 55 long. Slender proximal part about 30 long and swollen distal part 25–30 long. Fixed cheliceral digit 5 long. Setae *dF* slightly serrate,15 long, setae *dG* filiform and smooth 20 long. Each branch of peritremes 65–70 long. *Idiosoma* 145 long and 190 wide. Dorsum with barely discernible propodonotal shield 50 long and 70 wide. On propodonotal shield, four pairs of setae present; setae situated most laterally 20 long, setae situated medially 15 long. Additional six serrate setae, 20–35 long on the remaining part of idiosoma present. Eyes present laterally to propodonotal shield. Genital area with two pairs of genital setae *g1* and *g2* and three pairs of pseudanal setae *ps1–ps3*. Setae *g1* and *g2* 5–10 long, *ps1–ps3* 15–20 long. *Legs*. Coxae in formula: 2–1–1. Coxae *1a*, *1b* and *2b* filiform and smooth, setae *3a* spur-like and serrate. Setae of tibiae-femora I–III as in female, except for lack of setae *v'TrI–III*.

Type material examined.

Female holotype and paratypes: two females, one deutonymph, one protonymph and five larvae (CES19110) from *Cnemaspis mysoriensis* (Jerdon) (Squamata: Gekkonidae), India, Karnataka state, Bangalore, IISc, 19.09.2019, coll. Achyuthan Srikanthan; six female paratypes from same host species and locality, 17.10.2019, coll. Achyuthan Srikanthan, two females from the same host, India, Karnataka state, Bangalore, IISc, 09.10.2019, coll. Caleb Daniel.

Molecular data.

The *COI* sequence data of 646 bp were generated from two females. Both specimens shared the same *COI* haplotype. The alignment of the hypervariable D2 region of the nuclear 28S rRNA of *G. mysoriensis* is 909 bp long and comprises eight sequences represented by one haplotype. The 18S region od rRNA is 1681 bp long and comprises nine sequences represented by one haplotype.

Etymology.

The species name is derived from the species name of the host.

Differential diagnosis.

This species is most similar to *Geckobia uenoi* from *Eublepharis splendens* Nakamura and Ueno, 1959 (Squamata: Eublepharidae) from the Tokunoshima Island [14,44]. In both species, the body is almost circular, and the dorsal setae slightly increase in length from the anterior to posterior part of the idiosoma. The arrangement of the idiosomal setae and

setation of tibia–coxae I–IV are the same. *Geckobia mysoriensis* sp. n. differs from *G. uenoi* in terms of the presence of serrate subcapitular setae *n*, densely serrate dorsal setae with long ciliations, the absence of the propodonotal shield and lack of coxal setae *4c*. In *G. uenoi*, the subcapitular setae *n* are smooth, the dorsal setae are slightly serrate with short ciliations, the propodonotal shield is present and coxal setae *4c* are absent.

Remarks. For the first time, an active protonymph in a species of the genus *Geckobia* has been observed. The protonymph (sample ID: 14_28_SF001 and 14_18SF) and deutonymph (sample ID: 24_SF001 and 24_SF001) of the species are represented by the same haplotype, whereas, simultaneously, the significant morphological differences between the mite stages are observed (e.g., size of idiosoma, shape of setae *dF* or leg chaetotaxy pattern). These findings suggest that the protonymph can be either an active or inactive feeding stage depending on the *Geckobia* species. So far, only in mites of the genus *Pterygosoma* and *Neopterygosoma* have active protonymphs been frequently found, e.g., [17], whereas in the remaining pterygosomatids they are commonly represented by inactive forms.

Geckobia treutleri sp. n. (Figure 12).

Female (holotype, range for one paratype). *Gnathosoma*. Chelicerae 130 (135) long; swollen cheliceral part 50 (55) long and slender distal part 80 (80) long. Movable cheliceral digit three-pronged. Fixed cheliceral digit spinous, 5 (5) long. Setae *dF* and *dG* filiform and smooth, 55 (50) and 65 (65) long, respectively. Subcapitular seta *n* filiform and smooth, 60 (65) long. Each branch of peritremes 85 (85) long. *Idiosoma* 320 (295) long and 350 (325) wide. Propodonotal shield absent. Small eyes present laterally. Dorsum with numerous serrate setae, 30–50 (25–50) long, distributed as in Figure 12a. Venter (Figure 12b) with about 28 short and plumose setae in anterior part, 10–15(10–20) long and longer serrate setae, 25–50 (30–50) long in posterior part. Genital series represented by one slightly serrate seta *g1* and seven serrate pseudanal setae *ps1–ps7*. Seta *g1* 20–25 (25) long; setae *ps1–ps7* 45 (50), 40 (40), 30 (35), 20 (30), 20 (25), 30 (30) and 25 (25) long, respectively. Coxae in formula: 2–2–2–2. Setae *1a* and *1b* filiform and smooth, setae *2a*, *2b*, *3a*, *3b*, *4a*, *4b* spur-like and serrate at tip. Setae of tibiae I–IV (5–5–5–5), genua I–IV (0–0–0–1), femora I–IV (2–1–1–1), trochanters I–IV (1–1–1–1[0]). All setae of trochanter–genua I–IV filiform and smooth. Setation of tarsi I: 14 setae (*ft*, *tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *it′*, *it″*, *u′*, *u″*, *vs′*, *vs″* and *pl′*) and solenidion *ω1*; tarsi II: 10 setae (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*) and *ω1*; tarsi III and IV with 10 setae each (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*). Setae *it′*, *it″*, *tc′* and *tc″* of legs I in form of euphatidia. Setae *pl′* smooth, setae *tc′* and *tc″* of legs II–IV and all setae *vs′*, *vs″*, *a′*, *a″* slightly serrate. Setae *ft* smooth and about 5 long. Solenidion *ω1* of legs I about 25 long.

Type material.

Female holotype and one female paratype (CES19111) from *Hemidactylus treutleri* Mahony, 2009 (under toepads), (Squamata: Gekkonidae) (CES19102), India, Karnataka, Yerramaranahalli, 13°32′55.4″ N, 77°39′18.5″ E, 12.11.2019, coll. P. Karanth.

Molecular data.

The alignment of the D2 region of 28rRNA of *G. treutleri* is 927 bp long and comprises two sequences represented by two haplotypes differing in terms of three nucleotide positions (0.02%, SD = 0.001, K2P). The 18S region of rRNA was approximately 850 bp long and comprises two sequences represented by two haplotypes differing in one nucleotide position (0.12%, SD = 0.001).

Etymology.

The species name is derived from the species name of the host.

Differential diagnosis.

This species is most similar to *Geckobia keegani* Lawrence 1953 from *Hemidactylus frenatus* on Philippine Island [45], Australia [46] and Costa Rica [47]. In both species, the idiosoma is circular; the dorsal and ventral setae have the same general shape; the setation of tibiae I–IV, genua I–III, femora I–IV and trochanters I–IV is the same and legs I–IV are subequal in length. *G. treutleri* differs from *G. keegani* by the presence of smaller idiosoma (320 long and 350 wide), setae in the posterior part of the idiosoma, one pair of genital

setae, seta *lGIV* and the absence of the propodonotal shield. In *G. keegani*, the idiosoma is bigger (513–616 long and 496–630 wide), setae in the posterior part of idiosoma are absent, four pairs of genital setae are present, seta *lGIV* is absent and the propodonotal shield is present.

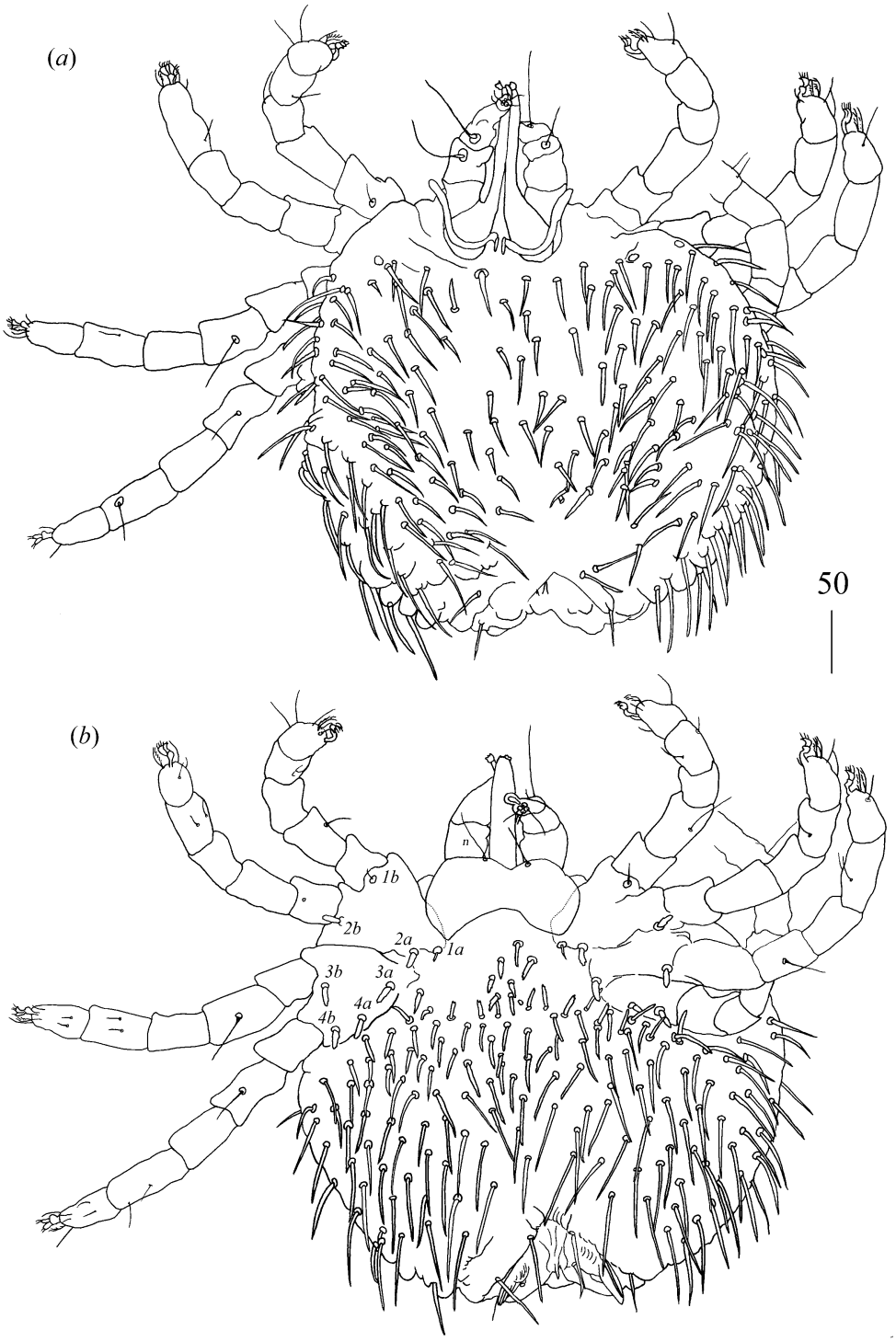

Figure 12. *Geckobia treutleri* sp. n. female: (**a**) in dorsal view; (**b**) in ventral view. Scale bar represents μm.

Geckobia unica sp. n. (Figure 13).

Female (holotype). *Gnathosoma*. Chelicerae 80 long. Swollen, proximal part of cheliceral base and slender distal part subequal in length, 40 long. Movable cheliceral digit three-pronged. Fixed cheliceral digit spinous, about 5 long. Palpal femur with filiform

serrate setae *dF* and *dG* 40 and 55 long, respectively. Setae *dF* only slightly thicker than setae *dG*. Palpal tibia with three smooth setae (*dTi*, *l′Ti* and *l″Ti*) and curved claw. Palpal tarsi with four smooth setae. Subcapitular seta *n* filiform and serrate, about 35 long. Each branch of peritremes with barely visible chambers about 75 long. Hypostome with ornamented apex. *Idiosoma* 340 long and 335 wide. Dorsum. Propodonotal shield well outlined, reniform, 110 long and 195 wide, sparsely punctate but in some parts covered by small unsclerotized lacunae (as in Figure 13a). On propodonotal shield, small eyes situated on lateral margins and 34 stout and plumose setae present. Setae situated antero-medially 25–30 long; setae situated posteriorly and laterally 35–40 long. Posterior to propodonotal shield longer setae (40 long) that decrease in length to median part of idiosoma (25–35 long) and then increase in length in the posterior part (40–55 long). Postero-lateral parts and most posterior part with numerous slightly serrate (50–60 long) and blunt-pointed setae. Venter (Figure 13b) with 26 short and plumose setae (10–15 long) situated anteriorly and numerous slightly flattened setae (60 long) with slightly serrated tip situated posteriorly. Genital region. Genital setae represented by four pairs of setae *g1–g4*. Setae *g1–g3* slightly serrate and 50, 45 and 20 long, respectively. Setae *g4* (30 long) thinner than *g1–g3* and situated on valvae. Pseudanal setal series represented by nine setae *ps* about: 75, 35, 40, 55, 60, 55, 45, 60 and 55 long, respectively, *Legs*. Coxal setation: *1a*, *1b*, *2a*, *2b*, *3a*, *3b*, *4a*, *4b* and *4c* arranged in formula: 2–2–2–3. All coxal setae thick and plumose, except for filiform, slightly serrate setae*1a* and *1b*. Leg chaetotaxy as follows: tibiae I–IV (5–5–5–5), genua I–IV (1 + k–0–1[0]–0), femora I–IV (3–2–2–2) and trochanters I–IV (1–1–1–2). Setae *d′TiI–IV*, *d″TiI*, *v′TiI–IV*, *v″TiI–IV*, *l′TiI–IV*, *vFI–II*, *lGI* and *lGIII* filiform and smooth; setae *l′FI–IV* filiform and slightly serrate; setae *vFIII* and *vFIV* thick and serrate, and *TrI–IV* filiform and serrate. Setation of tarsi I: 14 setae (*ft*, *tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *it′*, *it″*, *u′*, *u″*, *vs′*, *vs″* and *pl′*) and solenidion *ω1*; tarsi II: 10 setae (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*) and *ω1*; tarsi III and IV with 10 setae each (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*). Solendion *ω1* (about 25 long) of legs I longer than seta *ft* (about 5 long). Setae *tc′*, *tc″*, *it′*, *it″* of leg I represented by euphatidia; *tc′* and *tc″* of legs II–IV, *u′*, *u″*, *vs′*, *vs″*, *a′*, *a″* and *pl′* of legs I–IV filiform.

Type material.

Female holotype (CES19114) from *Hemidactylus* cf. *parvimaculatus* India, Karnataka state, Bangalore, NCBS campus, 30 October 2019, coll. Chaitanya R.

Molecular data.

The *COI* sequence data of 639 bp is generated from the holotype female. The D2 alignment of *G. unica* is 918 bp long, and the 18S region of rDNA is 1680 bp.

Etymology.

The species name is derived from the Latin adjective "*unique*" which means "one, uncommon" and refers to unique setation of mite's trochanter IV.

Differential diagnosis

This species is most similar to *G. bataviensis* Vitzthum, 1926 from *Hemidactylus frenatus* [48]. In both species, the idiosoma is almost circular; the propodonotal shield and the eyes are present; dorsal and ventral setae are plumose; palpal setae *dF* and *dG* are serrate; the chaetotaxy of coxae I–IV, tibiae I–IV and femora I–IV is the same, and four genital setae are present. In *G. unica* sp. n., the propodonotal shield is punctate, reniform and convex in its posterior margin; 34 setae are present on the propodonotal shield; dorsal setae decrease in length to the median part of the idiosoma and then increase in length in the posterior part of the idiosoma; subcapitular setae *n* are serrate, and leg setae *lGIII* and *lTrIV* are present. In *G. bataviensis*, the propodonotal shield is smooth and concave in its posterior margin, 40 setae are present on the propodonotal shield, the dorsal setae increase in length posteriorly, subscapular setae *n* has barely discernible serration and setae *lGIII* and *lTrIV* are absent.

Geckobia brevicephala sp. n. (Figures 14 and 15).

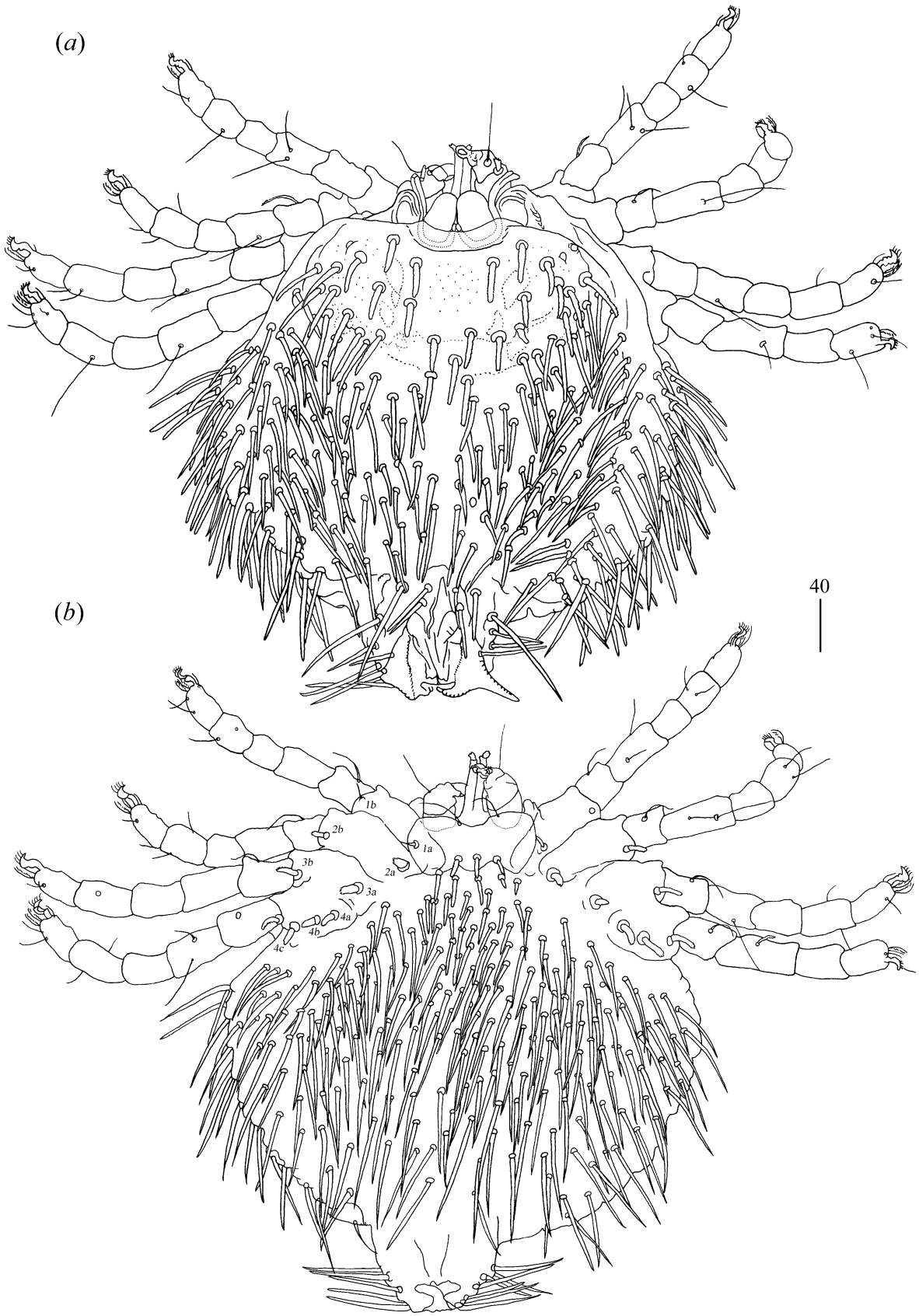

Figure 13. *Geckobia unica* sp. n. female: (**a**) in dorsal view; (**b**) in ventral view.

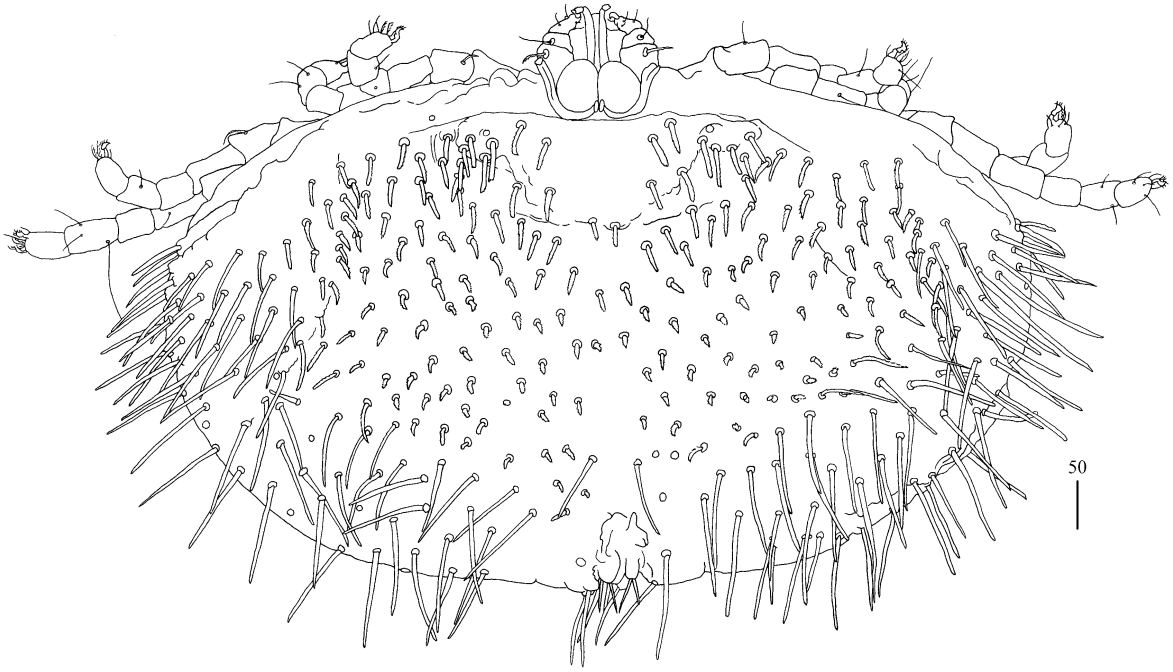

Figure 14. *Geckobia brevicephala* sp. n. female in dorsal view.

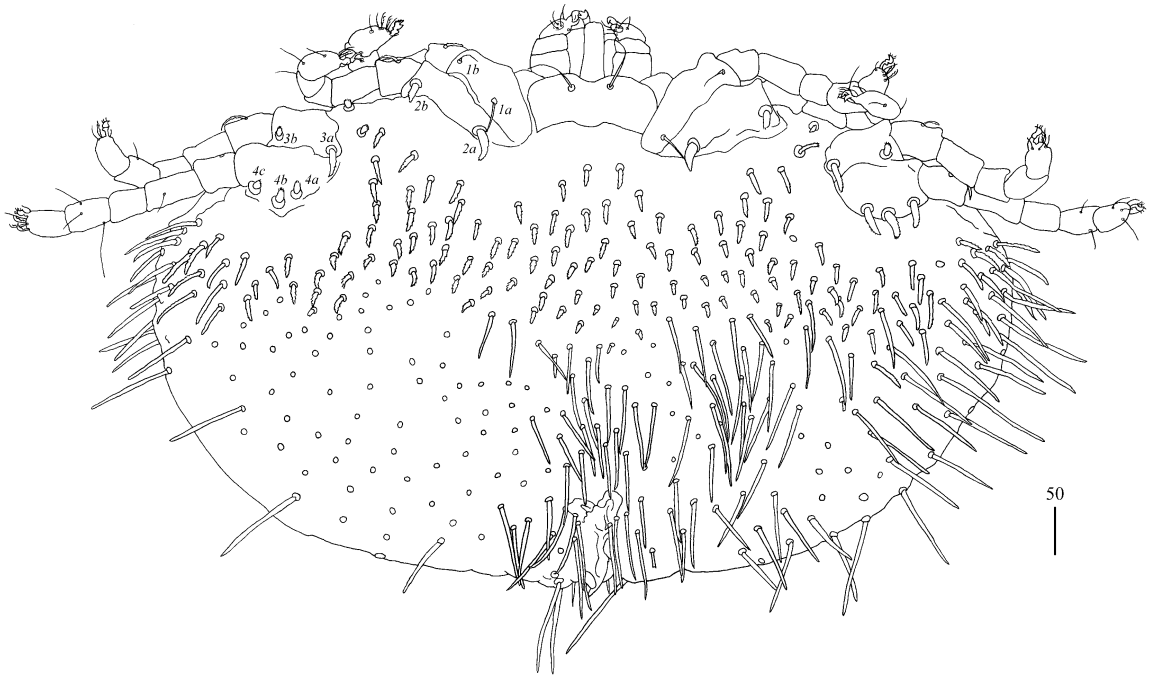

Figure 15. *Geckobia brevicephala* sp. n. female in ventral view.

Female (holotype, range for one paratype). *Gnathosoma.* Chelicerae 90 (90) long; swollen cheliceral part 40 (40) long and slender distal part 50 (55) long. Fixed cheliceral digit rounded. Setae *dF* and *dG* serrate, 25 (30) and 40 (40) long, respectively. Setae *dF* slightly thicker than *dG*. Subcapitular setae *n* with barely discernible serration, about 60 (60) long. Tibial setae (*v*, *l′* and *l″*) smooth. Palp tarsi with four slightly serrate setae. Each branch of peritremes 70 (75) long. *Idiosoma* 405 (415) long and 665 (670) wide. Dorsum. Propodonotal shield 190 (200) wide and 85 (90) long, punctuate (as in Figure 14) and with 18 plumose setae, subequal in length, 25–30 (25–30) long. Small eyes present laterally on propodonotal shield. Posteriorly to propodonotal shield, two rows of plumose setae, about 20 (20) long, resembling those on propodonotal shield, present. Medial part of idiosoma

with short plumose setae, about 10 (15) long. Most posterior part of idiosoma and lateral parts with serrate setae 70–75 (70–80) long. Venter. Antero-medial part with numerous plumose setae that slightly decrease in length from anterior, 15 (15) long, to posterior part of idiosoma 10 (10) long (Figure 15). Posterior and postero-lateral parts of idiosomal venter with slightly serrate flattened setae 40–55 long. Genital region. Setae *g1* and *g2* 30 (30) long, setae *g3* 20 (25) long. Pseudanal setae *ps1–ps10* 65–80 (70–80) long. *Legs*. Coxal setation: *1a*, *1b*, *2a*, *2b*, *3a*, *3b*, *4a*, *4b* and *4c* arranged in formula: 2–2–2–3. All coxal setae thick and plumose, except for filiform, slightly serrate setae *1a* and *1b*. Between coxae I and II, two thick plumose setae present. Leg chaetotaxy as follows: tibiae I–IV (5–5–5–5), genua I–IV (1–0–0–0), femora I–IV (3–2–1–2) and trochanters I–IV (1–1–1–1). Setae *d′TiI–IV*, *d″TiI*, *v′TiI–IV*, *v″TiI–IV*, *l′TiI–IV*, *vFI–IV*, *dFI–IV*, *dGI*, *vGI* long filiform and smooth, *vFI–II*, *vFIV* short and slightly serrate, *lFI* short and slightly serrate and setae *lTrI–IV* densely serrate. Setation of tarsi I: 14 setae (*ft*, *tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *it′*, *it″*, *u′*, *u″*, *vs′*, *vs″* and *pl′*) and solenidion *ω1*; tarsi II: 10 setae (*tc′*, *tc″*, *P′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*) and *ω1*; tarsi III and IV with 10 setae each (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*). Solendion *ω1* of legs I (about 25 long) longer than seta *ft* (about 5 long). Setae *tc′*, *tc″*, *it′* and *it″* of leg I represented by euphatidia; *tc′* and *tc″* of legs II–IV, *u′*, *u″*, *vs′* and vs. of legs I–IV, *pl′*, *a′*, *a″* of legs I–IV filiform.

Type material.

Female holotype and one female paratype (CES 19113) from *Hemidactylus frenatus*, India, Karnataka state, Bangalore, IISc campus, 19.09.2019, coll. Caleb Daniel.

Molecular data.

The D2 alignment of *G. brevicephala* is 918 bp long and comprises two sequences represented by one haplotype. The 18S region of rRNA is 1679 bp long and comprises two sequences represented by one haplotype.

Etymology.

The species name is derived from the Latin word *brevis* which means "short" and *cephale* which means "head" and refers to the short gnathosoma of the species.

Differential diagnosis.

This species is very similar to *Geckobia gibbonsi* Bertand and Ineich, 1987 taken from a gecko *Lepidodactylus* sp. from Eua Island of Tonga [49]. In both species, the idiosoma is much wider than long, the propodonotal shield is present, the eyes are present and the chaetotaxy of legs I–IV is the same. This new species differs from *G. gibbonsi* in terms of the shape of the propodonotal shield, which is straight in its anterior and posterior part; 18 setae and eyes are present on the shield and short setae in the middle of the idiosomal dorsum. In *G. gibbonsi*, the propodonotal shield is concave in the anterior and posterior parts, 14 setae are present on the shield, the eyes are situated outside the shield and the dorsal setae increase in length from the anterior to posterior part of the idiosomal dorsum.

3.2.2. New Data of Already Described Species

Geckobia indica Hirst, 1917 (Figures 16–20).

G. indica Hirst, 1917: 139; 1926: 185 Figure 8; Haitlinger 2005: 96.

Redescription.

Female (range for five specimens). Chelicerae 90–95 long. Swollen cheliceral part 45–50 long and slender distal part 45–50 long. Movable cheliceral digit three-pronged. Fixed cheliceral digit spinous, about 5 long. Setae *dF* thick and densely serrate, serrate *dG* slender and slightly serrate (as in Figure 16c). Setae *dF* and *dG* subequal in length and 25–30 long. Subcapitular setae *n* slightly serrate and about 40 long. Hypostomal apex smooth. Each branch of peritremes 75–85 long. *Idiosoma* 290–325 long and 430–460 wide. Dorsum (Figure 16a). Propodonotal shield almost smooth, 95 long and 240 wide. Inconspicuous eyes present laterally between most anterior setae. About 23–26 pairs of plumose setae (20–40 long) present on propodonotal shield. Posterior to propodonotal shield row of setae resembling setae on propodonotal shield (20–30 long) present. Below shorter plumose setae (about 10–15 long) present. Most posteriorly slender slightly serrate setae 50–70 long

present. Venter (Figure 16b) with short plumose setae about 15–20 long situated anterolaterally. Longer numerous setae (60–70 long) situated posteriorly and laterally. Genital area. Genital setal series represented by four pairs of slightly serrate setae *g1–g4*, about 30 long. Pseudanal setal series represented by eight blunt-pointed serrate setae *ps1–ps8* 60–75 long. *Legs*. Coxae in formula: 2–2–2–3. Setae *1a* and *1b* filiform and smooth, setae *2a* and *2b* serrate, setae *3a*, *3b*, *4a*, *4b* and *4c* spur-like and serrate. Setae of tibiae I–IV (5–5–5–5), genua I–IV (1–0–0–0), femora I–IV (3–2–2–2) and trochanters I–IV (1–1–1–1). Setae *l′TiI–IV*, *l″TiI–IV*, *v′TiI–IV*, *v″TiI–IV*, *dTiI–IV*, *vFI* and *vGI* smooth; setae *dFIIV* and *lFI–IV* slightly serrate and setae *vTrI–IV* serrate. Setation of tarsi I: 14 setae (*ft*, *tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *it′*, *it″*, *u′*, *u″*, *vs′*, *vs″* and *pl′*) and solenidion *ω1* (Figure 16d); tarsi II: 10 setae (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*) and *ω1*; tarsi III and IV with 10 setae each (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*). Setae *it′*, *it″*, *tc′* and *tc″* of legs I in form of euphatidia. Setae *a′*, *a″*, *u′* and *u″* of legs I–IV serrate. Setae *pl′*, *tc′* and *tc″* of legs II–IV and setae *vs′* and *vs″* of legs I–IV smooth. Setae *ft* smooth and about 5 long. Solenidion *ω1* of legs I about 25 long.

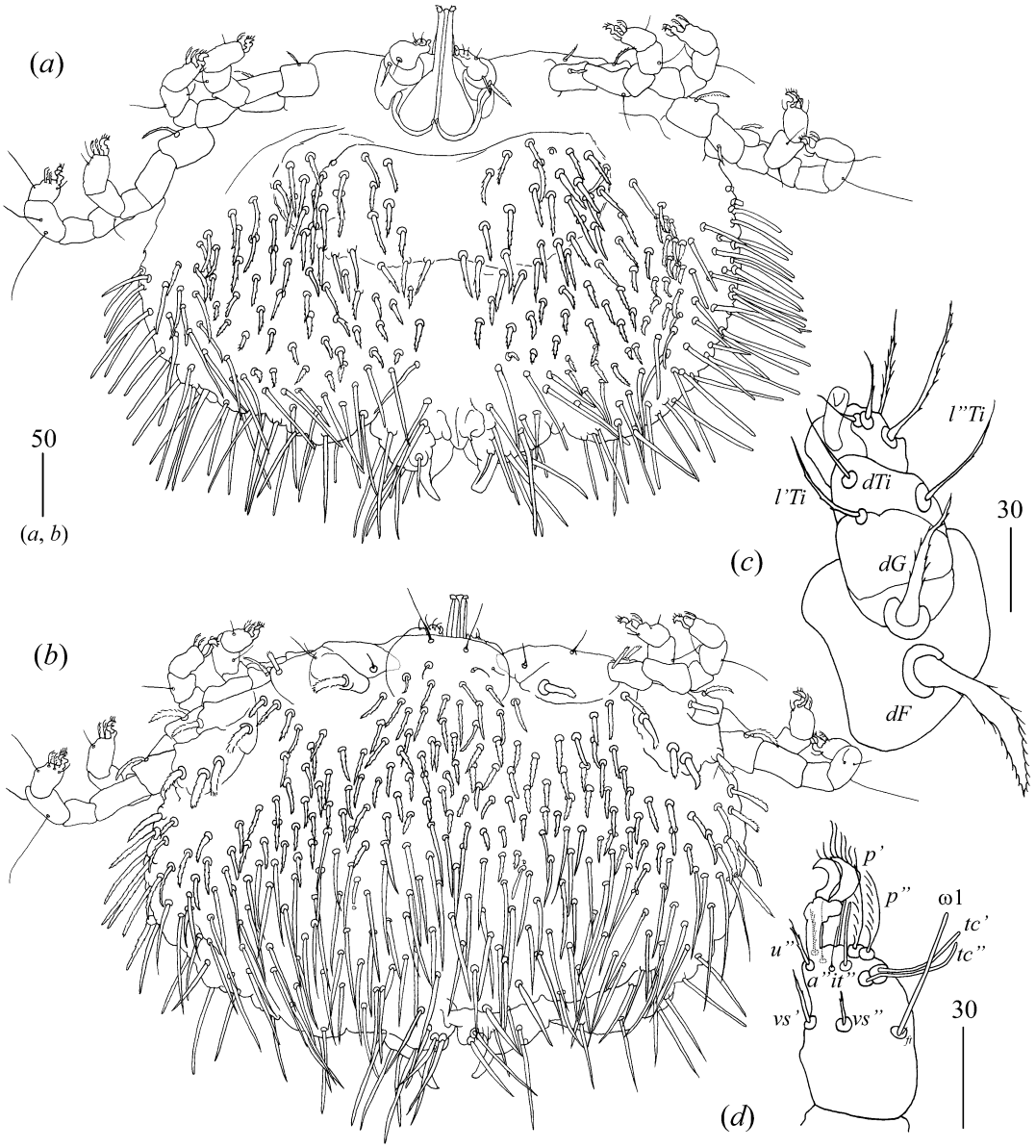

Figure 16. *Geckobia indica* Hirst, 1917, female: (**a**) idiosoma in dorsal view; (**b**) idiosoma in ventral view; (**c**) palps in dorsal view; (**d**) tarsi I in lateral view.

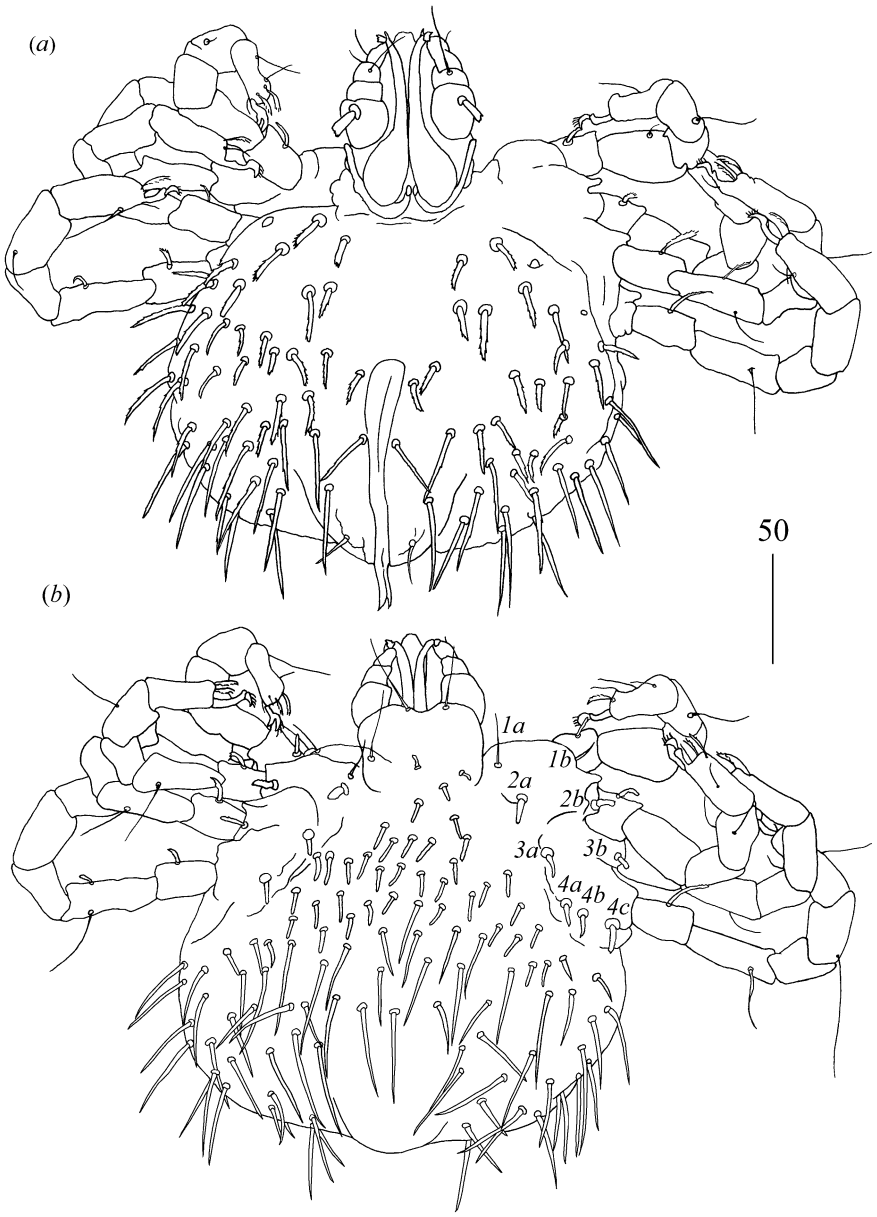

Figure 17. *Geckobia indica* Hirst, 1917, male: (**a**) in dorsal view; (**b**) in ventral view.

Description.

Male (range for three specimens). *Gnathosoma.* Chelicerae about 75 long; swollen cheliceral part about 35 long and slender distal part about 40 long. Setae *dF* thick and serrate, about 15 long; setae *dG* filiform and smooth, about 30 long. Fixed cheliceral digit spinous, 5 long. Subcapitular seta *n* filiform with barely discernible serration about 40 long. Each branch of peritremes about 50 long. *Idiosoma* 180–205 wide and 145–185 long with weakly outlined propodonotal shield in anterior part. Dorsum (Figure 17a) with about 20 pairs of short serrate setae (15–25 long) in anterior part of idiosoma and with 23 pairs of longer serrate setae (about 35 long) in posterior part. Venter (Figure 17b) with 20–34 pairs of short serrate setae (5–15 long) situated antero-medially and 20–25 pairs of longer setae (20–45 long) situated posteriorly. Aedeagus 90–120 long, bifurcated at the end. Genital cone with one pair of smooth and slightly serrate setae (about 15 long) situated most anteriorly, two pairs of smooth spine-like setae (about 10 long) situated medially and one pair of longer smooth setae (15–20 long) situated most posteriorly. *Legs* as in female.

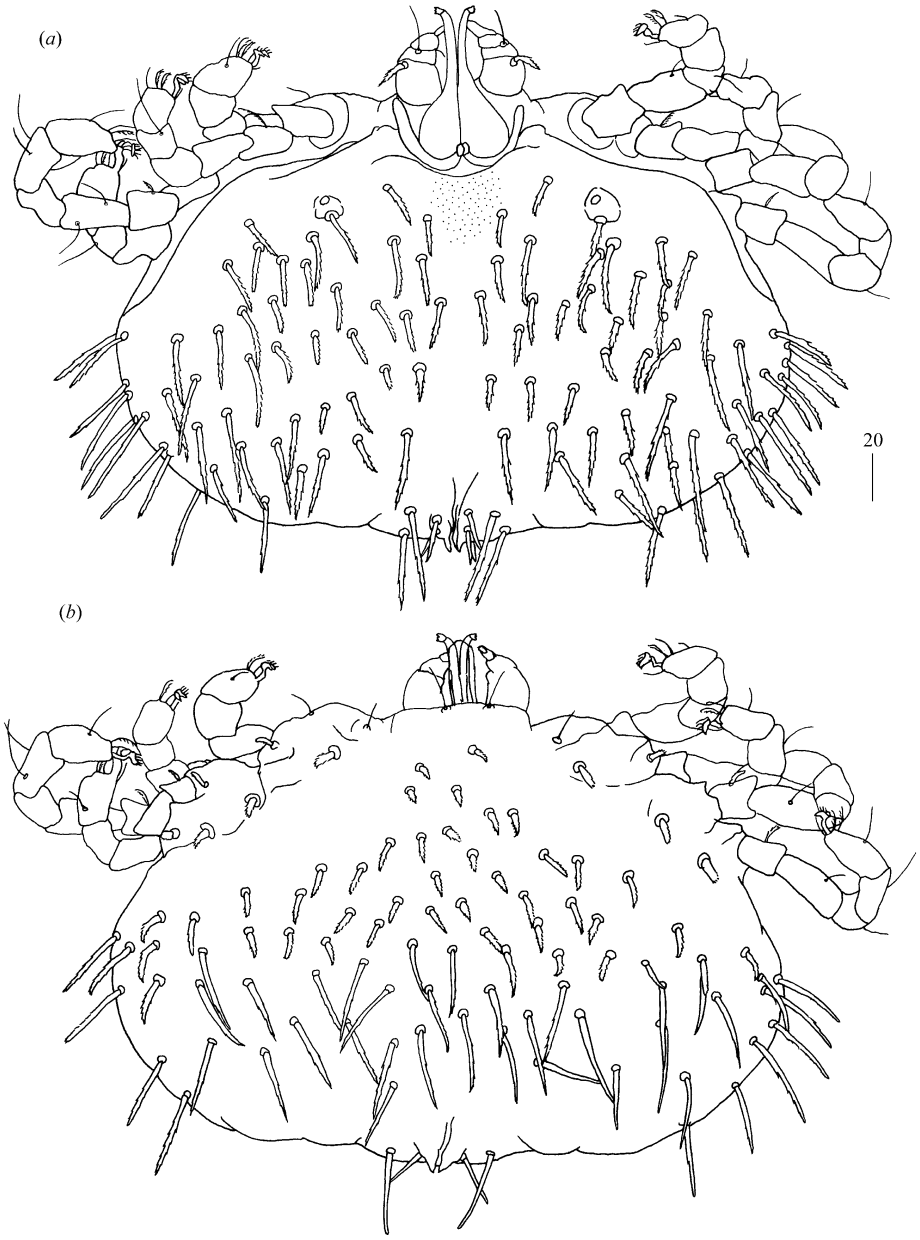

Figure 18. *Geckobia indica* Hirst, 1917 deutonymph: (**a**) in dorsal view; (**b**) in ventral view.

Deutonymph (range for two specimens). Chelicerae 70–75 long. Swollen cheliceral part 30–35 long and slender distal part about 40 long. Fixed cheliceral digit spinous and 5 long. Setae *dG* filiform and smooth, 35 long; setae *dF* slightly serrate and 20 long. Each branch of peritremes 45 long. *Idiosoma* 220–255 long and 320–330 wide. Dorsum (Figure 18a) propodonotal shield (about 90 wide and 65 long) weakly outlined, with minute punctations in anterior part and five serrate setae, 20–35 long. Laterally to propodonotal shield, small platelets (15 long and 15 wide) with eye and one pair of serrate setae about 20 long present. Posterior to propodonotal shield short plumose setae (about 15 long) present in medial part of idiosoma. These setae increase in size from medial to posterior and lateral parts of idiosoma, 20–40 long. Venter (Figure 18b) with about 35 short plumose setae in antero-medial part (10–30 long) and about 45 longer serrate setae (40–70 long) in posterior part. Genital setae series represented by three pairs of slightly serrate setae *g1–g3* 25, 20 and 15 long, respectively. Setae *ps1–ps3* serrate and 45, 40 and 25 long, respectively. *Legs* as in female.

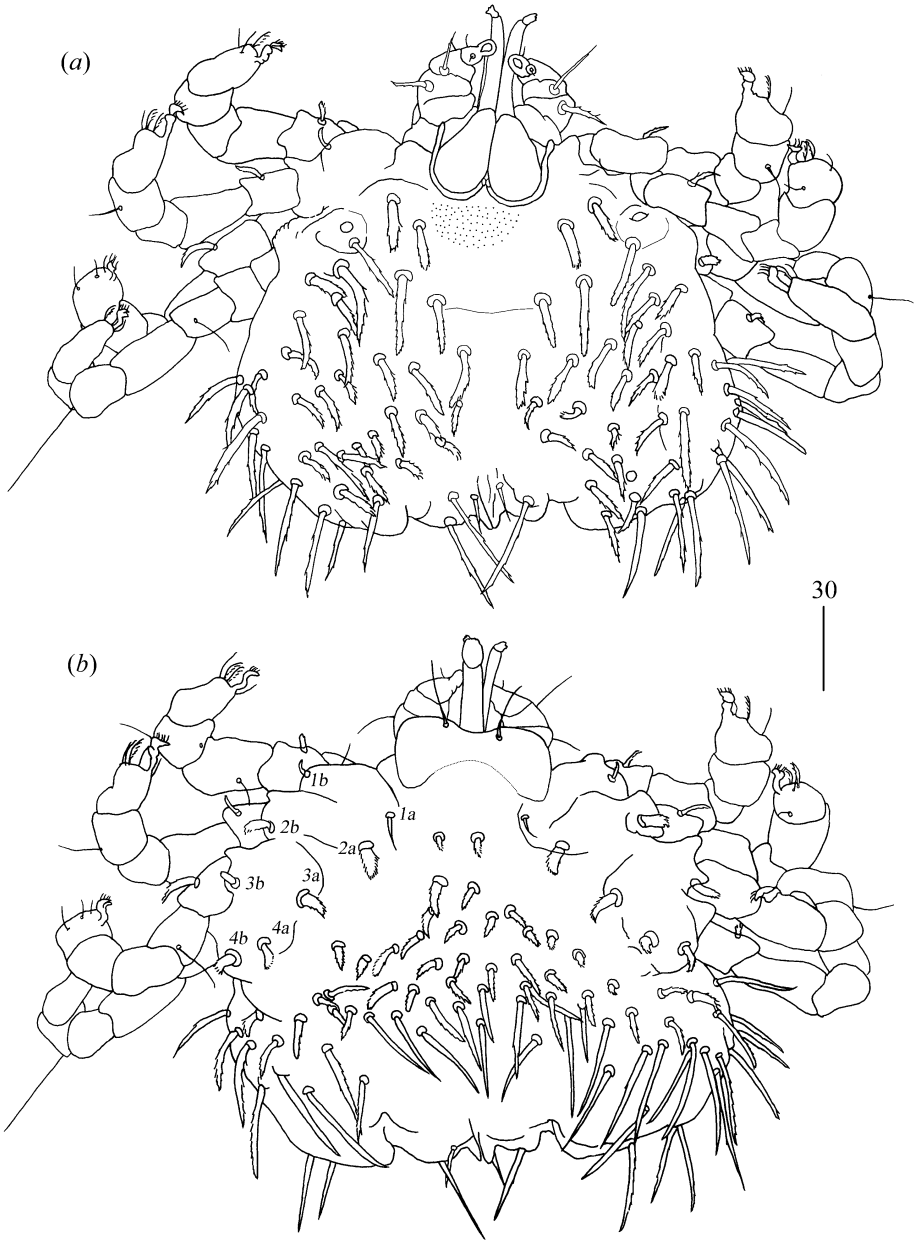

Figure 19. *Geckobia indica* Hirst, 1917 protonymph: (**a**) in dorsal view; (**b**) in ventral view.

Protonymph (range for three specimens). *Gnathosoma* as in female (Figure 20a). Chelicerae 55–70 long. Swollen cheliceral part 30 long and slender cheliceral part 40 long. Setae *dG* filiform, 25–30 long; setae *dF* serrate, 20–25 long. Subcapitular setae *n* 30 long. Each branch of peritremes 45 long. *Idiosoma* 150–195 long and 185–225 wide. Dorsum (Figure 19a). Propodonotal shield about 85 wide and 55 long; punctate only in its anterior part and with five pairs of serrate setae situated laterally. Small eyes present on lateral platelets (20 wide 15 long), accompanied by one serrate seta, about 25 long. Posterior to propodonotal shield serrate, longer setae (30–35 long) present. These setae longer than setae in posterior (20–25 long) part. Venter (Figure 19b) with about 20 pairs of plumose short setae 5–15 long. These setae increase in size from anterior to medial part of idiosoma. From medial to posterior part of idiosomal venter, 21 pairs of longer serrate setae (20–40 long) present. Genital setal series represented by slightly serrate setae *g1–g3.* Setae *g1* 15 long; setae *g2* and *g3* 10 long. Pseudanal setal series represented by serrate setae *ps1–ps3* 40, 30–35 and 20 long, respectively. *Legs* as in female.

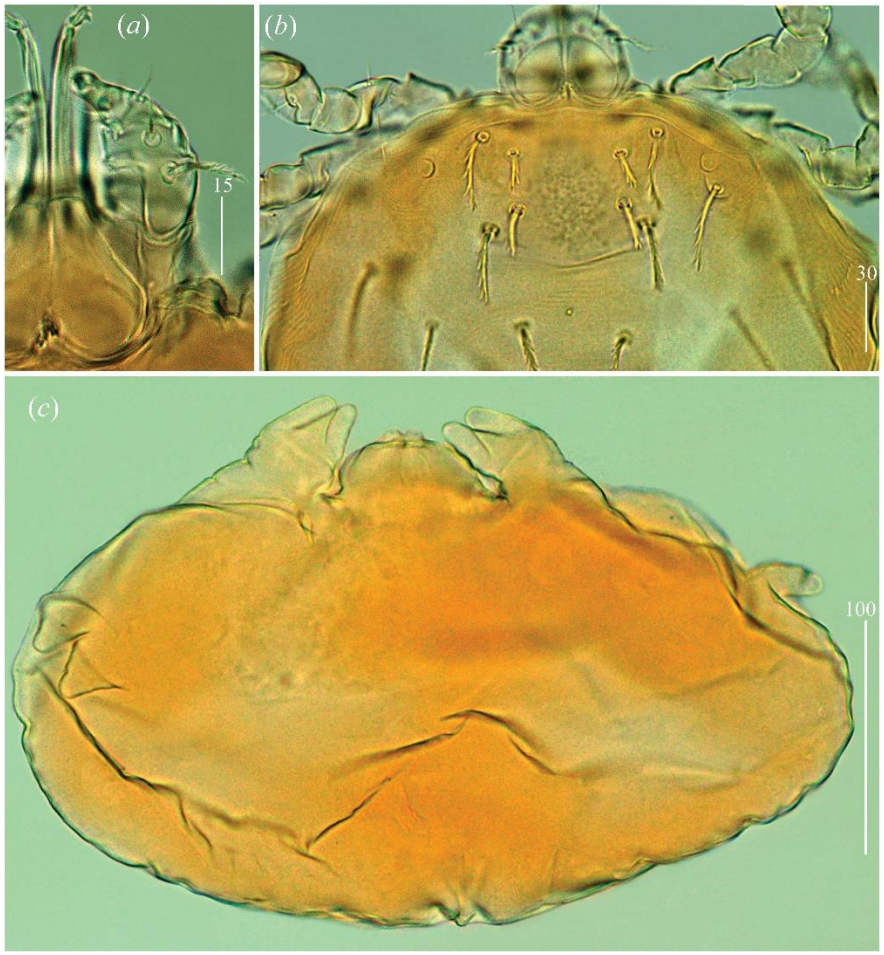

Figure 20. *Geckobia indica* Hirst, 1917: (**a**) part of gnathosoma of protonymph in dorsal view; (**b**) outlined propodonotal shield in larva; (**c**) chrysalis (tritonymph) in ventral view.

Nymphchrysalis (range for one specimen). *Idiosoma* 165 long and 360 wide with barely discernible coxae.

Larva (ranges for five specimens). *Gnathosoma*. Chelicerae 45–50 long. Swollen cheliceral part and slender distal part subequal in length, 20–25 long. Setae *dF* serrate and 15 long; setae *dG* filiform and smooth 20 long. Subcapitular seta *n* absent. Each peritremal branch about 25 long. *Idiosoma* 145–190 long and 180–225 wide. Dorsum with 11 serrate setae. Propodonotal shield 65 wide and 50 long with four pairs of setae: setae situated medially shorter, 15–20 long; setae situated laterally longer, about 25 long (Figure 20b). Eyes present on barely discernible lateral platelets with accompanied serrate seta about 30 long. Six pairs of setae situated in posterior half of idiosoma, 30–35 long. Genital area with three genital setae *g1–g3* (10–15 long) and two pseudanal setae *ps1* and *ps2* (20–25 long). *Legs*. Coxae in formula: 2–1–1. Coxae *1a*, *1b* and *2b* filiform and smooth; setae *3a* spur-like and serrate. Setae of tibiae I–III (5–5–4) genua I–III (1–0–0) femora I–III (3–2–2) and trochanters I–III (0–0–0). Setation of tarsi I–III as in female, except for lack of setae *p″* on tarsi I.

Type material (not examined).

Types from *Hemidactylus gleadowi* Murray (Squamata: Gekkonidae), ASIA: India, the northernmost portion of Sind ("upper Sind" according to Hirst 1917).

Type material deposition.

Unknown (it is not stated in the paper, i.e., [10,43]).

Non-type material (examined).

Five females, three males, two deutonymphs, three protonymphs, three males, five larvae and one nymphchrysalis (CES19112) from *Hemidactylus treutleri* Mahony (Squamata:

Gekkonidae) (CES19104), India, Karnataka, Yerramaranahalli 13°32′55.4″ N, 77°39′18.5″ E, 12 November 2019, coll. P. Karanth.

Molecular data.

The *COI* sequence data of 661 bp were generated from one female. The D2 alignment of *G. indica* is 918 bp long and comprises two sequences represented by two haplotypes differing in terms of two nucleotide positions (0.32%, SD = 0.002, K2P). The 18S region of rRNA is 1688 bp and 844 long and comprises two sequences represented by two haplotypes differing in terms of two nucleotide positions (0.36%, SD = 0.002, K2P).

Host and distribution.

This species was collected from *Hemidactylus gleadowi* Murray [43] and "*Hemidactylus gleadowi* (=*Hemidactylus brookii* Gray)" from India [10], the undetermined *Hemidactylus* sp. from Sri Lanka [7] and *Hemidactylus treutleri* Mahony (new host) (present study) from India, Karnataka state, Yerramaranahalli (new locality) (present study).

Remarks.

This species was originally described by Hirst [43] based on females collected from the ventral scales of a gecko *Hemidactylus gleadowi* Murray, 1884 from India. The species description was incomplete; only detailed chaetotaxy of idiosomal dorsum and venter was presented. The author neither provides the drawing of the species nor the description of the chaetotaxy of gnathosoma, legs and genital region. Later on, in 1926, Hirst presented a figure with a dorsal view of *Geckobia indica* and mentioned that the mites were collected from "*Hemidactylus gleadowi* (=*H. brooki*)" [10]. In 1964, Jack, based on loaned-type specimens, described the chaetotaxy of trochanters-tibiae I–IV of *Geckobia indica*; however, the chaetotaxy of coxae I–IV and tarsi I–IV was not presented [16]. Then, Haitlinger [7] collected *G. indica* from undetermined *Hemidactylus* sp. from Sri Lanka, exceeding the species distribution. Moreover, the measurements of many structures of the species were presented for the first time [7]. Here, we present a full description of the species and provide detailed figures based on the material collected from *Hemidactylus treutleri*. Additionally, males and immature stages are described for the first time. However, the neotype is not designated for this species because the specimens are not taken from the type of host and locality (see Article 75.3.6 of ICZN [50,51]).

Geckobia bataviensis Vitzthum, 1926 (Figures 21 and 22).

G. bataviensis Vitzthum, 1926: 122 Figure 76; Haitlinger 1998: 161 Figures 1–13; Prawasti Farajallahrika and Raffiudin 2013: 83 Figure 3; Jacinavicius, Bassini-Silva, Oda, Kaiser 2021: 1 Figures 1 and 2.

G. gleadoviana Hirst, 1926: 185 Figure 9; Haitlinger 2005: 96.

G. nepalii Hiregaudar, Joshee and Soman, 1959: 66 Figure 2; Haitlinger 2005: 96.

G. cosymboti Cuy, 1979: 156 Figure 1.

Material examined.

Two females from *Hemidactylus frenatus* Duméril and Bibron (Gekkonidae) (CES/08/035) India, Kerala state, Peechi, 24.01.2008, coll. P. Karanth; one female from the same host species from India, Karnataka, Bangalore, IISc, 14 September 2019, coll. Caleb Daniel; one female from the same host species and locality, 19 September 2019, coll. Caleb Daniel; one female from the same host species and locality, 19 September 2019, coll. Caleb Daniel; one female from the same host species and locality, 9 October 2019, coll. Caleb Daniel; one specimen from the same host species and locality, 19 October 2019, coll. Caleb Daniel; one female from the same host species, India, Karnataka, Bangalore, NCBS campus, 29 October 2019, coll. Caleb Daniel, one female from the same host species and locality, 29 October 2019, coll. Caleb Daniel; one female from the same host species and locality, 29 October 2019, coll. Caleb Daniel; one female from the same host species, India, Karnataka, Yerramaranahalli, 13°32′55.4″ N 77°39′18.5″ E, 12.11.2019, coll. P. Karanth; one female from *H. parvimaculatus* India, Karnataka, Bangalore, NCBS campus, 29 October 2019, coll. Chaitanya R.

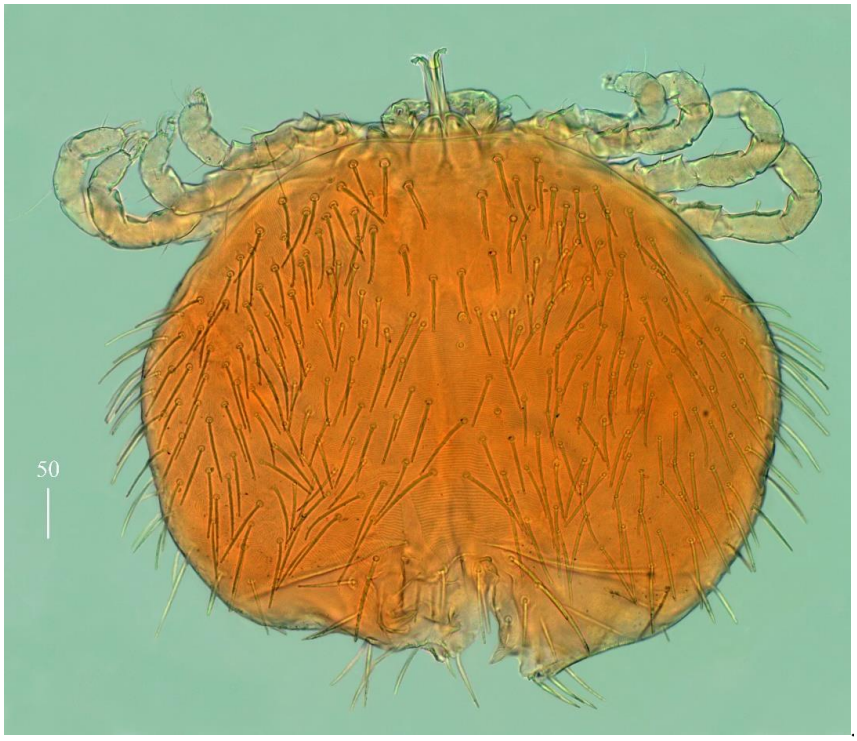

Figure 21. *Geckobia bataviensis* Vitzhum, 1926 female in dorsal view.

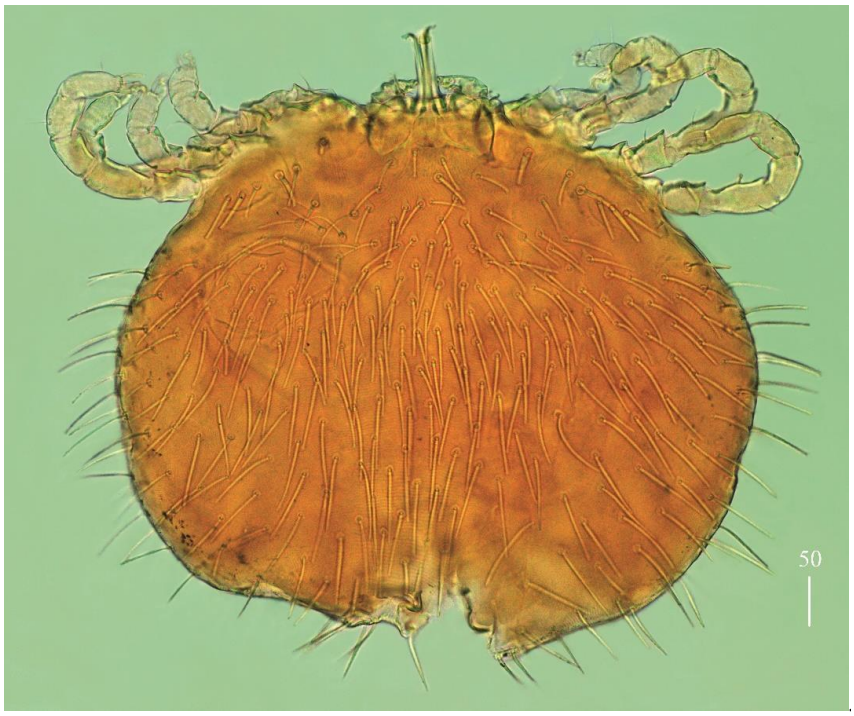

Figure 22. *Geckobia bataviensis* Vitzhum, 1926 female in ventral view.

Molecular data.

The *COI* sequence data of 660–668 bp were generated from three females of *G. bataviensis* and represented by two haplotypes. The pairwise comparison of the two *COI* haplotypes is high and amounts to 4.2% (SE = 0.009). The nuclear data, including 918 positions for the D2 region of 28S rRNA, are obtained for 11 specimens of *G. bataviensis* and represented by one haplotype. The 18S region of rRNA is 1679 bp long and comprises 12 sequences represented by 7 haplotypes. Intraspecific K2P divergence of 18S region of rDNA in *G.*

bataviensis is 0.28% (SD = 0.001), and the pairwise distance between the haplotypes ranges from 0.1 to 1.05% (SD = 0.002).

Remarks.

We adopt the convention of Domrow [46] regarding *G. gleadoviana* Hirst, 1926, *G. nepalii* Hiregauder, Joshee and Soman, 1959 and *G. cosymboti* Cuy, 1973 as junior synonyms of *G. bataviensis* Vitzthum, 1926. We reject the view of Haitlinger that these might be subspecies of *G. bataviensis* [15] or separate species [7]. The author in his latter paper [7] shows in Table 1 the differences between the of *G. gleadoviana* and *G. nepalii* (e.g., the species differs in length of the idiosomal and gnathosomal structures) all of which are exclusively quantitative characteristics which cannot be the only differences between species. However, we observe significant variability in size between engorged and non-engorged females of *G. bataviensis* (compare Figures 21 and 22 with, for example, Figure 76 in the original description or Figure 2 in [11]); therefore, quantitative characteristics cannot be the only differences between the species.

Geckobia phillipinensis Lawrence, 1953 (Figures 23–26).

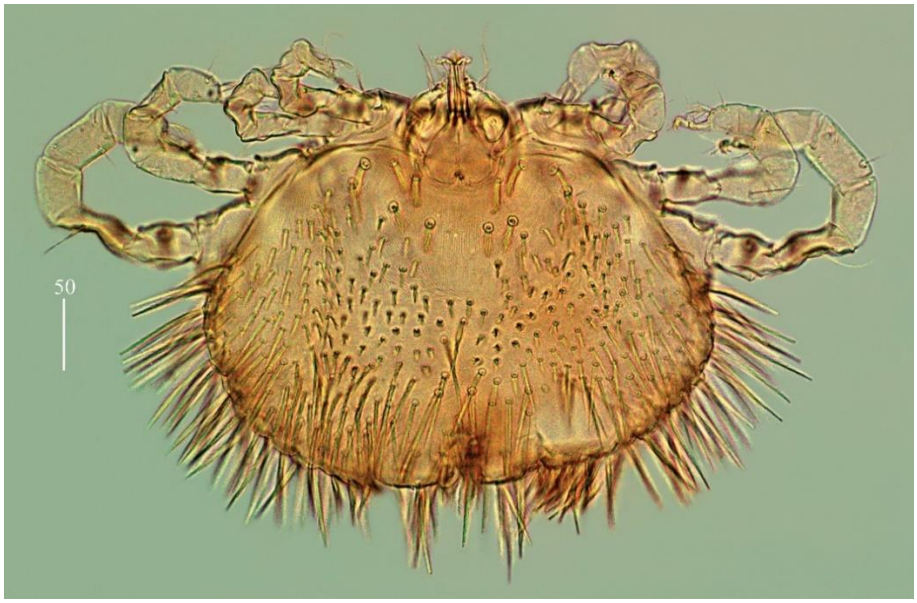

Figure 23. *Geckobia phillipinensis* Lawrence, 1953 female in dorsal view.

Geckobia phillipinensis Lawrence, 1953: 12 Figures 4 and 5.

Diagnosis.

Female (range for five specimens). *Gnathosoma*. Swollen cheliceral part shorter than slender distal part. Subcapitular setae *n* slightly serrate. Setae *dF* serrate, setae *dG* filiform and smooth. Fixed cheliceral digit spinous. Hypostome with small depression present at apex. *Idiosoma* 230–270 long and 360–405 wide. Dorsum (Figure 23) with six thick setae situated antero-laterally. In median and lateral parts short plumose setae present. Posterior, lateral and peripheral setae numerous and serrate. Venter (Figure 24). Antero-medial and lateral part of idiosoma with short plumose setae. Posterior half of idiosoma with lanceolate setae with minute serration on the surface. Most posterior peripheral setae more elongate and narrower than setae in medial part. Genital area represented by four slender genital setae *g1–g4* and eight densely serrate pseudanal setae *ps1–ps8*. Coxal setae *1a* and *1b* filiform; setae *2b* and *3b* thick and serrate; setae *2a*, *3a*, *4a*, *4b* and *4c* spur-like and serrate.

Description.

Male (range for six specimens). *Gnathosoma*. Chelicerae 60–70 long. Slender cheliceral part 30–40 long and swollen basal part 30 long. Fixed cheliceral digit about 5 long. Dorsal palpal setae *dF* thick and serrate, 10–15 long. Setae *dG* filiform and smooth, 25–40 long. Subcapitular setae *n* filiform and smooth, 30–50 long. Each branch of peritremes about

45 long. *Idiosoma* 130–175 long and 170–190 wide. Dorsum with about 22 pairs of setae situated as in Figure 25a. Setae situated anteriorly longer (40–45 long) than setae situated posteriorly (about 35 long). Eyes present. Venter (Figure 25b) with 14 short serrate setae (10–15 long) situated medially and 32 longer serrate setae (15–30 long) situated in posterior half of idiosoma. Aedeagus 100–125 long. Genital cone with three setae 15, 10 and 5 long, respectively. One serrate seta situated laterally to genital cone, about 15 long, present. *Legs*. Coxal setation: *1a*, *1b*, *2a*, *2b*, *3a*, *3b*, *4a* and *4b* arranged in formula: 2–2–2–2. Setae *1a* and *1b* filiform with barely discernible serration, setae *2b* serrate, setae *3b* slightly serrate, setae *2a*, *3a*, *4a* and *4b* spur-like, thick and serrate. Setae of tibiae I–IV (5–5–5–5), genua I–IV (1–0–0–1), femora I–IV (3–2–2–12) and trochanters I–IV (1–1–1–1). Setae *l′TiI–IV*, *l″TiI–IV*, *v′TiI–IV*, *v″TiI–IV*, *dTiI–IV*, *dFI–IV*, *vFI–III*, *lGI* and *lGIV* filiform and smooth; setae *vFIV* slender and slightly serrate; setae *lFI*, *lTrI–IV* thick and serrate. Setae *dFI–IV* much longer than *vFI–IV*. Setation of tarsi I: 14 setae (*ft*, *tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *it′*, *it″*, *u′*, *u″*, *vs′*, *vs″* and *pl′*) and solenidion *ω1*; tarsi II: 10 setae (*tc′*, *tc″*, *P′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*) and *ω1*; tarsi III and IV with 10 setae each (*tc′*, *tc″*, *p′*, *p″*, *a′*, *a″*, *u′*, *u″*, *vs′* and *vs″*). Setae *tc′*, *tc″*, *it′* and *it″* of legs I in form of euphatidia. Setae *pl′* smooth, setae *tc′* and *tc″* of legs II–IV and all setae *vs′*, *vs″*, *a′* and *a″* slightly serrate. Setae *ft* smooth, about 5 long. Solenidion *ω1* of legs I about 25 long. Length of legs I–IV as follows: 120, 130, 160 and 195 long, respectively.

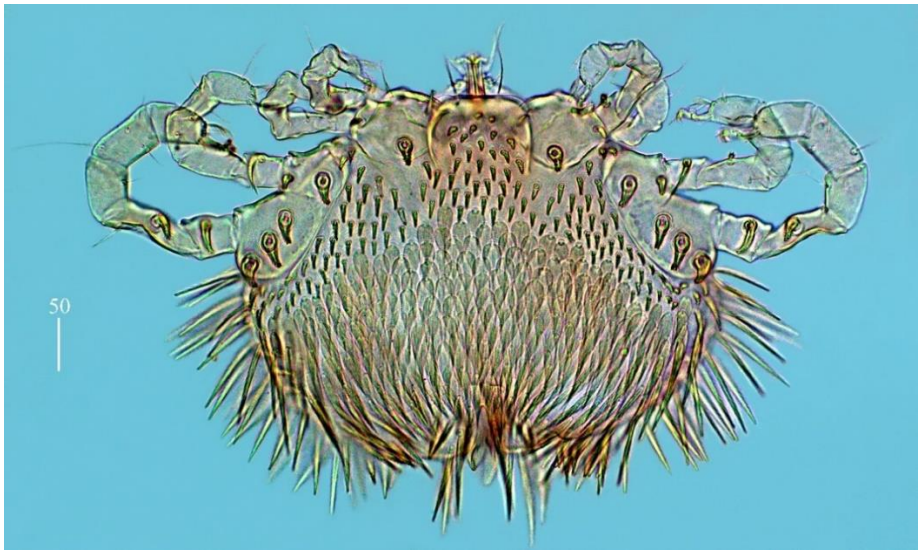

Figure 24. *Geckobia phillipinensis* Lawrence, 1953 female in ventral view.

Figure 25. *Geckobia phillipinensis* Lawrence, 1953 male: (**a**) dorsal view; (**b**) ventral view.

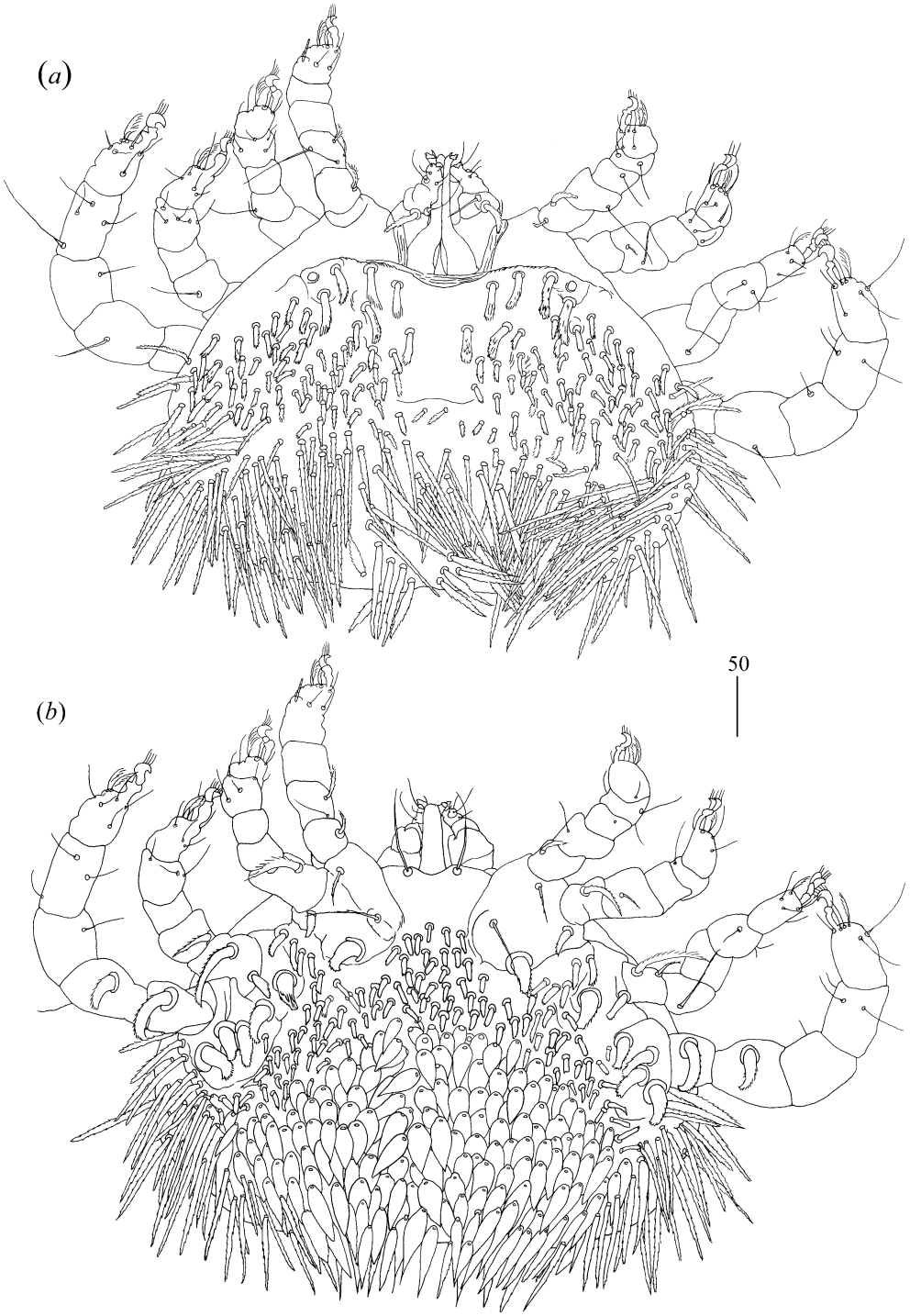

Figure 26. *Geckobia phillipinensis* Lawrence, 1953 deutonymph: (**a**) in dorsal view; (**b**) in ventral view.

Deutonymph (range for three specimens). *Gnathosoma*. Chelicerae 85 long; slender cheliceral part 50 long and swollen basal part 35 long. Palpal femora with dorsal densely serrate seta *dF*, 20 long; palpal genua with filiform and smooth dorsal seta *dG* 35 long. Subcapitular setae *n* filiform and serrate 50 long. Each branch of peritremes 50 long. *Idiosoma* 220–245 long and 380–395 wide. Dorsum (Figure 26a). Propodonotal shield (about 85 wide and 75 long) barely discernible, smooth and slightly concave in its anterior part. Four pairs of serrate thick setae (20–25 long) situated antero-medially on propodonotal shield. Anterior setae situated near propodonotal shield plumose and 10–15 long. Posterior half of idiosoma with thick densely serrate setae 50–70 long. Eyes present on small lateral

platelets and accompanied by two densely serrate setae, 20 long. Lateral setae densely serrate and 55–70 long. Venter (Figure 26b). Anterior part with numerous slightly serrate setae, about 10 long. Medial part with lanceolate setae, about 25 long and posterior part with longer, narrower and serrate setae, 50–60 long. Genital area with five pseudanal thick, flattened and densely serrate setae 35–30 long. *Legs* as in female.

Material examined.

Five females, six males and three deutonymphs (CES19115) from *Hemidactylus frenatus*, India, Karnataka state, Bangalore, NCBS campus, 29.10.2019, coll. Chaitanya R., Karanth P.

Molecular data.

The D2 alignment of *G. phillipinensis* is 910 bp long and comprises two sequences represented by one haplotype. The 18S region of rDNA is 1709 long and comprises four sequences represented by three haplotypes differing in one–two nucleotides (0.09%, SE = 0.0001). The amplification of *COI* was unsuccessful using both, universal and specific primers [35,52]. In all cases, *Wolbachia* endosymbiont was detected.

Host and distribution.

G. phillipinensis was described from *Hemidactylus frenatus* from the Philippine Islands [45] and India (new record).

3.3. Prevalence and Topical Specificity

A total of 208 lizards belonging to two genera and six species of the family Gekkonidae were examined. In 77 (37%) lizards, at least one mite species was found. Most of the host specimens (=179) were from IISc. Among the IISc host specimens, 44 were *H. frenatus* and 21 were *C. mysoriensis*, and together they hosted up to four mite species. The prevalence of the mites differed significantly between the host species: it was highest in *Hemidactylus frenatus* (61%), followed by *Cnemaspis mysoriensis* (47%) and *H. parvimaculatus* (12%). No mites of the genus *Geckobia* were collected from *H. leschenaultii* on IISc, although on the host species, *Pimeliaphilus hemidactyli* Fajfer and Karanth, 2021 was found [41].

In NCBS, 20 host specimens belonging to three species (*Hemidactylus frenatus*, *H. parvimaculatus* and *C. mysoriensis*) were checked for mites, of which nine were infested by three *Geckobia* species (prevalence = 45%): *G. bataviensis*, *G. phillipinensis* and *G. unica*. Additionally, four geckos of *Hemidactylus giganteus* and five geckos of *H. treutleri* were checked for mites in Yerramaranahalli. As a result, three species of *Geckobia* spp. were found, of which two were new to science: *G. gigantea* and *G. treutleri.*

The data for the preferred attachment site of all *Geckobia* spp. in the examined lizards were also collected. Most mites were almost completely hidden under the lizards' scales (Figure 27a,b and Figure 28b,c,e) (i.e., *G. brevicephala*, *G. treutleri*, *G. indica* and females of *G. phillipiensis*). The former species was found under the ventral scales of the host's tail, whereas the three latter were found under the belly scales. The males of *G. phillipinensis* were found in the tympanum (Figure 28d) where they moved freely and were not firmly attached to the skin.

Mites of *G. mysoriensis* were attached to exposed sites of the hosts (Figure 27c): the head, neck and lateral sites of the lizard's body as they are morphologically unable to shelter under scales (their idiosoma is rounded). *G. unica* and mites of *G. bataviensis* were found on the toes of hosts at the base of the claws (mostly distal phalanges) (Figure 28a), whereas *G. gigantea* was found under the lamellae of hindlimbs where they might have protection from the itching activity of the hosts.

3.4. Genetic Distance and Molecular Delimitation of the Geckobia *Species*

The final alignment for species delimitation was comprised of 710 nucleotide position (nps) for seven *COI* sequences. The nucleotide sequences were translated into amino acid sequences, and no stop codons or frameshifts were observed. In the *COI* dataset, 236 out of 710 nps were variable.

The pairwise K2P interspecies distances of the *COI* gene fragment ranged from 7.1 to 32.7% (Table 4), and the intraspecies variation ranged from 0 to 4.2%. The greatest genetic

intraspecies distances occurred in *G. bataviensis*. The *COI* distance within the genus has the largest values among analysed pterygosomatid genera (Table 5).

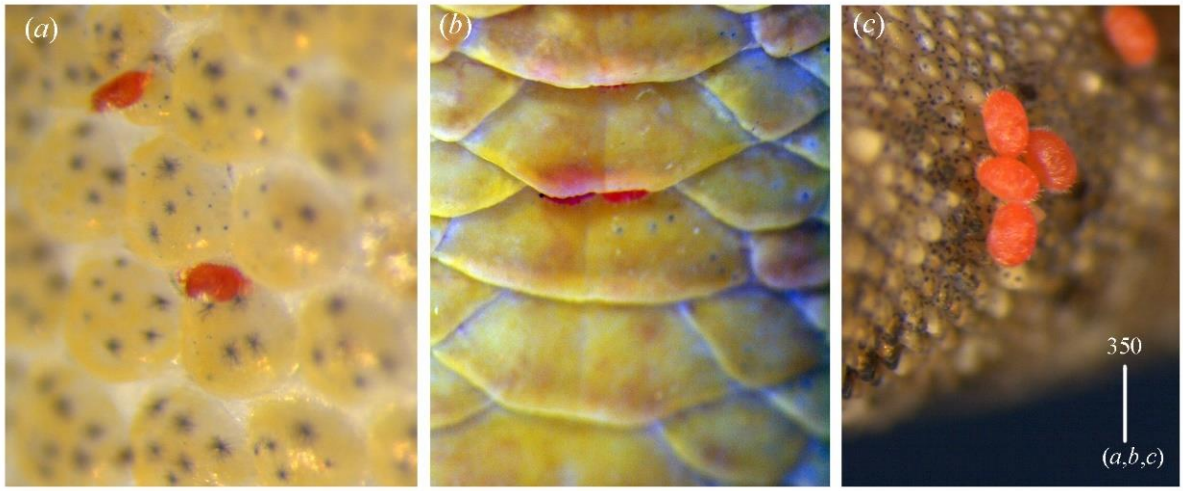

Figure 27. (**a**) *Geckobia* sp. attached to venter of Hemidactylus frenatus Duméril and Bibron; (**b**) *Geckobia brevicephala* sp. n. attached to venter scales of a tail of H. frenatus; (**c**) *Geckobia mysoriensis* sp. n. attached to lateral sides of *Cnemaspis mysoriensis* (Jerdon).

Figure 28. (**a**) *Geckobia bataviensis* Vitzhum, 1926 on toe of Hemidactylus frenatus Duméril and Bibron; (**b**) *Geckobia* sp. attached to H. cf. frenatus; (**c**) female of *Geckobia phillipinensis* under the ventral scales of *H. frenatus*; (**d**) *Geckobia indica* Hirst, 1917 under the ventral scales of *Hemidactylus treutleri* Mahony; (**e**) males of *Geckobia phillipinensis* Lawrence, 1953 in tympanum of *H. frenatus*.

Table 4. Estimates of evolutionary divergences between COI sequences of Geckobia species.

	1.	**2.**	**3.**	**4.**
1. *G. bataviensis* Vitzhum, 1926	-	-	-	-
2. *G. indica* Hirst, 1917	18.7 (0.02)	-	-	-
3. *G. mysoriensis* sp. n.	32.2 (0.03)	32.7 (0.03)	-	-
4. *G. unica* sp. n.	7.1 (0.01)	17.5 (0.02)	30.0 (0.03)	-

Table 5. Kimura two-parameter distances (presented as percentages with standard error estimates in parentheses) between D2 sequences and COI among genera and between genera.

	D2 28S rRNA				***COI***			
Mite Genus	**within Genus**	**between Genera**			**within Genus**	**between Genera**		
		1.	2.	3.		1.	2.	3.
1. *Geckobia* spp.	14.0 (0.17)	-	40.2 (0.02)	32.6 (0.02)	21.8 (0.01)	-	32.5 (0.02)	35.6 (0.02)
2. *Pterygosoma* spp.	22.9 (0.01)	-	-	43.1 (0.02)	20.1 (0.02)	-	-	30.7 (0.02)
3. *Pimeliaphilus* spp.	0.00 (0.00)	-	-	-	0.00 (0.00)	-	-	-

For D2 of 28S rRNA, the interspecies distances ranged from 0.66 to 10.06% (average = 6.9%) (Table 6), and the intraspecies variation ranged from 0 to 2.1 (average = 0.3). The greatest intraspecies genetic variation occurred in *G. mysoriensis*. For 18S, the intraspecies distances ranged from 0 to 0.36% (average = 0.17%), and the interspecies variation ranged from 0.18 to 1.75% (SD = 0.00, average = 0.86%) (Table 7). The greatest genetic intraspecies variation occurred in *G. indica*.

Table 6. Kimura two-parameter distances (presented as percentages with standard error estimates in parentheses) between D2 sequences of analysed *Geckobia* species. The analysis included 45 sequences.

Mite Species	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**
1. *G. bataviensis* Vitzhum, 1926	-	-	-	-	-	-	-	-
2. *G. brevicephala* sp. n.	2.66 (0.01)	-	-	-	-	-	-	-
3. *G. gigantea* sp. n.	7.18 (0.01)	7.53 (0.01)	-	-	-	-	-	-
4. *G. indica* Hirst, 1917	3.06 (0.01)	1.82 (0.00)	7.58 (0.01)	-	-	-	-	-
5. *G. mysoriensis* sp. n.	9.37 (0.01)	9.00 (0.01)	10.06 (0.01)	8.43 (0.01)	-	-	-	-
6. *G. phillipinensis* Lawrence, 1953	8.97 (0.01)	8.99 (0.01)	9.26 (0.01)	9.30 (0.01)	7.47 (0.01)	-	-	-
7. *G. treutleri* sp. n.	4.16 (0.01)	5.09 (0.01)	9.05 (0.01)	5.09 (0.01)	10.75 (0.01)	9.94 (0.01)	-	-
8. *G. unica* sp. n.	0.66 (0.00)	2.21 (0.01)	7.05 (0.01)	2.61 (0.01)	8.99 (0.01)	8.84 (0.01)	4.05 (0.01)	-

Table 7. Kimura two-parameter distances (presented as percentages with standard error estimates in parentheses) between 18S sequences of analysed *Geckobia* species. The analysis included 35 sequences.

Mite Species	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**
1. *G. bataviensis* Vitzhum, 1926	-	-	-	-	-	-	-	-
2. *G. brevicephala* sp. n.	0.43 (0.00)	-	-	-	-	-	-	-
3. *G. gigantea* sp. n.	0.87 (0.00)	0.60 (0.00)	-	-	-	-	-	-
4. *G. indica* Hirst, 1917	0.67 (0.00)	0.24 (0.00)	0.75 (0.00)	-	-	-	-	-
5. *G. mysoriensis* sp. n.	1.75 (0.00)	1.30 (0.00)	1.46 (0.00)	1.44 (0.00)	-	-	-	-
6. *G. phillipinensis* Lawrence, 1953	0.98 (0.00)	0.71 (0.00)	0.82 (0.00)	0.65 (0.00)	0.73 (0.00)	-	-	-
7. *G. treutleri* sp. n.	1.22 (0.00)	0.70 (0.00)	1.22 (0.00)	0.68 (0.00)	1.72 (0.00)	0.95 (0.00)	-	-
8. *G. unica* sp. n.	0.24 (0.00)	0.18 (0.00)	0.51 (0.00)	0.33 (0.00)	1.41 (0.00)	0.77 (0.00)	0.82 (0.00)	-

The ABGD of *COI* delimited three initial partitions with prior intraspecific divergence (*P*) varying from 0.1 to 10% (Figure 29). Barcode gaps were observed at K2P distances of 2–4%, 9–15%, 8–20%, 22–31% and 33–35%. Initial partitions were identical at 17 molecular operational taxonomic units (MOTUs), which was consistent with our prior morphospecies, except for the population of *G. bataviensis*, which was grouped with *G. unica*. For D2 of 28S rRNA, ABGD delimited eight MOTUs, with *P* varying from 0.1 to 5.9%, corresponding to

our morphologically identified eight species. Barcode gaps were observed at K2P distances of 3–4% and 6–7%.

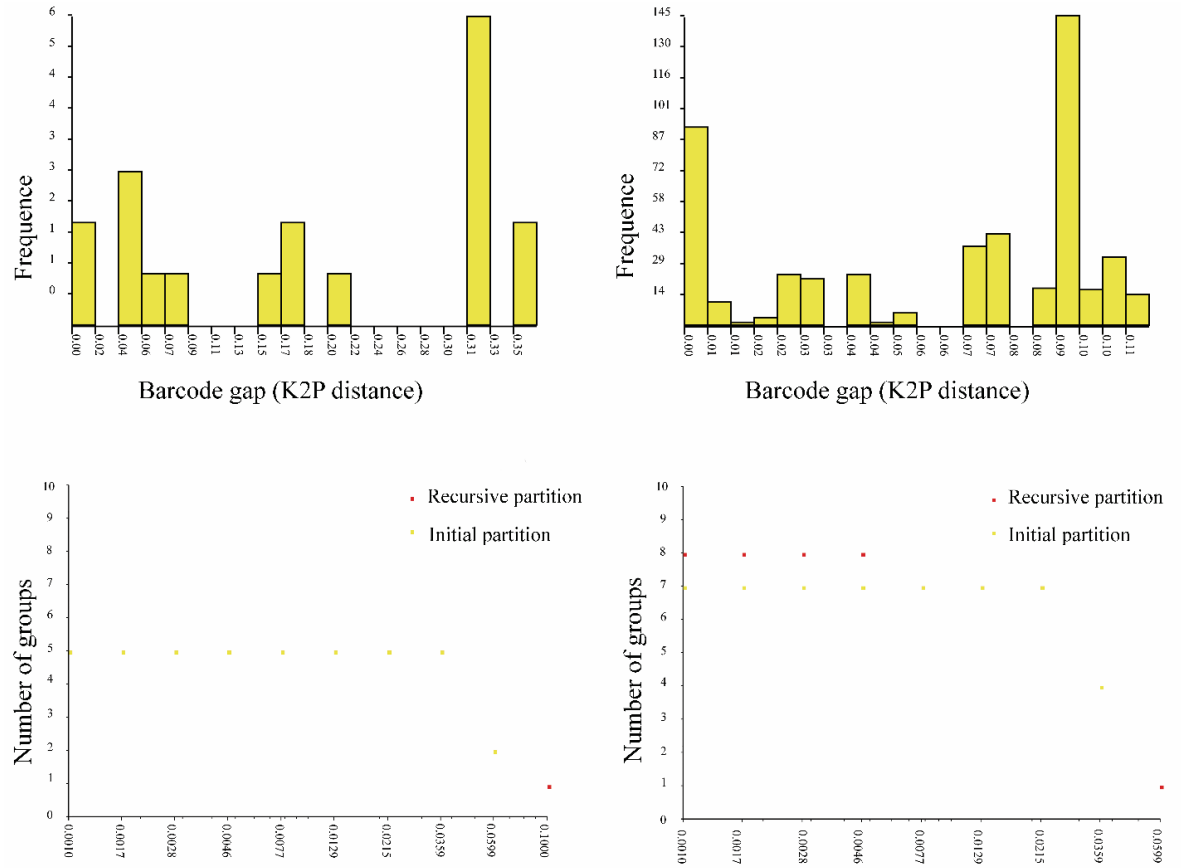

Figure 29. ABDG delimitation of *Geckobia* species: (**a**) COI gene; (**b**) 28S gene.

Genetic distance of *COI* within the genus *Geckobia* was much higher than the genetic distance of D2 of 28S rRNA fragment (Table 5). The *COI* genetic distance between genera *Geckobia* and *Pimeliaphilus* was higher (35.6, SD = 0.02) than between *Geckobia* and *Pterygosoma* (32.5, SD = 0.02), whereas the D2 of 28S rRNA fragment shows that the genetic distance between the former genera was lower (32.6, SD = 0.02) than between the latter genera (40.2, SD = 0.02).

3.5. Relationship between Analysed Species

The *COI* alignment was 671 nps long and comprises seven sequences of *Geckobia* species (ingroup), two sequences of *Pterygosoma* spp. and one sequence of *Pimeliaphilus hemidactyli* (outgroup). The BI and ML analyses revealed that the analysed *Geckobia* are a monophyletic taxon with strong support (BPP = 1) (Figure 30). According to our analysis, the first subclade (*G. indica* + *G. unica* + *G. bataviensis*) associated with *Hemidactylus* geckos represents a separate branch, whereas the mites of the second subclade (*G. mysoriensis*) associated with *Cnemaspis mysoriensis* form the second branch.

The D2 of 28S rRNA alignment was 945 bp long and comprises 32 sequences of *Geckobia* species (ingroup) and one sequence of *Pimeliaphilus hemidactyli* (outgroup). The ML and BI analyses showed very similar topologies (Figure 31). All species formed well-supported monophyletic groups (BPP = 0.96–1.00), with a basic division into eight *Geckobia* species. The first clade contains a subclade of *G. brevicephala* + *G. indica* as sister to *G. treutleri* + *G.unica* + *G.bataviensis*, with the genetic distance between the two subclades being 3.2%. The second well-supported clade (BS = 85, BPP = 1) includes *G. gigantea* as sister to *G. phillipinensis* + *G mysoriensis*. In general, we observed good support for the

external branches but weaker support for the deeper nodes, which is a common feature of the phylogenetic reconstruction of the datasets.

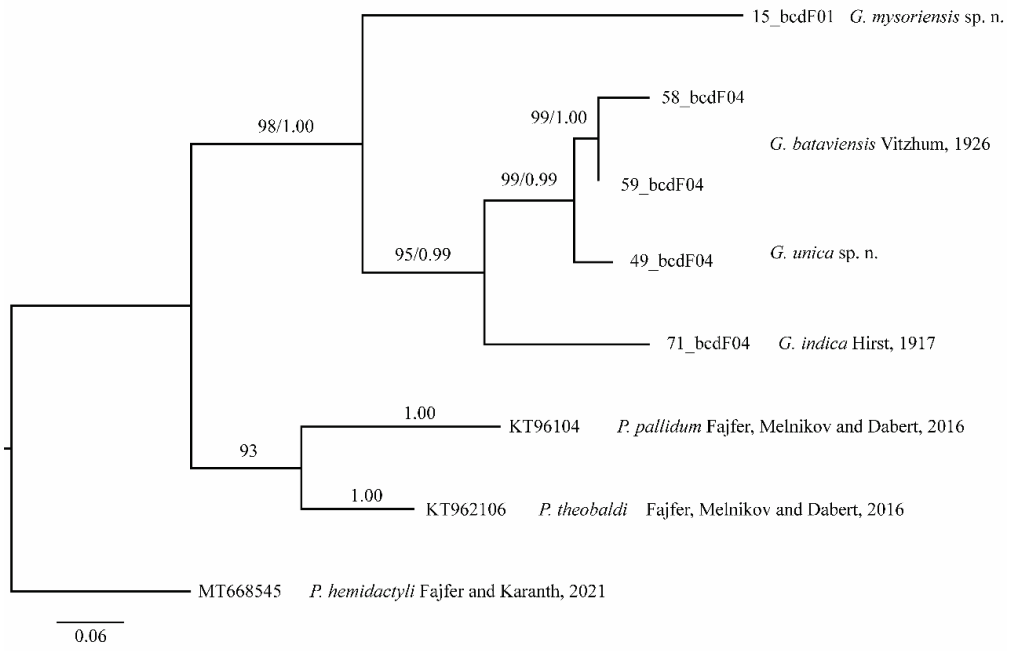

Figure 30. Maximum likelihood of COI mt DNA tree of the analysed *Geckobia* spp. Bayesian analysis yielded almost identical tree topology with only branches of the two Pterygosoma not resolved (polytomous). The support of branches is given as bootstrap value for 1000 replications followed by posterior probability index.

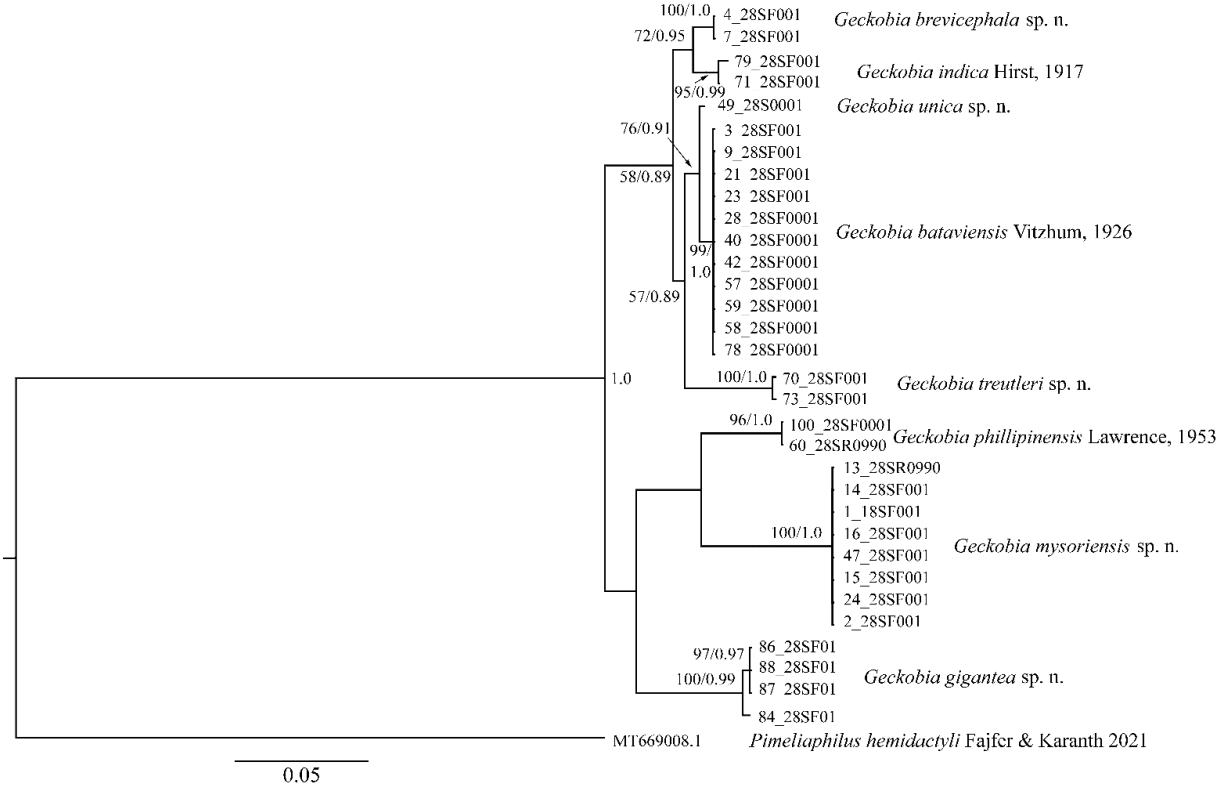

Figure 31. Maximum likelihood of 28S rRNA tree of the analysed *Geckobia* spp. Bayesian analysis yielded almost identical tree topology. The support of branches is given as bootstrap value for 1000 replications followed by posterior probability index.

4. Discussion

The present study is the first record of *Geckobia* mite's diversity investigated at a local scale using an integrative taxonomy approach. Our results generally confirm morphological species delimitations but also show that *Geckobia* species' richness is underestimated, and molecular methods provide complimentary information, although they are not essential to delimit the species boundaries. At the beginning of our research, we expected to find cryptic species, but our studies showed that the analysed *Geckobia* mite species have variable appearance and many unique morphological features which provide enough data for correct species delimitation by a specialist. However, the original morphological description of many already described species of the genus are vague, which results in problems with correct species delimitations based solely on morphological characteristics. Our results show that molecular data reliably complement morphological species identification and have many advantages, especially when used to identify multiple species simultaneously. The distance-based species delimitation methods have shown that, for each marker, the estimates of intraspecific genetic divergence between sequences representing *COI* and the D2 region of the 28S rRNA are 0.0–4.1% and 0.0–2.1%, respectively which is relatively high compared to other parasitic acariform taxa, e.g., [20], but seems to be typical in scale mites [42]. As the D2 of the 28S rDNA marker region shows distinct barcoding gaps and a clear species identification threshold, we recommend using it together with *COI* for identifying scale mites at species level.

The distance- and morphology-based results were confirmed by applying a phylogenetic approach. A monophyly of individuals belonging to the same species was evident. Both the distance-based method and phylogeny-based species delimitation revealed eight morphologically identified species: *Geckobia gigantea* sp. n., *Geckobia mysoriensis* sp. n., *Geckobia treutleri* sp. n., *Geckobia indica* Hirst, 1917, *Geckobia bataviensis* Vitzhum, 1926, *Geckobia phillipinensis* Lawrence, 1953, *Geckobia unica* sp. n. and *Geckobia brevicephala* sp. n. In one case, the *COI* ABGD results differed from the other methods by grouping two morphologically distinct species—*G. bataviensis* and *G. unica*—as one species. However, the species are phylogenetically sister taxa with high support values; therefore, we suspect that ABGD erroneously grouped the respective sequences and underestimated the number of species. Moreover, the ABGD initial and recursive partition of 28S grouped *G. unica* either as a separate species or together with *G. bataviensis.* The possibility of differentiation shown by recursive partition, together with the results of the phylogenetic analysis and morphological analysis, allows the placement of *G. unica* as a single species. Nevertheless, this taxa differentiation is based on a single individual. Therefore, further individuals from different host populations should be collected.

Although the results suggest the monophyly of the *Geckobia* mites used in this study, we cannot draw any certain conclusion regarding the whole genus. The representatives of the genus *Geckobia* are recorded from all gecko families [1], and according to the concept of Bochkov and Mironov [6] (based on parasitological data on associations of the genus *Geckobia* with the geckons), the mites might have parasitised on a common ancestor of these hosts (infraorder Gekkonomorpha). Nonetheless, the recent findings of *Geckobia* on iguanas (Liolaemidae) [2] challenge this thesis and indicate the need for further research including the mite species taken from host taxa distributed worldwide. To investigate species relationships and fully resolve the mite's taxonomy, we indicate the necessity of complementing the standard barcoding marker of *COI* with at least one additional gene marker (e.g., 28S or 18S), as proposed by [53].

Our findings show that in addition to the taxon assignment limitations when using *COI* alone, the primer bias problem needs to be considered when scale mites are targeted in molecular studies, as universal *COI* primers show unsatisfactory amplification performance. We attempted to address this problem by combining more specific *COI* primers commonly used in other acariform mites, e.g., [35,42,52], or even designing new primers for this mite genus. However, our attempts were unsuccessful, and only a few *COI* sequences from analysed *Geckobia* spp. were obtained. Instead of the *COI* sequences, in some cases the

intercellular symbiont *Wolbachia* was detected while using specific primers. *Wolbachia* are bacteria vertically inherited by transovarial transmission, which generally acts as reproductive parasite in arthropods, inducing a wide range of phenotypic effects, such as: parthenogenesis, feminisation, male-killing and cytoplasmic incompatibility, the inability of infected males to successfully fertilise eggs from uninfected females [54]. Our routine observations, as well as analysis of literature, show that the sex ratio in different scale-mite species (or even within different populations of the same species), is highly variable. Most populations are strongly female-biased, and in extreme cases, the host individuals are inhabited exclusively by females. Furthermore, males were never observed in at least half of all known pterygosomatid species, suggesting that they reproduce through parthenogenesis. So far, pterygosomatids have not been examined for the presence of any bacteria that potentially influence reproduction mechanisms. Consequently, we can only suspect that the sex-ratio biases observed in these scale mites are induced by *Wolbachia*. Interestingly, arthropods with a limited diet, such as vertebrate blood, often harbour endosymbiotic bacteria to obtain essential nutrients, e.g., [55], which shows an even more interesting area for future research on scale mites.

We found that *Geckobia* spp., in the presence of closely related host species living in sympatry on IISc, shows a high degree of host specificity even though the mites have good dispersal ability and can potentially switch between different host species during accidental encounters. Our results suggest a high level of host specificity for the mites that are infesting geckos with no evidence of host switching. It is plausible that factors such as odour signals (such as pheromones) could be used by the mites to differentiate between host species. Therefore, *Geckobia* mites seem to spend their entire lives on the same individual or switch the hosts only during their mating. However, our studies were limited to the restricted area of IISc campus; therefore, the host-specificity pattern may change after checking multiple hosts' species living outside IISc.

Geckobia mites live on various parts of the host's body, and species differ in the type of microhabitat they inhabited. *G. brevicephala*, *G. treutleri*, *G. indica* and females of *G. phillipiensis* are completely hidden under the scales; therefore, and they all possess short chelicerae, and their idiosoma is considerably wider than long. This is partly congruent with the observation of Hirst [10] made on Asian *Geckobia* species. The author [10,43] also noticed that the species living under the lizard's scales have scale-like setae on the venter instead of typical setae. However, this is true only for *G. phillipinensis*; the remaining three species of *Geckobia* have typical smooth and long setae in the posterior part of the idiosoma. The rest of the mite species investigated are morphologically unable to take shelter under the scales. Their idiosoma is almost as wide as it is long, and the chelicerae and legs are longer when compared to the species sheltering beneath the scales. Therefore, they inhabit exposed sites of the host's body (e.g., *G. mysoriensis*), attach to the hosts' toes at the base of the claws (e.g., *G. bataviensis* and *G. unica*) or hide in the tympanum (e.g., males of *G. phillipinensis*). Interestingly, inhabiting two separate niches on one individual host by one *Geckobia* species (i.e., females of *G. phillipinensis* are completely hidden under the scales, whereas males move freely in the tympanum) has not been observed for the genus *Geckobia* before. Moreover, in several instances, one host individual harboured more than one *Geckobia* species (Table 3). However, the mite species did not come into direct competition and were associated with different body regions of hosts.

In conclusion, this is the first attempt to combine three types of data for integrative taxonomical investigations of *Geckobia* mites—morphological, molecular and ecological—to investigate diversity and species delineation in *Geckobia* mites. Through comprehensive analysis on a local scale, the presented studies have revealed that the scale-mite fauna is substantially more diverse than expected. Previous records of *Geckobia* mites include 23 species described from Asian geckos (13 spp.), of which six species have been reported on three host species in India [1,6,7]. Our study almost doubled the hitherto known number of species in India, but we assume that *Geckobia* diversity can even mirror the diversity of its vast range of hosts. There is a need for similar investigations on a wider scale

to understand the global diversity of the mites. Undoubtedly, the integrative approach proposed here can be used in the future to not only reveal the further hidden biodiversity of *Geckobia* mites but also help build more reliable phylogenetic hypotheses, as the hypotheses of a pterygosomatid's phylogenies are based solely on morphology and built only for two genera [56,57].

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Data Availability Statement: The molecular data that support this study are available online (NCBI). The remaining material is stored in the Centre for Ecological Science (IISc, Bangalore) and will be shared upon reasonable request to Praveen Karanth.

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