Web mite *Schizotetranychus krungthepensis* on sugarcane in India: molecular evidence for occurrence and the way forward

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Following the detection of an incongruity in the nomenclature of sugarcane web mite *Schizotetranychus andropogoni* (Hirst) (Acari: Tetranychidae), we collected web mite samples from commercial hybrids of sugarcane and *Saccharum spontaneum* in parts of Tamil Nadu and Kerala States, India. Acarologists identified these samples as *Schizotetranychus krungthepensis* Naing & Auger (Acari: Tetranychidae), originally described from Thailand in 2014. To provide molecular evidence to distinguish *S. krungthepensis* from *S. andropogoni*, we subjected sequences of 5.8S rRNA and mitochondrial cytochrome oxidase I (mtCOI) genes of both species available in NCBI database to Molecular Evolutionary Genetics Analysis (MEGA6). The analysis produced phylogenetic trees with distinct clusters for *S. andropogoni* and *S. krungthepensis*, albeit with some exceptions, thus providing evidence to consider *S. krungthepensis* a species distinctly different from *S. andropogoni*. In view of the possible threat of *S. krungthepensis* to sugarcane cultivation in the country, we outline the basic course of action needed to manage the pest if it were to assume more serious proportions than the native species it appears to be displacing.

Keywords: Competitive exclusion, management, phylogenetic analysis, *Schizotetranychus andropogoni*, *Schizotetranychus krungthepensis*, sugarcane.

SUGARCANE, the second most important commercial crop in Indian agriculture after cotton, occupies about 4.60 Mha with production and productivity figures of 370.50 Mt and 80.50 t/ha respectively[1]. Among the ten species of phytophagous mites reported on sugarcane, *Schizotetranychus andropogoni* (Hirst) (Acari: Tetranychidae) is sporadic on cultivated sugarcane *Saccharum officinarum*, besides attacking *Andropogon annulatus*, *Chloris incompleta*, *Dichanthium annulatum*, *Sorghum bicolor*, *Sorghum arundinaceum*, *Saccharum spontaneum*, *Cajanus cajan* and *Zinnia* sp.[2–6].

Essentially a pest of sugarcane in India, Pakistan, Thailand and Mexico, *S. andropogoni* occurs in the Indian states of Bihar, Delhi, Odisha, Punjab, Tamil Nadu and West Bengal, predominantly in northern parts[4] and as a minor pest in South India[7]. Surveys conducted by us in sugarcane experimental plots and growers' farms in the South Indian State of Tamil Nadu over the past few years indicated sporadic spurts of the mite in a spatially and temporally discontinuous manner. However, the mite appears to occur more regularly in Punjab and Gujarat[8].

Schizotetranychus andropogoni, known as white patchy mite/sugarcane leaf spotted mite/web mite, constructs numerous small, oval colonies or webs on either side of the midrib on the abaxial surface of leaves[9]. All stages of the mite in varying numbers may be seen in the colonies numbering 1300–1500 per leaf, whose feeding causes white blotches or webs which later turn brown and are finally blown off[3,4]. In what appears to be a nomenclatural inconsistency, Sithanantham and David[7] treated and referred *S. andropogoni* as *Oligonychus sacchari* (McGregor) and described *O. sacchari* as causing webs when, in fact, the *Oligonychus* sp. is known to cause reddish spots which later coalesce to form large red patches covering the entire leaf[9]. Subsequent researchers continued to consider *S. andropogoni* as the sugarcane mite that produces web-nests on leaves[10,11].

To address the nomenclatural problems referred to above and establish the true identity of sugarcane web mite, we sought the help of mite taxonomists who identified specimens collected and submitted by us as *Schizotetranychus krungthepensis* Naing and Auger (Acari: Tetranychidae), which was described originally from sugarcane in Thailand only recently[12]. To obtain further clarity and distinguish *S. krungthepensis* from *S. andropogoni*, we subjected 5.8S ribosomal RNA (rRNA) and mitochondrial cytochrome oxidase I (mtCOI) gene sequences of both *S. krungthepensis* and *S. andropogoni* available in GenBank to molecular phylogeny analysis, whose complementary role in the taxonomic classification of mite species has been well established[13]. Against the backdrop of the limited information available on *S. krungthepensis* as a pest of sugarcane in Thailand[12,14], we hypothesize about its probable origin and current status in India vis-à-vis the native *S. andropogoni*. We also outline the need for concerted studies on

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Table 1. Details of web mite *Schizotetranychus krungthepensis* collected from two states of India

Date of collection	Place of collection	Host	Molecular characterization	NCBI accession no.	Date of submission
Tamil Nadu					
20 October 2015	ICAR-SBI[@], Coimbatore	Sugarcane	5.8S ribosomal RNA gene, partial sequence; ITS2, complete sequence; 28S ribosomal RNA gene, partial sequence	KU183503	22 November 2015
20 October 2015	ICAR-SBI, Coimbatore	Sugarcane	COI gene, partial cds; mitochondrial	KX681450	9 August 2016
20 October 2015	Coimbatore	Sugarcane	COI gene, partial cds; mitochondrial	KU310625	16 December 2015
27 September 2018[#]	Tiruchirapalli	Sugarcane	5.8S ribosomal RNA gene; ITS2, partial sequence	MK189301	14 November 2018
Kerala					
4 April 2016	Kannur	Sugarcane	COI gene, partial cds; mitochondrial	KX346706	28 May 2016
19 July 2016	Agali	*Saccharum spontaneum*	COI gene, partial cds; mitochondrial	KY094493	7 November 2016
19 July 2016	Agali	*S. spontaneum*	5.8S ribosomal RNA gene; ITS2, partial sequence	KY398122	23 December 2016

[@]Experimental plots of ICAR-Sugarcane Breeding Institute, Coimbatore. [#]Date of collection not given in the accession data.

S. krungthepensis in India to generate basic information that would enable its management if *S. krungthepensis* becomes a more serious pest than the native *S. andropogoni* it appears to be displacing.

Materials and methods

Mite sample collection and morphological identification

We inspected farms of sugarcane growers and experimental plots of the ICAR-Sugarcane Breeding Institute (ICAR-SBI), Coimbatore, Tamil Nadu, and collected mite-infested leaf samples. At the ICAR-Sugarcane Breeding Institute Research Centre (ICAR-SBIRC), Kannur, Kerala, where the world collection of sugarcane germplasm is maintained, we sampled infested leaves randomly from the entire germplasm. At ICAR-SBIRC, Agali, Kerala, we collected samples from *S. spontaneum* accessions that harboured colonies of the mite. After disturbing the webs with dissecting needles to expose live nymphs and adults, we collected the mite stages with a camel hair brush in 70% ethyl alcohol. We sent these samples, together with a few 20 cm leaf bits hosting active colonies, to the Project Coordinator, All India Network Project (AINP) on Agricultural Acarology, University of Agricultural Sciences (UAS), Bengaluru, Karnataka, for taxonomic identification. Table 1 presents details of samples collected from experimental plots of ICAR-SBI as early as 2015 and those collected from sugarcane growers' farms at Coimbatore in 2016 and Tiruchirappalli, Tamil Nadu, in 2018.

Nucleotide sequences of S. krungthepensis and S. andropogoni

We compiled NCBI accessions enumerating eight 5.8S rRNA and 18 mtCOI gene sequences of *S. krungthepensis* samples from the present study (Table 1) and those deciphered from samples collected by other researchers in different parts of the country (Table 2). Besides, we utilized six 5.8S rRNA and nine mtCOI gene sequences of *S. andropogoni* available in the NCBI database for comparative analysis (Table 3). Examination of accessions indicated that sequencing of genes of both species was carried out by AINP on Agricultural Acarology, UAS, Bengaluru, and no other sequences were available in the NCBI database.

Prediction of open reading frame and amino acid sequence for mtCOI

We used a total of 27 mtCOI gene sequences, 18 of *S. krungthepensis* (Tables 1 and 2) and nine of *S. andropogoni* (Table 3), for predicting open reading frame (ORF) using the OrfPredictor tool (http://bioinformatics.ysu.edu/tools/OrfPredictor.html). The tool scans the start codon AUG in all six reading frames and continues until termination occurs at the stop codon UGA, UAA or UAG. With all six reading frames, the tool provides an output file containing protein coding DNA sequences extracted from the input sequences and translates sequences from the six frames. We used the tool to predict the correct reading frame from all possible six reading frames and selected the longest coding frame[15]. Out of the 27 genes, we did not find ORF for the sequence Sa-MN904505-Mandya-KN. Using the universal genetic code, we translated the ORFs into amino acid sequences based on their triplet codon.

Phylogenetic analysis based on coding sequences of 5.8S rRNA, mtCOI and amino acids

We used Molecular Evolutionary Genetics Analysis (MEGA 6) tool to construct phylogenetic trees of *S. krungthepensis* and *S. andropogoni* for 5.8S rRNA, mtCOI and amino acid sequences separately. We subjected the ORF predicted

Table 2. Details of *S. krungthepensis* in sugarcane from different Indian States compiled from the NCBI database

Date of collection	Place and state of collection	Molecular characterization	NCBI accession no.	Date of submission
29 June 2016	Ludhiana, Punjab	COI gene, partial cds; mitochondrial	KX681449	9 August 2016
29 June 2016	Ludhiana, Punjab	COI gene, partial cds; mitochondrial	KX669022	4 August 2016
13 December 2018	Kalyani, West Bengal	5.8S ribosomal RNA gene; ITS2, partial sequence	MK796406	17 April 2019
13 December 2018	Kalyani, West Bengal	COI gene, partial cds; mitochondrial	MT233403	24 March 2020
13 December 2018	Kalyani, West Bengal	COI gene, partial cds; mitochondrial	MT502245	21 May 2020
13 December 2019	Kalyani, West Bengal	COI gene, partial cds; mitochondrial	MT510644	23 May 2020
15 June 2018	Cuddalore, Tamil Nadu	5.8S ribosomal RNA gene, partial sequence; ITS2, complete sequence; large subunit ribosomal RNA gene, partial sequence	MH607647	12 July 2018
15 June 2018	Cuddalore, Tamil Nadu	COI gene, partial sequence; mitochondrial	MT237188	24 March 2020
23 June 2018	Cuddalore, Tamil Nadu	COI gene, partial cds; mitochondrial	MH940222	22 September 2018
11 August 2018	Cuddalore, Tamil Nadu	COI gene, partial sequence; mitochondrial	MN181463 (unverified)	11 July 2019
22 September 2020	Tamil Nadu	CO1 gene, partial cds; mitochondrial	MW509526	20 January 2021
15 October 2020	Tamil Nadu	5.8S ribosomal RNA gene and ITS2, partial sequence	MW460714	11 January 2021
4 June 2019	Anakapalle, Andhra Pradesh	5.8S ribosomal RNA gene, partial sequence; ITS2, complete sequence; large subunit ribosomal RNA gene, partial sequence	MN238811	27 July 2019
4 June 2019	Anakapalle, Andhra Pradesh	COI gene, partial cds; mitochondrial	MT023132	5 February 2020
4 June 2019	Anakapalle, Andhra Pradesh	COI gene, partial cds; mitochondrial	MT233402	24 March 2020
10 July 2017	Bengaluru, Karnataka	COI gene, partial cds; mitochondrial	MG581970[@]	27 November 2017
24 June 2020	Mandya, Karnataka	COI gene, partial cds; mitochondrial	MT776314	18 July 2020
24 June 2020	Mandya, Karnataka	COI gene, partial cds; mitochondrial	MT776315	18 July 2020
24 June 2020	Mandya, Karnataka	5.8S ribosomal RNA gene; ITS2, partial sequence	MT777446	19 July 2020

[@]Collected from potted plants in polyhouse.

Table 3. Details of web mite *Schizotetranychus andropogoni* recorded in different Indian states compiled from the NCBI database

NCBI accession no.	Date of collection	Place and state of collection	Host
5.8S rRNA			
KX025157	30 April 2014	Hiriyur, Karnataka	*Cyrtococcum* sp.
KM580501	1 July 2014	Hiriyur, Karnataka	Grass weed
MH607648	16 June 2018	Bengaluru, Karnataka	Grass
MK386955	17 November 2018	Mandya, Karnataka	*Dichanthium annulatum*
MH763641	23 June 2018	Cuddalore, Tamil Nadu	Grass weed
MK189303	Not available	Not available	Not available
COI			
KM580508	1 July 2014	Hiriyur, Karnataka	Grass weed
MF929081	11 March 2017	Hiriyur, Karnataka	*Cyrtococcum* sp.
MF929080	11 March 2017	Hiriyur, Karnataka	*Cyrtococcum* sp.
MF929079	11 March 2017	Hiriyur, Karnataka	*Cyrtococcum* sp.
MN181465	12 October 2018	Hiriyur, Karnataka	*Ischaemum* grass
MH686294	18 June 2018	Bengaluru, Karnataka	*Ischaemum thomsonianum*
MN904505	17 November 2018	Mandya, Karnataka	*D. annulatum*
MT422536	17 November 2018	Mandya, Karnataka	*D. annulatum*
MN181464	17 November 2018	Mandya, Karnataka	*D. annulatum*

sequences to multiple sequence alignment (MSA) and used the alignment file to construct a distance matrix and estimate evolutionary distances among them.

Nucleotide distances estimate the number of nucleotide substitutions per site between nucleotide sequences. We used the pairwise distance matrix for a maximum likelihood method of phylogeny tree construction and bootstrapping with 1000 replications to ensure a more promising phylogeny tree[16]. We inferred the evolutionary history of the taxa analysed using the maximum likelihood method based on the JTT matrix-based model[17] and 1000 bootstrap replicates[18], allowing branches corresponding to partitions reproduced in less than 50% bootstrap replicates to collapse. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches of the phylogram[16].

Results

Identification and nature of damage

All mite samples we collected and submitted were identified as *S. krungthepensis* by the Project Coordinator, AINP on Agricultural Acarology, UAS, Bengaluru. In the field, a heavy attack of *S. krungthepensis* in sugarcane could be discerned from a distance as distinct white streaks on the leaves (Figure 1 *a*). On closer examination, several parallel rows of oval white webs could be seen on the abaxial surface of the leaves (Figure 1 *b*). The near-equidistant colonies constructed by the mite appear thin and transparent in the beginning (Figure 2 *a*), housing several eggs (Figure 2 *b*) and nymphs (Figure 2 *c*), but turn semi-transparent gradually. Mature stages could be seen not only moving inside young and old webs, but also roaming freely on the leaf surface (Figure 2 *d* and *e*).

Figure 1. Field symptoms of web mite *Schizotetranychus krungthepensis* in sugarcane: (***a***) colonized leaves in the canopy and (***b***) parallel rows of webs on the underside of a leaf.

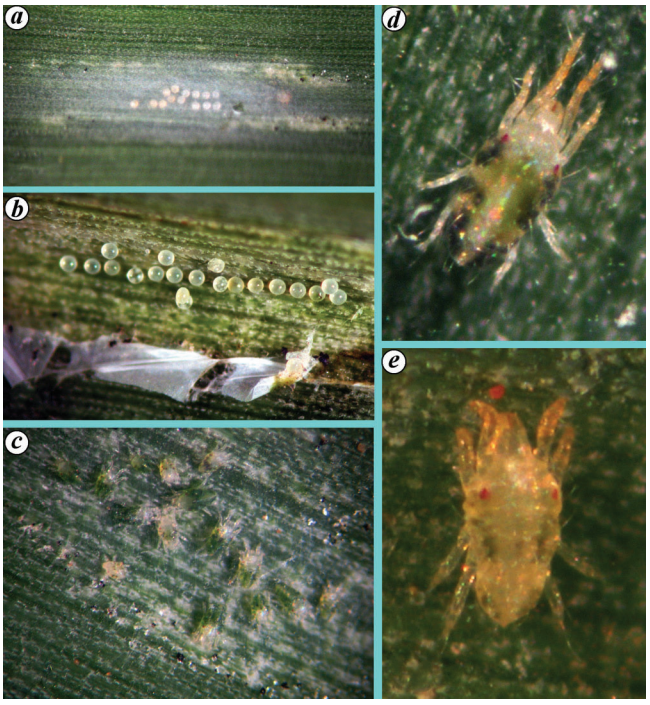

Figure 2. Life stages of *S. krungthepensis* in sugarcane: (***a***) eggs inside the web, (***b***) exposed eggs, (***c***) young nymphs inside the web and (***d***), (***e***) mature stages.

While Table 1 presents details of the samples collected by us and identified as *S. krungthepensis*, Table 2 enumerates the occurrence of *S. krungthepensis* on sugarcane in a few other Indian states, namely Punjab, Karnataka, West Bengal, Tamil Nadu and Andhra Pradesh, from June 2016 to June 2020 (Figure 3). The identification of specimens collected during our surveys at Coimbatore (October 2015) and Kannur (April 2016) as *S. krungthepensis*, whose sequences are available in the NCBI database, constitutes the first record of its occurrence in India.

Phylogenetic analysis of gene sequences

In the phylogenetic analysis with 5.8S rRNA sequences of six *S. andropogoni* and eight *S. krungthepensis* collections, the genetic distance of Sa-KX025157-Hiriyur-KN was highest with Sk-MK796406-Kalyani-WB (0.651), followed by Sk-KY398122-Agali (0.638) and Sk-MW460714-TNAU-SM04 (0.638) (Table 4). The genetic distances among *S. krungthepensis* were far lower. Genetic distances were not so clear-cut for several other combinations either between or within the two species. Genetic distances among mtCOI sequences of 9 *S. krungthepensis* and 18 *S. andropogoni* collections also varied considerably (Table 5). Distances were low within each of the two species and high for some combinations, and vice versa for several other combinations.

The phylogenetic tree based on 5.8S rRNA sequences, on the other hand, clearly separated the collections of *S. andropogoni* and *S. krungthepensis* into distinct clusters

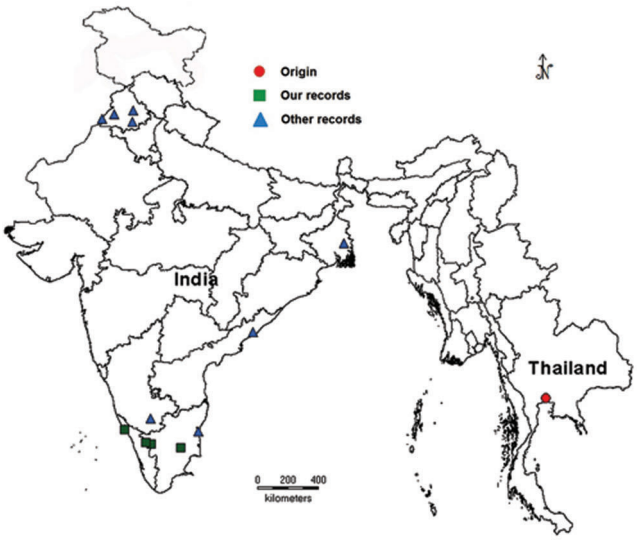

Figure 3. Current distribution of *S. krungthepensis* in India.

Table 4. Estimate of genetic distances among 5.8S rRNA sequences of *S. andropogoni* and *S. krungthepensis* compiled from the NCBI database based on Kimura 2-parameter model in MEGA6

NCBI accession no.	Sa-MK 386955	Sa-KX 025157	Sa-KM 580501	Sa-MK 189303	Sa-MH 763641	Sa-MH 607648	Sk-MN 238811	Sk-KY 398122	Sk-MW 460714	Sk-MT 777446	Sk-MK 796406	Sk-MK 189301	Sk-MH 607647	Sk-KU 183503
Sa-MK386955 (Mandya-KN)*	–													
Sa-KX025157 (Hiriyur-KN)	0.601	–												
Sa-KM580501 (Hiriyur-KN)	0.005	0.601	–											
Sa-MK189303 (UAS(B)-KN)	0.005	0.601	0.000	–										
Sa-MH763641 (Cuddalore-TN)	0.008	0.607	0.003	0.003	–									
Sa-MH607648 (Bengaluru-KN)	0.008	0.607	0.003	0.003	0.005	–								
Sk-MN238811 (Anakapalli-AP)	0.101	0.631	0.095	0.095	0.098	0.098	–							
Sk-KY398122 (Agali-KL-authors)	0.105	0.638	0.098	0.098	0.101	0.101	0.003	–						
Sk-MW460714 (TNAU-SM04)	0.111	0.638	0.104	0.104	0.107	0.107	0.011	0.014	–					
Sk-MT777446 (Mandya-KN)	0.105	0.631	0.098	0.098	0.101	0.101	0.003	0.005	0.014	–				
Sk-MK796406 (Kalyani-WB)	0.133	0.651	0.126	0.126	0.130	0.130	0.036	0.039	0.047	0.033	–			
Sk-MK189301 (Trichy-TN-authors)	0.105	0.631	0.098	0.098	0.101	0.101	0.003	0.005	0.014	0.000	0.033	–		
Sk-MH607647 (Cuddalore-TN)	0.101	0.631	0.095	0.095	0.098	0.098	0.000	0.003	0.011	0.003	0.036	0.003	–	
Sk-KU183503 (Coimbatore-TN-authors)	0.101	0.631	0.095	0.095	0.098	0.098	0.000	0.003	0.011	0.003	0.036	0.003	0.000	–

*Prefix denotes species: Sa, *Schizotetranychus andropogoni*; Sk, *Schizotetranychus krungthepensis*, suffix denotes place and state of collection: AP, Andhra Pradesh; KL, Kerala; KN, Karnataka; PB, Punjab; TN, Tamil Nadu; WB, West Bengal.

Table 5. Estimate of genetic distances among mtCOI sequences of the web mites *S. andropogoni* and *S. krungthepensis* compiled from the NCBI database based on Kimura 2-parameter model in MEGA6

NCBI accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13
Sa-MN904505-Mandya-KN[@]	–												
Sa-MT422536-Mandya-KN	0.771	–											
Sa-MF929081-Hiriyur-KN	0.149	0.797	–										
Sa-MF929080-Hiriyur-KN	0.793	0.102	0.825	–									
Sa-MF929079-Hiriyur-KN	0.545	0.847	0.500	0.845	–								
Sa-KM580508-Hiriyur-KN	0.785	0.090	0.813	0.034	0.842	–							
Sa-MN181465-Hiriyur-KN	0.054	0.771	0.157	0.793	0.562	0.785	–						
Sa-MN181464-Mandya-KN	0.768	0.011	0.800	0.112	0.845	0.102	0.768	–					
Sa-MH686294-Bangalore-KN	0.786	0.090	0.813	0.034	0.837	0.000	0.786	0.102	–				
Sk-MT502245-Kalyani-WB	0.819	0.182	0.838	0.227	0.859	0.191	0.819	0.205	0.191	–			
Sk-MT023132-Anakapalli-AP	0.821	0.191	0.838	0.227	0.860	0.200	0.821	0.205	0.200	0.000	–		
Sk-KX681450-Coimbatore-TN-authors	0.270	0.750	0.296	0.766	0.523	0.753	0.277	0.753	0.753	0.779	0.779	–	
Sk-KX681449-Ludhiana-PB	0.273	0.753	0.316	0.769	0.577	0.756	0.273	0.756	0.756	0.782	0.782	0.051	–
Sk-MW509526-TNAU-SM04	0.339	0.771	0.364	0.780	0.636	0.785	0.379	0.768	0.774	0.783	0.786	0.012	0.070
Sk-MT776314-Mandya-KN	0.827	0.195	0.844	0.224	0.867	0.195	0.827	0.200	0.195	0.000	0.011	0.784	0.787
Sk-MT776315-Mandya-KN	0.283	0.756	0.298	0.772	0.532	0.759	0.289	0.759	0.759	0.785	0.785	0.000	0.051
Sk-MT510644-Kalyani-WB	0.819	0.182	0.838	0.227	0.859	0.191	0.819	0.205	0.191	0.000	0.000	0.779	0.782
Sk-MT237188-Cuddalore-TN	0.302	0.759	0.320	0.768	0.561	0.774	0.302	0.756	0.762	0.783	0.786	0.000	0.051
Sk-MT233403-Kalyani-WB	0.300	0.759	0.320	0.768	0.551	0.774	0.300	0.756	0.762	0.783	0.786	0.000	0.051
Sk-MT233402-Anakapalli-AP	0.300	0.759	0.320	0.768	0.551	0.774	0.300	0.756	0.762	0.783	0.786	0.000	0.051
Sk-MN181463-Cuddalore-TN	0.331	0.753	0.353	0.763	0.627	0.769	0.372	0.750	0.756	0.778	0.780	0.000	0.060
Sk-MH940222-Cuddalore-TN	0.278	0.750	0.291	0.765	0.523	0.753	0.284	0.753	0.753	0.778	0.778	0.000	0.051
Sk-MG581970-Bangalore-KN	0.342	0.753	0.357	0.759	0.615	0.778	0.351	0.747	0.765	0.788	0.790	0.012	0.073
Sk-KX346706-Kannur-KL-authors	0.299	0.747	0.346	0.763	0.562	0.750	0.299	0.750	0.750	0.776	0.776	0.047	0.072
Sk-KU310625-Coimbatore-TN-authors	0.821	0.202	0.838	0.239	0.860	0.200	0.821	0.216	0.200	0.011	0.022	0.779	0.782
Sk-KY094493-Agali-KL-authors	0.829	0.198	0.844	0.226	0.869	0.207	0.829	0.202	0.198	0.000	0.012	0.784	0.787
Sk-KX669022-Ludhiana-PB	0.817	0.184	0.833	0.244	0.857	0.205	0.817	0.198	0.205	0.046	0.057	0.773	0.776

(*Contd*)

Table 5. (*Contd*)

NCBI accession no.	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Sa-MN904505-Mandya-KN[@]														
Sa-MT422536-Mandya-KN														
Sa-MF929081-Hiriyur-KN														
Sa-MF929080-Hiriyur-KN														
Sa-MF929079-Hiriyur-KN														
Sa-KM580508-Hiriyur-KN														
Sa-MN181465-Hiriyur-KN														
Sa-MN181464-Mandya-KN														
Sa-MH686294-Bangalore-KN														
Sk-MT502245-Kalyani-WB														
Sk-MT023132-Anakapalli-AP														
Sk-KX681450-Coimbatore-TN-authors														
Sk-KX681449-Ludhiana-PB														
Sk-MW509526-TNAU-SM04	–													
Sk-MT776314-Mandya-KN	0.790	–												
Sk-MT776315-Mandya-KN	0.023	0.789	–											
Sk-MT510644-Kalyani-WB	0.783	0.000	0.785	–										
Sk-MT237188-Cuddalore-TN	0.010	0.790	0.010	0.783	–									
Sk-MT233403-Kalyani-WB	0.010	0.790	0.010	0.783	0.000	–								
Sk-MT233402-Anakapalli-AP	0.010	0.790	0.010	0.783	0.000	0.000	–							
Sk-MN181463-Cuddalore-TN	0.014	0.790	0.012	0.778	0.000	0.000	0.000	–						
Sk-MH940222-Cuddalore-TN	0.011	0.782	0.009	0.778	0.000	0.000	0.000	0.000	–					
Sk-MG581970-Bangalore-KN	0.036	0.790	0.024	0.788	0.010	0.010	0.010	0.027	0.012	–				
Sk-KX346706-Kannur-KL-authors	0.024	0.781	0.047	0.776	0.020	0.020	0.020	0.012	0.047	0.025	–			
Sk-KU310625-Coimbatore-TN-authors	0.786	0.011	0.785	0.011	0.786	0.786	0.786	0.780	0.778	0.790	0.776	–		
Sk-KY094493-Agali-KL-authors	0.793	0.000	0.789	0.000	0.793	0.793	0.793	0.793	0.782	0.793	0.781	0.012	–	
Sk-KX669022-Ludhiana-PB	0.780	0.046	0.779	0.046	0.780	0.780	0.780	0.780	0.772	0.790	0.770	0.057	0.047	–

[@]Prefix denotes species: Sa, *Schizotetranychus andropogoni*; Sk, *Schizotetranychus krungthepensis*, suffix denotes place and state of collection: AP, Andhra Pradesh; KL, Kerala; KN, Karnataka; PB, Punjab; TN, Tamil Nadu; WB, West Bengal.

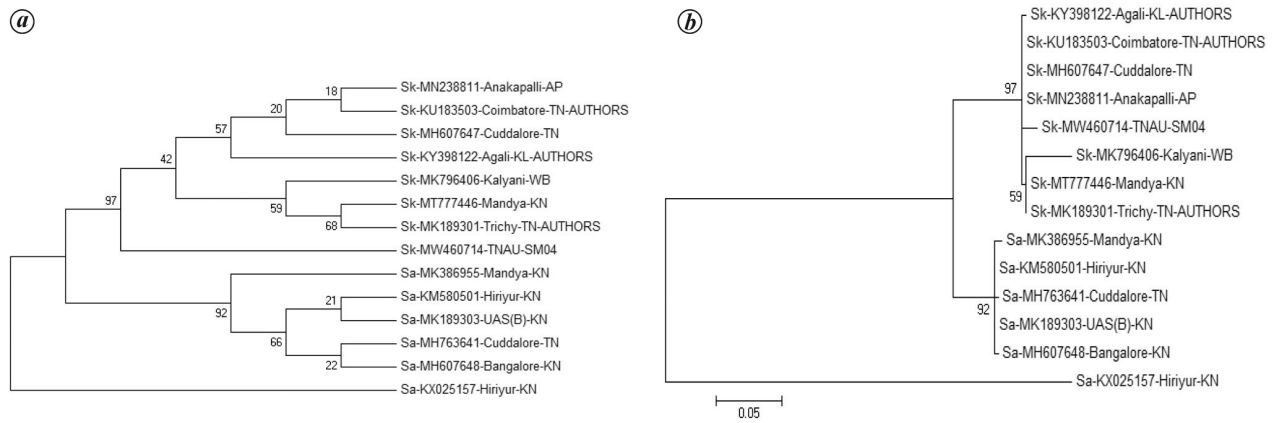

Figure 4. ***a***, Maximum likelihood phylogenetic tree of web mites *Schizotetranychus andropogoni* and *Schizotetranychus krungthepensis* based on 5.8S rRNA gene sequence. Bootstrap values based on 1000 replications are indicated at the nodes. Each taxonomic unit is denoted by the NCBI accession number prefixed with species abbreviation and suffixed with place of collection. ***b***, Collapsed branches corresponding to partitions reproduced in less than 50% bootstrap replicates.

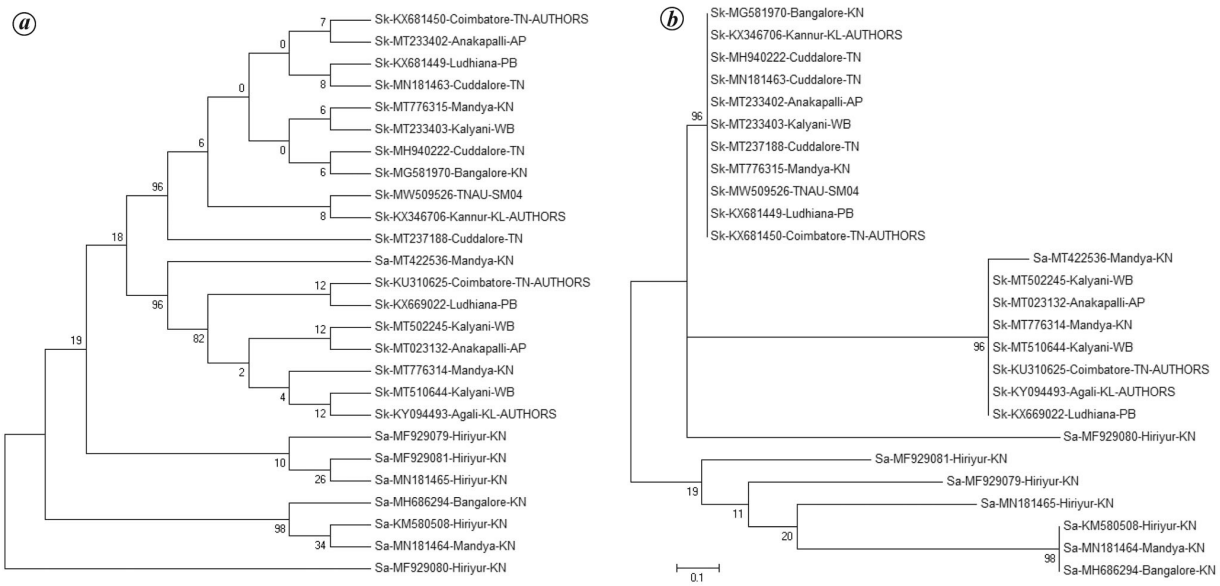

Figure 5. ***a***, Maximum likelihood phylogenetic tree of *S. andropogoni* and *S. krungthepensis* based on mtCOI coding sequence. Bootstrap values based on 1000 replications are indicated at the nodes. Each taxonomic unit is denoted by the NCBI accession number prefixed with species abbreviation and suffixed with place of collection. ***b***, Collapsed branches corresponding to partitions reproduced in less than 50% bootstrap replicates.

except for Sa-KX025157-Hiriyur-KN (Figure 4 *a* and *b*). Similarly, the phylogenetic tree based on mtCOI coding sequences of the two species formed different clusters under *S. krungthepensis* and *S. andropogoni* (Figure 5 *a* and *b*), except Sa-MT422536-Mandya-KN, which clustered along with *S. krungthepensis* with high bootstrap support of 96%. Phylogenetic analysis of mtCOI peptide sequences more or less confirmed the clustering obtained with mtCOI coding sequences, the exception once again being Sa-MT422536-Mandya-KN which tended to cluster with *S. krungthepensis* sequences (Figure 6 *a* and *b*). The amino acid sequence alignment showed greater variability among *S. andropogoni* sequences than *S. krungthepensis* sequences (Figure 7); conversely, the *S. krungthepensis* sequences appeared highly conserved.

Discussion

The damage symptoms and colonization pattern of *S. krungthepensis* observed in the present study resembled those of *S. andropogoni* described earlier[4]. A recent taxonomic publication that records several species of mites in Coimbatore, including *S. krungthepensis*[19], provides no description of the species. Although the two species were distinguished morphologically (Dr N. Srinivasa, pers. commun.), no taxonomic publication with a detailed description or drawings of *S. krungthepensis* specimens collected in India is available, except for the checklist of Srinivasa *et al*.[20]. In the light of the difficulties involved in morphological identification of mites, sequence-based methods not only help resolve incongruities in accurate identification but also help develop

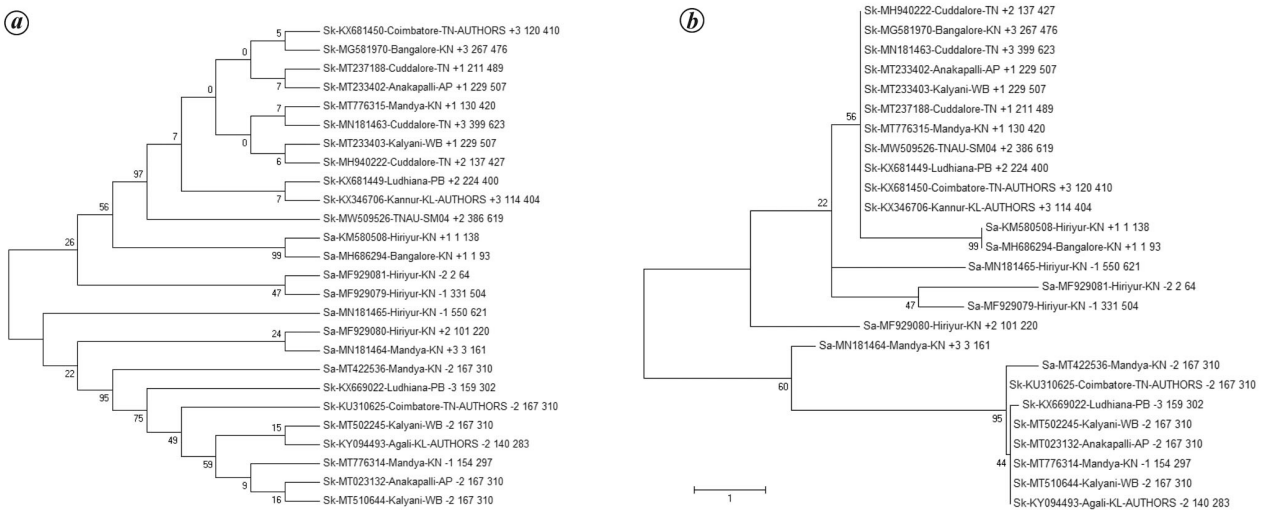

Figure 6. ***a***, Maximum likelihood phylogenetic tree of *S. andropogoni* and *S. krungthepensis* based on mtCOI gene-translated peptide sequence. Bootstrap values based on 1000 replications are indicated at the nodes. Each taxonomic unit is indicated by the NCBI accession number and place of collection. ***b***, Collapsed branches corresponding to partitions reproduced in less than 50% bootstrap replicates.

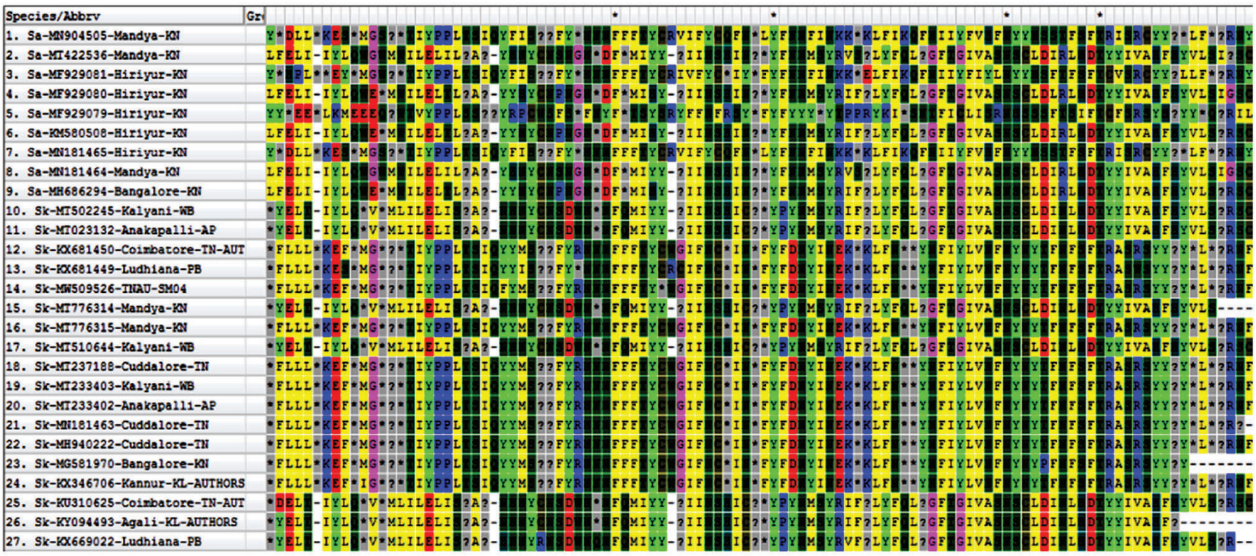

Figure 7. Alignment of amino acid sequences of *S. andropogoni* and *S. krungthepensis* showing conserved sequences.

DNA barcoding of mites[13]. Such methods are being increasingly used in the identification of genera and species under Tetranychidae. For example, Matsuda *et al.*[21,22] demonstrated that almost all species of Japanese *Oligonychus* and *Tetranychus* could be identified using the mtCOI gene of mitochondrial DNA. They used 18S and 28S rRNA genes to infer phylogenetic relationships within the sub-family Tetranychinae[23]. Fine resolution and reclassification of halacarid mites could be done earlier based on molecular phylogenetic analysis[24]. Following Matsuda *et al.*[23], we performed phylogenetic analysis of multi-locus genes, namely 5.8S rRNA and mtCOI genes, and compared the trees to differentiate *S. krungthepensis* and *S. andropogoni* in the present study. The phylogenetic tree based on the 5.8S rRNA gene clustered the two species separately. Further, *S. krungthepensis* sequences were tightly clustered and showed higher within-group similarity than *S. andropogoni*. The *S. andropogoni* sample collected from Karnataka (Sa-KX025157-Hiriyur) occupied a single clade independent of other *S. andropogoni* samples. The phylogenetic tree based on mtCOI clustering distinguished the two species, except for the sample Sa-MT422536-Mandya-KN, which clustered with *S. krungthepensis* clade, suggesting that it could be closer to *S. krungthepensis* than *S. andropogoni*. Phylogeny with translated peptide sequences showed a clustering pattern in congruence with that obtained for the mtCOI coding sequences. The analysis showed some variability among *S. andropogoni* with Sa-MT422536-Mandya-KN clustering with *S. krungthepensis*. Although molecular analysis seemed to suggest that it is appropriate to reclassify

Sa-MT422536-Mandya-KN as *S. krungthepensis*, such variation among samples of *S. andropogoni* and their occurrence in different clades could be partly due to differential host origin (Table 3). Despite considerable within-species and between-species variability in genetic distances, the overall results of the phylogenetic analysis indicated that *S. krungthepensis* is evolutionarily different from *S. andropogoni*. Also, *S. krungthepensis* samples collected by us at Coimbatore in October 2015 (NCBI accessions nos. KU183503, KX681450 and KU310625) (Table 1), whose morphological identity was confirmed by AINP on Agricultural Acarology, UAS, Bengaluru[20], constitute the first report of the mite species in India.

Schizotetranychus krungthepensis was described as a new species from *S. officinarum* in Ram Intra, Bangkok, Thailand, in November 2010 (Krungthep in Thai means the City of Angels) with detailed taxonomic description and characters to distinguish it from other *Schizotetranychus* spp., including *S. andropogoni*[12]. The only biological observations were that this species produces web-nests on the undersurface of leaves, adult females are yellowish-green with two pairs of maculae and eggs are white, translucent but turn yellow with age. In a subsequent compilation, the authors[14] provided notes and colour plates of female, male and aedeagus for several species of tetranychid mites, including *S. krungthepensis*. The limited information suggests that the mite lacks widespread distribution in Thailand in sugarcane, the only host recorded so far, and is not a serious pest of any other crop. A third possibility is that the species may have existed in Bangkok long before its collection in 2010 and identification in 2014 (ref. 12), a hypothesis that can be verified only if repositories with old collections exist in Thailand. A systematic survey of sugarcane and other crops may reveal its current distribution and economic importance in Thailand.

In India, current records of *S. krungthepensis* occurrence are from Ludhiana, Faridkot, Fazilka and Sangrur regions of Punjab, as documented by AINP on Agricultural Acarology, UAS, Bengaluru[25]. The mite was listed as one of 8–10 invasive spider mite species identified by molecular systematics[26], based on a previous report[25]. The publication that documents the occurrence of *S. krungthepensis* in India based on reports of AINP on Agricultural Acarology, UAS, Bengaluru and NCBI accessions, including our records, merely states that its invasive entry remains uncertain[19]. Assuming that *S. krungthepensis* is an exotic pest that originated in Thailand, its detection on sugarcane in different parts of India raises several hypothetical questions: (i) From where and when did the mite species enter India? (ii) After reaching India, did *S. krungthepensis* coexist with the native *S. andropogoni*, remain incognito due to its non-explosive manifestation and begin to displace *S. andropogoni* competitively as the recent spurt in mite occurrence or detection indicates?

Many phytophagous mite species, including *S. andropogoni*, are common to India and Thailand, with original records of occurrence and descriptions, of at least some, from either country[4]. These mite species may have strayed either way in the contiguous Asian subcontinent by different modes, including the clandestine transport of small quantities of seed material of prospective varieties. Confirmation of the identity of sugarcane mite in India as *S. andropogoni* in a taxonomic study in 2011 (ref. 9) and collection in 2010 and description in 2014 of *S. krungthepensis* in Thailand[12] indicate the possible origin of the latter as Thailand, not discounting the likelihood of its anonymous presence in other sugarcane-growing southeast Asian countries, including India. These observations also suggest post-2010/2011 as the probable time of entry of *S. krungthepensis* into India. This does not discard the possibility that the mite may have entered the country several years ago, even before it was collected and identified in Thailand, remained unnoticed for some years and spread spatially in a progressive manner before its detection in the recent past (Tables 1 and 2). While the sequence information of the Thailand population of *S. krungthepensis*, currently not available in the NCBI databases/public domain, and its phylogenetic analysis vis-à-vis Indian populations could establish the origin of the species as Thailand, examination of undescribed mite collections of the past decade, if available in Indian repositories, and re-examination of mite specimens regarded as *S. andropogoni* in recent non-taxonomic studies[10,11] may shed light on the probable time and place of entry. These studies would also help examine a different possibility that *S. krungthepensis* may have originated in India, moved to Thailand and remained undetected for long until its collection and description. Notwithstanding the identification of *S. krungthepensis* as distinctly different from *S. andropogoni* by AINP acarologists based on morphological characters that distinguish them[12,14,20] and evidence for molecular separation obtained in the present study, there appears to be a need to resolve the issue further by documenting the descriptions of Indian collections of *S. krungthepensis*.

If *S. krungthepensis* entered India some years ago, it might have masqueraded as *S. andropogoni* and perhaps been ignored by acarologists and entomologists due to similar field symptoms (Figure 1) and life stages (Figure 2). The non-explosive manifestation of *S. andropogoni* until a decade ago is suggestive of an equilibrium state, which sugarcane pests and natural enemies exhibit by maintaining their populations at the carrying capacity of the environment in tropical India due to uniform climate, favourable crop growth, and spatial and temporal continuity of food[27]. It is possible that *S. krungthepensis* began exploiting the vast and unoccupied foliage niche, coexisted with the native *S. andropogoni* for some time and began displacing the latter, as the recent intermittent spurts indicate.

In the past, sugarcane in India endured pest invasions within the country, more so from the subtropical to tropical region, leading to expansion of their geographical range but not the establishment of all as major pests. For example, the predominantly subtropical root borer *Polyocha depressella*

Swinhoe (Lepidoptera: Pyralidae)[28] appeared in tropical Tamil Nadu first in the early 1990s (ref. 29) and flared up yet again in 2015 (ref. 30). Both outbreaks, separated by almost two and a half decades, dissipated in the next one or two years probably due to discontinuance of susceptible varieties and indirect impact of biocontrol agents deployed against other target pests[30], besides other reasons.

This contrasts with the expansion of the invasive sugarcane woolly aphid *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae), a pest of the crop in North East India, in tropical States during 2002–04 (ref. 31), belying model-based predictions of its limited dispersal[32]. The proliferation of the aphid to near-epidemic levels[33] necessitated coordinated efforts predicated primarily on biological control[34,35]. Deployment of fortuitously introduced predators or native predators initially[34], followed by introduction and establishment of the parasitoid *Encarsia flavoscutellum* Zehntner (Hymenoptera: Aphelinidae) from the home of the aphid established equilibrium with the host[35,36].

A third example is the subtropical leaf miner *Asamangulia cuspidata* Maulik (Coleoptera: Chrysomelidae: Cassidinae: Hispini)[28] which reappeared recently in Tamil Nadu in a mild form; substantial activity of the parasitoid complex that accompanied the hispa continues to maintain the host at low levels till date[37].

Thus, natural or applied biological control obviously regulated host populations of woolly aphid and leaf miner, whereas lack of information on natural enemy status in tropical India precludes such conclusion on root borer *P. depressella*. Similarly, at present, it is difficult to visualize the status of *S. krungthepensis* from this angle. Yet, the absence of major outbreaks since its detection in 2015 leads to the speculation that the mite has been maintaining its population at the carrying capacity of the vast and vacant sugarcane foliage niche under the activity of natural enemies, about which no information is currently available.

The exotic fall armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) that entered India in mid-2018 with maize as its primary host soon spread to sugarcane[38,39]. However, the attack remained sporadic, confining to the tillering phase, apparently due to inherent defence mechanisms in the post-tillering phase and the absence of a fruiting body, unlike in maize[40]. Notwithstanding the entry of FAW into sugarcane fields, the detection of *S. krungthepensis* in 2015 appears to be the first record of an exotic pest exclusive to sugarcane in India, assuming that the mite originated in Thailand. Nonetheless, the possibility that the mite may have been introduced into either country through illegal transport of potential varieties underlines the need for a fool-proof quarantine system to prevent disastrous consequences to sugarcane agriculture. The present detection of *S. krungthepensis* in Thailand and India almost simultaneously should sound warning bells for sugarcane-growing countries, particularly Asia.

Regardless of the similarities in the biology of *S. krungthepensis* and *S. andropogoni* and the hypothetical possibilities discussed above, the former deserves an action plan for generating basic and applied knowledge to help manage it in the event of outbreaks.

Systematic surveys are needed in sugarcane belts of India to determine the actual status of *S. krungthepensis* vis-à-vis *S. andropogoni*. Since the limited data available presently suggest discrete distribution, such surveys are likely to reveal three scenarios, viz. competitive exclusion of the native species, the coexistence of both species, or the presence of native species alone in yet-to-be invaded areas. Internal quarantine and regulatory measures to prevent the movement of cane for seed or crushing, though difficult to enforce or practice, are likely to slow down the spread of the mite to areas with the third scenario.

Despite the current low-intensity levels and sporadic flare-ups, damage and crop loss caused by the mite need to be assessed in research plots and growers' farms since its attack has been observed to cause about 11.79% loss in leaf area[41]. Determination of accurate crop losses would justify appropriate interim chemical control measures.

Biological information, especially natural enemy profile, needs to be generated to determine the inadvertent entry of natural enemies alongside the mite or the adaptation of native natural enemies. These studies would also unravel, at least partly, the causes for its dominance and apparent replacement of the native species. In the event of an epidemic and the absence of local natural enemies, it may be imperative to import biological control agents from Thailand in the classical mode. However, a lack of relevant information on the natural enemy complex in Thailand may hinder this approach and warrant independent surveys by acarologists from India.

Since studies with *S. spontaneum* and Indian sugarcane hybrids revealed sources of resistance against the mite for possible introgression into hybrids for commercial cultivation[41], screening of sugarcane germplasm should be a part of the management programme envisaged for the mite.

Conflict of interest: The authors declare that they have no conflict of interest.

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ACKNOWLEDGEMENTS. We thank Dr N. Srinivasa (University of Agricultural Sciences, Bengaluru) for the identification of mite specimens. Gene sequences of *Sk* and *Sa* for phylogenetic analysis were downloaded from the public domain NCBI-GenBank. We thank the Director, ICAR-Sugarcane Breeding Institute, Coimbatore for encouragement and support and the two anonymous reviewers for their insightful comments that helped improve the manuscript.

Received 12 April 2022; revised accepted 1 July 2022

doi: 10.18520/cs/v123/i8/1038-1049