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DNA BARCODING AND ELUCIDATION OF CRYPTIC DIVERSITY IN THRIPS (THYSANOPTERA)

K. B. Rebijith[1,*], R. Asokan[1,*], V. Krishna[2], H. H. Ranjitha[1], N. K. Krishna Kumar[3] and V. V. Ramamurthy[4]
[1]Division of Biotechnology, Indian Institute of Horticultural Research, Bangalore, India

[2]Department of Biotechnology and Bioinformatics, Kuvempu University, Jnanasahyadri, Shankaraghatta, Shimoga, India

[3]Indian Council of Agricultural Research (ICAR), New Delhi, India

[4]Network Project on Insect Biosystematics, Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi

[†]Corresponding authors; E-mail: asokaniihr@gmail.com, rebijith@gmail.com

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Abstract

Accurate and timely identification of invasive insect pests underpins most biological endeavors ranging from biodiversity estimation to insect pest management. In this regard, identification of thrips, an invasive insect pest is important and challenging due to their complex life cycles, parthenogenetic mode of reproduction, sex and color morphs. In the recent years, DNA barcoding employing 5′ region of the mitochondrial Cytochrome Oxidase I (CO-I) gene has become a popular tool for species identification. In this study, we employed CO-I gene sequences for discriminating 151 species of thrips for the first time. Analyses of the intraspecific and intrageneric distances of the CO-I sequences ranged from 0.0 to 7.91% and 8.65% to 31.15% respectively. This study has revealed the existence of cryptic species in *Thrips hawaiiensis* (Morgan) (Thysanoptera: Thripidae) and *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae) for the first time, along with previously reported cryptic species such as *Thrips palmi* Karny (Thysanoptera: Thripidae), *T. tabaci* Lindeman, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), *Scirtothrips dorsalis* Hood. We are proposing, the feasibility of hosting an independent integrated taxonomy library for thrips and indicate that it can serve as an effective system for species identification, this approach could potentially play a key role in formulating effective insect pest management strategies.

Key Words: DNA barcoding, CO-I, thrips, intra and inter-specific distances

Resumen

La identificación precisa y oportuna de las plagas de insectos invasivos sustenta la mayoría de los esfuerzos biológicos desde la estimación de la diversidad biológica hasta el control de plagas de insectos. En este sentido, la identificación de los insectos plaga invasivos tales como trips es importante y un reto al nivel mundial debido a sus ciclos de vida complejos, el modo de reproducción partenogenética y los morfos de sexo y de color. En los últimos años, los códigos de barras de ADN empleando la región 5 'del mitocondrial citocromo oxidasa I gen (CO-I) se ha convertido en una herramienta popular para la identificación de especies. En este estudio, se emplearon secuencias de genes CO-I para discriminar 151 especies de trips por primera vez. El análisis de las distancias intra e inter-específicas de las secuencias de CO-I variaron desde 0.0 hasta 10.12% y 3.73% al 53.15%, respectivamente. Este estudio ha revelado la prevalencia de especies crípticas en *Thrips hawaiiensis* (Morgan) (Thysanoptera: Thripidae) y *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae) por primera vez, junto con los reportados previamente especies crípticas, como *Thrips palmi* (Thysanoptera: Thripidae) , *T. tabaci* Lindeman, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) y *Scirtothrips dorsalis* Hood. Estamos proponiendo, la factibilidad de organizar una biblioteca de taxonomía integrada independiente para los trips e indicamos que puede servir como un sistema eficaz para la identificación de especies, un enfoque que potencialmente podría jugar un papel clave en la formulación de estrategias eficaces de control de plagas de insectos.

Palabras Clave: código de barras del ADN, CO-I, thrips, distancias intra e interespecíficas

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Thrips (Thysanoptera) include major sap sucking insect pests, which limit crop productivity and nutritional security, by direct feeding or indirectly by transmitting plant pathogenic viruses (Rebijith et al. 2011). Among the known 6,000 species of *Thrips* (Thripidae) (Mound & Morris 2007) nearly 1% are pests of agricultural crops and the following 14 species are reported as vectors of Tospovirus: *Thrips tabaci* Lindeman, *T. palmi* Karny, *T. setosus* Moulton, *Scirtothrips dorsalis* Hood, *Frankliniella occidentalis* (Pergande), *F. schultzei* (Trybom), *F. bispinosa* (Morgan), *F. cephalica* (Crawford), *F. fusca* (Hinds), *F. gemina* Bagnall, *F. intonsa* (Trybom), *F. zucchini* Nakahara Monteiro, *Ceratothripoides claratris* (Shumsher) and *Dictyothrips betae* Uzel (Ullman et al. 1997; Mound 2005; Jones 2005; Pappu et al. 2009; Ciuffo et al. 2010; Hassani-Mehraban et al. 2010). Most of them are highly polyphagous, have overlapping host ranges, thus being collected together which makes their identification difficult, even though morphological identification keys and web-based identification systems are available. Difficulty in identification of thrips not only exists in the developmental stage, but also between polyphagous thrips species, e.g. *Thrips flavus* Schrank found to be morphologically very similar to *Thrips palmi* Karny (Glover et al. 2010). Morphological examination of *Thrips* to species level is restricted to adult specimens, as there are no adequate keys for identification of egg, larvae, or pupae (Kadirvel et al. 2013). Thrips are notorious for eliciting taxonomic problems mainly because of minute size, polymorphism (Murai et al. 2001), lack of solid morphological characters (Kadirvel et al. 2013), co-existence of different species on the same host plant, high intraspecific variations observed in thrips populations (Mound 2011) and need for taxonomic expertise (Brunner et al. 2004).

In order to implement an integrated pest management (IPM) strategy, a simple, accurate, general and easily applicable method is required to facilitate the identification of *Thrips* spp. Subsequent studies on thrips have demonstrated the utility of CO-I sequences being effective in identification of thrips species (Timm 2008; Glover et al. 2010) and also in unraveling cryptic species as in the case of *F. occidentalis* and *Pseudophilothrips gandolfoi* (Rugman-Jones et al. 2010; Mound et al. 2010).

Considering all the above mentioned factors, it is even more important to properly identify invasive quarantine pest species introduced at the ports-of-entry for early detection and risk analysis, where speed and accuracy are paramount (Glover et al. 2010). In this connection, Hebert et al. (2003a,b) proposed the concept of DNA barcoding, a powerful tool to identify all metazoan species employing a short standardized 658 bp fragment of the 5′ end of the mitochondrial cytochrome oxidase-I (CO-I) gene. DNA barcoding can be employed as the most effective approach for molecular identification of species independent of life stages, sex and polymorphism (Asokan et al. 2011), discriminating cryptic species (Glover et al. 2010; Rebijith et al. 2013), biotypes (Shufran et al. 2000), haplotypes (Zhang et al. 2011) and host and geographic associated genetic differences (Brunner et al. 2004; Rebijith et al. 2011). DNA barcoding can also play an important role in insect pest management program, where both selection and timing of the management practices can be affected by polymorphism and host adaptation/ suitability (Rebijith et al. 2013; Brunner et al. 2004).

In this study, molecular data have been generated and acquired (from NCBI and BOLD) to investigate the use of CO-I DNA barcoding in exploring diversity of thrips and is the first attempt to provide some understanding of the relationship between different *Thrips* spp. Thus, the purpose of the present study was to discriminate 996 sequences (CO-I) representing 151 thrips species globally and to record the presence of cryptic species and host or geographic associated genetic forms among thrips, if any.

Materials and Methods

Taxon Sampling and Morphological Identification

All the specimens were collected and stored in 95% ethanol during 2008-2012, and kept at -20 °C until processed. In each case, adults were examined using various characters described by Mound et al. 1996; Moritz et al. 2004. Prior to molecular work, all the thrips species were morphologically identified. In total, 151 thrips specimens representing 8 species were used for CO-I sequencing in this study (Table 1) and other sequences were retrieved from BOLD and NCBI-GenBank (see Supplementary Table 1). A summary of the current scientific classification of each thysanopteran species is given in Supplementary Table 2. These supplementary tables are available online in Florida Entomologist 97(4) (2014) at http://purl.fcla.edu/fcla/entomologist/browse.

DNA Isolation and Polymerase Chain Reaction (PCR)

A single specimen of each species of *Thrips* was digested overnight at 60 °C in lysis solution (10 mM Tris-HCl- pH-7.60, 20 mM NaCl, 100 mM Na_2 EDTA- pH-8.0, 1% Sarkosyl and 0.1 mg/mL proteinase K). DNA was extracted from the supernatant employing a non-destructive method (Mound and Morris, 2007a); while at the same time voucher specimens were mounted on glass slides and deposited with the National Pusa Collection (NPC), Indian Agricultural Research Institute (IARI), New Delhi, India. Standard

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TABLE 1. ANALYSED SAMPLES OF THRIPS SPECIES FROM THE CURRENT STUDY WITH DESCRIPTIONS OF THE SAMPLING LOCATIONS, GENBANK ACCESSION NUMBERS, NAMES OF THRIPS SPECIES AND HOST PLANTS, DATES AND VOUCHER SPECIMEN DETAILS.

Sl. No	Location	Locality	Accession Number	Name of the species	Host plant	Year of Collection	Specimen voucher
1	Maharastra	DOGR, Pune	KF015428	*Thrips tabaci*	*Allium cepa*	2010	KBRT-1
2	Karnataka	Bangalore, IIHR	KF015429	*Thrips tabaci*	*Allium cepa*	2011	KBRT-2
3	Coimbatore	Settiveedhi	KF015430	*Thrips tabaci*	*Allium cepa*	2012	KBRT-3
4		Muthugoundapalyam	KF015431	*Thrips tabaci*	*Allium cepa*	2012	KBRT-4
5	Tamil nadu	TNAU, Coimbatore	KF015432	*Thrips tabaci*	*Allium cepa*	2012	KBRT-5
6		Erode	KF015433	*Thrips tabaci*	*Allium cepa*	2012	KBRT-6
7		Trichy	KF015434	*Thrips tabaci*	*Allium cepa*	2012	KBRT-7
8		Bidar	KF015435	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-11
9		Bidar	KF015436	*Thrips palmi*	*Momordica charantia*	2011	KBRT-12
10		Bijapur	KF015437	*Thrips palmi*	*Macrotyloma uniflorum*	2011	KBRT-13
11		Gulbarga	KF015438	*Thrips palmi*	*Cucumis sativus*	2011	KBRT-14
12		Raichur	KF015439	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-15
13		Bagalkot	KF015440	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-16
14		Bagalkot	KF015441	*Thrips palmi*	*Luffa acutangula*	2011	KBRT-17
15		Belgaum	KF015442	*Thrips palmi*	*Momordica charantia*	2011	KBRT-18
16		Belgaum	KF015443	*Thrips palmi*	*Cucumis sativus*	2011	KBRT-19
17		Belgaum	KF015444	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-20
18		Belgaum	KF015445	*Thrips palmi*	*Luffa acutangula*	2011	KBRT-21
19		Dharwad	KF015446	*Thrips palmi*	*Cucumis sativus*	2011	KBRT-22
20		Dharwad	KF015447	*Thrips palmi*	*Luffa acutangula*	2011	KBRT-23
21		Dharwad	KF015448	*Thrips palmi*	*Momordica charantia*	2011	KBRT-24
22		Gadag	KF015449	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-25
23		Koppa	KF015450	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-26
24		Koppa	KF015451	*Thrips palmi*	*Momordica charantia*	2011	KBRT-27
25		Bellary	KF015452	*Thrips palmi*	*Cucumis sativus*	2010	KBRT-28
26		Bellary	KF015453	*Thrips palmi*	*Cucumis melo*	2010	KBRT-29
27		Bellary	KF015454	*Thrips palmi*	*Luffa acutangula*	2010	KBRT-30
28		Karwar	KF015455	*Thrips palmi*	*Citrullus lanatus*	2010	KBRT-31
29		Karwar	KF015456	*Thrips palmi*	*Solanum melongena*	2010	KBRT-32
30		Haveri	KF015457	*Thrips palmi*	*Citrullus lanatus*	2010	KBRT-33
31		Haveri	KF015458	*Thrips palmi*	*Vicia faba*	2010	KBRT-34
32		Davanagere	KF015459	*Thrips palmi*	*Luffa acutangula*	2010	KBRT-35
33		Davanagere	KF015460	*Thrips palmi*	*Luffa acutangula*	2010	KBRT-36

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Sl. No	Location	Locality	Accession Number	Name of the species	Host plant	Year of Collection	Specimen voucher
34		Chitradurga	KF015461	*Thrips palmi*	*Vicia faba*	2010	KBRT-37
35		Chitradurga	KF015462	*Thrips palmi*	*Luffa acutangula*	2010	KBRT-38
36		Tumkur	KF015463	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-39
37		Tumkur	KF015464	*Thrips palmi*	*Cucurbita maxima*	2011	KBRT-40
38		Shimoga	KF015465	*Thrips palmi*	*Solanum melongena*	2011	KBRT-41
39		Shimoga	KF015466	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-42
40		Shimoga	KF015467	*Thrips palmi*	*Luffa acutangula*	2011	KBRT-43
41		Udupi	KF015468	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-44
42		Mangalore	KF015469	*Thrips palmi*	*Solanum melongena*	2011	KBRT-45
43		Mangalore	KF015470	*Thrips palmi*	*Momordica charantia*	2011	KBRT-46
44		Madikeri	KF015471	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-47
45		Madikeri	KF015472	*Thrips palmi*	*Momordica charantia*	2012	KBRT-48
46		Hassan	KF015473	*Thrips palmi*	*Cucumis sativus*	2012	KBRT-49
47		Hassan	KF015474	*Thrips palmi*	*Momordica charantia*	2012	KBRT-50
48		Kolar	KF015475	*Thrips palmi*	*Solanum melongena*	2012	KBRT-51
49		Kolar	KF015476	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-52
50		Madikeri	KF015477	*Thrips palmi*	*Vicia faba*	2012	KBRT-53
51		Madikeri	KF015478	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-54
52		Mysore	KF015479	*Thrips palmi*	*Cucurbita maxima*	2012	KBRT-55
53		Mysore	KF015480	*Thrips palmi*	*Vicia faba*	2012	KBRT-56
54		Mandya	KF015481	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-57
55		Chamrajnagar	KF015482	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-58
56		Bangalore	KF015483	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-59
57		Balussery	KF015484	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-60
58		Thamarassery	KF015485	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-61
59		Thamarassery	KF015486	*Thrips palmi*	*Solanum melongena*	2011	KBRT-62
60		Ulliyeri	KF015487	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-63
61		Karumala	KF015488	*Thrips palmi*	*Solanum melongena*	2011	KBRT-64
62		Malapparamba	KF015489	*Thrips palmi*	*Solanum melongena*	2011	KBRT-65
63		Sulthan Bathery	KF015490	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-66
64		Kalpetta	KF015491	*Thrips palmi*	*Solanum melongena*	2011	KBRT-67
65		Malappuram	KF015492	*Thrips palmi*	*Solanum melongena*	2011	KBRT-68
66		Kannur	KF015493	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-69
67		Thrissur	KF015494	*Thrips palmi*	*Phaseolus vulgaris*	2011	KBRT-70

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Sl. No	Location	Locality	Accession Number	Name of the species	Host plant	Year of Collection	Specimen voucher
68		Palakkad	KF015495	*Thrips palmi*	*Solanum melongena*	2011	KBRT-71
69		Kolam	KF015496	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-72
70		Thiruvananthapuram	KF015497	*Thrips palmi*	*Solanum melongena*	2011	KBRT-73
71		Nava India	KF015498	*Thrips palmi*	*Phaseolus vulgaris*	2011	KBRT-74
72		Muthugoundapalayam	KF015499	*Thrips palmi*	*Citrullus lanatus*	2011	KBRT-75
73		Settiveedhi	KF015500	*Thrips palmi*	*Phaseolus vulgaris*	2012	KBRT-76
74		Salem	KF015501	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-77
75		Trichy	KF015502	*Thrips palmi*	*Phaseolus vulgaris*	2012	KBRT-78
76		Trichy	KF015503	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-79
77		Madurai	KF015504	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-80
78		Erode	KF015505	*Thrips palmi*	*Citrullus lanatus*	2012	KBRT-81
79	Bangalore	IIHR	KF015506	*Scirtothrips dorsalis*	*Capsicum annum*	2011	KBRT-82
80		Muthugoundapalyam	KF015507	*Scirtothrips dorsalis*	*Capsicum annum*	2010	KBRT-83
81		Balussery	KF015508	*Scirtothrips dorsalis*	*Capsicum annum*	2010	KBRT-84
82		Nagpur	KF015509	*Scirtothrips dorsalis*	*Gossypium hirsutum*	2010	KBRT-85
83		Mandya	KF015510	*Scirtothrips dorsalis*	*Capsicum annum*	2011	KBRT-86
84		Bangalore	KF015511	*Megalurothrips usitatus*	*Phaseolus vulgaris*	2011	KBRT-8
85		Bijapur	KF015512	*Megalurothrips usitatus*	*Phaseolus vulgaris*	2011	KBRT-9
86		Hassan	KF015513	*Megalurothrips usitatus*	*Phaseolus vulgaris*	2011	KBRT-10
87		Hebbal	KF606949	*Frankliniellas schultzei*		2012	KBRT-101
88		IIHR	KF606950	*Frankliniellas schultzei*		2012	KBRT-102
89		Coimbatore	KF606951	*Frankliniellas schultzei*		2012	KBRT-103
90		Salam	KF606952	*Frankliniellas schultzei*		2012	KBRT-104
91		Bangalore	KF606953	*Anaphothrips sudanensis*		2012	KBRT-106
92		Bangalore	KF606954	*Franklinothrips vespitormis*		2012	KBRT-105
93		Hallery Estate, Makkandur Kodagu	HM153744	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-39
94		Spices Board Nursery, Aigoor	HM153743	*Sciothrips cardamomi*	*Elettaria cardamomum*		ORP-2010-38
95		Sreelakshmi Estate, Kodagu	HM153742	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-37
96		Greenfield Estate, Virajpet	HM153741	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-36
97		Kalarikkal Estate, Siddapur Virajpet	HM153740	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-35
98		Cowribatta Estate, Siddapur Virajpet	HM153739	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-34
99		Yemmigundi Estate, Siddapur	HM153738	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-33
100		IISR Coorg	HM153737	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-32

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Sl. No	Location	Locality	Accession Number	Name of the species	Host plant	Year of Collection	Specimen voucher
101		Cherigala, Madikeri Coorg	HM153736	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-31
102		Bhagamandala-Coorg	HM153735	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-30
103		Bidarahalli, Mudigere	HM153734	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-29
104		Ballupet, Hassan	HM153733	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-28
105		Hebbanahalli, Sakleshpur	HM153732	*Sciothrips cardamomi*	*Elettaria cardamomum*	2008	ORP-2010-27
106		Rajakadu Estate, Padagiri	HM153731	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-26
107		Poabson Estate, Nelliyampathy	HM153730	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-25
108		Poabson Estate, Nelliyampathy	HM153729	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-25
109		Palagapandy Estate, Palakkad	HM153728	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-23
110		Minnampara, Nelliyampathy	HM153727	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-22
111		Devikulam, Idukki	HM153726	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-21
112		Upputhara, Idukki	HM153725	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-20
113		Vellathooval, Idukki	HM153724	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-19
114		Vakeri, Wayanad	HM153723	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-18
115		Vaduvanchal, Wayanad	HM153722	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-17
116		Kumily, Idukki	HM153721	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-16
117		Vandiperiyar, Idukki	HM153720	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-15
118		Peerumedu, Idukki	HM153719	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-14
119		Myladumpara, Idukki	HM153718	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-13
120		Parathode, Idukki	HM153717	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-12
121		Santhanpara, Idukki	HM153716	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-11
122		Thariode North, Wayanad	HM153715	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-10
123		Kattappana, Idukki	HM153714	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-09
124		Konnathady, Idukki	HM153713	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-08
125		Vathykudy, Idukki	HM153712	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-07
126		Chakkupallam, Idukki	HM153711	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-06
127		Kamakshy, Idukki	HM153710	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-05
128		Chinnakkanal, Idukki	HM153709	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-04
129		Erattayar, Idukki	HM153708	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-03
130		Pampadumpara, Idukki	HM153707	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-02
131		Kolapally, Wayanad	HM153706	*Sciothrips cardamomi*	*Elettaria cardamomum*	2009	ORP-2010-01
132		Tata Estate, Urulikkal, Tamilnadu	HQ230353	*Sciothrips cardamomi*	*Elettaria cardamomum*	2010	ORP-2010-45
133		Anali Estate, Valparai, Tamilnadu	HQ230352	*Sciothrips cardamomi*	*Elettaria cardamomum*	2010	ORP-2010-44
134		MSP plantation, Yercaud, Tamilnadu	HQ230351	*Sciothrips cardamomi*	*Elettaria cardamomum*	2010	ORP-2010-43

TABLE 1. (CONTINUED) ANALYSED SAMPLES OF THRIPS SPECIES FROM THE CURRENT STUDY WITH DESCRIPTIONS OF THE SAMPLING LOCATIONS, GENBANK ACCESSION NUMBERS, NAMES OF THRIPS SPECIES AND HOST PLANTS, DATES AND VOUCHER SPECIMEN DETAILS.

Sl. No	Location	Locality	Accession Number	Name of the species	Host plant	Year of Collection	Specimen voucher
135		TNDS Estate, Kodaikanal	HQ230350	*Sciothrips cardamomi*	*Elettaria cardamomum*	2010	ORP-2010-42
136		Sakthi Estate, Valparai, Tamilnadu	HQ230349	*Sciothrips cardamomi*	*Elettaria cardamomum*	2010	ORP-2010-41
137		Plenty Valley, Valparai, Tamilnadu	HQ230348	*Sciothrips cardamomi*	*Elettaria cardamomum*	2010	ORP-2010-40
138	India	Belagaum, Karnataka	EF117834	*Thrips palmi*	*Cucumis sativus*	2007	
139	India	Bijapur, Karnataka	EF117833	*Thrips palmi*	*Dolichos biflorus*	2007	
140	India	Raichur, Karnataka	EF117832	*Thrips palmi*	*Citrullus lanatus*	2007	
141	India	Bellary, Karnataka	EF117831	*Thrips palmi*	*Cucumis sativus*	2007	
142	India	Bellary, Karnataka	EF117830	*Thrips palmi*	*Luffa acutangula*	2007	
143	India	Bellary, Karnataka	EF117829	*Thrips palmi*	*Cucumis melo*	2007	
144	India	Belagaum, Karnataka	EF117828	*Thrips palmi*	*Citrullus lanatus*	2007	
145	India	Dharwad, Karnataka	EF117827	*Thrips palmi*	*Cucumis sativus*	2007	
146	India	Dharwad, Karnataka	EF117826	*Thrips palmi*	*Momordicha charantia*	2007	
147	India	Dharwad, Karnataka	EF117825	*Thrips palmi*	*Luffa acutangula*	2007	
148	India	Belagaum, Karnataka	EF117824	*Thrips palmi*	*Luffa acutangula*	2007	
149	India	Belagaum, Karnataka	EF117823	*Thrips palmi*	*Momordicha charantia*	2007	
150	India	Kunigal, Karnataka	EF117822	*Thrips palmi*	*Cucurbita moschata*	2007	
151	India	Kunigal, Karnataka	EF117821	*Thrips palmi*	*Citrullus lanatus*	2007	

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TABLE 2. INTRASPECIFIC K2P GENETIC DIVERGENCE AMONG THRIPS SPECIES.

Species	Average distance (D) (%)	Standard Error
P. dracaenae	0.36	0.0034
H. adolfifriderici	0.00	0.00
S. staphylinus	0.31	0.0021
C. brunneus	0.00	0.00
C. ericae	0.00	0. 00
C. manicatus	3.49	0.0098
C. meridionalis	0.00	0.00
E. americanus	0.24	0.0016
F. cephalica	0.00	0.00
F. intonsa	0.43	0.0022
F. tenuicornis	0.00	0.00
F. schultzei	0.10	0.0193
M. usitatus	0.16	0.0027
O. biuncus	0.00	0.00
O. ignobilis	2.20	0.0107
O. loti	0.00	0.00
O. meliloti	0.00	0.00
O. ulicis	0.12	0.0012
O. sylvanus	1.09	0.0075
K. robustus	0.00	0.00
L. lefroyi	1.09	0.0076
O. ajugae	1.48	0.0075
S. cardamomi	0.29	0.0009
S. aurantii	4.02	0.0161
S. citri	6.50	0.0211
S. perseae	4.58	0.0114
T. inconsequens	1.09	0.0079
T. alatus	0.54	0.0053
T. angusticeps	1.09	0.0076
T. flavidulus	0.31	0.0022
T. fuscipennis	0.00	0.00
T. flavus	1.63	0.0042
T. hawaiiensis	5.40	0.0118
T. major	3.96	0.0075
T. minutissimus	0.87	0.0054
T. nigropilosus	0.36	0.0021
T. obscuratus	2.87	0.0089
T. setosus	2.23	0.0092
T. trehernei	1.40	0.0058
T. urticae	0.78	0.0037
T. validus	0.00	0.00
T. vulgatissimus	0.27	0.0016
S. dorsalis	4.58	0.0104
T. palmi	4.52	0.0114
T. tabaci	7.91	0.0163

protocols were employed for Polymerase Chain Reaction (PCR), cloning and sequencing (Hajibabaei et al. 2006).

PCR was performed in a thermal cycler (ABI-Applied Biosystems, Veriti, USA) using the following cycling parameters; an initial denaturation step at 94 °C for 5 min followed by 35 cycles at 94 °C for 30 s, an annealing step at 48 °C for 45 s, an extension step at 72 °C for 45 s and a final extension step at 72 °C for 20 min using the CO-I specific primers: LCO-1490 ; 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ and HCO-2198; 5′- TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ (Hebert et al. 2003a; Hebert et al. 2003b). The total reaction volume of 25 µL contained 10 µM of each primer, 2.5 µL of 10X buffer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP and 1.0 U of Taq DNA polymerase (Fermentas Life Sciences, United Kingdom). The amplified products were resolved on 1.2% agarose gel, stained with ethidium bromide (10µg/mL) and visualized in a gel documentation system (UVP).

Sequencing and Sequence analyses

Each PCR product was purified using Gel extraction Kit (Nucleospin® Extract II, Macherey Nagel, Germany), cloned by ligation into PTZ57R/T vector (Fermentas Life Sciences, UK) and used to transform competent *Escherichia coli* (DH5α) cells. Blue- White colony, selection was carried out and plasmids were isolated using GenJET™ plasmid MiniPrep kit (Fermentas Life Sciences, United Kingdom), according to the manufacturer's protocol from the overnight culture of positive clones cultured in Luria Broth. Sequencing was performed in an automated sequencer (ABI prism® 3730 XL DNA Analyzer; Applied Biosystems, USA) using M13 universal primers, in both forward and reverse directions. A homology search was done using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov/) and sequence alignment was performed using BioEdit version 7.0.9.0 (Hall et al. 1999). In this study, we analyzed all the available thrips CO-I sequences from NCBI-GenBank for understanding and determining the intraspecific variations in each species (Meyer et al. 2005). All the sequences generated from this study are available at BOLD (www.barcodingoflife.com) and in NCBI-GenBank. The complete details such as *Thrips* species, host plants, date of collection and voucher specimen number are given in Supplementary Table 1 along with the NCBI-GenBank extracted sequences.

CO-I sequences were aligned using the Clustal W program integrated in BioEdit.7.0 (Hall et al. 1999). The sequences were further analyzed employing MEGA.5.0 (Kumar et al. 1993) to obtain conspecific and congeneric distances, whilst Neighbor-Joining trees were constructed employing the Kimura-2-parameter (K2P) distance model (Kimura et al. 1980; Saitou et al. 1987). Node support were assessed with 1000 bootstrap pseudoreplicates.

RESULTS

The dataset consisted of CO-I sequences from 996 individuals representing 151 species of Thrips

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(Thysanoptera: Insecta), of which 151 sequences were produced in this study (Supplementary Table. 1, Supplementary Table. 2, Table. 1 respectively). Sequence analysis revealed that, 459 characters were variable among which 406 was parsimony informative. Evidence of nuclear copies was not found in any of the sequences subjected for analyses, which was supported by the absence of stop codons and the base composition was similar with no indels (Rebijith et al. 2012). Majority of the nucleotide substitutions occurred in the wobble position (third position) of the triplet codon (48.9%, 218 sites). Nucleotide frequencies were 29.46% (A), 39.38% (T), 13.87% (G) and 17.29% (C) and base composition found to be biased towards Adenine and Thymine, which together constituted 69.2% as is typical for other invertebrate genes (Wang et al. 2011; Rebijith et al. 2013).

Neighbor Joining Analysis

The CO-I dataset resulted in a single NJ tree representing 151 thrips species, which formed distinct haplotype clusters (Fig. 1). The intraspecific and intrageneric sequence divergence ranged from 0.0 to 7.91% and 8.65% to 31.15%, respectively (Table 2 and Table 3, respectively). This discrete barcoding gap between intra and inter specific distances (Hebert et al. 2004) allowed us to clearly distinguish all the thrips species employed in this study. Besides being direct pests, many of the thrips species are known to vector Tospoviruses (Mound et al. 1996). In this regard, it is important to analyze the diversity within thrips species such as, *Thrips palmi*, *T. tabaci*, *Frankliniella occidentalis*, *Scirtothrips dorsalis, F. schultzei*, *Thrips hawaeiinsis*, *S. perseae* and *Chirothrips manicatus,* etc. For the first time, we were able to record the existence of possible cryptic species within 2 *Thrips* spp. viz. *T. hawaiiensis* and *S. perseae* along with previously reported cryptic species viz., *T. palmi* (Rebijith et al. 2011; Glover et al. 2010), *T. tabaci* (Brunner et al. 2004), *F. occidentalis* (Rugman-Jones et al. 2010), *S. dorsalis* (Rebijith et al. 2011; Kadirvel et al. 2013) based on the 'CO-I- 10X barcoding Rule' proposed by Hebert et al. 2004. All of them were supported by the calculated intra and inter specific genetic divergence (Table. 4) for different sub clusters as shown in Fig. 2 (A – F).

Thrips palmi

Thrips palmi was represented by 145 specimens forming 2 lineages. One lineage (n = 102) was consisted of the Indian *T. palmi* population collected from various host plants, and the second lineage (n = 43) represented the world population with *T. palmi* representatives from China, Dominican Republic, Thailand, Japan and United Kingdom. There was much less variation (0.4% to 0.7%) within these lineages, however 8.2% difference (hereafter, D) was observed between them (Fig. 2A, Table 4), which is indicative of geographically isolated cryptic species.

Thrips tabaci

A total of 146 of *T. tabaci* specimens were analyzed and found to form 2 clades. The first clade comprised a *T. tabaci* population representing various geographical locations across the globe from different host plants. However, the second clade represented a population collected only from the tobacco plant, *Nicotiana tabacum.* As an indicator of cryptic species, there was less variation evident within these clades (0.3% to 0.9%), whereas 9.0% D observed between them (Fig. 2B, Table 4).

Scirtothrips dorsalis

Scirtothrips dorsalis was represented by 39 specimens forming 4 lineages, among which no perfect lineages - neither with geographical locations nor host associated genetic differences - were formed. However, very little variation (0 to 0.96%) was observed within these lineages, and 1.84% to 10.07% D observed between them, which was indicative of the cryptic speciation in *S. dorsalis* (Fig. 2C, Table 4).

Frankliniella occidentalis

Frankliniella occidentalis was represented by 224 specimens, which formed 5 lineages of which none was > 500 bp in length. However, the genetic variation within and between the major 2 clades were 0 to 0.10% and 1.11 to 6.2%, respectively (Fig. 2D, Table 4).

Thrips hawaiiensis

Thrips hawaiiensis was represented by 22 specimens forming 5 lineages (Fig. 2E) with genetic variation 0 to 0.5% within these lineages and 3.4% to 9.5% between them (Table 4).

Scirtothrips perseae

Scirtothrips perseae was represented by 47 specimens forming 3 lineages, of which the first, second and third were represented by 6, 4 and 37 specimens respectively, with intra and inter subcluster values ranging from 0.07% to 0.91% and 5.1 to 11.6%, respectively (Fig. 2F, Table 4).

Discussion

Rapid or timely and accurate identification of invasive pests, such as thrips, is important and chal-

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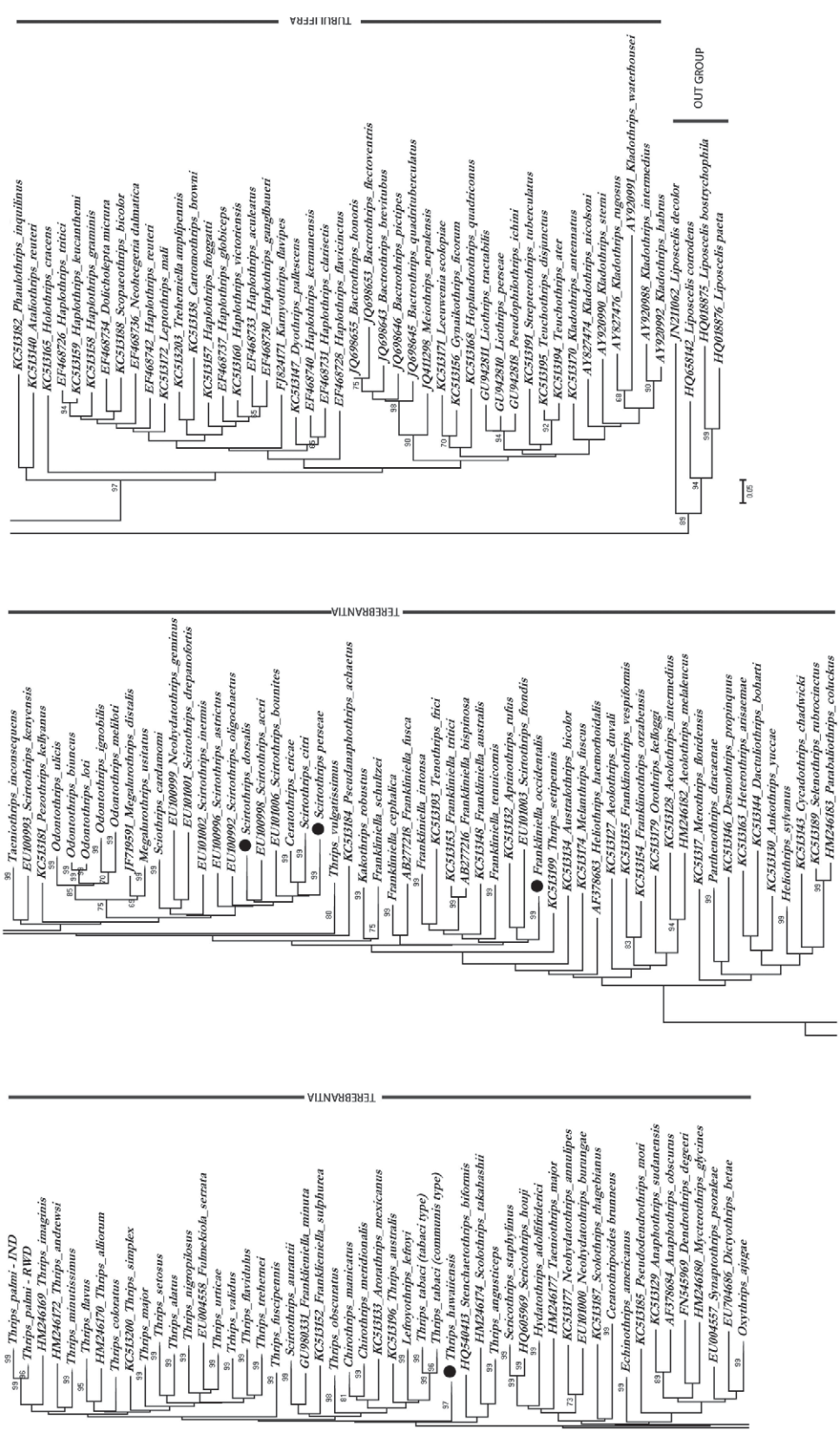

Fig. 1. Neighbor-Joining tree with bootstrap support (2,000 replicates) showing clusters of thrips species for CO-I sequences. Distinct clades for 151 species of thrips can be seen in the figure, in which both *T. palmi* and *T. tabaci* consist of two groups and 4 other species viz.*T. hawaiiensis*, *S. dorsalis*, *S. perseae and F. occidentalis* are each marked with a dark circle as an indicator of possible cryptic species. Dendrograms for these 5 species are shown in Figs. 2A, 2B, 2C, 2D and 2F, respectively. Bootstrap value > 65% only showed in the figure.

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TABLE 3. INTRAGENERIC K2P GENETIC DIVERGENCE WITHIN DIFFERENT GENERA OF THYSANOPTERA.

Genus	Distance	Std. Error
Scirtothrips	21.63	0.0275
Thrips	20.97	0.0264
Megalurothrips	10.56	0.0273
Neohydatothrips	27.99	0.0455
Chirothrips	09.87	0.0254
Frankliniella	20.51	0.0262
Odontothrips	12.51	0.0208
Haplothrips	12.21	0.0173
Kladothrips	23.13	0.0321
Liothrips	08.65	0.0236
Bactrothrips	10.98	0.0182
Anaphothrips	31.15	0.0571
Aeolothrips	29.45	0.0484
Sericothrips	08.94	0.0235

lenging worldwide, as these pests can cause crop damage either by direct feeding or by transmitting plant pathogens (German et al. 1992; Hebert et al. 2004). In this regard, classical taxonomy has its own strength, however DNA barcoding employing CO-I has the added advantage of not being limited by polymorphism, sex, and life stage of the target species (Rebijith et al. 2013). Our study is the first attempt to examine a large number of thrips species on a global scale, which include large number of genera represented by multiple species, where we expected that lower intra specific distances may cause problems in delimiting species. However, barcoding employing the CO-I gene allowed us to accurately discriminate all 151 species of thrips. Thus, CO-I DNA barcodes proved to be an invaluable tool for delimiting thrips species, an approach complementing classical taxonomy in the context of effective plant quarantine and biological control initiatives (Rugman-Jones et al. 2006).

Genetic Divergence

DNA barcoding has become an important tool in species identification, and has improved our ability to understand diversity among populations through to higher level taxa (Puckridge et al. 2013). Researchers employed a 2% genetic divergence cutoff value for species identification. However, many exceptional cases are reported with lower interspecific genetic divergences, yet most of the cases were still able to be correctly dissected out amongst most of the species (Pfannenstiel et al. 2008). By employing this 2% cutoff criterion for species delimitation, we aptly identified all thrips species in this study.

The mean K2P genetic distance values found for conspecific and congeneric comparison were 3.5% and 17.3% respectively, which were found to be on par with previous studies (Glover et al. 2010). Additionally, we analyzed a large number of closely related species, e.g., species of Genus *Thrips*, our observed mean congeneric divergence value was slightly smaller than in previous studies (Glover et al. 2010). This could be either due to the possible recent species radiation of *Thrips* similar to that of fresh water fish fauna (Albert et al. 2011; Bermingham et al. 1998; Montoya-Burgos et al. 2003), or the possible evolutionary rate variation of CO-I among different species employed in this study.

Advantages of DNA Barcoding in Taxonomy

Classical taxonomy employing morphological characters cannot be used for all species of thrips in all life stages because of insufficient phenotypic variation (Brunner et al. 2004). In addition, the presence of unusual morphological forms of species on different hosts, minute size, co-existence of different species on the same host, complex life cycles, color morphs, and parthenogenetic mode of reproduction, etc., add to the difficulties of precise identification. Furthermore, morphological examination of thrips to species requires adult specimens as there are no reliable keys for identification of immature stages, but even these are often difficult for a non-expert to use. In this regard, DNA barcoding can be an added advantage and an effective tool for molecular species identification (Brunner et al. 2004; Glover et al. 2010;

TABLE 4. LIST OF SPECIES WITH HIGHER INTRASPECIFIC K2P DIVERGENCES.

Species	Intraspecific divergence (%)			Number of subclusters	Inter-subclusters divergence	Intra-subclusters divergence
	Min	Max	Mean			
Thrips palmi	0.0	12.3	3.9	2	8.2	0.4 to 0.7
Thrips tabaci	0.0	13.8	3.33	2	9.0	0.3 to 0.9
Scirtothrips dorsalis	0.0	18.62	3.97	4	1.84 to 10.07	0 to 0.96
Thrips hawaiiensis	0.0	8.9	4.7	5	3.4 to 9.4	0 to 0.5
Scirtothrips perseae	0.0	11.4	3.9	3	5.1 to 11.6	0.07 to 0.91
Frankliniella occidentalis	0.0	10.32	1.76	5	0.02 to 0.10	1.1 to 6.2

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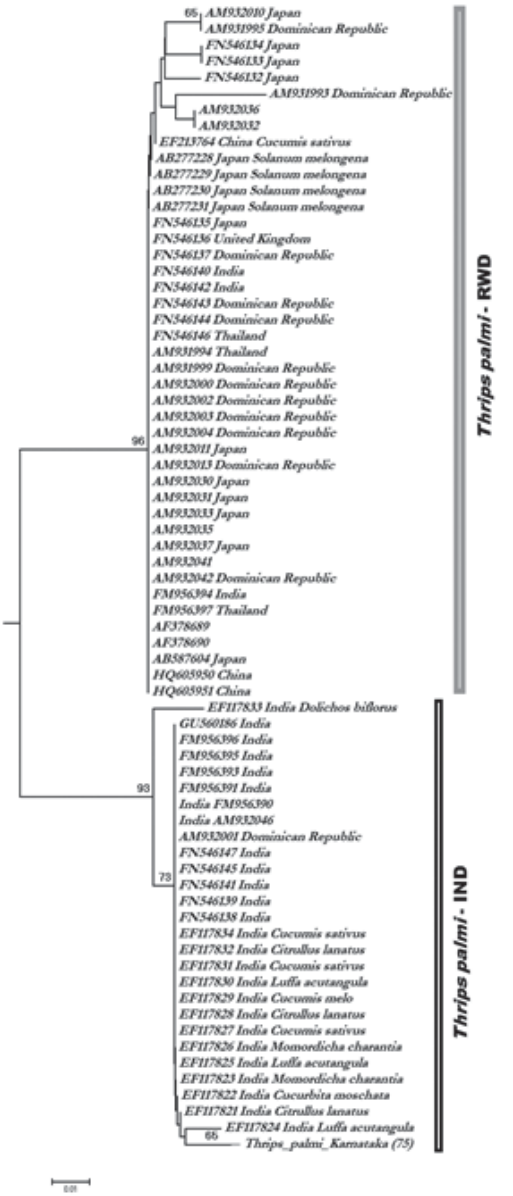

Fig. 2A. Neighbor-Joining tree with bootstrap support (2,000 replicates) showing clustering of *T. palmi* species for CO-I sequences. Two distinct groups can be seen Group-I (*Thrips palmi*- RWD, represents the *T. palmi* populations from various countries viz. Japan, China, Dominican Republic, Thailand and U.K.) and Group-II (*Thrips palmi*-IND, which clearly associated with Indian subcontinent), with 100% bootstrap support.

Lee et al. 2010), elucidation of biotypes or cryptic species, and host associated genetic differences (Shufran et al. 2000; Brunner et al. 2004) and species discovery (Foottit et al. 2010) in insects. Additionally, DNA barcoding could also be used in the identification of unknown thrips species that co-exist in a cropping system, since one particular Tospovirus disease may sometimes be vectored by more than one species of thrips (Wijkamp et al. 1995; Kadirvel et al. 2013; Amin et al. 1981).

In the recent past, DNA barcoding has become an effective tool in revealing cryptic and potentially new species, which has increased our knowledge of biodiversity (Rebijith et al. 2013; Puckridge et al. 2013). In this study, 7 species showed conspecific genetic divergence values ≥ 2%, and were subdivided into further sub clusters (Fig. 3 a- h). Hebert et al. (2004) proposed the 'CO-I DNA 10X barcoding rule', whereby identification of cryptic species is possible if the 2 lineages diverge by 10 times or more the average intra sub cluster variability within these lineages. In this study, such a sub cluster analysis has revealed tight clusters with significant larger mean values (2.57% to 18.02%), and smaller mean values within subclusters (0.00 to 3.28%) (Table 5). Such deep conspecific divergence has been reported previously in DNA barcoding and most of the entities are considered as cryptic species (Handfield & Handfield et al. 2006; Smith et al. 2006; Gomez et al. 2007; Pfenninger et al. 2007; April et al. 2011). The possible reasons for such higher conspecific genetic divergences are (i) representation of a novel species (ii) misidentification of species, and (iii) host/geographic preference of the organism (Brunner et al. 2004). However, according to Shao et al. (2003), the 10X estimation may be too low for *Thrips*, since they are known to have greater mitochondrial gene rearrangements and molecular evolution.

On the other hand, 2 *Thrips* species, viz. *T. palmi* and *T. tabaci,* require special attention because of their geographic and host associated genetic differences. *Thips palmi* formed 2 lineages; of which one lineage is unique to India (IND) and second lineage represents the rest of the world (ROW) (Rebijith et al. 2011). Geographic isolation and genetic drift can contribute strongly to intraspecific phylogeographic structure (Avise et al. 1987). Such allopatric lineages reinforce the fact that both these populations (IND and ROW) have independent evolutionary histories, which could be explained by restricted gene flow due to many physical and chemical barriers (April et al. 2011). Whereas in the case of *T. tabaci*, the 2 biotypes viz. 'tabaci' and 'communis', can be clearly distinguished with a character on the abdomen of the second larval stage (Zawirska et al. 1976). Brunner et al. (2004) proved the existence of 3 host associated genetic groups, viz. L1, L2 (Leak plant- 'communis type' sensu Zawirska) and T (Tobacco plant-'tabaci type') and proposed that when host fidelity is perfect, reproductive isolation is complete. In recent studies Jacobson et al. (2013) grouped *T. tabaci* into two entities based on their reproductive mode. However, our results showed 2 lineages, one with tobacco plants and the second is with various host plants such as onion, leak, etc., on par with previous studies (Za-

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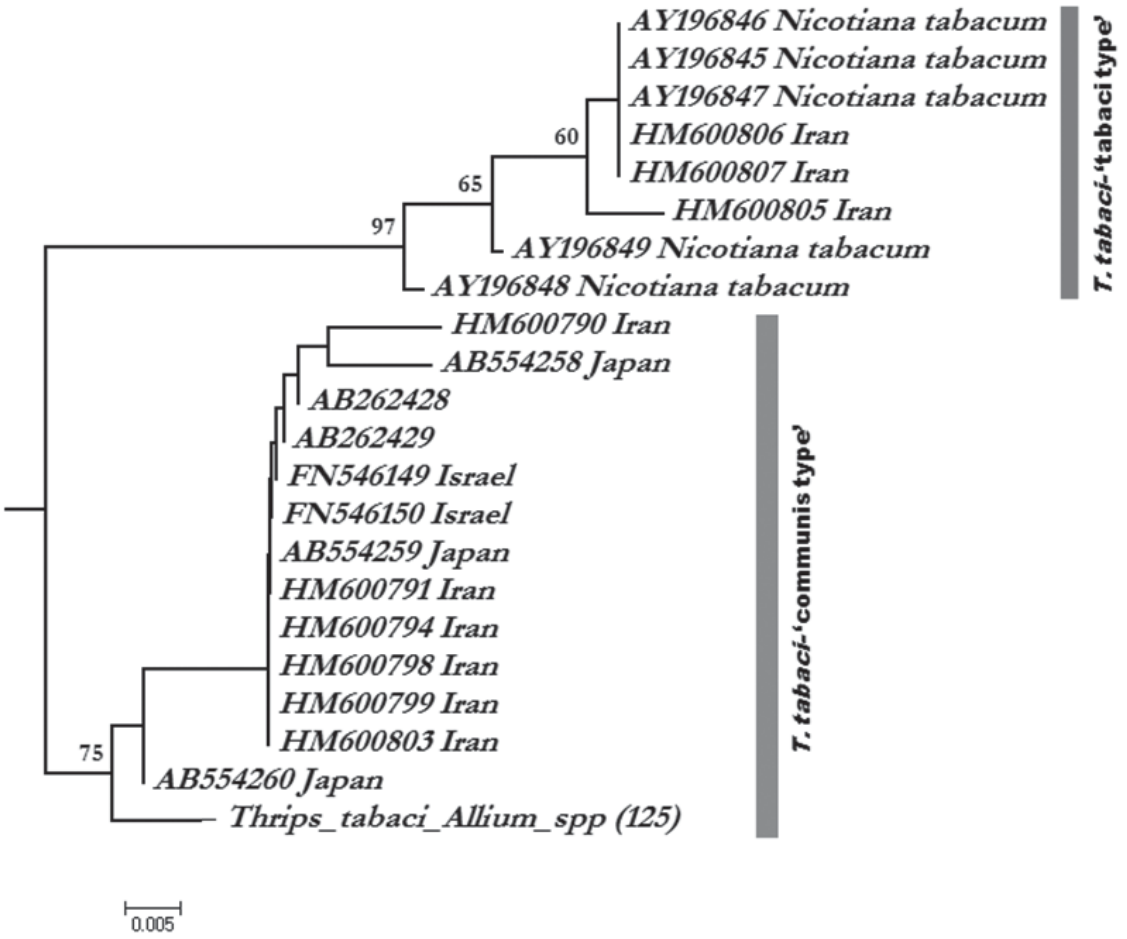

Fig. 2B. Neighbor-Joining tree with bootstrap support (2,000 replicates) showing clustering of *T. tabaci* species for CO-I sequences. Two distinct groups can be seen Group-I (*Thrips tabaci*- 'tabaci type'- represents the *T. tabaci* populations from host plant tobacco, *Nicotiana tabacum* and Group-II (*Thrips tabaci*- 'communis type'- which clearly associated with various host plants such as onion, leek, etc.) with 100% bootstrap support.

wirska et al. 1976; Brunner et al. 2004). In a nutshell, we conclude that all these cases represent cryptic species, but both *T. palmi* and *T. tabaci* can be subdivided and are strong candidates for novel species.

DNA Barcoding in Biosecurity

One of the major threats to crop production and productivity is the spread of invasive species such as thrips, aphids, whiteflies and planthoppers, etc., within and outside the country. Many of the monitoring programs are challenged by both quantity and quality of materials at the port of entry as well as the taxonomic breadth of intercepted organisms. Furthermore, morphological examination of majority of agricultural invasive pests such as thrips to species level is usually restricted to (i) adult specimens as there are no adequate keys for eggs, larvae or pupae (ii) polymorphism and (iii) sexual forms. At this juncture, CO-I based molecular identification has an added advantage of not being limited by any of the above factors, and DNA barcoding has shown success in discriminating arthropods of quarantine importance such as Tephritidae, Lymantriidae (Armstrong et al. 2005), *Spodoptera* spp. (Nagoshi et al. 2011), *Liriomyza* spp. (Scheffer et al. 2006) and Thysanoptera (Qiao et al. 2012). Thus, DNA barcoding can play a vital role in the international bio-security as an additional diagnostic protocol for quarantine pests at the port of entry (FAO 2006). DNA barcoding technique has been employed in New Zealand since 2005 for the identification of Tephritidae and Lymantriidae (Armstrong et al. 2005). Thrips species used in the

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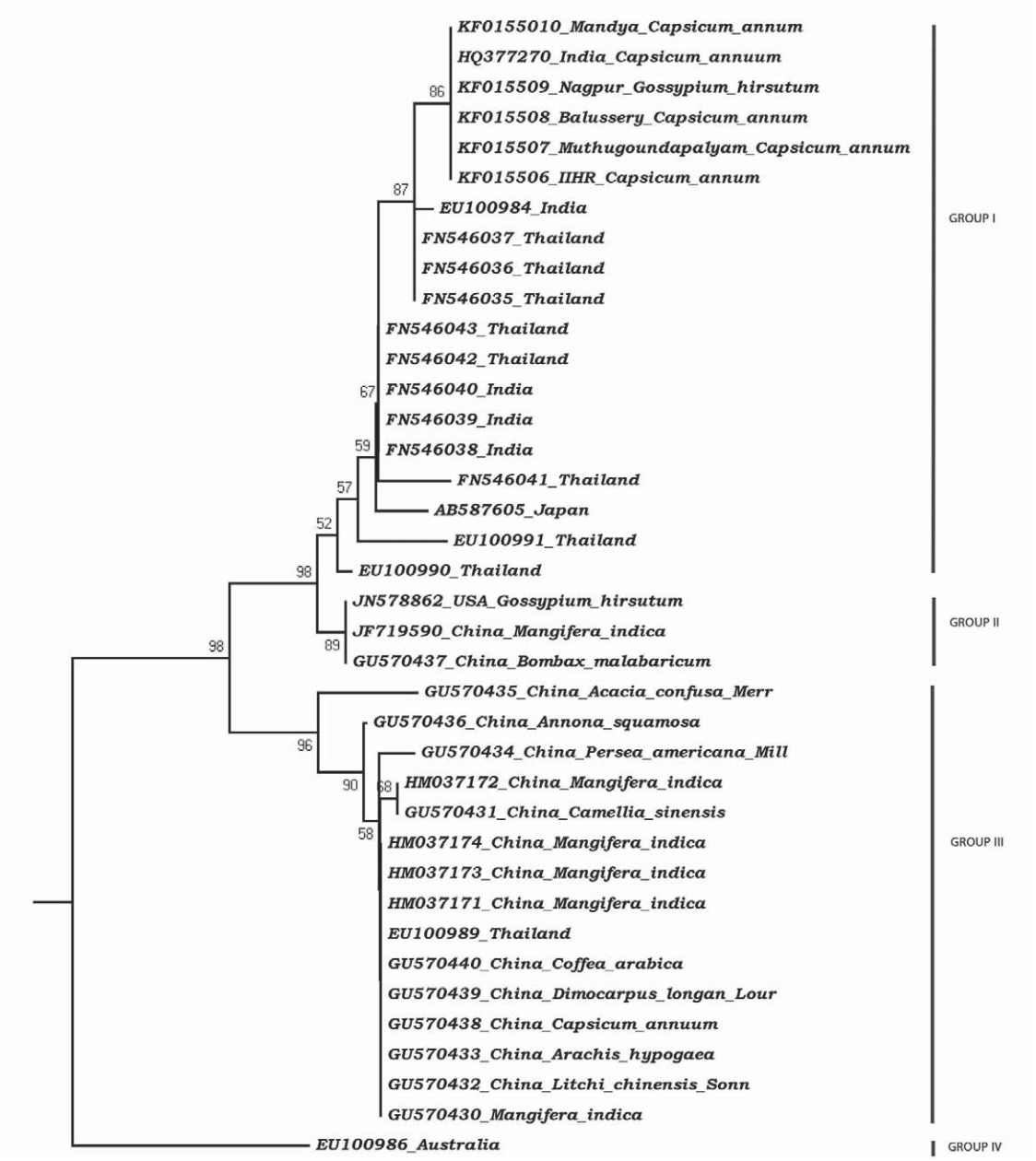

Fig. 2C. Neighbor-Joining tree with bootstrap support (2,000 replicates) showing clustering of *S. dorsalis* species for CO-I sequences. Four distinct groups can be seen, among which group-III corresponds to *S. dorsalis* collected on various host plants from China. Sequences generated from this study formed group I along with other sequences from Japan and Thailand.

current study were differentiated on the basis of DNA barcodes, which proved to be an invaluable tool for identification of Thysanoptera invasive insect pests, an approach complementing classical taxonomy. Having all these advantages, DNA barcoding was included as an effective diagnostic tool for invasive insect pest in the International Plant Protection Convention (IPPC) (FAO 2006).

Implications of DNA Barcoding for Insect Pest Management

Thrips continue to pose dual problems by either direct feeding or by transmitting plant pathogenic tospoviruses in both field and green house conditions (Mound et al. 1996; Rebijith et al. 2011). Control of thrips with insecticides is a

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Fig. 2D. Neighbor-Joining tree with bootstrap support (2,000 replicates) showing five distinct clusters for *F. occidentalis* CO-I sequences. Number in bracket indicates, the individuals formed that cluster.

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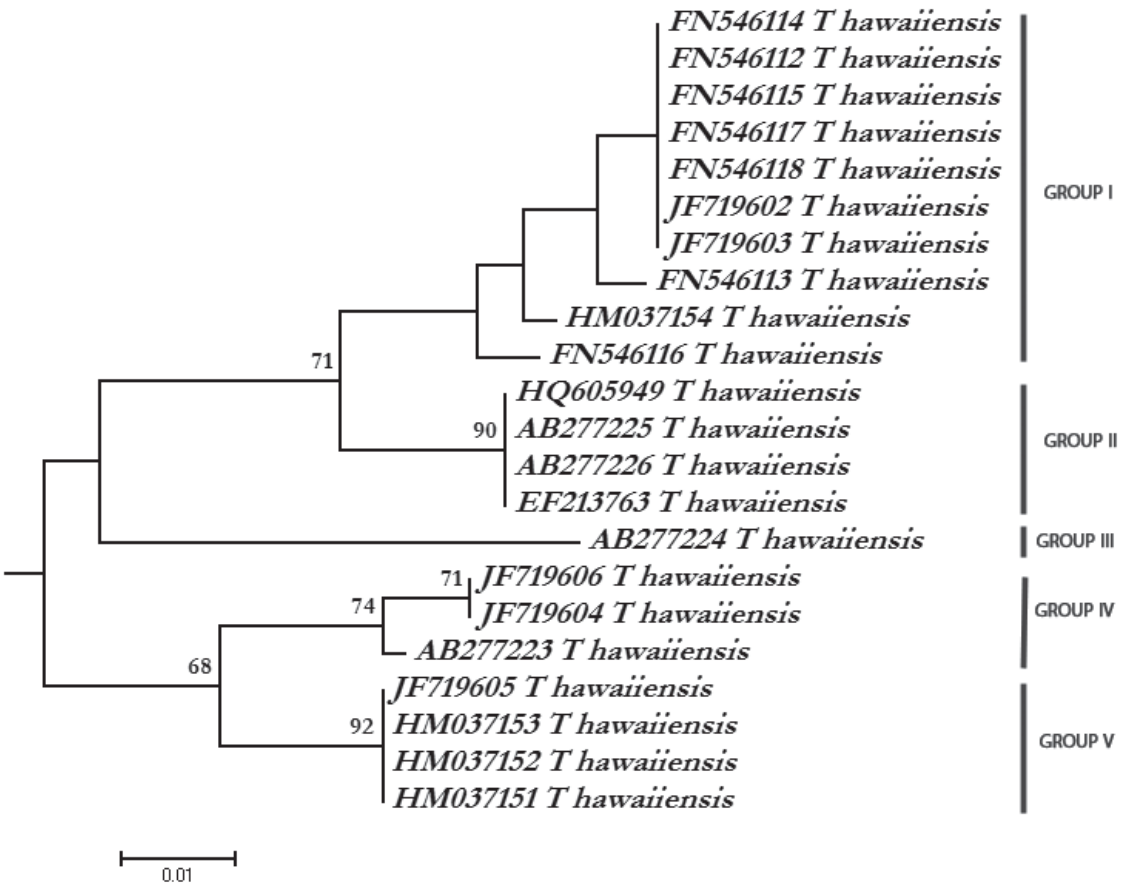

Fig. 2E. Neighbor-Joining tree with bootstrap support (2,000 replicates) showing five clusters for *T. hawaiiensis* CO-I sequences.

difficult task due to their parthenogenetic mode of reproduction (Rebijith et al. 2013), mode of life cycles (Brunner et al. 2004), etc. Eggs and pupae are protected either in leaf tissue or in soil/leaf litter and the larvae & adults are protected within buds and flowers (Glover et al. 2010). Yet, farmers employ insecticides as a primary control measure for the management of thrips, which could ultimately lead to the insecticide resistance.

Plant disease management requires accurate identification of species to understand the biology, population structure and ecology of the species (Rebijith et al. 2013). In this connection, *T. palmi* and *T. tabaci*, which infest watermelon to which they transmit Watermelon Bud Necrosis Virus, WBNV, and onion to which they transmitting Iris Yellow Spot Virus, IYSV, respectively, demand quick control measures using pesticides in order to limit the spread of these most potent viruses. On the other hand, *Thrips flavus* and *Thrips nigropilosus* (morphologically very similar to *T. palmi* and *T. tabaci*), can be managed effectively by employing biological control agents known as entomopathogenic nematodes (EPNs), such as *Thripenema fuscum* **n. sp**. (Asokan and Rebijith, unpublished data).

Reducing unnecessary pesticide usage through proper species identification can save growers money, and reduce chances of development insecticide resistance, as well as be more environmentally protective.

Conclusions

In this study, DNA barcoding proved to be an effective tool that can be employed for species identification, elucidation of cryptic species, biotypes and also in the discovery of new species. We trust that our work will serve as a rapid, precise, independent identification approach for the discrimination of thrips species of different life stages and color morphs, both for the species presently studied, and in the future, for other pest species of agricultural, horticultural and forestry interest and importance. This will in turn help in elucidation of the epidemiology of tospoviruses, their

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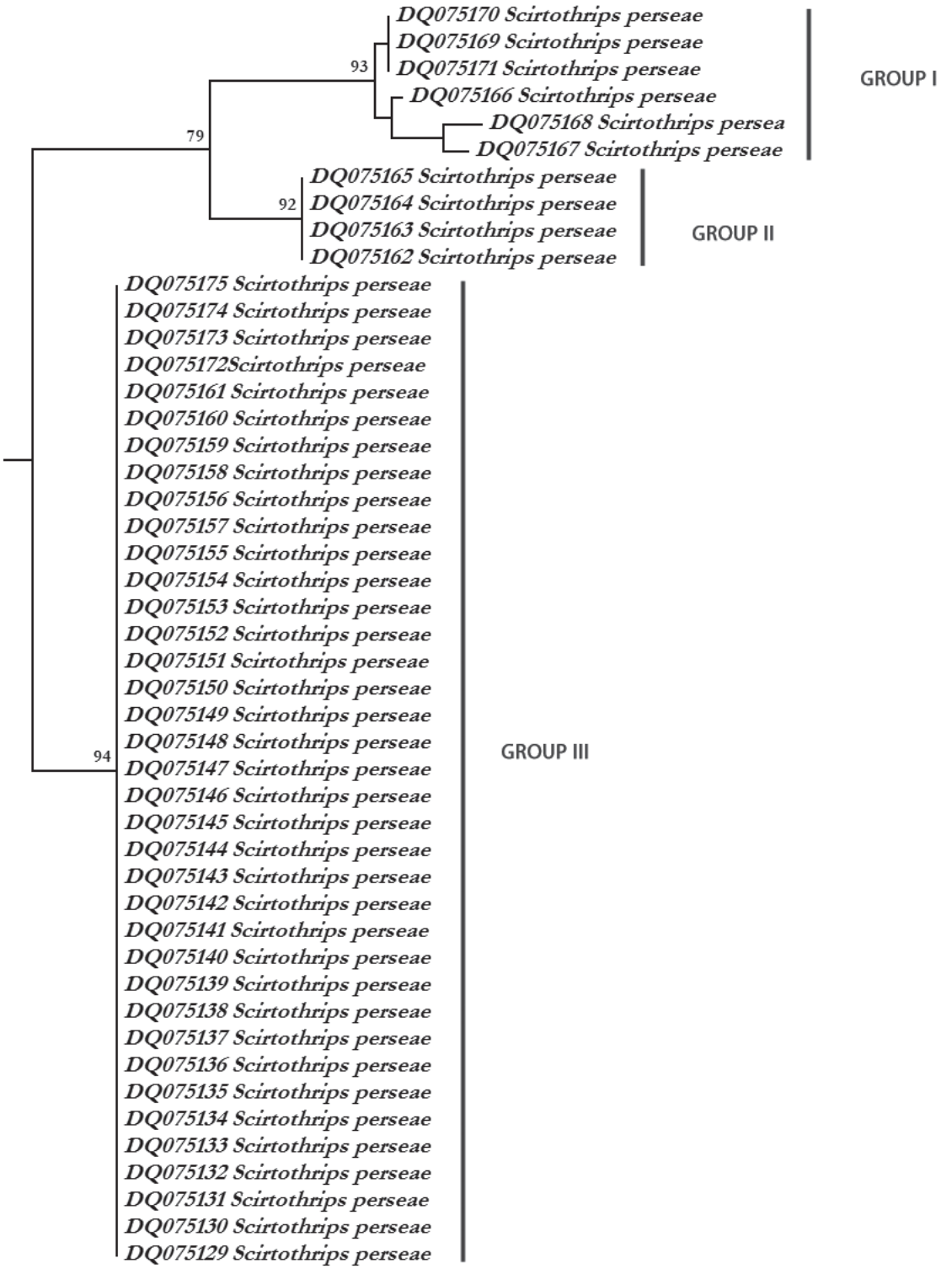

Fig. 2F. Neighbor-Joining tree with bootstrap support (2,000 replicates) showing three clusters for *S. perseae* CO-I sequences.

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management and serve as a potentially valuable tool in quarantine at ports-of-entry. Moreover, as our study has revealed, the existence of cryptic thrips species in *Thrips hawaiiensis* and *Scirtothrips perseae* and shows that further studies on the evolution of these particular species (and doubtless others too) are required before we can certain that we are dealing with sensu stricto taxa rather than sensu lato taxa.

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