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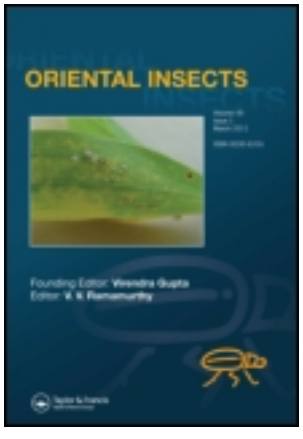

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Molecular diversity of cardamom thrips *Sciothrips cardamomi* (Ramakrishna) (Thripidae: Thysanoptera)

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The cardamom thrips *Sciothrips cardamomi* (Ramakrishna) is a destructive pest, and it is observed in all major Indian ecotypes of cardamom, viz. Vazhukka, Malabar and Mysore. Molecular studies can complement its morphological distinctions as it could be applied for identification of its intraspecific populations. Herein, identification of the adults based on cytochrome *c* oxidase subunit 1 is discussed, and the molecular diversity deciphered among the 45 intraspecific populations from the three cardamom ecotypes is explained. Although these fall in two clades, there are no significant variations among these intraspecific populations occurring in cardamom from India.

Keywords: cardamom; *Sciothrips cardamomi*; molecular diversity; cytochrome *c* oxidase subunit 1; intraspecific populations

Introduction

Cardamom, *Elettaria cardamom* Maton, is a native of the evergreen forests of Western Ghats in Southern India and is of three major ecotypes, viz. Vazhukka, Malabar and Mysore of India. The cardamom thrips *Sciothrips cardamomi* (Ramakrishna) is the most destructive and persistent pest (Gopakumar and Chandrasekhar 2002). It was first described by Ramakrishna Ayyar (1935) from Anamalai Hills of Tamil Nadu in India as *Taeniothrips cardamomi*. Later Bhatti (1969) transferred it to *Sciothrips*. It is also known from Sri Lanka as cardamom (Dharmadasa et al. 2008) and other zingiberaeceous plants from Hawaii (Mau and Kessing 2007). The molecular diversity of its intraspecific populations from the three cardamom ecotypes deciphered using mitochondrial cytochrome *c* oxidase subunit 1 (COX-1) is discussed herein.

Materials and methods

Specimens of *S. cardamomi* were field collected from cardamom plants from Kerala, Karnataka and Tamil Nadu (Table 1), and these were preserved in 70% ethyl alcohol in vials at −20°C. These were identified using Bhatti (1969), prior to molecular studies

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Table 1. Details of *S. cardamomi* populations studied.

GenBank Accession No.	Geographic region	Collector name	Collection date	Specimen designation	Host
HM153744	Makkathur, Kodagu, Karnataka	TKJ, KKS, ESS	18-11-2009	ORP-2010-39	Vazhukka
HM153743	Aigoor, Kodagu, Karnataka	TKJ, KKS, ESS	18-11-2009	ORP-2010-38	Vazhukka
HM153742	Somwarpet, Kodagu, Karnataka	TKJ, KKS, ESS	18-11-2009	ORP-2010-37	Vazhukka
HM153741	Siddapur, Virajpet, Kodagu, Karnataka	TKJ, KKS, ESS	19-11-2009	ORP-2010-36	Vazhukka
HM153740	Siddapur, Virajpet, Kodagu, Karnataka	TKJ, KKS, ESS	19-11-2009	ORP-2010-35	Vazhukka
HM153739	Siddapur, Virajpet, Kodagu, Karnataka	TKJ, KKS, ESS	19-11-2009	ORP-2010-34	Vazhukka
HM153738	Siddapur, Virajpet, Kodagu, Karnataka	TKJ, KKS, ESS	19-11-2009	ORP-2010-33	Vazhukka
HM153737	Appangala, Kodagu, Karnataka	SD, TKJ, KKS, ESS	17-11-2009	ORP-2010-32	Vazhukka
HM153736	Madikeri, Kodagu, Karnataka	TKJ, KKS, ESS	17-11-2009	ORP-2010-31	Vazhukka
HM153735	Bhagamandala, Kodagu, Karnataka	TKJ, KKS, ESS	18-11-2009	ORP-2010-30	Vazhukka
HM153734	Bidarahalli, Mudigere, Karnataka	ESS	05-12-2009	ORP-2010-29	Malabar
HM153733	Ballupet, Hassan, Karnataka	ESS	01-12-2009	ORP-2010-28	Malabar
HM153732	Hebbanahalli, Sakleshpur, Karnataka	ESS	02-12-2009	ORP-2010-27	Malabar
HM153731	Padagiri, Nelliyampathy, Kerala	ESS	03-02-2010	ORP-2010-26	Vazhukka
HM153730	Poabson Estate, Nelliyampathy, Kerala	ESS	02-02-2010	ORP-2010-25	Malabar
HM153729	Poothundu Estate, Palakkadu, Kerala	ESS	03-02-2010	ORP-2010-24	Vazhukka
HM153728	Seethargundu, Palakkadu, Kerala	ESS	03-02-2010	ORP-2010-23	Vazhukka
HM153727	Minnampara, Palakkadu, Kerala	ESS	03-02-2012	ORP-2010-22	Vazhukka
HM153726	Devikulam, Idukki, Kerala	ESS	23-12-2009	ORP-2010-21	Vazhukka
HM153725	Upputhara, Idukki, Kerala	ESS	22-12-2009	ORP-2010-20	Vazhukka
HM153724	Vellathooval, Idukki, Kerala	ESS	23-12-2009	ORP-2010-19	Vazhukka
HM153723	Vakeri, Wayanadu, Kerala	TKJ, KKS, ESS	30-12-2009	ORP-2010-18	Malabar
HM153722	Vaduvanchal, Wayanadu	TKJ, KKS, ESS	30-12-2009	ORP-2010-17	Vazhukka
HM153721	Kumily, Idukki, Kerala	ESS	22-12-2009	ORP-2012-16	Vazhukka
HM153720	Vandiperiyar, Idukki, Kerala	ESS	22-12-2009	ORP-2012-15	Vazhukka
HM153719	Peerumedu, Idukki, Kerala	ESS	22-12-2009	ORP-2012-14	Vazhukka
HM153718	Myladumpara, Idukki, Kerala	ESS	24-12-2009	ORP-2012-13	Vazhukka
HM153717	Parathode, Idukki, Kerala	ESS	24-12-2009	ORP-2012-12	Malabar
HM153716	Santhanpara, Idukki, Kerala	ESS	24-12-2009	ORP-2012-11	Malabar
HM153715	Thariode North, Wayanadu, Kerala	TKJ, KKS, ESS	08-10-2009	ORP-2012-10	Vazhukka
HM153714	Kattappana, Idukki, Kerala	ESS	22-12-2009	ORP-2012-09	Vazhukka
HM153713	Konnathady, Idukki, Kerala	ESS	23-12-2009	ORP-2012-08	Vazhukka

HM153712	Vathykudy, Idukki, Kerala	ESS	23-12-2009	ORP-2012-07	Vazhukka
HM153711	Chakkupallam, Idukki, Kerala	ESS	22-12-2009	ORP-2012-06	Malabar
HM153710	Kamakshmy, Idukki, Kerala	ESS	22-12-2009	ORP-2012-05	Vazhukka
HM153709	Chinnakkanal, Idukki, Kerala	ESS	21-12-2009	ORP-2012-04	Vazhukka
HM153708	Erattayar, Idukki, Kerala	ESS	23-12-2009	ORP-2012-03	Vazhukka
HM153707	Pampadumpara, Idukki, Kerala	ESS	21-12-2009	ORP-2012-02	Vazhukka
HM153706	Kolappally, Wayanadu, Kerala	TKJ, KKS, ESS	08-10-2009	ORP-2012-01	Vazhukka
HQ230353	Urulikkal, Tamil Nadu	ESS	30-04-2010	ORP-2012-45	Vazhukka
HQ230352	Valparai, Tamil Nadu	ESS	29-04-2010	ORP-2012-44	Vazhukka
HQ230351	Yercaud, Tamil Nadu	ESS	02-06-2012	ORP-2012-43	Vazhukka
HQ230350	Kodaikanal, Tamil Nadu	ESS	30-04-2010	ORP-2012-42	Vazhukka
HQ230349	Valparai, Tamil Nadu	ESS	29-04-2010	ORP-2012-41	Mysore
HQ230348	Valparai, Tamil Nadu	ESS	29-04-2012	ORP-2012-40	Vazhukka

TKJ – T.K. Jaacob, KKS – K.K. Sasidharan, ESS – E.S. Sujeesh and SD – S. Devasahayam.

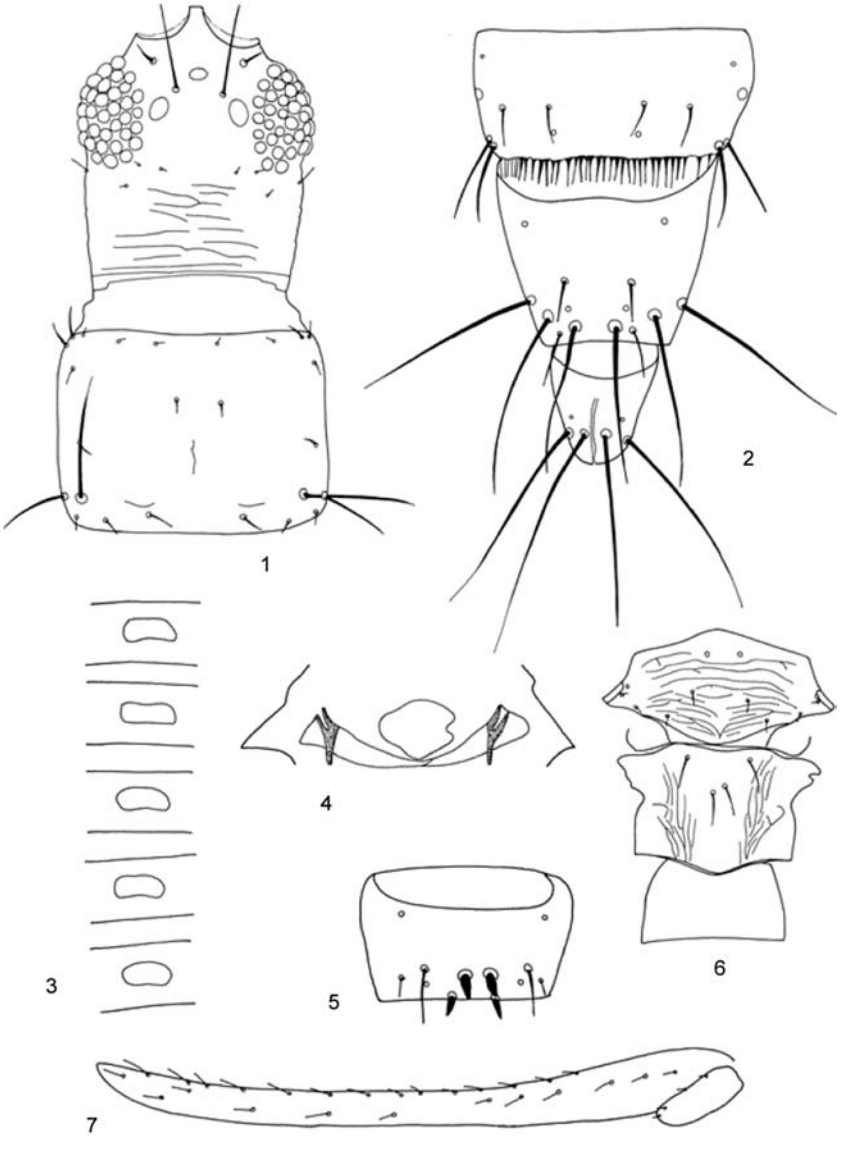


Figure 1–7. *S. cardamomi*: 1, head and prothorax; 2, abdominal tergites VIII–X; 3, male, pore plate on abdominal sternites III–VII; 4, half part of prosternum; 5, male, abdominal tergite IX; 6, mesonota and metanota; 7, fore wing.

(Figures 1–7). Total DNA was isolated using the 'salting out' procedure adapted from Rugman-Jones et al. (2006). Individual specimens were pierced through one side of the abdomen using a sterilised minute pin and placed in 0.5 ml PCR tubes containing 100 µl of 10 mM Tris (pH 7.4), 100 mM NaCl, 10 mM EDTA, 1% SDS (TNES). These tubes were incubated at 37°C for 24 h, and the proteins were precipitated with 5 M NaCl for 30 s of vigorous shaking. These proteins were pelleted in a microfuge at 13,000 rpm for 5 min, and the supernatant was transferred to a new microfuge tube. Then the DNA was precipitated from the supernatant by adding one volume of ice-cold 100% ethyl alcohol and incubated

for 1 h at −20°C. It was then pelleted by centrifugation, washed in ice-cold 70% ethyl alcohol, air dried and finally dissolved in 20 μl of sterile distilled water.

PCR was carried out in a thermal cycler (ABI-Applied Biosystems, Veriti, Maywood Avenue, USA) as follows: 94°C for 4 min as initial denaturation followed by 35 cycles of 94°C for 30 s, 47°C for 45 s, 72°C for 45 s and 72°C for 20 min as final extension using universal COX-1 primers (Folmer et al. 1994). PCR was performed in 25 μl of total reaction volume containing 20 picomoles of each primer, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP and 0.5 U of Taq DNA polymerase (Fermentas Life Sciences, EU). The amplified products were resolved in 1.0% agarose gel, stained with ethidium bromide (10 μg/ml) and visualised in a gel documentation system (UVP, Cambridge, UK). The voucher specimens are deposited in the National Pusa Collection, Division of Entomology, Indian Agricultural Research Institute, New Delhi (Table 1).

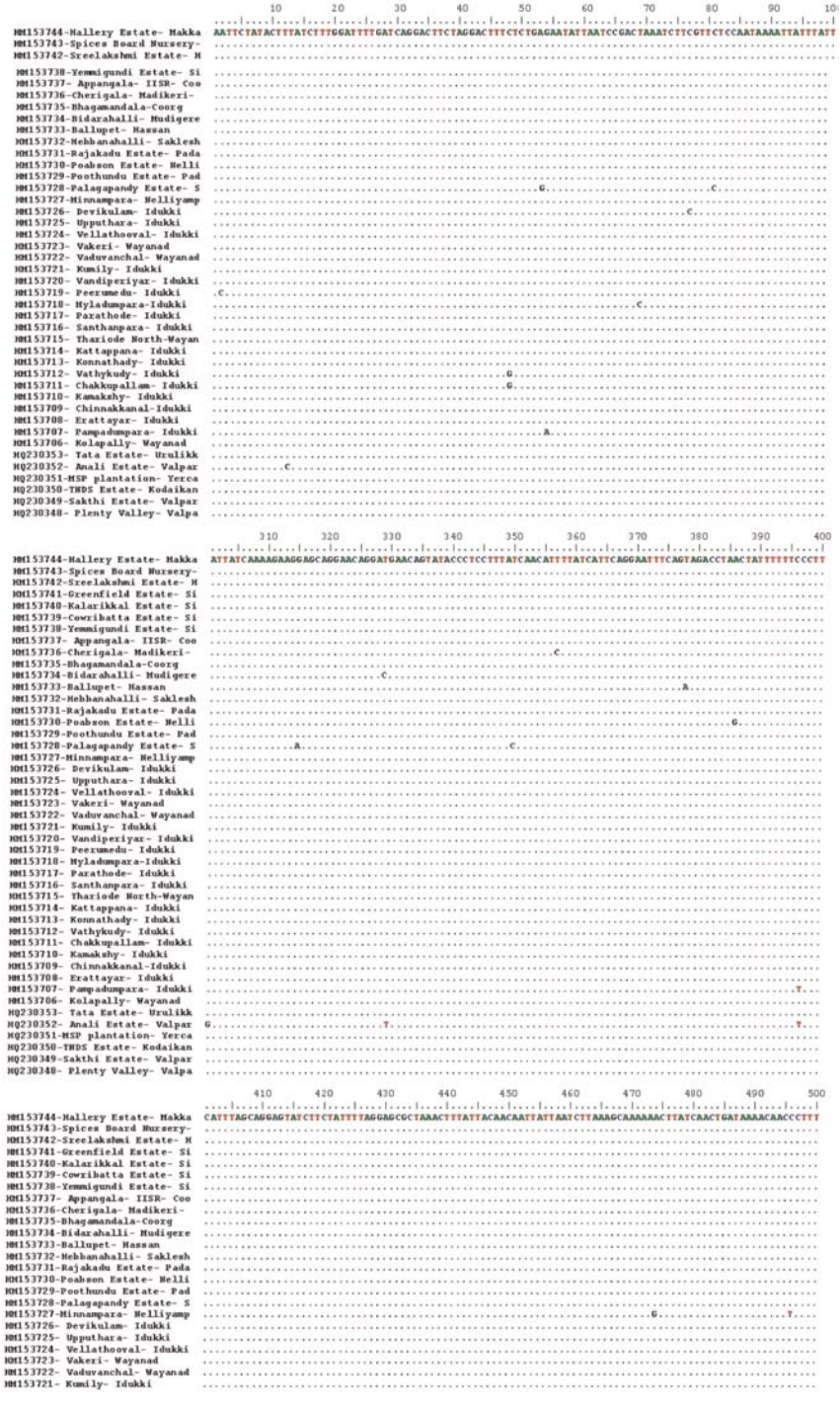
PCR amplified products were ligated into T/A cloning vector PTZ57R/T, which was used for transforming *Escherichia coli* DH5α by the standard protocols (Sambrook and Russel 2001) using Fermentas cloning kit. The transformed cells were spread on Luria Bertani (LB) agar plates containing X-gal (300 μg/ml), Isopropyl β-D-1-thiogalactopyranoside (IPTG) (120 μg/ml) and ampicillin (100 μg/ml). The plates were then incubated overnight at 37°C to screen blue and white colonies, and all the white colonies (colonies harbouring the insert) were maintained on Luria Bertani Agar (LBA) containing ampicillin (100 mg/ml), again incubated overnight at 37°C and stored at 4°C until further use. Plasmids were isolated using GeneJET Plasmid Miniprep Kit (Fermentas Life Sciences, EU) according to manufacturer's protocol, from overnight cultures of the five randomly selected clones multiplied in LB broth. Cloning was confirmed by colony PCR, and recombinant plasmids were compared with colony without insert.

Sequencing was carried out in an automated sequencer (ABI Prism 310; Applied Biosystems, USA) using M13 universal primers both in forward and reverse directions. Homology search was carried out using BLAST (http://www.ncbi.nlm.nih.gov), and the differences in nucleotide sequences were determined using the sequence alignment editor 'BioEdit'. The sequences are deposited in NCBI GenBank (Accession Nos. HM153706–HM153744 and HQ230348–HQ230353). Tree construction was done by neighbour joining and maximum parsimony using MEGA 4.0 (Kumar et al. 1993).

Results and discussion

The COX-1 sequencing resulted in 655-bp sequences for the 45 intraspecific populations of *S. cardamomi* collected from the locations of Kerala, Karnataka and Tamil Nadu. Molecular identifications were corroborated with morphological identification (Figures 1–7). Multiple sequences of COX-1 were aligned using ClustalX with default settings (Version 1.83, 2003; Thompson et al. 1997), and comparison of these sequences revealed insignificant variations (Figure 8). Figure 8 shows that fifty-three characters were variable, of which 11 were parsimony informative (Figure 8). There were no evidence of stop codons within the sequences showing the absence of nuclear copies, and the base pair composition was similar with no indels.

The maximum composite likelihood estimate of nucleotide substitution patterns for sequences was performed using MEGA 4.0 (Tamura et al. 2007). The reliability of the clustering pattern in the trees was determined by the bootstrap test with 1000 replications. Each entry shows the probability of substitution from one base (row) to another base (column) instantaneously. Rates of transitional substitutions obtained are shown in bold and those of transversional substitutions in italics (Table 2). The nucleotide frequencies



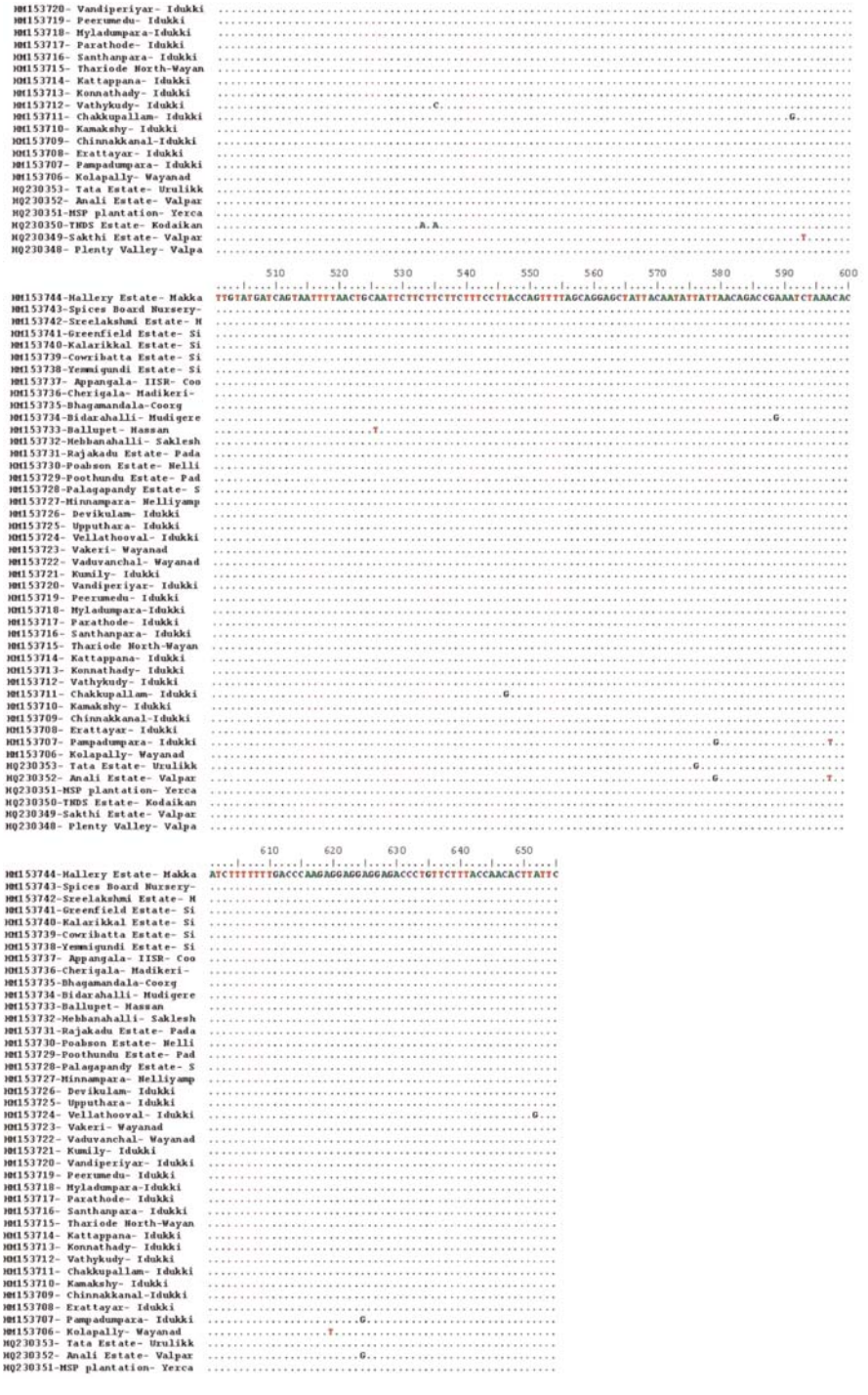


Figure 8. Consensus sequence of 655 bp from the mitochondrial COX-1 gene for *S. cardamomi*. Dots indicate nucleotides that are identical throughout the species compared.

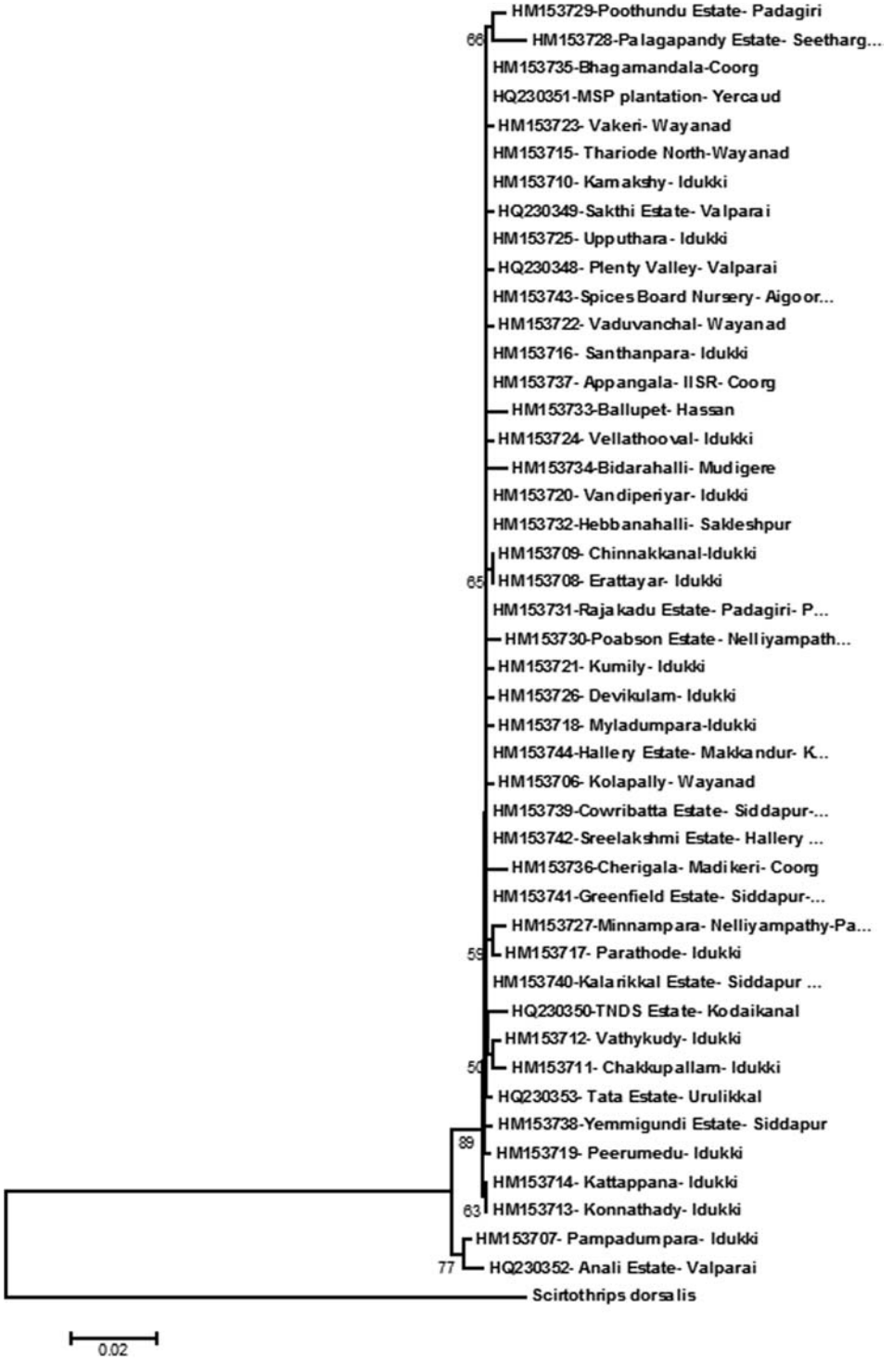


Figure 9. Minimum Evolution (ME) tree with bootstrap support (1000 replicates) showing clustering of *S. cardamomi* species for COX-I sequences. *Scirtothrips dorsalis* was used as an out group.

Table 2. *S. cardamomi* populations – maximum composite likelihood estimate of the nucleotide substitution patterns.

	A	T	C	G
A	–	*4.12*	*2.18*	**11.91**
T	*3.6*	–	**12.07**	*1.4*
C	*3.6*	**22.81**	–	*1.4*
G	**30.59**	*4.12*	*2.18*	–

Note: Each entry shows the probability of substitution from one base (row) to another base (column) instantaneously. Only entries within a row should be compared. Rates of transitional substitutions are shown in **bold** and those of transversional substitutions in *italics*.

were 0.319 (A), 0.364 (T), 0.193 (C) and 0.124 (G). The base composition of the COX-1 gene fragment was biased towards adenine (A) and thymine (T), which together constituted 68.4%. The overall transition (ti)/transversion (tv) bias of nucleotide sequence was $R=2.222$, where $R = [\mathrm{A} \times \mathrm{G} \times k_1 + \mathrm{T} \times \mathrm{C} \times k_2]/[(\mathrm{A} + \mathrm{G}) \times (\mathrm{T} + \mathrm{C})]$. Codon positions included were 1st + 2nd + 3rd + noncoding. All positions containing gaps and missing data were eliminated from the data sets (complete deletion option), and the calculations were conducted in MEGA 4.0 (Tamura et al. 2007). Summary statistics for the different substitutional changes are shown in Table 2.

Among the ecotypes of cardamom, viz. Vazhukka, Malabar and Mysore, the latter with erect panicles is more severely infested by the thrips, followed by Vazhukka (semi-erect panicles), while in the Malabar type (prostrate panicles), the damage was comparatively low (Gopakumar and Chandrasekhar 2002). The sequence alignment for the 45 intraspecific populations and the phylogram reveal that there are two minor clades (Figure 9), of which the clade I represents the populations from Kerala, Karnataka and Tamil Nadu; the clade II has two populations, one from Pampadumpara in Idukki District of Kerala and the other from the Anali Estate, Valparai District of Tamil Nadu. These results indicate that the populations of *S. cardamomi* from the locations analysed belong to a single species. This is in contrast to the intraspecific populations of *Thrips tabaci*, in which the host-associated genetic differences were observed (Brunner et al. 2004), and three distinct haplotype groups had been documented using COX-1 sequences (Toda and Murai 2007). Such results on *S. cardamomi* show that there are no appreciable nucleotide differences in its intraspecific populations.

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