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GENETIC DIVERSITY OF MAJOR POLYPHAGOUS SPIDER MITE SPECIES ACROSS HOST PLANTS AND SPATIAL DISTRIBUTION

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ABSTRACT

Different agroecosystems of southern Karnataka sampled for plant associated mites recorded six genera of tetranychid mites viz., *Aponychus, Eutetranychus, Oligonychus, Petrobia, Schizotetranychus* and *Tetranychus*. Neutrality test revealed a lowest haplotype diversity (0.993) of *T. neocaledonicus* population and Tajima's D and Fu's Fs test values were negatively significant for *T. fijiensis, E. orientalis* and *S. baltazari* populations, indicating the greater expansion of populations. Genetic analysis with ITS2 (rDNA) sequences revealed highest similarity between mite genera *Petrobia* and *Aponychus* showing lowest genetic distance of 0.51. *Schizotetranychus* has sister relationship with *Eutetranychus* which in turn clustered with *Petrobia* and *Aponychus. Tetranychus* and *Oligonychus* genera with lower genetic distance of 0.60 are clustered together. The study confirmed the clustering of morphologically related genera and lower genetic distances observed between related genera, expressed sister relationship. Molecular phylogenetic analysis of species under genus *Oligonychus* showed clustering in line with the morphology-based species taxonomic keys, while polyphagous species under the genus *Tetranychus* did not express form this grouping. Genetic diversity study of ten polyphagous mite species across their host plants and geographical occurrence revealed grouping according to locations in the phylogenetic tree. However, populations of *Oligonychus tylus* with a narrow host range showed close relationship for host plants than geographical locations which was evident in phylogenetic tree branching.

Key words: Tetranychid mites, *Aponychus, Eutetranychus, Oligonychus, Petrobia, Schizotetranychus, Tetranychus* genetic diversity, ITS2, neutrality test, phylogenetic analysis, host plants, cluster analysis, host range, polyphagous

Acari is a species rich subclass under the class Arachnida of subphylum Chelicerata under the phylum Arthropoda with more than 50,000 described species worldwide (Zhang, 2003). The Acarine diversity is such that, the currently described species represent only a small fraction of the present species and half to one million species in the world are yet to be discovered (Walter and Proctor, 1999). Apart from this vast diversity, spider mites are known to exhibit morphological variations and phenotypic plasticity, perceived as environmental variations (Meyers and Bull, 2002). Hence, molecular methods are increasingly being accepted for accurate taxonomic identification of mites as well as delineation of species. DNA sequences of mitochondrial COI (Cytochrome Oxidase I) gene and ITS (Internal Transcribed Spacer) regions of rDNA genes are widely used for phylogenetic analyses at different taxonomic levels, to ascertain intergeneric relationships in Tetranychidae (Navajas et al., 1996), interspecific studies (Navajas et al., 1997, 1998; Navajas and Boursot, 2003; Hinomoto et al., 2007b) and interpopulation analyses within a species (Navajas, 1998; Navajas et al., 1999; Hinomoto and Takafuji, 2001&2004; Hinomoto et al., 2001, 2007a). Using ITS2 DNA sequences Navajas et al. (1992) investigated phylogenetic relationships between six tetranychid mite species viz., *Eotetranychus carpini E. pruni, Tetranychus pacificus, T. mcdanieli, T. turkestani* and *T. urticae*. Detailed reassessments of the data could be used in studying the biogeography of the spider mite genera and for understanding their evolutionary relationships. Indian tetranychid mite fauna is lacking in such studies, except for the molecular phylogenetic analysis of *Tetranychus urticae* and *Tetranychus macfarlanei* by Zeity (2015). Considering the diversity of spider mites both within and between species, present study focused on the genetic diversity of few agriculturally important tetranychid mite pests was conducted using ITS2 sequences owing to its importance in the studies concerning intraspecific relationships. The noncoding

rDNA spacer ITS2 comprise fastest-evolving tool used frequently for validation of species status (Abdel-Mawgood, 2012) and an effective diagnostic tool for quarantine and decision making (David et al.,2007). Intraspecific variation among the populations of 10 economically important polyphagous spider mite species viz., *Eutetranychus orientalis* (Klein), *Oligonychus biharensis* (Hirst), *Oligonychus tylus* Baker and Pritchard, *Schizotetranychus baltazari* Rimando, *Tetranychus fijiensis* Hirst, *Tetranychus ludeni* Zacher, *Tetranychus macfarlanei* Baker and Pritchard, *Tetranychus neocaledonicus* Andre and *Tetranychus truncatus* Ehara was studied. Outcome of the study be extrapolated to understand their genetic relationship, expansion of pest status and the pest scenario in relevance to changing agricultural practices and the climate change as well.

MATERIALS AND METHODS

Mite samples collected from different locations and host plants were suitably preserved in absolute alcohol in airtight plastic vials (Table 1). After initial extraction of genomic DNA using CTAB method, ITS2 sequence region was amplified using 5.8S (5'-GGGTCGATGAAGAACGCAGC-3') and 28S (5'-ATATGCTTAAATTCAGCGGG-3') primers. PCR was performed in 25- µl reaction volume with 10X Scigenomics® Taq buffer (15 mM $MgCl_2$, 100 mM Tris pH 9), 2.5 mM of each nucleotide dNTP, 10 pmol. each of 5.8S and 28S primers, Genei® Taq polymerase (5 units/µl) and 1 µL DNA template in Bio-Rad® DNA Engine thermocycler, with 1 cycle of initial denaturation for 60 s at 94 °C followed by 35 cycles of denaturation (60 s at 94 °C), annealing (90 s at 52 °C) and extension (72 °C for 10 min), with a final extension period of 72°C for 10 min units. Amplified DNA segments were sequenced (Applied Biosystems® ABI 3130 XL), subjected to BLAST analysis and were then deposited in NCBI GenBank database (Accession No. given in supplementary data as Supplementary Table S1). The genetic diversity of populations was calculated using DnaSP 5.0 and neutrality tests, including Tajima's D (Tajima 1989) and Fu and Li's F (Fu 1997) were implemented for each population in DnaSP 5.0 software. Phylogenetic analysis with rDNA (ITS2) sequences of different populations across hosts and locations was carried out. The sequences were aligned using MEGA-X 64 software and genetic distances were calculated. Molecular phylogenetic reconstructions were executed using UPGMA (for inter and intrageneric studies) and Maximum Likelihood method (for intraspecific studies) with 1000 bootstrapping replications. Further, the aligned sequences were used in the assessment of genetic diversity by computing divergence as well as pairwise distance values.

RESULTS AND DISCUSSION

Neutrality test and genetic diversity analysis of spider mite populations revealed that the genetic diversity of 10 spider mite species collected from geographic regions in Karnataka. The haplotype diversity (Hd) of *T. neocaledonicus* population was the lowest (0.993) compared to other populations (1.000) and similar phenomenon was also detected for nucleotide diversity (Pi) of 0.71102, followed by 0.71236 for *T. truncatus* population. Under the hypothesis of selective neutrality and population equilibrium, Tajima's D and Fu's Fs test values tend to be negative under an excess of recent mutations, which is regarded as evidence of population expansion (Tajima, 1989; Fu, 1997). Tajima's D and Fu's Fs tests values of populations showed negative, indicating recent mutations and population expansion. The populations of *T. fijiensis*, *E. orientalis* and *S. baltazari* were significant for both tests, indicating that the expansion of these species was greater than that of the other species (Table 1). The *O. biharensis* and *O. tylus* populations also showed significant signs of expansion based on Tajima's D test; the other populations showed no significant signs of expansion. Genetic diversity data of tetranychid populations relevant to Indian situations of host plants and geographical locations is obsolete of source. The lowest haplotype diversity (Hd) of *T. neocaledonicus* population and negatively significant values for Tajima's D and Fu's Fs test for *T. fijiensis, E. orientalis* and *S. baltazari* populations in the present study indicates the greater population expansion than that of the other groups as claimed by Cai *et al.* (2019). Similar trend was observed in the field surveys, where *T. neocaledonicus* species found taking over the host plants of *T. urticae,* which was considered as serious mite pest for decades. Also, *E. orientalis* and *S. baltazari* species found expanding their host range in the field study confirming the results of genetic diversity analysis.

ITS2-rDNA sequences of six genera of Tetranychidae viz., *Aponychus, Eutetranychus, Oligonychus, Petrobia, Schizotetranychus* and *Tetranychus* were subjected to phylogenetic analysis using *Raoiella* representing mite Family Tenuipalpidae as out group. Highest similarity was evident between *Petrobia* and *Aponychus, Tetranychus* and the out group *Raoiella*. Genus *Schizotetranychus* showed sister relationship with *Eutetranychus* which clustered with genera *Petrobia*

Table 1. Details of GPS location and GenBank accession number of mite populations analysed

Sl. No.	Species	Location	GPS coordinates		Host plants	GenBank Accession
			Latitude (N)	Longitude (E)		
1.	*Aponychus corpuzae* Rimando	Bengaluru	13°04'40"	75°25'03"	*Bambusa* sp.	KT361606
2.	*Eutetranychus orientalis* (Klein)	Tarikere	13°45'13"	75°52'06"	*Nerium oleander*	MW981326
		Chitradurga	14°13'00"	76°23'49"	*Wrightia tinctoria*	OM248464
			14°13'00"	76°23'49"	*Azadirachta indica*	OM214534
3.	*Oligonychus bellarensis* sp. nov.	Ballari	15°10'23"	76°19'31"	*Saccharum officinarum*	MG677944
4.	*Oligonychus biharensis* (Hirst)	Harihara	14°30'27"	75°49'24"	*Militia pinnata*	MW709398
		Koppa	13°31'50"	75°21'28"	*Rosa chinensis*	MZ618719
		Thirthahalli	13°41'56"	75°13'59"	*Rosa chinensis*	MZ615524
		Hassan	12°58'17"	76°15'42"	*Rosa chinensis*	MW909770
5.	*Oligonychus plegas* Baker & Pritchard	Bengaluru	13°04'38"	77°34'39"	*Megathyrsus maximus*	MN986931
6.	*Oligonychus thelytokus*	Chikkamagaluru	13°31'50"	75°21'28"	*Syzygium jambos*	MZ604933
7.	*Oligonychus tylus* Baker & Pritchard	Honnalli	14°14'00"	75°38'33"	*Zea mays*	MZ618679
			14°14'00"	75°38'33"	*Pennisetum glaucum*	MW709399
		Hassan	12°58'17"	76°15'42"	*Cocos nucifera*	MW909769
8.	*Petrobia hartii*(Ewing)	Mudigere	13°07'15"	75°37'01"	*Oxalis* sp.	MW714337
9.	*Schizotetranychus baltazari* Rimando	Channagiri	14°07'42"	75°53'08"	*Azadirachta indica*	MW14336
		Hiriyur	13°53'06"	76°29'26"	*Murraya koenigii*	OM248459
		Chitradurga	14°13'00"	76°23'49"	*Azadirachta indica*	OM214535
10.	*Schizotetranychus* sp.	Chitradurga	14°11'35"	76°23'38"	*Bambusa* sp.	OM219636
11.	*Tetranychus bambusae* Wang and Ma	Hassan	12°58'17"	76°15'42"	*Bambusa* sp.	MW909782
12.	*Tetranychus fijiensis* Hirst	Mudigere	13°05'00"	75°39'40"	*Cocos nucifera*	MT582419
		Shivamogga	13°58'33"	75°34'40"	*Areca catechu*	MW459972
		Tarikere	13°45'13"	75°52'06"	*Areca catechu*	MW980051
		Chikkamagaluru	13°16'43"	75°48'06"	*Citrus* sp.	MN963777
13.	*Tetranychus lombardinii* Baker and Pritchard	Shivamogga	13°58'35"	75°33'06"	*Jasminum* sp.	MW980043
14.	*Tetranychus ludeni* Zacher	Hassan	12°58'17"	76°15'42"	*Parthenium hysterophorus*	MW911625
		Munirabad	15°17'47"	76°19'00"	*Ocimum* sp.	MN963774
		Shikharipura	14°14'05"	75°22'15"	*Bidens pilosa*	MZ618720
		Soraba	14°17'51"	75°15'32"	*Parthenium hysterophorus*	MZ540269
		Kadur	13°32'54"	76°00'36"	*Impatience balsamina*	MW704096

(contd. Table 1)

15.	*Tetranychus macfarlanei* Baker and Pritchard	Shivamogga	13°58'33"	75°34'40"	*Abelmoschus esculentus*	MW714334
			13°58'33"	75°34'40"	*Vicia faba*	MN963776
			13°58'33"	75°34'40"	*Impatience balsamina*	MT582418
			13°58'33"	75°34'40"	*Vigna unguiculata*	MT580908
			13°58'33"	75°34'40"	*Abelmoschus esculentus*	MW714335
			13°58'33"	75°34'40"	*Glycine max*	MT576576
			13°58'33"	75°34'40"	*Boerhavia diffusa*	MW332262
			13°58'33"	75°34'40"	*Tagetes erectus*	MW784003
		Bavikere	13°45'13"	75°52'06"	*Desmodium ganageticum*	MW459984
		Honnalli	14°14'00"	75°38'33"	*Pennisutum glaucum*	MZ618639
		Chamarajanagar	11°55'32"	76°56'44"	*Abelmoschus esculentus*	MT023425
		Hassan	12°58'17"	76°15'42"	*Phaseolus vulgaris*	MW911626
			12°58'17"	76°15'42"	*Lucas* sp.	MW911628
		Bidadi	12°47'55"	77°23'28"	*Abelmoschus esculentus*	MN794988
		Bramhavara	13°25'27"	74°45'24"	*Piper betel*	OM680002
16.	*Tetranychus neocaledonicus* Andre	Bengaluru	13°04'23"	77°34'56"	*Acalypha wilkasiana* var. inferno	MT023426
		Bengaluru	13°04'10"	77°36'45"	*Tagetes erectus*	MT020380
		Mudigere	13°05'00"	75°39'40"	*Piper betel*	MT576583
		Chikkamagaluru	13°16'43"	75°48'06"	*Hibiscus rosa-chinensis*	MW704021
		Tarikere	13°45'13"	75°52'06"	*Tinospora cardifolia*	MZ620637
		Mandya	12°34'06"	70°50'09"	*Macrotyloma uniflorum*	MW439310
			12°34'06"	70°50'09"	*Polyscias scutellaria*	MZ6020636
		Honnalli	14°14'00"	75°38'33"	*Tectona grandis*	MZ618670
		Davanagere	14°21'20"	75°44'30"	*Vigna mungo*	MW704022
		Thirthahalli	13°41'56"	75°13'59"	*Clitoria turnatea*	MZ618715
			13°41'56"	75°13'59"	*Syndrella nodiflora*	MZ618680
			13°41'56"	75°13'59"	*Codiem variegatum*	MZ618721
		Soraba	13°41'56"	75°13'59"	*Tinospora cardifolia*	MZ620637
		Hassan	12°58'17"	76°15'42"	*Acalypha wilkasiana*	MW915439
			12°58'17"	76°15'42"	*Rosa chinensis*	MW916311
			12°58'17"	76°15'42"	Dicot weed	MW911835
			12°58'17"	76°15'42"	*Carica papaya*	MW916308

(contd. Table 1)

(contd. Table 1)

17.	*Tetranychus okinawanus* Ehara	Hebri	13°07'57"	74°46'19"	*Hibiscus rosa-sinensis*	OM248465
18.	*Tetranychus truncatus* Ehara		13°16'43"	75°48'06"	*Parthenium hysterophorus*	MW332245
		Chikkamagaluru	13°16'43"	75°48'06"	*Amaranthus* sp.	MW748069
			13°16'43"	75°48'06"	*Crassocephalum crepediodus*	MW789347
		Harihara	14°30'27"	75°49'24"	*Tinospora cardifolia*	MZ542294
		Bhadravati	13°59'18"	75°41'43"	*Vicia faba*	MW704097
		Mudigere	13°06'53"	75°37'55"	*Solanum melongena*	MW332246
		Surahonne	14°08'22"	75°33'07"	*Rosa chinensis*	MW488928
		Soraba	14°17'51"	75°15'32"	*Tinospora cardifolia*	MZ618617
		Shikharipura	14°14'05"	75°22'15"	*Solanum melongena*	MZ618678
			14°14'05"	75°22'15"	*Tinospora cardifolia*	MZ618717
		Kaapu	13°00'30"	74°58'27"	*Amaranthus dubius*	MW704097
19.	*Tetranychus udaipurensis* Gupta and Gupta	Tarikere	13°45'13"	75°52'06"	*Ricinus communis*	MW979818
20.	*Tetranychus urticae* Koch	Shikharipura	14°14'05"	75°22'15"	*Solanum lycopersicum*	MZ618718

Neutrality test and genetic diversity metrics for populations of spider mite species

Population	Hd± SD	Pi	Tajima's D	Fu's Fs test
Eutetranychus orientalis	1.000± 0.177	0.73672	-2.11817*	-0.95124*
Oligonychus biharensis	1.000± 0.177	0.73100	-2.06570 *	-0.92096
Oligonychus tylus	1.000± 0.177	0.73621	-2.05782*	-0.92096
Schizotetranychus baltazari	1.000± 0.177	0.73200	-2.08446*	-0.95609*
Tetranychus fijiensis	1.000± 0.177	0.73262	-2.12850*	-0.98502*
Tetranychus ludeni	1.000± 0.126	0.71720	-1.65189	-0.77852
Tetranychus macfarlanei	1.000± 0.027	0.73015	-0.87879	0.52857
Tetranychus truncatus	1.000± 0.039	0.71236	-1.07616	0.04190
Tetranychus neocaledonicus	0.993± 0.996	0.71102	-0.66557	0.76135

Hd: haplotype diversity; SD: standard deviation; Pi: nucleotide diversity; *: significant difference ($p < 0.001$)

and *Aponychus*. While, *Oligonychus* clustered with *Tetranychus* and the outgroup *Raoiella* (Fig. 1). Genetic distance was lowest for *Aponychus* with *Petrobia* (0.51) and *Eutetranychus* (0.61), which clustered together. These genera were morphologically similar and excepting *Petrobia,* other two genera together form a tribe Eurytetranychini. *Tetranychus* and *Oligonychus* had lower divergence value of 0.60 and clustered together. Genetic distance of the out group *Raoiella* was lowest with *Tetranychus* (0.53) with which it had clustered as a sister group. While it was highest with *Petrobia* (1.12). The study revealed clustering of morphologically related genera together and lower genetic distances observed between related genera showing their sister relationship. This phylogenetic analysis of ITS2 region (rDNA sequences) of six genera confirmed the clustering of more morphologically related genera together. Lower genetic distances were observed between related genera evidencing their sister relationship.

Phylogenetic analysis of species under *Oligonychus* genus with *T. macfarlanei* as outgroup showed clustering of *O. tylus* and *Oligonychus bellarensis* sp. nov. together i.e., prompting to morphology-based species taxonomic keys. *O. biharensis* and *O. thelytokus* which were morphologically near similar having similar host range appeared in the common clade (Fig. 2). No similarity was observed in species clustering under the genus *Tetranychus* for their morphological taxonomic keys. But *T. bambusae* which is a grass feeding species clustered outside all other polyphagous species associated with broad leaved plants (Fig. 3). Genetic distances of *E. orientalis* populations harbouring neem, *W. tinctoria* and *N. oleander* at different locations of Chitradurga and Tarikere were estimated by using Mega-X software. The genetic distance within *E. orientalis* ranged from 0.053 to 0.087. The lowest distance between *N. oleander* and *W. tinctoria* host populations (0.053) and the maximum distance among populations from hosts of *Azadirachta* and *W. tinctoria* (0.87). Genetic distances between *E. orientalis* and out-group *Eutetranychus* sp. ranged from 0.056 to 0.085, of which least was in *N. oleander* population and maximum in *W. tinctoria* population. Phylogenetic tree of *E. orientalis* revealed deeper relationship among populations for host plants. Populations of *N. oleander* and *W. tinctoria* clustered together. Similarly, populations from neem plants clustered (with bootstrap value of 96) with the out group *Eutetranychus* sp. which also associated with neem plants in other locations (Fig. 4).

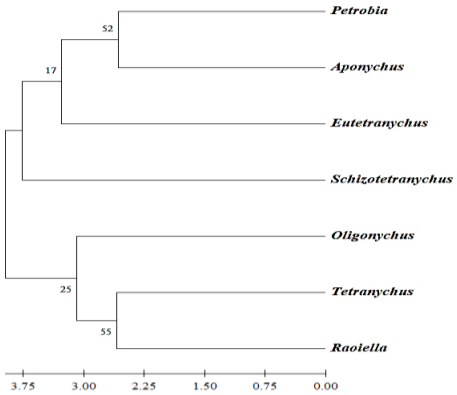

Fig. 1. Phylogenetic tree of tetranychid mite genera collected in the study for the ITS2 sequences using UPGMA method

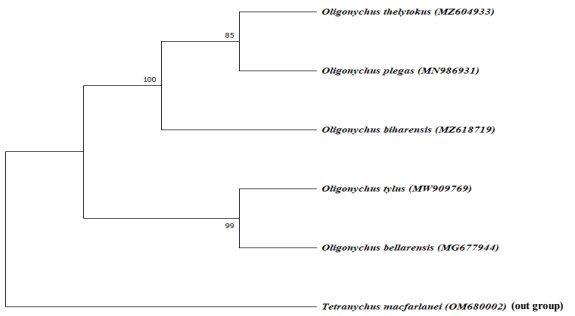

Fig. 2. Phylogenetic grouping of the genus *Oligonychus* of ITS2 sequences using UPGMA

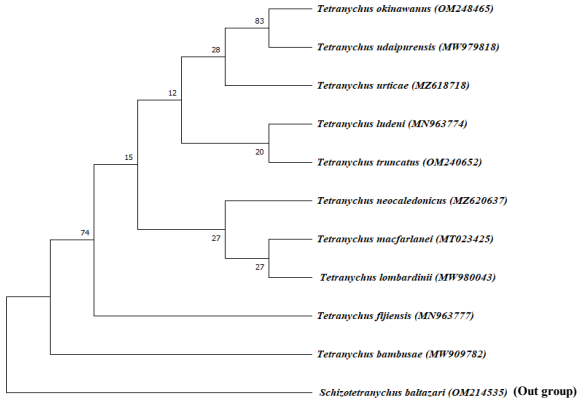

Fig. 3. Phylogenetic grouping of the genus *Tetranychus* of ITS2 sequences using UPGMA

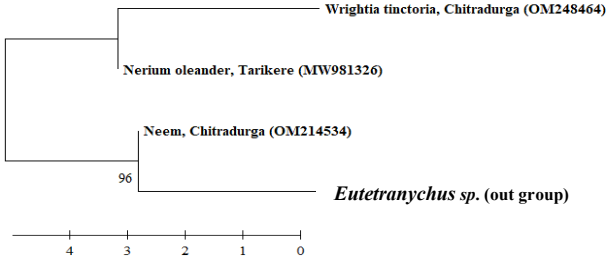

Fig. 4. Phylogenetic tree inferred from ITS2 sequences of *Eutetranychus orientalis* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

For *Oligonychus biharensis* (Hirst, fgenetic distance within the species ranged from 0.051 to 0.068, with a lowest of 0.051 for population on *Pongamia* from Davangere and on rose plant from Thirthahalli followed by Hassan (0.057) population. Distance within *O. biharensis* species and out group *O. thelytokus* was the lowest for the populations of rose from Hassan (0.053) followed by rose population from Koppa (0.056). Genetic distance was the highest for populations of *Pongamia* (0.088). The variation in the distance of populations from same host plant across different locations signifies the genetic diversity of *O. biharensis* attributed to the difference in locations. Phylogenetic analysis exhibited similar pattern, as the populations on rose plant in different locations clustered together (bootstrap value = 78) showing sister relationship. Also, Koppa and Thirthahalli rose populations fall in the same cluster (Fig. 5) owing to closer geographical distribution compared to distant location evident with Hassan population. For *Oligonychus tylus* Baker and Pritchard: Among the populations of *O. tylus,* genetic distance ranged from 0.054 to 0.080, the lowest distance between bajra and maize populations from Honnalli and the highest of 0.080 distance between populations occurring on coconut. This suggested that genetic distance between *O. tylus* populations occurring on grasses was lower (0.054-0.064) compared to that occurring on tall coconut tree (0.080), indicating its diversity for host plants. Genetic distance of *O. tylus* and the out-group *O. biharensis* was low for maize population (0.056) and high for bajra population. Phylogenetic analysis of ITS2 sequences confirmed the genetic diversity of *O. tylus* for host plants. Bajra and maize populations showed sister relationship with bootstrap value of 60 which clustered with *I. cylindrica* population (bootstrap value = 46). Whereas, coconut population clustered as a group well separated from population occurring on plants of grass species (Fig. 6).

With *Schizotetranychus baltazari* Rimando, genetic distance between populations ranged from 0.067 to 0.14, with the minimum distance observed between neem and curry leaf populations from locations of Hiriyur and Chitradurga. It was maximum for neem population from Channagiri location. This confirmed the genetic diversity of *S. baltazari* populations for geographical locations. The out group *Schizotetranychus* sp. exhibited minimum genetic distance (0.075) for neem population (from Channagiri) and maximum of 0.181 for curry leaf population (from Hiriyur). *Schizotetranychus baltazari* populations occurring on curry leaf and neem from different locations in Chitradurga district clustered with bootstrap value of 96 and separated from Channagiri population (Fig. 7).

Regarding *Tetranychus fijiensis* Hirst, arecanut population from Shivamogga and citrus population from Chikkamagaluru had lower genetic distance of

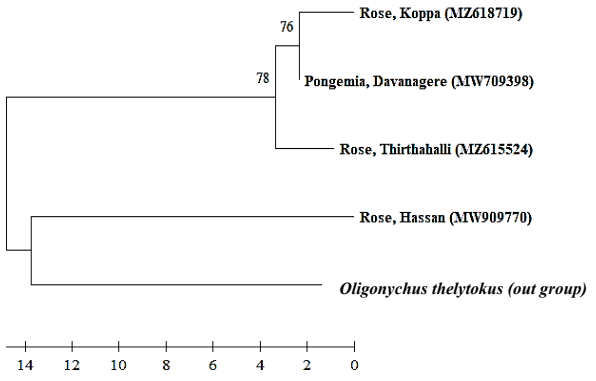

Fig. 5. Phylogenetic tree inferred from ITS2 sequences of *Oligonychus biharensis* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

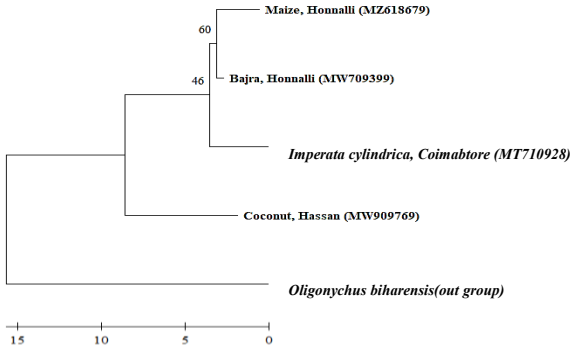

Fig. 6. Phylogenetic tree inferred from ITS2 sequences of *Oligonychus tylus* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

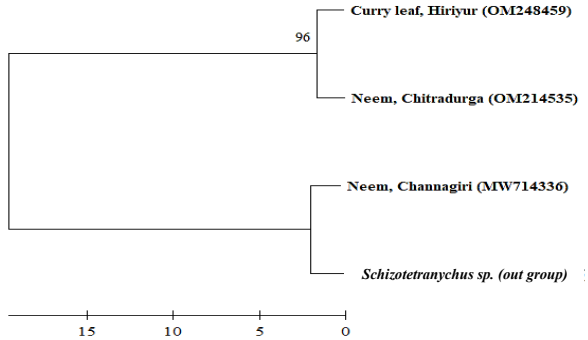

Fig. 7. Phylogenetic tree inferred from ITS2 sequences of *Schizotetranychus baltazari* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

0.050, while higher distance observed among coconut populations from Mudigere and arecanut population from Tarikere. This genetic difference could probably due to the environmental factor, the former two populations were from Southern Transitional zone, while the latter populations were from two extreme environments of hilly (Mudigere) and dry zones (Tarikere), accounting the genetic diversity of *T. fijiensis* to geographical locations. The out-group *T. ludeni* showed maximum genetic distance for citrus populations from Chikkamagaluru (0.094) and highest to arecanut population from Shivamogga (0.045). Phylogenetic analysis exhibited a distinct separation of *T. fijiensis* from the out-group *T. ludeni.* Arecanut population from Tarikere and citrus population from Chikkamagaluru clustered (with bootstrap value of 88) and formed a sister clade with arecanut population from Shivamogga (Fig. 8). Coconut population from Mudigere exhibited sister relationship with all other populations. With *Tetranychus ludeni* Zacher, populations showed a wider range of genetic distance (0.052 to 0.090). Lower distance of 0.052 was between *Ocimum* population from Munirabad and *Parthenium* population from Hassan.While the highest (0.090) distance was between balsam population from Kadur and black-jack population from Shikharipura. The outgroup *T. fijiensis* showed highest genetic distance of 0.102 with black-jack population and the while lowest distance of 0.056 was with *Parthenium* weed plant population from Hassan. Similarly, the populations with lower genetic distances clustered together in the dendrogram with the outgroup *T. fijiensis*. *Ocimum* population from Munirabad; parthenium population from Hassan and parthenium population from Soraba; balsam population from Kadur together with bootstrap values of 54 and 31, respectively (Fig. 9). Black jack

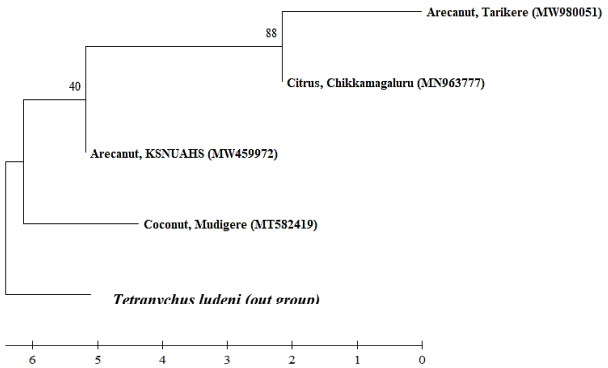

Fig. 8. Phylogenetic tree inferred from ITS2 sequences of *Tetranychus fijiensis* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

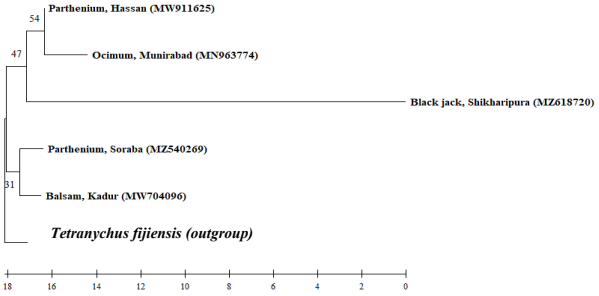

Fig. 9. Phylogenetic tree inferred from ITS2 sequences of *Tetranychus ludeni* by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

population formed a sister group with populations from former two host plants of *Ocimum* and parthenium.

As far as Tetranychus macfarlanei Baker and Pritchard is concerned, genetic distance across 15 populations ranged from 0.038 to 0.096. Among these populations, French bean population exposed highest distance with *D. gangeticum* population and the lowest with okra population. Genetic distance of *T. macfarlanei* and the out-group *T. fijiensis* ranged from 0.042 to 0.077. It was the lowest for okra (Shivamogga) population and highest for *Leucas* population. Phylogenetic analysis showed sister relationship among okra and balsam populations from Shivamogga, cowpea and soybean populations from Shivamogga (Fig. 10) and between bajra and *Lucas* population (Hassan). These populations showed lower genetic distances of 0.055, 0.044 and 0.043, respectively and clustered accordingly. Clustering of populations from different host plants from the same location indicated the similarity for locations and or divergence for host plants. With *Tetranychus neocaledonicus* Andre, genetic distance between the populations*s* ranged from 0-0.036. *T. cardifolia* population exhibited no variation for hibiscus population, while it was the lowest with *Acalypha* population (0.015). Rose (Hassan) and undetermined weed (Hassan) populations showed no variation with zero distance. Highest distance was observed between papaya and *Acalypha* populations of Hassan. This confirmed the genetic similarity of *T. neocaledonicus* populations harbouring different host plants in the same location and was diverse for populations occurring on different host plants. The out-group *T. udaipurensis* exhibited the lowest genetic distance of 0.015 for horse gram population, followed by marigold population (0.017), while distance was maximum for *Acalypha* (Hassan) population. Phylogenetic analysis of 18 populations of *T. neocaledonicus* showed distinct

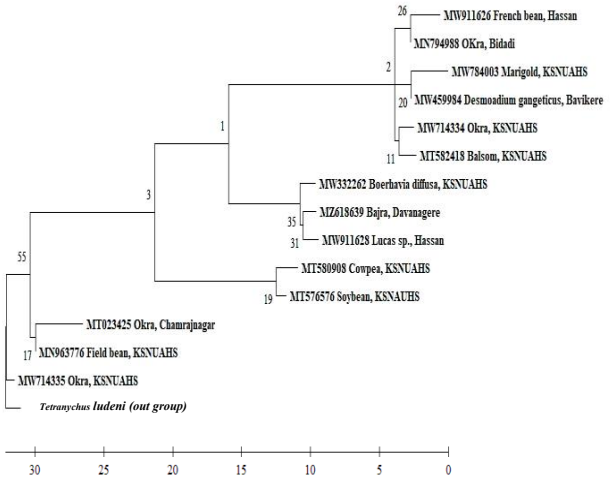

Fig. 10. Phylogenetic tree inferred from ITS2 sequences of Tetranychus macfarlanei by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

clustering of *T. cardifolia* (Soraba) and hibiscus (Amble) hosts; *Clitoria* and *Acalypha* (Hassan); rose (Hassan) and weed (Hassan); *Synedrella* (Thirthahalli) and horse gram (Mandya); croton (Thirthahalli) and black gram (Honnalli) showing sister relationship. The genetic distances corresponding to these populations were low 0, 0.032, 0, 0.022, 0.018 and hence clustered in a sister clade. Of which closer populations with zero distances i.e., *T. cardifolia* (Soraba); hibiscus and rose (Hassan); weed (Hassan) clustered with a high bootstrap value of 99 and 96, respectively (Fig. 11). The populations from Hassan, Mandya and Bengaluru clustered as a separate clade and those from Davanagere, Shivamogga and Chikkamagaluru formed separate clade. This confirmed the genetic similarity in *T. neocaledonicus* populations to geographical locations and its diversity for host plants.

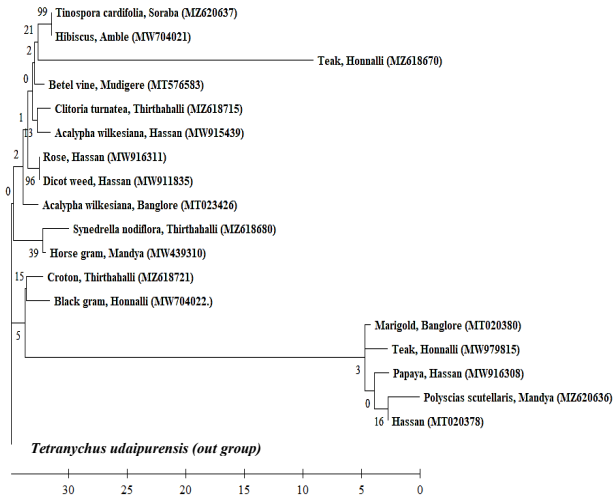

Fig. 11. Phylogenetic tree inferred from ITS2 sequences of Tetranychus neocaledonicus by Maximum Likelihood method. (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from 1000 samples)

Tetranychus truncatus Ehara revealed that the genetic distance of the populations ranged from 0 to 0.117 with zero distance between rose and *T. cardifolia* populations, followed by *Amaranthus* populations from Kaapu and Chikkamagaluru (0.052). While the highest distance of 0.117 was observed between populations of field bean & *T. cardifolia*. Genetic distance between the populations of *T. truncatus* and the out group (*T. neocaledonicus*) was the lowest (0.057) for field bean population (Bhadravati) and the highest (0.117) for *Amaranthus* population (Kaapu), thus showed their nearness and relationships, respectively. The phylogenetic analysis showed distinct clustering of populations from Southern Transitional zones, hilly & coastal zones as separate clades. Populations of Shikaripura, Sorahonne, Davangere and Chikkamagaluru formed a clade with bootstrap value of 19 and Mudigere and Kaapu in a clade with Bhadravati and Chikkamagaluru populations (bootstrap value = 27), branching represented Southern Transitional zone with populations from Soraba, Shikaripura and Chikkamagaluru (bootstrap value = 67). Rose (Surahonne) and *T. cardifolia* (Shikharipura) populations with zero genetic distance clustered with bootstrap value of 92 (Fig. 12). This confirmed the diversity of *T. truncatus* for host plants and similarity for location.

Variation in ITS2 sequences for populations of most of the tetranychid (spider mite) species was more related to locations indicating their genetic separation by geographical borders that are barriers of gene flow and prompting the population structure. As a result, different clades in the phylogenetic tree were closer

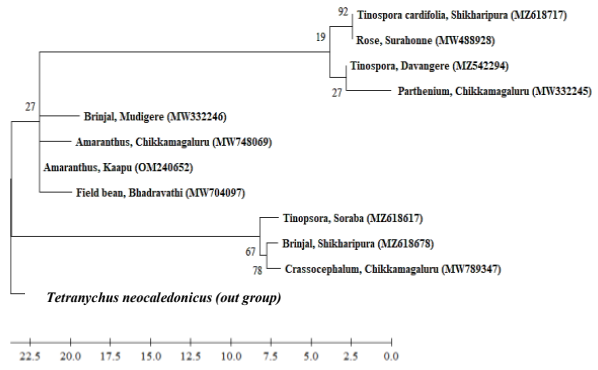

Fig. 12. Phylogenetic tree inferred from ITS2 sequences of Tetranychus truncatus by Maximum Likelihood method (Scale bar indicates number of substitutions; Number near the branches the bootstrap values from1000 samples)

to near-locations. For populations of *Tetranychus turkestani* geographical locations of France appeared to be a factor determining its genetic structure with no distinct variations for populations harbouring different host plants (Bailly *et al* 2004). However, in our study *Oligonychus tylus* populations showed close relationship for host plants than for geographical locations with clades in the phylogenetic tree distinctly separated for host plants such as bajra, maize and *I. cylindrica*. In Japan Nishimura *et al.* (2007) distinguished different populations of *Tetranychus kanzawai* (using rDNA fragments of ITS1 region) and attributed significant variations to associated host plants. Mirza *et al* (2020) also concluded that genetic variation in ten different haplotypes of *E. orientalis* was more for host plants in Saudi Arabia. Host plant selection by herbivores could be a major factor separating different gene pools of tetranychid mites inhabiting specific host plants. The spatial distribution of mite populations and strength of gene flow are essential in ascertaining their future pest invasiveness. Spider mites display their astounding dispersal mechanism and management of weeds which serve as efficient host plants of spider mites during the off-cropping season may be of prime significance. Ecologically linked molecular studies would explicate the genetic structure of populations, that could be used in predicting pest outbreaks and thus may be utilised in scheming effective management strategies.

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AUTHOR CONTRIBUTION STATEMENT

Contributed in molecular confirmation, diversity studies (SM, NS, CC, RHP); Field surveys and host studies (SM, RK, RHP).

CONFLICT OF INTEREST

No conflict of interest.

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