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Full Length Research Paper

Genetic diversity of mushroom mite (*Luciaphorus* sp.) infesting cultivated mushrooms in the Northeast of Thailand

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A pygmephorid mite, *Luciaphorus perniciosus* Rack, is widely spread in several commercial mushroom production settings in Thailand. This mushroom mite was collected from *Lentinus squarrosulus* (Mont.) Singer compost and basidiocarps from several farms located in 4 different provinces (Maha Sarakham, Kalasin, Roi Et and Ubon Ratchathani) of the Northeast of Thailand. The other specimens were provided by the Department of Agriculture, Thailand, which were previously collected from Ubon Ratchathani province. These mite samples could not be differentiated into sub-groups by external morphological examination, even though they appeared to have some minor morphological characteristics different from the prototype *L. perniciosus*. The RAPD-PCR profile and genetic distance information of the 5 populations could categorise these mites into 3 groups; mites in Group 1 which were collected from Maha Sarakham; Group 2 from Roi Et and Ubon Ratchathani-Bangkok and Group 3 from Kalasin, Ubon Ratchathani, Roi Et and Ubon Ratchathani-Bangkok. The results thus indicate that although all mites in this study were *L. perniciosus*, they are genetically diverse.

Key words: Mushroom mites, *Luciaphorus perniciosus*, diversity, morphology, molecular genetics.

INTRODUCTION

Cultivation of edible mushrooms in tropical regions of Asia is receiving an increased marketing interest particularly in China, Taiwan, India and Thailand. These countries already have a long history in this endeavor, and as a result, they have several enterprises capable of producing large volumes of various mushroom species. Unfortunately, several species of mushroom mites have been found to affect cultivated mushrooms worldwide (Gurney and Hussey, 1967; Wicht and Snetsinger, 1971; Clift and Toffolon, 1981a, b; Gao et al., 1986; Wu and Zhang, 1993). Mushroom mites can damage mushrooms by feeding on the mycelium and causing significant yield loss if not controlled (Wu and Zhang, 1993). Mite infestation often results in significant losses of yield and occasionally damages the entire crop (Wu et al., 1988).

One of the most destructive mushroom mites in Thailand is the pygmephorid (*Luciaphorus perniciosus* Rack). This mite was first reported as *Luciaporus hauseri* Mahunka and found to feed on Jew's ear fungus, *Auricularia polytricha* (Mont.) Sacc. (Jitrat, 1981). In 1983, Rack screened a large number of this mushroom mites infesting *A. polytricha* in Bangkok, Thailand, and he then described them as *L. perniciosus*. The life cycle of this mite was also presented by Kantaratanakul and Jitrat (1984).

L. perniciosus has been found to destroy commercial production of several mushroom species, including *Lentinus squarrosulus* (Mont.) Singer, *L. polychrous* Lev., *Auricularia auricular* (Bull.) Wettst. and *Flammulina*

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velutipes Karst. in the North and Northeast of Thailand. This mite has been shown to be widespread in several provinces, including Maha Sarakham, Kalasin, Roi Et, Khon Kaen and Ubon Ratchathani (Bussaman et al., 2004). It destroys every stages of the mushroom growth by retarding the mycelial growth and suppressing the sporophore formation (Charanasri et al., 1985). This mite can rapidly reproduce and spread airborne to other distant mushroom houses.

Since *L. perniciosus* is widely spread in Thailand, especially in the Northeast where several species of commercial mushrooms are cultivated, moreover, a better understanding of genetic variation of *L. perniciosus* is clearly needed in order to establish a good control measure that helps to decrease the use of chemical pesticides normally used by the mushroom farmers. This study is thus aimed to verify the genetic diversity of *Luciaphorus* sp. endemic in the Northeast of Thailand using molecular techniques and conventional taxonomic method.

MATERIALS AND METHODS

Mite samples

Mites were collected from *L. squarrosulus* compost and basidiocarps from various farms in 4 provinces; Maha Sarakham (MS), Kalasin (KS), Roi Et (RE), and Ubon Ratchathani (UB) of the Northeast Thailand. The other specimens were provided by the Department of Agriculture, Bangkhen, Bangkok, Thailand, which were then collected from Ubon Ratchathani province (UBbkk) and were used for comparison.

Morphological identification

This study used *Luciaphorus* sp. acquired from 38 specimens (females and males) which were mounted on microscopic slides in Hoyer's medium as described by Henderson (2001). Mite morphology and characteristics were observed using phase-contrast microscope. The specimens were also observed by Scanning Electron Microscope, Hitachi S-3500N and compared with the original description by Rack (1983).

Molecular classification

DNA extraction (modified method described by Weeks et al. [2000])

Twenty-five mites from each sample taken from mushroom composts were crushed in 100 µl of CTAB buffer using a glass homogenizer. The homogenized suspension was vortex for 10 s and incubated at 65°C for 15 min. Equal volume of chloroform: isoamyl-alcohol (24:1) was added and thoroughly mixed. The mixture was then centrifuged at 10,000 g for 15 min and the supernatant containing DNA was transferred to a sterile tube. DNA was precipitated by 2 volume of ice-cold absolute ethanol. Finally, DNA pellet was dissolved in 20 µl TE buffer.

RAPD analysis

RAPD reactions were performed using each of the 10-mer primers for adding to 25 µl of the reaction mixture. The reaction mixture, containing 4 mM dNTPs (Promega), 10X PCR buffer (Promega), 25 Mm $MgCl_2$, 10 pmole primer (Operon Technologies), and 5 units of Taq-polymerase (Promega), was added to DNA template. DNA template was then amplified using thermocycler (Hybrid programmable thermal cycler). All PCR reactions were performed with an initial denaturation at 94°C for 3 min, followed by 45 cycles of denaturation at 94°C for 1 min, then annealing at 36°C for 1 min and extension at 72°C for 2 min. The final extension was performed at 72°C for 5 min.

The amplified DNA products were separated using 2% TBE agarose gel electrophoresis. The gel was stained with ethidium bromide and the gel patterns visualized and photographed using a Gel Documentation System, GDS 8000 (UVP Inc., California, USA). A total of twenty primers (Kits A from Operon Technologies, Alameda, California) were screened, one primer at a time, for high discriminatory power in an initial survey (Table 1).

Data analysis

Each RAPD fragment is treated as an independent character (a locus). Size of each RAPD band was estimated by comparing with a 100 bp ladder and the data were recorded in a binary matrix to represent the absence (0) or presence (1) of a particular band. The index of similarity between individuals (S_{xy}) was calculated using the formula:

$$S_{xy} = 2n_{xy} /(n_x + n_y)$$

whereby n_x and n_y represent the number of RAPD bands in the x and y individuals and n_{xy} represents the number of shared bands between the individuals (Nei and Li, 1979).

The within sample similarity (S) is calculated as an average of S_{xy} across all possible comparisons between the individuals within a population. The between population similarity (S') is calculated using the within similarity values between the random pairs of individuals from populations for adding to the equation:

$$S'_i = 1 + S_{ij} -(Si+Sj)/2.$$

Si and Sj are the values of S for population i and j, respectively. S_{ij} is also converted to measure the genetic distances (D_{ij}) using the equation:

$D_{ij} = 1- S'_{ij}$ (Lynch, 1990).

The neighbour-joining tree was constructed from the genetic distances within species between the samples using unweighted pair group method with arithmetic mean (UPGMA) of Phylip version 3.5 c. (Felsenstion, 1993).

RESULTS AND DISCUSSION

Morphological study

The mushroom mites collected from the 4 provinces of

Table 1. Description of mite samples collected from commercial mushroom farms in the Northeast, Thailand.

No	Mite species	Mite color	Province	Habitat
1	*Luciaphorus* sp.	Pink	Ban Hun, Maha Sarakham (MS)	Basidocarps or mushroom compost
2	*Luciaphorus* sp.	Yellow	Ban Noon Sung, Kalasin (KS)	Basidocarps or mushroom compost
3	*Luciaphorus* sp	White	Ban Na Ki Lan, Ubon Ratchathani (UB)	Basidocarps or mushroom compost
4	*Luciaphorus* sp.	White	Amphur Muang, Roi Et (RE)	Basidocarps or mushroom compost
5	*Luciaphorus* sp.	White	Ubon Ratchathani (UBbkk stock, collected by Dept. of Agriculture, Bangkok.)	Mushroom spawns

the Northeast of Thailand are shown in Table 1. A colony of *L. perniciosus* provided by Department of Agriculture, Bangkhen, Bangkok, Thailand, was also used in the study as a positive control.

The results from morphological studies indicated that the mushroom mites found in the Northeast provinces of Thailand belong to the Genus *Luciaphorus* which consists of 3 species. Mahunka (1981) established the genus *Luciaphorus* with *L. hauseri* as the type species. This type species was collected from underneath the fallen tree barks in the forest of St. Lucia, Hungary. The other two species of genus *Luciaphorus* were found in Jew's ear mushroom in Thailand and China and subsequently named *L. perniciosus* and *L. auriculariae* by Rack (1983) and Gao et al. (1990), respectively. Rack noted that *L. perniciosus* is different from *L. hauseri* by having a wider sensilla, slightly longer peritreme, stronger dorsal setae, and shorter setae h_1 and h_2.

The morphological characters of Thai mushroom mite described here have revealed the features of female and male mites that do not actually fit to the descriptions of *L. perniciosus* previously reported by Rack (1983). This new species is closely related to *L. perniciosus* but it also has different characteristics in that the female mite is bigger; sensilla are wider and finely ripped with cup-like shape; setae h_1 and h_2 are at least three times longer than the other dorsal setae; peritreme is in W- shape; and 3c central and 1c setae are smooth and not forked (Table 2). For male mite, P_i is inside the plate and 4a is not longer than the other setae (1a, 2a, 3a,).

Genetic variation by RAPD

Screening for suitable primers

Twenty primers of arbitrary nucleotide sequences were used to amplify DNA fragments from the genomic DNA derived from 5 mushroom mite isolates. As summarized in Table 3, two primers, OPA03 and OPA04, successfully amplified the DNA of the mushroom mite and therefore they were selected for genetic diversity analysis. The results performed by these chosen primers were very well reproducible and gave easy-scoring patterns.

RAPD profile and genetic variation of mushroom mite samples

High level of genetic polymorphism was repeatedly observed in 5 sources of *Luciaphorus* sp. based on RAPD analysis using primer OPA03. Both high and low intensity bands were detected, with the minor bands assumed to be non-specific amplification products due to primer-DNA template mismatching (Williams et al., 1990). Therefore only the high intensity bands were considered for genetic variation analysis. DNA profiles were shown to consist of 58 RAPD fragments (Table 4) ranging between 400 to 2,600 bp (Figure 1), indicating a high polymorphism in the mite genetic pool. Since the primer OPA03 generated different patterns of the shared bands, depending on the isolates of mushroom mite, the RAPD profile can be used for mite identification. Based on the number of shared bands, each band was scored and calculated to investigate genetic diversity of mite isolates collected within and among provinces. The genetic distances of mites from the same location showed a variation ranged from 0.1 to 1.1 units whereas the mites from different location showed larger variation ranging from 0.8 to 1.5 units.

Genetic similarities and distance of mushroom mite strains

The similarity index between the pair of investigated

Table 2. The morphological characters of *Luciaphorus* sp. from sampled locations compared with *L. perniciosus*.

Location	sex	Body length (µm)	Body width (µm)	Peritreme	1c setae	Sensillus	h_1 and h_2 of female compared to other dorsal setae
MS	Male	140	75	-	-	-	-
	Female	138	80	W-shape	Unforked	Cup-shape	> 3x
UB	Male	138	75	-	-	-	-
	Female	135	78	W-shape	Unforked	Cup-shape	> 3x
RE	Male	135	75	-	-	-	-
	Female	132	76	W-shape	Unforked	Cup-shape	> 3x
KS	male	138	75	-	-	-	-
	Female	136	80	W-shape	Unforked	Cup-shape	> 3x
UBbkk (stock)	Male	135	75	-	-	-	-
	Female	130	78	W-shape	Unforked	Cup-shape	> 3x
L .perniciosus	Male	140	80	-	-	-	-
	Female	130	75	V-shape	Forked	Club-shape	3x

MS = Maha Sarakham, UB = Ubon Rachathani, RE = Roi Et, KS = Kalasin, UBbkk (stock) = stock colony collected from Ubon Rachathani.

Table 3. The RAPD amplification results using 20 arbitary nucleotide sequences to amplify *Luciaphorus* sp. DNA.

Primer	Sequence 5' to 3'	Amplification result
OPA-01	CAGGCCCTTC	-
OPA-02	TGCCGAGCTG	-
OPA-03	AGTCAGCCAC	+++
OPA-04	AATCGGGCTG	++
OPA-05	AGGGGTCTTG	-
OPA-06	GGTCCCTGAC	-
OPA-07	GAAACGGGTG	+
OPA-08	GTGACGTAGG	+
OPA-09	GGGTAACGCC	+
OPA-10	CAATCGCCGT	-
OPA-11	CAATCGCCGT	+

Table 3. Contd.

OPA-12	TCGGCGATAG	-
OPA-13	CAGCACCCAC	-
OPA-14	TCTGTGCTGG	+
OPA-15	TTCCGAACCC	+
OPA-16	AGCCAGCGAA	+
OPA-17	GACCGCTTGT	-
OPA-18	AGGTGACCGT	-
OPA-19	CAAACGTCGG	-
OPA-20	GTTGCGATCC	-

-Amplification was not successful. + amplification was successful but amplified bands were faint or complex. ++ amplification was successful. Amplified bands were moderately intense. +++ amplification was successful. Amplified bands were intense with suitable profile.

Table 4. The size and number of amplified RAPD bands of *Luciaphorus* sp. DNA using primer OPA03.

Samples	Size-range (bp)	No. of RAPD bands
Ban Hun (MS)	500-2600	12
Ban Noon Sung (KS)	400-2072	12
Ban Na Ki Lan (UB)	500-2600	12
Amphur Muang (RE)	500-2600	10
UBbkk (stock Collected by Department of Agriculture, Bangkhen)	500-2600	12
Overall	400-2600	58

samples (Sij) was calculated and represented as an average of all possible comparisons of individuals between the pairs of samples. The similarity level was further adjusted by subtracting with “within sample similarity effect (Saij)”. Finally the levels of genetic distance (Daij) were calculated by the following formula:

Daij = 1-Saij

The genetic distances of *Luciaphorus* sp. based on RAPD analysis indicated that mites from UB, RE and KS are closely related intraspecifically (Figure 2). The resulting phylogenetic tree divided *Luciaphorus* sp. into 3 closely related groups; Group I is the mites collected from Maha Sarakham (MS), Group II from Roi Et (RE) and Ubon Ratchathani (UBbkk) and the Group III from Kalasin (KS), Ubon Ratchathani (UB), Roi Et (RE) and Ubon Ratchathani (UBbkk).

Because prior knowledge of DNA sequence is not required to perform RAPD-PCR assay, RAPD profiling is a convenient and useful tool for this study. However, RAPD-PCR assay occasionally resulted in variation of band intensity even though using the same DNA template. Therefore RAPD-PCR assays should be performed in at least duplicates to prevent such variation (Black, 1993). Moreover, differences in the concentration of the template DNA, primer, $MgCl_2$ and Taq DNA polymerase have also been shown to result in different band patterns (Black, 1993; Ellsworth et al., 1993: MacPherson et al., 1993). Hence, fresh materials are indeed required and should be handled with extreme cautions to minimize the likelihood of degradation and variation of the results. Also, it should be noted that the differences of thermocycler and Taq polymerase in different laboratory settings can affect the results; therefore, the obtained result might not be used for universal comparison (MacPherson et al., 1993; Schierwater and Ender, 1993).

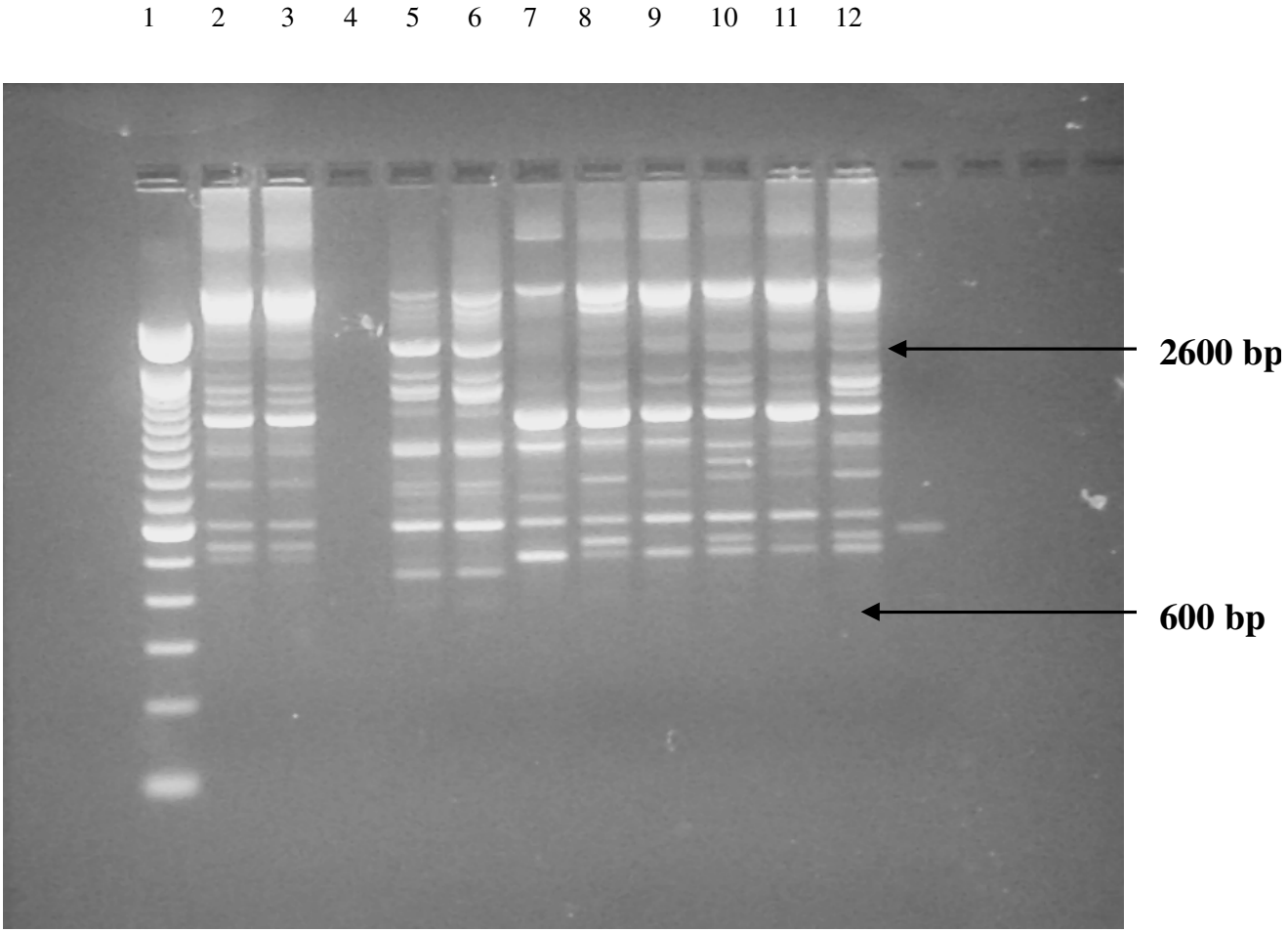

Figure 1. Ethidium Bromide stained RAPD gel patterns of mite spp. DNA using OPA3 primer. Lane 1 = 100 bp DNA markers, Lane 2-3 = mite- DNA from Ban Hun (Maha Sarakarm, MS), Lane 5-6 = mite- DNA from Ban Noon Sung (Kalasin, KS), Lane 7-8 = mit-DNA from Ban Na Ki Lan (Ubon Ratchathani, UB), Lane 9-10= mite-DNA from Amphur Muang (Roi Et, RE) and Lane 11-12= mite-DNA from Ubon Ratchathani (UBbkk, stock collected by Department of Agriculture, Bangkhen, Thailand.).

Suggestively, each laboratory should screen for the primers which can be used as markers for reliable detection of the species of its interest.

The RAPD-PCR assay may be useful for differentiation of the mushroom mite species other than those used in this study. This DNA analysis can be performed faster than the behavioral tests, mating experiments or intensive morphological study, and it thus would facilitate the detection and identification of the mite species.

Conclusion

Mite isolates in this study have been found to be slightly different from *L. perniciosus* described by Rack (1983). Moreover, data of RAPD pattern and genetic distance of mite samples indicated that *Luciaphorus* mite collected from Maha Sarakharm (MS) is relatively unique in the respect to RAPD pattern, but still related to all other mite isolates. Despite the current evidence, to confirm that all *Luciaphorus* mites collected from the Northeast Thailand are new species, more intensive morphological and genetic information remain to be investigated.

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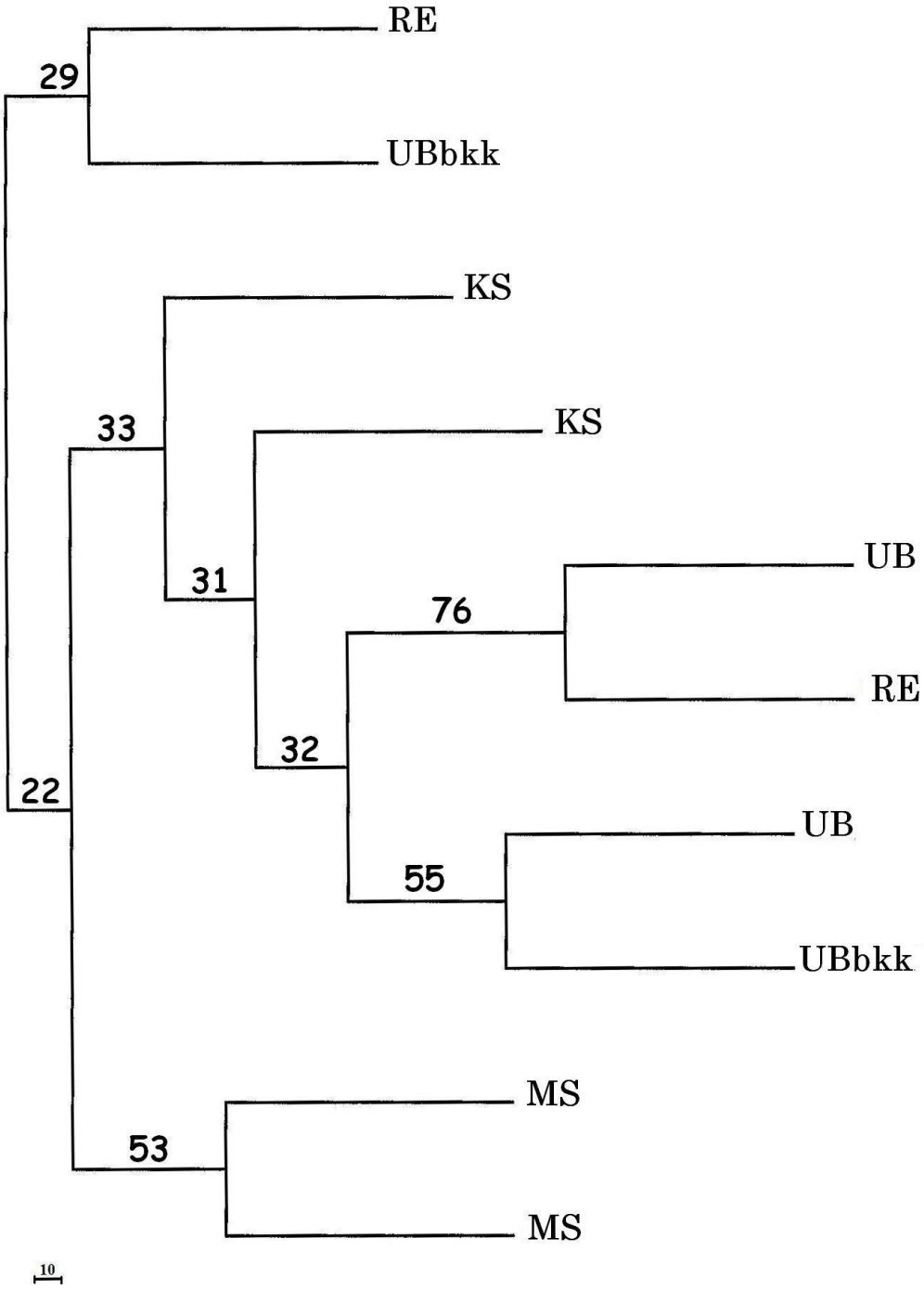

Figure 2. The genetic distance between the five populations of mite spp. as determined by using neighbour-joining analysis of the percentage of shared RAPD-PCR markers. The numbers above branches indicate bootstrap proportion.

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