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REVIEW ARTICLE ON MOLECULAR CHARACTERIZATION OF ASTIGMATID MITES (TYROPHAGUS PUTRESCENTIAE) USING ITS2 AND COI REGIONS

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Abstract-Identification of astigmatid mites based on their morphological characteristics is difficult because of the similarity of their organs, especially in immature mites. The ribosomal second internal transcribed spacer (ITS2) and the mitochondrial cytochrome oxidase subunit (COI) regions are usually used as barcodes. The ITS2 and COI regions of three species of astigmatid mites (Tyrophagus putrescentiae,, Dermatophagoides farinae, Dermatophagoides pteronyssinus) were obtained by polymerase chain reaction. The lengths of the ITS2 sequences varied from 310 to 482 bp, while the COI regions were 367or 368 bp long. The intra- and interspecific genetic distances were relatively smaller for the COI sequences than those for the ITS2 sequences. The genetic distances between species were obviously higher than those within species. In terms of ITS2 sequences, T. putrescentiae had the smallest intraspecific genetic distances.,whereas A. ovatus had the largest. D. farinae and D. pteronyssinus had the smallest interspecific genetic distances for ITS2 because they belong to the same genus. Differences in the ITS2 region were mainly found in terms of its length because of the deletions/ insertions of single nucleotides at several sites. Thus, the ITS2 and COI regions can be applied as barcodes to identify different species of astigmatid mites.

Key words: Astigmatid, Internal transcribed space (ITS)

1. INTRODUCTION

Information regarding mite genomics would greatly assist the development of novel control strategies by molecular approaches. Astigmatid mites identification by their morphological characteristics is difficult (Vargas et al., 2005). Similarity between their organs,bearing the same morphological characteristics (van Bronswijk et al., 1974; Colloff and Spieksma 1992). Recents in molecular biology techniques has enabled the identification of different mites species by sequence analysis (Suarez-Martinez et al., 2005; Prakash et al., 2006). The central part of the mitochondrial (COI) cytochrome oxidase subunit I region is highly conserved and frequently used for investigating intra- and interspecific variations (Ros and Breeuwer 2007). The ribosomal second internal transcribed spacer region (ITS2) is used to identify related species (Manonmani et al., 2001; Shaw et al., 2002; Hung et al., 2004). Data from ITS2 and COI regions of astigmatid mites were obtained, analyzed and results obtained were in close accordance of morphological studies.

2. MITES

For the proposed study, stock culture of T. putrescentiae will be maintained in laboratory on wheat flour: yeast (4:1) at 27 ± 1^{0}C and 80-85% RH. Dermatophagoides pteronyssinus and Dermatophagoides farina were reared in our lab at 75% on an appropriate medium). Specimens of Euroglyphus maynei were obtained by washing out slides stored in our laboratory. All mites were identified based on their morphological characteristics.

Mite Species	ITS2 Sequences Length (bp)
T. putrescentiae	234bp

3. EXTRACTION OF DNA

For each DNA extraction method, 60 miteswere tested, except in the case of DNA extracted one year before where sample sizes ranged from 22 to 60 specimens. Extractions were performed on individual mites, frozen as described. The concentration of each DNA sample was determined by OD 260 and OD 280 measurements, the purity was evaluated by the ratio OD260/OD280.

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3.1 Cethyl Trimethyl Ammonium Bromide (CTAB) Method

Individual mites were crushed with a sterile plastic pestle in a 1.5 mL microcentrifuge tube bottom containing 200 µL of extraction buffer, (2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0, 0.2% 2-mercaptoethanol), preheated to 65^0C. This homogenate was incubated at 65^0C for 1h. Proteins were removed with one volume of chloroform/isoamyl alcohol. DNA was then precipitated from the aqueous layer by adding one volume of isopropanol, letting the sample stand for 2 or 3 h at –20 ◦C and spun at 18 000 *g* for 30 min. The DNA pellet was then washed with 300 µL of ethanol 70%, dried for 10 to 15 min at 50 ◦C, and resuspended in 20 µL of ultrapure water. Tubes were placed for 2 h at 4 ◦C and DNA were resuspended again before being stored at –20 ◦C.

Table-3.1Regions for forward and Reverse

Regions	Forward	Reverse
ITS2 region	5′-CGACTTTCGAACGCATATTGC-3′	5′-GCTTAAATTCAGGGGGAATCTCG-3’
COI region	5′-GTTTTGGGATATCTCTCATAC-3′	5′-GAGCAACAACATAATAAGTATC-3′

4. DNA LIGATION AND SEQUENCING

The PCR products were collected and then ligated with the vector pUC19 using a DNA-Ligation Kit ver. 2 follows the manufacturer's instructions. Sequence analysis of PCR products was performed by In vitrogen employees.
The reaction conditions were the following:

- For the ITS2 region: denaturation was at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. Final extension was at 72°C for 7 min.
- For the COI region: Denaturation was at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min. Final extension took place at 72°C for 7 min.

4.1 Genetic Distances

When using the ITS2 sequences, the distances within/between species were much larger compared to those of the COI sequences, probably due to the much lower conservation of their lengths.

Table-4.1 Genetic Distances

Regions	Intraspecific Distance	Interspecific Distance
ITS2	0.0223553	0.654321
COI	0.0551234	0.055532

DISCUSSION

Astigmatid mites were compared and the lengths of the ITS2 sequences varied between 310 and 482 bp, whereas the COI sequences were 367 or 368 bp long. Analyses showed that such a method could be potentially used to identify astigmatid mites with similar morphological characteristics. (Webster et al., 2004; Marrelli et al., 2006; Thanwisai et al., 2006; Ben-David et al., 2007). ITS2 and COI regions were highly conserved in mites from different areas. Furthermore, the intraspecific variation among the ITS2 sequences of specimens. The intra- and interspecific genetic distances were relatively larger for theITS2 sequences than those for the COI sequences. Noge et al., (2005). Proposed that the genetic distances within were obviously smaller than those between species. In terms of ITS2 sequences, A. ovatus had the largest T. putrescentiae had the smallest intraspecific genetic distances. D. farinae and D. pteronyssinus had the smallest interspecific genetic distances for ITS2 because they belong to the same genus. Differences in the ITS2 region were mainly found in terms of its length because of the deletions/ insertions of single nucleotides at several sites. Thus, the ITS2 and COI regions can be applied as barcodes to identify different species of astigmatid mites. Alasaad et al., (2009) proposed that the astigmatid mites t morphological characteristics is difficult, the ITS2 and COI regions can be used to identify them. However, these genetic regions are not useful in distinguishing geographically different species. ITS2 and COI regions can also be used to identify many other parasites such as protozoans (Yang et al.,2 008), trematodes (Farjallah et al., 2009; Al-Kandari and Al-Bustan 2010), nematodes and parasitic arthropods (Suwannamit et al., 2009).

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REFERENCES

[1] Alasaad S, Soglia D, Spalenza V, Maione S, Soriguer RC, Perez JM, Rasero R, Degiorgis MPR, Nimmervoll H, Zhu XQ, Rossi L (2009). ITS-2 rDNA suitable marker for genetic characterization of Sarcoptes mites from different wild animals in different geographic areas Vet. Parasitol, 159:181–185.

[2] Al-Kandari WY, Al-Bustan SA (2010). Molecular identification of Probolocoryphe uca(Sarkisian, 1957; Digenea: Microphallidae) from Kuwait Bay using ITS1 and ITS2 sequences. Parasitol. Res, 106:1189–1195.

[3] Ben-David T, Melamed S, Gerson U, Morin S (2007). ITS2 sequences as barcodes for identifying and analyzing spider mites (Acari: Tetranychidae). Exp. Appl. Acarol, 41:169–181.

[4] Colloff MJ, Spieksma FTM (1992). Pictorial keys for the identification of domestic mites. Clin. Exp. Allergy, 22:823–830.

[5] Farjallah S, Sanna D, Amor N, Ben Mehel B, Piras MC, Merella P, Casu M, Curini-Galletti M, Said K, Garippa G (2009). Genetic characterization of Fasciola hepatica from Tunisia and Algeria based on mitochondrial and nuclear DNA sequences. Parasitol. Res, 105:1617–1621.

[6] Hung YT, Chen CA, Wu WJ, Lin CC, Shih CJ (2004). Phylogenetic utility of the ribosomal internal transcribed spacer 2 in Strumigenys spp. (Hymenoptera: Formicidae). Mol. Phylogenet. Evol, 32:407–415.

[7] Manonmani A, Townson H, Adeniran T, Jambulingam P, Sahu S, Vijayakumar T (2001). rDNA-ITS2 polymerase chain reaction assay for the sibling species of Anopheles fluviatilis. Acta. Trop, 78:3–9.

[8] Marrelli MT, Sallum MA, Marinotti O (2006) .The second internal transcribed spacer of nuclear ribosomal DNA as a tool for Latin American anopheline taxonomy - a critical review. Mem. Inst. Oswaldo. Cruz, 101:817–832.

[9] Miyamoto J, Ishii A, Sasa M (1975). A successful method for mass culture of the house dust mite, Dermatophagoides pteronyssinus. J. Exp. Med, 45:133–138.

[10] Noge K, Mori N, Tanaka C, Nishida R, Tsuda M, Kuwahara Y (2005). Identification of astigmatid mites using the second internal transcribed spacer (ITS2) region and its application for phylogenetic study. Exp. Appl. Acarol, 35:29–46.

[11] Prakash A, Walton C, Bhattacharyya DR, O'Loughlin S, Mohapatra PK, Mahanta J (2006). Molecular characterization and species identification of the Anopheles dirus and An. minimus complexes in north-east India using r-DNA ITS-2. Acta. Trop, 100:156–161.

[12] Ros VID, Breeuwer JAJ (2007). Spider mite (Acari: Tetranychidae) mitochondrial COI phylogeny reviewed: host plant relationships, phylogeography, reproductive parasites and barcoding. Exp. Appl. Acarol, 42:239–262.

[13] Shaw M, Murrell A, Barker SC (2002). Low intraspecific variation in the rRNA internal transcribed spacer 2 (ITS2) of the Australian paralysis tick, Ixodes holocyclus. Parasitol. Res, 88:247–252.

[14] Suarez-Martinez EB, Montealegre F, Sierra-Montes JM, Herrera RJ (2005). Molecular identification of pathogenic house dust mites using 12S rRNA sequences. Electrophoresis 26:2927–2934.

[15] Suwannamit S, Baimai V, Otsuka Y, Saeung A, Thongsahuan S, Tuetun B, Apiwathnasorn C, Jariyapan N, Somboon P, Takaoka H, Choochote W (2009). Cytogenetic and molecular evidence for an additional new species within the taxon Anopheles barbirostris (Diptera: Culicidae) in Thailand. Parasitol. Res, 104:905–918.

[16] Thanwisai A, Kuvangkadilok C, Baimai V (2006). Molecular phylogeny of black flies (Diptera: Simuliidae) from Thailand, using ITS2 rDNA. Genetica 128:177–204.

[17] Van Bronswijk JE, de Cock AW, Oshima S (1974). The genus Blomia Oudemans (Acari: Glycyphagidae) I. Description of Blomia tropicalis sp. n. from house dust in tropical and sub-tropical regions. Acarologia 15:477–489.

[18] Vargas M, Garcia-Varela M, Laclette JP, Perez TM (2005). Application of ITS-2 sequences as markers for identification and phylogenetic inference within the genus Geomylichus (Acari: Listrophoridae). Exp. Appl. Acarol, 35:223–238.

[19] Webster LMI, Thomas RH, McCormack GP (2004). Molecular systematics of Acarus siro s. lat., a complex of stored food pests. Mol. Phylogenet. Evol, 32:817–822.

[20] Yang Y, Jian W, Pozio E (2008).Analysis of cytochrome c-oxidase (COI) gene of mitochondrial DNA from the Trichinella spp. in China. Parasitol. Res, 103:1355–1359.

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