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28S Ribosomal DNA of Plant Infesting Mites: a Gene Applicable to Higher-level Acari Phylogeny
【Research report】

應用核醣體28S rDNA探討植食性蟎類高階親緣關係【研究報告】

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Abstract

The nuclear 28S rDNA sequence has been widely used in higher phylogenetics of arthropod, but has seldom been applied to Acari. In this study, ~3000 bp of 28S rDNA sequences from nine tetranychids, two tenuipalpids and two acarid mites were used to probe the essence of this gene and its applicability to the delineation of phylogenetic relationship of these species. Sequences of 28S rDNA across Acari, though with variable region, were not randomly distributed, with the expansion segments being more variable than the core regions and with an increasing sequence divergence with taxonomic distances. Scatter plots of total substitutions (Tvs) against transversions (Tv) or transitions (Ts) in 28S region revealed similar and linear evolving processes for both Tv and Ts. Phylogenetic inferences from both parsimony and clustering methods suggest members of the same family and genus were grouped together; a close relationship between genera Panonychus and Oligonychus, and between genera Tetranychus and Petrobia. Analyses of the nearly complete sequences of Acari 28S rDNA have shown that its expansion region may be more useful in the resolution of lower taxonomic relationship, and its core region may be more reliable for that of higher category.

摘要

核醣體28S rDNA序列已廣泛應用於節肢動物高階的親緣分析，但在蟎蜱的應用上卻仍罕見。本文分析9種葉蟎、2種擬葉蟎及2種根蟎28S rDNA約3000個核苷酸的序列特性，並評估此基因在蟎蜱高階親緣關係的應用。28S rDNA序列在蟎蜱的變異並不是逢機的，其核心區段變異較其兩側的展延區小，且分類關係離越遠其序列差異就越大。散佈圖顯示，轉換取代與顛換取代有類似的線性變化；支序分析及群聚分析的親緣關係顯示，同科及同屬內的成員都群聚的很好，全爪蟎屬與小爪蟎屬的親緣關係近，與葉蟎屬較近的是岩蟎屬。28S rDNA的序列特性顯示，其核心序列適合分析較高階的蟎蜱親緣，而展延區則可應用於低階的分析。

Key words: spider mite, false spider mite, bulb mite, 28S rDNA
關鍵詞: 葉蟎、擬葉蟎、根蟎、28S rDNA。

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ABSTRACT

The nuclear 28S rDNA sequence has been widely used in higher phylogenetics of arthropod, but has seldom been applied to Acari. In this study, ~3000 bp of 28S rDNA sequences from nine tetranychids, two tenuipalpids and two acarid mites were used to probe the essence of this gene and its applicability to the delineation of phylogenetic relationship of these species. Sequences of 28S rDNA across Acari, though with variable region, were not randomly distributed, with the expansion segments being more variable than the core regions and with an increasing sequence divergence with taxonomic distances. Scatter plots of total substitutions (Tvs) against transversions (Tv) or transitions (Ts) in 28S region revealed similar and linear evolving processes for both Tv and Ts. Phylogenetic inferences from both parsimony and clustering methods suggest members of the same family and genus were grouped together; a close relationship between genera *Panonychus* and *Oligonychus*, and between genera *Tetranychus* and *Petrobia*. Analyses of the nearly complete sequences of Acari 28S rDNA have shown that its expansion region may be more useful in the resolution of lower taxonomic relationship, and its core region may be more reliable for that of higher category.

Key words: spider mite, false spider mite, bulb mite, 28S rDNA

Introduction

Family Tetranychidae consists of more than 1200 species, including some of the world's major agricultural pests (Bolland *et al*., 1998) which often cause severe damage to their host plants (Jeppson *et al*., 1975). Taxonomic recognition of these mites using morphological features is not always easy due to their small size (≤ 1mm) and with only a few, variable characters available, such as aedeagus, peritreme

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and empodium (Krantz, 1978; Zhang and Liang, 1997). The most popular molecular markers used in the identification and phylogenetic study of spider mites are the internal transcribed space 2 (ITS2) of the ribosomal DNA region and partial sequences of the mitochondrial cytochrome oxidase subunit I (COI). A comparative analysis of ITS2 in ten economically important tetranychid mites belonging to genera *Eotetranychus*, *Mononychellus* and *Tetranychus* has been reported (Navajas *et al*., 1992, 1997). In addition, the results of Noge *et al*. (2005), using ITS2 region to infer the phylogeny of 73 specimens of Astigmata, concluded that this region is useful for studying low-level phylogenetic relationships.

Lately, molecular data have been increasingly applied to higher-level phylogenetic studies of Acari (Navajas and Fenton, 2000; Cruickshank, 2002). Commonly used markers in these studies include the mitochondrial ribosomal DNA, *e.g.*, mt16S rDNA, (Black and Piesman, 1994; Murrell *et al*., 2000; Suarez-Martinez *et al*., 2005) and nuclear ribosomal DNA, *i.e*., 18S rDNA, (Black *et al*., 1997; Mangold *et al*., 1998; Dobson and Barker, 1999; Murrell *et al*., 2001; Klompen *et al*., 2006). Although the nuclear 28S rDNA sequence has been used widely in higher- level phylogenetic work of arthropod (Wheeler *et al*., 2001; Schulmeister, 2003; Mallatt *et al*., 2004; Cryan, 2005; Mallatt and Giribet, 2006; Klompen *et al.*, 2006), only a few studies have chosen its partial sequence in their assessment of the higher phylogeny in Acari. Crampton *et al*. (1996) proposed that the D1 region (expansion divergent region containing 141bp) of 28S rDNA could be used to study the long- standing Ixodida phylogeny. Maraun *et al*. (2003, 2004), using 325 bp in the D3 region to study the oribatid relationship, concluded that this region of 28S rDNA was not reliable for the higher phylogeny inference. More recently, Klompen *et al.* (2006) used partial sequences in the D3- D5 and D9-D10 regions of 28S rDNA to address the phylogeny of parasitiform mites, and suggested that this nuclear rRNA gene is very well suited for analyzing higher level relationships of Parasitiformes.

Up till now, no phylogenetic analysis of phytophagous mites, such as spider mites, false spider mites, and bulb mites has been done. For the first time, we herein report the evolutionary relationship of a number of spider mites, false spider mites, and bulb mites and an assessment of the applicability of 28S rDNA in the higher phylogeny of Acari. We sequenced nearly complete 28S rDNA from stem 26 to stem 97, including the D3 to D11 regions (Kjer *et al*., 1994), and constructed the phylogenetic relationship of four tetranychid genera, two tenuipalpids and two acarid mites. Sequence divergence revealed increasing DNA variation with the taxonomic distance. Phylogenetic results have shown that taxa in the same family or genus are well grouped, yet the resolution among species within the genus is low.

Materials and Methods

Material sources and rearing conditions

Mites were collected from the field and reared individually on host plants in a glasshouse at 25ºC for several generations in order to get homogenous DNA materials. Taxa of mites used in this study, their collection records or sources, and their host plants are summarized in Table 1. *Panonychus ulmi* from the quarantine center was reared on branches of apple tree and kept at 20ºC under a 14L-10D photoperiod in the laboratory. In addition, bulb mites were reared in the laboratory with artificial medium (Bot and Meyer, 1967), and *Rhizoglyphus echinopus* was acquired from the quarantine center.

Examination of morphological characters

The method of mounting tetranychid mites for light microscopy was modified from Gutierrez (1985), and that used for

Table 1. Scientific names of the thirteen mites used in this study, their collecting localities, host plants, and abbreviation

Taxon	Family	Locality*	Host Plant
Oligonychus coffeae (Neitner)	Tetranychidae	Yunlin	*Thea sinensis* L.
Panonychus citri (McGregor)	Tetranychidae	Pingtung	*Carica papaya* L.
Panonychus ulmi (Koch)	Tetranychidae	Taichung*	*Malus pumila* Mill
Petrobia harti (Ewing)	Tetranychidae	Kaohsiung	*Oxalis corniculata* L.
Tetranychus hydrangeae Pritchard & Baker	Tetranychidae	Pingtung	*Ficus Iyrata* Warb
Tetranychus kanzawai Kishida	Tetranychidae	Pingtung	*Glycine max* Merr
Tetranychus piercei McGregor	Tetranychidae	Pingtung	*Musa colocasie*
Tetranychus truncatus Ehara	Tetranychidae	Pingtung	*Coiocasia esculenta* L.
Tetranychus urticae Koch	Tetranychidae	Tiachung	*Phaseolus vulgaris* L.
Tenuipalpus pacificus Baker	Tenuipalpidae	Pingtung	*Phalaenopsis aphrodite*
Raoiella indica Hirst	Tenuipalpidae	Pingtung	*Phoenix hanceana*
Rhizoglyphus robini Claparede	Acaridae	Taichung	*Allium fistulosum*
Rhizoglyphus echinopus (Fumouze & Robin)	Acaridae	Taichung*	*Allium cepa*

*Materials from quarantine center.

the scanning electron microscope was modified from Crooker (1985). Taxonomic characters of spider mites used by Krantz (1978) and Tseng (1990), *i.e.*, empodium I (the distal part of leg I), peritreme (part of the respiratory system) of the adult female, and aedeagus (part of the male genitalia) were recorded. Also, taxonomic characters of soma setae in tenuipalpids, chaetotaxy of legs, and aedeagus in bulb mites were examined.

DNA isolation and PCR amplification

Adult mites (100 individuals from a single parthenogenetic female in a pool) were homogenized in a 1.5 ml microtube with the Nuclei Lysis Solution, and genomic DNA was extracted using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI) as described before (Hsu *et al*., 2005). The recovered DNA pellet was dissolved in 50 μl of distilled water and stored at -20°C.

A 3 kb fragment in the central part of 28S rDNA gene was PCR-amplified and sequenced with four paired primer sets, *i.e.*, 28Sa-28Sb, 28Sc-28Sd, 28Se- 28Sf and 28Sg-28Sh (Hsu *et al*., 2005). The polymerase chain reaction (PCR) was performed in a final volume of 50 μl containing 1 μl of DNA solution, 0.2 mM dNTP, 20 pmole of each primer, 2 units of SuperTaq polymerase (HT Biotechnology, Taiwan), and 1X SuperTaq buffer; PCR programming conditions were 94°C for 2 min as first denaturation step, followed by 35 cycles of 94°C for 50 sec, 52°C for 50 sec, 72°C for 50-70 sec, and 72°C for 10 min as the final extension.

Cloning and sequencing of PCR products

The resultant PCR product was purified directly with Qiaquick PCR Purification Kit or Qiaquick Gel Extraction Kit (QIAGEN, Germany) from the excised gel containing the target DNA fragment in accordance with the manufacturer's instructions. Amplified fragments of 28Sab, 28Scd, 28Sef, and 28Sgh from each species, with a few exceptions as stated below, were sequenced directly using a *Taq* Dye Terminator Cycle Sequencing Kit by ABI 377A sequencer (Applied Biosystems, Foster City, CA). Amplified fragments of 28Sab from *Panonychus citri*, 28Scd from *Pan. citri*, *Tetranychus hydrangeae*, and 28Sef from *Oligonychus coffeae*, *Tet. truncatus*, *Tet.*

piercei, and *Tet. urticae* could not be sequenced directly, and were ligated into a pGEM-T easy vector using TA cloning kit (Promega, Madison, WI) and transformed into competent cells (JM109 of *Ecoli*). For each amplified product, at least ten white bacterial colonies were chosen randomly and cultured in ampicillin-containing 2YT medium. Plasmids were then extracted using Qiaprep Spin Miniprep Kit (QIAGEN, Germany) and the insertion was checked by enzyme digestion. Universal primers of M13 Forward and Reverse, T7 and SP6 primers were used in the sequencing reaction for 2 to 4 clones. These compiled sequences were submitted to GenBank under the accession numbers AY750691-AY750699 and AB287404-287407.

DNA sequence analysis

Sequences of fragments 28Sab, 28Scd, 28Sef, and 28Sgh for each species were used as query sequences to search for their homogeneous sequences under the Blastn algorithm in the NCBI website (http://www.ncbi.nlm.nih.gov). DNA sequences of these mites were aligned using the Pileup program of GCG (http://bioinfo.nhri.org.tw) and confirmed visually. Divergent regions across the aligned Acari 28S rDNA were examined dependent on whether the nucleotide was identical to or different from the corresponding nucleotide of the consensus sequence at a given alignment position (InforMax Inc. 2001). Phylogenetic analyses were performed using MEGA3 (Kumar *et al.*, 2004) and PAUP 4.0 (Swofford, 2002) packages. Divergence between sequences was estimated using proportion distance model. The phylogenetic tree was constructed using the maximum parsimony and neighbor-joining methods, and 1000 replications were performed in bootstrap analyses.

Results

Sequence composition of Acari 28S rDNA

Sequences of the central part of 28S rDNA gene in thirteen mite species are algned. The length of 28S rDNA sequences among taxa ranged from 2564 to 3115 bases. With gaps added to the alignment, a total of 3265 positions were analyzed and 974 positions (30%) were variable. Insertion fragments were observed commonly in genera *Oligonychus*, *Panonychus* and *Petrobia*, and two insertions of more than 100 bp were found in tenuipalpid mites (positions 1053-1222 and 2474-2683). DNA sequence of 28S rDNA has been separated into the core region and the expansion divergent segment. Sequences in the latter are known to be less-conserved than those in the former (Hancock and Dover, 1988; Kjer *et al.*, 1994).

The work herein found that variable sites were not randomly distributed over the examined 28S rDNA region. Sequences of the expansion segments, except the D11 region, were more divergent than those of the core regions (Fig. 1). While over 80% of the bases in the cores were conserved in Acari, most regions in the expansion segments were variable.

Sequence divergence in Acari 28S ribosomal DNA

Unlike the bias found in mtDNA, the base composition of Acari 28S rDNA, *i.e.*, 26.6% G, 27% A, 25.6% T, and 20.8% C, did not vary significantly among species. The uncorrected nucleotide divergence among species of a given genus was 0.2-1.2%, that among genera of the same family was 6-12.6%, that among families in the same suborder was 11.5% to 15.9%, and that among suborders was 16.7-19.1%. More significant gaps were found when sequences of 28S ribosomal DNA in different category levels of Acari were compared. Plot analysis of total substitutions (Tvs) versus transversions (Tv) and transitions (Ts) revealed that the mutation rate of Tv was similar to that of Ts, and that the substitutional saturation due to multiple hits had as yet not been observed

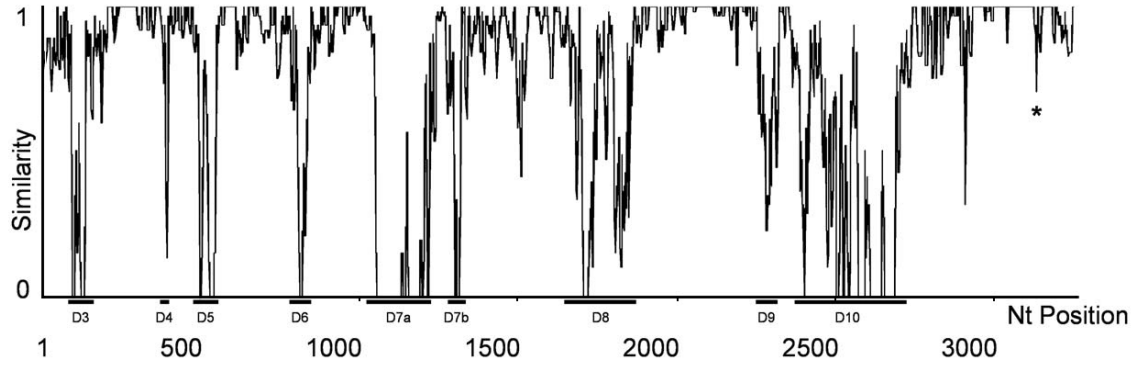

Fig. 1. Variable regions are not randomly distributed across the Acari 28S rDNA sequences. Relative position of expansion variable segments from the D3 to D10, except the D11 segment (*) are leveled beneath the horizontal axis.

in Acari (Fig. 2). Sequence divergence was increasingly obvious with the taxonomic distance. These results suggest that both Tv and Ts might provide useful phylogenetic information.

Phylogenetic analysis of Acari 28S ribosomal DNA sequences

Maximum parsimony analysis using 974 variables resulted a tree of 1559 steps, with a consistency index of 0.845 and a retention index of 0.837. As shown in Figure 3A, members of the same family and genus are grouped together. Bootstrap analysis indicates interior nodes above the generic level with high support. Therefore, the relationship between *Panonychus* and *Oligonychus* genera is strongly supported, and so is the relationship between *Tetranychus* and *Petrobia*, although with lower bootstrap support.

A dendrogram, based on Kimura two-parameter substituted model, resulting from a neighbor-joining analysis with 1000 bootstrap replications is shown in Fig. 3B. Members of the same genus, family, and suborder were grouped together and received significant bootstrap support. The phylogenetic tree reveals the close relationships between *Panonychus* and *Oligonychus*, and between *Petrobia* and *Tetranychus*. However, phylogenetic relationships among *Tetranychus* species are poorly resolved except for the lineage of *Tet. hydrangeae* and *Tet. kanzawai* in the parsimony tree.

Discussion

Molecular phylogenetic studies of Acari using mitochondrial *COI* and the internal ribosomal ITS2 region have focused mostly on the lower taxonomic taxa (Fenton *et al*., 2000; Navajas and Fenton, 2000; Cruickshank, 2002). Sequences of *COI* gene from eight genera of tetranychid spider mites revealed that genera *Petrobia* and *Bryobia* are the most divergent among the tetranychid genera (Navajas *et al*., 1996, Navajas *et al.*, 1998). However, rapid saturation of transitional substitution and the third codon positional substitution in *COI* gene result in low bootstrap values in some generic nodes. Phylogenetic inferences from 28S rDNA sequence, such as in the current study, reveal robust relationships among tetranychid genera, although the phylogenetic confidence of *Petrobia* to *Tetranychus* is somewhat lower in parsimony analysis.

Crampton *et al*. (1996) indicated that the D1 region of 28S rDNA may be used to study the long-standing Ixodida phylogeny. However, the results of Maraun *et al*.

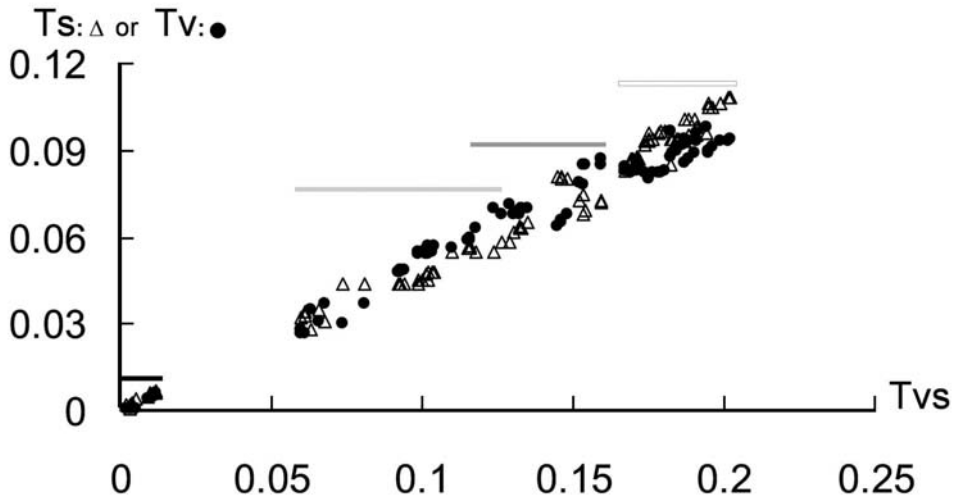

Fig. 2. Scatter plots of total substitutions (Tvs) versus transversion (Tv, circles) and transition (Ts, triangles) substitutions. Both Tv and Ts have a similar linearly evolved pattern in 28S rDNA. Distinct gap is found in different hierachical levels. Black, gray-hatched, gray, and blind bars indicate, respectively, the variation ranges among species of a given genus, among genera of a given family, between families within the suborder, and between suborders.

(2004) indicated that the D3 of 28S rDNA region was not a good tool for elucidating the relationship among families of oribatid mites. The current study, using the nearly complete sequences of Acari 28S rDNA shows that the sequence in the expansion region is more differentiated than that of the core region. This finding suggests that the expansion region, especially the D7a and D10, is useful in the resolution of lower taxonomic studies, and the core region is reliable at a higher category.

The empodium and peritreme of *Oligonychus* are very similar to those of *Panonychus*. However, the same characters of *Pet. harti* have been identified as being extremely different from those of *Tetranychus*. The evolutionary changes of the morphological characters in Tetranychidae as described by Gutierrez and Helle (1985), *i.e.*, simple straight aedeagus, claw-like empodium, and anastomosing peritreme in *Pet. harti*, were considered to represent the original ancestral state. According to Gutierrez and Helle (1985), both anastomosing and simple peritremes were found in different lineages of Tetranychinae, including Tenuipalpoidini, Tetranychini, and Eurytetranychini. Similarly evolved aedeagus, shaped as a straight or curved stylet, were also found in Hystrichonychini, Tenuipalpoidini, and Eurytetranychini. Both ancestral and derived characters found in the same lineage reveal the possibility of symplesiomorphy retaining or adaptively evolving process in tetranychoids.

Moreover, we are confident that the tetranychid DNAs were not contaminated nor that misleading products were obtained for the following reasons: (i) the resulting tetranychid DNAs were searched in GenBank (Blastn in website: www.ncbi.nlm.nih.gov), and patterns of the highest similarity were those from other arachnid members; (ii) phylogenetic analysis of sequences of the two bulb mites of another Acari suborder showed that these sequences were located in the outset position (Fig. 3); and (iii) 28S ribosomal DNA sequences of two species, *Raoiella indica* Hirst and

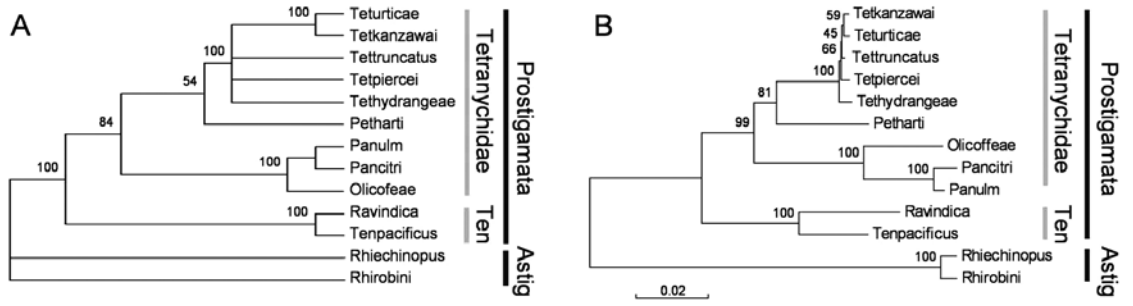

Fig. 3. The phylogenetic tree inferred from 28S rDNA region. Values (percentages) on branches are the results of 1000 bootstrap replications. The parsimony tree was obtained under a branch-bound search (A). Neighbor-Joining clustering analysis was used, based on Kimura 2-parameter distance model (B). The gray and black rectangle-boxes indicate the clusters of each family and suborder, respectively. Astig and Ten mean Astigmata and Tenuipalpidae, respectively. Scale indicates branch length and scientific names are cited in Table 1.

Tenuipalpus pacificus Baker, of Tenuipalpidae, which belong to the same prostigmata suborder, exhibited close affinity to tetranychids in the phylogenetic analysis.

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References

Black, W. C., and J. Piesman. 1994. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. Proc. Natl. Acad. Sci. USA 91: 10034-10038.

Black, W. C., J. S. H. Klompen, and J. E. Keirans. 1997. Phylogenetic relationships among tick subfamilies (Ixodida: Ixodidae: Argasidae) based on the 18S nuclear rDNA gene. Mol. Phylogenet. Evol. 7: 129-144.

Bolland, H. R., J. Gutierrez, and C. H. W. Flechtmann. 1998. World Catalogue of the Spider Mite Family (Acari: Tetranychidae). Koninklijke Brill Press, Leiden, Netherlands.

Bot, J., and M. K. P. Meyer. 1967. An artificial method for acarid mites. J. Entomol. Soc. South Afr. 29: 1-199.

Crampton, A., I. McKay, and S. C. Barker. 1996. Phylogeny of ticks (Ixodida) inferred from nuclear ribosomal DNA. Int. J. Parasitol. 26: 511-517.

Crooker, A. R. 1985. Histological techniques. pp 359-381. *In*: W. Helle, and M. W. Sabelis, eds. Spider Mites, Their Biology, Natural Enemies and Control, Vol 1A. Elsevier, Amsterdam.

Cruickshank, R. H. 2002. Molecular markers for the phylogenetics of mites and ticks. Syst. Appl. Acarol. 7: 3-14.

Cryan, J. R. 2005. Molecular phylogeny of Cicadomorpha (Insecta: Hemiptera: Cicadoidea, Cercopoidea and Membracoidea): adding evidence to the controversy. Syst. Entomol. 30: 563-574.

Dobson, S. J, and S. C. Barker. 1999. Phylogeny of the hard ticks (Ixodidae) inferred from 18S rRNA indicates that the genus *Aponomma* is paraphyletic. Mol. Phylogenet. Evol. 11: 288-295.

Fenton, B., A. N. Birch, G. Malloch, P. G. Lanham, and R. M. Brennan. 2000. Gall mite molecular phylogeny and its relationship to the evolution of plant host specificity. Exp. Appl. Acarol. 24: 831-861.

Gutierrez, J. 1985. Mounting techniques. pp. 351-354. *In*: W. Helle, and M. W. Sabelis, eds, Spider Mites, Their Biology, Natural Enemies and Control, Vol 1A. Elsevier, Amsterdam.

Gutierrez, J., and W. Helle. 1985. Evolutionary changes in the Tetranychidae. pp. 91-107. *In*: W. Helle, and M. W. Sabelis, eds, Spider Mites, Their Biology, Natural Enemies and Control, Vol 1A. Elsevier, Amsterdam.

Hancock, J. M., and G. A. Dover. 1988. Molecular coevolution among cryptically simple expansion segments of eukaryotic 26S/28S rRNAs. Mol. Biol. Evol. 5: 377-391.

Hsu, K., T. Hua, N. T. Chang, and W. B. Yeh. 2005. Molecular Identification of important spider mites using multiplex PCR and PCR-RFLP. Formosa Entomol. 25: 211-223.

InforMax, Inc. 2001. Vector NTI Suite User's Manual, Version 7. InforMax Inc. Bethesda, MD.

Jeppson, L. R., H. H. Keifer, and E. W. Baker. 1975. Mites Injurious to Economic Plants. California University Press, Los Angeles, California.

Kjer, K. M., G. D. Baldridge, and A. M. Fallon. 1994. Mosquito large subunit ribosomal RNA: simultaneous alignment of primary and secondary structure. Biochim. Biophys. Acta 1217: 147-155.

Klompen, H., M. G. Lekveishvili, and W. C. Black IV. 2006. Phylogeny of Parasitiform mites (Acari) based on rRNA. Mol. Phylogen. Evol. 43: 936-951.

Krantz, G. W. 1978. A Manual of Acarology, 2nd ed. Oregon State Univerity Book Stores, Corvallis.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief. Bioinform. 5: 150-163.

Mallatt, J. M., and G. Giribet. 2006. Further use of nearly complete 28S and 18S rRNA genes to classify Ecdysozoa: 37 more arthropods and a kinorhynch. Mol. Phylogen. Evol. 40: 772-794.

Mallatt, J. M., J. R. Garey, and J. W. Shultz. 2004. Ecdysozoan phylogeny and Bayesian inference: first use of nearly complete 28S and 18S rRNA gene sequences to classify the arthropods and their kin. Mol. Phylogenet. Evol. 31: 178-191.

Mangold, A. J., M. D. Bargues, and S. Mas-Coma. 1998. 18S rRNA gene sequences and phylogenetic relationships of European hard-tick species (Acari: Ixodidae). Parasitol. Res. 84: 31-37.

Maraun, M., M. Heethoff, S. Scheu, R. A. Norton, G. Weigmann, and R. H. Thomas. 2003. Radiation in sexual and parthenogenetic oribatid mites (Oribatida, Acari) as indicated by genetic divergence of closely related species. Exp. App. Acarol. 29: 1265-1277.

Maraun, M., M. Heethoff, K. Schneider, S. Scheu, G. Weigmann, J. Cianciolo, R. H. Thomas, and R. A. Norton. 2004. Molecular phylogeny of oribatid mites (Oribatida, Acari): evidence for multiple radiations of parthenogenetic lineages. Exp. App. Acarol. 33: 183- 201.

Murrell, A., N. J. H. Campbell, and S. C. Barker. 2000. Phylogenetic analyses of the rhipicephaline ticks indicate that the genus *Rhipicephalus* is paraphyletic. Mol. Phylogenet. Evol. 16: 1-7.

Murrell, A., N. J. H. Campbell, and S. C. Barker. 2001. A total-evidence phylogeny of ticks provides insights into the evolution of life cycles and biogeography. Mol. Phylogenet. Evol.

21: 244-258.

Navajas, M., and B. Fenton. 2000. The application of molecular markers in the study of diversity in acarology: a review. Exp. App. Acarol. 24: 751- 774.

Navajas, M., J. Gutierrez, and T. Gotoh. 1997. Convergence of molecular and morphological data reveals phylogenetic information on *Tetranychus* species and allows the restoration of the genus *Amphitetranychus* (Acari: Tetranychidae). Bull. Entomol. Res. 87: 283-288.

Navajas, M., D. Cotton, S. Kreiter, and J. Gutierrez. 1992. Molecular approach in spider mites (Acari: Tetranychidae): preliminary data on ribosomal DNA sequences. Exp. App. Acarol. 15: 211-218.

Navajas, M., J. Gutierrez, J. Lagnel, and P. Boursot. 1996. Mitochondrial cytochrome oxidase I in tetranychid mites: a comparison between molecular phylogeny and changes of morphological and life history traits. Bull. Entomol. Res. 86: 407-417.

Navajas, M., J. Lagnel, J. Gutierrez, and P. Boursot. 1998. Species-wide homogeneity of nuclear ribosomal ITS2 seqences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. Heredity 80: 742-752.

Noge, K., N. Mori, C. Tanaka, R. Nishida, M. Tsuda, and Y. Kuwahara. 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.

Schulmeister, S. 2003. Simultaneous analysis of basal Hymenoptera (Insecta): introducing robust-choice sensitivity analysis. Biol. J. Linn. Soc. 79: 245-275.

Suarez-Martinez, E. B., F. Montealegre, J. M. Sierra-Montes, and R. J. Herrera. 2005. Molecular identification of pathogenic house dust mites using 12S rRNA sequences. Electrophoresis 26: 2927-2934.

Swofford, D. L. 2002. PAUP* 4.0: Phylogenetic Analysis Using Parsimony, Beta Version 10, Sinauer Associates, MA.

Tseng, Y. H. 1990. A monograph of the mite family Tetranychidae (Acarina: Trombidiformes) from Taiwan. Taiwan Museum, Special Publ. No. 9. 224 pp.

Wheeler, W. C., M. Whiting, Q. D. Wheeler, and J. M. Carpenter. 2001. The phylogeny of extant hexapod orders. Cladistics 17: 113-169.

Zhang, Z., and L. Liang. 1997. An Illustrated Guide to Mites of Agricultural Importance. Tongji University Press, Shanghai, China.

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應用核醣體28S rDNA探討植食性蠊類高階親緣關係

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摘　要

核醣體 28S rDNA 序列已廣泛應用於節肢動物高階的親緣分析，但在蠊蜱的應用上卻仍罕見。本文分析 9 種葉蠊、2 種擬葉蠊及 2 種根蠊 28S rDNA 約 3000 個核苷酸的序列特性，並評估此基因在蠊蜱高階親緣關係的應用。28S rDNA 序列在蠊蜱的變異並不是逢機的，其核心區段變異較其兩側的展延區小，且分類關係離越遠其序列差異就越大。散佈圖顯示，轉換取代與顛換取代有類似的線性變化；支序分析及群聚分析的親緣關係顯示，同科及同屬內的成員都群聚的很好，全爪蠊屬與小爪蠊屬的親緣關係近，與葉蠊屬較近的是岩蠊屬。28S rDNA 的序列特性顯示，其核心序列適合分析較高階的蠊蜱親緣，而展延區則可應用於低階的分析。

關鍵詞：葉蠊、擬葉蠊、根蠊、28S rDNA。

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