Phylogeny of Mite Taxa (Acari:Sarcoptiformes) Based on Small Subunit Ribosomal RNA Sequences

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We analyzed the phylogenic relationships of 23 partial 18S rDNA sequences of 22 species (1 species has 2 strains) belonging to Sarcorptiforms include 4 new sequences, using several tools. Although geographic distributions are quite far from, sequence similarity of two strains of *Dermatophygoides pteronyssinus* isolated from Japan and New Zealand were very high. This result suggests that mite migration by animals including human occurred in the two continents. We investigated the Endeostigmata taxonomic relationship between the Prostigmata and Oribatida subgroups using small fragments (340 -400 bp) of their 18S rDNA sequences. But Endeostigmata was not grouped with Oribatida or Prostigmata. In conclusion, it is first reported phylogenic relationship for classified mites included in Sarcoptiformes using 18S rDNA sequence analysis and its system is a very powerful tool for classification of mites.

Key words – Mite, 18S rDNA, sarcoptiformes, phylogenic tree.

Mites belong to subphylum Cheliceriformes (Chelicerata) and the class Arachnida within phylum Arthropoda. Arachnida usually possess four pairs of legs and chelate mouthparts[2]. The three superorders Opilioacarida, Acariformes, and Parasitoformes are usually recognized as acarologists[7]. The superorder Opilioacarida is a small group of large mites that resemble "daddy long-leg spiders" (Opiliones). The superorder Parasitiformes has three orders: Ixodida (Metastigmata), containing the parasitic tick species; Holothyrida, containing the water mites; and Mesostigmata, including mite species that are parasitic of birds, mammals, and plants. All of these orders are free-living and predacious. Orders within the Acariformes include Prostigmata (about 14,000 species), Orbatida or Cryptostigmata (about 7,000 species), Astigmata (about 5,000 species in 70 families), and Endeostigmata (their position and status are still subjects of much debate)[7].

Most mites cause harm to humans directly or indirectly. Especially, according to reports of the Voorhost *et al.*[12] and Oshima[10], mites have been known to play a major role in dust allergens, and the medical importance of mites has been emphasized. About 50% of the species of Astigmata are parasites of birds and mammals[9]. Also, the family Pyloglyphidae contains about 18 genera and 46 species of mites; among these species, 13 have been collected from houses and barn dust. These mites and mite products can cause severe allergies in sensitive persons.

The phylogenetic relationships of mites have been investigated on the sole basis of morphological features. But these classification tasks were very tedious and difficult because of the small sizes of mites. Only a few reports on the molecular phylogeny of mites have been reported. Molecular phylogeny is a very powerful method for revealing the genetic and evolutionary relationships of species that are difficult to classify by morphological features. Salomone *et al.*[11] successfully classified Steganacaridae (Acari, Oribatida, living in the Canary Islands) by their mitochondrial DNA sequence data. Molecular phylogeny based on ribosomal gene analysis has been a valuable tool for investigating the evolutionary relationships among other organisms[1,3,6].

Prior to the present study, there has been no molecular phylogenic study based on small subunit ribosomal RNA (18S rRNA) sequences of mites. We analyzed, for the first time, the phylogenic relationships of 23 partial 18S rDNA sequences of 22 species (1 species has 2 strains) belonging to Sarcorptiforms include 4 new sequences, using several tools.

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Table 1. Species subjected to phylogenetic analysis and accession numbers.

Order	Suborder	Superfamily	Family	Species	Accession No.
Astigmata		Acaroidea	Acaridae	*Acarus siro*	AF022023
				Rhizoglyphus sp.	AF287236
				Tyrophagus puterescentiae	DQ025510
		Glycyphagoidea	Chortoglyphagidae	*Chortoglyphus arcuatus*	AF022028
		Histiostomatoidea	Histiostomatidae	*Histiostomata* sp.	AF022032
		Analgoidea	Pyroglyphidae	*Dermatophagoides pteronyssinus* J*	DQ025511
				Dermatophagoides pteronyssinus N†	DQ025512
				Dermatophagoides farinae	DQ025509
Endostigmata			Alicorhagiidae	*Alicorhagidia* sp.	AF022024
Orbatida	Brachypylina	Gustavioidea	Xenillidae	*Xenillus tegeocranus*	AF022042
		Liodoidea	Liodidae	*Liodes* sp.	AF022035
		Ceratozetoidea	Euzetidae	*Euzetes globulosus*	AF022030
	Desmonomata	Crotonioidea	Nothridae	*Nothrus sylvestris*	AF022039
		Nanhermannoidea	Nanhermanniidae	*Nanhermannia* sp.	AF022037
		Trhypochthonioidea	Trhypochthoniidae	*Allonothrus russeolus*	AF022025
				Archegozetes longisetosus	AF022027
				Trhypochthonius tectorum	AF022041
	Enarthronota	Hypochthonoidea	Hypochthoniidae	*Hypochthonius rufulus*	AF022033
	Mixomata	Collohmannioidea	Collohmanniidae	*Collohmannia* sp.	AF022029
		Lohmannioidea	Lohmannia	*Lohmannia banksi*	AF022036
				Meristolohmannia meristacaroid	AF287234
		Nehypochthonoidea	Nehypochthoniidae	*Nehypochthonius porosus*	AF022038
		Phthiracaroidea	Steganacaridae	*Steganacarus magnus*	AF022040

*; collected from Japan.
†; collected from New Zealand.

Materials and Methods

Mite strains

We used 18S rRNA sequences from 24 taxa: 8 from the order Astigmata, one from the order Endeostigmata, and 15 from the order Oribatida. We obtained 20 sequences from the GenBank database (the GenBank accession numbers are shown in Table 1). We generated 18S rRNA sequences from 4 mite isolates: one strain of *Dermatophagoides pteronyssinus* from New Zealand, the other strain of *D. pteronyssinus* and *D. farinae* from Japan, and *Tyrophagus puterscentiae* from Korea. These mites were kindly provided by Dr. HS Nam (Soonchunhyang University, Korea) and Dr. TS Yong (Yonsei University, Korea).

DNA Preparation

Genomic DNA was isolated from 100 mg of mites. The mites were crushed and homogenized in a 10 ml lysis buffer (10 mM EDTA, 50 mM Tris-HCl, and 0.5% SDS). The lysates were incubated at 37°C for 1 hr. After centrifugation, the supernatant was transferred to new tube and proteinase K (final concentration: 0.2 mg/ml) was added. After incubation at 56°C for 3 hrs, DNA was purified with one phenol and one phenol-chloroform extraction, and DNA was precipitated by ethanol. The pellet was resuspended in 100 μl of distilled water.

PCR Amplification and Sequencing

Oligonucleotide primers for amplify of 18S rDNA were made from the published nucleotide sequences (Table 1). PCR amplifications were carried out on a DNA Thermal Cycler 9600 (Perkin-Elmer Cetus) with the Takara DNA Amplification kit (Takara, Japan). The composition of the reaction mixture was as follows: 10 mM of Tris-HCl (pH 8.8), 50 mM of KCl, 0.1% Triton X-100, 1.5 mM of $MgCl_2$, deoxynucleoside triphosphates (0.2 mM each), 0.2 μM per primer, and 1 U of Taq polymerase in a total volume of 49 μl. A total of 1 μl of DNA was added to the reaction mixture, which was centrifuged briefly before 50 μl of mineral oil was layered onto the surface. The PCR program is consisted of an initial denaturation step at 95°C for 3 min followed by 35 cycles of DNA denaturation at 94°C for 30 sec, primer annealing at 55°C for 30 sec, and primer extension at 72°C for 1 min. After the final cycle, the prod-

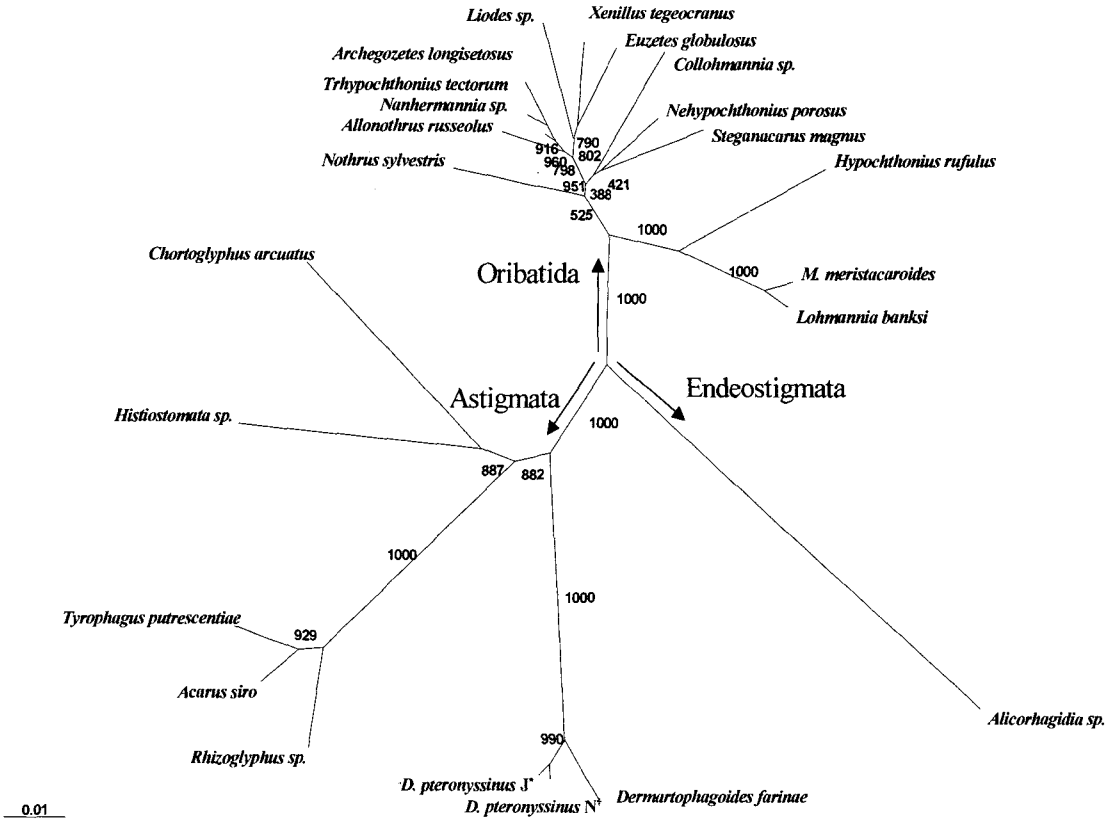

Fig. 1. Phylogenetic relationships of mites belonging to Sarcorptiforms on 18S rDNA sequences. The numbers are maximum bootstrap values based on 1000 replicates. J* is collected from Japan. N† is collected from New Zealand.

Table 2. Dissimilarity (below diagonal) and number of different nucleotides (above diagonal) of mites belonging to Astigmata.

No.	Species	1	2	3	4	5	6	7	8
1	*Dermartophagoides farinae*	-	29	25	182	188	178	172	157
2	*D. pteronyssinus* N	0.019	-	7	163	176	177	173	146
3	*D. pteronyssinus* J	0.018	0.005	-	171	176	167	175	148
4	*Acarus siro*	0.119	0.115	0.113	-	58	42	183	162
5	*Rhizoglyphus* sp.	0.124	0.116	0.116	0.038	-	50	174	173
6	*Tyrophagus putrescentiae*	0.118	0.113	0.116	0.028	0.034	-	173	167
7	*Histiostomata* sp.	0.114	0.113	0.114	0.119	0.113	0.114	-	147
8	*Chortoglyphus arcuatus*	0.104	0.097	0.098	0.107	0.113	0.11	0.098	-

ucts were stored at 4°C. The PCR products were analyzed by electrophoresis with 0.7% agarose gels in a TBE buffer. To determine the sequence analysis, the PCR products were ligated with a pGEM T-easy vector (Promega, Madison, WI, USA) and introduced into *E. coli* DH5α cells. The sequencing reactions were performed using a double-stranded plasmid preparation by dideoxy chain termination with T7 and Sp6 primers.

Phylogenetic Analysis

Multialign of 24 18S rDNA partial sequences was performed using Clustal W (ver. 1.82) available on the ExPASy web site (http://www.expasy.org). Phylogenetic tree is showing the genetic relationships among the 24 mites were constructed using the Neighbor- Joining method.

In addition, bootstrap analyses (1000 replicates) were done with data sets.

Results and Discussion

Phylogeny studies inferred from the 18S rRNA gene

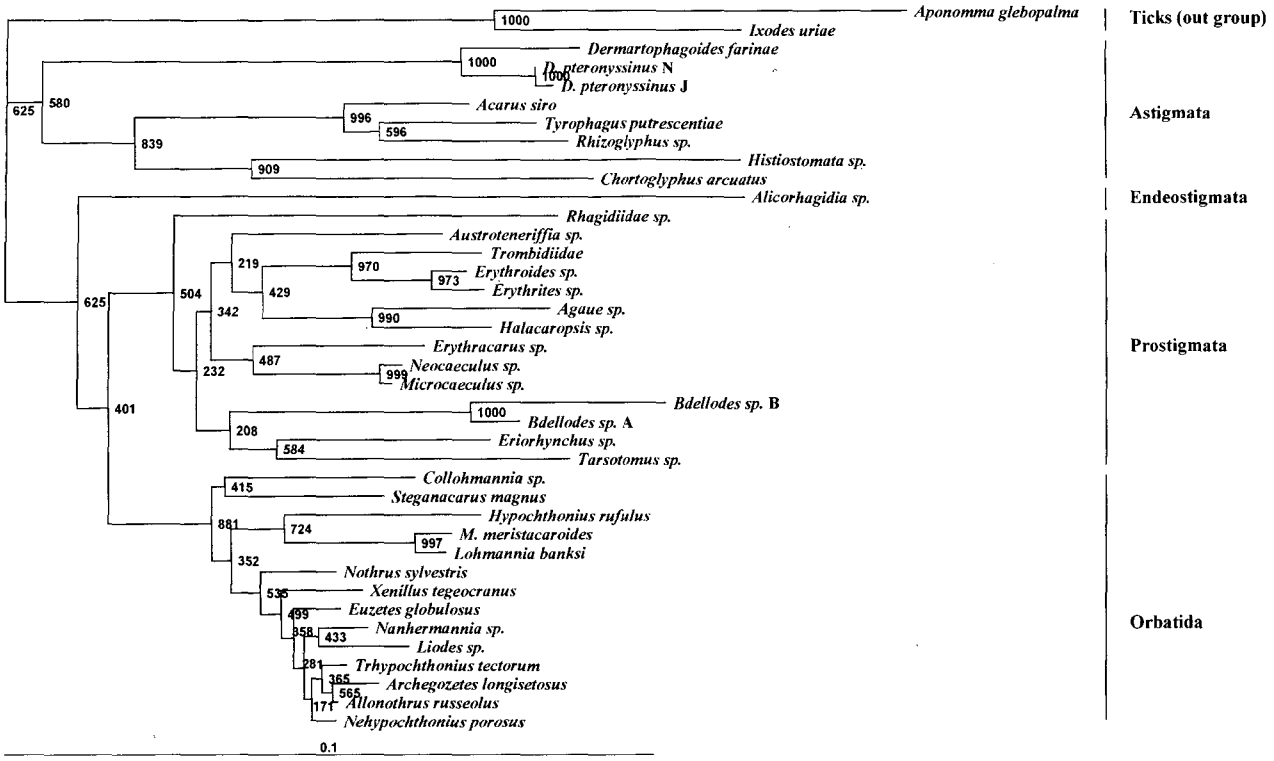

Fig. 2. Maximum likelihood tree for mites were calculated from partial 18S rDNA sequences. The numbers are maximum bootstrap values based on 1000 replicates of stepwise random addition. Two ticks (*Aponomma glebopalma and Ixodes uriae)* were used as outgroups.

sequences of various organisms were used to reveal the genetic relationship among them that are difficult to classify by morphological data. 18S rRNA sequences are much conserved from *E. coli* to humans. In this study, we successfully classified 23 species belonging to Sarcorptiforms and coinciding with previous taxonomy (Fig. 1). The interspecies branches of Oribatida were much shorter than those of Astigmata and Endeostigmata. This result shows that the Oribatida species have a closer relationship to each other than do other species.

It is very difficult and tedious to identify and classify mites species or taxa. In many cases, mites are crushed by a vacuum apparatus in the collecting stage and it is especially difficult to identify mites in case of several mites being mixed in collect on samples. Molecular phylogeny can more identify and classify easy the genus or species of unknown mites than that of morphology. The 18S rDNA sequences of the 23 mite species were analyzed by computer program according to the restriction fragment patterns that were made by several restriction enzymes. The deduced restriction fragment patterns made by the *Taq* I restriction enzymes of the 23 mite 18S rDNA sequences could be successfully classified into their subgroups: Astigmata, Endeostigmata and Oribatida. The sizes of the largest fragments of mites from the Oribatida subgroups were very similar (717-726 bp), but those from the other subgroups were various (504-1128 bp). Of the mites from Astigmata (except *Chortoglyphus arcuatus* [504 bp]), the sizes of the largest fragments were above 800 bp. Even when various mites were mixed in the collection sample, we could easily identify individual mites using the 18S rDNA PCR-RFLP method.

The dissimilarity of the rDNA sequences of the Asigmata subgroup is shown in Table 2. Two strains of *Dermatophygoides pteronyssinus,* from Japan and New Zealand respectively, were analyzed in this study. Interestingly, although these two geographic regions are quite far from each other, their 18S rDNA sequences were found to be quite similar: only 7 bp from each other among within range of 1507 bp (Table 2). This result suggests that mite migration by persons and animals occurred in the two regions. To know more about the exact evolutionary events of *D. pteronyssinus,* more molecular taxo-

nomic data about mites from other geographic sites is necessary.

Endeostigmata taxa still cause much debate. Historically they are considered to be closely related to Prostigmata [4,5], with which they comprise the taxon Actinedida, more recent studies[7,9]and suggest for most endeostigmatid groups a closer relationship to Oribatida. In this study, we investigated the Endeostigmata taxonomic relationship between the Prostigmata and Oribatida subgroups using small fragments (340-400 bp) of their 18S rDNA sequences. But Endeostigmata was not grouped with Oribatida or Prostigmata (Fig. 2). To understand the evolutionary events of Endeostigmata, more sequences of mites belonging to Endeostigmata must be obtained.

In conclusion, it is first reported phylogenic relationship for classified mites included in Sarcoptiformes using 18S rDNA sequence analysis and its system is a very powerful tool for classification of mites.

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