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Phylogenetic analysis of house dust mites

Research Article

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Abstract: House dust mites live in house dusts and affect the health of humans. Among the many species, *Dermatophagoides farinae, D. pteronyssinus*, and *Euroglyphus maynei* have been found to be commonly associated with Ig-E-mediated allergic diseases. As a result, there is increasing effort to develop methods for the diagnosis and treatment of diseases caused by these species. The purpose of the current study was to explore the evolutionary relationships among house dust mites. After adult *D. farinae* were separated and isolated for total RNA extract, the cDNA coding for Der f 1 and Der f 2 were cloned and sequenced. Then amino acid sequences for group 1 and 2 allergens of two of the most common house dust mites, *D. pteronyssinus, E. maynei*, were obtained from databases. Interestingly, homological analysis of amino acid sequences showed that both Der p 1 and Der p 2 from *D. pteronyssinus* had more similarities to Eur m 1 and Eur m 2, respectively, than they had to Der f 1 and Der f 2 from *D. farinae*. In the phylogenetic trees, *D. pteronyssinus* clustered with *E. maynei*, but not with *D. farinae*, although *D. pteronyssinus* and *D. farinae* belong to the same genus according to morphological taxonomy. It was previously assumed that *D. pteronyssinus* was more similar to *E. maynei* than to *D. farinae* at evolutionary levels.

Keywords: *Dermatophagoides farinae* • *Dermatophagoides pteronyssinus* • *Euroglyphus maynei* • *Taxonomy* • *Phylogenetic analysis*

1. Introduction

Mites are small arthropods approximately half a millimeter in body size, creamy white in color, and members of the class Arachnida. So far, more than 30 species of mites have been found in house dusts and recorded in locations throughout the world [1,2]. However, *D. farinae*, *D. pteronyssinus*, and *E. maynei* are the three most common species, comprising up to 90% of the house dust mite fauna [3,4]. Many publications have suggested that these house dust mites are responsible for many allergic diseases such as asthma, rhinallergosis, atopic dermatitis, and chronic urticaria [5-9]. The group 1 and 2 allergens from these mites have been reported to be major allergens in house dust mite extracts. These allergens belong to the family of cysteine proteases and HE1 homologues [8,9]. Homology, alignment, and phylogenetic tree have been largely restricted to only group 1 or 2. Although phylogenetic studies of house dust mites have been reported on the basis of these two allergens, no study has combined the sequences of both genes, and few have included all three common mite species. In this study, using sequences from both allergens from all three species, we found a higher degree of similarity between *D. pteronyssinus* and *E. maynei* than between *D. pteronyssinus* and *D. farinae*, even though the latter two species are described in the same genus.

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2. Material and Methods

2.1. *D. farinae* culture and isolation of adult mites

To isolate house dust mites, we first obtained house dusts from the floors of rice and flour shops in Haikou City, Hainan Island, and then isolated house dust mites under a stereomicroscope. All of the mites that potentially belonged to *D. farinae* were put into a culture chamber. After approximately 2 months, the entire culture in the chamber was examined by stereomicroscope to select for *D. farinae*. Subsequently, mites regarded to be *D. farinae* were cultivated in small chambers for pure culture. Two months later, mites were taken from the chambers to identify their species. If the mite was confirmed to be *D. farinae*, all of the mites in the chamber were treated as *D. farinae*. These mites were then cultivated on a large scale with relative humidity of 75%, temperature of 25°C, and a culture medium comprised of yeast, wheat flour, and rice. The pure adults were isolated according to our previous protocols [10]. The entire culture was placed on glass plates, and after 30 minutes, the culture media were removed manually. The adult mites, larval mites, and some media were collected in a small ceramic cup with a small writing brush. Under a lamp, the adults moved rapidly to the basal part of the ceramic cup. Under a microscope, they were removed with a writing brush, and about 600 mites were chosen for total RNA isolation.

2.2. Total RNA isolation

About 600 live adult mites were rapidly frozen with liquid nitrogen, and 1 ml of RNA isolator (TaKaRa Biotech Co. Led, Code No.D312) was added. The mites in RNAiso were homogenized with a PowerGen 125 Tissue Homogenizer (Fisher Scientific), starting at 5,000 RPM, with a gradual increase to approximately 20,000 RPM over a period of 30 to 60 seconds at room temperature. This process was continued until a homogeneous solution could be seen. After homogenization, all materials in the Tissue Homogenizer were transferred to an eppendorf tube, and 0.2 ml chloroform was added. The total RNA of adult *D. farinae* was extracted according to the manufacturer's protocol.

2.3. Cloning and DNA sequencing of Der f 1

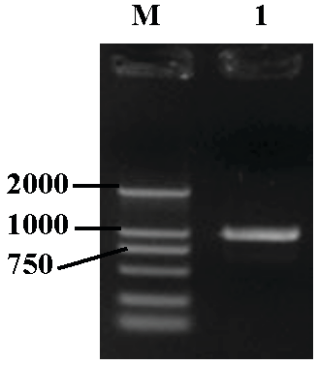
For nested-PCR amplification of the Der f 1 gene, the oligonucleotide primers F (5'GGATCCATGAAATTCGTTTTGGCCATTG3'), R0(5'TCGCAAGAGTAGTTGTTTTTAT3'), and R (5'CTCGAGTCACATGATTACAACATATGGATATT3') were designed and constructed on the basis of the sequence in the Genbank AB034946. For effective cloning, the end of primer F was added to a BamHI site at the 5' end of the coding sequencing (underlined), and the end of primer R was added to a XhoI site at the 3' end of the gene fragment. First, the reverse transcription polymerase chain reaction (RT-PCR) amplification with the total RNA as template was performed with the One Step RNA PCR Kit (TaKaRa Biotech Co. Led, Code No.DRR024A). The final concentrations per reaction (50 μl final volume) for the ingredients were as follows: 20 pmol of primers F and R0 each (1 μl each), total RNA (4 μl), 2×One Step RNA PCR Buffer (25 μl), 2.5mM of the dNTP Mixture (2 μl), 40 u/μl of the RNase Inhibitor (1 μl), 5 U/μl of the M-MLV Reverse Transcriptase XL (0.5 μl), 5 U/ul of the Ex Taq (1 μl), and DEPC H_2O (14.5 μl). RT-PCR was performed in the PCR Thermal Cycler Dice (TaKaRa Biotech Co. Led, Code TP600) with RT of 30 min at 50°C and PCR with an initial incubation of 2 min at 94°C, followed by 30 cycles of 15 s at 98°C, 30 s at 57°C, and 1 min at 72°C. After a final incubation of 5 min at 72°C, amplicons were analyzed by agarose gel electrophoresis (1.0%) with the image device ImageMaster® VDS. To obtain the gene fragment encoding Der f 1, the above amplicons were treated as template, and the primers F and R were used in the second PCR with PrimeSTARTM HS DNA Polymerase kit (TaKaRa Biotech Co. Led, Code No.DR010A). The final concentration for each ingredient per reaction (50 μl final volume) was as follows: 20 pmol of the primers F and R each (0.5 μl each), the above amplicons (1 μl), 2×PrimerSTARTM Buffer (25 μl), 2.5 mM of the dNTP Mixture (2 μl), 2.5 U/μl of the PrimeSTARTM HS DNA Polymerase (0.5 μl), and the DEPC H_2O (18.5 μl). PCR was performed in the same PCR Thermal Cycler Dice with an initial incubation of 5 min at 94°C, followed by 30 cycles of 10 s at 98°C, 10 s at 57°C, and 1 min at 72°C. After a final incubation of 5 min at 72°C, the PCR product was analyzed by agarose gel electrophoresis (1.0%) with ImageMaster® VDS. After PCR amplification and gel electrophoresis, DNA was recovered from the gel with Agarose Gel DNA Purification Kit Ver.2.0 (TaKaRa Biotech Co. Led, Code No. DV805). The purified DNA was added to a poly(A) tail with the DNA A-Tailing Kit (TaKaRa Biotech Co. Led, Code No.D404) and refined with DNA Fragment Purification Kit Ver.2.0 (TaKaRa Biotech Co. Led, Code No.DV807). The cloned gene fragments were then linked to the plasmid pMD19-T simple (TaKaRa Biotech Co. Led, Code No. D104). The recombinant plasmids were transformed into *E. coli* JM109 (TaKaRa Biotech Co. Led, Code No.D9052) and identified by the blue/white screening on Luria-Bertani (LB) plates containing 100 μg/ml ampicillin. Plasmids were purified by the MiniBEST Plasmid Purification Kit

Ver.2.0 according to the manufacturer's specifications (TaKaRa Biotech Co. Led, Code No.DV801A). The presence of insert DNA was confirmed by restriction analysis, DNA sequencing with the BcaBEST M13-47 forward primer, and the BcaBEST RV-M reverse primer on a MegaBACE 1, 000 DNA sequencer. After digestion with BamHI and XhoI, the plasmid was analyzed by agarose gel electrophoresis (1.0%) with ImageMaster® VDS.

2.4. Cloning and DNA sequencing of Der f 2

For PCR amplification of cDNA coding for Der f 2, two primers were designed and synthesized in based on the sequence in the Genbank (AB195580), one primer was F (5'GGATCCATGATTTCC AAAATCTTGTGCCTTTC3') and the other was R (5'CTCGAG TTAATCACGGATTTTACCAT GGG 3'). These two primers contained a BamHI and an XhoI site at their 5' end (underlined) respectively. Firstly, reverse transcription with the total RNA as template was performed with 3'-Full RACE Core Set Ver.2.0 (TaKaRa Biotech Co. Led, Code No.D314) in the PCR Thermal Cycler Dice (TaKaRa Biotech Co. Led, Code TP600) for 30 min. The final concentration of each component per reaction (10 μl final volume) was as follows: Total RNA (3 μl), 10×RNA PCR Buffer (1 μl), 10 mM of the dNTP Mixture (1 μl), 25mM of $MgCl_2$ (2 μl), 5 U/μl of the M-MLV Reverse Transcriptase XL (0.25 μl), 40 U μl of the RNase Inhibitor (0.25 μl), 20 pmol of primer R (0.5 μl), Random primer (1 μl), and DEPC H_2O (1 μl). Secondly, the RT products were used as a template for PCR in the same thermal Cycler Dice with an initial incubation of 3 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C. The reaction system included RT products (2 μl), 2×GC Buffer (5 mM Mg^{2+} Plus, 25 μl), 5 U/μl of the LA Taq (0.5 μl), 1×cDNA dilution Buffer (8 μl), 20 pmol of the primers F and R each (1 μl each), and dH_2O (12.5 μl). PCR amplification products were cloned by ligation into plasmid pMD19-T simple (TaKaRa Biotech Co. Led, Code No. D104). The recombinant plasmids were transformed into *E. coli* Competent Cells JM109 (TaKaRa Biotech Co. Led, Code No.D9052) with the blue/white selection on Luria-Bertani (LB) plates containing 100 μg/ml ampicillin. The presence of insert DNA was confirmed by restriction analysis and DNA sequencing with the BcaBEST M13-47 forward primer and the BcaBEST RV-M reverse primer on a MegaBACE 1000 DNA sequencer. After digestion by restriction enzymes BamHI and XhoI, DNA was electrophoretically analyzed on agarose gel by standard techniques.

Figure 1. Analysis of the nested-PCR products for Der f 1 by agarose electrophoresis.



As described in "Materials and Methods", after the total RNA was isolated from mites with RNA isolator, Der f 1 was amplifiedby RT-PCR. The PCR product was analyzed on 1% agarose gel containing ethidium bromide. Lane M, DNA Marker DL2, 000; lane 1, the RT-PCR products.

2.5. Phylogenetic tree construction

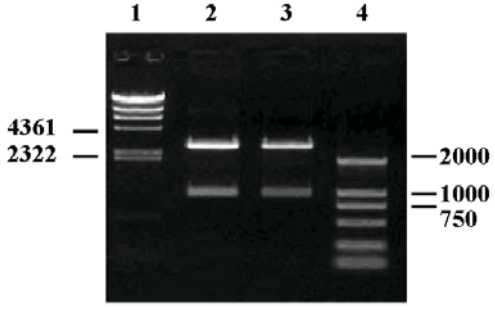
The amino acid sequences for both Der f 1 and Der f 2 were deduced by Translate Tools from their cDNA sequences. Their homologous partners based on the deduced amino acid sequences were determined by comparing the sequences with those in all non-redundant GenBank CDS translations+PDB+ SwissProt+PIR+PRF excluding environmental samples using BLASTp in NCBI (National Center for Biotechnology Information). Then, the sequences for the most common house dust mites, i.e. *D. pteronyssinus*, *E. maynei*, were chosen and exported with format Fasta for similarity analysis with the translated amino acid sequences with VECTOR NTI 9.0 software. The phylogenetic trees were constructed with Mega 3.0. In the case of several sequences existing for the same species of mites, we chose the one with the highest similarity. *Psoroptes ovis* was used to root the trees.

3. Results

3.1. Cloning and sequence of Der f 1 cDNA

From adult mites, the total RNA was obtained with the RNAiso reagents. On the basis of the nucleotide sequence in the GenBank (AB034946), primers were constructed, and the cDNA fragments were amplified by nested-PCR. By agarose gel electrophoresis, one product about 966 bp was observed (Figure 1). The recovered PCR product was linked to plasmid pMD19-T simple and was transformed into *E. coli* Competent Cells JM109. Transformants were identified by blue/white screening, and the recombinant plasmids were extracted and analyzed by restriction digests with enzymes BamHI and XhoI (Figure 2). Two plasmids

Figure 2. Restriction enzyme analysis of the recombinant plasmids pMD19-T-Der f 1.



As described in "Materials and Methods", after the total RNA was isolated from mites using RNA isolator and Der f 1 was amplified by RT-PCR, the Der f 1 cDNA was cloned into pMD19-T vector to generate pMD19-T-Der f 1. After blue/white screening, the positive clones were determined by restriction enzyme digestion with BamHI and XhoI, followed by separation on 1% agarose gel containing ethidium bromide. Lane M1, λ-Hind λ DNA Marker; lane M2, DNA Marker DL2,000; lane 1 and 2, recombinant plasmids were digested with BamHI and XhoI.

containing the targeted cDNA fragment were screened and sequenced with primers BcaBEST Primer M13-47 and BcaBEST Primer RV-M. The plasmids and the restriction sites were removed from the sequencing results before translation and analyses. We found one bp mutation at position 23 bp, from C to T. This result suggests a high level of similarity to the previously published sequence of Der f 1 (BLAST level of similarity, 99.99%). According to the ORF (Open Reading Frame) Finder in the NCBI Web server, a complete ORF was found from the start codon ATG to stop codon TGA with a full length of 966 bps. The translated amino acid sequence is shown in Figure 3.

3.2. Cloning and sequence of Der f 2 cDNA from China

On the basis of the nucleotide sequence in the Genbank (AB195580), primers were constructed, and the cDNA fragments were amplified by PCR. The recovered PCR product was linked to the plasmid vector pMD19-T simple, and the recombinant plasmid was transformed into *E. coli* Competent Cells JM109. Positive colonies

Figure 3. The translated amino acid sequence of Hainan Der f 1 allergen.

```
atgaaattcgttttggccattgtctctttgttggtattgagcactgtttatgctcgtcca
 M  K  F  V  L  A  I  V  S  L  L  V  L  S  T  V  Y  A  R  P
gcttcaatcaaaacttttgaagaattcaaaaaagccttcaacaaaaactatgccaccgtt
 A  S  I  K  T  F  E  E  F  K  K  A  F  N  K  N  Y  A  T  V
gaagaggaagaagttgcccgtaaaaacttttggaatcattgaaatatgttgaagctaac
 E  E  E  E  V  A  R  K  N  F  L  E  S  L  K  Y  V  E  A  N
aaaggtgccatcaaccatttgtccgatttgtcattggatgaattcaaaaaccgttatttg
 K  G  A  I  N  H  L  S  D  L  S  L  D  E  F  K  N  R  Y  L
atgagtgctgaagcttttgaacaactcaaaactcaattcgatttgaatgccgaaacaagc
 M  S  A  E  A  F  E  Q  L  K  T  Q  F  D  L  N  A  E  T  S
gcttgccgtatcaattcggttaacgttccatcggaattggatttacgatcactgcgaact
 A  C  R  I  N  S  V  N  V  P  S  E  L  D  L  R  S  L  R  T
gtcactccaatccgtatgcaaggaggctgtggttcatgttgggctttctctggtgtcgcc
 V  T  P  I  R  M  Q  G  G  C  G  S  C  W  A  F  S  G  V  A
gcaactgaatcagcttatttggcctaccgtaacacgtctttggatctttctgaacaggaa
 A  T  E  S  A  Y  L  A  Y  R  N  T  S  L  D  L  S  E  Q  E
ctcgtcgattgcgcatctcaacacggatgtcacggcgatacaataccaagaggcatcgaa
 L  V  D  C  A  S  Q  H  G  C  H  G  D  T  I  P  R  G  I  E
tacatccaacaaaatggtgtcgttgaagaaagaagctatccatacgttgcacgagaacaa
 Y  I  Q  Q  N  G  V  V  E  E  R  S  Y  P  Y  V  A  R  E  Q
caatgccgacgaccaaattcgcaacattacggtatctcaaactactgccaaatttatcca
 Q  C  R  R  P  N  S  Q  H  Y  G  I  S  N  Y  C  Q  I  Y  P
ccagatgtgaaacaaatccgtgaagctttgactcaaacacacacagctattgccgtcatt
 P  D  V  K  Q  I  R  E  A  L  T  Q  T  H  T  A  I  A  V  I
attggcattaaagatttgagagcttttcaacattatgatggacgaacaatcattcaacat
 I  G  I  K  D  L  R  A  F  Q  H  Y  D  G  R  T  I  I  Q  H
gacaatggttatcaaccaaactatcatgccgtcaacattgtcggttacggaagtacacaa
 D  N  G  Y  Q  P  N  Y  H  A  V  N  I  V  G  Y  G  S  T  Q
ggcgtcgattattggatcgtacgaaacagttgggatactacctggggtgatagcggatac
 G  V  D  Y  W  I  V  R  N  S  W  D  T  T  W  G  D  S  G  Y
ggatatttccaagccggaaacaacctcatgatgatcgaacaatatccatatgttgtaatc
 G  Y  F  Q  A  G  N  N  L  M  M  I  E  Q  Y  P  Y  V  V  I
atgtga
 M  -
```

On the basis of the cDNA sequence of Hainan group 1 allergen, its amino acid sequence was deduced using Translate Tools (http://www.expasy.org).

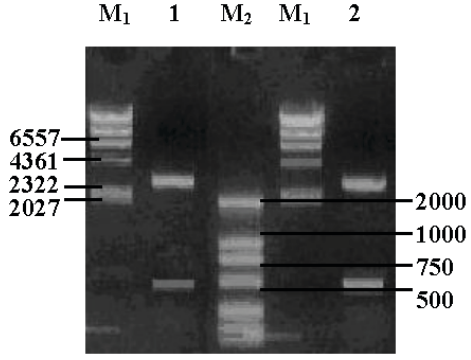
that were identified by blue/white screening and contained the recombinant plasmids were subjected to plasmid extraction and analyzed by restriction digests with BamHI and XhoI (Figure 4). Two plasmids containing the targeted cDNA fragment were screened and sequenced with two primers of BcaBEST Primer M13-47 and BcaBEST Primer RV-M. According to the ORF Finder in the NCBI Web server, a complete ORF was found from the start codon ATG to stop codon TGA with a full length of 528 bps. Figure 5 shows the translated amino acid sequence.

Table 1. Amino acid sequences of group 1 and 2 allergens for the most common house dust mites.

Allergens	Accession code	Identity	E value (10,000)	References
EUR m1	P25780	86.0%	2.5e-161	Smith,W., et al.(1999)
Der p 1	P08176	82.9%	4.2e-155	Chua,K.Y., et al (1993)
Pso o 1	CAK32515	65.2%	3e-122	Nisbet,A.J.(2007)
EUR m2	AAC82350	82.6%	6.8e-57	Smith,W., et al (1999)
Der p 2	ABA39437	87.6%	1.2e-60	Piboonpocanun,S.et al.(2006)
Pso o 2	Q965E2	40.7%	1.4e-26	Temeyer,K.B., et al.(2002)

By BLASTn searches on NCBI, sequences coding for group 1 and group 2 allergens for different species of house dust mite were chosen. The identity and E value between our Der f 1, Der f 2 sequences and each of these sequences were computed by LALIGN. http://www.ch.embnet.org/software/LALIGN_form.html)

Figure 4. Restriction enzyme analysis of the recombinant plasmids pMD19-T-Der f 2.



As described in "Materials and Methods", after the total RNA was isolated from mites using RNA isolator and Der f 2 was amplified by RT-PCR, the Der f 2 cDNA was cloned into pMD19-T vector to generate pMD19-T-Der f 2. After blue/white screening, the positive clones were determined by restriction enzyme digestion with BamHI and XhoI, followed by separation on 1% agarose gel containing ethidium bromide. Lane M1, λ-Hind λ DNA Marker; lane M2, DNA Marker DL2,000; lane 1 and 2, recombinant plasmids were digested with BamHI and XhoI

Figure 5. The translated amino acid sequence of Hainan Der f 2 allergen.

```
atgatttccaaaatcttgtgcctttcattgttggtagcagccgttgttgccgatcaagtc
 M  I  S  K  I  L  C  L  S  L  L  V  A  A  V  V  A  D  Q  V
gatgttaaagattgtggtaagtttgtttgtgtgatacatttttttttctttttcatttattc
 D  V  K  D  C  G  K  F  V  C  V  I  H  F  F  S  F  H  L  F
aacactaaacacaatttttcttttttcttgtttatattcatatagccaacaatgaaatcaaa
 N  T  K  H  N  F  L  F  L  V  Y  I  H  I  A  N  N  E  I  K
aaagtaatggtcgatggttgccatggttctgatccatgcatcatccatcgtggtaaacca
 K  V  M  V  D  G  C  H  G  S  D  P  C  I  I  H  R  G  K  P
ttcacttttggaagccttattcgatgccaaccaaaacactaaaaccgctaaaattgaaatc
 F  T  L  E  A  L  F  D  A  N  Q  N  T  K  T  A  K  I  E  I
aaagccagcctcgatggtcttgaaattgatgttcccggtatcgataccaatgcttgccat
 K  A  S  L  D  G  L  E  I  D  V  P  G  I  D  T  N  A  C  H
tttatgaaatgtccattggttaaaggtcaacaatatgatatcaaatatacatggaatgtg
 F  M  K  C  P  L  V  K  G  Q  Q  Y  D  I  K  Y  T  W  N  V
ccgaaaattgcaccaaaatctgaaaacgttgtcgttacagtcaaacttatcggtgataat
 P  K  I  A  P  K  S  E  N  V  V  V  T  V  K  L  I  G  D  N
ggtgttttggcttgcgctattgctacccatggtaaaatccgtgattaa
 G  V  L  A  C  A  I  A  T  H  G  K  I  R  D  -
```

On the basis of the cDNA sequence of Hainan group 2 allergen, its amino acid sequence was deduced with use of Translate Tools (http://www.expasy.org).

Table 2A. Amino acid sequence similarity for group 1 allergens of the most common house dust mites.

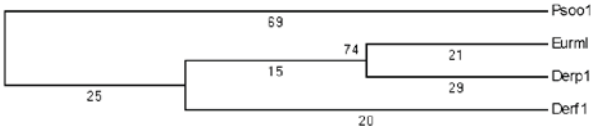
	Der f 1	Der p 1	Eur m l	Pso o 1
Derf1		83	84	65
Derp1			86	63
Eurml				64
Psoo1				

by VCETOR NTI 9.0 software

Table 2B. Amino acid sequence similarity for group 2 allergens of the most common house dust mites.

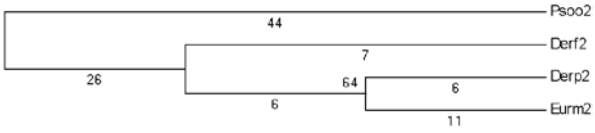
	Der f 2	Der p 2	Eur m 2	Pso o 2
Der f 2		68	67	31
Der p 2			87	44
EUR m2				46
Pso o 2				

Figure 6. A phylogenetic tree for group 1 allergens of different house dust mite species.



After alignment of the indicated group 1 allergens, a phylogenetic tree was constructed as described in "Materials and Methods".

Figure 7. A phylogenetic tree for group 2 allergens of different house dust mite-species.



After alignment of the indicated group 2 allergens, a phylogenetic tree was constructed as described in "Materials and Methods".

3.3. Similarity and phylogenetic analysis of house dust mite

The most similar sequences for the group 1 and 2 allergens of the most common mite species were selected for analyses by Blastp in NCBI. The similarity indexes are shown in Table 1. The results showed that Der p 1 shared 84% identical residues with EUR m1 and 83% with Der f 1. Similarly, Der p 2 shared 87% identical residues with EUR m2 and 68% with Der f 1 (Table 2). On the basis of the results from both gene fragments, it appeared that *D. pteronyssinus* is more similar to *E. maynei* than to *D. farinae* in both its group 1 and 2 allergens. Specifically, Der p 1 and Der p 2 clustered in the phylogenetic trees with Eur m 1 and Eur m 2, respectively, but not with Der f 1 and Der f 2, although the *D. pteronyssinus* and *D. farinae* belong to the same genus (Figure 6 and 7).

4. Discussion

Mites belong to the class Arachnida and are characterized by four pairs of legs. They are divided into 11 subclasses based on the number of body regions and abdominal segmentation patterns. The term "house dust mite" generally refers to those species within the family Pyroglyphidae indexed in the mite suborder Astigmata. Pyroglyphidae is divided into two subfamilies: the Pyroglyphinae that includes the genera *Pyroglyphus* and *Euroglyphus* and Dermatophagoidinae containing the genus *Dermatophagoides*. This classification is based on morphologic differences. Accordingly, mites with short scapular setae as well as with an acute anterior margin of the prodorsum belong to the subfamily Pyroglyphinae. In contrast, mites with long external scapular setae and a rounded, intact anterior margin of the prodorsum

belong to the subfamily Dermatophagoidinae [11]. The robustness of this classification system has not been tested with molecular sequences.

Voorhorst et al (1964) and Oshima (1964) first suggested that mites contribute to the house dust allergy problem [12,13]. Since then, significant research efforts have focused on the IgE-binding components for house dust mites. By immunological analysis of mite extracts, more than 30 IgE-binding components have been demonstrated in allergic patients, and group 1 and 2 allergens were found responsible for high titres of IgE and Th2 cytokines in 80% of allergic patients [9]. Immunological and molecular evidence has shown that the group 1 allergens might have cysteine peptidase active sites and group 2 allergens a MD-2-related lipid recognition domain. These proteins have shown significant sequence conservation among the different mite species [8]. In this study, we cloned and sequenced the group 1 and 2 allergens from adult *D. farinae*. We unexpectedly found that sequence analysis showed that *D. pteronyssinus* and *E. maynei* were evolutionarily more similar to each other than to *D. farinae*. First, the amino acid sequence of Der p 1 had an 84% similarity to Eur m 1, while it showed only 83% sequence similarity to Der f 1. As well, the amino acid sequence of Der p 2 had an 87% similarityto Eur m 2 and only 68% similarity to Der f 2. Second, *D. pteronyssinus* was clustered with *E. maynei*, but not *D. farinae*, with a bootstrap support of 74% in the phylogenetic tree for group 1 allergens and 64% in the tree for group 2 allergens. This finding suggested that the taxonomy for mites and tick on the basis of morphology does not correctly reflect their true evolutionary history.

In brief, the phylogenetic analysis in this study showed that *D. pteronyssinus* was evolutionarily more similar to *E. maynei* than to *D. farinae*, even though *D. pteronyssinus* and *D. farinae* belong to the same genus as identified by morphological characteristics.

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