Phylogenetic Placement of the Spider Genus Nephila (Araneae: Araneoidea) Inferred from rRNA and MaSp1 Gene Sequences

Authors: Hong-Chun, Pan, Kai-Ya, Zhou, Da-Xiang, Song, and Yang, Qiu

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Phylogenetic Placement of the Spider Genus *Nephila* (Araneae: Araneoidea) Inferred from rRNA and *MaSp*1 Gene Sequences

Pan Hong-Chun, Zhou Kai-Ya*, Song Da-Xiang and Qiu Yang

Institute of Genetic Resources, College of Life Sciences, Nanjing Normal University, 122 Ninghai Road, Nanjing 210097, China

ABSTRACT—The family status of the genus *Nephila*, which belongs to Tetragnathidae currently but Araneidae formerly, was reexamined based on molecular phylogenetic analyses. In the present study, 12S and 18S rRNA gene fragments of eight species of spiders were amplified and sequenced. In addition, 3'-end partial cDNA of major ampullate spidroin-1 (*MaSp*1) gene of *Argiope amoena* was cloned and sequenced, and the 3'-end non-repetitive region's cDNA sequence of *MaSp*1 gene and the predicted amino acid sequence of C-terminal non-repetitive region of *MaSp*1 were aligned with some previously known sequences. The resulting phylogeny showed that Araneidae and Tetragnathidae are not a sister group in the superfamily Araneoidea, and the genus *Nephila* is closer to the genera of the family Araneidae rather than to those of Tetragnathidae. We suggest that the genus *Nephila* should be transferred back to Araneidae. Or the subfamily Nephilinae might be elevated to family level after it was redefined and redelimited. Furthermore, the study showed that 3'-end non-repetitive region's cDNA sequence of *MaSp*1 gene and C-terminal non-repetitive region's amino acid sequence of *MaSp*1 are useful molecular markers for phylogenetic analysis of spiders.

Key words: genus *Nephila*, superfamily Araneoidea, rRNA gene, major ampullate spidroin-1(*MaSp*1), molecular phylogeny

INTRODUCTION

The phylogenetic placement of the genus *Nephila* Leach has been changed several times together with the subfamily Nephilinae since it was established in 1815. Generally, the following placement of the genus has been appeared in different classifications. The Nephilinae was placed in the family Argiopidae along with the subfamilies Argiopinae, Araneinae, Tetragnathinae etc. (Simon, 1894; Pocock, 1900; Petrunkevitch, 1928), or in the family Araneidae along with the subfamilies Argiopinae, Araneinae etc., excluding Tetragnathinae (Roewer, 1942; Brignoli, l983). In the later case Tetragnathinae was elevated to family level as Tetragnathidae. In 1980, Levi placed the genus *Nephila* in the subfamily Metinae and placed Metinae, Araneinae and Tetragnathinae in the family Araneidae. Meanwhile, in the paper he also mentioned the genus might belong to a separate subgroup or subfamily (Levi, 1980). Later, Levi & Eickstedt (1989) pointed out that the genus *Nephila* have certain apomorphic tetragnathid characters in their palpal structures, and suggested that the placement of *Nephila* under Araneidae was incorrect. Subsequently, their suggestions were also supported by some morphological and behavioral studies (Coddington, 1990; Hormiga *et al*., 1995; Griswold *et al*., 1998). In most current papers *Nephila* has been treated as a genus of Nephilinae in the Tetragnathidae along with Tetragnathinae, and/or Metinae, Leucauginae etc. (Coddington and Levi, 1991; Platnick, 1989, 1997, 2003; Dippenaar-Schoeman and Jocqué, 1997).

To date, whether nephilines should be placed in Tetragnathidae or Araneidae is still a problem remains to be unsolved. The purpose of the present study was to test the two different placements of the genus *Nephila* by means of molecular phylogenetic analysis. In addition, the use of the 3'-end non-repetitive region's DNA sequence of *MaSp*1 gene and the C-terminal non-repetitive region's amino acid sequences of *MaSp*1 as molecular markers for phylogenetic analysis of spiders is also discussed.

* Corresponding author: Tel. +86-25-83598388;
FAX. +86-25-83598215.
E-mail: kyzhounj@jlonline.com

MATERIALS AND METHODS

Samples

A total of nine species of spiders representing six families according to current classification were collected from China and India (Table 1). Samples were preserved in 99.9% ethanol.

DNA extraction, amplification and sequencing of 12S and 18S rRNA gene fragments

Total genomic DNA was extracted from leg tissue following standard proteinase digestion and phenol-chloroform extraction procedures as described by Sambrook *et al*. (1989). A fragment of the mitochondral 12S rRNA gene was amplified by using primers 12St-L: 5'-GGTGGCATTTTATTTTATTAGAGG-3' (Croom *et al*., 1991) and 12Sbi-H: 5'-AAGAGCGACGGGCGATGTGT-3' (Simon *et al*., 1994), and a fragment of the nuclear 18S rRNA gene was also amplified by using primers 18S-ai: 5'-CCTGAGAAACGGC-TACCACATC-3' and 18S-b0.5: 5'-GTTTCAGCTTTGCAACCAT-3' (Tautz *et al*., 1988). The polymerase chain reaction (PCR) was performed on PE 2400 (Perkin Elmer) thermocycler system in 30 μl volumes containing 1–3 μl of genomic DNA, 10 pM of each primer, each of four deoxynucleoside triphosphates at 250 μM, 1.5 mM $MgCl_2$, and 2 units of *Taq* Polymerase. PCR was carried out under the following conditions: hot-start at 95°C for 5 min; repeated 30 cycles; denaturation at 95°C for 40 s, primer annealing at 55°C for 50 s and primer extension at 72°C for 2 min; final extension at 72°C for 10 min. PCR products were separated on 2% agarose gel, and purified with DNA gel extraction kit (Vitagene). The purified DNA was sequenced using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM 310 Genetic Analyzer. An additional sequence of 12S rDNA was retrieved from GenBank (Table 1).

Cloning and sequencing of the 3'-end cDNA fragment of *MaSp*1 gene

Silk glands were dissected from *Argiope amoena* abdomens. The entire gland was removed and frozen immediately in liquid nitrogen. The gland was then crushed in a mortar and pestle under liquid nitrogen. All solutions, instruments and glassware were treated to inhibit RNAase activity. Total RNA was isolated by using Trizol®Reagent (Gibco), mRNA was isolated from total RNA with Oligotex™ mRNA Purification Kit (Qiagen). Single-strand cDNA was synthesized directly from mRNA using SMART™ cDNA Library Construction Kit (Clontech). Double-strand cDNA was synthesized by long-distance PCR using two adaptor primers offered by SMART™ cDNA Library Construction Kit (3' PCR Primer: 5'-TTCTAGAGGCCGAGGCGGCC-3'; 5' PCR Primer: 5'-AGCAGTG-GTATCAACGCAGAGT-3).

Based on repetitive region's amino acid sequence (GGQG-

Table 1. Specimens used in this study[1)]

Species	Locality	12S rRNA gene	18S rRNA gene	*MaSp*1 gene
Araneidae				
Araneus diadematus				U47854[2)]
Argiope amoena	Anhui, China	AY164669	AY425717	AY263390
Argiope trifasciata				AF350266[2)]
Gasteracantha kuhli	Tami Nadu, India	AY164670	AY425718	
Tetragnathidae				
Nephila clavata	Anhui, China	AY164671	AY425721	
Nephila pilipes[3)]		AF145035[2)]		
Nephila clavipes				U20329[2)]
Nephila senegalensis				AF350279[2)]
Nephila madagascariensis				AF350277[2)]
Leucauge magnifica	Guangxi, China	AY164672		
Tetragnatha maxillosa	Anhui, China	AY164673	AY425723	
Tetragnatha versicolor				AF350286[2)]
Tetragnatha kauaiensis				AF350285[2)]
Linyphiidae				
Hylyphantes graminicola	Jiangsu, China	AY425715	AY425720	
Theridiidae				
Achaearanea tepidariorum	Jiangsu, China	AY425713	AY425716	
Pisauridae				
Dolomedes tenebrosus				AF350270[2)]
Theraphosidae				
Ornithoctonus huwena	Guangxi, China	AY164666	AY425722	
Liphistiidae				
Heptathela hangzhouensis	Hubei, China		AY425719	

[1)] Placement of the main genera follows Song *et al*. (2001)
[2)] These data were retrieved from GenBank.
[3)] *Nephila pilipes*, senior synonym of *N. maculata*

GYGGL) of *MaSp*1 in spiders (Xu and Lewis, 1990), one primer (P_{MaSp1}: 5'-GGAGGACAAGGTGGATATGGCGGATTAGG-3') was designed and synthesized. Double-strand cDNA of silk gland in *Argiope amoena* was used as a template for 3' RACE. The 3' end cDNA fragment of *MaSp*1 gene was amplified by the primer pair 3' PCR Primer and P_{MaSp1}. A total of 30 μl PCR reaction mixture was composed of 3 μl of 1:100 dilution of double-strand cDNA of silk gland, 10 pM of each primer, each of four deoxynucleoside triphosphates at 250 μM, 1.5 mM $MgCl_2$, and 2 units of *Taq* Polymerase. Amplification was carried out on PE2400 (Perkin Elmer) thermocycler system under the following conditions: hot-start at 95°C for 5 min; repeated 30 cycles: denaturation at 95°C for 40 s, primer annealing at 50°C for 40 s and primer extension at 72°C for 2 min; final extension at 72°C for 10 min.

PCR products were separated on 2% agarose gel, purified with DNA gel extraction kit (Vitagene), and ligated into PMD18-T vector using T/A Cloning Kit (TaKaRa). The cloned DNA was transformed and replicated in *Escherichia coli* (JM109), plasmid DNA was purified using Plasmid Mini Kit (Watson). After identified by PCR using M13 and M13 Reserve as primers, positive clones were sequenced on LI-COR DNA sequencer (LI-COR) also using M13 and M13 Reserve as primers. A further eight sequences were retrieved from GenBank (Table 1).

Data analysis

The predicted C-terminal non-repetitive region's amino acid sequences of *MaSp*1 were obtained from the 3'-end cDNA fragment of *MaSp*1 gene by using DNAclub. The DNA sequences of 12S and 18S rRNA gene fragments, *MaSp*1 gene fragments and predicted C-terminal non-repetitive region's amino acid sequences of *MaSp*1 were aligned respectively with CLUSTAL × 1.8 (Thompson *et al*., 1997). The neighbor-joining (NJ) method was applied to infer relationships among taxa on the basis of a pairwise matrix of the distance from Kimura's two-parameter model, using MEGA 2.1 (Kumar *et al*., 2000). The maximum-parsimony (MP) analyses were conducted using heuristic search option of PAUP 4.0b (Swofford, 2000). And the Maximum-likelihood (ML) analyses were performed by use of DnaSP 3.5 and PHYLIP vers. 3.5c computer packages (Felsenstein, 1993).

RESULTS

12S rDNA data set

The aligned sequences of 12S rDNA fragments for nine species of spiders consisted of a total of 269 sites, including 191 variable and 102 parsimony informative sites. The fragments have a high average A/T content of about 75.5%; this result is consistent with the statement that arthropod mitochondrial genomes in general tend to be highly A+T biased (Crozier and Croizer, 1993). The majority of transition values were lower than transversion ones. Sequence differences (transition + transversion) among these spiders ranged from 0.6% to 33.9%, with an average of 24.7%. It is noteworthy that average sequence difference (23.0%) between the genus *Nephila* and other tetragnathid genera was somewhat higher than that between *Nephila* and the araneid genera (21.4%) (Table 2).

NJ, MP and ML trees of the 12S rDNA fragments with *Ornithoctonus huwena* as outgroup indicate that the Araneidae, *Nephila*, Linyphiidae and Tetragnathidae clustered together, while the theridiid genus (*Achaearanea*) formed another single clade (Fig. 1). In NJ and ML trees, two species of *Nephila* clustered with the araneid clade, the linyphiid genus (*Hylyphantes*) and two tetragnathid genera (*Tetragnatha* and *Leucauge*) formed another monophyletic clade; but in the MP tree, the linyphiid genus clustered with the group which consists of the araneid genera (*Gasteracantha*, *Argiope*) and *Nephila*.

18S rDNA data set

The aligned sequences of 18S rDNA fragments for eight species of spiders consisted of 738 total sites, including 190 variable and 58 parsimony informative sites. In contrast to the 12S rDNA fragments, the 18S fragments have a lower average A/T content about 49.6%, and the majority of transition values were higher than transversion ones (Table 3).

NJ, MP and ML trees of the 18S rDNA fragments with *Heptathela hangzhouensis* and *Ornithoctonus huwena* as outgroups generated very similar tree topologies one another (Fig. 2). The theridiid genus (*Achaearanea*) clustered with the group which consists of *Nephila* and the araneid genera (*Gasteracantha*, *Argiope*), while the tetragnathid genus (*Tetragnatha*) and the linyphiid genus (*Hylyphantes*) formed another monophyletic clade.

*MaSp*1 gene data set

The sequences of the cDNA fragment (GenBank Accession no. AY263390) of *MaSp*1 gene can be divided into two regions: (1) a repetitive region that codes for an alternating alanine-rich and glycine-rich domain and (2) a non-repetitive coding region. The predicted C-terminal non-repetitive region of *MaSp*1 consisted of predicted 102 amino acids, its sequence was rather homologous to published amino acid sequences of other eight species of spiders, for example, the amino acid sequence of *Argiope amoena* had a homology of 72.4% to that of *Nephila clavipes* (Fig. 3).

The aligned 3'-end non-repetitive region's cDNA sequences of *MaSp*1 gene for nine species of spiders consisted of 310 total sites, including 215 variable and 149 parsimony informative sites. The *MaSp*1 gene fragments have a lower average A/T content about 54.6%, and the majority of transition values were higher than transversion ones. The average sequence difference (18.1%) between the genus *Nephila* and other tetragnathid genera was markedly higher than that between *Nephila* and the araneid genera (13.2%) (Table 4).

NJ and MP trees based on the 3'-end non-repetitive region's cDNA sequences of *MaSp*1 gene or the predicted amino acid sequences of C-terminal non-repetitive region of *MaSp*1 with *Dolomedes tenebrosus* as an outgroup are also in agreement with the traditional view that genus *Nephila* should be placed in the family Araneidae rather than Tetragnathidae (Fig. 4). Three species of the genus *Nephila* is sister to other two araneid genera *Araneus* and *Argiope*. And then the clade is sister to genus *Tetragnatha* of the family Tetragnathidae.

Table 2. Percent difference of base substitution (upper triangle) and values of transitions/transversions (lower triangle) for 12S rRNA gene fragment among 9 species of spiders

	1	2	3	4	5	6	7	8	9
1. *Leucauge magnifica*		16.9	19.2	23.6	24.0	23.3	23.3	27.5	29.5
2. *Tetragnatha maxillosa*	0.43		20.8	24.3	25.2	22.7	22.4	28.8	31.6
3. *Hylyphantes graminicola*	0.40	0.27		21.1	23.6	20.1	19.8	27.5	29.7
4. *Gasteracantha kuhli*	0.45	0.52	0.61		17.6	20.9	21.2	28.4	31.0
5. *Argiope amoena*	0.63	0.76	0.68	0.77		22.0	21.7	32.6	33.9
6. *Nephila pilipes*	0.28	0.29	0.29	0.51	0.64		0.6	28.8	31.9
7. *Nephila clavata*	0.26	0.30	0.29	0.50	0.66	1.00		28.4	31.9
8. *Achaearanea tepidariorum*	0.37	0.29	0.3	0.39	0.57	0.25	0.25		32.9
9. *Ornithoctonus huwena*	0.53	0.46	0.55	0.64	0.61	0.67	0.64	0.47	

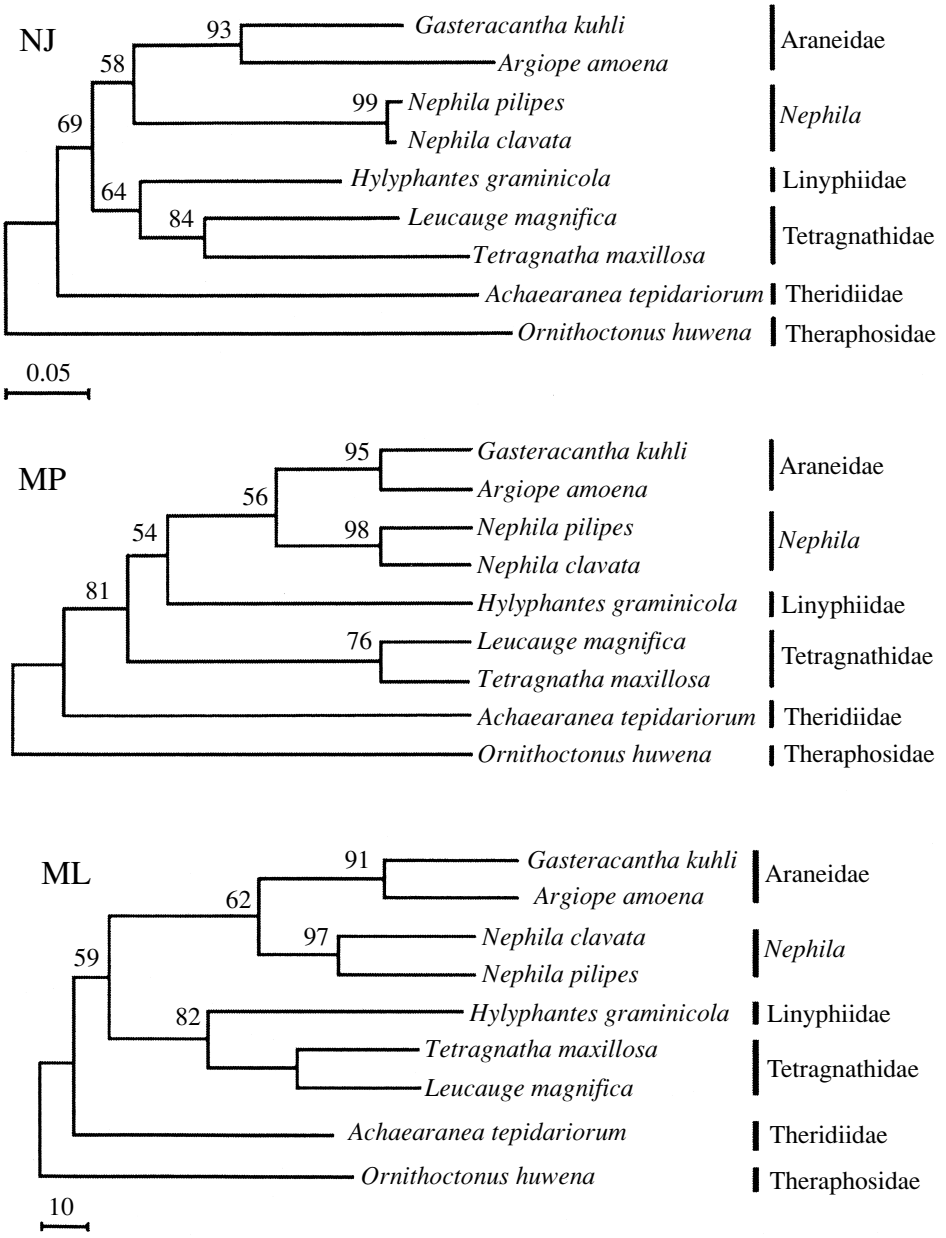

Fig.1. 1Neighbor-joining (NJ), maximum parsimony (MP) and maximum-likelihood (ML) trees based on 12S rDNA sequence data of nine species of spiders. Bar equals 0.05 (NJ) and 10 (ML) units of Kimura's two-parameter distance. Numbers above branches in these trees are Bootstrap values at least 50% of the 1,000 bootstrap replications.

Table 3. Percent difference of base substitution (upper triangle) and values of transitions/transversions (lower triangle) for 18S rRNA gene fragment among 8 species of spiders

	1	2	3	4	5	6	7	8
1. *Heptathela hangzhouensis*		1.5	4.6	5.0	6.5	6.2	7.2	17.0
2. *Ornithoctonus huwena*	1.75		5.0	5.8	6.9	7.2	7.3	17.0
3. *Hylyphantes graminicola*	1.62	1.47		3.8	7.5	5.7	6.8	16.3
4. *Tetragnatha maxillosa*	1.18	1.26	1.8		8.0	6.4	7.2	16.1
5. *Gasteracantha kuhli*	1.09	1.04	1.39	1.27		4.7	7.5	18.4
6. *Argiope amoena*	1.00	1.04	1.10	0.96	1.33		7.3	16.7
7. *Achaearanea tepidariorum*	0.61	0.54	0.85	0.71	0.96	0.74		17.5
8. *Nephila clavata*	1.08	1.05	1.03	1.05	1.14	0.95	0.98	

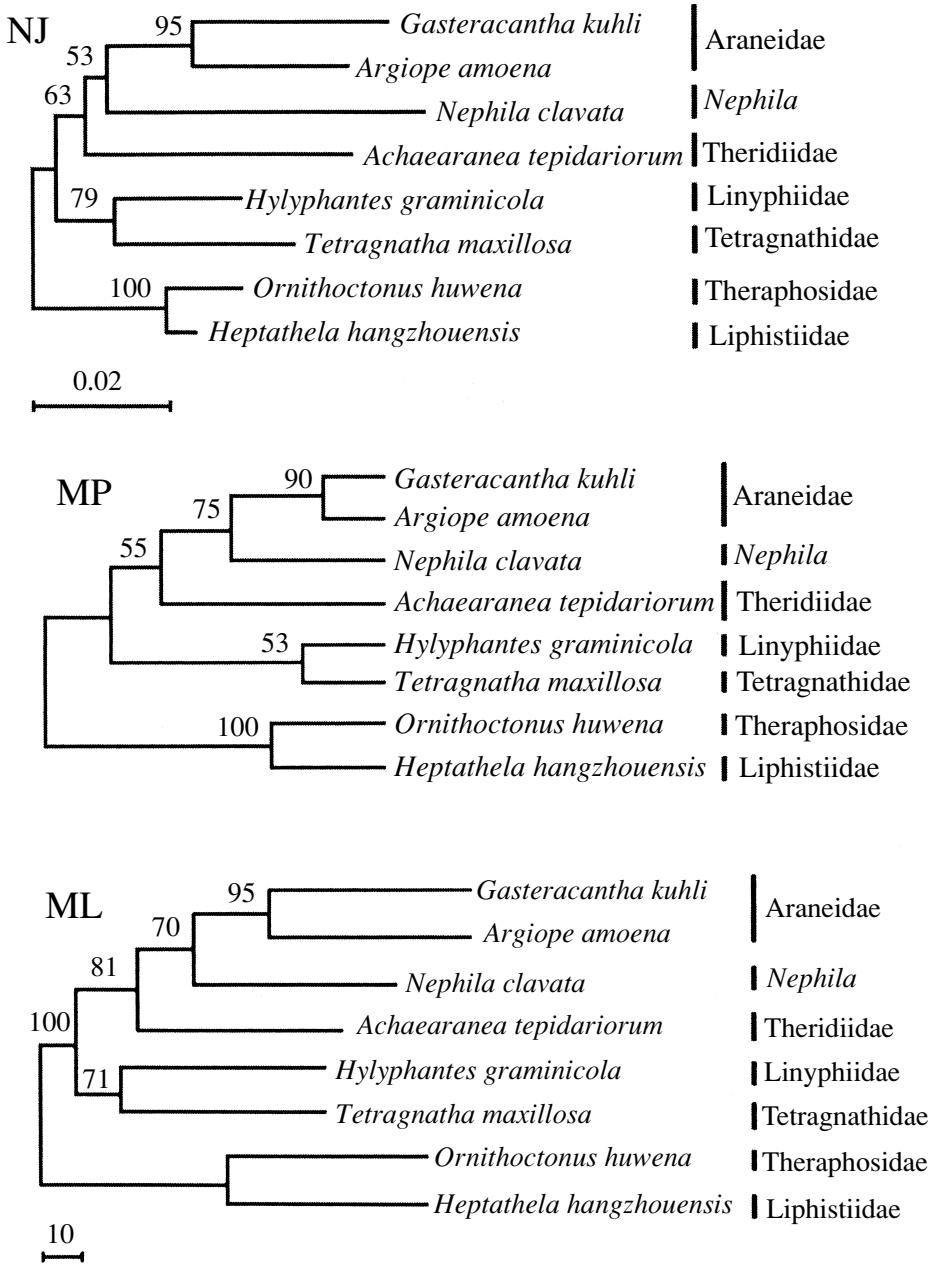

Fig. 2. Neighbor-joining (NJ), maximum parsimony (MP) and maximum-likelihood (ML) trees based on 18S rDNA sequence data of eight species of spiders. Bar equals 0.02 (NJ) and 10 (ML) units of Kimura's two-parameter distance. Numbers above branches in these trees are Bootstrap values at least 50% of the 1,000 bootstrap replications.

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Tetragnatha versicolor      NAVSRLSSPASNARISSAVSALASGG-ASSPGYLSSIISNVVSQVSSNNDG
Tetragnatha kauaiensis      NAVSLLSSPASNARISSAVSALASGA-ASGPGYLSSVISNVVSQVSSNSGG
Nephila clavipes            AAASRLSSPQASSRVSSAVSNLVASG-PTNSAALSSTISNVVSQIGASNPG
Nephila madagascariensis    AAASRLSSPQASSRVSSAVSNLVASG-PTNSAALSSTISNAVSQIGASNPG
Nephila senegalensis        AAASRLSSPEASSRVSSAVSNLVSSG-PTNSAALSSTISNVVSQIGASNPG
Argiope trifasciala         AAASRLSSPGAASRVSSAVTSLVSSGGPTNSAALSNTISNVVSQISSSNPG
Argiope amoena              AAASRLSSPGAASRVSSAVTSLVSSGGPTNSAALSNTISDVVSQISASNPG
Araneus diadematus          AAASRLSSPSAAARVSSAVS-LVSNGGPTSPAALSSSISNVVSQISASNPG
Dolomedes tenebrosus        NMVSRLSSPEAASRVSSAVSSLVSNG-QVNVDALPSIISNLSSSISASATT

Tetragnatha versicolor      LSGCDTVVQALLEVAAALVHVLASSNIGQVNLNTAGYTSQL##########
Tetragnatha kauaiensis      LVGCDTLVQALLEAAAALVHVLASSSGGQVNLNTAGYTSQL##########
Nephila clavipes            LSGCDVLIQALLEVVSALIQILGSSSIQGVNYGSAGQATQIVGQSVYQALG
Nephila madagascariensis    LSGCDVLIQALLEVVSALIHILGSSSIGQVNYGSAGQATQ###########
Nephila senegalensis        LSGCDVLIQALLEVVSALVHILGSSSIGQVNYGSAGQATQ###########
Argiope trifasciala         LSGCDVLVQALLEIVSALVHILGSANIGQVNSSGVGRSASIVGQSINQAFS
Argiope amoena              LSGCDVLVQALLEIVSALVHILGSANIGQVNSSSAGQSASLVGQSVYQALS
Araneus diadematus          LSGCDILVQALLEIISALVHILGSANIGPVNSSSAGQSASIVGQSVYRALS
Dolomedes tenebrosus        ASDCEVLVQVLLEVVSALVQIVCS############################
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Fig. 3. Allignment of the C-terminal non-repetitive regions predicted Amino acid sequences of *MaSp*1 of *Argiope amoena* and other eight species of spiders. The mark (#) indicates where amino acid sequence has not revealed, the short line (–) indicates where an amino acid is deleted.

Table 4. Percent difference of base substitution (upper triangle) and values of transitions/transversions (lower triangle) for 3'-end non-repetitive region's cDNA sequence of *MaSp*1 gene among 9 species of spiders

	1	2	3	4	5	6	7	8	9
1. *Tetragnatha kauai*		7.1	23.5	23.2	20.6	21.6	21.9	19.4	30.3
2. *Tetragnatha versicolor*	0.83		15.2	16.5	14.8	15.8	15.8	13.9	22.9
3. *Argiope trifasciata*	1.28	0.96		4.2	8.1	14.2	12.3	10.6	28.4
4. *Argiope amoena*	1.25	1.04	1.17		6.5	11.6	10.7	9.0	28.1
5. *Araneus diadematus*	0.94	1.00	1.50	1.50		26.8	12.6	11.0	28.1
6. *Nephila clavipes*	1.03	0.69	0.83	0.80	0.69		1.0	1.6	28.4
7. *Nephila madagascariensis*	1.19	0.81	1.00	1.06	1.29	0.50		1.3	23.5
8. *Nephila senegalensis*	1.14	0.87	1.06	1.00	1.62	0.25	1.00		22.3
9. *Dolomedes tenebrosus*	0.81	0.82	1.05	0.89	0.85	0.83	1.09	1.03	

DISCUSSION

The phylogenetic position of the genus *Nephila*

Although the genus *Nephila* possesses morphological characters that are believed to be synapomorphies of the family Tetragnathidae (Roewer, 1942; Brignoli, 1983; Song *et al.*, 2001), it also has many characters that are not shared by other tetragnathid genera. These characters include the labium longer than wide, transverse grooves on the book lung covers, and the metatarsi and tarsi together longer than the patellae and tibiae (Levi and Eickstedt, 1989). On the other hand, it also bears many characters in common with the family Araneidae, such as a distinct epigynum, characteristic integral structure of male palpal organ and striking sexual size dimorphism (female giantism). Hence, it seems to be difficult to judge the phylogenetic position of the genus *Nephila* by means of morphological methods alone.

In the present study, the NJ, MP and ML trees based on the sequences of 12S rRNA or 18S rRNA gene fragments, and the NJ and MP trees based on the 3'-end non-repetitive region's cDNA sequences of *MaSp*1 gene or the C-terminal non-repetitive region's amino acid sequences of *MaSp*1, revealed that the phylogenetic position of the genus *Nephila* is closer to the araneid genera than to tetragnathid. It is notable that the NJ, MP and ML trees of the 12S and 18S rDNA fragments corroborated the statements that Araneidae and Tetragnathidae do not form a sister group in the superfamily Araneoidea (Coddington and Levi, 1991; Hausdorf, 1999). Although a linyphiid genus or a theriddiid genus included in the analyses intervened between Araneidae and Tetragnathidae in those reconstructed phylogenetic trees (Figs. 1 and 2), the genus *Nephila* was always connected directly with the araneid genera. Our results showed that *Nephila* should be transferred back to the family Araneidae,

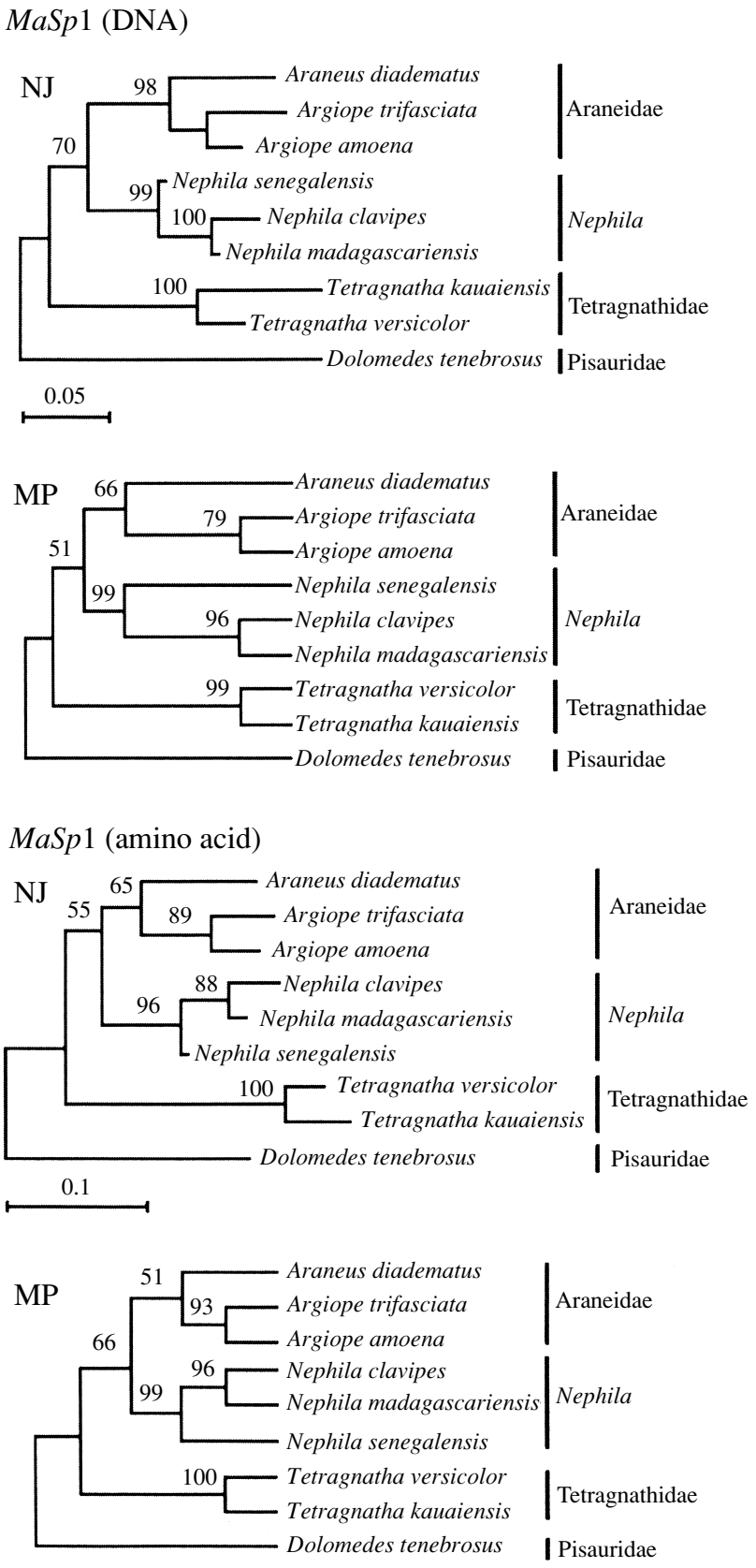

Fig. 4. The neighbour-joining (NJ) and Maximum parsimony (MP) trees resulting from analysis of the 3'-end non-repetitive regions cDNA sequences of *MaSp*1 gene and C-terminal non-repetitive regions amino acid sequences of *MaSp*1 of nine species of spiders. Bootstrap values at least 50% of the 1,000 bootstrap replications are shown above branches in these trees.

or the genus and its subfamily might be recognized as a separate clade in the superfamily Araneoidea and should be removed from either Araneidae or Tetragnathidae. In the later case, the subfamily Nephilinae might be elevated to family level after being redefined and redelimited.

The use of the C-terminal non-repetitive region of *MaSp*1 as a molecular marker

As the web frame and lifelines of spiders, dragline silk plays an important role in spider's life. Dragline silk is a two-protein fiber and, *MaSp*1 (major ampullate spidroin-1) and *MaSp*2 (major ampullate spidroin-2) genes which are active in the major ampullate gland are thought to be responsible for the dragline silk. *MaSp*1 gene encodes a kind of protein molecule that contains the repetitive region and C-terminal non-repetitive region (Xu and Lewis, 1990; Van Beek *et al*., 2002). The repetitive region exhibits a pattern of alternating alanine-rich, crystal-forming blocks that impart the silk's unmatched strength in the natural world and glycine-rich amorphous blocks implicated in providing elasticity in the silk filament. In contrast to the repetitive region, the C-terminal non-repetitive region is hydrophilic, thus it proved to be necessary to maintain the soluble or liquid crystalline state of silk molecules along the gland duct before being secreted from silk gland (Anthoula *et al*., 2002). The alignment of predicted amino acid sequences of C-terminal non-repetitive region of *MaSp*1 of nine species of spiders shows this region has considerably conserved amino acid sequences (Fig. 3).

The results from the NJ and MP trees based on the 3'-end non-repetitive region's cDNA sequences of *MaSp*1 gene or the C-terminal non-repetitive region's amino acid sequences (Fig. 4) agree with the phylogenetic relationships among these spiders in traditional classifications. They are also similar to the results based on sequences of 12S and 18S rRNA gene fragments (Figs. 1 and 2) on the phylogenetic placement of *Nephila*. At least, it indicates that both the 3'-end non-repetitive region's cDNA sequences of *MaSp*1 gene and the C-terminal non-repetitive region's amino acid sequence of *MaSp*1 have a certain value in phylogenetic analysis of spiders. In addition, the *MaSp*1 gene has no intron, and consists of a single exon alone (Beckwitt *et al*, 1998; Hayashi and Lewis, 2000). Therefore, amplification of the 3'-end partial DNA of *MaSp*1 gene directly from genomic DNA of spider is feasible. That is to say, not via complicated reverse transcription from mRNA, the 3'-end non-repetitive region's DNA sequences of *MaSp*1 gene and the C-terminal non-repetitive region's amino acid sequence of *MaSp*1 can be obtained conveniently and economically.

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