J Mol Evol (2005) 60:57–71
DOI: 10.1007/s00239-004-0010-2

JOURNAL OF MOLECULAR EVOLUTION

The Mitochondrial Sequences of *Heptathela hangzhouensis* and *Ornithoctonus huwena* Reveal Unique Gene Arrangements and Atypical tRNAs

Yang Qiu, Daxiang Song, Kaiya Zhou, Hongying Sun

Jiangsu Key Lab for Bioresource Technology, College of Life Sciences, Nanjing Normal University, Nanjing 210097, P.R. China

Received: 19 March 2004 / Accepted: 29 July 2004 [*Reviewing Editor:* Dr. Rafael Zardoya]

Abstract. We have sequenced the complete mitochondrial genomes of the spiders *Heptathela hangzhouensis* and *Ornithoctonus huwena*. Both genomes encode 13 protein-coding genes, 22 tRNA genes, and 2 ribosomal RNA genes. *H. hangzhouensis*, a species of the suborder Mesothelae and a representative of the most basal clade of Araneae, possesses a gene order identical to that of *Limulus polyphemus* of Xiphosura. On the other hand, *O. huwena*, a representative of suborder Opisthothelae, infraorder Mygalomorphae, was found to have seven tRNA genes positioned differently from those of *Limulus*. The *rrnL–trnL1–nad1* arrangement shared by the araneomorph families Salticidae, Nesticidae, and Linyphiidae and the mygalomorph family Theraphosidae is a putative synapomorphy joining the mygalomorph with the araneomorph. Between the two species examined, base compositions also differ significantly. The lengths of most protein-coding genes in *H. hangzhouensis* and *O. huwena* mtDNA are either identical to or slightly shorter than their *Limulus* counterparts. Usage of initiation and termination codons in these protein-coding genes seems to follow patterns conserved among most arthropod and some other metazoan mitochondrial genomes. The sequences of the 3′ ends of *rrnS* and *rrnL* in the two species are similar to those reported for *Limulus*, and the entire genes are shortened by about 100–250 nucleotides with respect to *Limulus*. The lengths of most tRNA genes from the two species are distinctly shorter than those of *Limulus* and the sequences reveal unusual inferred tRNA secondary structures. Our finding provides new molecular evidence supporting that the suborder Mesothelae is basal to opisthothelids.

Key words: *Heptathela hangzhouensis* — *Ornithoctonus huwena* — Mitochondrial genome — Gene rearrangement — tRNA secondary structure

Correspondence to: Kaiya Zhou; *email:* kyzhounj@jlonline.com

Introduction

The order Araneae ranks seventh in global animal diversity based on its described and anticipated species (Coddington and Levi 1991), next to the five largest insect orders (Coleoptera, Hymenoptera, Lepidoptera, Diptera, Hemiptera) and Acari among the Arachnida. To date, approximately 38,432 species of spiders have been named and placed in 3542 genera and 110 families (Platnick 2004). Statistical analysis suggests that about 20% of the world fauna has been described, implicating that there may be as many as 190,000 distinct spiders in all (Coddington and Levi 1991).

Mitochondrial genome sequence and gene arrangement comparisons have emerged as powerful tools during the past decade for resolving ancient phylogenetic relationships. Although partial sequences of *rrnS*, *rrnL*, *nad1*, *cox1*, and tRNAs from spiders have been documented (Gillespie et al. 1994; Hedin 1997a, b, 2001; Hedin and Maddison 2001; Zehethofer and Sturmbauer 1998; Fang et al. 2000; Masta 2000; Piel and Nutt 2000; Bond et al. 2001; Vink et al. 2002; Vink

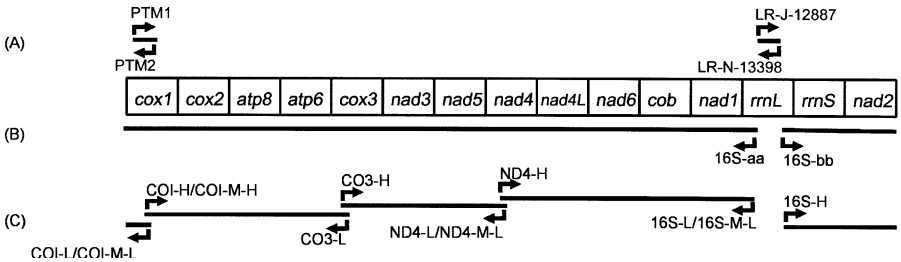

Fig. 1. Schematic of sequencing strategy. Horizontal lines indicate amplified fragments. **A** The partial portions of the *rrnL* and *cox1* genes of the two species were amplified with two pairs of universal primers: LR-J-12887/LR-N-13398 and PMT1/PMT2. **B** For *Ornithoctonus huwena*, the complete mitochondrial genome was amplified with a pair of primers, 16S-aa and 16S-bb, designed based on the sequence of *rrnL*. **C** In *O. huwena*, the *rrnL–cox1* region was amplified with the primers 16S-H and COI-L, the *cox1–cox3* region was amplified with the primers COI-H and CO3-L, the *cox3–nad4* region was amplified with the primers CO3-H and ND4-L, and the *nad4–rrnL* region was amplified with the primers ND4-H and 16S-L. In *Heptathela hangzhouensis*, the *rrnL–cox1* region was amplified with the primers 16S-H and COI-M-L, the *cox1–cox3* region was amplified with the primers COI-M-H and CO3-L, the *cox3–nad4* region was amplified with the primers CO3-H and ND4-M-L, and the *nad4–rrnL* region was amplified with the primers ND4-H and 16S-M-L.

and Paterson 2003; Hormiga et al. 2003; Maddison and Hedin 2003; Arnedo et al. 2004), the complete mitochondrial sequence has not been reported for any of the spiders. The number of complete mtDNAs of Arthropoda is limited to 40, of which 6 are from Chelicerata. Moreover, the sampling of order in Chelicerata is extremely biased. Of the six sequenced mtDNAs, five are from Acari, and the sixth one is from Xiphosura, the horseshoe crab *Limulus*.

Morphological and auxiliary parameters were used to form three major monophyletic groups of Araneae: Mesothelae, Mygalomorphae, and Araneomorphae. The viewpoint of Platnick and Gertsch (1976), based on a cladistic analysis using 14 characteristics, is accepted by most in the arachnological field: Mesothelae as a basal suborder and Mygalomorphae and Araneomorphae as two infraorders under the suborder Opisthothelae. This classification is also supported by a recent study using both morphological and molecular characters (Giribet et al. 2002). Two spiders, *Heptathela hangzhouensis* (suborder Mesothelae, family Liphistiidae) and *Ornithoctonus huwena* (suborder Opisthothelae, infraorder Mygalomorphae, family Theraphosidae), were selected as the representatives of Araneae for studies of mitochondrial gene structure.

In this article, the gene sequences and arrangements of mitochondrial genomes of the two spiders are compared with those of other chelicerates. The complete mitochondrial sequences of *H. hangzhouensis* and *O. huwena* allowed us to analyze nucleotide composition and codon usage patterns and to identify structural features that may be involved in regulating mtDNA replication. We observed truncations in tRNA and rRNA genes and overlaps among genes in the two species, which may reflect a trend of mitochondrial genome minimization. The mismatches in the tRNA aminoacyl acceptor arms of the two species may indicate involvement of editing mechanisms (Yokobori and Pääbo 1995a, b; Lavrov et al. 2000b).

Materials and Methods

Of the two spider species used in this report, *H. hangzhouensis* was collected from Xianning County, Hubei Province (29.8 N, 114.2 E), and *O. huwena* was collected from Ningming County, Guangxi Province (22.1 N, 107.0 E), in China. Voucher specimens are stored at Nanjing Normal University at −70°C. Total cellular DNA was prepared using whole bodies of *H. hangzhouensis* and from leg muscles of *O. huwena*, as described previously by Hwang et al. (2001).

Prior to long PCR, partial regions of *rrnL* and *cox1* from the total DNAs of the two spiders were amplified (Fig. 1A) and sequenced, using the primer pairs LR-J-12887/LR-N-13398 (Simon et al. 1994) and PMT1/PMT2 (Folmer et al. 1994). The primer pair 16S-aa/16S-bb (Table 1) was designed based on the partial *rrnL* sequence of *O. huwena* and the pair of primers (16S-aa and 16S-bb) reported by Hwang et al. (2001). The complete mitochondrial genome of *O. huwena* was amplified with this primer pair using the Expand Long Template PCR System (Roche) except for 249 bp of the *rrnL* segment that had been sequenced (Fig. 1B). The PCR product was purified using the Gel Extraction Mini Kit (Watson Biotechnologies). Using the purified long-PCR amplification product as template, the mitochondrial genome of *O. huwena* was amplified in four pieces (Fig. 1C) with TaKaRa Ex Taq (TaKaRa Biotechnology). Among the four pairs of primers, four primers (16S-H and 16S-L, COI-L and COI-H) (Table 1) were designed based on the sequences of *rrnL* and *cox1* of *O. huwena*, two primers (CO3-L and ND4-H) were designed based on C3-N-5460 and N4-J-8944 (Simon et al. 1994), and two primers (CO3-H and ND4-L) were designed based on the sequences of *cox1–cox3* and *nad4–rrnL* of *O. huwena*. For *H. hangzhouensis*, using total cellular DNA as the template, the entire mitochondrial genome was amplified in four pieces (Fig. 1C) with TaKaRa Ex Taq. Among the four pairs of primers, four primers (16S-H and 16S-M-L, COI-M-L and COI-M-H) (Table 1) were designed based on the sequences of *rrnL* and *cox1* of *H. hangzhouensis*, two primers (CO3-L and ND4-H) were designed based on C3-N-5460 and N4-J-8944, and two primers (CO3-H and ND4-M-L) were designed based on the sequences of *cox1–cox3* and *nad4–rrnL* of *H. hangzhouensis*.

The four amplification products (*rrnL–cox1*, *cox1–cox3*, *cox3–nad4* and *nad4–rrnL*) of the two spiders were sequenced by primer walking using an ABI 3700 and LI-COR automated DNA sequencer. Sequences were assembled with the DNASTAR software package (DNASTAR, Inc.) and aligned with Clustal_X (Version 1.8) (Thompson et al. 1997), DNAClub (Chen, DNAclub, Inc.), and MEGA (Version 2.1) (Kumar et al. 2001). Protein-coding and ribosomal genes were identified using the NCBI Internet

Table 1. Primer sequences and their target genes/genome regions

Species	Primer	Sequence	Reference
Ornithoctonus huwena and Heptathela hangzhouensis	*rrnL*		
	LR-J–12887	5'-CCGGTCTGAACTCAGATCACGT-3'	Simon et al. (1994)
	LR-N–13398	5'-CGCCTGTTTACCAAAAACAT-3'	Simon et al. (1994)
	cox1		
	PMT1	5'-GGTCAACAAATCATAAAGATATTGG-3'	Folmer et al. (1994)
	PMT2	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	Folmer et al.(1994)
Ornithoctonus huwena	Complete mtDNA		
	16S-aa	5'-CTTACCAAAAGTGAGATTGTGACCTCGATGTTG-3'	Hwang et al. (2001)
	16S-bb	5'-AATCATTATGCTACCTTAGCACTATTCGCGGC-3'	Hwang et al. (2001)
	rrnL–cox1		
	16S-H	5'-GCAAATCATTATGCTACCTTAGCAC-3'	This study
	COL-L	5'-TTCGAGGAAAAGCCATATCAGGAGC-3'	This study
	cox1–cox3		
	COI-H	5'-TAGGTGCTCCTGATATGGCTTTTCC-3'	This study
	CO3-L	5'-CTACATCGACAAAATGTCAGTATCA-3'	Simon et al. (1994)
	cox3–nad4		
	CO3-H	5'-GGAGCCTTTCAATCCTTTTCAAGTTCC-3'	This study
	ND4-L	5'-CGTCTACAAGCAGGAATTTACCTTC-3'	This study
	nad4-rrnL		
	ND4-H	5'-CTCGAGGAGCTTCAACATGAGCYTT-3'	Simon et al. (1994)
	16S-L	5'-CCAACCCTTCAGAGATGC-3'	This study
Heptathela hangzhouensis	*rrnL–cox1*		
	16S-H	5'-GCAAATCATTATGCTACCTTAGCAC-3'	This study
	COI-M-L	5'-AAATTGATGAGGCTCCWGCTAAATG-3'	This study
	cox1–cox3		
	COI–M-H	5'-TAGGGGGCACCAGATATAGCTTTTCC-3'	This study
	CO3-L	5'-CTACATCGACAAAATGTCAGTATCA-3'	Simon et al. (1994)
	cox3–nad4		
	CO3-H	5'-GGAGCCTTTCAATCCTTTTCAAGTTCC-3'	This study
	ND4-M-L	5'-GGTTCTTTACCTTTGTTGTTGGGAC-3'	This study
	nad4–rrnL		
	ND4–H	5'-CTCGAGGAGCTTCAACATGAGCYTT-3'	Simon et al. (1994)
	16S-M-L	5'-GCTCAATGCATATGTAAATAGCCGC-3'	This study

Note. Y = C/T, W = A/T.

Blast Search function. Transfer RNA genes were identified visually as sequences with potential tRNA secondary structure and specific tRNAs were identified by their anticodon sequences. The complete sequences of the mtDNA of *H. hangzhouensis* and *O. huwena* have been submitted to GenBank under accession numbers AY309258 and AY309259, respectively.

Results

Genome Size and Structure

The complete mtDNA sequences of *H. hangzhouensis* and *O. huwena* are 14,215 and 13,874 bp, respectively. Both contain the 37 genes typical for animal mtDNA. The size of the mitochondrial genome of *O. huwena* is relatively short in metazoa but longer than that of the nematode *Caenorhabditis elegans* (13,794 bp [Okimoto et al. 1992]). The majority of genes on both genomes are either immediately contiguous or overlapping. In addition to the large noncoding region, 71 and 12 noncoding nucleotides are present in *H. hangzhouensis* and *O. huwena*, respectively. In *H. hangzhouensis*, protein-coding genes account for 75.7% (10,759 bp) of the genome, rRNA genes for 12.8% (1,817 bp), tRNA genes for 9.0% (1,285 bp), and noncoding DNA for 2.9% (411 bp). Similarly, in *O. huwena*, protein-coding genes account for 77.3% (10,719 bp) of the genome, rRNA genes for 12.4% (1,714 bp), tRNA genes for 8.9% (1,240 bp), and noncoding DNA for 2.9% (408 bp). In *H. hangzhouensis* and *O. huwena*, 57 and 207 bp, which overlap among genes, were counted twice in the above calculation, respectively.

The gene arrangement of *H. hangzhouensis* appears identical to that of *Limulus* (Lavrov et al. 2000a); However, *O. huwena* and certain species of suborder Opisthothelae, infraorder Araneomorphae (Hedin 1997b; Masta 2000; Hormiga et al. 2003), share a partial mitochondrial gene arrangement that may reflect a derivative status. Compared with the basal arthropod mitochondrial gene arrangement in *Limulus*, a total of seven tRNA genes were found to be rearranged in *O. huwena* (Fig. 2).

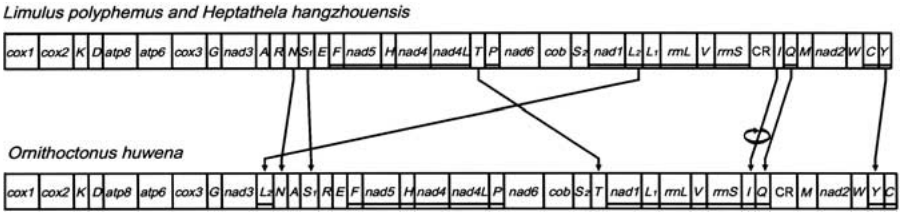

Fig. 2. Comparison of gene arrangements in mtDNA of *Limulus polyphemus*, *Heptathela hangzhouensis*, and *Ornithoctonus huwena*. Genes are transcribed from left to right except those underlined, which are transcribed oppositely. Transfer RNA genes are labeled with single-letter amino acid abbreviations except for those encoding leucine and serine, which are labeled *L1* (tag), *L2* (taa), *S1* (gct), and *S2* (tga). Abbreviations of protein-coding genes and rRNA genes: *cox1–cox3*, cytochrome oxidase subunits 1–3; *cob*, cytochrome b; *nad1–nad6*, NADH dehydrogenase subunits 1–6; *nad4L*, NADH dehydrogenase subunit 4L; *atp6* and *atp8*, ATP synthase subunits 6 and 8; *rrnL* and *rrnS*, large and small ribosomal subunit RNAs. Long arrows are drawn from the location of each tRNA gene in the ancestral arrangement to its location in *O. huwena*. The rotating arrow indicates inversion. CR indicates the control region (the large noncoding region).

Base Composition

The A + T content of *H. hangzhouensis* and *O. huwena* is 72.2 and 69.8%, respectively, lower than that of all other chelicerates (Navajas et al. 2002) except for *Limulus*, with an A + T content of 67.6% (Lavrov et al. 2000a). We designated the strand bearing more protein-encoding sequence the α strand, and its complementary strand the β strand. *H. hangzhouensis* has the nucleotide composition of the α strand: A = 5014 (35.27%), T = 5252 (36.95%), C = 2438 (17.15%), and G = 1511 (10.63%); *O. huwena* has the nucleotide composition of the α strand: A = 4438 (31.99%), T = 5246 (37.81%), C = 1375 (9.91%), and G = 2815 (20.29%). The base compositional difference between the two strands can be measured as GC- and AT-skews, where GC-skew = (G − C)/(G + C) and AT-skew = (A−T)/(A + T) (Perna and Kocher 1995). For the *H. hangzhouensis* α strand, GC-skew = −0.23 ana AT-skew = −0.02. For the *O. huwena* α strand, GC-skew = 0.34 and AT-skew = −0.08. Although lower than the two absolute values of *Limulus*, the absolute GC-skew of the two-species α strand and the AT-skew of the *O. huwena* α strand are comparatively high for an arthropod mtDNA, reflecting their base compositional differences and an obvious bias between the two strands. The opposite directions of GC-skews of α strands in *H. hangzhouensis* and *O. huwena* mitochondrial genomes may suggest that the β strand is replicated first in *H. hangzhouensis* and that the α strand is replicated first in *O. huwena*. Because the parental strand is displaced by its daughter strand and remains single-stranded until the lagging strand is synthesized, the strand displaced earlier will presumably be more susceptible to hydrolytic deamination. As replication of DNA is strand-asymmetric due to antiparallel alignment of the complementary strands, mutations are expected to occur in a strand-asymmetric manner (Tanaka and Ozawa 1994; Saccone et al. 1999). Therefore, the difference of nucleotide composition between the two strands may be related to the asynchronous synthesis of the two mitochondrial strands.

Protein-Coding Genes

Size and Sequence Similarity

The lengths of most protein-coding genes in *H. hangzhouensis* and *O. huwena* mtDNAs are either identical to or slightly smaller than their *Limulus* counterparts. Sequence comparison among *H. hangzhouensis*, *O. huwena*, and *Limulus* revealed *cox1* as the most conserved gene and *atp8*, *nad2*, and *nad6* as the least conserved genes (Table 2). The protein-coding genes of the two species have an arrangement commonly observed among arthropods (Crease 1999).

Translation Initiation and Termination Codons

The 5′ ends of protein-coding genes of the two species were inferred to be at the first legitimate start codon (ATN, GTG, TTG, GTT [Wolstenholme 1992]) in the open reading frame (ORF), except that of *cox2* in *H. hangzhouensis*, which appears to use the infrequent start codon CTG (Table 2), and *cox1* in *O. huwena*, which appears to use the nonstandard start codon TTA (Table 2). The unusual start codons were determined for *cox1* and *cox2* based on the sequence alignment among our two sequences and the 5′ ends of *cox1* and *cox2* in *Limulus*. Unusual start codons CTG and TTA were also assigned in a similar fashion in *Balanoglossus* (Castresana et al. 1998) and *Limulus* (Lavrov et al. 2000a), respectively. Regardless of the actual initiation codon, all proteins were assumed to start with formyl-Met, as has been demonstrated for other mitochondrial systems (Smith and Marcker 1968; Fearnley and Walker 1986).

Only 10 protein-coding genes in the two species are inferred to have complete termination codons (Table 2). Among them, the termination codons of *atp8* and *nad1* in both *O. huwena* and *H. hangzhouensis* are entirely within the sequences of the down-

Table 2. Comparison of Mitochondrial Proteins of *Heptathela hangzhouensis, Ornithoctonus huwena*, and *Limulus polyphemus*

	No. of amino acids[a]			% amino acid identity			Predicted initiation and termiation codons in			
				Heptathela/	*Ornithoctonus*/	*Ornithoctonus*/				
Protein	*Limulus*	*Heptathela*	*Ornithoctonus*	*Limulus*	*Limulus*	*Heptathela*	*Heptathela*		*Ornithoctonus*	
ATP6	224	220	222	51.3	41.5	40.5	ATG(−7)[b]	T(1)	ATG(−7)	TAG(0)
ATP8	51	49	50	31.4	31.4	18.4	ATA(0)	TAA(−7)	ATG(−9)	TAA(−7)
COX1	511	510	511	82.2	72.4	72.5	TTG(−2)	TAA(3)	TTA(−6)	TAG(0)
COX2	228	222	222	58.3	54.4	52.7	CTG(3)	T(0)	ATG(0)	T(AG)[c](0)
COX3	261	257	261	66.3	57.1	59.1	TTG(0)	TAG(11)	TTG(0)	T(0)
COB	377	373	378	60.2	56.8	55.5	ATA(6)	T(AA)(0)	GTG(O)	T(1)
NAD1	310	304	303	47.4	46.5	46.7	ATG(5)	TAG(−10)	ATT(−12)	TAA(−25)
NAD2	338	320	306	35.5	24.6	22.8	ATA(−3)	T(AA)(0)	ATT(7)	TA(A)(0)
NAD3	114	109	107	54.4	41.2	38.5	ATA(6)	TA(G)(0)	ATA(−l)	T(−3)
NAD4	445	433	425	45.6	40.0	39.0	ATG(0)	T(0)	ATT(0)	T(AA)(0)
NAD4L	99	93	92	44.4	29.3	33.3	ATC(−2)	TA(A)(0)	ATC(−7)	T(0)
NAD5	571	545	546	43.3	38.0	39.1	ATT(−2)	T(1)	ATC(−12)	T(0)
NAD6	153	143	142	32.7	25.5	30.1	ATT(11)	TAA(6)	ATT(−8)	TAG(0)

[a]This is the number of amino acid residues coded for by these genes. The numbers of amino acids in *Limulus polyphemus* proteins were taken from Lavrov (2000a).
[b]The numbers in parentheses after the initiation and termination codon are the numbers of noncoding nucleotides upstream and downstream of a gene. Negative numbers indicate that the genes are overlapping.
[c]Nucleotides in parentheses indicate the potential for a complete termination codon overlapping the downstream gene.

stream genes. But only the protein-coding gene terminus of *atp8* was inferred to be located within the sequence of the downstream gene encoded on the same strand. Although four genes (*cob*, *nad2*, *nad3*, and *nad4L*) in *H. hangzhouensis* and three genes (*cox2*, *nad2*, and *nad4*) in *O. huwena* do not have complete termination codons, their next one or two nucleotides of downstream gene constitute a stop codon which could be used as such if translation occurred before the genes adjacent to the 3′ ends of these genes were cleaved from the common transcript (Ojala et al. 1981). In three genes (*cox2*, *nad4*, and *nad5*) of *H. hangzhouensis* and the four (*cox3*, *cob*, *nad3*, and *nad5*) of *O. huwena*, a solitary T at the inferred 3′ end of a gene abuts the 5′ end of a downstream tRNA gene, having no downstream gene to complete truncated stop codons. The mRNA is probably polyadenylated to form a UAA stop codon after the downstream tRNA is cleaved from the polycistronic transcript (Lavrov et al. 2000a). The gene atp6 of *H. hangzhouensis* and the gene *nad4L* of *O. huwena* have a truncated stop codon (T), have no downstream gene to complete them, and do not abut tRNA. In mammals the transcription products of mtDNA are primarily polycistronic. Individual gene transcripts are produced by precise cleavage, and protein-coding gene transcripts ending in U acquire complete termination codons by polyadenylation (Ojala et al. 1981). So these truncated stop codons may also serve as functional stop codons, as similar scenarios have been reported in *Limulus* (Lavrov et al. 2000a).

A significant degree of physical overlap has been observed in the genes from both species. There are overlaps between many genes of the two species, maybe because of their condensed genomes. However, the overlap between protein-coding genes only happened in *atp8–atp6*. Among them, a 7-nt (nucleotide) overlap is present between them, which has been reported in all arthropod mtDNA sequences published except that of *Apis* (Crozier and Crozier 1993), where the overlap is 19 nt. Transcriptional mapping analysis in several species of animals has demonstrated the presence of bicistronic transcripts for the *atp8–atp6* gene pairs (Ojala et al. 1980; Berthier et al. 1986). It was also demonstrated that both of these genes are fully translated into proteins (Fearnley and Walker 1986). Taanman (1999) has suggested that the *atp8* mRNA, if single, may be too short to be translated efficiently. Therefore there may be some selective advantage in having *atp8–atp6* adjacent (Lavrov et al. 2000a).

Codon Usage

The frequencies of nucleotides in all three codon positions for each of the strands are presented in Fig. 3. In *H. hangzhouensis*, the percentages of T's and G's are lower in the α strand than in the β strand, whereas the percentages of A's and C's are higher. The more frequent T's and infrequent A's in the α strand are an exception in third codon positions. In *O. huwena*, the percentage of T's and G's is higher in the α strand than in the β strand, whereas the percentages of A's and C's are lower. The exceptions are

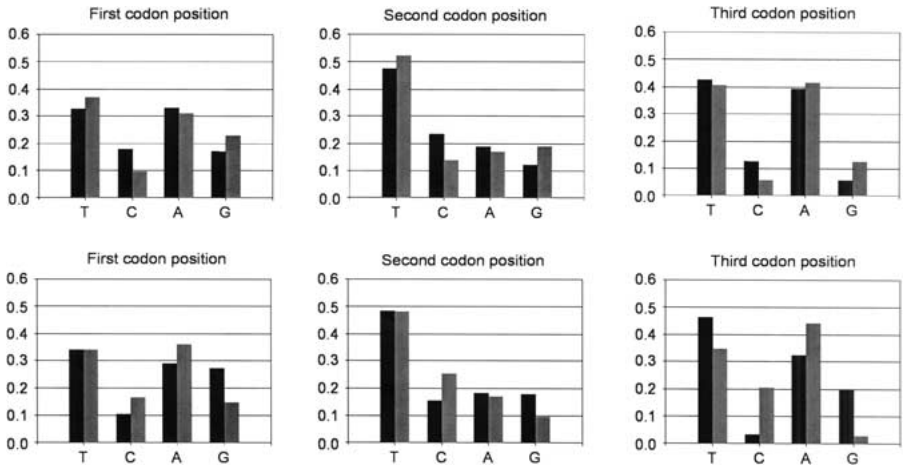

Fig. 3. Nucleotide composition comparison of the first, second, and third codon positions of protein-coding genes encoded by each strand of mtDNAs of *Heptathela hangzhouensis* (above) and *Ornithoctonus huwena* (below). The darker shaded bars show the nucleotide composition of the α strand and the lighter shaded bars show the nucleotide composition of the β strand.

the equal frequency of T's at the first codon position between the two strands and the high percentage of A's at second codon position in the α strand.

Selection may explain the high frequency of T at second codon positions in both strands, since codons with T in such position specify predominantly hydrophobic (nonpolar) amino acids, which are essential for the membrane-associated proteins encoded by mtDNA (Lavrov et al. 2000a). In *H. hangzhouensis*, the frequencies of G and C at third codon position in the two strands are greatly reduced. In *O. huwena*, the frequency of C at third codon position in the α strand and G at third codon position in the β strand are greatly reduced. Both reductions appear to reflect the mutational pattern in the genome, as nucleotides at third codon positions are under the least selective pressure.

We compared individual amino acid frequency in each strand and found significant differences for 8 of the 20 amino acids in *H. hangzhouensis* and in *O. huwena* (Tables 3 and 4). However, when we grouped amino acids based on the chemical nature of their side chains (nonpolar, polar, acidic, or basic) and compared the frequency of each group in each strand, we found no significant difference in *H. hangzhouensis* and three of the four groups in *O. huwena* (Tables 3 and 4). Only the group of acidic amino acids in *O. huwena* was significantly different in frequency between the strands.

Ribosomal RNA Genes

The putative sizes of the inferred *rrnS* and *rrnL* in *H. hangzhouensis* are 698 and 1119 nt, respectively, while those in *O. huwena* are 666 and 1048 nt, respectively. Although the entire rRNA genes of the two species are truncated about 100–250 nt with respect to *Limulus*, *H. hangzhouensis* and *Limulus* share 75% sequence similarity for the last 594 nt of the 3′ end of the *rrnL* and 73% sequence similarity for the last 419 nt of the 3′ end of the *rrnS*. Similarly, *O. huwena* and *Limulus* share 71% sequence similarity for the last 497 nt of the 3′ end of the *rrnL* and 66% sequence similarity for the last 422 nt of the 3′ end of the *rrnS*. Furthermore, *rrnL* of *H. hangzhouensis* and *O. huwena* share highly conserved stem-and-loop-region sequence motifs including the peptidyl transferase center, as reported by Masta (2000) and Hedin and Maddison (2001). Masta (2000) also found truncated *rrnL* in the spiders, which have tRNAs lacking the TΨC arm and suggested that normal functioning of tRNAs with a TV-replacement loop may require changes in ribosomal RNA structures.

Transfer RNA

Based on the sequence similarity with *Limulus* and the potential secondary structures of these tRNA genes, there are 22 putative tRNA genes in each species, as there are in most other published metazoan mtDNAs. In *H. hangzhouensis*, 9 tRNA genes are β strand-encoded tRNAs and 13 tRNA genes are α strand-encoded tRNAs, which is identical in *Limulus*. In *O. huwena*, however, 10 tRNA genes are encoded by the β strand and 12 tRNA genes are encoded by the α strand, while 7 have been translocated to other positions (Fig. 2), compared with *Limulus*.

When these tRNA genes of *H. hangzhouensis* and *O. huwena* are folded into putative secondary structures (Figs. 4 and 5), the tRNA genes of *H. hangzhouensis* have fewer mismatches in the aminoacyl acceptor arms compared with those of *O. huwena*. Most of the tRNAs appear to be truncated and lack one of the arms found in canonical tRNAs. Instead, these arms are replaced by a TV-replacement loop, as previously described for nematodes (Wolstenholme et al. 1987; Okimoto and Wolstenholme 1990; Navajas et al. 2002) and some mollusks (Terrett et al. 1996; Yamazaki et al. 1997). At the same time, the loss of the TΨC arm or DHU arm was also found in the

Table 3. Amino Acid Composition of Protein-Coding Genes in *Heptathela hangzhouensis*

	α strand protein[a]		β strand protein[b]		Both strands			
	No.	%	No.	%	No.	%	OR[c]	χ^2 test
Nonpolar								
Alanine								
GCN	93	4.22	42	3.05	135	3.77	1.40	3.06
Isoleucine								
ATY	257	11.67	113	8.22	370	10.34	1.47	9.73*
Leucine								
Total	316	14.34	245	17.82	560	15.65	0.77	6.53
CTN	137	6.22	54	3.93	190	5.31	1.62	8.38*
TTR	179	8.13	191	13.89	370	10.34	0.55	27.21*
Methionine								
ATR	146	6.62	129	9.38	275	7.69	0.69	8.36*
Phenylalanine								
TTY	215	9.76	130	9.45	345	9.64	1.04	0.08
Proline								
CCN	113	5.13	27	1.96	139	3.88	2.70	21.85*
Tryptophan								
TGR	63	2.86	37	2.69	99	2.77	1.06	0.10
Valine								
GTN	100	4.54	92	6.69	192	5.37	0.66	7.31*
Total	1303	59.15	815	59.27	2115	59.11	0.99	0.01
Polar								
Asparagine								
AAY	94	4.27	42	3.05	136	3.80	1.41	3.27
Cysteine								
TGY	8	0.36	21	1.53	29	0.81	0.23	14.17*
Glutamine								
CAR	50	2.27	15	1.09	65	1.82	2.11	6.48
Glycine								
GGN	106	4.81	112	8.15	218	6.09	0.57	15.44*
Serine								
Total	224	10.17	152	11.05	376	10.51	0.91	0.63
AGN	49	2.22	65	4.73	114	3.19	0.46	21.76*
TCN	175	7.94	87	6.33	262	7.32	1.28	3.01
Threonine								
ACN	127	5.76	24	1.75	150	4.19	3.44	32.63*
Tyrosine								
TAY	72	3.27	41	2.98	113	3.16	1.10	0.22
Total	681	30.91	407	29.60	1087	30.38	1.06	0.48
Acidic								
Aspartate								
GAY	33	1.50	22	1.60	55	1.54	0.94	0.06
Glutamate								
GAR	44	2.00	46	3.35	90	2.52	0.59	6.11
Total	77	3.50	68	4.95	145	4.05	0.70	4.40
Basic								
Arginine								
CGN	32	1.45	21	1.53	53	1.48	0.95	0.03
Histidine								
CAY	59	2.68	14	1.02	73	2.04	2.68	11.43*
Lysine								
AAR	51	2.32	50	3.64	101	2.82	0.63	5.24
Total	142	6.45	85	6.18	227	6.34	1.05	0.09
Grand total	2203		1375		3578			

[a]ATP6, ATP8, COX1, COX2, COX3, COB, NAD2, NAD3, NAD6.
[b]NAD1, NAD4, NAD4L, NAD5.
[c]Odds ratio; the proportion of an amino acid (or a group of amino acids) to all other amino acids on the α strand over the same proportion on the β strand.
[d]Test of the difference in the frequency of an amino acid or a group of amino acids on the two strands. Asterisks indicate a probability < 0.01.

Table 4. Amino Acid Composition of Protein-Coding Genes in *Ornithoctonus huwena*

	α strand protein[a]		β strand protein[b]		Both strands			
	No.	%	No.	%	No.	%	OR[c]	χ^2 test
Nonpolar								
Alanine								
GCN	75	3.41	72	5.27	147	4.12	0.63	7.07*
Isoleucine								
ATY	161	7.32	200	14.64	361	10.13	0.46	44.59*
Leucine								
Total	262	11.91	207	15.15	469	13.16	0.76	6.72*
CTN	32	1.45	107	7.83	139	3.90	0.17	87.91*
TTR	230	10.46	100	7.32	330	9.26	1.48	8.97*
Methionine								
ATR	196	8.91	79	5.78	275	7.71	1.59	10.70*
Phenylalanine								
TTY	216	9.82	140	10.25	356	9.99	0.95	0.15
Proline								
CCN	84	3.82	50	3.66	134	3.76	1.05	0.06
Tryptophan								
TGR	83	3.77	17	1.24	100	2.81	3.11	19.23*
Valine								
GTN	230	10.46	31	2.27	261	7.32	5.03	77.20*
Total	1307	59.44	795	58.20	2102	58.96	1.05	0.22
Polar								
Asparagine								
AAY	59	2.68	53	3.88	112	3.14	0.68	3.84
Cysteine								
TGY	12	0.55	11	0.81	23	0.65	0.68	0.88
Glutamine								
CAR	28	1.27	21	1.54	49	1.37	0.83	0.43
Glycine								
GGN	165	7.50	48	3.51	213	5.97	2.23	22.45*
Serine								
Total	208	9.46	195	14.28	403	11.30	0.63	17.29*
AGN	95	4.32	37	2.71	132	3.70	1.62	5.91
TCN	113	5.14	158	11.57	271	7.60	0.41	45.80*
Threonine								
ACN	72	3.27	67	4.90	139	3.90	0.66	5.74
Tyrosine								
TAY	90	4.09	37	2.71	127	3.56	1.53	4.53
Total	634	28.83	432	31.63	1066	29.90	0.88	2.20
Acidic								
Aspartate								
GAY	53	2.41	22	1.61	75	2.10	1.51	2.56
Glutamate								
GAR	71	3.23	25	1.83	96	2.69	1.79	6.12
Total	124	5.64	47	3.44	171	4.80	1.68	8.49*
Basic								
Arginine								
CGN	35	1.59	18	1.32	53	1.49	1.21	0.43
Histidine								
CAY	49	2.23	25	1.83	74	2.08	1.22	0.64
Lysine								
AAR	50	2.27	49	3.59	99	2.78	0.63	5.26
Total	134	6.09	92	6.73	226	6.34	0.90	0.55
Grand total	2199		1366		3565			

[a]ATP6, ATP8, COX1, COX2, COX3, COB, NAD2, NAD3, NAD6.
[b]NAD1, NAD4, NAD4L, NAD5.
[c]Odds ratio, the proportion of an amino acid (or a group of amino acids) to all other amino acids on the α strand over the same proportion on the β strand.
[d]Test of the difference in the frequency of an amino acid or a group of amino acids on the two strands. Asterisks indicate a probability < 0.01.

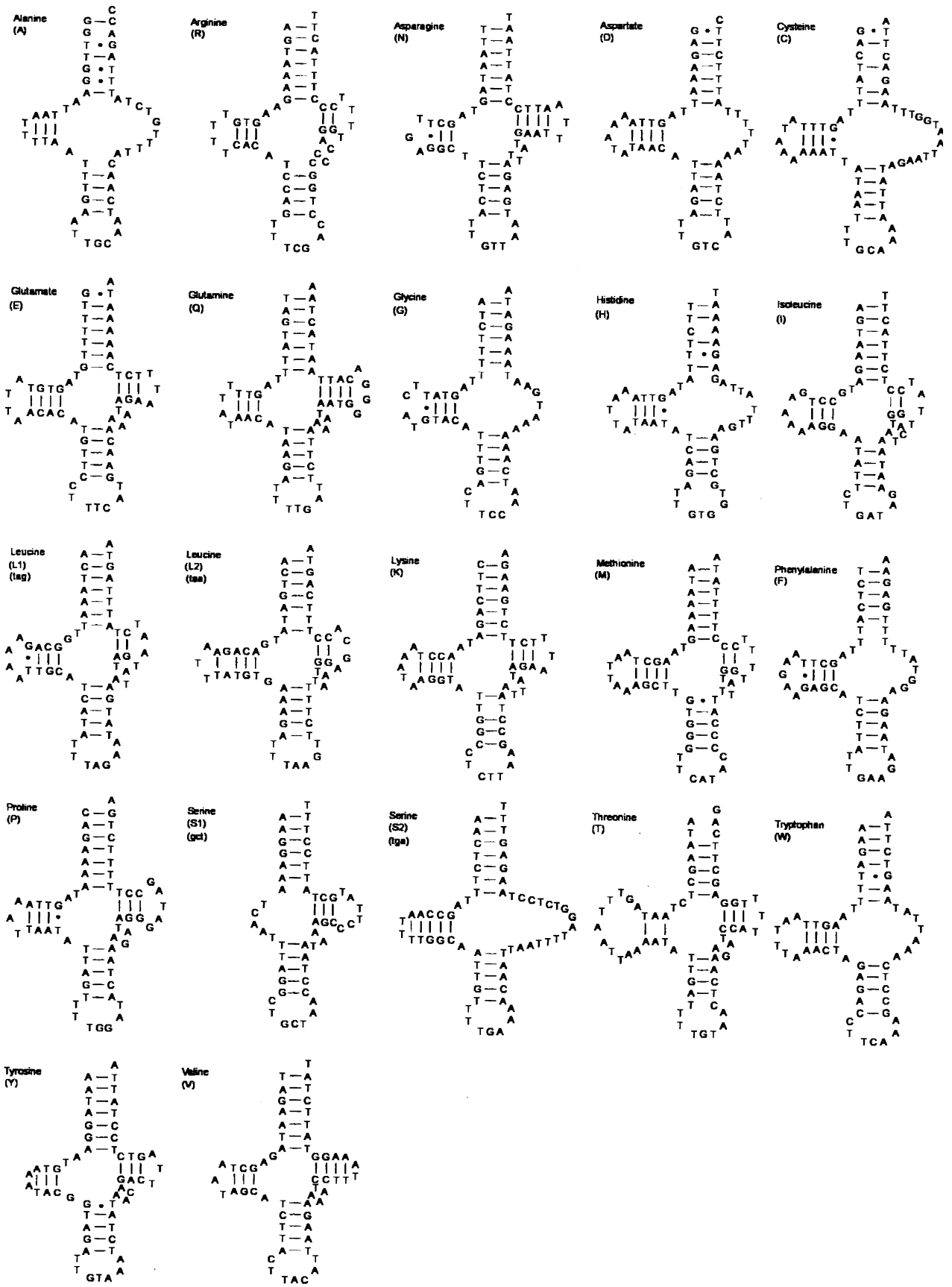

Fig. 4. Anticipated secondary structures of the 22 inferred tRNAs of *Heptathela hangzhouensis*. AT/GC pairs are indicated by dashes, and GT pairs are indicated by dots.

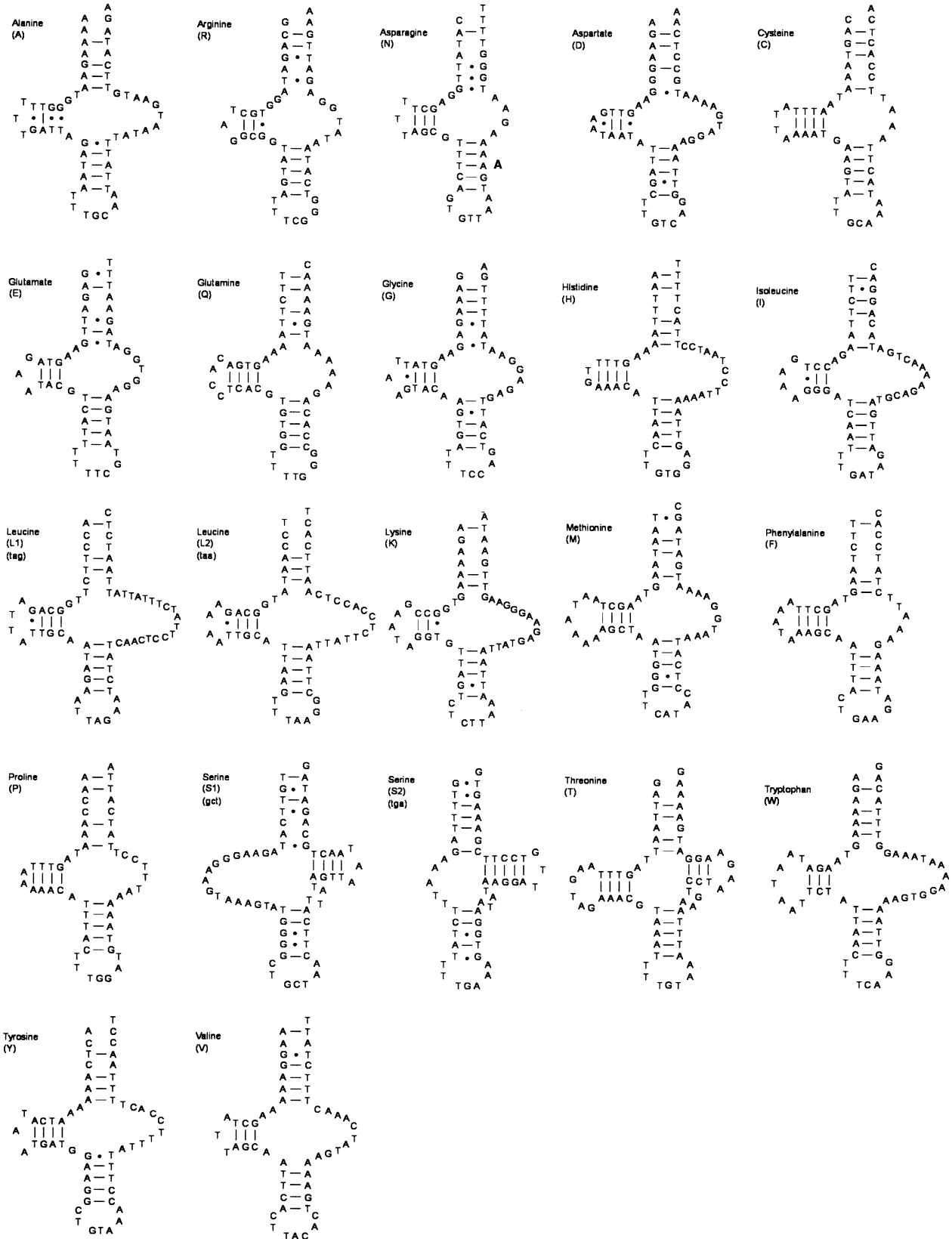

Fig. 5. Anticipated secondary structures of the 22 inferred tRNAs of *Ornithoctonus huwena*. AT/GC pairs are indicated by dashes, and GT pairs are indicated by dots.

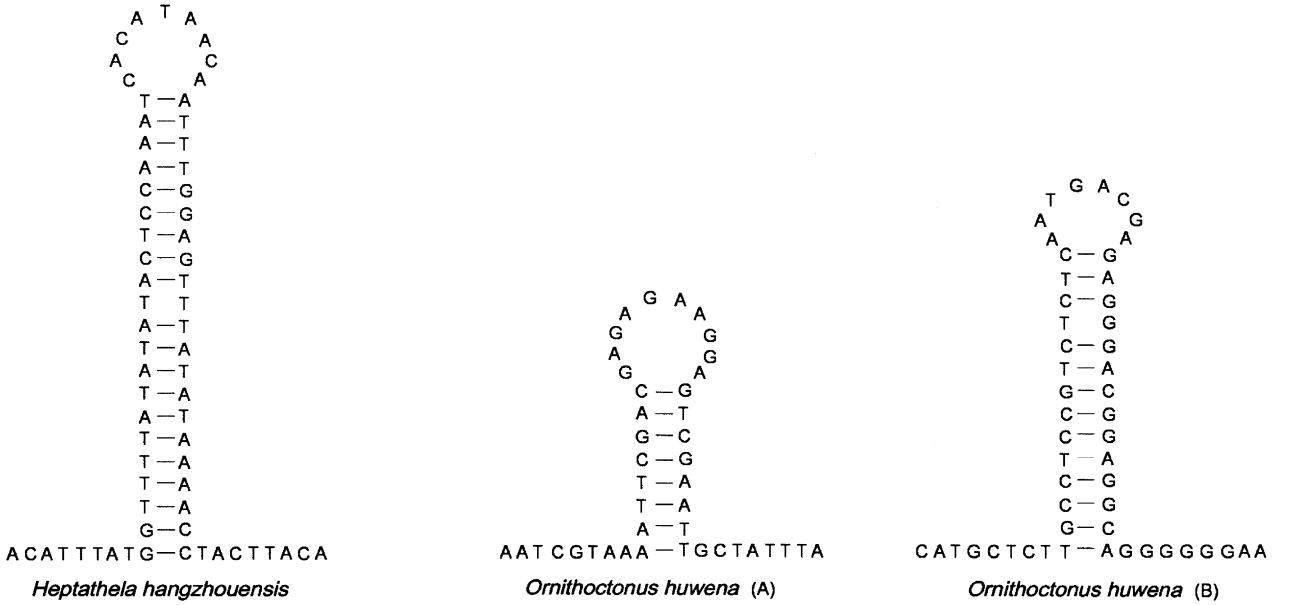

Fig. 6. The potential stem–loop structures in the large noncoding regions of *Heptathela hangzhouensis* and *Ornithoctonus huwena*. **A** The potential stem–loop structure close to *rrnS* in *O. huwena*. **B** The other stem–loop structure in *O. huwena* near the 3′ flanking of the 26-nt stem–loop structure.

Arthropoda (Mitchell et al. 1993; Hedin 1997b; Masta 2000; Lavrov et al. 2000b; Hormiga et al. 2003). It is most important that the tRNA secondary structures proposed for the two species may not be unusual among spiders. Mitochondrial sequences for $tRNA^{Leu(CUN)}$ and $tRNA^{val}$ for Salticidae (Masta 2000) have been reported, and $tRNA^{Leu(CUN)}$ for Nesticidae has been published by Hedin (1997b). All of them can be folded into secondary structures lacking the TΨC arm. Both Salticidae and Nesticidae are in the derived suborder Araneomorphae of the order Araneae; we found that the mygalomorph spider and the mesothelid spider share this attribute. Our observations from the mygalomorph spider and the mesothelid spider further enlarged this family of tRNAs.

Large Noncoding Region

The longest noncoding region in *H. hangzhouensis* mtDNA is 340 bp. It is located between *rrnS* and *trnI* and is significantly more AT-rich (80.6%) than the rest of the genome as previously reported in *Limulus*. Within this region, a 51-nt sequence close to *rrnS* has the potential to form a stem-loop structure with a 21-bp stem and a 9-bp loop (Fig. 6). In *O. huwena*, the largest noncoding region is 396 bp long. It is located between *trnQ* and *trnM* and is 68.2% A + T, which is slightly less AT-rich than the rest of the genome. The absence of such a higher A + T content had also been found in four mites (Navajas et al. 2002), the hexapod *Triatoma dimidiata* (Reduviidae) (Dotson and Beard 2001), and the myriapod *Thyropygus* sp. (Harpagophoridae) (Lavrov et al. 2002). These comparatively lower A + T contents may imply a higher efficiency of enzymatic complex involved in the DNA denaturation before replication–transcription (Navajas et al. 2002). Within *O. huwena*, a 26-nt sequence close to *rrnS* has the potential to form a stem–loop structure with an 8-bp stem and a 10-bp loop (Fig. 6A). The other stem–loop structure may exist in *O. huwena* (Fig. 6B) with a 14-nt stem and an 8-nt loop, to the 3′ flanking of the 26-nt stem–loop structure. A feature of the sequences of stem–loop structures in the two species is the ability to form a secondary structure that is less stable. The function of the region, if any, is unknown. However, similar stem–loop structures in the region have been shown to initiate replication in vertebrates (Sumida et al. 2001). Whether the large noncoding regions in spiders serving the same function remains elusive.

Discussion

Gene Rearrangement

In general, a large number of possible gene rearrangements make it improbable that the same order would arise independently; shared–derived arrangements most likely indicate common ancestry (Boore et al. 1995). Thus, gene rearrangement can also be used as an effective parameter to establish phylogenetic relationships.

Moritz (1987) proposed the most commonly recognized model for gene order change. Figure 7 shows

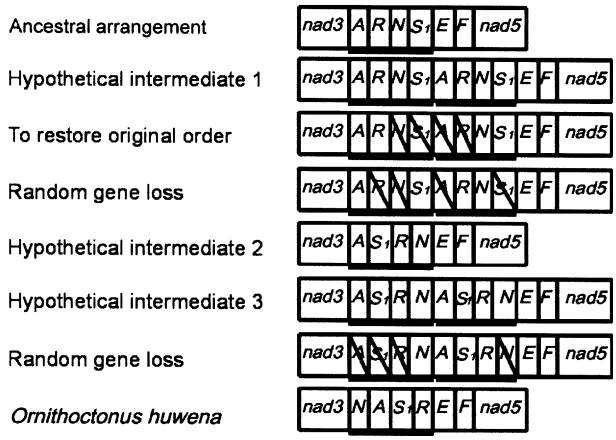

Fig. 7. Proposed mechanism of gene rearrangements in *Ornithoctonus huwena*, compared with the arrangement in *Limulus polyphemus* and *Heptathela hangzhouensis*. The hypothetical duplication of the *trnA–trnR–trnN–trnS1* and *trnA–trnS1–trnR–trnN* that could have predated the rearrangement, and the paths of gene loss from this hypothetical duplication that would lead to a return to the original order or that found in *O. huwena*.

a model modified for the gene rearrangement (*trnN–trnA–trnS1–trnR*) of *O. huwena*, compared with the arrangement in *Limulus* and *H. hangzhouensis*. The mechanism of the rearrangement (*trnW–trnY–trnC*) is similar to what took place in *trnN–trnA–trnSl–trnR* (genes underlined are transcribed in the reverse direction). First, the portions of the mitochondrial genome may have become duplicated, perhaps by slipped-strand mispairing or by erroneous termination of replication (Boore and Brown 1998). Next, one copy of each of the duplicated genes is lost by deletion. Depending on which copy of these genes is lost, the original arrangement may be restored or a rearrangement may occur. Three transpositions (*trnL2*, *trnT*, and *trnQ*) may take place by a duplication of a large region followed by loss of all but one or a few genes in a single or several deletion events (Lavrov et al. 2002). The inverted *trnI* may have arisen by local small inversion, a kind of intramitochondrial recombination, necessarily invoking breakage and rejoining of the mitochondrial genome (Dowton and Austin 1999). The finding of subgenomic minicircle in a nematode (Lunt and Hyman 1997) indicates the presence of such intramitochondrial recombination.

To date, seven complete mtDNA gene arrangements have been published for Chelicerata. The gene arrangements in *Ixodes hexagonus* (Arachnida: Ixodida: Ixodidae) (AF081828), *Ixodes persulcatus* (Arachnida: Ixodida: Ixodidae) (AB073725), and *Ornithodoros moubata* (Arachnida: Ixodida: Argasidae) (AB073679) are identical to those in *Limulus* and *H. hangzhouensis*. In an other two species of Ixodidae, *Rhipicephalus sanguineus* (Arachnida: Ixodida: Ixodidae) (AF081829) and *Boophilus microplus* (Arachnida: Ixodida: Ixodidae) (AH007623), an eight-gene block has been translocated to the region between *nad3* and *nad5* (Fig. 8), *trnC* has reversed to the region between *trnL1* and *trnM*, and *trnL1* has been translocated to the region between *trnS2* and *trnC* (Black and Roehrdanz 1998; Campbell and Barker 1998) (Fig. 8). Navajas et al. (2002) have found translocations of *rrnS*, *trnQ*, *trnY*, *trnP* and *trnC* and translocations and inversions of *trnH* and *trnS2* in *Varroa destructor* (Arachnida: Mesostigmata: Varroidae) (AJ493124) (Fig. 8). These results, along with our data, revealed four different mitochondrial gene arrangements in chelicerates. The rearrangement of an eight-gene block in *R. sanguineus* and *B. microplus* and the three tRNA rearrangements in *O. huwena* were found between *nad3* and *nad5*. This may imply that the region between *nad3* and *nad5* is a hot spot of rearrangement in chelicerates. Another hot spot is around the large noncoding region such as in many vertebrates. As Boore (1999) suggested, the most common changes in arthropods are genes near the large noncoding region or the tRNAs in the region between *nad3* and *nad5* that is *trnA–trnR–trnN–trnS1–trnE–trnF* in *Drosophila*. Because rearrangements often occur near the region where replication is initiated by erroneous termination of replication, provided that the large noncoding region probably initiates replication, we suggest that the second-strand origin in arthropod mtDNA is perhaps near the region between *nad3* and *nad5* (Boore 1999), while the first-strand origin is like around the large noncoding region.

We also studied the phylogenetic relationship among the three high-level taxa of Araneae using the partial mitochondrial gene rearrangement. The *rrnL–trnL1–trnL2–nad1* arrangement shared by *Limulus* and the mesothelid spider *H. hangzhouensis* is thought to be ancestral for arthropods (Boore et al. 1995). In the mygalomorph spider *O. huwena*, *trnL2* has been translocated to the position between *trnN* and *nad3* and exhibits an *rrnL–trnL1–nad1* arrangement. The latter arrangement is also shared by araneomorph taxa: *Habronattus* sp. of Salticidae (Masta 2000), *Nesticus* sp. of Nesticidae (Hedin 1997b), and *Orsonwelles* sp. of Linyphiidae (Hormiga et al. 2003). The *rrnL–trnL1–nad1* arrangement therefore is a putative synapomorphy joining the Mygalomorphae with the Araneomorphae. However, the location of the *trnL2* in the two araneomorphs is still unknown; further study on the location *trnL2* in the two taxa is needed.

Transfer RNA

Masta (2000) first discovered the modified truncated structure of spiders tRNAs. Presumably, the tRNAs lacking the TψC arm in Araneae have certain tertiary

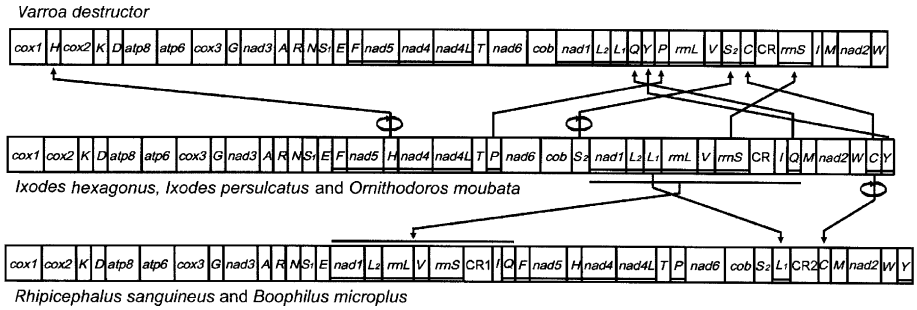

Fig. 8. Comparisons of gene arrangements in mtDNAs of six chelicerates (*Ixodes hexagonus*, *Ixodes persulcatus*, *Ornithodoros moubata*, *Rhipicephalus sanguineus*, *Boophilus microplus*, and *Varroa destructor*). Genes are labeled just as in Fig. 2.

interactions to maintain their functionality (Masta 2000). Kumazawa et al. (1996) proposed that selective pressure to shorten the TψC arms was balanced by selective pressure to maintain TψC loops because of functional interaction with DHU loops. Even for nematode tRNAs completely lacking the TψC arm, Watanabe et al. (1994) had shown evidence of tertiary interactions between the TV-replacement loop and the DHU arm, suggesting that these tRNAs maintain their functionality. On the other hand, Ohtsuki et al. (2001, 2002) found the atypical EF-Tul to be special for nematode mitochondrial tRNAs that lack the TψC arm and the EF-Tu2 to bind only to tRNAs that lack the DHU arm. The two translation elongation factor Tu (EF-Tu) homologues may suggest the co-evolution of a structurally simplified RNA and the cognate RNA-binding protein and may explain the structural divergence in animal mitochondrial tRNAs such as these special tRNAs in Araneae. Based on the co-evolutionary viewpoint, there might be special tRNA-binding proteins in Araneae to make tRNAs lacking the TΨC arm or DHU arm functional.

Among the tRNAs of the two spiders, only 14 inferred tRNA genes in *H.hangzhouensis* encode a fully paired aminoacyl acceptor stem. The other 30 genes encode tRNAs with up to four mismatches in the aminoacyl acceptor stem, as previously reported for spiders tRNAs, with up to three mismatches (Hedin 1997b; Masta 2000), and centipede tRNAs, with up to five mismatches in the aminoacyl acceptor stem (Lavrov et al. 2000b). Because a well-paired aminoacyl acceptor stem is required for proper tRNA functioning, Lavrov et al. (2000b) studied cDNA sequences from tRNAs of *Lithobius forficatus* and found the editing of up to 5 nt at their 3' ends. This editing appears to occur by a novel mechanism, with the 5' end of the aminoacyl acceptor arm being used as a template for the *de novo* synthesis of the 3' end, presumably by an RNA-dependent RNA polymerase. Therefore, tRNAs from the two spiders we have analyzed may also undergo posttranscriptional processing to acquiring their functionality. Such post-transcriptional editing mechanisms (Yokobori and Pääbo 1995a, b; Lavrov et al. 2000b) may correct potential mismatches in the aminoacyl acceptor stems.

The extensive overlaps with the downstream genes in *O. huwena* also occurred in *L. forficatus* (Lavrov et al. 2000b), but the overlaps in *O. huwena* are much longer than those of *L. forficatus*. The excision of the unedited pre-tRNAs from polycistronic transcript must be an active process and the enzymes involved should recognize the unusual secondary structures formed by these sequences. When overlaps of tRNA genes are excessively long, excisions at different points may yield one or the other functional tRNA in a mutually exclusive manner.

Mitochondrial genome may be particularly susceptible to accumulation of mutations, and mitochondrial tRNAs have been shown to accumulate deleterious mutations relative to their nuclear tRNA counterparts (Lynch 1996). Macey et al. (1997) proposed that replication slippage might cause loss of tRNA arms. Yamazaki et al. (1997) suggested that mt-tRNA genes would have changed their secondary structure due to reduction of their chain lengths under the pressure for genome minimization. The truncated structures of the tRNAs from our study provide additional support to these ideas.

While this paper was under review, a paper reporting complete mitochondrial genome of the spider *Habronattus oregonensis* was published. In that paper, Masta and Boore (2004) revealed seven rearranged tRNAs, the extremely truncated tRNAs, and the overlaps among many of the mitochondrial gene sequences. The rearrangements in *H. oregonensis* occurred similarly as in *O. huwena*, except for *trnI* being translocated to a different position compared with those of *O. huwena*. Because both *O. huwena* and *H. oregonensis* are mygalomorph spiders and share most rearrangements, their finding appears to be consistent with our findings that the rearrangements join the mygalomorph with the araneomorph and that Mesothelae is basal to opisthothelids. In *H. or-*

egonensis, most tRNAs are extremely truncated, and many of the gene sequences overlap with one another and are truncated as in *O. huwena*.

Acknowledgments. The authors wish to thank Fengxiang Liu of Hubei University for providing the specimens of *H. hangzhouensis*. This work was supported by the National Natural Science Foundation of China (Key Project No. 30130040).

References

Arnedo MA, Coddington J, Agnarsson I, Gillespie RG (2004) From a comb to a tree: Phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes. Mol Phylogenet Evol 31(1):225–245

Berthier F, Renaud M, Alziari S, Durand R (1986) RNA mapping on *Drosophila* mitochondrial DNA: Precursors and template strands. Nucleic Acids Res 14(11):4519–4533

Black WC IV, Roehrdanz RL (1998) Mitochondrial gene order is not conserved in arthropods: Prostriate and metastriate tick mitochondrial genomes. Mol Biol Evol 15(12):1772–1785

Bond JE, Hedin MC, Ramirez MG, Opell BD (2001) Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider *Aptostichus simus*. Mol Ecol 10(4):899–910

Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Res 27(8):1767–1780

Boore JL, Brown WM (1998) Big trees from little genomes: Mitochondrial gene order as a phylogenetic tool. Curr Opin Genet Dev 8(6):668–674

Boore JL, Collins TM, Stanton D, Daehler LL, Brown WM (1995) Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. Nature 376(6536):163–165

Campbell NJ, Barker SC (1998) An unprecedented major rearrangement in an arthropod mitocondrial genome. Mol Biol Evol 15(12):1786–1787

Castresana J, Feldmaier-Fuchs G, Yokobori S, Satoh N, Pääbo S (1998) The mitochondrial genome of the hemichordate *Balanoglossus carnosus* and the evolution of deuterostome mitochondria. Genetics 150(3):1115–1123

Coddington JA, Levi HW (1991) Systematics and evolution of spiders (Araneae). Annu Rev Ecol Syst 22:565–592

Crease TJ (1999) The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). Gene 233(1–2):89–99

Crozier RH, Crozier YC (1993) The mitochondrial genome of the honeybee *Apis mellifera*: Complete sequence and genome organization. Genetics 133(1):97–117

Dotson EM, Beard CB (2001) Sequence and organization of the mitochondrial genome of the Chagas disease vector, *Triatoma dimidiata*. Insect Mol Biol 10(3):205–215

Dowton M, Austin AD (1999) Evolutionary dynamics of a mitochondrial rearrangement "hot spot" in the Hymenoptera. Mol Biol Evol 16(2):298–309

Fang K, Yang CC, Lue BW, Chen SH, Lue KY (2000) Phylogenetic corroboration of superfamily Lycosoidae spiders (Araneae) as inferred from partial mitochondrial 12S and 16S ribosomal DNA sequences. Zool Stud 39(2):107–113

Fearnley IM, Walker JE (1986) Two overlapping genes in bovine mitochondrial DNA encode membrane components of ATP synthase. EMBO J 5(8):2003–2008

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for ampliation of mitochondrial cytochrome coxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3(5):294–299

Gillespie RG, Croom HB, Palumbi SR (1994) Multiple origins of a spider radiation in Hawaii. Proc Natl Acad Sci USA 91(6):2290–2294

Giribet G, Edgecombe GD, Wheeler WC, Babbitt C (2002) Phylogeny and systematic position of Opiliones: a combined analysis of chelicerate relationships using morphological and molecular data. Cladistics 18(1):5–70

Hedin MC (1997a) Molecular phylogenetics at the population/species interface in cave spiders of the southern Appalachians (Araneae: Nesticidae: *Nesticus*). Mol Biol Evol 14(3):309–324

Hedin MC (1997b) Speciational history in a diverse clade of habitat-specialized spiders (Araneae: Nesticidae: *Nesticus*): Inferences from geographic-based sampling. Evolution 51(6):1927–1943

Hedin MC (2001) Molecular insights into species phylogeny, biogeography, and morphological stasis in the ancient spider genus *Hypochilus* (Araneae: Hypochilidae). Mol Phylogenet Evol 18(2):238–251

Hedin MC, Maddison WP (2001) A combined molecular approach to phylogeny of the jumping spider subfamily dendryphantinae (Araneae: Salticidae). Mol Phylogenet Evol 18(3):386–403

Hormiga G, Arnedo M, Gillespie RG (2003) Speciation on a conveyor belt: sequential colonization of the Hawaiian islands by *Orsonwelles* spiders (Araneae, Linyphiidae). Syst Biol 52(l):70–88

Hwang UW, Park CJ, Yong TS, Kirn W (2001) One-step PCR amplification of complete arthropod mitochondrial genomes. Mol Phylogenet Evol 19(3):345–352

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: Molecular evolutionary genetics analysis software. Bioinformatics 17(12):1244–1245

Kumazawa Y, Ota H, Nishida M, Ozawa T (1996) Gene rearrangements in snake mitochondrial genomes: highly concerted evolution of control-region-like sequences duplicated and inserted into a tRNA gene cluster. Mol Biol Evol 13(9):1242–1254

Lavrov DV, Boore JL, Brown WM (2000a) The complete mitochondrial DNA sequence of the horseshoe crab *Limulus polyphemus*. Mol Biol Evol 17(5):813–824

Lavrov DV, Brown WM, Boore JL (2000b) A novel type of RNA editing occurs in the mitochondrial tRNAs of the centipede *Lithobius forficatus*. Proc Natl Acad Sci USA 97(25):13738–13742

Lavrov DV, Boore JL, Brown WM (2002) Complete mtDNA sequences of two millipedes suggest a new model for mitochondrial gene rearrangements: Duplication and nonrandom loss. Mol Biol Evol 19(2):163–169

Lunt DH, Hyman BC (1997) Animal mitochondrial DNA recombination. Nature 387(6630):247

Lynch M (1996) Mutation accumulation in transfer RNAs: Molecular evidence for Muller's ratchet in mitochondrial genomes. Mol Biol Evol 13(1):209–220

Macey JR, Larson A, Ananjeva NB, Papenfuss TJ (1997) Replication slippage may cause parallel evolution in the secondary structures of mitochondrial transfer RNAs. Mol Biol Evol 14(1):30–39

Maddison WP, Hedin MC (2003) Jumping spider phylogeny (Araneae: Salticidae). Invertebr Syst 17:529–549

Masta SE (2000) Mitochondrial sequence evolution in spiders: Intraspecific variation in tRNAs lacking the TψC arm. Mol Biol Evol 17(7):1091–1100

Masta SE, Boore JL (2004) The complete mitochondrial genome sequence of the spider *Habronattus oregonensis* reveals rearranged and extremely truncated tRNAs. Mol Biol Evol 21(5):893–902

Mitchell SE, Cockburn AF, Seawright JA (1993) The mitochondrial genome of *Anopheles quadrimaculatus* species A: Complete

nucleotide sequence and gene organization. Genome 36(6): 1058–1073

Monnerot M, Solignac M, Wolstenholme DR (1990) Discrepancy in divergence of the mitochondrial and nuclear genomes of *Drosophila teissieri* and *Drosophila yakuba*. J Mol Evol 30(6):500–508

Moritz C, Dowling TE, Brown WM (1987) Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. Annu Rev Ecol Syst 18:269–292

Navajas M, Le Conte Y, Solignac M, Cros-Arteil S, Cornuet JM (2002) The complete sequence of the mitochondrial genome of the honeybee ectoparasite mite *Varroa destructor* (Acari: Mesostigmata). Mol Biol Evol 19(12):2313–2317

Ohtsuki T, Watanabe YI, Takemoto C, Kawai G, Ueda T, Kita K, Kojima S, Kaziro Y, Nyborg J, Watanabe K (2001) An "elongated" translation elongation factor Tu for truncated tRNAs in nematode mitochondria. J Biol Chem 276(24):21571–21577

Ohtsuki T, Sato A, Watanabe Y, Watanabe K (2002) A unique serine-specific elongation factor Tu found in nematode mitochondria. Nat Struct Biol 9(9):669–673

Okimoto R, Wolstenholme DR (1990) A set of tRNAs that lack either the TΨC arm or the dihydrouridine arm: Towards a minimal tRNA adaptor. EMBO J 9:3405–3411

Okimoto R, Macfarlane JL, Clary DO, Wolstenholme DR (1992) The mitochondrial genomes of two nematodes, *Caenorhabditis elegans* and *Ascaris suum*. Genetics 130:471–498

Ojala D, Merkel C, Gelfand R, Attardi G (1980) The tRNA genes punctuate the reading of genetic information in human mitochondrial DNA. Cell 22:393–403

Ojala D, Montoya J, Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondria. Nature 290 (5806):470–474

Perna NT, Kocher TD (1995) Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J Mol Evol 41:353–358

Piel WH, Nutt KJ (2000) One species or several? Discordant patterns of geographic variation between allozymes and mtDNA sequences among spiders in the genus *Metepeira* (Araneae: Araneidae). Mol Phylogenet Evol 15(3):414–418

Platnick NI (2004) The world spider catalog, version 3.5. 2004. The world spider catalog, version 4.5. American Museum of Natural History; http://research.amnh.org/entomology/spiders/ catalog/ INTR01.html

Platnick NI, Gertsch WJ (1976) The suborders of spiders: A cladistic analysis (Arachnida, Araneae). Am Mus Novit 2607:1–15

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–701

Smith AE, Marcker KA (1968) N-Formylmethionyl transfer RNA in mitochondria from yeast and rat liver. J Mol Biol 38:241–243

Sumida M, Kanamori Y, Kaneda H, Kato Y, Nishioka M, Hasegawa M, Yonekawa H (2001) Complete nucleotide sequence and gene rearrangement of the mitochondrial genome of the Japanese pond frog *Rana nigromaculata*. Genes Genet Syst 76:311–325

Taanman JW (1999) The mitochondrial genome: Structure, transcription, translation and replication. Biochim Biophys Acta 1410:103–123

Tanaka M, Ozawa T (1994) Strand asymmetry in human mitochondrial DNA mutations. Genomics 22(2):327–335

Terrett JA, Miles S, Thomas RH (1996) Complete DNA sequence of the mitochondrial genome of *Cepaea nemoralis* (Gastropoda: Pulmonata). J Mol Evol 42(2):160–168

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Vink CJ, Paterson AM (2003) Combined molecular and morphological phylogenetic analyses of the New Zealand wolf spider genus *Anoteropsis* (Araneae: Lycosidae). Mol Phylogenet Evol 28:576–587

Vink CJ, Mitchell AD, Paterson AM (2002) A preliminary molecular analysis of phylogenetic relationships of Australasian wolf spider genera (Araneae, Lycosidae). J Arachnol 30:227–237

Watanabe YI, Tsurui H, Ueda T, Furushima R, Takamiya S, Kita K, Nishikawa K, Watanabe K (1994) Primary and higher order structures of nematode (*Ascaris suum*) mitochondrial tRNAs lacking either the T or D stem. J Biol Chem 269:22902–22906

Wolstenholme DR (1992) Animal mitochondrial DNA: Structure and evolution. Int Rev Cytol 141:173–216

Wolstenholme DR, Macfarlane JL, Okimoto R, Clary DO, Wahleithner JA (1987) Bizarre tRNAs inferred from DNA sequences of mitochondrial genomes of nematode worms. Proc Natl Acad Sci USA 84(5):1324–1328

Yamazaki N, Ueshima R, Terrett JA, et al. (1997) Evolution of pulmonate gastropod mitochondrial genomes: Comparisons of gene organizations of *Euhadra, Cepaea* and *Albinaria* and implications of unusual tRNA secondary structures. Genetics 145(3):749–758

Yokobori S, Pääbo S (1995a) tRNA editing in metazoans. Nature 377(6549):490

Yokobori S, Pääbo S (1995b) Transfer RNA editing in land snail mitochondria. Proc Natl Acad Sci USA 92(22):10432–10435

Zehethofer K, Sturmbauer C (1998) Phylogenetic relationships of Central European wolf spiders (Araneae: Lycosidae) inferred from 12S ribosomal DNA sequences. Mol Phylogenet Evol 10(3):391–398