

The complete mitochondrial genome of the blue-tailed damselfly *Ischnura elegans* (Odonata: Coenagrionidae)—a climate-sensitive indicator species in South Korea

Seung Hyun Lee¹, Jeong Sun Park¹, Jee-Young Pyo¹, Sung-Soo Kim², and Iksoo Kim^{1,*}

¹Department of Applied Biology, College of Agriculture & Life Sciences, Chonnam National University, Gwangju 61186, Republic of Korea

²Research Institute for East Asian Environment and Biology, Seoul 05207, Republic of Korea

Abstract

The blue-tailed damselfly, *Ischnura elegans* Van der Linden, 1820 (Odonata: Coenagrionidae), is a climate-sensitive indicator species in South Korea. In this study, we sequenced the complete mitochondrial genome (mitogenome) of *I. elegans* collected from South Korea for subsequent population genetic analysis, particularly to trace population movements in response to climate change. The 15,963 base pair (bp)-long complete mitogenome of *I. elegans* has typical sets of genes including a major non-coding region (the A+T-rich region), and an arrangement identical to that observed in ancestral insect species. The *ATP6*, *ND3* and *ND1* genes have the TTG start codon, which, although rare, is the canonical start codon for animal mitochondrial tRNA. The A/T content was 71.4% in protein-coding genes, 72.1% in tRNAs, 72.9% in the whole genome, 74.7% in *srRNA*, 75.3% in *lrRNA*, and 83.8% in the A+T-rich region. The A+T-rich region is unusually long (1,196 bp) and contains two subunits (192 bp and 176–165 bp), each of which is tandemly triplicated and surrounded by non-repeat sequences. Comparison of the sequence divergence among available mitogenomes of *I. elegans*, including the one from the current study, revealed *ND2* as the most variable gene, followed by *COII* and *COI*, suggesting that *ND2* should be targeted first in subsequent population-level studies. Phylogenetic reconstruction based on all available mitogenome sequences of Coenagrionidae showed a strong sister relationship between *I. elegans* and *I. senegalensis*.

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Int. J. Indust. Entomol. 46(2), 41–54 (2023)

Received : 3 Apr 2023
Revised : 22 May 2023
Accepted : 31 May 2023

Keywords:

Ischnura elegans,
Mitochondrial genome,
Coenagrionidae

Introduction

The blue-tailed damselfly, *Ischnura elegans* Van der Linden, 1820 (Odonata: Coenagrionidae), is widely distributed in Europe and Asia (Dijkstra and Lewington, 2006; Kim *et al.*, 2020). It inhabits ponds, lowland marshes rich in aquatic plants and organic deposits, and standing and slow-flowing waters (Jung,

2012). Adults of this species are observed in Korea during May–September (Jung, 2012).

The species is popular for scientific studies because it displays adult dimorphism and hybridization with other species and has been nominated as a climate change-sensitive species. *Ischnura elegans* has three color morphs in Europe—a male-like and two non-male-like colors (Sánchez-Guillén *et al.*, 2005). In Korea,

*Corresponding author.

Iksoo Kim, Ph.D.

Department of Applied Biology, College of Agriculture & Life Sciences, Chonnam National University, Gwangju 61186, Republic of Korea

Tel: +82-62-530-5117 / FAX: +82-62-530-2069

E-mail: ikkim81@chonnam.ac.kr

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two female morphs, one that has a blue color similar to the male and the other that has a green color at the eighth abdominal segment, have been reported (Jung, 2007). *Ischnura elegans*, which is ecologically, morphologically, and genetically similar to *I. graellsii*, hybridizes with *I. graellsii* in Spain, where the two species have overlapping distributions (Sánchez-Guillén *et al.*, 2011). Moreover, *I. elegans* has extended its range in Europe in accordance with climate change during the last 10 years (Hickling *et al.*, 2005; Wellenreuther *et al.*, 2011).

In Korea, *I. elegans* is listed as a climate-sensitive biological indicator species (National Institute of Biological Resources, 2017). The available records indicate that *I. elegans* is mostly present in the middle region of South Korea; however, a recent field observation indicated the presence of *I. elegans* in Muju, which is rather South of the previously recorded spots (Kim *et al.*, 2020). Nevertheless, considering the range shift of *I. elegans* in Europe, the South Korean populations are also expected to move northward and become more abundant in the northern regions of South Korea in the future (Hickling *et al.*, 2005; Wellenreuther *et al.*, 2011).

Recently, molecular marker-based studies on the range-shifting damselflies have popularly been conducted for diverse purposes, such as understanding the adaptability of a species to new environments using single nucleotide polymorphisms (SNPs), studying the introgression between species with overlapping distributional ranges using microsatellite DNA and mitochondrial gene sequences, and tracing the changes in population genetic structure using the mitochondrial *COI* gene sequences (Sánchez-Guillén *et al.*, 2011; Troast *et al.*, 2016; Dudaniec *et al.*, 2018). Considering that *I. elegans* is a climate-sensitive species in South Korea, studies on Korean *I. elegans* populations are urgent. Therefore, we sequenced the complete mitochondrial genome (mitogenome) of *I. elegans* to accumulate genomic information and develop variable markers, which are informative in population genetic studies based on a comparison of available mitogenome sequences from different geographical regions (Feindt *et al.*, 2016; Song *et al.*, 2019).

Materials and Methods

Sample and sequencing

In 2019, an *I. elegans* adult female was collected from

Yongin City, Gyeonggi Province, Korea (37°18'14" N, 127°14'04" E). DNA was extracted from one of its legs using a commercial kit (Qiagen, Hilden, Germany), and three long overlapping fragments (*COI-ND4*, *ND5-lrRNA*, and *lrRNA-COI*) were amplified using the primers designed in this study based on sequence alignment of corresponding regions of available mitogenomes (Feindt *et al.*, 2016; Song *et al.*, 2019). The primer sequences used were LF1-F (5'-CAGGAATAGTAGGGACTGC-3') and LF1-R (5'-CATCTTTGATTACCAAAGGC-3') for *COI-ND4*; LF2-F (5'-AGCACTTTTAGTTATAGCAGC-3') and LF2-R (5'-ATTTATAGTACCTTTTGTATC-3') for *ND5-lrRNA*; LF3-F (5'-GGACATGTTTTTGATAAACA-3') and LF3-R (5'-AATTCCAATTGCAATTATAGC-3') for *lrRNA-COI*.

The PCR products were purified using a PCR purification Kit (Bioneer, Daejeon, Korea) and sequenced using the barcode-tagged sequencing technology (Celeomics, Inc., Seoul, Korea) and an Illumina MiSeq platform (Illumina, San Diego, CA, USA). The leftover tissue samples and DNA were deposited as voucher specimens at the Chonnam National University, Gwangju, Korea (Accession no. CNU11497).

Genome construction

Genome construction and the annotation of protein-coding genes (PCGs), tRNAs, rRNAs, and the A+T-rich region were performed through *de novo* assembly using a conspecies (Feindt *et al.*, 2016) and MITObim ver. 1.9 (Hahn *et al.*, 2013) following the protocols presented by Cameron (2014). Start and stop codons of the PCGs were further confirmed by alignment against two mitochondrial PCGs of *I. elegans* (Feindt *et al.*, 2016; Song *et al.*, 2019). However, conflicts in the boundary delimitation between public and current data were found in some places, such as the 3'-end of *ND5*, 5'-end of *ND1*, and both 3'- and 5'-ends of *srRNA*. In this case, further alignment with other species of *Ischnura* was performed for clarity (Lorenzo-Carballa *et al.*, 2014; Jiang *et al.*, 2021). The nucleotide sequences of the *Ischnura* PCGs were translated based on the invertebrate mitochondrial DNA genetic code.

Genome analysis

The A/T content of each gene and the whole genome was calculated using DNASTAR (Madison, Wisconsin, USA). The nucleotide divergence of 13 PCGs and two rRNAs among the three *I. elegans* mitogenomes, including the one from the current

study, was calculated based on unrooted pairwise distances estimated using PAUP ver. 4.01b10 (Swofford, 2002).

Phylogenetic analysis

Phylogenetic analysis was performed using the concatenated sequences of 13 PCGs and two rRNA genes (12,489 bp, excluding gaps) from all available mitogenome sequences in the Coenagrionidae family. The Bayesian inference (BI) method, which was implemented in CIPRES Portal v. 3.1 (Miller *et al.*, 2010), was used for phylogenetic analysis. For the analysis, two independent runs of four incrementally heated Markov and Monte Carlo chains (one cold chain and three hot chains) were simultaneously run for one million generations, with tree sampling conducted every 1,000 generations. The first 25% of the sampled trees were discarded as burn-in; the maximum running time was 48 h. The average of the split frequencies under 0.01 was the criterion to decide the reach of convergence from two simultaneous runs. Confidence values were obtained from the Bayesian posterior probabilities (BPPs). After comparing the Akaike Information Criterion (AIC) scores (Akaike, 1974) using Modeltest ver. 3.7 (Posada and Crandall, 1998), the general time-reversible (GTR) substitution model with gamma-distributed rate heterogeneity (G) and invariable sites (I) (GTR + I + G) was selected and applied to analyze BI.

Results and Discussion

General genomic characteristics

The 15,963 base pair (bp)-long complete mitogenome of *I. elegans* is composed of typical gene sets (two rRNAs, 22 tRNAs, and 13 PCGs) and a major non-coding A+T-rich region (GenBank acc. no. OQ693852; Table 1). Ten PCGs had the typical ATN start codon, whereas the *ATP6*, *ND3* and *ND1* genes had an atypical TTG codon. The TTG start codon is atypical, but has often been designated as an initiation codon for the mitochondrial genes of invertebrates; it has been detected in other Coenagrionidae species: *ATP6*, *ND3*, and *ND1* genes of *I. elegans* (KU958378; Feindt *et al.*, 2016), *I. asiatica* (OM310774; Jeong *et al.*, 2023), and *I. pumilio* (KC878732; Lorenzo-Carballa *et al.*, 2014); *ATP6* and *ND3* genes of *I. elegans* (MK951668; Song *et al.*, 2019) and *Paracercion v-nigrum* (MK951669; Song *et al.*, 2019); *ND3* and *ND1* genes of *I. senegalensis* (MT787567; Jiang *et al.*, 2021), *Enallagma cyathigerum* (MF716899; Zhang

et al., 2017), and *Ceriagrion fallax* (MW092110; Shao *et al.*, 2021). Furthermore, several insect groups, including Coleoptera, have been reported to have TTG as a start codon (Mitchell *et al.*, 1993; Bae *et al.*, 2004; Hong *et al.*, 2008). Eight PCGs ended with typical stop codons such as TAA and TAG, whereas *COI*, *COII*, *COIII*, *ND5*, and *ND4* ended with an incomplete one, presenting either only as a single thymine or TA (Table 1). The most common interpretation of this phenomenon is that the complete functional stop codon, TAA, is created via post-transcriptional polyadenylation (Ojala *et al.*, 1981).

The current *I. elegans* mitogenome has 93 bp-long intergenic spacer sequences interleaved in ten junctions, ranging in size from 1 bp (*trnN* – *trnS_I* and *ND1* – *trnL_I* junctions) to 39 bp (*trnY* – *COI*), and 40 bp-long overlapping sequences in 10 junctions, ranging in size from 1 bp (*trnQ* – *trnM*, *ATP6* – *COIII*, and *ND6* – *CytB*) to 13 bp (*ATP8* – *ATP6*) (Table 1). The gene arrangement of the *I. elegans* mitogenome is identical to that of all species in Coenagrionidae, including two conspecifics (data not shown).

The A/T content was 83.8% in the A+T-rich region, 75.3% in *lrRNA*, 74.7% in *srRNA*, 72.9% in the whole genome, 72.1% in tRNAs, and 71.4% in PCGs (Table 2). The two sequences of *I. elegans* from different geographical regions also have the same order of A/T content. However, other species of Coenagrionidae have the same order of A/T content in the A+T-rich region and PCGs, but the order differs in other types of genes (Table 2).

tRNAs

All tRNAs, except *trnS_I*, which lacks the DHU loop, fold into the expected cloverleaf secondary structure (Fig. 1). The incomplete *trnS_I* structure has been detected frequently in the mitogenomes of other animals, including insects (Wolstenholme, 1992; Kim *et al.*, 2022; Park *et al.*, 2022). The *I. elegans* tRNAs ranged from 62 bp (*trnR*) to 72 bp (*trnK*) in size (Table 1). All *I. elegans* tRNAs possessed an invariable 7 bp-long aminoacyl stem, 7 bp-long anticodon loop, and 5 bp-long anticodon stem, and most tRNA size variations resulted from length variations in the DHU and TΨC arms (Fig. 1). *Ischnura elegans* tRNAs have 37 unmatched bp, of which, 25 are G-U bp, while 12 are non-Watson-Crick bp (Fig. 1). The 12 non-Watson-Crick bp are distributed either in the anticodon stem (six) or aminoacyl acceptor stem (six). *trnS_I* has two non-Watson-Crick bp at the aminoacyl acceptor stem and one at the anticodon stem; *trnT* has two non-Watson-Crick bp at the aminoacyl stem; and *trnR* has

Table 1. Summary of *Ischnura elegans* mitochondrial genome.

Gene	Nucleotide number	Size	Anticodon	Start codon	Stop codon	O/S
<i>tml</i>	1-67	67	GAT 31-33			
<i>tmQ</i>	65-132	68	TTG 102-100			+3
<i>tmM</i>	132-200	69	CAT 162-164			+1
<i>ND2</i>	201-1196	996		ATT	TAA	
<i>tmW</i>	1195-1263	69	TCA 1225-1227			+2
<i>tmC</i>	1256-1321	66	GCA 1289-1287			+8
<i>tmY</i>	1322-1389	68	GTA 1358-1356			
<i>COI</i>	1429-2962	1534		ATG	T-tRNA	-39
<i>tmL₂</i>	2963-3028	66	TAA 2992-2994			
<i>COII</i>	3029-3716	688		ATG	T-tRNA	
<i>tmK</i>	3717-3788	72	CTT 3747-3749			
<i>tmD</i>	3789-3854	66	GTC 3819-3821			
<i>ATP8</i>	3855-4016	162		ATC	TAA	
<i>ATP6</i>	4004-4687	684		TTG	TAA	+13
<i>COIII</i>	4687-5473	787		ATG	T-tRNA	+1
<i>tmG</i>	5474-5538	65	TCC 5504-5506			
<i>ND3</i>	5539-5892	354		TTG	TAG	
<i>trnA</i>	5891-5957	67	TGC 5920-5922			+2
<i>trnR</i>	5958-6019	62	TCG 5986-5988			
<i>trnN</i>	6023-6089	67	GTT 6054-6056			-3
<i>trnS₁</i>	6091-6157	67	GCT 6117-6119			-1
<i>trnE</i>	6161-6227	67	TTC 6192-6194			-3
<i>trnF</i>	6226-6291	66	GAA 6259-6257			+2
<i>ND5</i>	6304-8020	1717		ATT	T-tRNA	-12
<i>trnH</i>	8021-8086	66	CAC 8053-8055			
<i>ND4</i>	8087-9429	1343		ATG	TA-tRNA	
<i>ND4L</i>	9423-9716	294		ATG	TAA	+7
<i>trnT</i>	9719-9784	66	TGT 9748-9750			-2
<i>trnP</i>	9795-9862	68	TGG 9830-9828			-10
<i>ND6</i>	9865-10380	516		ATC	TAA	-2
<i>CytB</i>	10380-11513	1134		ATG	TAA	+1
<i>trnS₂</i>	11514-11578	65	TGA 11543-11545			
<i>ND1</i>	11599-12549	951		TTG	TAA	-20
<i>tmL₁</i>	12551-12618	68	TAG 12589-12587			-1
<i>lrRNA</i>	12619-13903	1285				
<i>trnV</i>	13904-13974	71	TAC 13942-13940			
<i>srRNA</i>	13975-14767	793				
A+T-rich region	14768-15963	1196				

Gene names that are *not underlined* indicate a forward transcriptional direction, whereas *underlines* indicate a reverse transcriptional direction. tRNAs, except those encoding leucine and serine, are denoted as one-letter symbols in accordance with the IUPAC-IUB single-letter amino acid codes. O/S, number of overlapping (+)/intergenic spacer (-) sequences.

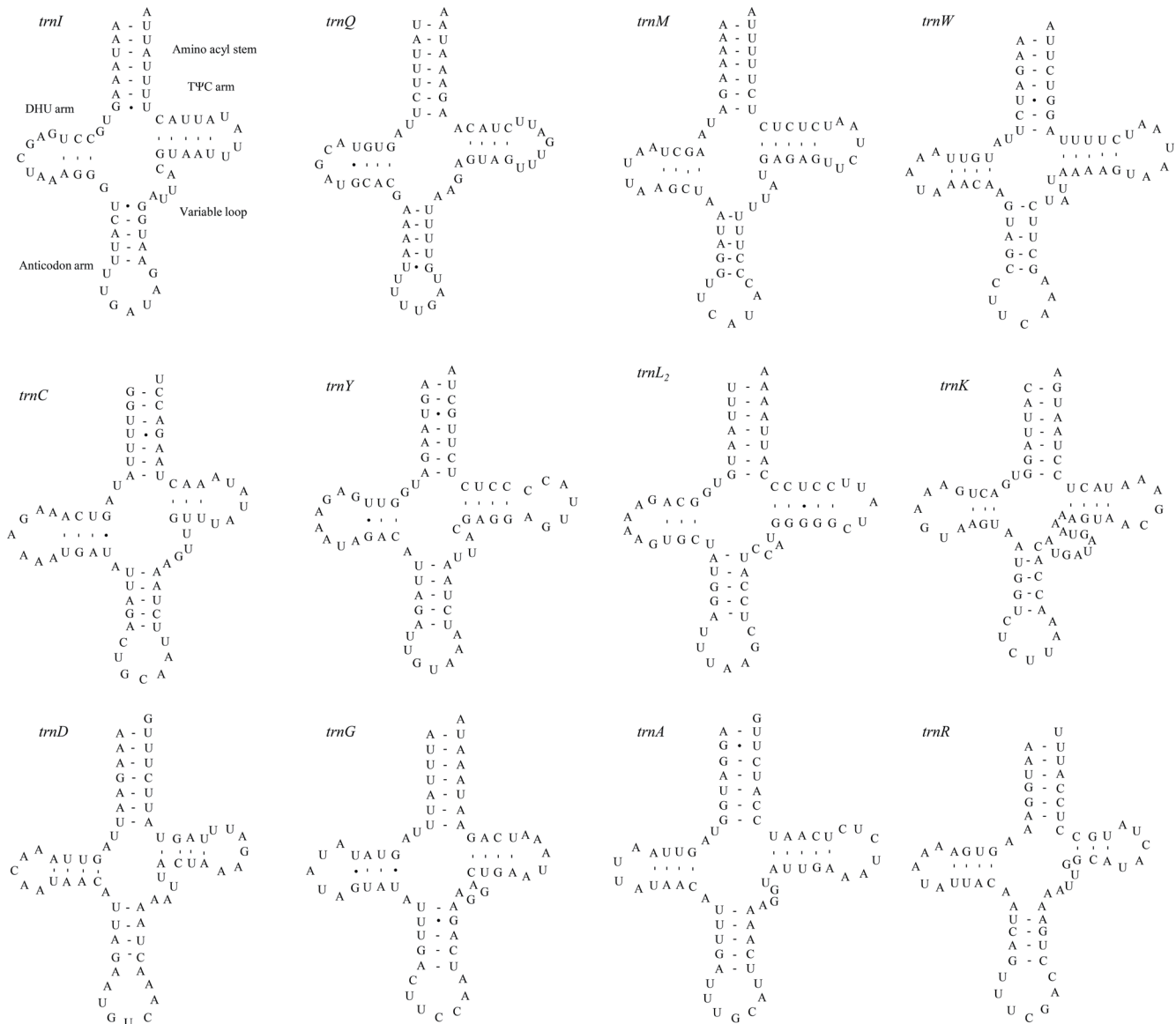


Fig. 1. Predicted secondary cloverleaf structures of the *Ischnura elegans* tRNA genes. Dashes (-) indicate Watson–Crick base pairing, and centered dots (•) indicate G-U base pairing. The tRNA arms in each image (clockwise from the top) are the amino acid acceptor arm, TΨC arm, anticodon arm, and dihydrouridine (DHU) arm.

one non-Watson–Crick bp at the aminoacyl acceptor stem and one at the anticodon stem. However, the majority of tRNAs do not have any non-Watson–Crick bp or only one such mismatch, indicating that *I. elegans* tRNAs have highly conserved stem regions.

A+T-rich region

The A+T-rich region of *I. elegans* is 1,196 bp long (Table 1) and identical to the mitogenome of *I. elegans* from northern Germany; however, it greatly differs from the mitogenome of *I. elegans* collected from China, which is only 255 bp long

(Table 2). A scrutinized examination of the A+T-rich regions of *I. elegans* from Korea and Germany revealed that they have identical lengths (1,196 bp) and are composed of two subunits (subunits A and B) with three tandem repeats each (Fig. 2A, 2B). The 192-bp long subunit A is composed of three tandem repeats, identical in sequence and length among repeats and between the two geographic samples of *I. elegans* (Fig. 2C). In contrast to the samples from Korea and Germany, the Chinese sample has only a single copy of each subunit, and the length of each subunit is substantially shorter: 76 bp in subunit A and 131 bp in subunit B (Fig. 2); however, no substitution was detected

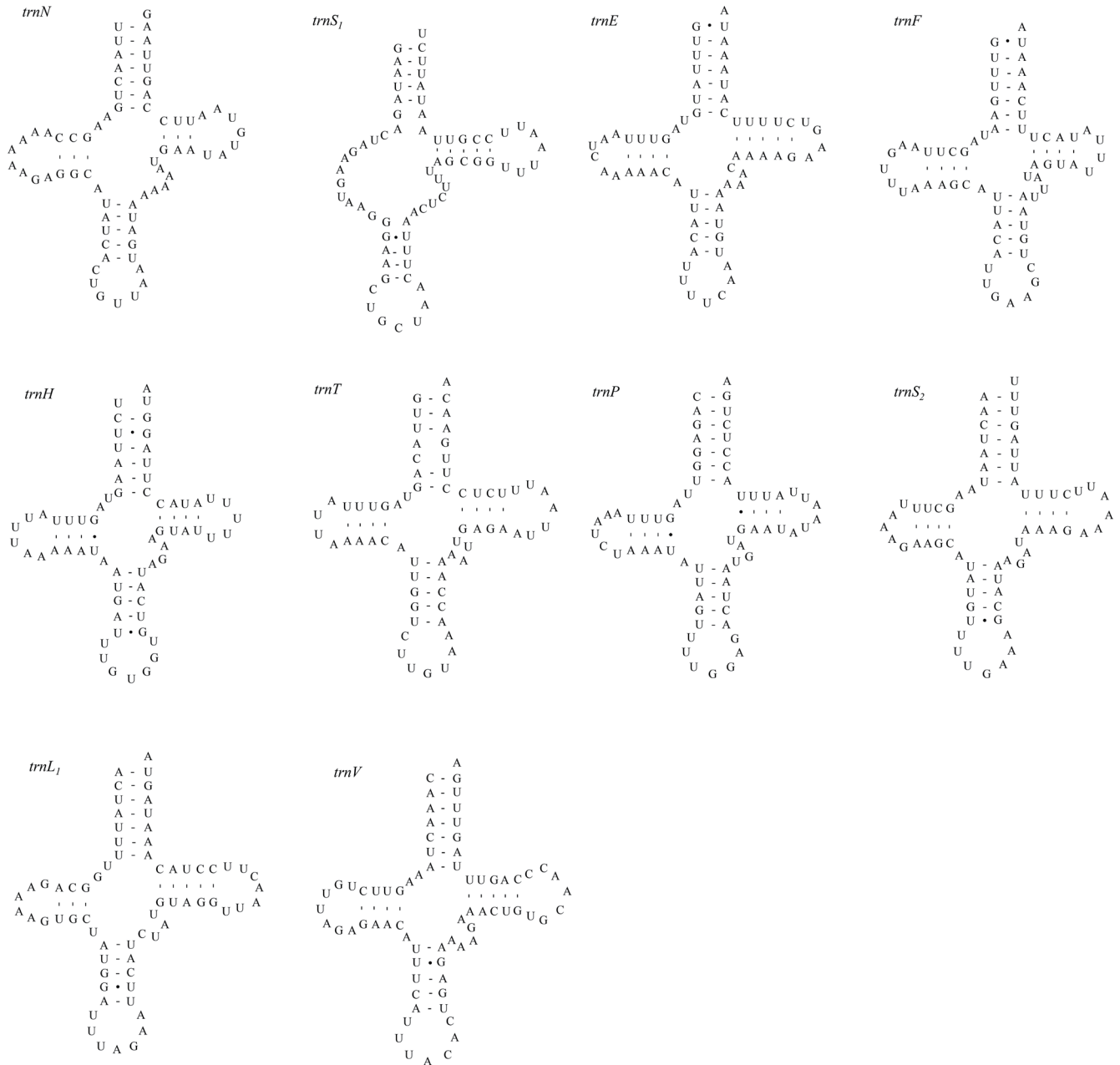


Fig. 1. Continued

between the geographic samples. Considering these results, the origins of *I. elegans* differ between Korea–Germany and China. If not, technical implications in sequence assembly may have caused such difference. More geographic samples of *I. elegans* from these regions should be analyzed in connection with the biogeographic history of *I. elegans* for further scrutinized inference. Nevertheless, it may not be typical to have such a huge length difference without any nucleotide substitution within conspecies.

Variability among *Ischnura elegans* mitochondrial genes

A comparison of the 13 PCGs and two rRNAs of three *I. elegans* mitogenomes revealed variable degrees of sequence divergence among genes (Table 3). For this analysis, we included the *I. asiatica* mitogenome, which was sequenced using the species collected from South Korea (OM310774, Jeong *et al.*, 2023). Our pairwise comparison of public *COI* sequences, including those of *I. asiatica* and the current *I.*

Table 2. Characteristics of mitochondrial genomes of the Coenagrionidae family./

Taxon	Size (bp)	A/T (%)	PCG ^b		srRNA		lrRNA		tRNA		A+T-rich region		GenBank accession no.	References
			No. co- dons ^a	A/T (%)	Size (bp)	A/T (%)	Size (bp)	A/T (%)	Size (bp)	A/T (%)	Size (bp)	A/T (%)		
Coenagrionidae														
<i>Ceriagrion fallax</i>	15,350	74.0	3,709	73.0	752	75.1	1,300	77.4	1,480	73.1	602	85.4	MW092110	Shao et al. (2021)
<i>Enallagma cyathigerum</i>	16,661	74.2	3,721	72.4	760	74.5	1,286	75.9	1,488	72.7	1,906	85.0	MF716899	Zhang et al. (2017)
<i>Ischnura asiatica</i>	15,769	72.8	3,714	71.4	809	74.7	1,284	75.3	1,479	72.1	981	85.5	OM310774	Jeong et al. (2023)
<i>Ischnura elegans</i>	15,963	72.9	3,710	71.4	793	74.7	1,285	75.3	1,476	72.1	1,196	83.8	OQ693852	This study
<i>Ischnura elegans</i>	15,962	72.9	3,709	71.3	793	74.7	1,284	75.3	1,484	72.1	1,196	83.8	KU958378	Feindt et al. (2016)
<i>Ischnura elegans</i>	14,990	72.1	3,708	71.4	755	73.8	1,284	75.3	1,479	72.1	255	81.2	MK951668	Song et al. (2019)
<i>Ischnura pumilio</i>	15,250	73.1	3,713	72.1	782	74.9	1,290	76.0	1,482	72.7	489	86.3	KC878732	Lorenzo-Carballa et al. (2014)
<i>Ischnura senegalensis</i>	15,762	72.9	3,712	71.8	753	74.5	1,286	75.6	1,482	71.9	309	93.9	MT787567	Jiang et al. (2021)
<i>Paracercion v-nigrum</i>	15,366	71.7	3,705	70.2	745	74.0	1,296	75.77	1,485	71.7	648	88.1	MK951669	Song et al. (2019)

^aTermination codons were excluded from the total codon count. ^bProtein coding genes.

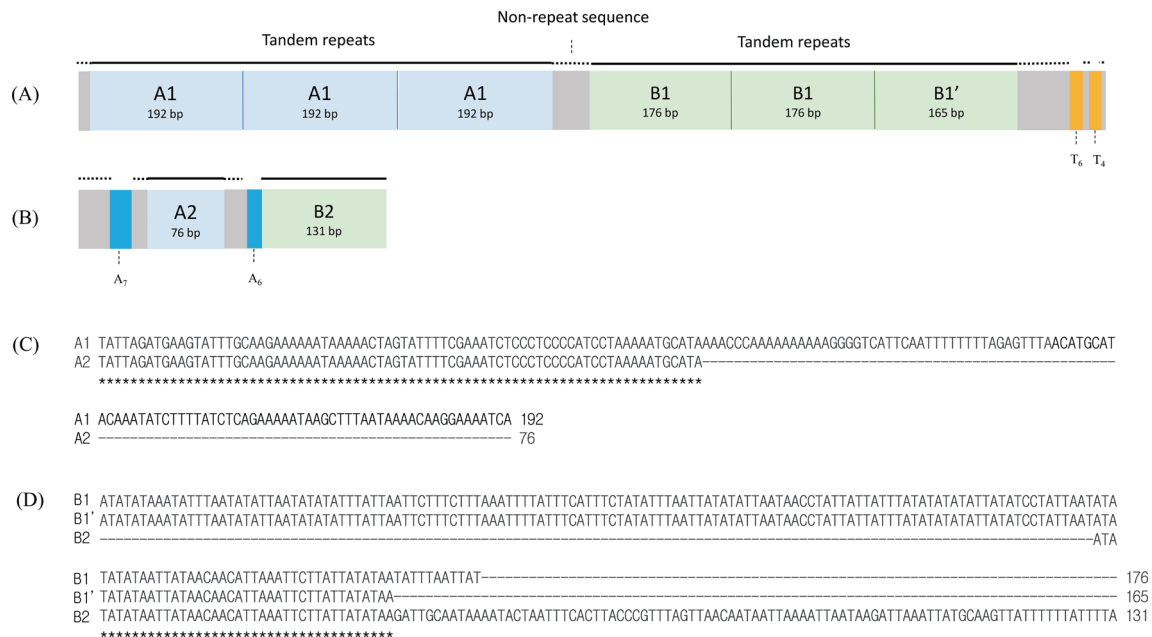


Fig. 2. The A+T-rich region of the *Ischnura elegans* mitogenome. Schematic map of the A+T-rich region of *I. elegans* mitogenome from (A) the present study and Feindt *et al.* (2016) and (B) Song *et al.* (2019). (C) Sequence alignment of subunit A. (D) Sequence alignment of subunit B. T_n and A_n, number of T and A nucleotides, respectively.

Table 3. Percent sequence divergence in 13 PCGs and two rRNAs from *Ischnura elegans* and *I. asiatica*.

	ND2	COI	COII	ATP8	ATP6	COIII	ND3	ND5	ND4	ND4L	ND6	CytB	ND1	lrRNA	srRNA
Maximum	0.302 (3)	0.131 (2)	0.291 (2)	0 (0)	0 (0)	0.127 (1)	0 (0)	0 (0)	0.075 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0.078 (1)	0.126 (1)
Average	0.181 (1.5)	0.065 (1)	0.146 (1)	0 (0)	0 (0)	0.064 (0.5)	0 (0)	0 (0)	0.038 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	0.039 (0.5)	0.063 (0.5)

The number within parentheses indicates the actual number of nucleotide differences.

Table 4. Pairwise comparisons among COI sequences of *Ischnura elegans* and *I. asiatica* (451 bp) obtained from public and current data.

GenBank Num.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. OM310774	asiatica	-	15.97	15.97	15.30	15.52	15.74	15.74	15.97	0	0	0.22	0	0.22	0	0	0	0	0.22	0	0	0.22	0	0
2. MH449998	asiatica	72	-	0.89	0.67	0.44	0.89	0.22	0.44	15.97	15.97	15.74	16.00	15.74	15.97	15.97	15.97	15.97	15.74	15.97	15.97	15.74	16.00	15.97
3. KF257113	asiatica	72	4	-	1.11	0.89	1.33	0.67	0.89	15.97	15.97	15.74	16.00	15.74	15.97	15.97	15.97	15.97	15.74	15.97	15.97	15.74	16.00	15.97
4. KC135957	asiatica	69	3	5	-	0.67	1.11	0.44	0.67	15.30	15.30	15.08	15.33	15.08	15.30	15.30	15.30	15.30	15.08	15.30	15.30	15.08	15.33	15.30
5. MK818665	asiatica	70	2	4	3	-	0.44	0.22	0.44	15.52	15.52	15.30	15.57	15.30	15.52	15.52	15.52	15.52	15.30	15.52	15.52	15.30	15.56	15.52
6. LC366722	asiatica	71	4	6	5	2	-	0.67	0.89	15.74	15.74	15.52	15.78	15.52	15.74	15.74	15.74	15.74	15.52	15.74	15.74	15.52	15.78	15.74
7. KX263694	asiatica	71	1	3	2	1	3	-	0.22	15.74	15.74	15.52	15.78	15.52	15.74	15.74	15.74	15.74	15.74	15.74	15.74	15.52	15.78	15.74
8. AB708497	asiatica	72	2	4	3	2	4	1	-	15.97	15.97	15.74	16.00	15.74	15.97	15.97	15.97	15.97	15.74	15.97	15.97	15.74	16.00	15.97
9. This study	elegans	0	72	72	69	70	71	71	72	-	0	0.22	0	0.22	0	0	0	0	15.97	0	0	0.22	0	0
10. KU958378	elegans	0	72	72	69	70	71	71	72	0	-	0.22	0	0.22	0	0	0	0	0	0	0	0.22	0	0
11. MK951668	elegans	1	71	71	68	69	70	70	71	1	1	-	0.22	0.44	0.22	0.22	0.22	0.22	0	0.22	0.22	0.44	0.22	0.22
12. MW490567	elegans	0	72	72	69	70	71	71	72	0	0	1	-	0.22	0	0	0	0	0.22	0	0	0.22	0	0
13. MW490515	elegans	1	71	71	68	69	70	70	71	1	1	2	1	-	0.22	0.22	0.22	0.22	0	0.22	0.22	0	0.22	0.22
14. MW490501	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	-	0	0	0	0.22	0	0	0.22	0	0
15. MW490468	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	-	0	0	0	0	0	0.22	0	0
16. MW490463	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	-	0	0	0	0	0.22	0	0
17. MW490459	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	-	0	0	0	0.22	0	0
18. MW490445	elegans	1	71	71	68	69	70	70	71	1	1	2	1	0	1	1	1	1	-	0.22	0.22	0	0.22	0.22
19. MW490434	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	-	0	0.22	0	0
20. MW490420	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	-	0.22	0	0.22
21. MW490415	elegans	1	71	71	68	69	70	70	71	1	1	2	1	0	1	1	1	1	0	1	1	-	0.22	0
22. MW490413	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	-	0
23. MW490412	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	-
24. MW490402	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
25. MW490382	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
26. MW490371	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
27. MW490337	elegans	2	72	72	69	70	71	71	72	2	2	3	2	3	2	2	2	2	3	2	2	3	2	2
28. MW490314	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
29. MW490310	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
30. MW490302	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
31. MW490215	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
32. MW490169	elegans	1	71	71	68	69	70	70	71	1	1	2	1	0	1	1	1	1	0	1	1	0	1	1
33. MW490135	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
34. MW490113	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
35. MT298469	elegans	1	71	71	68	69	70	70	71	1	1	2	1	2	1	1	1	1	2	1	1	2	1	1
36. MT298468	elegans	1	71	71	68	69	70	70	71	1	1	2	1	2	1	1	1	1	2	1	1	2	1	1
37. MT298467	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
38. MT298466	elegans	1	71	71	68	69	70	70	71	1	1	2	1	2	1	1	1	1	2	1	1	2	1	1
39. MT298465	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
40. MT298464	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
41. MT216300	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
42. HM376192	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
43. MH449993	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
44. MH449982	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
45. KF369415	elegans	0	72	72	69	70	71	71	72	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0
46. KF257118	elegans	3	74	74	71	72	73	73	74	3	3	4	3	4	3	3	3	3	4	3	3	4	3	3

Table 4. Continued

Haplotype	Species	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
1. OM310774	asiatica	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
2. MH449998	asiatica	15.97	15.97	15.97	15.97	15.97	15.97	15.97	16.00	15.74	15.97	15.97	15.74	15.74	15.97	15.74	15.97	15.97	15.97	15.97	15.97	15.97	15.97	16.41
3. KF257113	asiatica	15.97	15.97	15.97	15.97	15.97	15.97	15.97	16.00	15.74	15.97	15.97	15.74	15.74	15.97	15.74	15.97	15.97	15.97	15.97	15.97	15.97	15.97	16.41
4. KC135957	asiatica	15.30	15.30	15.30	15.30	15.30	15.30	15.30	15.33	15.08	15.30	15.30	15.08	15.08	15.30	15.08	15.30	15.30	15.30	15.30	15.30	15.30	15.30	15.74
5. MK818665	asiatica	15.52	15.52	15.52	15.52	15.52	15.52	15.52	15.56	15.30	15.52	15.52	15.30	15.30	15.52	15.30	15.52	15.52	15.52	15.52	15.52	15.52	15.52	15.97
6. LC366722	asiatica	15.74	15.74	15.74	15.74	15.74	15.74	15.74	15.78	15.52	15.74	15.74	15.52	15.52	15.74	15.52	15.74	15.74	15.74	15.74	15.74	15.74	15.74	16.19
7. KX263694	asiatica	15.74	15.74	15.74	15.74	15.74	15.74	15.74	15.78	15.52	15.74	15.74	15.52	15.52	15.74	15.52	15.74	15.74	15.74	15.74	15.74	15.74	15.74	16.19
8. AB708497	asiatica	15.97	15.97	15.97	15.97	15.97	15.97	15.97	16.00	15.74	15.97	15.97	15.74	15.74	15.97	15.74	15.97	15.97	15.97	15.97	15.97	15.97	15.97	16.41
9. This study	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
10. KU958378	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
11. MK951668	elegans	0.22	0.22	0.22	0.67	0.22	0.22	0.22	0.22	0.44	0.22	0.22	0.44	0.44	0.22	0.44	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.89
12. MW490567	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.68
13. MW490515	elegans	0.22	0.22	0.22	0.67	0.22	0.22	0.22	0.22	0	0.22	0.22	0	0	0.22	0	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.89
14. MW490501	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
15. MW490468	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
16. MW490463	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
17. MW490459	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
18. MW490445	elegans	0.22	0.22	0.22	0.67	0.22	0.22	0.22	0.22	0	0.22	0.22	0	0	0.22	0	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.89
19. MW490434	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
20. MW490420	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
21. MW490415	elegans	0.22	0.22	0.22	0.67	0.22	0.22	0.22	0.22	0	0.22	0.22	0	0	0.22	0	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.89
22. MW490413	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
23. MW490412	elegans	0	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
24. MW490402	elegans	-	0	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
25. MW490382	elegans	0	-	0	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
26. MW490371	elegans	0	0	-	0.44	0	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
27. MW490337	elegans	2	2	2	-	0.44	0.44	0.44	0.44	0.67	0.44	0.44	0.67	0.67	0.44	0.67	0.44	0.44	0.44	0.44	0.44	0.44	0.44	1.12
28. MW490314	elegans	0	0	0	2	-	0	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
29. MW490310	elegans	0	0	0	2	0	-	0	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
30. MW490302	elegans	0	0	0	2	0	0	-	0	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
31. MW490215	elegans	0	0	0	2	0	0	0	-	0.22	0	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
32. MW490169	elegans	1	1	1	3	1	1	1	1	-	0.22	0.22	0.44	0.44	0.22	0.44	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.89
33. MW490135	elegans	0	0	0	2	0	0	0	0	1	-	0	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
34. MW490113	elegans	0	0	0	2	0	0	0	0	1	0	-	0.22	0.22	0	0.22	0	0	0	0	0	0	0	0.67
35. MT298469	elegans	1	1	1	3	1	1	1	1	2	1	1	-	0	0.22	0	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.89
36. MT298468	elegans	1	1	1	3	1	1	1	1	2	1	1	0	-	0.22	0	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.89
37. MT298467	elegans	0	0	0	2	0	0	0	0	1	0	0	1	1	-	0.22	0	0	0	0	0	0	0	0.67
38. MT298466	elegans	1	1	1	3	1	1	1	1	2	1	1	0	0	1	-	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.89
39. MT298465	elegans	0	0	0	2	0	0	0	0	1	0	0	1	1	0	1	-	0	0	0	0	0	0	0.67
40. MT298464	elegans	0	0	0	2	0	0	0	0	1	0	0	1	1	0	1	0	-	0	0	0	0	0	0.67
41. MT216300	elegans	0	0	0	2	0	0	0	0	1	0	0	1	1	0	1	0	0	-	0	0	0	0	0.67
42. HM376192	elegans	0	0	0	2	0	0	0	0	1	0	0	1	1	0	1	0	0	0	-	0	0	0	0.67
43. MH449993	elegans	0	0	0	2	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	-	0	0	0.67
44. MH449982	elegans	0	0	0	2	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	-	0	0.67
45. KF369415	elegans	0	0	0	2	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	-	0.67
46. KF257118	elegans	3	3	3	5	3	3	3	3	4	3	3	4	4	3	4	3	3	3	3	3	3	3	-

Numbers above the diagonal are percent distance values; numbers below the diagonal are absolute distance values. Sources of public data are presented in Sup. Table 1. Note that the OM310774 sequence is listed as *I. asiatica* but has a high sequence identity to *I. elegans*.

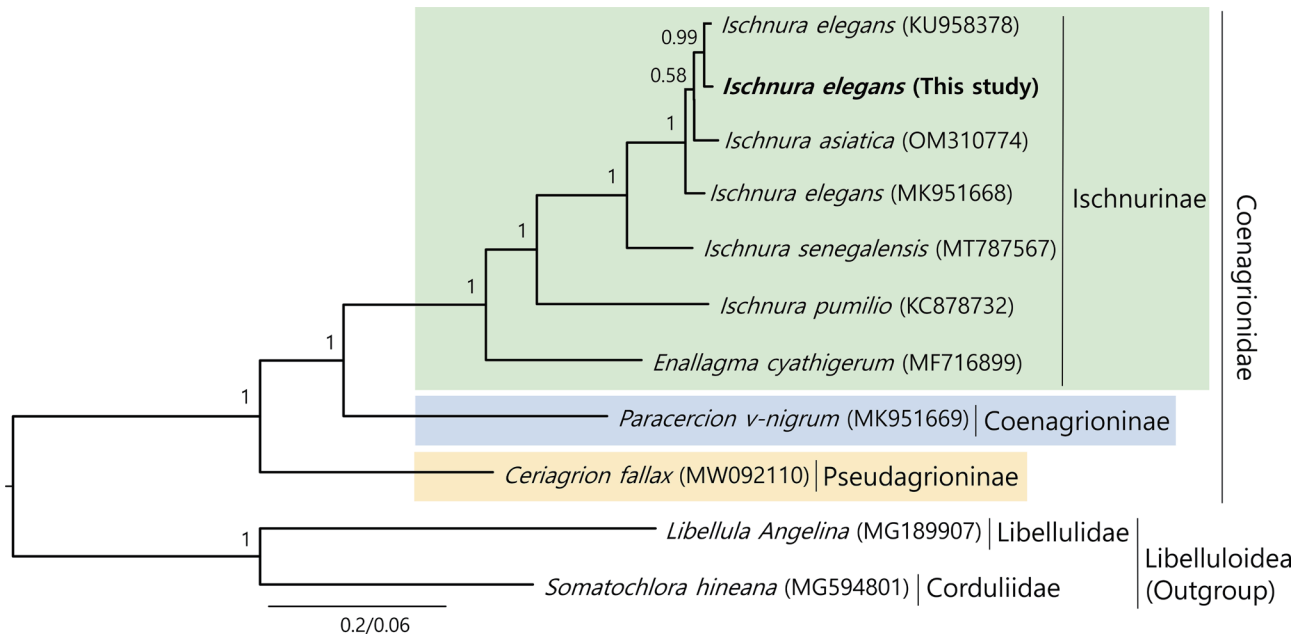


Fig. 3. Phylogenetic tree obtained using the Bayesian inference method. The numbers at each node specify Bayesian posterior probabilities. Two species in Libelluloidea were used as outgroups. The GenBank accession number of each species is provided in parentheses after the scientific names.

elegans mitogenome, strongly indicated that *I. asiatica* (Jeong *et al.*, 2023) should be *I. elegans* because of its substantially high sequence similarity to *I. elegans* (Table 4; Sup. Table 1). Furthermore, for further accurate estimation of divergence, we realigned all PCGs and rRNAs of *I. elegans* and *I. asiatica* mitogenomes and revised the boundaries of the genes as follows: we shortened 3 bp at the 5'-end of *COI* and 12 bp at the 3'-end of *ND5* and expanded 18 bp at the 5'-end of *ND1*, 5 bp at the 5'-end of *srRNA*, and 33 bp at the 3'-end of *srRNA* in the mitogenome of *I. elegans* from China; shortened 3 bp at the 5'-end of *COI*, 12 bp at the 3'-end of *ND5*, 3 bp at the 3'-end of *CytB*, and 21 bp at the 3'-end of *srRNA* and expanded 5 bp at the 5'-end of *srRNA* in the mitogenome of *I. asiatica* (the specific alignment of each gene can be provided upon request).

Seven out of 15 genes (*ND2*, *COI*, *COII*, *COIII*, *ND4*, *lrRNA*, and *srRNA*) showed variation among the three *I. elegans* and one *I. asiatica* mitogenomes, ranging in maximum sequence divergence from 0.075% (1 bp, *ND4*) to 0.302% (3 bp, *ND2*) (Table 3). *COI* gene, which includes the DNA barcoding region, ranked as the third variable gene at 0.131% of maximum sequence variation (2 bp), next to *COII*, which showed 0.291% of maximum sequence variation (2 bp). Thus, although the current comparison is not extensive enough to reach a conclusion, *ND2* and *COII*, which showed at least

twice higher maximum sequence divergence are more suitable than *COI* as candidates for population-level studies using mitochondrial gene segments. Our comparison of 38 *I. elegans* *COI* sequences (451 bp) downloaded from public data revealed a maximum sequence divergence of 1.12% (5 bp; Table 4). Considering that these sequences originated extensively from its range (Korea, China, Japan, Europe, and the USA), sequence divergence among geographic samples appears to be poor compared to other intra-specific variations detected in other insects, including those belonging to Odonata (Kang *et al.*, 2012). For example, the damselfly species, *Forcepsioneura sancta* and *F. aff. lucia*, collected only from Brazil showed a maximum sequence divergence of 5.0% in 14 individuals and 1.1% in six individuals, respectively, in 658 bp of *COI* (Pimenta *et al.*, 2019). Furthermore, the sequence divergence of dragonflies (*Libellula quadrimaculata*) collected from 22 localities across its range in Europe, Asia, and North America (USA and Canada) showed a maximum sequence divergence of only <1% between samples within Europe; however, it was 0.2–2.3% between samples within North America and 1–6% between samples from different continents (Artiss, 2004). Although a robust conclusion can only be made after extensive analysis of more *I. elegans* samples, the current data indicate that the *ND2* or *COII* genes of the damselfly *I. elegans* should

be studied first as population markers for higher sequence variation among geographic samples.

Phylogenetic relationships

Phylogenetic analysis among species of Coenagrionidae supported the monophyletic Coenagrionidae, Ischnurinae, and *Ischnura* with the highest nodal supports (BPP = 1.0; Fig. 3). The current *I. elegans* sample formed a strong sister group with that from Germany (BPP = 0.99), but *I. elegans* from China was interrupted by *I. asiatica*, which, considering the sequence divergence of *COI*, was possibly misidentified (Table 4). Thus, further scrutinized analysis of *I. asiatica* samples from Korea may help infer the relationship between *I. elegans* and *I. asiatica*. After excluding *I. asiatica*, a closer relationship, with the highest nodal support, was observed between *I. elegans* and *I. senegalensis*, leaving *I. pumilio* as the most basal lineage of *Ischnura*. A previous phylogenetic study using mitogenomes also supported the sister relationship between *I. elegans* and *I. senegalensis*, leaving *I. pumilio* as the most basal lineage of *Ischnura* and the sister relationship between *Ischnura* and *E. cyathigerum* with the highest nodal support (Jiang *et al.*, 2021). The sister relationship between *I. elegans* and *I. senegalensis* is further supported by the results of an extensive phylogenetic analysis of Odonata using the sequences of 1,147 bp regions of two mitochondrial (*COI* and *16S rRNA*) and nuclear (*28S rRNA* and *elongation factor-1 α*) genes from 71 odonate species (Kim *et al.*, 2014).

Acknowledgement

This study was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry through the Agriculture, Food, and Rural Affairs Convergence Technologies Program and Educating Creative Global Leader Program, funded by the Ministry of Agriculture, Food, and Rural Affairs (Grant no. 321001-03).

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Sup. Table 1. List of COI sequences obtained from public data.

GenBank No.	Species	Country	References
1. OM310774	<i>asiatica</i>	-	Jeong et al. (2023)
2. MH449998	<i>asiatica</i>	Beijing, China	Dumont (2013)
3. KF257113	<i>asiatica</i>	-	Kim et al. (2014)
4. KC135957	<i>asiatica</i>	Pocheon, South Korea	Unpublished
5. MK818665	<i>asiatica</i>	-	Willink et al. (2019)
6. LC366722	<i>asiatica</i>	Ibaraki, Tsukuba, Japan	Futahashi. (2014)
7. KX263694	<i>asiatica</i>	-	Ning et al. (2016)
8. AB708497	<i>asiatica</i>	Toyama,Fuchu, Japan	Karube (2012)
9. This study	<i>elegans</i>	Yongin, South Korea	This study
10. KU958378	<i>elegans</i>	-	Feindt et al. (2016)
11. MK951668	<i>elegans</i>	-	Song et al. (2019)
12. MW490567	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
13. MW490515	<i>elegans</i>	Leksdalsvatnet, Norway	Geiger et al. (2021)
14. MW490501	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
15. MW490468	<i>elegans</i>	Evros, Alexandropolis, Greece	Geiger et al. (2021)
16. MW490463	<i>elegans</i>	Wadolek Lake, Poland	Geiger et al. (2021)
17. MW490459	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
18. MW490445	<i>elegans</i>	Nord-Trondelag, Norway	Geiger et al. (2021)
19. MW490434	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
20. MW490420	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
21. MW490415	<i>elegans</i>	Nord-Trondelag, Norway	Geiger et al. (2021)
22. MW490413	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
23. MW490412	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
24. MW490402	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
25. MW490382	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
26. MW490371	<i>elegans</i>	Wadolek Lake, Poland	Geiger et al. (2021)
27. MW490337	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
28. MW490314	<i>elegans</i>	Wadolek Lake, Poland	Geiger et al. (2021)
29. MW490310	<i>elegans</i>	Burgenland, Austria	Geiger et al. (2021)
30. MW490302	<i>elegans</i>	Mecklenburg-Vorpommern, Germany	Geiger et al. (2021)
31. MW490215	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
32. MW490169	<i>elegans</i>	Nord-Trondelag, Norway	Geiger et al. (2021)
33. MW490135	<i>elegans</i>	Evros, Greece	Geiger et al. (2021)
34. MW490113	<i>elegans</i>	Bavaria, Germany	Geiger et al. (2021)
35. MT298469	<i>elegans</i>	Lazio, Viterbo, Italy	Galimverti et al. (2021)
36. MT298468	<i>elegans</i>	Tuscany, Grosseto, Italy	Galimverti et al. (2021)
37. MT298467	<i>elegans</i>	Apulia, Lecce, Italy	Galimverti et al. (2021)
38. MT298466	<i>elegans</i>	Piedmont, Torino, Italy	Galimverti et al. (2021)
39. MT298465	<i>elegans</i>	Trentino-Alto Adige, Trento, Italy	Galimverti et al. (2021)
40. MT298464	<i>elegans</i>	Montenegro	Galimverti et al. (2021)
41. MT216300	<i>elegans</i>	River Odra, Leszkwice, Poland	Rewicz et al. (2021)
42. HM376192	<i>elegans</i>	Germany	Unpublished
43. MH449993	<i>elegans</i>	Batumi Georgia, U.S.A.	Dumont (2013)
44. MH449982	<i>elegans</i>	Affligem, Belgium	Dumont (2013)
45. KF369415	<i>elegans</i>	Netherlands	Dijkstra et al. (2014)
46. KF257118	<i>elegans</i>	-	Kim et al. (2014)

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