

Discovery of the vagrant darter, *Sympetrum vulgatum* (Odonata: Libellulidae) in Korea

Jeong Sun Park^{1,†}, Jong Moon Kim^{2,†}, Hyeong Gi Jeon², Sung Soo Kim², Jee-Young Pyo¹, 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

Among the 17 species of *Sympetrum* found on the Korean peninsula *Sympetrum vulgatum* Linnaeus, 1758 had not been recorded since its first record in 1932 by Doi, suggesting the potential for misidentification. We observed a total of four specimens on September 21, 25, 30, and November 1 2022 in Goseong, Gangwon-do and collected each one male and female. We described the morphological and ecological characteristics of this species, conducted molecular analysis using mitochondrial *COI*, and addressed the controversial past record regarding its presence on the Korean Peninsula.

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Introduction

Sympetrum Newman, 1833 (Libellulidae) consists of over 60 species and is found on every continent except for Australia (Needham *et al.*, 2000). In Korea, 17 species are distributed, but *S. frequens* Sélys, 1883 has recently been synonymized with *S. depressiusculum* Sélys, 1841 in Korea (Pyo *et al.*, 2023). Among them the vagrant darter, *S. vulgatum* Linnaeus, 1758 is widely distributed across the northern Eurasian continent, spanning from northeastern Asia to the northeastern and central parts of Europe (Hinojosa *et al.*, 2017). This species comprises four subspecies: *S. v. vulgatum*, *S. v. ibericum*, and *S. v. decoloratum* distributed in Europe; and *S. v. imitans* distributed in Asia (Hinojosa *et al.*, 2017). In China and Russia, *S. vulgatum* inhabits high-latitude or high-altitude regions (Hinojosa *et al.*, 2017). In Japan, the species has been confirmed as migrants, detected for about ten

times along the coastal regions from the North Sea to Fukuoka in Kyushu since 2002 (Takuma, 2021).

The *S. striolatum* Charpentier, 1840 known as a species similar to *S. vulgatum* is common and widely distributed in central Europe (Hogreve and Suhling, 2023). In Korea, *S. striolatum* is found frequently in coastal areas and inland regions in Korea, whereas *S. vulgatum* was recorded to have been discovered on Mt. Samseong, Gyeongseong (an old name of Seoul) by Doi (1932), but no additional report on its' discovery has been recorded since then (Lee, 2001; Kim *et al.*, 2020), suggesting the potential of misidentification (Lee, 2001; Kim *et al.*, 2020).

Recently, we discovered each two males and females in Gangwon-do, adjacent to the coastal regions of the East Sea and each one male and female were collected. In this study, we report information on the taxonomic history, morphology, *COI* sequences, and ecology of this species.

†These authors contributed equally to this paper.

*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: +82-62-530-2079

E-mail: ikkim81@chonnam.ac.kr

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Materials and Methods

Samples and morphological analysis

Two male adults (September 25 2022 and November 1 2022) and two female adults (September 21 2022 and September 30 2022) of *S. vulgatum* were discovered in Sinpyeong-ri, Toseongmyeon, Goseong-gun, Gangwon Province, South Korea, adjacent to the East Sea. Each one male (voucher specimen no. CNU16260) and female (CNU16261) were collected (Fig. 1). Additionally, four individuals of *S. striolatum* adults were collected in Jeju Island in South Korea (CNU17121, CNU17122, CNU17123, and CNU17124; Fig. 1). These samples were collected using insect nets and photographs were taken by Jong Moon Kim and Hyeong Ki Jeon with Samsung Galaxy S7 (SM-G930K), Nikon Coolpix P950, and Nikon D300 with Nikkor 105 mm micro VR. Ecological information of the places, where the species were observed were recorded. Once collected, external morphology of *S. vulgatum*, including abdomen and hindwing lengths, genitalia shape, and color or pattern of various parts were examined.

DNA extraction, amplification, and sequencing

Total DNA was extracted from one or two hind legs using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA), following the manufacturer's instructions. To amplify a 451 bp fragment (497 bp including primer sites) of the mitochondrial *COI* gene, which is often used for species identification in Odonata, primers were adapted from Futahashi *et al.* (2018) for the forward primer and Hayashi *et al.* (2005)

for the reverse primer: *COI*-S1n (forward primer) 5'-ATA ATT GGR GGR TTY GGA AAC TG-3' and *COI*-AS1 (reverse primer) 5'-CCA AAR AAT CAA AAT AAR TGT TG-3'. PCR amplification was performed using the following conditions: an initial denaturation step at 94°C for 5 min, 35 amplification cycles (denaturation at 94°C for 1 min, annealing at 50–57°C for 1 min, and extension at 72°C for 1 min), and a final extension step at 72°C for 7 min using AccuPower® PCR PreMix (Bioneer, Daejeon, South Korea). To ensure the accuracy of the PCR, distilled water was used instead of DNA as a negative control. Electrophoresis was performed in 0.5× Tris-acetate EDTA buffer on 1% agarose gels to confirm successful DNA amplification. PCR products were then purified using a PCR purification kit (Bioneer). All PCR products were directly sequenced in both directions (Macrogen Co., Seoul, South Korea) on an ABI 3730xl automated DNA sequencer (PE Applied Biosystems, Foster City, CA, USA). GenBank accession numbers are as follows: *S. vulgatum*, PP869367 for male (voucher specimen no. CNU16260) and PP869368 for female (CNU16261); *S. striolatum*, PP869363 for CNU17121, PP869364 for CNU17122, PP869365 for CNU17123, and PP869366 for CNU17124.

Sequence analysis

COI sequences of the six individuals were aligned using MAFFT ver. 7 (Kato and Standley, 2013). The nucleotide sequences of the *COI* gene were translated using the genetic code for invertebrate mitochondrial DNA to detect the potential presence of pseudogene sequences. However, none of the sequences exhibited any indication of pseudogene. Furthermore, to verify the accuracy of each sequence, a BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 22 April 2024) was performed.

Public data

To understand sequence divergence and phylogenetic relationships among *Sympetrum*, 31 *COI* sequences, representing 31 species of the *Sympetrum* were downloaded from the GenBank database. If multiple sequences for each species exist, only one sequence per species was randomly selected and downloaded. Six species, which provide substantially shorter than 451 bp were excluded, for accurate analysis for sequence divergence and phylogenetic inference. Additionally, *S. janeae* and *S. obtrusum*, which provided an identical sequence were excluded from the analysis.



Fig. 1. *Sympetrum* species. (A) Male, *S. vulgatum*; (B) Female, *S. vulgatum*; (C) Male, *S. striolatum*; (D) Female, *S. striolatum*.

Phylogenetic analysis

To understand the degree of sequence divergence the unrooted pairwise distances were calculated using PAUP ver. 4.01b (Swofford, 2002). This analysis was performed using a total of 37 sequences, consisted of 31 species of the *Sympetrum* downloaded from the GenBank and each two and four sequences for *S. vulgatum* and *S. striolatum*, respectively, from this study. Alignment was performed using MAFFT ver. 7 (Kato and Standley, 2013) and further adjusted using Gblock 0.91b (Talavera and Castresana, 2007). Clustal X ver. 1.83 (Thompson *et al.*, 1997) was employed to alter the file format for phylogenetic analysis. Phylogenetic analyses were performed using the maximum-likelihood (ML) methods. The GTR+I+G was selected as the best substitution model using Modeltest, which is incorporated into PhyloSuite ver. 1.2.3. (Xiang *et al.*, 2023). IQ-TREE (Nguyen *et al.*, 2015), which is incorporated into PhyloSuite ver. 1.2.3 (Xiang *et al.*, 2023) was employed. Branch support was assessed using 1,000 replicates of the Ultrafast Bootstrap (UFBoot; Minh *et al.*, 2013) and 1,000 replicates of the Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT; Guindon *et al.*, 2010). *Leucorrhinia intacta* was used as an outgroup (GenBank accession number JF839418; Unpublished). The tree was visualized using FigTree ver. 1.42 (<https://tree.bio.ed.ac.uk/software/figtree/>).

Results

Species information

Sympetrum vulgatum Linnaeus, 1758

Libellula vulgata Linnaeus, 1758, Syst Nat 10: 543. TL: Sweden.

Specimens examined. 30. IX. 2022 Goseong-gun, Gangwon Province, South Korea 1♀ (Female) (voucher specimen no. CNU16261), 1. XI. 2022 Goseong-gun, Gangwon Province, South Korea 1♂ (Male) (CNU16260) (Fig. 2).

Morphology

The male abdomen and hindwing lengths were 29 mm and 32 mm, respectively, while the female abdomen and hindwing lengths were 31 mm and 33 mm, respectively, indicating a slightly larger body size in females (Fig. 2). The dorsum of is yellowish-brown, with a thin basifrontal stripe extending towards the mouth. The metathorax of mature adult exhibits a red coloration for males and

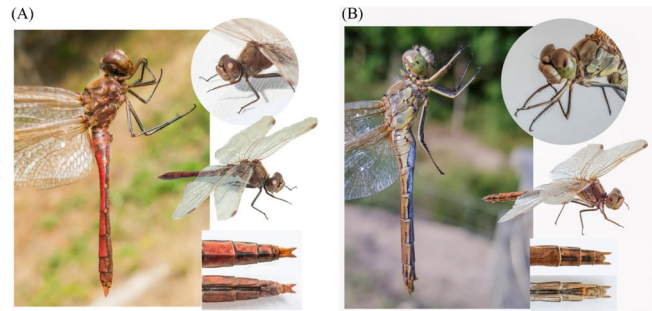


Fig. 2. Lateral aspect of *Sympetrum vulgatum*. (A) Male; (B) Female.

a greenish-brown coloration for females, without distinct patterns. A slight, thin black line is present above the humeral suture and the 2nd lateral suture, while above the 1st lateral suture, the black line extends only halfway from below, remaining thin. The inner side of the fore femur becomes black, while the outer side of tibia is yellowish-brown. The abdomen of the male is relatively flat and nearly patternless, while in females, there are thin black stripes along the sides, resembling rods. The two superior appendages of the male form a V-shape when viewed from above and the end of superior appendage is stout and appear truncated, extending posterior. The ventral plate of the 9th abdominal segment of the female extends to three-fourths length of the 9th segment with a rounded central end. The basal of wings has a reddish-yellow color, which extends to the pterostigma along the costa. The costa is yellowish-brown, and the pterostigma is reddish-dark brown. Matured males have an entirely red color, while females become red only in the dorsum of abdomen, with the rest being greenish-brown.

This species is similar to *S. striolatum* in morphology, but differences exist as follows (Takuma, 2021): 1) The basifrontal stripe more extends toward the mouth part. 2) The outer parts of the femur and tibia are yellowish-brown. 3) The superior appendages of male adult are longer, extending beyond the inferior appendages. 4) There is hardly any black pattern on the side parts of the 8th-9th abdominal segments. 5) The valvula vulvae of the female adult is longer, with its end pointing vertically downward.

Past records of *S. vulgatum* in Korea

In Korea, *S. vulgatum* was first recorded by Doi (1932). This record was later revealed to be *S. striolatum* through the following process: 1) Doi (1932) initially recorded *S. vulgatum* using the specimens collected on Mt. Samseong, Gyeongseong (an old name of Seoul), but no detailed records such as collection date, sex and so on were provided. 2) Asahina (1990) met Doi

in Gyeongseong in 1942, examined his specimens and gave him advice on misidentified species. 3) In compiling a list of dragonflies in Korea, Doi (1943) included *S. striolatum*, a species very similar to *S. vulgatum*. The description of *S. vulgatum* in this list was labeled as “マンシウアカネ,” meaning Manchuria dragonfly, instead of “イソアカネ,” which refers to the Seashore dragonfly, following Asahina's opinion. Coincidentally, the collection site of *S. striolatum* was recorded to be Mt. Samseong, Gyeongseong, which is the same as the collection site of *S. vulgatum* recorded by Doi (1932). The list of Cho (1958) was equal to that of Doi. 4) Asahina (1990) later corrected *S. vulgatum*, which was recorded by Doi (1932, 1943) to *S. striolatum* in a review of the list of dragonflies in Korea. 5) Lee (2001) and Kim *et al.* (2020) compiled a list of Korean dragonflies, incorporating the revisions made by Asahina (1990).

It may not be easy to reach to a conclusion because there is no leftover specimen, which was used by Doi to record *S. vulgatum*. Nevertheless, the past recording process of *S. vulgatum* in Korea seems to suggest objective information. Based on the information mentioned above and the fact that there was no subsequent record for the species for long time in South Korea, we strongly suppose that the same specimen collected in the same site was mistakenly recorded into both *S. striolatum* and *S. vulgatum* by Doi. Nonetheless, Cho (1958) and the national species list in Korea still list *S. vulgatum*, without reflecting the past records of *S. vulgatum* in Korea.

Description of collection site and ecological overview of *S. vulgatum*

Sympetrum vulgatum was discovered in Goseong, Gangwon Province, which is the northernmost region of South Korea, closer northward to North Korea. This location is a mountain area with nearby coast located about 2-3 km away. The species was detected where there are small ponds in the vicinity, which supply water to neighboring waterways: paddy fields and farmland are scattered, well-maintained dirt roads run next to ponds, and the surrounding vegetation is rich with broadleaf trees (Fig. 3). Although ecological activities such as mating or oviposition were not observed, hunting behavior was observed to be solitary. Other species found in the area include the Siberian hawkler *Aeshna crenata*, the jumbo dragonfly *Anotogaster sieboldii*, the oriental brilliant emerald *Somatochlora exuberata*, the globe skimmer *Pantala flavescens*, the white-tailed skimmer *Orthetrum albistylum*, the greater blue skimmer *O. melania*,



Fig. 3. The view of the observation area of *Sympetrum vulgatum*.

the scarlet skimmer *Crocothemis servilia*, and the lesser emperor *Anax parthenope*. In addition, several species of *Sympetrum*, such as *S. depressiusculum*, *S. infuscatum*, *S. baccha*, *S. risi*, *S. parvulum*, *S. cordulegaster*, *S. speciosum*, *S. croceolum*, *S. eroticum*, *S. kunkeli*, and *S. striolatum* were found. Generally, species of *Sympetrum* are most commonly found in habitats with slow or stagnant water, such as marshes, ponds, mid-upper reaches of streams, or wetlands, often perching on reeds or other plants (Pilgrim and von Dohlen, 2012). *Sympetrum striolatum* and *S. vulgatum* are known to inhabit saline brackish water zone in Europe and *S. striolatum* particularly seems to have a reproductive advantage on slightly increased salinity over tap water condition, showing higher hatching and survival rates (Hogreve and Suhling, 2023).

Sympetrum vulgatum seems to be a migrant species, as the species was not found at the same area in 2023 even though we visited the place for seven times. Although the precise migration route of *S. vulgatum* remains unknown, it is speculated to have migrated through the East Sea from the Russian Far East. The higher number of observation records in Japan compared to South Korea suggests that the species follows the migration path of westerlies from late summer to autumn, which is the dispersal period of the species. In South Korea, there are occasions that the westerlies shift to east wind during spring and summer (<https://www.a-ha.io/questions/4873eaf1b62d3b12acfb38dda8ce7b2e>). Currently, this species inhabits the Russian Far East (Malikova and Kosterin, 2019), but the number is small (Onishko *et al.*, 2023).

Sequence divergence and phylogenetic analysis

The two *S. vulgatum* individuals sequenced in this study were identical, but two bp differ to that available in GenBank, which was originated from The Netherlands (Table 1; acc. no.

Table 1. COI-based pairwise comparisons of *Sympetrum* species.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1. <i>S. striolatum</i> (GNU17121)	-	0.00	0.44	0.00	0.22	0.22	10.42	10.42	10.87	14.86	14.63	18.40	15.08
2. <i>S. striolatum</i> (GNU17122)	0	-	0.44	0.00	0.22	0.22	10.42	10.42	10.87	14.86	14.63	18.40	15.08
3. <i>S. striolatum</i> (GNU17123)	2	2	-	0.44	0.67	0.67	9.98	9.98	10.42	14.41	14.19	17.96	14.63
4. <i>S. striolatum</i> (GNU17124)	0	0	2	-	0.22	0.22	10.42	10.42	10.87	14.86	14.63	18.40	15.08
5. <i>S. striolatum</i> (MW208384)	1	1	3	1	-	0.44	10.64	10.64	11.09	15.08	14.41	18.18	15.30
6. <i>S. nigrescens</i> (EF636215)	1	1	3	1	2	-	10.20	10.20	10.64	14.63	14.86	18.18	14.86
7. <i>S. vulgatum</i> (GNU16260)	47	47	45	47	48	46	-	0.00	0.44	11.97	12.64	16.19	11.53
8. <i>S. vulgatum</i> (GNU16261)	47	47	45	47	48	46	0	-	0.44	11.97	12.64	16.19	11.53
9. <i>S. vulgatum</i> (EF636246)	49	49	47	49	50	48	2	2	-	11.97	13.08	16.41	11.75
10. <i>S. baccha</i> (KF257091)	67	67	65	67	68	66	54	54	54	-	14.41	17.74	11.97
11. <i>S. corulegaster</i> (AB709116)	66	66	64	66	65	67	57	57	59	65	-	18.18	12.20
12. <i>S. corruptum</i> (GU711566)	83	83	81	83	82	82	73	73	74	80	82	-	15.74
13. <i>S. costiferum</i> (JF839431)	68	68	66	68	69	67	52	52	53	54	55	71	-
14. <i>S. croceolum</i> (AB709117)	64	64	62	64	65	65	55	55	57	70	65	77	56
15. <i>S. danae</i> (JF839317)	67	67	65	67	68	68	47	47	49	62	64	68	49
16. <i>S. danvianum</i> (KF257097)	67	67	65	67	68	68	54	54	56	63	61	67	56
17. <i>S. depressiusculum</i> (MT298644)	72	72	70	72	73	71	50	50	50	59	58	73	47
18. <i>S. eroticum</i> (KF257077)	71	71	69	71	70	72	62	62	62	37	69	83	55
19. <i>S. flaveolum</i> (MT298646)	72	72	70	72	71	71	57	57	57	74	68	78	65
20. <i>S. fonscolombii</i> (KF257098)	79	79	77	79	78	78	71	71	73	73	84	71	75
21. <i>S. frequens</i> (KF257076)	74	74	72	74	75	73	52	52	52	59	60	74	49
22. <i>S. gracile</i> (AB709166)	72	72	70	72	73	73	60	60	62	60	64	76	55
23. <i>S. infuscatum</i> (KF257078)	60	60	58	60	61	61	60	60	62	58	61	63	49
24. <i>S. internum</i> (JF839245)	74	74	72	74	73	75	55	55	57	74	66	60	58
25. <i>S. kunkeli</i> (KF257092)	68	68	66	68	69	69	50	50	50	34	66	79	54
26. <i>S. maculatum</i> (AB709173)	71	71	69	71	72	70	60	60	62	66	65	71	60
27. <i>S. madidum</i> (KM529557)	66	66	64	66	65	65	52	52	54	67	58	81	64
28. <i>S. meridionale</i> (MW208386)	61	61	59	61	62	60	54	54	56	64	67	75	62
29. <i>S. pallipes</i> (KF839438)	71	71	69	71	70	72	52	52	54	69	61	62	57
30. <i>S. parvulum</i> (KF257088)	66	66	64	66	67	65	48	48	48	33	61	73	53
31. <i>S. pedemontanum</i> (KF257095)	59	59	57	59	58	58	42	42	42	47	46	68	53
32. <i>S. risi</i> (KF257127)	72	72	70	72	73	71	64	64	66	67	72	74	57
33. <i>S. sanguineum</i> (MW208383)	52	52	50	52	51	51	41	41	43	54	55	67	50
34. <i>S. semicinctum</i> (JN294514)	61	61	59	61	60	62	58	58	59	55	53	64	25
35. <i>S. speciosum</i> (KF257089)	76	76	74	76	75	77	55	55	57	63	55	67	54
36. <i>S. uniforme</i> (KF257087)	62	62	60	62	63	63	52	52	54	70	63	77	55
37. <i>S. vicinum</i> (HM413479)	72	72	70	72	73	71	50	50	51	57	63	74	57

Table 1. Continued

Species	14	15	16	17	18	19	20	21	22	23	24	25
1. <i>S. striolatum</i> (CNU17121)	14.19	14.86	14.86	15.97	15.74	15.97	17.52	16.41	15.97	13.30	16.41	15.08
2. <i>S. striolatum</i> (CNU17122)	14.19	14.86	14.86	15.97	15.74	15.97	17.52	16.41	15.97	13.30	16.41	15.08
3. <i>S. striolatum</i> (CNU17123)	13.75	14.41	14.41	15.52	15.30	15.52	17.07	15.97	15.52	12.86	15.97	14.63
4. <i>S. striolatum</i> (CNU17124)	14.19	14.86	14.86	15.97	15.74	15.97	17.52	16.41	15.97	13.30	16.41	15.08
5. <i>S. striolatum</i> (MW208384)	14.41	15.08	15.08	16.19	15.52	15.74	17.30	16.63	16.19	13.53	16.19	15.30
6. <i>S. nigrescens</i> (EF636215)	14.41	15.08	15.08	15.74	15.97	15.74	17.30	16.19	16.19	13.53	16.63	15.30
7. <i>S. vulgatum</i> (CNU16260)	12.20	10.42	11.97	11.09	13.75	12.64	15.74	11.53	13.30	13.30	12.20	11.09
8. <i>S. vulgatum</i> (CNU16261)	12.20	10.42	11.97	11.09	13.75	12.64	15.74	11.53	13.30	13.30	12.20	11.09
9. <i>S. vulgatum</i> (EF636246)	12.64	10.87	12.42	11.09	13.75	12.64	16.19	11.53	13.75	13.75	12.64	11.09
10. <i>S. baccha</i> (KF257091)	15.52	13.75	13.97	13.08	8.20	16.41	16.19	13.08	13.30	12.86	16.41	7.54
11. <i>S. cordulegaster</i> (AB709116)	14.41	14.19	13.53	12.86	15.30	15.08	18.63	13.30	14.19	13.53	14.63	14.63
12. <i>S. corruptum</i> (GU711566)	17.07	15.08	14.86	16.19	18.40	17.30	15.74	16.41	16.85	13.97	13.30	17.52
13. <i>S. costiferum</i> (JF839431)	12.42	10.87	12.42	10.42	12.20	14.41	16.63	10.87	12.20	10.87	12.86	11.97
14. <i>S. croceolum</i> (AB709117)	-	11.09	14.19	11.53	15.74	14.86	17.07	12.20	13.75	12.42	14.86	13.97
15. <i>S. danae</i> (JF839317)	50	-	9.98	9.09	13.53	14.41	16.19	9.76	12.64	11.09	11.97	12.64
16. <i>S. darwinianum</i> (KF257097)	64	45	-	11.53	15.08	15.30	14.63	12.20	10.64	10.20	13.97	13.53
17. <i>S. depressiusculum</i> (MT298644)	52	41	52	-	14.41	13.53	15.74	0.67	12.86	11.09	12.42	12.86
18. <i>S. eroticum</i> (KF257077)	71	61	68	65	-	17.07	18.18	14.41	15.52	12.86	15.30	6.87
19. <i>S. flaveolum</i> (MT298646)	67	65	69	61	77	-	15.74	13.30	15.74	15.97	13.30	14.86
20. <i>S. fonscolombii</i> (KF257098)	77	73	66	71	82	71	-	16.19	13.97	14.86	17.07	17.07
21. <i>S. frequens</i> (KF257076)	55	44	55	3	65	60	73	-	12.86	11.53	12.42	12.86
22. <i>S. gracile</i> (AB709166)	62	57	48	58	70	71	63	58	-	9.31	15.97	13.30
23. <i>S. infuscatum</i> (KF257078)	56	50	46	50	58	72	67	52	42	-	13.08	12.64
24. <i>S. internum</i> (JF839245)	67	54	63	56	69	60	77	56	72	59	-	15.08
25. <i>S. kunkelii</i> (KF257092)	63	57	61	58	31	67	77	58	60	57	68	-
26. <i>S. maculatum</i> (AB709173)	71	56	14	56	75	65	65	59	53	55	71	67
27. <i>S. madidum</i> (KM529557)	65	60	55	59	78	60	70	62	69	66	68	68
28. <i>S. meridionale</i> (MW208386)	72	58	59	70	65	71	68	70	69	60	68	65
29. <i>S. pallipes</i> (KF839438)	65	52	59	53	66	58	77	55	68	57	8	65
30. <i>S. parvulum</i> (KF257088)	59	50	56	57	26	71	76	57	60	48	64	21
31. <i>S. pedemontanum</i> (KF257095)	61	56	53	52	53	54	73	53	64	52	60	47
32. <i>S. risi</i> (KF257127)	65	60	57	56	66	69	79	54	46	32	70	61
33. <i>S. sanguineum</i> (MW208383)	55	56	58	54	62	65	73	54	63	54	58	54
34. <i>S. seminctum</i> (JN294514)	55	45	48	53	54	74	75	55	58	45	59	58
35. <i>S. speciosum</i> (KF257089)	59	46	51	48	65	69	73	48	63	53	49	66
36. <i>S. uniforme</i> (KF257087)	5	52	65	52	73	64	75	55	62	58	68	63
37. <i>S. vicinum</i> (HM413479)	64	61	56	56	67	62	70	56	68	59	67	63

Table 1. Continued

Species	26	27	28	29	30	31	32	33	34	35	36	37
1. <i>S. striolatum</i> (GNU17121)	15.74	14.63	13.53	15.74	14.63	13.08	15.97	11.53	13.53	16.85	13.75	15.97
2. <i>S. striolatum</i> (GNU17122)	15.74	14.63	13.53	15.74	14.63	13.08	15.97	11.53	13.53	16.85	13.75	15.97
3. <i>S. striolatum</i> (GNU17123)	15.30	14.19	13.08	15.30	14.19	12.64	15.52	11.09	13.08	16.41	13.30	15.52
4. <i>S. striolatum</i> (GNU17124)	15.74	14.63	13.53	15.74	14.63	13.08	15.97	11.53	13.53	16.85	13.75	15.97
5. <i>S. striolatum</i> (MW208384)	15.97	14.41	13.75	15.52	14.86	12.86	16.19	11.31	13.30	16.63	13.97	16.19
6. <i>S. nigrescens</i> (EF636215)	15.52	14.41	13.30	15.97	14.41	12.86	15.74	11.31	13.75	17.07	13.97	15.74
7. <i>S. vulgatum</i> (GNU16260)	13.30	11.53	11.97	11.53	10.64	9.31	14.19	9.09	12.86	12.20	11.53	11.09
8. <i>S. vulgatum</i> (GNU16261)	13.30	11.53	11.97	11.53	10.64	9.31	14.19	9.09	12.86	12.20	11.53	11.09
9. <i>S. vulgatum</i> (EF636246)	13.75	11.97	12.42	11.97	10.64	9.31	14.63	9.53	13.08	12.64	11.97	11.31
10. <i>S. baccha</i> (KF257091)	14.63	14.86	14.19	15.30	7.32	10.42	14.86	11.97	12.20	13.97	15.52	12.64
11. <i>S. cordulegaster</i> (AB709116)	14.41	12.86	14.86	13.53	13.53	10.20	15.97	12.20	11.75	12.20	13.97	13.97
12. <i>S. corruptum</i> (GU711566)	15.74	17.96	16.63	13.75	16.19	15.08	16.41	14.86	14.19	14.86	17.07	16.41
13. <i>S. costiferum</i> (JF839431)	13.30	14.19	13.75	12.64	11.75	11.75	12.64	11.09	5.54	11.97	12.20	12.64
14. <i>S. croceolum</i> (AB709117)	15.74	14.41	15.97	14.41	13.08	13.53	14.41	12.20	12.20	13.08	1.11	14.19
15. <i>S. danae</i> (JF839317)	12.42	13.30	12.86	11.53	11.09	12.42	13.30	12.42	9.98	10.20	11.53	13.53
16. <i>S. darwinianum</i> (KF257097)	3.10	12.20	13.08	13.08	12.42	11.75	12.64	12.86	10.64	11.31	14.41	12.42
17. <i>S. depressiusculum</i> (MT298644)	12.42	13.08	15.52	11.75	12.64	11.53	12.42	11.97	11.75	10.64	11.53	12.42
18. <i>S. eroticum</i> (KF257077)	16.63	17.30	14.41	14.63	5.77	11.75	14.63	13.75	11.97	14.41	16.19	14.86
19. <i>S. flaveolum</i> (MT298646)	14.41	13.30	15.74	12.86	15.74	11.97	15.30	14.41	16.41	15.30	14.19	13.75
20. <i>S. fonscolombii</i> (KF257098)	14.41	15.52	15.08	17.07	16.85	16.19	17.52	16.19	16.63	16.19	16.63	15.52
21. <i>S. frequens</i> (KF257076)	13.08	13.75	15.52	12.20	12.64	11.75	11.97	11.97	12.20	10.64	12.20	12.42
22. <i>S. gracile</i> (AB709166)	11.75	15.30	15.30	15.08	13.30	14.19	10.20	13.97	12.86	13.97	13.75	15.08
23. <i>S. infuscatum</i> (KF257078)	12.20	14.63	13.30	12.64	10.64	11.53	7.10	11.97	9.98	11.75	12.86	13.08
24. <i>S. internum</i> (JF839245)	15.74	15.08	15.08	1.77	14.19	13.30	15.52	12.86	13.08	10.87	15.08	14.86
25. <i>S. kunckei</i> (KF257092)	14.86	15.08	14.41	14.41	4.66	10.42	13.53	11.97	12.86	14.63	13.97	13.97
26. <i>S. maculatum</i> (AB709173)	-	13.75	14.86	14.86	13.53	13.30	13.30	14.19	11.97	13.97	15.97	13.30
27. <i>S. madidum</i> (KM529557)	62	-	13.97	14.19	14.19	10.64	16.41	13.08	13.75	13.30	14.19	13.75
28. <i>S. meridionale</i> (MW208386)	67	63	-	14.86	12.64	12.20	15.97	10.64	12.20	14.19	15.74	14.41
29. <i>S. pallipes</i> (KF839438)	67	64	67	-	13.75	12.64	15.74	12.42	12.42	10.87	14.19	13.75
30. <i>S. parvulum</i> (KF257088)	61	64	57	62	-	10.20	12.42	11.97	11.53	12.64	13.53	12.20
31. <i>S. pedemontanum</i> (KF257095)	60	48	55	57	46	-	13.97	10.87	9.98	12.86	13.08	11.09
32. <i>S. risi</i> (KF257127)	60	74	72	71	56	63	-	14.19	13.97	14.63	14.63	15.52
33. <i>S. sanguineum</i> (MW208383)	64	59	48	56	54	49	64	-	11.97	11.97	11.97	13.30
34. <i>S. semicinctum</i> (JN294514)	54	62	55	56	52	45	63	54	-	11.09	12.20	13.53
35. <i>S. speciosum</i> (KF257089)	63	60	64	49	57	58	66	54	50	-	13.53	12.86
36. <i>S. uniforme</i> (KF257087)	72	64	71	64	61	59	66	54	55	61	-	13.53
37. <i>S. vicinum</i> (HM413479)	60	62	65	62	55	50	70	60	61	58	61	-

Numbers above the diagonal are percent distance values; numbers below the diagonal are absolute distance values. GenBank accession numbers are provided within parenthesis for the sequences obtained from GenBank, but *S. vulgatum* and *S. striolatum* sequences, which were obtained in this study (written with boldface) provide voucher specimen numbers within parenthesis.

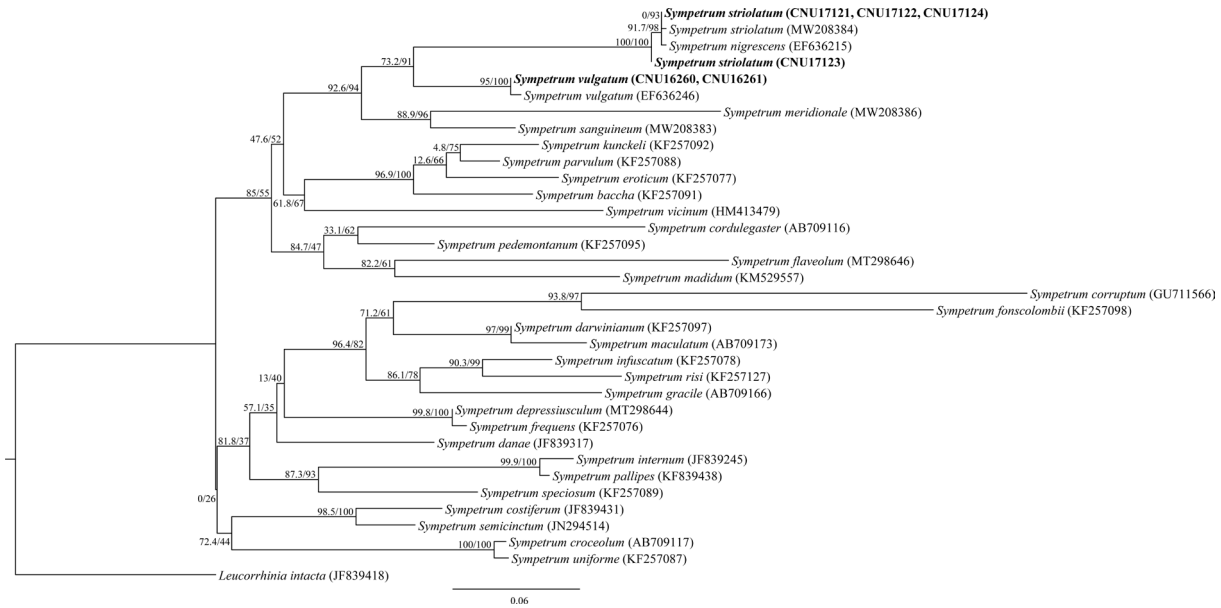


Fig. 4. Phylogeny of *Sympetrum* species using Maximum-likelihood method. The numbers at each node specifies SH-aLRT/UFBoot values. GenBank accession numbers are provided within parenthesis for the sequences obtained from GenBank, but *S. vulgatum* and *S. striolatum* sequences, which were obtained in this study (written with boldface) provide voucher specimen numbers within parenthesis. The scale bar indicates the number of substitutions per site.

EF636246; Pilgrim and von Dohlen, 2007). The four individuals of *S. striolatum* sequenced in this study differ 0-2 bp and these differ by 1-3 bp to that available in GenBank, which was originated in Australia (Table 1; acc. no. MW208384; Haring *et al.*, 2020). Between *S. vulgatum* and *S. striolatum* sequence divergence ranged from 9.98% (45 bp) to 11.09% (50 bp) (Table 1). Such range of divergence is often found in other species pairs, ranging from 6.87% [31 bp; between *S. kunckeli* (KF257092) and *S. eroticum* (KF257077)] to 18.18% (82 bp; between *S. fonscolombii* (KF257098) and *S. eroticum* (KF257077)].

On the other hand, *S. nigrescens* (EF636215) showed only 0.22-0.67% (1-3 bp) difference to *S. striolatum*, but this result is consistent with the previous research that the two species should be considered as the same species on the basis of morphological and molecular data (Pilgrim and von Dohlen, 2007; Parkers *et al.*, 2009). Also, *S. depressiusculum* (MT298644) and *S. frequens* (KF257076) showed 0.67% (3 bp) difference and Pyo *et al.* (2023) claimed that *S. depressiusculum* and *S. frequens* occurring in South Korea should be classified into a single species *S. depressiusculum* on the basis of morphology, two mitochondrial gene sequences, and one nuclear region sequences using the samples from The Netherlands and Russia for *S. depressiusculum* and the samples from Japan for *S. frequens* as well as five populations of *Sympetrum* collected in South

Korea. Similarly, the species pair, *S. croceolum* (AB709117) and *S. uniforme* (KF257087), *S. maculatum* (AB709173) and *S. darwinianum* (KF257097), and *S. pallipes* (KF839438) and *S. internum* (JF839245) also showed very low sequence divergence at 1.11% (5 bp), 3.10% (14 bp), and 1.77% (8 bp), respectively (Table 1). However, previous studies, which included these pairs in the phylogenetic analyses did not comment on this issues, possibly due to the reasonable distinction between the species pairs (Pilgrim and von Dohlen, 2007, 2012).

Phylogenetic analysis showed that *S. striolatum* and *S. vulgatum* form the sister group with the higher nodal supports (SH-aLRT = 73.2, UFBoot = 91), but each species including current samples formed well supported inclusive group with higher nodal supports (UFBoot = 100, SH-aLRT = 100 for *S. striolatum* and SH-aLRT = 100, UFBoot = 95 for *S. vulgatum*; Fig. 4). This result suggests that *S. striolatum* and *S. vulgatum* resemble superficially to each other, but each species is distinct genetically.

Discussion

Sympetrum vulgatum, which was recorded by Doi (1932) has long been included in the national species list in South Korea.

However, there have been no additional discovery records after the first erroneous record of the species. Consequently, we assert that the discovery of this species in Goseong, Gangwon Province, in September 2022 constitutes the first confirmed record of *S. vulgatum* in South Korea. In Japan, the species has been collected in coastal regions bordering the East Sea. In this study, we also collected this species at a coastal area adjacent to the East Sea. Thus, we believe that this species could have been migrated from the Russian Far East. Collectively, the focus of this study lies on elucidating the first true entity of *S. vulgatum* in South Korea through actual sampling, morphological examination, tracing species record, genetic analysis, and ecological description of the species. Through this study, it has become clear that *S. vulgatum* inhabits South Korea. However, there is no available information on the ecology, such as distribution and habitat, of *S. vulgatum* in South Korea. Various ecological studies of *S. vulgatum* in South Korea are needed in the future to update the species distribution information and to track changes in biodiversity due to factors such as climate change.

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