



DNA barcode library of South Korean limoniid crane flies (Diptera: Limoniidae)

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Introduction

Limoniidae is one of the largest groups in Diptera and comprises 10,700 species worldwide (Oosterbroek, 2022). In the South Korean fauna, limoniid crane flies are represented by 127 species belonging to 32 genera in three subfamilies. Identification of this family has been mainly based on morphological characteristics, but their taxonomy has not been solved thoroughly due to individual variations, sexual dimorphism, and cryptic species. Therefore, DNA barcodes have recently been used to support the species delimitation. Here, we constructed DNA barcode library of Korean Limoniidae for the first time.

Materials & Methods

- Specimens were collected by sweep-net or Malaise trap. Specimens are stored in 80% or 100% ethanol for molecular works.
- All materials are deposited at Korea University Entomological Museum, Seoul, Korea or Yeongwol Insect Museum, Yeongwol, Korea.
- Total genomic DNA was extracted from one leg or some parts of it of the specimens using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. Polymerase chain reaction (PCR) was performed to amplify using primers LCO1490 (5' - GGT CAA CAA ATC ATA AAG ATA TTG G - 3') (Folmer et al. 1994) and COIA-r (5' - CCC GGT AAA ATT AAA ATA TAA ACT TC - 3') (Simon et al. 1994) on a 676 bp region of COI gene.
- A protocol for amplification and sequencing of the COI gene was generally followed Suh et al. (2019).
- Phylogenetic relationships were conducted by neighbor-joining (NJ) using Kimura-2-parameter model with 1000 bootstrap replicates in MEGA X.

Results

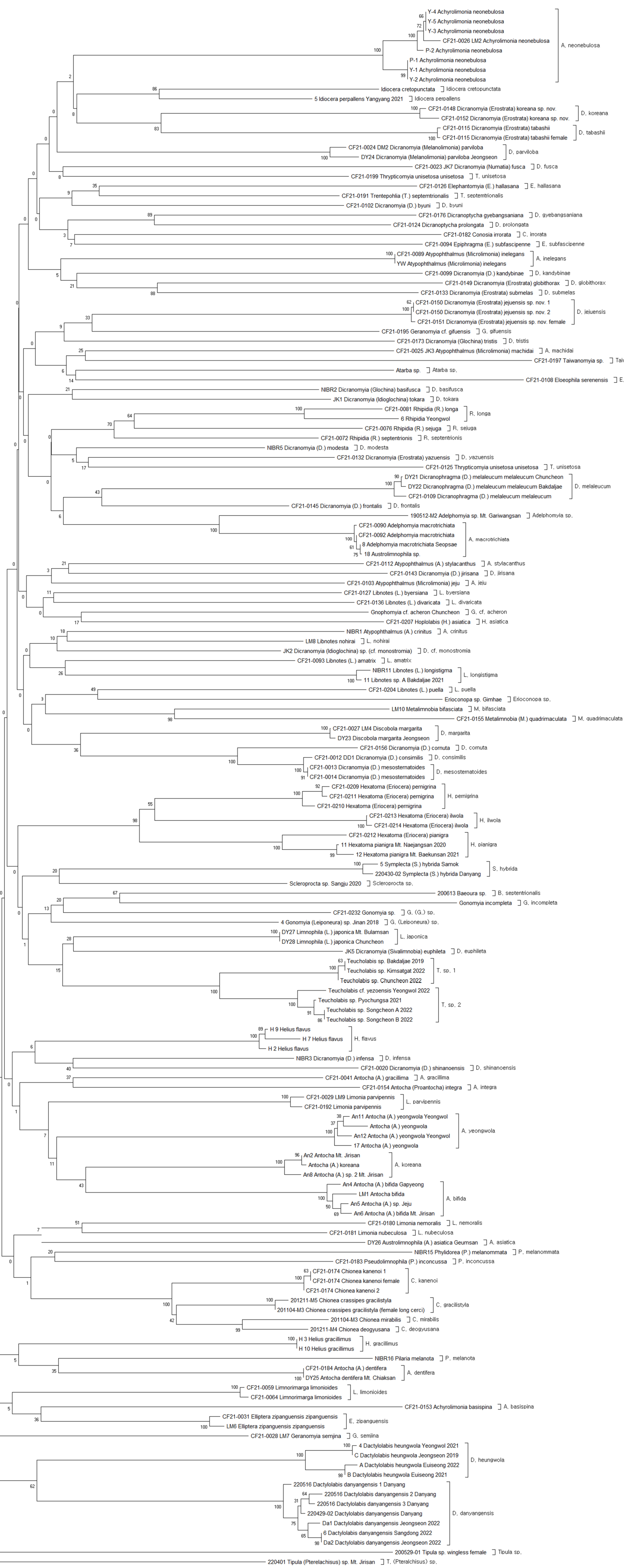


Fig. 1. NJ tree of Limoniidae in South Korea based on COI sequences

Family Limoniidae

Subfamily Chioneinae

Subfamily Dactylolabinae

Subfamily Limnophilinae

Subfamily Limoniinae

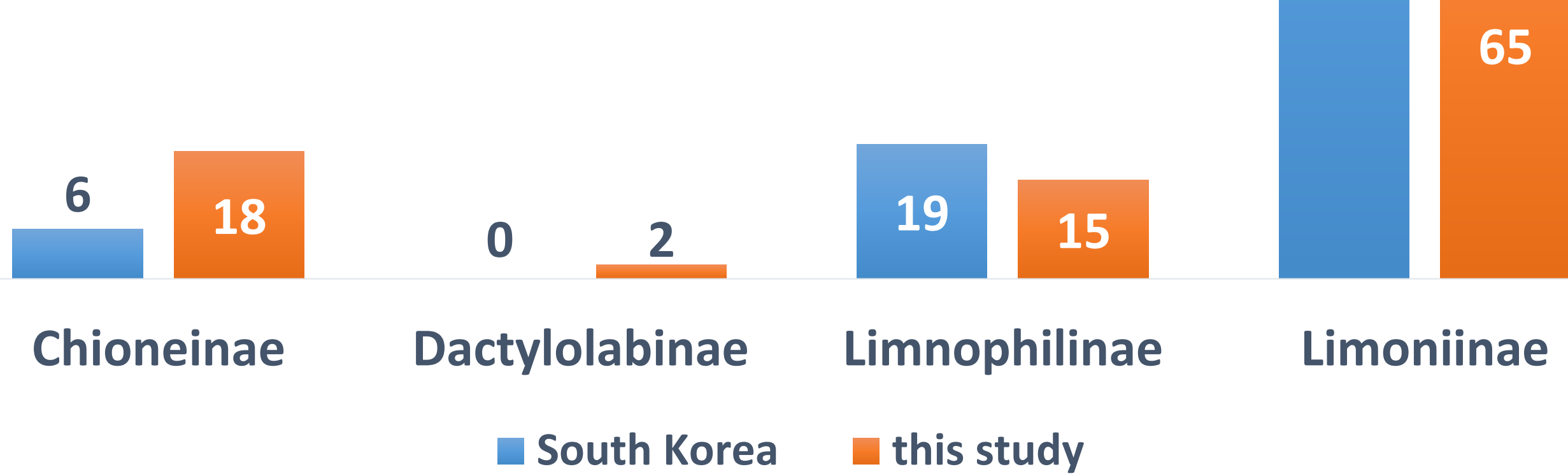


Fig. 2. Number of species recorded in South Korea and used in this study

Table 1. Intra- and interspecific genetic distances of South Korean Limoniidae (except *Dicranomyia* (*D.*) *consimilis* and *D. (D.) mesosternatoides*)

	Mean intraspecific genetic distance (%)	Mean interspecific genetic distance (%)	Maximum intraspecific genetic distance (%)	Minimum interspecific genetic distance (%)
Chioneinae	0.36	13.58	0.31	5.04
Dactylolabinae	1.10	12.36	1.45	12.36
Limnophilinae	0.57	15.21	2.07	7.11
Limoniinae	0.62	14.04	0.63	4.14
Limoniidae	0.58	14.48	2.07	4.14

- In this study, 162 COI sequences from 100 species in 37 genera, which are including 16 species new to South Korea, were obtained.
- There is no range of overlap between intra- and interspecific genetic distances (maximum intraspecific genetic distance: 2.07% in *Hexatoma* (*Eriocera*) *pianigra*; minimum interspecific genetic distance: 4.14% between *Dicranomyia* (*D.*) *cornuta* and *D. (D.) mesosternatoides*).

Conclusions

- Most of limoniid crane flies in South Korea can be discriminated by DNA barcodes with high interspecific genetic distances.
- In the case of *Dicranomyia* (*D.*) *consimilis* and *D. (D.) mesosternatoides*, regarded as the same species in NJ tree, we need further experiments to prove whether they are identical or not.
- The establishment of a Limoniidae DNA barcode library for South Korea will improve the taxonomy and biodiversity of this family.
- Furthermore, since COI marker alone is insufficient to understand the phylogenetic relationship in Limoniidae, it is necessary to reveal it using different region of markers.

References

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