Taxonomic review of Mongolian Ephemerellidae (Ephemeroptera)

Sang Kyu Shin[p1], Jeong Mi Hwang[2] , Badamdorj Bayartogtokh[3], Yeon Jae Bae[c1,2]

1. *Department of Environmental Science and Ecological Engineering, Graduate School, Korea University, Seoul 02841, South Korea*
2. *Korean Entomological Institute, Korea University, Seoul 02841, South Korea*
3. *School of Arts and Sciences, National University of Mongolia, Ulaanbaatar 14200, Mongolia*

Correspondence email: yjbae@korea.ac.kr

Abstract

The mayfly family Ephemerellidae (Ephemeroptera) is particularly diverse and abundant in the cold mountain streams in the Northern Hemisphere, such as Mongolian alpine streams. Although Mongolian mayfly fauna has been continuously investigated since 1970s, knowledge on mountainous ephemerellids is still waning. In this study, we reviewed Mongolian ephemerellids using materials from our long-term investigation from 2009-2022. As a result, we reviewed 16 species of Mongolian Ephemerellidae, including two new records of *Drunella ishiyamana* Matsumura, 1931, and *Cincticostella nigra* (Uéno, 1928). With DNA barcode analysis, special attention was paid on the cryptic diversity of the species of *Drunella* Needham, 1905. Further comprehensive morphological and phylogenetic studies of *Drunella* are needed to revel cryptic species within the genus.

Materials and Methods

Mongolian samples were collected between 2009~2022, excluding 2011, 2020 and 2021. Ephemerellidae nymphs were sampled qualitatively using hand nets (mesh size 1mm) and preserved in 80% ethanol in the field then transferred to 95% ethanol for future molecular analysis. Identification of species was conducted based on morphological character keys of Kluge (1997) and Bae (2010).

58 *COI mtDNA* sequences were used for phylogenetic analysis, including 11 new sequences obtained from Mongolian and Korean samples and 47 GenBank sequences from Korea and Japan. One *COI* sequence from *Teloganopsis punctisetae* was used as an outgroup. Genomic DNA was extracted from a foreleg of the samples using DNeasy Blood and Tissue Kit (Qiagen, Germanton, NC, USA). A 658 bp *mtDNA* fragment was then polymerase chain reaction amplified under the following conditions: initial denaturation of 3 minutes at 94°C, 36 cycles of 30s denaturation at 94 °C, 30s of annealing at 47 °C, and 1 min of extension at 72 °C; and a final extension of 5 min at 72 °C. Verified PCR products were purified using Exonucleas I and Shrimp Alkaline Phosphate and then sequenced by Macrogen INC sequencing (Republic of Korea) on a ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic tree was reconstructed using 58 *COI mtDNA* sequences from 10 species (including the outgroup). Sequence alignment was performed using Clustral-W multiple sequence alignment package in MEGA 10.2.6 and edited manually. Genetic sequence divergence was estimated using p-distance in MEGA 10.2.6. TrN+G+I model of nucleotide substitution was selected as the best fit for our data by jModelTest v.2.1.10 under the Akaike information criteria.

Results and Discussion

Checklist of the Ephemerellidae of Mongolia

Family Ephemerellidae Klapálek, 1909

Subfamily Ephemerellinae Lestage, 1917

Genus *Cincticostella* Tshernova, 1972

1. *Cincticostella nigra* (Uéno, 1928)

Ephemerella nigra Uéno, 1928: 44

Cincticostella nigra Tshernova, 1972: 614

Remarks

New record in Mongolia; Materials examined: 2 nymph (in ethanol), Tuul River , Ulaanbaatar, Mongolia, VII-30-2014, hand net, Coll. YJ Bae.

Genus *Drunella* Needham, 1927

2. *Drunella cryptomeria* (Imanishi, 1937)

Ephemerella cryptomeria Imanishi, 1937: 328

Drunella cryptomeria Tiunova, 1984: 49

3. *Drunella ishiyamana* Matsumura, 1931

Drunella ishiyamana Matsumura, 1931: 1470

Remarks

New record in Mongolia; Materials examined: 21 nymphs (in ethanol), Tuul River, Bayanzurkh, Ulaanbaatar, Mongolia, 47°49'45.9"N 107°19'24.3"E, VI-27-2022, hand net, Coll. SH Park, SK Shin, G. Altangerel.

4. *Drunella latipes* (Tshernova, 1952)

Ephemerella latipes Tshernova, 1952: 273

Drunella latipes Allen, 1980

Remarks

Tshernova et al. (1986) synonymized *D. latipes* to *D. cryptomeria*

5. *Drunella lepnevae* (Tshernova, 1949)

Ephemerella lepnevae Tshernova, 1949: 152

Cincticostella lepnevae Tiunova, 1984: 50

Drunella lepnevae Potikha, 1985

6. *Drunella submontana* (Brodsky, 1930)

Ephemerella submontane Brodsky, 1930: 683

Drunella submontana Landa & Soldan, 1983: 200

7. *Drunella triacantha* (Tshernova, 1949)

Ephemerella triacantha Tshernova, 1949: 151

Drunella triacantha Tiunova, 1984: 48

8. *Drunella trispina* (Uéno, 1928)

Ephemerella trispina Uéno, 1928: 45

Drunella trispina Allen, 1980: 80

Genus *Ephemerella* Walsh, 1862

9. *Ephemerella aurivillii* Bengtsson, 1908

Chitonophora aurivillii Bengtsson, 1908

Ephemerella aurivillii McDunnough, 1931: 197

10. *Ephemerella mucronata* (Bengtsson, 1909)

Chitonophora mucronata Bengtsson, 1909: 8

Ephemerella mucronate Tshernova, 1952: 281

11. *Ephemerella nuda* Tshernova, 1949

Ephemerella nuda Tshernova, 1949: 156

12. *Ephemerella zapekinae* Bajkova, 1967

Ephemerella zapekinae Bajkova, 1967: 329

Genus *Serratella* Allen, 1980

13. *Serratella ignita* (Poda, 1761)

Ephemera ignita Poda, 1761: 97

Ephemerella ignita Eaton, 1871: 98

Serratella ignita Tiunova, 1984: 47

14. *Serratella setigera* (Bajkova, 1965)

Ephemerella setigera Bajkova, 1965

Serratella setigera Tiunova & Belov, 1984: 74

Genus *Teloganopsis* Ulmer, 1939

15. *Teloganopsis punctisetae* (Matsumura, 1931)

Drunella punctisetae Matsumura, 1931: 1471

Ephemerella punctisetae Edmunds, 1959: 546

Uracanthella punctisetae Ishiwata, 2001: 63

Teloganopsis punctisetae Jacobus & McCafferty, 2008: 241

Genus *Uracanthella* Belov, 1979

16. *Uracanthella lenoki* (Tshernova, 1952)

Ephemerella lenoki Tshernova, 1952: 275

Uracanthella lenoki Belov, 1979: 575

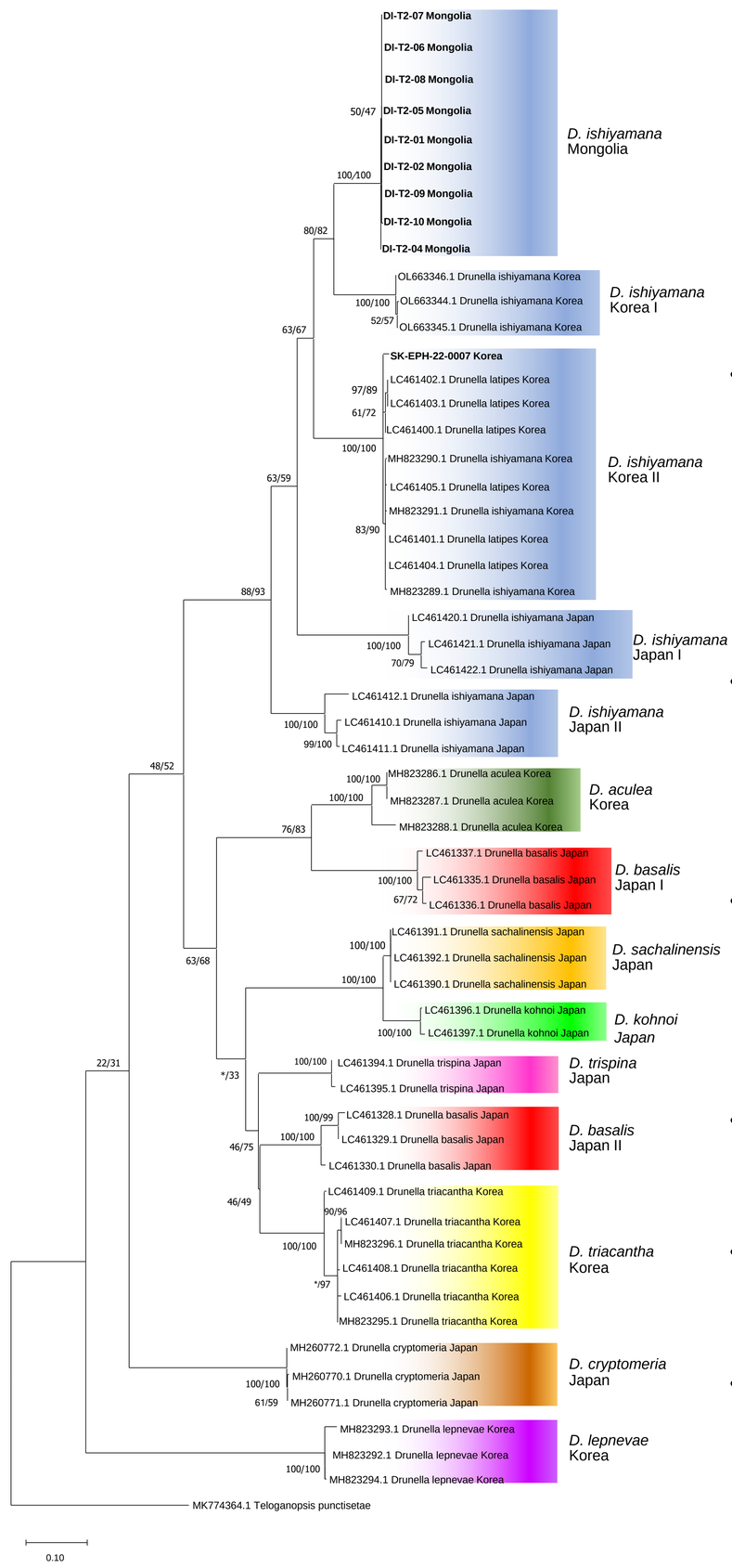

Fig. 1. Maximum likelihood tree of *Drunella* species of Mongolia, Korea and Japan. 58 *COI mtDNA* gene sequences from 10 species were used. Sequences newly obtained in current study are highlighted in bold. Branch values indicate neighbour-joining (NJ) and maximum likelihood (ML) bootstrap values, respectively. Trees were bootstrapped using 1000 repetitions. Asterisks (*) indicate branches not supported by NJ analysis.

- Mongolian Ephemerellidae samples from 2009~2014 and 2022 were examined and identified based on morphological characteristics. Two new recorded species (*Drunella ishiyamana*, *Cincticostella nigra*) were identified and added to the list of Mongolian Ephemerellidae. Further examination of our samples seems likely to reveal more new records or possibly new species in Mongolia.
- Identifying Ephemerellidae species with only morphological characteristics has its limits because of intraspecific morphological variations. This has caused problems with determining species groups, as in the case of *Drunella* species (*D. cryptomeria*, *D. latipes*, *D. ishiyamana*).
- Previous studies revealed cryptic diversity in *D. ishiyamana* and *D. basalis* (Jo and Tojo, 2019), as shown as *D. basalis* clade I and II, and *D.ishiyamana* clade Korea II, Japan I and II.
- Performing phylogenetic analysis by including our samples revealed further cryptic diversity within *D. ishiyamana*, as shown as clade Mongolia and Korea I, with the end result showing five distinct clades of *D. ishiyamana*.
- Korean *D. latipes* can be seen assimilated with *D. ishiyamana* clade Korea II, suggesting that these samples are possibly *D. ishiyamana*.
- Mean distance of genetic divergence was quite high, with the average mean distance within *D. ishiyamana* clades being 16.49%.
- High mean distance and multiple clades of several *Drunella* species being revealed hint towards possible cryptic species, however, is not enough evidence to define new species or subspecies.
- Further phylogenetic analysis using multiple DNA markers and more samples, as well as extensive morphological study, is needed to properly explain the cryptic diversity found within *Drunella* and Ephemerellidae species.

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