DNA barcode analysis of chironomid larvae (Diptera: Chironomidae) from large rivers in South Korea to faciliate freshwater biomonitoring and public health surveillance

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Introduction

Chironomid larvae are among the dominant benthic macroinvertebrates in all types of water systems in South Korea. They may pass through pipes in rivers (raw water) and occur in drinking/tap water, thus creating public health issues. However, little is known about the larval stages of chironomids in large South Korean rivers. Therefore, we examined larval–adult associations in chironomids inhabiting major rivers used as water sources. The samples from the four major rivers yielded 61 mitochondrial *COI* sequences belonging to 18 species, including *Hydrobaenus kondoi* Saether, 1989, which was reported for the first time in the Korean Peninsula. Further, morphological identification of the larvae was conducted, and a pictorial taxonomic key to Chironomidae species in large rivers in South Korea was developed to facilitate freshwater biomonitoring research.

Materials & Methods

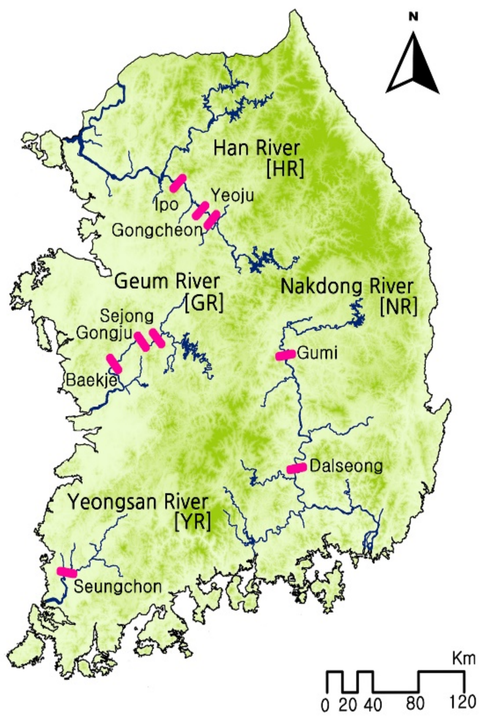


Figure 1. Sampling sites in major four rivers of south Korea. Abbreviations: H (Han river,); IP (Ipo weir); YJ, Yeoju weir; GC, Gangcheon weir; Geum River, G: SJ, Sejong weir; GJ, Gongju weir; BJ, Baekje weir; Yeongsan river, Y: SC, Seungchon weir; Nakdong River, N: GM, Gumi weir; DS, Dalseong weir.

- Adults: sweeping, light-trap
- Larvae: ponar grab
- Mar. 2015 to Aug. 2016

- Adults: antennae, head, wings and hypopygium mounted with Hoyer's solution and Euparal
- Larvae: Head, 7th - 9th abdomen segments mounted with solutions
- Remaining parts preserved in 99.5% ethanol for DNA extraction
- Genomic DNA extraction: DNeasy® Blood and Tissue Kit (Quiagen Genomics Inc., Dusseldolf, Germany)
- Primer information: Universal primer: LCO1490; HCO2198
- PCR condition:

		denaturation		annealing	extension	final extension	preservation
PCR condition	Tm.	94℃	94℃	48℃ [gradient: 46-53]	72℃	72℃	4℃
	Cycle	1	35			1	1
	time	5 mins	1 min	1:30 min	1 min	5mins	∞

Results

Subfamily	Species
Tanypodinae	*Procladius choreus* (Meigen, 1804)
Orthocladiinae	*Hydrobaenus kondoi* Saether, 1989 *
	Propsilocerus akamusi (Tokunaga, 1938)
Chironominae	*Benthalia carbonaria* (Meigen, 1804)
	Chironomus circumdatus (Kieffer, 1916)
	Chironomus nipponensis Tokunaga, 1940
	Chironomus plumosus (Linnaeus, 1758)
	Dicrotendipes nervosus (Staeger, 1839)
	Glyptotendipes tokunagai Sasa, 1979
	Microchironomus tener (Kieffer, 1918)
	Lipiniella moderata Kalugina, 1970
	Polypedilum nubeculosum (Meigen, 1804)
	Stenochironomus sp.
	Tanytarsus ahyoni Ree & Jeong, 2010
	Tanytarsus kiseogi Ree & Jeong, 2010
	Chironominae sp. 1
	Chironominae sp. 2
	Chironominae sp. 3

Asterisks (*) indicate species recorded as new to the South Korean fauna. GenBank accession numbers with daggers (†) indicate the specimens whose sequences were retrieved from GenBank.

Table 1. List of larval Chironomidae taxa collected from four large rivers in South Korea and identified using a DNA barcode library in this study

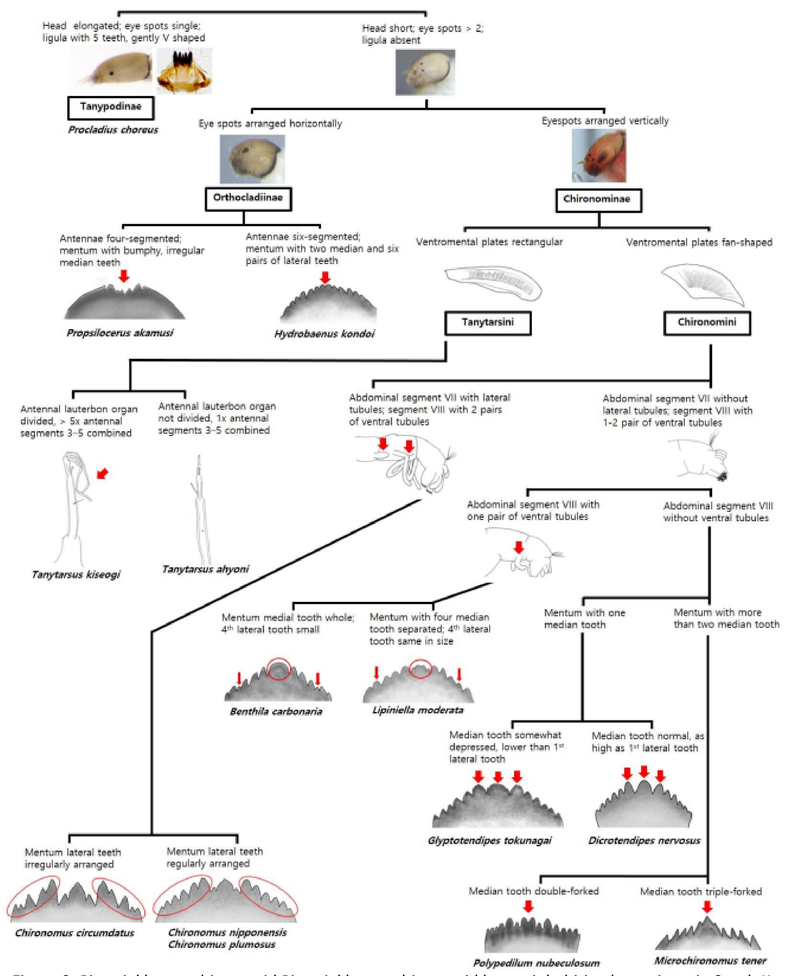
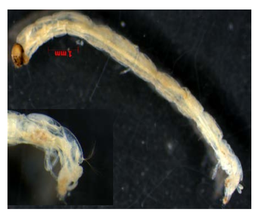
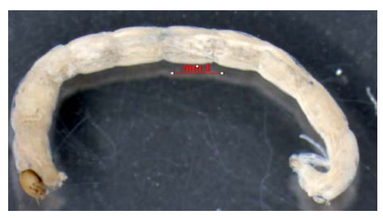


Figure 2. Pictorial key to chironomid Pictorial key to chironomid larvae inhabiting large rivers in South Korea.

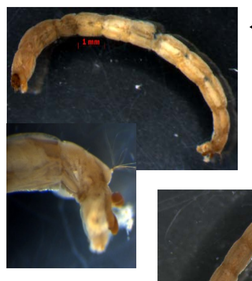
Results



▲ *Chironomus nipponensis*

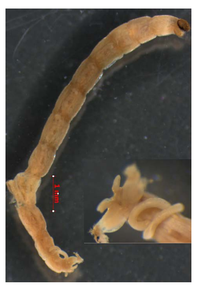


▲ *Chironomus plumosus*

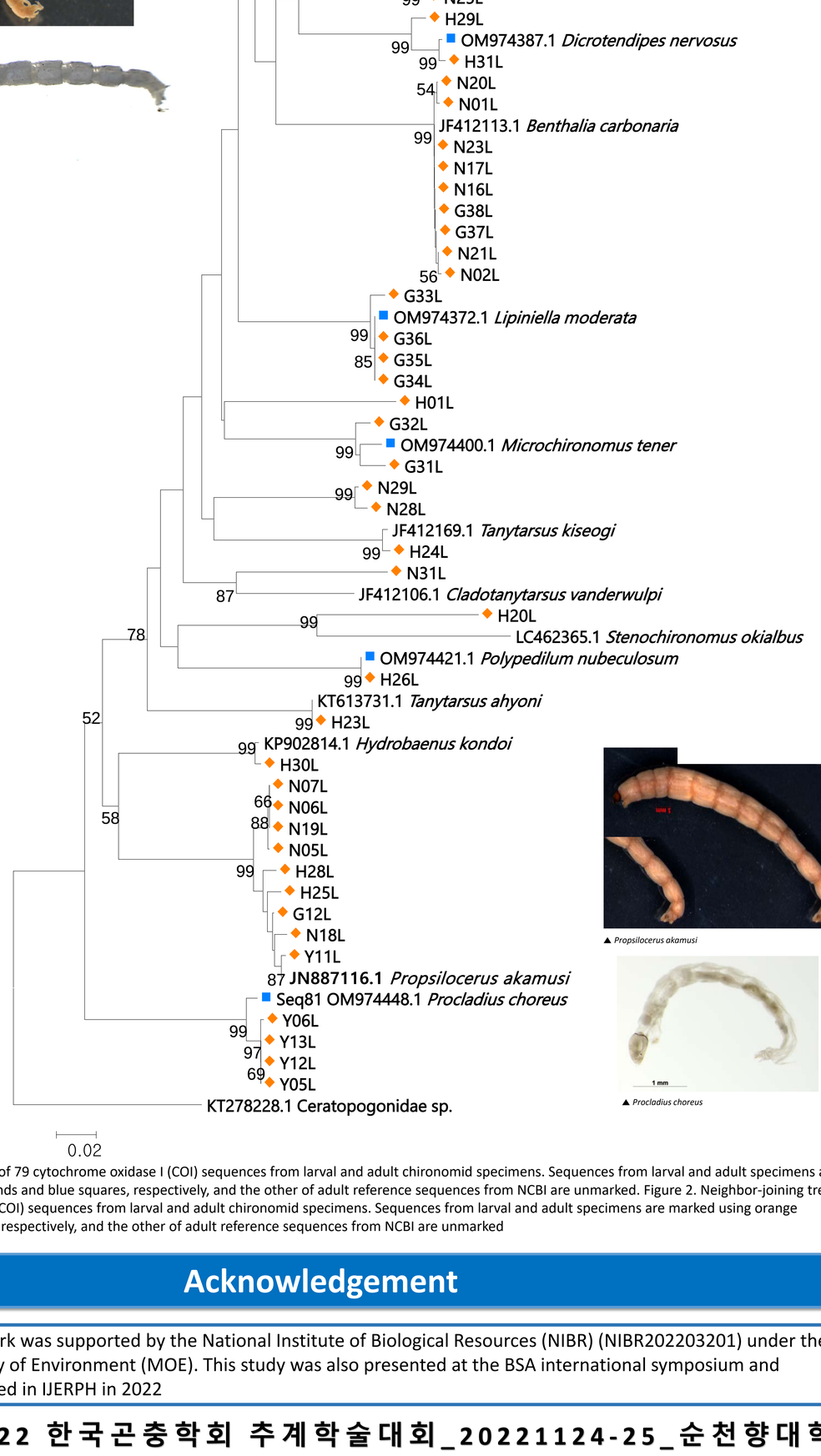


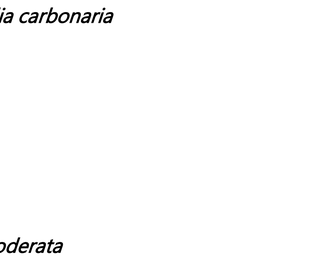
◀ *Dicrotendipes nervosus*

Glyptotendipes tokunagai ▶



▲ *Hydrobaenus kondoi*





▲ *Propsilocerus akamusi*

▲ *Procladius choreus*

Figure 3. Nabor-joining tree of 79 cytochrome oxidase I (COI) sequences from larval and adult chironomid specimens. Sequences from larval and adult specimens are marked using orange diamonds and blue squares, respectively, and the other of adult reference sequences from NCBI are unmarked. Figure 2. Neighbor-joining tree of 79 cytochrome oxidase I (COI) sequences from larval and adult chironomid specimens. Sequences from larval and adult specimens are marked using orange diamonds and blue squares, respectively, and the other of adult reference sequences from NCBI are unmarked

Conclusions

- This study identified 18 species within this family and successfully associated larval morphotypes with known adult species for the first time
  - species delimitation problems solved
  - high-resolution identification of larval specimens can be achieved
  - *Hydrobaenus kondoi* species recorded for the first time by DNA barcoding
- Our newly developed chironomid larvae pictorial key in large South Korean rivers may facilitate biomonitoring of large rivers in Northeast Asia, can be used by the public to enable a rapid response to environmental issues such as larval infestation of drinking water, helping alleviate a problem that has greatly affected countries such as Korea in the past.

Acknowledgement

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