



A CAMPAIGN TO DNA BARCODE CHINESE STONEFLIES (INSECTA: PLECOPTERA)

Zhi-Teng Chen

School of Grain Science and Technology, Jiangsu University of Science and Technology,
Zhenjiang 212004, China.
E-mail: 741208116@qq.com

ABSTRACT

A DNA barcode library for Chinese stoneflies would increase the speed and accuracy of species identification, help associate life stages, improve our basic understanding of the phylogeny of the group, and build a roadmap for future systematic, conservation, biodiversity, and population genetic work in the region. However, no such library exists in China. This study synthesizes all published Cytochrome Oxidase Subunit 1 (COI) sequence data available from online databases and published literature. This contribution outlines a proposal for establishing a DNA barcode library for Chinese stoneflies.

Keywords: biodiversity, rapid identification, molecular database, COI gene

INTRODUCTION

Plecoptera is an order of hemimetabolous insects whose nymphs inhabit clean water and are the most environmentally sensitive of aquatic insects (DeWalt & Ower 2019). The accelerating urbanization of China is rapidly degrading water quality across the country. Consequently, stoneflies are also rapidly disappearing, some being lost before we find and describe them. Accurate and rapid identification of the ubiquitous aquatic insects, including stoneflies, is a vital precondition for water quality monitoring and biodiversity surveys (Macher et al. 2016). However, the traditional way based on morphology is time-consuming, requiring professional taxonomic skill (Zhou et al. 2009).

DNA barcoding is a well-developed technique that uses a short fragment of the Cytochrome Oxidase Subunit 1(COI) gene for species delimitation (Hebert et al. 2003). Three main

functions of DNA barcodes have been used in Plecoptera, including rapid identification, association of different life stages, and population genetics (DeWalt 2011, Mynott 2015, Boumans et al. 2016). Many entities have conducted studies of DNA barcodes of stoneflies and established DNA barcode libraries (Zhou et al. 2009, Gattolliat et al. 2016, Morinière et al. 2017). However, in the Barcode of Life Data System (BOLD), a global library for DNA barcode sequences, most sequences originate from Europe, North America, Japan and New Zealand; very few sequences are from China (Fig. 1). This data gap hinders the ability of Chinese scholars to advance the study of stoneflies in a number of scientific endeavors. Chinese researchers must begin to erase this deficit if we are to document and protect our fauna and the habitats on which they depend. In this study, I have summarized and accumulated all available COI sequences from Chinese

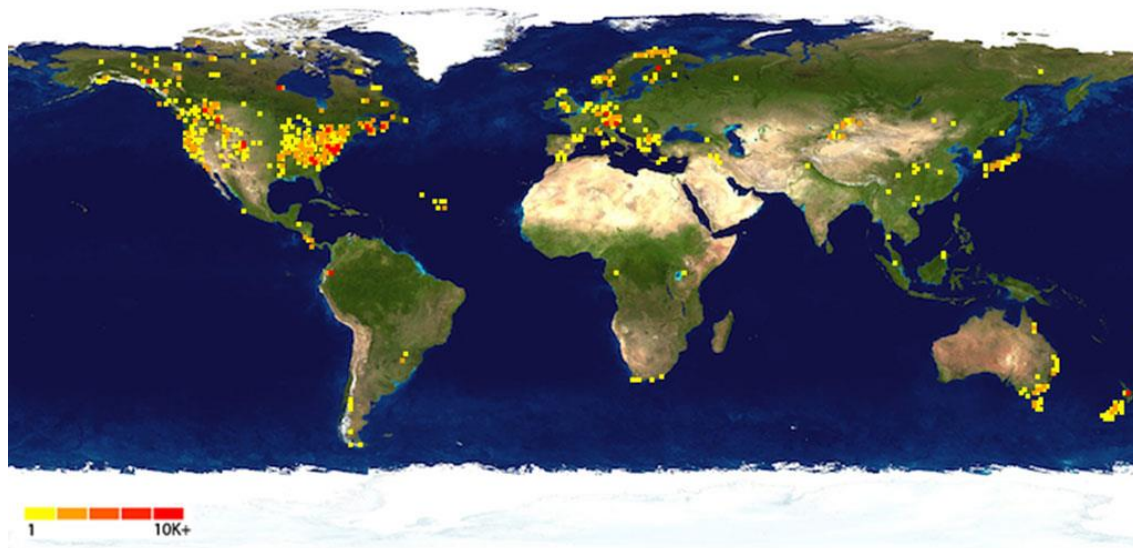


Fig. 1. Barcoding data sources in Genbank and BOLD databases.

stoneflies from Genbank, BOLD, and published literature. I herein propose a collaborative campaign to DNA barcode all stonefly species in China.

MATERIALS AND METHODS

Public COI sequences were downloaded from Genbank (<https://www.ncbi.nlm.nih.gov/>) and the BOLD System (<http://www.boldsystems.org/>). All obtained sequences were temporarily stored on a server in the Insect Collection of Jiangsu University of Science and Technology (ICJUST), Jiangsu Province, China. In next steps, these data and newly obtained sequences will be submitted to the project entitled “DNA barcodes of Chinese Plecoptera” in the BOLD system, along with all relevant information and images of sequenced specimens.

RESULTS AND DISCUSSION

The currently available COI sequences of Chinese stoneflies are mostly obtained from partial or whole mitochondrial genomes. Only a few studies from China have combined the molecular data with morphological characters (Li et al. 2017, Chen 2019). Approximately 658 stonefly species representing 67 genera and 10

families are known from China (DeWalt & Ower 2019, Yang et al. 2015, Yang & Li 2018). Only 36 species of 26 genera have COI sequences (Table 1, Fig. 2). Perlidae, Nemouridae and Leuctridae have relatively more available COI sequences, whereas Pteronarcyidae and Taeniopterygidae have not been sequenced (Fig. 3). The number of COI sequences for each family is found to have a positive correlation with the number of species in each family, suggesting that availability of specimens has more to do with which species are sequenced rather than the existence of a larger objective.

Previous molecular contributions to Chinese stoneflies, especially the mitochondrial genomic contributions made mainly by Drs. Yu-Zhou Du, Wei-Hai Li and Ding Yang and students, have contributed a lot to the taxonomy of the Plecoptera of China (Table 1). However, no comprehensive DNA barcode study has been considered or conducted for Chinese stoneflies, the reason for the campaign proposed in this study.

The limited DNA barcode sequences and metadata are all deposited in Genbank and BOLD, but only some of them are available for public use. A new BOLD campaign to build a

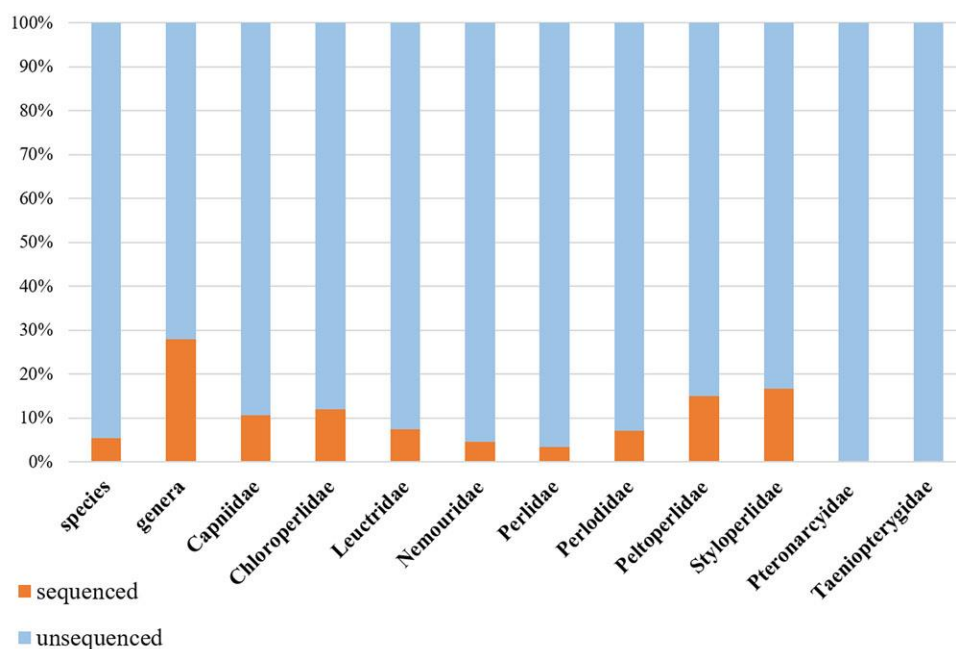


Fig. 2. Percentage of Chinese species, genera, and species within families that have been barcoded. The percentages reflect only the publicly available data from Genbank and BOLD Systems.

DNA barcode library for Chinese stoneflies and other aquatic insects (especially Ephemeroptera and Trichoptera), should be established with the joint effort of researchers from China and worldwide. The remaining stonefly species known from China should be sequenced to facilitate: 1) rapid identification; 2) association of life stages; and 3) studies of evolutionary history and biogeography of Chinese stoneflies. This campaign must build a highly vetted resource that notes where voucher specimens for each sequence may be borrowed for re-examination, allows for submission of multiple taxonomically informative images that aid in verification of species identification, and that will provide open access for other researchers as data are published. All these attributes are already available in BOLD Systems in a global, collaborative platform.

In the next stages, the following phases are expected to be realized with our joint efforts: 1) relevant scholars join in the new project in BOLD with full access to the database; 2) develop a jointly maintained database of freshly collected species contained within major stonefly

collections in China so that a plan for collecting and sequencing may be advanced; 3) sequence at least five individuals from across the range of each species; 4) submit all DNA sequences and photos of specimens to the new project in BOLD along with detailed geographical and biological information; and 5) analyze and publish these data to support the goals mentioned above. Please join me in this collaborative project that will help us attack taxonomic, ecological, and water quality issues with species-level resolution.

The proposed work will require at least 5 years to complete. The development of a joint specimen database and barcode library will demonstrate a level of collaboration heretofore not experienced by stonefly researchers in China. This work will also push Chinese researchers to adopt museum and specimen database standards that will help preserve type and non-type specimens for the future and allow sharing of biodiversity data with the Global Biodiversity Informatics Facility (<https://www.GBIF.org>), increasing the visibility of Chinese studies of stoneflies and the researchers who conducted

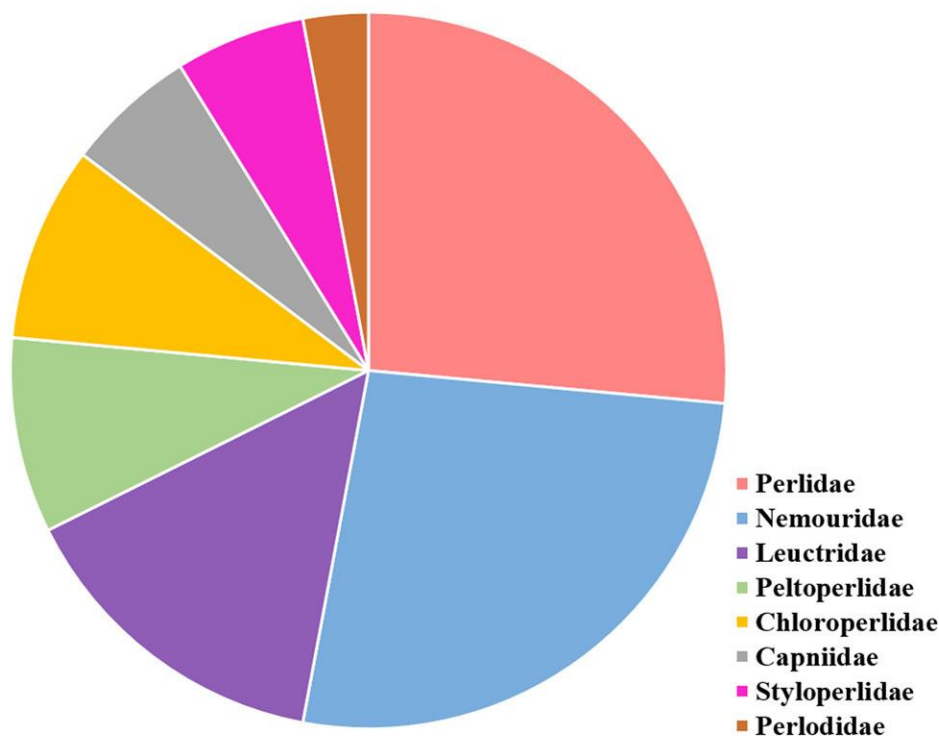


Fig. 3. Percentage of all sequenced Chinese stoneflies relative to families in which they are classified.

Table 1. Information of available DNA barcodes for Chinese stoneflies.

Family	Species	Accession No.	Reference
Capniidae	<i>Capnia zijinshana</i> Du & Chen, 2016	NC_034661	Chen & Du 2017a
	<i>Mesocapnia daxingana</i> Chen & Du, 2016	KY568983	Wang et al. 2017a
Chloroperlidae	<i>Haploperla ussurica</i> Navas, 1934	XJDQD193-18	BOLD
	<i>Suwallia teleckojensis</i> (Šámal, 1939)	NC_037754	Wang et al. 2018a
	<i>Sweltsa longistyla</i> (Wu, 1938)	KM216826	Chen & Du 2015
Leuctridae	<i>Paraleuctra cuihuashana</i> Chen, 2019	MK995183- MK995195	Chen 2019
	<i>Paraleuctra orientalis</i> (Chu, 1928)	MK995196- MK995198	Chen 2019
	<i>Rhopalopsle duyuzhoui</i> Sivec & Harper, 2008	KY304497	Li et al. 2017
	<i>Rhopalopsle longispina</i> Yang & Yang, 1991	KY304503	Li et al. 2017
	<i>Rhopalopsle sinensis</i> Yang & Yang, 1993	KY304499	Li et al. 2017
	<i>Amphinemura yao</i> Mo, Yang, Wang & Li, 2017	NC_044749	Cao et al. 2019a
	<i>Indonemoura jacobsoni</i> (Klapálek, 1912)	NC_044750	Cao et al. 2019a
Nemouridae	<i>Indonemoura scalprata</i> (Li & Yang, 2007)	MK132455	Gamboa et al. 2019
	<i>Mesonemoura tritaenia</i> Li & Yang, 2007	NC_044720	Cao et al. 2019a
	<i>Nemoura nankinensis</i> Wu, 1926	NC_034939	Chen & Du 2017b
	<i>Nemoura papilla</i> Okamoto, 1922	MK290826	Cao et al. 2019b
	<i>Nemoura securigera</i> Klapálek, 1907	MK132393	Gamboa et al. 2019

	<i>Sphaeroneurina grandicauda</i> (Wu, 1973)	NC_044754	Cao et al. 2019a
	<i>Sphaeroneurina hamistyla</i> (Wu, 1962)	NC_044755	Cao et al. 2019a
Perlidae	<i>Acroneuria hainana</i> Wu, 1938	KM199685	Huang et al. 2015
	<i>Agnentina brevipennis</i> (Navás, 1912)	XJDQD106-18	BOLD
	<i>Caroperla siveci</i> Li & Wang, 2013	MG677942	Cao et al. 2019c
	<i>Etrocorema hochii</i> (Wu, 1938)	MK905888	Liu et al. 2019
	<i>Kamimuria chungnanshana</i> Wu, 1938	NC_028076	Wang et al. 2015
	<i>Kamimuria klapaleki</i> (Wu & Claassen, 1934)	MN400755	Chen et al. 2019
	<i>Kamimuria wangi</i> Sun & Du, 2012	KC894944	Qian et al. 2014
	<i>Neoperlops gressitti</i> Banks, 1939	MN400756	Zhang et al. 2019
	<i>Sinacroneuria dabieshana</i> Li & Murányi, 2014	MK492253	Cao et al. 2019d
Perlodidae	<i>Isoperla eximia</i> Zapekina-Dulkeit, 1975	NC_038167	Wang et al. 2018b
	<i>Isoperla lunigera</i> (Klapálek, 1923)	XJDQD196-18	BOLD
	<i>Skavala compacta</i> (McLachlan, 1872)	XJDQD135-18	BOLD
Peltoperlidae	<i>Cryptoperla stilifera</i> Sivec, 1995	KC952026	Wu et al. 2014
	<i>Microperla geei</i> Chu, 1928	MN096323	Cao et al. 2019e
	<i>Soliperla wanglanga</i> Huo & Du, 2017	NC_038189	Chen & Du 2018
Styloperlidae	<i>Cerconychia sapa</i> Stark & Sivec, 2007	MF100783	Wang et al. 2018c
	<i>Styloperla spinicercia</i> Wu, 1935	NC_034809	Wang et al. 2017b

them. Development of this kind of community and research tools may also improve funding of stonefly and aquatic insect taxonomy since a barcode library is useful in meeting so many basic and applied scientific needs.

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