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The Utility of DNA Barcoding for the Species Identification of Larval Fish in the Lower Ing River, Thailand

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Abstract

The species identification of larval fish is very important for sustainable fishery resource management. However, identification based on morphological characters is very difficult, complex and error-prone. DNA barcoding with the sequence of cytochrome c oxidase I (COI) gene was used to identify larval fish species from 10 stations in the tributaries of the lower Ing River. One hundred and six samples were collected between May 2016 and April 2017. The average length of the COI nucleotide sequences was approximately 640 bp. A total of 99 nucleotide sequences were identified in 35 species, 31 genera, 19 families and 9 orders, with 97-100% identity with entries in both the GenBank and BOLD databases. The genetic distance within species ranged from 0.000 to 0.004. However, seven samples were identified at only the genus level because their sequences had not been reported in any databases. Based on IUCN conservation status, most species were classified as least concern (77.14%). Approximately 69.23% of all species were related to human uses in fisheries, aquaculture or aquariums, whereas 30.77% of species were not assessed. *Trichopsis vittata* (family Osphronemidae) (90%) had the most frequency of occurrence, followed by *Oryzias minutillus* (family Adrianichthyidae) (70%) and *Trichopodus trichopterus* (family Osphronemidae) (70%).

Introduction

The Ing River is a major water source of the Phayao and Chiang Rai provinces in northern Thailand and is a tributary of the Mekong River. It flows northwards from Kwan Phayao, Phayao Province through the Mekong River in Chiang Rai Province for approximately 240 kilometers. There are 86 fish species in the upper Mekong River at the Thai-Laos border, and 66 of these species migrate to lay eggs in its tributaries, such as the Ing River. The Ing River has a warmer water temperature and is a more suitable ecosystem for laying eggs than the cooler Mekong River, which flows from the Himalayas (Thai Baan Research, 2006). In addition, the Ing River also has a large variety of fish species that includes 82 fish species belonging to 57 genera and 22 families (Valunpion & Suvarnaraksha, 2013). Therefore, the Ing River and its tributaries most likely contain the most diverse group of larval fish species.

The species identification of larval fish is very important for fishery resource management in various water sources for predicting the changes in fish populations and calculating the size of fish stocks (Termvidchakorn, 2003). However, the appearance of larval fish is completely different from that of adult fish. Also, species identification based on morphological characteristics, such as the numbers of muscles, the notochord and fin rays, body shape, and eye shape (Termvidchakorn, 2003) is uaually difficult. Moreover, the accuracy may be quite low; for example, a total of

100 larval fish were identified based on morphology in five laboratories in Taiwan. The average accuracy was quite low: 80.1, 41.1 and 13.5% at the family, genus and species levels, respectively (Ko *et al*., 2013). A total of 354 larval fish samples were morphologically identified. Within these samples, 67.8% could be identified at the family level and 30% at the genus level, while the identification at the species level was not possible (Azmir, Esa, Amin, Yasin, & Yusof, 2017).

DNA barcoding with the partial nucleotide sequence of the cytochrome c oxidase I (COI) gene serves as the core of a global bio-identification system for animals (Hebert, Cywinska, Ball, & deWaard, 2003). All species can be differentiated by their COI sequences with a low average distance within species of 0.39% (Ward, Zemlak, Innes, Last, & Hebert, 2005). In fish, DNA barcoding has been very successful for species identification because of the universal primers described by Ward *et al*. (2005) and Ivanova, Zemlak, Hanner, and Hebert (2007) that were very effective for the amplification of the COI sequences of most species. Furthermore, DNA barcoding was used for the identification of several larval fish species, including the members of Acanthuridae and Holocentridae families (Hubert, Delrieu-Trottin, Irisson, Meyer, & Planes, 2010) and the genus *Pseudoblennius* (Kwun, 2018).

The objective of this study was to identify larval fish species collected from 10 stations in the tributaries of the lower Ing River using DNA barcoding. The samples were identified to obtain their scientific name after comparing their COI sequences with the reported sequences of organisms in databases. In addition, the human uses for and the distribution of each species were also determined. The results of this study would be useful for the creation of a database to manage fish resources in the future.

Materials and Methods

Larval Fish Collection

The larval fish were collected at 10 stations (Figure 1) in the tributaries of the lower Ing River in Chang Rai Province, northern Thailand between May 2016 and April 2017. The samples were obtained by using plastic nylon nets with 16×16 mesh/inch that were 3×1.2×1.2 m in size. The nets were towed many times close to marginal areas to obtain the most samples. All samples were anesthetized in 0.2 g/L of MS-222 (Sigma, Missouri, USA) dissolved in water, preserved in absolute ethanol and transported to the laboratory. Samples with similar morphological characteristics were grouped together under a stereo microscope and photographed.

DNA Barcoding

A total of 106 genomic DNA samples from representative larval fish were extracted from muscle

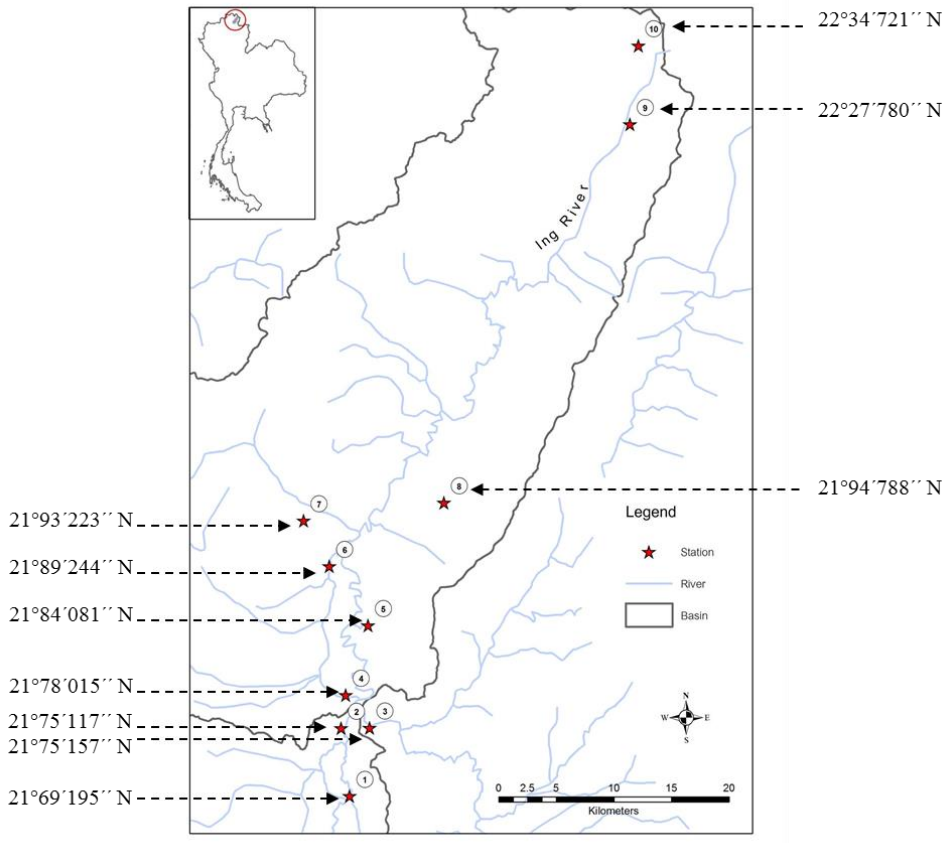

Figure 1. Collection stations in the tributaries of the lower Ing River, northern Thailand.

tissues using proteinase K digestion followed by the standard phenol chloroform method (Sambrook & Russell, 2001). The quality of the extracted DNA was determined on a 1% agarose gel. The fragments of the COI gene were amplified with four primers (FishF1, FishF2, FishR1, and FishR2) that were described by Ward *et al*. (2005) using PCR. A total volume of 25 µl of a PCR mixture contained 1× *Taq* buffer, 2.5 mM $MgCl_2$, 0.4 µM of each primer, 1 µM dNTPs, 0.625 U of *Taq* DNA polymerase (RBC Bioscience Corp., New Taipei, Taiwan) and 50-100 ng of the extracted DNA. The thermal conditions included initial denaturation for 2 min at 95°C followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 54°C, extension for 1 min at 72°C and an extension for 10 min at 72°C. The PCR products were visualized by 1% agarose gel electrophoresis under UV light.

The amplified PCR products were purified with the HiYield™ Gel/PCR DNA Fragments Extraction kit (RBC Bioscience) according to the manufacturer's instructions. All purified PCR products were sequenced in one direction with the FishF1/FishF2 primers complementary to the 5' ends of the COI gene fragments by Macrogen Inc. in South Korea.

Species Identification

All sequences were searched for open reading frames using ORF finder program (https://www.ncbi.nlm.nih.gov/orffinder/). The scientific name of each sample was obtained by comparing its COI sequence with reference sequences in the GenBank (https://www.ncbi.nlm.nih.gov/) database using the BLASTn program (Altschul, Gish, Miller, Myers, & Lipman, 1990) and the BOLD database (http://www.boldsystems.org/). Sequence similarity greater than 97% was the criterion for identification at the species level (Wong & Hanner, 2008) and a similarity lower than that was used for identification at the genus level. All COI sequences with similarities less than 97% were aligned together using the ClustalW program (Thompson, Higgins, & Gibson, 1994). The similar sequences were considered the same genus. Furthermore, the ClustalW program was also used to align the COI sequences of each species to determine the existing haplotypes. The genetic distances within each species were calculated with the Kimura 2-parameter (K2P) model in MEGA version 4.0 (Tamura, Dudley, Nei, & Kumar, 2007). All sequences were deposited in the GenBank database.

Larval Fish Diversity

From the comparison of the COI sequences to databases, the fish species were classified based on the fish taxonomy of Nelson, Grande, and Wilson (2016). The conservation status of each fish species was determined on the IUCN webpage (https://www.iucnredlist.org/). In addition, the human uses for each fish were determined with the FishBase webpage (http://www.fishbase.org/). The frequency of occurrence (V, %) of each species was calculated according to Joganzen & Faizova (1978) and Čivas & Kesminas (2011).

Results

DNA Barcoding for Species Identification

A total of 106 nucleotide sequences were successfully amplified using four primers. No deletion, insertion or stop codon was observed in any of the sequences after trimming. The average length of the amplified COI genes was 640 bp and ranged from 627 to 648 bp. From the comparison with reference sequences in the GenBank and BOLD databases, 99 COI gene sequences were classified into 9 orders, 19 families, 31 genera and 35 species with 97-100% identity (Table 1, 2). However, 7 samples could not be identified at the species level and could be classified only at the genus level, including *Danio* sp. (1 sample), *Opsarius* sp. (1 sample), *Brachygobius* sp. (3 samples) and *Dentex* sp. (2 samples), which were 84-93% identity and had no match in the GenBank and BOLD databases, respectively (Table 2).

The existing haplotypes of each species ranged from 1 to 3. The genetic distance within species ranged from 0.000 to 0.004. The 106 COI sequences were deposited in the GenBank database under the accession number MK628319-MK628424 (Table 2).

Larval Fish Diversity

The order Cypriniformes was the most dominant taxon among fish found in the tributaries of the lower Ing River and contained the highest percentage of fish, 38.46% (Figure 2). The second most populated taxon was the order Anabantiformes (17.95%), followed by the orders Siluriformes and Gobiiformes (10.26%), Synbranchiformes, Cyprinodontiformes and Beloniformes (5.13%). The three orders Cichliformes, Spariformes, and Osteoglossiformes contained the lowest percentage of fish (2.56%).

A total of 35 species were classified by their IUCN status as a species of least concern (27 species, 77.14%), followed by not evaluated species (7 species, 20.00%) and data deficient species (1 species, 2.86%) (Table 1).

In terms of the human uses for larval fish species as determined by FishBase, several larval fish species were used for many purposes, including fisheries, aquaculture or aquariums (Table 1). There were only 8 larval fish species (20.51%), such as *Notopterus notopterus*, *Barbonymus gonionotus*, and *Hemibagrus nemurus*, that were used for all purposes. Only two purposes and one purpose were identified for 10 (25.64%) and 9 (23.08%) species, respectively. The

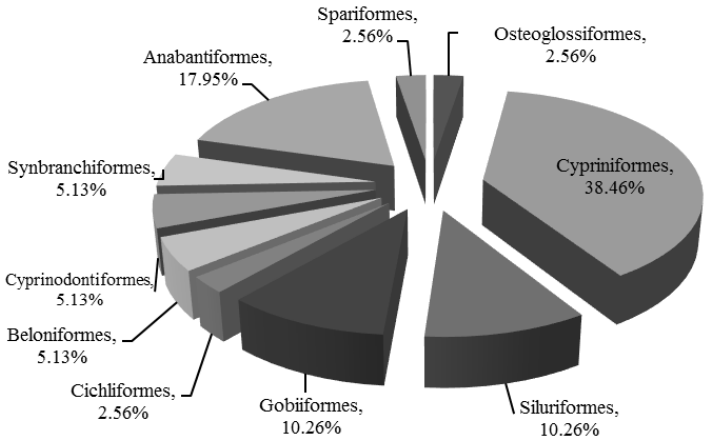

Figure 2. The percentage of larval fish species collected from the tributaries of the lower Ing River comprising different orders.

human uses for the remaining species (30.77%) could not be assigned by the database.

Of the 10 different stations sampled, stations 5 and 7 had the most larval fish species, followed by stations 1, 6 and 4 (Table 3). The fewest species were found at stations 3 and 10. Furthermore, the most frequently found larval fish was *Trichopsis vittata* (90%), followed by *Oryzias minutillus* (70%) and *Trichopodus trichopterus* (70%) (Table 3).

Discussion

Because the morphological characteristics of larval fish are completely different from those of adult fish, the species identification of these larval fish is very difficult, especially for nontaxonomic experts. Currently, DNA barcoding is a popular tool for identifying the species of organisms. DNA barcoding can efficiently identify larval fish from several water sources, including the eastern

Table 1. Classification of larval fish species in the tributaries of the lower Ing River, their IUCN statuses and their human uses

Order	Family	Genus	Species	No. of samples	IUCN status	Human uses
Osteoglossiformes	Notopteridae	*Notopterus*	*N. notopterus*	1	LC	Fisheries, Aquaculture, Aquariums
Cypriniformes	Cyprinidae	*Amblypharyngodon*	*A. chulabhornae*	2	LC	NA
		Barbonymus	*B. gonionotus*	8	LC	Fisheries, Aquaculture, Aquariums
		Cyclocheilichthys	*C. armatus*	1	LC	NA
		Danio	*D. roseus*	5	LC	NA
			Danio sp.	1	NA	NA
		Esomus	*E. metallicus*	14	LC	Fisheries
		Henicorhynchus	*H. siamensis*	3	LC	Fisheries, Aquariums
		Labiobarbus	*L. siamensis*	3	LC	Fisheries
		Opsarius	*Opsarius* sp.	1	NA	NA
		Puntigrus	*P. partipentazona*	3	LC	Aquariums
		Puntius	*P. cf. sophore*	4	LC	Aquariums
			P. brevis	1	LC	NA
		Rasbora	*R. borapetensis*	3	LC	Aquariums
		Systomus	*S. orphoides*	2	NE	NA
	Cobitidae	*Pangio*	*P. anguillaris*	1	NE	Aquariums
Siluriformes	Loricariidae	*Pterygoplichthy*	*P. anisitsi*	1	NE	Fisheries, Aquariums
			P. pardalis	2	NE	Fisheries, Aquariums
	Bagridae	*Hemibagrus*	*H. nemurus*	3	LC	Fisheries, Aquaculture, Aquariums
	Clariidae	*Clarias*	*C. batrachus*	1	LC	Fisheries, Aquaculture, Aquariums
Gobiiformes	Eleotridae	*Oxyeleotris*	*O. marmorata*	1	LC	Fisheries, Aquaculture, Aquariums
	Gobiidae	*Brachygobius*	*Brachygobius* sp.	3	NA	NA
		Gobiopterus	*G. lacustris*	3	NE	NA
	Ambassidae	*Parambassis*	*P. ranga*	2	LC	Fisheries, Aquariums
Cichliformes	Cichlidae	*Oreochromis*	*O. niloticus*	5	LC	Fisheries, Aquaculture

Table 1. Continued

Order	Family	Genus	Species	No. of samples	IUCN status	Human uses
Beloniformes	Adrianichthyidae	*Oryzias*	*O. minutillus*	3	LC	NA
	Zenarchopteridae	*Dermogenys*	*D. pusilla*	4	NE	Fisheries, Aquariums
Cyprinodontiformes	Poeciliidae	*Gambusia*	*G. affinis*	3	LC	Fisheries, Aquariums
		Poecilia	*P. reticulata*	3	NE	Fisheries, Aquariums
Synbranchiformes	Synbranchidae	*Monopterus*	*M. javanensis*	1	LC	Fisheries, Aquaculture, Aquariums
	Mastacembelidae	*Mastacembelus*	*M. favus*	3	LC	NA
Anabantiformes	Anabantidae	*Anabas*	*A. testudineus*	1	DD	Fisheries, Aquaculture, Aquariums
	Osphronemidae	*Trichopodus*	*T. microlepis*	4	LC	Fisheries, Aquariums
			T. trichopterus	2	LC	Fisheries, Aquaculture, Aquariums
		Trichopsis	*T. vittata*	2	LC	Aquariums
	Channidae	*Channa*	*C. gachua*	1	LC	Aquariums
			C. striata	2	LC	Fisheries, Aquaculture, Aquariums
	Pristolepididae	*Pristolepis*	*P. fasciata*	1	LC	Fisheries, Aquariums
Spariformes	Sparidae	*Dentex*	*Dentex* sp.	2	NA	NA

LC: Least concern, DD: Data deficient, NE: Not evaluated and NA: Not assessed

Atlantic Ocean (Ardura, Morote, Kochzius, & Garcia-Vazquez, 2016), Bahia, northeastern Brazil (Brandão *et al*., 2016) and the mangroves of peninsular Malaysia (Azmir *et al*., 2017), at the species level. Moreover, the accuracy of species-level identification with DNA barcoding was higher than that with the morphological method (Overdyk, Holm, Crawford, & Hanner, 2016; Azmir *et al*., 2017).

A total of 99 samples were identified as 35 species with more than 97% similarity based on the general rule of Wong and Hanner (2008). However, if the similarity was less than 96%, it would be considered at the genus level (Chen *et al*., 2013). Seven samples were identified at only the genus level because the similarities were between 84-93% and the COI nucleotide sequences of the relevant species have not been reported in any databases. Thus, increasing the number of COI nucleotide sequences in databases will be important and useful for identifying unknown fish species (Sarma & Mankodi, 2017).

The average length of the 106 COI sequences was 640 bp, which was shorter than that reported in other studies such as Ward *et al*. (2005), Pegg, Sinclair, Briskey, and Aspden (2006) and Brandão *et al*. (2016). Although the amplified COI sequences were bidirectionally sequenced using both forward and reverse primers in these studies, the 106 sequences in the current study were sequenced only in the forward direction. However, 130 bp mini-barcodes successfully identified several organisms at the species level (Meusnier *et al*., 2008). One to three haplotypes were found as well as a low genetic distance within species that ranged from 0.000 to 0.004 (0-0.4%) was observed for each species. All species were differentiated by their COI sequence with a 0.39% distance (Ward *et al*., 2005). This study indicated that DNA barcoding is an effective approach to identify larval fish species in the tributaries of the lower Ing River.

Most larval fish species found in the tributaries of the lower Ing River belonged to the order Cypriniformes, which is the most diverse order in Southeast Asia (Nelson *et al*., 2016). Regarding their IUCN conservation status, the majority of fish species were classified as a species of least concern (77.14%), which is similar to the findings of previous studies that identified 72% (Joadder, Galib, Haque, & Chaki, 2015) and 59% (Pramanik, Hasan, Bisshas, Hossain, & Biswas, 2017) of species to be species of least concern in the Padma and Meghna Rivers in Bangladesh, respectively.

Several larval fish species are used in fisheries, aquaculture or aquariums in the adult stage. However, some fish are alien aquatic species in Thailand, although FishBase assessed the human uses of some of these species, such as *Pterygoplichthys pardalis*, *P. anisitsi* and *Oreochromis niloticus* (Termvidchakorn, Vidthayanon, Getpetch, Sorrak, & Paradonpanichakul, 2003). Members of the genus *Pterygoplichthys* are invasive alien species that affect native species through egg predation, especially *P. pardalis* (Chaichana & Jongphadungkiet, 2012). In addition, *O. niloticus* is a noninvasive species that successfully adapts to and is widely distributed in various aquatic habitats. However, environmental change may cause this species to grow faster than native fish and interrupt the recovery of ecological balance (Termvidchakorn *et al*., 2003).

In general, the frequency of occurrence is an index that indicates the ability of a species to live or spread in different environments (Keawkhiew, Keawtip, Seetakoses, & Montien-art, 2013). The most common species was *Trichopsis vittata,* followed by *Trichopodus*

Table 2. Larval fish species identification in the GenBank and BOLD databases, length of COI sequences, accession no., no. of haplotypes and genetic distances within each species

No.	GenBank		BOLD		Identified species	Length of COI gene (bp)	Accession no.	No. of haplotypes	Genetic distance
	Species	%Identity	Species	%Identity					
1	*Notopterus notopterus*	99	*Notopterus notopterus*	99.68	*N. notopterus*	630	MK628319	1	_
2	*Amblypharyngodon chulabhornae*	99	*Amblypharyngodon chulabhornae*	99.68	*A. chulabhornae*	636	MK628320-MK628321	1	0.000
3	*Barbonymus gonionotus*	99	*Barbonymus gonionotus*	99.63-100	*B. gonionotus*	648	MK628322-MK628329	3	0.003
4	*Cyclocheilichthys armatus*	99	*Cyclocheilichthys armatus*	99.51	*C. armatus*	630	MK628330	1	_
5	*Danio roseus*	99	*Danio roseus*	100	*D. roseus*	636	MK628331-MK628335	1	0.000
6	*Danio roseus*	93	No match	_	*Danio* sp.	630	MK628336	1	_
7	*Esomus metallicus*	99	*Esomus metallicus*	99.22-99.38	*E. metallicus*	648	MK628337-MK628350	3	0.001
8	*Henicorhynchus siamensis*	99	*Henicorhynchus siamensis*	99.84	*H. siamensis*	630	MK628351-MK628353	1	0.000
9	*Labiobarbus siamensis*	99	*Labiobarbus siamensis*	99.02	*L. siamensis*	630	MK628354-MK628356	1	0.000
10	*Opsarius koratensis*	90	No match	_	*Opsarius* sp.	630	MK628357	1	_
11	*Puntigrus partipentazona*	99	*Puntigrus partipentazona*	99.02	*P. partipentazona*	642	MK628358-MK628360	1	0.000
12	*Puntius cf. sophore*	99	*Puntius cf. sophore*	100	*P. cf. sophore*	648	MK628361-MK628364	1	0.000
13	*Puntius brevis*	99	*Puntius brevis*	99.17	*P. brevis*	639	MK628365	1	_
14	*Rasbora borapetensis*	99	*Rasbora borapetensis*	99.68-99.84	*R. borapetensis*	636	MK628366-MK628368	2	0.001
15	*Systomus orphoides*	100	*Systomus orphoides*	100	*S. orphoides*	633	MK628369-MK628370	1	0.000
16	*Pangio anguillaris*	97	*Pangio anguillaris*	97	*P. anguillaris*	636	MK628371	1	_
17	*Pterygoplichthys anisitsi*	99	*Pterygoplichthys anisitsi*	100	*P. anisitsi*	642	MK628372	1	_
18	*Pterygoplichthys pardalis*	99	*Pterygoplichthys pardalis*	99.84-100	*P. pardalis*	642	MK628373-MK628374	2	0.002
19	*Hemibagrus nemurus*	99	*Hemibagrus nemurus*	99.52-99.68	*H. nemurus*	648	MK628375-MK628377	2	0.001
20	*Clarias batrachus*	99	*Clarias batrachus*	100	*C. batrachus*	636	MK628378	1	_
21	*Oxyeleotris marmorata*	100	*Oxyeleotris marmorata*	99.68	*O. marmorata*	633	MK628379	1	_
22	*Brachygobius kabiliensis*	87-88	No match	_	*Brachygobius* sp.	636	MK628380-MK628382	2	0.004
23	*Gobiopterus lacustris*	99-100	*Gobiopterus lacustris*	99.68-100	*G. lacustris*	636	MK628383-MK628385	2	0.002
24	*Parambassis ranga*	98	*Parambassis ranga*	98.05-98.21	*P. ranga*	639	MK628386-MK628387	2	0.002
25	*Oreochromis niloticus*	100	*Oreochromis niloticus*	100	*O. niloticus*	639	MK628388-MK628392	1	0.000
26	*Oryzias minutillus*	99	*Oryzias minutillus*	99.06	*O. minutillus*	639	MK628393-MK628395	2	0.002
27	*Dermogenys pusilla*	99	*Dermogenys pusilla*	100	*D. pusilla*	642	MK628396-MK628399	1	0.000
28	*Gambusia affinis*	100	*Gambusia affinis*	100	*G. affinis*	648	MK628400-MK628402	1	0.000
29	*Poecilia reticulata*	99	*Poecilia reticulata*	100	*P. reticulata*	639	MK628403-MK628405	2	0.003
30	*Monopterus javanensis*	99	*Monopterus javanensis*	98.53	*M. javanensis*	630	MK628406	1	_
31	*Mastacembelus favus*	100	*Mastacembelus favus*	100	*M. favus*	639	MK628407-MK628409	1	0.000
32	*Anabas testudineus*	100	*Anabas testudineus*	100	*A. testudineus*	639	MK628410	1	_
33	*Trichopodus microlepis*	100	*Trichopodus microlepis*	100	*T. microlepis*	648	MK628411-MK628414	1	0.000
34	*Trichopodus trichopterus*	100	*Trichopodus trichopterus*	100	*T. trichopterus*	633	MK628415-MK628416	1	0.000
35	*Trichopsis vittata*	100	*Trichopsis vittata*	100	*T. vittata*	636	MK628417-MK628418	1	0.000
36	*Channa gachua*	99	*Channa gachua*	99.84	*C. gachua*	639	MK628419	1	_
37	*Channa striata*	99	*Channa striata*	99.68	*C. striata*	642	MK628420-MK628421	1	0.000
38	*Pristolepis fasciata*	100	*Pristolepis fasciata*	99.34	*P. fasciata*	627	MK628422	1	_
39	*Dentex tumifrons*	84	No match	_	*Dentex* sp.	636	MK628423-MK628424	2	0.003

Table 3. Distribution and frequency (V, %) of occurrence of larval fish species in 10 different stations from the tributaries of the lower Ing River

Species	Stations										V, %
	1	2	3	4	5	6	7	8	9	10	
Notopterus notopterus	-	-	-	-	-	+	-	-	-	-	10
Amblypharyngodon chulabhornae	+	-	-	-	+	-	+	-	-	-	30
Barbonymus gonionotus	+	-	-	-	+	+	+	+	-	-	50
Cyclocheilichthys armatus	-	-	-	-	+	-	-	-	-	-	10
Danio roseus	-	-	-	+	+	-	+	+	+	+	60
Danio sp.	-	-	-	-	-	-	-	-	+	-	10
Esomus metallicus	+	-	-	+	+	-	+	+	+	-	60
Henicorhynchus siamensis	-	+	-	-	-	-	+	-	-	-	20
Labiobarbus siamensis	+	+	-	-	+	+	-	-	-	-	40
Opsarius sp.	-	-	+	-	-	-	-	-	-	-	10
Puntigrus partipentazona	-	-	+	-	-	+	-	-	-	-	20
Puntius cf. sophore	-	+	-	-	+	-	-	+	+	-	40
Puntius brevis	+	-	-	-	-	-	-	-	-	-	10
Rasbora borapetensis	+	+	+	+	-	+	-	-	-	+	60
Systomus orphoides	-	-	-	-	-	-	-	-	+	-	10
Pangio anguillaris	+	-	-	-	-	-	-	-	-	-	10
Pterygoplichthys anisitsi	-	-	-	+	-	-	-	-	-	-	10
Pterygoplichthys pardalis	-	-	-	-	-	-	+	-	-	-	10
Hemibagrus nemurus	-	-	-	-	+	-	+	-	-	-	20
Clarias batrachus	-	-	-	-	-	-	+	-	-	-	10
Oxyeleotris marmorata	+	+	+	-	-	-	+	-	-	-	40
Brachygobius sp.	+	-	-	-	+	+	+	-	-	-	40
Gobiopterus lacustris	+	+	-	+	+	+	-	-	-	+	60
Parambassis ranga	+	-	+	-	+	+	+	-	-	-	50
Oreochromis niloticus	-	+	-	+	-	+	+	+	-	-	50
Oryzias minutillus	-	-	-	+	+	+	+	+	+	+	70
Dermogenys pusilla	+	+	-	+	-	-	-	-	-	+	40
Gambusia affinis	-	-	-	+	-	-	-	+	+	-	30
Poecilia reticulate	-	-	-	-	-	-	-	+	-	-	10
Monopterus javanensis	-	-	-	-	-	-	-	+	-	-	10
Mastacembelus favus	-	-	-	-	-	+	+	-	-	-	20
Anabas testudineus	-	-	-	-	+	-	-	-	-	-	10
Trichopodus microlepis	+	-	-	+	+	-	-	-	-	-	30
Trichopodus trichopterus	+	+	-	+	+	-	+	+	+	-	70
Trichopsis vittata	+	+	+	+	+	+	+	-	+	+	90
Channa gachua	-	-	-	-	-	-	-	+	-	-	10
Channa striata	+	+	-	+	-	+	+	+	-	-	60
Pristolepis fasciata	-	-	+	-	-	+	-	-	-	-	20
Dentex sp.	-	-	-	-	+	+	-	-	-	-	20
Total species	16	11	7	13	17	15	17	12	9	6	

+ Found, - Missed

trichopterus, which can survive in oxygen-poor water because these species have an accessory air-breathing organ called the labyrinth that allows them to directly breathe the air from the surface of the water (Suvarnaraksha, 2015). Thus, these species were widely found in various water sources, even in polluted water. Moreover, *Oryzias minutillus* was also widely observed in many stations, and this species can live in a variety of habitats, such as shallow ponds, ditches and paddy fields (Ngamniyom, 2012). Because the station 3, 9 and 10 are shollow and narrow streams, less fish species were found. However, most larval fish species were found at station 5 because this station is near the Ing River in the Thoeng District, Chiang Rai Province, where is a wide stream and has water all year round. In addition, most adult fish species were collected in that area (Valunpion & Suvarnaraksha, 2013). This result indicated that this area is suitable to lay eggs and serve as a nursery for the fish.

Conclusion

DNA barcoding is an efficient approach for identifying larval fish collected from 10 stations in the tributaries of the lower Ing River. This method successfully identified 93.4% of 106 samples at the species level, whereas 7 samples (6.6%) were identified at only the genus level. These results of this study will be used for a DNA barcode database to plan the designation of conservation areas for spawning and the nursing of fish for sustainable fishery resource management in the future.

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