Fish diversity of a spring field in Hopong Town, Taunggyi District, Shan State, Myanmar (the Salween River Basin), with genetic comparisons to some “species endemic to Inle Lake”

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Abstract

Hopong, a small town in the Salween (Thanlwin) River Basin, Myanmar, is located 35 km northeast of Inle Lake, a famous ancient lake with numerous endemic fish species. We surveyed the fish fauna of a spring pond in Hopong in 2016, 2019 and 2020 and identified 25 species. Of these, seven, including Inlecypris auropurpureus and Sawbwa resplendens, had been considered endemic to Inle Lake and at least three species were genetically unique. Eight were suspected or definite introduced species, including Oreochromis
*niloticus* and *Gambusia affinis*. We were unable to identify a nemachilid species of the genus *Petruichthys*, which would need a taxonomic examination. The Hopong area is being developed rapidly and, hence, it is crucial to conserve its native fish species and the freshwater ecosystems.

**Keywords**

Burma, *Inlecypris auropurpureus*, *Microrasbora rubescens*, mitochondrial DNA (mtDNA), *Physoschistura brunneana*, *Sawbwa resplendens*

**Introduction**

Hopong is a small town in the Salween (Thanlwin) River Basin, situated 15 km east of Taunggyi, the capital of Shan State, Myanmar (Fig. 1). The town has several natural wetlands and springs. *Danio (Celestichthys) margaritatus*, a minute cyprinid fish, is a symbolic fish species from this area (Roberts 2007, Conway et al. 2008). However, the fields of Hopong have been developed rapidly with extensive road construction and urbanisation (Roberts 2007).
Hopong is about 35 km northeast of Inle Lake, a famous ancient lake with many endemic fish species (Annandale 1918, Kano et al. 2016, Win 2018). The Hopong area and Inle Lake are both in the Salween River Basin, so are close together geographically; however, they belong to different drainages separated by highlands and are ca. 400 km apart by river (Fig. 1). The endemism of fishes in Inle Lake has not been validated, because studies of the fish fauna of the middle–upper Salween Basin are insufficient. Thus, the ichthyofauna of the Hopong area is of biogeographical interest, especially in terms of comparison with that of Inle Lake, including the validation of species endemic to Inle Lake.

Generally, studies of freshwater fish biodiversity in Myanmar are insufficient; most are fragmentary reports on fauna of specific regions or new species. Annandale (1918) and Hora and Mukerji (1934) reported the fish fauna of Inle Lake and Shan State, respectively. Musikasinthorn (1998a) first reported *Channa panaw* from the Irrawaddy and Sittang River Basins, Myanmar. Britz (2003) described a new species of miniature fish, *Danionella mirifica*, from northern Myanmar. Roberts (2012) reported a new species of arowana, *Scleropages inscriptus*, from the Malay Peninsula, Myanmar. Recently, Vidthayanon et al. (2005) provided a rough list of inland fish species of Myanmar. Data on the freshwater fish biodiversity of Myanmar are generally limited and more information is required.

Here, we report the results of a freshwater fish inventory for a spring field in Hopong conducted in 2016, 2019 and 2020. The list contains 25 species, including seven “species endemic to Inle Lake”. We provide DNA barcoding (mitochondrial COI sequences) data for the species, which clarify their genetic uniqueness and should promote biogeographical research in this area.

**Methods**

Sampling was conducted nine times in Hopong from March 2016 to March 2020 (Fig. 2). Fish were collected with hand-nets, throwing nets and fish traps. Except for several individuals that were only photographed or recorded on-site and released, the collected specimens were photographed fresh (Kano and Nakajima 2014), fixed in 10% formalin and then transferred to 70% ethanol. The specimens and their tissue samples were catalogued and deposited at the Research Laboratory of Ichthyology, Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand (RLIKU) and Kyoto University, Kyoto, Japan (tissue samples). All specimens were assigned IDs associated with the records of location (latitude, longitude and region name), collection date, DNA sequence accession numbers etc. The data were registered in the integrated Monsoon Asia “ffish.asia” online database of freshwater organism biodiversity (Watanabe et al. 2009, Kano et al. 2013) and can be retrieved at [https://ffish.asia/Hopong2020](https://ffish.asia/Hopong2020).

To obtain DNA barcoding data (partial mitochondrial COI sequences) for the species collected from Hopong (102 specimens from 19 species including all native species), total DNA was extracted using a Genomic DNA Purification kit (Promega) or Monarch Genomic DNA Purification Kit (New England Biolabs). The COI gene was amplified by PCR using the primer pair FishF1 (5’-TCA ACC AAC CAC AAA GAC ATT GGC AC-3’) and FishR1 (5’-
TAG ACT TCT GGG TGG CCA AAG AAT CA-3’ (Ward et al. 2005). The amplification consisted of an initial denaturation step (94°C, 2 min); 30 cycles of 94°C for 30 s, 56–60°C for 30 s and 72°C for 60 s; and a final extension (72°C, 7 min). The PCR products were purified with Illustra ExoStar (GE Healthcare) and sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems) using an amplification primer and the BigDye Terminator Cycle sequencing FS Ready Reaction kit ver. 3.1 (Applied Biosystems). The DNA sequences (640 bp) were deposited at DDBJ/EMBL/GenBank (accession numbers: LC190268–LC190330, LC190383, LC190395–LC190405, LC645163–LC645189).

The obtained sequences were aligned using MAFFT (Katoh and Standley 2013) at Unipro UGENE (Okonechnikov et al. 2012). Sequence data for the same or related species from Inle Lake and the surrounding rivers, as reported by Kano et al. (2016), were included in the analysis for comparison with the Hopong populations. To visualise the relationships amongst populations, haplotype networks, based on the TCS algorithm (statistical parsimony; Clement et al. 2000), were constructed for five genera endemic to this region—Inlecypris, Microrasbora, Sawbwa, Petruichthys and Physoschistura—using POPART (Leigh and Bryant 2015). The mean uncorrected sequence differences amongst different populations were calculated using MEGA 7 (Kumar et al. 2016).
Results

The survey uncovered 25 species (Table 1). We collected *Danio margaritatus* in a shallow wetland habitat, as described by Roberts (2007). We also obtained *Devario sondhii*, which was reported by Hora and Mukerji (1934) with a specimen obtained in the Hopong area. We also found a likely undescribed species of *Pteruichthys*, which we tentatively labelled “*Pteruichthys* sp. (Hopong)”. Of the 25 species, seven had been treated as “species endemic to Inle Lake”: *Danio erythromicron, Inlecypris auropurpureus, Microrasbora rubescens, Sawbwa resplendens, Pteruichthys brevis, Channa harcourtbutleri* and *Mastacembelus caudicellatus* (Annandale 1918, Kano et al. 2016). Eight were suspected or definite introduced species: *Cyprinus rubofuscus, Esomus danica, Parambassis lala, Oreochromis niloticus, Trichogaster labiosa, Oryzias uwai, Gambusia affinis* and *Poecilia reticulate* (Annandale 1918, Hora and Mukerji 1934, Musikasinthorn 1998b, Kano et al. 2016). Three of these (i.e. *E. danica, T. labiosa, O. uwai*) are distributed widely in Southeast Asia; hence the possibility that these species are native cannot be completely ruled out.

| Order          | Family       | Species                        | N (DNA barcoding) | Accession number                  |
|---------------|-------------|--------------------------------|-------------------|-----------------------------------|
| Cypriniformes |             | *Cyprinus rubofuscus* †          | 1 (0)             | —                                 |
|               | Cyprinidae  | *Danio erythromicron* †          | 33 (12)           | LC190280, LC190284– LC190286, LC190314, LC645170– LC645175, LC645189 |
|               |             | *Danio margaritatus*             | 21 (9)            | LC190268, LC190311– LC190313, LC190315, LC190395– LC190398 |
|               |             | *Devario brawni*                | 6 (4)             | LC190317, LC190318, LC190320, LC645169 |
|               |             | *Devario sondhii*               | 21 (20)           | LC190281, LC190289– LC190304, LC645177–LC645179 |
|               |             | *Esomus danica* †               | 2 (1)             | LC645176 |
|               |             | *Inlecypris auropurpureus* †     | 19 (13)           | LC190269– LC190279, LC645167, LC645168 |
|               |             | *Microrasbora rubescens* †       | 66 (4)            | LC190305– LC190308 |
|               |             | *Pethia stoliczkana*             | 30 (2)            | LC190282, LC190283 |
|               |             | *Sawbwa resplendens* †           | 3 (2)             | LC645184 |
|               |             | *Systomus sp. cf. rubripinnis*    | 3 (2)             | LC190316, LC645185 |
| Order          | Family            | Species                          | N (DNA barcoding) | Accession number               |
|---------------|-------------------|----------------------------------|-------------------|--------------------------------|
|               |                   | Lepidocephalichthys berdmorei    | 9 (3)             | LC190324–LC190326              |
| Nemacheilidae | Petruichthys brevis † | 18 (7)                           |                   | LC190287, LC190288, LC190309, LC190310, LC645165, LC645166, LC645186 |
|               | Petruichthys sp. (Hopong) | 2 (1)                           |                   | LC645164                      |
|               | Physoschistura brunneana † | 5 (4)                           |                   | LC190399–LC190402              |
|               | Physoschistura rivulicola | 16 (9) | LC190319, LC190321–LC190323, LC190403–LC190405, LC645181, LC645182 |
| Cyprinodontiformes |               | Poeciliidae Gambusia affinis * | 1 (1)             | LC645188                      |
|               |                   | Poecilia reticulata *            | 1 (0)             | —                              |
| Cichliformes  | Ambassidae        | Parambassis laa *                | 10 (2)            | LC645180, LC645187            |
|               | Cichlidae         | Oreochromis niloticus *          | 1 (0)             | —                              |
| Beloniformes  | Adrianichthyidae  | Oryzias uwai *                   | 11 (0)            | —                              |
| Synbranchiformes | Synbranchidae    | Monopterus javanensis           | 1 (1)             | LC190383                      |
|               | Mastacembelidae   | Mastacembelus caudiocellatus †   | 2 (1)             | LC645163                      |
| Anabantiformes | Osphronemidae     | Trichogaster labiosa *           | 7 (0)             | —                              |
|               | Channidae         | Channa harcourtbutleri †        | 30 (5)            | LC190327–LC190330, LC645183   |

* Putative introduced species; † Assumed as endemic to Inle Lake

Haplotype networks of the populations of three species from the Hopong area (Inlecypris auropurpureus [Fig. 3A], Microrasbora rubescens [Fig. 3B] and Physoschistura rivulicola [Fig. 3C]) showed genetic uniqueness compared to Inle Lake populations, even within species. Inlecypris auropurpureus, Microrasbora rubescens and Physoschistura rivulicola showed clear genetic divergence (4.1%, 3.2% and 2.1%, respectively, in mean uncorrected sequence differences). By contrast, Sawbwa resplendens (Fig. 3D) and Petruichthys brevis (Fig. 3E) showed no clear genetic divergence amongst local populations (0.3% and < 0.1%, respectively). Petruichthys sp. (Hopong) showed an obviously distinct genetic profile from the sympatric P. brevis (12.6% mean uncorrected sequence difference; Fig. 3E).
A total of eleven freshwater fish species have been reported from Hopong in previous papers (Hora and Mukerji 1934, Roberts 2007, Kullander et al. 2017). All of them, except for *Barbus hexastichus* (valid as *Neolissochilus hexastichus*), are judged to be included in the 25 species reported in this study. Hora and Mukerji (1934) reported *Barbus hexastichus* from Hopong as having two pairs of barbels, a light brown body colour and a black round...
spot at the base of the caudal fin. However, this record should be reconsidered since its main distribution range is in India (Talwar and Jhingran 1991). In fact, *Neolissochilus nigrovittatus*, which is distributed around Inle Lake (Talwar and Jhingran 1991, Kano et al. 2016), also fits the description of the fish by Hora and Mukerji (1934). Further survey and examination are necessary for the *Neolissochilus* species in this area.

Our results indicated that at least seven "species endemic to Inle Lake" (Annandale 1918, Kano et al. 2016, Win 2018) have wider distributions than previously thought, beyond the Inle Lake Basin (Table 1). Artificial introduction from Inle Lake cannot explain their wide distributions because of the genetic divergence of the Hopong populations from the Inle Lake populations, except for *Sawbwa resplendens* (only one DNA sample and details unknown) and *Petruichthys brevis* (Fig. 3D and E). Therefore, Hopong shares fish fauna with the Inle Lake Basin, but the populations of several fish species in Hopong are genetically unique and require detailed taxonomic examinations.

*Danio margaritatus* is symbolic of Hopong (Roberts 2007). Conway et al. (2008) reported that *D. margaritatus* is distributed not only in Hopong (the type locality of the species; Roberts 2007), but also in wider areas of Myanmar and Thailand following Clarke (2007) and Hary (2007). However, these sources are not formal scientific reports (website and local report, respectively) and, in fact, we could not access them. Thus, at present, it cannot be determined whether *D. margaritatus* is distributed only in the Hopong area or more widely. If the former is the case, conservation of *D. margaritatus* and its habitat, i.e. shallow ponds/wetlands with plants and clear water from springs (Fig. 2B; Roberts 2007), is very important. *Devario sondhii* might also be endemic to the Hopong area; more information on its distribution is necessary to determine its conservation status.

*Petruichthys* sp. (Hopong) is genetically distinguishable from the sympatric *P. brevis* (Fig. 3E). Morphologically, its body is smaller and the male is redder than that of *P. brevis*. Taxonomic examination of this fish is required.

The Hopong area is being developed rapidly (Roberts 2007). In addition, several invasive alien fish have become established, including *Oreochromis niloticus* and *Gambusia affinis*. This strongly suggests that the native freshwater fishes and other native freshwater organisms in Hopong are now severely threatened. As explained above, Hopong has a unique fish fauna that includes endemic species and species shared with Inle Lake, an unique ancient lake in Southeast Asia. Therefore, wetland and biodiversity conservation in Hopong are a high priority.

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