

## The Mae Khlong Basin as the potential origin of Florida’s feral bullseye snakehead fish (Pisces: Channidae)

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**Abstract.** The “bullseye” or “cobra” snakehead is a large, predatory freshwater fish of South Asian origin. Until recently, only one bullseye species has been recognised, *Channa marulius*, but new studies on the ‘Marulius group’ have revealed that there are at least four species with different native ranges in rivers of mainland South Asia, as well as *Channa marulioides* (the “emperor snakehead”) from Sundaland. New data, generated as part of this wider taxonomic investigation and reported here for the first time, reveal that Marulius group specimens collected in the Mae Khlong Basin of Western Thailand are similar genetically and in appearance to the recently revalidated *Channa aurolineata*, that occurs in Myanmar’s Salween, Sittang and Ayeyarwady River Basins. Thus, the Mae Khlong bullseye snakehead should now rightly be referred to by the scientific name *C. aurolineata*. Furthermore, *C. aurolineata* from western Thailand are a direct DNA barcode match to publicly available data for the feral bullseye snakehead population in Florida, North America. This identifies the Mae Khlong Basin as the probable source for the feral population in Florida. Identifying Thailand as the likely origin for the feral North American population may further have implications for understanding historical invasion pathways into the USA.

**Key words.** COI, DNA barcode, freshwater biogeography, invasive species, species identification, Southeast Asia

### INTRODUCTION

Freshwater snakehead fishes of the family Channidae include three genera, the African *Parachanna* (three valid species), the Asian *Channa* (47 species) and the recently discovered Asian *Aenigmachanna* (1 species) (Britz et al., 2019). Some of the larger Asian *Channa* species represent an important component of wild freshwater fisheries throughout their natural geographical range and are prized as gamefishes (Courtenay & Williams, 2004), while others can be valuable in the aquarium trade (e.g., Raghavan et al., 2013).

*Channa marulius* (Hamilton, 1822) was first described from India, and for a long time all Asian snakehead fishes of similar appearance have been referred to using this scientific name. However, stimulated by initial genetic studies (Adamson et al., 2010; Serrao et al., 2014; Conte-Grand et al., 2017), a series of recent genetic and taxonomic papers has recognised that there are actually at least four distinct ‘Marulius group’ species distributed in rivers across mainland Southeast Asia: *Channa pseudomarulius* (Günther, 1861) found in west flowing drainages of the Western Ghats region of South India; *C. marulius* in large parts of India and in west flowing

rivers of the Rakhine Yoma in western Myanmar (Britz et al., 2017); *Channa aurolineata* (Day, 1870) in the Chindwin, Ayeyarwaddy and Salween drainages in Myanmar (Adamson & Britz, 2018); and *Channa auroflammea* Adamson, Britz & Lieng, 2019 from the Mekong drainage in Laos and Cambodia. A fifth member of the group, *Channa marulioides* (Bleeker, 1851), is found in Malaysia and western Indonesia.

One of the Marulius group snakehead fishes has also established a feral population outside of its native range in Florida, North America (Benson et al., 2018). This population, commonly known as the “Florida bullseye snakehead” has been referred to scientifically as *C. marulius*, although it has been shown to be quite genetically different from *C. marulius* collected from the type locality in India (Serrao et al., 2014; Conte-Grand et al., 2017).

A Marulius group fish is known to occur in Thailand, although little scientific information has been published on it. Now that a more complete understanding of the Marulius group species is available, it is possible to determine the identity of specimens from Thailand with reference to genetic and morphological data for valid species that occur in drainages to the West (Salween [Thanlwin] Basin) and East (Mekong Basin). In providing data from Thai Marulius group fishes, for the first time it is also possible to genetically compare Mae Khlong Marulius group specimens to the feral bullseye populations in Florida and to identify a likely origin of the American introduction.

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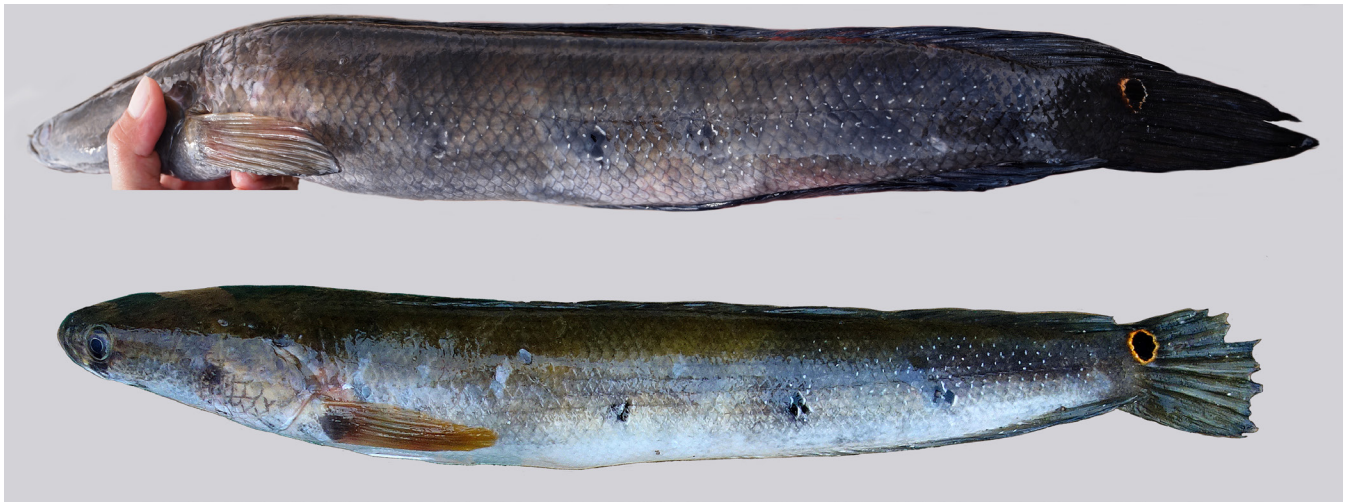


Fig. 1. Comparison of colouration of fresh specimens of Mae Khlong Marulius group snakehead (BMNH 2015.1.1.94, 468 mm SL, above) and of topotype of *C. aurolineata* (BMNH 2018. 4.24.1–5, 330 mm SL, below). Note numerous small white spots, especially on posterior part of body and white scale margins of black scales forming midlateral blotches.

## MATERIAL & METHODS

**Sampling.** During field sampling in November 2014, three freshly dead specimens of *Channa* of the Marulius group were encountered at a fish landing site upstream of the Srinakarin [Srinagarind] Dam, Mae Khlong Catchment, Northwest Thailand. The fish had been wild caught at the northern end of the Dam Reservoir (approx. 14.64N, 99.04E). Tissue samples were taken from all three specimens (BMNH 2015.1.1.92-94), and their DNA sequences for a 654 base pair region of the mitochondrial cytochrome oxidase *c* subunit 1 gene (CO1 “DNA barcodes”) were obtained using standard fish barcoding primers (Ward et al., 2005) following methods outlined previously (Britz et al., 2017). One of these specimens was preserved and is now housed at the Natural History Museum in London (BMNH 2015.1.1.94). A second preserved specimen from the Mae Khlong was obtained from Maurice Kottelat’s private collection (CMK) to enable comparison in meristic characters with other members of the Marulius group.

**Morphology.** Counts of vertebrae, dorsal and anal fin rays (from x-radiographs), and of lateral line scales (not including pored scales on caudal-fin base) were taken for the two available Thai specimens (BMNH 2015.1.1.94, SL 468mm, Srinakarin Dam, Mae Khlong Basin; CMK17516, SL 223mm, Kanchanaburi, Mae Khlong Basin) in the same manner as has been done for other members of the Marulius group (Britz et al., 2017; Adamson & Britz, 2018; Adamson et al., 2019). This information, along with photographs taken at point of collection in 2014 at Srinakarin Dam, is reported here primarily to add to the available information on the Thai Marulius group, which is otherwise limited.

**DNA barcodes.** Marulius group CO1 DNA sequence data were compiled from recent snakehead barcoding studies and taxonomic work (see supplementary table). The three new sequences from the Thai specimens were included. The final barcode library we compiled had sequences from 92 individuals, including representatives of all five currently

recognised Marulius group species. To the best of our knowledge, this represents the full suite of diversity that has been observed among Marulius group barcodes to date.

After alignment in Geneious software (version 7.1.9, <https://www.geneious.com>), Mega software (version X, Kumar et al., 2018) was used to calculate uncorrected pairwise distances and establish which existing barcodes were the most similar to the new Thai samples and to the feral North American samples, with data summarised in graphs. To further visualise the barcode diversity and divergence within the Marulius group, an un-rooted neighbour joining tree was constructed in Mega software, using uncorrected pairwise distances. Barcodes that were less than 2% divergent are shown as single leaves in the resulting figure for ease of interpretation.

## RESULTS

The Marulius group fishes from the Srinakarin Reservoir, Mae Khlong Basin in western Thailand appear to be very similar, both genetically and in colouration, to *C. aurolineata* from Myanmar’s Salween, Sittang and Ayeyarwady-Chindwin River Basins. Furthermore, the fishes from Thailand have an identical DNA barcode to that of Florida’s non-native bullseye population.

**Colouration.** The overall colour pattern of the Mae Khlong specimens (Fig. 1) matches that of *C. aurolineata* reported and illustrated in Adamson et al. (2018). Both share the series of black midlateral blotches in which the black scales have a bright white margin, and in fishes from both areas there are numerous small bright white spots on the scales that surround these blotches. These white scale margins and small white spots that were observed in the fresh specimen disappeared in the preservation process and so are no longer visible in BMNH 2015.1.1.94.

Photographs (Fig. 2) of freshly caught, adult Marulius group snakeheads from Nam Tjon River, Srinakarin Dam,

Table 1. Meristic information for the two Mae Khlong specimens, in comparison to other members of the Marulius group from mainland Asia.

	BMNH 2015.1.1.94	CMK 17516	<i>C. aurolineata</i>	<i>C. auroflammea</i>	<i>C. marulius</i>	<i>C. pseudomarulius</i>
Total vertebrae	63, 1 with double neural spine	63	63–66	58–61	59–63	55
Lateral-line scales	67	65	65–71	61–65	62–65	58–61
Dorsal-fin rays	55	52	55–58	52–54	52–56	47–50
Anal-fin rays	35	36	35–38	33–36	33–37	29–33



Fig. 2. Variation in colour pattern observed in adult *Marulius* group snakeheads from the Nam Tjon River, Srinakarin Dam, Mae Khlong Basin (left, photos Jean-Francois Helias), and from the Florida population (right, photos Steven Ryan). Note absence of white margin on black scales in series of midlateral blotches in upper and presence of these in lower images.

Mae Khlong, illustrate that these white margins are not universally present in the population. Comparative photos of adult individuals from the Florida population demonstrate presence of a similar variation in this scale colour pattern.

**Meristic information.** A summary of the vertebral, scale and fin-ray counts from the two preserved Mae Khlong specimens we studied is provided in Table 1, in comparison to the four *Marulius* Group species for which comparative data are available.

**DNA barcodes.** All three Mae Khlong *Marulius* group fishes had the same DNA barcode sequence (accessioned in genbank: MK599316-MK599316), and this was exactly the same as all barcodes available for Florida’s non-native population. Although there was no direct match to the barcode of any described species in the wider geographical range of the group in Southeast Asia, the Mae Khlong barcode was similar to barcodes of *C. aurolineata* in neighbouring Myanmar. The similarity with *C. aurolineata* equated to just over 2% uncorrected pairwise distance, in contrast to the 6.62% difference that was revealed between the Mae Khlong and more easterly distributed Mekong Basin *C. auroflammea*. Generally, among members of the *Marulius* group, uncorrected CO1 divergences below 2% are indicative of previously described intraspecific variation, while barcode divergences of between 6% and 12% can be expected between different species (see Fig 3). Within Myanmar, the level of

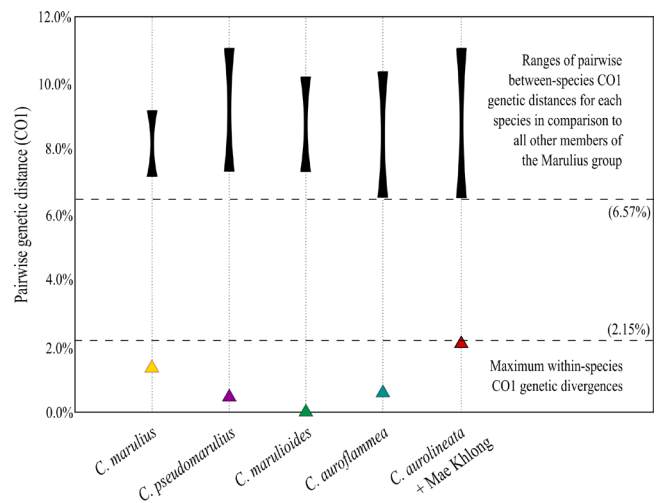


Fig 3. The maximum intra-specific pairwise genetic distances (closed triangles), and range of inter-specific divergences (black bars) observed among CO1 barcodes for members of the *Marulius* group, calculated from the DNA barcode library compiled in this study.

intraspecific divergence among *C. aurolineata* barcodes was only 0.3% (Adamson & Britz, 2018). When the Mae Khlong CO1 haplotype is included as a new *C. aurolineata* barcode, the maximum level of within-species divergence rises to 2.15%, a value still well below the 6% level of difference that would appear likely to indicate different species within the *Marulius* group. The genetic distance between all members

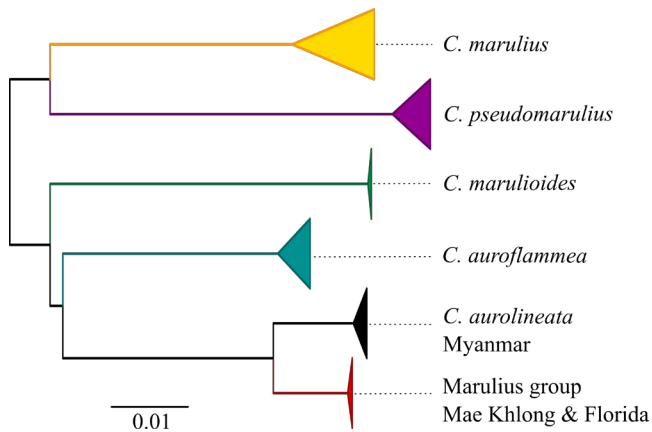


Fig 4. Neighbour-joining tree estimated from uncorrected pairwise distances of CO1 DNA barcodes, to illustrate genetic diversity within and difference between species of the Marulius group. Scale bar 0.01 = 1% divergence in DNA sequence. Nodes with multiple sequences less than 2% divergent from each other have been collapsed. The Mae Khlong and Florida DNA barcodes are 2.15% different from *Channa aurolineata* sequences from Myanmar.

of the Marulius group is further illustrated in Fig 4, where short branches between *C. aurolineata* and Mae Khlong (+ Florida) DNA barcodes show how similar they are to each other, in comparison to all other species in the group.

## DISCUSSION

***Channa aurolineata* in Thailand.** Long prized by anglers, the Marulius group fishes of western Thailand are seldom encountered by the scientific community. This small study adds to the restricted knowledge about this group of channids in Thailand. The genetic barcodes and notes on its appearance clearly identify the Mae Khlong fish as conspecific with the recently revalidated *C. aurolineata*. However, the small number of specimens we have been able to examine means we that are unable to provide clear ranges for meristic characters for the Mae Khlong population.

The Mae Khlong Basin sits on the western border of Thailand, with headwaters in the Tenasserim Ranges that border Myanmar. Just to the north, the Salween flows on the other side of the mountains, while the Mekong and Chao Phraya Rivers both claim tributaries on the eastern side of this range. The freshwater fauna of the Mae Khlong is little studied, and in the past has sometimes been considered more closely allied to the fauna of more easterly drainages (e.g., Kottelat, 1989; Zakaria-Ismail, 1994). In the current example, the affinity to the fauna in western drainages is clear, and although the genetic divergence observed between Salween and Mae Khlong fishes was greater than between Salween and Ayeyarwady individuals, all were much more closely related to each other than they were to the eastern Mekong Basin Marulius group species, pointing to a shared biogeographical connection between the rivers of Myanmar and western Thailand (see Kottelat, 1989).

We understand that a Marulius group fish may also be present in some parts of Central Thailand's Chao Phraya Basin, but

as we are yet to encounter a specimen we cannot confirm whether this is the same as the Mae Khlong *C. aurolineata*, or potentially the same as the more easterly distributed Mekong species, *C. auroflammea*. Likewise, we are aware of a Marulius group fish in the Tanintharyi River directly west of the Mae Klong (see Adamson & Britz, 2018) but are unable to confirm its identity.

***Channa aurolineata* in Florida.** Without comprehensive surveys of diversity within native ranges, it is often hard to establish the origin of feral populations. This study revealed 100% DNA barcode identity between natural Thai populations and the feral Florida population, giving a strong indication that the Florida population was established from a founding population of Thai origin. Knowing the potential origin may help identify vectors for movement of prohibited species into mainland USA, and thus enable better policing of human mediated invasion pathways to prevent future introductions. This comes too late to halt the invasion of the Florida bullseye, that is now present and steadily expanding its non-native range across the canal system of southeastern Florida, where it was first detected in 2000 (Benson et al., 2018).

To date, the Florida bullseye snakehead has been referred to by the scientific name *C. marulius*, although recent work by Serrao et al. (2014) and Conte-Grand et al. (2017) has already exposed large genetic difference between the Florida population and individuals from the natural range of *C. marulius* in India. Now that the Marulius group as a whole is better understood, we can say with some certainty that the snakehead population in Florida waters is actually *C. aurolineata*, a species first described by Day (1870), but only recently revalidated as part of geographically wide-ranging investigations of the Marulius group across its natural range (Adamson & Britz, 2018). In Thailand, confirmed here as the likely source of the Florida population, *C. aurolineata* is a popular target for international angling tourists, and perhaps this might, as previously suggested by Courtenay & Williams (2004), explain the motivation behind the original introduction of this fish into North America.

## ACKNOWLEDGEMENTS

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Supplementary information: List of samples compiled to create the Marulius group DNA barcode library.

Genbank	Sample ID	Bold BIN	Reference	Specimen Origin	Species Name
<i>Channa marulius</i> (35)					
KY425546/ MF496845	LR1437	ABA8625	Britz et al., 2017; Conte-Grande et al., 2017.	India	<i>C.marulius</i>
KY425543/ MF496849	LR2301		Britz et al., 2017; Conte-Grande et al., 2017.	India	<i>C.marulius</i>
KY425545/ MF496842	LR0129		Britz et al., 2017; Conte-Grande et al., 2017.	India	<i>C.marulius</i>
KY425545/ MF496845	LR1200		Britz et al., 2017; Conte-Grande et al., 2017.	India	<i>C.marulius</i>
KY425555/ MF496846	LR1603		Britz et al., 2017; Conte-Grande et al., 2017.	India	<i>C.marulius</i>
KY425548/ MF496847	LR1608		Britz et al., 2017; Conte-Grande et al., 2017.	India	<i>C.marulius</i>
KY425554/ MF496852	LR6591		Britz et al., 2017; Conte-Grande et al., 2017.	Myanmar	<i>C.marulius</i>
KY425557/ MF496848	LR2263		Britz et al., 2017; Conte-Grande et al., 2017.	Myanmar	<i>C.marulius</i>
KY425547	BNHS347		Britz et al., 2017.	India	<i>C.marulius</i>
KY425549	43		Britz et al., 2017.	India	<i>C.marulius</i>
KY425550	ChLeMa1		Britz et al., 2017.	India	<i>C.marulius</i>
KY425551	BNHS338		Britz et al., 2017.	India	<i>C.marulius</i>
KY425552	BNHS342		Britz et al., 2017.	India	<i>C.marulius</i>
KY425553	BNHS344		Britz et al., 2017.	India	<i>C.marulius</i>
KY425554	BNHS348		Britz et al., 2017.	India	<i>C.marulius</i>
AB968638	Unpublished		Cui & Xu, unpublished	na	<i>C.marulius</i>
KF511552	BOF19F		Dhar & Ghosh, 2015	India	<i>C.marulius</i>
JX983244	–		Khedkar et al., 2014	India	<i>C.marulius</i>
JX260840	MUND01		Kalyankar et al., unpublished	India	<i>C.marulius</i>
JX260841	MUND02		Kalyankar et al., unpublished	India	<i>C.marulius</i>
KJ847102	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847103	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847104	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847105	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847106	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847107	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847108	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847109	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847110	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847111	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>

Genbank	Sample ID	Bold BIN	Reference	Specimen Origin	Species Name
KJ847112	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847113	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847114	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847115	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
KJ847116	Unpublished		Barman et al., unpublished	India	<i>C.marulius</i>
<b><i>Channa pseudomarulius</i> (21)</b>					
KY425558/ MF496882	LR1213	AAI7187	Britz et al., 2017; Conte-Grande et al., 2017.	India	<i>C.pseudomarulius</i>
KY425560/ MF496883	LR2312		Britz et al., 2017; Conte-Grande et al., 2017.	India	<i>C.pseudomarulius</i>
KY425565/ MF496884	LR2443		Britz et al., 2017; Conte-Grande et al., 2017.	India	<i>C.pseudomarulius</i>
KY425559	ChMaPr3		Britz et al., 2017.	India	<i>C.pseudomarulius</i>
KY425561	ChMaPa1		Britz et al., 2017.	India	<i>C.pseudomarulius</i>
KY425562	ChMaPa3		Britz et al., 2017.	India	<i>C.pseudomarulius</i>
KY425563	ChMaPa4		Britz et al., 2017.	India	<i>C.pseudomarulius</i>
KY425564	ChMaPr2		Britz et al., 2017.	India	<i>C.pseudomarulius</i>
KY425566	ChMaPr1		Britz et al., 2017.	India	<i>C.pseudomarulius</i>
KY425567	ChMaPa2		Britz et al., 2017.	India	<i>C.pseudomarulius</i>
KY425568	ChMaPa5		Britz et al., 2017.	India	<i>C.pseudomarulius</i>
EU342199	–		Benziger et al., 2011.	India	<i>C.pseudomarulius</i>
EU342200	–		Benziger et al., 2011.	India	<i>C.pseudomarulius</i>
HM117192	NE-CM1		Larkra et al., 2010.	India	<i>C.pseudomarulius</i>
HM117193	NE-CM2		Larkra et al., 2010.	India	<i>C.pseudomarulius</i>
HM117194	NE-CM3		Larkra et al., 2010.	India	<i>C.pseudomarulius</i>
HM117195	NE-CM4		Larkra et al., 2010.	India	<i>C.pseudomarulius</i>
HM117196	NE-CM5		Larkra et al., 2010.	India	<i>C.pseudomarulius</i>
KJ937341	NRS048-12		Serrao et al., 2014.	India	<i>C.pseudomarulius</i>
KJ937348	NRS047-12		Serrao et al., 2014.	India	<i>C.pseudomarulius</i>
KJ937388	NRS049-12		Serrao et al., 2014.	India	<i>C.pseudomarulius</i>
<b><i>Channa aurolineata</i> (10)</b>					
MH375690/ MF496843	LR1037	Close to ABW0012 (florida)	Conte-Grande et al., 2017; Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>
MH375691	LR09373		Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>
MH375692	EA0229		Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>
MH375693	EA0266		Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>
MH375694	EA0350		Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>
MH375695	EA0543		Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>
MH375696	EA1251		Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>
MH375697	EA1252		Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>

Genbank	Sample ID	Bold BIN	Reference	Specimen Origin	Species Name	
MH375698	EA1253		Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>	
MH375699	EA1254		Adamson & Britz, 2018.	Myanmar	<i>C.aurolineata</i>	
<b><i>Channa marulioides</i> (6)</b>						
KJ937378	DSCHA002-07	AAC6049	Serrao et al., 2014.	na	<i>C.marulioides</i>	Mis-ID
MF496837	LR1257		Conte-Grande et al., 2017.	Indonesia	<i>C.marulioides</i>	
MF496838	LR1684		Conte-Grande et al., 2017.	Aquarium	<i>C.marulioides</i>	
MF496838	LR2050		Conte-Grande et al., 2017.	Malaysia	<i>C.marulioides</i>	
MF496840	LR2295		Conte-Grande et al., 2017.	Aquarium	<i>C.marulioides</i>	
MF496841	LR2488		Conte-Grande et al., 2017.	Aquarium	<i>C.marulioides</i>	
<b><i>Channa auroflammea</i> (10)</b>						
MF496850	LR2311	No bin	Conte-Grande et al., 2017.	Aquarium	<i>C.auroflammea</i>	
MF496851	LR2444		Conte-Grande et al., 2017.	LaoPDR	<i>C.auroflammea</i>	
MK423209	EA141		Adamson et al., 2019.	Cambodia	<i>C.auroflammea</i>	
MK423210	EA145		Adamson et al., 2019.	Cambodia	<i>C.auroflammea</i>	
MK423211	EA562		Adamson et al., 2019.	Cambodia	<i>C.auroflammea</i>	
MK423212	EA575		Adamson et al., 2019.	Cambodia	<i>C.auroflammea</i>	
MK423213	EA674		Adamson et al., 2019.	LaoPDR	<i>C.auroflammea</i>	
MK423214	EA675		Adamson et al., 2019.	LaoPDR	<i>C.auroflammea</i>	
MK423215	CMK27769		Adamson et al., 2019.	LaoPDR	<i>C.auroflammea</i>	
MK423216	CMK27770		Adamson et al., 2019.	LaoPDR	<i>C.auroflammea</i>	
<b><i>Channa Mae Khlong</i> (3)</b>						
MK599316	EA1075_ BMNH2015.1.1.94		This study	Thailand	Marulius group	
MK599317	EA1076_ BMNH2015.1.1.95		This study	Thailand	Marulius group	
MK599318	EA1077_ BMNH2015.1.1.96		This study	Thailand	Marulius group	
<b><i>North American bullseye</i> (7)</b>						
KJ937352	NRSC053-12	ABW0012	Serrao et al., 2014.	Florida	Florida snakehead	
KJ937369	NRSC001-11		Serrao et al., 2014.	Canada (aquarium)	Ontario snakehead	
KJ937407	NRSC055-12		Serrao et al., 2014.	Florida	Florida snakehead	
KJ937410	NRSC056-12		Serrao et al., 2014.	Florida	Florida snakehead	
KJ937419	NRSC054-12		Serrao et al., 2014.	Florida	Florida snakehead	
KJ937432	NRSC052-12		Serrao et al., 2014.	Florida	Florida snakehead	
KJ937440	NRSC051-12		Serrao et al., 2014.	Florida	Florida snakehead	

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