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Research Article

Occurrence of a reproducing wild population of *Channa aurolineata* (Pisces: Channidae) in the Manatee River drainage, Florida

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Abstract

We report on the discovery of a wild, reproducing population of *Channa aurolineata* (Pisces: Channidae) in west-central Florida (USA), and first documented occurrence of snakeheads in the Gulf Coast region. *Channa aurolineata* is a large, predatory fish of the bullseye snakehead "Marulius group" species complex from Asia. Adult and juvenile specimens were captured in June 2020 in a 1.8-hectare pond that connects during high water to a small stream within the Manatee River-Tampa Bay Basin. The pond site is 250-km from the only other wild *C. aurolineata* population in the USA (present in southeast Florida since ca. 2000) and is considered a separate introduction and not the result of natural dispersal. Morphological and molecular comparisons revealed high overlap between the two Florida populations, evidence humans may have transported fish between sites. To verify identification, we compared Florida samples to *C. aurolineata* from Thailand and found mtDNA-COI barcode sequences to be identical or to differ by only a single base pair. Life body coloration of Florida samples matched their Asian counterparts, but Florida specimens averaged fewer dorsal fin rays (53.6 vs. 56.0), anal fin rays (34.2 vs 36.1), lateral line scales (65.3 vs. 67.4), and vertebrae (62.1 vs. 64.3), differences implying possible founder effect or sampling bias. Existence of this invasive predator is a concern because of the risk of spread and negative ecological effects, including an observation of terrestrial hunting behavior. In 2020–2021, several hundred *C. aurolineata* were removed from the pond by nets and electrofishing, and surveys suggested the population had not spread to nearby waters. In May 2021 the pond was treated with rotenone and 48 more specimens were recovered. No additional snakeheads have been sighted since the piscicide operation, although verification of eradication will require monitoring of the watershed.

Key words: goldline snakehead, invasive fish, taxonomy, rotenone, eradication, terrestrial feeding, cytochrome c oxidase subunit I (COI), USFWS-LEMIS database

Introduction

The Channidae (snakeheads) is a family of predatory freshwater fishes naturally distributed in parts of Asia and Africa. In North America, at least five different snakehead species of Asian origin have been reported from open waters (i.e., the wild) and as many as four of these are considered established (Courtenay and Williams 2004; Cudmore and Mandrak 2009; Adamson and Britz 2019; Benson 2019). Recent taxonomic assessments of the Channidae describe the family as composed of at least three genera (i.e., *Channa*, *Parachanna*, and *Aenigmachanna*) (Adamson and Britz 2018; Rüber et al. 2020). Recent estimates of the total number of valid snakehead species range widely, anywhere from 47 (Rüber et al. 2020) to 56 (Fricke et al. 2021). Such conflicting numbers highlight the difficult and confusing condition of the family's taxonomy and the great uncertainty regarding the group's species-level diversity (Rüber et al. 2020). The unstable situation presents major difficulties for stakeholders interested in positive identification of specimens; a problem exacerbated in dealing with introduced populations whose native origin is unclear.

Channa aurolineata (Day, 1870) is a member of the *Channa* "Marulius group", a complex of five or more large (60–120 cm SL), closely-related species native to southern Asia (Adamson and Britz 2018, 2019; Rüber et al. 2020). Although some channid taxa may possess eye spots on the dorsal fin or caudal peduncle (Courtenay and Williams 2004), members of the Marulius group (herein also referred to as Marulius snakeheads) display a conspicuous ocellus on the upper part of the caudal fin during at least one stage of their life cycle, a condition purported to be a synapomorphy (Adamson and Britz 2018). Previously, *Channa aurolineata* was considered a junior synonym of *C. marulius* (Hamilton, 1822), but recently Adamson and Britz (2018) provided strong morphological and genetic evidence that *aurolineata* is a distinct species, distinguishable from other Marulius snakeheads. The type locality of *C. aurolineata* is Mawlamyine (Moulmein) in Mon State, Myanmar and the species' natural distribution includes the Chindwin, Irrawaddy, Sittang, Salween, and Mae Klong river basins of Myanmar and Thailand (Adamson and Britz 2018, 2019).

Snakeheads of Asian origin have been widely introduced around the globe, a situation generating concern because of their frequency of establishment and the possibility of negative ecological consequences (Lever 1996; Courtenay and Williams 2004; Cudmore and Mandrak 2009; Odenkirk and Chapman 2019; Froese and Pauly 2021). In 2000, snakeheads reported as *Channa marulius* were found in the United States in the Cypress Creek Canal system of Broward County in southeast Florida, herein referred to as "SE Florida" (Courtenay and Williams 2004). Initial sampling showed the SE Florida population to be relatively abundant, widespread, and biologists suspected that the species had been present at least two to three years prior to its discovery (Courtenay and Williams 2004; Shafland et al. 2008). The SE Florida record represented the first snakehead population documented as established in North America (Benson et al. 2018; Robins et al. 2018; Benson 2019) and, prior to the current study, the only reported non-native occurrence of a Marulius snakehead anywhere in the world. Recently,

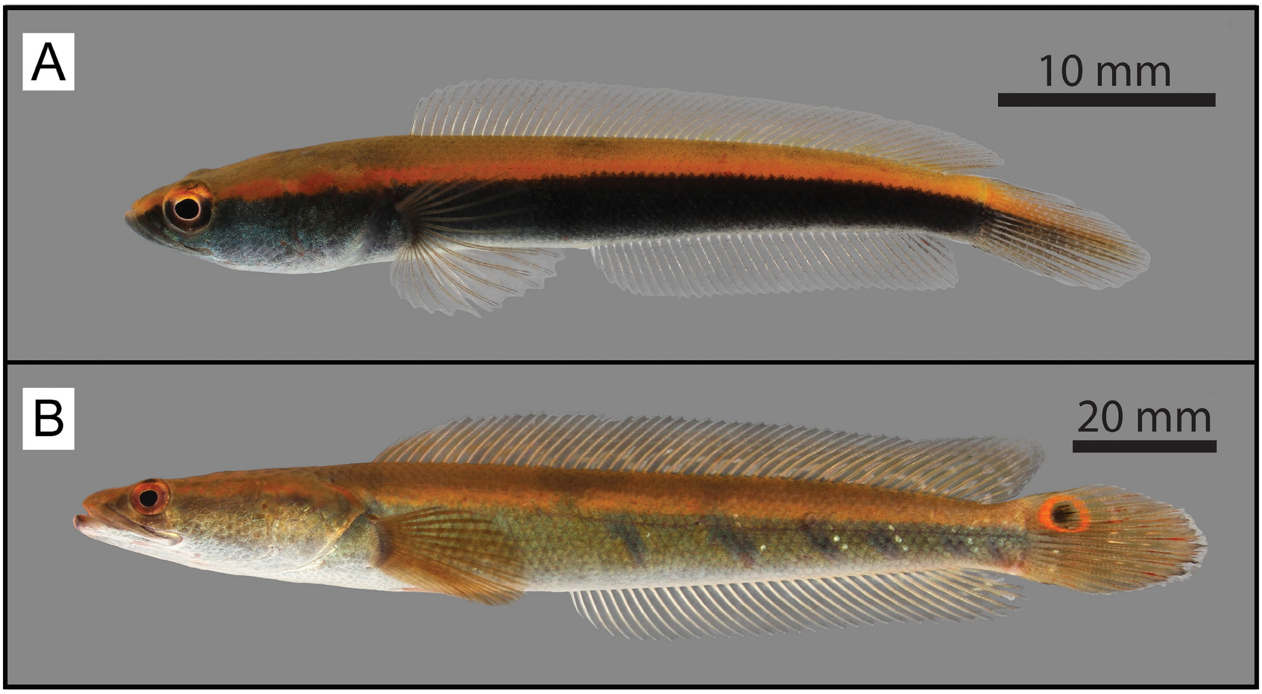

Figure 1. Photographs of juvenile goldline snakehead *Channa aurolineata* collected from retention pond ("MC Channa pond") in Manatee River drainage of Florida in Summer 2020: (A) 40 mm SL; and (B) 131.9 mm SL. Preserved museum voucher catalog number: UF 245635. Photographs by Zachary S. Randall.

Adamson and Britz (2019) compiled a DNA barcode library as part of a taxonomic investigation of Marulius snakeheads from western Thailand and found matching DNA barcodes between western Thailand and SE Florida, concluding that the introduced population in SE Florida should be referred to as *C. aurolineata* rather than *C. marulius*.

In this paper, we report on a second, geographically distinct non-native population of *Channa aurolineata* in Florida. We became aware of this new population in June 2020 when one of the authors (MK) netted adult and juvenile *C. aurolineata* specimens while sampling a large retention pond in the Manatee River basin, a Gulf Coast drainage in the west-central part of the state (Figures 1–4). We document the existence of this newly discovered population and present results of morphological and molecular analyses to validate identity and assess likely introduction origin. Our study includes a comparison of Florida samples from both Manatee County and SE Florida as well as *Channa* material from Asia. We provide information on the invaded environment, report original field observations, describe the current status and geographic distribution of snakeheads in Florida, while discussing introduction pathways, potential range expansion, and ecological threats associated with snakehead introduction. In addition, we summarize efforts to eradicate the Manatee County *C. aurolineata* population.

Materials and methods

Study area

The Manatee County water body where *C. aurolineata* was discovered is a large, unnamed, freshwater pond, herein referred to as the "MC Channa pond".

Figure 2. An adult goldline snakehead *Channa aurolineata* (*in situ*) inhabiting pond (see Figure 4) in the Manatee River drainage in Manatee County, Florida, 21 June 2020 (Field# LGN 20-18). Photograph by Leo G. Nico.

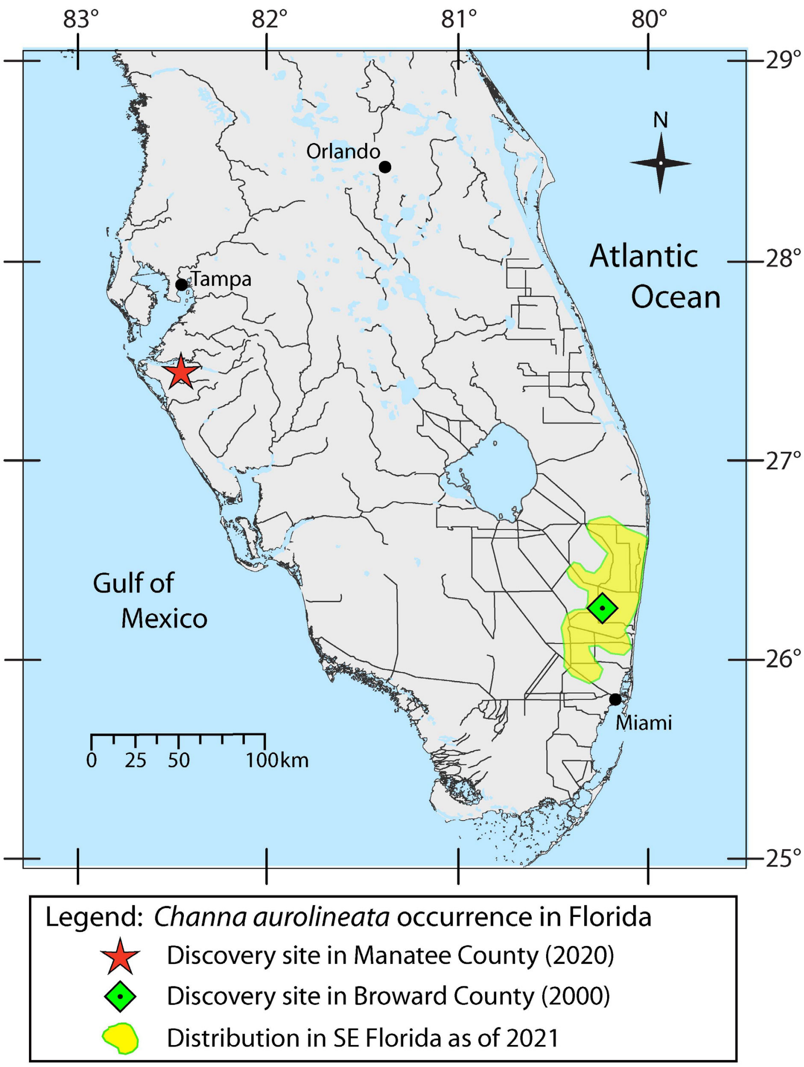

Figure 3. Map of peninsular Florida showing location of MC Channa pond (red star), Manatee County, where *Channa aurolineata* was documented in 2020. Also shown is the southeast Florida location where the species was discovered in 2000 and its approximate expanded, distribution as of 2021 (source: U.S. Geological Survey, nas.er.usgs.gov).

Figure 4. Southern portion of the 1.8-hectare MC Channa pond (Manatee River drainage) in Manatee County, Florida, where *Channa aurolineata* was discovered in the Summer of 2020. Photograph by Leo G. Nico.

The pond is located in the Williams Creek watershed, a tributary of the Braden River in the lower Manatee River drainage of west-central peninsular Florida, USA (27.4555N; 82.4582W) (Figures 3, 4). Williams Creek is a small meandering stream that drains about 7 km^2 (DelCharco and Lewelling 1997). The Manatee River is a lowland, coastal river (72-km long) with a drainage area of approximately 932 km^2, lying mostly in Manatee County, with portions in Sarasota and Hillsborough counties (Chen 2012). The Manatee River is one of many streams, large and small, feeding Tampa Bay, a major estuary of the Gulf of Mexico.

The MC Channa pond has a markedly elongate shape (360-m long by 40-m wide), with a surface area of about 1.8 ha and average depth of approximately 2.1 meters. During our field investigation, the deep, central portions of the pond were mostly open water with a few large mats of floating plants, primarily water lilies (*Nymphaea* sp.). Pond margins were a mosaic of emergent aquatic and wetland plants, grasses, shrubs, and trees. A dense stand of emergent wetland vegetation, mostly cattail (*Typha* sp.) and arrowhead (*Sagittaria* sp.), covered approximately 0.25 ha of the pond's extremely shallow north section near an outflow culvert. On 21 June 2020 and 10–11 May 2021, readings taken near shore with a YSI 85 meter showed water temperatures ranging from 27.1 to 30.5 °C, conductivity 154 to 211 µS/cm, and salinity of 0.1 ppt. Pond water was moderately clear (0.3 to 0.8 m visibility) and slightly tannic in color. On 21 June 2020, a Hach colorimeter test kit gave a pH reading of 8.

Manatee County government documents describe the pond as a recent construct (post mid-1990s) created by the deepening of an existing wetland for stormwater retention, presumably in preparation for the rural area's transition toward commercial development. During a visit to the MC Channa pond in early 2020, the site was at a high-water stage and outflowing to Williams Creek via a culvert at its northern extreme. However, the same pond-to-creek culvert area was completely dry during subsequent visits to the site between June 2020 and May 2021. In addition to the outflow culvert, nearby parking lots contained several storm drains designed to collect and convey rainwater to the MC Channa pond via underground pipes. It has not been verified whether this pipe drainage system is linked to any other, nearby water bodies.

The climate of the Manatee County region is subtropical, characterized by high temperatures, high average annual rainfall, intermittent widespread flooding, and periodic winter cold fronts (DelCharco and Lewelling 1997). Air temperatures data over the years 1991–2020 showed average monthly readings ranging from a low of 16.7 °C (January) to a high of 28.7 °C (August) (Florida Climate Center 2021).

Sampled sites and methods

Following initial capture of *Channa aurolineata* at the MC Channa pond site in June 2020, we intensively sampled fishes on multiple occasions, including the pond site and nearby waters during the remainder of 2020 and into May 2021. The main purpose was to ascertain the distribution and abundance of the snakehead population. Our search included repeated visual surveys of the general area both at night (with spotlights) and during daylight hours. The first snakeheads taken from the MC Channa pond were captured at night and early morning hours in nearshore areas using hand nets (e.g., landing nets). Subsequent sampling was organized by Florida Fish and Wildlife Conservation Commission (FWC) authorities and involved use of boat-mounted electrofishing gear to sample the MC Channa pond, nearby deeper ponds, and the Braden River (near its confluence with Williams Creek). Backpack electrofishing units and various types of hand nets were used to sample Williams Creek, ditches, and other shallow-water habitats.

Eradication rotenone operation

Snakeheads were not detected during surveys of sites outside the MC Channa pond, suggesting that the pond population was confined to the pond site and had yet to disperse. Consequently, FWC authorities judged eradication feasible and decided that use of the fish toxicant rotenone would be an effective and rapid removal method. After receiving permission from local land owners to renovate the pond's entire fish population utilizing rotenone, FWC staff assembled a team of biologists to plan the logistics. On the morning of 10 May 2021, the commercially-available liquid rotenone formulation Prenfish (USEPA reg. no. 89459-85) was dispensed throughout the pond via boats and backpack sprayers. A sufficient quantity of the chemical was applied to achieve an effective dose of 0.2 mg/L active rotenone throughout the water body. Additionally, rotenone was applied to several stormwater drainage pipes connected to the pond in an attempt to eradicate any snakeheads possibly living inside that would escape the main pond treatment. Immediately following rotenone application, several days were spent recovering fish incapacitated or killed by the piscicide. All snakeheads recovered during the rotenone treatment were counted, measured, and weighed. Most snakeheads (one was preserved as a voucher) and other recovered fishes were transported to a nearby county landfill where combined total weight of all fishes was estimated.

Original and comparative material

A subset of juvenile and adult snakehead specimens taken from the MC Channa pond during our 2020–2021 visits were preserved as vouchers. Most of this material was deposited and cataloged in the ichthyological collection of the Florida Museum of Natural History-University of Florida (UF), in Gainesville, Florida. We also had access to *Channa* specimens and tissues from the SE Florida population previously deposited at UF. A subsample of the vouchered material was used to obtain morphological data and as sources of tissues for molecular analysis. Morphological, meristic, and DNA information on *Channa aurolineata* from the two non-native Florida populations represent original data generated during the present study. Data on *C. aurolineata* and *C. marulius* specimens from Asia used for comparative purposes are from the published literature (Britz et al. 2017; Adamson and Britz 2018), supplemented with unpublished data provided by E. Adamson (Natural History Museum, London, *written communication*, 2021).

Florida *C. aurolineata* material examined for morphological comparison are listed below by museum catalog number. Included are specimens collected by us at the MC Channa pond, as well as *C. aurolineata* collected by others in SE Florida. The number and size range (standard length [SL] in mm) of specimens is given in parentheses. Any UF material that served as the source of tissues used in COI (cytochrome oxidase subunit 1) barcode sequencing are displayed in **bold** (one of these lots represents tissues only and does not have an associated cataloged whole voucher specimen, however it is supported by photographs of the specimens from which the tissues were taken). Cataloged lots containing the 10 Florida *C. aurolineata* specimens used in vertebral counts are indicated by an asterisk (*).

MC Channa pond, Manatee River drainage, Manatee County, FL: **UF 245637** (1, 546); UF 247187 (8, 242–563); **UF 245609** (1, 483); and **UF 245635** (248, 29.5–131.9).

SE Florida samples from Broward and Palm Beach counties: UF 129392 (3, 176–223); UF 190143 (1, 210); **UF 237863** (8, 207–289); UF 238351 (5, 268–307); UF 238392 (4, 314–660); UF 238608 (1, 116); UF 238610 (3, 144–184); UF 129478* (7, 81–121); UF 191248* (4, 177–276); UF 237965* (1, 208); UF 238607* (3, 168–211); UF 238609* (3, 138–190); UF 241571* (4, 110–161); **UF 182635** (6, 412–612); **UF 187100** (1, 412); **UF 237264** (tissue only).

In addition to the Florida specimen data, meristics data for 20 *Channa aurolineata* from multiple sites in Asia (Myanmar) were provided by E. Adamson (Natural History Museum, London). Vouchers of that material are deposited in the following institutions: BMNH, Natural History Museum, London; USNM, National Museum of Natural History-Smithsonian Institution, Washington, D.C.; ZRC, Lee Kong Chian Natural History Museum, Singapore; and NMW, Naturhistorisches Museum Wien.

Morphology and meristics

We measured SL to nearest mm and counted dorsal fin rays, anal fin rays, and lateral line scales on 64 *C. aurolineata* specimens from the two Florida populations (Neilson et al. 2022). Scale and fin counts were made on the left side with a Leica MZ 75 dissecting microscope or for larger specimens, a Magni-Focuser visor. Lateral-line scale counts included all pored scales, as well as any unpored scales, encountered along the single scale row starting from behind the gill opening and ending at the caudal flexion (pored scales posterior to the flexion were excluded). Dorsal and anal fin rays (including final two rays articulating on a single pterygiophore) were counted individually. Vertebrae counts from 10 specimens were obtained using CT scans and were generated using a Phoenix v|tome|x M scanner (GE Measurement & Control, Boston, USA) at the University of Florida's Nanoscale Research Facility. X-ray data were processed using datos|x software v. 2.3 and segmented and visualized using VG StudioMax v. 3 (Volume Graphics, Heidelberg, Germany). The scan data are freely available for download at MorphoSource (media IDs 000170160–000170165). Photographs of selected live and freshly dead juvenile and adult specimens were taken to document body color patterns and for comparison with published images of *Channa*.

Molecular methods

Sequenced *Channa* material unique to this study consisted of seven samples, four from SE Florida and three from the MC Channa pond population. Fin tissues from the seven specimens were preserved in 95% ethanol and total genomic DNA extracted using AutoGenPrep 965 (Autogen, Holliston, MA). COI was amplified and sequenced using the primers developed by Ward et al. (2005). Polymerase chain reaction was performed in 10 µl reactions using the following reagents and volumes: H20 (3.2 µl), genomic DNA (1 µl), BSA (0.1 µl), DMSO (0.1 µl), forward and reverse primers 10 nM (0.3 µl each), GoTaq Hot Start Master Mix (Promega, Madison, WI; 5.0 µl). Bidirectional Sanger sequencing was performed at the National Museum of Natural History's Laboratories of Analytical Biology using ABI 3730xl Genetic Analyzer (Thermo Fisher, Waltham, MA).

The newly sequenced material was analyzed in conjunction with the Channidae barcode library compiled by Adamson and Britz (2019) that contains COI mtDNA sequence data from 92 individuals across all five currently recognized Marulius snakehead species and all *Channa* sequence data of Serrao et al. (2014). We generated a new sequence alignment by combining the Adamson and Britz library with our new data and re-ran the molecular analyses using MEGA X (Kumar et al. 2018). Sequences were aligned using MUSCLE (Edgar 2004) implemented in MEGA X using the default settings. Uncorrected pairwise distances were calculated to identify genetic affinities for Florida snakehead samples. An unrooted neighbor joining tree was generated using uncorrected pairwise distances in MEGA X, and a TCS haplotype network of all *C. aurolineata* sequences was generated in PopART (Clement et al. 2002; Leigh and Bryant 2015) to help visualize divergence.

Results

Fish tentatively identified as snakeheads were observed in the MC Channa pond site in early 2020. In June 2020 we collected specimens and confirmed that a *Channa* species occupied the site. Our subsequent morphological and molecular analyses revealed the pond population to be *Channa aurolineata*.

Morphology and meristics

Samples of *Channa aurolineata* collected in Florida were found to have a lower average number of dorsal and anal fin rays, pored lateral line scales, and vertebrae than *C. aurolineata* samples from Asia. The dorsal (Table 1) and anal (Table 2) fin-ray counts of Florida *C. aurolineata* were more similar to *C. marulius* than to the ray counts of Asia *C. aurolineata* samples. The numbers of pored lateral line scales (Table 3) and vertebrae (Table 4) of sampled Florida *C. aurolineata* were intermediate between *C. marulius* and native range *C. aurolineata*. Coloration of adult and subadult *C. aurolineata* from the MC Channa pond resembles specimens described by Adamson and Britz (2018) in that they possess dark midlateral blotches composed of 2–3 rows of black scales usually rimmed with a white posterior margin. Adult and larger juvenile *C. aurolineata* from the Manatee County pond site all possessed a conspicuous ocellus on the upper caudal fin base, a characteristic of members of the Marulius group.

DNA barcodes

Newly sequenced DNA barcodes were generated for four individuals from SE Florida and three individuals from Manatee County (GenBank accession

Table 1. Frequency distribution of dorsal fin rays for Florida (introduced) and Asian (native) populations of *Channa aurolineata* versus *C. marulius*. Modal values indicated in ***bold italics***, N = sample size, and X = average. Data on Florida populations based on original material of the present study. Number of lateral line scales and vertebrae reported for *C. aurolineata* holotype (BMNH 1889.2.1.3804) indicated by asterisk (*). Information on *C. aurolineata* from Asia (including holotype) and for *C. marulius* is from Britz et al. (2017) and Adamson and Britz (2018), supplemented with data provided by E. Adamson (Natural History Museum, London, unpublished data, 2021).

	Number of dorsal fin rays													
	47	48	49	50	51	52	53	54	55	56	57	58	N	X
C. aurolineata (Florida)	1			1		7	17	***22***	13	3			64	53.6
Manatee County				1		1	3	***4***	1				10	53.2
Southeast Florida	1					6	14	***18***	12	3			54	53.7
C. aurolineata (Asia)						1			5	***7***	6	1	20	56.0
C. marulius						8	***18***	5	4	1			36	53.2

Table 2. Frequency distribution of anal fin rays for Florida (introduced) and Asian (native) populations of *Channa aurolineata* versus *C. marulius*. Abbreviations and data sources following Table 1.

	Number of anal fin rays								
	32	33	34	35	36	37	38	N	X
C. aurolineata (Florida)	4	14	***21***	19	4	1		63	34.2
Manatee County	1	2	3	***4***				10	34.0
Southeast Florida	3	12	***18***	15	4	1		53	34.2
C. aurolineata (Asia)			1	4	***9***	4	2	20	36.1
C. marulius		10	***15***	10		2		37	34.2

Table 3. Frequency distribution of lateral line scales for Florida (introduced) and Asian (native) populations of *Channa aurolineata* versus *C. marulius*. Abbreviations and data sources following Table 1.

	Number of lateral line scales (pored and not pored)											
	62	63	64	65	66	67*	68	69	70	71	N	X
C. aurolineata (Florida)	3	5	11	***18***	12	8	5	2			64	65.3
Manatee County	1	1	1	***4***	1	2					10	64.9
Southeast Florida	2	4	10	***14***	11	6	5	2			54	65.4
C. aurolineata (Asia)				***5***	4	2	3	2	1	3	20	67.4
C. marulius	11	***13***	9	3							36	63.1

Table 4. Frequency distribution of vertebrae for Florida (introduced) and Asian (native) populations of *Channa aurolineata* versus *C. marulius*. Abbreviations and data sources following Table 1.

	Number of vertebrae											
	58	59	60	61	62	63	64*	65	66	67	N	X
C. aurolineata (Florida)				2	***6***	1	1				10	62.1
C. aurolineata (Asia)						6	***7***	5	2		20	64.3
C. marulius		2	3	***4***	3	1					13	60.8

numbers OL986013–OL986019; Neilson et al. 2022). One individual from Manatee County and two individuals from SE Florida shared an identical DNA barcode sequences to existing sequences of *C. aurolineata* from the Mae Klong basin of Thailand (see supplemental material in Adamson and Britz 2019 for full list of accession numbers). Two individuals each from Manatee County and SE Florida shared a DNA barcode sequence that differed from the Mae Klong sequences by a single base pair. Mean pairwise distance between Manatee County and SE Florida was 0.00%. Mean pairwise distances between Mae Klong *C. aurolineata* and the Florida populations were 0.029% (SE Florida) and 0.10% (Manatee County). Mean pairwise distances between Myanmar *C. aurolineata* were 2.17% (SE Florida) and

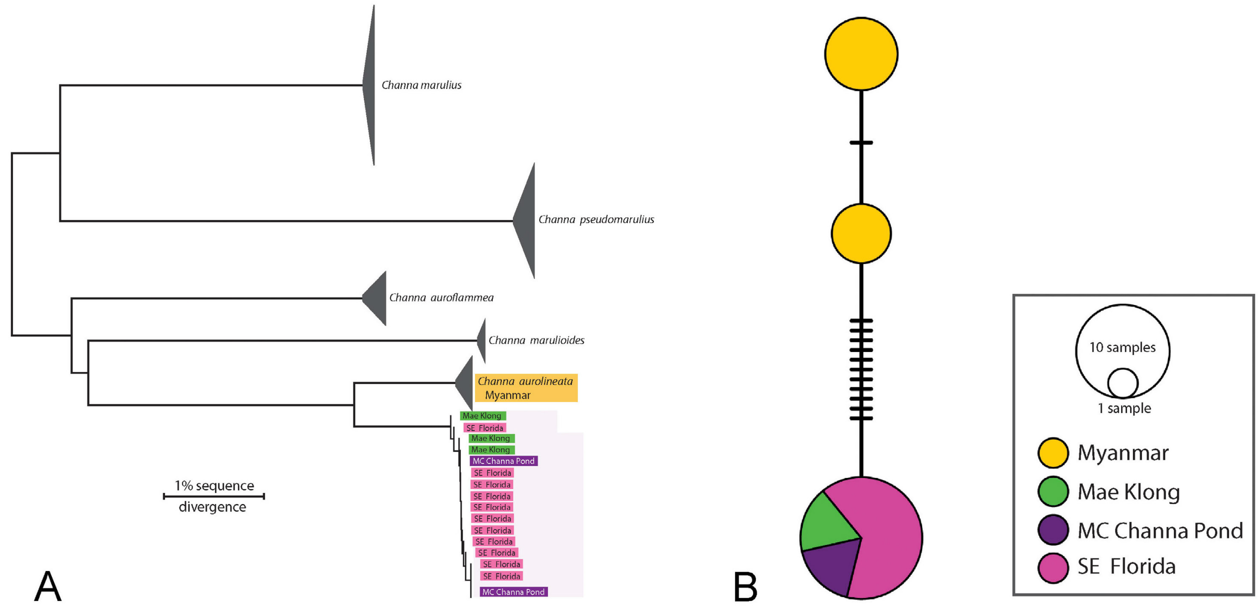

Figure 5. Graphical representations of the molecular data and divergence of *Channa aurolineata* samples from their native range in Asia and samples from the introduced Florida populations of Manatee County (i.e., MC Channa pond) and southeastern Florida. (A) is a neighbor-joining tree derived from uncorrected *p*-distances of CO1 DNA sequence barcodes with inclusion of dataset and analyses of Adamson and Britz (2019). Nodes for all taxa (except those containing sequences from Florida and Thailand's Mae Klong basin) with less than 2% sequence divergence are collapsed into wedges. (B) shows a TCS haplotype network of CO1 DNA sequence barcodes. Circles represent haplotypes differing by a single mutational difference. Small bars on each connecting edge indicate one additional mutational step. Circle size indicates number of samples containing that haplotype. See text for additional information.

2.23% (Manatee County), similar to the mean pairwise distance of ~ 2.1% reported by Adamson and Britz (2019) between *C. aurolineata* from Thailand and Myanmar. The neighbor joining tree (not shown) based on uncorrected pairwise distance was identical to that of Adamson and Britz (2019), with all newly sequenced Florida individuals grouped in a clade matching Adamson and Britz's "Marulius group Mae Khlong & Florida" clade. A haplotype network constructed from all *C. aurolineata* sequences identified two main groups separated by ~12 substitutions, one containing *C. aurolineata* from Myanmar and a second containing individuals from Thailand and Florida (Figure 5).

Field surveys and observations

Approximately 373 *C. aurolineata*, ranging in length from 15.5 to 582 mm SL, were taken from the MC Channa pond over the period 3 June 2020 to 13 May 2021. Most were captured with hand nets, by boat electrofishing, and with use of rotenone. We found *C. aurolineata* to be most common along the pond's margins and in vegetated areas. There were no confirmed sightings or evidence of snakeheads in any sites (i.e., streams, ditches, ponds) outside the MC Channa pond even though the general area was subject to repeated and intensive sampling and numerous visual surveys during 2020–2022. The only possible exception was a 2020 sighting of a single fish in a nearby pond that was conceivably a snakehead. That same pond underwent several subsequent visual surveys and sampling by an electrofishing boat without detecting any snakeheads.

The MC Channa pond snakeheads were considered a reproducing population. Evidence included the diverse array of sizes of juveniles and adults collected over the course of the entire study and the existence of multiple and rather distinct size classes during certain parts of year, particularly during months following apparent spawning events. Moreover, walks around the MC Channa pond in June 2020 revealed presence of adults with young in several shallow-water sites around the pond's perimeter. In each case, the site was occupied by a single pair of large adult *C. aurolineata* guarding a school of young snakehead.

Any one school consisted of fry or small juveniles approximately the same size. The school with the smallest members consisted of fry about 10-mm total length (TL); guarded schools with the largest cohorts were composed of juveniles 100-mm TL or more. Schools containing larger individuals were also observed to move more freely, sometimes wandering into deeper water or areas more than 15 meters from where first sighted. None of the adult pairs were seen to occupy a nest or found with eggs but the adults were regularly seen in the mouths of culvert pipes, suggesting they may have been using the pipes as nesting sites.

Terrestrial movement and feeding were also documented by one of the authors (MK) while reconnoitering the MC Channa pond on a rainy night in 2020. At that time, several *C. aurolineata* (about 20 to 40 cm TL) were observed on land in the wet grass, all within about one meter from the water's edge. Some of the fish were seen attacking and consuming native green treefrogs (*Hyla cinerea*), after which the snakehead would return to the water, locomoting by undulating its entire body. During their terrestrial feeding bout, a snakehead used a rapid sideways thrust of the head, lunging toward a nearby frog and then using its jaws to grasp the prey. Whenever a lantern's light was directed toward snakehead during their terrestrial excursion, the startled fish began performing a series of body flips.

Rotenone treatment of MC Channa pond

A total of 48 *C. aurolineata* were recovered from the 10 May 2021 rotenone treatment of the pond site in Manatee County (Figure 6A). On the day of rotenone application, six snakeheads killed or slowed by the toxicant were netted. An additional 42 snakeheads, all dead, were recovered during the following days (May 11–13). The retrieved snakehead carcasses were either found floating at the water surface or found on land near the pond margin. Some of the specimens found on shore apparently succumbed attempting to escape the piscicide. The *C. aurolineata* killed by rotenone, all presumed to be adults or near adult size, ranged from 281 to 582 mm SL (335 to 702 mm TL) and in weight from 220 to 1,824 g. The relationship between weight (W) and TL of the rotenone-killed specimens was: $W = 0.000008 * TL^{2.9272}$ ($r^2 = 0.9848$, $n = 46$). The SL-TL relationship was $SL = 0.8252 * TL + 3.3774$

Figure 6. *Channa aurolineata* and other non-native fishes recovered from the MC Channa pond in Manatee County, Florida, during the rotenone snakehead-eradication operation conducted 10–13 May 2021. Seven of the eight non-native species taken are shown: (A) Goldline snakehead, *C. aurolineata*; and (B) from top to bottom, Goldline snakehead; brown hoplo, *Hoplosternum littorale*; Asian swamp eel, *Monopterus javanensis*; jewelfish *Hemichromis letourneuxi*; Jack Dempsey, *Rocio octofasciata*; walking catfish, *Clarias batrachus*; and suckermouth armored catfish, *Pterygoplichthys disjunctivus*. Blue tilapia not shown. Photographs by Leo G. Nico.

(r^2 = 0.9968. n = 46). Approximately 317 kg (700 lbs) of dead fish, including snakeheads and other non-native and native fishes, were netted from the pond during several days of rotenone post-treatment. Carcasses were transported to a nearby Manatee County landfill. The rotenone-killed snakeheads (n = 48) accounted for 33 kg of the total, slightly over 10% of the total fish biomass.

Pond ichthyofauna

In addition to *Channa aurolineata* and the family Channidae, non-native fish taxa taken from the MC Channa pond during the 2020–2021 collections included seven other species of five additional families (Figure 6B). These included: Callichthyidae—brown hoplo, *Hoplosternum littorale* (Hancock, 1828); Loricariidae—vermiculated sailfin catfish, *Pterygoplichthys disjunctivus* (Weber, 1991); Clariidae—walking catfish, *Clarias batrachus* (Linnaeus, 1758); Synbranchidae—Asian swamp eel, *Monopterus javanensis* Lacepède, 1800; and Cichlidae—jewelfish *Hemichromis letourneuxi* Sauvage, 1880; blue tilapia, *Oreochromis aureus* (Steindachner, 1864); and Jack Dempsey, *Rocio octofasciata* (Regan, 1903). At least 11 fish species native to Florida were also collected, including members of the families Lepisosteidae (1 species), Cyprinidae (2), Fundulidae (2), Poeciliidae (2), and Centrarchidae (4).

Discussion

With the aid of humans, as many as nine different snakehead species have established or possibly established populations outside their native ranges

(Lever 1996; Courtenay and Williams 2004; Benson 2019; Froese and Pauly 2021). Some, notably *Channa striata* (Bloch, 1793) and *C. argus* (Cantor, 1842), have been widely introduced around the world and proven to be highly successful in colonizing new environments. In the continental United States, over the period 1968–2020 at least four different snakehead species have been reported from open waters and two, *C. argus* and *C. aurolineata*, are firmly established in the wild (Benson 2019; this study). Among the Marulius group (*sensu* Rüber et al. 2020), the *C. aurolineata* populations in Florida are the only Marulius snakeheads documented as introduced outside their home range of Asia.

Nomenclature

"*Channa marulius*" is known by several different names within the ornamental fish trade, examples include the "cobra snakehead" and "great snakehead." Courtenay and Williams (2004) promoted use of "bullseye snakehead" as the official English common name while recognizing that *C. marulius* represented a complex rather than a single, distinct species. Recent morphological and molecular analyses confirmed *C. aurolineata* as a distinct species within the Marulius complex (Adamson and Britz 2018). Because no two species should have the same common name, we propose "goldline snakehead" as the official English name for *C. aurolineata*. The term "goldline" is derived from Day's (1870) description of the holotype (and only specimen in the original type series), a 94-mm long juvenile whose life colors Day described as "… dark purplish black, with an orange stripe commencing at the snout and passing through the eye along the side and above the lateral line to the upper half of the tail" (see images of juvenile specimens from the Manatee County site in Figure 1). The name "bullseye snakehead" should continue as the common name for *C. marulius* (*sensu stricto*).

Identification and origin of the Florida MC Channa pond population

Genetic and morphological data gathered during the current study verified that the Manatee County *Channa* population is *C. aurolineata* and very similar to the snakehead population found in SE Florida. The SE Florida population, initially identified as *Channa marulius*, was first reported in October 2000 in Broward County and subsequently expanded its range via the region's freshwater canal networks into adjacent counties of Miami-Dade and Palm Beach (see discussion of invasion history in Shafland et al. 2008; Benson et al. 2018; Benson 2019). Previous DNA barcoding studies which included samples from the SE Florida population suggested hidden diversity within *C. marulius* (Serrao et al. 2014; Conte-Grand et al. 2017). Subsequently, *Channa aurolineata* was removed from synonymy with *C. marulius* and recognized as a distinct species by Adamson and Britz

(2018). A follow-up study revealed identical DNA barcode sequences between *C. aurolineata* specimens from Thailand and samples from SE Florida, results that suggested the likely source of the southeast Florida introduction (Adamson and Britz 2019). Our study builds on those earlier findings by establishing possible links between the two Florida populations.

The lack of congruence in modal or average number of meristic features exhibited by *C. aurolineata* within Florida and *C. aurolineata* examined from their native range could be the result of a founder effect or sampling biases (Tables 1–4). A larger sample of *C. aurolineata* from native populations, possibly including a greater proportion of large adult specimens, would be useful to detect sampling bias effect. Support for a founder effect is suggested by the presence of nearly identical DNA barcodes and low pairwise distances between Florida specimens sequenced in the present study and from the Mae Klong basin specimens from Adamson and Britz (2019), although this interpretation is hindered by the low number of individuals sequenced overall. A wider geographic characterization of molecular diversity from both the native and introduced range of *C. aurolineata* would be required for identifying the ultimate source of the Florida populations.

Based on their local abundance and wide range of sizes, it is likely the *C. aurolineata* population inhabiting the MC Channa pond had been present at the site a number of years prior to their discovery in 2020. However, their apparent restriction to the single pond would seem to suggest that the introduction occurred relatively recently, perhaps only three or four years prior. In contrast, the *C. aurolineata* population in SE Florida has existed over two decades. The two Florida populations are approximately 250-km apart. Consequently, the Manatee County population almost certainly represents a separate introduction rather than the result of natural dispersal. Morphological and genetic similarities argue for the likelihood of the SE Florida population being the source.

Introduction pathway

An introduction pathway describes the processes that result in the introduction of non-native species from one geographic area to another (Hulme et al. 2008). Available evidence indicates that all or most snakeheads reaching the United States from Asia entered the country by either of two primary pathways: the international live-food trade and the ornamental fish trade (Courtenay and Williams 2004; Odenkirk and Chapman 2019). However, how and why imported snakehead stocks ultimately gained access to open waters in the United States remain unclear. Eyewitnesses rarely come forward. In general, releases of live snakeheads into the wild are presumed related to their perceived value as food or sport fishes, although some captive snakeheads may have been set free as part of religious ceremonies (Courtenay and Williams 2004; also see Nico et al. 2019). While many international shipments of live snakeheads into the United States occurred prior to 2002 when importation was legal, release of non-native fish into U.S. waters is generally considered an illegal action.

Prohibitions against import and release

Both the United States and Florida (among other states) have regulations in place against snakeheads. Florida legislative prohibitions against all *Channa* species have been in place since at least the mid-1970s (Conroy 1975) and the entire family was added to the state's Prohibited Non-native Aquatic Species list in 1979 (Florida Administrative Code [FAC] Rule 68A-23.008). Current Florida legislation, in effect since the 1990s, prohibits the import, sale, possession, and transport of "snakeheads (family Channidae, all species)" (Florida Wildlife Code Title 39 – FAC 1994-1995; FAC Rule 68-5.006 of 2021). In 2002, the United States Fish and Wildlife Service (USFWS) added the family Channidae to the Federal Government's list of injurious wildlife under the Lacey Act 18 U.S.C. section 42, an action that prohibits their import into the country and any of its territories and possessions (USFWS 2002). Although state and federal regulations have reduced the likelihood of new snakehead stocks being imported and subsequently introduced into the wild, documented violations of these prohibitions exist (Courtenay and Williams 2004). Moreover, despite restrictions against import and possession, non-native wild populations already present in U.S. waters, such as those in SE Florida, remain a source for unauthorized transfers and releases by humans.

LEMIS data

The USFWS Law Enforcement Management Information System (LEMIS) compiles detailed information gathered from declaration forms on live fish and other wildlife imported into the country. Prior to about 2000, LEMIS import records were entirely in paper form and retained only a few years before being discarded (Nico et al. 2019). Since about 2000, LEMIS import records have been recorded and largely retained in electronic form. LEMIS data for the years 2000–2014 have recently become publicly available (Eskew et al. 2019, 2020) and mining of this information reveals that 178 separate shipments of live snakeheads were imported into the United States and its territories during that 15-year period. Fish are typically declared on custom forms either as total weight or as numbers of individuals per shipment. The LEMIS data on live snakehead indicates approximately 62,496 individuals and an additional 10,294 kg were imported over the years 2000–2014. Most of the live snakeheads were registered as having come from the wild, with only a few reported as being captive reared or bred. Most of these live snakehead imports were recorded as originating in China (145 shipments), although other Asian countries of origin listed included Thailand (9), India (8), Indonesia (8), Vietnam (3), Macao (3), and South Korea (1). All live snakehead imports were coded in the LEMIS database as being for commercial purposes, a broad category that does not differentiate between animals intended for the food trade versus the pet trade. However, names of export/import businesses in the LEMIS system indicate most of the 178 shipments (159) were almost certainly part of the live-food trade, with perhaps 19 others connected to the ornamental fish trade. "*Channa marulius*" was not listed as a live import, but most shipments (118 of 178) were identified only as "*Channa* sp." rather than to species level. Although the U.S. government prohibited the import of live snakeheads as of 2002, the LEMIS database indicates as many as 16 shipments containing some 3,940 live snakeheads were imported during the years 2003–2010. We have no information to indicate if any of these fish were confiscated. These 16 shipments appeared to include imports associated with both the live-food trade and the ornamental fish trade. Receiving ports for these 2003–2010 shipments included Los Angeles (8 shipments), New York city (4), Atlanta (1), Denver (1), San Francisco (1), and Saipan (1).

Live-food trade

Marulius snakeheads are cultured as a food fish in several Asian countries, mainly India, Pakistan, and Bangladesh (Bardach et al. 1972; Talwar and Jhingran 1992; Siddiquee et al. 2015). However, sources suggest the practice is generally small scale, low-tech, and heavily dependent on captures of wild young that are then grown out in captivity (Talwar and Jhingran 1992). In parts of Asia, Marulius snakeheads intended for use as food are marketed alive (Rainboth 1996). According to Pethiyagoda (1991), the Marulius species native to Sri Lanka is considered a food fish, but not known to be bred in captivity and is seldomly exported. When the import of live snakeheads into the United States was legal (prior to 2002), surveys revealed that a few different snakehead species made their appearance in U.S. ethnic food markets (Courtenay and Williams 2004; Odenkirk and Chapman 2019; LGN *pers. obs.*). Although live individuals of some snakehead species were common at the time, Marulius snakeheads were relatively rare in the live food trade and largely insignificant as an imported food fish compared to that of other channids. For instance, Courtenay and Williams (2004) unearthed only a single "*Channa marulius*" record from a U.S. live-food market. That information was based on observations by the ichthyologist Leo Smith (University of Kansas Biodiversity Institute and Natural History Museum, *written communication*, November 2021) who recently reaffirmed to us that on 14 May 2001 he encountered the taxa in a market in New York City's Chinatown and purchased two live specimens which he genetically sequenced confirming his initial identification that they belonged to the Marulius group. One of us (LGN) surveyed more than two hundred ethnic food markets across the USA and Canada during the period 2001–2019

Figure 7. Juvenile snakeheads of the *Channa* Marulius group in aquaria. The fish shown were one of perhaps three snakehead species on display and for sale in the live pet section of the Chatuchak Market, Bangkok, Thailand on 14 January 2006. Photograph by Leo G. Nico.

without encountering a single live Marulius snakehead (for details see Nico et al. 2019). In contrast, live *Channa* of the Asiatica, Striata, and Micropeltes groups were more frequently observed in markets, although these taxa seem to have disappeared from markets after the U.S. government-imposed prohibitions on importation (LGN *pers. obs.*; Leo Smith, University of Kansas Biodiversity Institute and Natural History Museum, *written communication*, November 2021).

Ornamental fish trade

Reported maximum sizes of the different *Channa* species range from about 10 cm to over 1 m SL (Rüber et al. 2020). Only a small percentage of hobbyists are considered snakehead enthusiasts and their interest, at least in more recent years, appears focused on the so-called “dwarf” species (Brede and Kleinbölting 2012; Bitter 2021). Nevertheless, several large snakehead species are reared in the aquarium and occasionally appear in the pet trade, mainly those with colorful juveniles, including members of the Marulius group (Pethiyagoda 1991; Schmidt 2001; Courtenay and Williams 2004; LGN *pers. obs.*; Figure 7). Although there is scant information on the subject, there are records of Marulius snakeheads being in the trade for over a hundred years. For instance, *Channa marulius* (*sensu lato*) is recorded as imported into Germany as an ornamental as early as 1906 (Novák et al. 2020). Writing for the trade magazine Tropical Fish Hobbyist, Schmidt (2001) described the rearing of “*Channa marulia*” in aquaria, emphasizing the species’ extremely aggressive behavior and rapid growth. Within their purported native range, *Channa marulius* (*sensu lato*) is listed among species important in the aquarium fish culture of Bangladesh (Siddiquee et al. 2015) and the taxa also appears on lists of fishes exported by the ornamental fish industry of India (e.g., Jayalal and Ramachandran 2012). Admittedly, mention of Marulius occurrence in the U.S. pet trade is poorly documented. Ross Socolof (1996: 195), the long-time Florida ornamental fish farmer, noted that "snakeheads" were part of tropical fish shipments from Singapore to dealers in New York city as early as about 1950. Unfortunately, Socolof (1996) did not report the species involved. Regardless, as with other aquarium fish that reach large sizes, release of snakeheads into the wild by a hobbyist may occur once the pet fish outgrows the home aquarium, especially given the tendency of larger Marulius snakeheads to attack and kill all or most tankmates (see Schmidt 2001).

Sport fishing

Within their native ranges in Asia and a few places where introduced, several of the larger snakehead species are popular as recreational and sport fishes and a few are known to attract anglers from around the world (Jaggs 2007; Love and Genovese 2019; Chocklett 2021). In India and Bangladesh, Marulius snakeheads have been described as a favorite or important sport fish (Talwar and Jhingran 1992; Siddiquee et al. 2015), although in Thailand they are not among local snakeheads most sought after by anglers (Jaggs 2007). In contrast, in South Florida *C. aurolineata* has achieved considerable attention from freshwater anglers due to its increased abundance and distribution, large size, and tendency to surface fight when hooked (Hart 2016). Indeed, local guides promote recreational fishing of the region's *C. aurolineata* by noting that there are no catch limits while claiming that anglers can catch as many as 15 snakeheads per outing, occasionally 40, in a single day (Isackson 2018). As part of government efforts to control snakehead populations, some state agencies in the United States encourage recreational harvest (Love and Genovese 2019). However, the increased popularity of snakeheads as a sport fish in the United States has raised concerns that such programs may also lead to unauthorized transplants (Courtenay and Williams 2004; Benson 2019). The state of Florida places no limits on the number of snakeheads an angler may take and captures are not to be released back into the water nor transported live.

Origin of the Manatee County population

How and why *C. aurolineata* was introduced to the MC Channa pond site is not known with certainty, but our morphological and genetic analyses indicate the population's likely source is the non-native snakehead population long-established (since ca. 2000) in SE Florida. Less likely sources are live-food markets or the aquarium trade since possession of live snakeheads has been illegal in Florida well over two decades. The possibility of natural dispersal is precluded because of the distance and lack of any direct hydrological connections between the two Florida sites (Figure 3). As such, the Manatee County population can be considered a separate introduction, although it is likely that humans, either directly or indirectly, transferred live individuals from SE Florida waters to Manatee County. It has been hypothesized that snakeheads present in U.S. waters likely resulted from the release of unwanted aquarium pets or of fish purchased at live food markets, and that motives behind such introductions perhaps involved a desire to establish a wild population that can then be harvested as food or for sport (Courtenay and Williams 2004; Snakehead Plan Development Committee 2014). Because non-native snakeheads in SE Florida have become a popular target for recreational anglers, there is a possibility that anglers transferred live *C. aurolineata* from that part of the state to the Manatee County area. It is also possible that introduction of snakeheads into some sites is attributable to religious ceremonies since that pathway has been proposed for other non-native fish species (Nico et al. 2019). However, the general intent of such religious practices is to set captive animals free, not to transfer animals already in the wild to new areas. The long prohibition in Florida against food markets or aquarium dealers from having live snakeheads also argues against the likelihood that religious practitioners would encounter captive snakeheads. Snakeheads are very hardy fish. In particular, because of their air-breathing ability they are capable of surviving transport in containers with little or no water (Innes 1966). Such characteristics also increases the likelihood that snakeheads moved and released into new habitats have a high chance of surviving transport and release.

Natural history, behavior, and potential impacts

Little or no biological information exists specific to *C. aurolineata* in its native range. The published literature on other members of the Marulius snakeheads is also relatively scant especially with regard to wild populations. The small amount of information available on Marulius snakehead ecology, reproduction, and natural diets are based primarily on studies of populations in India (e.g., Devaraj 1973) and a few other sites far outside the native range of *C. aurolineata*. FWC biologists have investigated the non-native *C. aurolineata* population of SE Florida, but results of that research have not yet been published except for a summary abstract (Gestring et al. 2019).

Snakeheads have been widely introduced around the world and their realized and potential ecological impacts have been discussed (Lever 1996; Courtenay and Williams 2004). Similar to the threats posed by other introduced piscivores, the focus tends to be on possible direct (e.g., predation) and indirect (e.g., competition) effects on native fish populations. As a predator and competitor, introduced snakeheads are of particular concern because of their large size and highly aggressive nature, their broad tolerance to a range of environmental conditions, reproductive potential, and high dispersal abilities. In Asia, the natural diets of Marulius snakeheads reportedly consist of fishes, frogs, tadpoles, snakes, and various invertebrates (Bhuiyan 1964; Rahman 2005). There are also numerous anecdotal reports of Marulius taking water birds and rodents (Pethiyagoda 1991). However, quantitative dietary studies are lacking. Gestring et al. (2019) briefly summarized the diet of *C. aurolineata* (reported as *C. marulius*) inhabiting SE Florida waters, noting that they preyed primarily on small fishes, crustaceans, and insects. The available information indicates that Marulius snakeheads, like other members of the family, are opportunistic feeders, consuming nearly any type of animal prey they encounter and are able to overcome.

Marulius snakeheads, similar to most channids, are highly proficient air breathers and able to tolerate hypoxic waters and to survive long periods of drought (Graham 1997). Several *Channa* species are known to conduct terrestrial excursions (Liem 1987; Lee and Ng 1991; Graham 1997; Courtenay and Williams 2004). The ability of introduced snakeheads to move over land, even if only under certain conditions (e.g., rainy periods), is concerning since their dispersal is not completely dependent on water routes. In addition, snakeheads traveling on land have the potential to invade naturally fishless, aquatic habitats and prey on native fauna. Our sighting of *C. aurolineata* feeding on adult frogs on land is of academic interest in part because, with a few exceptions (e.g., Liem 1987 reporting on *Clarias*), most descriptions of terrestrial feeding by air-breathing fishes are based on studies of animals in laboratory settings (Van Wassenbergh 2019). Snakeheads are similar to swamp eels of the family Synbranchidae, another group of invasive, air-breathing fishes (Nico et al. 2019). Like swamp eels, *C. aurolineata* (and other channids) have physiological and behavioral traits that make them ideal for international shipping and market survival and undoubtedly increase their chances of survival when transported and released by humans into novel environments. Swamp eels survive long periods out of water but only if their body is kept moist (Nico et al. 2019). In contrast, we have observed *C. aurolineata* to survive many hours out of water even in completely dry condition, likely because their body is protected by heavy and densely packed scales that slows dehydration.

Eradication efforts

Repeated use of electrofishing gear and nets on multiple occasions in late 2020 and early 2021 removed several hundred *C. aurolineata* from the MC Channa pond. These efforts substantially reduced but did not eliminate the pond population. Treatment of the entire pond with rotenone in May 2021 removed an additional 48 snakeheads. The eradication efforts were deemed a success, as no additional snakeheads have been sighted since the piscicide operation. Included among post-treatment assessments was an electrofishing survey of the pond conducted by FWC biologists on 11 January 2022.

However, verification of eradication will require periodic monitoring of the pond and entire watershed.

Potential geographic range

Although the *C. aurolineata* in Florida's Manatee County represented a reproducing population and was relatively abundant within the MC Channa pond, their apparent absence in any surrounding sites suggests that the species had been present only a few years, at most, prior to their discovery in 2020. Nevertheless, if not eradicated and given enough time, their continued existence would likely result in spread into other parts of the Manatee River system and beyond. Previous researchers have attempted to predict the potential non-native ranges of different snakehead taxa in North America using the ecological niche modeling algorithm GARP (genetic algorithm for rule-set prediction) (Herborg et al. 2007; Cudmore and Mandrak 2009). Their resulting maps depicting areas of high habitat match levels for "*Channa marulius*" predict a potential distribution that would include much of the far southern United States (i.e., Florida and portions of Georgia, Alabama, Mississippi, Louisiana, Texas, California, and Arizona), most of Mexico, and all of the Caribbean. However, such environment suitability models rely on available native geographic distribution data of the targeted species. Because the native distribution of *C. aurolineata* is a small portion of the overall Asian range of the entire Marulius group, their potential range in North America would be expected to be less using GARP analysis.

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Author's contribution

MEN – research conceptualization, investigation and data collection, data analysis and interpretation, writing-original draft, writing-review and editing; LGN – research conceptualization, sample design and methodology, field investigation and data collection, writing-original draft, writing-review and editing; RHR – investigation and data collection, data analysis and interpretation, writing-original draft; MK – research conceptualization, investigation and data collection; ZSR – investigation and data collection; EJ – field investigation and data collection, lead on rotenone project, writing-review; JMP – data collection, writing-review.

Ethics and permits

In general, sampling done with nets and by electrofishing was performed under the authority of permit number FNC-21-002. The rotenone operation was a project planned and overseen by fishery biologists with the Florida Fish and Wildlife Conservation Commission (FWC). The FWC is the Florida agency that issues scientific collector's permits for the state and its fishery biologists have blanket authority to sample fishes. Most snakeheads used in this study were captured during joint operations between the authors and FWC biologists. A few snakeheads collected in 2020 were obtained from a licensed fisher and those specimens were deposited and cataloged in the Florida Museum of Natural History. The authors, when participating in the capture and preservation of *Channa* and other fishes, followed animal care and use protocols described in IACUC number USGS/WARC/GNV 2018-08 and amendments.

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