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Author(s): Thomas M. Orrell and Lee Weigt
Source: Proceedings of the Biological Society of Washington, 118(2):407-415. 2005.
Published By: Biological Society of Washington
DOI: http://dx.doi.org/10.2988/0006-324X(2005)118[407:TNSCAA]2.0.CO;2
URL: http://www.bioone.org/doi/full/10.2988/0006-324X%282005%29118%5B407%3ATNSCAA%5D2.0.CO%3B2

PROCEEDINGS OF THE BIOLOGICAL SOCIETY OF WASHINGTON
118(2):407–415. 2005.

The Northern Snakehead *Channa argus* (Anabantomorpha: Channidae), a non-indigenous fish species in the Potomac River, U.S.A.

Thomas M. Orrell and Lee Weigt

(TMO) Smithsonian Institution, P.O. Box 37012, National Museum of Natural History, Washington, D.C. 20013-7012, e-mail: orrellt@si.edu;
(LW) Laboratories of Analytical Biology, Smithsonian Institution, Suitland, Maryland 20746, e-mail: lweigt@lab.si.edu

Abstract.—Mitochondrial sequence variation was examined in the northern snakehead, *Channa argus* (Cantor, 1842), a species of fish native to Asia and recently collected in the eastern United States. There are seven unique haplotypes in 29 specimens studied, with no haplotype shared between areas of introduction. One haplotype was shared by 15 individuals from the Potomac River system (both males and females <480 mm total length), indicating possible breeding within the system. A single large adult male from the Potomac River system has a unique haplotype. All Crofton, Maryland, specimens had one haplotype. Two haplotypes were found in Meadow Lake, FDR Park, Philadelphia, Pennsylvania. Unique haplotypes were found in Wheaton, Maryland, and Shrewsbury, Massachusetts. These results support the conclusion that there were several independent introductions of the northern snakehead into these waters, and that no two introductions came from the same maternal source.

In May 2004, a northern snakehead *Channa argus* (Cantor, 1842) was caught by an angler in Little Hunting Creek, a tributary to the Potomac River in Alexandria, Virginia, U.S.A. This was the first of many northern snakeheads captured from both Virginia (VA) and Maryland (MD) Potomac River tributaries between May and August 2004. These fish represent the first reported population of the non-indigenous *C. argus* in a major, temperate, North American waterway. In addition to those specimens taken from the Potomac River tributaries, a single specimen was taken from a pond in Wheaton, MD in April 2004, and three specimens were taken from Meadow Lake, FDR Park, Philadelphia, Pennsylvania (PA) in July 2004.

Channa argus is native to China, Manchuria, southern Siberia, and Korea and has been introduced in Japan, other areas of Asia, and eastern Europe (Courtenay and Williams 2004, Berra 2001). The northern snakehead is primarily known as a food fish and was regularly imported and sold live in the market, but it was not known to have been sold as an aquarium fish (Courtenay and Williams 2004). In the past decade, there have been reports of this species from open waters in California, Florida, and Massachusetts (MA), but there has been no evidence of established populations from these introductions (Courtenay and Williams 2004, Fuller et al. 1999, Hartel et al. 2002).

The northern snakehead is an obligate air breather; it utilizes a suprabranchial organ and a bifurcate ventral aorta that permits aquatic and aerial respiration (Ishimatsu and Itazaw 1981, Graham 1997). Young of this species may be able to move overland for short distances using wriggling motions

(Courtenay and Williams 2004). The preferred habitats of this species are stagnant water with mud substrate and aquatic vegetation, or slow muddy streams; it is primarily piscivorous but is known to eat crustaceans, other invertebrates, and amphibians (Okada 1960). The northern snakehead is a freshwater species and cannot tolerate salinities in excess of 10 parts per thousand (Courtenay and Williams 2004). The northern snakehead is capable of spawning more than once in a breeding season (Courtenay and Williams 2004) and builds spawning nests in aquatic vegetation. Females discharge eggs over the nest, which are externally fertilized by males (Okada 1960).

The first reported breeding population in the United States was discovered in a retention pond in Crofton, Anne Arundel County, MD, in May 2002; it was exterminated by the Maryland Department of Natural Resources in September 2002. Despite significant monitoring, no northern snakehead specimens have been reported from the Little Patuxent River, the drainage nearest the Crofton retention pond (Courtenay and Williams 2004).

It has been suggested that the source of the Potomac River population of *Channa argus* was the retention pond in Crofton, MD; people were seen dip-netting juveniles from the pond prior to the release of rotenone, an ichthyocide, in September 2002 (B. Wajda, Virginia Department of Game and Inland Fisheries, pers. comm.). If the source of the Potomac River specimens was the Crofton pond, it is unlikely, even given the limited ability of the species to move overland, that any individual could have moved from the Crofton pond to the locations of capture in the Potomac River system without human intervention. The Little Patuxent River drains into the Patuxent River, which flows directly into the Chesapeake Bay. Individuals would have to migrate from the Crofton pond to the Little Patuxent River, then enter the Chesapeake Bay and subsequently move to the Potomac River. However, the saline environment of the Chesapeake Bay would act as a barrier to migration. The alternative route to the Potomac River would have required *C. argus* to migrate from the Crofton pond to the Little Patuxent River and then to the Anacostia River drainage before reaching the Potomac River drainage. However, no *C. argus* has been reported from the Little Patuxent River. A single 493 mm female specimen was captured in Pine Lake, Wheaton Regional Park, MD, which has limited access to the Northwest Branch of the Anacostia River. Shortly after this specimen was caught, barrier screens were erected at both inflow and outflow areas of the pond. No other specimen of *C. argus* has been caught in Pine Lake and none has been found in the Anacostia River despite continued monitoring.

It is likely that these and other U.S. introductions are the result of deliberate or unintentional release. This paper addresses the following questions about *C. argus* using comparative DNA sequences: 1) are specimens caught in the Potomac River in 2004 putative offspring/siblings of those discovered in a Crofton, MD retention pond in 2002, and 2) are those in the Potomac River breeding?

Materials and Methods

Voucher specimens, where available, were saved and deposited at the National Museum of Natural History, Smithsonian Institution (USNM) or at the Museum of Comparative Zoology, Harvard University (MCZ). Collection data, museum catalogue and GenBank accession numbers are given in Table 1 and MD and VA collection locations are mapped in Fig. 1. Specimens were collected by anglers using tackle, or by fisheries biologists using electro-fishing gear or the ichthyocide rotenone, and nets from the following locations: Potomac River, VA and MD (n = 16, size range 327–634 mm TL); Crofton Pond, Crofton, MD (n = 8, size range 90–672 mm TL); FDR Park, Philadelphia, PA (n = 3, size range

Table 1.—Collection information, catalog and GenBank accession numbers.

Tissue No.	GenBank No.	Catalog No.	Collection data (length in mm = total length)
N0129	AY714750	USNM uncat	Potomac, Pohick Bay/Accotink (VA) 365 mm
N0775	AY714751	USNM 377165	Potomac, Marshall Hall (VA) 327 mm
N0776	AY714752	USNM 377166	Pine Lake, Wheaton Regional Park (MD) 493 mm female
N0777	AY714753	USNM 377167	Potomac, Little Hunting Cr (VA) 327 mm
N0778	AY714754	USNM 377168	Potomac, Occoquan (VA) 340 mm
N0779	AY714755	USNM 377169	Crofton Pond (MD) 104 mm juv.
N0780	AY714756	USNM 377170	Crofton Pond (MD) 105 mm juv.
N0781	AY714757	USNM 377171	Crofton Pond (MD) 90 mm juv.
No782	AY714758	USNM uncat	Potomac, Dogue Cr (VA) 363 mm
No783	AY714759	USNM uncat	Potomac, Little Hunting Cr (VA) 378 mm female—gravid
No784	AY714760	USNM uncat	Potomac, Kane Creek (VA) 450 mm
N0785	AY714761	USNM uncat	Potomac, Little Hunting Cr (VA) 634 mm male
N0786	AY714762	USNM uncat	Crofton Pond (MD) 545 mm
N0787	AY714763	USNM uncat	Crofton Pond (MD) 672 mm
N0788	AY714764	USNM uncat	Potomac, Dogue Cr (VA) 434 mm female
N0789	AY714765	USNM uncat	Potomac, Dogue Cr (VA) 418 mm female—gravid
N0790	AY714766	USNM uncat	Potomac, Dogue Cr (VA) 378 mm male
N0791	AY714767	USNM uncat	Potomac, Dogue Cr (VA) 412 mm
N0792	AY714768	USNM uncat	Potomac, Dogue Cr (VA) 452 mm female
N0793	AY714769	USNM uncat	Potomac, Pohick Bay (VA) 478 mm female
N0794	AY714770	USNM uncat	Crofton Pond (MD) 93 mm juv.
N0795	AY714771	USNM uncat	Crofton Pond (MD) 90 mm juv.
N0796	AY714772	USNM uncat	Crofton Pond (MD) 114 mm juv.
N0797	AY714773	USNM uncat	Potomac, Pomonkey Cr (MD) 455 mm
N0798	AY714774	USNM uncat	Potomac, Mattawoman Cr (MD) 432 mm
N0799	AY714775	MCZ 160176	Newton Pond, Shrewsbury (MA) 342 mm
N0800	AY714776	no voucher	Meadow Lake, FDR Park, Philadelphia (PA) 382 mm
N0801	AY714777	no voucher	Meadow Lake, FDR Park, Philadelphia (PA) 334 mm
N0802	AY714778	no voucher	Meadow Lake, FDR Park, Philadelphia (PA) 325 mm female—gravid

325–382 mm TL); Pine Lake, Wheaton, MD (n = 1, 493 mm TL); and Newton Pond, Shrewsbury, MA (n = 1, 342 mm TL).

All specimens were identified following the best available key to species (Courtenay et al. 2004). White muscle tissue was dissected from fresh or frozen samples and placed into a buffer solution of 0.25 M disodium ethylenediaminetetraacetate (EDTA), 20% dimethyl sulfoxide (DMSO), saturated sodium chloride (NaCl), at pH 8.0 (Seutin et al. 1990), and stored at room temperature. Total genomic DNA was isolated from approximately 0.05–0.1 g of tissue using phenol-chloroform extraction (Sambrook et al. 1989). PCR primers (SHF9: 5′-ATCGGACAAGTCGCTCTTTCCTCT-3′ and SHR2: 5′-TGCGGATACTTGCATGTGTAAGT-3′) targeted 1269 bp of the mitochondrial genome including 65 bp of 3′ end of cytochrome *b* the complete THR and PRO tRNAs, the complete control region (D-loop), complete PHE tRNA, and 39 bp of 5′ end of 12S RNA. PCR reactions included 1 ul of DNA template; 2 ul (10 uM) of each primer; 5 ul of diH_2O; 10 ul of Diamond Mix (Bioline USA, Inc; Randolph, MA). A DNA Engine Tetrad© (MJ Research; Waltham, MA) thermal-cycler was used for PCR amplification with the following cycle parameters: initial denaturation (94°C for 2 min); 35 cycles [denaturation (94°C for 30 sec); annealing (63°C for 15 sec); extension (72°C for 1 min)]; and final extension (72°C for 2 min). PCR purification was performed using 8 ul EXO-SAP (USB; Cleveland, OH) with a 15 min in-

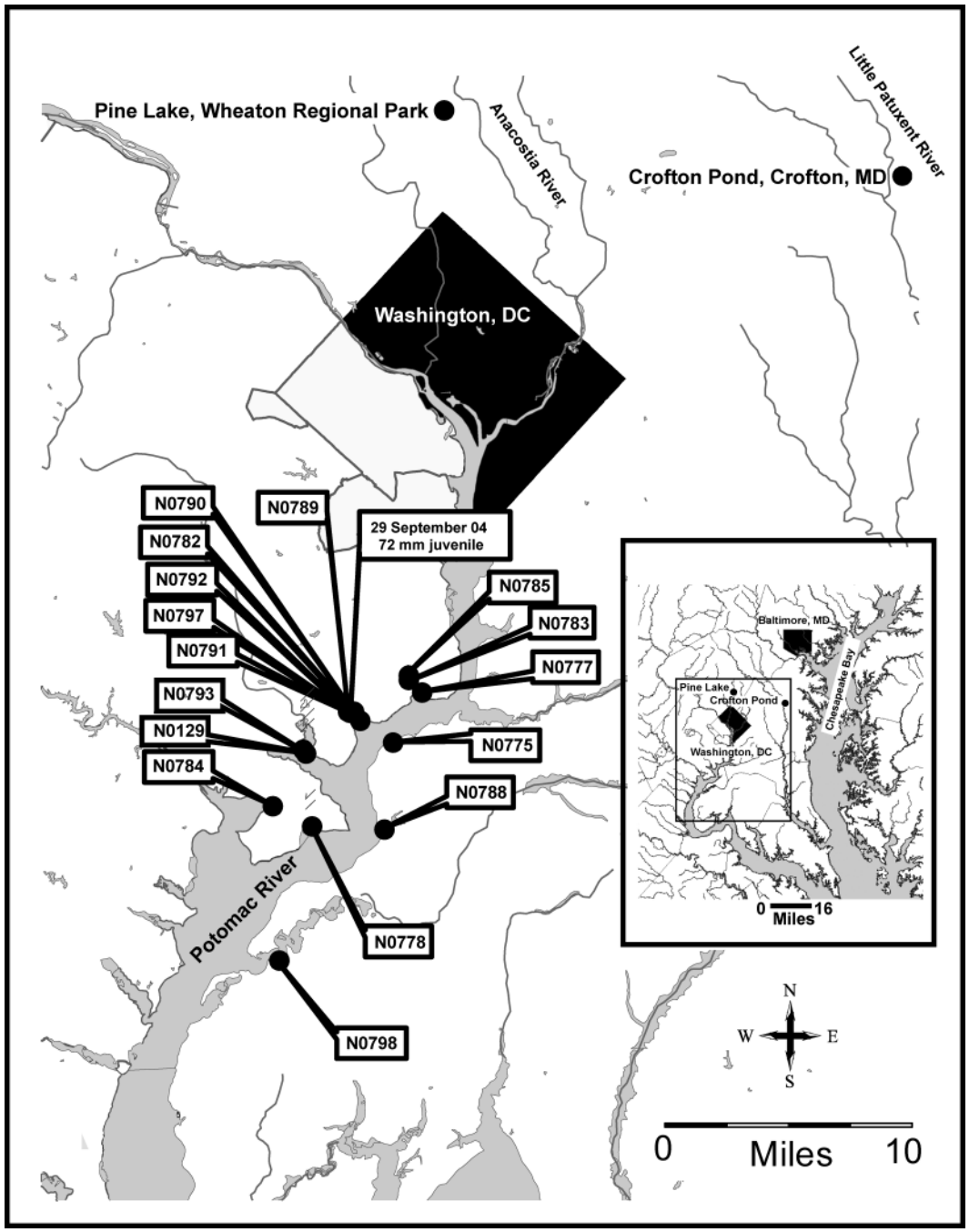

Fig. 1. Map of the Washington, D.C., area indicating the areas of collection of the Maryland and Virginia northern snakehead samples.

Haplotype	Nucleotide at position number									Area of Occurrence
	349*	418*	573*	668*	684*	687*	772*	1022*	1136**	
H1	C	C	T	G	T	T	A	G	G	Potomac, MD + VA
H2	A	C	T	G	C	T	A	G	A	Crofton, MD
H3	A	C	T	G	T	T	A	A	A	Wheaton, MD
H4	A	C	T	A	T	T	A	G	A	FDR Park, PA
H5	A	C	C	G	T	T	A	G	A	FDR Park, PA
H6	A	T	T	G	T	C	G	G	A	Potomac, VA
H7	A	C	T	G	T	C	G	G	A	Shrewsbury, MA

Fig. 2. Haplotypes, variable nucleotide positions, and areas of occurrence of haplotypes. Key: * = variable positions found in D-loop; ** variable position found in tRNA-Phe.

cubation at 37°C followed by deactivation at 80°C for 15 min. Purified PCR product (2 ul) was used in the cycle sequencing reactions with both the original sequencing primers and internal D-loop primers (SHF1: 5′-GGGGTT TCACAGAATGAAC TAT-3′ and SHR1: 5′-ATAGTTCATTCTGTGA-AACCCC-3′). Cycle sequencing reactions: 2 ul of DNA template; 2 ul of primer (10 uM); 2 ul of BigDye v3.1 (Applied Biosystems, Inc (ABI); Foster City, CA) ;10 ul of water; 4 ul of BigDye dilution buffer (5×). Cycle sequencing profile consisted of 35 cycles of denaturation (94°C for 30 sec), annealing (55°C for 15 sec) extension (60°C for 4 min). Cycle sequencing products were purified via Sephadex© G-50 column filtration in Multiscreen plates (Millipore: Billerica, MA) and run on an ABI 3100 DNA sequencer (50 cm or 80 cm array as per manufacturer's instructions). Sequences were imported, edited, trimmed and aligned with Sequencher (GeneCodes, Inc; Ann Arbor, MI) and exported as PAUP* (Swofford 2003) Nexus files. MEGA2 (Kumar et al. 2001) was used to compute p-distance (pairwise distances) and standard error and PAUP* was used to generate a maximum likelihood tree of genetic relationships. Modeltest, V.3.06 (Posada and Crandall 1998) was used to select the best-fit for maximum likelihood analysis.

Results and Discussion

Double stranded sequencing resulted in a 1164 base pair (bp) fragment that spanned 40 bp of the cytochrome *b* gene, complete tRNA-Thr (72 bp), tRNA-Pro (69 pb), D-loop (907 bp) and tRNA-Pre (69 pb), and 12S rDNA (6 bp). Sequences are available in GenBank (Accession Numbers AY714750 to AY714778). Comparisons of the resulting sequences yielded nine variable positions, eight in D-loop and one in tRNA-Phe (Fig. 2). These variable sites revealed seven haplotypes (H), with three (H1, H2 and H5) shared by more than one specimen. The greatest pairwise distance was between H1 and H6 ($p = 0.0043 \pm 0.0019$), both haplotypes from the Potomac River.

The hierarchical likelihood ratio test in Modeltest indicated that the Hasegawa-

Kishino-Yano model (Hasegawa et al. 1985) was the best-fit for the data (assumed nucleotide frequencies of A = 0.3348, C = 0.2343, G = 0.1692, T = 0.2617). A maximum likelihood heuristic search in PAUP* produced a single tree with a negative log likelihood score ($-\ln L$) of 1640.4 (Fig. 3). No haplotype was shared between areas. H1 was the haplotype for all Potomac River specimens (both males and females) less than 480 mm TL. H6 was found only in the largest Potomac River specimen, a 634 mm TL male. H2 was carried by all Crofton, MD, specimens. H5 was shared by two Meadow Lake, FDR Park, PA, specimens. Unique haplotypes were Wheaton, MD (H3), FDR Park, PA (H4), and Shrewsbury, MA (H7).

No haplotype was shared between the Potomac River and Crofton, MD, samples, which negates the hypothesis that the Crofton pond population was the source of the Potomac River introduction. Because only a single haplotype (H1) was found in all Potomac River specimens less than 480 mm TL (both males and females), it is likely that these fish are the offspring (cohorts) of either a single pair of breeding adults or the offspring from multiple adult female siblings. This breeding may have taken place in the Potomac River, but also could have occurred in vitro (aquarium), in an aquaculture pond, or in the wild, with subsequent release into the Potomac River. Although the large male from the Potomac River (N0785) had a unique haplotype (H6), it might still be related to the other Potomac River specimens. Because the mitochondrial genome is maternally inherited, it cannot be determined if N0785 was the sire to all or some of the Potomac River specimens that share H1. Future analysis with nuclear DNA markers (sequence or highly polymorphic microsatellites) might elucidate parentage. The genetic data reported here are consistent with the hypothesis that the Potomac River population is breeding, which is further supported by the capture of a 72 mm juvenile from Dogue Creek in Fairfax County, VA, on 29 September 2004. The Dogue Creek specimen is not included in this study, as it has not yet been processed.

The Wheaton, MD, specimen had a unique haplotype and is not closely related to Potomac River or Crofton, MD, fish. The presence of more than one haplotype in FDR Park, PA, indicates that the specimens captured there are offspring of more than one female, but nothing can be determined as to whether these specimens are from an established (breeding) population in Meadow Lake or if they represent the release of genetically distinct individuals into the lake.

The presence of seven unique haplotypes, with no haplotype shared between areas, supports the hypothesis that there were several independent introductions of the northern snakehead into these waters, and that no two introductions came from the same original parent source. An alternate hypothesis is that the northern snakehead has become established in east coast US rivers with sufficient time to diversify into new habitats and for fixed genetic differences to evolve between populations, explaining the lack of shared haplotypes between areas. The latter hypothesis is unlikely, as there is no record of the northern snakehead in east coast rivers prior to 2004. The most parsimonious explanation is that each introduction is a unique and recent event.

The impact of the northern snakehead in these waterways cannot yet be determined, but the species could threaten populations of other fishes and insects. Future monitoring of native species and ecosystems, combined with historical records of community structure, is needed to measure the impact. The northern snakehead is not the first exotic fish species to become established in the Potomac River system. Others include: flathead catfish, *Pylodictis olivaris* (Rafinesque, 1818); common carp, *Cyprinus carpio* Linnaeus, 1758; goldfish, *Carassius auratus* (Linnaeus, 1758); blue catfish, *Ictalu-*

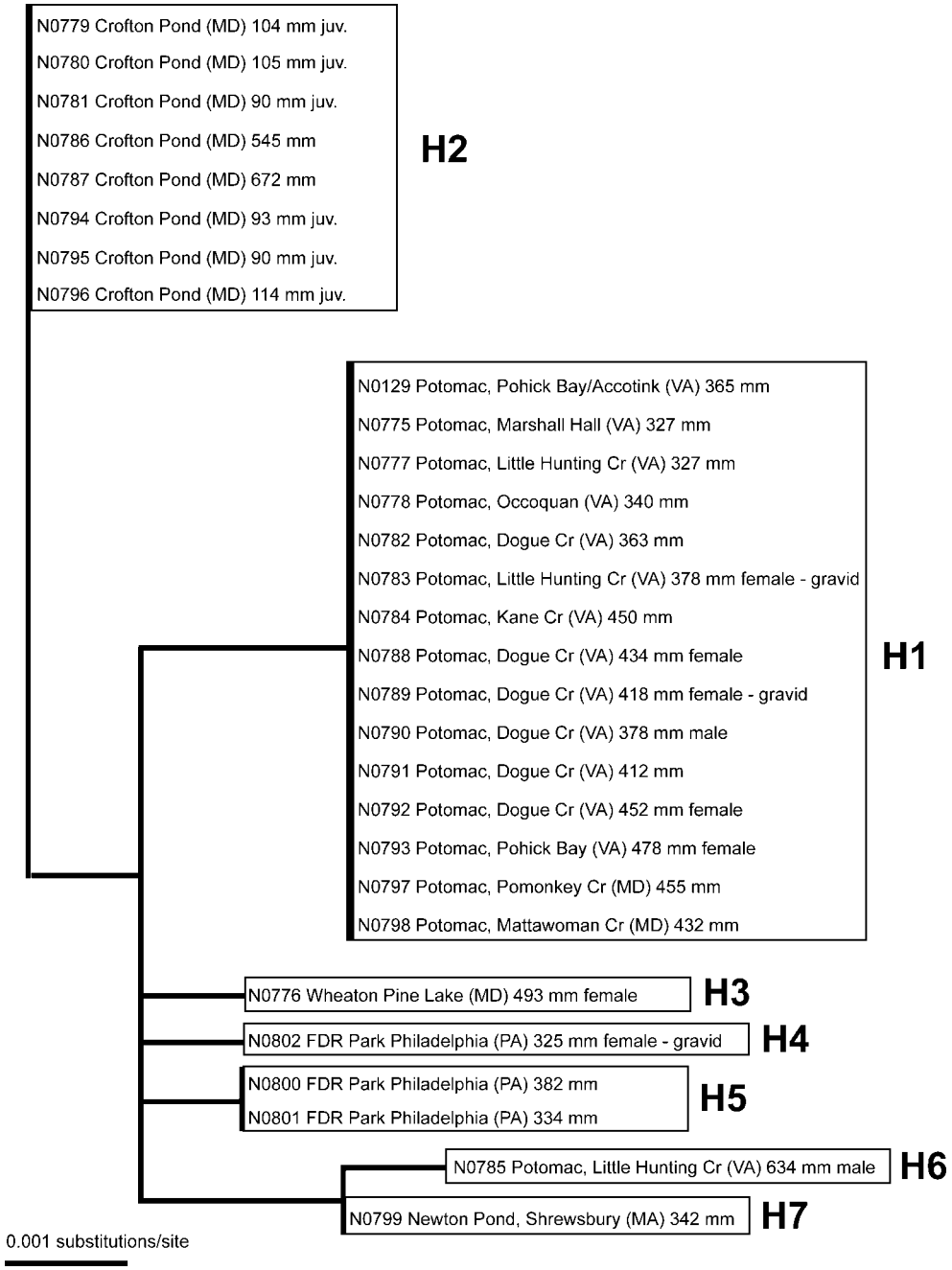

Fig. 3. Maximum likelihood tree (HKY model) of *Channa argus* mtDNA haplotypes. Tree is rooted to the haplotypes collected in Crofton, MD, 2002.

rus furcatus (Lesueur, 1840); and largemouth bass, *Micropterus salmoides* (Lacepède, 1802) (Fuller et al. 1999). The largemouth bass was introduced into the Potomac River in the 1800s and likely caused the extirpation of the native trout-perch *Percopsis omiscomaycus* (Walbaum, 1792) in the that river (Jenkins and Burkhead 1994).

These genetic data might provide a baseline by which to trace expansions should *C. argus* extend its range from the areas of initial introduction, although the specimens reported here may represent unique haplotypes that are now removed from the system. *Channa argus* is native to a latitude between 24 and 53°N, can survive a broad temperature range (0° to >30°C), and is capable of living under ice (Courtenay and Williams 2004, Okada 1960). If it becomes established in North America, *C. argus* could live as far north as Quebec, Canada, and spread throughout much of the contiguous United States. Alarmingly, as this paper was being prepared for submission, a 457 mm TL specimen was captured in mid-October 2004 in Chicago's Burnham Harbor, Lake Michigan.

In the future, we would like to expand our study to include samples of *C. argus* from across its native range and from aquaculture and aquarium samples to better understand the species' genetic diversity. In addition, we would like to include nuclear DNA markers, possibly to elucidate parentage.

Acknowledgments

We thank the Laboratories of Analytical Biology for financial and laboratory support; the VA Department of Game and Inland Fisheries (G. Martel, B. Wajda, M. DiLuigi, D. Watts, J. Odenkirk); the MD Department of Natural Resources (D. Cosden, S. Early, T. and M. Groves, B. Lundsford, R. Williams); the U.S. Geological Survey (W. Courtenay and J. Williams); the PA Fish and Boat Commission (B. Chikotas); MCZ (K. Hartel); and the MA Department of Fish and Wildlife (T. Richards).

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Associate Editor: Edward O. Murdy