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Genetic Characterization and Identification of Multiple Swamp Eel Introductions

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When invasions occur, the most fundamental questions that can be asked are: (1) How did these invasions occur? (2) What is the identity of the invader or invaders? (3) How many species are involved? (4) What is known about the factors that control the abundance and distribution of these species over their native range? These questions have not been answered satisfactorily in the case of recent Asian swamp or rice eel invasions. In the last decade, synbranchid eels, tentatively identified as belonging within the genus *Monopterus*, have been found in aquatic habitats in several regions of the southeastern United States (Fuller et al. 1999). Populations have been identified from near Atlanta, Georgia as well as Tampa, North Miami, and, most recently, Homestead, Florida, in close proximity to Everglades National Park. Swamp eels have many characteristics that make them potentially disruptive to native ecosystems and threatening to native species. They are aquatic predators. They are air breathers capable of dispersal over land, and able to survive for long periods out of water. They have high fecundity, and are protogynous hermaphrodites. These characteristics suggest that swamp eels have the potential to severely disrupt already threatened native aquatic communities.

Proper characterization of the ecological and evolutionary diversity of introduced swamp eel populations and identification of native source populations are the necessary first steps in developing control and management strategies. These data will indicate the nature and extent of management units within introduced swamp eels, and by understanding the factors controlling swamp eel distribution over their native range, allow us to predict potential range expansion of current populations. The conservation and biological control literature is filled with examples of difficulties resulting from failure to properly account for genetic diversity and variation of superficially similar taxa. In biological control, there are many examples of the introduction of incorrect or misidentified biotypes that are morphologically indistinguishable from the targeted species, but which differ in key traits from the correct biological control agent. This has led to the failure of efforts for biological control of pest insects in several well-documented cases (Gonzalez and Gilstrap 1992). In fishes, various misidentified species of tilapia have been introduced as food and game, often with negative impacts. One complication of control and management of tilapia has been difficulty in identification of species and the formation of hybrids with different or unknown environmental tolerances and feeding biology (Courtenay and Robins,1989; Fuller et al. 1999).
We have been pursuing a combination of molecular laboratory and field-based ecological studies in order to address these fundamental questions concerning the nature of swamp eel invasions: Are introduced swamp eel populations the result of a single event with several subsequent sub-introductions and/or dispersals, or do they result from independent introductions? What is (are) the source(s) of introduced populations? What

is the level of genetic diversity and genetic distinctness among these populations? What are the relationships among introduced and native populations?

The goals of this project were to determine the source populations of invasive swamp eels in the Southeastern United States and to look for evidence of hybridization among these introduced populations. Additional goals include archiving collections of these introduced populations early in their introduction, and the development of high-resolution markers that may be used to track pathways of introduction, from native source populations, through local sources to invasive populations. Finally basic distributional data and life history information, including feeding, reproduction, and growth were collected, as well as morphometric data to aid in discrimination of introduced populations. Below we present results for specific research objectives.

Objectives:

(1) To identify native source populations for introductions. We have generated over 200 sequences for a portion of the 16S gene from native populations, introduced populations, and market samples. We have also sequenced a portion of the 12S gene from all unique 16S haplotypes to enhance support for clades recovered. A preliminary phylogenetic tree from these data is below (Figure 1)

Significant results are as follows:

- The four known North American introduced populations of Synbranchid eels represent at least 3 separate introductions from differing native-range sources:
    - Tampa and North Miami populations from China.
    - Homestead population from SE Asia.
    - Atlanta population from Japan or Korea.
- The degree of genetic divergence (and morphological difference in the case of cuchia), and robust phylogenetic support for clades suggests that introduced U.S. populations represent at least three and possibly four or more species.
- Swamp eels from several of the major clades are being sold in U.S. markets
- One species, "*Monopterus" cuchia*, is being sold live in the U.S., but is not yet known to be introduced into nature. Phylogenetic analyses indicate that *Monopterus* is not a monophyletic group. The genus *Amphipnous* will need to be resurrected for cuchia and it's relatives. Morphological characters support this distinction.
- These distinctions are important because clades vary in life history traits relevant to environmental impact, management, and spread of invasive populations. For example, Schofield and Nico (unpublished data, 2003) have demonstrated that salinity tolerance varies significantly among swamp eel clades, with implications for the spread of these populations in Florida.
- Populations in close geographic proximity may represent separate introductions of genetically distinct forms. This has significant management implications.

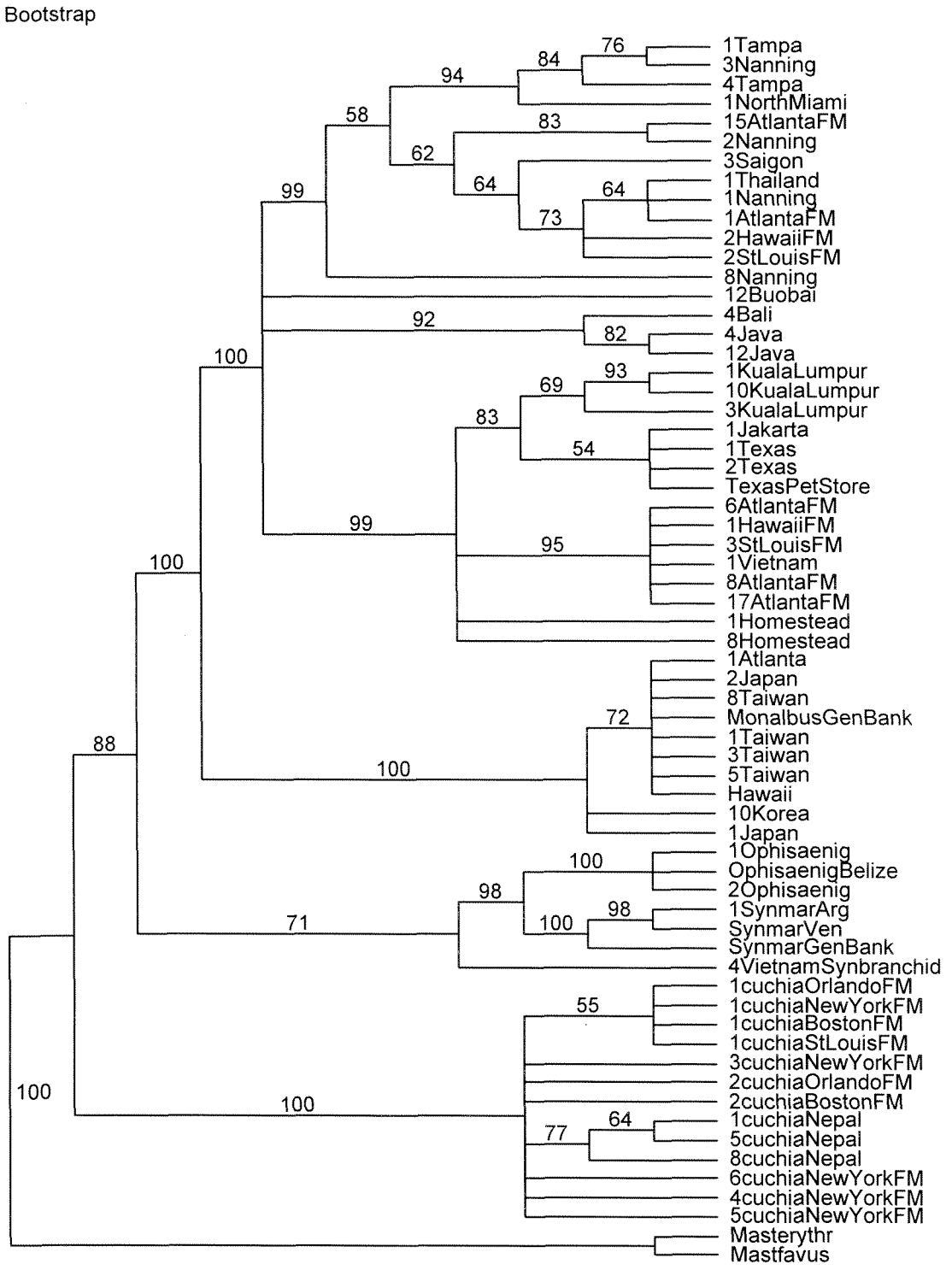

Figure 1 Bootstrap distance tree for 64 swamp eels sampled from native range, introduced, or market populations based on 1001 bases of 12S and 16S mitochondrial DNA. Market populations are identified by the suffix "FM". "Monopterus" cuchia specimens are labeled as "cuchia", *Ophisternon aenigmaticum* samples as "Ophisaenig", *Synbranchus marmoratus* as "Synmar". The unidentified Vietnam synbranchid is likely *Ophisternon bengalense*, but final determination is pending. Two mastacembelids, *Mastacembelus*

erythrotaenia, and *Mastacembelus favus* are used to root the tree. All other individuals are considered to be within *Monopterus*, and are labeled by locality.

(2) Determine with a nuclear marker whether there has been hybridization among the introduced lineages determined on the basis of mtDNA variation. We have generated sequence for two nuclear gene regions, having developed taxon-specific primers for the RAG2 gene and using previously-developed primers for the LDHA6 intron. The phylogenetic trees for these genes are largely congruent with the mitochondrial tree (Figure2). These data provide no evidence for hybridization among the major clades. Nuclear data also confirm that *Monopterus* is not monophyletic, and the distinctness of the "cuchia" clades, as well as the Atlanta/Japan/Korea clade.

(3) Archive samples which capture the genetic diversity of these early founding populations.

We have tissue samples of 50 to 100 individuals for each of the three Florida populations, and are continuing to arrange for a sample of this size for the Atlanta population. We have also archived samples from fish markets, and other commercial sources to document potential commercial pathways of introduction.

(4) Develop high-resolution genetic markers for *Monopterus*. We have developed 18 tetranucleotide microsatellite (GACA. GATA) loci for swamp eels. From these loci we have selected the 8 most promising loci (ease of scoring alleles, level of variation) for analyses of swamp eel populations with support from the ANS program.

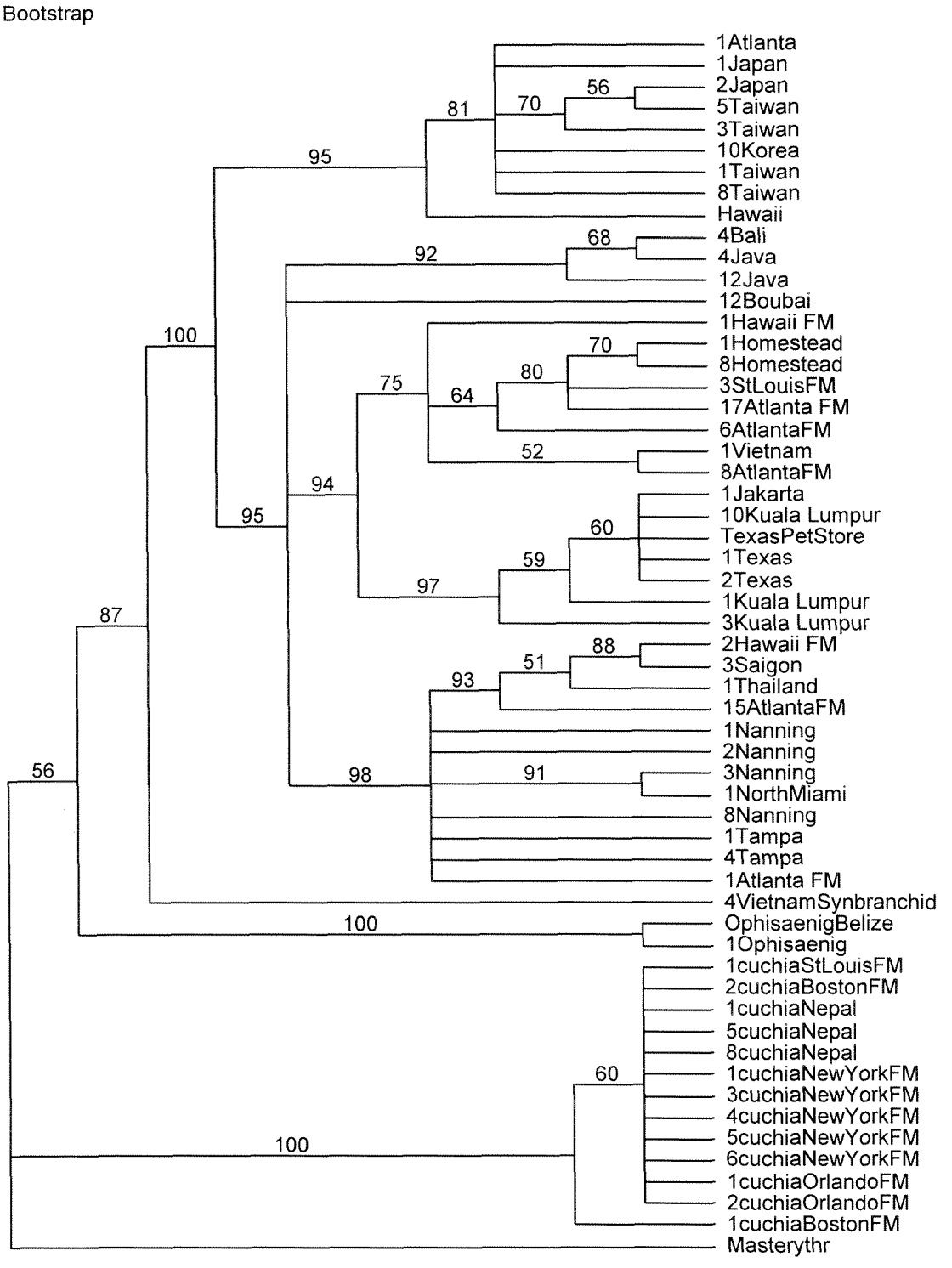

Figure 2 Bootstrap distance tree for 59 swamp eels sampled from native range, introduced, or market populations based on 1185 bases of the nuclear RAG2 gene. Labeling is as in figure 1. We have not yet generated RAG2 sequence for *Synbranchus marmoratus*. Note close correspondence to clades in figure 1.

(5) Field Sampling – During the period 2002-2003 we conducted additional field sampling of the Florida populations to better document existing eel distribution and to determine possible changes in their range. Sampling downstream of Structure S-18C on C-111 Canal revealed swamp eels adjacent to Everglades National Park. This area should be monitored closely by the National Park Service as it is the most likely location for eels to enter the Everglades ecosystem. Because of difficulties in obtaining scientific collecting permits from the state to sample in south Florida, much of our field work during the period was focused in the Tampa area where we did discover that the Tampa population was more widespread and more abundant than previously thought. To date, we have not sampled areas downstream from the salinity barrier of Snake Creek Canal. The delay is result of permit as well as logistic and equipment problems.

(6) Reproductive Phenology – We are in the process of analyzing length-frequency data of swamp eels from two of the introduced populations. As part of other ongoing projects, additional samples are being measured.

(7) Feeding Ecology – Recent research indicates there have been four introductions into southeastern US waters of Asian swamp eels (Genus: *Monopterus*), represented by three genetically distinct forms. Little information on the diets and predator-prey interactions of *Monopterus* eels is available from either the native or introduced ranges. Those data are needed to estimate ecosystem-level effects caused by eel introductions and to prepare science-based risk assessments. Presently, eels occur mainly in disturbed habitats such as canals, from which we collected samples across seasons by electrofishing. We have stockpiled large numbers of preserved eels and removed the gastro-intestinal tracts from several hundred preserved specimens for investigation of eel diets. We used gut analysis to determine relative contributions of prey in eel diets from the three known populations in Florida. We also used stable-isotope analysis of muscle tissue to estimate trophic positions of the eels and to corroborate gut-data results. We compared eel diets and trophic position with those of native fishes to determine the role of eels in the aquatic food web. Asian swamp eels are predators that feed on a variety of fishes, crustaceans, oligochaetes, and aquatic insects. Diet differences among the populations probably stem from prey differences at the sampling sites. Diet analyses from samples taken at different seasons are continuing.

(8) Age and Growth – As part of a related project, otoliths have been taken from a sample of >100 swamp eels from the North Miami population. Dr. Debra Murie, University of Florida, is assisting us in analyzing the otoliths. As part of our age validation work, in early 2001 we successfully recaptured three eels that had been tagged and chemically marked 12 months before. We did not have success in recapturing marked eels during sampling conducted in early 2002 and we did not sample the release area in 2003.

(9) Morphometric Analysis – To date, we have sufficient material to make progress on morphometric analysis of eels from introduced populations. To better understand and document variation in eel body and head shape and eel color patterns, we are taking several digital photographs (ventral, dorsal, and lateral views) of each eel in our

collection. One of us (LGN) will visit the Smithsonian Institute in Washington D.C. and make use of a new digital x-ray imaging device. A trip is planned for 2004. We investigated the possibility of purchasing digital x-ray imaging equipment for the USGS facility in Gainesville, but the cost was prohibitive (> $100,000).

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