Genetic Diversity in a Morphologically Conservative Invasive Taxon: Multiple Introductions of Swamp Eels to the Southeastern United States

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Abstract: *Genetic analysis of introduced populations, especially in morphologically conservative taxa, can clarify introduction histories, identify management units and source populations, provide a more realistic estimate of the frequency of successful invasion, and suggest strategies for preventing further introductions. In the last 7 years, populations of swamp eels, referred to the Asian genus* Monopterus *(Family Synbranchidae) on the basis of external morphology, have been discovered in aquatic habitats near Atlanta, Georgia; Tampa, Florida; North Miami, Florida; and most recently in close proximity to Everglades National Park in Homestead, Florida. Swamp eels are large predators capable of dispersal over land and have the potential to disrupt already threatened ecosystems. We analyzed mitochondrial DNA sequences from four known populations in the continental United States and samples from Malaysia, Indonesia, Vietnam, and two locations in China to determine introduction histories, source populations, genetic diversity, and relationships among populations. Our results indicate that there have been at least three independent introductions of genetically distinct forms. Introduced populations in close proximity (separated by <40 km) are genetically distinct. The level of sequence difference among introduced populations reaches levels seen among sister families of teleost fishes for the same region of the mitochondrial genome. These genetically distinct introduced populations in all likelihood represent at least two and possibly three species. Regardless of species status, these genetically distinct lineages may be expected to vary in ecological or life-history traits, representing different potential threats to the ecosystems where they have been introduced. Given the success of swamp eels in invading many habitats around the world, further study of these eels is warranted to elucidate the characteristics of successful invaders and invasions.*

Diversidad Genética en un Taxón Invasivo Morfológicamente Conservativo: Introducciones Múltiples de Anguila de Pantano en el Sureste de Estados Unidos

Resumen: *El análisis genético de poblaciones introducidas, especialmente en taxones morfológicamente conservativas, puede clarificar la historia de las introducciones, identificar unidades de manejo y poblaciones fuente, proporcionar una estimación más realista de la frecuencia de invasiones exitosas y sugerir estrategias para prevenir introducciones futuras. En los últimos 7 años, se han descubierto poblaciones de anguilas de pantano, referidas al género Asiático* Monopterus *(Familia Synbranchidae) con base en su morfología externa, en los hábitats acuáticos cerca de Atlanta, Georgia; Tampa, Florida; Miami Norte, Florida y más recientemente muy cerca del Parque Nacional Everglades en Homestead, Florida. Las anguilas de pantano son grandes depredadores capaces de dispersarse por tierra y tienen un potencial para alterar ecosistemas de por si amenazados. Analizamos secuencias de ADN mitocondrial de cuatro poblaciones conocidas en Estados Unidos y muestras de Malasia, Indonesia, Vietnam y dos localidades en China para determinar la historia de las introducciones, poblaciones fuente, diversidad genética y relaciones entre las poblaciones. Nuestros resultados*

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indican que ha habido por lo menos tres introducciones independientes de formas genéticamente distintas. Las poblaciones introducidas en sitios cercanos (separados por <40 km) son genéticamente distintos. La diferencia de secuencias entre poblaciones introducidas alcanza niveles vistos entre familias hermanas de peces teleósteos para la misma región del genoma mitocondrial. Muy probablemente, estas poblaciones introducidas y genéticamente diferentes representan por lo menos dos y posiblemente tres especies. Independientemente del estado de la especie, se puede esperar que estos linajes genéticamente distintos varíen en cuanto a características ecológicas y de historia de vida, representando diferentes amenazas potenciales para los ecosistemas en donde han sido introducidos. Debido al éxito de las anguilas de pantano al invadir muchos hábitats en el mundo, se requiere mayor estudio de estas anguilas para dilucidar las características de invasores exitosos e invasiones.

Introduction

When biological invasions occur, ecosystems may be disrupted and the persistence of indigenous species threatened. To gauge the effect of an invasion on indigenous species and ecosystems and to plan prevention of future invasions, the most fundamental questions that can be asked are (1) How did these invasions occur? (2) How many species are involved? (3) What is the identity of the invader or invaders? (4) Where did these species come from? (5) What is known about the factors that control the abundance and distribution of these species over their native range? Answers to these questions may go a long way toward predicting the effects of biological invasions, as well as suggesting prevention, control, and management strategies (Courtenay & Stauffer 1984; Fuller et al. 1999).

Determination of the ecological and evolutionary diversity of introduced populations and identification of native source populations are therefore the necessary first steps in characterizing a recent invasion. Information on genetic variation clarifies the nature and extent of management units among populations of invasive species and can test competing hypotheses about the nature of introductions. Source-population data gives us information on factors controlling the distribution of invasive species over their native range and allows us to estimate the potential range of expansion of introduced populations as well as potential controls on this expansion. Examples of difficulties resulting from failure to properly account for genetic diversity and variation of superficially similar taxa are plentiful in the literature. Incorrect or misidentified biotypes have been introduced that are morphologically indistinguishable from the proper biotype but that differ in key traits from the correct biological control agent, leading to failure of biological control of pest insects in well-documented cases (e.g., Gonzalez & Gilstrap 1992). In several cases, proper matching of the climate requirements of different host races of parasitioids to the site of introduction has been critical to successful biological control (Gonzalez et al. 1979; Diehl & Bush 1984). In fishes, various misidenti-

fied species of tilapia have been introduced as food, game, or biological control agents, often with negative effects (e.g., Courtenay & Hensely 1979). Likewise, difficulties with species identification and formation of interspecies hybrids with different or unknown environmental tolerances and feeding habits have complicated the control and management of tilapia (Courtenay & Robins 1989; Fuller et al. 1999). An increasing appreciation of the importance of taxonomy for understanding and managing invasive species is reflected in a draft strategy by the European Platform for Biodiversity Research, which identified extending systematics and updating taxonomic knowledge of invasive species as research topics of high priority (Scott 2001).

In the last decade, swamp or rice eels, tentatively identified as belonging within the synbranchid genus *Monopterus*, have been found in aquatic habitats in several regions of the southeastern United States (Fuller et al. 1999). Swamp eels have many characteristics that make them potentially disruptive to already threatened native ecosystems. They are large predators, with adults reaching nearly 1 m in length, and they feed on a variety of animals, including fish. Piscivores are more likely to alter freshwater fish communities (Moyle & Light 1996), especially in cases such as the Everglades where species number is low and environmental variability and severity are high. Many swamp eels are air breathers, able to survive for long periods out of water, and capable of dispersal over land (Liem 1987; Graham 1997). Swamp eels produce mucus copiously, preventing dessication, and burrow as water levels drop (Liem 1967), making them extremely tolerant of drought. Hydrologic fluctuations, which appear to be an important factor controlling introduced fish populations in the Everglades (Trexler et al. 2001), are therefore unlikely to limit populations of swamp eels.

Given their amphibious abilities, synbranchids are capable of becoming established in habitats beyond the range of most other large fishes. Thus, invading eels are a potential threat to normally isolated populations of small fishes, frogs, crayfishes, and other small aquatic organisms. In addition, introduced swamp eels may also

compete for food with native fishes and wading birds. Swamp eels are relatively secretive and possibly nocturnal, often living in burrows or associated with dense mats of aquatic vegetation, complicating surveys and eradication of populations. There is evidence that synbranchids exhibit parental care, and some populations include individuals that undergo sex reversal, changing from female to male (Liu 1944; Liem 1963; Matsumoto & Iwata 1997).

Swamp eels were first discovered in North America near Atlanta, Georgia, in 1994 (Fig. 1:1), although population size structure suggests that the population may have been first introduced circa 1990 (Starnes et al. 1998). In 1997, two populations were discovered in Florida, one in Manatee County, near Tampa, and the other in Miami-Dade and Broward counties in North Miami (Fig. 1: 2, 3). More recently in late 1999, a population was discovered in the Homestead area of Miami-Dade County, near Everglades National Park (ENP) (Fig 1:4). If the Homestead population had dispersed from North Miami, this would have entailed a rapid spread through 40 km of canals and water-control structures in a short period of time. Such rapid dispersal would suggest that there is little hope of containing or eradicating the population to prevent its spread to ENP. Field sampling, however, failed to recover eels from intervening canals.

Our initial question was, therefore, are the North Miami and Homestead populations genetically distinct? As sampling proceeded, we broadened the scope of our enquiry, using DNA sequence data to answer the following questions: Are introduced populations the result of a single event with subsequent "hopping" (sensu Kolar & Lodge 2001), 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?

Methods

Systematics and Distribution of Swamp Eels

The Synbranchidae is a family of eel-like percomorph fishes widely distributed throughout Central and South America, the Caribbean, tropical west Africa, and from the Indian subcontinent throughout Asia and the Indo-Australian archipelago to Australia. The native range of the genus *Monopterus* is restricted to the Old World and includes much of southern and eastern Asia, from the Indo-Malayan Archipelago, the East Indies, and Sulawesi, through Vietnam, Thailand, Laos, much of eastern China, and perhaps as far north as Vladivostok in far-eastern Russia. To the west it naturally occurs in Burma, India, Sri Lanka, Nepal, and Pakistan. One member of the genus
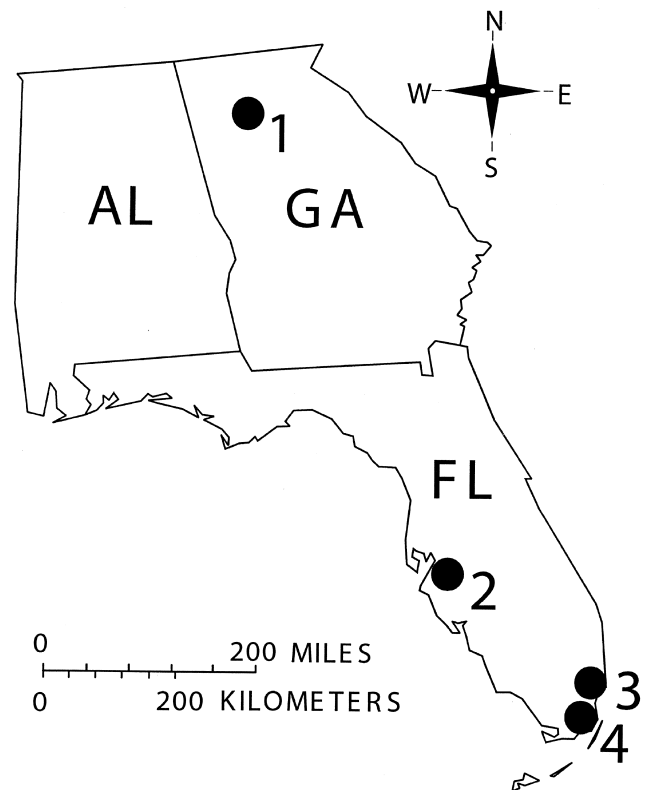

Figure 1. Locations of known swamp eel populations in the southeastern United States: Atlanta, Georgia (GA), 1; Tampa, Florida (FL), 2; North Miami, Florida, 3; Homestead, Florida, 4. See Table 1 for detailed locality information.

is also known from West Africa (Rosen & Greenwood 1976; Bailey & Gans 1998).

Based on the triangular shape and size of the external gill opening and other morphological characteristics, all populations introduced into the waters of the continental United States were initially assigned to *Monopterus albus*, commonly known as the Asian swamp eel, rice eel, or belut (Starnes et al. 1998; Fuller et al. 1999). The assignment is provisional, however, because of the lack of useful morphological characters to separate *M. albus* consistently from other members of the genus and the general confusion surrounding synbranchid taxonomy and systematics (e.g., Rosen & Greenwood 1976). In fact, because of its broad geographic distribution and reported regional morphological variation, *M. albus* is particularly problematic, and it is conceivable that what is referred to as *M. albus* actually is a species complex. *M. albus* naturally occurs in southern and eastern Asia.

Swamp eels have been introduced outside their native range in places other than the continental United States. It is unclear, however, whether populations along the periphery of synbranchid native ranges represent introductions. Swamp eels were introduced into the region

near Hami, Xinjiang Province, China, by the provincial army during the late nineteenth century Chin Dynasty (Wang et al. 1994). According to Matsumoto et al. (1998), *M. albus* was introduced to Nara Prefecture in Japan from Korea in the early 1900s. Some believe that the small population present in northeastern Australia is the result of an introduction (Merrick & Schmida 1984). Finally, a synbranchid identified as *M. albus* has become established on Oahu, Hawaii, from founding populations brought to the islands by Asian immigrants prior to 1900 (Brock 1960; Devick 1991).

Sampling, DNA Extraction, Amplification, and Sequencing

We sampled swamp eels from each of the four known localities in the continental United States, as well as China, Vietnam, the Malay Peninsula, and the East Indies (Table 1). Asian samples were either caught in the wild or obtained from local markets. *Synbranchus marmoratus* (family Synbranchidae) was sampled from Venezuela as an outgroup. Two of the swamp eels sampled from Vietnam were more closely related to *Synbranchus marmoratus* than to any other swamp eels sam-

pled and were therefore treated as additional outgroups (listed as unidentified synbranchid in Table 1). Specimens were either frozen or preserved in 70–95% ethanol. Voucher specimens for the United States populations are stored at the Florida Museum of Natural History, United States National Museum, and the North Carolina State Museum of Natural History.

We investigated relationships among populations of swamp eels by phylogenetic analysis of a portion of the 16S rRNA gene. This gene had been successfully employed for analyzing relationships from the level of populations to families and above (Palumbi 1996; Sullivan et al. 2000) and is therefore useful for investigating relationships of unknown phylogenetic depth. We extracted total genomic DNA from approximately 0.1 g of muscle tissue, following Collins et al. (1996). Polymerase chain reaction (PCR) amplifications were performed in 50 μL of a solution containing 10–100 ng of genomic DNA, 200 μM of each dNTP, 1 unit Taq Polymerase (Promega), 1.5 mM MgCL$_2$, each primer at 0.5 μM, and buffer supplied by the manufacturer. The primers, designed by Kocher and Palumbi (Palumbi 1996), amplify an approximately 615-bp portion at the 3′ end of the

Table 1. Localities sampled for swamp eels.

Locality*	Sample size	Taxonomic designation
Tampa, Florida		
Manatee County, Tampa Bay Basin, Frog Creek drainage, approxmately 27°35′N, 82°29′W	11	*Monopterus*
North Miami, Florida		
Miami-Dade County, Turnpike Pond, Miramar (*n* = 2), Snake Creek Canal, C-9 (*n = 8)	10	*Monopterus*
Homestead, Florida		
Miami-Dade County, SW 296th Street & 205th Avenue, 25°29.47′N, 80°31.46′W (*n* = 1), C-113 Canal (*n* = 9)	10	*Monopterus*
Atlanta, Georgia		
Fulton County, pond(s) of Chattahoochee Nature Center, south-southwest of Roswell, 34°00.3′N, 84°22.8′W	10	*Monopterus*
Nanning, China		
Guanxi Province, Nanning and Nanhu markets, approximately 22°48′N, 108°20′E	9	*Monopterus*
Buobai, China		
Guanxi Province, Buobai (or Bobai) county market, approximately 22°12′N, 109°52′E	2	*Monopterus*
Ca Mau, Vietnam		
Southwestern Vietnam, approximately 8°46′N, 104°59′E	4	*Monopterus*
Kuala Lumpur, Malaysia		
Selangor Province, market in town of Petaling Jaya, approximately 3°7′N, 101°42′E	10	*Monopterus*
Jakarta, Indonesia		
Senen market, Jakarta-Pusat (central Jakarta), approximately 6°11′S, 106°50′E	2	*Monopterus*
Ca Mau, Vietnam		
southwestern Vietnam, approximately 8°46′N, 104°59′E	2	unidentified synbranchid
Venezuela		
Apure State, Orinoco River Basin, Apure River drainage, Caño Caicara 07°33.51′N, 69°15.51′W	1	*Synbranchus marmoratus*

*Localities named for nearby major city, when possible, for ease of recognition.

16S (large subunit) mitochondrial ribosomal RNA gene. The PCR cycling parameters (MJ Research PTC 200, Watertown Massachusetts) for the initial double-stranded amplification were 94° C for 30 seconds, 47°–49° C for 30 seconds, and 72° C for 45 seconds, repeated for 37 cycles. Free dNTPs and unincorporated dye-labeled nucleotides and primers were removed with the GeneClean kit (Bio 101 Company, Vista California). The PCR products were quantified on a Dynaquant fluorometer (Pharmacia Biotech Inc., Piscataway, New Jersey). Cycle sequencing of 30–50 ng of the double-stranded PCR product was carried out with each primer and Big Dye terminators following the manufacturer's recommendation (PE ABI, Foster City, California). Sequences were determined on an ABI 377 automated DNA sequencer using 48-cm well-to-read plates.

To increase geographic and taxonomic sampling, we also attempted to determine sequences from formalin-fixed, ethanol-preserved museum specimens of swamp eels. We extracted DNA with a protocol developed for formalin-fixed tissue (Chase et al. 1998). Tissue samples were analyzed from *Monopterus albus* (University of Michigan Museum of Zoology numbers UMMZ 232761 and UMMZ 194392) collected in 1995 and 1969, respectively, and *Monopterus cuchia* (UMMZ 208372) collected in 1977. These extractions yielded DNA fragments up to 250–500 bp in length, based on agarose electrophoresis and subsequent staining with ethidium bromide. We were, however, unable to amplify a product over a range of conditions with the 16S primers mentioned above. We therefore designed internal primers that amplified 250–375 base-pair regions of 16S. These primer pairs produced PCR products that unfortunately proved to be contaminants. Further optimization will be required to produce results for this gene region in formalin-fixed specimens of swamp eel.

Data Analysis

We aligned sequences with CLUSTAL W (Thompson et al. 1994) at the following settings: gap opening cost 10, gap extension 2.5, and transition-transversion bias. We subsequently examined alignments by eye to locate errors resulting from pairwise progressive alignment methods. We investigated relationships among mitochondrial haplotypes with maximum parsimony (MP) and minimum evolution (ME) in PAUP* 4.0 (Swofford 2000). We carried out a branch-and-bound search of all unique haplotype-locality combinations using parsimony informative characters, with all characters unordered and equally weighted. Gaps were treated as missing data. We based our parsimony bootstrap proportions on 100 replicates of branch-and-bound searches including all unique haplotype-locality combinations. We also carried out a heuristic search with all 71 sequences to illustrate levels of within-population haplotype variation.

We tested for base compositional equilibrium across taxa using a chi-square test implemented in PAUP* 4.0 (Swofford 2000). Patterns of nucleotide substitution mapped onto the set of most parsimonious trees (MacClade 4.0; Maddison & Maddison 2000) indicated an excess of transitions relative to transversions and different rates of change for the two types of transversion. Thus, Tamura-Nei distances based on all characters were used for distance analyses. Minimum-evolution bootstrap trees were based on 100 replicates, with starting trees obtained via random-sequence addition, 10 random-sequence additions per replicate, and tree bisection-reconnection branch swapping.

Results

The 71 synbranchid eels sequenced for a portion of the 16S mitochondrial rRNA gene yielded 18 unique haplotype-locality combinations. After removal of primer regions, these sequences varied in length from 556 to 578 base pairs (average 575). Sequences have been deposited in GenBank under the accession numbers AF512841–AF512858. Base composition did not vary significantly across taxa, with average base frequencies of 23% A, 21% C, 26% G, and 30% T. Using the parameters described above, we obtained an alignment of 586 positions. This alignment had an average of only 2.5 gaps per sequence, with the majority of these resulting from indels in *Synbranchus* and the unidentified Vietnamese synbranchid relative to the remaining taxa.

Introduced populations had low levels of within-population haplotype diversity and small haplotype-to-haplotype distances (Table 2; Fig. 2). The Atlanta and North Miami populations were represented by single haplotypes, whereas the Tampa population had two haplotypes that differed by a single substitution. The Homestead population also contained two haplotypes, these differing pairwise by four nucleotide substitutions (patristic distance was six).

Nucleotide-sequence divergence varied widely among populations. One haplotype found in the Tampa population was identical to the North Miami haplotype, and the other Tampa haplotype differed from North Miami by a single nucleotide substitution. No haplotypes were shared among the remaining introduced populations. Uncorrected percent sequence difference among other introduced populations ranged from 3.5% to >10% (Table 2).

A branch-and-bound parsimony search of the 18 unique haplotype-locality combinations with informative characters yielded 24 most-parsimonious trees of 128 steps with ensemble retention indices of 0.870. This tree indicated strong bootstrap support for a clade of haplotypes from Tampa, North Miami, and Nanning, China (clade C; Fig. 3). A second well-supported clade was composed of Homestead, Vietnamese, Indonesian,

Table 2. Percent sequence difference matrix for pairwise comparisons among the 18 unique 16S rRNA haplotype-locality combinations of swamp eels.

Haplotype	Haplotype distance matrix number																
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*
1Tampa	—																
3Nanning	0.17	—															
4Tampa	0.17	0.17	—														
1NorthMiami	0.17	0.17	0.00	—													
2Nanning	0.52	0.52	0.35	0.35	—												
8Nanning	0.87	0.87	0.70	0.70	0.35	—											
1Nanning	0.70	0.70	0.52	0.52	0.17	0.52	—										
11Buobai	2.96	2.96	2.78	2.78	2.44	2.78	2.26	—									
1Homestead	4.01	4.01	3.84	3.84	3.49	3.49	3.67	3.32	—								
1Jakarta	3.31	3.31	3.14	3.14	2.96	2.96	3.14	3.14	0.53	—							
1Vietnam	3.48	3.48	3.30	3.30	2.96	2.96	3.13	3.13	0.52	0.35	—						
8Homestead	3.65	3.65	3.48	3.48	3.13	3.13	3.30	3.30	0.70	0.87	0.52	—					
1KualaLumpur	4.17	4.17	4.00	4.00	3.65	3.65	3.83	3.48	1.22	0.70	1.04	1.57	—				
10KualaLumpur	4.00	4.00	3.83	3.83	3.48	3.48	3.65	3.30	1.05	0.52	0.87	1.39	0.17	—			
3KualaLumpur	4.01	4.01	3.84	3.84	3.49	3.49	3.66	2.96	0.70	0.52	0.52	1.05	0.52	0.35	—		
1Atlanta	10.17	10.17	9.99	9.99	9.81	9.81	9.99	10.35	10.18	10.34	10.69	10.52	10.51	10.34	10.35	—	
Unidentified synbranchid	17.01	17.01	16.83	16.83	16.83	16.83	17.00	17.18	16.69	16.50	16.83	17.00	16.29	16.47	16.86	14.66	—
Synbranchus	17.06	17.05	16.87	16.87	16.87	17.06	17.06	16.52	17.64	17.66	17.79	17.79	17.06	17.24	17.64	15.18	12.22

and Malaysian haplotypes (clade B). These two clades formed a third well-supported clade, along with the Buobai haplotype. This clade was in turn the sister group to the Atlanta haplotype (clade A). The unidentified synbranchid was basal to this clade, and, when additional percomorph fish sequences were added to the analysis (data not shown), was a sister to *Synbranchus marmoratus* (Fig. 3).

Discussion

Introduction Histories and Invasibility

Improving our understanding of the "epidemiology of invasions" (Mack et al. 2000) is critical if we are to control current invasions and, perhaps more significantly, prevent future invasions. Genetic data have much to offer in this regard, indicating, for example, the number of independent invasions and the geographic distribution of distinct invading populations (Bastrop et al. 1998; Kreiser et al. 2001). In addition, the nature of population expansion of invading species may be elucidated by population genetic studies (Wilson et al. 1999). When genetic sampling includes potential native populations and local source populations (e.g., pet stores, food markets), precise pathways of introduction may be determined with important lessons for preventing future introductions.

In the case of the swamp eels, the geographic distribution and phylogenetic relationships among haplotypes indicate that there have been at least three introductions

to North America from distinct source populations. The North Miami and Tampa populations possess closely related haplotypes and therefore could be derived from a common source. Additional data must be collected to determine whether these populations represent independent introductions or a single introduction and subsequent spread or transplant of individuals from the initial site. The Tampa and North Miami populations are genetically similar to haplotypes sampled from Nanning, indicating southern China as a potential source. The population from Homestead is nested within a clade of haplotypes sampled from Indochina, the Malay Peninsula, and the East Indies, identifying this broad region as a source. The Homestead population is clearly distinct from the North Miami population and is not part of a contiguous population extending from North Miami to Homestead. It is thus a recent introduction of limited distribution, making it a promising candidate, although by no means guaranteed, for successful eradication (Mack et al. 2000; Myers et al. 2000). Based in part on the results of our study, a program aimed at eradicating or controlling the Homestead swamp eel population is being pursued (J. Curnutt, personal communication). The Atlanta population is phylogenetically distinct from all sampled populations, and its source could not be determined from the available data.

Another important goal in studies of invasive species is the identification of probable invaders (Kolar & Lodge 2001) to prevent their introduction. The prevention of invasions is both more likely to succeed and more cost-effective than extirpation of established populations (Mack
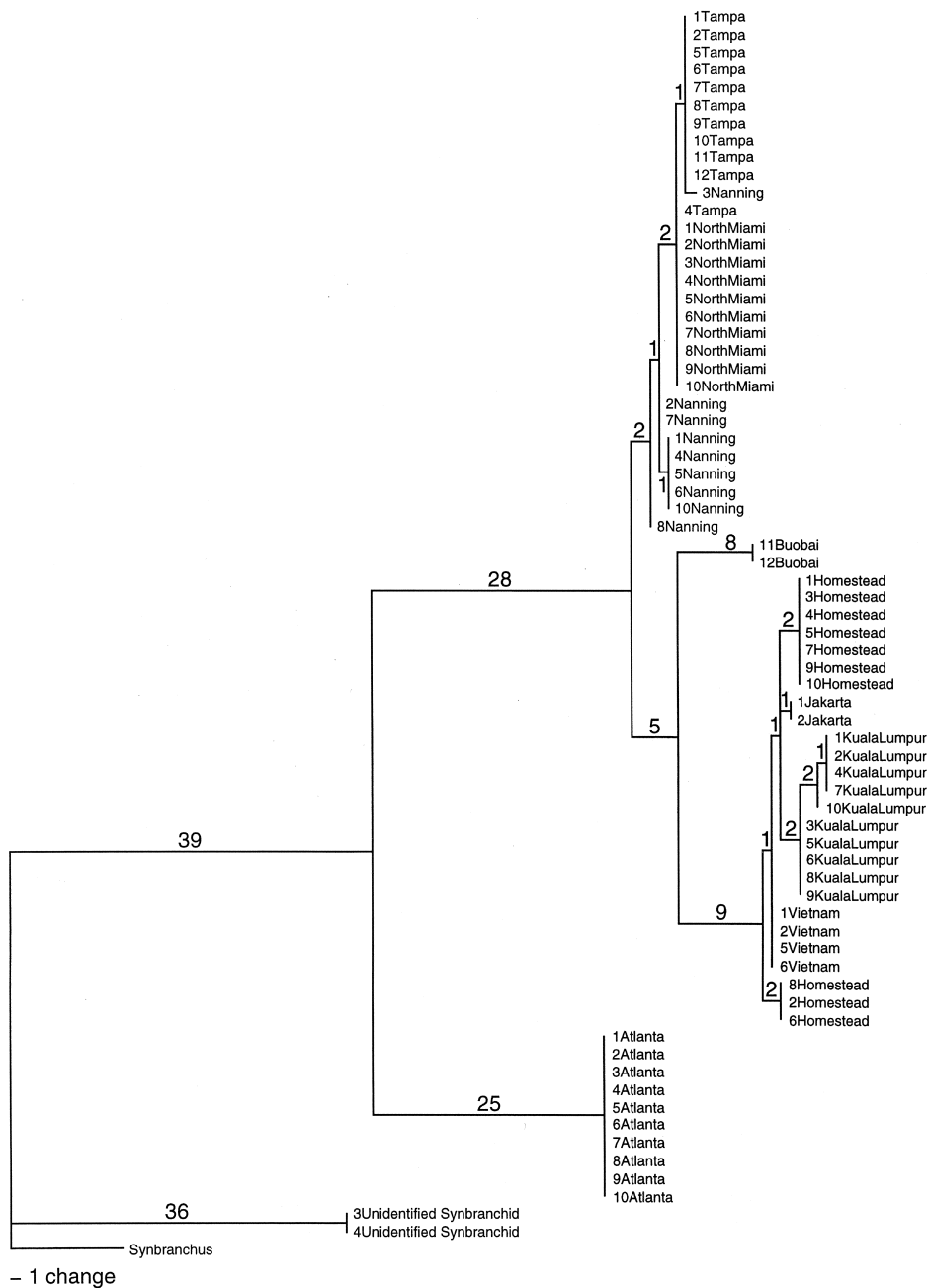
Figure 2. One of 210 most-parsimonious trees resulting from a heuristic search of parsimony-informative characters for the portion of the 16S gene sequenced from all swamp eels sampled. Branch lengths are proportional to the number of nucleotide substitutions. Branch lengths are also given above internal branches. Numerals preceding locality names are within-locality sample-identification numbers. The ensemble retention index for characters on this tree is 0.976.

et al. 2000). Our study and published distributional data indicate that swamp eels have successfully invaded new habitats around the globe a minimum of six to eight times, depending on whether the Japanese and Australian occurrences are considered indigenous or nonindigenous. Swamp eels must therefore be added to the small list of taxa such as the European starling (*Sturnus vulgaris*), black rat (*Rattus rattus*), lantana (*Lantana camara*), and water hyacinth (*Eichhornia crassipes*) that are guilty of "winning repeatedly in a high stakes lottery" of successful invasion (Mack et al. 2000). The occurrence of four successful introductions of swamp eels to the southeastern United States in 10 years is not, how-

ever, entirely consistent with the view of invasion success as a rare stochastic event. One explanation for this discrepancy is that the number of invasion events is much greater than is currently recognized and that rates of invasion are routinely underestimated in the literature. This may be especially true in meta-analyses, in which, for example, populations of swamp eels in Florida and Georgia could be most parsimoniously explained as resulting from a single introduction.

An alternative explanation is that some characteristic of swamp eel invasions may predispose them to success. Unfortunately, it has proven difficult to discern clear correspondences between species traits, environmental
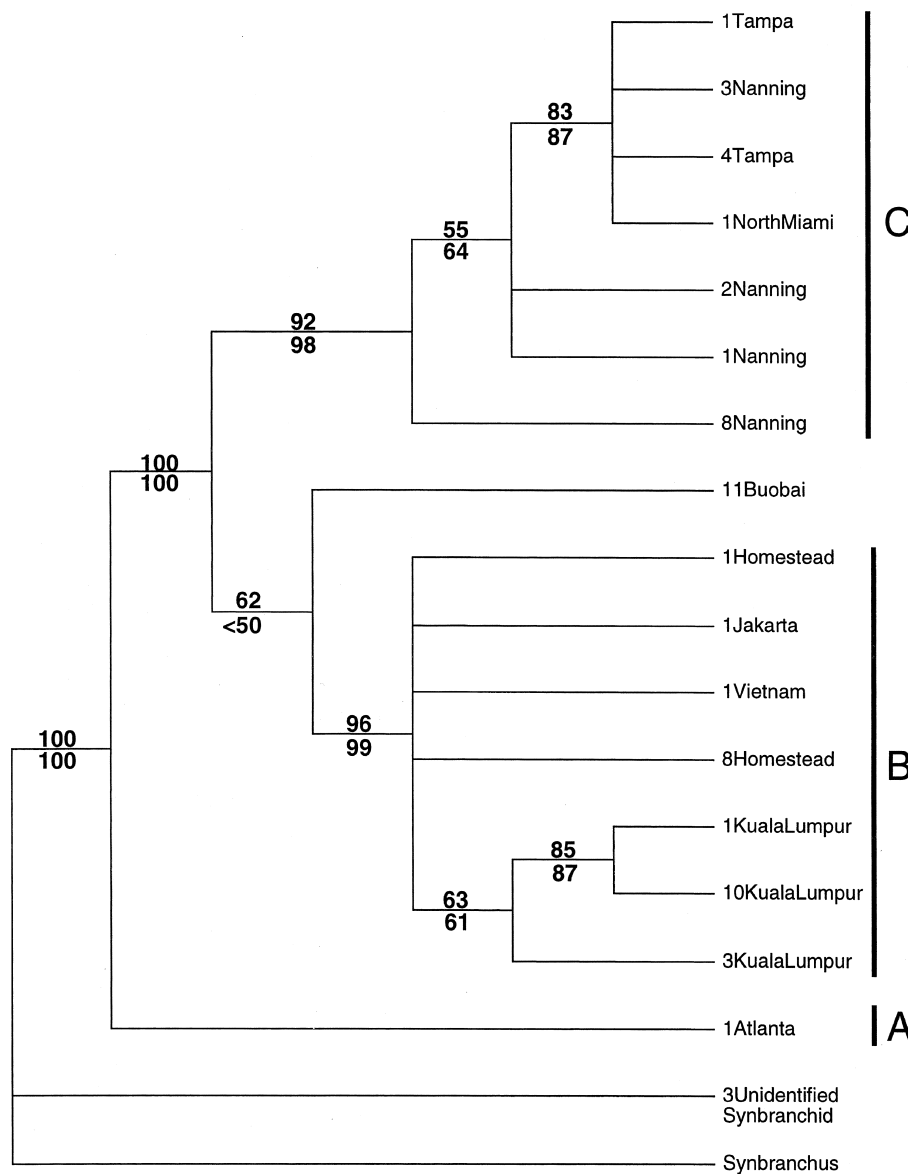
Figure 3. Strict consensus of the 24 most parsimonious trees resulting from a branch-and-bound search of parsimony-informative characters for the portion of the 16S gene sequenced from all unique swamp-eel haplotype-locality combinations. The ensemble retention index for characters on these 24 trees is 0.870. Bootstrap proportions are given above (parsimony) and below (minimum evolution) branches. Numerals preceding locality names are within-locality sample-identification numbers. Three clades with strong bootstrap support are labeled A, B, and C.

parameters, dispersal mechanisms, and the likelihood of invasion (Mack et al. 2000; Kolar & Lodge 2001). Whether the success of swamp eels is due to some innate properties of the clade, the fit between swamp eel biology and habitats in the southeastern United States, or the advantage of human-mediated dispersal remains to be determined. Molecular analyses may help sort out these alternatives because correct taxonomic apportionment and clarification of introduction histories may give a more accurate estimate of the numbers of invasions and the correspondence of traits to frequency of invasion.

It is not possible at this point to specify the agents of these independent introductions. Swamp eels have been sold at aquarium stores in Atlanta (Starnes et al. 1998), suggesting that Atlanta introduction may have resulted

from an aquarium release. At least one Florida introduction is in close proximity to a formerly active fish farm. Finally, swamp eel is a popular food item in many Asian cuisines, favored for its fine-textured meat and commonly sold in local markets.

Distinctness of Introduced Populations, Genetic Diversity, and Taxonomic Status: Implications for Management

Genetic diversity of introduced populations may give important clues to the size of the founding populations. In addition, populations with limited genetic diversity may be more susceptible to biological control than genetically diverse populations (Nissen et al. 1995). Genetic diversity within each of the four introduced populations

of swamp eel was generally low, as would be expected of populations that were likely founded from a small number of individuals. Although the Homestead population possessed only two haplotypes, these differed by four nucleotide substitutions, approaching the maximum within-population haplotype pairwise distance (5, Nanning) found among "native" samples. Genetic distance among introduced populations was remarkably high, with the Atlanta populations differing by >10% in uncorrected pairwise distance from the other North American populations. The difference between the A clade and the B and C clades exceeds that seen among intergeneric sister taxa in selected comparisons and is within the range seen between sister families of teleost fishes (Fig. 4). Moreover, it exceeds the amount of sequence difference seen among three pairs of snook species (family Centropomidae) inferred to have been isolated by the final emergence of the Isthmus of Panama approximately 3.5 million years ago (Donaldson & Wilson 1999; Tringali et al. 1999). The sequence difference between the B and C clades surpasses that seen among some of the snook geminates and ranges to the high end of the comparisons among intrageneric sister species analyzed (Fig. 4). The level of genetic difference among these populations suggests that they are unlikely to belong to a single species.

Species may, of course, vary in traits relevant to their ability to invade new habitats as well as in their effects on habitats and native species within those habitats. Unfortunately, the taxonomy and classification of swamp eels are problematic, primarily because of the simple external morphology of the family. The difficulties have been succinctly summarized by Rosen and Greenwood (1976): "Few groups of teleostean fishes have had so long and obscure a taxonomic history as the swamp eels . . . these fishes have a virtually featureless superficial anatomy and are rather variable in the very few external characters that can be measured or described precisely." The situation is particularly confusing within the genus *Monopterus*. Intraspecific variation and geographic ranges are not well characterized. Two named species, for example, are known only from holotypes, and Rosen and Greenwood (1976) suspect that several species may ultimately be found within the range of putatively broadly distributed taxa. It is therefore not surprising that it has proven difficult to assign the forms introduced into the southeastern United States with confidence to one or more species with proper binomials. Starnes et al. (1998) identified eels taken from near Atlanta as *Monopterus albus.* According to W. C. Starnes (personal communication), that determination was made according to the work of Rosen and Greenwood (1976) and was based on gill aperture, jaw morphology, and overall appearance. Those same characters were the basis for the tentative assignment of all Florida specimens to *M. albus* (Fuller et al. 1999).
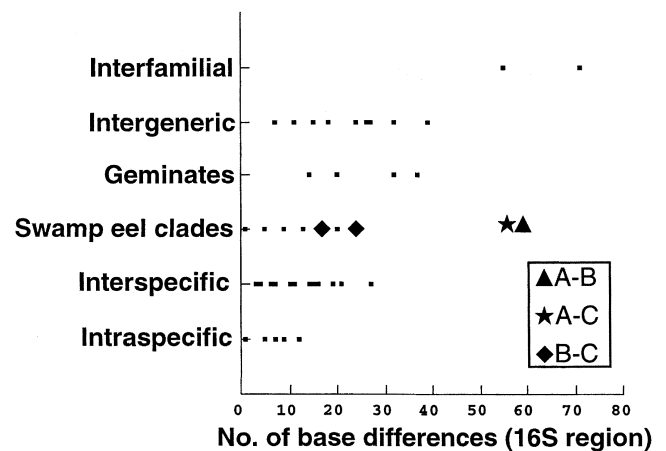

Figure 4. A comparison of the numbers of base differences between pairs of swamp-eel 16S haplotypes to numbers of differences over the same gene region in other pairs of fish taxa at a variety of taxonomic levels. Comparisons range from intraspecific to interfamilial. Interspecific comparisons are between terminal sister taxa (pairs of most closely related living species). The A, B, and C refer to clades of swamp eels from Fig. 3. Other swamp eel comparisons are within-clade and between clades B and C and the Buobai clade. Geminates refers to geminate species of snook thought to have been isolated by the emergence of the Isthmus of Panama 3.5 million years ago (Tringali et al. 1999). One of the geminates has speciated following the geminate split, so there are four comparisons for the three geminate pairs. Other data are from Duvernell and Aspinwall (1995), Simons and Mayden (1999), and Sullivan et al. (2000).

The proper generic and specific names that apply to the species being called *Monopterus albus* and the number of species that may ultimately prove to reside under this binomial are, however, in doubt. The binomials *Fluta alba* (Zuiew)(= *Monopterus albus*) and *Monopterus javanensis* Lacépède have also been used for what many authors consider a single species, although Rosen and Greenwood (1976) believe at least two species may be involved, one in China and another with a more southerly distribution, including Java. In his publication on the fishes of China, Nichols (1943) noted that "*Fluta alba*" specimens from south China were differentiable from other Chinese material he examined. The type specimen of *Monopterus javanensis* is from Java, whereas the type specimen of *Fluta alba* is presumed to be from Asiatic Russia. If these forms represent different species, it is possible that clade B, including specimens from Java, may include part or all of *Monopterus javanensis.* Clade C, including specimens from mainland China, might represent all of part of *Monopterus albus.* The type specimens on which these names are based are no

longer in existence, however, making a conclusive resolution unlikely. We have not sampled any individuals over the native range of *Monopterus* that are similar in mtDNA sequence to the Atlanta population.

In summary, the difficulties with synbranchid taxonomy and classification are profound and beyond the scope of this paper. For reasons we discuss below, however, it is imperative that the substantial variation recognized on the basis of mtDNA sequences not be obscured by grouping these forms under a single name, or simply as *Monopterus* spp. Until the taxonomy and classification of this group are satisfactorily resolved, we suggest that the introduced forms be known informally as *Monopterus* clade A, *Monopterus* clade B, and *Monopterus* clade C.

How many species are represented in these introductions? The answer depends on one's species concept. A phylogenetic species concept could certainly be applied to the distinct lineages shown in Fig. 3, but from the perspective of management of genetically distinct invasive populations, a biological species concept emphasizing the limits of gene exchange seems appropriate. We currently have no information on the potential for interbreeding among these populations. The admittedly approximate yardstick of degree of difference among introduced populations relative to recognized species (Fig. 4) suggests that, at a minimum, clade A is a distinct species. Clades B and C are more different over the sampled region of the 16S gene than many distinct species, including some thought to have been isolated for 3.5 million years. In addition, they are geographically separated over their native range based on current sampling. Of course, many more than three species may prove to be represented by the specimens sampled for this study once swamp eels are fully characterized over their native range.

The number of species diagnosed is ultimately less important than the recognition that these genetically distinct forms may vary in traits that will influence the effect of their introduction on native species and ecosystems. Just as biologists recognize evolutionarily significant units to conserve the genetic diversity of endangered or threatened indigenous species (Moritz 1994), those studying biotic invasions need to recognize distinct evolutionary lineages of invading taxa, evolutionarily significant invasion units (ESIUs). Swamp eels are conservative in external morphology but vary in other significant ways, including salinity tolerance from fresh to hypersaline waters, and habitat from clear flowing streams to sluggish or standing water with dense vegetation; they are cavernicolous and epigean (Tyler & Feller 1996; Bailey & Gans 1998). They vary in the degree of development of the lung-like suprapharyngeal diverticula (Rosen & Greenwood 1976), which affects the efficiency of aerial respiration and hence their ability to disperse over land and to tolerate low-oxygen conditions. Swamp eels vary in sex ratio during development, with some species or populations producing primary males and females and others producing only primary females that transform as adults to males (Liem 1968; Chan et al. 1972). Finally, swamp eels have a range from Japan and Russia south to Australia, and therefore will likely vary in temperature tolerance.

Variation in these traits has two important implications for conservation. First, these variations will affect the ability of introduced populations to persist in an area or undergo range expansion. For this reason, the ability to relate introduced populations to populations over the native range of *Monopterus* will be advantageous. Knowledge of the ecology and life history of these eels in their native habitat, and information on geographic range, temperature tolerance, predators, pathogens, and other checks on population size and growth may prove useful for management, control, and estimation of possible range expansion of introduced populations. Second, results of studies of the ecology, climatic tolerances, life-history traits, or susceptibility to biological controls of one introduced population should not be assumed to apply, in part or whole, to other introduced populations. For example, because numerous introduced species in Florida are from the tropics, temperature tolerance appears to be the most important factor controlling the demography of many invasive fish species in the state (Shafland & Pestrak 1982). Populations of the Mayan cichlid (*Cichlasoma urophthalmus*) exemplify this tendency, growing during typical years, only to crash precipitously during extreme cold events (Trexler et al. 2001). Based on the fact that the Atlanta populations of introduced swamp eels overwinter at latitude 34°N, one might conclude that cold events will have little effect on swamp eel populations in southern Florida. Because the Atlanta population represents a distinct lineage, however, its climatic tolerance may be irrelevant to the survival of introduced populations in peninsular Florida. The ecology of each genetically distinct population of swamp eels must be studied independently.

An important caveat to our study is that we have characterized these populations for a single maternally inherited marker. Because these introductions have likely resulted from the aquarium trade or the use of swamp eels as food items, we cannot rule out the possibility that there may have been some hybridization among populations of swamp eels characterized by distinct mtDNA lineages. This could occur by mixing of populations while in captivity, resulting in hybrid offspring. The absence of mixing of the major clades in any of the introduced or market-purchased populations argues against this possibility, as does the congruence of clades to broad-scale geographic distribution, with a southern clade B and a northern clade C. Nevertheless, these lineages should be characterized for one or more nuclear markers to determine definitively whether there has been any recent mixing of these lineages in captivity.

Our results suggest that genetic characterization of multiple populations of a putative invasive species, and identification of source populations, may provide data critical to the proper control, management, and determination of the frequency and nature of introductions, especially in the case of morphologically conservative taxa. The costs of these analyses have declined with technological advances and should no longer be considered extravagant or required only in exceptional circumstances. Analysis of genetic variation should be a routine part of an integrated approach to the prevention, control, and management of invasive species. Swamp eels have invaded habitats around the globe, suggesting that further investigation is merited to elucidate the characteristics of successful invaders and invasions.

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Note Added in Proof

Seiji Matsumoto kindly provided us with several samples of *Monopterus* from Nara Prefecture in Japan. These individuals are identical to the Atlanta haplotype for the 16S region we analyzed in this paper (unpublished data). This indicates Japan or Korea as the most likely source of the Atlanta population.

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