

Genetic Diversity of Invasive Species in the Great Lakes Versus Their Eurasian Source Populations: Insights for Risk Analysis

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Combining DNA variation data and risk assessment procedures offers important diagnostic and monitoring tools for evaluating the relative success of exotic species invasions. Risk assessment may allow us to understand how the numbers of founding individuals, genetic variants, population sources, and introduction events affect successful establishment and spread. This is particularly important in habitats that are “hotbeds” for invasive species—such as the North American Great Lakes. This study compares genetic variability and its application to risk assessment within and among three Eurasian groups and five species that successfully invaded the Great Lakes during the mid 1980s through early 1990s; including zebra and quagga mussels, round and tubenose gobies, and the ruffe. DNA sequences are compared from exotic and native populations in order to evaluate the role of genetic diversity in invasions. Close relatives are also examined, since they often invade in concert and several are saline tolerant and are likely to spread to North American estuaries. Results show that very high genetic diversity characterizes the invasions of all five species, indicating that they were founded by very large numbers of propagules and underwent no founder effects. Genetic evidence points to multiple invasion sources for both dreissenid and goby species, which appears related to especially rapid spread and widespread colonization success in a variety of habitats. In contrast, results show that the ruffe population in the Great Lakes originated from a single founding population source from the Elbe River drainage. Both the Great Lakes and the Elbe River populations of ruffe have similar genetic diversity levels—showing no founder effect, as in the other invasive species. In conclusion, high genetic variability, large numbers of founders, and multiple founding sources likely significantly contribute to the risk of an exotic species introduction’s success and persistence.

KEY WORDS: Founder effect; Great Lakes; nonindigenous species; population genetics; risk assessment

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1. INTRODUCTION

The genetic character of a nonindigenous species introduction is regarded as fundamental to its invasive success^(1–5) and provides important data for comparative environmental risk assessment. There are three basic components to the use of genetic information as part of a risk assessment involving invasive populations. The first component involves estimating the probability of critical events that are likely to lead to the success of invasive populations. Pertinent genetic

data that may assist in this estimation include the level of genetic variability, the genotypic composition, number of individuals and genotypes introduced, and number and variety of founding sources. These factors are the focus of the present study. Once the probabilities of the underlying “risk events” are identified, to complete a risk assessment, it is necessary to: (1) estimate the probability of those events; and (2) evaluate the impacts or consequences, in terms of losses (to the native ecosystem, other species, and the environment) given the likelihood of the event (i.e., the establishment, spread, and persistence of the invasive population). The combination of the probability of the event and the consequential damage should the event occur is a measure of the “risk” and, if done quantitatively, can provide actual probabilities. The results of such a risk assessment, even if only qualitative, are extremely valuable in risk management to analyze protective actions that could be implemented, in this case, to prevent the occurrence, establishment, and/or spread of an invasion.

1.1. Genetic Analysis of Exotic Species in the Great Lakes

The North American Great Lakes have been ecologically restructured by waves of invaders that were accidentally introduced from ships’ ballast water, with several high-impact introductions clustering during the mid 1980s to early 1990s—including the dreissenid mussel, neogobiin gobies, and ruffe invasions examined here. The Great Lakes have been a “hotbed” for invasive species—presumably due to high shipping traffic, a history of pollution and ecological disturbance, the presence of open niches in an ecologically young system dating to the Pleistocene Ice Ages, facilitative interactions by co-evolved invaders—such as the predator-prey relationship between the dreissenids and the round goby—and a donor-recipient pathway from the Ponto-Caspian region.^(1,6–8)

In this study, we compare the population genetic and systematic relationships of five exotic species invasions in the Great Lakes, including: (1) dreissenid mussels—the zebra mussel *Dreissena polymorpha* and the quagga mussel *D. bugensis*, (2) neogobiin fishes—the round goby *Apollonia* (formerly *Neogobius*) *melanostomus* and the freshwater tubenose goby *Proterorhinus semilunaris* (name changed with division from the marine tubenose goby *P. marmoratus* per Stepien and Tumeo⁽⁹⁾ and Neilson and Stepien (in progress)), and (3) the ruffe percid fish *Gymnocephalus cernuus*. All these invasions occurred during

the mid 1980s to early 1990s from Eurasian source populations having phylogenetic species origins in the Ponto-Caspian region.^(1,9–12) All have been successful at establishment and spread, with *D. polymorpha* and *A. melanostomus* being the most widespread and numerous. Conventional genetic theory^(2,13) predicts that founding populations, such as these, would have markedly lower genetic variability than contained in the source populations, due to few genotypes being introduced—comprising a central hypothesis surmising a “founder effect” that is critically examined here. We analyze a common suite of genetic characters that may characterize and/or differentiate these five invasions and explore their application to risk assessment.

1.2. Genetic Characteristics of Exotic Invasions

Ecological analyses reveal that successful exotic species often display a broad range of environmental tolerance, such as to a variety of salinity and temperate regimes, possess a history of invasiveness in other areas, and grow and reproduce rapidly.⁽¹⁴⁾ A large number of introduced individuals and high genetic variability are predicted to increase the risk of establishment, spread, and adaptation to new habitats^(1,2,15,16)—hypotheses that are critically examined here. Similarly, temporal and spatial waves of introductions originating from multiple founding sources may fuel the genetic diversity of an invasion, enhancing its ability to adapt to new and changing environments. Ballast water may be exchanged by ships in a variety of ports, mixing the gene pools of different founding sources, and introducing a unique combination of genotypes to new areas.

However, it is likely that most invasions are founded by very small populations having limited gene pools due to the difficulty of surviving transport, encountering favorable environmental conditions, securing habitat and resources, surviving the stress of new competitors and predators, finding a mate, and successfully reproducing. Typically, new populations thus would be likely to show marked founder effects (low genetic variability compared to source population areas)—a central hypothesis examined in the present study by comparing the genetic characters and diversity of exotic versus native populations. Low genetic variability in new populations from founder effects would be especially apparent in the mitochondrial DNA genome due to its smaller effective population size, more rapid extinction of lineages, and lack of recombination.⁽¹⁷⁾ Here, we analyze results from both

mtDNA and nuclear DNA analyses in a comparative approach and discuss their relation to risk assessment.

2. MATERIALS AND METHODS

Species examined, samples, sampling site coordinates, and types of genetic data analyzed are indicated in Table I, with North American and Eurasian sampling locations shown on the maps in Figs. 1 and 2. The present study focuses on comparing levels of genetic variation within and among five invasive species in the Great Lakes versus their native and invasive populations in Eurasia, and is part of larger ongoing separate analyses of the extent of variation within each species by our Great Lakes Genetics Laboratory. Previous results for the five species (dreissenids,^(1,11,12) neogobiins,^(9,18) and ruffe^(10,19)) here are compared with new genetic databases for each, offering increased resolution power for understanding the role of genetic variation in invasions. Previously collected data are re-analyzed here using a common format and computer programs in order to facilitate the comparisons.

2.1. Samples and Genes Examined for Dreissenid Mussels

The population genetic characters of the dreissenids zebra mussel *Dreissena polymorpha* and quagga mussel *D. bugensis* are compared from North American and Eurasian samples using mitochondrial DNA (mtDNA) cytochrome *b* gene sequences, as well as previously collected data for nuclear RAPDs (Randomly Amplified Polymorphic DNA) variation (originally reported in Reference 1). In the earlier nuclear DNA RAPDs study, genetic variation of 280 zebra mussel samples were analyzed for 63 putative RAPDs loci.⁽¹⁾ Those results are compared with mtDNA cytochrome *b* gene sequence data from 188 individuals, including 111 samples from throughout their North American invasive range and 77 from Eurasian locations (Table I; Figs. 1 and 2). New sampling locations include those collected during a research expedition by C. Stepien around the northern Black Sea.

Variation in quagga mussels *D. bugensis* originally were analyzed using 52 nuclear RAPDs loci for 136 individuals, including 111 from locations in the lower Great Lakes region and 25 individuals from the central Dnieper River.⁽¹⁾ The earlier study lacked the range of Eurasian samples available for the more recent investigation. The present study also utilizes new mtDNA cytochrome *b* sequence data from 78 individuals, including 49 from the lower Great

Lakes and 29 representing their present Eurasian range (encompassing recent invasion locations in the Caspian Sea and Volga River).⁽²⁰⁾ In addition, sequences from the closely related saline form *D. rostriformis* are compared with *D. bugensis*, since the former are predicted to invade North American estuarine systems.⁽¹²⁾ Samples of the remaining species in the genus—*D. stankovici*—are included in order to interpret patterns of intra- versus interspecific variation in dreissenids. Phylogenetic trees include the sister genera to *Dreissena* (*Congerina* and *Mytilopsis*) as outgroups (for systematic details see References 11, 12, and 21).

2.2. Populations and Genes Analyzed for Neogobiin Gobies

Genetic diversity in the round goby *Apollonia* (formerly *Neogobius*—see Reference 9) *melanostomus* originally was surveyed using sequences from the left domain of the mtDNA control region for 75 individuals, including 35 samples from a native Eurasian site (western Black Sea at Varna, Bulgaria) and an invasive Eurasian location (Gulf of Gdansk, Poland), as well as 40 samples from five sites in the lower Great Lakes (reported in Reference 18). Those findings are compared here with new results from sequencing the mtDNA cytochrome *b* gene, supplemented with data reported by Dougherty, Moore, and Ram.⁽²²⁾ We survey variation among 110 individuals, including 59 from Eurasian locations and 51 samples from the lower Great Lakes (Table I).

The present study analyzes variation in mtDNA cytochrome *b* gene sequences for 20 samples of the freshwater tubenose goby *Proterorhinus semilunaris* from the lower Great Lakes and rivers draining into the Black Sea, in comparison with 22 representatives of its sister species the marine tubenose goby *P. marmoratus*. Recent results⁽⁹⁾ demonstrate that the freshwater and marine clades of this taxon are separate species, and the historic name *P. semilunaris* thus has been resurrected for the freshwater clade. In another previous study,⁽¹⁸⁾ 12 samples of *P. semilunaris* were sequenced from the Lake St. Clair region for the mtDNA control region (when Eurasian samples were unavailable). The present article compares results of the previous work with newly collected data.

2.3. Samples and Genes Tested for Ruffe

Samples analyzed for the Eurasian ruffe *Gymnocephalus cernuus* encompass 120 individuals from

Table 1. Taxa Examined, Collection Locations, and DNA Data Analyzed (Refer to Maps of Figs. 1 and 2)

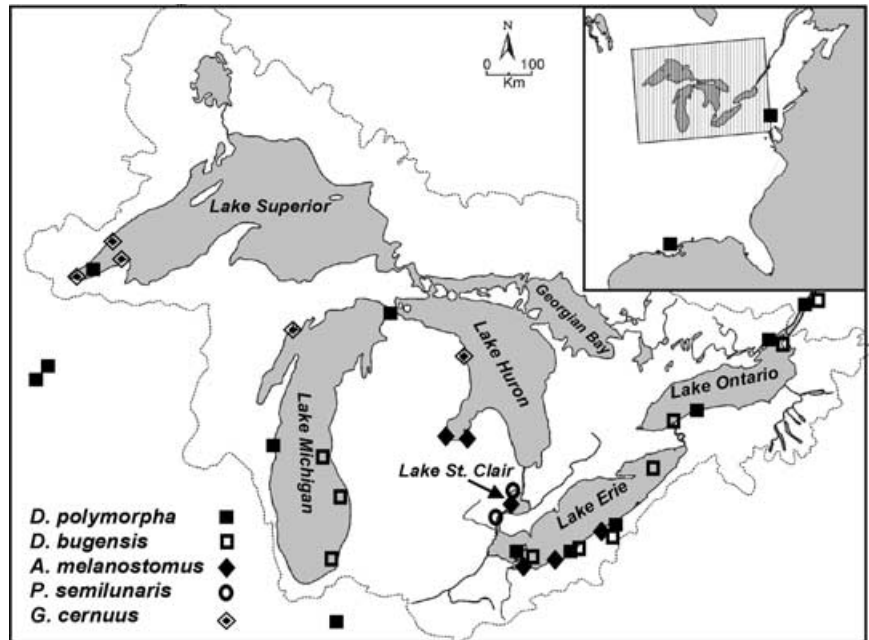
Species	Grouped Location in the Analyses	Body of Water	Location	Latitude	Longitude	Sample Size	
						Cytochrome <i>b</i>	RAPDS
Dreissenids <i>Dreissena polymorpha</i>	w. & c. Europe	Ebro River	Catalonia, Spain	41°25'N	0°39'E	5	—
		Small lake	Naar Rupel, Belgium	51°00'N	4°00'E	1	—
		Lake IJsselmeer	Amsterdam, Netherlands	52°46'N	5°14'E	5	24
	n. w. Black Sea Rivers	Rhine River	Vuren, Netherlands	51°20'N	4°24'E	6	21
		Lake Dybrzk	Chojnice, Poland	52°50'N	19°00'E	5	18
		n. Danube River	Budapest, Hungary	47°29'N	19°00'E	5	19
		s. Danube River	Vikove, Ukraine	45°30'	29°40'E	5	—
		Dniester River	Belgorod-Dnestrovskii, Ukraine	46°15'N	30°20'E	5	—
		S. Bug River	Nikolaev, Ukraine	47°00'N	32°08'E	5	—
	n. c. Black Sea Rivers	n. Dnieper River	Kiev, Ukraine	50°27'N	30°30'E	6	—
		c. Dnieper River	Dnipropetrovs'k, Ukraine	48°15'N	34°00'E	2	19
		s. Dnieper River	Kherson, Ukraine	46°38'N	32°34'E	5	—
	s. Volga River region	Kakhovskii Canal	Crimea, Ukraine	45°46'N	33°28'E	5	—
		s. Volga River	Obukhovskaya Protoka, Russia	44°21'N	48°12'E	6	—
		Moskva River	Moscow, Russia	55°30'N	37°05'E	5	—
		n. Volga River	Rybinsk, Russia	58°17'N	37°28'E	1	—
		n. Volga River	Kostroma, Russia	57°50'N	41°10'E	3	5
	n. Black Sea Rivers	n. Volga River	City of Tver, Russia	56°51'N	35°54'E	2	—
		Dniester Liman	Belgorod-Dnestrovskii, Ukraine	46°15'N	30°20'E	2	—
Volga River system	s. Dnieper River	Kherson, Ukraine	46°38'N	32°34'E	3	—	
	c. Dnieper River	Dnipropetrovs'k, Ukraine	48°15'N	34°00'E	—	25	
	n. Dnieper River	Kiev, Ukraine	50°27'N	30°30'E	5	—	
	Kakhovskii Canal	Crimea, Ukraine	45°46'N	33°28'E	3	—	
	n. Volga River	Rybinsk, Russia	58°17'N	37°28'E	7	—	
	n. Caspian Sea	Lagan, Russia	45°35'N	47°45'E	2	—	
	Bolda River, Volga River delta	Bol'shaya Bolda, Russia	46°80'N	48°34'E	3	—	
Gulf of Gdansk	Kuibyshev Reservoir	Togliatti, Russia	53°27'N	49°24'E	3	—	
	Gulf of Gdansk	Gdynia, Poland	54°20'N	18°40'E	—	20	
n. Black Sea Rivers	Danube River	Vienna, Austria	48°13'N	16°22'E	9	—	
	Danube River	Prahovo, Yugoslavia	44°17'N	22°35'E	23	—	
	n. Dnieper River	Kiev, Ukraine	50°27'N	30°30'E	5	—	
	w. Black Sea	Varna, Bulgaria	43°14'N	27°58'E	15*	15	
	n. c. & n. e. Black Sea	Odessa, Ukraine	46°28'N	30°44'E	2	—	
n. e. Black Sea	n. e. Black Sea	Sevastopol, Crimea, Ukraine	44°34'N	33°34'E	6	—	

(continued)

Table I. Continued.

Species	Grouped Location in the Analyses	Body of Water	Location	Latitude	Longitude	Sample Size	Sample Size	
<i>Proterorhinus semilunaris</i>	n. Black Sea Rivers	Danube River	Prahovo, Yugoslavia	44°17'N	22°35'E	3	—	
			Biliaivka, Ukraine	46°28'N	30°13'E	1	—	
			Dniester River	50°27'N	30°30'E	5	—	
<i>P. marmoratus</i>	n.c. Black Sea	n.c. Black Sea	Tytilul Estuary, Ukraine	46°50'N	31°10'E	6	—	
			Odessa, Ukraine	46°28'N	30°44'E	10	—	
			n.e. Black Sea	44°34'N	33°34'E	6	—	
Ruffe <i>Gymnocephalus cernuus</i>	Loch Lomond	Loch Lomond	Inversnaid, Scotland	56°41'N	3°11'W	12	LdhA6 12	
	Bassenthwaite Lake	Bassenthwaite Lake	Cumbria, England	54°25'N	2°59'W	12	12	
	Elbe River	Elbe River	Magdeburg, Germany	51°30'N	6°43'E	11	11	
	Morava River	Morava River	Morava, Czech Republic	48°44'N	16°54'E	8	8	
	Danube River	Danube River	Gabcikovo, Slovakia	47°48'N	17°35'E	13	13	
	St. Petersburg	Neva River and Komsomolskoe Lake	St. Petersburg, Russia	59°57'N	30°20'E	15	15	
	Ob' River	Ob' River	Novosibirsk, Siberia, Russia	55°09'N	82°58'E	12	12	
	North America							
	Dreissenids <i>D. polymorpha</i>	Upper Great Lakes	n. Mississippi River Lake Zumbro w. Lake Superior	Lake Pepin, WI, USA	44°57'N	92°53'W	5	Cytochrome b 5
				Millville, MN, USA	44°07'N	92°22'W	5	5
Duluth-Superior Harbor, MN, USA				46°50'N	92°07'W	5	24	
Mississippi River Middle Great Lakes		s. Mississippi River w.c. Lake Michigan Lake Wawasee	Baton Rouge, LA, USA	30°24'N	91°11'W	5	26	
			Sheboygan, WI, USA	43°45'N	87°42'W	7	—	
			Syracuse, IN, USA	39°53'N	86°16'W	5	—	
Lake Erie		n.w. Lake Huron w. Lake Erie	Mackinac Straits, MI, USA	45°14'N	85°11'W	5	28	
			Put-in-Bay, South Bass Island, OH, USA	41°50'N	83°00'W	24	31	
			Cleveland, OH, USA	41°30'N	81°42'W	5	—	
			Olcott, NY, USA	43°00'N	79°00'W	5	22	
Hudson River	Hudson River 1995	Cape Vincent, NY, USA	44°20'N	76°10'W	5	21		
		Catskill, NY, USA	44°20'N	72°33'W	5	—		
		Stuyvesant, NY, USA	42°20'N	73°50'W	12	22		
St. Lawrence River Lake Michigan	St. Lawrence River	East Chatham, NY, USA	42°27'N	74°03'W	13	—		
		Becancour, Quebec, Canada	46°24'N	72°23'W	5	—		
		Sable Point, MI, USA	44°08'N	86°54'W	3	—		
<i>D. bugensis</i>	Lake Michigan	St. Joseph, MI, USA	44°06'N	86°29'W	1	—		
		Muskegon, MI, USA	43°13'N	86°20'W	2	—		

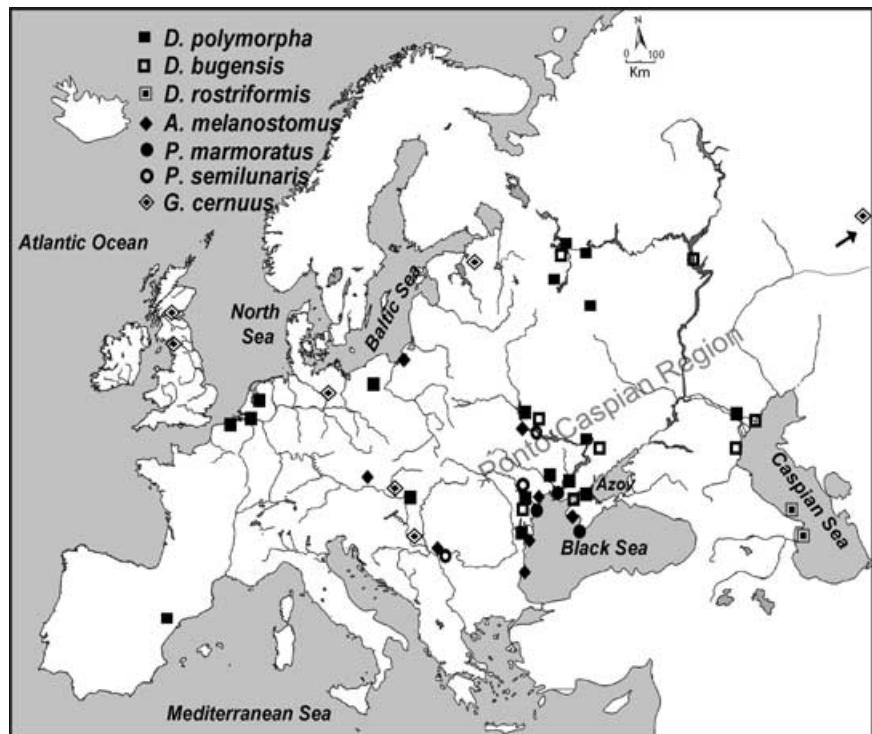
Fig. 1. Map showing locations of sampling sites from the invasive ranges of dreissenid mussels, neogobiin gobies, and the ruffe percid fish in the North American Great Lakes region and outgroup populations.



11 population sites across its native (Eurasian) and anthropogenically introduced (North American Great Lakes and northwestern Europe) ranges (Table I and Figs. 1 and 2), using DNA sequences from the en-

tire mtDNA control region and the sixth intron of the nuclear lactate dehydrogenase A gene (LdhA6). We include samples from the ruffe's 2002 appearance in Lake Michigan, as well as representatives

Fig. 2. Map showing locations of sampling sites from the current (invasive and native) ranges of dreissenid mussels, neogobiin gobies, and the ruffe percid fish in Eurasia. The Ponto-Caspian region includes the Black, Azov, and Caspian Seas and their drainages.



from its present range in Lakes Huron and Superior. In addition, results are compared with sequences from its Eurasian congeners—Balon's ruffe *G. baloni* and the yellow pope *G. schraester*—as well as the yellow perch *Perca flavescens* as an outgroup. Prior work has indicated that *Perca* is the genus sister to *Gymnocephalus*.^(10,23)

2.4. Genetic Data Collection and Analysis

Samples were either frozen live in liquid nitrogen or on dry ice and stored at -80°C , or were placed directly in 95% ethanol while alive and then stored at room temperature. Guts were removed and genomic DNA was extracted from muscle tissues and purified using the DNeasy tissue kit and protocol (Qiagen, Inc., Valencia, CA). We amplified the mitochondrial DNA cytochrome *b* regions for dreissenid mussels (429 bp) and neogobiin gobies (451 bp), the mitochondrial DNA control region for the round goby (750 bp) and the ruffe (1,024 bp), and the nuclear *LdhA6* intron for the ruffe (194 bp) using conserved primers and PCR (the polymerase chain reaction) as described in previous studies by the Stepien laboratory.^(9,10,12,18,19) PCR products were purified with a QIAquick kit (Qiagen, Inc., Valencia, CA). DNA sequencing reactions were performed separately in both directions (for independent verification) using the PCR primers and cycle sequencing, following manufacturer's directions (CEQ DTCS kit, Beckman-Coulter, Inc., Fullerton, CA). Sequences were determined using a Beckman-Coulter CEQ 8000 capillary autosequencer. Sequences were aligned using Se-Al⁽²⁴⁾ and manual adjustments were made employing amino acid alignments and parsimonious procedures (see Reference 9). Sequences were deposited in Genbank (see Table II for accession numbers). Results here are discussed in comparison with our earlier analyses for dreissenids,^(1,11,12,21) neogobiin gobies,^(9,18) and ruffe.^(10,19)

Phylogenetic relationships among the DNA haplotypes were analyzed using the neighbor joining algorithm⁽²⁵⁾ with Kimura⁽²⁶⁾ 2-parameter genetic distances, the pairwise deletion option, and 1,000 bootstrap replications in MEGA 2.1.⁽²⁷⁾ Population genetic data analyses were run in Arlequin version 2.001⁽²⁸⁾ in order to compute genetic diversity indices, conformance to Hardy-Weinberg equilibrium expectations (in the case of the diploid RAPD data), *Fst* analog estimates and corresponding probability values (from 1,000 permutations), migration

Nm estimates, and hierarchical partitioning of variation (presented here as % Variance) using AMOVA (Analysis of Molecular Variance).⁽²⁹⁾ Statistical measures of genetic variability used for comparisons of invasive versus native populations in this study included haplotypic diversity/ gene diversity/ heterozygosity (*h*) and nucleotide diversity (π) (see Reference 30).

Haplotype diversity is equivalent to gene diversity in analyses involving multiple loci. Haplotype/ gene diversity is based on the number and frequencies of alleles at a locus, regardless of their sequence relationships and the number of sequence differences.⁽³⁰⁾ This measure is defined as the probability that two randomly chosen haplotypes/genotypes in the sample are different. Haplotype/ gene diversity (*h*) was calculated from the sum of squares of allele frequencies, and is close in value to heterozygosity in randomly mating populations.⁽³¹⁾ Its sampling variance was calculated according to Nei.⁽³⁰⁾

Nucleotide diversity or average gene diversity at the nucleotide level (π) is based on the mean number of sequence differences between individuals in a population, regardless of the number of different genotypes. Nucleotide diversity was calculated as the mean number of base differences between two genomes (haplotypes or alleles), divided by the number of base pairs compared.⁽³⁰⁾ Its sampling variance was calculated following Nei.⁽³⁰⁾ These measures were determined in grouped analyses for the North American and Eurasian populations, as well as among separate population samples (see Table I), in order to compare the mean amount of genetic diversity per sampling location, as well as among exotic versus native populations and between the continents. In cases of lower sample size (see Table I), some samples were pooled with nearby locations.

2.5. Relating the Genetic Data to Risk Assessment

We relate our genetic data findings to risk assessment using the elements developed by the Aquatic Nuisance Species Task Force,⁽³²⁾ summarized in Reference 33. This comparative genetic approach for analyzing population genetic relationships of taxa introduced to the Great Lakes via ballast water during the late 1980s through early 1990s may shed light on the common genetic characteristics rendering the invasions successful. Differences in the degree and distribution of genetic variation among species may

Table II. Genetic Diversity and Divergence Comparisons Between Samples from North America and Eurasia for Five Species that are Invasive in the North American Great Lakes and Native to the Ponto-Caspian Region of Eurasia

	Taxon				
	Zebra Mussel <i>D. polymorpha</i>	Quagga Mussel <i>D. bugensis</i>	Round Goby <i>A. (N.) melanostomus</i>	Freshwater Tubenose Goby <i>P. semilunaris</i>	Ruffe <i>G. cernuus</i>
DNA Regions Analyzed	mtDNA cyt b — Nuclear RAPDs	mtDNA cyt b — Nuclear RAPDs	mtDNA cyt b — mtDNA Control	mtDNA cyt b — mtDNA Control	mtDNA Control — Nuclear LdhA6
<i>N</i> individuals sampled	188 — 280	78 — 136	110 — 75	20 — 10	118 — 118
<i>N</i> total haplotypes/alleles	19 — 126	15 — 104	7 — 11	4 — 1	5 — 15
GenBank accession numbers	DQ072111726 — NA (data in Reference 1)	DQ072130-7 — NA (data in Reference 1)	AY884582-3 U53673-7 — AP082970-4	AY88572-5 — AP082969	AF25355-9 — AY34781-3
Divergence between continents <i>Fst</i>	0.002 NS — 0.065 NS	0.039 <i>p</i> < 0.017 — 0.021 NS	0.163 <i>p</i> < 0.0001 — 0.519 <i>p</i> < 0.0001	0.078 NS — NA	0.319 <i>p</i> < 0.0001 — 0.188 <i>p</i> < 0.0001
Overall migration between continents <i>Nm</i>	253.65 — 3.46	12.79 — 8.67	2.55 — 0.46	Panmixie — NA	1.73 — 1.12
% Variation between continents	0.11% NS — 6.50% <i>p</i> < 0.0001	0.57% NS — NA	15.25% NS — 49.77% <i>p</i> < 0.0001	0.01% NS — NA	90.68% NS — 11.58% <i>p</i> < 0.016
% Variation among sites within continents	5.65% <i>p</i> < 0.023 — 12.46% <i>p</i> < 0.0001	10.52% <i>p</i> < 0.0001 — 6.8% <i>p</i> < 0.0001	6.24% <i>p</i> < 0.0001 — 6.23% <i>p</i> < 0.0001	34.18% <i>p</i> < 0.0001 — NA	8.70% <i>p</i> < 0.0001 — 6.84% <i>p</i> < 0.0001
% Variation within sampling sites	93.28% <i>p</i> < 0.0001 — 81.04% <i>p</i> < 0.0001	88.91% <i>p</i> < 0.0001 — 93.2% <i>p</i> < 0.0001	82.11% <i>p</i> < 0.0001 — 44% <i>p</i> < 0.0001	65.81% <i>p</i> < 0.0001 — NA	.60% <i>p</i> < 0.0001 — 81.58% <i>p</i> < 0.0001

Note: NS = not significant, NA = not available, *p* = statistical probability.

also account for their differential spread and success patterns. Coupling of risk assessment and genetic analyses may ultimately help us to understand how to circumvent further spread of these particular exotic species and prevent their congeners and relatives from also being introduced.

3. GENETIC RESULTS AND DISCUSSION

3.1. Genetic Variation Within and Among Zebra Mussel Populations and Relatives

The present study identifies a total of 19 mtDNA cytochrome *b* haplotypes among 188 individual

Table III. Allelic Variation and Genetic Variability Comparisons Within and Among Sites from North America and Eurasia

DNA region(s) analyzed	Zebra Mussel North America		Zebra Mussel Eurasia		Quagga Mussel North America		Quagga Mussel Eurasia		Round Goby North America		Round Goby Eurasia		Tubenose Goby North America		Tubenose Goby Eurasia		Ruffe North America		Ruffe Eurasia	
	mtDNA	cyt <i>b</i>	mtDNA	cyt <i>b</i>	mtDNA	cyt <i>b</i>	mtDNA	cyt <i>b</i>	mtDNA	cyt <i>b</i>	mtDNA	cyt <i>b</i>	mtDNA	cyt <i>b</i>	mtDNA	cyt <i>b</i>	mtDNA	cyt <i>b</i>	mtDNA	cyt <i>b</i>
<i>N</i> individuals per continent	111	77	49	29	51	59	11	31	11	11	31	83	35	83	35	83	35	83	35	83
<i>N</i> regions per continent	7	5	3	2	2	6	1	3	2	2	6	1	1	3	2	7	2	7	2	7
<i>N</i> DNA alleles/haplotypes per continent and % of total	14 74%	12 63%	15 65%	14 61%	4 57%	5 71%	3 75%	4 100%	3 75%	4 71%	5 71%	3 75%	3 75%	4 100%	1 20%	4 100%	1 20%	4 100%	1 20%	5 100%
Mean <i>N</i> alleles/haplotypes per region (and range) per continent	4.4 (3–8)	5.4 (3–7)	6.7 (5–10)	8 (6–10)	2.7 (2–3)	1.8 (1–3)	3 (3)	2.5 (2–3)	3 (3)	1.8 (1–3)	1.8 (1–3)	3 (3)	3 (3)	3 (3)	1 (1)	2.5 (2–3)	1 (1)	1 (1)	1 (1)	1.2 (1–2)
<i>N</i> haplotypes and % of total shared with the other continent	7 50%	7 58%	6 40%	6 43%	2 50%	2 29%	3 100%	3 75%	2 50%	2 100%	2 18%	3 100%	3 100%	3 100%	1 100%	3 75%	3 100%	4 100%	4 100%	6 17%
Haplotype/gene diversity (<i>h</i>) ± sampling variance	0.672 ± 0.041	0.691 ± 0.050	0.887 ± 0.028	0.884 ± 0.021	0.512 ± 0.060	0.132 ± 0.060	0.655 ± 0.112	0.806 ± 0.089	0.512 ± 0.060	0.132 ± 0.060	0.865 ± 0.050	0.563 ± 0.046	0.655 ± 0.112	0.806 ± 0.089	0.000 ± 0.000	0.658 ± 0.026	0.000 ± 0.000	0.658 ± 0.026	0.560 ± 0.041	0.732 ± 0.025
Mean haplotype/gene diversity (<i>h</i>) per region within continent (and range)	0.579 (0.080–0.800)	0.693 (0.451–0.873)	0.861 (0.800–0.905)	0.884 (0.867–0.900)	0.505 (0.404–0.606)	0.272 (0.063–0.467)	0.655 (0.655)	0.684 (0.667–0.700)	0.505 (0.404–0.606)	0.272 (0.063–0.467)	0.865 ± 0.050	0.563 ± 0.046	0.655 (0.655)	0.684 (0.667–0.700)	0.000 (0.000)	0.658 ± 0.026	0.000 (0.000)	0.658 ± 0.026	0.560 ± 0.041	0.732 ± 0.025
Nucleotide diversity (π) on continent	0.0042 ± 0.0027	0.0040 ± 0.0027	0.0048 ± 0.0031	0.0054 ± 0.0034	0.0012 ± 0.0011	0.0003 ± 0.0005	0.0124 ± 0.0074	0.0156 ± 0.0092	0.0012 ± 0.0011	0.0003 ± 0.0005	0.0040 ± 0.0020	0.0059 ± 0.0043	0.0124 ± 0.0074	0.0156 ± 0.0092	0.0000 ± 0.0000	0.0062 ± 0.0045	0.0000 ± 0.0000	0.0062 ± 0.0045	0.0045 ± 0.0025	0.0045 ± 0.0025
Mean nucleotide diversity (π) within regions on continent	0.0041	0.0039	0.0048	0.0048	0.0028	0.0004	0.0124	0.0053	0.0028	0.0004	0.0059 ± 0.0043	0.0124 ± 0.0074	0.0053	0.0053	0.0000	0.0062 ± 0.0045	0.0000	0.0062 ± 0.0045	0.0045 ± 0.0025	
Mean divergence among sites within continent (<i>F_{ST}</i>)	0.069	0.066	0.164	0.182	0.062	0.482	NA	0.347	0.062	0.482	0.031	0.035	0.062	0.347	0.000	0.0063	0.0063	0.0063	0.0063	0.926
	0.095	0.189	0.084	NA	0.311	0.035	NA	0.311	0.311	0.035	0.035	0.035	0.035	0.347	0.000	0.0063	0.0063	0.0063	0.0063	0.108

* Indicates based on RAPDs data and is overall *N* of alleles. Note: NS = not significant, NA = not available, *p* = statistical probability.

zebra mussels (Table II), of which 14 are found in invasive North American habitats and 12 throughout Eurasia (where fewer individuals have been analyzed to date; Table III). Only 50% of the mtDNA haplotypes found to date in North America are also identified at present in Eurasia, and only 58% of those in Eurasia are discerned in North America. Thus, a much greater sampling in both areas is needed, as this is certainly due to the limited numbers sequenced in the present study. Nevertheless, overall haplotype diversity levels in both continents are very high for both types of data—mtDNA sequence and nuclear RAPDs variation (Tables II and III)—indicating that the invasion of North America was extremely large, involving a huge number of propagules and multiple founding sources.

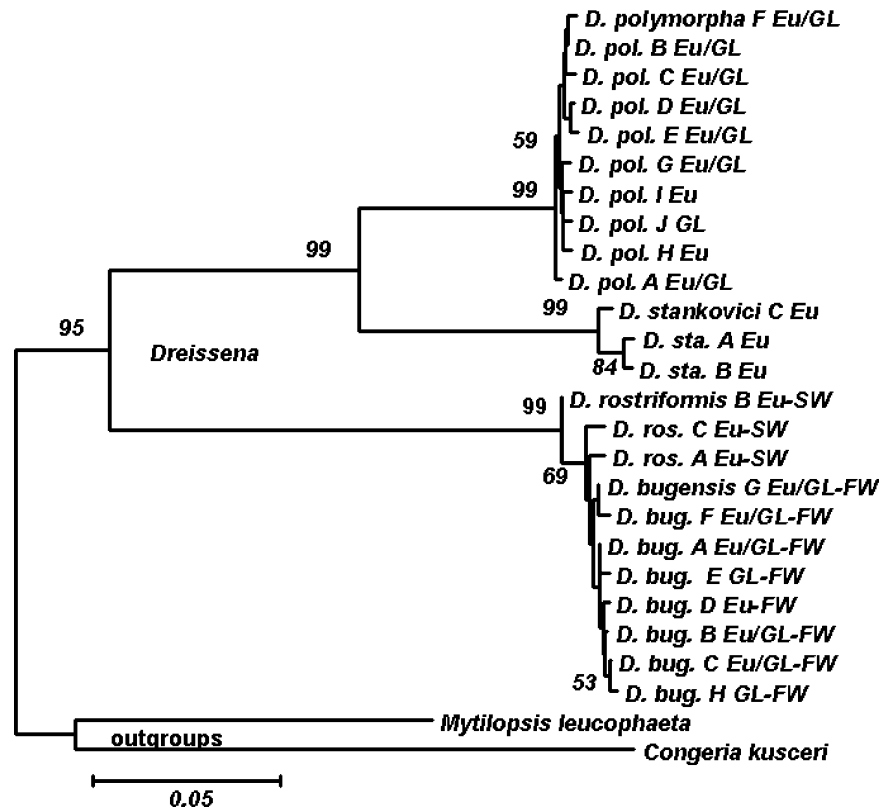
In addition, nucleotide diversity levels on both continents are very similar and migration levels between them are very high—consistent with the hypotheses of a large invasion and no apparent founder effects (Tables II and III). Overall divergence values between the pooled data for the continents of North America and Eurasia as measured by *Fst* are low and not significant (Table II), supporting the hypothesis of high gene flow “fueling” the invasion. Divergence

levels among individual locations on each continent are greater (Table III) than those between the continents overall (Table II), showing that the distribution of genetic variation differs among sites and is not homogeneous. Results for the mtDNA cytochrome *b* and the nuclear RAPDs data sets (Tables II and III) reveal very similar trends.

Genetic divergences among Eurasian sites were larger than those among North American sites for both the mtDNA and the nuclear RAPDs data sets (Table III), reflecting their longer phylogeographic history and time for vicariant evolutionary differentiation among locations. However, genetic differences among North American sites also were appreciable—consistent with the multiple independent invasion hypothesis formulated by Stepien *et al.*^(1,12) AMOVA % variation levels between the continents and among sites within the continents showed significant differentiation levels, supporting the hypothesis of population genetic structure across their invasive as well as their native ranges (Table II).

The neighbor-joining tree (Fig. 3) shows the most common haplotypes that characterized three or more individuals (lettered A–J), which were all closely related in sequence. Of these, seven of the ten most

Fig. 3. Neighbor-joining tree depicting the evolutionary relationships among *Dreissena* mtDNA cytochrome *b* gene sequence haplotypes; including the primary types of zebra mussel *D. polymorpha* and quagga mussel *D. bugensis* (numbering three or more individuals; thus rare haplotypes are excluded here). We compare these species with variation among all four species in the genus *Dreissena*—including the saline variant *D. rostriformis* (which appears little diverged from *D. bugensis*; see Reference 12) and *D. stankovici* (endemic to lakes in Macedonia and Albania, Reference 12)—as well as its sister genera *Congerina* and *Mytilopsis*.^(11,12) The tree was constructed using Kimura⁽²⁶⁾ 2-parameter genetic distances in MEGA 2.1,⁽²⁷⁾ with pairwise deletion and 1,000 bootstrap replications (bootstrap support values greater or equal to 50% are indicated at nodes).



common zebra mussel haplotypes (70%) were found in both North American and Eurasian locations. Its sister species *Dreissena stankovici* from lakes in Albania and Macedonia shows greater divergence among haplotypes than does *D. polymorpha* (Fig. 3).

3.2. Genetic Diversity Within and Among Quagga Mussel Populations and Relatives

Fifteen mtDNA cytochrome *b* haplotypes of quagga mussels were found among 78 individuals sampled (Table II), with 15 types in North America and 14 in Eurasia, and only 40% and 43% of these respectively were shared with the other continent (Table III). Relationships among the most common haplotypes (A–H) are depicted in Fig. 3. As in the case of the zebra mussel, additional individuals need to be analyzed in order to gain a more complete understanding of this wealth of genetic variation among native and invasive dreissenid populations. Haplotypic/gene diversity levels were very high in the quagga mussel—even greater than those found in the zebra mussel—according to both the mtDNA and the nuclear DNA data (Table III). Similarly, nucleotide diversity levels were higher in the quagga mussel for both genomes (Table III). As in the zebra mussel, the distribution of genetic variation was not significant between the continents in an AMOVA analysis (Table II) since so much variation was introduced, but was significant among sites within continents (Table III).

The saline taxon *D. rostriformis* is very closely related to *D. bugensis*, and their taxonomic distinctiveness is unclear—thus *D. bugensis* may be a variant of *D. rostriformis* (Fig. 3; see Reference 12). This relationship is being further tested by our laboratory, and we have recommended that they each continue to be recognized as separate species pending those results.⁽¹²⁾ Close divergence between them is based on single nucleotide synapomorphies in both mitochondrial cytochrome *b* gene and 16S rDNA sequences,⁽¹²⁾ in addition to slight morphological distinctions described by Rosenberg and Ludayskiy.⁽³⁴⁾ This close relationship suggests that estuarine systems in North America may be vulnerable to invasion by colonists of *D. rostriformis* or possibly saline-tolerant strains of *D. bugensis* (which have not been tested for). Our study finds that *D. bugensis* now is quite common in the Volga River system and the upper Caspian Sea region, where it is hypothesized to have anthropogenically spread from the Black Sea region.⁽²⁰⁾ Historically, the taxa may have been vicariantly isolated

in the Black Sea (*D. bugensis*) and the Caspian Sea (*D. rostriformis*) drainages since the mid-Pleistocene epoch.⁽¹²⁾

3.3. Population Genetic Relationships of the Round Goby

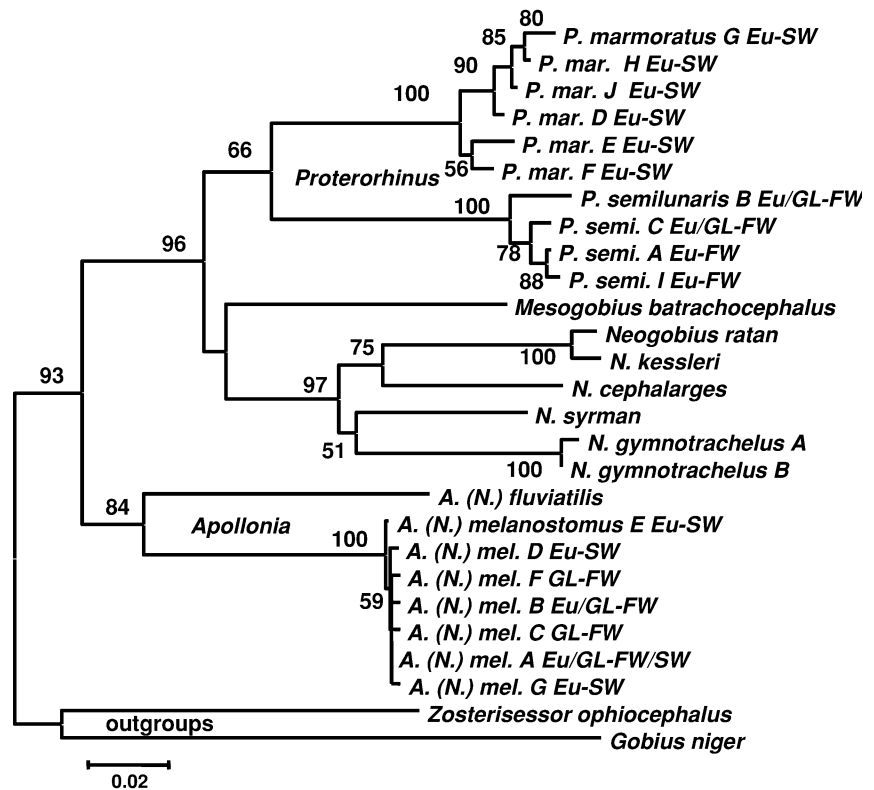
Both the mtDNA cytochrome *b* gene and the control region were sequenced for samples of round gobies, with greater sample sizes and diversity of locations in the cytochrome *b* analyses. As in other studies of fishes (summarized by Reference 35), the noncoding control region has greater levels of divergence and haplotypic diversity than does the coding cytochrome *b* gene. Both data sets reveal significant overall *Fst* divergences between pooled data for the continents of North America versus Europe (Table II). *Fst* values are higher and migration values lower than those characterizing dreissenid mussel populations (Table II). AMOVA variation patterns show significant divergence between the continents for the mtDNA control region data (but not for the cytochrome *b* gene data, which is likely related to the fact that the earlier control region data were based on only two widely separated geographic locations—one a native and one an introduced population; Table II). Significant differences are discerned among locations within both continents, revealing appreciable geographic structure across their native and invasive ranges.

Both data sets showed that the North American populations overall are as genetically diverse as those in Eurasia—in terms of numbers of haplotypes per location and haplotype/gene diversity. Unlike dreissenids, fewer overall numbers of round goby haplotypes were introduced to the Great Lakes. However, individual locations in the Great Lakes had as many or more haplotypes as those found in individual native Eurasian locations (Table III). As seen in Fig. 4, both fresh and euryhaline tolerant genotypes have been introduced to the Great Lakes, suggesting the potential for the latter to spread to salt marsh habitats. This needs to be further tested.

3.4. Population Variation in the Freshwater Tubenose Goby Versus Its Marine Sister Group

Sample sizes for tubenose gobies are limited at present, since prior analyses showed that they comprise separate marine and freshwater species.⁽⁹⁾ The freshwater tubenose goby data show no significant *Fst* divergence and high migration values between samples from the Great Lakes and rivers draining

Fig. 4. Neighbor-joining tree depicting the evolutionary relationships among neogobiin mtDNA cytochrome *b* gene sequence haplotypes, including round goby *Apollonia* (*Neogobius*) *melanostomus*, freshwater tubenose goby *Proterorhinus semilunaris* (formerly part of *P. marmoratus*), and the marine tubenose goby *P. marmoratus* (including samples from the type locality at Sevastopol, Crimea, Ukraine). We compare these species with variation among other neogobiin species and the remaining neogobiin genera, including monkey goby *Apollonia* (*Neogobius*) *fluviatilis*, syrman goby *Neogobius syrman*, racer goby *N. gymnotrachelus*, ratan goby *N. ratan*, ginger goby *N. cephalarges*, bighead goby *N. kessleri*, and knout goby *Mesogobius batrachocephalus*. Outgroups include two members of the subfamily Gobiinae, grass goby *Zosterisessor ophiocephalus* and black goby *Gobius niger*. The tree was constructed using Kimura⁽²⁶⁾ 2-parameter genetic distances in MEGA 2.1,⁽²⁷⁾ with pairwise deletion and 1,000 bootstrap replications (bootstrap support values greater or equal to 50% are indicated at nodes).



into the Black Sea (Table II). However, there is considerable genetic divergence among individual native river locations in Eurasia (Tables II and III). The samples from the Great Lakes contained as many haplotypes or more than were found in the individual Eurasian river sites, indicating that a large number of propagules were introduced into the Great Lakes. Overall, haplotype and nucleotide diversity values were almost as large in the Great Lakes as for pooled Eurasian data. Those values for individual sites in the Great Lakes were equivalent and greater, respectively, than the mean values for Eurasian river sites. Results indicate that overall genetic diversity of tubenose goby in the Great Lakes is as high or larger than in a typical native Eurasian location, and the variety of genotypes suggests multiple founding sources. As in dreissenids and round gobies, a large number of propagules founded the Great Lakes invasion by the tubenose goby and there is no appreciable founder effect.

The neighbor-joining tree (Fig. 4) shows marked separation between the freshwater tubenose goby *P. semilunaris* and the marine/estuarine *P. marmoratus*, along with relatively high divergences among haplotypes. Since congeners are often also successful in

vasions, it appears likely that *P. marmoratus* may be successfully introduced to North American coastal estuaries.

3.5. Genetic Patterns Among Ruffe Populations and Congeners

Most sampling locations (and all individuals from the Great Lakes) are monotypic for a single mtDNA control region haplotype, with Eurasian populations showing marked allopatric structure and divergence among locations (Fig. 5; Table III). Only five mtDNA control region haplotypes are identified across Eurasia, and only one of these is found in North America (Fig. 5; Tables II and III). All locations except for one (the Ob' River in Siberia) are monotypic, and all Eurasian sites show marked genetic divergence among locations (Fig. 5). The North American invasive populations comprise only mtDNA haplotype A, which also is monotypic in samples from the Elbe, Morava, and Danube Rivers. This result indicates that the North American population was founded from this region, whose location is pinpointed further using DNA sequencing of the nuclear LdhA6 intron. Similarly, invasive populations sampled in Bassenthwaite

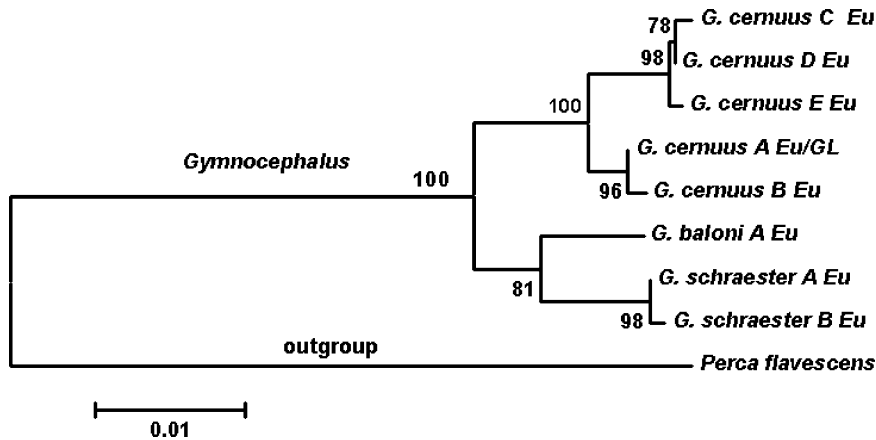


Fig. 5. Neighbor-joining tree depicting the evolutionary relationships among *Gymnocephalus* mtDNA control region sequence haplotypes; including the ruffe *G. cernuus*, Balon's ruffe *G. baloni*, and yellow pope *G. schraester*. We compare variation in *Gymnocephalus* with its sister genus *Perca*.^(10,23) The tree was constructed using Kimura⁽²⁶⁾ 2-parameter genetic distances in MEGA 2.1,⁽²⁷⁾ with pairwise deletion and 1,000 bootstrap replications (bootstrap support values greater or equal to 50% are indicated at nodes).

Lake, England and Loch Lomond, Scotland are both monotypic for haplotype B. Phylogenetic relationships among *G. cernuus* haplotypes are shown in the tree in Fig. 5.

Analysis of the nuclear *LdhA6* intron reveals greater diversity than found in mtDNA sequences. Four haplotypes are identified in North America, which do not significantly differ in genetic composition among locations from their present-day range in the upper Great Lakes. The invasive population in the North American Great Lakes genetically matches the Elbe River drainage region in northwestern Europe, sharing identical haplotypes at about the same frequencies. This particular genetic composition appears unique to only the Elbe River and Great Lakes samples, and they show no significant difference—suggesting that the Elbe River drainage region was the founding source for the single population of ruffe in the Great Lakes.

There are 15 *LdhA6* haplotypes identified from Eurasia, with a mean of 4.4 haplotypes per sampling location—ranging from one (the Morava River) to seven haplotypes (St. Petersburg) per site (Table II). The four sites sampled in North America each house three to four *LdhA6* haplotypes. Invasive populations sampled in Europe (Bassenthwaite Lake, England and Loch Lomond, Scotland) each contain five haplotypes, of which three are in common. Thus, invasive sites in both North America and Europe house numbers of haplotypes equivalent to native population sites (Table III).

Most population sites in Eurasia, in contrast to those in North America, are significantly divergent in genotypic composition, indicated by significant *Fst* values. Only one region of Eurasia (the Elbe River drainage) appears to have genetically contributed to the North American invasion. This makes the inva-

sion of the ruffe in the Great Lakes much different from those by dreissenids or gobies, since only a single source area founded the invasion. A large number of propagules were introduced, as all genotypes native to the Elbe River are represented in approximately the same frequencies in North America. Unlike the dreissenid and round goby invasions, there are no significant differences in genetic composition among sites in the Great Lakes although there is considerable divergence among Eurasian locations (Table III). In the Great Lakes, all genotypes of ruffe have spread fairly homogeneously and there is no differentiation among areas. All later colonization areas for ruffe in the Great Lakes appear to have spread from the original introduction in the St. Louis Harbor region of Lake Superior. As in the other Great Lakes invaders, no founder effects are apparent.

3.6. Overall Genetic Trends in the Great Lakes Invasions

Our results indicate that “founder effects” are not apparent in the invasions of zebra mussels, quagga mussels, round gobies, tubenose gobies, and ruffe—that is, the introductions were so large and extensive that the populations established in the Great Lakes have as much or greater genetic variability as that found in native Eurasian sites. These results indicate that large numbers of propagules founded the invasions and, except for the ruffe, multiple founding site sources were involved. Multiple founding sources likely significantly boost the probability of establishment success, spread, and adaptability to new habitats (also see Reference 15). The ruffe alone was founded from a probable single location—the vicinity of the Elbe River drainage. This may be related to the slower

Table IV. Risk Assessment and Relation to Genetic Studies of Exotic Species (Adapted from Reference 9)

Element (Per ANSTF Guidance)	Important Considerations in Assessing Element	Application of Data Collected in This Study
Section 1: Probability of organism establishment		
Estimate probability of the organism being on, with, or in the pathway (ballast water in this case).	Organism’s temporal and spatial association with the pathway.	Genetic data show that highly successful Ponto-Caspian invasions in the Great Lakes are characterized by (1) a large number of introduced genotypes, with genetic diversities of invasive populations comparable to that of native population sites, (2) equivalent effective population sizes of native and introduced populations, indicating that the introductions did not undergo “genetic bottlenecks,” and (3) considerable genetic differentiation and large number of haplotypes. Results indicate that multiple founding source populations were involved for zebra and quagga mussels and the round goby. Hence, related populations and species in the areas of origin likely have a high probability to be in ballast water and to survive transport.
Estimate probability of the organism surviving in transit.	Organism’s hitchhiking ability in commerce, ability to survive during transit, stage of lifecycle during transit, number of individuals expected to be associated with the pathway; or whether it is deliberately introduced (e.g., biocontrol agent or fish stocking).	In addition, the invasive population areas for zebra mussels, quagga mussels, and round gobies, including those in different Great Lakes, show genetic divergence and match different source populations, suggesting that different founding sources contributed differentially to the new populations. It thus is likely that several nearly simultaneous invasions occurred from several different sources in these cases.
Estimate probability of the organism colonizing and maintaining a population.	Organism’s ability to come into contact with an adequate food resource, probability of encountering appreciable abiotic and biotic environmental resistance, and the ability to reproduce in the new environment. Introduction of co-evolved predator and prey species such as the round goby and zebra mussel may result in ecological facilitation and enhanced invasive success.	The ruffe, however, matched only the Elbe River source population. All the genotypes in the Elbe were present in the North American Great Lakes. Thus, the ruffe had comparable genetic diversity to a native site but evidence supported that there was a single source population that founded the invasion. This may account for the ruffe’s restriction to the Upper Great Lakes in its invasive habitat.
Estimate probability of the organism spreading beyond the colonized area.	Ability for natural dispersal, ability to use human activity for dispersal, ability to readily develop races or strains, and the estimated range of probable spread.	The risk consequence indicated by genetic data—that is, a large number of genotypes, high genetic diversity, and significant divergence among colonizing population sites—appears to be extremely high in terms of our unfortunate inability to “control” an invasion once it has become established, as is the case with dreissenid mussels as well as the round goby.
Section 2: Consequence of establishment		
Estimate economic impact if established.	Economic importance of hosts, damage to crop or natural resources, effects to subsidiary industries, exports, and control costs.	Genetic data not applicable.
Estimate environmental impact if established.	Ecosystem destabilization, reduction in biodiversity, reduction or elimination of keystone species, reduction or elimination of endangered/threatened species, effects of control measures, and impacts on the human environment (e.g., human parasites or pathogens would also be captured under this element).	
Estimate impact from social and/or political influences.	None provided.	

spread of the ruffe and its confinement to the upper Great Lakes. The invasion by the ruffe coincides with the opening of ports on the Elbe River (which drains north into the North and Baltic Seas) to trade with North America, during the "reunification" of the former West and East Germany. All genotypes identified from the Elbe River area were introduced, involving a large number of propagules from a single source location.

The "rain" of propagules shown by these comparative genetic investigations indicates that the Great Lakes ballast water introductions have been widespread, numerous, and commonplace. Due to the large number of propagules and multiple founding sources in most cases, these invasions have great genetic diversity and hence are very adaptable to new habitats. Additionally, the large size of the new populations would have circumvented Allee effects and susceptibility to stochastic factors (see References 13 and 15), leading to successful population growth. Moreover, invasive ranges for zebra mussels, quagga mussels, and round gobies in the Great Lakes also show significant genetic structuring among lakes and locations. This appears to be due to differential patterns of introduction from different Eurasian source populations, and may also be influenced by spread patterns from established sites.

4. APPLICATION OF THE GENETIC RESULTS TO RISK ASSESSMENT

The U.S. National Oceanic and Atmospheric Administration (NOAA) is responsible for managing invasive species in the Great Lakes through the Non-indigenous Aquatic Nuisance Prevention and Control Act (NANPCA) summarized in Reference 33. The U.S. Department of Commerce (through NOAA) and the U.S. Department of the Interior (through the Fish and Wildlife Service) co-chair the Aquatic Nuisance Species Task Force. This task force has developed a generic nonindigenous aquatic organism risk analysis review process that includes estimation of risks from the introduction of nonindigenous aquatic organisms. The objective of this generic process is to provide standardization for evaluating the risk of introducing nonindigenous organisms into a new environment and determining the correct risk management steps needed to mitigate that risk. The process provides a framework by which scientific, technical, and other relevant information can be organized into a format that is proposed to be understandable and useful to managers and decisionmakers.

A significant advantage of the genetic data collected in this study is their application in determining the risk of multiple invasion events and invasions from related taxa. Table IV lists the elements as detailed in the guidance document⁽³²⁾ and provides an example of how the genetic data collected in this study could be used to produce a qualitative estimate of risk for each of the seven elements shown.

An important step in the risk analysis process involves examining what kind of risk management could be implemented to eliminate or reduce the impact of the hazard (in this case, the establishment of an invasive species). The best solution is to prevent the introduction altogether. International cooperative efforts for stemming ballast water introductions have moved strongly in this direction. Genetic data can be used in conjunction with mathematical models to predict likely source areas⁽³⁶⁾ as well as areas with a high probability of being invaded.⁽³⁷⁾ The type of genetic data presented in the present study can greatly assist in targeting efforts in this regard because it is likely that related species have similar risk for entry and establishment. For example, it appears highly likely that the round goby will spread to salt marsh and estuarine habitats in North America, where they will encounter abundant native prey in *Mytilus* mussels. Their genotypic diversity and divergence patterns in the Great Lakes suggest that genotypes already here will likely be successful in more saline habitats. It also is likely that *Dreissena rostriformis* and the marine tubenose goby *Proterorhinus marmoratus* will be introduced and become established in North American salt marsh and estuarine habitats.

In conclusion, qualitative risk assessment using genetic characters of invasive populations indicates that high genetic variability, large number of founders, and introduction of several invasion founding source populations lead to high probability of establishment and persistence. The combination of DNA variation data and risk assessment procedures offers an important diagnostic and monitoring tool for evaluating the relative success of exotic species invasions.

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