

Invasion genetics of Ponto-Caspian gobies in the Great Lakes: a ‘cryptic’ species, absence of founder effects, and comparative risk analysis

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Received 1 January 2004; accepted in revised form 27 August 2004

Key words: cytochrome *b*, exotic species, founder effect, Great Lakes, *Neogobius*, nonindigenous, Ponto Caspian, *Proterorhinus*, risk analysis, round goby, tubenose goby

Abstract

Genetic variability and structure of nonindigenous vs native populations are compared for the Eurasian round goby *Neogobius melanostomus* and the tubenose goby *Proterorhinus marmoratus*, which both invaded Lake St. Clair of the North American Great Lakes about 1990. The round goby spread rapidly to all of the Great Lakes and the tubenose goby largely has been restricted to Lake St. Clair, with some recent range extension into western Lake Erie. Risk analyses may indicate whether genetic variability of colonizers is predictive of their relative invasive and establishment successes. The present investigation examined DNA sequence variation across the left domain of the mitochondrial DNA cytochrome *b* gene in round and tubenose gobies from Eurasian and Great Lakes locations. We also sequenced six additional *Neogobius* species (including the monkey *N. fluviatilis*, racer *N. gymnotrachelus*, and bighead *N. kessleri* gobies that have been ‘on the move’ in Europe) and the knout goby *Mesogobius batrachocephalus* from the Black Sea in order to develop diagnostic genetic characters to identify them in case of future and/or undetected invasions and to delineate their phylogenetic relationships. Results show that a diverse number of haplotypes characterize round and tubenose goby populations from both North America and Eurasian sites, fitting a risk analysis prediction of high genetic variability in their successful introductions. Phylogenetic results indicate that the current genus *Neogobius* is paraphyletic and that the subgenus *Apollonia* thus should be elevated to the level of genus, containing *Apollonia (N.) melanostomus* (the round goby) and *A. (N.) fluviatilis* (the monkey goby). In addition, there appear to be two separate species of *Proterorhinus marmoratus*, a marine *P. marmoratus* Pallas 1814 in the Black Sea (matching the original type locality), and a ‘cryptic’ freshwater species in the Danube and Dnieper Rivers and probably other Eurasian freshwater habitats, as well as invasive in the Great Lakes. We suggest resurrecting the name *P. semilunaris* Heckel 1837 for the freshwater species (a taxon that was originally described from rivers draining into the Aegean Sea and the Danube River, but was later placed in synonymy with *P. marmoratus*).

Introduction

Identifying nonindigenous species often is problematic – since many come from diverse taxonomic groups and areas of the world that have been little studied using modern phylogenetic systematic methodology. DNA sequence tools now allow us to develop diagnostic characters for identifying species, elucidating their founding populations, and determining whether variants belong to ‘cryptic’ taxa. DNA sequence comparisons between native and invasive populations also are important for interpreting whether new colonizations arise from spread or from independent introductions, testing how many source populations were introduced, evaluating the extent of founder effects in new populations, as well as making valid ecological comparisons.

The genetic composition of an exotic population is believed to be fundamental to its success (Williamson 1996). High genetic variability appears positively correlated with an exotic population’s ability to invade, become established, and adapt to new habitats (Williamson 1996; Garcia-Ramos and Rodriguez 2002; Stepien et al. 2002). However, invasive populations traditionally have been believed to be characterized by low genetic diversity, due to founder effects and bottlenecks (Williamson 1996; Holland 2000). Low genetic variability in new populations would be especially apparent in the mitochondrial genome (as examined here) due to its smaller effective population size, more rapid extinction of lineages, and lack of recombination (Avice 2000). Analyses that compare the levels of genetic diversity, as well as the number and proportion of shared genotypes, between founding source and invasive populations allow us to interpret the role of genetic variability governing invasive success (Stepien et al. 2002).

In addition, genetic data also may provide important evidence for evaluating and comparing the invasive ‘risk’ of a given species. Risk analysis as applied to invasive species is a complex process. In summary, the process involves first identifying the events that are likely to lead to the establishment, spread, persistence, and continued growth of invasive populations – then estimating the likelihood, or probability, of these

events. As applied to an examination of the risk of a non-indigenous species invasion, this means determining what level of genetic variability, specific genetic composition, number of individuals introduced, and number of founding source populations will result in relative invasive success. Once the hazard is identified and its probability of occurrence quantified, the consequence of the event must be calculated (in this case, the impact on the native ecosystem and environment). The combination of the probability of the event and the consequence of the event should it occur constitutes the vulnerability or ‘risk’. Once risk is identified and quantified, then risk management options can be considered. These actions then could be implemented to prevent the invasion or subsequent spread of an invasive species once it is introduced.

The risk of nonindigenous species invasions appears particularly high from some particular donor ecosystem regions to specific recipient ecosystem areas, which may result in ‘waves’ of invasions. For example, the Eurasian Ponto-Caspian donor region (Figure 1) has provided many exotic species to the North American Great Lakes over the past 20 years via accidental ballast water introductions. Examples of Great Lakes invasions by Ponto-Caspian taxa include the dreissenid zebra and quagga mussels, as well as the round and tubenose gobies examined here (Mills et al. 1993; Ricciardi and MacIsaac 2000; Grigorovich et al. 2002). Their successful establishment may be attributed to common vector pathways (in this case, shipping routes and ballast water exchange practices; MacIsaac et al. 2001), high number of introduced propagules from native areas with similar habitats for source and sink populations, facilitative interactions among co-evolved invaders – such as round gobies eating zebra mussels (Ghedotti et al. 1995; Simberloff and Van Holle 1999; Ricciardi and MacIsaac 2000), and relatively high genetic diversity – as tested here and suggested by previous work (Dillon and Stepien 2001; Stepien et al. 2002, 2003).

Following the zebra mussel’s appearance in 1988, two members of the Ponto-Caspian subfamily Neogobiinae – the round goby *Neogobius melanostomus* and the tubenose goby *Proterorhinus marmoratus* – invaded Lake St.

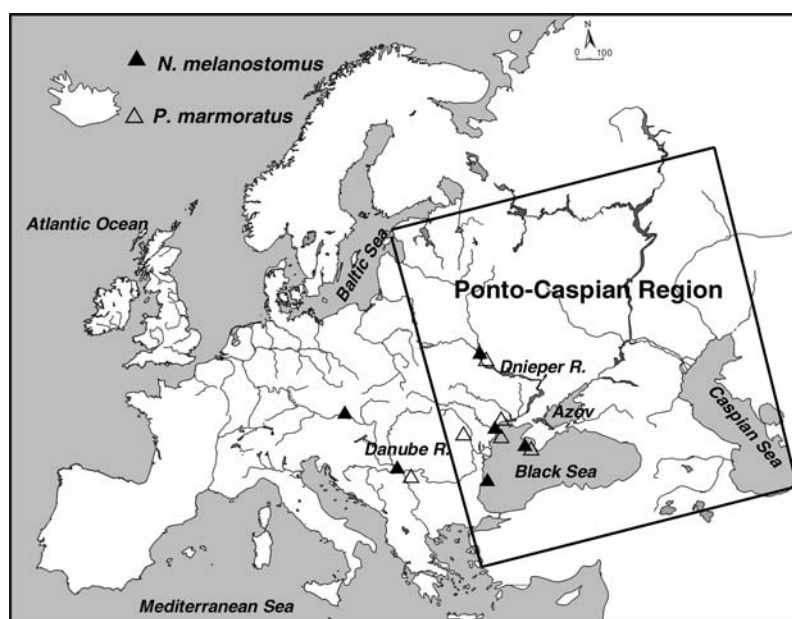


Figure 1. Location of the Ponto-Caspian region and sampling sites for the round goby and the tubenose goby.

Clair in 1990 (Crossman et al. 1992). Like the zebra mussel, the round goby rapidly spread to all five Great Lakes and is now one of the most abundant benthic species (Jude 1997, 2001). The round goby *N. melanostomus* has been more successful at spreading to new habitats in North America and Eurasia and is much more common than is the tubenose goby *P. marmoratus* in both regions (Holcik 1991; Charlebois et al. 1997). Models for predicting invaders (Kolar and Lodge 2001, 2002; Muller 2001) find that congeners with histories of invasions are more likely to invade together or in close sequence (as for neogobiins).

The round and tubenose gobies belong to the species-rich but poorly known subfamily Neogobiinae (Simonovic 1999), which is native to the Ponto-Caspian region (including the Marmara, Black, Azov, Caspian, and Aral Seas; Figure 1). The Neogobiinae comprises about 18 species in three genera (*Neogobius*, *Mesogobius*, and *Proterorhinus*) (see Table 1). Research collections by C. Stepien along the northern Black Sea, Crimean Coast, and Sea of Azov in 2002 commonly found four to seven sympatric neogobiin species in given native locations. Neogobiins are highly adaptable to a variety of habitats, making them excellent invaders (Charlebois et al. 1997; Ahnelt et al. 1998). Several other neogobiin spe-

cies, including the monkey goby *N. fluviatilis*, the bighead goby *N. kessleri*, and the racer goby *N. gymnotrachelus* have been 'on the move' in Eurasian waterways, due to the construction of canals, shipping, and habitat disturbances (Holcik 1991; Ahnelt et al. 1998; Kvach 2001; Kautman 2001). It thus appears likely that additional neogobiin species will invade North America from common shipping ports, or are already 'cryptically' present – but not yet identified. DNA sequence markers will enable their correct and rapid identification if and when they invade.

The objectives of the present study were to: (1) develop DNA markers for distinguishing among neogobiin species, (2) test for 'cryptic' invasive taxa, (3) determine whether the existing taxonomic classifications are systematically correct, (4) compare genotypic composition and levels of genetic variability in the invasive Great Lakes versus native populations, and (5) evaluate the use of these genetic data for risk assessment of invasive species.

Materials and methods

Specimens were collected either by hook-and-line or netting, measured and identified, and then

Table 1. Taxonomic status of the neogobiinae examined in the present study (status based on Simonovic 1999; Froese and Pauly 2004; and the California Academy of Sciences 2004).

Genus	Species	Described by	Common name	Distribution and habitat	Name change proposed by these results
<i>Mesogobius</i>	<i>batrachocephalus</i>	Pallas (1814)	Knout goby	Brackish water; Black and Azov Seas and estuaries	–
<i>Neogobius</i>	<i>cephalargus</i>	Pallas (1814)	Ginger goby	Fresh and brackish water; Black and Azov Seas	–
<i>N.</i>	<i>fluviatilis</i> ^a	Pallas (1814)	Monkey goby	Fresh and brackish; rivers entering Black and Caspian Seas	<i>Apollonia fluviatilis</i>
<i>N.</i>	<i>gymnotrachelus</i> ^a	Kessler (1857)	Racer goby	Fresh and brackish; nw Black and Azov Seas and Dniester R., invasive in Danube R.	–
<i>N.</i>	<i>kessleri</i> ^a	Gunther (1861)	Bighead goby	Fresh and brackish; n. Black Sea and rivers, invasive in Danube R.	–
<i>N.</i>	<i>melanostomus</i> ^a	Pallas (1814)	Round goby	Freshwater, prefer brackish; Black, Azov, Caspian and Marmora Seas and rivers; many introduced sites, e.g., Gulf of Gdansk, upper Danube R. and Great Lakes	<i>Apollonia melanostomus</i>
<i>N.</i>	<i>ratan</i>	Nordmann 1840	ratan goby	Black Sea, near Odessa, Ukraine	–
<i>N.</i>	<i>syrman</i>	Nordmann 1840	Syrman goby	Brackish and marine; Black and Azov Seas	–
<i>Proterorhinus</i>	<i>marmoratus</i>	Pallas 1814	Marine tubenose goby	Brackish and marine and estuaries; Black, Azov and Caspian Seas	–
<i>Proterorhinus</i>	<i>marmoratus</i> ^a	Pallas (1814)	Freshwater tubenose goby	Freshwater rivers; Native to the Danube, Dniester, and Dnieper Rivers (and possibly others) of the Ponto-Caspian region; introduced to Great Lakes	<i>P. semilunaris</i> Heckel 1837

^aCurrently invasively expanding range.

placed directly in 95% ethanol and stored at room temperature. Round gobies analyzed totaled 87 specimens (Table 2), including: 22 from Lake Erie, Ohio, USA; 29 from Lake St. Clair, Michigan, USA (sequenced and originally analyzed by Dougherty et al. 1996); nine from the Danube River at Vienna, Austria; 23 from the Danube River at Prahovo, Yugoslavia; five from the northern Dnieper River at Kiev, Ukraine; 15 from the western Black Sea at Varna, Bulgaria (sequenced and originally analyzed by Dougherty et al. 1996); two from the north-central Black Sea at Odessa, Ukraine; and five from the north-east Black Sea location at Sevastopol, Ukraine (Figure 1). The latter two sampling sites (Odessa and Sevastopol) were grouped in the analyses. Two of the specimens of round gobies from Lake Erie did not have the characteristic dorsal fin spot, and were possible monkey goby (*N. fluviatilis*) suspects.

A total of 44 tubenose goby specimens were analyzed (Table 2), including 11 from the Lake St. Clair region (two of which had been previously sequenced by Dougherty et al. 1996); three from the Danube River at Prahovo, Yugoslavia; one from the Dniester River at Biliavka, Ukraine; five from the Dnieper River at Kiev, Ukraine; six from the Tyligul River estuary north of Odessa, Ukraine in the north-central Black Sea area; 10 from the north-central Black Sea at Odessa, Ukraine; and six from the northeastern Black Sea site at Sevastopol, Ukraine (Figure 1). The Sevastopol site collection was made by Stepien at the type locality of the original description of the tubenose goby *Proterorhinus marmoratus* (Pallas 1814 and California Academy of Sciences website 2004), rendering it an important benchmark systematic comparison.

DNA sequence data were collected for seven additional related taxa for the purpose of

Table 2. Sampling locations and haplotype frequencies for comparative study of variation in the mtDNA cytochrome *b* gene in the round and tubenose gobies.

Species	Body of water	Location	Latitude	Longitude	<i>n</i> fish	Haplotype(s) and relative frequencies
<i>N. (A.) melanostomus</i> Round goby	Lake St. Clair	near Detroit, MI, USA	42°22' N	82°39' W	29*	22A, 5B, 2C, 0.76A, 0.17B, 0.07C
	Lake Erie	South Bass Island, OH, USA	41°50' N	83°00' W	22	11A, 9B, 1C, 1F, 0.50A, 0.40B, 0.05C, 0.05F
	Danube River	Vienna, Austria	48°13' N	16°22' E	23	9A 1.00A
	Danube River	Prahovo, Yugoslavia	44°17' N	22°35' E	19	22A, 1B, 0.96A, 0.04B
	n. Dnieper River	Kiev, Ukraine	50°27' N	30°30' E	5	5A, 1.00A
	w. Black Sea	Varna, Bulgaria	43°14' N	27°58' E	15*	13A, 1D, 1E 0.86A, 0.07D, 0.07E
	n. c. Black Sea	Odessa, Ukraine	46°28' N	30°44' E	2	2A, 1.00A
	n. e. Black Sea	Sevastopol, Crimea, Ukraine	44°34' N	33°34' E	5	4A, 16 0.80A, 0.206
<i>Proterorhinus</i> Tubenose goby	Lake St. Clair	near Detroit, MI, USA	42°22' N	82°39' W	11*	6A, 3B, 2C 0.55A, 0.27B, 0.18C
	Danube River	Prahovo, Yugoslavia	44°17' N	22°35' E	3	1A, 2C 0.33A, 0.67C
	Dniester River	Biliaivka, Ukraine	46°28' N	30°13' E	1	1A 1.0A
	Dnieper River	Kiev, Ukraine	50°27' N	30°30' E	5	3B, 1C, 1D 0.60B, 0.20C, 0.20D
	n.c. Black Sea	Tyligul estuary, Ukraine	46°50' N	31°10' E	6	1G, 4H, 1J 0.17G, 0.66H, 0.17J
	n.c. Black Sea	Odessa, Ukraine	46°28' N	30°44' E	10	7E, 3F 0.70E, 0.30F
	n.e. Black Sea	Sevastopol, Crimea, Ukraine	44°34' N	33°34' E	6	3E, 3I 0.50E, 0.50I

*Data collected by Dougherty et al. (1996).

elucidating the correct phylogenetic relationships for the targeted species, as well as to develop diagnostic sequences for identifying possible 'cryptic' invasive neogobiin species in the Great Lakes and other regions (Table 1). These additional taxa included two specimens each of the ginger goby *Neogobius cephalarges*, monkey goby *N. fluviatilis*, racer goby *N. gymnotrachelus*, bighead goby *N. kessleri*, ratan goby *N. ratan*, syrman goby *N. syrman*, and knout goby *Mesogobius batrachocephalus*. All specimens were collected from the Danube, Dniester, and/or Dnieper rivers draining into the Black Sea, Ukraine and the Black Sea (the sites in Figure 1).

Genomic DNA was extracted from muscle tissue and purified using the DNeasy tissue kit and protocol (Qiagen, Inc., Valencia, CA). We amplified the mitochondrial DNA cytochrome *b* gene from neogobiin gobies (1150 bp), and here report on sequence data from 453 bp of the left domain. We relate our data with those previously published by Dougherty et al. (1996) on the same DNA region for two tubenose goby specimens from Lake St. Clair and for 37 round gobies

from Lake St. Clair and the western Black Sea at Varna, Bulgaria (Figure 1). The cytochrome *b* gene codes for a transmembrane protein functional in the electron transport chain of cellular respiration in mitochondria, and has been widely used in fish population genetic and species relationship studies (reviewed by Stepien and Kocher 1997). Variation at these lower taxonomic levels, as analyzed in the present study, is largely found in the third codon 'wobble' position (reviewed in Palumbi 1997; Stepien and Kocher 1997).

The cytochrome *b* gene was amplified using the polymerase chain reaction (PCR), with conserved primers adapted from Dougherty et al. (1996), including Gludg-L14724 (Meyer et al. 1990), H15149 (Kocher et al. 1989), and CB₆Thr-H (Martin et al. 1992). PCR parameters included 1 min denaturation at 94 °C, 30 s annealing at 50 °C, and 2 min extension at 72 °C, for 38 cycles, followed by a final extension step for 5 min. The PCR products were purified using Exosap kits (U.S. Biochemical Corp., Inc., Solon, OH). DNA sequencing reactions were performed separately in both directions (for independent verification) using the PCR prim-

ers and cycle sequencing, following manufacturer's directions (CEQ DTCS kits, Beckman-Coulter, Inc., Fullerton, CA). Sequences were determined using a Beckman-Coulter CEQ 8000 capillary autosequencer at the Cleveland State University DNA Analysis Facility. Sequences were aligned using Se-Al: Sequence Alignment Editor (Rambaut 1996) and manual adjustments were made employing amino acid alignments and parsimonious procedures.

Phylogenetic relationships among neogobiins were analyzed from 453 bp of the left domain of the cytochrome *b* gene using maximum parsimony and a branch-and-bound search in PAUP* 4.0 vers. B10 (Swofford et al. 1996; Swofford 2003), with the Mediterranean gobies *Gobius niger* and *Zosterisessor ophiocephalus* (belonging to the putative 'sister' group, Gobiinae; Simonovic 1999) designated as outgroups (and also sequenced by us). Relative support of the data set for relationships were evaluated using 1000 bootstrap replications (Felsenstein 1985) and a branch-and-bound search (Swofford et al. 1996). Multiple most-parsimonious trees were evaluated using strict and 50% majority rule consensus analyses (Margush and McMorris 1981; see Swofford et al. 1996). Kimura (1980) two-parameter genetic distances were calculated between each pair of taxa, clustered using the neighbor-joining (Saitou and Nei 1987) algorithm, and evaluated with 1000 bootstrap replications in MEGA vers. 2.1 (Molecular Evolutionary Genetics Analysis; Kumar et al. 2001).

Comparative measures of genetic variability for sampling sites included gene diversity = haplotypic diversity (h), nucleotide diversity (π), and the mean number of pairwise differences among haplotypes (all following Nei 1987), calculated in Arlequin 2.2 (Schneider et al. 2003). Relationships among population sites for the round and tubenose gobies, respectively, were evaluated using Wright's (1978) *Fst* analog estimates and corresponding probability values (from 1000 permutations). Global tests of differentiation between pairs of samples were determined following Raymond and Rousset (1995), based on an exact nonparametric procedure and probabilities estimated using the Markov chain method. This method should yield

similar results to the *Fst* divergence values. Comparative population sizes (θ) between pairs of sampling sites and probability values for significant differences were estimated following Gaggiotti and Excoffier (2000). Hierarchical partitioning of genetic variation (% Variance) among groups of populations, population samples within groups, and variation within sampling sites were evaluated using Analysis of Molecular Variance (AMOVA) in Arlequin 2.2 (Schneider et al. 2003). Migration estimates (Nm) between sampling sites were calculated according to Slatkin (1995).

We compared the results from this study with earlier results obtained by the Stepien laboratory from some of the populations of round and tubenose gobies using mtDNA control region sequences (Dillon and Stepien 2001). We related our genetic data findings to risk analysis using the elements developed by the Aquatic Nuisance Species Task Force (ANSTF 1996; summarized in Nicholls and Tumeo 2001).

Results

Phylogenetic relationships of Neogobiins and distinguishing genetic markers

DNA sequences from 453 bp of the left domain of the mtDNA cytochrome *b* gene generated species-specific characters, which can be used to identify them at all life history stages. Sequences are deposited in the NIH GenBank (<http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html>) as Accession numbers AY884588 for the ginger goby *N. cephalarges*, AY884589 for the monkey goby *Neogobius* (to be changed to *Apollonia*; see further Results and Discussion) *fluviatilis*, AY884589 for the racer goby *N. gymnotrachelus*, AY884587 for the bighead goby *N. kessleri*, AY884585 for the ratan goby *N. ratan*, AY884586 for the syrman goby (*N. syrman*), and AY884590 for the knout goby *Mesogobius batrachocephalus*. In addition, haplotypes A-J for the tubenose goby were deposited in GenBank as Accession numbers AY884572–884581. Haplotypes A–D number AY884572–884575 (with A as AY884572 corresponding to A in Dougherty et al. 1996) and

constitute the freshwater clade. Haplotypes E–J number AY884576–884581 and include the marine and estuarine *P. marmoratus*. Haplotypes F and G for the round goby *N.* (to be changed to *Apollonia* here; see further Results and Discussion) *melanostomus* are Accession numbers AY884582–3. Haplotypes A for *P. marmoratus* (to be designated as *P. semilunaris*; see further Results and Discussion) and A–E for *N.* (now *Apollonia*) *melanostomus* were previously deposited in GenBank by Dougherty et al. (1996). In addition, cytochrome *b* gene sequences for the gobiin outgroup species the black goby *Gobius niger* and the grass goby *Zosterisessor ophiocephalus* were deposited by us as GenBank Accession numbers AY884591 and AY884592, respectively.

Phylogenetic relationships among 22 taxa; including seven haplotypes of the round goby *Neogobius* (to be changed to *Apollonia*) *melanostomus*, six haplotypes of tubenose goby *Proterorhinus*, seven other neogobiin species (see Table 1), and two outgroups; from maximum

parsimony analysis using a branch-and-bound search revealed two most parsimonious trees (Figure 2). The trees had 493 steps and 148 variable characters were parsimony-informative. Three primary clades were revealed: (1) *N.* (*A.*) *melanostomus* as the sister group to *N.* (*A.*) *fluviatilis*, which in turn forms the sister group to all remaining Neogobiinae, including; (2) *Proterorhinus* (with *Mesogobius batracocephalus* located intermediate between clades 2 and 3, and the relationships between those genera were unresolved between the two most-parsimonious trees), and (3) the remaining *Neogobius* species; with *N. cephalarges* as the basal taxon, and *N. ratan* and *N. kessleri* as the sister group to a clade comprising *N. gymnotrachelus* and *N. syrman*. Results indicated that the neogobiin genus *Neogobius* is paraphyletic, consisting of two separate clades that are not each other's sister group (see Figure 2). The neighbor-joining tree (Figure 3) details genetic distance relationships among the *Proterorhinus* haplotypes.

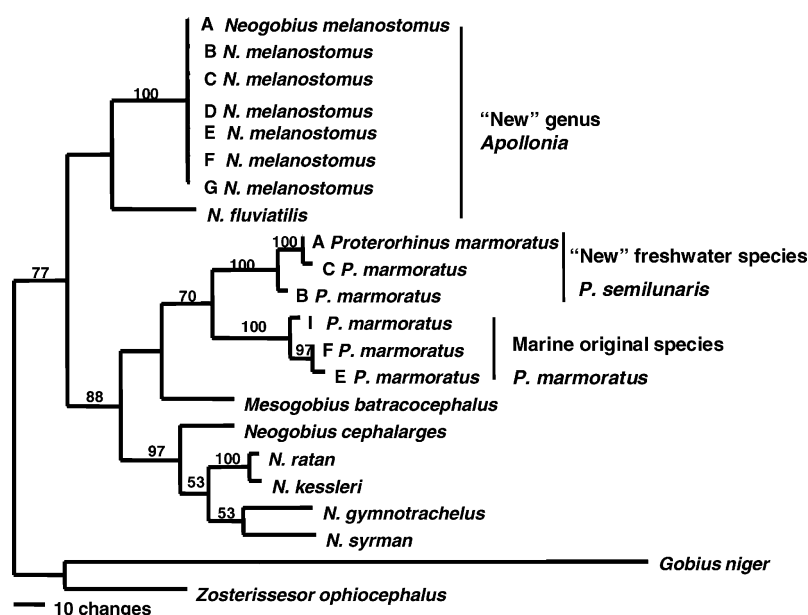


Figure 2. Consensus of two most-parsimonious trees of neogobiin relationships from partial cytochrome *b* sequences using PAUP* branch-and-bound search and 1000 bootstrap replications (numbers at nodes). (Swofford 2003; 453 bp). 493 steps, CI = 0.68, RI = 0.75, CI excluding uninformative characters = 0.56, 148 parsimony informative characters. Taxa names to the right of the vertical bars are proposed name changes based on our results. Note: *Neogobius* is paraphyletic, indicating that the subgenus *Apollonia* should be elevated to include *N.* (*A.*) *melanostomus* and *N.* (*A.*) *fluviatilis*. Tree discerns two putative species for *P. marmoratus* – the top clade is the ‘new/to be resurrected’ freshwater species (defined by 15 synapomorphies) and the bottom clade is marine (including the type locality of Sevastopol, Crimea, Black Sea; defined by 17 synapomorphies). Additional haplotypes of *P. marmoratus* are included in Figure 3.

A 'cryptic' species of tubenose goby

Our results showed that the tubenose goby *Proterorhinus marmoratus* as presently recognized comprises two genetically divergent species (Figures 2 and 3). Haplotypes A–D (found in Lake St. Clair of the Great Lakes and eastern European rivers that drain into the Black Sea) significantly diverged from a clade comprising haplotypes E–J (found in the Black Sea and its brackish estuaries) (Figure 3). The marine taxon (E–J) was characterized by 15 nucleotide synapomorphies, and the freshwater taxon (A–D) had 17 synapomorphies.

The two tubenose goby species each had two to three haplotypes per sampling location. All locations samples had similar gene diversity values (Table 3A). The freshwater and marine clades diverged significantly in *Fst* (Table 4) and global differentiation tests (Tables 4 and 5), and 71% of the overall AMOVA variation in the group was found between the two clades ($P < 0.0001$). Together, these results indicate significant divergence at the species level for the two clades of tubenose gobies.

Population genetics of tubenose gobies

In the freshwater taxon, two haplotypes were shared between each pair of primary sampling locations, with A comprising 55% of the sample

in Lake St. Clair and 33% of the sample from the Danube River at Prahovo, Yugoslavia – as well as the single sample analyzed from the Dniester River (Table 2). In addition, type B represented 27% of the Lake St. Clair sample and 60% from the Dnieper River location. Haplotype C made up 67% of the individuals from the Danube River, 20% in the Dnieper River, and 18% of the Great Lakes sample. All haplotypes in the freshwater collections were closely related (Figures 2 and 3).

The two Black Sea sampling locations of the marine taxon (corresponding to the original *P. marmoratus*) shared haplotype E (Table 2), which constituted 70% of the sample in the north-central Black Sea (at Odessa, Ukraine) and 50% of the individuals from the north-eastern Black Sea location (at Sevastopol, Ukraine). In addition, the Odessa site contained 30% of the F haplotype and the Sevastopol location had 50% of the I type. The estuarine sample from the Tyligul River estuary north of Odessa had three unique haplotypes that were most closely related to type I from the Sevastopol region of the Crimea in the Black Sea. The sample from the Tyligul estuary thus was more closely related to the marine types than to the freshwater haplotypes (Table 2).

Nucleotide diversity values were highest for sites from the Black Sea, which also had larger pairwise distances among their haplotypes than did the freshwater locations (Table 3A). The

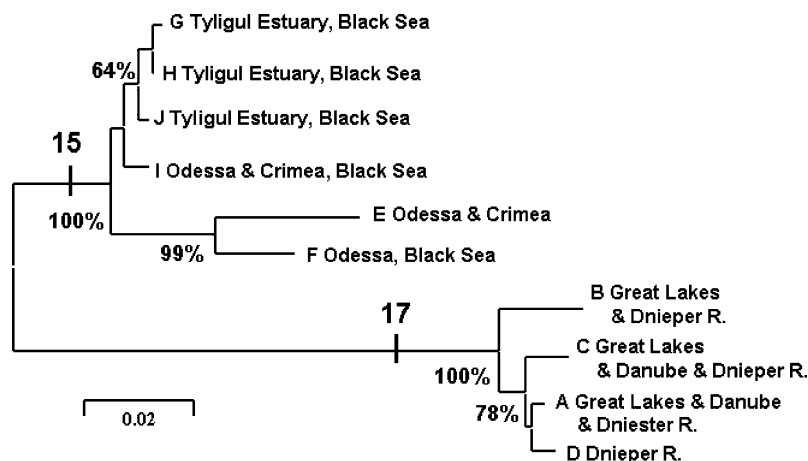


Figure 3. Neighbor-joining tree of relationships among *Proterorhinus* haplotypes from partial cytochrome *b* sequences, constructed using Kimura (1980) two-parameter genetic distances in MEGA 2.1 (Kumar et al. 2001), with 1000 bootstrap replications (support for nodes indicated as %). Vertical bars denote the number of nucleotide synapomorphies distinguishing the two primary clades.

Table 3. Genetic diversity measures at primary sampling sites for the tubenose (A) and round gobies (B).

Genetic Diversity measure	Lake St. Clair, Michigan, USA	Danube River, Prahovo, Yugoslavia	Dnieper River Kiev, Ukraine	Tyligul Estuary, Odessa, Ukraine	NC Black Sea, Odessa, Ukraine	NE Black Sea, Sevastopol, Ukraine
<i>A. Tubenose goby</i>						
<i>N</i> haplotypes	3	2	3	3	2	2
Gene diversity (<i>h</i>)	0.654 ± 0.112	0.667 ± 0.314	0.700 ± 0.210	0.600 ± 0.215	0.467 ± 0.132	0.600 ± 0.129
Nucleotide diversity (π)	0.0123 ± 0.0072	0.0059 ± 0.0053	0.0167 ± 0.0109	0.0044 ± 0.0033	0.0394 ± 0.0217	0.0720 ± 0.0425
Mean <i>N</i> pairwise differences	5.563 ± 2.895	2.667 ± 1.919	7.500 ± 4.223	2.004 ± 1.303	17.733 ± 8.617	32.400 ± 16.546
<i>B. Round goby</i>						
	Lake St. Clair Michigan, USA	Lake Erie Ohio, USA	Danube River at Vienna, Austria and Prahovo, Yugoslavia	Dnieper River Kiev, Ukraine	w Black Sea Varna, Bulgaria	n Black Sea Odessa and Sevastopol, Ukraine
<i>N</i> haplotypes	3	4	2	1	3	2
Gene diversity (<i>h</i>)	0.404 ± 0.100	0.606 ± 0.062	0.063 ± 0.058	0.000 ± 0.000	0.467 ± 0.132	0.286 ± 0.196
Nucleotide diversity (π)	0.0096 ± 0.0010	0.0015 ± 0.0014	0.0001 ± 0.0003	0.0000 ± 0.0000	0.0006 ± 0.0008	0.0006 ± 0.0009
Mean <i>N</i> pairwise differences	0.429 ± 0.399	0.688 ± 0.0543	0.063 ± 0.0004	0.000 ± 0.000	0.267 ± 0.309	0.286 ± 0.341

Table 4. Genetic divergences between pairs of sampling sites for the tubenose goby. *Fst* values and probabilities are below the diagonal and *Nm* estimates of migration are above the diagonal (Slatkin 1995).

Sampling location	Lake St. Clair, Great Lakes, Michigan, USA	Rivers draining into Black Sea (Danube, Dniester, and Dnieper) Yugoslavia and Ukraine	NC Black Sea Odessa and Tyligul Estuary, Ukraine	NE Black Sea Sevastopol, Ukraine
Lake St. Clair	–	infinite	0.150	0.118
Black Sea Rivers	0.078 <i>P</i> < 0.811 N.S.	–	0.165	0.140
NC Black Sea	0.769** <i>P</i> < 0.00001	0.752** <i>P</i> < 0.00001	–	1.76
NE Black Sea	0.809** <i>P</i> < 0.00001	0.782** <i>P</i> < 0.00001	0.221 <i>P</i> < 0.048*	–

N. S., not significant.

***Fst* significant after Bonferroni correction for multiple post-hoc tests.

*Significant at *P* < 0.05 level, without Bonferroni correction (e.g., needs larger sample size to determine).

Table 5. Comparative population sizes and differentiation between pairs of sampling sites for the tubenose goby. Below diagonal: Ancestral population sizes (θ) comparisons and probability values (Gaggiotti and Excoffier 2000). Above diagonal: Global test of differentiation (Raymond and Rousset 1995).

Sampling location	Lake St. Clair, Great Lakes Michigan, USA	Black Sea Rivers (Danube, Dniester, and Dnieper) Yugoslavia and Ukraine	NC Black Sea Odessa and Tyligul Estuary, Ukraine	NE Black Sea Sevastopol, Ukraine
Lake St. Clair	–	<i>P</i> < 0.474 N.S.	<i>P</i> < 0.0001**	<i>P</i> < 0.004**
Danube and Dnieper Rivers	5.479 <i>P</i> < 0.872 N.S.	–	<i>P</i> < 0.0010**	<i>P</i> < 0.001**
NC Black Sea	26.372 <i>P</i> < 0.770 N.S.	27.564 <i>P</i> < 0.937 N.S.	–	<i>P</i> < 0.050*
NE Black Sea	35.551 <i>P</i> < 0.986 N.S.	27.509 <i>P</i> < 0.983 N.S.	32.461 <i>P</i> < 0.167 N.S.	–

N.S., not significant.

*Significant at *P* < 0.05 level, without Bonferroni correction (e.g., may need a larger sample size to determine).

** Significant after Bonferroni correction for multiple post-hoc tests.

largest nucleotide diversity characterized the Sevastopol, Ukraine location. The global test of genetic differentiation between the Black Sea sites suggested negligible divergence, with migration estimated as less than two individuals per generation (Tables 4 and 5). The samples from the Danube, Dniester, and Dnieper Rivers were combined for analysis of pairwise differentiation, due to low sample sizes at present (Table 4). Estimates of ancestral population sizes (θ) did not significantly differ between pairs of sampling sites, suggesting that the populations were approximately equivalent (Table 5). AMOVA tests showed that 12.24% of the overall genetic variation was found among the pairs of populations in the two regions (freshwater and marine), which was significant ($P < 0.001$).

Population genetics of round gobies

Seven haplotypes of round gobies were detected, of which A (originally described by Dougherty et al. 1996) was the most common overall and was prevalent in all sampling locations – freshwater and marine, as well as nonindigenous and native sites (Table 2). Haplotype A predominated in all European locations, including marine and freshwater sites. Sites in the Great Lakes had less proportional representation of type A. All other haplotypes diverged from A by a single point mutation each (Figure 2), two at first codon positions and four at third codon positions. Lake St. Clair in the Great Lakes housed three haplotypes (A, B, and C), and Lake Erie contained four types (A, B, C, and F), with type B being much more common in Lake Erie than in Lake St. Clair (Table 2). Two round gobies without the characteristic dorsal spot from central Lake Erie were haplotypes A and F. Those samples also corresponded morphologically to *N. melanostomus*.

Haplotype B was very rare in the Danube River sample at Prahovo, Yugoslavia and neither types C nor F were detected in the European locations. The sampling location in the western Black Sea (Varna, Bulgaria) also had three types (A, D, and E; described by Dougherty et al. 1996), of which the latter two were unique to that area. Most other locations sampled in Europe had two haplotypes, with sites from the

Danube River at Vienna, Austria and the north-central Black Sea at Odessa, Ukraine appearing monomorphic for type A (which may be due to low sample size). The north-eastern Black Sea sample (Sevastopol, Crimea, Ukraine) housed a rare haplotype (G), in addition to type A.

Gene diversity (Table 3B) was greatest in the Lake Erie population, and was next highest in the sample from Lake St. Clair and in the western Black Sea at Varna, Bulgaria. Nucleotide diversity and the mean number of pairwise differences also were highest in the two Great Lakes locations. In contrast, the European freshwater locations in the Danube and Dnieper Rivers had very low values of gene diversity, nucleotide diversity, and mean numbers of pairwise differences (Table 3B). The sample in Lake St. Clair appeared close to those from the northern Black Sea and the Dnieper River in genetic composition, whereas the Lake Erie sample was least divergent from the Dnieper River (Tables 6 and 7).

Global tests (Raymond and Rousset 1995) showed significant pairwise differentiation between samples from the Great Lakes vs the Danube River and between Lake Erie and the western Black Sea (Table 7), with the Danube River population estimate being appreciably smaller. Migration (Nm) estimates between pairs of sampling sites were appreciable (Table 6), especially between Lake St. Clair and the Dnieper River, the Danube and the Dnieper Rivers, and the Black Sea locations. Migration was least between the Danube River and Lake Erie, and between the western Black Sea site and Lake Erie. These results may suggest that the Lake St. Clair and Lake Erie populations of round gobies have different founding sources, but larger sample sizes are needed.

Estimates of ancestral population sizes (θ) showed that the Danube River sample differed significantly from the Dnieper River as well as the northern Black Sea, with the Danube River population being smaller (Table 7). Other significant differences occurred between the northern Black Sea vs the Dnieper River and the western Black Sea, with the northern Black Sea population estimated as larger in size (Table 7).

AMOVA analyses showed that 10% of the genetic variation in the round goby was partitioned among the three groups (Great Lakes,

Table 6. Genetic divergences between pairs of sampling sites for the round goby. *Fst* values and probabilities (below diagonal) and *Nm* estimates of migration above diagonal (Slatkin 1995) for the round goby.

Sampling location	Lake St. Clair, Michigan, USA	Lake Erie, Ohio, USA	Danube River at Vienna, Austria and Prahovo, Yugoslavia	Dnieper River Kiev, Ukraine	w Black Sea, Varna, Bulgaria	n Black Sea, Odessa and Sevastopol, Ukraine
Lake St. Clair	–	7.285	6.981	Infinite	7.668	10.545
Lake Erie	0.0642 <i>P</i> < 0.099 N.S.	–	1.169	2.878	1.718	2.210
Danube River	0.067 <i>P</i> < 0.037*	0.300 <i>P</i> < 0.0001**	–	Infinite	17.049	3.859
Dnieper River	0.026 <i>P</i> < 0.543 N.S.	0.148 <i>P</i> < 0.157 N.S.	0.999 <i>P</i> < 0.101 N.S.	–	Infinite	Infinite
w Black Sea	0.061 <i>P</i> < 0.054 N.S.	0.225 <i>P</i> < 0.005**	0.028 <i>P</i> < 0.256 N.S.	0.100 <i>P</i> < 0.999 N.S.	–	178.750
n Black Sea	0.045 <i>P</i> < 0.198 N.S.	0.184 <i>P</i> < 0.051 N.S.	0.115 <i>P</i> < 0.307 N.S.	0.055 <i>P</i> < 0.999 N.S.	0.003 <i>P</i> < 0.703 N.S.	–

N. S., not significant.

*Significant at *P* < 0.05 level, without Bonferroni correction (e.g., needs larger sample size to determine).

***Fst* significant after Bonferroni correction for multiple post-hoc tests.

Table 7. Comparative population sizes and differentiation between pairs of sampling sites for the round goby, *Neogobius (Apollonia) melanostomus*. Below diagonal: Ancestral population sizes (θ) comparisons and probability values (Gaggiotti and Excoffier 2000). Above diagonal: Global test of differentiation using exact *P*-values (Raymond and Rousset 1995).

Sampling location	Lake St. Clair, Michigan, USA	Lake Erie, Ohio, USA	Danube River at Vienna, Austria and Prahovo, Yugoslavia	Dnieper River, Kiev, Ukraine	w Black Sea, Varna, Bulgaria	n Black Sea, Odessa and Sevastopol, Ukraine
Lake St. Clair	–	<i>P</i> < 0.109 N.S.	<i>P</i> < 0.038*	<i>P</i> < 0.070 N.S.	<i>P</i> < 0.059 N.S.	<i>P</i> < 0.191 N.S.
Lake Erie	0.880 <i>P</i> < 0.146 N.S.	–	<i>P</i> < 0.000**	<i>P</i> < 0.300 N.S.	<i>P</i> < 0.002**	<i>P</i> < 0.059 N.S.
Danube River	0.669 <i>P</i> < 0.171 N.S.	0.738 <i>P</i> < 0.149 N.S.	–	<i>P</i> < 1.000 N.S.	<i>P</i> < 0.244 N.S.	<i>P</i> < 0.324 N.S.
Dnieper River	0.000 <i>P</i> < 1.000 N.S.	0.000 <i>P</i> < 1.000 N.S.	0.000 <i>P</i> < 0.0001**	–	<i>P</i> < 1.000 N.S.	<i>P</i> < 1.000 N.S.
w Black Sea	0.469 <i>P</i> < 0.437 N.S.	0.694 <i>P</i> < 0.258 N.S.	0.289 <i>P</i> < 1.000 N.S.	0.000 <i>P</i> < 1.000 N.S.	–	<i>P</i> < 0.709 N.S.
n Black Sea	0.457 <i>P</i> < 0.402 N.S.	0.693 <i>P</i> < 0.209 N.S.	0.009 <i>P</i> < 0.0001**	0.000 <i>P</i> < 0.0001**	1.482 <i>P</i> < 0.0001**	–

N.S., not significant.

*Significant at *P* < 0.05 level, without Bonferroni correction (e.g., needs larger sample size to determine).

**Significant after Bonferroni correction for multiple post-hoc tests.

European Rivers (Danube and Dnieper), and the Black Sea), which was not significant. Variation within the pairs of samples per group comprised 4.72% overall, which was not significant. The majority of the genetic variation in the round goby (85.26%) was found within the population samples.

Discussion

Systematic revision of Neogobiinae and diagnostic species characters

The phylogenetic trees indicated that the neogobiin genus *Neogobius* is paraphyletic, and

thus the subgenus name *N. Apollonia* (Vasil'eva et al. 1993), which contains the species *N. melanostomus* and *N. fluviatilis*, needs to be elevated to the level of genus (Harold Ahnelt, pers. commun.). According to our phylogenetic analyses, the newly elevated genus *Apollonia* is the sister group to all of the remaining neogobiins that have been analyzed (including *Proterorhinus*, *Mesogobius*, and the newly restricted *Neogobius* clade (see Figures 2 and 3). Within *Apollonia*, a sister relationship was discerned between *A. (N.) melanostomus* and *A. (N.) fluviatilis*, which is congruent with relationships based on osteological characters by Vasil'eva et al. (1993) and Simonovic (1999). Three primary clades were resolved within the subfamily Neogobiinae, as follows: (1) *A. (N.) melanostomus* and *A. (N.) fluviatilis* = new genus *Apollonia* (elevated from the subgenus level of Vasil'eva et al. 1993), (2) *Proterorhinus* – now to be designated as two species, and (3) the remaining species of *Neogobius*. Distinguishing nucleotide characters for all species can be used to elucidate among neogobiins at all life history stages and to identify invasive 'unknown' taxa (see GenBank Accession numbers in Results).

Phylogenetic results revealed marked and significant genetic divergence between the freshwater and marine clades of tubenose gobies, indicating that they comprise two separate species. The original type locality for the tubenose goby *P. marmoratus* Pallas (1814) is from Sevastopol, Crimea, Ukraine – which also was sampled in our study. The 'newly-found' 'cryptic' species appears to have originally been described as *P. semilunaris* Heckel (1837) according to records at California Academy of Sciences (2005) and consultation with Dr Harald Ahnelt (pers. commun.). At present, we thus recommend that *Proterorhinus semilunaris* Heckel (1837), which is the oldest name for a freshwater tubenose goby, be resurrected for the freshwater tubenose goby. The species *Proterorhinus semilunaris* was originally described from the Maritsa River in the Aegean region (Heckel 1837) and then from a stream near the Danube River estuary (Nordmann 1840). It was placed in synonymy with *P. marmoratus* by Berg (1949), along with other freshwater and marine names for variants of *Proterorhinus*. Specimens of tubenose goby from the North American Great Lakes region, as well as those from freshwater

European habitats – including the Danube, Dniester, and Dnieper Rivers – are thus likely *P. semilunaris*, pending further examination and description. We also recommend that the common names 'freshwater tubenose goby' be used for *P. semilunaris* and 'marine tubenose goby' for *P. marmoratus*.

Our results demonstrated a marked genetic divergence distinguishing these species, with *P. semilunaris* in freshwater habitats and *P. marmoratus* in estuarine and saline waters. This description is being completed by Stepien and graduate student Matthew Neilson, based on morphological and genetic analysis of specimens and syntypes of *P. semilunaris*, obtained from the Vienna Museum of Natural History.

Genetic diversity in invasive vs native population sites

Results from the present study showed that both the tubenose goby and round goby samples from the Great Lakes have high genetic diversity values. Both nonindigenous species introductions revealed genetic diversity values equivalent to native European populations. There thus was no evidence apparent for founder effects or bottlenecks in either species. These results suggest that the populations of round and tubenose gobies in the Great Lakes were founded by a very large number of diverse individuals. Similar results were shown by our studies of genetic diversity in invasive zebra and quagga mussel populations in the Great Lakes vs native areas in Eurasia (Stepien et al. 2002, 2003, 2005).

In addition, samples of round gobies from the adjacent Great Lakes St. Clair and Erie differed somewhat, which may suggest different founding sources but needs to be tested with larger sample sizes. However, an earlier study by Dillon and Stepien (2001) of those populations using the more-quickly evolving mtDNA control region sequences revealed significant differences between the Lake St. Clair and Lake Erie samples. These congruent results from two different gene regions suggest a possibility of separate founding events for each lake. Similarly, zebra and quagga mussel populations in the Great Lakes showed significant genetic divergences among sites, as well as differential relationships with putative Eurasian

founding source populations, suggesting that more than one colonization source was involved. Thus, in both the round goby and the zebra mussel – two of the largest and most successful invasions in the Great Lakes – multiple founding sources and nearly simultaneous introductions in different areas from different colonizing source populations may have characterized the invasions. This high genetic variability appears to have led to their ability to quickly adapt to a variety of conditions in their new habitats, as well as to spread into new areas.

After colonization, spatial segregation of the dispersal genotypes may foster local adaptation, increasing reproductive isolation of the new populations and reducing between-location migration (Mathias et al. 2001). Such spatial segregation may have resulted from multiple introductions from different Eurasian source populations in different areas of the Great Lakes for zebra and quagga mussels (Stepien et al. 2002, 2003, 2005) and the round goby (Dillon and Stepien 2001; Stepien et al. 2005), as revealed by significant genetic divergences among population areas. In addition to physical isolation, local differential selection might limit their later dispersal and introgressive hybridization among areas, enhancing the genetic and ecological differences among the populations (Bernatchez 2001). These factors may lead to increased divergence among regions of an invasion that have some geographic barriers to gene flow.

Characteristics of successful invasions and predictions

Regions with histories of anthropogenic and/or natural disturbances are more susceptible to successful nonindigenous species invasions (Williamson 1996, Ross et al. 2001). Both the geologically young Great Lakes and the Ponto-Caspian regions have histories of climatic instability, dramatic basin and waterway changes, and more recently – heavy metal, pesticide, fertilizer, and run-off pollution. The round goby has particularly high pollution tolerance (Moskal'kova 1996; Landrum and Fisher 1998), which may aid its success in new and marginal habitats. In addition, results of ecological studies predict that

invasion-prone communities have lower overall species diversities and offer greater niche opportunities (Elton 1958; Case 1990; Moulton and Pimm 1993; Shea and Chesson 2002).

The round goby prefers brackish waters in its native habitats (Charlebois et al. 1997), although it is confined to freshwater in its present North American invasive range. Our results found that round gobies sampled from freshwater habitats had greater genetic diversity than in marine habitats. The most common haplotype 'A' was abundant in both freshwater and marine areas. The round goby thus appears likely to extend its range into North American salt marsh and estuarine habitats, where it may significantly impact marine and estuarine benthic fish communities. The round goby will encounter an abundant food supply in native blue mussels along North American coasts, to which they are pre-adapted (since they feed on native *Mytilus* congeners in their Black and Caspian Sea marine/estuarine habitats; personal observation and field data collected by C. Stepien).

Both North American invasive species also have been extending their ranges and invading new areas in Eurasia, including traveling up the Danube River (Ahnelt et al. 1998), where some samples were analyzed in the present study (Figure 1). In contrast to the Great Lakes samples, both the Danube and Dnieper River samples of the round goby had lower genetic diversity values, which may indicate a founder effect. In addition, the round goby invaded the Gulf of Gdansk, Baltic Sea, Poland in 1990 (Skora and Stolarski 1993) – about the same time as its discovery in the Great Lakes. Our mtDNA sequence study from the control region (Dillon and Stepien 2001) discerned that the Gulf of Gdansk and North American invasions had separate founding sources, which are presently unknown, since the sites shared no haplotypes and both had few in common with the NW Black Sea region. The genetic diversity of the Gulf of Gdansk samples were about half that of the Great Lakes samples (Dillon and Stepien 2001). The sources for each invasive population are being sought from statistical comparisons with our collections spanning the Ponto-Caspian region in further study by the Stepien laboratory.

Implications for risk analysis and invasive species management

The US National Oceanic and Atmospheric Administration (NOAA) is charged with the management of the Great Lakes aquatic invasive species through the Non-indigenous Aquatic Nuisance Prevention and Control Act (NANPCA) (summarized in Nicholls and Tumeo, 2001). The US Department of Commerce (through NOAA) and the US 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 non-indigenous aquatic organism risk analysis review process for estimating risks from the introduction of non-indigenous aquatic organisms. The objective of this generic process is to provide a standardized process for evaluating the risk of introducing non-indigenous 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 decision makers.

A significant advantage of the genetic data collected in this study is their application in determining the risk of invasion from related species. Table 8 lists the elements as detailed in the guidance document (ANSTF 1996) 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 examination of 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. The type of genetic data presented in this study can greatly assist in targeting efforts in this regard when one recognizes 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 is also likely that other neogobiin taxa will be introduced and established, as there is a high probability that there will be multiple introductions as clearly indicated by the genetic data in this study. Our investigation, however, indicates that atypical round goby *A. (N.) melanostomus* morphotypes in the Great Lakes are *A. (N.) melanostomus* and not the monkey goby 'sister species' *A. (N.) fluviatilis*. This suggests that the genetic differences between these two species may warrant further investigation and could provide additional genetic bases for the analysis of "invasiveness."

Conclusions and summary

This investigation developed species and population identification genetic markers to allow invasive neogobiin taxa to be described accurately at all life history stages (including eggs and larvae). Correct species and founding population source identifications are necessary for monitoring the invasions, making valid ecological comparisons, and formulating methods of prevention and control. This genetic study, as well as others by the Stepien laboratory to date reveal that invasions in the Great Lakes by Ponto-Caspian species – including the zebra mussel, quagga mussel, round goby, and tubenose goby – are characterized by high levels of genetic diversity, comparable to that of native populations, which appears important to their invasive successes (Dillon and Stepien 2001; Stepien et al. 2002, 2003, 2005, and here).

Risk analysis of the 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. In addition, the populations of round gobies in the Great Lakes – as

Table 8. Application of genetic data to risk assessments.

Element (per ANSTF guidance)	Important considerations in assessing element	Application of data collected in this study
<i>Section I: Probability of organism establishment</i>		
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. This strongly suggests that more than one founding source population was involved and that each likely had separate origins. Hence, related species in the areas of origin could be considered to 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 life cycle 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).	
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.	
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.	Similar results have been found in zebra and quagga mussels (Stepien et al. 2002, 2003, 2005). 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.
<i>Section II: Consequence of Establishment</i>		
Estimate economic impact if established.	Economic importance of hosts, damage to crop or natural resources, effects to subsidiary industries, exports, and control costs.	The risk consequence indicated by genetic data – i.e., 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.
Estimate environmental impact if established	Ecosystem destabilization, reduction in biodiversity, reduction or elimination of keystone species, reduction or elimination of endangered/threatened species, and effects of control measures, 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	Genetic data not applicable

well as zebra and quagga mussels (Stepien et al. 2002, 2003) – have appreciable population structure, which may enhance their persistence and ability to adapt to new environments. It is likely that the round goby will spread to estuarine and salt marsh habitats in North America from the Great Lakes region. It is also predicted that other neogobiin congeners will invade from the Ponto-Caspian region, and be successful, if ballast water regulations are not strictly enforced.

Acknowledgements

National Science Foundation grant DEB-0349964, the Lake Erie Protection Fund # 02-04, the USEPA, and the Cleveland State University Environmental Risk Analysis Project through the Centre for Environmental Science, Technology and Policy generously supported this work. We greatly appreciate the help of Igor Grigorovich, Jeffrey Gunderson, John Hageman, Juraj Holcik, Douglas Jensen, David Jude, Kevin Kayle, Carey Knight, Julia Kornijchuck, Yuriy Kvach, Vladimir Kovac, J. Ellen Marsden, Jeffrey Ram, Svetlana Rudnicka, Mariusz Sapota, Predrag Simonovic, and A. Smirnov in obtaining specimens. We additionally thank Elizabeth Whippo-Cline, Sandra Justin, Jeffrey Busch, and Jeffrey Reutter for logistical assistance. Technician Clifford Taylor and graduate students Matthew Neilson, Alison Dillon-Klika, W. Calvin Borden, Toriano Bowens, and Kora Dabrowska helped with raw data acquisition at various stages of the project. Graduate student Alex Ford and colleague Igor Grigorovich helped with the map designs. Melissa Blum of the CSU DNA Analysis Facility ran the DNA sequencer. In addition, we thank Harald Ahnelt for nomenclature advice, as well as information about historical taxonomy and morphology.

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