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Phylogenetic Relationships of Extant Esocid Species (Teleostei: Salmoniformes) Based on Morphological and Molecular Characters

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The phylogenetic relationships of extant species of *Esox* were investigated using both morphological and molecular data. The complete mtDNA cytochrome *b* gene (*cytb*) and the second intron of the RAG1 gene were sequenced from multiple specimens of each species and analyzed using maximum parsimony and maximum likelihood. The resulting cladograms were compared with each other and to the morphological cladogram for congruence. Data from all three sources strongly support the monophyly of the genus, and the monophyly of the subgenera *Esox* (i.e., pikes) and *Kenoza* (i.e., pickerels). Our data support the sister-group relationship between *Esox reicherti* and *Esox lucius* (the Amur and Northern Pike, respectively). Incongruent results between the morphological and RAG1 data and the *cytb* data, with respect to pickerel interrelationships, suggest hybridization and introgression among pickerel species. Additional research is necessary to explore these results further. This study represents the first study to integrate both morphological and molecular data into a phylogenetic analysis of *Esox*. It aims to provide a better understanding of esocid evolution and lay the foundation for the interpretation of fossil material assigned to *Esox*. It also provides preliminary genetic evidence of hybridization among the pickerels.

THE genus *Esox*, Linnaeus 1758 (i.e., pikes and pickerels) is a group of freshwater fishes known for their duck-billed snouts, elongated bodies, and voracious feeding behavior. Extant members of the group are found in North America, Europe, and Asia and constitute important commercial and recreational fisheries. Although the Recent distribution of *Esox* is somewhat restricted, fossil representatives are found throughout the Northern Hemisphere (Grande, 1999) and China (Chang and Zhou, 2002), with its oldest known member collected in Canada, dating to the Late Cretaceous (Wilson et al., 1992).

The family Esocidae, which is coextensive with the genus *Esox*, has been the subject of taxonomic debate for many years. Jordan and Evermann (1896) subdivided *Esox* into three subgenera: *Lucius* Geoffroy, 1767; *Kenoza* Jordan and Evermann, 1896; and *Mascalongus* Jordan, 1878. Nelson (1972), and subsequently Sytchevskaya (1976), found no justification for the retention of the subgenus *Mascalongus*, which consisted exclusively of *Esox masquinongy* and, based on their examination of the cephalic sensory canal system and the internal morphology, divided the genus into two subgenera: *Esox*, representing the pikes, and *Kenoza*, representing the pickerels.

Species within the subgenus *Esox* are restricted to latitudes further north, and include the following: *Esox lucius* (the Northern Pike) exhibits a circumpolar distribution through the eastern and central United States, throughout Canada, Europe, and into Asia; *Esox masquinongy* (Muskie or Muskellunge) is found in eastern and central North America; and *Esox reicherti* (the Amur Pike) is restricted to the Amur River basin of northeastern Asia. *Esox lucius* is the type species for the genus. The species of *Kenoza* include *Esox niger* (the Chain Pickerel) distributed through the eastern part of North America with some introductions into the central and midwestern United States (Crossman, 1978) and *Esox americanus*. *Esox americanus* contains two subspecies: *Esox americanus americanus* (the Redfin Pickerel), restricted to the eastern coast of the United States; and *Esox americanus vermiculatus* (the Grass Pickerel) distributed from northwestern New York to the Gulf Coast and as far west as eastern Texas. An intergrade zone has been reported for the two subspecies between western Florida and western Mississippi (Crossman, 1978).

Although several studies have been published examining the taxonomic status (e.g., Crossman and Casselman, 1969; Casselman et al., 1986; Rab and Crossman, 1994) and distribution patterns (e.g., Crossman, 1966; Crossman, 1978; Maes et al., 2003) of various species of *Esox*, surprisingly few studies have examined species-level interrelationships. Those studies that did (e.g., Nelson, 1972; Sytchevskaya; 1976; Grande, 1999) were narrowly focused, often excluding taxa from analysis or examined an inadequate sample size that did not reflect the geographic

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range and possible morphological variation within each species. Previous studies also relied exclusively on morphological characters known to be problematic in groups such as *Esox* that exhibit high phenotypic similarity and are reported to hybridize (Casselman et al., 1966).

This study addresses the species-level relationships of extant esocid fishes by analyzing character information from both morphological and molecular sources. Previously proposed morphological characters were reevaluated in light of an increased geographic sample size and added to new character information proposed here. Only those characters that showed consistent measurable variation among species were used in the analysis. In addition to morphology as a source of characters, the entire mitochondrial DNA cytochrome *b* (*cytb*) coding region and intron 2 of the nuclear RAG1 gene were PCR-amplified and sequenced from multiple specimens of each species. The resulting cladograms were compared for congruence. The use of multiple data sources allows for comparative and independent evaluations of phylogenetic relationships. The goal of this paper is to provide a better understanding of extant *Esox* interrelationships on both the morphological and molecular levels that will, in turn, serve as the foundation for future studies (e.g., the inclusion of fossil *Esox* taxa into a phylogenetic framework, and the examination of population structure within *Esox* species).

Materials and Methods

Morphological methods.—Over 400 specimens (including multiple specimens of each taxon, see Materials Examined) were obtained for this study from multiple sites throughout the geographic range of each species. Meristic data (e.g., fin-ray counts) were taken from all specimens following Hubbs and Lagler (1949). Standard measurements were made following Grande and Bemis (1998). Total lengths (TL) and standard lengths (SL) were taken from each specimen. Because of reports of meristic intraspecific asymmetry (Crossman, 1960), measurements were made from the left side of the fish. To examine internal morphology (e.g., vertebral counts and caudal fin morphology), formalin-preserved specimens were either x-rayed or cleared and differentially stained for cartilage and bone using a modified version of Dingerkus and Uhler (1977). Specimens were examined, illustrated, and photographed using a Wild M3 or MZ8 stereomicroscope with a drawing attachment and digital camera. Institutional abbreviations used are those of Leviton et al. (1985) with the exception of LU (Loyola University). Statistical analyses were performed using SYSTAT for DOS (vers. 6 ed., Evanston, IL, 1994, unpubl.).

Character information published in the literature (e.g., Nelson, 1972; Patterson and Johnson, 1995; Johnson and Patterson, 1996; Grande, 1999) was reevaluated based on our specimens, and only those characters that either showed clear morphological distinctions or statistically significant meristic differences among species were used for phylogenetic analysis.

Molecular methods.—DNA was isolated from fresh, frozen, or alcohol-preserved muscle, heart, or fin clippings using the DNeasy Tissue Kit (Qiagen) or the High Pure PCR Template Preparation Kit (Roche). Multiple specimens of each species were obtained for molecular analyses. PCR amplicons were generated using Taq DNA polymerase (Promega). For *cytb,* a primer pair flanking the coding region was used. The forward primer was 5′-ATGACTTGAAGAACCACCGTTG and the reverse primer was either 5′-ATTTAACCTTCGATCTTCGGATTAC or 5′-CTAGGGGGGATTTTAACCTC. Standard PCR reaction conditions (0.8 mM dNTP's, 1.5–2.5 mM MgCl2, 0.2 μM primers) were used. The PCR temperature cycling profile was 30 sec at 94 C, 30 sec at 55 C, 75 sec at 72 C for 30 cycles.

For RAG1, a primer pair flanking the second intron was used. These primers were designed specifically for this study based on sequences from esocids of RAG1 exons that flank the intron (López et al., 2004). The exon sequences used for primer design were checked against known RAG1 sequences from teleosts to ensure that the primers target the functional RAG1 gene. The forward primer was 5′-GAACGTGARGCCATGATGCAAGGT and the reverse primer was 5′-TGGCTRCAGCTCAGRAAYGTGTTGAC. The PCR temperature cycling profile was 30 sec at 94 C, 30 sec at 57 C, 60 sec at 72 C for 36 cycles. In cases where flanking primers gave weak signals, even at reduced annealing temperatures, internal forward (5′-GGGGTGC AATTAACAGATATTCC) and reverse (5′-GGC TCTTTAAGCTCTTTGAGAT) primers were used with the flanking primers, and the annealing temperature was lowered to 53 C to generate a pair of shorter, overlapping amplicons. PCR products were evaluated by gel electrophoresis on 1% agarose and amplicons were purified using Qiaquick spin columns (Qiagen). DNAs were sequenced on an Applied Biosystems 3700 automated DNA sequencer at the University of Chicago Cancer Research DNA Sequencing Facility. The sequencing primers for cyt*b*

were forward: CBF1: ATGACTTGAAGAACCAC CGTTG; CBF2: TTCGTCATTGCAGCAGCCA; CBF3: TCTCCGTAGATAATGCAACCTT. Reverse: CBR1: ATTTAACCTTCGATCTTCGGAT TAC; CBR2: GGAATTTTGTCTGCGTCAGAGT; CBR3: CCAATAATGATAAAGGGTGTTC; CBR4: GCCCACGAAGGCAGTTATT. The sequencing primers for RAG1 were forward: RAGF2: GGG GTGCAATTAACAGATATTCC; RAGF3: GATTC ACCCCGCTGTTCCAT; RAGF4: CTGGTGTCT GTTTTCATCTGC. Reverse: RAGR2: GGCTCT TTAAGCTCTTTGAGAT; RAGR3: AAGTGGTG CTGATGTTGTTTTG. Sequences were assembled using LaserGene 6.0 (DNAStar, Inc.) and aligned using CLUSTAL X v. 1.62 (Thompson et al., 1997) with default settings (Gap opening 10, gap extension 0.05, transition weight 0.5). In cases where the RAG1 gene was heterozygous (as inferred from double peaks in electropherograms) at a single site, separate alleles are represented. In cases where the RAG1 heterozygotes differed at more than one site, the International Union of Pure and Applied Chemistry (IUPAC) designations of nucleotide base codes for the ambiguous base calls were used.

The CLUSTAL alignments can be found at http://www.luc.edu/faculty/hlaten/. Tissue and voucher specimens were deposited at Loyola University Chicago and the Field Museum of Natural History for each fish sequenced. GenBank accession numbers and locality data are given in Materials Examined.

Phylogenetic methods.—The interrelationships among extant *Esox* species were examined by means of phylogenetic analysis (Hennig, 1966). Character polarity was determined by outgroup comparison (Nixon and Carpenter, 1993). *Umbra limi* and *Novumbra hubbsi* were chosen as outgroup taxa for both the morphological and molecular analyses.

A total of 38 morphological characters were used in this study. Characters were assigned discrete character states in either a binary or multistate coding scheme. Derived character information (Table 1, Appendix 1) was analyzed by means of maximum parsimony, outgroup rooting, and the branch-and-bound option of PAUP* v.4.0b10 (D. Swofford, unpubl.). All characters were run as unordered. For evaluation of the robustness of the results, 1000 bootstrap replicates were performed, and only groups present in half of the resulting trees were retained (i.e., 50% majority-rule consensus).

Both molecular datasets were analyzed using parsimony and likelihood optimality criteria as implemented in PAUP* v.4.0b10 (D. Swofford,

TABLE 1. MORPHOLOGICAL DATA MATRIX. Numbers on first row refer to the character list in Appendix 1. Character states are represented by 0, 1, 2, 3, 4. Question marks represent unknown characters.

	5	10	15	20	25	30	35	38
Umbra limi	00000	00000	00000	00000	00000	00001	00000	000
Novumbra hubbsi	10101	01000	0?000	00100	00000	?0000	00000	000
Esox masquinongy	13211	11011	10111	31400	32112	02012	33101	300
Esox lucius	12211	11011	10111	21301	22102	02012	22102	201
Esox reichertii	12211	11011	11111	21301	22102	02012	22102	200
Esox niger	11221	10011	10101	11310	11001	11111	22110	110
Esox americanus vermiculatus	11221	10111	10101	11210	11001	11111	11110	100
Esox americanus americanus	11221	10111	10101	11210	11001	11111	11110	100

unpubl.). To determine the optimal parsimony tree or trees for the *cytb* dataset, we conducted a heuristic search with 1000 random taxon addition replicates with random starting trees. To determine the optimal parsimony tree or trees for the RAG1 intron dataset, we conducted a branch-and-bound search because the smaller number of sequences in this dataset allowed for the calculation of an optimal solution in reasonable time. To estimate the relative support in the sequence data for different nodes on the tree under the parsimony optimality criterion, we performed bootstrap analyses (full heuristic search, 1000 replicates, 50% majority-rule consensus) and calculated decay indices (Bremer, 1988) for the nodes on the bootstrap consensus tree using TreeRot v.2 (M. D. Sorenson, unpubl).

We used the program ModelTest v. 3.06 (Posada and Crandall, 1998) to select the likelihood model settings that best fit each of the datasets. We then used those likelihood model parameters in the estimation of the maximum likelihood (ML) trees for each dataset. To determine ML trees for each dataset we conducted a heuristic search with 10 random taxon addition replicates with random starting trees. To expedite the likelihood analysis of the much larger *cytb* dataset, we selected a subset of taxa (n = 17) by eliminating identical and very similar sequences (< 1% divergence). Comparing the topologies of the MP and ML trees allowed us to determine whether there were aspects of the resulting phylogenetic hypothesis that were dependent on the type of analysis employed and, therefore, best left unresolved pending further study.

We did not perform combined sequence or total evidence analyses because hybridization has been documented among the pickerels (Crossman and Buss, 1965). By conducting independent analysis of each dataset we can potentially detect evidence of past hybridization from any conflicting results obtained from mitochondrial, nuclear, and morphological characters. If hybridization has had a lasting effect in pickerel morphology and genetics then combined analyses will obscure this evidence and may produce ambiguous results.

Results

Phylogeny based on morphology.—One most parsimonious tree (CI = 0.92, 64 steps) was found (Fig. 1). Of the 38 characters analyzed, 32 were parsimony informative. The monophyly of the genus *Esox* (100%) is supported by eight derived character states including the presence of a posttemporal canal, depressible teeth, toothplates on basibranchials 1 and 2, notched scales along the lateral line, and an expansion of the anterior supraneural (Fig. 2). The monophyly of the subgenus *Esox* (100%) is supported by eight derived character states including the presence of three epurals in the caudal-fin skeleton (Fig. 3), high vertebral centra counts (57–68), and a fusion of respective epineurals to the bases of neural arches one through four. The sister-group relationship between *E. reicherti* and *E. lucius* is strongly supported as indicated by a bootstrap value of 91%. They overlap in virtually all meristics and measurements taken (Table 2) and as sister taxa share a distinctive head scale and eye stripe pattern, five mandibular canal pores, and a distinctive vomerine tooth patch and cleithrum morphology.

The subgenus *Kenoza* (85%) is supported by 12 derived character states, including four mandibular canal pores, an interrupted infraorbital canal, a predorsal to preanal fin length ratio of one, a unique association between the anterior epicentral and epineural intermuscular bones and the presence of notched scales between the

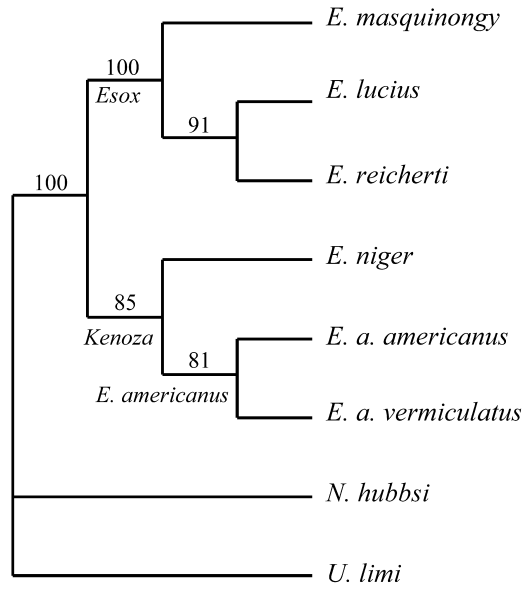
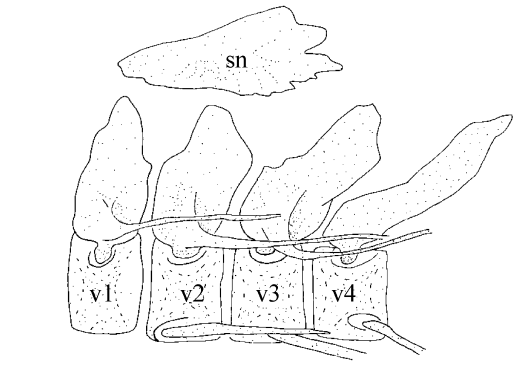
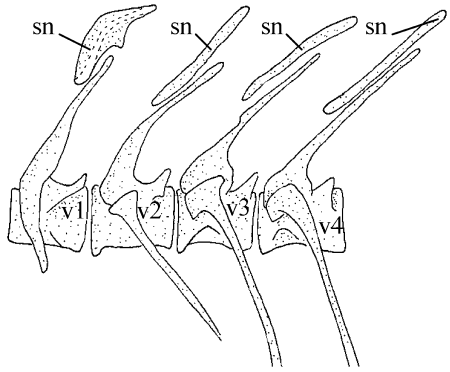


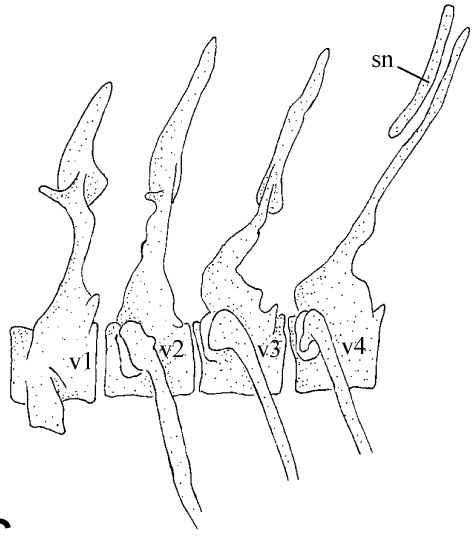
Fig. 1. Morphological cladogram (64 steps, CI = 0.92). Bootstrap values are given at each node. Number in brackets indicates character state. Characters are discussed in the text. The nodes are supported by the following synapomorphies: *Esox*: 6(1), 9(1), 10(1), 11(1), 15(1), 17 (1), 29 (1), 33(1); subgenus *Esox*: 4(1), 13(1), 14(1), 22(2), 23(1), 25(2), 27(2), 30(2); *Esox lucius* + *Esox reicherti*: 2(2), 16(2), 20(1), 21(2), 35(2), 36(2); *Kenoza*: 2(1), 4(1), 16(1), 21(1), 22(1), 25(1), 26(1), 27(1), 28(1), 30(1), 34(1), 36(3); *Esox americanus*: 8(1), 18(1), 31(1), 32(1).



A.

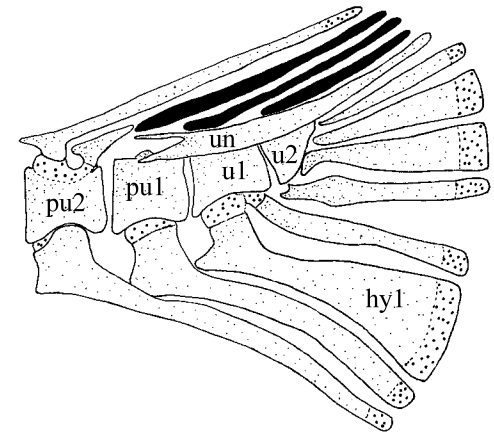


B.

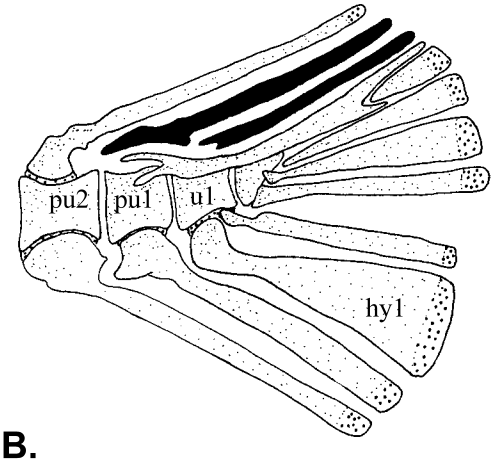


C.

Fig. 2. Illustrations of anterior vertebral region in (A) *Esox reicherti* (SL = 135 mm, CU 64229), (B) *Umbra limi* (SL = 74 mm, FMNH 99738), (C) *Novumbra hubbsi* (composite drawing from UMMZ 187427, SL = 48 mm, and UAMZ 3714, Wilson and Veilleux, 1982). sn, supraneural, v1–v4, vertebra 1 through 4. Anterior directed to the left.



A.



B.

Fig. 3. Caudal fin skeleton of (A) *Esox masquinongy* (SL = 133 mm, CU 9118), (B) *Esox americanus vermiculatus* (SL = 128 mm, FMNH 7187). hy1, hypural 1, pu1, preural centrum 1; pu2, preural centrum 2; u1, ural centrum 1. Epurals drawn in black. Anterior directed to left.

pelvic fins. The sister-group relationship between *E. a. vermiculatus* and *E. a. americanus* (81%) is supported and based on an elongation of the maxillae, a mean branchiostegal-ray count of 12 and 8–9 pelvic-fin rays. Subspecies status is retained for *E. a. americanus* and *E. a. vermiculatus* pending further study.

TABLE 2. MERISTIC AND MEASUREMENT DATA.

	Branchiostegal ray number range (mean, *n*)	Most common branchiostegal patterns	Pelvic-fin rays range (mean, *n*)	Total centra range (mean, *n*)	Abdominal centra range (mean, *n*)	Caudal centra range (mean, *n*)	Total lateral-line scales range (mean, *n*)	Notched lateral line scales range (mean, *n*)
E. masquinongy	16–19	8 + 9	12–13	65–68	45–48	18–21	130–168	42–59
	(17, 53)	9 + 9	(13, 15)	(66, 55)	(46, 49)	(19, 55)	(142, 47)	(52, 20)
E. lucius	13–16	6 + 8	10–11	57–63	39–44	17–21	107–138	39–53
	(14, 80)	7 + 8	(11, 14)	(60, 75)	(42, 76)	(19, 76)	(120, 75)	(45, 25)
E. reichertii	13–14	6 + 8	10–11	62–66	44–47	17–19	105–139	36–52
	(13, 5)		(11, 5)	(63, 5)	(45, 5)	(18, 5)	(120, 5)	(41, 5)
E. niger	14–17	6 + 9	10	49–55	33–39	16–18	87–140	36–42
	(15, 47)	7 + 9	(10, 21)	(52, 50)	(36, 50)	(16, 50)	(122, 46)	(40, 15)
E. a. americanus	11–13	4 + 7	8–9	46–51	32–37	13–17	86–106	70–105
	(12, 28)	5 + 7	(9, 30)	(49, 23)	(34, 68)	(15, 23)	(95, 27)	(83, 50)
E. a. vermiculatus	11–15	4 + 7	8–9	46–54	30–36	13–18	78–115	30–44
	(12, 71)	5 + 7	(9, 10)	(49, 71)	(33, 23)	(15, 70)	(100, 67)	(34, 32)
U. limi	4–5	3 + 1	6–7	34–38	20–22	14–17	31–36	0
	(4, 7)		(6, 10)	(36, 34)	(20, 28)	(16, 21)	(?, 34)	
N. hubbsi	8		6	37–38	18–19	18	52–58	0
	(8, 1 + lit.)		(6, 1 + lit.)	(literature)	(literature)	(1 + literature)	(literature)	

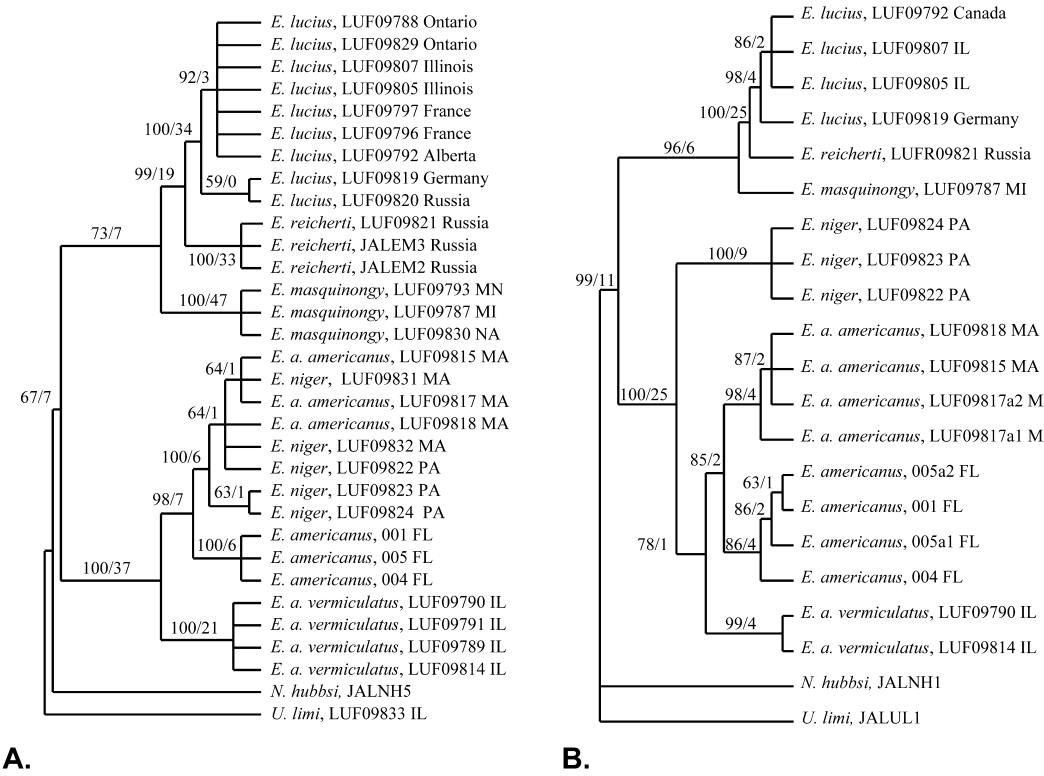


Fig. 4. (A) MP bootstrap consensus tree based on cytochrome *b* data. (B) MP bootstrap consensus tree based on based on RAG 1 intron 2 sequence data. Bootstrap and decay values, in that order, are given at each node. Catalog numbers followed by geographic localities are given for each specimen.

Phylogeny based on DNA sequences.—The *cytb* region sequenced includes the full coding sequence of that mitochondrial gene (1137 bp with the stop codon) plus 17 bp that encode part of the Threonine tRNA located at the 3′ end of *cytb*. The alignment of *Esox cytb* sequences contains 306 variable sites and 299 parsimony informative (PI) sites. Inclusion of the outgroup taxa increases these numbers to 419 and 318, respectively. The RAG1 intron 2 fragment sequenced for this study ranges in length between 877 bp in *E. a. americanus* to 960 bp in *Umbra limi*. For the RAG1 intron sequences, there were 131 variable sites and 100 PI sites. Including the outgroups, these numbers increase to 352 and 119, respectively. There was no evidence of substitution saturation in either of the two sets of sequences. Sequence divergence among specimens of *Esox* ranges between 0–17.9% and 0–9.5% for the mitochondrial and nuclear sequences, respectively.

The *cytb* dataset generated nine most parsimonious trees, each with 752 steps. The strict consensus of these MP trees (not shown) closely matches the topology of the bootstrap consensus tree shown in Figure 4A. The only differences between the two topologies are (1) in the MP consensus tree, *E. lucius* LUF09819 and LUF09820 do not form a clade; and (2) the *E. a. vermiculatus* specimens LUF09789 and LUF09791 form a clade. The ML model that best fits the *cytb* sequences is the general time reversible (GTR) with rate variation among sites (G) and invariant sites (I). The *cytb* ML tree differs from the parsimony-based results (see below). For the RAG1 intron 2 sequence data, there is a single most parsimonious tree of 401 steps. The topologies of the RAG1 intron MP tree, ML tree and parsimony bootstrap consensus tree are identical (Fig. 4B). The ML model that best fits the RAG1 data is the GTR+G.

Both sets of sequences support a close relationship between *E. lucius* and *E. reicherti* (bootstrap values 99% and 100%, decay indices 19 and 25; for *cytb* and RAG1, respectively) and the monophyly of the subgenus *Kenoza* (bootstrap 100%, decay indices 37 and 25). The subgenus *Esox* is found to be monophyletic in the *cytb* MP bootstrap and optimal consensus trees and in all the RAG1 intron MP and ML trees (optimal and bootstrap). In the *cytb* ML tree, *E. masquinongy* is placed as the sister group to all other

esocids thus making the subgenus *Esox* paraphyletic. The mitochondrial sequences from *E. masquinongy* show similar levels of divergence when compared to other species of the subgenus *Esox* and to the pickerels (subgenus *Kenoza*). This may explain the low level of support for this clade in parsimony analysis (bootstrap 73%, decay index 7) and its absence in the ML tree.

The mitochondrial sequences obtained from specimens of *E. a. americanus* and *E. niger* form a clade where sequences from both taxa are found interspersed (Fig. 4A). This arrangement is strongly supported (bootstrap 100%, decay index 6) by MP both ML analyses. In contrast, the RAG1 intron sequences from all specimens of *E. americanus* form a monophyletic clade that constitutes the sister group to the clade formed by sequences from *E. niger* (Fig. 4B). This arrangement better corresponds to the taxonomy of this species and to the results of the morphology-based analyses. The *Kenoza* clade also receives strong support from the nuclear sequences (100%).

The sequences from individuals of *E. americanus* collected from Illinois and Florida do not form a monophyletic group in the MP and ML analyses. In the trees supported by *cytb* MP and RAG1 intron MP and ML, the Florida specimens form the sister group to the clade that contains all the *E. a. americanus* and *E. niger* sequences. The Florida specimens were difficult to diagnose to the subspecies level because of the presence of an intermediate number of notched scales between the pelvic fins.

Discussion

The morphological and molecular evidence support hypotheses of relationships between species of *Esox* that are in general but not complete agreement with each other (Figs. 1, 4) and are congruent with the esocid classification proposed by Nelson (1972). The monophyly of the genus *Esox* has strong morphological and molecular support from the RAG1 intron data but only marginal support according to the *cytb* data (69%). Similarly, the subgenera *Esox* and *Kenoza* are strongly to moderately supported by all three datasets. Within the subgenus *Esox*, the sister species relationship between *E. lucius* and *E. reicherti* is robustly and consistently supported by all three sources of evidence. Both the morphological and genetic data generated in this study suggest that *E. masquinongy* is the result of an early speciation event in the history of the subgenus *Esox*.

Within the subgenus *Kenoza*, the morphological and RAG1 intron sequence data support a sister-group relationship between the two subspecies of *E. americanus*. This sister-group relationship, along with the position of *E. niger* within *Kenoza*, however is not supported by the *cytb* sequence data. In fact, these data group our specimens of *E. niger* and *E. a. americanus* together in a clade, and specimens from Florida, identifiable only as *E. americanus*, are placed as the sister group to the *E. niger* + *E. a. americanus* clade (Fig 4A). These discrepancies among phylogenetic hypotheses generated by the different datasets may be the result of inaccurate phylogenetic inference or differences between the history captured by the mitochondrial sequences and that captured by the nuclear sequences and morphological data. Under the former scenario, the incongruence in taxon placement observed among trees is the result of homoplasy. This implies that one or more of our cladograms are in error. In the case in question, this explanation, however, seems unlikely because the most salient incongruence is caused by the placement of mitochondrial sequences from *E. niger* relative to those from *E. a. americanus*, and the sequences from these two species show very low levels of divergence (0–3.5%). Further, because it is improbable that sequences from two species would converge to identity as a result of homoplasy, we do not consider homoplasy a likely explanation for the incongruence between the different datasets. An alternative explanation for the incongruence is that differential retention of ancestral mitochondrial polymorphisms by the three lineages in question has rendered mitochondrial sequences inaccurate indicators of phylogeny. Again, the presence of identical and near identical haplotypes in populations of *E. niger* and *E. a. americanus* makes this explanation unlikely, specially when considering that all nuclear alleles from these two lineages are clearly and invariably distinct. Finally, hybridization has been documented among all pickerels (Crossman and Buss, 1965) and as stated by Crossman (1978:21), ''Where chain pickerel is sympatric with another of the pickerels extensive hybridization occurs.'' Hybridization has the potential to alter the historical signal captured by DNA sequences. In the case in question, we think that the different placement of *E. niger* specimens in the nuclear and mitochondrial trees is the result of past hybridization events that resulted in some *E. niger* and *E. a. americanus* populations sharing closely related mitochondrial genomes.

Our sample size, does not allow us to determine whether this shared genetic pool extends over the entire range of the two species or whether it is restricted to a particular geograph-

ic area. Based only on the *cytb* cladograms, we are unable to determine which of the mitochondrial genomes from *E. niger* or *E. a. americanus* is represented in the specimens sampled. But considering the RAG1 intron cladogram, the placement of *E. niger* specimens as the sister group of all *E. americanus* specimens suggests that we did not find *E. niger* mitochondrial genomes and that all of the *E. niger* specimens sampled carry mitochondrial genomes belonging to the *E. a. americanus* lineage. Further, because none of the nuclear sequences from specimens *E. niger* were placed in the *E. a. americanus* clade, it appears that the events that affected the mitochondrial genomes did not leave a lasting effect on the nuclear genomes of these two taxa. One question that arises from these observations is whether there remain populations of *E. niger* that carry the original *E. niger* mitochondrial genome. If not, then why did the *E. a. americanus* mitochondrial DNA replace that of *E. niger*? To answer these questions, it will be necessary to widen the geographic area of the populations sampled to include the entire distribution range of both taxa.

With respect to the Florida *E. americanus* specimens, these specimens were collected in an area where *E. a. americanus* and *E. a. vermiculatus* are sympatric and their morphological characters precluded clear subspecies identification. For example, two characters that clearly separate *E. a. americanus* from *E. a. vermiculatus* in zones of nonoverlap are the number of notched scales along the lateral line and between the pelvic fins (characters 33, 34). Among the Florida specimens examined, the number of notched scales is significantly reduced from that found in *E. a. americanus* but greater in number than that found in *E. a. vermiculatus*. Crossman (1966) also found a lack of clear morphological characters separating populations of *E. a. americanus* and *E. a. vermiculatus* within sympatric zones. In our study, the sequence data are in agreement with the morphology in placing the Florida specimens in a distinct clade. This arrangement may be evidence of a distinct and previously unrecognized population of *E. americanus* of unknown taxonomic rank. Alternatively, it may be indicative of hybridization between the two subspecies of *E. americanus*. According to Crossman (1978) the areas of sympatry represent a secondary mixing of both stocks that originally diverged during the formation of the Appalachian Mountains. Our data indicate that the status of these populations needs further study.

Materials Examined

Morphological material.—Specimens marked with an asterisk are specimens used for both morphological and molecular analyses. *Esox masquinongy*: 86 specimens (SL: 64–179 mm): CU 9116, 9118, 19154 (alcohol, c&s); FMNH 85991, 105931 (alcohol); FMNH 51273, 72177, 73806, LUF 09787*, 09830* (alcohol fixed), 09793* (frozen), 09826 (alcohol). *Esox lucius*: 91 specimens (SL: 8–400 mm): FMNH 142, 144, 3160, 4007, 6304, 6460, 6724, 7406, 10064, 18090, 43024, 75232, 79584, 91381 (alcohol, c&s), FMNH 32734, 9760, 9964, 73641 (dried skeleton); LUF 09792*, 09805*, 09807* (frozen), 09808, 09809, 09811, 09825 (alcohol, c&s); MCZ 6516, 6524, 6542, 25550, 26540 (alcohol); UF 82643 (alcohol); UMMZ 173710, 174626, 185115, 201197, 201213, 201226, 201320, 201335, 205365; USNM 22013, 021606, 122013, 064655, 068224, 068225 (alcohol). *Esox reicherti*: 6 specimens (SL: 65–225 mm): CU 64227, 64228, 64229, 64232 (alcohol, c&s); FMNH 109221 (alcohol). *Esox niger*: 67 specimens (SL: 63–247 mm): FMNH 697, 712, 714, 6724, 10418, 13349, 13361, 13627, 15568, 15572, 21814, 21815, 32719–32726, 32720, 32721, 32722, 32723, 32726, 32727–32732, 37026, 37027, 60693 (alcohol), FMNH 21811 (c&s); LUF 082291, 082292 082293 (c&s), 09831* (alcohol fixed). *Esox americanus*: 28 specimens (SL: 122–145 mm): FMNH 10489, 11392, 13347, 31768, 42852 (alcohol, c&s). UF 130792.001–011 (alcohol fixed, 001*, 005*, 004*). *Esox americanus americanus*: 44 specimens (SL: 64.5–194 mm): FMNH 1264, 1991, 10417, 10489, 15569, 21817, 21823, 21831, 21832, 21819, 31773, 31774, 31775, 37025 (alcohol); LUF 09815*, 09817*, 09818* (alcohol fixed); UMA F10424, 24-288-3-14 (c&s). *Esox americanus vermiculatus*: 146 specimens (SL: 65–190 mm): FMNH 299, 736, 2176, 2923, 6404, 6720, 6722, 6723, 7143, 7187, 10052–10055, 10064, 10237, 10539, 10632, 10635, 10656, 13528, 31768, 42256, 42287, 42468, 42966, 61214, 61154, 63035, 63906, 63907, 79335, 80336, 88728, 88891, 99908,100821 (alcohol, c&s); LUF 082298 (c&s), 09789*, 09790*, 09791*, 09814* (alcohol fixed). *Umbra limi*: 46 specimens (SL: 45–94 mm): FMNH 1378, 1563, 3084, 3928, 6403, 13927, 13928, 13930, 13931, 13933, 42077, 60932, 99738 (alcohol, c&s); LUF 01892* (frozen). *Novumbra hubbsi*: 2 specimens (SL: 46.5 mm, disarticulated): UMMN 179398, 187427 (c&s).

Molecular material.—Specimens marked with an asterisk are specimens used for both morpho-

logical and molecular analyses. GenBank numbers for RAG1 sequences are in bold. All other GenBank numbers correspond to *cytb* sequences. *Esox masquinongy*: 3 complete specimens: LUF09787* (alcohol fixed, Plum lake, Gogebic, MI, GenBank numbers AY497456, AY506519); LUF09830* (alcohol fixed, Illinois, GenBank number AY497455); LUF09793* (frozen, MN fish hatchery, GenBank number AY497455). *Esox lucius*: 9 specimens: LUF09805* (complete frozen, Lake Marie, Lake County, IL, GenBank numbers AY497449, AY506522); 09807* (complete frozen, Des Plains R. Lake County, IL, GenBank numbers AY497450, AY506523); 09788 (tissue, Lake of the Woods, Ontario, Canada, GenBank number AY49744) ; 09829 (tissue, Big Rideau, Ontario, Canada, GenBank number AY497453); 09792* (complete alcohol, Alberta, Canada, GenBank numbers AY497446, **AY506520**); 09796 (tissue, France, GenBank number AY497447); 09797 (France, GenBank number AY497448); 09819 (tissue, Germany, GenBank numbers AY497451, AY506521); 09820 tissue, St. Petersburg, Russia, GenBank number AY497452). *Esox reicherti*: 3 specimens: LUF09821 (tissue, Amur River, Khabarovsk, Russia, GenBank numbers AY497442, AY506524); JALEM2 (tissue, Amur River, Khabarovsk, Russia, GenBank number AY497443); JAEM3 (tissue, Khabarovsk, Russia, GenBank number AY497444). *Esox niger*: 5 specimens: LUF09822 (tissue, Nockamixon Lake, Bucks County, PA, GenBank numbers AY497437, AY506511); 09823 (tissue, Promised Land Lake, Pike County, PA, GenBank numbers AY497438, AY506512); 09824 (tissue, Lake Jean, Luzerne County, PA, GenBank numbers AY497439, AY506510); 09831* (complete alcohol fixed, Amherst, MA, GenBank number AY497440); 09832* (complete alcohol fixed, Amherst, MA, GenBank number AY497441). *Esox americanus*: 3 complete alcohol specimens: UF 130792* (Alachua County, FL, GenBank numbers 001: AY497434, AY506528; 004: AY497435, AY506526; 005: AY497436, AY506525, AY506527). *Esox americanus americanus*: 3 complete alcohol fixed specimens: LUF09815* (Dighton, MA, GenBank numbers AY497431, AY506518); 09817* (Dighton, MA, GenBank numbers AY497432, AY506517, AY506515); 09818* (Hampden County, MA, GenBank numbers AY497433, AY506516). *Esox americanus vermiculatus*: 4 complete alcohol fixed specimens: LUF09789–91* (Cache River, Johnson City, IL, GenBank numbers AY497427; AY497428, AY506513; AY487429); 09814* (Des Plaines River, IL, GenBank numbers AY497430, AY506514). *Umbra limi*: 2 specimens: LUF09833* (complete alcohol, Cook County, IL, GenBank number AY497458); JALUL1 (tissue, Dubuque, IA, GenBank number AY380548). *Novumbra hubbsi*: tissue samples: JALNH5 (Greys Harbor, WA, GenBank number AY497457), JALNH1 (Greys harbor, WA, GenBank number AY380546).

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APPENDIX 1

CHARACTER LIST

The following is a summary of the morphological characters used in the phylogenetic analysis and a description of the coding scheme used for the various character state (the character state matrix is given in Table 1).

1. *Mandibular canal:* absent [0]; present [1]. A mandibular canal that runs anterior to posterior along the ventral side of the dentary was observed in all species of *Esox*. A shortened mandibular canal was observed in *N. hubbsi*. This character is polymorphic for *Umbra* in that a mandibular canal is absent in *U. limi* and *U. pygmaea*, but present in *U. krameri* (Nelson, 1972).
2. *Number of mandibular canal pores*: 0–2 [0]; 4 [1]; 5 [2]; 8–9 [3]. *Umbra krameri* (Nelson, 1972) and *N. hubbsi* exhibit two mandibular canal pores. Four pores were observed in the mandibular canal for all *Kenoza* species. Five canals were observed in *E. lucius* and *E. reicherti*, whereas *E. masquinongy* exhibited 8–9. According to Nelson (1972), the reduction in the number of pores in umbrids and *Kenoza* species is from the posterior end of the canal.
3. *Preopercular canal pores*: 3–4 [0]; 5 [1]; 6 [2]. Six preopercular canal pores were consistently observed in all *Esox* species and subspecies. Three to four pores were observed in *Umbra*. *Novumbra hubbsi* exhibits five pores, representing the loss of one pore and neuromast from the ascending part of the canal (Nelson, 1972).
4. *Infraorbital canal*: incomplete/reduced [0]; continuous and complete [1]; discontinuous but complete [2]. In pikes (i.e., *E. masquinongy*: 7–9

pores, *E. lucius* and *E. reicherti*: 7–8 pores) the infraorbital pores are enclosed in an uninterrupted series of canals with no reduction in the number of pores (i.e., complete). Although no reduction in the number of infraorbital pores is exhibited in pickerels (8–9 pores), the infraorbital canal is interrupted in two places and considered discontinuous. In both *Umbra* and *Novumbra*, portions of the infraorbital canal is replaced by a pitline and the number of pores is reduced. This condition is, thus, considered incomplete/reduced. In *Umbra*, the canal is eliminated posteriorly (0–3 pores retained anteriorly), whereas in *Novumbra* the posterior portion of the canal is retained with two pores and the anterior portion is lost. The center portion of the canal is lost in both genera. Nelson (1972) considered the umbrid condition (i.e., the reduction of sensory canals to pitlines) a derived condition with pickerels showing the ''tendency'' toward canal reduction (p. 8). Lopez et al. (2004) caution that the directional evolutionary mode suggested by Nelson (1972) needs additional justification.

5. *Temporal canal pores*: 2 [0]; 3[1]; All *Esox* species have three temporal canal pores while all members of the genus *Umbra* exhibit two. *Novumbra hubbsi* was observed to have three temporal canal pores. According to Nelson (1972), the posterior pore and neuromast are lost in *Umbra*.
6. *Posttemporal canal*: absent [0]; present [1]. All *Esox* species have a posttemporal canal. A posttemporal canal was not observed in *Umbra* and *Novumbra*.
7. *Extrascapular canal*: absent [0]; present [1]. The subgenus *Esox* exhibits an extrascapular canal with three pores. An extrascapular canal with three pores is retained in *Novumbra*. An extrascapular canal is absent in *Umbra*, and, as shown in Nelson (1972), this canal is reduced to pitlines.
8. *Posterior tip of maxillae*: maxillae do not reach or extend beyond the midpoint of the orbit [0]; maxillae extend beyond the midpoint of the orbit [1]. Both subspecies of *E. americanus* have extremely long maxillae that extend beyond the midpoint of the orbit. In *E. niger*, the maxillae do not even approach the anterior margin of the orbit. Although the pikes have longer maxillae than *E. niger*, their maxillae never extend to the midpoint of the eye. Short maxillae were also observed in the outgroups examined.
9. *Palatine/premaxilla articulation*: anterior part of palatine does not articulate with the premaxilla [0]; anterior part of the palatine articulates with the premaxilla to form a toothed biting surface of the upper jaw [1]. In Esocidae, the anterior tip of the toothed palatine articulates with the medial end of the premaxilla. Together they form a biting surface of the upper jaw. In *Umbra* and *Novumbra*, the anterior portion of the palatine articulates with the maxilla, not the premaxilla, and the palatine is not part of the biting surface.
10. *Mandibular length/head length ratio*: 16–37% [0]; 52–70% [1]: In species of *Esox* the mandibular to head length ratio is over 50%. The mandibular length was measured from the anterior margin of the premaxilla to the posterior tip of the supramaxilla. Head length was measured from the tip of the snout to the posterior margin of the opercle. The high mandibular length to head length ratio in Esocidae is correlated with the characteristically long head shape in these fish.
11. *Palatine with maxillary articulation process*: absent [0]; present [1]. All *Esox* species exhibit a process on the anteriolateral margin of the palatine that articulates with the maxilla. This process was not found in *Umbra* and *Novumbra*.
12. *Parietals*: separated by supraoccipital and not in contact with each other [0]; positioned in front of the supraoccipital and in contact with each other and [1]. In most esocids and *Umbra*, the parietals are small and separated from each other by the supraoccipital. They never meet along the dorsal midline. In *E. reicherti* however, the parietals meet along the midline and are separated the frontals from the supraoccipital. The parietal position relative to the supraoccipital in *Novumbra* is problematic. Wilson and Veilleux (1982) described the parietals in *Novumbra* as being separated by the supraoccipital, whereas Sytchevskaya (1976) described the parietals as meeting along the dorsal midline. We were unable to definitively determine the position of the parietals in the specimens examined here; therefore, *Novumbr*a was coded as unknown.
13. *Posttemporal fossa covered by parietals*: absent [0]; present [1]. Our observations agree with those of Sytchevskaya (1976). The posttemporal fossa is covered by the parietals in all *Esox* species and not in the outgroups examined.
14. *Length of vomer/length of parasphenoid*: vomer less than or equal to 50% of parasphenoid length [0]; vomer greater that 50% of parasphenoid length [1]. In comparison to the pickerels and the outgroups, *E. masquinongy*, *E. lucius*, and *E. reicherti* exhibit a very long vomer relative to the length of the parasphenoid. In the pikes the length of the vomer is always more that 50% of the length of the parasphenoid. In *E. niger* (e.g., FMNH 21811), the vomer length is 50% of the parasphenoid length. In *E. a. vermiculatus* (e.g., FMNH 21811), the vomer length is 43% that of the parasphenoid length. Parasphenoid and vomer lengths were measured from adult cleared-and-stained and skeletonized specimens.
15. *Depressible tooth morphology*: absent [0]; present [1]. Esocidae is diagnosed in part by the presence of depressible teeth on the dentary, vomer, and pal-

atine. Depressible teeth are not found in *Umbra* and *Novumbra*. In all *Esox* species, depressible teeth are found on the anterior part of the dentary, whereas large fixed canine-like teeth are positioned along the posterior border. Depressible teeth are found on the entirety of the palatines and vomers of all species except *Esox masquinongy*. In *E. masquinongy*, a few fixed fanglike teeth are positioned near the anterior end of the vomer and palatine. On the vomer, depressible teeth are positioned between the fixed fangs, making the anterior dentition of *E. masquinongy* distinct from *E. lucius* and *E. reicherti*, where the anterior vomerine teeth are of equal length and always depressible. Fink (1981) classified esocid type of dentition as type 4 tooth attachment and considered depressible teeth to be derived for actinopterygians. Wilson et al. (1992) argued, however, that fixed teeth could have evolved from depressible ones because depressible teeth are present in Cretaceous esocids, and the umbrid *Paleoesox*, and that a combination of fixed and depressible teeth are present in Tertiary and Recent esocids.

16. *Vomerine teeth*: vomerine tooth patch small and with a few teeth: [0]; tooth patch less than 50% of vomer length, consisting of small patch of anterior teeth and few teeth along the neck of vomer [1]; tooth patch more than 50% of length of vomer with anterior teeth of same size [2]; tooth patch with a few anterior teeth including large canines that narrow to a single lateral row [3]. The vomerine tooth patch in *Umbra* and *Novumbra* is virtually insignificant and consists of only a few anterior teeth. We have found three distinctive tooth patch morphologies among esocids that appear consistent throughout the range of each species. Relative to the outgroups, the tooth patch in pickerels is larger but is always less than 50% of the vomer length. It consists of larger teeth of about equal size anteriorly, and few teeth positioned along the shaft of the vomer. *Esox lucius* and *E. reicherti* share a common tooth patch morphology in that the vomer consists of a dense anterior patch of similarly sized teeth and numerous teeth along the shaft of the vomer. These teeth gradually decrease in size posteriorly and the tooth patch is at least 50% of the vomer length. *Esox masquinongy* has a distinctive arrangement of vomerine teeth. Unlike other esocids, the vomerine teeth in *E. masquinongy* consists of four or five fanglike teeth along the anterior front row. The teeth in the center of this row are smaller in size. These teeth form a single row of teeth that runs almost half the length of the vomer. The vomerine tooth patch morphology was used by Casselman et al. (1986) to distinguish *E. masquinongy* from *E. lucius*.
17. *Toothplates on basibranchials 1 and 2*: absent [0]; present [1]. Toothplates are present on basibranchials 1 and 2 in all *Esox* species and absent in *Umbra* and *Novumbra*.
18. *Branchiostegal rays*: 4–5 [0]; 8 [1]; mean: 12 (range: 11–13) [2]; mean: 14 (range: 13–17) [3]; mean: 17 (range: 16–19) [4]. Of the specimens examined, *Umbra* exhibits 4–5 rays and *Novumbra* exhibits 8. Among *Esox* species, *E. americanus* (both subspecies *americanus* and *vermiculatus*) exhibit the lowest branchiostegal ray number [2], *E. niger*, *E. lucius*, and *E. reicherti* exhibit condition [3], whereas *Esox masquinongy* exhibit condition [4]. A one-way ANOVA revealed significant differences in total branchiostegal ray number among taxa ($F_{0.05,\ 7,\ 276} = 174.098$, $P < 0.001$). Tukey's HSD multiple comparison indicated that *E. masquinongy* has statistically more branchiostegal rays than any of the other taxa examined (Tukey $P < 0.001$). Although Crossman (1960) cautions that branchiostegal ray patterns can be variable within a species (i.e., variation may exist in the number of rays on the anterior and posterior ceratohyals), we have found that each species exhibits a particular pattern or patterns that are more common than others. See Table 2 for species ranges and ray patterns.
19. *Predorsal length/preanal length ratio*: less than 1.00 [0]; 1 [1.00]. The subgenus *Kenoza* displays a predorsal/preanal length ratio of 1.00. Pikes, *Umbra*, and *Novumbra* have a predorsal/preanal length ratio of less than one, meaning that the dorsal fin is closer to the head than in the pickerels. Our data support those of Grande (1999) in using this character to help distinguish pikes from pickerels.
20. *Cleithra shape*: inner ridge straight with straight dorsal spine [0]; curvature of inner ridge and posterior indentation of dorsal spine [1]. *Esox lucius* and *E. reicherti* share a distinctive morphology of the cleithrum consisting of a curved inner ridge, a posteriorly indented dorsal spine and a greater than ninety degree angle made from the intersection of the inner rib and dorsal spine. This character was introduced by Casselman et al. (1986) to distinguish *E. masquinongy* from *E. lucius*. We have found that *E. reicherti* also has this morphology, which is distinct from that found in all other taxa examined.
21. *Total vertebrae*: 34–38 [0]; mean: 50 (range: 46–55) [1]; mean: 61.5 (range: 57–66) [2]; mean: 66 (range: 65–68) [3]. Among *Esox* species, a clear gap in total vertebral number was observed separating the subgenus *Kenoza* [1] from the subgenus *Esox* (Table 2). The subgenus *Esox* can be divided further, with *E. reicherti* and *E. lucius* forming a group [2]. A one-way ANOVA revealed significant differences among taxa with respect to the total number of vertebrae ($F_{0.05,\ 8,\ 302} = 2134.9$, $P < 0.001$). Tukey's HSD multiple comparison in-

dicated that *E. masquinongy* has statistically more vertebrae than any of the other taxa examined (Tukey $P < 0.001$). *Esox masquinongy* was thus coded as [3].

22. *Abdominal vertebrae*: 18–22 [0]; mean: 34.3 (range: 32–39) [1]; mean: 44.3 (range: 39–48) [2]. The subgenus *Kenoza* [1] can be separated from the subgenus *Esox* [2] by the mean number of abdominal vertebrae. The overlap in ranges between the subgenera is caused by one specimen of *E. lucius* (MCZ 6524A) with an abdominal vertebral count of 39. If this specimen is eliminated from these data, the ranges do not overlap (range: 41–48). No clear divisions could be observed separating species within each subgenus. (Table 2).
23. *Caudal vertebrae*: mean: 15.5 (range: 13–18) [0]; mean: 18.6 (range: 17–21) [1]. *Esox masquinongy*, *E. lucius*, and *E. reicherti* share as a group a higher caudal vertebrae count in comparison with the subgenus *Kenoza* and the outgroups. A clear gap in the mean caudal vertebral counts separate the pikes from the pickerels and outgroups (Table 2).
24. *Abdominal centra*: striated [0]; smooth [1]. As discussed by Casselman et al. (1986) and confirmed by this study, the common condition for *Esox* is for the abdominal centra to be strongly striated with a very deep dorsal aortic groove. In *E. masquinongy* however, the centra are only slightly striated and the dorsal aortic groove is shallow. This corresponds to an asymmetrical location of the dorsal aorta in *E. masquinongy*, which in turn corresponds with an asymmetrical location of the dorsal aortic groove in the centra.
25. *Fusion of epineural to base of neural arch*: absent [0]; v1–v2 [1]; v1–v3/v4 [2]. As discussed by Patterson and Johnson (1995), in *Esox*, epineurals are fused to the bases of their corresponding neural arches. This condition is not found in *Umbra* and *Novumbra*. Within *Esox*, only the first two epineurals are fused to their corresponding neural arches in *Kenoza*. In *E. lucius* and *E. reicherti*, we observed a fusion of the first four epineurals with their neural arches. In some specimens of the *E. masquinongy* examined, only the first three epineurals were fused to their corresponding neural arches. Additional material is necessary to determine whether this morphology is the common condition in this species.
26. *Epipleural intermuscular bones*: no association between epicentral and epineural intermuscular bones [0]; close association between epicentral and epineural intermuscular bones [1]. As discussed by Patterson and Johnson (1995), *Kenoza* species are diagnosable by a unique connection between the epicentral and epineural intermuscular bones. In *E. niger* and both species of *E. americanus*, we observed that the anteroventral tips of the epineurals of vertebrae 3–8 are attached to the corresponding rib by means of the epicentral ligament, supporting Patterson and Johnson (1995). This condition was not observed in the subgenus *Esox* and was unobservable in the *Novumbra* specimens examined.
27. *Coossification of the rib and parapophysis of the anterior vertebrae*: absent [0]; coossification of rib and parapophysis in membrane bone of vertebra two [1]; coossifications of rib an parapophysis of vertebrae 2–4 [2]. In *Kenoza*, the rib and parapophysis of vertebra two are coossified, whereas the subgenus *Esox* exhibits coossification of the ribs and parapophyses of vertebrae 2–4 (Patterson and Johnson, 1995).
28. *Expansion of the second neural arch*: absent [0]; present [1]. As discussed by Patterson and Johnson (1995) and observed by us, the second neural arch is expanded in the transverse plane and rostrocaudally in *Kenoza*. As a result of this expansion, neural arch two extends over vertebra three. Such an expansion was not observed among pikes and outgroups.
29. *Expansion of the anterior supraneural*: absent [0]; present [1]. In all extant esocids, an expanded supraneural is positioned above the anterior neural arches (Fig. 2A). We observed some variation among *Esox* species in the position of this supraneural and the corresponding neural arches ventral to it. For example, in *E. niger* (e.g., FMNH 21181) and *E. a. vermiculatus* (e.g., FMNH 7187), this supraneural sits directly above neural arches two and three; in *E. lucius* (e.g., LUF 082290) and *E. reicherti* (CU 64299), the supraneural appears to be expanded anteriorly and sits directly above neural arches one, two and three. Finally, in *E. masquinongy* (e.g., CU 9118), this supraneural is positioned directly above neural arches one through four. According to Johnson and Patterson (1996) *Esox* exhibits a supraneural condition that results from the independent formation of the first supraneural relative to the remaining supraneurals. The remaining supraneurals from in an anterior to posterior direction, beginning anterior to the neural spine of vertebra eight or nine in *Kenoza* and 10 or 11 for the subgenus *Esox*. In *Esox*, there is at least a four vertebrae gap between the first and second supraneurals. In *Umbra*, supraneural one develops anterior to the first neural spine with no gap between the first and second supraneurals (Fig. 2B). In *Novumbra* (Fig. 2C) no supraneural is present above neural arches two and three, and the first supraneural are positioned anterior to the neural spine of four.
30. *Epurals in the caudal fin skeleton*: 1 [0]; 2 [1]; 3 [2]. *Esox masquinongy*, *E. lucius*, and *E. reicherti* exhibit three epurals in the caudal fin skeleton, whereas *Umbra* and the pickerels have two. One epural was

observed in the caudal skeleton of *Novumbra* (Fig. 3).

31. *Pelvic-fin ray count*: 6–7 [0]; 8–9 [1]; 10–11[2]; 12–13 [3]. Redfin and grass pickerels (*E. americanus*) have a pelvic-fin ray count of 8–9, whereas *E. niger*, *E. lucius*, and *E. reicherti* consistently exhibit a pelvic-fin ray count of 10–11. *Esox masquinongy* exhibits a higher pelvic-fin ray count, and no overlap was observed with any other species. A pelvic-fin ray count of 6 or 7 was observed for the *Umbra* and *Novumbra* specimens examined. (Table 2).
32. *Total lateral-line scale count*: 31–58 [0]; mean: 82 (range: 78–115) [1]; mean: 125 (range: 87–139) [2]; mean: 142 (range: 130–168) [3]. Based on a one-way ANOVA ($F_{0.05,\ 7,137.2}$, $P < 0.001$) followed by a Tukey's multiple comparison test, three *Esox* subgroups were statistically identified: *E. americanus* (both subspecies) [1], *E. niger* + *E. lucius* + *E. reicherti* [2], and *E. masquinongy* [3]. No significant differences were found within each subgroup ($P > 0.05$). Lateral-line scale counts for *Umbra* were made from direct observations of specimens, but the lateral-line count for *Novumbra* is from Wilson and Veilleux (1982) since only cleared-and-stained material was available for this study (Table. 2).
33. *Notched scales along the lateral line*: absent [0]; present [1]. Scales along the lateral line are of two types in *Esox* species. One is the typical cycloid scale common among ecocoids, but the other is a cardioid, or notched scale (Casselman et al., 1986). The two types of scales appear to be randomly scattered along the lateral line and the proportions of notched scales in most species are fewer in number than the typical cycloid scales (range: 30–60). In *Esox a. americanus* however, the number of notched scales were more numerous (range: 70–105) and in many cases out numbered the typical lateral line scales. In *E. a. americanus* specimens examined, not only were numerous notched scales present along the lateral line but they were also all found over the body. In other esocids, notched scales on the body were few.
34. *Notched scales between the pelvic fins*: absent [0]; present [1]. Notched scales positioned between the pelvic fins were observed in all species of the subgenus *Kenoza*. Notched pelvic scales were not observed in the subgenus *Esox* or *Umbra*. This character was unobservable in the *Novumbra* specimens examined, and to our knowledge notched scales have not been reported for the genus in the literature; therefore, *Novumbra* was coded as [0]. Within the subgenus *Kenoza*, the number of notched scales varies. Of the *E. niger* specimens examined, the number of notched scales between the pelvic fins ranged from 2–10. *Esox americanus vermiculatus* had the fewest number of notched scales and never exceeded five in a single specimen. Specimens of *E. a. americanus* had the highest number of notched scales (10–36, x = 21), which is probably correlated with the numerous notched scales all over the body. Specimens of *E. americanus* examined from Florida, however, had about 5–8 notched scales between the pelvic fins. The uncharacteristic number of notched scales accompanied by a reduction in the number of notched scales along the lateral line and unpigmented paired fins precluded us from assigning these fish to subspecies.
35. *Cheek scale pattern*: opercle and cheek fully scaled [0]; opercle partially scaled and cheek fully scaled [1]; opercle and cheek partially scaled [2]. The cheeks and opercles of the subgenus *Kenoza* and *Umbra* are fully scaled. *Esox lucius* and *E. reicherti* both have a partially scaled opercle and a fully scaled cheek. *Esox masquinongy* has both the opercle and cheek partially scaled. Notched scales were not observed on the cheek or opercle of any specimen examined.
36. *Eye stripe*: no eye stripe [0]; eye stripe extending from the ventral margin of the orbit to the ventral margin of the head [1]; eye stripe extending from the ventral margin of the orbit to two-thirds of the ventral margin of the head [2]; eye stripe extending from the ventral margin of orbit to half the ventral margin of the head [3]. No eye stripe was observed in the outgroups. *Kenoza* species exhibit a complete eye stripe that extends from the eye to the ventral margin of the head. *Esox lucius* and *E. reicherti* exhibit an incomplete eye stripe extending closer to the ventral margin (about two-thirds) of the head, and *E. masquinongy* exhibits condition [3].
37. *Netlike pigmentation pattern*: absent [0]; present [1]. *Esox niger* exhibits a unique pigmentation pattern that consists of a network of iridescent "chains" that flank the sides. This pigmentation pattern was not observed in any other taxon examined.
38. *Color pattern consisting of whitish or yellow spots along the sides*: absent [0]; present [1]. *Esox lucius* also exhibits a unique color pattern that consists of light spotting on a dark green background. No other *Esox* species has this adult color pattern.