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MITOCHONDRIAL-DNA VARIATION IN PALLID AND SHOVELNOSE STURGEONS, *SCAPHIRHYNCHUS* (PISCES: ACIPENSERIDAE)

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ABSTRACT

Sequencing analysis was used to characterize a mtDNA D-loop amplicon of 838 bp. Four pallid sturgeons, *Scaphirhynchus albus,* and four shovelnose sturgeons, *S. platorynchus,* from four states (Illinois, Louisiana, Montana, North Dakota) were used for this analysis. DNA sequencing of the nine fish revealed eleven polymorphic sites and six distinct haplotypes. No fixed nucleotide substitutions were observed between pallid and shovelnose sturgeons.

† † †

Pallid (*Scaphirhynchus albus* Forbes & Richardson), shovelnose (*S. platorynchus* Rafinesque), and Alabama (*S. suttkusi* Williams & Clemmer) sturgeons represent a morphologically distinct genus indigenous to the Mississippi and Mobile river drainages of North America (Bailey and Cross 1954). The shovelnose sturgeon is relatively common and inhabits most major rivers of the Mississippi River drainage, while the pallid sturgeon is endangered and restricted to the Missouri and the lower Mississippi River (Birstein 1993, Dryer and Sandoval 1993). The Alabama sturgeon is very rare and is restricted to the Mobile River basin of Alabama and Mississippi (Williams and Clemmer 1991). While the pallid and shovelnose sturgeons are morphologically distinct (Keenlyne and Henry 1994), hybrids are known to occur (Carlson et al. 1985, Keenlyne et al. 1994). The issue of the genetic distinction of pallid and shovelnose sturgeons has been controversial and was recently reviewed by Wirgin et al (1997). While several studies (Phelps and Allendorf 1983, Genetic Analysis, Inc. 1994) found no genetic differentiation between the species, others (Campton et al. 1995) suggest they are distinct species. The purpose of this study was to determine if pallid and shovelnose sturgeons sampled from four US states can be differentiated using DNA sequencing of the mitochondrial D-Loop region.

METHODS AND MATERIALS

Fin tissue from five pallid and four shovelnose sturgeons from four US states was obtained during 1995 (Table 1). Tissue samples were preserved by freezing at -70°C. Voucher specimens are maintained at the Genetic and Forensic Analysis Laboratory of the Nebraska Game and Parks Commission, Lincoln, Nebraska, USA. DNA was isolated from a 5 × 5 mm portion of each sample using the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). The extracted DNA was stored at -20°C. PCR was conducted using the primers Proline-tRNA-2 (5'-ACCCTTAACTCCCAAAGC-3') and Phenyl-tRNA-1 (5'-GTGTTATGCTTTAGTTAAGC-3') (Bernatchez et al. 1992) per Szalanski et al. (in press). Amplified DNA was purified using Micron 50 microconcentrator (Amicon Inc, Beverly, MA) and resuspended to a volume of 10.0 µl using nanopure water.

Purified and concentrated DNA, approximately 2.5 ng/100 bp, was sent to the DNA Sequencing Facility, Iowa State University (Ames, IA) for direct sequencing

Table 1. Samples of *Scaphirhynchus platorynchus* and *S. albus* used in this study.

Sample ID	Species	State	River
SS635	*S. platorynchus*	MT	Yellowstone
SS638	*S. platorynchus*	MT	Yellowstone
SS641	*S. platorynchus*	MT	Yellowstone
SS644	*S. platorynchus*	MT	Yellowstone
PS109	*S. albus*	ND	Missouri
PS115	*S. albus*	ND	Missouri
PS129	*S. albus*	IL	Mississippi
PS560	*S. albus*	IL	Mississippi
PS572	*S. albus*	LA	Atchafalaya

in both directions. Consensus sequences for each sequenced individual were derived using GCG (Genetics Computer Group, Madison, WI) GAP program. The GenBank accession numbers for each sequence are AF176341 and AF224873 to AF224880. Sequences were aligned with GCG PILEUP program using paddlefish, *Polyodon spathula* (GenBank accession number AF176340), as the outgroup taxon. Parsimony analyses on the alignments were conducted with PHYLIP v3.57c (Felsenstein 1993) on phylogenetically informative characters only, with gaps being treated as a fifth character state. Bootstrapping was performed by generating 1000 data sets with the SEQBOOT program. Most-parsimonious trees were constructed using DNAPARS, and a majority rule and combinable competent consensus of these trees were constructed using the CONSENSE program in PHYLIP. The DNADIST program of PHYLIP was used to calculate genetic distances according to the Kimura 2-parameter (Kimura 1980) and maximum likelihood models of sequence evolution. Trees were constructed from these distances with the NEIGHBOR program to create neighbor-joining (Saitou and Nei, 1987) trees.

RESULTS

Polymerase chain reaction resulted in an 838 bp amplicon for the nine sturgeons sequenced. Among the nine individuals, 11 nucleotide positions are polymorphic, of which eight are transitions and three are transversions. Six mitochondrial haplotypes were observed. Genetic distance among all sturgeons sequenced ranged from 0.0 to 0.84%, with a mean of 0.26%. Alignment of the *Scaphirhynchus* and *Polyodon* mtDNA sequences resulted in a total of 941 characters, including gaps. Distance analysis resulted in a cladogram similar to the one derived from parsimony analysis (Fig. 1). Neither analysis resulted in a distinct clade for pallid and shovelnose sturgeons.

DISCUSSION

Although the examined sturgeons had nucleotide polymorphisms at 11 nucleotide sites of the mitochon-

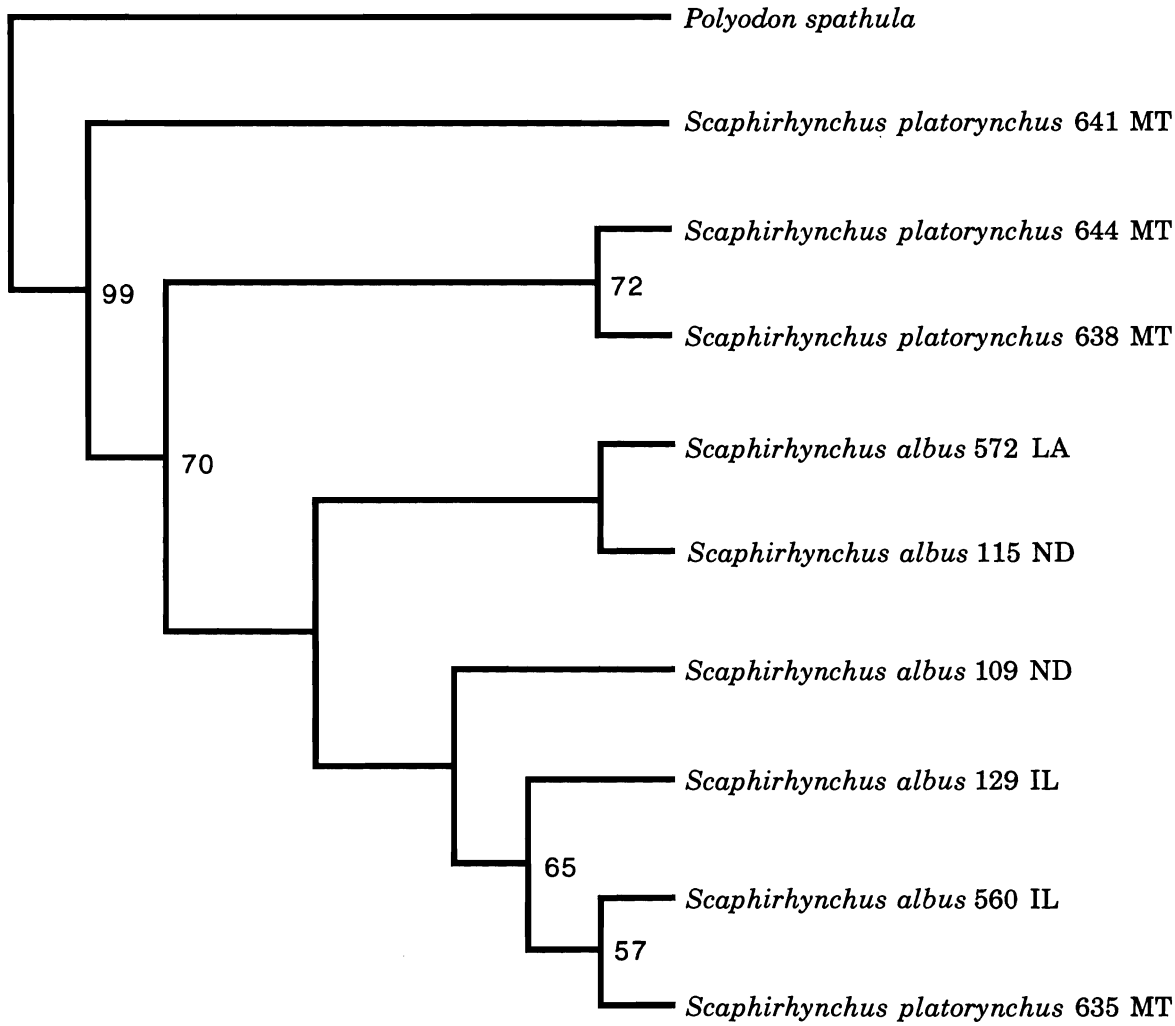

Figure 1. Maximum parsimony consensus cladogram generated by PHYLIP from 5 pallid and 4 shovelnose sturgeon D-Loop sequences, using paddlefish as the outgroup taxon. Only bootstrap percentages >50% are provided.

drial D-Loop region, shovelnose and pallid sturgeons are indistinguishable by our results. Because of the limited sample size of each population in our study, no conclusions could be drawn regarding the extent of the variation and possible interpopulational differences. This lack of genetic differentiation between pallid and shovelnose sturgeons has been observed in several other studies. Phelps and Allendorf (1983), using allozyme analysis, observed a lack of genetic evidence supporting pallid and shovelnose sturgeons as different species. PCR-RFLP analysis of nuclear DNA amplicons by Genetic Analysis, Inc. (1994) showed no significant difference between species for the only variable locus. Campton et al. (1995) sequenced approximately 435 bp of mitochondrial DNA of pallid and shovelnose sturgeons from the upper Missouri River and found a difference in haplotype frequencies between shovelnose and pallid sturgeons, but the haplotypes overlapped such that maximum parsimony analysis did not result in two species-congruent clades. Avise (1994) suggested that divergence between genetic and phenotypic data may represent introgressive hybridization or recent divergence of pallid and shovelnose sturgeons. While other nuclear and mitochondrial markers that are commonly used as phylogenetic tools showed low levels of variation when applied to Acipenserifomes (Birstein et al. 1997), the examination of nuclear ribosomal DNA internal transcribed spacer regions or other nuclear markers may yield different results and should be a goal of future research.

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