2000

Determining the phylogenetic relationships among the northern pike (Esox lucius), the muskellunge (Esox masquinongy), and the silver pike

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Determining the Phylogenetic Relationships Among the Northern Pike (*Esox lucius*), the Muskellunge (*Esox masquinongy*), and the Silver Pike.

Thesis Submitted to

The Graduate College of

Marshall University

In partial fulfillment of the

Requirements for the Degree of

Masters of Science Program

by

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10 April, 2000

This thesis was accepted on APRIL 10TH 2000
Month Day Year

as meeting the research requirements for the master's degree.

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ACKNOWLEDGEMENTS

I would like to thank Dr. Charles C. Somerville for his expertise and guidance throughout graduate school and this project. This thesis would not have been possible without his contributions. I must also thank him for all of his patience and one-on-one attention that he so willingly provided. I would also like to extend my appreciation to my graduate committee, Dr. Donald Tarter, Dr. Loren Miller, and Dr. Michael Little for their suggestions during the preparation of this manuscript. I would also like to thank Dr. Loren Miller for providing two silver pike samples and coming to Marshall University from the University of Minnesota for my oral exam and thesis defense.

I would like to thank Mr. Edward Theland of the Iowa Department of Natural Resources and the operators of the Marten River Lodge and guide Bill Tregenza for providing silver pike samples. I thank the Marshall University Graduate College and the NASA Grant Committee for funding.

I extend much love and thanks to my wife, Melissa, for her help and support through graduate school and preparation of this thesis. Special thanks to my daughter, Taylor, for providing much needed breaks to read her books, and to a good friend, Apple, for providing breaks from that. A deep appreciation is extended to my mother, Brenda, for her support, as well as providing a computer and quiet place to work, and to my father and step-mother, Steve and Janet, for their support during my academic career.

To these persons, my sincere thanks.

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Keywords: Silver pike, northern pike (*Esox lucius*), muskellunge (*E. masquinongy*), mitochondrial 16 rRNA, phylogeny.

Prioritization of taxa has become a necessity due to limited resources available for conservation. Conservation efforts are often concentrated on those threatened and endangered species with the highest taxonomic importance. The silver pike is a rare game fish, often accounting for less than 1% of a total pike population where it is found. Although silver pike are normally found in association with northern pike, they have distinct morphological characteristics and have not been shown to hybridize with the northern pike. Still, many fisheries biologists consider the silver pike to be a color variant of the northern pike, and to date the silver pike has not been assigned to a taxonomic unit. This study employs the use of a molecular marker on the mitochondrial genome to determine the phylogenetic relationships between the northern pike, silver pike and muskellunge. Mitochondrial DNA was amplified from individual scales, cloned into the pCR®2.1 vector and sequenced at the Marshall Core Facility. The sequences were aligned using ClustalX (Thompson et al., Nuc. Acids Res. **22**:4673), and phylogenetic distances and tree topologies were inferred using the programs of the PHYLIP package (Felsenstein, Cladistics **5**:164). Sequence data was sufficient to distinguish between the northern pike and muskellunge. No sequence variation was found between the silver pike and northern pike. Resulting tree topologies were unable to distinguish between the silver pike and northern pike in all cases. Molecular data supports the hypothesis that the silver pike is a color variant of the northern pike and not a separate species.

CHAPTER I

INTRODUCTION

Silver Pike

The silver pike is a member of the family Esocidae and most closely resembles the northern pike (*Esox lucius*) in morphological characters. The silver pike is often referred to as a blue pike in Canada. This common name often leads to confusion with a subspecies of the walleye (*Stizostedion vitreum glaucum*) from the Great Lakes region, which is believed to be extinct. Few publications exist on the silver pike. The only peer reviewed journal article from Lawler (1960) stated that the silver pike is a color mutation of the northern pike. Scott and Crossman (1973), Eddy and Underhill (1974), and Becker (1983) briefly discuss the silver pike, mainly pointing out the obvious color difference between the two fish. The majority of citations originally stem from an earlier publication by Eddy and Surber (1943).

The correct common name is the silver pike rather than the silver muskellunge once used by Minnesota fisherman (Eddy and Underhill, 1974). The most distinct difference between the northern pike and the silver pike is that of color (Fig. 1). The northern pike has a basic color arrangement of light yellow to whitish bean shaped spots about as long as the diameter of the eye on a dark, brilliant green to olive background. The body has the appearance of being flecked with gold, caused by a tiny gold spot on the tip of the exposed edge of most body scales. The dorsal, caudal and anal fins are green to yellow and sometimes orange or pale red with large irregular black markings.

Paired fins are usually unmarked and buff to dusky (Scott and Crossman, 1973). The silver pike has deep blue color in the dorsal area, shading through lighter blue and gray devoid of bean shaped spots on the flanks, to white on the ventral surface. The flanks of the silver pike are more specifically dark silver or gray and sometimes flecked with gold. The fins appear to be almost clear with a tint of orange and red and may be speckled with black, particularly the ventral fins (Lawler, 1960). Occasionally, silver pike are found with faint spots on the caudal peduncle, similar to those found on the northern pike but less distinct (Eddy and Underhill, 1974). Silver pike are distinguishable from the northern pike even after death as their silver color intensifies rather than fades (Lawler, 1960).

Although coloration is the most obvious difference between silver pike and northern pike, there are several other subtle morphological differences. First, in a sample of 33 silver pike compared to 189 northern pike, two silver pike had a higher lateral line scale count than the maximum recorded for the northern pike (Lawler, 1960). Though this is not statistically significant, it supports anecdotal information from fisherman that suggest silver pike have finer scales than the northern pike, similar to a rainbow trout (*Onkorhynchus mykiss*). Silver pike have consistently narrow body width in individuals between 150 to 400 mm, while silver pike between 400 to 550 mm were not different from northern pike of the same size. Interorbital width was the most substantial morphological difference between the northern and silver pike. Although the diameter of the eye can not be measured as accurately as a bony structure, interorbital width confirmed that eye diameter was larger in the silver pike than in the northern pike

(Lawler, 1960). When interorbital distances were plotted arithmetically against the standard length, it was possible to draw a line through all the points above which only northern pike occurred and below which only silver pike occurred (Lawler, 1960; Fig. 2). In the same study, measurements of the mandible and maxillary of the silver pike were plotted against the standard lengths to show that 23 of the 28 silver pike had shorter mandibles than did northern pike. It was also shown that 24 of the 26 silver pike had shorter maxillary lengths than northern pike. This suggests that silver pike have a shorter snout length than do northern pike, though no statistical analysis was performed. Bernardo (1998) stated that silver pike have a caudal fin shape similar to that of the muskellunge, the tips being more pointed than those of northern pike, which are noticeably more rounded. This was also noted in observations made during the present study. Although the average size of the silver pike is similar to that of the northern pike, it rarely reaches the maximum sizes of the northern pike. Silver pike seldom exceed ten pounds (Eddy and Underhill, 1974), though larger fish have been reported. Lawler (1960) also noted that at each age the average length and weight of silver pike was greater than that of northern pike.

There are also morphological similarities between the silver pike and the northern pike. As is common with the northern pike, the opercle of the silver pike is only scaled on the upper half while the cheek is completely scaled. The submandibular pore count resembled that of the northern pike with five on both sides totaling ten (Lawler, 1960). Branchiastegal ray counts fell within the range of the counts for northern pike as did fin ray counts and average head lengths for all specimens observed. Stomach contents

showed that silver pike feed on foods similar to that of northern pike (Lawler, 1960). Food items included perch (*Perca flavescens*), common sucker (*Castotomus commersoni*), spottail shiner (*Notropis hudsonius*), nine spine stickleback (*Pungitius pungitius*), and troutperch (*Percopsis omiscomaycus*).

Silver pike captured and placed in aquaria appeared to be exceptionally hardy. One specimen went 22 days before eating, although ample food was supplied. The same fish was dipped twice in formaldehyde to kill *Saprolegnia*, and still lived for 47 days after capture (Lawler, 1960). In another account by Lawler (1960), a silver pike was caught, held by the eyes to dislodge a hook, placed on the deck of the boat where it remained for two hours before being placed in an aquarium, and survived without the appearance of ill effect. Scott and Crossman (1973) also reported that silver pike is similar to northern pike except for an apparent increased hardiness.

Several breeding experiments have been reported but not extensively documented. Silver pike specimens were incidentally obtained while collecting brood stock muskellunge for the Nevis Fish Hatchery from Lake Belletaine, Minnesota. These silver pike were propagated along with the true muskellunge and stocked in nearby lakes for several years. Breeding experiments at the Spirit Lake Hatchery, Iowa (Eddy and Underhill, 1974) found that silver pike breed true in that all offspring were similar to the parents. When silver pike were crossed with northern pike, the offspring had a peculiar black mottling similar to that of the black crappie (*Pomoxis nigromaculatus*) and subsequent generations could not be obtained (Runnström, 1949). This suggests that the offspring from a silver pike-northern pike cross are sterile. When the silver pike was

crossed with the muskellunge the resulting F1 generation resembled a tiger muskellunge which is a cross between the muskellunge and a northern pike. Since no specimens having the black mottled pattern of a silver pike-northern pike hybrid have been taken in the field, it is believed that the silver pike and northern pike do not hybridize in nature.

No research has been done on spawning times or behaviors of silver pike, although silver pike have been captured with spawning muskellunge (Eddy and Underhill, 1974) and spawning pike (Lawler, 1960). All members of the family Esocidae spawn in the same manner. In general, species within this family spawn in the spring in heavily vegetated flood plains. Eggs are scattered at random over the vegetation as large females and smaller males roll bringing vents into proximity while eggs and milt are extruded. Rapid thrusting of tails further scatter the settling eggs, which are not protected by either parent. Because the eggs of these species are scattered in such a manner, the presence of spawning silver pike in the general location of spawning northern pike would lead to an increased occurrence of the silver pike-northern pike hybrids. It is difficult to distinguish the difference between a silver pike-muskellunge and a northern pike-muskellunge hybrid, so occurrence of this hybrid would not be as easily detected. Due to the lack of parental care of fertilized eggs and fingerlings, and the fast growth rate of the northern pike fingerlings, subsequent spawning runs of other fish provide excellent forage for the voracious northern pike fingerlings. This is the fate of many muskellunge fry (Scott and Crossman, 1973) where the muskellunge and northern pike occur together, and may also be the fate of silver pike, explaining decreased population densities.

The most limiting factor on the research of the silver pike is its small population density in relation to the total pike population. Of the total pike population of Heming Lake in Manitoba, Canada, it was estimated that only 0.2 percent were silver pike (Lawler, 1960). From 1952 to 1958, 19,420 pike were taken from Heming Lake, of which only 42 were silver pike. Sixty pike were taken from Angler's Lake, Manitoba, in 1958. Only two of the 60 fish were silver pike. The following year the same ratio applied. In 1959 at Beaverlodge Lake in Mackenzie, Canada, one silver pike was caught out of 151 total pike (Lawler, 1960). From a small lake near the Marten River in Ontario, out of 104 pike caught, four were silver pike (personal observations). Early spawning times, faster growth, better food conversion, and early dominance of forage fish have been cited to explain the northern pike's domination of other Esocids where habitats overlaps (Scott and Crossman, 1973).

Discussions on the classification of the silver pike are clouded by confusion and misunderstanding among fisheries biologists. Becker (1983) stated that hybridization between the silver pike and muskellunge leads to offspring similar to muskellunge-northern pike hybrids, which is speckled like a crappie. The muskellunge-northern pike hybrid, known as the tiger muskellunge, is characterized by having a strong barring pattern on its sides (Page and Burr, 1991). Eddy and Surber (1943) stated that the muskellunge-silver pike hybrids do resemble muskellunge-northern pike hybrids, but it is the silver pike-northern pike hybrids that are characterized by having the crappie-like black mottling. Anecdotal reports from fisheries biologist have reported that silver pike are dominant where they are found and only occur in isolated areas. This contradicts

distribution described by Lawler (1960) and Eddy and Surber (1943) and adds to the confusion among fisheries scientists. The lack of research and familiarity with this fish has resulted in its being arbitrarily categorized as a color mutation of the northern pike based on distribution and morphological characteristics.

Molecular Chronometers

The use of molecules as "documents of evolutionary history," or "molecular chronometers" was first introduced in 1965 by Emile Zuckerandl and Linus Pauling (Wolfgang and Schleifer, 1999; Woese, 1987). The advent of molecular chronometers provides taxonomists with a powerful tool to complement phenotypic techniques. Genotypic information is more readily, reliably, and precisely interpreted than phenotypic patterns allowing for increased information on evolutionary relationships (Woese, 1987). In addition, molecular characters are less likely related to adaptive evolution than are morphological characters (Briolay et al., 1998). Despite the benefits inherent in phylogenies derived from sequence data, it is important to remember that genetic information yields hypotheses to be tested and either strengthened or rejected on the basis of other kinds of data (Woese, 1987). In cases where molecules and morphology disagree, provisional morphology-based constraints on the analysis of molecular data offer a practical means of integrating the two types of data (Normark et al., 1991).

Mitochondrial DNA (mtDNA) mutates at a rate that is ten times faster than that of nuclear DNA (Wiesner et al., 1991; Patarnello et al., 1993). This rapid mutation rate

makes mitochondrial DNA a powerful tool in estimating the degree of divergence between closely related species (Wilson et al., 1985; Avise et al., 1987). In addition, the ease with which mitochondrial genes can be isolated and sequenced coupled with special features of mitochondria such as lack of introns, maternal inheritance, absence of recombination events, and haploidy also contribute to its reputation as a reliable tracer of evolutionary history (Zardoya and Meyer, 1996). A marvel of genetic economy, the mitochondrial genome encodes 22 tRNAs, 13 mRNAs, and 2 rRNAs in a 16.5-to 17.5- kb closed circular molecule (Digby et al., 1992; Lake, 1998).

Molecules whose sequences change randomly in time can be considered chronometers (Woese, 1987). In order to yield useful information, the clocklike behavior of molecular chronometers should measure, and be representative of, the overall rate of evolution. The amount of change within a sequence that accumulates (proportionate to genetic distance) is the product of the rate (in occurrence of fixed mutations) multiplied by the time over which these mutations occur. Because distance can not be measured from the original to final states of evolution due to the lack of original states, distances are measured between extant forms. The distance from original states can then be estimated given comparable rates of change in each line.

Ribosomal RNA has been termed the ultimate molecular chronometer (Woese, 1987) and was chosen for this study for several reasons. They are found in all organisms. Their large size provides several domains changing at varying rates; this allows for different levels of evolution to be evaluated. The functional constancy and genetic stability assures a relatively good clocklike behavior. The 16S rRNA contains highly

variable regions that change fast enough to provide information on closely related species (Mangold et al., 1997). There has also been a large sequence database describing a wide spectrum of phylogenetically diverse organisms developed over the past decade.

Mitochondrial 16S rRNA gene has developed the reputation of being a reliable tracer of evolutionary history. Another common molecule used for determining phylogenetic relationships is cytochrome *b*. Some studies indicate, however, that phylogenetic relationships among deep branching lineages are poorly resolved using cytochrome *b* sequences (Briolay et al., 1998). Patarnello et al. (1994) used 16S rRNA gene sequence variation combined with that of cytochrome *b* to infer phylogeny of the brown trout (*Salmo trutta*). They found that the 16S rRNA gene alone gave identical results to those of the combined sequences.

This study employs the use of the mitochondrial 16S rRNA gene as a molecular chronometer to determine the phylogenetic relationships between the northern pike, silver pike and muskellunge. Comparison of mtDNA sequences has become a common way to establish phylogenetic relationships among closely related genera (Woese, 1987; Mangold et al., 1997; Parker and Kornfield, 1996; Normark et al., 1991; Ritchie et al., 1997; Patarnello et al., 1993; Danzmann and Ihssen, 1995; Matthee and Robinson, 1997; Murphy and Collier, 1997; Briolay et al., 1998; Gilles et al., 1998). The use of morphological characteristics such as body size, color, behavior, ecophysiology, and meristics alone to classify higher vertebrates is becoming more questionable, due to the phenotypic plasticity of many similar species. Adding the use of molecular chronometers to existing phenotypic techniques to distinguish between phenotypic variants, subspecies,

and true Linnean species may lead to fewer inappropriate taxonomic divisions.

In recent phylogenetic studies, DNA segments of 300-600 base pairs have been found to be sufficient to infer evolutionary relationships (Kocher et al., 1989; Patarnello et al., 1993). For this study, a 370 bp region of the 16S rRNA gene was used. The northern pike and muskellunge, two clearly distinct species, were used as a control to establish a phylogenetic distance sufficient to delineate species. According to Mangold et al. (1997), a difference of 1.3 percent was sufficient to delineate between two tick species *Rhipicephalus sanguineus* and *R. turanicus*. The high percentage of similarity between these two species suggests they recently diverged within the genus *Rhipicephalus*.

T Vectors

The use of T vectors allows the generation of unambiguous sequence data. T vectors are linearized plasmids containing single 5' T overhangs. This allows for the direct and efficient cloning of PCR products. This is possible due to the inherent activity of *Taq* polymerase to add a single dATP nucleotide to the 3' ends of PCR products under standard PCR conditions. Standard primer annealing sites (M13-20, M13 reverse, T3, and T7) on either side of the inserted amplicon in these vectors allow for efficient sequencing.

Sequence Analysis

Once sequence data have been collected and aligned their meaning or results

must be interpreted. The two most common and accepted ways to develop phylogenetic trees from sequence data, are known as distance matrix and maximum parsimony methods. The distance matrix technique considers all possible pair-wise alignments, i.e., it compares the fraction of positions that are different between the two sequences. The differences are then calculated into a distance and recorded in a matrix. Distance matrices often underestimate true distance due to the occurrence of multiple mutational events at a single site. This has been taken into account by algorithms, which increase the calculated distance as a function of increased sequence divergence. The programs FITCH, KITSCH, and NEIGHBOR are for dealing with data which come in the form of distance matrix. The FITCH and NEIGHBOR programs fit a tree which has the branch lengths unconstrained. The KITSCH program assumes that an "evolutionary clock" is valid, according to which the true branch lengths from the root of the tree to each tip are the same.

In contrast, maximum parsimony and maximum likelihood techniques analyze primary sequence data without the initial cluster analysis of matrices of binary distance values. Maximum parsimony treats each position individually, assuming that the most correct phylogenetic tree is the most stringent, in terms of differences between sequences. Tree topologies are determined directly from sequence alignments and are based on finding the shortest possible tree length. For each tree topology, parsimony methods calculate the minimum number of nucleotide changes that are required to explain the observed pattern. The number of changes are summed for each tree topology, and the topology having the smallest total number of changes is considered the best

estimate of phylogeny (Yang, 1996). Maximum parsimony is recommended when the rate of evolution is more or less constant among lineages and the amount of evolution is small (Yang, 1996).

This study employs the use of a 370 base pair sequenced region of the 16S rRNA gene from the mitochondrial genome to determine the phylogenetic relationships between the northern pike, muskellunge, and the silver pike. Sequence data will be interpreted using both distance matrix and maximum parsimony methods.

CHAPTER II

TAXONOMY AND DISTRIBUTION

Taxonomy

Eukaryota: Chordata: Vertebrata: Osteichthyes: Actinopterygii: Teleostei: Esociformes: Esocidae: *Esox*: *lucius*.

Distribution

In 1897, E. E. Prince gave the first professional assessment of a fish collected from Sharbot Lake, Ontario that resembled a northern pike but lacked the characteristic coloration (Eddy and Surber, 1943). In 1930 biologists collected several specimens of what local fisherman called silver muskellunge from Lake Belletaine near Nevis, Minnesota while collecting brood stocks of muskellunge for the Nevis Fish Hatchery. This was the first time the silver pike appeared while collecting muskellunge at this sight, and local fisherman reported that it had not appeared in their catches until that year (Eddy and Underhill, 1974). For several years afterwards the silver pike was raised along with true muskellunge and stocked in nearby lakes, thus increasing its range throughout much of Minnesota. Years later a specimen was taken from Detroit Lake, where they were not introduced, suggesting silver pike were not confined to Lake Belletaine. In the summer of 1952, a silver pike was found in Manitoba from Heming Lake. Further collections of silver pike from this lake were intensely recorded for seven years. Lawler (1960) noted that the silver pike was also present in several lakes adjacent to Heming

Lake. In 1959 a silver pike was collected from Beaverlodge Lake in Mackenzie, Canada by a field party of the Arctic Unit of the Fisheries Research Board of Canada. This fish represented the northernmost known record of the silver pike range. The silver pike is not confined to North America. Runnström (1949) reported that G. Svärdson had accounts of a silver colored pike in Sweden on which he had performed breeding experiments. Since these early reports, the silver pike has been reported sporadically throughout much of the circum-polar distribution inhabited by the northern pike (Scott and Crossman, 1973). Scott and Crossman (1973) also indicated that the silver pike has been found only in conjunction with the northern pike (Fig. 3)

CHAPTER III

MATERIALS AND METHODS

Primer Design

The mitochondrial genome coding for the 12S rRNA, 16S rRNA, and tRNA-Val genes of ten species, the fin whale (*Balaenoptera physalus*, Arnason et al., 1991), blue whale (*Balaenoptera musculus*, Arnason and Gullberg, 1993), cow (*Bos taurus*, Anderson et al., 1982), rat (*Rattus norvegicus*, Gadaleta et al., 1989), mouse (*Mus musculus*, Bibb et al., 1981), opossum (*Didelphis virginiana*, Janke et al., 1994), chicken (*Gallus gallus*, Desjardins and Morais, 1990), trout (*Oncorhynchus mykiss*, Zardoya et al., 1995), loach (*Crossostoma lacustre*, Tzeng et al., 1992), and carp (*Cyprinus carpio*, Chang et al., 1994) were obtained from GenBank and aligned using ClustalX (Thompson, 1994; Appendix A). Examination of the 16S rRNA gene alignments revealed a number of hypervariable regions flanked by highly conserved sequences. This orientation is important because it allows for the construction of PCR primers to anneal to the adjacent conserved areas and amplify through hypervariable regions.

Once the 16S rRNA gene sequence was chosen, 13 sequences from different genera in the family Cyprinidae were aligned using Clustal X (Thompson et al., 1994) along with one sequence from the northern pike (Appendix B). Sequences were then analyzed to find the region that provided the most variability and still provided enough conservation for primer annealing. Each base pair (bp) location along the 16S rRNA gene (1722 bp long) was compared among all species and assigned a number. If all bases

were the same across all species the row was assigned a zero. If that location contained only two different bases (ex. A and G) that row was assigned a one, if there was three bases (ex. A, G, and T) the row was assigned a two, all four bases scored a three (Fig. 4). If a site within the sequence of the 16S rRNA gene is capable of having any one of the four bases and still remains functional, the site is considered highly variable. Next, all sites that were completely conserved across all species for at least 18 consecutive bp were noted as possible primer locations. Then, the variability between primer sites was calculated. Two sites were found that contained highly variable regions flanked by good primer locations. The two sites were separated by a 23 bp conserved area. It was then decided to combine the two areas to provide a single 338 bp target region. This area had a 30 bp-conserved region on the 5′ end and a 45 bp conserved region on the 3′ end.

All possible permutations of the upstream and downstream primer sequences were analyzed using the Lazergene Primer Select program (DNAstar, Madison, WI). Of 20 potential primers, one forward primer and one reverse primer were found to have acceptably low self-annealing properties. These primer sequences were used to amplify the DNA from fish tissues. The forward primer was 5'-GGT AGC GCA ATC ACT TGT CT-3'. The reverse primer was 5'-TAT CCC TAG GGT AAC TTG GT-3'. Primers were synthesized at the Marshall University DNA Core Facility. Their designation and properties are listed in Tables 1 and 2. Sequences that would be amplified by the designed primers for members of the family Cyprinidae and the northern pike were then taken from GenBank and aligned using Clustal X (Thompson et al., 1994) to determine efficacy of this DNA region for phylogenetic reconstruction (Fig. 5).

DNA Isolation

DNA samples were prepared for the fish listed in Table 3. All tools used in preparation of fish scales for DNA amplification were first placed in 1 N HCl, rinsed with sterile water, placed in 10 N NaOH, rinsed with sterile water and soaked in 95 percent ethanol until used. All procedures were carried out under a laminar flow hood. Fresh fish specimens were prepared by first swabbing the area where a scale would be removed with 95 percent ethanol to minimize surface contamination. When the area was completely dry, a single scale was removed with a pair of flame-sterilized tweezers and placed in a 1.5 ml sterile centrifuge tube with 100 μl of 5 percent chelex and 1 μl of Proteinase K. Tubes were then placed in a dry bath at 56°C for at least 1.5 hours, preferably overnight. Following incubation at 56°C, tubes were boiled for 8 min then immediately centrifuged at 16,200 *x g* for 5 min and stored at -20°C until used. Scales were unavailable for silver pikes three and four, northern pikes two and three, and muskellunge four and five. In this case, muscle tissue was used and prepared in a similar manner. A sterile wooden applicator was used to obtain a very small amount of tissue from sample. By twisting the wooden applicator tip in the tissue sample, a small amount of tissue (~10 mg) could be obained. Subsequent procedures remained the same for both scales and tissue samples.

Amplification

Amplification mixtures were prepared using a PCR core kit as specified by the

manufacturer (Boehringer Mannheim, Germany). Taq DNA polymerase was not added until tubes were placed in the thermal cycler and heated to 94°C for a minimum of 1 min. Cycle one was 5 min at 94°C and allowed ample time for addition of Taq polymerase. Cycle two consisted of 1 min at 94°C for denaturation, 1 min at 37°C to allow primers to anneal, and 1 min at 72°C for primer extension and was repeated 30 times. Cycle three used the same denaturation and primer annealing times and temperatures but had a 6 min extension time. Upon completion of amplification tubes were stored at -20°C. Agarose gel electrophoresis was used to confirm the presence of an amplicon. To aid in determination of band size, one lane contained 0.5 μg/μl of a 1 Kb DNA ladder (Promega, Madison, WI) in 4 μl water with 1μl of 10X stop buffer (See Table 1). Gels were run with a FisherBiotech Electrophoresis Systems FB 105 voltage regulator and FB MSU-1 Small Horizontal Gel System. Gels were then viewed using a Spectroline® Transilluminator (Model TR-302, 302nm ultraviolet) and photographed using a Fisher Scientific Photo-Documentation Camera (FB-PDC-34), a Tiffen®40.5 mm deep yellow filter with Polaroid type 667, 3000 iso black and white film.

Cloning

Amplification products were cloned into either the pCR®2.1 (Invitrogen, Carlsbad, CA) vector or the pT-NOT vector (Max-Planck-Institut Für Immunbiologie, Freiburg, Germany; Table 3) using a protocol modified from the TA Cloning Kit® (Invitrogen, Carlsbad, CA). Fresh amplification product was added to 12.5 ng/μl of pCR®2.1 vector in a weight ratio of 3:1 with 1 μl of 10X ligation buffer. The volume of

the mixture was then adjusted to 9 μl with sterile dd water and 1 μl of T4 DNA Ligase was added. Ligation reactions were incubated overnight at 14°C and stored at -20°C.

Plasmids were transformed using a modified protocol included in the TA Cloning Kit®. Competent cells of INVαF' strain designation, 0.5 M β-mercaptoethanol (β-ME), and the ligation mixture were thawed on ice. After thawing, 2μl of 0.5 M β-ME, and 5 μl of ligation reaction mixture were added to 25 μl of competent cells and mixed gently by stirring with the pipette tip. Competent cells were then incubated on ice for 30 min before being heat shocked at 42°C for 30 sec. Trypticase soy broth (TSB) (1 ml) was added and competent cells were incubated for 1hr at 37°C. The cells were then spread on Luria Bertani (LB) agar plates containing 50 μg/ml of ampicillin and 40 μl of X-Gal (40 mg/ml) and incubated overnight at 37°C.

The pCR®2.1 vector contains the *lacZ*α gene. This allows for blue-white screening. Bacterial cells that contain an uninterrupted *lacZ*α gene produce colonies that are blue in color. If bacteria take up plasmids that have a *lacZ*α gene that has been interrupted by insertion of PCR amplicon, the colonies are white. Nine white colonies and one blue colony were transferred from LB plates to 5 ml TSB with 50 μg/ml of ampicillin and incubated overnight at 37°C in a waterbath shaker. The cell suspension (1.5 ml) was pelleted in a 1.5 ml microfuge tube by centrifuging 2 min at 10,000 *x g*. The pellet was then resuspended in 200 μl of cell resuspension buffer (Table 1) and lysed with 200 μl of NaOH-SDS for 3 min. The suspension was then neutralized with 200 μl of 3M Na-acetate and incubated at room temperature for 4 min before centrifuging 10 min at 10,000 *x g*. The supernatant was collected, 1 ml of 95 percent ethanol was added

and the sample was centrifuged at 16,000 *x g* for 30 min at 4°C. The supernatant was discarded and the pellet was then rinsed three times with 70 percent ethanol and one time with 95 percent ethanol. The pellet was dried and resuspended in 400 µl of water and 200 µl of 7.5 M Ammonium Acetate. This solution was incubated at room temperature for 10 min then centrifuged for 15 min at 16,000 *x g*. The supernatant was collected, 1 ml of 95 percent ethanol was added and the sample was centrifuged at 16,000 *x g* for 30 min at 4°C. The supernatant was discarded and the pellet was then rinsed three times with 70 percent ethanol and one time with 95 percent ethanol. The pellet was dried and hydrated in 50 µl of water. Agarose gel electrophoresis was used to confirm the presence of plasmids containing an amplicon.

Samples containing plasmids with inserts were desalted using the Wizard®*Plus* Minipreps DNA Purification System (Promega, Madison,WI). Purified plasmids were sequenced at the Marshall University DNA Core Facility (Huntington, WV). Both strands were determined for all sequences to assure accuracy. Sequences were aligned using Clustal X (Thompson et al., 1994). Phylogenetic relationships were analyzed using distance matrix and maximum parsimony methods.

All phylogenetic analyses were performed using programs of the PHYLIP package (Felsenstein, 1989). Sequence alignments were bootstrapped using the SEQBOOT program. One hundred bootstrapped data sets were used to assess reproducibility. The resulting output file was analyzed first using DNAPAR. DNADIST was used to generate distance matrices for the NEIGHBOR, FITCH, and KITSCH treeing programs. The distance matrix file was then run in the NEIGHBOR, FITCH and the

KITSCH programs using global rearrangement, randomized input, and 100 multiple data sets with all other settings remaining as default. The resulting files were then run in CONSENSUS to determine the consensus tree topology and trees were visualized using Treeview (Page, 1996; Figs. 7,8,9, and 10). For all trees the four muskellunge sequences were set as outgroup taxa.

Specimen Collection

One northern pike sample was collected from the Great Miami River near Dayton, Ohio and three northern pike specimens were collected from Ramsey Lake in Ontario, Canada. Two northern pike were collected from the Marten River, also in Ontario, which also provided one silver pike. Muscle tissue from two silver pike from Young Lake, Minnesota was obtained from Dr. Loren Miller from the University of Minnesota. Two silver pike specimens were obtained from Mr. Ed Thelend at the Spirit Lake Fish Hatchery, Iowa. Three muskellunge samples were obtained from the Morehead Fish Hatchery, Kentucky and two were collected form the Green River near Cave City, Kentucky (Fig. 6, Table 3).

CHAPTER IV

RESULTS

Alignment of sequences within the Cyprinidae family amplified with designed primers were capable of distinguishing between genera in the same family (Fig. 5). High bootstrap values were found between specimens of the same species. The ability of the primers to distinguish between genera in the same family confirms the efficacy of the primers.

A total of 370 bp were sequenced from each of the 12 specimens. There was one variation observed among the five silver pike, a transversion at site 159. Three sites were not conserved between all northern pike, transitions at sites 143, 207, and 326. There was also a single transition at site 197 found among the muskellunge. There were a total of 32 variable sites found among all species consisting of 21 transitions and 11 transversions. This high ratio of transition mutations to transversion mutations was also found in a study on mitochondrial 16S rDNA sequence variation of brown trout (Patarnello et al., 1994). There were 28 variable sites between the pikes and the muskellunge with a mean sequence difference of 7.6 percent. Nucleotide divergence between the muskellunge and the pikes was calculated by dividing the number of substitutions by the total number of nucleotides examined (Patarnello et al., 1994). Neither the maximum parsimony or distance matrix programs separated the northern pike from the silver pike (Figs. 7,8,9, and 10). Low bootstrap values were found among the silver pike and the northern pike in all trees. The grouping of the silver pike with

northern pike was not consistent through all trees and varied from one tree to another. No two silver pike or two northern pike were grouped together consistently.

CHAPTER V

DISCUSSION

This study uses mtDNA sequences in an attempt to classify the silver pike. In the eighteenth century, Carolus Linnaeus developed an order for classifying organisms which he termed taxonomy. Linnaeus described species in terms of their morphology. Physiology, biochemistry, life history, and behavior were soon accepted ways to classify organisms. The advent of DNA sequencing for use as a molecular chronometer gives biologist a powerful tool for classifying organisms. The problem comes when deciding what molecules are best or exactly how much divergence is necessary to reclassify an organism. As sequence databases grow and more organisms are examined this technique will grow in acceptance and the parameters will become more defined.

The first objective in primer design was to find a region within the mitochondrial genome that would provide sufficient variability for phylogenetic analysis and still be flanked by highly conserved areas for primer adhesion during the polymerase chain reaction (PCR). Although it was originally hoped to target intergenic regions for sequence determination and analysis, these regions proved to be too small to provide sufficient discriminatory information. The 16S rRNA gene was chosen for analysis after comparison of the 12S and 16S rRNA and the tRNA valine genes. The 16S rRNA gene provided functional constancy and genetic stability to assure a relatively good clocklike behavior, highly variable regions which mutate fast enough to provide information on

closely related species (Mangold et al., 1997), and a comprehensive sequence database describing a wide spectrum of phylogenetically diverse organisms.

Phylogenetic trees constructed from sequence data did not differentiate the silver pike from the northern pike. In all instances the four muskellunge samples were grouped together separate from all other samples (Figs. 7,8,9, and 10). The silver pike and the northern pike sequences were never grouped separate from each other but always intermingled. Low bootstrap numbers between silver pike and northern pike indicate the very low sequence variation between the two. By comparing sequences once they were aligned it was found that none of the differences between the northern pike and the silver pike were conserved throughout all the silver pike or all of the northern pike as was found in the muskellunge. Although there are significant differences between the northern pike and the silver pike in morphological characteristics, these differences are not apparent within the mitochondrial genome. Because the sequenced area was able to differentiate between the northern pike and the muskellunge, it contained enough variability to distinguish between two species within the same genus. This area was unable to differentiate between the northern pike and silver pike. Since none of the differences between the silver pike and northern pike occurred throughout all silver pike, the differences are not the result of divergence from the northern pike as a separate species, but instead are likely to be variations within a single population or individual. Use of specimens from several geographical locations removed the chance that variations among species are do to population varience and not speciation. The geographical distribution of specimens is a possible explanation for intraspecific sequence differences

at single sites. The phylogenetic trees developed in this study confirmed previous research based on morphological characteristics.

The minor sequence differences between the silver pike and the northern pike contrasts with the high degree of phenotypic variation and the inability of these fish to produce fertile hybrids. Similar situations have been reported in several species of fish. Despite at least three phenotypes with very different ecologies, the arctic char (*Slavelinus alpinus*) has very low genetic differentiation between these phenotypes (Vrijenhoek et al., 1987: Snorrason et al., 1988). In contrast, despite their similar morphological characteristics, brown trout from the River Brenta, Ireland, compared to brown trout from the Stura and Ripa Stream, Ireland, display an appreciable degree of genetic differentiation (Patarnello et al., 1994). Cichlid fishes have also displayed differing rates of morphological and molecular evolution (Meyer et al., 1990; Sturmbauer and Meyer, 1992). These studies, present included, further support the idea that phenotypic characteristics evolve at different rates among different species.

The silver or blue color phase of the silver pike may be a similar genetic variation found in other types of fish. In two species of fish, the northern pike and the walleye (*Stizostedion vitreum*) there is a blue color phase which is devoid of many dominant color traits. In addition to this color difference there appears to be other morphological differences, mainly the diameter of eye and overall size. From the late 1800's to the mid 1900's, a species known as the blue pike (*Stizostedion glaucum*) was extensively harvested from the great lakes. The blue pike was similar to the walleye in appearance except its characteristic blue coloration, larger eyes and smaller overall size, the same

characteristics found in the silver color phase of the northern pike. In 1926 Dr. Carl Hubbs classified the blue pike as a separate species of the walleye (Manns and Quinn, 1999). The blue pike, prized for its soft flesh and small size, was quickly fished to extinction. It was placed on the Endangered Species List in 1971 though it was believed to be extinct. As is the case with the silver pike, the blue pike has received little research. Several fish have been collected that appear to be blue pike resulting in research being done to compare archived samples to recently obtained samples. If the blue pike was fished to extinction, it is possible that the color mutation once again occurred, starting another population of the true breeding variant. If the mutation occurs frequently enough to cause repopulation of blue pike in the Great Lakes, it would explain the large range of the silver pike. Recently, the blue pike was reclassified as a subspecies of the walleye (*Stizostedion vitreum glaucum*).

The word species means "kind" or "appearance" in Latin. According to Campbell (1996) a biological species is defined by its reproductive isolation from other species in a natural environment. Mayr (1942) proposed that species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups. Although the silver pikes very little divergence from the northern pike in the present study, the inability for the two morphs to hybridize adheres to the basic definition of a species.

Although the silver pike is not considered a subspecies, nor does this study suggest that, it does display several characteristics consistent to a subspecies. The definition of a subspecies according to Campbell (1996) is a population or group of

populations that live in one area and have minor differences from conspecific populations found elsewhere in the species geographical range. Mayr (1953) views a subspecies as being geographically defined aggregates of local populations which differ taxonomically from other subdivisions of a species. Although geographical ranges do not separate the silver pike and northern pikes, they are separated by life history traits (i.e., possible spawning times or niche utilization).

Though no taxonomic importance would be achieved, to classify the silver pike as a sibling species of the northern pike would provide a classification status of the silver pike. A Sibling species according to Mayr (1942) is another class of difficulties caused by pairs of even larger groups of sympatric, related species which are so similar that they are consider as belonging to one species until a more satisfactory analysis clears up the mistake. Mayr (1942) also states that there is no reason to believe that sibling species evolve in a manner that is in the least different from that of other species. Biologist dedicate their lives to defining what amount of evolution constitutes a species, to try to sum it up in a single chapter would be foolish.

The present study concurs with past studies and places the silver pike as a color variant of the northern pike. Nonetheless, fisheries biologists are given a chance to study the exact point at which a new species is evolving from a current species. It is as though we are witnessing the silver pike in its evolutionary descent from the northern pike. Species that are destined not to survive are common, evolving species that die out unstudied because of how they are classified should not be.

CHAPTER VI

SUMMARY AND CONCLUSION

Summary

The silver pike is a member of the family Esocidae and most closely resembles the northern pike (*Esox lucius*) in morphological characteristics. The most distinct difference between the silver pike and the northern pike is color, though other more subtle differences do occur. Breeding experiments have been reported on the silver pike, though not well documented. The silver pike always breeds true when crossed with other silver pike. When the silver pike is crossed with the northern pike, the offspring that survive are unable to produce a F2 generation, suggesting their sterility. The F1 generation of silver pike, northern pike hybrids are mottled like a crappie, a color morph of that pike has not been documented in nature. This suggest that the silver pike and northern pike do not hybridize in nature. The silver pike has only been documented in sympatric populations with northern pike, where it is found it often accounts for less than one percent of the total pike population.

Molecular chronometers provide taxonomists with a powerful tool to complement phenotypic techniques. The rapid mutation rate of mitochondrial DNA provides a reliable tool in estimating the degree of divergence among closely related species. The 16S ribosomal RNA gene has been termed the ultimate molecular chronometer due to its large size, genetic stability, highly variable regions, and large sequence database.

Conclusion

This study employed the use of a 370 base pair sequenced region of the 16S rRNA gene from the mitochondrial genome to determine the phylogenetic relationships between the northern pike, muskellunge, and the silver pike. Sequence data was interpreted using both distance matrix and maximum parsimony methods. The sequenced area was able to delineate between the northern pike and the muskellunge though no differences were found between the northern pike and the silver pike sequences. Phylogenetic trees constructed from sequence data did not differentiate the silver pike from the northern pike. The silver pike and northern pike sequences were never grouped separate from each other but always intermingled. Low bootstrap numbers between the silver pike and northern pike indicate the very low sequence variation between the two. Although there are significant morphological differences between the silver pike and the northern pike in morphological characteristics, these differences are not apparent in the 16S rRNA gene of the mitochondrial genome. The present study concurs with past studies and places the silver pike as a color variant of the northern pike.

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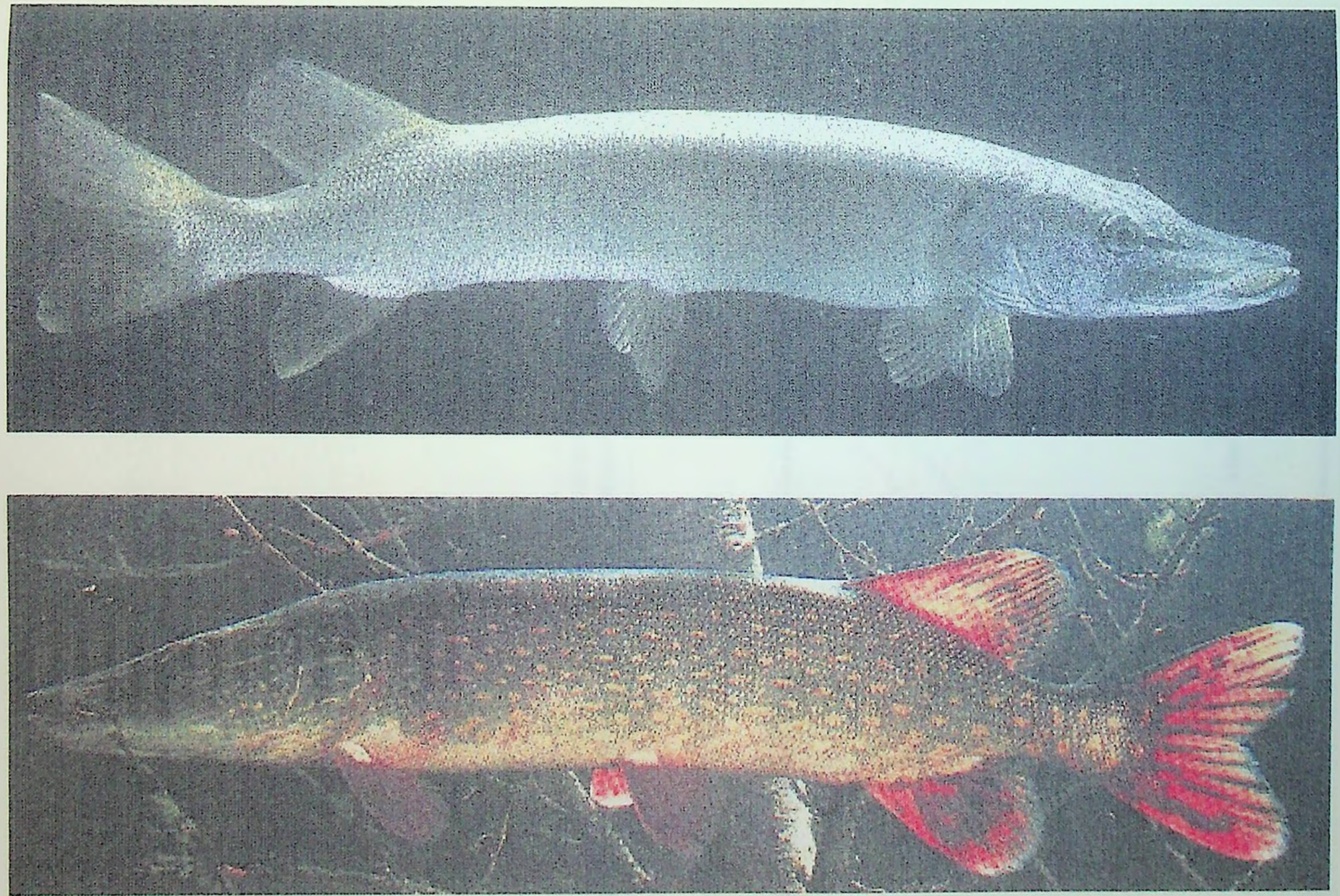


Figure 1. Silver pike (top) in comparison of northern pike (bottom) showing distinct color difference (Sternberg, 1992).

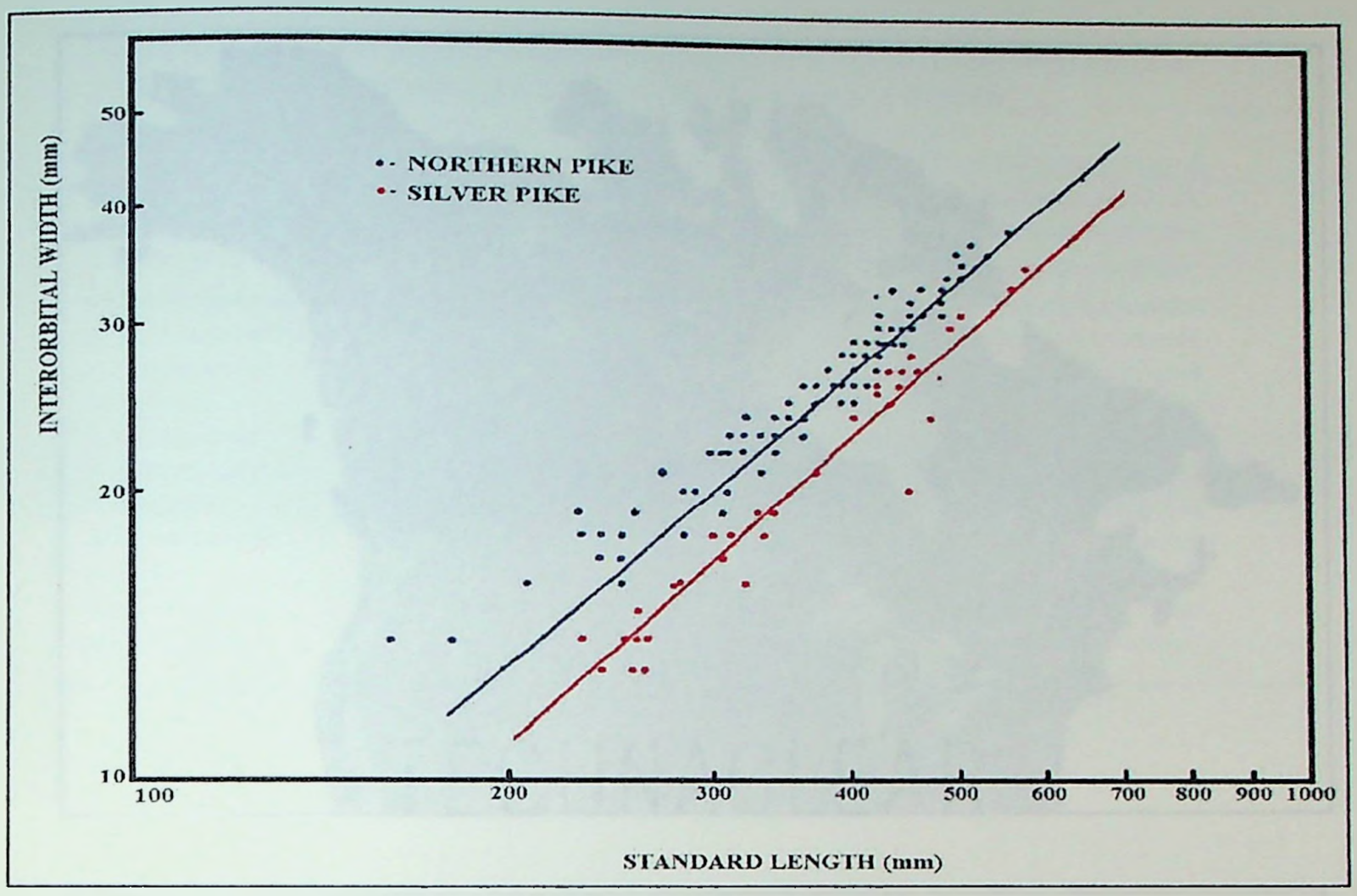


Figure 2. Relation between interorbital width and standard length of normal and silver pike from Heming Lake, Manitoba (Lawler, 1960)

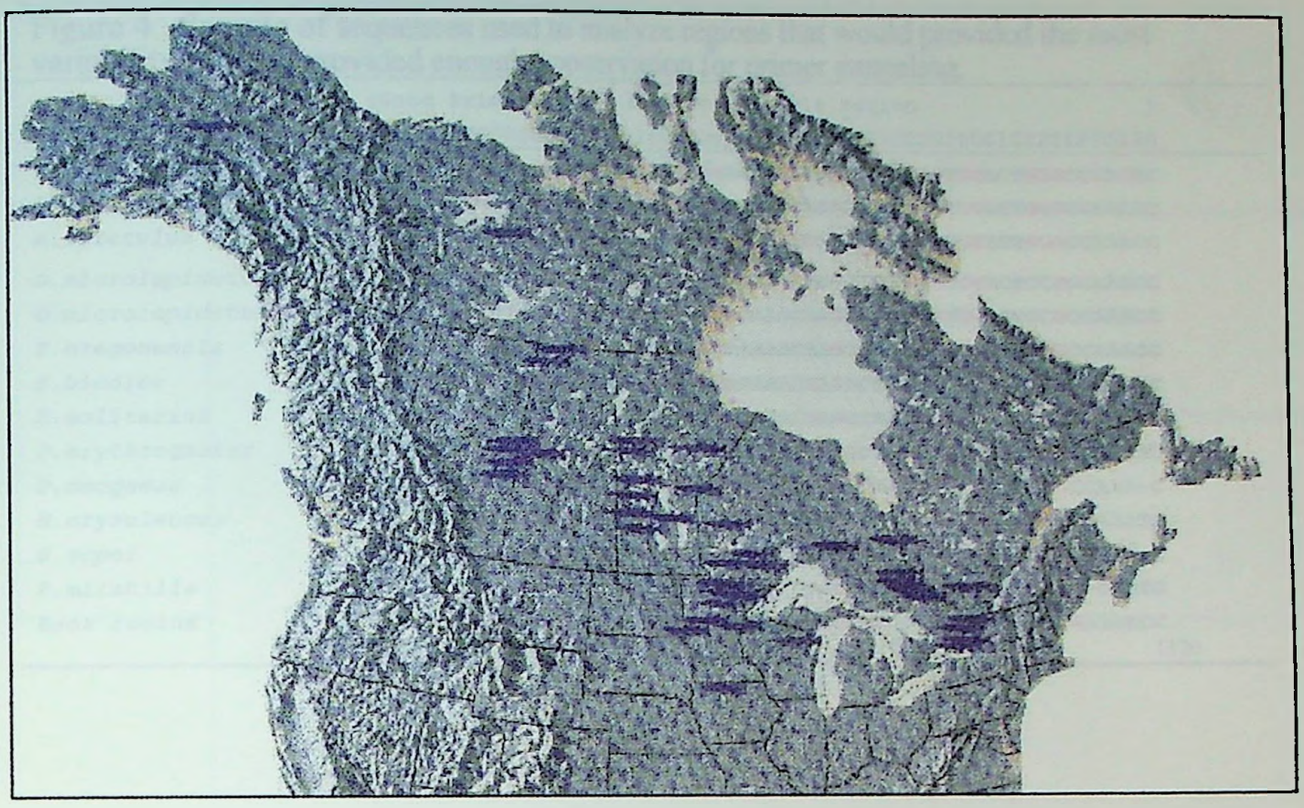
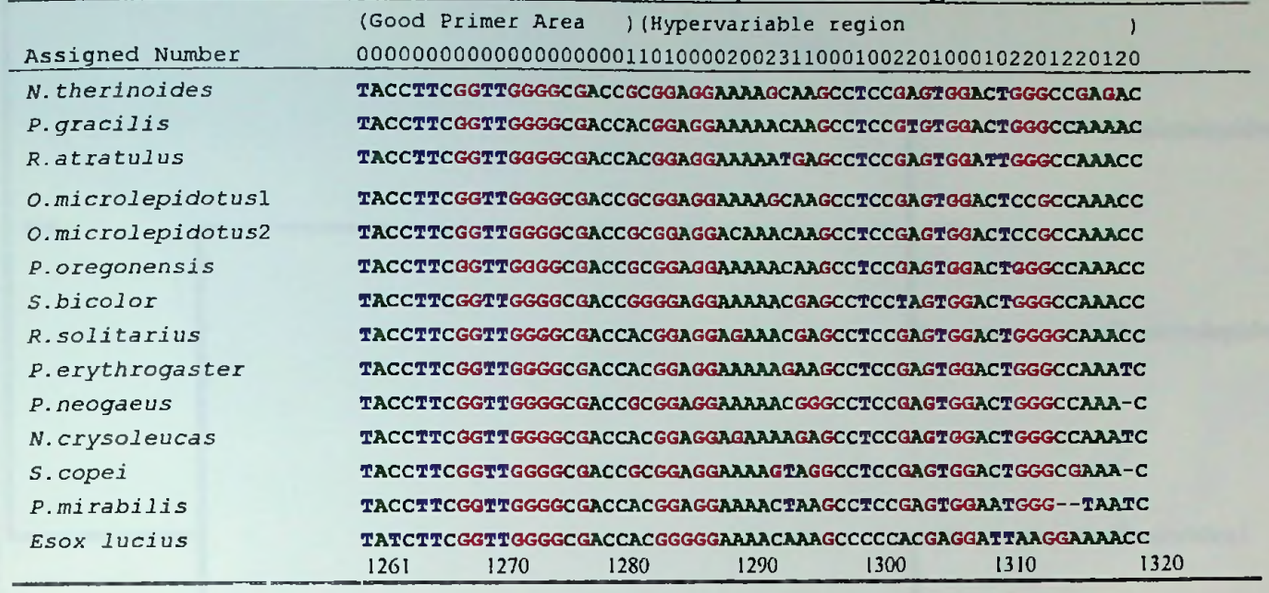


Figure 3. Distribution of silver pike (Bernardo, 1998)

Figure 4. Sample of sequences used to analyze regions that would provided the most variability and still provided enough conservation for primer annealing.



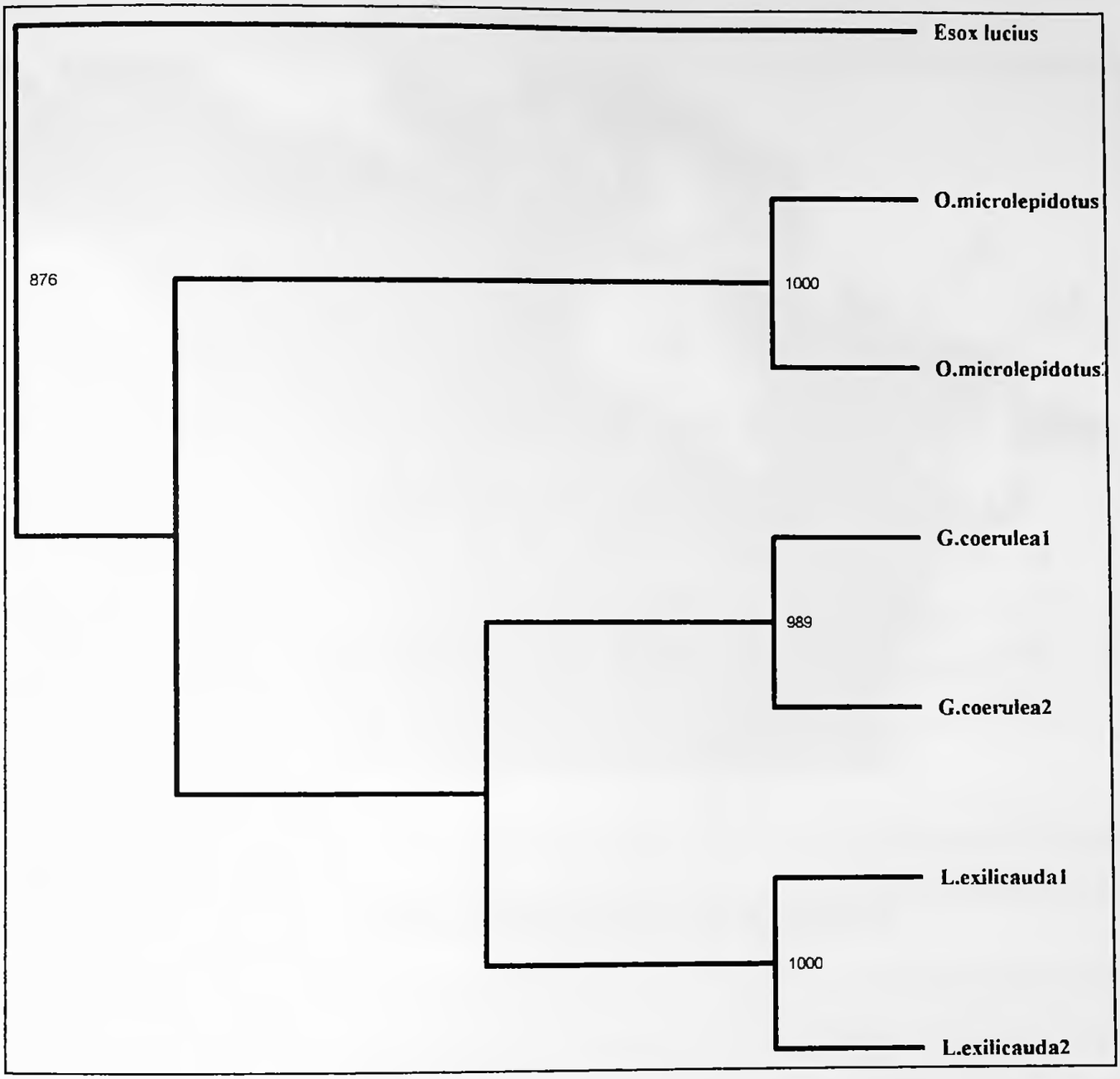


Figure 5. Phylogenetic tree of the family Cyprinidae showing accuracy of clustering obtained with the proposed amplicon.

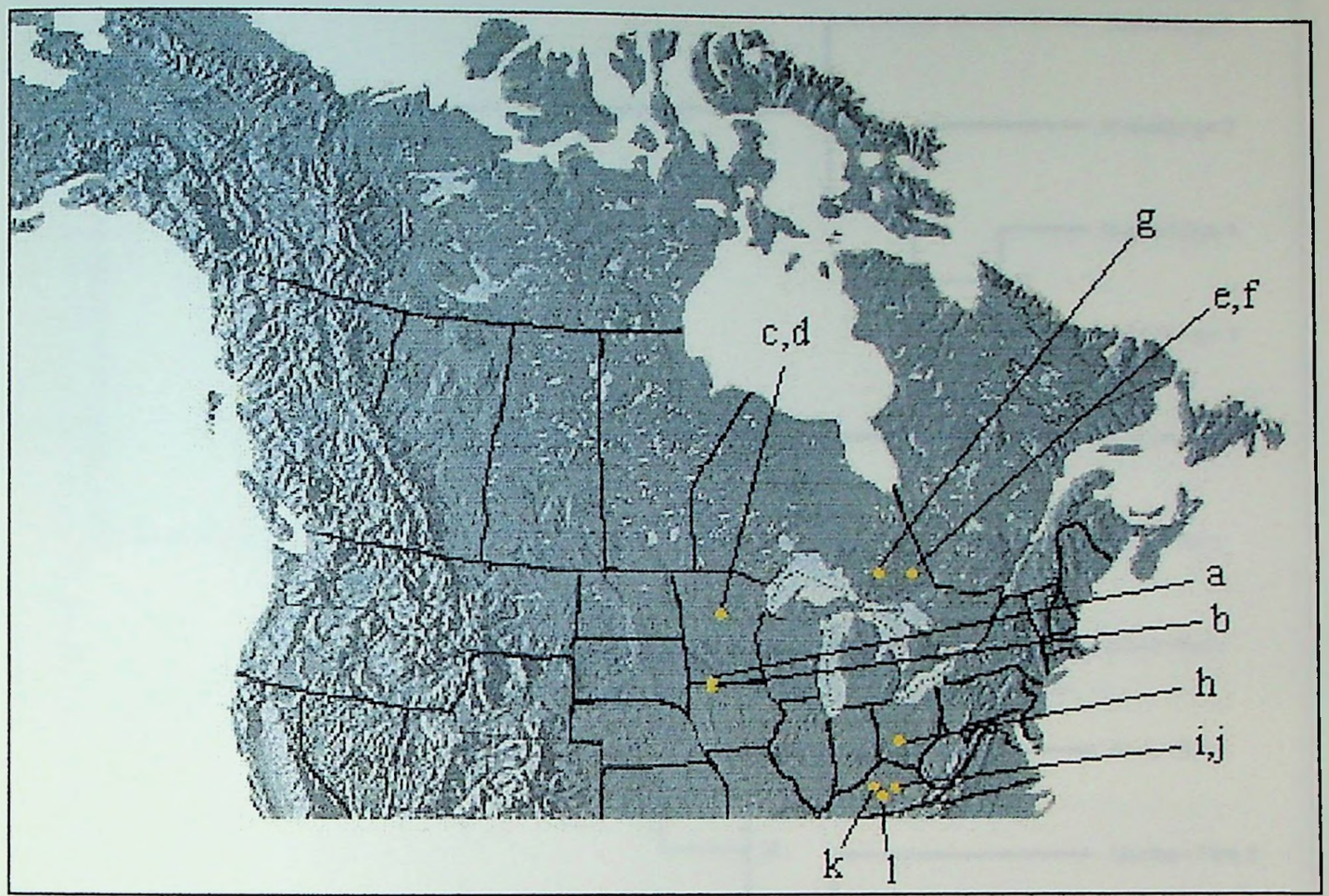


Figure 6. Sample collection locations. Letters represent specimens (Table 3).

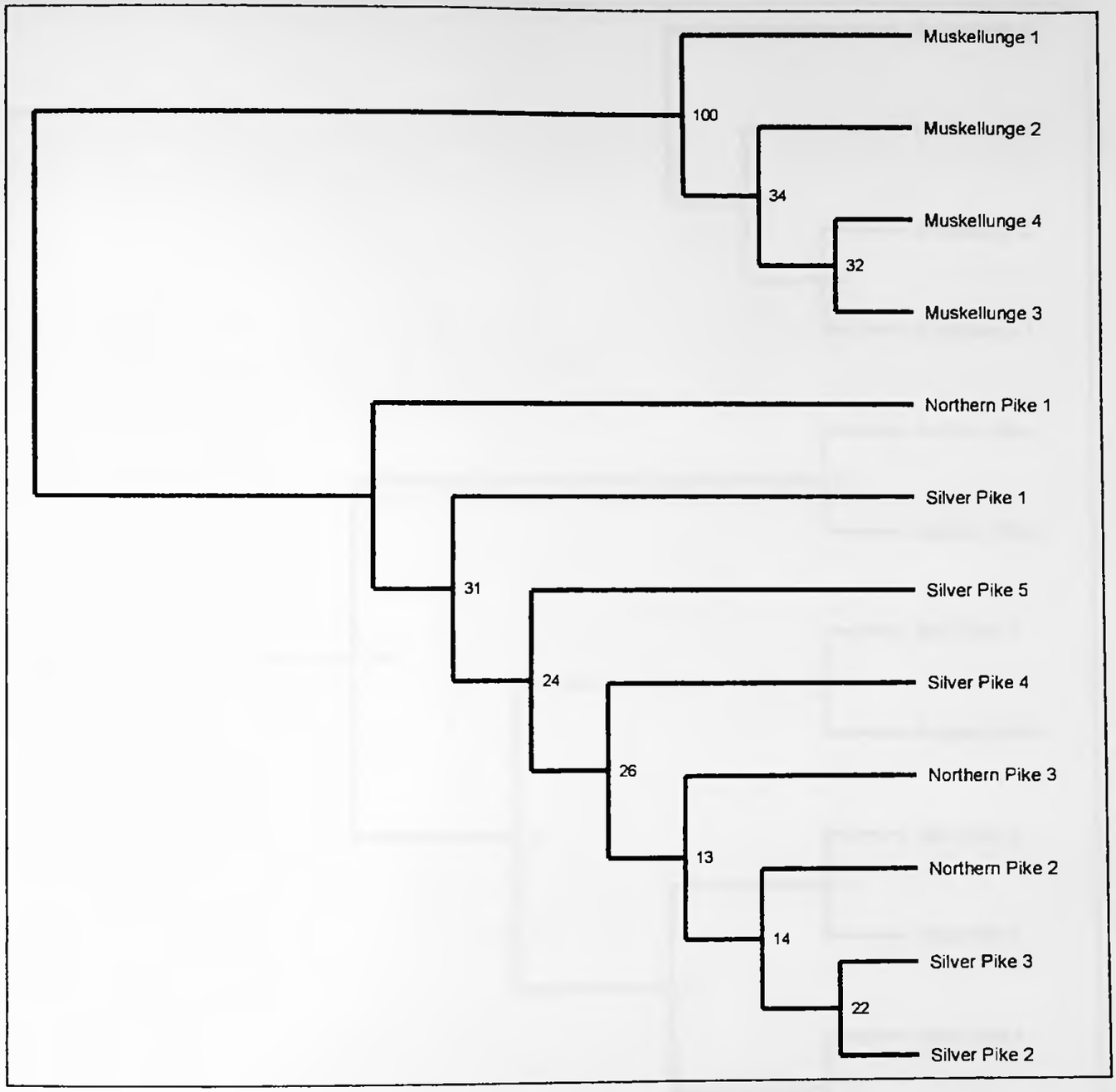


Figure 7. Neighbor joining phylogenetic tree of silver pike, northern pike, and muskellunge using sequenced data. Internal labels are bootstrap values after 100 replications. Muskellunge were set as outgroup.

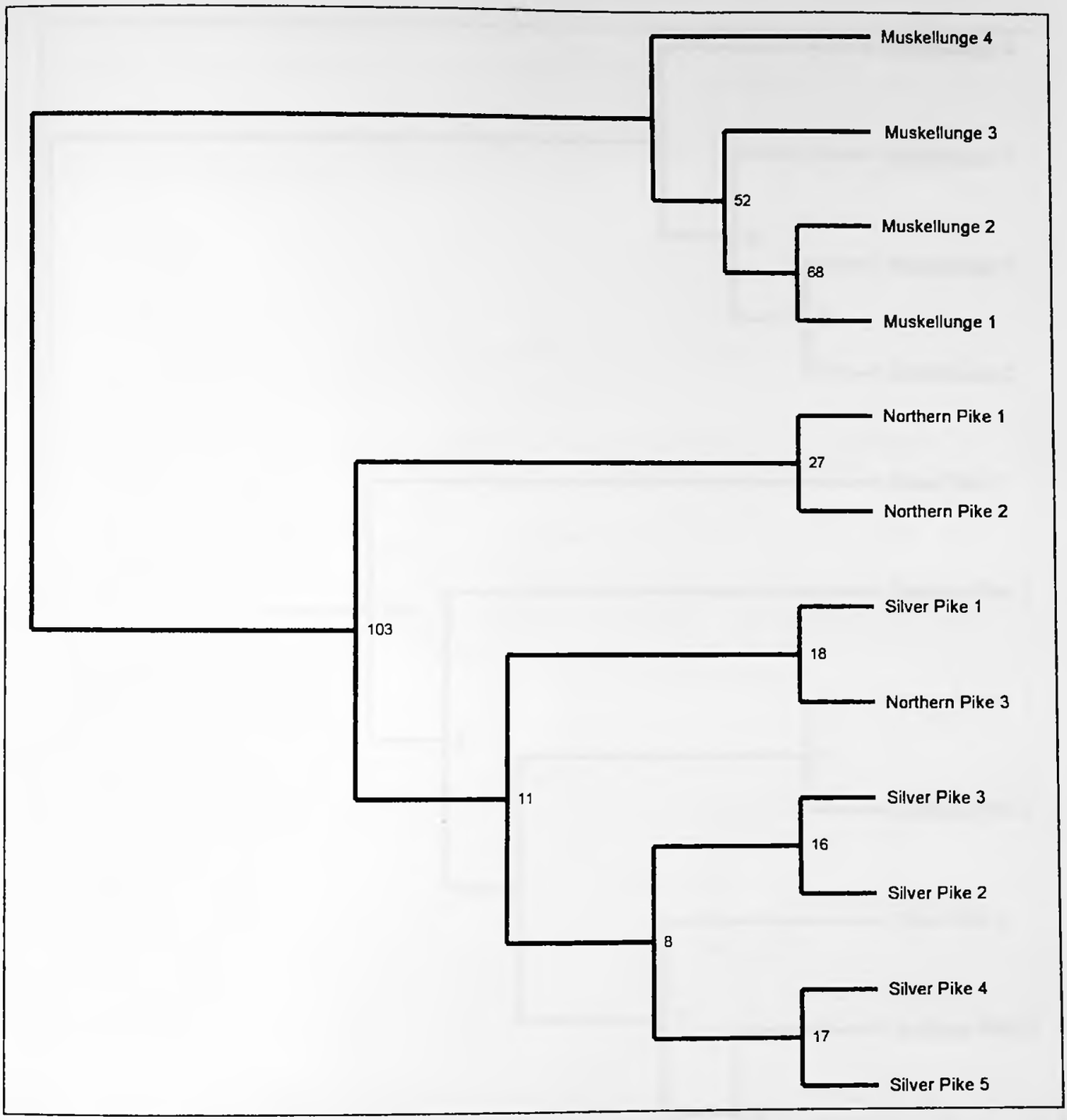


Figure 8: Maximum parsimony phylogenetic tree of silver pike, northern pike, and muskellunge using sequenced data. Internal labels are bootstrap values after 100 replications. Muskellunge were set as outgroup.

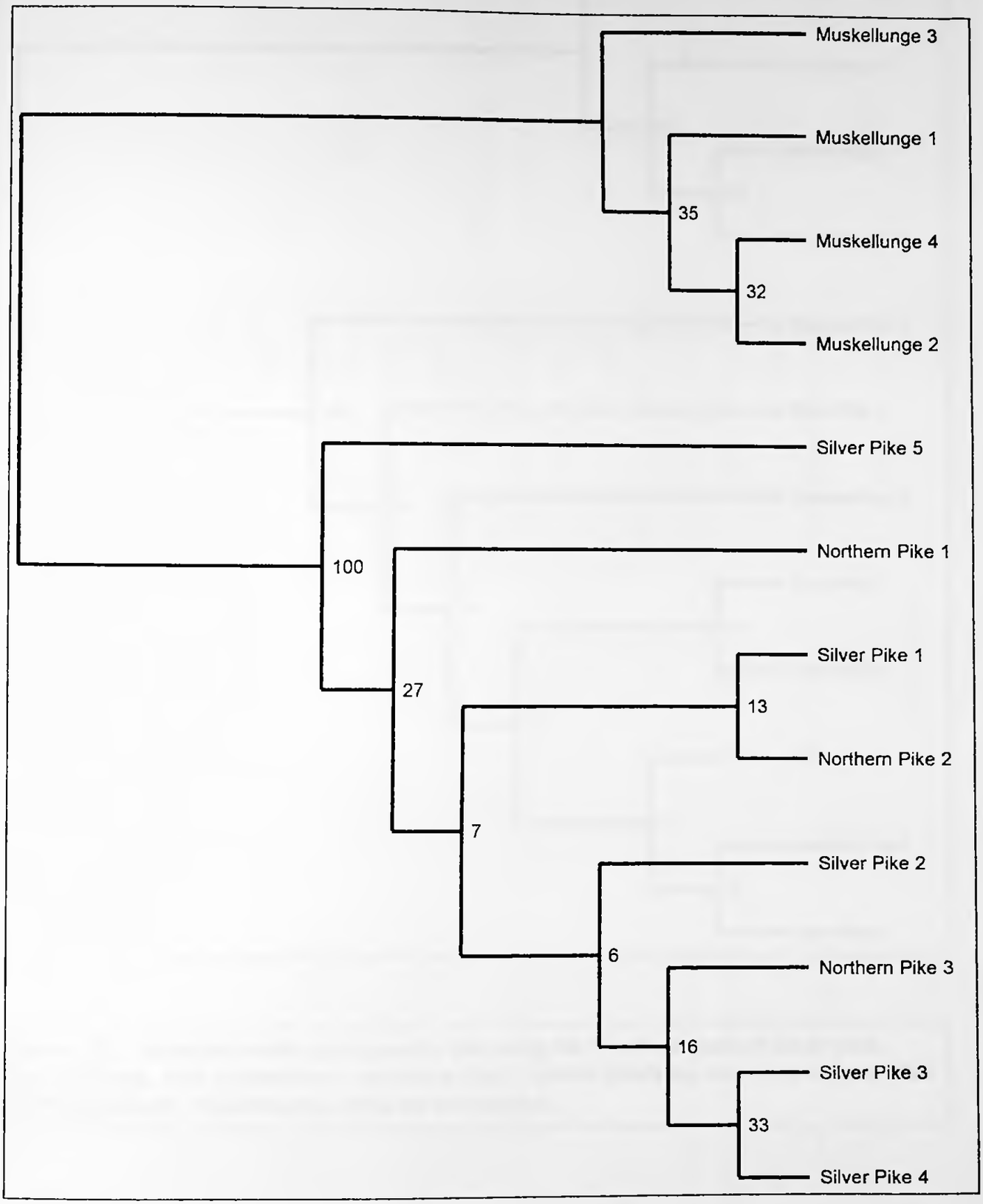


Figure 9. Distance matrix phylogenetic tree using the Fitch program of silver pike, northern pike, and muskellunge sequence data. Internal labels are bootstrap values after 100 replications. Muskellunge were set as outgroup.

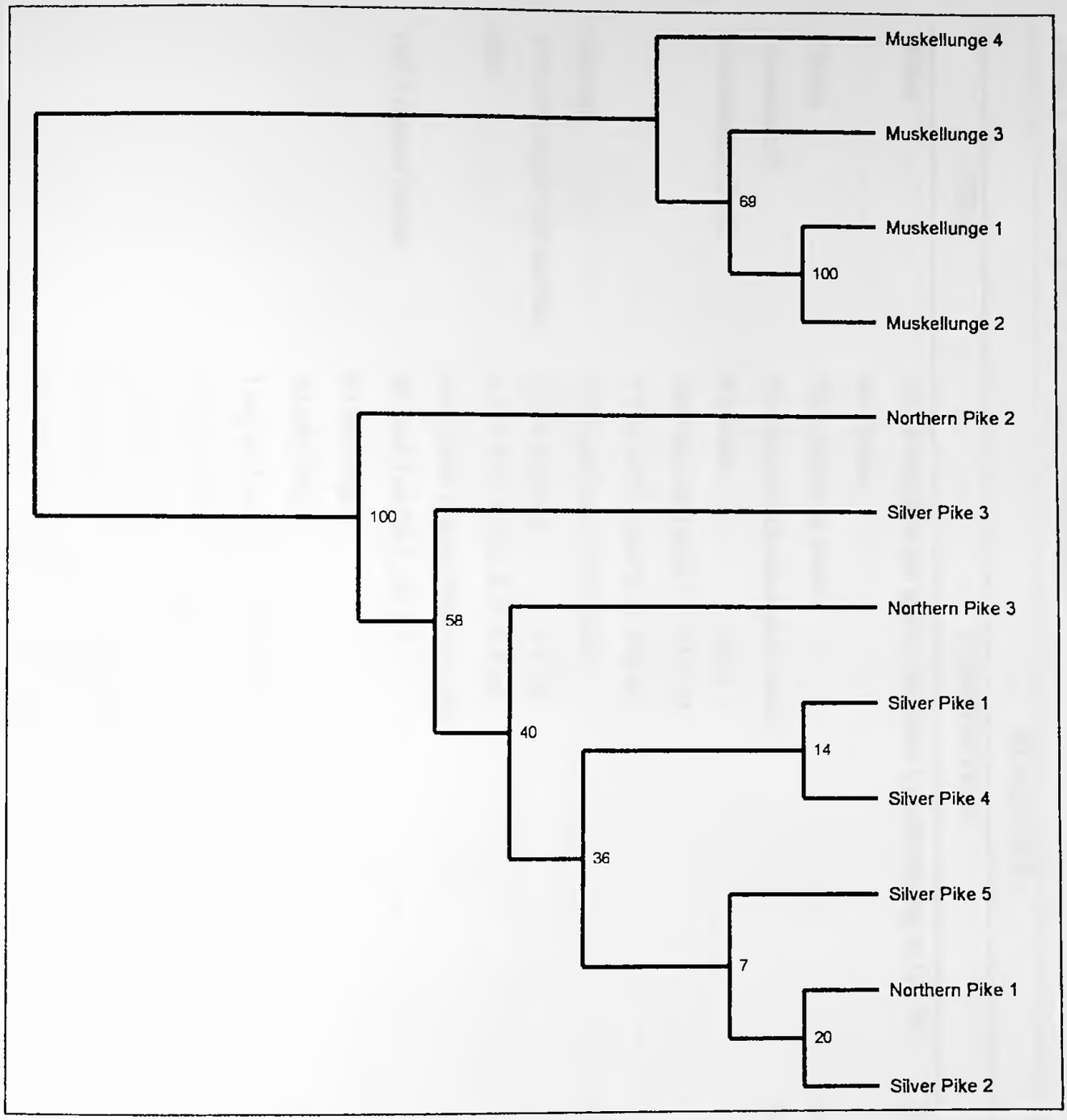


Figure 10. Distance matrix phylogenetic tree using the Kitsch program of silver pike, northern pike, and muskellunge sequence data. Internal labels are bootstrap values after 100 replications. Muskellunge were set as outgroup.

Table 1. Materials used in laboratory experiments.

REAGENTS		
ITEM	COMPOSITION	COMPANY
Water	16-18 megohm-cm purity, sterilized by autoclaving at 125 °C for 20 min	Barnsted E-Pure, Dubuque, IA
Chelex	5% Chelex in water	Carolina Biological Supply Company, NC
Proteinase K	20 mg/ml stock solution in water	Boehringer Mannheim, Germany
Tris-Acetate (TAE)	Tris base 242 g Glacial acetic acid 57.1 ml 0.5 M EDTA (pH 8.0) 100 ml	
Agarose	1% Agarose in TAE buffer	BIO-RAD, Hercules, CA
10X stop buffer for agarose gels	100% glycerol 0.7 ml 0.5 M EDTA (pH 8.0) 0.3 ml Add Brom Phenol Blue for color	
10X Ligation Buffer	60 mM Tris-HCl, pH 7.5 60 mM $MgCl_2$ 50 mM NaCl 1 mg/ml bovine serum albumin 70 mM β-Mercaptoethanol 1 mM ATP 20 mM ditiothreitol 10 mM spermidine	

Table 1. Continued.

ITEM	COMPOSITION	COMPANY
	REAGENTS	
T4 DNA Ligase	4.0 Weiss units/µl	Invitrogen, Carlsbad, CA
Cell resuspension buffer	50 mM Tris, (pH 7.5)	Promega, Madison, WI
	10 mM EDTA	
	100 mg/ml Rnase	
NaOH-SDS	0.2 M NaOH	
	1% Sodium Dodecyl Sulfate (SDS)	
	COMPETENT CELLS	
Competent cells	*E. coli*, strain INVαF' (F' *end*A1 *rec*A1 *hsd*R17 (r_k^-, m_k^+) *sup*E44	Invitrogen, Carlsbad, CA
	thi-1 *gyr*A96 *rel*A1 ϕ80*lac*Z)M15)(*lac*ZY A-*arg*F)U 169λ$^-$)	

Table 2. Oligonucleotide Primers. Oligonucleotide analysis data calculated using OLIGO 4.0 primer analysis software (NBI).

NAME	SEQUENCE	OLIGONUCLEOTIDE ANALYSIS
SF1-68-1 (Forward)	GGT AGC GCA ATC ACT TGT CT	Td = 62.0° (nearest neighbor method) Tm = 68.2° (%GC method) Tm = 60.0° (2*(A+T)+4*(G+C)) nmol/OD = 5.35 (nearest neighbor extn. coeff.) µg/OD = 33.1 Composition = A + T 10 (50%) C + G 10 (50%)
SF1-68-2 (Reverse)	TAT CCC TAG GGT AAC TTG GT	Td = 57.5° (nearest neighbor method) Tm = 66.2° (%GC method) Tm = 58.0° (2*(A+T)+4*(G+C)) nmol/OD = 5.24 (nearest neighbor extn. coeff.) µg/OD = 32.5 Composition = A + T 11 (55%) C + G 9 (45%)

Table 3. Locations of specimen collection.

Specimen	Location	Latitude	Longitude
Silver Pike 1*	Spirit Lake, IA (a)	43.28 N	95.06 W
Silver Pike 2	Okoboji Lake, IA (b)	43.29 N	95.07 W
Silver Pike 3	Young Lake, MN (c)	46.34 N	94.08 W
Silver Pike 4	Young Lake, MN (d)	46.34 N	94.08 W
Silver Pike 5	Marten River, ON, Canada (e)	46.22 N	79.25 W
Northern Pike 1	Marten River, ON, Canada (f)	46.22 N	79.25 W
Northern Pike 2	Lake Ramsey, ON, Canada (g)	46.37 N	80.48 W
Northern Pike 3	Great Miami River, OH (h)	40.07 N	84.14 W
Muskellunge 1	Morehead Fish Hatchery, KY (i)	38.22 N	83.57 W
Muskellunge 2	Morehead Fish Hatchery, KY (j)	38.22 N	83.57 W
Muskellunge 3	Green River, KY (k)	37.11 N	86.07 W
Muskellunge 4	Green River, KY (l)	37.16 N	85.37 W

Letters in parentheses indicate specimen sampling locations shown in Fig. 1. * Indicates specimens cloned into the PT-NOT vector, all others were cloned into the pCR®2.1 vector.

APPENDIX A: The mitochondrial genome coding for the 12S rRNA, 16S rRNA, and tRNA-Val of ten species, the fin whale (*Balaenoptera physalus*, Arnason et al., 1991), blue whale (*Balaenoptera musculus*, Arnason and Gullberg, 1993), cow (*Bos taurus*, Anderson et al., 1982), rat (*Rattus norvegicus*, Gadaleta et al., 1989), mouse (*Mus musculus*, Bibb et al., 1981), opossum (*Didelphis virginiana*, Janke et al., 1994), chicken (*Gallus gallus*, Desjardins and Morais, 1990), trout (*Oncorhynchus mykiss*, Zardoya et al., 1995), loach (*Crossostoma lacustre*, Tzeng et al., 1992), and carp (*Cyprinus carpio*, Chang et al., 1994) as determined from GenBank annotations and aligned using ClustalX (Thompson, 1994). Conserved base pairs through all species are indicated by *. Location of 12S rRNA is bp 1-943, tRNA Valine 944-1014, and 16S rRNA 1015-2754.

```
Fin Whale       AAAAGGTTTGGTCCCAGCCTTTCTATTAGTTCTTAACAGACTTACACATGCAAGTATCCA
Blue Whale      AAAAGGTTTGGTCCCAGCCTTTCTATTAGTTCTTAACAAACTTACACATGCAAGCGTCTA
Cow             AATAGGTTTGGTCCCAGCCTTCCTGTTAACTCTTAATAAACTTACACATGCAAGCATCTA
Rat             AAAAGGTTTGGTCCTGGCCTTATAATTAATTGGAGGTAAGATTACACATGCAAACATCCA
Mouse           AAAAGGTTTGGTCCTGGCCTTATAATTAATTAGAGGTAAAATTACACATGCAAACCTCCA
Loach           AAAAGGCTTGGTCCTGACTTTACTATCAGCCGTAGCACAACTTACACATGCAAGTCTCCG
Carp            AAAAGGCATGGTCCCGACCTTATTATCAGCTCTAACTCAACTTACACATGCAAGTCTCCG
Trout           AAAAGGCTTGGTCCTGACTTTACTATCAGCTCTAACTGAACTTACACATGCAAGTCTCCG
Chicken         AAAAGACTTAGTCCTAACCTTTCTATTGGTTTTTGCTAGACATATACATGCAAGTATCCG
Opossum         AATAGGTTTGGTCCTAGCCTTATTATTAGTTCTAATTAGACCTACACATGCAAGTTTCCG
                 * **    * ****    * **    *                  ** ********   **

                1         10        20        30        40        50        60

Fin Whale       CATCCCAGTGAGAACGCCCTCTAAA--TCATA--AAGATTAAAAGGAGCGGGTATCAAGC
Blue Whale      CATCCCGGTGAGAATGCCCTCTAAA--TCACA--AAGATCAAAAGGAGCAGGTATCAAGC
Cow             CACCCCAGTGAGAATGCCCTCTAGG--TTATT--AAAACTAAGAGGAGCTGGCATCAAGC
Rat             TAAACCGGTGTAAAATCCCTTAAAGATTTGCC--TAAAACTTAAGGAGAGGGCATCAAGC
Mouse           TAGACCGGTGTAAAATCCCTTAAACATTTACT--TAAAATTTAAGGAGAGGGTATCAAGC
Loach           CAGCCCCGTGAGGATGCCCTTAATCCCTGC----CCGGGGACGAGGAGCGGGCATCAGGC
Carp            CAACCCAGTGAATATGCCCTCAATCCCCCCA---CCCGGGACGAGGAGCGGGCATCAGGC
Trout           CATTCCTGTGAGGATGCCCTTAATCCCTGC----CCGGGGACGAGGAGCCGGCATCAGGC
Chicken         CATCCCAGTGAAAATGCCCCCAAACCTTTCTTC-CCAAGCAAAAGGAGCAGGTATCAGGC
Opossum         CTACCCAGTGAGAATGCCCTTTAAGTCTTATAAATTAAGCAAAAGGAGCTGGTATCAGGC
                    ** ***    *  ***   *                      *****  ** **** **

                61        70        80        90        100       110       120

Fin Whale       ACGCTAGCACTAGCAGCTCACAACGCCTCGCT-TAGCCACGCCCCCACGG-GACACAGCA
Blue Whale      ACGCTAGTACTAGCAGCTCACAACGCCTCGCT-TAGCCACACCCCCACGG-GACACAGCA
Cow             ACACAC---CCTGTAGCTCACGACGCCTTGCT-TAACCACACCCC-ACGG-GAAACAGCA
Rat             ACAT-A----ATATAGCTCAAGACGCCTTGCC-TAGCCACACCCCCACGG-GACTCAGCA
Mouse           ACATTA----AAATAGCTTAAGACACCTTGCC-TAGCCACACCCCCACGG-GACTCAGCA
Loach           TCAATT--ATTT--AGCCCAAGACGCCTTGCC-ACGCCACACCCCCAAGGGAACTCAGCA
Carp            ACAAAC--ATT---AGCC-AAGACGCCTAGCC-AAGCCACACCCCCAAGG-AATTCAGCA
Trout           ACGCCC--AGGC--AGCCCACGACGCCTTGCT-AAGCCACACCCCCAAGGAAACTCAGCA
Chicken         ACACTC--AGCAGTAGCCCAAGACGCCTTGCTTAAGCCACACCCCCACGGGTACTCAGCA
Opossum         ACACAAA---ATGTAGCCGATAACACCTTGCT-TTACCACACCCCCACGG-GAGACAGCA
                 *              ***  *  ** *** **     **** **** * **  *  *****

                121       130       140       150       160       170       180

Fin Whale       GTGATAAAAATTAAGCTATAAACGAAAG-TTCGACTAAGTCATG-TTAATTTAAGGGTTG
Blue Whale      GTGATAAAAATTAAGCTATAAACGAAAG-TTTGACTAAGTTATG-TTAAT--AAGGGTTG
Cow             GTGACAAAAATTAAGCCATAAACGAAAG-TTTGACTAAGTTATA-TTAATT--AGGGTTG
Rat             GTGATAAATATTAAGCAATGAACGAAAG-TTTGACTAAGCTAG--TACCTCTCAGGGTTG
Mouse           GTGATAAATATTAAGCAATAAACGAAAG-TTTGACTAAGTTA---TACCTCTTAGGGTTG
Loach           GTGATAAAAATTAAGCCATGAGCACTAAGCTTGACTTAGTTAGT-GTTAAG--AGGGCCG
Carp            GTGATAAACATTAAGCCATAAGTGAAAA-CTTGACTCAGTTAGT-GTTAAG--AGGGCCG
Trout           GTGATAAATATTAAGCCATAAGCGAAAG-CTTGACTTAGTTAAG-GTTAAG--AGGGCCG
Chicken         GTAATTAACCTTAAGCAATAAGTGTAAA-CTTGACTTAGCCATA-GCAACCC-AGGGTTG
Opossum         GTGATTAAAATTAAGCAATAAACGAAAG-TTTGACTAAGTCATAATTTACATTAGGGTTG
                ** *   **   ****** ** *      *    * **** **  *             ****  *

                181       190       200       210       220       230       240
```

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Fin Whale       GTAAACTTCGTGCCAGCCACCGCGGTCATACGATCGACCCAAATTAATAGAAGCACGGCG
Blue Whale      GTAAACTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAATTAATAGAAACACGGCG
Cow             GTAAATCTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAGCTAACAGGAGTACGGCG
Rat             GTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAACTAATT-ATTTTCGGCG
Mouse           GTAAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAACTAATT-ATCTTCGGCG
Loach           GTAAAACTCGTGCCAGCCACCGCGGTTATACGAGAGGCCCTAGTTGATAGGTG--CGGCG
Carp            GTAAAACTCGTGACAGCCACCGCGGTTAGACGAGAGGCCCTAGTTGATATTACAACGGCG
Trout           GTAAAACTCGTGCCAGCCACCGCGGTTATACGAGAGACCCTAGTTGATAACTAC-CGGCG
Chicken         GTAAATCTTGTGCCAGCCACCGCGGTCATACAAGAAACCCAAATCAATAGCTACCCGGCG
Opossum         GTCAATTTCGTGCCAGCCACCGCGGTCATACGATTAACCCAAATTAATAAATA-ACGGCG
                ** **  * *** ************** * ** *    *** *    *         *****
                241       250       260       270       280       290       300

Fin Whale       TAAAGAGTGTTAAGGAGCCACATGA-AA---TAAAGTCAAACCTTAATTAAGCTGTAAAA
Blue Whale      TAAAGAGTGTTAAGGAGTCTCATAG-AA---TAAAGTCAAACCTTAATTAAGCTGTAAAA
Cow             TAAAACGTGTTAAAGCACCATACCA-AA---TAGGGTTAAATTCTAACTAAGCTGTAAAA
Rat             TAAAACGTGCCAACTATAAATCTCATAA---TAGAATTAAAATCCAACTTATATGTGAAA
Mouse           TAAAACGTGTCAACTATAAATAAATAAA---TAGAATTAAAATCCAACTTATATGTGAAA
Loach           TAAAGGGTGGTTAAGGAGAGCAAGAAT----TAAAGCCAAGGGACCTCTTGGCCGTCATA
Carp            TAAAGGGTGGTTAAGGATAAACAAAAA----TAAAGTCAAATGGCCCCTTGGCCGTCATA
Trout           TAAAGAGTGGTTATGGAAAATATTTAA----TAAAGCCGAACACCCCCTCAGCCGTCATA
Chicken         TAAAGAGTGGCCACATGTTATCTGCACCAGCTAAGATTAAAATGCAACCAAGCTGTCATA
Opossum         TAAAGAGTGTTTAAGTTATATACAAAAA---TAAAGTTAATAATTAACTAAACTGTAGCA
                ****  ***   *                    **      *              **   *
                301       310       320       330       340       350       360

Fin Whale       AGCCCTAATTAAAAT-TAAGCCAAACTACGAAAGTGACTT----TAAT-ATA---ATCTG
Blue Whale      AGCCATAATTAAAAT-TAAGCCAAACTACGAAAGTGACTT----TAAT-ATG---ATCTG
Cow             AGCCATGATTAAAAT-AAAAATAAATGACGAAAGTGACCC----TACA-AT----AGCCG
Rat             ATTCATTGTTAGGACCTAAGCCCAATAACGAAAGTAATTC----TAATCATT---TATAT
Mouse           ATTCATTGTTAGGACCTAAACTCAATAACGAAAGTAATTC----TAGTCATT---TATA-
Loach           CGCTTCTGAGTATCC-AAAGCCA-AATACGAAAGTAGCTT----TAATAAAACCCACCTG
Carp            CGCTTCTAGGAGTCC-GAAGCCCTAATACGAAAGTAACTT----TAATAAA-CCCACCTG
Trout           CGCACCTGGGAGCAC-GAAGACCTACTGCGAAAGCAGCGT----TTAACTA---TGCCTG
Chicken         AGCCTAAGATCCACC-TAAACCC-AACCCAAATCCATCTTAGCCTCAACGA-TTAATTTT
Opossum         CGTTCTAGTTAATAT-TAAAATACATAATAAAAATGACTT----TAAT-AT----CACCG
                               **     *     **              *
                361       370       380       390       400       410       420

Fin Whale       ATCACACGACAGCTAAGATCCAAACTGGGATTAGATACCCCACTATGCTTAGTCGTAAAC
Blue Whale      ATCACACGACAGCTAAGATCCAAACTGGGATTAGATACCCCACTATGCTTAGCCATAAAC
Cow             A-CGCACTATAGCTAAGACCCAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAAC
Rat             AATGCACGATAGCTAAGACCCAAACTGGGATTAGATACCCCACTATGCTTAGCCCTAAAC
Mouse           -ATACACGACAGCTAAGACCCAAACTGGGATTAGATACCCCACTATGCTTAGCCATAAAC
Loach           ACCCCACGAAAGCTGAGAAACAAACTGGGATTAGATACCCCACTATGCTCAGCTATAAAC
Carp            ACCCCACGAAAGCTGAGAAACAAACTGGGATTAGATACCCCACTATGCTCAGCCGTAAAC
Trout           ACCCCACGACAGCTAAGAAACAAACTGGGATTAGATACCC-ACTATGCCTAGCCGTAAAC
Chicken         AACCCACGAAAGCTAGGACCCAAACTGGGATTAGATACCCCACTATGCCTAGCCCTAAAT
Opossum         ACTACACGAAAACTAAGACACAAACTGGGATTAGATACCCCACTATGCTTAGTAATAAAC
                    *** * * **  **  ********************** *******  **   ****
                421       430       440       450       460       470       480

Fin Whale       CCCAATAGTCACA--AAACAAGACTATTCGCCAGAGTACTACTAGCAACA--GCCTAAAA
Blue Whale      CCCAGTAGTCACA--AAACAAGACTATTCGCCAGAGTACTACTAGCAACA--GCTTAAAA
Cow             ACAGATAATTACAT-AAACAAAATTATTCGCCAGAGTACTACTAGCAACA--GCTTAAAA
Rat             CTTAATAATTAAACCTA-CAAAATTATTTGCCAGAGAACTACTAGCTACA--GCTTAAAA
Mouse           CTAAATAATTAAATTTAACAAAACTATTTGCCAGAGAACTACTAGCCATA--GCTTAAAA
Loach           CTAGACGTTTAATC-ACAACAA-ACGTCCGCCAGGGTACTACGAGCGTCA--GCTTAAAA
Carp            TCAGACATCCAGCT-ACAATTAGATGTCCGCCAGGGTACTACGAGCATTA--GCTTAAAA
Trout           CTTGATAGAAATAT-ACAATTG-ATATCCGCCAGGGAACTACAAGCGCCA--GCTTAAAA
Chicken         CTAGATACCTCCCA-TCACACATGTATCCGCCTGAGAACTACGAGCACAAACGCTTAAAA
Opossum         TAAAATAATTTAAC-AAACAAAATTATTCGCCAGAGAACTACTAGCAATT--GCTTAAAA
                               *  *** * * ***** ***      ** *****
                481       490       500       510       520       530       540
```

```
Fin Whale       CTCAAAGGACTTGGCGGTGCCTCATACCCATCTAGAGGAGCCTGTTCTGTAACCGATAAA
Blue Whale      CTCAAAGGACTTGGCGGTGCTTCATACCCCTCTAGAGGAGCCTGTTCTGTAACCGATAAA
Cow             CTCAAAGGACTTGGCGGTGCTTTATATCCTTCTAGAGGAGCCTGTTCTATAATCGATAAA
Rat             CTCAAAGGACTTGGCGGTACTTTATATCCATCTAGAGGAGCCTGTTCTATAATCGATAAA
Mouse           CTCAAAGGACTTGGCGGTACTTTATATCCATCTAGAGGAGCCTGTTCTATAATCGATAAA
Loach           CCCAAAGGACTTGGCGGTGCCTTAGACCCCCCTAGAGGAGCCTGTTCTAGAACCGATAAC
Carp            CCCAAAGGACCTGACGGTGTCTCAGACCCCCCTAGAGGAGCCTGTTCTAGAACCGATAAC
Trout           CCCAAAGGACTTGGCGGTGCCTCAGACCCACCTAGAGGAGCCTGTTCTAGAACCGATAAC
Chicken         CTCTAAGGACTTGGCGGTGCCCCAAACCCACCTAGAGGAGCCTGTTCTATAATCGATAAT
Opossum         CTCAAAGGACTTGGCGGTGCCCTAAACCCACCTAGAGGAGCCTGTTCTATAATCGATAAA
                * * ****** ** ****     * * **  ****************  ** ******
                541       550       560       570       580       590       600

Fin Whale       CCCCGATCAACCTCACCAACCCTTGCTACTTCAGTCTATATACCGCCATCTTCAGCAAAC
Blue Whale      CCCCGATTAACCTCACCAACCCTTGCTACTTCAGTCTATATACCGCCATCTTCAGCAAAC
Cow             CCCCGATAAACCTCACCAATTCTTGCTAATACAGTCTATATACCGCCATCTTCAGCAAAC
Rat             CCCCGTTCTACCTTACCCCTTCTCGCTAATTCAGCCTATATACCGCCATCTTCAGCAAAC
Mouse           CCCCGCTCTACCTCACCATCTCTTGCTAATTCAGCCTATATACCGCCATCTTCAGCAAAC
Loach           CCCCGTTAAACCTCACCACTTCTAGTCATCCCCGCCTATATACCGCCGTCGTCAGCTTAC
Carp            CCCCGTTCAACCTCACCACTTCTAGCCACCCCAGCCTATATACCGCCGTCGTCAGCTTAC
Trout           CCCCGTTCAACCTCACCACCCCTTGTTTTACCCGCCTATATACCACCGTCGTCAGCTTAC
Chicken         CCACGATTCACCCAACCACCCCTTGCCAGCACAGCCTACATACCGCCGTCGCCAGCCCAC
Opossum         CCCCGATAAACCAGACCTTATCTTGCCAATACAGCCTATATACCGCCATCGTCAGCTAAC
                ** ** *  ***  ***    ** *      * * *** ***** ** **  ****  **
                601       610       620       630       640       650       660

Fin Whale       C-CTA-AAGGGAGA---AAAGTAAGCATAACCATCCTACATAAAAACGTTAGGTCAAGGT
Blue Whale      C-CTA-AAAGGGAAC-GAAAGTAAGCATAATCATCCTACATAAAAACGTTAGGTCAAGGT
Cow             C-CTA-AAAAGGAAA-AAAAGTAAGCGTAATTATGATACATAAAAACGTTAGGTCAAGGT
Rat             C-CTA-AAAAGGCAC-TAAAGTAAGCACAAGAA-CAAACATAAAAACGTTAGGTCAAGGT
Mouse           C-CTA-AAAAGGTAT-TAAAGTAAGCAAAAGAATCAAACATAAAAACGTTAGGTCAAGGT
Loach           C-CTG-TGAAGGCTC-AATAGTAAGCAAAGTGGGCACAACCCAAAACGTCAGGTCGAGGT
Carp            C-CTG-TGAAGGTAATAAAAGTAAGCAAAATGGGCACAACCCAAAACGTCAGGTCGAGGT
Trout           C-CTG-TGAAGGCCC-CATAGTA-GCAAAATGGGCAAAACC-AAAACGTCAGGTCGAGGT
Chicken         CTCTAATGAAAGAAC-AACAGTGAGCTCAATAGCCCCTCGCTAATAAGACAGGTCAAGGT
Opossum         CTTTA--AAAAGAAT-TACAGTAAGCAAAATCATACAACATAAAAACGTTAGGTCAAGGT
                *   *              * ***  **  *                ** * *  ***** ****
                661       670       680       690       700       710       720

Fin Whale       GTAACCCATGGGTTGGGAAGTAATGGGCTACATTTTCTAAGCTAAGAACATCCCCTATAC
Blue Whale      GTAACCAATGGGTTGGGAAGAAATGGGCTACATTTTCTAAACTAAGAATATCCC-TATAC
Cow             GTAACCTATGAAATGGGAAGAAATGGGCTACATTCTCTACACCAAGAGAATCAA------
Rat             GTAGCCAATGAAGCGGAAAGAAATGGGCTACATTTTCTTTTCCCAGAGAACATT------
Mouse           GTAGCCAATGAAATGGGAAGAAATGGGCTACATTTTCTTATA--AAAGAACATT------
Loach           GTAGCGTACGAAGTGGGAAGAGATGGGCTACATTTTCTATAAT---AGAATAAG------
Carp            GTAGCGCATGAAGTGGGAAGAAATGGGCTACATTTTCTAATAT---AGAATATT------
Trout           GTAGCGCATGAGGTGGGAAGAAATGGGCTACATTCTCTAAATT---AGAGCACT------
Chicken         ATAGCCTATGGGGTGGGA-GAAATGGGCTACATTTTCTAACAT---AGAACAA-------
Opossum         GTAGCATATGATAAGGAAAGTAATGGGCTACATTCTCTACTAT---AGAGCATA------
                  ** *  * *    ** * *  ************ ***         *
                721       730       740       750       760       770       780

Fin Whale       TCACACGAAAG-TTTTTATGAAACTT-AAAAACTAAAGGAGGA-TTTAGTAGTAAATCAA
Blue Whale      --ACACGAAAG-TTTTTATGAAACTT-AAAAACCAAAGGAGGA-TTTAGTAGTAAATCAA
Cow             --GCACGAAAG-TTATTATGAAACC--AATAACCAAAGGAGGA-TTTAGCAGTAAACTAA
Rat             ----ACGAAAC-C-TTTATGAAACT--AAAGGACAAAGGAGGA-TTTAGTAGTAAATTAA
Mouse           ----ACTATAC-CCTTTATGAAACT--AAAGGACTAAGGAGGA-TTTAGTAGTAAATTAA
Loach           ----ACGAATA-GCATCATGAAAACTTAATGCTTGAAGGAGGA-TTTAGTAGTAAAAAGG
Carp            ----ACGAACATGCACCATGAAAC---AATGCTTGAAGGAGGA-TTTAGTAGTAAAAGGG
Trout           ----ACGAACC-ACGCTGTGAAATC--AGCGTCCGAAGGTGAAATTTAGCAGTAAACAGA
Chicken         ----ACGAAAA-AGGATGTGAAACCC-GCCCTTAGAAGGAGGA-TTTAGCAGTAAAGTGA
Opossum         ----ACGAATC-ATATTATGAAACTAAAATGCTTGAAGGAGGA-TTTAGTAGTAAATTAA
                    ** *          *****                 **** * * ***** ******
                781       790       800       810       820       830       840
```

```
Fin Whale       GAGCAGAG------TGCTT-GATTGAATAAGGCCATGAGGGCACGCACACACCGCCCGTC
Blue Whale      GAGCAGAG------TGCTT-GATTGAATAAGGCCATGAAG-CACGCACACACCGCCCGTC
Cow             GAATAGAG------TGCTT-AGTTGAATTAGGCCATGAAG-CACGCACACACCGCCCGTC
Rat             GAATAGAG------AGCTT-AATTGAATAGAGCAATGAAG-TACGCACACACCGCCCGTC
Mouse           GAATAGAG------AGCTT-AATTGAATTGAGCAATGAAG-TACGCACACACCGCCCGTC
Loach           AAATAGAG------TGTCC-TTTTGAACCCGGCTCTGAGG-CGCGTACACACCGCCCGTC
Carp            AAGTAGAG------TGTCCCTTTTGAACCCGGCTCTGAGA-CGCGTACACACCGCCCGTC
Trout           AAACAGAG------AGTTC-TCTTGAAACTGGCTCTGAGG-CGCGCACACACCGCCCGTC
Chicken         GATCATACCCCCTAAGCTC-ACTTTAAGACGGCTCTGAGG-CACGTACATACCGCCCGTC
Opossum         GAATAGAG------AGCTT-AATTGAATTAGGCAAT-AGGGCGCGCACACACCGCCCGTC
                  *   * *         *      ** **    **   * *      ** *** **********
                841       850       860       870       880       890       900

Fin Whale       ACCCTCCTCAAGTACCCCAGCTATAAACCCCAGTTC-GTTAACTCAGGCCAAGCAA-TTA
Blue Whale      ACCCTCCTCAAGTACCCCAGCTA-AAGCCACAGTTC-ATTAACTCAGGCCAAGCAACTTA
Cow             ACCCTCCTCAAATAGATTCAGTGCATCTAACCCTAT-TTAAACGCA---CTAGC----TA
Rat             ACCCTCCTCAAAT---TAGATTGACATTCACATATACATAATTTCA---CTA-ACAAATT
Mouse           ACCCTCCTCAAAT---TAAATTAAACTTAACATAAT--TAATTTCT---AGACATCCGTT
Loach           ACTCTC--CCCT----GTAATA--GCAATCAATGTAAATAACAACA---AAGCA----CC
Carp            ACTCTC--CCCT----GTCAAAACGCACCAAAAATACATAATACAA---CAGCA----CT
Trout           ACTCTC--CCCAA---GTTCAA--CCTGTCCTTCTAACTAAGAAGT---TAACC----GA
Chicken         ACCCTCTTCACAA---GCCATCAACATCAATAAATATATACTTCCC---CTCCC----GG
Opossum         ACCCTCCTCAACA----TAATA----ATCCAACATACCTAATACAA---TTATT----CA
                ** ***  *                                  *
                901       910       920       930       940       950       960

Fin Whale       TACGAGAGGAGACAAGTCGTAACAAGGTAAGCATACCGGAAGGTGTGCTTGGACAAA--A
Blue Whale      TACGAGAGGAGACAAGTCGTAACAAGGTAAGCATACCGGAAGGTGTGCTTGGACAAA--A
Cow             CATGAGAGGAGACAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGGATAAA--T
Rat             TATGAGAGGAGATAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGGAATAA--T
Mouse           TATGAGAGGAGATAAGTCGTAACAAGGTAAGCATACTGGAAAGTGTGCTTGGAATAA--T
Loach           AACAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCACTTGGATCAAACC
Carp            GACAAGGGGAGGCAAGTCGTAACACGGTAAGT-TACCGGAAG-TGCACTTGGATCAAACC
Trout           ACAAAGGGGAGGCAAGTCGTAACATGGTAAGTGTACCGGAAGGTGCGCTTGGAATAA--C
Chicken         CTAAAGACGAGGCAAGTCGTAACAAGGTAAGTGTACCGGAAGGTGCACTTAGACTA---C
Opossum         TTAAAGAGGAGAAAAGTCGTAACACGGTAAGTGTACTGGAAAGTGCACTTGGAATA----
                   **   ***  *********** ******  *** ****  **  *** **  *
                961       970       980       990       1000      1010      1020

Fin Whale       CAAGATATAGCTTAA----ACAAAGCATGTAGTTTACACCTAGAAGATTCCACAG---CC
Blue Whale      CAAGATATAGCTTAA----ACAAAGCATCTAGTTTACACCTAGAAGATTCCACAG---CC
Cow             CAAGATATAGCTTAA----ACAAAGCATCCAGTTTACACCTAGAAGACTTCATTC---AT
Rat             CACAGTGTAGCTTAAT--CACAAAGCATCTGGCCTACACCCAGAAGAATTCATAA---AA
Mouse           CATAGTGTAGCTTAAT--ATTAAAGCATCTGGCCTACACCCAGAAGATTTCATGA---CC
Loach           CAGGGCGTGGCTGAGAT-AGTTAAGCACCTCCCTTACACCGAGAAGACATCCATG-CGAG
Carp            CAGGGTGTGGCTGAGTT-AGTCAAGCATCTCACTTACACCGAGAAGACATCCATG-CAAA
Trout           CAGAGTGTAGCTAAAAT-AGGAAAGCACCTCCCTTACACCGAGAAGACATCCGTG-CAAA
Chicken         CAAGGCGTAGCTATAAC-TTCAAAGCATTCAGCTTACACCTGAAAGATACCCTCAACAGA
Opossum         CAAAATGTAGCTTGATTTATTAAAGCATTTAGTTTACACCTAAAAGATTTCAGTC--TAA
                **      * ***           *****        ******   ****   *
                1021      1030      1040      1050      1060      1070      1080

Fin Whale       CGTGTATATCTTGA---ACTAGCC--CTAGCCC-ACACCCTC--CC-------CACCTCT
Blue Whale      CGTGCATATCTTGA---ACCAGCT--CTAGCCC-ACCCCCAC--CC-------CCCTTTT
Cow             TATGAATATCTTGA---ACTAGAC--CTAGCCC-AAAGATAC--CCT----CTCGACTAA
Rat             AATGAACACTTTGA---ACTAACC--CTAGCCCTACAACCA---AC-------CAACATA
Mouse           AATGAACACTCTGA---ACTAATC--CTAGCCCTAGCCCTAC--AC-------AAATATA
Loach           CTGGATCGCCCTGA---GCCAAACAGCTAGCTTAACAACCTAG-ACAACTAAACAACATA
Carp            TTGGATCGCCCTGA---GCCAAACAGCTAGCTTAACTACTTA--ATAACTAAACAATATA
Trout           TCGGGTCACCCTGA---GCTGACTAGCTAGCCAACATATTTGGTCCAACACCACAACATA
Chicken         CAAGGTCGCCTTGACTTGCCCCCCCTCTAGCCCGACAAACTCGTAC----CCTTAACATA
Opossum         TCTGACCATTTTGA---ACTAACC--ACAGCCCTAAAATCAT---------ATCAAATTA
                   *       ***    *          ***
                1081      1090      1100      1110      1120      1130      1140
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Fin Whale       ACTACCACAAATCAA----TCAAATAAAACATTTACCATCCCTTCAA---AGTATAGGAG
Blue Whale      ACTACCATAGACCAA----TCAAATAAAACATTCACCAACCTTCTAA---AGTATAGGAG
Cow             ACAACCAAGATAGAA----TAAAACAAAACATTTAATCCCAATTTAA---AGTATAGGAG
Rat             ACTAA-ACCCCCACA----TAAACTAAAACATTTAACTCA----AAA---AGTATTGGAG
Mouse           ATTAT-ACTATTATA----TAAATCAAAACATTTATCCTAC--TAAA---AGTATTGGAG
Loach           AATAAAACAACAAAA-CCTTAAAAAATAAACTAAACCATTTTTCCACCTTAGTACGGGCG
Carp            AATAAAACAAAATAAGCCTGACACCAAAAACTAAACCATTTTTTTACCTGAGTATGGGAG
Trout           CATACCCCAATAAAACTTAGAATTAAGTCAACAAACCATTTTTCCACCTTAGTAGGGGCG
Chicken         AAAAACTTACCTCCCCCTCTTAACCAAAACATTATAAATTGTCCC-----AGTATAGGCG
Opossum         ACTAA--CTACTTTT----TCAATTTAAACCATTTTAATTATCCT-----AGTATAGGTG
                                                    **** ** *
                1141      1150      1160      1170      1180      1190      1200

Fin Whale       ATAGAAATT--TAAATATCAGTGGCGCTATAGA----GATAGTACCGTAAGG-AAA-GAT
Blue Whale      ATAGAAATT--TAAACATCAGTGGCGCTATAGA----AATAGTACCGTAAGGGAAA-GAT
Cow             ATAGAAATC--TAAGTAC----GGCGCTATAGA----GAAAGTACCGCAAGGGAAC-GAT
Rat             AAAGAAATT--TACTTACCA--AGAGCTATAGA----GAAAGTACCGCAAGGGAAATGAT
Mouse           AAAGAAATTCGTACAT-CTA--GGAGCTATAGA----ACTAGTACCGCAAGGGAAA-GAT
Loach           ACGGAAAAGGATCCGA-----TTAAGCGATAGA----AAAAGTACCGCAAGGGAAA-GCT
Carp            ACAGAAAAGGTTCC-A-----CAAAGCGATAGA----AATAGTACCGCAAGGGAAA-GCT
Trout           ACCGAAAAGGAGATAA-----TTGAGCAACAGA----AAAAGTACCGCAAGGGGAA-GCT
Chicken         ATAGAAAAGACTACCC-----CGGCGCAATAGAGGCTAACTGTACCGCAAGGGAAA-GAT
Opossum         ATAGAAAAG-ATATAATA----GGAGCTATAGT---TTATAGTACCGCAAGGGAAA-AAT
                *   ****                      ** * **         ****** ****  *    *
                1201      1210      1220      1230      1240      1250      1260

Fin Whale       GAAAGAAAAAC-CT---------AAAAGTAATAAAAAG-CAAAGCTTACCACTTGTACCT
Blue Whale      GAAAGAAAAAC-CC---------AAAAGTAATAAAAAG-CAAAGCTTACCCCTTGTACCT
Cow             GAAAGAAAAAAACT---------AAAAGTATAAAAAAG-CAAAGATTACCCCTTGTACCT
Rat             GAAAGACTAAT--T---------TAAAGTAAAAACAAGACAAAGATTAAACCT-GTACCT
Mouse           GAAAGACTAAT--T---------AAAAGTAAGAACAAG-CAAAGATTAAACCTTGTACCT
Loach           GAAAAAGAAATGAAACAACCCATATAAGCACCACAAAG-CAGAGACACAACCTCGTACCT
Carp            GAAAGAGAAATGAAATAACCCATATAAGCACTAAAAAG-CAAAGATTAAACCTCGTACCT
Trout           GAAAGAGAATTGAAATAACCCATTTAAGCCTAGAGAAG-CAGAGATTAAATCTCGTACCT
Chicken         GAAATAGCAATGAAA--ACC---ATAAGCAAAAAACAG-CAAAGACCAACCCTTGTACCT
Opossum         GAAAGATAAAT------------TATAGTAATTAAAAG-CAAAGATTAACTCTTGTACCT
                **** *  *                **         ** ** **       ** ******
                1261      1270      1280      1290      1300      1310      1320

Fin Whale       TTTGCATAATGACTTAACTAGTAATA-AATTAGCAAAGAGACCTTAAGTTAAATTACCCG
Blue Whale      TTTGCATAATGACTTAACTAGTAATA-ACTTAGCAAAGAGACCTTAAGTTAAACTACCCG
Cow             TTTGCATAATGAATTAACTAGTATAAGACTTAACAAAATGAATTTTAGCTAAGCAGCCCG
Rat             TTTGCATAATGAATTAACTAG-AAAATCCTTAACAAAAAGAATTTAAGCTAAGAACCCCG
Mouse           TTTGCATAATGAACTAACTAG-AAAACTTCTAACTAAAAGAATTACAGCTAGAAACCCCG
Loach           TTTGCATCATGATTTAGCCAGAACAC--CCAAGCAAAGAGACCTTTAGTTTGAAACCCCG
Carp            TTTGCATCATGATTTAGCCAGTACAC--CCAAGCAAAGAGACCTTTAGTTTGAAACCCCG
Trout           TTTGCATCATGATTTAGCCAGCACAC--CTGAGCAAAGAGAACTTTAGTTTAGGCCCCCG
Chicken         TTTGCATCATGATTTAGCAAGAACAA--CCAAGCAAAGTGAGCTAAAGTTTGCCTTCCCG
Opossum         TTTGCATAATGATTTAGCCAGTCAAC--ACGGACAAAAAGAA-TTATGCCCGACATCCCG
                ******* ****  ** * **                * **  **  *   *        ****
                1321      1330      1340      1350      1360      1370      1380

Fin Whale       AAACC-AGACGAGCTACTTATGAGCAGC--ACCTA-GAACGAACTCATCTATGTGGCAAA
Blue Whale      AAACC-AGACGAGCTACTTATGAGCAGT--ACCTA-GGACGAACTCATCTATGTGGCAAA
Cow             AAACC-AGACGAGCTACTCACAAACAGTTTACCAA-GAACTAACTCATCTATGTGGCAAA
Rat             AAACC-AAACGAGCTACCTAAAAACAAT--TTCAT-GAATCAACCCGTCTATGTAGCAAA
Mouse           AAACC-AAACGAGCTACCTAAAAACAAT--TTTAT-GAATCAACTCGTCTATGTGGCAAA
Loach           AAACC-AAGTGAGCTACCCCGGGACCGCCAAC-AT-GGGCCAACCCATCTCTGTGGCAAA
Carp            AAACC-AGGTGAGCTACCCCGAGACAGCCTATTAT-GGCCCACCCCGTCTCTGTGGCAAA
Trout           AAACT-AGACGAGCTACTCCGGGACAGCCTATTGTAGGGCCAACCCGTCTCTGTGGCAAA
Chicken         AAACCCAAGCGAGCTACTTGCGAGCAGCTAAAATTTGAGCGAACCCGTCTCTGTTGCAAA
Opossum         AAATT-AAGTGAGCTACTATAAGACAGT-TACTAATGAACCAACTCATCTATGTAGCAAA
                ***   *   *******       *                 *   * * * *** *** *****
                1381      1390      1400      1410      1420      1430      1440
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Fin Whale       ATAGTGAGAAGACTTATAAGTAGAGGTGAAAAGCCTAACGAGCCTGGTGATAGCTGGTTG
Blue Whale      ATAGTGAGAAGACTTATAAGTAGAGGTGAAAAGCCTAACGAGCCTGGTGATAGCTGGTTG
Cow             ATAGTGAGAAGATTTGTAAGTAGAGGTGACATGCCTAACGAGCCTGGTGATAGCTGGTTG
Rat             ATAGTGGGAAGATTTTTAGGTAGAGGTGAAAAGCCTATCGAGCTTGGTGATAGCTGGTTG
Mouse           ATAGTGAGAAGATTTTTAGGTAGAGGTGAAAAGCCTATCGAGCTTGGTGATAGCTGGTTG
Loach           AGAGTGGGGAGAGCCCCGGGTAGAGGTGATAGACCTACCGAACTTGGTGATAGCTGGTTA
Carp            AGAGTGGGAAGAGCTCCGGGTAGAAGTGACAGACCTACCGAACCTGGTGATAGCTGGTTG
Trout           AGAGTGGGACGAGCCCCGAGTAGAGGTGATAAACCTATCGAGCCTAGTTATAGCTGGTTG
Chicken         AGAGCGGGATGACTTGCCAGTAGAGGTGAAAAGCCTACCGAGCTGGGTGATAGCTGGTTA
Opossum         ATAGTGAGAAGATTTTATAGTAGAAGTGAAAAACCTATCGAACTTAATGATAGCTGGTTA
                * ** * *  **        ***** **** *  **** *** *    * **********
                1441      1450      1460      1470      1480      1490      1500

Fin Whale       TCCCTGAAAAGAATCTCAGTTCAACATTAAATAATACT------AAAAGCCCATG----C
Blue Whale      TCCATGAAAAGAATCTCAGTTCAACATTAAATGATACT------AAAAGCC-ATG----C
Cow             TCCAGAAAATGAATCTAAGTTCAGCTTTAAA-GATACC------AAAAATTCAAA----T
Rat             CCCAAAAAA-GAATTTCAGTTCAAACTTTAA-GCTTCC------ATCAGAACAA-----C
Mouse           CCCAAAAAAATGAATTTAAGTTCAATTTTAAA-CTTGCT------AAAAAAACAA-----C
Loach           TCTGGGAAATGAATAGAAGTTCAGCCTCGCACCCCTTGAGTTAAATAAGAAACACTCTAA
Carp            CCTAAGAAATGAATAGAAGTTCAGCCTCGTGCACCTC-----AAATCACACAAAC---AA
Trout           CTTAGGAAATGAATAGAAGTTCAGCCCCCCGGCTTTCTTAGGACCTTAAGGTAAAACTAA
Chicken         CCTGTCAAACGAATCTAAGTTC--CCCCTTAACCCACCCCCTAAAGACACCCACCTTTGT
Opossum         TCCAAAAAA-GAATTTAAGTTCAACTTTAAGTCCTATT------ACAATGCCTAT----T
                      *** ****   *****
                1501      1510      1520      1530      1540      1550      1560

Fin Whale       CAAG--CCTTAACGTATA-TTTAACTGTTAATCTAAAAAGGTACAGCTTTTTAGAAA-TG
Blue Whale      CAAG--CCTTGACGTACA-TTTAACTGTTAGTCTAAAAAGGTACAGCTTTTTAGATA-TG
Cow             AAAC--CCC-ACTGTAGC-TTTAAAAGTTAGTCTAAAAAGGTACAGCCTTTTAGAAA-CG
Rat             AAA---TCAAAATGTAAA-CTTAAAATATAGCCAAAAGAGGGACAGCTCTTTAGGAAACG
Mouse           AAAA--TCAAAAAGTAAG-TTTAGATTATAGCCAAAAGAGGGACAGCTCTTCTGGAA-CG
Loach           CATAACACAAAAAGAGAAACGCGAGAGTTAGTCAAAGGGGGTACAGCCCCTCTGATAAAG
Carp            AACAAGACTAAGAGAAACACATGAGAGTTAGTTAAAGGGGGTACAGCCCCTTTAACAAAG
Trout           TATT-GTCCCAAAGAAAC-CAGGAGAGTTAGTCAAAGGAGGTACAGCTCCTTTGAACAAG
Chicken         CAACCTTGAGAACGTTGGGGTTAAGAGCAATTCGATGGGGGTACAGCTCCATCGAAAAAG
Opossum         CAAA--GCACACAATAAG-CTTAAAAGTTAGTCAAAGAAGGGACAACTTCTTTGACCAAG
                *                                 *    *    ** *** *         *
                1561      1570      1580      1590      1600      1610      1620

Fin Whale       GGTACAACCTTGACTAG-AGAGTAA--AATCAAACA----------TAAAC-ATAGTTGG
Blue Whale      GGTACAACCTTCACTAG-AGAGTAA--AACCAAACA----------TACACCATAGTTGG
Cow             GATACAACCTTGACTAG-AGAGTAA--AATTTAACA----------CTACC-ATAGTAGG
Rat             GAAAAAACCTTAAATAG-TGAATAAACAACTACA-AT------CACTTAACCATTGTAGG
Mouse           GAAAAAACCTTTAATAG-TGAATAATTAACAAAACAG------CTTTTAACCATTGTAGG
Loach           AATACAACCTTAGCCAGGAGGATAAGGATCATACTTAACAAAACACGTCGTCTCAGTGGG
Carp            GACACAACCTTT-CCAGGAGGATAAAGATCATAATACATAAAACATACTGTTCTAGTGGG
Trout           GACACAACCTTA-ACAGGCGGCTAAGGATCATAGTTC-CAAGGTAACCTGTTACAGTGGG
Chicken         AACACAACCTCCTCCAG-CGGATAATAATCACCCCTC----------CCCGCACTGTGGG
Opossum         TATACAAACTACATTAG-AGGGTAATAATTAATA------------ATCCACATCGTTGG
                   * ** **      **  *  ***  *                          ** **
                1621      1630      1640      1650      1660      1670      1680

Fin Whale       CCTAAAAGCA-GCCATCAA-TTAAGAAAGCGTTCAAGCTCGACAACAAAATAATGTTTTA
Blue Whale      CCTAAAAGCA-GCCATCAA-TTAAGAAAGCGTTCAAGCTCGACAATAAAACAATGTTTTA
Cow             CCTAAAAGCA-GCCATCAA-TTAAGAAAGCGTTAAAGCTCAACAACAAAA-ATTAAATAG
Rat             CTTAAAAGCAAGCCATCAA-TAAAGAAAGCGTCAAGCCACATCATCTTACACACACACTA
Mouse           CCTAAAAGCA-GCCACCAA-TAAAGAAAGCGTTCAAGCTCAACAT--AAAATTTCAATTA
Loach           CCCAAAAGCA-GCCATCTG-ATCAGAAAGCGTTAAAGCTCAAACGACACAAAGTTTATT-
Carp            CCTAAAAGCA-GCCATCTA-AACAGAAAGCGTTAAAGCTCAGACAGAAAAAAGTTTATT-
Trout           CCTAAGAGCA-GCCACCTG-CACAGAAAGCGTTAAAGCTCAGACAGATACAAACCTCTT-
Chicken         CCTTCAAGCA-GCCACCAACAAAAGAGTGCGTCAAAGCTCCCTCATTAAAAAATCTAAA-
Opossum         CTTAAAAGCA-GCCATCAA-CTAAGAAAGCGTTAAAGCTCAAACTC-ACATTCCAATTTA
                *     **** **** *      ***  ****  *  * *
                1681      1690      1700      1710      1720      1730    1740
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Fin Whale       ATTCCAACATTAAGTAAATCAACTCCTAGCCTGACTATTGGACTAA-TCTATACAAATAT
Blue Whale      ATTCCAACATTAGACAAATCAACTCCTAGCATGATTATTGGACTAA-TCTATATAAACAT
Cow             ATTCCAACAACAAATGA-TTAACTCCTAGCCCCAATACTGGACTAA-TCTATTATAGAAT
Rat             ATTCCACAAACCTCAAA--AA-TTCCAAAATTACAAATTGGGCTAAATCTATGATCCTAG
Mouse           ATTCCATAATTTACACC--AACTTCCTAAACTTAAAATTGGGTTAA-TCTATAACTTTAT
Loach           ATTCTGATAAACAATCC--CACTCCCCTAAA--ATTACCAGACTAT-TCCATGCCCCCAT
Carp            ATCCCGATAAAAAAATCT--TACTCCCCTAAAT-ACTATTAGGCCAA-CCCATGCCCCCAT
Trout           ATCCTGATAAGAAATCC--CACCCCCCTAAC--CGTACTAAGCCGT-TCCATGCCCCCAT
Chicken         ACCCT--------ATTT--GACTCCCTCAAC--CAAAGCAGGTTAA-CCTATGAC--AAT
Opossum         ATACCATAAAAAAACTA--AAACCCCTAAAAT-ACTATTGGATGAT-TCTATGATATTAT
                *  *                 *   **          *           * **     *
                1741      1750      1760      1770      1780      1790      1800

Fin Whale       AGAAGCAATACTGTTAATATGAGTAACAAGAAA----T--TTTTCTCCTAGCACAAGCT-
Blue Whale      AGAAGCAACACTGTTAATATGAGTAACAAGAAA----T--TTTTCTCCTAGCACGAGCT-
Cow             AGAAGCAATAATGTTAATATGAGTAACAAGAAA----AA-TTTTCTCCTTGCATAAGTC-
Rat             TGA----ATACTGTTAATATGTG-AACAAGAA------CCAATCCACCAAGCACAAGTGC
Mouse           AGATGCAACACTGTTAGTATGAGTAACAAGAAT----TCCAATTCTCCAGACATACGCG-
Loach           GGAAGAAACTATGCTAAAATGAGTAACAAGGGGGTCATGGCCCCCTCCCGGCACAAGTG-
Carp            GGAAGAGATTATGCTAAAATGAGTAACAAGAAGGCCCGCCCTTCTTCCAAGAACAAGTG-
Trout           GGAAGAGATTATGCTAGAATGAGTAATAAGAGAGTACAA-CTCTCTCCCAGCACATGTG-
Chicken         AGAAGAATCAATGCTAAAATGAGTAATCTGGAA--CCTA---TCCTCC------------
Opossum         AGAATACATAATGCTAAAATTAGTAATAAGAAC-----CCCGTTCTCCTCGCACAAGCC-
                 **         **  **  **  *  **   *              **
                1801      1810      1820      1830      1840      1850      1860

Fin Whale       TACACCAGTAA--CTGATAATATACTGATAATTAA-CAGCAAATAAATA-A---------
Blue Whale      TATATTAGTAA--CTGATAATATACTAATAATTAA-CAGCAAATAAATA-A---------
Cow             TAAGTCAGTGC--CTGATAATACTCTGACCACTAA-CAGTCAATAAAAA-T---------
Rat             TAAGACAAC----CGGATAAC-CATTGTTAATTATTGAATC-ATAGGCA-T---------
Mouse           TATAACAACT---CGGATAAC-CATTGTTAGTTAATCAGACTATAGGCAAT---------
Loach           TAAGCCAGAC---CGGACCCACCACTGGCAATTAACGAACCCAAAAAAAGAGGGATGCAT
Carp            TAAGCCAAAC---CGGACCAACCATTGGCAACTAACGAACTCAATCAAAGAGAGCAATGT
Trout           TAAGTCGGAC---CGGACCCCCCACCGACAAATAACGAACCCAAACCAAGAGGGAACTGT
Chicken         TACGGCGTA-------AACTTACATTAATACATTATTAACAGAACTCAACT------TAT
Opossum         TAAGTTAGAATAACGGATATCCCACTAATAGTTAACAAAAACATAATAA-----------
                **                *                 *    *    *    *
                1861      1870      1880      1890      1900      1910      1920

Fin Whale       AACCCAACACTA--------AATTATTTATTAAA-ATAC---TGTTAACCCAACACAGGC
Blue Whale      AATCTAACACTA--------GACTATTTATTAAG-ACAC---TGTTAACCCAACACAGGA
Cow             AATCCAACAATA--------AACAATTTATTGATTATAC---TGTTAACCCAACACAGGA
Rat             AACCCAACAATAG-------AATTACCTATCCCTAACTC----GTTAGCCCAACACAGGC
Mouse           AATCACACTATA--------AATAATCCACCTATAACTTCTCTGTTAACCCAACACCGGA
Loach           GGAAGACAAAAAA--CCAAGAAAACCCCATGTTATAACAATC-GCTAACCCCACACTGGA
Carp            GAATTACAAAAAAACCCAAGAAAAATCCACAACTAAGCTATC-GTTACCCCCACACTGGA
Trout           AGGCCAGAACAAACACCAAGAAAAACCTACACCA-ACAAATC-GTTACCCCCACACAGGA
Chicken         ACCCCCACACTAA--C-AAGCAATACGTATTCCT---CAATCTGTTAAGCCAACCCAGGA
Opossum         CATTCATCAACC--------AGCCTCTTATACAAAATTT---TGTTAATCCAACACAGGT
                                    *                  * **  ** ** * **
                1921      1930      1940      1950      1960      1970      1980

Fin Whale       GTGCAT-TAAGGAAAGATTAAAAAAAGTAAAAGGAACTCGGCAAACACAAACCCCGCCTG
Blue Whale      GTGCAT-TAAGGAAAGATTAAAAAAAGTAAAAGGAACTCGGCAAACACAAACCCCGCCTG
Cow             GTGCATCTAAGGAAAGATTAAAAGAAGTAAAAGGAACTCGGCAAACACAAACCCCGCCTG
Rat             GTGCTT-TAAGGAAAGTTTAAAAAAGT--AAAGGAACTCGGCAAACACGAACCCCGCCTG
Mouse           ATGCCT-AAAGGAAAGATCCAAAAAGATAAAAGGAACTCGGCAAACAAGAACCCCGCCTG
Loach           GTGCCCCCA-GGAAAGACTAAAAGAAAAGGAAGGAACTCGGCAAACATAAGCCTCGCCTG
Carp            GTGCCCTAAAGGAAAGACTAAAAGAAAAGGAAGGAACTCGGCAAACACAAGCCTCGCCTG
Trout           GTGCCCCAAGGGAAAGACCCAAAGGAAGAGAAGGAACTCGGCAAACACAAGCCTCGCCTG
Chicken         GCGCCCACA--GGATGATTAAAACCTACAGAAGGAACTCGGCAAACCAAAGACCCGACTG
Opossum         GTGCATTAAAGGAAAGATATAAAAGAACAAAAGGAACTCGGCAAACATGAACCCCGCCTG
                  **     *  *  *  *     ***       ****************   *  * ** ***
                1981      1990      2000      2010      2020      2030      2040
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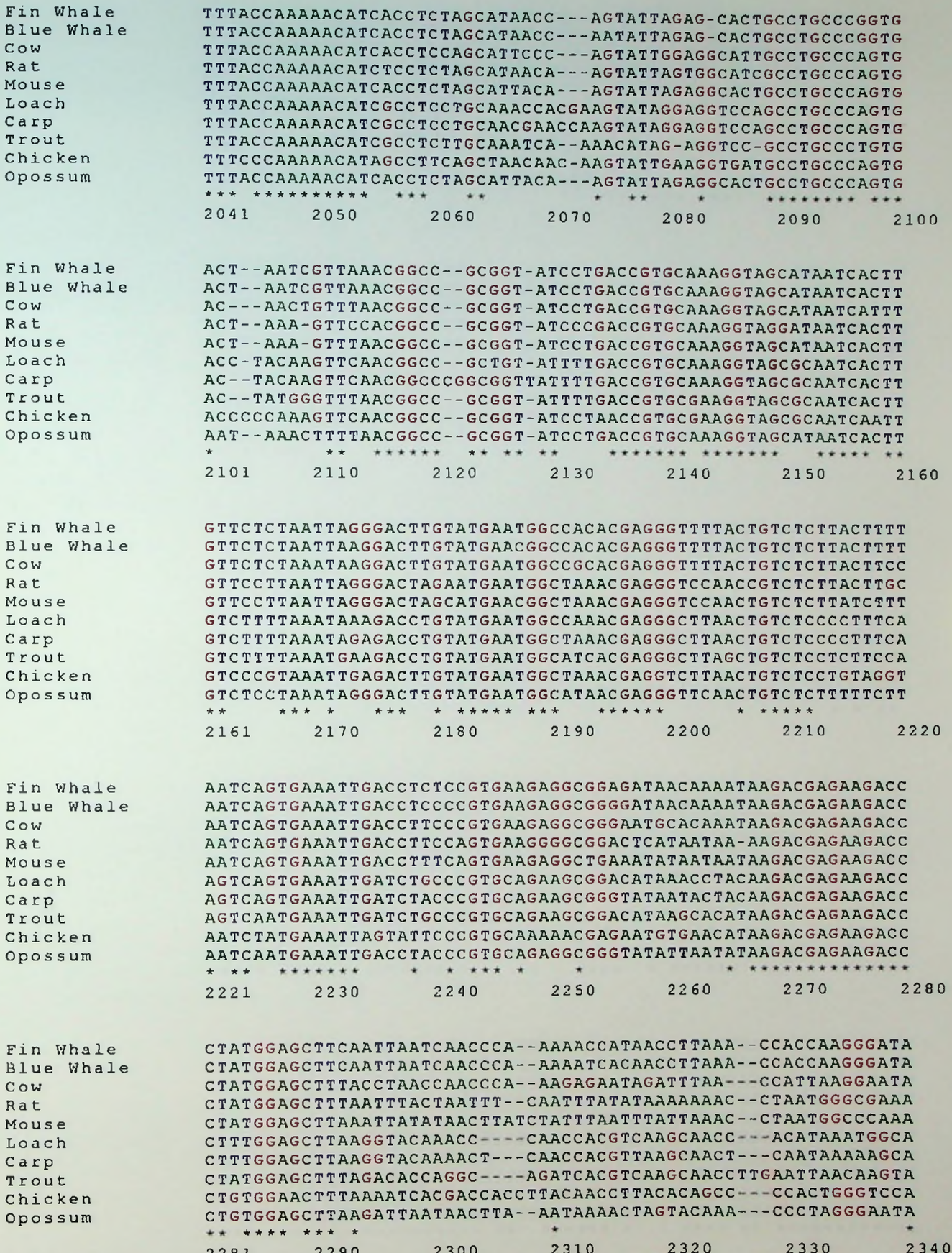
Fin Whale TTTACCAAAAACATCACCTCTAGCATAACC---AGTATTAGAG-CACTGCCTGCCCGGTG
Blue Whale TTTACCAAAAACATCACCTCTAGCATAACC---AATATTAGAG-CACTGCCTGCCCGGTG
Cow TTTACCAAAAACATCACCTCCAGCATTCCC---AGTATTGGAGGCATTGCCTGCCCAGTG
Rat TTTACCAAAAACATCTCCTCTAGCATAACA---AGTATTAGTGGCATCGCCTGCCCAGTG
Mouse TTTACCAAAAACATCACCTCTAGCATTACA---AGTATTAGAGGCACTGCCTGCCCAGTG
Loach TTTACCAAAAACATCGCCTCCTGCAAACCACGAAGTATAGGAGGTCCAGCCTGCCCAGTG
Carp TTTACCAAAAACATCGCCTCCTGCAACGAACCAAGTATAGGAGGTCCAGCCTGCCCAGTG
Trout TTTACCAAAAACATCGCCTCTTGCAAATCA--AAACATAG-AGGTCC-GCCTGCCCTGTG
Chicken TTTCCCAAAAACATAGCCTTCAGCTAACAAC-AAGTATTGAAGGTGATGCCTGCCCAGTG
Opossum TTTACCAAAAACATCACCTCTAGCATTACA---AGTATTAGAGGCACTGCCTGCCCAGTG
2041 2050 2060 2070 2080 2090 2100
Fin Whale ACT--AATCGTTAAACGGCC--GCGGT-ATCCTGACCGTGCAAAGGTAGCATAATCACTT
Blue Whale ACT--AATCGTTAAACGGCC--GCGGT-ATCCTGACCGTGCAAAGGTAGCATAATCACTT
Cow AC---AACTGTTTAACGGCC--GCGGT-ATCCTGACCGTGCAAAGGTAGCATAATCATTT
Rat ACT--AAA-GTTCCACGGCC--GCGGT-ATCCCGACCGTGCAAAGGTAGGATAATCACTT
Mouse ACT--AAA-GTTTAACGGCC--GCGGT-ATCCTGACCGTGCAAAGGTAGCATAATCACTT
Loach ACC-TACAAGTTCAACGGCC--GCTGT-ATTTTGACCGTGCAAAGGTAGCGCAATCACTT
Carp AC--TACAAGTTCAACGGCCCGGCGGTTATTTTGACCGTGCAAAGGTAGCGCAATCACTT
Trout AC--TATGGGTTTAACGGCC--GCGGT-ATTTTGACCGTGCGAAGGTAGCGCAATCACTT
Chicken ACCCCCAAAGTTCAACGGCC--GCGGT-ATCCTAACCGTGCGAAGGTAGCGCAATCAATT
Opossum AAT--AAACTTTTAACGGCC--GCGGT-ATCCTGACCGTGCAAAGGTAGCATAATCACTT
2101 2110 2120 2130 2140 2150 2160
Fin Whale GTTCTCTAATTAGGGACTTGTATGAATGGCCACACGAGGGTTTTACTGTCTCTTACTTTT
Blue Whale GTTCTCTAATTAAGGACTTGTATGAACGGCCACACGAGGGTTTTACTGTCTCTTACTTTT
Cow GTTCTCTAAATAAGGACTTGTATGAATGGCCGCACGAGGGTTTTACTGTCTCTTACTTCC
Rat GTTCCTTAATTAGGGACTAGAATGAATGGCTAAACGAGGGTCCAACCGTCTCTTACTTGC
Mouse GTTCCTTAATTAGGGACTAGCATGAACGGCTAAACGAGGGTCCAACTGTCTCTTATCTTT
Loach GTCTTTTAAATAAAGACCTGTATGAATGGCCAAACGAGGGCTTAACTGTCTCCCCTTTCA
Carp GTCTTTTAAATAGAGACCTGTATGAATGGCTAAACGAGGGCTTAACTGTCTCCCCTTTCA
Trout GTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTAGCTGTCTCCTCTTCCA
Chicken GTCCCGTAAATTGAGACTTGTATGAATGGCTAAACGAGGTCTTAACTGTCTCCTGTAGGT
Opossum GTCTCCTAAATAGGGACTTGTATGAATGGCATAACGAGGGTTCAACTGTCTCTTTTTCTT
2161 2170 2180 2190 2200 2210 2220
Fin Whale AATCAGTGAAATTGACCTCTCCGTGAAGAGGCGGAGATAACAAAATAAGACGAGAAGACC
Blue Whale AATCAGTGAAATTGACCTCCCCGTGAAGAGGCGGGGATAACAAAATAAGACGAGAAGACC
Cow AATCAGTGAAATTGACCTTCCCGTGAAGAGGCGGGAATGCACAAATAAGACGAGAAGACC
Rat AATCAGTGAAATTGACCTTCCAGTGAAGGGGCGGACTCATAATAA-AAGACGAGAAGACC
Mouse AATCAGTGAAATTGACCTTTCAGTGAAGAGGCTGAAATATAATAATAAGACGAGAAGACC
Loach AGTCAGTGAAATTGATCTGCCCGTGCAGAAGCGGACATAAACCTACAAGACGAGAAGACC
Carp AGTCAGTGAAATTGATCTACCCGTGCAGAAGCGGGTATAATACTACAAGACGAGAAGACC
Trout AGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGCACATAAGACGAGAAGACC
Chicken AATCTATGAAATTAGTATTCCCGTGCAAAAACGAGAATGTGAACATAAGACGAGAAGACC
Opossum AATCAATGAAATTGACCTACCCGTGCAGAGGCGGGTATATTAATATAAGACGAGAAGACC
2221 2230 2240 2250 2260 2270 2280
Fin Whale CTATGGAGCTTCAATTAATCAACCCA--AAAACCATAACCTTAAA--CCACCAAGGGATA
Blue Whale CTATGGAGCTTCAATTAATCAACCCA--AAAATCACAACCTTAAA--CCACCAAGGGATA
Cow CTATGGAGCTTTACCTAACCAACCCA--AAGAGAATAGATTTAA---CCATTAAGGAATA
Rat CTATGGAGCTTTAATTTACTAATTT--CAATTTATATAAAAAAAC--CTAATGGGCGAAA
Mouse CTATGGAGCTTAAATTATATAACTTATCTATTTAATTTATTAAAC--CTAATGGCCCAAA
Loach CTTTGGAGCTTAAGGTACAAACC----CAACCACGTCAAGCAACC---ACATAAATGGCA
Carp CTTTGGAGCTTAAGGTACAAAACT---CAACCACGTTAAGCAACT---CAATAAAAAGCA
Trout CTATGGAGCTTTAGACACCAGGC----AGATCACGTCAAGCAACCTTGAATTAACAAGTA
Chicken CTGTGGAACTTTAAAATCACGACCACCTTACAACCTTACACAGCC---CCACTGGGTCCA
Opossum CTGTGGAGCTTAAGATTAATAACTTA--AATAAAACTAGTACAAA---CCCTAGGGAATA
2281 2290 2300 2310 2320 2330 2340

```
Fin Whale       ACAAAACCTTATAT---GGGCTGACAAT--TTCGGTTGGGGTGACCTCGGAGTACAAAA-
Blue Whale      ACAAAATTTTATAT---GGGCTGACAAT--TTCGGTTGGGGTGACCTCGGAGCACAAAA-
Cow             ACAACAATCTCCAT---GAGTTGGTAGT--TTCGGTTGGGGTGACCTCGGAGAATAAAA-
Rat             ACAACAAAATTATG---AACTACCAAAT--TTCGGTTGGGGTGACCTCGGAGAATAAAA-
Mouse           A--ACTATAGTAT----AAGTTTGAAAT--TTCGGTTGGGGTGACCTCGGAGAATAAAA-
Loach           TAAAATTAGTGAGTAGTGGAATTTTAC---TTCGGTTGGGGCGACCACGGAGAAAAAAA-
Carp            AAAACCTTGTGGATCATGAGATTTTACC--TTCGGTTGGGGCGACCACGGAGGAAAGAA-
Trout           AAAACGCAGTAGACCCCTAGCCCATATGTCTTTGGTTGGGGCGACCGCGGGGGGAAAATT
Chicken         CCCACACA-TAAACCCCTGGTCGACATTT-TTCGGTTGGGGCGACCTTGGAGAAAAAAA-
Opossum         ACATTATTATTATT---AAGTT-ATATTC-TTTGGTTGGGGTGACCTCGGAGAACAAAA-
                                            *    ** ******** ****  ** *   * *
                2341      2350      2360      2370      2380      2390      2400

Fin Whale       AACCCTCCGA------GTGATT--AAAACTT----------AGGC-----CCA-------
Blue Whale      AATCCTCCGA------GTGATT--AAAACTT----------AGGC-----TCA-------
Cow             AATCCTCCGA------GCGATTTTAAAGACT----------AGAC-----CCA-------
Rat             AATCCTCCGA------ATGATT--ATAACCG----------AGTCGGTAACCGTGTCCGA
Mouse           AATCCTCCGA------ATGATT--ATAACCT----------AGAC--TTACAA-----GT
Loach           GATCCTCCGA-GTGGACTGAGCCAACAAGCTTAA--AACTACGAAAAACATTTCTTTCTC
Carp            AAGCCTCCAG-GTGGACTGGGAAAACCTCCT-AA--AACCAAGAGAGACATCTCTAAG-C
Trout           AAGCCCCCACTGTGGACTGGGGGGCACTGCCCCACCAGCCGAGAGCTACAGCTCTAAGCA
Chicken         AATCCTCCAA-ACCCACAGACCACAACTCTT-CA----CTAAGACCAACTCCTCAAAGTA
Opossum         AAACCTCCGA------ATGA-C--ATAACCT----------AGATTAACTAAT-------
                 * ** **         *                        *
                2401      2410      2420      2430      2440      2450      2460

Fin Whale       -CTAGCCAAAGTACAAT-ATCACTTATTGATCCAATCCT---TTGATCAACGGAACAAGT
Blue Whale      -CTAGCCAAAGTGTAAT-ATCACTTATTGATCCAATCTC---TTGATCAACGGAACAAGT
Cow             -CAAGTCAAATCACTCT-ATCGCTCATTGATCCAAAAAC---TTGATCAACGGAACAAGT
Rat             CCCAGTCAAGTAATACTAATATCTTATTGACCCAATTAT----TGATCAACGGACCAAGT
Mouse           CAAAGTAAAATCA-AC--ATATCTTATTGACCCAGATATATTTTGATCAACGGACCAAGT
Loach           TCTATCC--CTCTGACC-AAATATGATCCGACCTATAAA--GTCGATCAACGAACCAAGT
Carp            ACAGAAC--ATCTGACC-AAATATGATCCGGCTAACACATAGCCGATCAACGAACCAAGT
Trout           CCAGAAT--ATCTGACC-AAATATGATCCGGCGAACGCAT------TCAACGGACCGAGT
Chicken         CCAACAGTAACCAGACC-CAATATAATTGAGC----A------------ATGGACCAAGC
Opossum         CCAAGTG----CACAAAAGCCAGTAATTGACCCAAATAT----TGATCAACGGAACAAGT
                                  * **    *                    * * * * * **
                2461      2470      2480      2490      2500      2510      2520

Fin Whale       TACCCTAGGGATAACAGCGCAATCCTATTCTAGAGTCCATATCGACAAT--AGGGTTTAC
Blue Whale      TACCCTAGGGATAACAGCGCAATCCTATTCTAGAGTCCATATCGACAAT--AGGGTTTAC
Cow             TACCCTAGGGATAACAGCGCAATCCTATTCAAGAGTCCATATCGACAAT--AGGGTTTAC
Rat             TACCCTAGGG-TAACAGCGCGA-CCTATTTAAGAGTTCATATCGACAATT-AGGGTTTAC
Mouse           TACCCTAGGGATAACAGCGCAATCCTATTTAAGAGTTCATATCGACAATT-AGGGTTTAC
Loach           TACCCTAGGGATTACAGCGCAATCCTCTCCCAGAGTCCATATCGACGAG--AGGGTTTAC
Carp            TACCCTAGGGATAACAGCGCAATCCTCTCCCAGAGTCCATATCGACGAG--GGGGTTTAC
Trout           TACCCTAGGGATAACAGCGCAATCCTCTCCCAGAGTCCCTATCGACGAG--GGGGTTTAC
Chicken         TACCCCAGGGATAACAGCGCAATCTCCTCCAAGAGCCCATATCGACAAG--GAGGTTTAC
Opossum         TACCCCAGGGATAACAGCGCAATCCTATTTAAGAGCCCATATCGAAAATCTAGGGTTTAC
                ***** **** * ******* * *   *   **** * ******  *     *******
                2521      2530      2540      2550      2560      2570      2580

Fin Whale       GACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCTGCTATTAAGGGTTCGTTTGTT
Blue Whale      GACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCACTATTAAGGGTTCGTTTGTT
Cow             GACCTCGATGTTGGATCAGGACATCCTGATGGTGCAACCGCTATCAAAGGTTCGTTTGTT
Rat             GACCTCGATGTTGGATCAGGACATCCCAATGGTGCAGAAGCTATTAATGGTTCGTTTGTT
Mouse           GACCTCGATGTTGGATCAGGACATCCCAATGGTGTAGAAGCTATTAATGGTTCGTTTGTT
Loach           GACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGTT
Carp            GACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGTT
Trout           GACCTCGATGTTGGATCAGGACATCCTAATGGTGCAGCCGCTATTAAGGGTTCGTTTGTT
Chicken         GACCTCGATGTTGGATCAGGACAACCTAATGGTGCAACCGCTATTAAGGGTTCGTTTGTT
Opossum         GACCTCGATGTTGGATCAGGACATCCTAATGGTGCAACCGCTATTAAAGGTTCGTTTGTT
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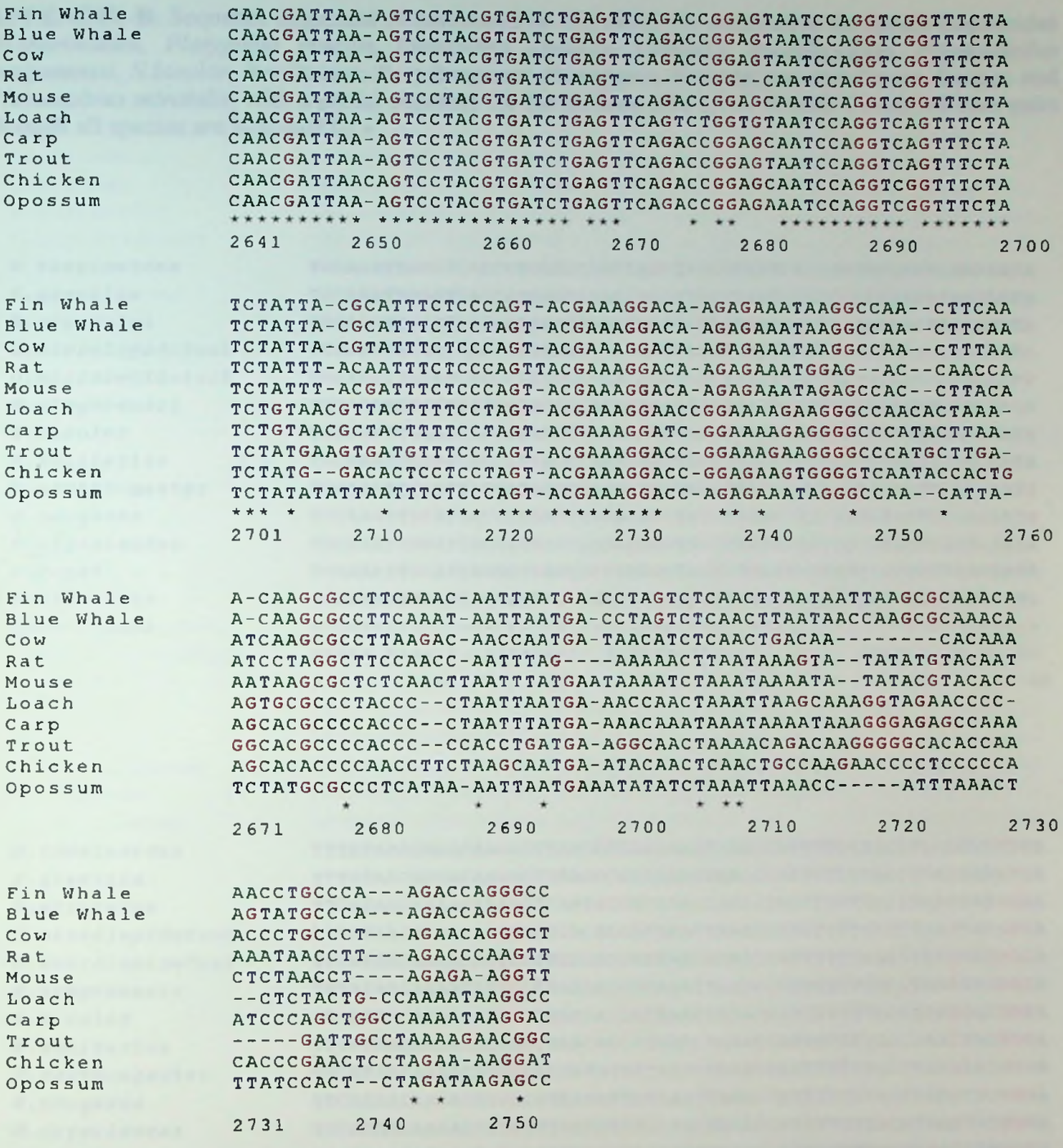
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Cow CAACGATTAA-AGTCCTACGTGATCTGAGTTCAGACCGGAGTAATCCAGGTCGGTTTCTA
Rat CAACGATTAA-AGTCCTACGTGATCTAAGT-----CCGG--CAATCCAGGTCGGTTTCTA
Mouse CAACGATTAA-AGTCCTACGTGATCTGAGTTCAGACCGGAGCAATCCAGGTCGGTTTCTA
Loach CAACGATTAA-AGTCCTACGTGATCTGAGTTCAGTCTGGTGTAATCCAGGTCAGTTTCTA
Carp CAACGATTAA-AGTCCTACGTGATCTGAGTTCAGACCGGAGCAATCCAGGTCAGTTTCTA
Trout CAACGATTAA-AGTCCTACGTGATCTGAGTTCAGACCGGAGTAATCCAGGTCAGTTTCTA
Chicken CAACGATTAACAGTCCTACGTGATCTGAGTTCAGACCGGAGCAATCCAGGTCGGTTTCTA
Opossum CAACGATTAA-AGTCCTACGTGATCTGAGTTCAGACCGGAGAAATCCAGGTCGGTTTCTA
2641 2650 2660 2670 2680 2690 2700
Fin Whale TCTATTA-CGCATTTCTCCCAGT-ACGAAAGGACA-AGAGAAATAAGGCCAA--CTTCAA
Blue Whale TCTATTA-CGCATTTCTCCTAGT-ACGAAAGGACA-AGAGAAATAAGGCCAA--CTTCAA
Cow TCTATTA-CGTATTTCTCCCAGT-ACGAAAGGACA-AGAGAAATAAGGCCAA--CTTTAA
Rat TCTATTT-ACAATTTCTCCCAGTTACGAAAGGACA-AGAGAAATGGAG--AC--CAACCA
Mouse TCTATTT-ACGATTTCTCCCAGT-ACGAAAGGACA-AGAGAAATAGAGCCAC--CTTACA
Loach TCTGTAACGTTACTTTTCCTAGT-ACGAAAGGAACCGGAAAAGAAGGGCCAACACTAAA-
Carp TCTGTAACGCTACTTTTCCTAGT-ACGAAAGGATC-GGAAAAGAGGGGCCCATACTTAA-
Trout TCTATGAAGTGATGTTTCCTAGT-ACGAAAGGACC-GGAAAGAAGGGGCCCATGCTTGA-
Chicken TCTATG--GACACTCCTCCTAGT-ACGAAAGGACC-GGAGAAGTGGGGTCAATACCACTG
Opossum TCTATATATTAATTTCTCCCAGT-ACGAAAGGACC-AGAGAAATAGGGCCAA--CATTA-
2701 2710 2720 2730 2740 2750 2760
Fin Whale A-CAAGCGCCTTCAAAC-AATTAATGA-CCTAGTCTCAACTTAATAATTAAGCGCAAACA
Blue Whale A-CAAGCGCCTTCAAAT-AATTAATGA-CCTAGTCTCAACTTAATAACCAAGCGCAAACA
Cow ATCAAGCGCCTTAAGAC-AACCAATGA-TAACATCTCAACTGACAA--------CACAAA
Rat ATCCTAGGCTTCCAACC-AATTTAG----AAAAACTTAATAAAGTA--TATATGTACAAT
Mouse AATAAGCGCTCTCAACTTAATTTATGAATAAAATCTAAATAAAATA--TATACGTACACC
Loach AGTGCGCCCTACCC--CTAATTAATGA-AACCAACTAAATTAAGCAAAGGTAGAACCCC-
Carp AGCACGCCCCACCC--CTAATTTATGA-AAACAAATAAATAAAATAAAGGGAGAGCCAAA
Trout GGCACGCCCCACCC--CCACCTGATGA-AGGCAACTAAAACAGACAAGGGGGCACACCAA
Chicken AGCACACCCCAACCTTCTAAGCAATGA-ATACAACTCAACTGCCAAGAACCCCTCCCCCA
Opossum TCTATGCGCCCTCATAA-AATTAATGAAATATATCTAAATTAAACC-----ATTTAAACT
2671 2680 2690 2700 2710 2720 2730
Fin Whale AACCTGCCCA---AGACCAGGGCC
Blue Whale AGTATGCCCA---AGACCAGGGCC
Cow ACCCTGCCCT---AGAACAGGGCT
Rat AAATAACCTT---AGACCCAAGTT
Mouse CTCTAACCT----AGAGA-AGGTT
Loach --CTCTACTG-CCAAAATAAGGCC
Carp ATCCCAGCTGGCCAAAATAAGGAC
Trout -----GATTGCCTAAAAGAACGGC
Chicken CACCCGAACTCCTAGAA-AAGGAT
Opossum TTATCCACT--CTAGATAAGAGCC
2731 2740 2750

APPENDIX B: Sequence alignment of mitochondrial 16S rRNA for members in the family Leuciscidae (*N.therinoides*, *Platygobio gracilis*, *Rhinichthys atratulus*, *Orthodon microlepidotus*, *Ptychocheilus oregonensis*, *S.bicolor*, *R.solitarius*, *P.erythrogaster*, *P.neogaeus*, *Notemigonus crysoleucas*, *S.copei*, and *Phenacobius mirabilis*, with a partial sequence for the northern pike (*Esox lucius*). Conserved base pairs through all species are indicated by *.

```
N.therinoides          TGCAAATTGGGTCGCCCTGAGCCAACCAGCTAGCTTAATCACCAGTATAATTCAACAATA
P.gracilis             TGCAAATTGGATCACCCTGAGCTAACCAGCTAGCTTAATCATCACTATAATTTAACAATA
R.atratulus            TGCAAGTTGGATCACCCTGAGCTAACCAGCTAGCTTGACTACTAATATAACTAAACTTTA
O.microlepidotus1      TGCAAGTTGGATCACCCTGAGCCAAACAGCTAGCTTAATTACTAATATAACCCAACAATG
O.microlepidotus2      TGCAAGTTGGATCACCCTGAGCCAAACAGCTAGCTTAATTACTAATATAACCCAACAATG
P.oregonensis          TGCAAATTGGGTCGCCCTGAGCCAAACAGCTAGCTTAATTACTAATATAATTCAACAATA
S.bicolor              TGCAAATTGGATCACCCTGAGCCAAACAGCTAGCCTAATTACTGATATAATTCAACAATA
R.solitarius           TGCAAATTGGGTCACCCTGAGCCAAACAGCTAGCTTAATTACTAATATAATTCAACAATA
P.erythrogaster        TGCAAATTGGATTACCCTGAGCCAAACAGCTAGCTTAGCCATCAATATAATTCAACAATG
P.neogaeus             TGCAAGTTGGATTACCCTGAGCCAAACAGCTAGCTTAACTACCAATATAATTCAACAATG
N.crysoleucas          TGCAAATTGGATCACCCTGAGCCAACCAGCTAGCTTGATTATTAATATAATTTAACAATA
S.copei                TGCAAATTGGATCACCCTGAGCCGACCAGCTAGCCTGATTATTAATATAATTTAACAATA
P.mirabilis            TGCAAATTGGATCACCCTGAGCCAACCAGCTAGCTTAACTAAAAATATAATGTAGCACTG
Esox lucius            ------------------------------------------------------------
                       ***** **** *  ********  * ******** *    *    *****   * *  *
                       1         10        20        30        40        50        60

N.therinoides          TTTATAACAAAACAAGACCTAACCCTACAAACTAAACCATTTTTTTACCTGAGTATGGGA
P.gracilis             TTTATAACAACACAAGACTTAACCCCACAAACTAAACCATTTTTTTACCTGAGTATGGGA
R.atratulus            TTCATAACAAAACAAAACCCAATGCCACAAACTAAACCATTTTTTTACCTAAGTATGGGA
O.microlepidotus1      TTCATAACAAAACATGACCTGACACCATAAACTAAACCATTTTTTTACCTAAGTACGGGA
O.microlepidotus2      TTCATAACAAAACATGACCTGACACCATAAACTAAACCATTTTTTTACCTAAGTACGGGA
P.oregonensis          TTTATAACAAAACATGGCCTAACACCATAAACTAAACCATTTTTTTACCTAAGTACGGGA
S.bicolor              TTCATAACAAAACATGGCCTAACACCATAAACTAAACCATTTTTTTACCTGAGTACGGGA
R.solitarius           TTTATAACAAAACATAACCTAACACCATAAACTAAACCATTTTTTTACCTAAGTACGGGA
P.erythrogaster        TTCATAACAAAACATGGCCCAATATTATAAACTAAACCATTTTTTTACCTAAGTACGGGA
P.neogaeus             TTCATAACAAAACATGACCTAATATTATAAATTAAACCATTTTTTTACCTAAGTACGGGA
N.crysoleucas          TTCATAACAAAACATGGCTTAACATTACAAACTAAACCATTTTTTTGCCTAAGTACGGGA
S.copei                TTTATAACAAAACACGGCCTAACGCCACAAATTAAACCATTTTTTTACCTGAGTATGGGA
P.mirabilis            TTTATAACCAACCAAGACCTAACCCCACCAACTAAACCATTTTTTTACCTAAGTATGGGA
Esox lucius            ------------------------------------------------------------
                       ** ***** ** **   *   *    *  ** ************** *** **** ****
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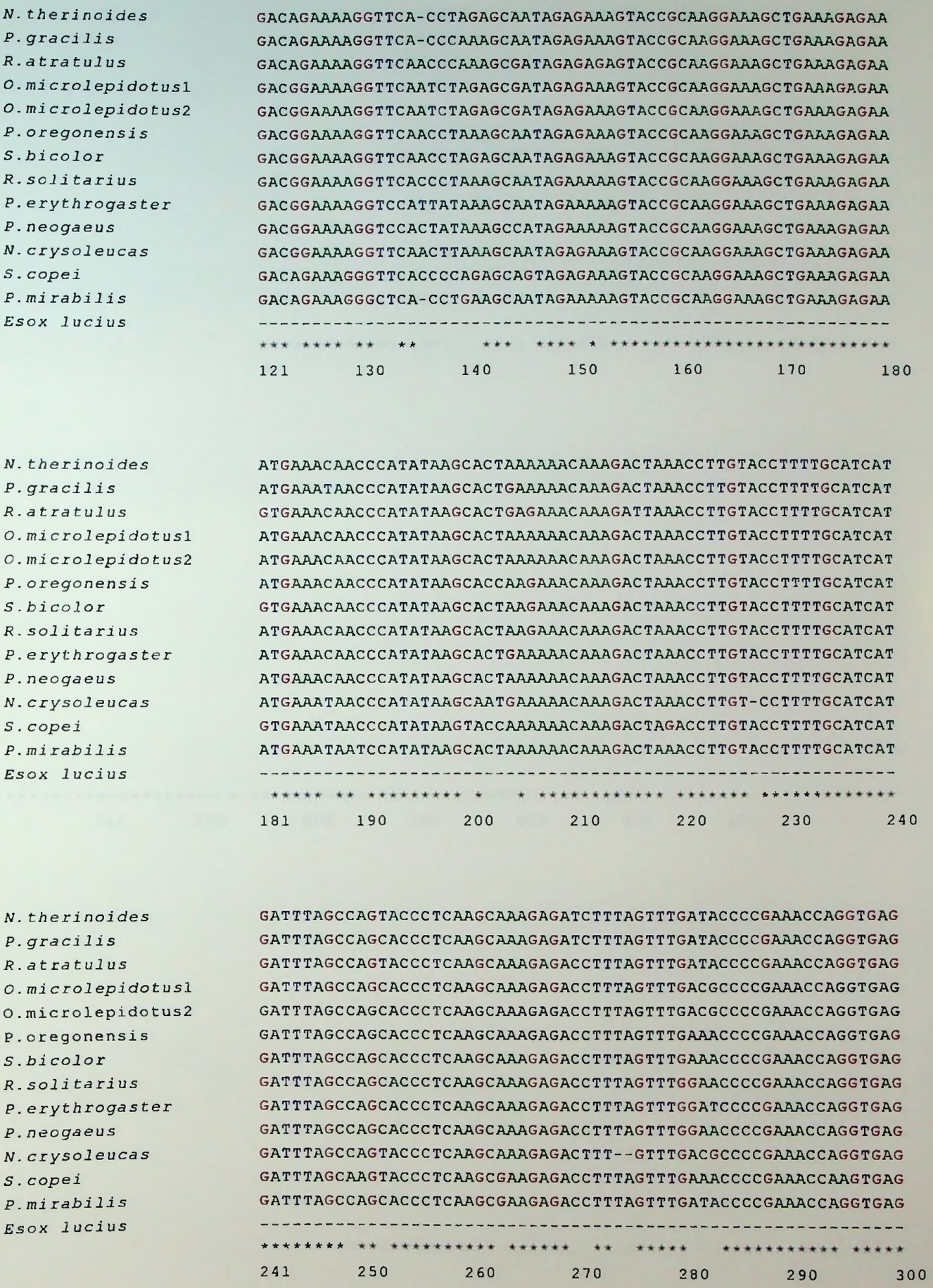
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R.atratulus GACAGAAAAGGTTCAACCCAAAGCGATAGAGAGAGTACCGCAAGGAAAGCTGAAAGAGAA
O.microlepidotus1 GACGGAAAAGGTTCAATCTAGAGCGATAGAGAAAGTACCGCAAGGAAAGCTGAAAGAGAA
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P.oregonensis GACGGAAAAGGTTCAACCTAAAGCAATAGAGAAAGTACCGCAAGGAAAGCTGAAAGAGAA
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N.crysoleucas GACGGAAAAGGTTCAACTTAAAGCAATAGAGAAAGTACCGCAAGGAAAGCTGAAAGAGAA
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P.mirabilis GACAGAAAGGGCTCA-CCTGAAGCAATAGAAAAAGTACCGCAAGGAAAGCTGAAAGAGAA
Esox lucius
121 130 140 150 160 170 180
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P.mirabilis ATGAAATAATCCATATAAGCACTAAAAAACAAAGACTAAACCTTGTACCTTTTGCATCAT
Esox lucius
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R.atratulus GATTTAGCCAGTACCCTCAAGCAAAGAGACCTTTAGTTTGATACCCCGAAACCAGGTGAG
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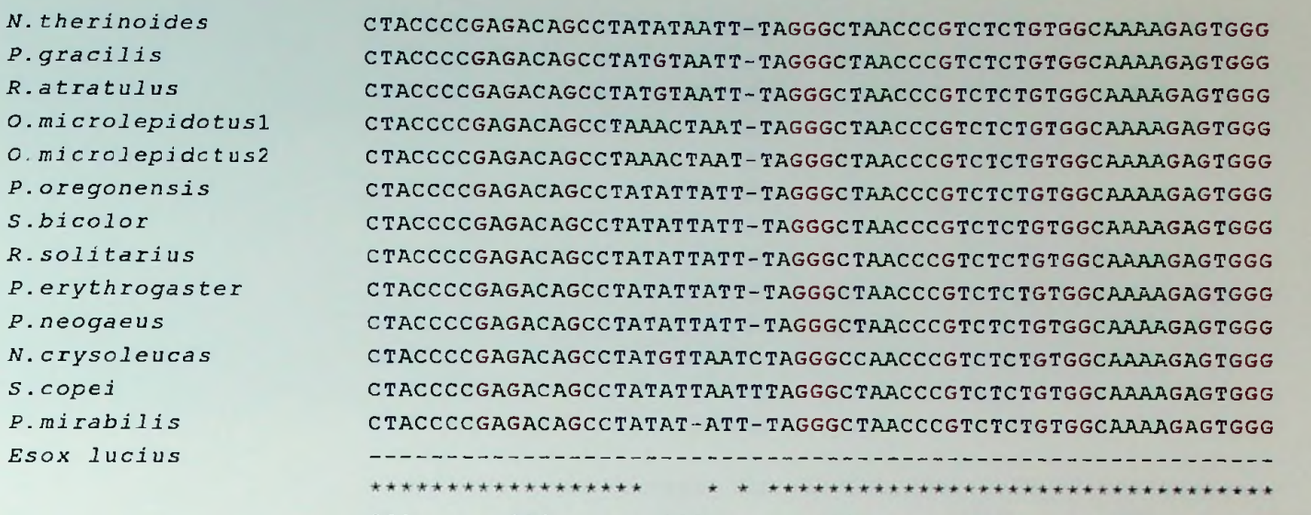
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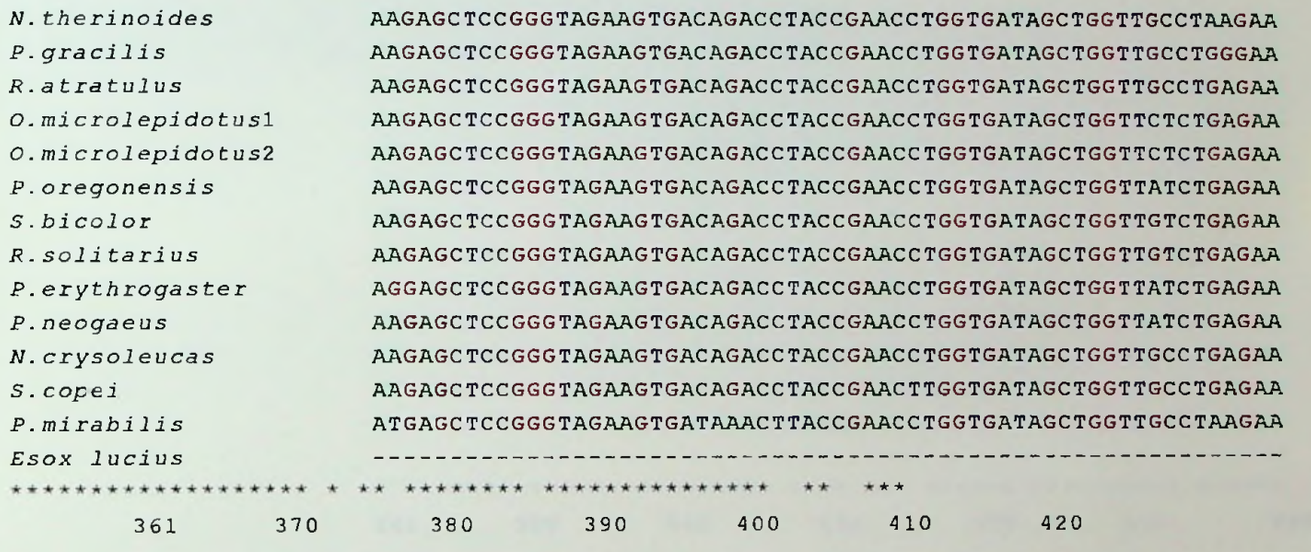
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R.atratulus AAGAGCTCCGGGTAGAAGTGACAGACCTACCGAACCTGGTGATAGCTGGTTGCCTGAGAA
O.microlepidotus1 AAGAGCTCCGGGTAGAAGTGACAGACCTACCGAACCTGGTGATAGCTGGTTCTCTGAGAA
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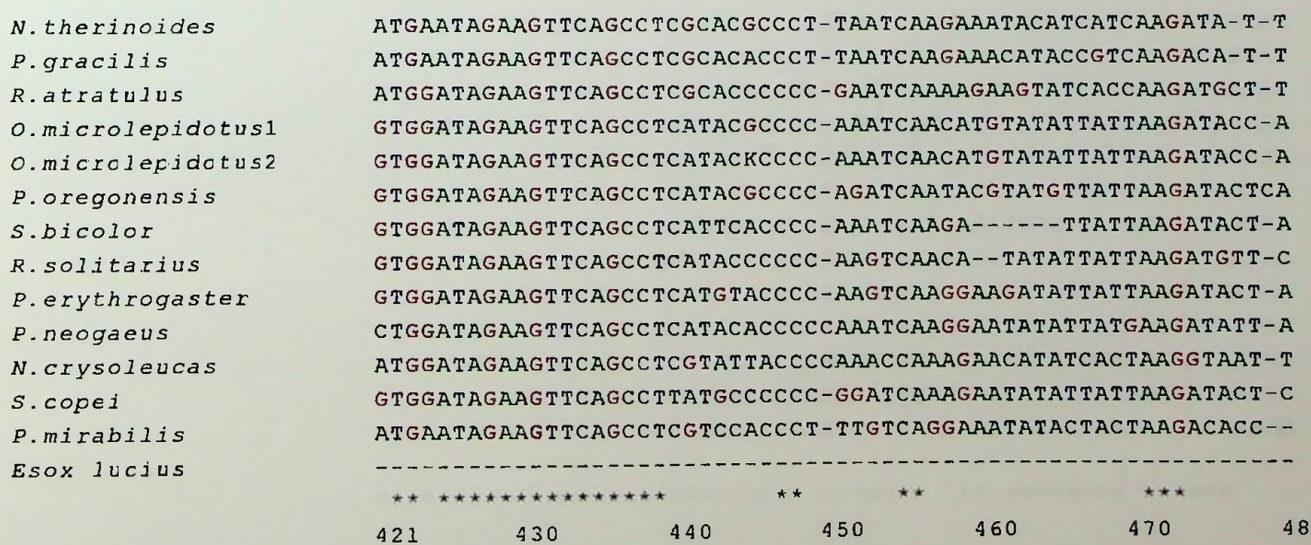
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Esox lucius ----------
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P.gracilis             ATGGAAAAATACGAGAGTTAGTTGAAGGGGGTACAGCCCCTTTAACAAAGGATACAACCT
R.atratulus            AAGGGAAAGTGCGAGAGTTAGTTGAAGGGGGTACAGCCCCTTCAACAAAGGATACAACCT
O.microlepidotus1      GGAGAAATATATGAGAGTTAGTTAAAAGGGGTACAGCCCTTTTAACAAAGGATACAACCT
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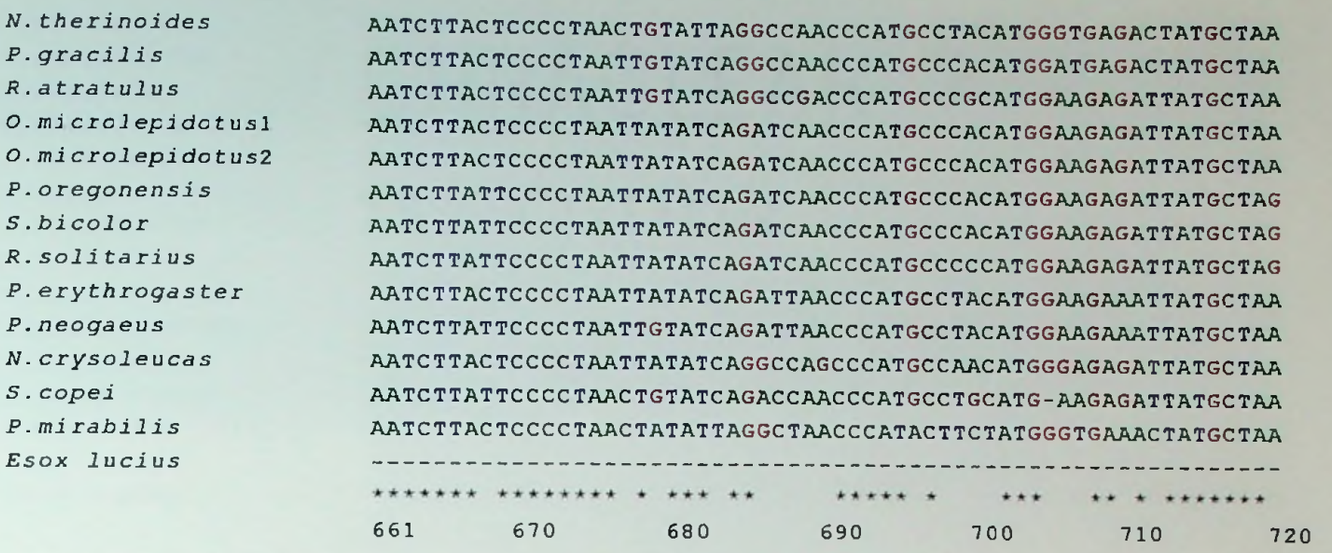
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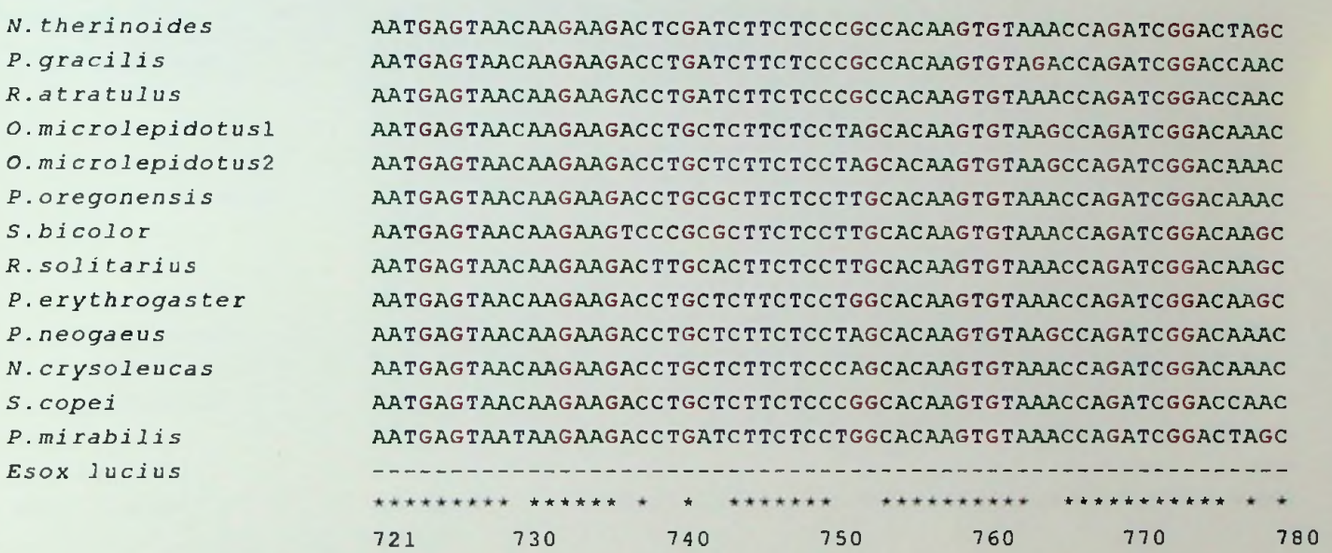
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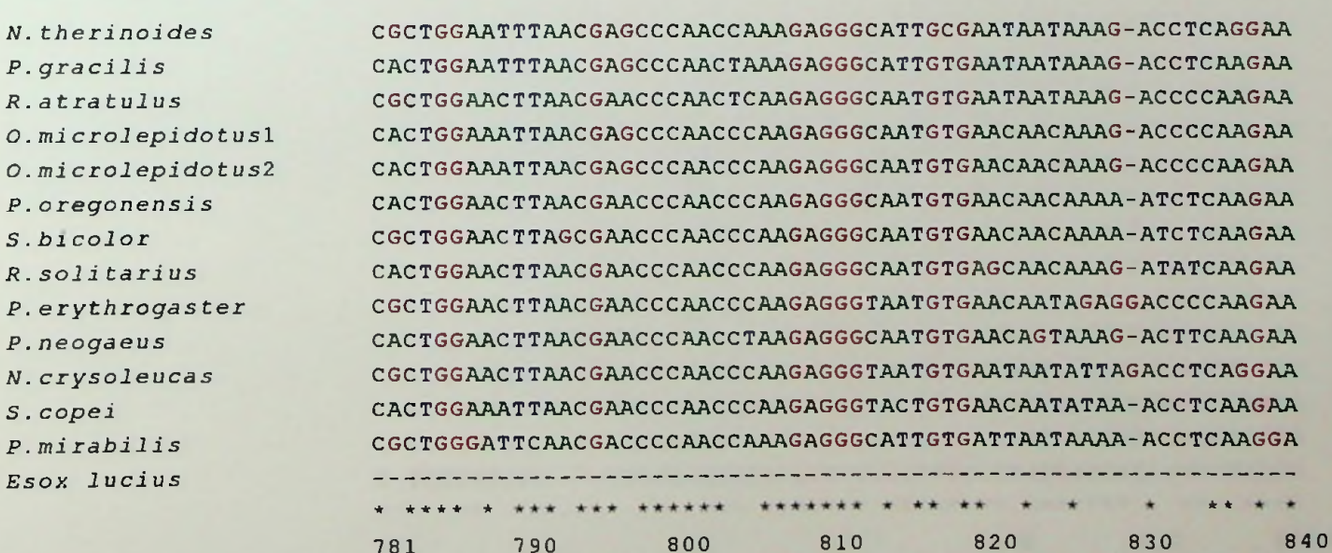
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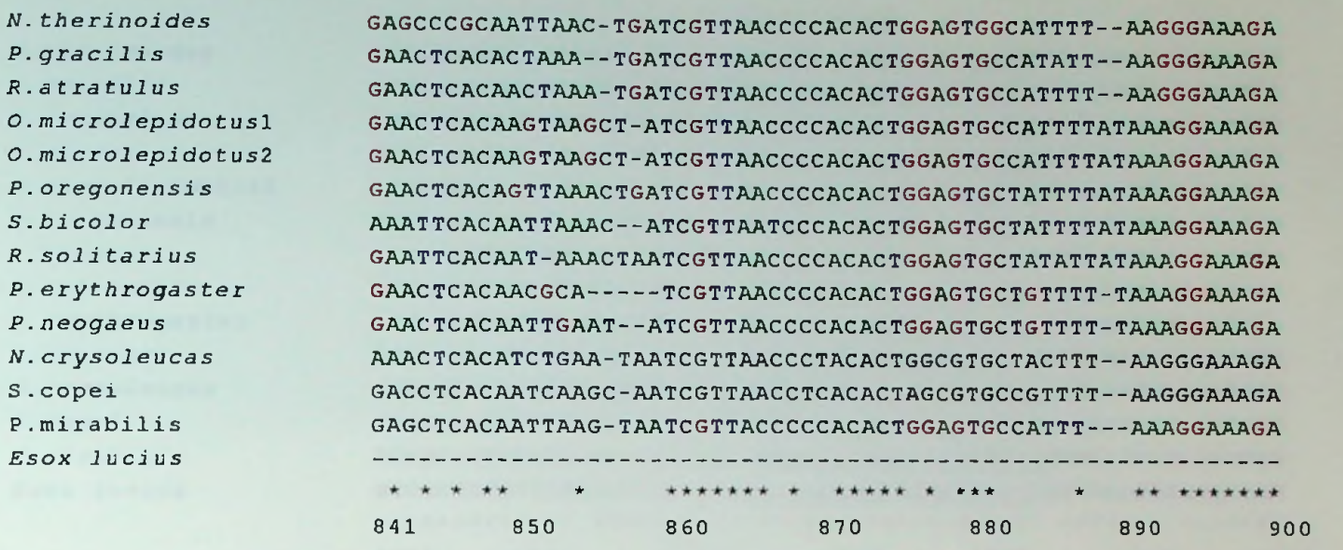
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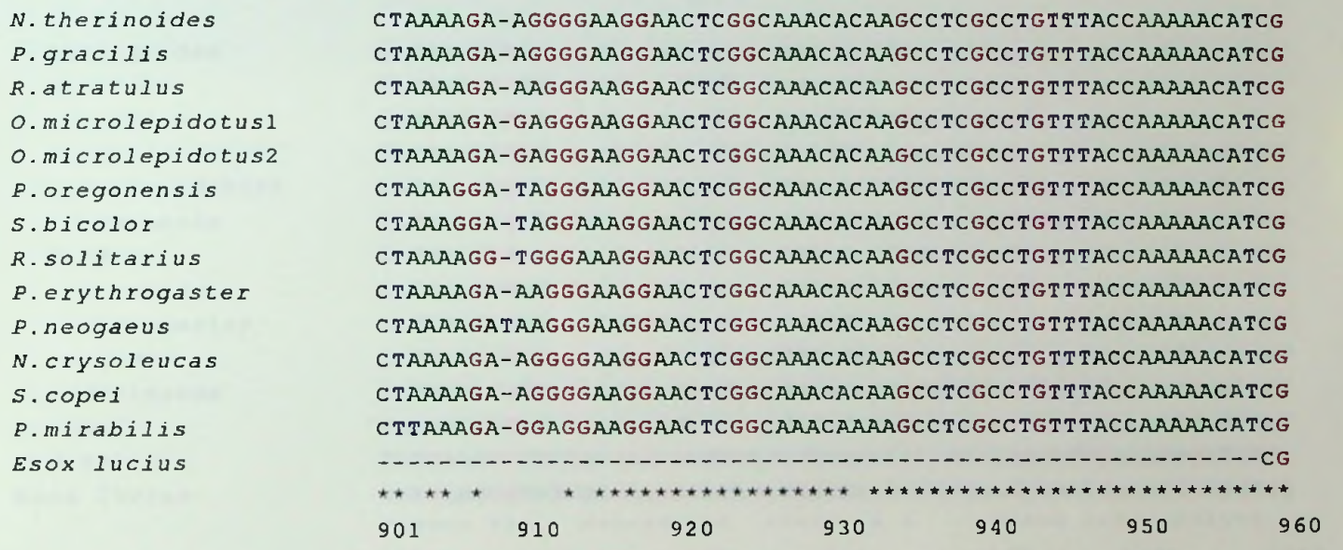
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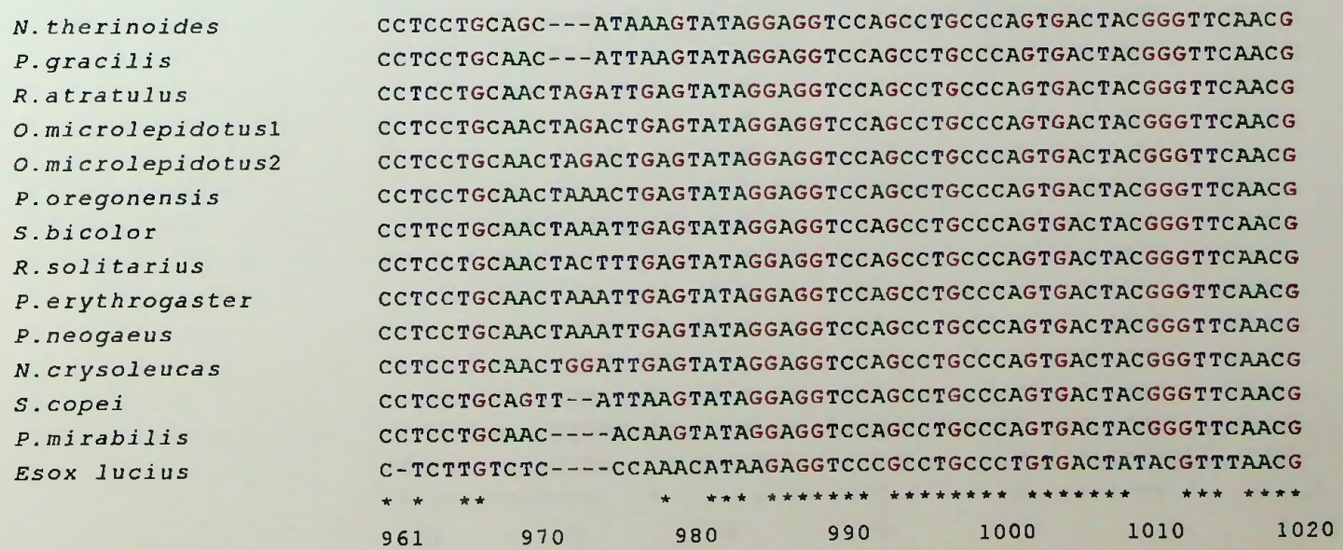
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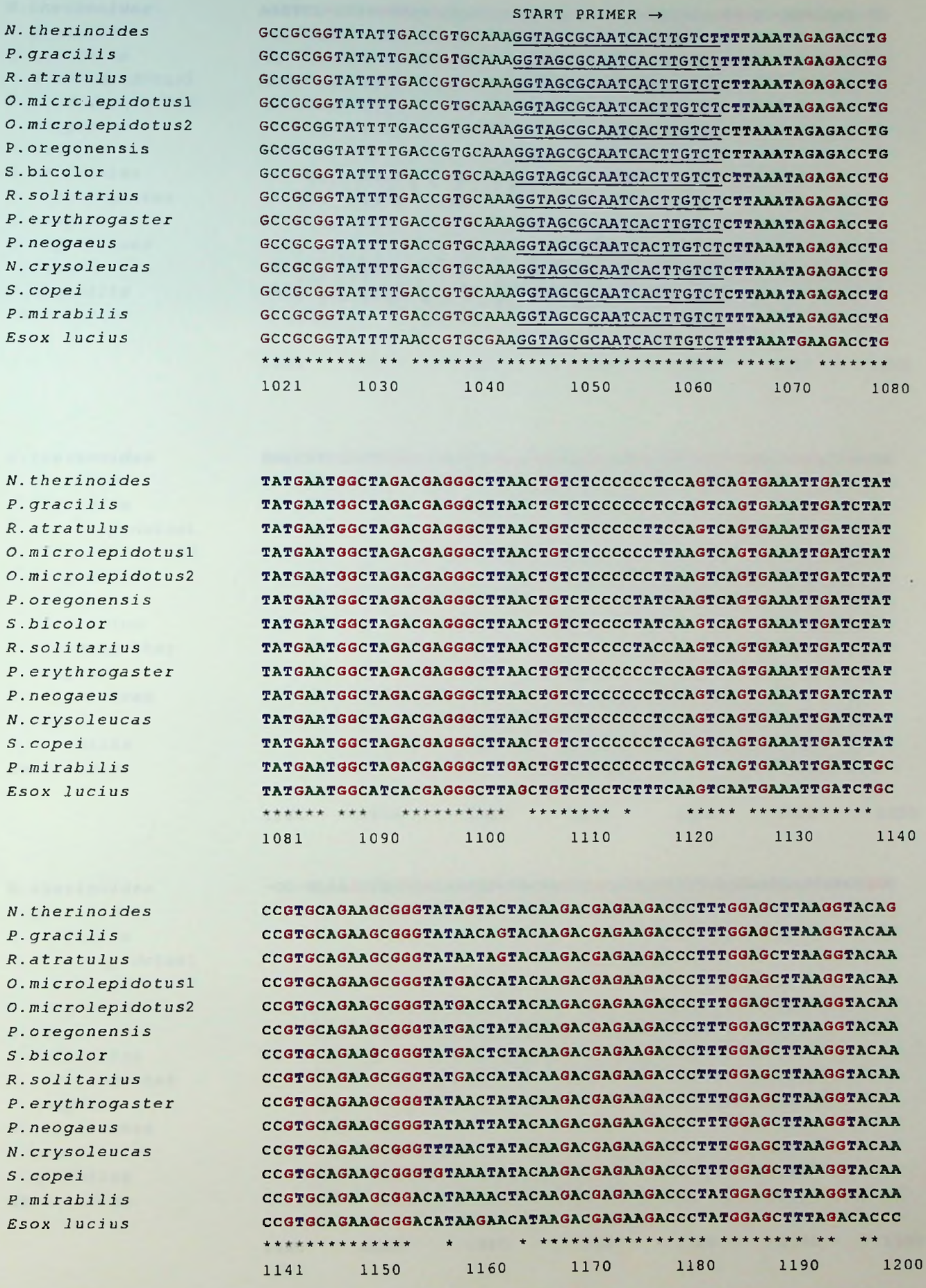
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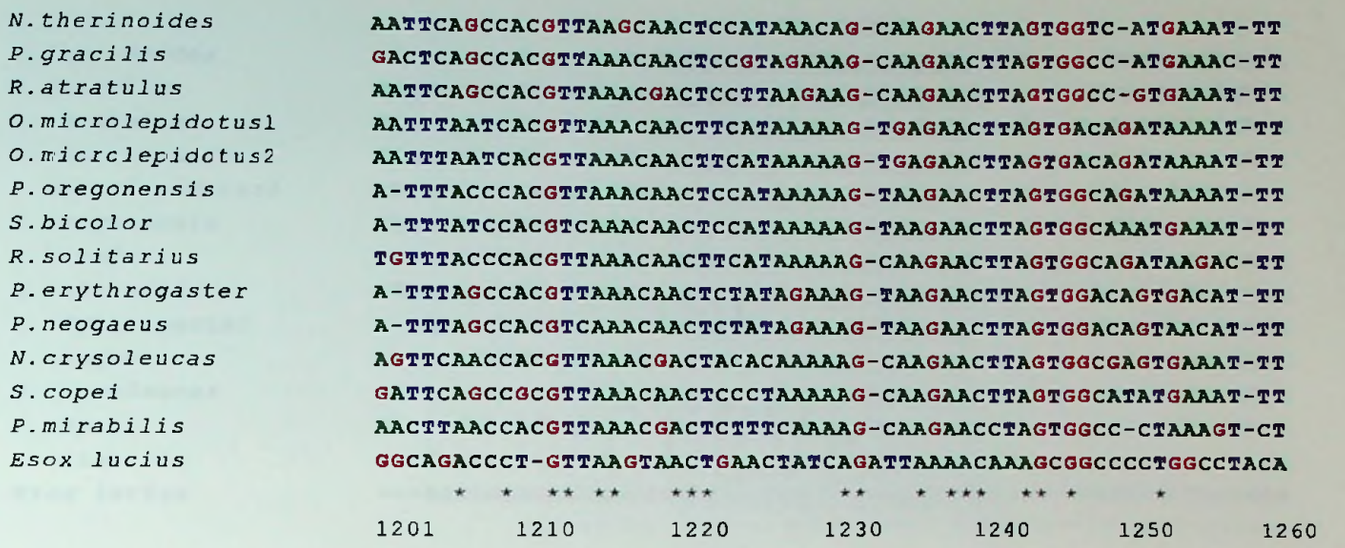
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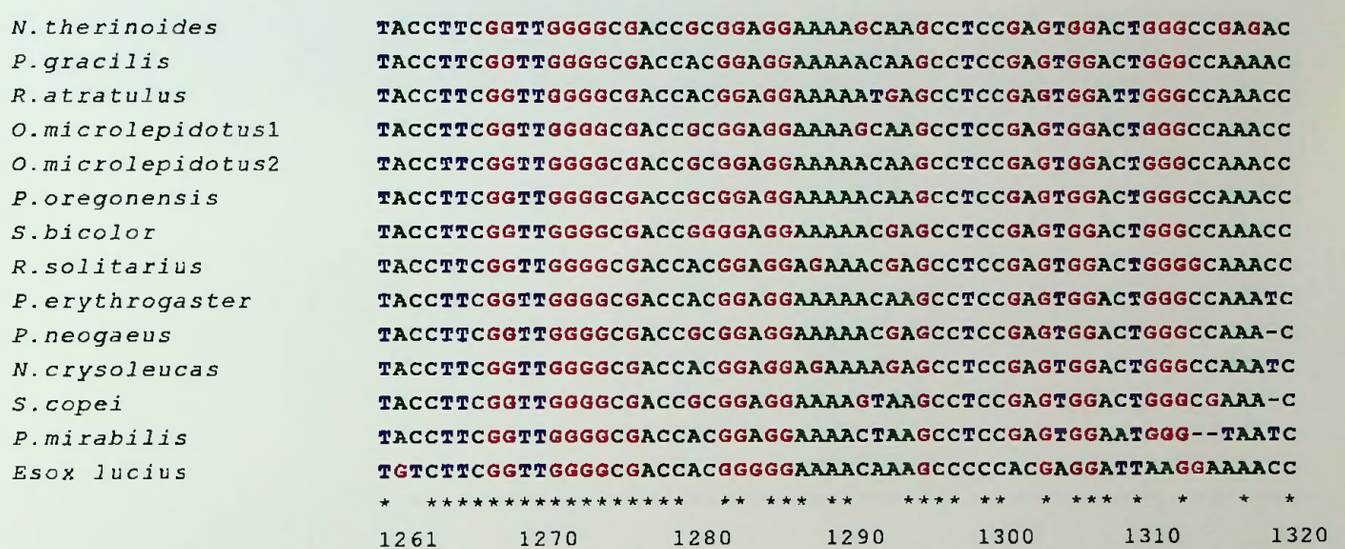
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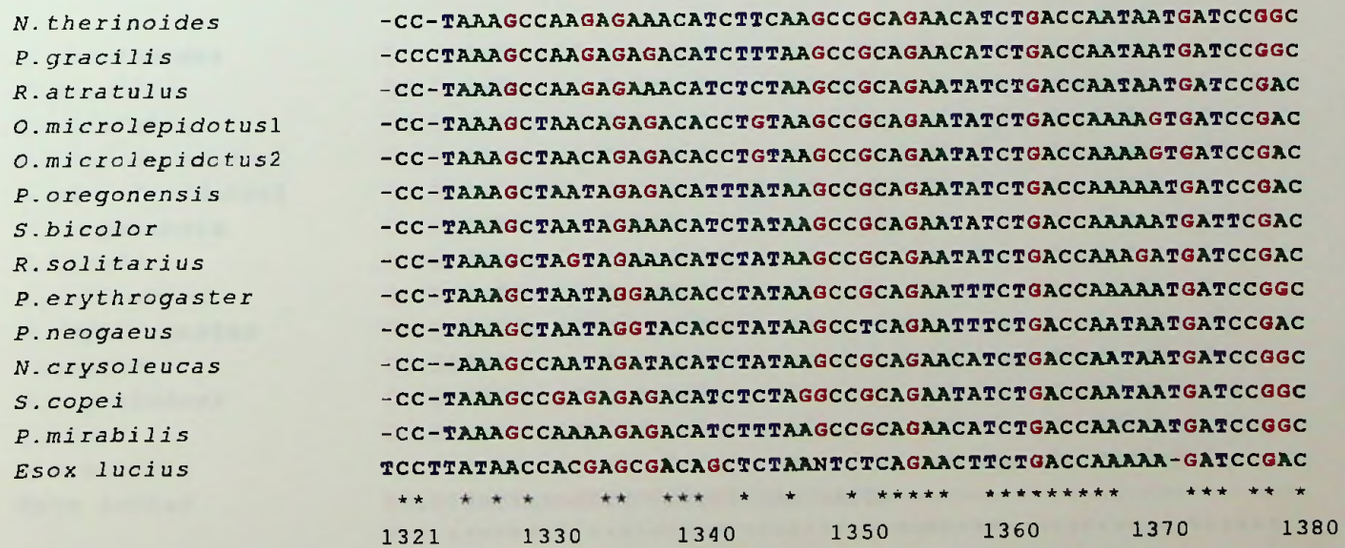
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R.atratulus -CC-TAAAGCCAAGAGAAACATCTCTAAGCCGCAGAATATCTGACCAATAATGATCCGAC
O.microlepidotus1 -CC-TAAAGCTAACAGAGACACCTGTAAGCCGCAGAATATCTGACCAAAAGTGATCCGAC
O.microlepidotus2 -CC-TAAAGCTAACAGAGACACCTGTAAGCCGCAGAATATCTGACCAAAAGTGATCCGAC
P.oregonensis -CC-TAAAGCTAATAGAGACATTTATAAGCCGCAGAATATCTGACCAAAAAATGATCCGAC
S.bicolor -CC-TAAAGCTAATAGAAACATCTATAAGCCGCAGAATATCTGACCAAAAATGATTCGAC
R.solitarius -CC-TAAAGCTAGTAGAAACATCTATAAGCCGCAGAATATCTGACCAAAGATGATCCGAC
P.erythrogaster -CC-TAAAGCTAATAGGAACACCTATAAGCCGCAGAATTTCTGACCAAAAATGATCCGGC
P.neogaeus -CC-TAAAGCTAATAGGTACACCTATAAGCCTCAGAATTTCTGACCAATAATGATCCGAC
N.crysoleucas -CC--AAAGCCAATAGATACATCTATAAGCCGCAGAACATCTGACCAATAATGATCCGGC
S.copei -CC-TAAAGCCGAGAGAGACATCTCTAGGCCGCAGAATATCTGACCAATAATGATCCGGC
P.mirabilis -CC-TAAAGCCAAAAGAGACATCTTTAAGCCGCAGAACATCTGACCAACAATGATCCGGC
Esox lucius TCCTTATAACCACGAGCGACAGCTCTAANTCTCAGAACTTCTGACCAAAAA-GATCCGAC
1321 1330 1340 1350 1360 1370 1380

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P.gracilis             TAATAAAGCCGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCTCTCCCAGA
R.atratulus            TAA-AAAGTCGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCTCTCCCAGA
O.microlepidotus1      CACAAGAGTCGATCAACGGACCAAGTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGA
O.microlepidotus2      CACAAGAGTCGATCAACGGACCAAGTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGA
P.oregonensis          CACAGTGATCGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGA
S.bicolor              CACAGGGGTCGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGA
R.solitarius           CTTAGCAGTCGATCAACGGACCAAGTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGA
P.erythrogaster        ---AAAAGTCGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGA
P.neogaeus             ---AGAAGTCGATCAACGGACCAAGTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGA
N.crysoleucas          -TGAAAAGGC-ATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCTCTCCCAGA
S.copei                C--GGAAGCCGATCAACGGACCAAGTTACCCTAGGGATAACAGCGCAATCCTCTCCCAGA
P.mirabilis            T-CTAGGGCCGATCAACGAACCAAGTTACCCTAGGGATAACAGCGCAATCCTCTCCCAGA
Esox lucius            ---ACCAGTCGATCAACGGACCAAGTTACCCTAGGGATAACAGCGCAATCCCCTCCCAGA
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N.therinoides          GCCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTGGTGGTGCA
P.gracilis             GCCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTGGTGGTGCA
R.atratulus            GCCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
O.microlepidotus1      GTCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
O.microlepidotus2      GTCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
P.oregonensis          GTCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
S.bicolor              GTCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
R.solitarius           GTCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
P.erythrogaster        GGCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
P.neogaeus             GGCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
N.crysoleucas          GTCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
S.copei                GCCCGTATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
P.mirabilis            GCCCATATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
Esox lucius            GTCCCTATCGACGAGGGGGTTTACGACCTCGATGTTGGATCAGGACATCCTAATGGTGCA
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R.atratulus            GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
O.microlepidotus1      GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
O.microlepidotus2      GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
P.oregonensis          GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
S.bicolor              GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
R.solitarius           GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
P.erythrogaster        GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
P.neogaeus             GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
N.crysoleucas          GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
S.copei                GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
P.mirabilis            GCCGCTATTAAGGGTTCGTTTGTTCAACGATTAAAGTCCTACGTGATCTGAGTTCAGACC
Esox lucius            GCCGCTATTAAGGGTTCGTTTGTTCAACGATTA--------------------------
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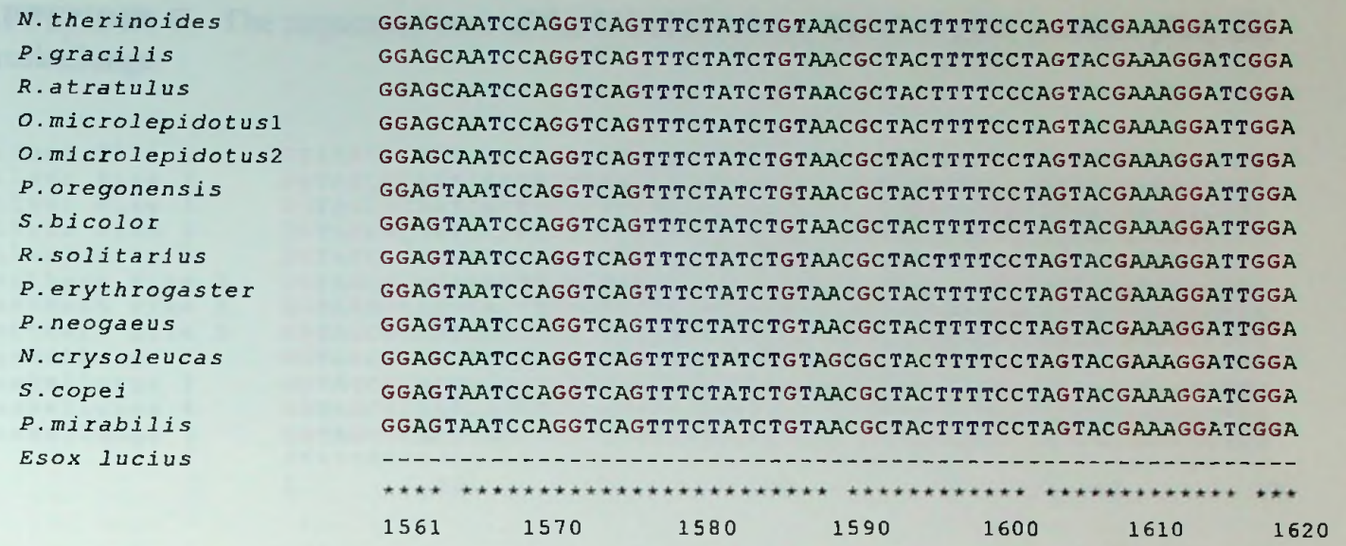
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P.gracilis GGAGCAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATCGGA
R.atratulus GGAGCAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCCAGTACGAAAGGATCGGA
O.microlepidotus1 GGAGCAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATTGGA
O.microlepidotus2 GGAGCAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATTGGA
P.oregonensis GGAGTAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATTGGA
S.bicolor GGAGTAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATTGGA
R.solitarius GGAGTAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATTGGA
P.erythrogaster GGAGTAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATTGGA
P.neogaeus GGAGTAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATTGGA
N.crysoleucas GGAGCAATCCAGGTCAGTTTCTATCTGTAGCGCTACTTTTCCTAGTACGAAAGGATCGGA
S.copei GGAGTAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATCGGA
P.mirabilis GGAGTAATCCAGGTCAGTTTCTATCTGTAACGCTACTTTTCCTAGTACGAAAGGATCGGA
Esox lucius ----------
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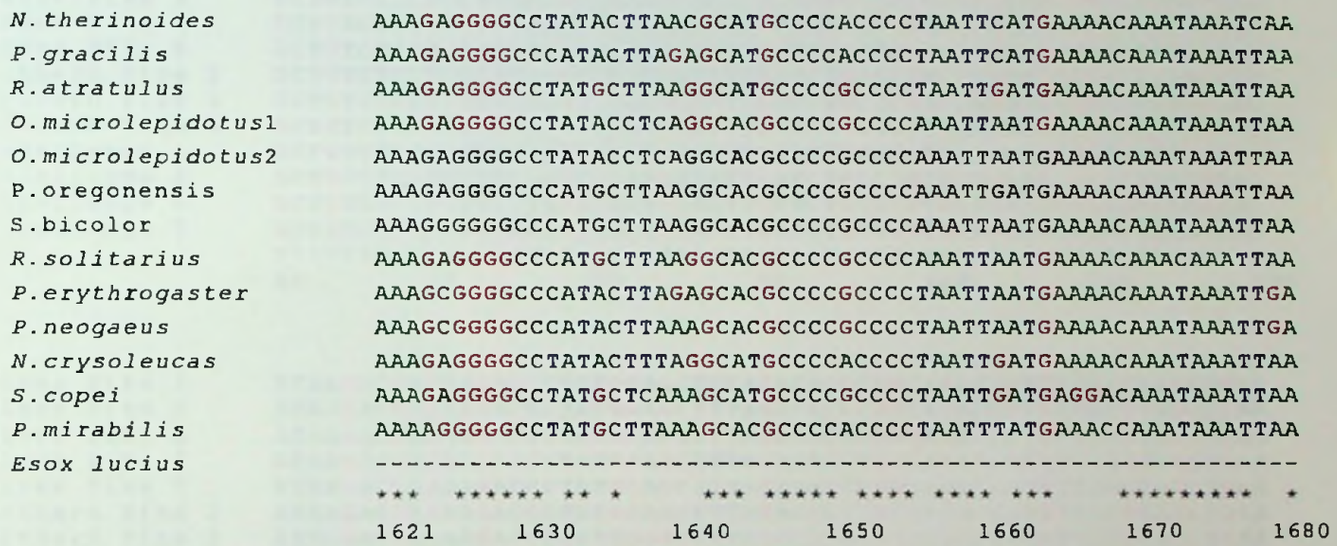
1561 1570 1580 1590 1600 1610 1620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P.gracilis AAAGAGGGGCCCATACTTAGAGCATGCCCCACCCCTAATTCATGAAAACAAATAAATTAA
R.atratulus AAAGAGGGGCCTATGCTTAAGGCATGCCCCGCCCCTAATTGATGAAAACAAATAAATTAA
O.microlepidotus1 AAAGAGGGGCCTATACCTCAGGCACGCCCCGCCCCAAATTAATGAAAACAAATAAATTAA
O.microlepidotus2 AAAGAGGGGCCTATACCTCAGGCACGCCCCGCCCCAAATTAATGAAAACAAATAAATTAA
P.oregonensis AAAGAGGGGCCCATGCTTAAGGCACGCCCCGCCCCAAATTGATGAAAACAAATAAATTAA
S.bicolor AAAGGGGGGCCCATGCTTAAGGCACGCCCCGCCCCAAATTAATGAAAACAAATAAATTAA
R.solitarius AAAGAGGGGCCCATGCTTAAGGCACGCCCCGCCCCAAATTAATGAAAACAAACAAATTAA
P.erythrogaster AAAGCGGGGCCCATACTTAGAGCACGCCCCGCCCCTAATTAATGAAAACAAATAAATTGA
P.neogaeus AAAGCGGGGCCCATACTTAAAGCACGCCCCGCCCCTAATTAATGAAAACAAATAAATTGA
N.crysoleucas AAAGAGGGGCCTATACTTTAGGCATGCCCCACCCCTAATTGATGAAAACAAATAAATTAA
S.copei AAAGAGGGGCCTATGCTCAAAGCATGCCCCGCCCCTAATTGATGAGGACAAATAAATTAA
P.mirabilis AAAAGGGGGCCTATGCTTAAAGCACGCCCCACCCCTAATTTATGAAACCAAATAAATTAA
Esox lucius ----------
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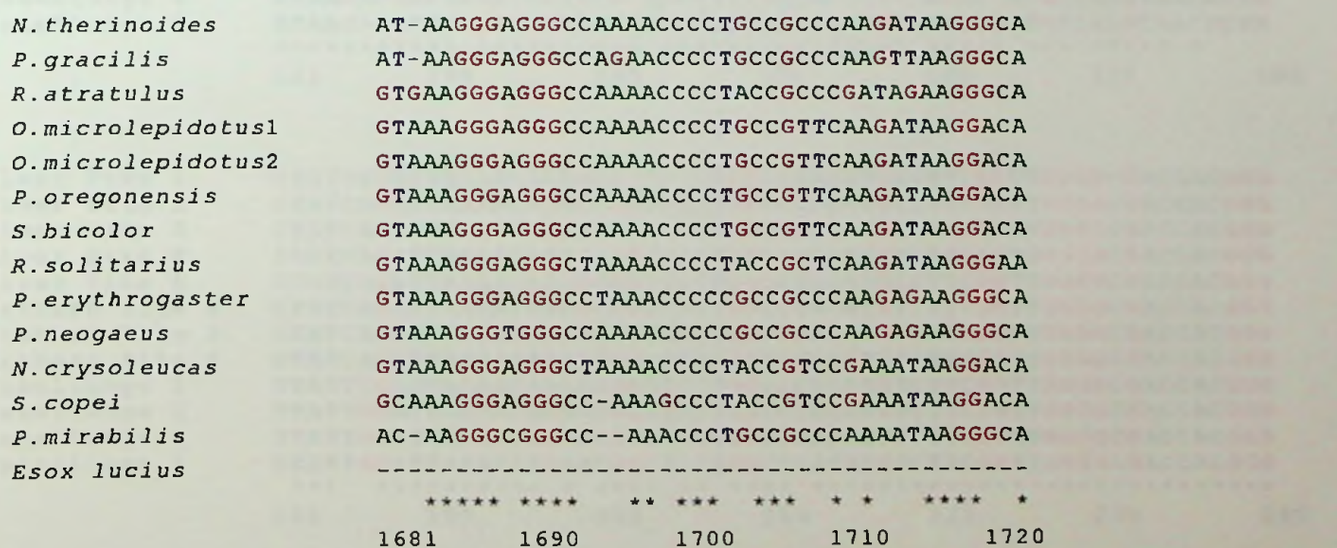
1621 1630 1640 1650 1660 1670 1680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P.gracilis AT-AAGGGAGGGCCAGAACCCCTGCCGCCCAAGTTAAGGGCA
R.atratulus GTGAAGGGAGGGCCAAAACCCCTACCGCCCGATAGAAGGGCA
O.microlepidotus1 GTAAAGGGAGGGCCAAAACCCCTGCCGTTCAAGATAAGGACA
O.microlepidotus2 GTAAAGGGAGGGCCAAAACCCCTGCCGTTCAAGATAAGGACA
P.oregonensis GTAAAGGGAGGGCCAAAACCCCTGCCGTTCAAGATAAGGACA
S.bicolor GTAAAGGGAGGGCCAAAACCCCTGCCGTTCAAGATAAGGACA
R.solitarius GTAAAGGGAGGGCTAAAACCCCTACCGCTCAAGATAAGGGAA
P.erythrogaster GTAAAGGGAGGGCCTAAACCCCCGCCGCCCAAGAGAAGGGCA
P.neogaeus GTAAAGGGTGGGCCAAAACCCCCGCCGCCCAAGAGAAGGGCA
N.crysoleucas GTAAAGGGAGGGCTAAAACCCCTACCGTCCGAAATAAGGACA
S.copei GCAAAGGGAGGGCC-AAAGCCCTACCGTCCGAAATAAGGACA
P.mirabilis AC-AAGGGCGGGCC--AAACCCTGCCGCCCAAAATAAGGGCA
Esox lucius ----------
***** **** ** *** *** * * **** *
1681 1690 1700 1710 1720

APPENDIX C: The sequenced area of the 16S rDNA from the silver pike, northern pike, and muskellunge.

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Silver Pike 1       GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Silver Pike 2       GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAANGGCATCACGAGGGCTTA
Silver Pike 3       GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Silver Pike 4       GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Silver Pike 5       GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Northern Pike 2     GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Northern Pike 3     GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Northern Pike 5     GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Muskellunge 1       GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Muskellunge 2       GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Muskellunge 4       GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
Muskellunge 5       GGTAGCGCAATCACTTGTCTTTTAAATGAAGACCTGTATGAATGGCATCACGAGGGCTTA
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                    1         10         20         30         40         50         60

Silver Pike 1       GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Silver Pike 2       GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Silver Pike 3       GCTGTCTCCTCTNTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Silver Pike 4       GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGANC
Silver Pike 5       GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Northern Pike 2     GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Northern Pike 3     GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Northern Pike 5     GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Muskellunge 1       GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Muskellunge 2       GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Muskellunge 4       GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
Muskellunge 5       GCTGTCTCCTCTTTCAAGTCAATGAAATTGATCTGCCCGTGCAGAAGCGGACATAAGAAC
                    ************ ************************************************ *
                    61         70         80         90         100        110        120

Silver Pike 1       ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCCTGTTAAGTAGCTGAA
Silver Pike 2       ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCCTGTTAAGTAGCTGAA
Silver Pike 3       ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGGAGACCCTGTTAAGTAGCTGAA
Silver Pike 4       ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCCTGTTAAGTAGCTGAA
Silver Pike 5       ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCCTGTTAAGTAGCTGAA
Northern Pike 2     ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCCTGTTAAGTAGCTGAA
Northern Pike 3     ATAAGACGAGAAGACCCTATGGGGCTTTAGACACCCGGCAGACCCTGTTAAGTAGCTGAA
Northern Pike 5     ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCCTGTTAAGTAGCTGAA
Muskellunge 1       ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCATGTCAAGTAACCCTG
Muskellunge 2       ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCATGTCAAGTAACCCTG
Muskellunge 4       ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCATGTCAAGTAACCCTG
Muskellunge 5       ATAAGACGAGAAGACCCTATGGAGCTTTAGACACCCGGCAGACCATGTCAAGTAACCCTG
                    ********************** ********************* ***** *** ***** *
                    121        130        140        150        160        170        180

Silver Pike 1       CTATCAGATTAAAACAAAGCGGCCCCTGGCCTACATGTCTTCGGTTGGGGCGACCACGGG
Silver Pike 2       CTATCAGATTAAAACAAAGCGGCCCCTGGCCTACATGTCTTCGGTTGGGGCGACCACGGG
Silver Pike 3       CTATCAGATTAAAACAAAGCGGCCCCTGGCCTACATGTCTTCGGTTGGGGCGACCACGGG
Silver Pike 4       CTATCAGATTAAAACAAAGCGGCCCCTGGCCTACATGTCTTCGGTTGGGGCGACCACGGG
Silver Pike 5       CTATCAGATTAAAACAAAGCGGCCCCTGGCCTACATGTCTTCGGTTGGGGCGACCACGGG
Northern Pike 2     CTATCAGATTAAAACAAAGCGGCCCCTGGCCTACATGTCTTCGGTTGGGGCGACCACGGG
Northern Pike 3     CTATCAGATTAAAACAAAGCGGCCCCTGGCCTACATGTCTTCGGTTGGGGCGACCACGGG
Northern Pike 5     CTATCAGATTAAAACAAAGCGGCCCCCGGCCTACATGTCTTCGGTTGGGGCGACCACGGG
Muskellunge 1       TTATTGGATTAAAACAAAACGGCTCCTGGCCCACATGTCTTCGGTTGGGGCGACCACGGG
Muskellunge 2       TTATTGGATTAAAACAAAACGGCTCCTGGCCCACATGTCTTCGGTTGGGGCGACCACGGG
Muskellunge 4       TTATTGGATTAAAACAAAACGGCTCCTGGCCCACATGTCTTCGGTTGGGGCGACCACGGG
Muskellunge 5       TTATTGGATTAAAACAGAACGGCTCCTGGCCCACATGTCTTCGGTTGGGGCGACCACGGG
                     ***  ********** * **** ** **** ****************************
                    181        190        200        210        220        230        240
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Silver Pike 2      GGAAAACAAAGCCCCCACGAGGATTAAGGAAAACCTCCTTATAACCACGAGCGACAGCTC
Silver Pike 3      GGAAAACAAAGCCCCCACGAGGATTAAGGAAAACCTCCTTATAACCACGAGCGACAGCTC
Silver Pike 4      GGAAAACAAAGCCCCCACGAGGATTAAGGAAAACCTCCTTATAACCACGAGCGACAGCTC
Silver Pike 5      GGAAAACAAAGCCCCCACGAGGATTAAGGAAAACCTCCTTATAACCACGAGCGACAGCTC
Northern Pike 2    GGAAAACAAAGCCCCCACGAGGATTAAGGAAAACCTCCTTATAACCACGAGCGACAGCTC
Northern Pike 3    GGAAAACAAAGCCCCCACGAGGATTAAGGAAAACCTCCTTATAACCACGAGCGACAGCTC
Northern Pike 5    GGAAAACAAAGCCCCCACGAGGATTAAGGAAAACCTCCTTATAACCACGAGCGACAGCTC
Muskellunge 1      GGAAAATATAGCCCCCATGCGGACTAAGGGTAACACCCTTATAACCATGAACTACAGCTC
Muskellunge 2      GGAAAATATAGCCCCCATGCGGACTAAGGGTAACACCCTTATAACCATGAACTACAGCTC
Muskellunge 4      GGAAAATATAGCCCCCATGCGGACTAAGGGTAACACCCTTATAACCATGAACTACAGCTC
Muskellunge 5      GGAAAATATAGCCCCCATGCGGACTAAGGGTAACACCCTTATAACCATGAACTACAGCTC
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Silver Pike 1      TAAGTCTCAGAACTTCTGACCAAAAAGATCCGACACCAGTCGATCAACGGACCAAGTTAC
Silver Pike 2      TAAGTCTCAGAACTTCTGACCAAAAAGATCCGACACCAGTCGATCAACGGACCAAGTTAC
Silver Pike 3      TAAGTCTCAGAACTTCTGACCAAAAAGATCCGACACCAGTCGATCAACGGACCAAGTTAC
Silver Pike 4      TAAGTCTCAGAACTTCTGACCAAAAAGATCCGACACCAGTCGATCAACGGACCAAGTTAC
Silver Pike 5      TAAGTCTCAGAACTTCTGACCAAAAAGATCCGACACCAGTCGATCAACGGACCAAGTTAC
Northern Pike 2    TAAGTCTCAGAACTTCTGACCAAAAAGATCCGACACCAGTCGATCAACGGACCAAGTTAC
Northern Pike 3    TAAGTCTCAGAACTTCTGACCAAAAAGGATCCGACACCAGTCGATCAACGGACCAAGTTAC
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Muskellunge 1      TAAGTCTCAGAAATTCTGACCAAAAAGATCCGACACCCGTCGATCAACGGACCAAGTTAC
Muskellunge 2      TAAGTCTCAGAAATTCTGACCAAAAAGATCCGACACCCGTCGATCAACGGACCAAGTTAC
Muskellunge 4      TAAGTCTCAGAAATTCTGACCAAAAAGATCCGACACCCGTCGATCAACGGACCAAGTTAC
Muskellunge 5      TAAGTCTCAGAAATTCTGACCAAAAAGATCCGACACCCGTCGATCAACGGACCAAGTTAC
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Silver Pike 1      CCTAGGGATA
Silver Pike 2      CCTAGGGATA
Silver Pike 3      CCTAGGGATA
Silver Pike 4      CCTAGGGATA
Silver Pike 5      CCTAGGGATA
Northern Pike 2    CCTAGGGATA
Northern Pike 3    CCTAGGGATA
Northern Pike 5    CCTAGGGATA
Muskellunge 1      CCTAGGGATA
Muskellunge 2      CCTAGGGATA
Muskellunge 4      CCTAGGGATA
Muskellunge 5      CCTAGGGATA
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                   361       370
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