

Molecular Phylogeny of Acipenseridae: Nonmonophyly of Scaphirhynchinae

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A phylogenetic hypothesis is presented illustrating relationships among 23 extant sturgeon species based on nucleotide data from five mitochondrial genes (cytochrome *b*, NADH5, control region, 16S, and 12S). The tree supports our previous hypotheses regarding relationships among species of *Acipenser* and *Huso*. The introduction of *Scaphirhynchus* and *Pseudoscaphirhynchus* to the analysis does not change these main relationships. The genera *Scaphirhynchus* and *Pseudoscaphirhynchus* do not form a monophyletic group corresponding to the subfamily Scaphirhynchinae or the tribe Scaphirhynchini. The genus *Scaphirhynchus* may be basal within the family Acipenseridae, whereas *Pseudoscaphirhynchus* appears to be embedded within *Acipenser* clustering with the stellate sturgeon, *Acipenser stellatus*. The morphological similarity between *Scaphirhynchus* and *Pseudoscaphirhynchus* could be a result of convergent evolution of different ancestral sturgeon forms in similar environmental conditions of distant river systems of North America and Central Asia.

ACCORDING to traditional classification, the Order Acipenseriformes (sturgeons and paddlefishes) consists of two families, the Acipenseridae, with genera *Acipenser*, *Huso*, *Scaphirhynchus*, and *Pseudoscaphirhynchus*, and the Polyodontidae, represented by two extant species, *Polyodon spathula* and *Psephurus gladius* (Berg, 1904, 1940). Recent studies confirm that the two families are monophyletic, sister taxa (Grande and Bemis, 1991, 1996; Bemis et al., 1997; but see Gardiner, 1984). Two subfamilies are further recognized within Acipenseridae: the Acipenserinae consisting of *Acipenser* and *Huso* and the Scaphirhynchinae, which includes *Scaphirhynchus* and *Pseudoscaphirhynchus* (Berg, 1911, 1940). Currently, the Scaphirhynchinae is regarded as monophyletic (Mayden and Kuhajda, 1996) with some authors suggesting the group is more appropriately recognized as the tribe Scaphirhynchini within the subfamily Acipenserinae (Grande and Bemis, 1996; Bemis et al., 1997; Findeis, 1997). The Scaphirhynchinae is generally believed to be the sister group of all other sturgeons (Berg, 1911; Mayden and Kuhajda, 1996) and the most basal lineage within the Acipenseridae (Bogdanov, 1882:106–110; Zograf, 1887; Schmalhausen, 1991). Other authors, however, suggest the group may be derived (Kessler, 1877; Berg, 1905; Findeis, 1993, 1997).

Two species of *Scaphirhynchus* and three species of *Pseudoscaphirhynchus* were originally described in the 19th century, with the two genera placed in the separate subfamily Scaphirhynchinae at the turn of the 20th century. The first species of Scaphirhynchinae was described by C. Rafinesque in his monograph on fishes of the

Ohio River (Rafinesque, 1820). This “shovelfish (currently shovelnose) sturgeon *Accipenser platorynchus*” was later placed in a new separate genus *Scaphirhynchus* as *Scaphirhynchus platorynchus* (Heckel, 1835). The pallid sturgeon, *Parascaphirhynchus albus*, was subsequently described from the same river system (Forbes and Richardson, 1905) and eventually recognized as *Scaphirhynchus albus* (Bailey and Cross, 1954). The third species, the Alabama sturgeon *Scaphirhynchus suttkusi*, was discovered in the Mobile River system and described much later (Williams and Clemmer, 1991).

The first description of a species of *Pseudoscaphirhynchus*, *Scaphirhynchus fedtschenkoi*, was based on a specimen caught in 1871 in the Syr Darya River in Central Asia (former Turkestan, currently Kazakhstan; Kessler, 1872). This specimen was originally defined as a member of the genus *Scaphirhynchus* because of its morphological similarity (e.g. shovel-shaped head and long caudal rod) to published drawings of *S. platorynchus* (Kessler, 1872, 1873). *Scaphirhynchus hermanni* and *Scaphirhynchus kaufmanni* were later described based on specimens caught in the Amu Darya River in Central Asia (former Turkestan, currently Uzbekistan and Turkmenistan; Bogdanov, 1874; Kessler, 1877). The three species were elevated to a separate genus *Pseudoscaphirhynchus* by Nikolsky (1900). The confirmation of the species of *Pseudoscaphirhynchus* and the unification of the subfamily Scaphirhynchinae was later accomplished by the Russian ichthyologist Berg (1904, 1905, 1911).

In their review of the subfamily Scaphirhynchinae, Bailey and Cross (1954:172) wrote “. . . we were uncertain whether the two genera

TABLE 1. PRIMERS AND CONDITIONS USED FOR PCR AMPLIFICATION OF GENE REGIONS. Forward primers are given first followed by reverse primers. All sequences are listed in 5' to 3' direction.

| Gene region ^a | Primer sequence | Primer name/reference | |
|-----------------------------|-----------------------------|--------------------------------|---------------------------|
| 18S ^b | TACCTGGTTGATCCTGCCAGTAG | 1F/Giribet et al., 1996 | |
| | AGGGTCCCTCTCCGGAATCGAAC | 3R/Giribet et al., 1996 | |
| | CCAGCAGCCGCGCTAATTC | 4F/Giribet et al., 1996 | |
| | CTTGGCAAATGCTTTTCGC | 5R/Giribet et al., 1996 | |
| | GCAATAACAGGTCTGTGATGCC | 7F/Giribet et al., 1996 | |
| | GATCCTTCCGCGAGTTACACCTAC | 9R/Giribet et al., 1996 | |
| | 12S | GGTGGCATTATTTATTATTAGAGG | 12Sa/Kocher et al., 1989 |
| | | CCGGTCTGAACTCAGATCACGT | 12Sb/Hedges et al., 1993 |
| | 16S | CGCCTGTTTACCAAAACAT | 16Sa/Palumbi et al., 1991 |
| CCGGTCTGAACTCAGATCACGT | | 16Sb/Palumbi et al., 1991 | |
| Control region ^c | TAACTGCAGAAGGCTAGGACCAAACCT | mod H00651/Kocher et al., 1989 | |
| | GCACCCAAAGCTGARRTTCTA | dIp 1.5/Baker et al., 1993 | |
| | CAGTCTGATTTTGGGGTTTGAC | HF400/P. Doukakis, unpubl. | |
| | TGTCAAACCCCAAAGCAGG | HB400/P. Doukakis, unpubl. | |
| | TTCTYGGCATGTGGGGWCAT | HF100/P. Doukakis, unpubl. | |
| NADH5 ^d | AATAGTTTATCCAGTTGGTCTTAG | ND5/Bembo et al., 1995 | |
| | TAACAACGGTGGTTTTCAAGTCA | ND5/6a/Nielsen et al., 1998 | |
| | AAGCCCATGAGTGGTAGG | RND5-3/Doukakis et al., 1999 | |
| Cyt <i>b</i> | CCATCCAACATCTCTGCTTGATGAAA | B1/J. Groth, pers. comm. | |
| | CCTCCAATTCATGTGAGTACT | S2A/Birstein et al., 1997 | |

^a Conditions for PCR amplification were 18S: 94° 0:15, 55° 0:15, 72° 0:15, 30 cycles; 12S and 16S: 94° 1:00, 55° 1:00, 72° 1:30, 33 cycles; control region and cyt *b*: 94° 1:00, 46° 1:00, 72° 1:50, 33 cycles; NADH5: 94° 1:00, 48° 1:00, 72° 1:30, 33 cycles.

^b 18S primers are listed sequentially as pairs for amplification purposes with forward (F) and reverse (R) primers.

^c The modified H00651 and dIp 1.5 were used to amplify the entire control region. HF400, HB400, HF100 are internal sequencing primers.

^d ND5 and ND5/6a are forward primers while RND5-3 is the reverse primer.

of 'shovelnose' sturgeons (*Scaphirhynchus* and *Pseudoscaphirhynchus*) owed their close resemblance to common ancestry or to convergence. . . . It is premature to deny categorically that the striking agreement in several features may be due to parallel modifications in animals that inhabit the channels of large silty rivers." The Mississippi-Missouri and Amu Darya/Syr Darya rivers inhabited by *Scaphirhynchus* and *Pseudoscaphirhynchus*, respectively, have strong currents and extremely muddy and silty water (Nikolsky, 1938; Hurley et al., 1987; Burke and Ramsey, 1995). These conditions differ considerably from the clear waters inhabited by *Acipenser* and *Huso* (Bemis and Kynard, 1997). Our preliminary molecular studies including exemplars from the two subfamilies found *Scaphirhynchinae* to be paraphyletic, with *S. albus* occupying a basal position sister to *P. kaufmanni*, which was sister to *Acipenser* plus *Huso* (Birstein et al., 1997). Further molecular studies examining all extant species of *Acipenser* used only *S. albus* as a representative of the *Scaphirhynchinae* and did not consider any species of *Pseudoscaphirhynchus* (Birstein and DeSalle, 1998). Here, we use an additional DNA sequence analysis and further taxon sampling to address the monophyly of the *Scaphirhynchinae* and *Acipenserinae* subfamilies and provide clarification

for species designations and relationships within the *Acipenseriformes*. We further assess our results in light of available morphological character information and historic biogeography. We studied only two of three species of *Pseudoscaphirhynchus*: currently, small populations of *Pseudoscaphirhynchus hermanni* and *Pseudoscaphirhynchus kaufmanni* still survive in the Turkmenian part of the Amu Darya River (Salnikov et al., 1996), whereas *P. fedtschenkoi* is practically extinct (Birstein, 1997).

MATERIALS AND METHODS

DNA extraction and sequencing.—DNA was extracted from all tissue specimens (see Materials Examined) using standard phenol chloroform techniques (see DeSalle et al., 1993) after overnight Proteinase K incubation. Generally, only one individual per species was considered. Five mitochondrial gene regions [12S, 16S, NADH5, control region, cytochrome *b* (cyt *b*)] and several regions within the nuclear 18S region were examined using the primers and conditions listed in Table 1. Low variability was found for 18S fragments between representative sequences of *A. baerii*, *S. albus*, *P. kaufmanni*, and *Polyodon spatulata*; hence, this region was not sequenced for all taxa nor included in the analysis. Sequenced

regions for all taxa consisted of 337 bp 12S, 523 bp 16S, 643 bp NADH5, 846 bp control region, and 526 bp *cyt b*. The *cyt b* gene sequences consisted of the three noncontiguous fragments as detailed in Birstein and DeSalle (1998). In some cases, sequences available through GenBank were used for the *cyt b* gene region (see Appendix 1).

All PCR amplification was conducted in a Perkin-Elmer 480 thermocycler (PE Biosystems, Foster City, CA; except for 18S amplifications using a PE 9600). PCR products were purified with the BIO 101 Gene Clean system (BIO 101, Inc., La Jolla, CA) before use in sequencing reactions. Automated sequencing employed fluorescent FS or BigDye sequencing chemistry and either an ABI 373 or 377 automated sequencer (PE Biosystems, Foster City, CA). Sequences were transferred to Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, MI) for initial trimming and editing. All sequences were deposited in GenBank under the accession numbers listed in Appendix 1. To verify our sequencing results on *P. kaufmanni*, we sent tissue samples to A. Ludwig (Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany). He independently sequenced the *cyt b* gene and control region and confirmed our results for this species.

Analysis.—No alignment program was needed for the protein coding *cyt b* and NADH 5 gene regions. Both gene regions were translated into amino acid code to ensure no stop codons were present. Alignment of the 12S and 16S regions was trivial with few indels and therefore objectively accomplished by eye. Although an 846 bp fragment of the control region was amplified, alignment was performed after exclusion of the heteroplasmic region (see Buroker et al., 1990). Alignment was accomplished using MALIGN Version 2.7 (Wheeler and Gladstein, 1994) employing gap:change costs of 2, 4, 6, 8, and 10 under the “build” algorithm. The five alignment matrices were then compared and alignment ambiguous regions were culled (as in Gatesy et al., 1993). This ultimately reduced the control region matrix to the 410 bp fragments.

All tree-building analyses were conducted using PAUP (vers. 4.0b2 D. L. Swofford, Sinauer Associates, Sunderland, MA, 1999, unpubl.) under the optimality criterion of maximum parsimony (MP) and maximum likelihood (ML) as a means of dataset exploration. We used *Polyodon spathula* and *Psephurus gladius* as outgroups. As all of the genes included are mitochondrial and, therefore, function as a single inherited linkage group, a combined evidence

approach was used in analyzing all gene regions simultaneously (Nixon and Carpenter, 1995). A combined evidence approach (molecules plus morphology) was also adopted for assessing the species relationships among the subset of species included in Mayden and Kuhajda (1996).

For the MP analyses, bootstrap support values were obtained using random searches and 1000 replicates. Gaps were coded both as characters and as missing in separate analyses. Bremer support values (Bremer, 1988, 1994) were calculated using AutoDecay (vers. 4.0, T. Eriksson, 1998, program distributed by the author, Department of Botany, Stockholm Univ. Press, Stockholm, Sweden). The Incongruence Length Difference (ILD; Mickevich and Farris, 1981; Farris et al., 1995) between data partitions was calculated by hand and tests of the ILD significance between major data partitions (i.e., ribosomal vs coding; coding vs noncoding; molecules vs morphology) used the Partition Homogeneity option in PAUP 4.0b2. These tree searches used a simple addition search to generate starting trees. To look at the contribution of molecular and morphological data to the total Bremer support value on the combined evidence tree, Partition Bremer Support (PBS) values were calculated using the methods outlined in Baker et al. (1998).

The use of ML required the specification of the explicit assumptions. The program ModelTest Version 2.0 (Posada and Crandall, 1988) was used to test the fit of several sequence evolution models on the given data using a likelihood ratio test framework. The ModelTest programs examined the fit of each model independently as well as with the addition of either a gamma distribution shape parameter (G), a proportion of invariable sites parameter (I) or both (G+I).

The difference between tree topologies was tested for significance using the Templeton (Templeton, 1983) and Kishino-Hasegawa (Kishino and Hasegawa, 1989) tests. These tests were used to compare the MP and ML to each other as well as to the shortest parsimony trees obtained under the constraint of Scaphirhynchinae monophyly.

RESULTS

After culling the alignment ambiguous sites of the control region, the total evidence data matrix consisted of 5 gene regions totaling 2439 bp for each of 25 taxa. The information content contained within the dataset under the parsimony criteria is shown in Table 2 and indicates the NADH5, control region, and *cyt b* were, overall, the most parsimony informative gene regions. Parsimony analysis using equal weight-

TABLE 2. INFORMATION CONTENT OF THE PRESENT DATASET UNDER THE PARSIMONY CRITERIA.

| Subset | # Chars | # Var (%) | # Inf (%) | # Trees | Steps |
|---------------------|---------|------------|------------|---------|-------|
| 16S | 527 | 95 (18.0) | 45 (8.5) | 55 | 157 |
| 12S | 337 | 12 (3.6) | 8 (2.4) | 12 | 17 |
| NADH5 | 643 | 211 (32.8) | 147 (22.9) | 50 | 376 |
| Cyt <i>b</i> | 526 | 169 (32.1) | 116 (22.1) | 8 | 389 |
| Control region | 410 | 231 (56.3) | 154 (37.6) | 3 | 550 |
| Morphology | 36 | 36 (100) | 32 (89.0) | 3 | 62 |
| Total (morph) | 2439 | 692 (28.4) | 423 (17.3) | 3 | 1337 |
| Total | 2439 | 718 (29.4) | 470 (19.3) | 9 | 1573 |
| Total (morph + mol) | 2475 | 728 (29.4) | 455 (18.4) | 1 | 1419 |

ing produced nine trees of the same topology regardless of the treatment of gaps (1573 steps, CI: 0.545, RI: 0.630). The strict consensus parsimony tree is shown in Figure 1A. Bootstrap values differed only slightly when gaps were coded as characters versus missing, whereas Bremer support values did not differ between the two coding schemes (Fig. 1A). Combined analysis of molecules and morphology produced a single tree (1419 steps, CI: 0.617, RI: 0.627) topologically similar to the MP tree but not to the strict consensus of the three trees obtained using only morphology (62 steps, CI: 0.855, RI: 0.909; Fig. 2). Significant incongruence was observed between all major data partitions (16S plus 12S vs NADH5 plus control region plus cyt *b*: $P = 0.001$; coding vs noncoding $P = 0.01$; molecules vs morphology $P = 0.001$). PBS values for the molecular and morphological comparison indicated conflict at only two of the 15 nodes, with some nodes only supported by morphology (no. 14) or molecules (nos. 1, 2, 6, 8, 10; Table 3).

ModelTest results indicated the HKY85 (Hasegawa et al., 1985) + gamma + invariant sites

approach ($ti/tv = 5.4073$, $p_{inv} = 0.5374$, $\gamma = 0.774$) was the most appropriate model for the dataset. The ML search using this model yielded a tree with score $-\ln L$ 10539.26 and a topology shown in Figure 1B. The ML and any of the nine MP topologies (ML: length 1586, CI 0.541, RI 0.623; MP: $-\ln L$ 10567.94, 1573 steps, CI: 0.545, RI: 0.630) were not significantly different under the Templeton test ($P = 0.01$) or KH test ($P = 0.01$). All of these trees were significantly different from the three trees obtained in searches constrained for Scaphirhynchinae monophyly ($-\ln L$ 10604.24, 1608 steps, CI: 0.534, RI: 0.611; $P < 0.001$).

DISCUSSION

Our new MP tree (Fig. 1A) supports the relationships between the species of *Acipenser* and

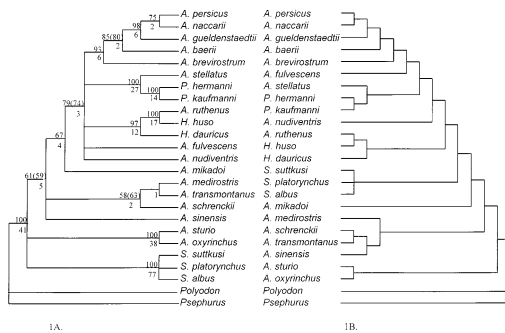


Fig. 1. Maximum parsimony strict consensus (A) and maximum likelihood (B) tree topologies. For A, bootstrap and Bremer support values are shown above and below the nodes, respectively. Bootstrap values that differed with the coding of gaps as characters are shown in parentheses.

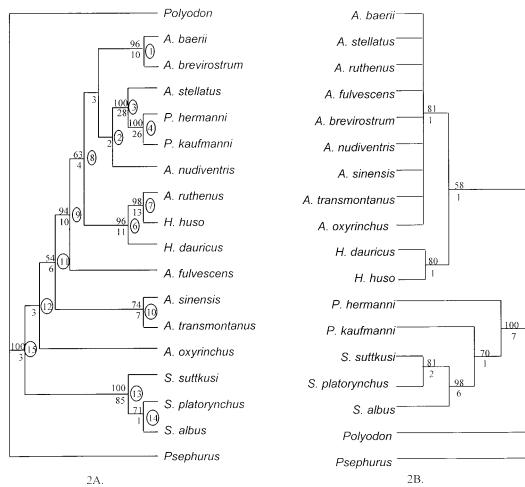


Fig. 2. Combined evidence (A) and morphology alone (B) maximum parsimony trees using data from Maiden and Kuhajda (1996). Bootstrap and Bremer support values are shown above and below the nodes, respectively. Numbers encircled refer to nodes referenced in Table 3.

TABLE 3. PARTITIONED BREMER SUPPORT FOR MOLECULAR AND MORPHOLOGICAL DATA. (*) indicates PBS conflict between the molecular and morphology data.

| Node | Molecules | Morphology | Total BS |
|------|-----------|------------|----------|
| 1 | 10 | 0 | 10 |
| 2 | 2 | 0 | 2 |
| 3 | 37.5 | -9.5 | 28* |
| 4 | 13 | 13 | 26 |
| 5 | 2.3 | 0.7 | 3 |
| 6 | 11 | 0 | 11 |
| 7 | 18 | -5 | 13* |
| 8 | 4 | 0 | 4 |
| 9 | 8 | 2 | 10 |
| 10 | 7 | 0 | 7 |
| 11 | 4 | 2 | 6 |
| 12 | 1 | 2 | 3 |
| 13 | 74 | 9 | 85 |
| 14 | 0 | 1 | 1 |
| 15 | 51 | 1 | 52 |

Huso discussed in Birstein and DeSalle (1998). The introduction of additional species of *Scaphirhynchus* and *Pseudoscaphirhynchus* does not change these basic relationships. Although the new MP and ML tree topologies are not significantly different (Fig. 1), they differ in their respective degrees of resolution and in the placement of certain taxa. Only the ML tree offers resolution for the placement of *A. fulvescens*, *A. nudiventris*, the *A. stellatus*/*P. kaufmanni*/*P. hermanni* clade and the *A. ruthenus*/*H. huso*/*H. dauricus* clade (Fig. 1B). Conflict between the ML and MP topologies is apparent in relationships among *A. transmontanus*, *A. medirostris*, *A. sinensis*, and *A. schrenckii* and the placement of the *Scaphirhynchus* node.

Although the MP and ML methods are known to conflict in cases of "long-branch attraction" (Felsenstein, 1981; Siddall, 1998; Siddall and Whiting, 1999), we do not feel this situation applies to the current dataset. If long-branch attractions were involved, our MP tree (Fig. 1A) would have firmly placed the long branches together with accompanying large support values. Although the branches leading to *Scaphirhynchus*, *A. oxyrinchus*, *A. sinensis* and the outgroups are the longest in the dataset (data not shown), using the methods of Siddall (1995), we did not find evidence that the placement of *Scaphirhynchus* in the MP tree is dictated by the presence of long branches.

The nonmonophyly of the subfamily Scaphirhynchinae (*Pseudoscaphirhynchus* + *Scaphirhynchus*) is congruent between the two trees (Fig. 1). The two species of the *Pseudoscaphirhynchus* group are within *Acipenser* and are closely relat-

ed to the stellate sturgeon *A. stellatus*, whereas the three *Scaphirhynchus* species form a separate monophyletic clade, which may (MP) or may not (ML) be basal to the Acipenseridae. A comparison of the ML and MP trees with the most parsimonious topology constraining Scaphirhynchinae monophyly indicates significant difference. Furthermore, no synapomorphic molecular characters could be identified to unite the subfamily Scaphirhynchinae.

Although the species of *Pseudoscaphirhynchus* and *Scaphirhynchus* share overall morphological similarity (Bailey and Cross, 1954; Nelson, 1994) the two genera are apparently united by only a few robust synapomorphic characters. The commonly cited synapomorphy of the Scaphirhynchinae associated with the absence of spiracles in adults (Bailey and Cross, 1954; Nelson, 1994), was recently shown to be ontogenetic in *Pseudoscaphirhynchus*. In *P. kaufmanni*, like in the species of *Acipenser*, spiracles appear during the early development (in larvae), but unlike in *Acipenser*, they close later and they are absent in adults (Schmalhausen, 1991; Dettlaff et al., 1993:181). Characters identified by Findeis (1997) require further examination as only two specimens of the dwarf form of *P. kaufmanni* (for discussion of the normal and dwarf morphs within *P. kaufmanni*, see Sagitov, 1969) and no *P. hermanni* or *P. fedtschenkoi* were compared with the species of *Scaphirhynchus*. Mayden and Kuhajda (1996) identified five of 36 characters as synapomorphies for the Scaphirhynchinae, one of which was the presence or absence of the spiracle in adults. Further review of the existing morphological descriptions and studies of individual taxa suggests these genera differ greatly (Berg, 1948; Bailey and Cross, 1954; Bemis et al., 1997).

The tree topology produced when molecular and morphological data (i.e., Mayden and Kuhajda, 1996) are combined also does not support the monophyly of the Scaphirhynchinae. The two data partitions were statistically significantly incongruent using the incongruence length difference test (Farris et al, 1995) and harbored conflict with respect to the PBS (Baker et al., 1998) at two nodes (Table 3, Fig. 2A). Not surprisingly, these two nodes are associated with the grouping of *Huso* and *A. ruthenus* and *Pseudoscaphirhynchus* and *A. stellatus* (Fig. 2A). We recognize that combining molecular and morphological data is often criticized because of the potential for "swamping" of morphological information with molecular (Hillis, 1987; Patterson et al., 1993; but see Baker et al., 1998) and agree that this is the most likely explanation for the pattern observed here.

Fossil information combined with the geological history of the current habitats of *Scaphirhynchus* and *Pseudoscaphirhynchus* add additional uncertainty to the monophyletic origin of the Scaphirhynchinae. Acipenseriforms likely originated in the Tethys Basin in the Jurassic, diversifying first in Europe and Asia and then later in North America (see Birstein and DeSalle, 1998). The ancestral form of *Scaphirhynchus* (i.e., †*Protoscaphirhynchus*) was found in Montana (Wilimovsky, 1956), in the same range as the fossil and recent polyodontids (McAlpin, 1947), supporting the scenario that *Scaphirhynchus* emerged from this ancestral freshwater lineage. No fossils can be directly linked to the Central Asian *Pseudoscaphirhynchus*, which presently inhabits the Amu Darya and Syr Darya Rivers. The Tethys Sea covered these river systems in the Upper Cretaceous and until approximately 15mybp (Smith et al., 1994; Veevers, 1994). Therefore, if the *Pseudoscaphirhynchus* emerged from a freshwater *Scaphirhynchus*-like ancestral form, that form must have survived in present day Central Asia in the Tethys and then Paratethys seas (see Hsü and Giovanoli, 1979/1980). The genus *Pseudoscaphirhynchus* could have evolved in this area from an Acipenserinae ancestor after the Amu Darya and Syr Darya Rivers entered into the Paratethys Sea or the Aral or Caspian Sea had been established, about 5.5 mybp to 0.02 mybp (see Birstein and DeSalle, 1998).

Our study offers little resolution regarding the species status and relationships among species of *Scaphirhynchus* and *Pseudoscaphirhynchus*. Individual species within both genera can be differentiated based upon morphological and life history characters (Tleuov and Sagitov, 1973; Keenlyne et al., 1994; Mayden and Kuhajda, 1996). The species of *Scaphirhynchus*, however, exhibit little genetic differentiation (review in Wirgin et al., 1997; Bischof and Szalanski, 2000; Campion et al., 2000). Our raw molecular data indicate very few differences among available species (for species divergence values, see Appendices 2–4). We stress that larger sample sizes of all species must be examined before concluding that these few differences observed uphold species designations.

The monophyly of the genus *Acipenser* is also not supported by our analysis as no synapomorphic molecular characters unite all *Acipenser*. Findeis (1997) came to a similar conclusion, finding no osteological characters uniting the genus, whereas Mayden and Kuhajda (1996) identified only one character defining the genus. Within *Acipenser*, our results support several previously identified relationships (Birstein and

DeSalle, 1998; Choudhury and Dick, 1998; Ludwig et al., 2000) but contradict assumptions that North American and Eurasian taxa form separate monophyletic groups (Brown et al., 1996; Krieger et al., 1996) and that phylogenetic groupings and relationships directly correspond with biogeographic information (Artyukhin, 1995). Sturgeon species from the Eurasian and American coasts of the Atlantic and Pacific oceans clearly have trans-Atlantic and trans-Pacific relationships, respectively (Figs. 1–2).

Molecular information apparently conflicts with available morphological matrices for sturgeons, particularly with respect to the monophyly of the Scaphirhynchinae. Although we recognize that phylogenetic hypotheses presented here require additional character information for improved resolution, our data are robust in testing this hypothesis and in suggesting morphological convergence. When nuclear markers are developed to accommodate the high ploidy levels characterizing sturgeons, and a comprehensive morphological matrix is constructed, a more fully resolved and well-supported analysis can be accomplished.

MATERIALS EXAMINED

We used the same tissue samples as in our previous papers (Birstein et al., 1997; Birstein and DeSalle, 1998; Doukakis et al., 1999), and additional samples from *P. hermanni*, *P. kaufmanni*, and *A. baerii baicalensis* collected in 1996–1997. Most samples were pieces of muscle tissue taken from fish and preserved in 80% EtOH; for *A. nudiventris* and *A. ruthenus*, blood samples were preserved in 100 mM Tris, 100 mM EDTA, and 2% SDS buffer and stored at -80°C . Fish specimens of *A. baerii baicalensis* were collected by G. Ruban at the Konakovo Hatchery (Russia; an artificially maintained stock); of *A. brevirostrum*, by B. Kynard in the Connecticut River (MA); of *A. fulvescens*, by F. Binkowski in the Great Lakes (WI); of *A. gueldenstaedtii* and *A. stellatus*, by R. Suciú in the Danube River (Romania); of *A. medirostris* and *A. transmontanus*, by J. North in the Columbia River (OR); of *A. mikadoi*, by Ye. Artyukhin in the Tumnin River (Russian Far East); of *A. naccarii*, by P. Bronzi in the Po River basin (Italy; aquaculture); of *A. nudiventris* (from the Aral Sea, Uzbekistan) and *A. ruthenus* (from the Volga River, Russia), by V. Birstein at the Moscow Aquarium; of *A. oxyrinchus desotoi*, by J. Waldman in the Pearl River (MS); of *A. persicus*, by M. Pourkazemi in the Southern part of the Caspian Sea (Iran); of *A. schrenkii* and *H. dauricus*, by V. Svirskii in the Amur River (Siberia, Russia); of *A. sinensis* and

Psephurus gladius, by Q. Wei in the Yangtze River (China); of *A. sturio*, by P. Williot in the Gironde River (France); of *H. huso*, by T. Gulyas in the Romanian part of the Black Sea; of *P. hermanni* and *P. kaufmanni*, by V. Salnikov in the Amu Darya River (Turkmenistan); of *S. albus* and *S. platyrhynchus*, by H. Bollig in the Yellowstone River (MT); of *S. suttkusi* (specimen 11158.01 at the University of Alabama Ichthyological Collection), by R. Mayden in the Alabama River (AL); of *Polyodon spathula*, by S. Mims in the Cumberland River (KY). All tissue samples and voucher information are kept in the collection of Sturgeon Conservation International, New York and requests for tissues or other voucher information should be directed to the first author of this paper.

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LITERATURE CITED

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APPENDIX 1. GENBANK ACCESSION NUMBERS OF SEQUENCES GENERATED AND USED IN THIS STUDY.

| Species | GenBank accession numbers | | | | |
|------------------------------|---------------------------|-------------|----------|----------|----------|
| | Cyt <i>b</i> | Contr. reg. | 12S | 16S | ND5 |
| <i>A. baerii baicalensis</i> | AF404779, 791, 803 | AF402844 | AF402882 | AF404836 | AF238776 |
| <i>A. brevisrostrum</i> | AF006124, 38, 73 | AF402848 | AF402866 | AF404838 | AF403083 |
| <i>A. fulvescens</i> | AF006125, 35, 78 | AF402847 | AF402885 | AF004954 | AF403082 |
| <i>A. gueldenstaedtii</i> | AF404797, 785, 809 | AF402857 | AF402895 | AF404844 | AF238841 |
| <i>A. medirostris</i> | AF006146, 71, X95053 | AF402843 | AF402881 | AF404835 | AF403079 |
| <i>A. mikadoi</i> | AF404782, 794, 806 | AF402851 | AF402889 | AF404841 | AF403086 |
| <i>A. naccarii</i> | AF404783, 795, 807 | AF402852 | AF402890 | AF404842 | AF238810 |
| <i>A. nudiventris</i> | AF006151, 52, 62 | AF402853 | AF402891 | AF004958 | AF403087 |
| <i>A. oxyrinchus desotoi</i> | AF006153, 54, 64 | AF402856 | AF402894 | AF004959 | AF403090 |
| <i>A. persicus</i> | AF404776, 790, 802 | AF402842 | AF402880 | AF404834 | AF238775 |
| <i>A. ruthenus</i> | AF006157, 68, X95055 | AF402846 | AF402884 | AF004962 | AF403081 |
| <i>A. schrenckii</i> | AF404781, 793, 805 | AF402850 | AF402888 | AF404840 | AF403085 |
| <i>A. sinensis</i> | AF006158, 59, 66 | AF402854 | AF402892 | AF004964 | AF403088 |
| <i>A. stellatus</i> | AF404780, 792, 804 | AF402845 | AF402883 | AF404837 | AF403080 |
| <i>A. sturio</i> | AF006134, 45, 76 | AF402849 | AF402887 | AF404839 | AF403084 |
| <i>A. transmontanus</i> | AF404784, 796, 808 | AF402855 | AF402893 | AF404843 | AF403089 |
| <i>H. dauricus</i> | AF006133, 44, 75 | AF402858 | AF402896 | AF004966 | AF403091 |
| <i>H. huso</i> | AF404786, 798, 180 | AF402859 | AF402897 | AF404845 | AF403092 |
| <i>P. hermanni</i> | AF404787, 799, 811 | AF402860 | AF402898 | AF404846 | AF403093 |
| <i>P. kaufmanni</i> | AF404788, 800, 812 | AF402861 | AF402899 | AF404847 | AF403094 |
| <i>S. albus</i> | U56983 | AF402864 | AF402902 | AF404850 | AF403097 |
| <i>S. platyrinchus</i> | U56988 | AF402863 | AF402901 | AF404849 | AF403096 |
| <i>S. suttkusi</i> | AF404789, 801, 813 | AF402862 | AF402900 | AF404848 | AF403095 |
| <i>Polyodon spathula</i> | AF006183, 84, X95059 | AF402865 | AF402903 | AF404851 | AF403098 |
| <i>Psephurus gladius</i> | AF006130, 67, 88 | AF402866 | AF402904 | AF404852 | AF403099 |

APPENDIX 2. SEQUENCE DIVERGENCE (UNCORRECTED *P*) BETWEEN TAXA. Upper half of matrix is based on the all gene region sequences combined. Lower half of matrix is based on 16S sequences.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1 <i>A. persicus</i> | *** | 0.05 | 0.0151 | 0.0465 | 0.036 | 0.0416 | 0.0269 | 0.073 | 0.0533 | 0.0478 | 0.005 |
| 2 <i>A. medirostris</i> | 0.0079 | *** | 0.0557 | 0.0671 | 0.0532 | 0.0635 | 0.0596 | 0.0676 | 0.0273 | 0.0407 | 0.0549 |
| 3 <i>A. baerii</i> | 0.0039 | 0.0079 | *** | 0.0473 | 0.0385 | 0.0428 | 0.0273 | 0.0717 | 0.0541 | 0.0448 | 0.0159 |
| 4 <i>A. stellatus</i> | 0.0178 | 0.0217 | 0.0178 | *** | 0.0507 | 0.0579 | 0.0537 | 0.0889 | 0.0659 | 0.0478 | 0.0482 |
| 5 <i>A. ruthenus</i> | 0.0099 | 0.0138 | 0.0099 | 0.0237 | *** | 0.0563 | 0.0449 | 0.0721 | 0.0545 | 0.0524 | 0.0385 |
| 6 <i>A. fulvescens</i> | 0.0158 | 0.0197 | 0.0158 | 0.0276 | 0.0217 | *** | 0.0505 | 0.0786 | 0.0597 | 0.0575 | 0.042 |
| 7 <i>A. brevisrostrum</i> | 0.0276 | 0.0316 | 0.0276 | 0.0335 | 0.0335 | 0.0394 | *** | 0.0756 | 0.0605 | 0.0525 | 0.0277 |
| 8 <i>A. sturio</i> | 0.0158 | 0.0158 | 0.0158 | 0.0276 | 0.0217 | 0.0158 | 0.0394 | *** | 0.0659 | 0.0721 | 0.0729 |
| 9 <i>A. schrenckii</i> | 0.0039 | 0.0079 | 0.0079 | 0.0217 | 0.0138 | 0.0197 | 0.0316 | 0.0118 | *** | 0.0419 | 0.0541 |
| 10 <i>A. mikadoi</i> | 0.0039 | 0.0039 | 0.0039 | 0.0178 | 0.0099 | 0.0158 | 0.0276 | 0.0018 | 0.0039 | *** | 0.0478 |
| 11 <i>A. naccarii</i> | 0.002 | 0.0059 | 0.002 | 0.0158 | 0.0079 | 0.0138 | 0.0256 | 0.0138 | 0.0059 | 0.002 | *** |
| 12 <i>A. nudiventris</i> | 0.0079 | 0.0118 | 0.0079 | 0.0217 | 0.0138 | 0.0197 | 0.0276 | 0.0158 | 0.0118 | 0.0079 | 0.0059 |
| 13 <i>A. sinensis</i> | 0.0138 | 0.0138 | 0.0138 | 0.0197 | 0.0197 | 0.0256 | 0.0335 | 0.0178 | 0.0099 | 0.0099 | 0.0118 |
| 14 <i>A. transmontanus</i> | 0.0119 | 0.0158 | 0.0158 | 0.0257 | 0.0217 | 0.0277 | 0.0356 | 0.0198 | 0.0079 | 0.0119 | 0.0139 |
| 15 <i>A. oxyrinchus</i> | 0.0394 | 0.0394 | 0.0394 | 0.0473 | 0.0454 | 0.0394 | 0.0592 | 0.0316 | 0.0355 | 0.0355 | 0.0375 |
| 16 <i>A. gueldenstaedtii</i> | 0 | 0.0079 | 0.0039 | 0.0178 | 0.0099 | 0.0158 | 0.0277 | 0.0158 | 0.0039 | 0.0039 | 0.002 |
| 17 <i>H. dauricus</i> | 0.0079 | 0.0118 | 0.0079 | 0.0178 | 0.0138 | 0.0178 | 0.0276 | 0.0138 | 0.0079 | 0.0079 | 0.0059 |
| 18 <i>H. huso</i> | 0.0335 | 0.0375 | 0.0335 | 0.0375 | 0.0355 | 0.0434 | 0.0493 | 0.0434 | 0.0375 | 0.0335 | 0.0316 |
| 19 <i>P. hermanni</i> | 0.0099 | 0.0138 | 0.0059 | 0.0158 | 0.0158 | 0.0197 | 0.0335 | 0.0158 | 0.0138 | 0.0099 | 0.0079 |
| 20 <i>P. kaufmanni</i> | 0.0099 | 0.0138 | 0.0059 | 0.0158 | 0.0158 | 0.0197 | 0.0335 | 0.0158 | 0.0138 | 0.0099 | 0.0079 |
| 21 <i>S. suttkusi</i> | 0.0178 | 0.0178 | 0.0138 | 0.0316 | 0.0237 | 0.0296 | 0.0414 | 0.0256 | 0.0178 | 0.0138 | 0.0158 |
| 22 <i>S. platyrinchus</i> | 0.0178 | 0.0178 | 0.0138 | 0.0316 | 0.0237 | 0.0296 | 0.0414 | 0.0256 | 0.0178 | 0.0138 | 0.0158 |
| 23 <i>S. albus</i> | 0.0178 | 0.0178 | 0.0138 | 0.0316 | 0.0237 | 0.0296 | 0.0414 | 0.0256 | 0.0178 | 0.0138 | 0.0158 |
| 24 <i>Polyodon spathula</i> | 0.0434 | 0.0454 | 0.0414 | 0.0592 | 0.0513 | 0.0493 | 0.0611 | 0.0533 | 0.0434 | 0.0414 | 0.0434 |
| 25 <i>Psephurus gladius</i> | 0.0276 | 0.0256 | 0.0296 | 0.0434 | 0.0355 | 0.0335 | 0.0454 | 0.0335 | 0.0237 | 0.0256 | 0.0276 |

APPENDIX 2. Extended.

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.0382 | 0.0679 | 0.0558 | 0.0857 | 0.0063 | 0.0424 | 0.0428 | 0.052 | 0.0491 | 0.0731 | 0.0717 | 0.0721 | 0.1069 | 0.1027 |
| 0.0546 | 0.0508 | 0.0302 | 0.0773 | 0.0528 | 0.0604 | 0.0608 | 0.0713 | 0.0701 | 0.0782 | 0.0771 | 0.0767 | 0.0969 | 0.0965 |
| 0.0365 | 0.0687 | 0.0582 | 0.0878 | 0.0155 | 0.044 | 0.0461 | 0.052 | 0.0499 | 0.0743 | 0.0737 | 0.0733 | 0.109 | 0.1081 |
| 0.0495 | 0.083 | 0.0704 | 0.0932 | 0.0478 | 0.057 | 0.0544 | 0.0264 | 0.026 | 0.0756 | 0.0758 | 0.075 | 0.1153 | 0.1073 |
| 0.0415 | 0.0691 | 0.0545 | 0.0865 | 0.039 | 0.0339 | 0.0163 | 0.0545 | 0.0524 | 0.0731 | 0.0712 | 0.0716 | 0.1077 | 0.106 |
| 0.0522 | 0.071 | 0.0672 | 0.0875 | 0.0412 | 0.0563 | 0.0617 | 0.0647 | 0.0606 | 0.0834 | 0.0823 | 0.0819 | 0.1151 | 0.1084 |
| 0.0412 | 0.0734 | 0.0621 | 0.0904 | 0.0281 | 0.0504 | 0.0512 | 0.0596 | 0.0579 | 0.077 | 0.0759 | 0.0755 | 0.1112 | 0.107 |
| 0.076 | 0.0801 | 0.0667 | 0.0471 | 0.0717 | 0.0797 | 0.0759 | 0.0898 | 0.0848 | 0.0857 | 0.0847 | 0.0843 | 0.1007 | 0.1032 |
| 0.0593 | 0.0483 | 0.0248 | 0.0774 | 0.0537 | 0.0604 | 0.0604 | 0.0705 | 0.0702 | 0.0732 | 0.0726 | 0.0722 | 0.099 | 0.094 |
| 0.0504 | 0.0612 | 0.0398 | 0.0853 | 0.049 | 0.0583 | 0.0582 | 0.0541 | 0.0516 | 0.0756 | 0.0754 | 0.0746 | 0.1027 | 0.0968 |
| 0.0399 | 0.0692 | 0.0566 | 0.0857 | 0.008 | 0.044 | 0.0452 | 0.0536 | 0.0512 | 0.0743 | 0.0737 | 0.0733 | 0.1068 | 0.1052 |
| *** | 0.0706 | 0.063 | 0.0909 | 0.0386 | 0.0416 | 0.0483 | 0.0542 | 0.0504 | 0.0779 | 0.0768 | 0.0764 | 0.1092 | 0.1087 |
| 0.0178 | *** | 0.0524 | 0.0878 | 0.0662 | 0.0747 | 0.0746 | 0.0889 | 0.0865 | 0.0845 | 0.0834 | 0.083 | 0.1019 | 0.0969 |
| 0.0198 | 0.0139 | *** | 0.0781 | 0.0562 | 0.065 | 0.0612 | 0.0721 | 0.0722 | 0.0752 | 0.0742 | 0.0738 | 0.0985 | 0.0968 |
| 0.0434 | 0.0414 | 0.0435 | *** | 0.0824 | 0.0912 | 0.0899 | 0.0996 | 0.0959 | 0.0952 | 0.0941 | 0.0937 | 0.1155 | 0.1163 |
| 0.0079 | 0.0138 | 0.0119 | 0.0395 | *** | 0.0453 | 0.0457 | 0.0533 | 0.0504 | 0.0744 | 0.0737 | 0.0733 | 0.1056 | 0.1035 |
| 0.0118 | 0.0138 | 0.0158 | 0.0375 | 0.0079 | *** | 0.0381 | 0.065 | 0.0609 | 0.079 | 0.0771 | 0.0776 | 0.1153 | 0.1103 |
| 0.0375 | 0.0394 | 0.0415 | 0.0611 | 0.0336 | 0.0335 | *** | 0.0629 | 0.0541 | 0.0764 | 0.075 | 0.0754 | 0.1132 | 0.1089 |
| 0.0099 | 0.0197 | 0.0218 | 0.0434 | 0.0099 | 0.0099 | 0.0355 | *** | 0.0155 | 0.0807 | 0.0809 | 0.0801 | 0.1141 | 0.114 |
| 0.0099 | 0.0197 | 0.0218 | 0.0434 | 0.0099 | 0.0099 | 0.0355 | 0 | *** | 0.0765 | 0.0763 | 0.0755 | 0.1154 | 0.1104 |
| 0.0217 | 0.0237 | 0.0257 | 0.0493 | 0.0178 | 0.0217 | 0.0473 | 0.0197 | 0.0197 | *** | 0.0025 | 0.017 | 0.1135 | 0.1067 |
| 0.0217 | 0.0237 | 0.0257 | 0.0493 | 0.0178 | 0.0217 | 0.0473 | 0.0197 | 0.0197 | 0 | *** | 0.0004 | 0.1123 | 0.1073 |
| 0.0217 | 0.0237 | 0.0257 | 0.0493 | 0.0178 | 0.0217 | 0.0473 | 0.0197 | 0.0197 | 0 | 0 | *** | 0.1119 | 0.1065 |
| 0.0493 | 0.0513 | 0.0514 | 0.0671 | 0.0434 | 0.0493 | 0.0671 | 0.0473 | 0.0473 | 0.0513 | 0.0513 | 0.0513 | *** | 0.086 |
| 0.0335 | 0.0316 | 0.0316 | 0.0513 | 0.0277 | 0.0296 | 0.0533 | 0.0355 | 0.0355 | 0.0394 | 0.0394 | 0.0394 | 0.0394 | *** |

APPENDIX 3. SEQUENCE DIVERGENCE (UNCORRECTED *P*) BETWEEN TAXA. Upper half of matrix is based on 12S sequences. Lower half of matrix is based on NADH5 sequences.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|------------------------------|--------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1 <i>A. persicus</i> | *** | 0.003 | 0 | 0.0089 | 0.0089 | 0.0121 | 0.003 | 0.0148 | 0.0089 | 0.0089 | 0 |
| 2 <i>A. medirostris</i> | 0.0701 | *** | 0.003 | 0.0059 | 0.0059 | 0.0091 | 0.0059 | 0.0119 | 0.0059 | 0.0059 | 0.003 |
| 3 <i>A. baerii</i> | 0.0156 | 0.07 | *** | 0.0089 | 0.0089 | 0.0121 | 0.003 | 0.0148 | 0.0089 | 0.0089 | 0 |
| 4 <i>A. stellatus</i> | 0.0452 | 0.086 | 0.04 | *** | 0.0059 | 0.0151 | 0.0119 | 0.0119 | 0.0119 | 0.0119 | 0.0089 |
| 5 <i>A. ruthenus</i> | 0.0374 | 0.067 | 0.037 | 0.056 | *** | 0.0151 | 0.0119 | 0.0178 | 0.0119 | 0.0119 | 0.0089 |
| 6 <i>A. fulvescens</i> | 0.0358 | 0.078 | 0.036 | 0.056 | 0.0482 | *** | 0.0091 | 0.0091 | 0.0092 | 0.0092 | 0.0121 |
| 7 <i>A. brevirostrum</i> | 0.0202 | 0.072 | 0.02 | 0.0498 | 0.0327 | 0.0404 | *** | 0.0119 | 0.0059 | 0.0059 | 0.003 |
| 8 <i>A. sturio</i> | 0.0936 | 0.092 | 0.1 | 0.1169 | 0.0904 | 0.1075 | 0.095 | *** | 0.0119 | 0.0119 | 0.0148 |
| 9 <i>A. schrenckii</i> | 0.0624 | 0.036 | 0.062 | 0.0732 | 0.053 | 0.067 | 0.0577 | 0.0843 | *** | 0 | 0.0089 |
| 10 <i>A. mikadoi</i> | 0.0452 | 0.086 | 0.04 | 0 | 0.056 | 0.056 | 0.0498 | 0.1169 | 0.0732 | *** | 0.0089 |
| 11 <i>A. naccarii</i> | 0.0016 | 0.072 | 0.017 | 0.0467 | 0.0389 | 0.0373 | 0.0218 | 0.095 | 0.0639 | 0.0467 | *** |
| 12 <i>A. nudiventris</i> | 0.0327 | 0.067 | 0.033 | 0.0435 | 0.0358 | 0.0404 | 0.0311 | 0.0982 | 0.0592 | 0.0435 | 0.0342 |
| 13 <i>A. sinensis</i> | 0.078 | 0.061 | 0.078 | 0.0919 | 0.0748 | 0.0919 | 0.0763 | 0.0952 | 0.0499 | 0.0919 | 0.0794 |
| 14 <i>A. transmontanus</i> | 0.0654 | 0.039 | 0.065 | 0.0762 | 0.056 | 0.0731 | 0.0638 | 0.0842 | 0.0093 | 0.0762 | 0.0669 |
| 15 <i>A. oxyrinchus</i> | 0.0935 | 0.089 | 0.093 | 0.1042 | 0.0871 | 0.1011 | 0.0949 | 0.0499 | 0.0779 | 0.1042 | 0.0949 |
| 16 <i>A. gueldenstaedtii</i> | 0.0016 | 0.068 | 0.017 | 0.0467 | 0.0389 | 0.0373 | 0.0218 | 0.0919 | 0.0608 | 0.0467 | 0.0031 |
| 17 <i>H. dauricus</i> | 0.0422 | 0.078 | 0.045 | 0.0546 | 0.0296 | 0.0468 | 0.0405 | 0.1046 | 0.0686 | 0.0546 | 0.0437 |
| 18 <i>H. huso</i> | 0.0374 | 0.067 | 0.037 | 0.056 | 0.0016 | 0.0482 | 0.0327 | 0.0904 | 0.053 | 0.056 | 0.0389 |
| 19 <i>P. hermanni</i> | 0.0623 | 0.092 | 0.058 | 0.0202 | 0.0731 | 0.0669 | 0.0669 | 0.1278 | 0.0795 | 0.0202 | 0.0638 |
| 20 <i>P. kaufmanni</i> | 0.0592 | 0.09 | 0.054 | 0.0171 | 0.0669 | 0.0638 | 0.0638 | 0.1215 | 0.0779 | 0.0171 | 0.0607 |
| 21 <i>S. suttkusi</i> | 0.0872 | 0.092 | 0.089 | 0.0824 | 0.0762 | 0.0964 | 0.0778 | 0.1184 | 0.0842 | 0.0824 | 0.0902 |
| 22 <i>S. platorynchy</i> | 0.0841 | 0.089 | 0.086 | 0.0824 | 0.0731 | 0.0933 | 0.0747 | 0.1153 | 0.081 | 0.0824 | 0.0871 |
| 23 <i>S. albus</i> | 0.0843 | 0.089 | 0.086 | 0.081 | 0.0732 | 0.0935 | 0.0748 | 0.1155 | 0.0812 | 0.081 | 0.0872 |
| 24 <i>Polyodon spathula</i> | 0.131 | 0.115 | 0.132 | 0.1323 | 0.1214 | 0.137 | 0.1308 | 0.1217 | 0.1091 | 0.1323 | 0.1339 |
| 25 <i>Psephurus gladius</i> | 0.1433 | 0.14 | 0.145 | 0.1353 | 0.14 | 0.1524 | 0.1415 | 0.1496 | 0.1262 | 0.1353 | 0.1462 |

APPENDIX 3. Extended.

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.003 | 0.0059 | 0.0089 | 0.0148 | 0 | 0.0089 | 0.0089 | 0.0089 | 0.006 | 0.0119 | 0.0119 | 0.0119 | 0.0178 | 0.0148 |
| 0 | 0.003 | 0.0059 | 0.0119 | 0.003 | 0.0059 | 0.0059 | 0.0059 | 0.0059 | 0.0089 | 0.0089 | 0.0089 | 0.0148 | 0.0119 |
| 0.003 | 0.0059 | 0.0089 | 0.0148 | 0 | 0.0089 | 0.0089 | 0.0089 | 0.006 | 0.0119 | 0.0119 | 0.0119 | 0.0178 | 0.0148 |
| 0.006 | 0.0089 | 0.0119 | 0.0119 | 0.0089 | 0.0059 | 0.0059 | 0 | 0 | 0.0089 | 0.0089 | 0.0089 | 0.0148 | 0.0119 |
| 0.006 | 0.0089 | 0.0119 | 0.0178 | 0.0089 | 0 | 0 | 0.0059 | 0.006 | 0.0148 | 0.0148 | 0.0148 | 0.0208 | 0.0178 |
| 0.0092 | 0.0061 | 0.0092 | 0.0152 | 0.0121 | 0.0151 | 0.0151 | 0.0151 | 0.0151 | 0.0122 | 0.0122 | 0.0122 | 0.0121 | 0.0152 |
| 0.006 | 0.003 | 0.0059 | 0.0119 | 0.003 | 0.0119 | 0.0119 | 0.0119 | 0.009 | 0.0089 | 0.0089 | 0.0089 | 0.0148 | 0.0119 |
| 0.012 | 0.0089 | 0.0119 | 0.0119 | 0.0148 | 0.0178 | 0.0178 | 0.0119 | 0.0119 | 0.0089 | 0.0089 | 0.0089 | 0.0089 | 0.0119 |
| 0.006 | 0.003 | 0 | 0.0119 | 0.0089 | 0.0119 | 0.0119 | 0.0119 | 0.0119 | 0.003 | 0.003 | 0.003 | 0.0089 | 0.0059 |
| 0.006 | 0.003 | 0 | 0.0119 | 0.0089 | 0.0119 | 0.0119 | 0.0119 | 0.0119 | 0.003 | 0.003 | 0.003 | 0.0089 | 0.0059 |
| 0.003 | 0.0059 | 0.0089 | 0.0148 | 0 | 0.0089 | 0.0089 | 0.0089 | 0.006 | 0.0119 | 0.0119 | 0.0119 | 0.0178 | 0.0148 |
| *** | 0.003 | 0.006 | 0.012 | 0.003 | 0.006 | 0.006 | 0.006 | 0.006 | 0.009 | 0.009 | 0.009 | 0.015 | 0.012 |
| 0.0763 | *** | 0.003 | 0.0089 | 0.0059 | 0.0089 | 0.0089 | 0.0089 | 0.0089 | 0.0059 | 0.0059 | 0.0059 | 0.0119 | 0.0089 |
| 0.0622 | 0.053 | *** | 0.0119 | 0.0089 | 0.0119 | 0.0119 | 0.0119 | 0.0119 | 0.003 | 0.003 | 0.003 | 0.0089 | 0.0059 |
| 0.098 | 0.0934 | 0.0778 | *** | 0.0148 | 0.0178 | 0.0178 | 0.0119 | 0.0019 | 0.0089 | 0.0089 | 0.0089 | 0.0089 | 0.0119 |
| 0.0342 | 0.0763 | 0.0638 | 0.0918 | *** | 0.0089 | 0.0089 | 0.0089 | 0.006 | 0.0119 | 0.0119 | 0.0199 | 0.0178 | 0.0148 |
| 0.0343 | 0.0873 | 0.0732 | 0.1013 | 0.0437 | *** | 0 | 0.0059 | 0.006 | 0.0148 | 0.0148 | 0.0148 | 0.0208 | 0.0178 |
| 0.0358 | 0.0748 | 0.056 | 0.0871 | 0.0389 | 0.0296 | *** | 0.0059 | 0.006 | 0.0148 | 0.0148 | 0.0148 | 0.0208 | 0.0178 |
| 0.0575 | 0.0981 | 0.0824 | 0.1151 | 0.0607 | 0.0717 | 0.0731 | *** | 0 | 0.0089 | 0.0089 | 0.0089 | 0.0148 | 0.0119 |
| 0.0544 | 0.0966 | 0.0809 | 0.1089 | 0.0575 | 0.0655 | 0.0669 | 0.0124 | *** | 0.0089 | 0.0089 | 0.0089 | 0.0149 | 0.0119 |
| 0.084 | 0.1028 | 0.0871 | 0.1089 | 0.0871 | 0.081 | 0.0762 | 0.0933 | 0.0871 | *** | 0 | 0 | 0.0059 | 0.003 |
| 0.0809 | 0.0997 | 0.084 | 0.1058 | 0.084 | 0.0779 | 0.0731 | 0.0933 | 0.0871 | 0.0031 | *** | 0 | 0.0059 | 0.003 |
| 0.081 | 0.0999 | 0.0841 | 0.1059 | 0.0841 | 0.078 | 0.0732 | 0.0919 | 0.0857 | 0.0016 | 0 | *** | 0.0059 | 0.003 |
| 0.1308 | 0.1216 | 0.1152 | 0.1308 | 0.1308 | 0.1325 | 0.1214 | 0.142 | 0.137 | 0.1292 | 0.1261 | 0.1264 | *** | 0.0089 |
| 0.1462 | 0.1309 | 0.1337 | 0.1555 | 0.1431 | 0.1448 | 0.14 | 0.148 | 0.143 | 0.1322 | 0.1322 | 0.1308 | 0.0872 | *** |

APPENDIX 4. SEQUENCE DIVERGENCE (UNCORRECTED P) BETWEEN TAXA. Upper half of matrix is based on cytochrome b sequences. Lower half of matrix is based on control region sequences.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1 <i>A. persicus</i> | *** | 0.0627 | 0.0114 | 0.0513 | 0.0456 | 0.0456 | 0.0342 | 0.0894 | 0.0589 | 0.0646 | 0.0057 |
| 2 <i>A. medirostris</i> | 0.1184 | *** | 0.0703 | 0.0703 | 0.0627 | 0.0437 | 0.0722 | 0.0817 | 0.0266 | 0.0589 | 0.0684 |
| 3 <i>A. baerii</i> | 0.0481 | 0.1235 | *** | 0.0551 | 0.0418 | 0.0532 | 0.0304 | 0.0932 | 0.0665 | 0.0646 | 0.0171 |
| 4 <i>A. stellatus</i> | 0.1151 | 0.1475 | 0.1227 | *** | 0.057 | 0.0627 | 0.0665 | 0.0951 | 0.0665 | 0.0913 | 0.057 |
| 5 <i>A. ruthenus</i> | 0.0801 | 0.1129 | 0.1013 | 0.1093 | *** | 0.0532 | 0.057 | 0.0875 | 0.0627 | 0.0722 | 0.0513 |
| 6 <i>A. fulvescens</i> | 0.1072 | 0.175 | 0.1043 | 0.1336 | 0.1578 | *** | 0.0551 | 0.0722 | 0.0361 | 0.0532 | 0.0513 |
| 7 <i>A. brevirostrum</i> | 0.0485 | 0.1087 | 0.0566 | 0.1077 | 0.0943 | 0.113 | *** | 0.0817 | 0.0722 | 0.0627 | 0.0399 |
| 8 <i>A. sturio</i> | 0.1448 | 0.1267 | 0.1204 | 0.1847 | 0.1364 | 0.1851 | 0.1408 | *** | 0.0856 | 0.0837 | 0.0951 |
| 9 <i>A. schrenckii</i> | 0.1372 | 0.0594 | 0.1261 | 0.1612 | 0.1394 | 0.1802 | 0.1385 | 0.129 | *** | 0.0627 | 0.0646 |
| 10 <i>A. mikadoi</i> | 0.1232 | 0.0189 | 0.1123 | 0.1416 | 0.1123 | 0.1662 | 0.1192 | 0.1153 | 0.0484 | *** | 0.0665 |
| 11 <i>A. naccarii</i> | 0.0187 | 0.1208 | 0.0453 | 0.1173 | 0.088 | 0.1016 | 0.0458 | 0.1364 | 0.1287 | 0.1203 | *** |
| 12 <i>A. nudiventris</i> | 0.1157 | 0.1266 | 0.1098 | 0.1179 | 0.0938 | 0.1425 | 0.1026 | 0.1614 | 0.1513 | 0.1236 | 0.1181 |
| 13 <i>A. sinensis</i> | 0.1663 | 0.1103 | 0.1659 | 0.2113 | 0.1685 | 0.1984 | 0.167 | 0.1744 | 0.1235 | 0.1124 | 0.1658 |
| 14 <i>A. transmontanus</i> | 0.123 | 0.043 | 0.1227 | 0.1547 | 0.1147 | 0.1765 | 0.1295 | 0.1177 | 0.0535 | 0.0481 | 0.12 |
| 15 <i>A. oxyrinchus</i> | 0.1632 | 0.1392 | 0.1713 | 0.1818 | 0.1762 | 0.1927 | 0.1651 | 0.1062 | 0.158 | 0.1493 | 0.1601 |
| 16 <i>A. gueldenstaedtii</i> | 0.0321 | 0.1127 | 0.0427 | 0.12 | 0.096 | 0.0963 | 0.0483 | 0.1445 | 0.1369 | 0.123 | 0.0293 |
| 17 <i>H. dauricus</i> | 0.0858 | 0.1238 | 0.0962 | 0.1256 | 0.0696 | 0.1477 | 0.0947 | 0.1529 | 0.1452 | 0.1312 | 0.0882 |
| 18 <i>H. huso</i> | 0.0801 | 0.1128 | 0.1067 | 0.104 | 0.0107 | 0.1577 | 0.0969 | 0.1364 | 0.1394 | 0.1123 | 0.088 |
| 19 <i>P. hermanni</i> | 0.1204 | 0.1583 | 0.1333 | 0.0587 | 0.128 | 0.1471 | 0.116 | 0.182 | 0.1692 | 0.1523 | 0.1253 |
| 20 <i>P. kaufmanni</i> | 0.1105 | 0.1538 | 0.1237 | 0.0566 | 0.1072 | 0.1319 | 0.1137 | 0.1721 | 0.1648 | 0.1478 | 0.1155 |
| 21 <i>S. suttkusi</i> | 0.1669 | 0.1912 | 0.169 | 0.177 | 0.1662 | 0.2015 | 0.1765 | 0.1749 | 0.1835 | 0.18 | 0.1636 |
| 22 <i>S. platorynchy</i> | 0.1632 | 0.1929 | 0.1707 | 0.1787 | 0.1627 | 0.2032 | 0.1781 | 0.1767 | 0.1852 | 0.1818 | 0.1653 |
| 23 <i>S. albus</i> | 0.1659 | 0.1902 | 0.168 | 0.176 | 0.1653 | 0.2005 | 0.1754 | 0.174 | 0.1824 | 0.1791 | 0.1627 |
| 24 <i>Polyodon spathula</i> | 0.2005 | 0.1801 | 0.2027 | 0.2373 | 0.2187 | 0.2434 | 0.205 | 0.1738 | 0.1929 | 0.1738 | 0.1947 |
| 25 <i>Psephurus gladius</i> | 0.2059 | 0.1852 | 0.224 | 0.2321 | 0.208 | 0.2542 | 0.2076 | 0.19 | 0.2063 | 0.1898 | 0.208 |

APPENDIX 4. Extended.

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.0418 | 0.0779 | 0.0684 | 0.1122 | 0.0038 | 0.0665 | 0.0532 | 0.0592 | 0.0589 | 0.0824 | 0.0817 | 0.0817 | 0.1294 | 0.1087 |
| 0.0646 | 0.0627 | 0.0399 | 0.0989 | 0.0665 | 0.076 | 0.0741 | 0.082 | 0.0817 | 0.0843 | 0.0817 | 0.0817 | 0.1181 | 0.103 |
| 0.038 | 0.0817 | 0.076 | 0.116 | 0.0152 | 0.0627 | 0.0494 | 0.0592 | 0.0627 | 0.0882 | 0.0875 | 0.0875 | 0.1371 | 0.1163 |
| 0.0627 | 0.0894 | 0.0837 | 0.1141 | 0.0513 | 0.0817 | 0.0646 | 0.0382 | 0.0418 | 0.0806 | 0.0798 | 0.0798 | 0.1257 | 0.1067 |
| 0.0608 | 0.0779 | 0.0684 | 0.1065 | 0.0456 | 0.0551 | 0.0304 | 0.0477 | 0.0608 | 0.0882 | 0.0856 | 0.0856 | 0.1219 | 0.1162 |
| 0.0608 | 0.0399 | 0.057 | 0.0894 | 0.0494 | 0.0665 | 0.057 | 0.0782 | 0.0741 | 0.0805 | 0.0779 | 0.0779 | 0.1256 | 0.082 |
| 0.0456 | 0.0875 | 0.0741 | 0.1141 | 0.038 | 0.0779 | 0.0684 | 0.0668 | 0.0665 | 0.0843 | 0.0817 | 0.0817 | 0.1314 | 0.1144 |
| 0.0875 | 0.1008 | 0.0894 | 0.0399 | 0.0856 | 0.1008 | 0.0837 | 0.0992 | 0.0913 | 0.0901 | 0.0875 | 0.0875 | 0.1276 | 0.1105 |
| 0.0741 | 0.0589 | 0.0551 | 0.1027 | 0.0627 | 0.0722 | 0.0665 | 0.082 | 0.0856 | 0.0805 | 0.0798 | 0.0798 | 0.1314 | 0.0992 |
| 0.076 | 0.0741 | 0.0418 | 0.1122 | 0.0684 | 0.0894 | 0.076 | 0.0954 | 0.0913 | 0.0997 | 0.097 | 0.097 | 0.1352 | 0.1106 |
| 0.0475 | 0.0837 | 0.0703 | 0.1141 | 0.0095 | 0.0722 | 0.0589 | 0.063 | 0.0646 | 0.0881 | 0.0875 | 0.0875 | 0.1295 | 0.1144 |
| *** | 0.0817 | 0.0798 | 0.1103 | 0.0456 | 0.0646 | 0.0646 | 0.0706 | 0.0665 | 0.0959 | 0.0932 | 0.0932 | 0.1295 | 0.1163 |
| 0.1775 | *** | 0.0779 | 0.1179 | 0.0817 | 0.0875 | 0.0856 | 0.1029 | 0.1027 | 0.0978 | 0.0951 | 0.0951 | 0.1142 | 0.1049 |
| 0.1501 | 0.1123 | *** | 0.1103 | 0.0722 | 0.0894 | 0.0837 | 0.0859 | 0.0951 | 0.094 | 0.0913 | 0.0913 | 0.1257 | 0.1125 |
| 0.1877 | 0.1703 | 0.1408 | *** | 0.1084 | 0.116 | 0.1065 | 0.1183 | 0.1122 | 0.1131 | 0.1103 | 0.1103 | 0.1429 | 0.1276 |
| 0.11 | 0.1524 | 0.1227 | 0.1492 | *** | 0.0703 | 0.0532 | 0.0592 | 0.0589 | 0.0824 | 0.0817 | 0.0817 | 0.1256 | 0.1067 |
| 0.0942 | 0.1769 | 0.131 | 0.1796 | 0.0962 | *** | 0.0551 | 0.0954 | 0.0894 | 0.1054 | 0.1027 | 0.1027 | 0.1448 | 0.1277 |
| 0.0992 | 0.1658 | 0.1093 | 0.1763 | 0.096 | 0.0696 | *** | 0.0706 | 0.0494 | 0.0844 | 0.0837 | 0.0837 | 0.1333 | 0.1162 |
| 0.1286 | 0.2193 | 0.1573 | 0.2031 | 0.1307 | 0.139 | 0.1227 | *** | 0.0363 | 0.0924 | 0.0915 | 0.0915 | 0.1165 | 0.1224 |
| 0.1158 | 0.2072 | 0.1474 | 0.1983 | 0.1209 | 0.1318 | 0.1072 | 0.0269 | *** | 0.0862 | 0.0837 | 0.0837 | 0.1276 | 0.1105 |
| 0.1806 | 0.1883 | 0.1608 | 0.1883 | 0.1744 | 0.1747 | 0.1608 | 0.1904 | 0.1831 | *** | 0.0058 | 0.0058 | 0.1423 | 0.1193 |
| 0.1822 | 0.19 | 0.1627 | 0.19 | 0.176 | 0.171 | 0.1573 | 0.192 | 0.1848 | 0.0027 | *** | 0 | 0.139 | 0.12 |
| 0.1795 | 0.1873 | 0.16 | 0.1873 | 0.1733 | 0.1736 | 0.16 | 0.1893 | 0.1821 | 0 | 0.0027 | *** | 0.139 | 0.12 |
| 0.2092 | 0.2005 | 0.176 | 0.2139 | 0.1973 | 0.2192 | 0.216 | 0.2427 | 0.244 | 0.2276 | 0.2293 | 0.2267 | *** | 0.1355 |
| 0.2226 | 0.1953 | 0.1814 | 0.2165 | 0.2134 | 0.2194 | 0.2027 | 0.2428 | 0.2443 | 0.2305 | 0.232 | 0.2294 | 0.1467 | *** |