Molecular Ecology (1999) 8, S117–S127

Molecular genetic analysis among subspecies of two Eurasian sturgeon species, *Acipenser baerii* and *A. stellatus*

P. DOUKAKIS,*† V. J. BIRSTEIN,* G. I. RUBAN‡ and R. DESALLE*
*American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA, †Department of Ecology/Evolutionary Biology, Graduate School of Arts and Science, Yale University, New Haven, CT 06511 USA, ‡Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii prospekt 33, Moscow, 117071 Russia

Abstract

Two species, the Siberian sturgeon, *Acipenser baerii*, and stellate sturgeon, *A. stellatus*, were studied using mitochondrial DNA (mtDNA) (D-loop, cytochrome *b* (cyt-*b*) and ND5/6 genes) sequencing to determine whether traditionally defined subspecies correspond to taxonomic entities and conservation management units. Initially, several mtDNA regions for each taxon (*A. baerii*: 737 bp D-loop, 750 bp ND5, 200 bp ND6, and 790 bp cyt-*b*; *A. stellatus*: 737 bp D-loop and 600 bp ND5) were examined. The D-loop was the most variable region and was sequenced for 35 *A. baerii* and 82 *A. stellatus* individuals. No fixed, diagnostic differences were found between any of the subspecies. Geographical structuring of haplotypes was observed within *A. baerii*, and gene flow estimates suggest isolation of the *A. baerii baicalensis* subspecies and the Yenisie and Lena River populations. No intraspecific subdivisioning was found within the genetic data for *A. stellatus*. The use of the phylogenetic criterion (fixed diagnostic differences) for identifying conservation units is compared to the rationale and results of other methods. Overall, morphologically and geographically based subspecies designations within Acipenseridae may not directly correspond to the biological entities appropriate for management and should not be used for conservation programmes without genetic support.

Keywords: *Acipenser baerii*, *Acipenser stellatus*, conservation, mtDNA, sturgeon, subspecies

Received 11 February 1999; revision received 19 May 1999; accepted 10 August 1999

Introduction

Habitat degradation and overexploitation for caviar have caused unprecedented population declines in many sturgeon and paddlefish species (Order Acipenseriformes) (Birstein 1993; Barannikova *et al.* 1995; Khodorevskaya *et al.* 1995, 1997; DeMeulenaer & Raymakers 1996). As a protective measure, several species-level conservation plans have recently been established, such as the 1998 CITES listing of all Acipenseriformes and the subsequent regulation of the caviar trade (Birstein 1993; Birstein *et al.* 1997; 1998a,b). Efforts must now focus on protecting the diversity residing within species by altering harvest and hatchery release programmes to sustain locally adapted gene pools.

Defining conservation units for sturgeons is particularly difficult. Similar to many fishes, sturgeons and paddlefishes exhibit a great deal of morphological plasticity and difficulty exists in defining good characters for designating species, subspecies and stocks (Bemis *et al.* 1997; Birstein & Bemis 1997). Recent studies indicate that many of the morphological and ecological characters used to initially define subspecies and stocks are ecophenotypic and are therefore not heritable indicators of genetically distinct entities. Geographical isolation may also be an unreliable proxy for defining intraspecific units due to the extensive history of population management and stock translocation for many sturgeon species. As the existing subspecies and stock designations are based on morphological, geographical and ecological characters, these units may not correspond to the appropriate management units. Under these circumstances traditionally defined subspecies and populations can be used as first approximations for management units and further analysed using a molecular genetic approach (Avise 1989, 1994; O'Brien & Mayr 1991; O'Brien 1994).

Correspondence: P. Doukakis. American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA. Fax: +1-212-769-5277; E-mail: doukakis@amnh.org

Many current fisheries management practices and protective programmes for sturgeons use traditionally defined subspecies. For the Siberian sturgeon, *Acipenser baerii*, Red Book and Convention on Migratory Species listings recognize and protect separate subspecies (*A. baerii baerii* (Ob River), *A.b. baicalensis* (Lake Baikal), and *A.b. stenorrhynchus* (Lena and Yenisie rivers)). *A. baerii* represents a typical potamodromous sturgeon species existing as a continuous series of resident populations in most Siberian river systems and in Lake Baikal (Ruban 1997, 1998). Subspecies of the anadromous stellate sturgeon, *A. stellatus*, correspond to isolated populations in the Caspian (*A. stellatus stellatus*), Black (*A.s. ponticus*), and Azov (*A.s. donensis*) seas (reviews in Berg 1948; Chugunov & Chugunova 1964; Movchan 1966; Shubina *et al.* 1989; Tsvetnenko 1993). Restocking plays a vital role in sustaining stellate sturgeon populations but few studies have considered the intraspecific units appropriate for these programmes (Chebanov 1996; Khodorevskaya *et al.* 1997). As these two sturgeon species differ greatly in distribution patterns and life-history characters, genetic study of subspecies designations offers a general case study in the overall utility of named subspecies or subspecies-defining characters within a management context.

Rapidly evolving mitochondrial DNA (mtDNA) regions are the most appropriate for intraspecific studies and have proven useful in studies of many fishes including sturgeons (Meyer 1993, 1994; Pourkazemi *et al.* 1994, 1995, 1997; Bembo *et al.* 1995; Bernatchez 1995; Toline & Baker 1995; Ong *et al.* 1996; reviewed in Wirgin *et al.* 1997b). The interpretation of these data for defining management units is, however, less straightforward. As our study primarily focuses on the subspecies level, the phylogenetic species concept and population aggregation analysis (PAA) (Davis & Nixon 1992) can be applied. Although we consider diagnostic nucleotides as unambiguous evidence of phylogenetic uniqueness and taxonomic validity, we also further explore alternative methods for defining units of conservation priority within this important group of fishes.

Table 1 Samples examined in current study

Subspecies*	N	Code	GenBank (D-loop sequence)	Sample	Collection location	Collector
A. baerii baerii	11	*A. b. b.* 1–11	AF168469–79	muscle	Ob River	G. Ruban
A.b. stenorrhynchus	4	*A. b. s.* Y1–4	AF168480–83	muscle	Yenisei River	G. Ruban
	10	*A. b. s.* L1–10	AF168484–93	muscle	Lena River (Konakovo Hatchery)†	G. Ruban, V. Birstein
	1	*A. b. s.* Lfc	AF168494	eggs(fc)	Lena River (Konakovo Hatchery)†	G. Ruban, V. Birstein
	2	*A. b. s.* Lcav	AF168595§	caviar(cav)	Lena River (Bordeaux, France, aquaculture),	R. Billard, P. Williot
A.b. baicalensis	7	*A. b. baic* 1–7	AF168496–502	muscle	Lake Baikal‡ (Konakovo Hatchery)	G. Ruban, V. Birstein
A.s. stellatu	4	*A. s. s.* 1–4	AF168503–06	muscle	Caspian Sea	A. Vlasenko
	4	*A. s. s.* 5–8	AF168507–10	muscle	Caspian Sea	P. Vecsei
	1	*A. s. s.* 9	AF168511	muscle	Volga River	A. Vlasenko
	1	*A. s. s.* 10	AF168512	muscle	Ural River	T. Gulyas
A.s. ponticus	32	*A. s. p.* 1–32	AF168513–44	blood	Danube River (Black Sea)	R. Suciu
	1	*A. s. p.* 33	AF168545	juvenile	Dnieper River (Black Sea)	J. Waldman
A.s. donensis	30	*A. s. d.* 1–30	AF168546–75	muscle	Sea of Azov (near Krasnodar)	N. Mugue
	9	*A. s. d.* 31–39	AF168576–84	muscle	Sea of Azov	P.Vecsei

*For subspecies of *A. baerii*, see Ruban (1997, 1998); for subspecies of *A. stellatus*, see Chugunov & Chugunova (1964); Tsvetnenko (1993).
†In the early 1970s, fertilized eggs of *A. baerii* from the Lena River population were brought to the Konakovo Hatchery (about 50 miles from Moscow) (Sokolov *et al.* 1976). Since then, the hatchery keeps a captive stock of the Lena River Siberian sturgeon. In the late 1970s, eggs from these sturgeon were sent to Bordeaux (France). Now there is a captive stock of *A. baerii* at the CEMAGREF Institute that is used for scientific studies and there are aquacultures of *A. baerii* at sturgeon farms in the same area near Bordeaux (Sabeau 1997).
‡In 1988, larvae of the Baikal sturgeon, *A. baerii baicalensis*, were brought to the Konakovo Hatchery (Podushka 1995). Currently, there is a small stock of mature Baikal sturgeon at this hatchery.
§Identical sequences.

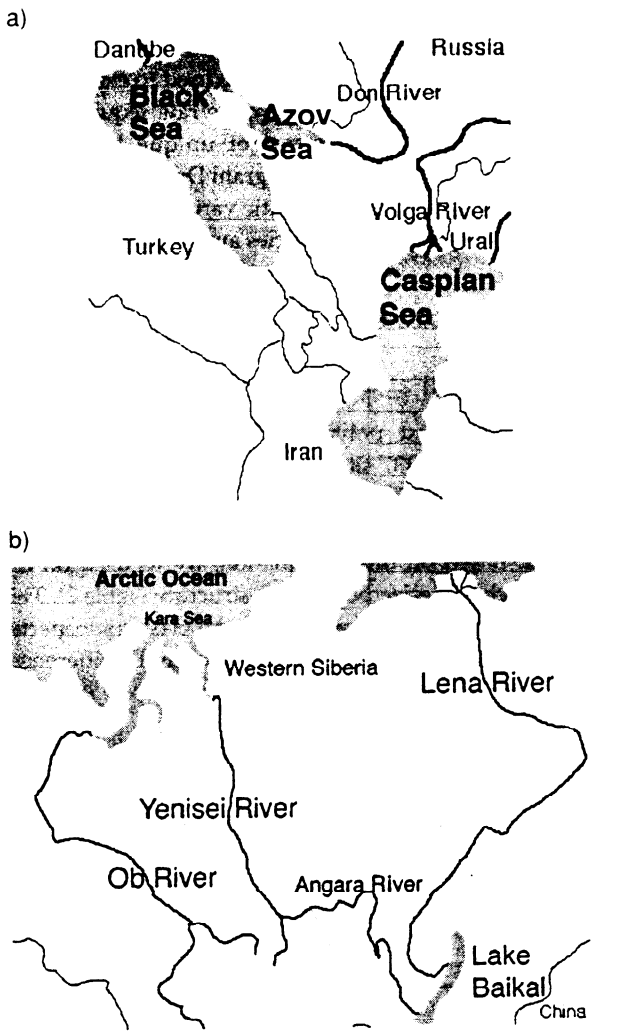

Fig. 1 Map illustrating the range of study species. (a) *Acipenser stellatus*; (b) *A. baerii*.

Materials and methods

Samples

The number and location of sturgeons used in this study are given in Table 1 and Fig. 1. Muscle tissue samples from *Acipenser baerii baerii* were obtained from specimens caught in the Ob River. Samples from *A. b. stenorrhynchus* were taken from the Yenisei River wild sturgeon, from the Lena River sturgeon kept at the Konakovo Hatchery (approximately 50 miles from Moscow, Russia) and from sturgeons aquacultured in France (caviar). Fishes at the Konakovo Hatchery and French aquaculture stocks originally were brought from the Lena River in the 1970s (Sokolov *et al.* 1976). The Baikal sturgeon samples were obtained from individuals of the unique stock of this subspecies kept at the Konakovo Hatchery (Podushka 1995).

Tissue and blood samples of *A. stellatus* were taken from individuals belonging to all subspecies (Shubina *et al.* 1989): (i) the Caspian Sea subspecies (sturgeons were caught in the sea and in the main rivers used by *A. stellatus* for spawning (Volga and Ural)); (ii) the Black Sea subspecies (the Danube and Dnieper river populations); and (iii) the Sea of Azov subspecies.

DNA extraction and manipulation

All tissue, egg, and blood samples were stored in 70% ethanol. DNA was extracted using standard phenol–chloroform extraction protocols (see DeSalle *et al.* 1993). For each of 15 *A. baerii* individuals, a total of approximately 2.4 kb of nucleotide data (737 bp D-loop + tRNA, 750 bp ND5, 200 bp ND6, and 790 bp cyt-*b*) were examined. For each of 11 *A. stellatus* individuals, 1.3 kb of nucleotide data (737 bp D-loop and 600 bp ND5) were initially studied. Additionally, 35 *A. baerii* and 82 *A. stellatus* individuals were examined for the D-loop only. cyt-*b* was not examined for *A. stellatus* because prior studies indicated little intraspecific variation (Birstein *et al.* 1998b). Also, primer incompatibility prohibited study of ND6 for *A. stellatus*.

PCR amplification primers consisted of the following: (1) control region: dLp1.5 (Baker *et al.* 1993) and H00651 (5′-TAACTGCAGAAGGCTAGGACCAAACCT-3′) (Kocher *et al.* 1989) or modified H00651 (5′-ATCTTAACATCTTCAGTG-3′); (2) ND5: ND5 (5′-AATAGTTTATCCAGTTGGTCTTAG-3′) (Bembo *et al.* 1995) and RND5–3 (5′-AAGCCCATGAGTGGTAGG-3′); (3) ND 5/6: ND6 (5′-TTACAACGATGGTTTTTCATAGTC-3′) (Bembo *et al.* 1995) and ND5–3 (5′-AGCAACCCTACCACTCATGGG-3′); (4) cyt-*b*: B1 (5′-CCATCCAACATCTCTGCTTGATGAAA-3′) (J. Groth, Department of Ornithology, AMNH) and S2A (5′-CCTCCAATTCATGTGAGTACT-3′). All PCR was conducted in a Perkin-Elmer 480 thermocycler (PE Biosystems, Foster City, CA, USA) under the following conditions: D-loop and cyt-*b*: 94 °C for 1 min, 46 °C for 1 min, 72 °C for 1 min 50 s for 33 cycles; both ND5/6 reactions: 94 °C for 1 min, 48 °C for 1 min, 72 °C for 1 min 30 s for 33 cycles. No initial denaturation step was used. For the ND 5/6 fragment, 200 bp of this region were sequenced using ND6 and 150 bp were sequenced using ND5–3. For the purposes of sequencing the larger amplified fragments of the D-loop and cyt-*b* internal primers were designed every 300 base pairs. All PCR products were purified with BIO 101 Gene Clean system (BIO 101, Inc., La Jolla, CA, USA) before sequencing. All sequencing protocols followed manufactureres (PE Biosystems) specifications for cycle sequencing reactions and ABI 373/377 operation (PE Biosystems). Sequences were initially aligned and analysed using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, MI, USA) and then transferred to MacClade version 3.01 (Maddison & Maddison

1992) and PAUP version 4.0b2 (Swofford 1999) for further analysis.

Data analysis

Population aggregation analysis (PAA) (Davis & Nixon 1992), based on the phylogenetic species concept (Cracraft 1989), was used to search for fixed, diagnostic characters within the hypothesized intraspecific units. PAA was used to differentiate between characters (fixed nucleotide states) and traits (variable or polymorphic sites) amongst the attribute data (nucleotide sequence data) (Davis & Nixon 1992). Characters (fixed nucleotide states) were held as evidence of phylogenetic uniqueness only if shared by all individuals within the hypothesized unit. A lack of diagnostic character differences dictated reliance on alternative, population genetics-based methodology (Vogler & DeSalle 1994).

Standard tree-building methods as implemented in PAUP 4.02b were employed to visualize geographical structuring of haplotypes and explore character support for defined subspecies. Heuristic searches were conducted using only phylogenetically informative characters in the tree bisection reconnection (TBR) branch-swapping option. Jacknife and bootstrap analyses were used to examine the robustness of clades. Additionally, the raw sequence data was examined for the presence of unique haplotypes within each subspecies. The program DnaSP2.2 was used to calculate the partition of genetic variation (G_{ST}) (Lynch & Crease 1990) for each subspecies and population.

Results and Discussion

Acipenser baerii

For *A. baerii*, most polymorphic sites were identified within the D-loop (37 sites) while few informative, polymorphic sites were found within the ND5 and cyt-*b* genes (3 sites and 1 site, respectively). The ND6 sequences were identical. Based on this information, we focused on the D-loop to characterize population structure (Table 2). This genetic evidence does not support the classic subspecies designations for *A. baerii* because no fixed differences

Table 2 Variable characters for *Acipenser baerii* for combined cyt-*b*, ND5 and D-loop data matrix

A.b. baicalensis 1	ATTTAGCGGCTCATGCTTTAAAGAAGGTTATATATGG
A.b. baicalensis 2	.....
A.b. baicalensis 3	.....
A.b. baicalensis 4	G....T.....
A.b. baicalensis 5	.....
A.b. baicalensis 6	.....
A.b. baicalensis 7	.....
A.b. baerii 1	G..C..T......CA.....A..GG.CGGA.
A.b. baerii 2	.....
A.b. baerii 3	G..C..T.A....C.....A..GG..GG..
A.b. baerii 4	G..C..T.....AA..GG..GG..
A.b. baerii 5	G..C..T......C.....A..GG..GG..
A.b. baerii 6	G..C..T.A....C.....A..GG..G...
A.b. baerii 7	G..C..T.A....C.....A..GG..GG..
A.b. baerii 8	GCC..CTAA.CT...T..CGG...G..C.GGG.GG..
A.b. baerii 9	......T.....G....C.....A..GG..GG..
A.b. baerii 10	......T..T.....A..GG..GG.A
A.b. baerii 11	G..C..T.A....C.....A..GG..GG..
A.b. stenorrhynchus Lena1	G..C..T.....A..GG..GG..
A.b. stenorrhynchus Lena2	G..C..T.....A..GG..GG..
A.b. stenorrhynchus Lena3	G..C..T.....A..GG..GG..
A.b. stenorrhynchus Lena4	G..C..T.....A..GG..GG..
A.b. stenorrhynchus Lena5	G..C..T.....A..GG..GG..
A.b. stenorrhynchus Lena6	G..C..T.....A..GG..GG..
A.b. stenorrhynchus Lena7	G..C..T.....A..GG..GG..
A.b. stenorrhynchus Lena8	G..C..T.....TG..A..GG..GG..
A.b. stenorrhynchus Lena9	G..C..T.....A..GG..GG..
A.b. stenorrhynchus Lena10	G..C..T.....A..GG..GG..
A.b. stenorrhynchus L.eggs	G..CC.T.....A..GG..GG..
A.b. stenorrhynchus L.cav(2)	G..C..T.....A..GG..GG..
A.b. stenorrhynchus Yen1	G..C..T......C.....G....A.CGG..GG..
A.b. stenorrhynchus Yen2	G..C..T......C.....G....A..GG..GG..
A.b. stenorrhynchus Yen3	G..C..T......C.....G....A..GG..GG..
A.b. stenorrhynchus Yen4	......T.....C.....A..GG..GG..

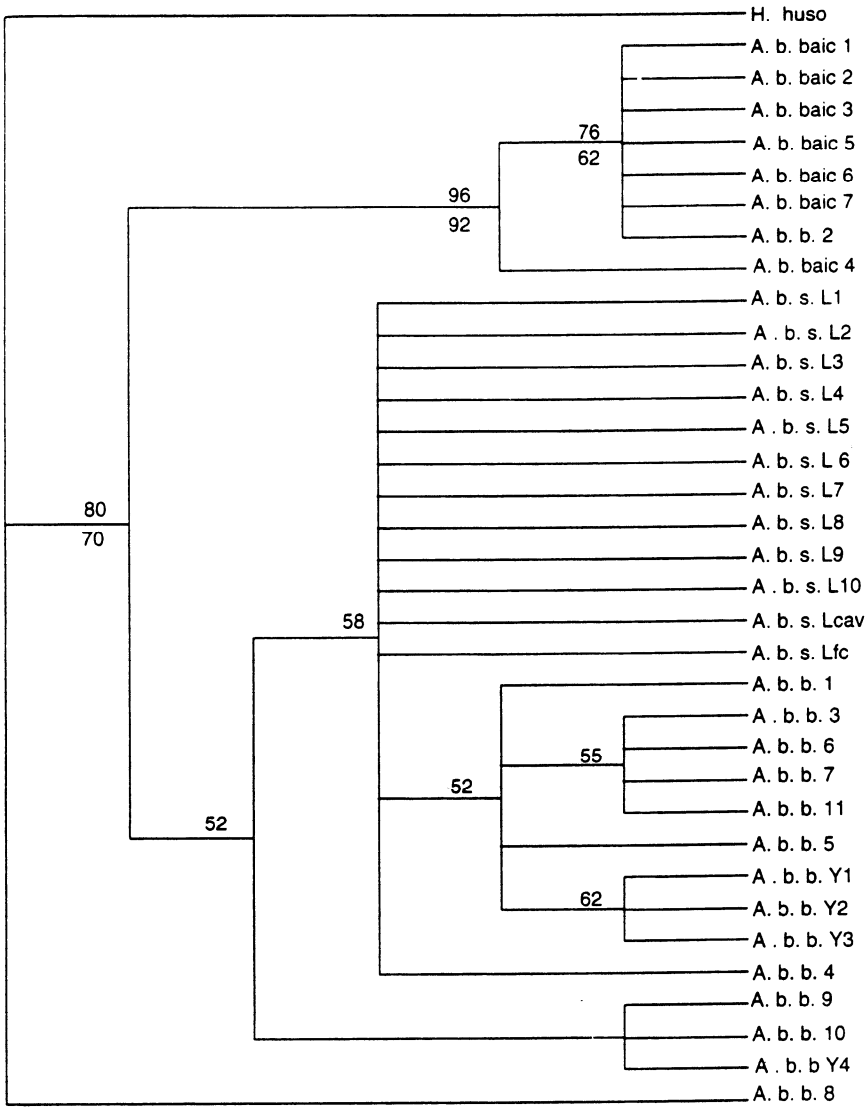

Fig. 2 Single parsimony tree obtained through heurisitc search of the D-loop data set using multiple individuals of *Acipenser baerii*. Bootstrap and jacknife values are given above and below the nodes, respectively. Nodes without values indicate less than 50% support. A. b. b. = *A. baerii baerii*; A. b. s. L = *A. baerii stenorrhynchus* Lena River; A. b. s. Y = *A. baerii stenorrhynchus* Yenisei River; A. b. baic = *A. baerii baicalensis*.

were observed. However, tree building, inspection of raw data and gene flow estimates indicate uniqueness of certain subspecies and populations. Seventeen of the 737 bp of the D-loop data were parsimony informative and a heurisitc search of these data yielded a single tree (Fig. 2). Visual inspection of the raw data (Table 2) combined with the results of the parsimony analysis suggest that the Lake Baikal subspecies and the Yenisei river population are distinct. *A.b. baicalensis* harbours a unique haplotype which is shared with only one specimen of *A.b. baerii* from the Ob River (no. 2). Had this individual (Ob no. 2) not been examined, it would have appeared as though *A.b. stenorrhynchus* and *A.b. baerii* could be grouped together to the exclusion of *A.b. baicalensis*. The Yenisei river population also harbours a haplotype which is unique to but not fixed within the population. As our current sample size is small, this apparent isolation might change with a more extensive study. Gene flow estimates (G_{ST}) (Fig. 3a) illustrate the highest levels of isolation between Lake Baikal *A. baerii* and other subspecies. Interestingly, high G_{ST} values were also observed between Lena and Yenisei River populations which belong to the same subspecies.

The lack of diagnostic differences between *A. baerii* subspecies is concordant with the results of previous studies. In these studies many of the original subspecies-defining characters of snout morphology, time and site of spawning, and diet were found to be environmentally controlled (Ruban 1992, 1997, 1998). The lack of diagnostic differences found in the electrophoretic spectra of muscle proteins between Ob, Yenisei, and Lena River populations further supports these findings (Kuz'min 1994). This concordance of genetic, morphometric and meristic information suggests that subspecies status may be unwarranted. Instead, it appears that there is or has recently been gene flow between *A. baerii* populations in the Ob, Yenisei, and

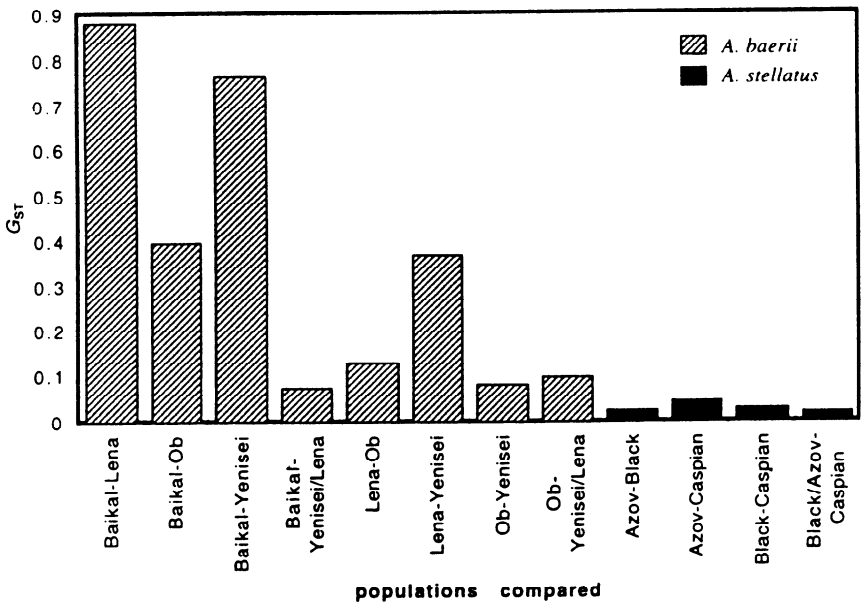

Fig. 3 Graphic representation of G_{ST} values between populations and subspecies of *Acipenser baerii* and *A. stellatus*. *A. baerii* is represented by cross-hatch bars and *A. stellatus* by solid black bars.

Lena River basins and that the Lake Baikal population is distinct from these other populations.

Geological factors have certainly played a role in shaping the intrapsecific structure of *A. baerii*. During the glaciation age, the Siberian rivers were repeatedly cut off from the seas and their basins were connected through a system of lakes (Arkhipov 1998; Grosswald 1998) with the last connection occurring approximately 18 000–20 000 (Laukhin 1997) or even 10 000–15 000 years ago (Arkhipov 1998). This relatively recent connection suggests that sufficient evolutionary time has not elapsed for fixation of nucleotide changes in different subspecies of *A. baerii*. A lack of fixed D-loop haplotype differences was also observed among postglacial populations for another potamodromous sturgeon species, the North American lake sturgeon, *A. fulvescens* (review in Ferguson & Duckworth 1997). Similarly, no fixed differences were found in the D-loop between a postglacial population and its parental population of the anadromous white sturgeon, *A. transmontanus* (Brown *et al.* 1992, 1993). The recolonization of the Fraser River by *A. transmontanus* from the Columbia River refugium occurred about 10 000–12 000 years ago, which is apparently not a sufficient time for fixed genetic divergence, although a difference in the frequency of shared haplotypes was apparently established.

Further studies are necessary before our results are directly utilized for management purposes due to the nature of our sampling. Although *A.b. baicalensis* could be considered a distinct conservation unit, our samples for this subspecies were obtained from a captive population started in the 1970s from one or possibly two breeding pairs. It is questionable whether the isolation observed here is representative of the natural Lake Baikal population or simply an artefact of the captive situation. Unfortunately, the wild population of *A.b. baicalensis* is potentially extinct (Afanasiev & Afanasieva 1996) and therefore obtaining wild-caught individuals for comparison may be impossible. Future restocking programmes for the Lake Baikal sturgeon will have to consider this issue as well as the low haplotypic diversity observed within the captive *A.b. baicalensis* population. The Ob River subspecies should be considered as a source population for such a restocking programme because it contains the Lake Baikal major haplotype (Ob no. 2) and has the greatest overall number of different haplotypes. A further investigation of the Yenisie and Lena river populations should also be conducted including larger sample sizes, finer-scale sampling and wild-caught Lena River individuals

A forensic method is currently needed for discriminating between the eggs and meat of aquacultured and wild-caught sturgeons. Currently, the populations of the nominal *A.b. baerii* in the Ob River and *A.b. stenorrhynchus* of the Yenisei River are especially affected by overfishing for meat and caviar (Ruban 1996, 1997; Sololov 1997). Caviar from aquacultured, Lena River Siberian sturgeon is now produced in France (Sabeau 1997) providing a viable alternative to wild-caught commercial caviar. Our studies suggest that the D-loop will not be useful for tracing the origin of harvest. Genetically tagging aquaculture populations may be the best way to identify eggs from aquacultured and wild *A. baerii* females.

A. stellatus

Our results also do not support subspecies designations within the stellate sturgeon. No fixed differences were

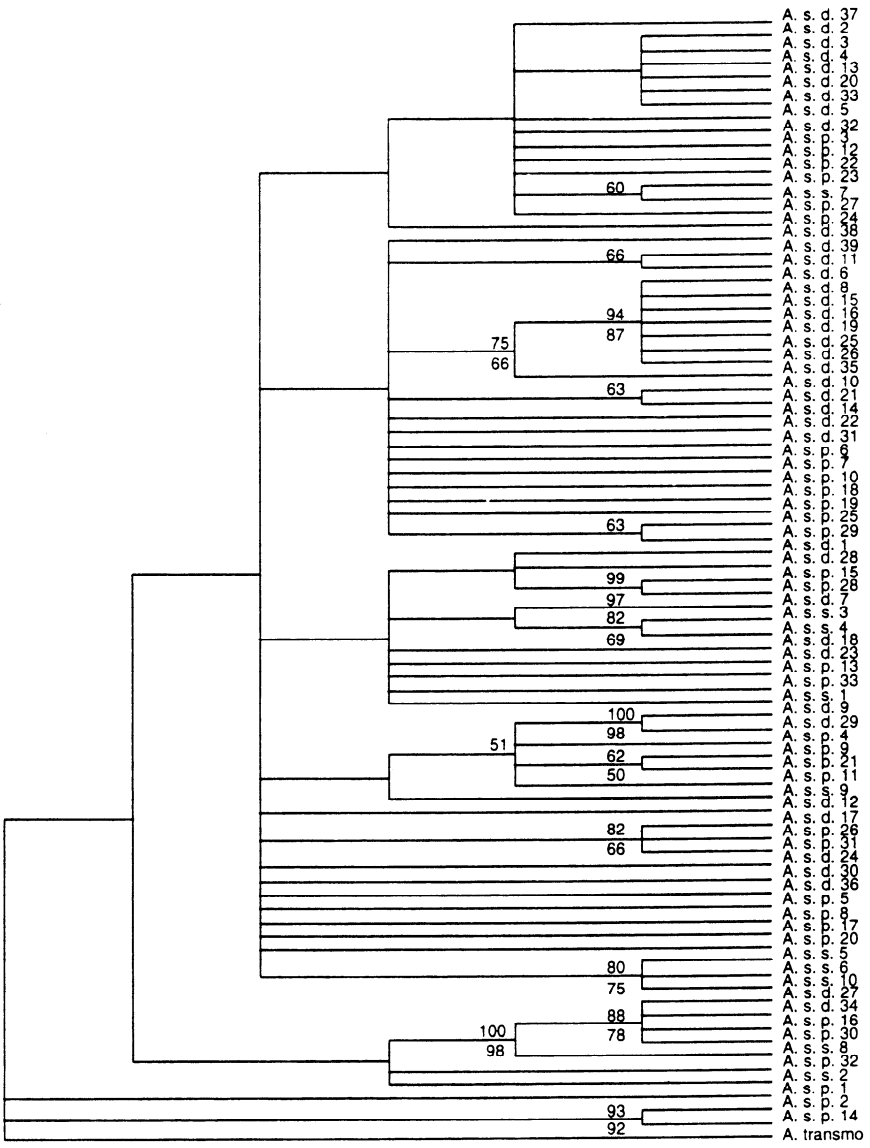

Fig. 4 Strict consensus of 18 900 trees obtained through heuristic search of the D-loop data set using multiple individuals of *Acipenser stellatus*. Bootstrap and jacknife values are given above and below the nodes, respectively. Nodes without values indicate less than 50% support. A. s. d. = *Acipenser stellatus donensis*; A. s. p. = *A. stellatus ponticus*; A. s. s. = *A. stellatus stellatus*.

observed within the control region sequences (sequence available upon request) and the ND5 gene region sequences were identical for the 11 screened individuals. The parsimony tree shown in Fig. 4, constructed using the 78 parsimony informative characters within the 737-bp D-loop data set, does not support structuring of haplotypes by geography and/or subspecies designation. This strict consensus of 18 900 trees is also not well supported under bootstrap and jacknife analysis. Low G_{ST} values confirm the PAA and tree-building results (Fig. 3b). The overall lack of structure grouping the Ural River/Volga River/Caspian Sea individuals, Black Sea individuals or Sea of Azov individuals suggests that these classically designated subspecies are genetically contiguous.

Our study corroborates historic skepticism regarding the use of subspecies nomenclature within *A. stellatus*. The Caspian Sea (*A. stellatus stellatus*) and the Sea of Azov (*A.s. donensis*) subspecies were originally based on average head proportions and anal and dorsal fin characters, and later on rates of growth and maturity (Chugunov & Chugunova 1964; Tsvetnenko 1993). Holcik & Jedlicka (1994) concluded that these original characters change with growth and feeding and that designations based on such attributes are questionable. Similarly, Birstein & Bemis (1997) agree that such taxonomic nomenclature should not be used without the support of genetic evidence. The results of our genetic study combined with the lack of differences found among muscle protein profiles from stellate sturgeon from the Volga River, Ural River, Caspian and Azov seas from other studies (Kuz'min 1994) do not support subspecies designations.

As the subspecies studied here are presently confined to isolated water bodies, gene flow through either geological or human-induced influences (translocation) must have

occurred fairly recently to explain our results. Repeated water connections between the Caspian and Azov–Black seas have been recorded over the last 1.5 million years, the last of which occurred approximately 20 000–40 000 years ago (Zubakov 1988). Studies of Caspian and Black Sea populations of a salmonid (also an anadromous species) and a brown trout *Salmo trutta* (Bernatchez & Osinov 1995; Bernatchez 1995; Osinov & Bernatchez 1996) also reported no fixed genetic differences between these two basins but, in contrast to our study, differences in geographical structuring of haplotypes were observed. The comparison of these results to ours suggests that restocking may have been a major influence shaping the intraspecific structure of *A. stellatus*.

Ongoing restocking programmes are the only means of sustaining viable populations for many populations of stellate sturgeon in the Caspian, Azov and Black seas. In the early 1960s, the Soviet Union started releasing juvenile sturgeon from 28 specially built hatcheries in response to the loss of spawning grounds due to river damming (Barannikova *et al.* 1995; Khodorevskaya *et al.* 1997). In the 1980s, approximately 100-million hatchery-raised juveniles were released annually into the Caspian Sea but this number has recently dropped by 50% because of economic problems in Russia. From 1961 to 1968, Caspian Sea *A. stellatus* were introduced into the Sea of Azov as part of a restocking effort (Tsvetnenko 1993). This particular interstock transfer has been regarded as largely unsuccessful due to the low viability of introduced Caspian Sea stellate sturgeon in the Sea of Azov environmental conditions. Our genetic data suggest that the restocking programme may have been more successful than previously thought. Restocking programmes still exist in the Caspian (Volga River, Russia; Kura River, Azerbaijan; Sefidrud River, Iran) and Azov (Kuban River, Russia) seas (Chebanov 1996; Khodorevskaya *et al.* 1997; R. Kasimov and M. Pourkazemi, personal communication). None of these restocking programmes have been accompanied by genetic stock analysis. Testing whether massive restocking has affected the premanagement genetic structure could be accomplished through examining museum specimens collected before restocking. Formalin preservation of many museum specimens may make this study difficult (Vachot & Monnerot 1996; Wirgin *et al.* 1997a), but would provide clues as to whether geology or human influences have caused the absence of population structure found here.

Fine-scale analysis is needed to further characterize *A. stellatus* stock structure. This species is heavily exploited for sevruga caviar, and dam construction and environmental pollution has led to a 60% loss of historic spawning grounds as well as range and population size decreases (Shubina *et al.* 1989; Birstein 1993; Khodorevskaya *et al.* 1997). Attention should focus on whether management units correspond to natal rivers used for spawning as the degree of homing fidelity within *A. stellatus* is still questionable. Individuals may assort randomly to different river systems for spawning within each sea. As the availability of reproductive age fish is a major hindrance to stellate sturgeon restocking programmes it is essential to examine this issue to address whether future interstock transfers would be detrimental. Commercially viable populations still exist in the Volga, Ural, Kura and Iranian rivers of the Caspian Sea, the Danube river of the Black Sea and the Kuban river of the Sea of Azov making these populations good candidates for initial study. The D-loop may be useful for this work, but ND5 does not appear to be sufficiently variable (see also Pourkazemi *et al.* 1994, 1995; Gilkolaei & Skibinski 1999). Mixed DNA fingerprinting such as that used for establishing intraspecies conservation units in salmonids (Angers & Bernatchez 1998; Cummings *et al.* 1998; Wenburg *et al.* 1998) might be appropriate to further characterize these populations.

Management units

Although there are several methods available for defining units of conservation, we have chosen to use the phylogenetic approach (using the PSC and PAA) to examine sturgeon subspecies. The appeal of this method lies in its simplicity, objectivity and practical utility for endangered species: diagnostic characters can be easily and unambiguously identified and few individuals are needed for initially screening for diagnosis. We recognize that this approach is limited, however, to studies of genetically isolated units and that it can only delimit the boundary below which population genetics-based methodology applies. Alternatively, we could have used several other approaches including haplotype or allele frequency differences, reciprocal monophyly (ESUs), or genetic distance measures (Avise 1994; Moritz 1994, 1995). Yet the interpretation and sampling problems associated with these methods make them less practical. No objective boundaries exist for delimiting taxonomic or conservation units based on haplotype frequency differences or degree of genetic distance (O'Brien & Mayr 1991; Wayne 1992; O'Brien 1994; Vogler & DeSalle 1994; Legge *et al.* 1995; also see Dizon *et al.* 1992; Waples 1995). Furthermore, the large sample sizes and temporal sampling strategy necessary for accurate and stable frequency estimates make these methods particularly difficult in studies of endangered species. Without knowing which method accurately reflects intraspecific structure and what degree of subdivision justifies separate conservation, the exercise of defining management units could become highly subjective.

Here we have attempted to maintain objectivity by using a phylogenetic approach to examine units which we assumed were genetically isolated. When we were

unable to define any strict phylogenetic conservation units, we resorted to a more subjective approach (gene flow and haplotype analysis). As conservation units cannot currently be defined based on gene flow values or the presence of private haplotypes, we could only use this information to subjectively identify several intraspecific units requiring further conservation attention and then discuss the data in this context. These data show that the potadromous *A. baerii* has more intraspecific structure than the anadromous *A. stellatus*. Overall, the genetic, morphological and ecological evidence combined indicate that the existing subspecies designations do not directly correspond to conservation management units and the morphological, ecological and geographical characters used to define these subspecies are not useful for identifying conservation units in sturgeons.

Although we appreciate the necessity of articulating objective methodology and criteria for designating units of conservation, extenuating circumstances may direct the actual conservation programmes for many species. Realistically, fisheries management and protection programmes for sturgeons will be dictated by economics and resource availability and not genetic information. Restocking programmes for *A. stellatus* illustrate this well: the possibility of population extinction will have to be weighed against the detrimental effects of interstock transfer, because obtaining breeders from all populations may be impossible. For *A. baerii*, the last remaining stock of the Lake Baikal subspecies may be so highly inbred that interstock transfer from the Ob River may be a better alternative than stocking using the captive Lake Baikal population. Future conservation efforts for sturgeons may be more effectively directed towards population viability and habitat renewal programmes.

Acknowledgements

We are extremely grateful to all colleagues who provided us with samples of sturgeon muscles, blood or eggs: Dr Rolland Billiard (National Museum of Natural History, Paris, France), Mr Tamas Gulyas (Propa-Gen, Hungary), Dr Nikolai Mugue (Koltsov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia), Dr Radu Suciu (Danube Delta Institute, Tulcea, Romania), Mr Paul Vecsei (Guelph University, Canada), Dr Anatolii Vlasenko (Caspian Fisheries Research Institute, Astrakhan, Russia), Dr John Waldman (Hudson River Foundation for Science and Environmental Research, New York, USA), and Dr Patrick Williot (CEMAGREF, Bordeaux, France). This work was supported in part through an EPA STAR graduate fellowship to P. Doukakis.

References

Afanasiev GA, Afanasieva BG (1996) Status of resources and natural reproduction of the Baikal sturgeon. *Rybovodstvo I Rybolovstvo*, **2**, 6–7 (in Russian).

Angers B, Bernatchez L (1998) Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related Brook *char* (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. *Molecular Biology and Evolution*, **15**, 143–159.

Arkhipov SA (1998) Stratigraphy and paleogeography of the Sartan glaciation in West Siberia. *Quarternary International*, **45/46**, 29–42.

Avise JC (1989) A role for molecular genetics in the recognition and conservation of endangered species. *TREE*, **4**, 279–281.

Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York.

Baker CS, Perry A, Bannister JL *et al.* (1993) Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proceedings of the National Academy of Sciences of the USA*, **90**, 8239–8243.

Barannikova IA, Burtsev IA, Vlasenko AD *et al.* (1995) Sturgeon fisheries in Russia. In: *Proceedings of the International Symposium on Sturgeons. September 6–11, 1993, Moscow* (eds Gershanovich AD, Smith TIJ), pp. 124–130. VNIRO Publishing House, Moscow.

Bembo DG, Carvalo GR, Snow M *et al.* (1995) Stock discrimination among European anchovies, *Eugraulis encrasicolas*, by means of PCR amplified mtDNA analysis. *Fishery Bulletin*, **94**, 31–40.

Bemis WE, Findeis E, Grande L (1997) An overview of Acipenseriformes. In: *Sturgeon Biodiversity and Conservation* (eds Birstein VJ, Waldman JR, Bemis WE), pp. 25–71. Kluwer Academic Publishers, Dordrecht.

Berg LS (1948) The freshwater fishes of the USSR and adjacent countries. In: *Akademiya Nauk USSR, Moscow and Lenningrad, Part 1*. 4th edn (in Russian. English translation published by Israel Program for Scientific Translations, Jerusalem).

Bernatchez L (1995) A role of molecular systematics in defining evolutionary significant units in fishes. In: *Evolution and the Aquatic Ecosystem: Defining Units in Population Conservation. Symposium 17* (ed. Nielsen JL), pp. 114–132. American Fisheries Society, Bethesda, Maryland.

Bernatchez L, Osinov A (1995) Genetic diversity of trout (genus *Salmo*) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. *Molecular Ecology*, **4**, 285–297.

Birstein VJ (1993) Sturgeons and paddlefishes: threatened fishes in need of conservation. *Conservation Biology*, **7**, 773–787.

Birstein VJ, Bemis WE (1997) How many species are there within the genus Acipenser? In: *Sturgeon Biodiversity and Conservation* (eds Birstein VJ, Waldman JR, Bemis WE), pp. 25–71. Kluwer Academic Publishers, Dordrecht.

Birstein VJ, Bemis WE, Waldman JR (1997) The threatened status of acipenseriform fishes: a summary. In: *Sturgeon Biodiversity and Conservation* (eds Birstein VJ, Waldman JR, Bemis WE), pp. 427–435. Kluwer Academic Publishers, Dordrecht.

Birstein VJ, Betts J, DeSalle R (1998a) Molecular identification of *Acipenser sturio* specimens: a warning note for recovery plans. *Biological Conservation*, **84**, 97–101.

Birstein VJ, Doukakis P, Sorkin B, DeSalle R (1998b) Population aggregation analysis of caviar producing species of sturgeons and implications for diagnosis of black caviar. *Conservation Biology*, **12**, 766–775.

Brown JR, Beckenbach AT, Smith MJ (1992) Influence of Pleistocene glaciations and human intervention upon mtDNA diversity in white sturgeon (*Acipenser transmontanus*) populations. *Canadian Journal of Fisheries and Aquatic Sciences*, **49**, 358–367.

Brown JR, Beckenbach AT, Smith MJ (1993) Intraspecific DNA sequence variation of the mitochondrial control region of white sturgeon (*Acipenser transmontanus*). *Molecular Biology and Evolution*, **10**, 326–341.

Chebanov MS (1996) Ecological foundations of the optimal artificial reproduction of sturgeons. *Rybolovstvo I Rybolovstvo*, **2**, 9–12 (in Russian).

Chugunov NL, Chugunova NI (1964) A comparative commercial and biological characteristics of the Sea of Azov acipenserids. *Trudy VNIRO*, **51**, 87–182 (in Russian).

Cracraft J (1989) Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. In: *Speciation and its Consequences* (eds Otte D, Endler JA), Sinauer Associates, Inc., Sunderland, MA.

Cummings SA, Brannon EL, Adams KJ, Thorgaard GH (1998) Genetic analysis to establish captive breeding priorities for endangered Snake River sockeye salmon. *Conservation Biology*, **11**, 662–669.

Davis JI, Nixon KC (1992) Populations, genetic variation and the delimitation of the phylogenetic species. *Systematic Biology*, **41**, 421–435.

DeMeulenaer T, Raymakers C (1996) Sturgeons of the Caspian Sea and investigations of the international trade in caviar. TRAFFIC International, Cambridge.

DeSalle R, Williams AK, George M (1993) Isolation and characterization of animal mitochondrial DNA. In: *Methods in Enzymology. Molecular Evolution: Producing the Biochemical Data*, Vol. 224 (eds Zimmer EA, White TJ, Cann RL, Wilson AC), pp. 176–204. Academic Press, San Diego, CA.

Dizon AE, Lockyer C, Perrin WF, DeMaster DP, Sisson J (1992) Rethinking the stock concept: a phylogeographic approach. *Conservation Biology*, **6**, 24–36.

Ferguson MM, Duckworth GA (1997) The status and distribution of lake sturgeon, *Acipenser fulvescens*, in the Canadian provinces of Manitoba, Ontario and Quebec: a genetic perspective. In: *Sturgeon Biodiversity and Conservation* (eds Birstein VJ, Waldman JR, Bemis WE), pp. 299–309. Kluwer Academic Publishers, Dordrecht.

Gilkolaei SR, Skibinski DOF (1999) PCR and direct sequencing of mtDNA from the ND5/6 gene region in persian sturgeon *Acipenser persicus* from the southern Caspian Sea. *Iranian Journal of Fisheries Science*, **1**, 23–34.

Grosswald MG (1998) Late-Weichselian ice sheets in Arctic and Pacific Siberia. *Quarternary International*, **45/46**, 3–18.

Holcik J, Jedlicka L (1994) Geographical variation of some taxonomically important characters in fishes: the case of the bitterling *Rhodeus sericeus*. *Environmental Biology of Fishes*, **41**, 147–170.

Khodorevskaya RP, Dovgopol GF, Zhuravleva OL (1995) Formation of commercial sturgeon (Acipenserida) stocks. In: *Proceedings of the International Symposium on Sturgeons. September 6–11, 1993, Moscow* (eds Gershanovich AD, Smith TIJ), pp. 137–150. VNIRO Publishing House, Moscow.

Khodorevskaya RP, Dovgopol GF, Zhuravleva OL, Vlasenko AD (1997) Present status of commercial stocks of sturgeons in the Caspian Sea basin. In: *Sturgeon Biodiversity and Conservation* (eds Birstein VJ, Waldman JR, Bemis WE), pp. 209–219. Kluwer Academic Publishers, Dordrecht.

Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA*, **86**, 6169–6220.

Kuz'min YV (1994) Comparative analysis of the fractional composition of sarcoplasmic muscle proteins of different representatives of sturgeons (Acipenseridae). *Voprosy Ikhtiologii*, **34** (4), 548–556 (in Russian; English translation. *Journal of Ichthyology*, **34** (9), 111–124).

Laukhin SA (1997) The Late Pleistocene glaciation in the Northern Chukchi Peninsular. *Quaternary International*, **41/42**, 33–41.

Legge JT, Roush R, DeSalle R *et al.* (1995) Genetic criteria for establishing evolutionary significant units in Cryan's buckmoths. *Conservation Biology*, **10**, 85–98.

Lynch M, Crease TJ (1990) The analysis of population survey data on DNA sequence variation. *Molecular Biology and Evolution*, **7**, 377–394.

Maddison WP, Maddison DR (1992) *Macclade, Version 3*. Sunderland, MA. Sinauer Associates, Inc.

Meyer A (1993) Evolution of mitochondrial DNA in fishes. In: *Biochemistry and Molecular Biology of Fishes*, Vol. 2 (eds Hochachka PW, Mommsen TP), pp. 1–38. Elsevier, Amsterdam.

Meyer A (1994) DNA technology and phylogeny of fish. In: *Genetics and Evolution of Aquatic Organisms* (ed. Beaumont AR), pp. 219–248. Chapman & Hall, London.

Moritz C (1994) Application of mtDNA analysis in conservation: a critical review. *Molecular Ecology*, **3**, 401–411.

Moritz C (1995) Use of molecular phylogenies for conservation. *Philosophical Transactions of the Royal Society of London B*, **349**, 113–118.

Movchan YV (1966) Intraspecies variability of morphological characters in the Sea of Azov and Black Sea sevruga and Russian sturgeon populations. Izdatelstvo Akademii Nauk SSSR, Kiev (in Russian).

O'Brien J (1994) A role for molecular genetics in biological conservation. *Proceedings of the National Academy of Sciences of the USA*, **91**, 5748–5755.

O'Brien SJ, Mayr E (1991) Bureaucratic mischief: recognizing endangered species and subspecies. *Science*, **251**, 1187–1188.

Ong T, Stabile J, Wirgin I, Waldman JR (1996) Genetic divergence between *Acipenser oxyrhynchus oxyrhynchus* and *A. o. destoi* as addressed by mtDNA sequence analyses. *Copeia*, **2**, 464–469.

Osinov AG, Bernatchez L (1996). Atlantic and Danubian phylogenetic groupings of brown trout *Salmo trutta* complex: genetic divergence, evolution, and conservation. *Voprosy Ikhtiologii*, **36** (6), 762–786 (in Russian; English translation. *Journal of Ichthyology*, **36** (9), 723–746).

Podushka SB (1995) It is necessary to change a method of sturgeon breeding in captivity. *Rybnoe Khozyaistvo*, **2**, 31–32 (in Russian).

Pourkazemi M, Skibinsky DOF, Beardmore JA (1994) Population genetics studies of four sturgeon species in Iranian coastal waters of the Caspian Sea using molecular and biochemical markers. *Sturgeon Quarterly*, **2**, 7.

Pourkazemi M, Skibinsky DOF, Beardmore JA (1995) Population structure of stellate sturgeon, *Acipenser stellatus* Pallas, in southern part of the Caspian Sea studied using mtDNA and allozyme analysis. *Sturgeon Quarterly*, **3**, 10.

Pourkazemi M, Skibinsky DOF, Beardmore JA (1997) Application of mtDNA D-loop region for the study of Russian sturgeon population structure from Iranian coastline of the Caspian Sea. Abstracts of the 3rd International Symposium on Sturgeon. Piacenza, Italy, July, 8–11, 1997.

Ruban GI (1992) Plasticity of development in natural and experimental populations of Siberian sturgeon, *Acipenser baerii* Brandt. *Acta Zoologica Fennica*, **191**, 43–46.

Ruban GI (1996) The Siberian sturgeon, *Acipenser baerii baerii*, population status in the Ob River. *Sturgeon Quarterly*, **4**, 8–10.

Ruban GI (1997) Species structure, contemporary distribution and status of the Siberian sturgeon, *Acipenser baerii*. In: *Sturgeon Biodiversity and Conservation* (eds Birstein VJ, Waldman JR, Bemis W), pp. 221–230. Kluwer Academic Publishers, Dordrecht.

Ruban GI (1998) On the species structure of the Siberian sturgeon *Acipenser baerii* Brandt (Acipenseridae). *Voprosy Ikhtiologii*, **38** (3), 307–327 (in Russian; English translation. *Journal of Ichthyology*, **38** (5), 345–365).

Sabeau L (1997) Sturgeon aquaculture in France. In: *Caspian Environment Program. Proceedings from the First Bio-Network Workshop. Bordeaux, November 1997* (eds Dumond HS, Wilson Wazniewicz), pp. 43–49. World Bank.

Shubina TN, Popova AA, Vasil'ev, VP (1989) *Acipenser stellatus* Pallas, 1771. In: *The Freshwater Fishes of Europe*, Vol. 1, *Part II. General Introduction to Fishes, Acipenseriformes* (ed. Holcik J), pp. 395–443. AULA-Verlag, Weisbaden.

Sololov VP (1997) Present status of the populations of Acipenser baerii in the upper reaches of the Ob. *Journal of Ichthyology*, **37**, 41–47.

Sokolov LI, Malyutin VS, Smol'yanov II, Burtsev IA (1976) The first result of the Lena River Siberian sturgeon farming for commercial purposes and for its acclimatization in the inner water bodies of the USSR. In: *Fish Farming and the Improvement of Fish Farming Biotechnology*, pp. 75–78. Izdatelstvo Timpul, Kishinev (in Russian).

Swofford DL (1999) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b2*. Sinauer Associates, Sunderland, MA.

Toline CA, Baker AJ (1995) Mitochondrial DNA variation and population genetic structure of the northern redbelly dace (*Phoxinus eos*). *Molecular Ecology*, **4**, 745–753.

Tsvetnenko YB (1993) The effectiveness and genetic consequences of the introduction of the stellate sturgeon, *Acipenser stellatus*, into the Azov basin from the Caspian sea. *Journal of Icthyology*, **33**, 1–10.

Vachot AM, Monnerot M (1996) Extraction, amplification and sequencing of DNA from formaldehyde-fixed specimens. *Ancient Biomolecules*, **1**, 3–16.

Vogler AP, DeSalle R (1994) Diagnosing units of conservation management. *Conservation Biology*, **8**, 354–363.

Waples RS (1995) Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act. In: *Evolution and the Aquatic Ecosystem: Defining Units in Population Conservation. Symposium 17* (ed. Nielsen JL), pp. 8–27. American Fisheries Society, Bethesda, MD.

Wayne RK (1992) On the use of morphological and molecular genetic characters to investigate species status. *Conservation Biology*, **6**, 590–592.

Wenburg JK, Bentzen P, Foote CJ (1998) Microsatellite analysis of genetic population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). *Molecular Ecology*, **7**, 733–749.

Wirgin I, Maceda L, Stabile J, Mesing C (1997a) An evaluation of introgression of Atlantic coast striped bass mitochondrial DNA in a Gulf of Mexico population using formalin-preserved museum collections. *Molecular Ecology*, **6**, 907–916.

Wirgin II, Stabile JE, Waldman JR (1997b) Molecular analysis in the conservation of sturgeons and paddlefish. In: *Sturgeon Biodiversity and Conservation* (eds Birstein VJ, Waldman JR, Bemis WE), pp. 221–230. Kluwer Academic Publishers, Dordrecht.

Zubakov VA (1988) Climatostratigraphic scheme of the Black Sea Pleistocene and its correlation with the oxygen-isotope scale and glacial events. *Quarternary Research*, **29**, 1–24.

P. Doukakis is currently a doctoral student at Yale University with interests in conservation genetics, management policy and environmental law. Dr V. J. Birstein is currently a Visiting Scientist at the American Museum of Natural History (AMNH) and is the author of more than 100 papers and several monographs on genetics and conservation biology. Dr G. I. Ruban is a Senior Research scientist at the Russian Academy of Sciences and is the world authority on Siberian sturgeon. Dr R. DeSalle is codirector of the Molecular Laboratories at the AMNH where he is a member of the conservation genetics program at the Center for Biodiversity and Conservation.
