Российская академия наук
Институт истории естествознания и техники им. **СИ. Вавилова** Санкт-Петербургский филиал

ЭВОЛЮЦИОННАЯ БИОЛОГИЯ: ИСТОРИЯ И ТЕОРИЯ

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В сборник включены статьи сотрудников сектора истории эволюционной теории и экологии СПбФ ИИЕТ РАН, а также статьи специалистов из других учреждений, активно сотрудничающих с СПбФ ИИЕТ РАН. Статьи посвящены актуальным проблемам современной эволюционной биологии и важным страницам ее истории.

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Предисловие

Сектор истории эволюционной теории и экологии Санкт-Петербургского филиала Института истории естествознания и техники РАН выпускает очередной сборник. В нем помещены статьи сотрудников СПбФ ИИЕТ РАН и известных специалистов в области эволюционной и молекулярной биологии. При подготовке сборника была поставлена цель ознакомить читателя с историко-биологическими исследованиями, которые ведутся в филиале, и с последними работами в эволюционной биологии, в которых ставится задача увязать традиционные подходы с самыми современными, ведущимися на молекулярно-генетическом и эпигенетическом уровнях. В одном томе охвачена большая тематика по теории эволюции и ее истории.

Настоящий сборник посвящен 100-летию со дня рождения выдающегося биолога-эволюциониста Ефима Иудовича Лукина.

N. V. Tomilin

On the role of «junk» DNA in regulation of gene expression and cell differentiation

Most of non-coding DNA in eukaryotic genomes plays an important role in regulation of gene expression and cell differentiation and, therefore, strongly contribute to phenotype. This DNA controls protein- and RNA-coding genes stimulating formation of compact chromatin or heterochromatin, which depends on its ability produce small interfering RNAs (tandem repeats, some families of mobile genes), as well as its ability to bind protein repressors of transcription (retrotransposons). Constitutive and facultative heterochromatin define unique 3D structure of cell nucleus and control gene expression during ontogenesis. System mutations in macroevolution probably also depend on rearrangements of non-coding DNA. Therefore, this DNA is not «parasitic» or «junk».

Vadim J. Birstein

Phytogeny and Evolution of Acipenseriformes: New Molecular and Genetic Data Create New Puzzles

The order Acipenseriformes is a group of ancient fishes that includes two families, the Polyodontidae (two extant species of paddlefishes, *Polyodon spathula* and *Psephrus gladius),* and the Acipenseridae (25 usually recognized extant species of *Acipenser, Huso, Pseudoscaphirhynchus,* and *Scaphirhynchus)* (Table 1). This is the most numerous group among all living «fossil» fishes (Gardiner, 1984). Acipenseriforms inhabit the Northern Hemisphere only, and the present biogeographic distribution of the extant species reflects ancient relationships among fish faunas of Eurasia and North America (Berg, 1909, 1949; Grande and Bemis, 1991, 1996; Wilson and Williams, 1992; J. Nelson, 1994). Practically all species are currently endangered or threatened (Birstein, 1993, 1999, 2001).

Morphology and osteology studies of sturgeons and paddlefishes during the last 15 years gave a new insight in the phylogenetic relationships of acipenseriforms (Grande and Bemis, 1991, 1996). However, the interrelationships within the group are still unclear. Even the number of sturgeon species is not well established (Birstein and Bemis, 1997). The increasing newly published molecular and genetic data provide new information for revisions of traditional hypotheses of interrelationships of the order with other groups of fishes and on the phylogeny of the group. In the present paper I review the new data in relation with different aspects of the acipenseriform evolution.

Position Within the Vertebrate System

For the first time the position of the Acipenseriformes within fishes was discussed in the classic works of Aleksei Sewertzoff (1926a, b, 1928) and Leo Berg (1948a). Recently, a cladistic approach was used (reviews in Patterson, 1982; Lauderand Liem, 1983; G. Nelson, 1989). According to contemporary views, the extant acipenseriforms form the monophyletic sister-group of all extant Neopterygii (Lepisosteiformes — several species of gars, Amiiformes — bowfin *Amia calva,* and numerous Teleostei;

see reviews in Bemis et al., 1997; Inoue et al., 2003). Most ichthyologists regard Polypteridae as the sister-group of Acipenseriformes and Neopterygii.

Several molecular studies support the view that Acipensiformes and Neopterygii represent a clade with Polypteridae being its sister-group. Perhaps, the data on insertions and deletions of particular gene and other nucleotide sequences and duplications that occurred in the course of jawed vertebrate evolution are the most informative. Fig. 1 presents such events for 13 molecular markers (Venkatesh et al., 2001) or, in the cladistic terms, synapomorphies, and a duplication of a cluster of the //ox-gene. Markers include coding sequences of the recombinase-activating proteins 1 and 2 (RAG1 and RAG2), proopiomelanocortin (POMC) genes, the DM20 and rhodopsin genes.

The coding sequence of the RAG1 gene could have introns la or lb (marked in Fig. 1 as RAG la and RAGlb), and both RAG1 and RAG2 have different single-amino-acid deletions (RAG 1 637T638; RAG2 81, 116T117, 253T254, and 449T450) and insertions (RAG1 5931594, 640sl641; RAG2 4911492) that are specific to distinct groups of vertebrates (Venkatesh et al., 2001). In contrast to the intronless RAG1 genes in the cartilaginous fishes such as sharks and rays, in the lobe-finned fishes (lungfish and coelacanth), as well as in tetrapods, the RAG1 genes in the bichir *{Polypterus* sp.), paddlefish *(Polyodon spathula),* sturgeon *(Acipensersp.),* gar *(Lepisosteusosseus),* and bowfin *(Amia calva)* contain a single intron (RAGlb), whereas RAG1 genes in teleosts contain two introns (RAG la and lb). In other words, the second intron was acquired in a teleost ancestor.

The gene for DM20 shows an alternative splicing in different groups of vertebrates (Venkatesh et al., 2001). DM20 produces a single transcript in cartilaginous fishes, lobe-finned fishes, ray-finned fishes *(Acipensersp., P. spathula,* teleosts), whereas in mammals, reptiles and birds a second transcript named proteolipid protein (PLP) is also produced. In amphibians (all three orders, Anura, Caudata, and Gymnophiona) only PLP is produced. Possibly, DM20 was an ancestral transcript, and the longer PLP transcript was generated in a common ancestor of tetrapods after it diverged from the lungfish lineage. In any case, the data for DM 20 support the existence of the (Acipenseriformes + Neopterygii) clade. Phylogenetic analysis of the data on the structure of a part of the molecule of high molecular weight immunoglobulin heavy chain, the C_H4 domain, also supports this clade (Ota et al., 2003; not shown in Fig. 1).

As for the rhodopsin gene, it contains an intron in cartilaginous fishes, lobe-finned fishes, and tetrapods (amphibians, reptiles, birds, and mammals), whereas in all ray-finned fishes, except of the bichir, the rhodopsin gene contains no intron (Venkatesh et al., 2001). In other words, the phylogenetic distribution of these introns supports that Polypteridae lays outside of the (Acipenseriformes + Lepisosteidae + Amiidae + Teleostei) clade.

The phylogenetic position of a duplication of the //ox genes cluster provides an additional proof that the Polypteridae is a sister-group of the (Acipenseriformes + Neopterygii) clade. Clusters of the //ox genes play a key role in the animal body plan development. The horn shark, *Heterodontusfrancisci,* mammals, as well as *Polypterus senegalus* have one *HoxA* cluster, while sturgeons and teleosts have duplicated *HoxA6* and *HOXAB* clusters (Wagner et al., 2003; Chiu et al., 2004). If these data are confirmed on a higher number of species, this will mean that a duplication of the //ox gene cluster occurred after the divergence of the Polypteridae from the (Acipensiformes + Neopterygii) lineage.

POMC has a two-aa deletion (POMC 242?243) characteristic for all tetrapods. Possibly, there was one more important insertion in the lineage of tetrapods. All fishes and amphibians have a particular molecular-cytogenetic feature in common. By using traditional differential staining methods, it is not possible to obtain the so-called G-bands in the chromosomes of fishes and amphibians, whereas the typical G-bands are easily revealed in the chromosomes of reptiles, birds, and mammals, i.e. amniotes (review in Birstein, 1982, 1987). As I have suggested in Birstein (1987), the G-band chromosomal regions of amniotes probably contain particular types of transposons or long interspersed nuclear elements that induce formation of chromosomal regions with more compact chromatin structure than between G-bands. After such a hypothetical G-band-organizing transposon inserted in the genome of the amniote ancestor (Fig. 1, big arrow), these elements could have been distributed throughout the ancestral genome and later they may have remained in the genomes of descendants of the three amniote lineages.

The basal position of Teleostei within the (Acipenseridae+Neopterygii) clade (Fig. 2A) is unusual. In the phylogenetic trees suggested by morphologists, Acipenseriformes is basal to the Neopterygii (G. Nelson, 1969, Olsen, 1984, Bemis et al, 1997; Fig. 2B). In the tree constructed for the 28S rRNA gene acipenseriforms are also basal to the Neopterrygii (Leet al., 1993; Lecointreetal., 1993; Fig. 2C). However, the basal position of teleosts (Fig. 2A) suggested by Venkatesh et al. (2001) on the basis

of gaining/losses of nuclear genes, is supported by the study of mitochondrial DNA (mtDN A). The same interrelationships within the (Acipenseriformes + Neopterygii) clade were inferred from an analysis of nucleotide sequences of 12 mitochondrial protein-coding genes and 22 transfer RNA genes (Inoue et al, 2003). Additionally, an analysis of amino acid sequences of several proteins also showed the closer relationships between acipenseriforms, gars, and bowfin, than between acpenseriforms and teleosts (Wang et al., 1999). Therefore, the hypothesis inferred from molecular data on positions of Teleostei and Acipenseriformes and should be seriously considered by morphologists.

Karyotypes and Polyploidy

According to the chromosome number, acipenseriform species comprise three groups: (1) species with approximately 120 chromosomes (polyodontids and species of all acipeserid genera); (2) with approximately 240 chromosomes (several *Acipenser* species), and (3) with possibly 500 chromosomes (two *Acipenser* species) (Birstein et al., 1993; 1997; see Table 1). The karyotypes of the 120-chromosome species consist of 60-80 mostly biarmed macrochromosomes, and of 40-60 very small, about 1 u in length, microchromosomes. The exact number of macro- and microchromosome depends on a species. The presence of microchromosmes is a characteristic feature of primitive karyotypes of the most ancient and generalized representatives of cartilaginous fishes, ray-finned fishes, amphibians, reptiles and birds. Representatives of the advanced groups within cartilaginous fishes (some sharks), fin-ray fishes (teleosts) and amphibians (advanced urodelians and anurans) do not possess microchomosomes. Apparently, during the course of evolution within these groups, microchromosmes were fused with or into macrochromosomes.

However, a high number of biarmed chromosomes in the acipenseriform karyotypes is considered an evolutionary advanced characteristic. Karyotypes of most of cartilaginous species consist of 62-104 acrocentric macrochrochromosomes and microchromosomes. Only in several derived species the chromosome number is lower; thus, in the shark *Narcine brasiliensis,* 2n = 28. The decrease in the chromosome number occurs due to two processes: the gradual «disappearance» of microchromosomes and centric fusions of acrocentrics into biarmed macrochromosomes (review in Birstein, 1987).

The karyotypes consisting of biarmed chromosomes are called symmetric, and the process of formation of such karyotypes is known

as symmetrization (Morescalchi, 1970). Since the karyotypes of 120-chromosome acipenseriforms retain a high number of microchromosomes, they remain asymmetric. Within tetrapods, primitive urodelians have karyotypes that resemble the 120-chromosome karyotypes of acipenseriforms.

The karyotypes of the 240-chromosome species (depending on a species, there are, in fact, between 240 and 270 chromosomes) basically represent duplicated 120-chromosome karyotypes. In general, the DNA content in these species (2C = 7.8 - 9.5 pg) is twice higher than in the 120-chromosome species (2C = 3.5 - 4.7 pg). In two polyodontid species it is similar to that in the 120-chromosome sturgeons, 2C ~ 4.0 pg (Table 1). Therefore, the 240-chromosome karyotypes originated through polyploidization events in different lineages of Acipenserinae.

The DNA content in the 120-chromosome *Huso huso* is unclear. Using flow cytometry, it was determined 2.4 pg in two individuals from the Volga River, which is low comparatively to *H. dauricus,* 3.7-3.8 pg, and to the other 120-chromosome species (Birstein etal., 1993). In *H. huso* from the Po River population that Fontana studied (1976) using Feulgen-stained nuclei, the DNA content was 3.6 pg, which is similar to the other 120-chromosome sturgeon species. It is hard to say why the individuals we studied had a low DNA content. Obviously, more individuals from different *H. huso* populations should be additionally studied.

Although the karyotypes of *A. mikadoi* and *A. brevirostrum* have not been described yet, the chromosome number in them deduced from the cellular DNA content, might be about 500. The DNA content in these species is approximately four-times higher than in the 120-chromosome species and two-times higher than in the 240-chromosome species. In other words, these species have a higher ploidy level than the 240-chromosome species. Without karyology data it is not possible to come to a conclusion if such polyploidization occurred on the level of DNA content only or the number of chromosome also was doubled to 500.

A Controversial Issue: the Ancestral Karyotype of Acipenseriformes

Despite the obvious existence of three ploidy levels in the Acipenseriformes, there is no consensus among researchers if the ancestral 120-chromosome karyotype was diploid or tetraploid. While studying the karyotype of *Polyodon spathula,* Dingercus and Howell (1976) assumed that the 120-chromosome species are tetraploids. The authors gathered macrochromosomes of/[3], *spathula* in groups of four and concluded that the species was tetraploid. Later a similar approach was applied to the karyotypes of several acipenserid

species (Vasiliev et al., 1982; Birstein and Vasiliev, 1982, 1987). It was proposed that the ancestral karyotype of approximately 60 macro- and microchromosomes was duplicated the early evolution oftheacipenseriform lineage.

In 1983, Viktor Arefjev described the 120-chromosome karyotype of the ship sturgeon, *A. nudivenris,* as diploid (Arefjev, 1983). Dr. Francesco Fontana of the University of Ferrara and his colleagues, who currently continue to actively study the karyology of acipenseriforms, also consider the 120-chromosome species to be diploids (reviews in Fontana, 1994, 2001, Fontana et al., 1999). They postulated that even if to assume that a duplication of the diploid ancestral genome had occurred in the acipenseriform lineage, a complete reestablishment of the diploid condition happened before the radiation within the order. Although no available data allow one to come to a definite conclusion, there are various data supporting tetraploid condition of the 120-chromosome species.

1) *The origin of the ancestral karyotype*

It makes sense to discuss the possible origin of the 120-chromosome acipenseriform karyotype in the phylogenetic context. Karyotypes of the representatives of two groups closest to acipenseriforms within the (Acipenseriformes + Neopterygii) clade, the Lepisosteidae and Amiidae, are considerably smaller than the 120-chromosome karyotypes of acipenseriforms (review in Birstein, 1987). The karyotype of the gar *Lepisosteus oculatus (L. productus),* 2n = 68, consists of meta- and acrocentric macrochromosomes plus microchromosomes, while there are no microchromosomes in the karyotype of *L. osseus,* 2n = 56 (Ohno et al., 1969; Ojima and Jamano, 1980). Therefore, symmetrization has taken place in this genus and microchromosomes were «lost». The DNA contents in the two species of gars are similar, 2C = 2.8 and 2.2 pg, correspondingly. There are no microchromosmes in the karyotype *of Ami a calva,* 2n = 46 (Ohno et al., 1969), which means that it is also symmetric, and the DNA content in *Amia,* 2C = 2.5pg, istwicesmallerthatinthe 120-chromosome acipenseriforms.

All these data support well the phylogenetic relationships in the (Acipenseriformes + Neopterygii) clade inferred from the combined data for mtDNA genes by Innoue et al. (2003) (Fig. 3). Acipenseriforms and lepisosteids have microchromosomes and their karyotypes might have derived from the same ancestral karyotype that possibly consisted of approximately 60 macro- and microchromosomes. Karyotypic symmetrization occurred in the Lepisosteidae, while the ancestral karyotype *of Amia calva* was, possibly, already symmetric. Since the DNA content in the lepisosteids and *Amia* is almost twice less than in the 120-chromosome acipenseriforms, there is a high probability that a diploidization of the ancestral genome occurred in the acipenseriform lineage.

Typical karyotypes of teleosts considerably differ from those of acipenseriforms, bichirs, and *Amia*. The modal chromosome number in the teleosts is 48-50 (review in Vasiliev, 1985), and the modal DNA content is about 2.0 pg (Hardie and Herbert, 2003). In terms of a small variation in the chromosome number teleosts resemble anurans with their constancy in 2n within genera and even families (review in Birstein, 1987). Karyotypes of almost all teleosts consist of acrocentric or subtelocentric chromosomes. Possibly, the compartmentalization of the genome in 24-25 chromosomes in teleostst was important for diversification of the group. If teleosts are basal to the three lineages of ancient fishes (Figs. 1, 2A), their ancestral karyotype comprised of macro- and microchromosomes experienced extensive evolutionary changes during the formation of the 48-50-chromosome «typical» karyotype of teleosts.

Like in sturgeons, polyploidization occurred in several lineages of teleosts. Thus, all salmonids evolved from a tetraploid ancestor. Later the ancestral chromosome number 100 decreased through centric fusions of acrocentrics in various genera to 52-90 (review in Vasiliev, 1985). Such symmetrization did not happen in sturgeons.

The structure of polypteriform karyotypes is not helpful for a discussion of an ancestral karyotype in the (Acipenseriformes + Neopterygii) clade. Karyotypes of six polypterid species are very similar, 2n = 36, and in only one species, *Polypterus weeksii,* 2n = 38 (review in Vervoort, 1980). All karyotypes are symmetric and consist of biarmed macrochromosomes. Therefore, even if to suggest that the ancestral polypterid karyotype was similar to those of the cartilaginous fishes and consisted of acrocentric macrochromosomes and microchromosomes, this hypothetical karyotype has undergone considerable changes and a karyotype consisting of 36-38 biarmed chromosomes evolved. The DNA content is high in polypterids: in *Polypteruspalmas,* 2n = 36, it is 7.39 pg (Hardie and Herbert, 2003), and in the other species it is even higher. Apparently, polypterids retained the DNA content similar to that in the cartilaginous fishes, which is very high, 2C = 5.9 - 32.8 pg (review in Birstein, 1978; see also Hardie and Herbert, 2003). Alternatively, it might have increased if the ancestral polypterids had the DNA content similar to that in the acipenseriform ancestors.

a*?"*

Although all fish karyological data better support the scheme of relatinships given in Fig. 3, they do not contradict the alternative phylogenetic relationships presented in Fig. 2B, C.

2) *A high number of duplicated genes in the 120-chromosome species*

Already in the first paper on the protein population study of *Polyodon spathula* (2n = 120) authors concluded that 6 % of the studied loci are expressed as duplicated genes (Carlson et al., 1982). Later two different loci for a number of genes of proteins and protein hormones were found in *Polyodon spathula, Scaphirhynchus albus, Huso dauricus,* and *A. ruthenus* (Andoh et al., 2000; Kim et al., 2000; Alabyev et al., 2000; Trabucchi et al., 2002). Therefore, at least some of protein genes are duplicated in the genomes of the 120-chromosome species.

3) *The number ofnucleolar organizer regions (NORs)*

From six to eight NORs were identified in the 120-chromosome sturgeon species (Table 2). This is an unusually high number for NORs in fishes. In the only cartilaginous species studied, the shark *Scyliorhinus canicula,* and in most of teleosts, the number of NORs is two per a karyotype (reviews in Birsteinand Vasiliev, 1987; Birstein, 1987). Only some of tetra- ortriploid teleost species (mostly salmonids) have 4-10 NORs.

In the 120-chromosome acipenserids, the number of NORs correlates with the highest number of active nucleoli per nucleus (Table 2). However, a functional diploidization in the number of NORs occurs: in about of 70 % of cell nuclei, only 2-3 nucleoli of potential six are formed. In the nuclei of teleosts, typically one-two nucleoli per nucleus are present. In the 240-chromosome acipenserids, the number of NORs and the modal number of functioning nucleoli are two-times higher than in the 120-chromosome species, which is expected (Table 2).

The unusually high number of NORs in the 120-chromosome species and the functional diploidization of the number of nucleoli support the possibility of a tetraploid origin of the ancestral acipenseriforms. Surprisingly, the number of the 5S rRNA loci sites in the acipenserids corresponds to the diploid/tetraploid, and not the tetraploid/octoploid hypothesis (Table 2).

4) *Other approaches*

Dr. Van Eenennaam et al. (1998) of the University of California conducted a study of the synaptonemal complex in spermatocytes of the white sturgeon, *A. transmontanus.* This is a presumably octoploid species (or a tetraploid according to Fontana) with 272 ± 3 chromosomes. Based on the observation of bivalent pairing in males, Van Eenennaam et al. suggested

that the diploidization process in this species was complete. This does not prove if the actual ploidy in this species is 4 or 8.

Unfortunately, females of/4, *transmontanus* have not been studied yet. In salmonid species that originated from a tetraploid ancestor (in fact, from an octoploid — see below) and then undergone karyotypic and functional diploidization, meiotic pairing of homologs and homeologs is sex-specific, with females exhibiting disomic inheritance, while males may exhibit residual tetrasomy (review in Allendorf and Danzman, 1997). Since meiotic pairing may be sex-specific also in sturgeons, for the final conclusion the meiotic pairing in the *A. transmontanus* females should be studied. And, obviously, it is necessary to study meiotic pairing in males and females of a 120-chromosome species.

The recent studies of inheritance of microsatellite loci in sturgeons gave also ambiguous results. Ludwig et al.(2001) showed several *A. fulvescens* (presumably, an octoploid, 8n = 262 ± 6) microsattelite loci to be inherited disomically in the 120-chromosome species (all *Acipenser, Huso,* and two *Scaphirhynchus* species in Table 1). The loci are tetrasomically inherited in the 240-chromosome species (all *Acipenser* species of this group except *A. dabryanus* in Table 1).

While using other microsatellite markers, a combination of disomically and tetrasomically inherited loci was found in the same *A. fulvescence* (Pyatskowit et al., 2001; McQuown et al., 2002) and in *A. transmontanus* (Rodzen and May, 2002). Apparently, the mode of inheritance of microsatellite loci may be locus-specific in a given species and cannot be assumed without inheritance data for that locus. Individual microsatellite alleles generally segregate one to one among the progeny.

However, Ludwig et al. (2001) additionally described exceptions to the rule of disomic and tetrasomic inheritance of microsatellite loci in *Acipenser.* A smaller number of loci did not follow disomic or tetrasomic inheritance pattern in both the 120- and 240-chromosome groups, but instead followed some higher order level of inheritance. This might be a proof of an ancient tetraploidization in a 60-chromosome acipenseriform ancestor of the 120-chromosome species.

Did a Genome Duplication Happen in the Ancestral Acipenseriform-Teleostei Lineage?

!,. A comparison of sequenced animal genomes resulted in the most .unexpected discovery that a whole-genome duplication occurred in the ancestral teleost lineage (Taylor et al., 2003; Christoffels et al., 2004; Jaillion

et al., 2004). Based on the data for a teleost with the smallest genome, *Tetraodon nigroviridis,* 2n = 42, 2C = 0.78 pg, and the genomes of other vertebrates (Table 3), Jaillon et al. (2004) suggested that the ancestral vertebrate genome was comprised of 12 chromosomes, was compact, and contained not significantly fewer genes than modem vertebrates.

In fact, karyotypes of cephalochordates that are considered vertebrate ancestors and whose genomes have only a single cluster of *Hox* genes (Wagner et al., 2003), are bigger. Karyotypes of *Branchiostoma lanceolatum* and *B.floridae* consist of 38 small-sized chromosomes, 1.2-4.0e in length, and the karyotype of the third species, *B. belcheri,* is smaller, 2n = 32 (review in Birstein, 1987). Additionally, the genome size in *B. lanceolatum,* C= 0.39 or 0.60 pg (according to different authors), is approximately the same as in *T. nigroviridis.* The size of chromosomes in *T. nigroviridis,* C = 0.39 - 0.50 pg, is comparable with that in *Branchiostoma:* the length of the largest pair is less than 3u (Fischer et al., 2000). The size of the single-copy DNA component in the genomes of *B. lanceolatum* and in *Fugu rubripes* (another teleost with a small genome, C = 0.42 pg), is similar, 0.28-0.41 and 0.33 pg, correspondingly (Table 3).

The discovery of the duplicated genie component in teleosts leads to the question if this duplication occurred in the ancestral teleostei lineage separately from the possible ancestral duplication in the ancestral genome of acipenseriforms or it was a duplication in the ancestral (Acipenseriformes + Neopterygii) lineage (see three possible positions in Fig. 3). If to compare the size of the single-copy DNA component in the genomes of the 120-chromosome sturgeon, *A. ruthenus,* and various teleosts, it becomes clear that in *A. ruthenus* the size of this component is approximately two-times larger than *Lepisosteus osseus* and the diploid (in fact, tetraploid) teleosts (Table 3). Only in teleosts of ancient tetraploid origin such as salmonids and in newly formed tetraploid cyprinid species the size of the single-copy DNA component is comparable with that in *A. ruthenus.* Therefore, it still appears that a separate duplication occurred in the acipenseriform lineage. However, the data on the size of the single-copy DNA component should be treated with caution because only about a third of this component in the *F. rubripes* genome is represented by genie sequences and the function of the other sequences comprising two thirds of the component is unknown (Aparicio et al., 2002).

According to the estimation of Vandepoele et al. (2004), the duplication of the whole genome that was discovered in teleosts happened about 320 million years ago (mya). If this approximate date is correct, the first

duplication definitely happened in the ancestral genome of the (Acipenseriformes + Neopterygii) clade [positions (1) and (2) in Fig. 3] since it is generally accepted that the Acipenseriformes originated in the Jurassic, between 200 and 175 mya (Bemis et al., 1997). If so, all members of the (Acipenseriformes+Neopterygii) clade should have been ancient tetraploids, whose ancestral karyotype was possibly comprised of 60 chromosomes. Alternatively, especially if this date is wrong, the duplication could have happened in the Teleostei lineage [position (3) in Fig. 3]. Only after sequencing genomes of a 120-chromosome acipenseriform, *Amia calva.* and one of the *Lepisosteus species* it will be possible to make a conclusion on the ancient ploidy level of these fishes. But in any case this duplication, apparently, had nothing to do with the possible tetraploidization of the presumed 60-chromosome karyotype in the Acipenseriform lineage.

An extensive study of the genome and karyotype of *Tetraodon nigroviridis* revealed a specific molecular character of heterochromatin in teleosts. In this species the pericentromeric regions and short arms of subtelocentric chromosomes are unusually highly saturated with transposons and pseudogenes comparatively to heterochromatin in tetrapods (Dasilva et al., 2002; Bouneau et al., 2003). At least one type of retrotranposons, *Zebulon,* was not found in the human genome and, therefore, might have been acquired in the teleost lineage. Apparently, transposons and pseudogenes compartmentalized in the heterochromatic areas of teleost chromosomes created instability in these areas, caused partial losses of DNA from these regions, and, possibly, participated in the restructuring of the 48-50 karyotypes.

Probably, in acipenseriforms with their slow molecular and cytogenetic evolution (Birstein, 1978; Birstein et al., 1997; Krieger and Fuerst, 2002) the heterochromatic areas of chromosomes are not enriched in such types of transposons. This potential molecular difference may describe the lack of microchromosome fusions and an unusually high frequency of polyploidization events in the acipenserids. Until now, only two conservative satellite DNA families comprised of short monomers, ////?£/! 11 and *Pstl* (169-173bpand 179-219 bp, correspondingly, depending on a species), localized in the heterochromatic areas of chromosomes of acipenseriforms, and not transposons, were found and characterized in detail (De la Herran et al., 2001, 2004; Robles et al., 2004). Although a *Acc56*1 family composed of 1.5 kb-long repeating units that theoretically could include transposons, was also identified in the genomes of *A. rutehnus, A. gueldenstaedtii* and *A. stellatus* (Mikhailova et al., 1995), its chromosome localization remains unknown.

Molecular Phytogeny of Acipenseridae

The origin and radiation of Acipenseriformes most likely occurred in the Tethys Sea basin that connected the Atlantic and Indian oceans (see Smith et al., 1994). The classic phylogeny of Acipenseriformes based on morphological differences considers two families within the order, the Polyodontidae (paddlefishes) and Acipenseridae (sturgeons), and two subfamilies within the Acipenseridae, Acipenserinae with the genera *Pseudoscaphirhynchus* and *Scaphirhynchus,* and Acipenserinae with the genera *Acipenserand Huso* (review in Bemis et al., 1997). All ichthyologists agree that the Acipenseridae and Polyodontidae diverged in the Jurassic, 200-175 mya, prior to the Late Cretaceous (Berg, 1948a; Yakovlev, 1977, 1986; Grande and Bemis, 1991; Jin, 1995). In contrast to other workers (review in Jin, 1985), Grande and Bemis (1991, 1996) concluded that paddlefishes and sturgeons are sister-taxa.

The split into two families was accompanied by changes in the genomes. Thus, in sturgeons (species of *Scaphirhinchus* and *Acipenser),* clusters of the 18S RNA genes have a unique intra-individual sequence variation (Kriegerand Fuerst, 2002). No variation was detected in *Polyodon spathula* or any other fish, including bichirs, gars, bowfin, and others. Also, new *HindlU* and *Pstl* families of satellite DNA appeared in the genomes of acipenserids (Robles et al., 2004). They were not found in the genome of *P. spathula.*

The number of species within *Acipenser is* still under discussion, and the molecular and genetic data make it possible to correct traditional concepts (Birstein and Bemis, 1997). For example, the DNA content in the Asian Sakhalin sturgeon, *A. mikadoi,* appeared to be twice higher that in the American green sturgeon, *A. medirostris,* and the other 240-chromosome sturgeon species (Table 1). For a long time these two species were considered conspecific and were described as *A. medirostris* (Berg, 1948b; Artyukhin and Andronov, 1990). Obviously, from the genetic point of view they cannot belong to the same species because of the difference in the DNA content (Birstein, 1993b). Later a difference in morphometric characteristics between the two species was also demonstrated (North et al., 2002). As I will discuss below, the two species occupy different positions in the phylogeneticy tree. Despite the difference, not all ichthyologists accepted the validity of the two species.

Theoretically, a molecular phylogeny analysis based on nuclear genes is the most reliable. However, the polyploidy within the order Acipenseriformes and a possibly tetraploid origin of the whole group makes it technically impossible to use nuclear genes for this purpose. Instead, various authors, including us, used mitochondrial genes for phylogeny studies of acipenseriforms (Birstein and DeSalle, 1998; Krieger et al., 2000; Fontanaetal.,2001; Ludwigetal., 2001; Simons etal., 2001). In our final research, we constructed a phylogenetic tree of Acipenseridae on the basis of combined data for nucleotide sequences of fragments of five mitochondrial genes, the 16S and 12S rRNA genes, NADH5, cytochrome *b* (cyt *b),* and control region, totaling 2,439 bp for each of 25 taxa included (details in Birstein et al., 2002). To construct the tree, we used 19 most commonly recognized species of the Acipenserinae (Birstein 1993a; Billiard and Lecointre, 2001; see Table 1). Asoutgoups, we chose twopolyodontid species, *Polyodon spathula and Psephurusgladius.* The maximum parsimony strict consensus tree for the species we studied is given in Fig. 4A. Three main conclusions can be inferred from this tree.

1. There are three major lineages within the family: (1) *Scaphirhynchus,* (2) *A. sturio-A. oxyrinchus,* and (3) all other *Acipenser* species and species traditionally ascribed to the genera *Huso* and *Pseudoscaphirhynchus.*

2. It is believed that *Scaphirhynchus* belongs to the subfamily Schphirhynchinae and occupies a basal position within the family (for instance, Maydenand Kuhajda, 1996; Bemis et al., 1997). In Fig. 4A, both *Scaphi rhynchus* and *A. sturio/A. oxyrinchus* lineages occupy basal position in the tree. The basal position *of Scaphirhynchus was* demonstrated in our previous phyologenetic analysis when we used combined sequence data for the 12S and 16S genes or for these genes plus the cyt *b* gene (Figs. 2 and 3 in Birstein and Desalle, 1998). If to use the data for the cyt 6 gene alone, there is no resolution between the *Scaphirhynchus* and *A. sturio/ A. oxyrinchus* lineages (Fig. IB in Birstein and DeSalle, 1998, Fig. 4 in Ludwig et al., 2001) or *A. sturio/A. oxyrinchus* is basal to *Scaphirhynchus* (Fig. 1 in Fontanaetal.,2001; Fig. 1 in Ludwigetal., 2001). When we to applied maximum likelihood analysis to the same dataset of combined sequence data for five mitochondrial genes, the genus *Scaphirhynchus* appeared to be imbedded within *Acipenser,* while *A. sturio/A. oxyrinchus* still occupied the basal position (Fig. 1B in Birstein et al., 2002). All these results point to a possibility that *Scaphirhynchus,* in fact, does not belong to a separate subfamily and it is an outshoot within Acipenserinae.

The unusual distribution of two satellite DNA families, *HindXW* and *Pstl,* in the genomes of various acipenseriforms supports this possibility. The *Hindlll* sequences are present in all species *of Acipenser, Huso,* and *Scaphirhynchus,* but the *Pstl* sequences are absent in *A. sturio* and

A. oxyrinchus (Robles et al., 2004). Two explanations of this situation could be given. First, if the genus *Scaphirhynchus* belongs to *Acipenser* and the *A. sturio/A. oxyrinchus* lineage is basal to *Scaphirhynchus* and other *Acipenser* species, the *Pst* sequences was gained (inserted?) after the divergence of the A *sturio/A. oxyrnchus* lineage and before the origin of the *ScaphirhynchusYmeage (Fig.* 4B). Second, if alternatively to consider the traditionally recognized two subfamilies within Acipenseridae, the *Pst* family of sequences was deleted from the genome of the *A. sturio/A. oxyrinchus* ancestor (Fig. 4A). It is unclear due to what molecular mechanism a family of sequences dispersed throughout a genome (localized in a various chromosomes) could have been lost. In any rate, the uncertainty of the position of *Scaphirhynchus* in the molecular phylogeny tree and the presence of *Pstl* family in the *Scaphirhynchus* genome provide a researcher with some doubts in the division of Acipenseridae into two subfamilies.

Traditionally, it is generally accepted that the split into Scaphirhynchinae and Acipenserinae occurred in the Middle-Late Jurassic, **175**—135 mya (reviews in Grande and Bemis, 1991; Jin, 1995). However, the fossil ancestor of the genus *Scaphirhynchus, Protoscaphirhynchus squamosus,* was described from the Upper Cretaceous, 95-65 mya (Wilimovsky, 1956). This date coincides with the age of numerous fossil species *of Acipenser* found both in North America and Central Asia (reviews in Birstein and DeSalle, 1998; Choudhury and Dick, 1998). The oldest known fossil representative of the acipenserids, *Asiacipenser kotelnikovi,* described from the Middle Jurassic (175-155 mya), was found in Central Asia (Nesov et al., 1990). This region was covered at the time by theTethys Sea, where the acipenseriforms presumably appeared and diversified. Therefore, it is possible that the diversification of the ancient acipenserids predated the split of the Scaphirhynchinae-Acipenserinae lineages. If so, the split might have happened later, in the Upper Cretaceous (Table 4).

Interestingly, the molecular data do not support the morphological discrimination between the three extant species of *Scaphirhynchus, S. albus, S. platorynchus,* and *S. sutkusi,* neither using mitochondrial genes (Fig. 2; also, Simons et al., 2001), nor nuclear markers — microsatellites (Campton et al., 2000; Tranah et al., 2001). The most probable explanation of this unusual fact is that *S. albus* and *S. platorynchus* are possibly reproductively distinct groups undergoing hybridization, especially in the lower Mississippi River (Tranah et al., 2004).

3. The genus *Pseudoscaphirhinchus* does not belong to Scaphirhynchinae and it is closely related to *A. stellatus* (Fig. 4). Traditionally, beginning

from the early publications of Leo Berg (1904, 1905, 1911), this genus, together with the genus *Scaphirhynchus,* was considered a member of the subfamily Scaphirhynchinae, and only species of *Huso* and *Acipenser* were included in the Acipenseridae (reviews in Mayden and Kuhajda, 1996; Bemis et al., 1997). Our results demonstrate that, contrary to this view, *Pseudoscaphirhynchus* is imbedded within the /fc//?e«.«?/-species, where it is clustered with *A. stellatus.*

Although the nonmonophyly of the subfamily Scaphirhynchinae appears unexpected, one should not forget that originally the two genera were combined on the basis of visual similarity of *Pseudoscaphirhynchus* with published drawings of the *Scaphirhynchus* individuals, and not on a comparison of morphology of specimens. In 1954, the American ichthyologists Bailey and Cross expressed the first doubts about close relatedness of the two genera: «<...> we were uncertain whether the two genera of "shortnose" sturgeons *{Scaphirhynchus* and *Pseudoscaphirhynchus)* owed their close resemblance to common ancestry or to convergence* (Bailey and Cross, 1954, p. 172). Our results confirm their suspicion. Apparently, the general morphological similarity of the species *of Scaphirhynchus and Pseudoscaphirhynchus* is a result of the convergence. According to the position of *Pseudoscaphirhynchus* in the tree, it is not a separate taxonomic unit and belongs to the genus *Acipenser.*

4. There are at least three main clades within *Acipenser* (including *Pseudoscaphirhynchus):* (a) an *A. sturio-A. oxyrinchus* clade, (b) an *A. schrenkii-A. transmontanus* clade, and (c) a clade that includes all Ponto-Caspian species (or, in Berg's terms, species of the Ponto-Caspian-Aral Province), as well as species of *Pseudoscaphirhyncus*and *Huso,* plus *A. brewostrum* and *A.fulvescens.* Although this study did not include *A. dabryanus,* our previous data showed that this species belongs to the third clade (Birstein and DeSalle, 1998). *Acipenser mikadoi* is the sister-species of the third clade.

(a) Previously we demonstrated the basal position of *A. sturio-A. oxyrinchus* within the *Acipenser* genus (Fig. 3 in Birstein and DeSalle, 1998). Once considered a subspecies of A *sturio,* later the American Atlantic sturgeon was described as a separate species, *A. oxyrinchus* (Vladykov and Greely, 1963). *Acipenser sturio* is probably the descendant of the most primitive forms within the extant lineage of *Acipenser* (Nesov and Kaznyshkin, 1983). Since the main geological changes in the North Atlantic Ocean area occurred during the Cretaceous (135-65 mya) (Smith et al., 1994), it is possible that the *A. sturio* lineage originated during the Middle Cretaceous, about 90 mya.

4.

,3i The detailed analysis of mitochondrial DNA (mtDNA) of these two species revealed a complicated history of the lineage. In 1997, we described two forms of mtDNA (they differed in six nucleotide changes in the studied region of the cyt *b* gene) in *A. sturio:* in specimens from the Gironde River population and from the Baltic Sea (Birstein et al., 1997a; Birstein and DeSalle, 1998). A detailed examination of numerous A *sturio and A. oxyrinchus* individuals from different populations and specimens from European museum demonstrated that, in fact, the second form belongs to *A. oxyrinchus* (Ludwig et al., 2000; 2002). Apaprently, *A. sturio* colonized the Baltic Sea after the Pleistocene, approximately 3,000 years ago, and after this, about 1,800 years ago, *A. oxyrinchus* also colonized the same area. Later, between 1,200 and 800 years ago, *A. oxyrinchus* completely replaced *A. sturio* in the Baltic Sea (Ludwig et al., 2002). Therefore, the sturgeon known as the Baltic sturgeon is not the same as the Atlantic sturgeon from the Gironde River and the Mediterranean.

(b) *Acipenser schrenskii-A. medirostris* clade contains three species with strong trans-Pacific relationships: *A. schrenkii* is an Asian species, and *A. medirostris* and *A. transmontcmus* are species of the East coast of North America. Apparently, the two American species originated from an Asian ancestor. *Acipensersinensis* is closely related to these three species. All four species have 240 chromosomes and, possibly, they originated from a 240-chromosome ancestor.

Leo Berg considered the two acipenserids of the Amur River basin, *A. schrenkii* and *Huso dauricus,* to have been relics of the Upper Tertiary fauna that characterized the entire northern hemisphere and that mostly disappeared as a result of cooling during the Quaternary, i.e. approximately between 20 and 2 mya(Berg, 1909, 1949). Apparently,/I. *sinensis,* like *A. schrenkii,* is also a relic.

(c) The third clade contains the Ponto-Caspian group in which traditionally *A. gueldenstaedtii, A. nudiventris, A. persicus, A. stellatus,* and *H. huso* are included, as well as the closely related to them Eurasian species *A. naccarii, A. ruthenus,* and *H. dauricus; Pseudoscaphirhynchus;* and two American species with trans-Atlantic relationships, *A. fulvescens* and *A. brevirostrum.* The clade includes species with 120, 240, and, possibly, 500 chromosomes.

This clade is the sister group to *A. mikadoi,* that, apparently, has a strong affiliation to the Ponto-Caspian species. Two polyploidization events occurred during the evolution of the *A. mikadoi* lineage. Since *A. mikadoi* and *A. medirosris* belong to two different clusters, their morphological similarity

is, apparently, a results of convergent evolution. Like *A. schrenkii, A. mikadoi* is, possibly, a relic of the previous fauna of the area. This explains the unusually very small species area of *A. mikadoi and* its spawning in only one river.

In Fig. 4, as in our previous study (Birstein and DeSalle, 1998), two species of *Huso, H. dauricus* and *H. huso,* are embedded within the genus *Acipenser.* Apparently, the genus *Huso* is not a separate taxonomic unit.

Traditionally, the two species of *Huso* were considered belonging to a separate genus (Berg, 1904, 1948b). Our data support the possibility that the two *Huso* species belong to the genus *Acipenser and* are closely related to *A. ruthenus,* despite a considerable difference in general morphology of *Huso* species (that are very big) and *A. ruthenus* (which is small).

The fact that *H. huso* and *A. ruthenus* might be closely related is supported by the ease with which they hybridize in captivity. Hybrids between these species are not only viable, but also fertile (review, for instance, in Arefjev, 1999). However, due to ecological differences, these species «do not hybridize in the natural conditions* (Nikolyukin, 1964, p. 145). The extreme morphological difference between these two related species is possibly a result of paedomorphosis, which played an important role in the evolution of acipenseriforms (Yakovlev, 1977; Grande and Bemis, 1991; Tsessarsky, 1992).

As I have already mentioned, in Fig. 4 the *Pseudoscaphirhynchus* species are clustered with *A. stellatus.* The latter species needs a comment. Recently, Fontana and his colleagues described a karyotype of *A. stellatus* from the Danube River consisting of 146 ± 6 chromosomes (Chicca et al., 2002). In the previous descriptions of the karyotype of specimens from the Caspian Sea the chromosome number was given as 118 ± 2 (Vasiliev, 1985; Birstein and Vasiliev, 1987; Nowruzfashkhami and Khosroshahi, 1999). The difference in the numbers determined by different authors is too high to consider it a technical mistake in counting small chromosomes. Possibly, this discrepancy reflects a population difference. However, our molecular comparison (the cyt *b* gene and D-loop) of *A. stellatus* from two populations, the Caspian Sea and the Danube River, did not reveal any difference between these populations (Doukakis et al., 2000). Obviously, the question about a possible cytogenetic population difference within *A. stellatus* should be studied in more detail.

The Ponto-Caspian group of *Acipenser/Huso/Pseudoscaphirhynchus* species has probably originated in the Tethys Sea or its derivative, the Paratethys Sea which covered the contemporary Black, Azov, Caspian, and Aral seas and was formed in the Middle Miocene, about 15 mya (Jones and Simons, 1996). The Caspian Sea has remained a refugium for sturgeon

species common to the Caspian, Aral, Azov, Black, and Mediterranean seas from 15 mya to about 1.5 mya. Probably, *Pseudoscaphirhynchus* originated from the *A. stellatus-Pseudoscaphirhynchs* ancestral lineage after the disruption of the Paratethys into several basins in the Late Miocene-Early Pliocene, between 5.5 and 3.5 mya (see Adams, 1981).

The last cluster consists of a group of very closely related Eurasian species, A *gueldenstaedtii, A. persicus, A. naccarii,* and *A. baerii,* and the American *A. brevirostrum*. All these species, except *A. brevirostrum,* possess 240 chromosomes, and *A. brevirosrum* has, possibly, 500 chromosomes (Table 1). Apparently, an additional polyplidization event occurred in the *A. brevirostrum* lineage.

No genetic difference between *A. gueldenstaedtii* and *A. persicus* was found, and *A. persicus* is, apparently, one of intraspecies forms (mtDNA haplotypes) *of A. gueldenstaedtii (Birstein* et al., 2000). The taxonomic status of *A. persicus* is unclear, and for a long time it was considered a subspecies *of A. gueldenstaedtii, A. g. persicus* (Berg, 1948b). Later it was promoted to the species level (Vlasenko et al., 1989). Obviously, molecular data do not support this view.

The rest three Eurasian species, *A. gueldenstaedtii, A. naccarii,* and *A. baerii,* are not only closely related, but *A. gueldenstaedtii* in the Caspian Sea consists of forms genetically related to the ancestral forms of *A. baerii* and *A. naccarii* (Birstein et al., 2000, 2005; Jenneckens et al., 2000). A possible close relatedness *of A. naccarii* to *A. sturio* suggested by some morphologists have no molecular support (Doukakisetal., 2000; De la Herran et al., 2004).

Different individuals from the *A. gueldenstaedtii* in the Caspian Sea population possess one of three forms of mtDNA: the *A. gueldenstaedtii*-like, i.e. known as *A. gueldenstaedtii* (most of the population), *A. baerii*-like (approximately 30 % of the population), and a low number of A *naccarii-\ike.* Individuals possessing the three forms are morphologically indistinguishable, i.e., according to the morphological criteria, they are *A. gueldenstaedtii* (Birstein et al., 2000, 2005). In the Black Sea, individuals with the *A gueldenstaedtii-\ike* and *A. naccarii-\ike* forms of mtDNA were found. There are three forms of mtDNA in individuals of A *naccarii,* an endemic of the Adriatic Sea: two forms of the *A. naccarii* mtDNA, and the *A. gueldenstaedtii-Yike A. naccarii* form (Ludwig et al., 2003).

We interpreted this complex situation the following way (Birstein et al., 2005). Apparently, the three ancestral forms of mtDNA evolved in the population of A *gueldenstaedtii* in the Caspian Sea. Individuals with the

ancestral A *baerii-Yikc* form migrated to the Siberian river basins through the post-glacial connection between the Caspian Sea and Siberian rivers, and later they evolved into the extant *A. baerii.* During the other geological events, individuals with the A *naccarii-\ike* form of mtDNA migrated to the Black, and then to the Adriatic Sea, where they evolved into A *naccarii.* If to accept this hypothesis, A *baerii* and A rtoccan'/should be rather young species. In any rate, the whole species complex A *gueldenstaedtii-A. naccarii-A. baerii* includes the three valid species, as well as the descendants of the ancestral forms of these species.

Traditionally, it is believed that the American shortnose sturgeon, A *brevirostrum,* which is basal to the group, is closely related to the lake sturgeon, *A. fulvescens,* that inhabits mostly the Great Lakes basin (Lee et al., 1980). The only conclusion one can make from Fig. 4 is that *A. fulvescens,* as well as the Ponto-Caspian A *nudiventris,* belongs to the same clade as A *brevirostrum,* and both species have trans-Atlantic relationships with the Ponto-Caspian species. Probably, these two species had common European ancestors in the Middle Eocene, approximately 50-40 mya, when Europe and North America became disconnected (Adams, 1981).

There no reason to consider A *fulvescens* to have been an ancestor of A *transmontanus* and A *medirostris* (see Brown et al., 1996). The lake sturgeon is related to the Ponto-Caspian group of species, whereas *A. transmontanus* and A *medirostrisbdong* to anotherclade and they are related to A *schrenkii,* i.e., they originated from an Asian ancestor.

Obviously, the molecular phylogeny provides a new insight into the relationships between species of the Acipenseridae. The hypothetical relationships constructed on the basis of biogeography data of the group (Choudhuri and Dick, 1998) are totally unsupported by our molecular research. Hopefully, our results will stimulate new comparative studies of this group of ancient fishes by ichthyologists and morphologists. The first anatomical research that supported our conclusion on the possible close relationship between *A. stellatus* and *Pseudoscaphirhynchus kaufmanni* has already been done (Hilton, in press).

More Troubles

At present, taxonomy and systematics become more and more dependent on the DNA data (Tautz et al., 2003). Several cryptic species have already been identified due to differences in mtDNA (Baker et al., 1996; Garcia-Rodriguez et al., 1998; Hebert et al., 2004). An ambitious project on providing all animal species with a «barcode» based on variations

in the mitochondrial cytochrome c oxidase subunit 1 (COI) gene is currently under the development (Blaxter, 2003; Hebert et al., 2003).

Evidently, sturgeons create a problem for this approach. The unusual genetic structure of *A. gueldenstaedtii* that includes three forms of mtDNA within the same species does not fit in the idea to provide this species with one barcode. The same concerns *A. naccarii with* its at least three forms of mtDNA, as well as *A. sturio* in the Baltic Sea. Also, it is problematic to give separate barcodes to the three *Scaphirhynchus* species that cannot be discriminated on the basis of mtDNA. At least partial (473 bp) sequences of the COI gene in *S. albus* and *S. platorynchus* are identical (accession Nos. M64919 and NC004420 in GenBank). Therefore, the barcoding cannot be applied to all sturgeon species.

The DN A data have also become crucial for conservation biology (DeSalle and Amato, 2004). In 1996, because of their endangered status, all sturgeon species were listed by the Convention on Importation and Trade of Endangered Species (CITES) in Appendix II (internationally controlled trade). One of the criteria for listing is that commercial products obtained from an endangered species must be identified. In case of sturgeons, this criteria concerns mostly caviar of three species, the beluga *H. huso* (beluga caviar), sevruga *A. stellatus* (sevruga caviar), and Russian sturgeon, *A. gueldenstaedtii* (osetra). A simple mtDNA-based test was developed for discriminating caviar of these three species (DeSalle and Birstein, 1996; Birstein et al., 1998, 1999). Later, when three mtDNA-forms were discovered within *A. gueldenstaedtii,* it appeared that the method allows one to easily identify caviar of//, *huso* and *A. stellatus,* but it is unable to discriminate between the caviar of the/). *baerii-\\Y&* Russian sturgeon and *A. baerii.* For such a discrimination, a more extensive research needs to be performed.

Caviar of *A. gueidenstaedtii* created a legal problem. A molecular laboratory of the US Fish and Wildlife Service (US FWS, the American analogue of Ministry of Environment) developed its own mtDNA-based method of caviar species identification. This method has never been per reviewed by independent scientists or published. Such a violation of scientific standards is based on a gap in the American law which does demand an independent reviewing of scientific methods used by the US FWS.

For several years, the US FWS has been presenting results obtained by its own method as evidence in the American federal courts. However, the method cannot discriminate the caviar from the *A. baerii-Yike* Russian sturgeon and that from *A. baerii.* If during the testing of a caviar shipment from the Caspian Sea area the US FWS laboratory identifies *A. baerii,* it concludes that

the shipment contained caviar of *A. baerii,* and not that of A *gueldenstaedtii.* As a result, the American businessman who has received the shipment, is accused of violating importation laws (caviar of a supposedly wrong species was declared at the customs). Such a shipment is confiscated and destroyed, and the businessman is usually tried. Any appeal to the common sense that caviar could not come from the Siberian sturgeon because there is no production of such type of caviar for export in Russia or to scientific data on the complex genetic structure of A *gueldenstaedtii does* not work. During the last five years, several caviar businessmen were sentenced to paying fines or even to short prison terms on the basis of US FWS results. Unfortunately, the CITES Secretariat supported this practice (Birstein, 2002).

The implementation of the CITES listing of sturgeon species had only one clear impact: it efficiently disrupts joint international efforts of scientists (Birstein and Doukakis, 2001). According to the CITES rules, any person can buy and bring 250 g of caviar to any country. However, a scientist working with DNA, cannot bring legally even one egg fixed in alcohol to another country without a special CITES permit from the local authorities. It is practically impossible to receive such a permit from, for instance, the Russian CITES authorities because of absurd rules the bureaucrats have created: of 11 documents necessary for a permit, at least 6 simply could not be physically obtained. Some other countries, for example Romania, issue permits easily. There is no will of the CITES officials to normalize this situation and to exclude scientific samples from being treated under a trade convention.

Conclusions

The fast growing number of new molecular and genetic data allows researchers to review previous concepts of phylogenetic relationships of the major groups of fishes based on morphology studies and to construct new possible relationships within the groups.

1. Using molecular data, a new hypothesis of the basal position of the Teleostei, and not Acipenseriformes, has been suggested recently. The karyology data for fishes, including acipenseriforms, support this model. However, the karyology data do not contradict more traditional models in which the order Acipenseriformes is basal to the Neopterygii, i.e. (Lepisosteiformes+Amiiformes+Teleostei).

2. Although still controversial, some molecular and genetic data point to a possibility that the ancestral karyotype in the acipenseriform lineage could have consisted of 60 macro- and microchromosomes.

3. Molecular data do not support the validity of the subfamily Scaphirhynchinae. The division of Acipenseridae into two subfamilies should be at tentively reevaluated.

4. Contrary to the traditional opinion of morphologists, the phylogeny of Acipenseridae based on the analysis of combined sequence data for five mitochondrial genes demonstrated the nonmonophyly of the subgenus *Scaphirhynchinae.* The genus *Pseudoscaphirhynchus* belongs not to Scaphirhynchinae, but to Acipenserinae, where it clasters with *A. stellatus.* The first comparison of anatomical details of the skull structure in *Pseudo scaphirhynchus kaufmanni* and *A. stellatus* supports our conclusion on the relatedness between these two species (Hilton, 2005).

5. There are three major lineages resolved in within the Acipenserinae: (a) *A. sturio/A. oxyrhinchus* (possibly, the oldest lineage in the subfamily), (b) a group of three *Acipenser* species *{A schrenkii, A. medirostris,* and *A. transmontanus)* with clearly trans-Pacific relationships, and (c) a group of the rest of *Acipenser, Huso,* and *Pseudoscaphirhynchus* with complicated trans-Atlantic relationships between Eurasian and American species *of Acipenser.* The cluster of Ponto-Caspian species (includes species of the three genera, *Acipenser, Huso,* and *Pseudoscaphirhynchus)* and their close *Acipenser* relatives is most numerous within the third group.

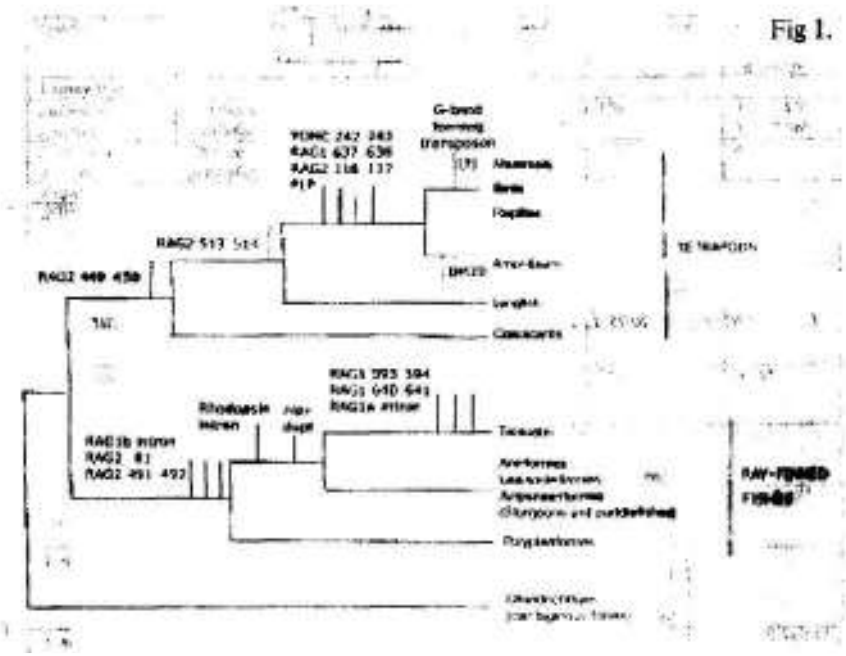
6. Polyploidization played an important role in of the evolution of the genus *Acipenser.* As a result, three levels of ploidy were created: (1) species with 120 chromosomes, (2) with 240 chromosomes, and (3), two species with, possibly, 500 chromosomes (deduced from the DNA content), polyploidization events occurred several times independently in various lineages of the genus.

7. Because of the unusual genetic structure of some of species possessing several mt DNA forms (the *A. guledenstaedtii-A. naccarii-A. baerii*complex, *A. sturio)* and the impossibility to discriminate three species within the genus *Scaphirhynchus* using mtDNA data, the «barcoding» characterization of all species cannot be applied to these species.

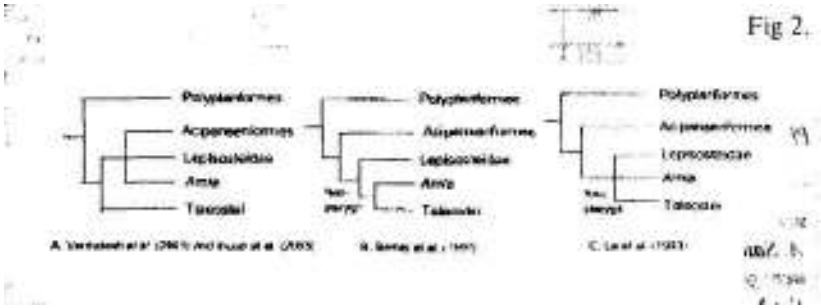
8. The analysis of molecular and genetic data present a challenge to tra ditional views of morphologists on the evolutionary relationships of Acipenseriformes with the other groups of ancient ray-finned fishes and Teleostei and suggest possible relationships within Acipenseridae. Ichthy ologists and vertebrate morphologists should take this formation into con sideration in their new studies.

9. The implementation of the CITES listing of sturgeons should be ba sed on the recently developed scientific knowledge about acipenseriforms

and their evolution and not on the outdated information. Specimens **for** the mtDNA research must not be covered by the CITES trade treaty.



Phylogenetic relationships of jawed verteberats inferred from molecular markers | (from Venkatesh et ai, 2001, slightly modified). Details are in the text.



Alternative hypotheses for four major lineages of ray-finned fishes.

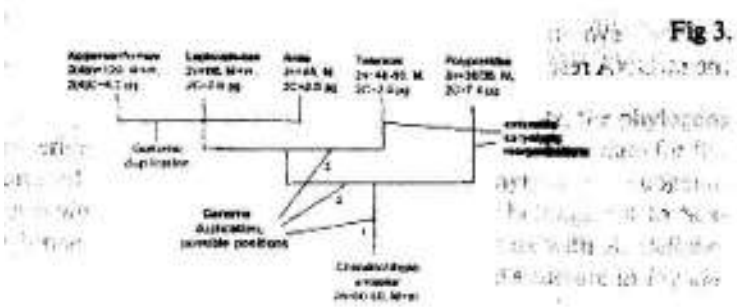


Fig 3.

Hypothetic evolution of the karyotype of an ancestral cartilaginous fish into the karyotypes of extant ray-finned fishes. Phylogentic relationships following Venkatesh et al.(2001) and Inoue et at. (2003). M, macrochromosomes, and, m, microchromosomes. The genome duplication found in the teleosts could have happened in positions I, 2, or 3. Another genome duplication possibly occurred in the acipenseriform lineage.

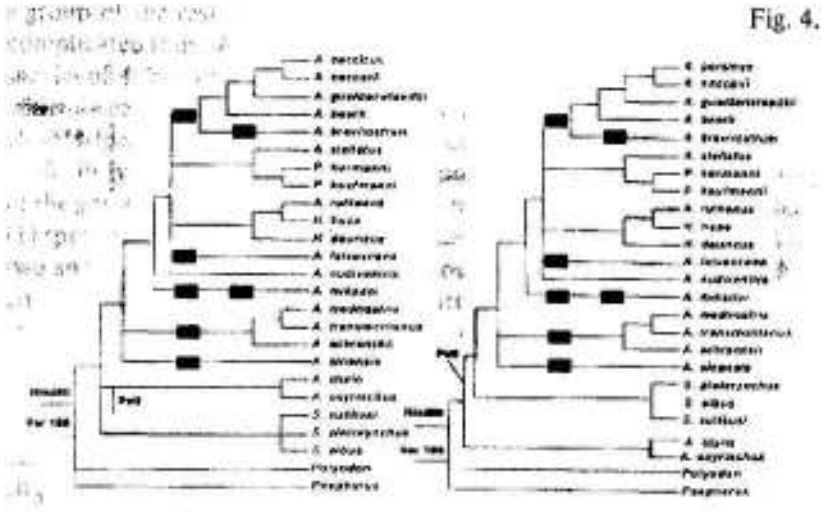


Fig. 4.

Phytogeny tree of acipenserids inferred from the combined mtDNA sequence data for five genes by maximum parsimony method (details in Birstein et ai, 2002). Data for the Hind///*and* Pst/*satellite DNA sequences (Robles et ai, 2004) and intraspecific variation in the ISSrRNA genes in acipenserids (Krieger and Fuerst, 2002) was added. A. Satellite DNAs* Hind/// *and* Pst / *and intraspecific variation in the 18SrRNA genes were gained in the ancestral genome of acipenserids. Later the Pst/ sequence was lost in the A.* sturio/A. oxyrinchus *lineage. B. Alternatevely, the Pis/satellite DNA sequence was gained in the* Scaphirhynchus/Acipenser/Huso *lineage*

Table 1. Species of the Acepenseriformes, their historical distribution, chromosome numbers, and nuclear DNA content

Species	English Name	Historical Distribution	Chromosome Number"	DNA Content. pj^Number'
Family Polyodontidae				
Polyoilon spat hula	American paddlefish	Mississippi River system, USA and Canada	120	3.17", 4.89[1], 3.9',3.96*
Psephurus Si ladins	Chinese paddlefish	Yangtze River system, China	120"	4.11*
Family Acipenseridae				
Acipenser baerii	Siberian sturgeon	Main Siberian rivers, Russia and China	249 ±5	8.29-8.3 r
A. brevirostntm	Shortnose sturgeon	Rivers, estuaries and ocean along east coast of North America from Indian River (Florida, USA) to Saint John River (New Brunswick, Canada)	500 *	13.08" (13.22)"
A. dabtyanus	Yangtze or Dabry's sturgeon	Yangtze River system, China	250"	8.26*
A. fulvescens	Lake sturgeon	Great Lakes (USA and Canada), their tributaries and lakes of southern Canada	262 ±6	8.90'
A. gueldenstaedtii	Russian sturgeon	Black, Azov, Caspian seas and rivers entering into them (all countries of the area)	250 ± 8	7.86-7.88"
A. medirostris	Green sturgeon	Pacific coast of North America from Aleutian Islands and Gulf of Alaska (USA) lo Ensenada (Mexico)	249 ±8	8.82"
A. mikadoi	Sakhalin sturgeon	Pacific Ocean from Amur River to northern Japan, Korea, and Bering Sea, Tumnin (Datta) River (Russia)	500"	13.93-14.73"
A. naccarii	Adriatic sturgeon	Adriatic Sea, Po, Adige, and Buna rivers	246 ±8	(5.7-6.3)"
A. nudiventris	Ship sturgeon	Aral, Caspian, Black seas and rivers entering into it (all countries of the area)	118±3	3.88-4.04"
A. oxvrinchus desotoi	Gulf sturgeon	Gulf of Mexico and northern coast of South America (USA, Mexico)	120"	4.55[1]
A. o. uxyrinchus	Atlantic sturgeon	Rivers, estuaries and ocean along east coast of North America from the St. Johns River (Florida, USA) to Hamilton Inlet (Labrador, Canada)	120"	(4.38)"
A. persicus	Persian sturgeon	Caspian Sea and rivers entering into them (Russia, Azerbaijan, Iran)	258 ± 2	Unknown
A. ruthenus	Sterlet	Drainages of main rivers entering the Caspian and Black seas (Volga, Danube) (all countries of the area) and Ob, Irtysh, Yenisey rivers (Siberia; Russia)	!18±2	3.74"

A. schrenckii	Amur River sturgeon	Amur River system {Siberia; Russia and China)	238 ±8	6.07"
A. sbifnsis	Chinese sturgeon	Yangtze River system (China)	264 ±4	9.07"
A. stellatus	Stellate or sevruga sturgeon	Caspian, Azov, Blaek and Aegean Seas and rivers entering into them (all countries of the area)	118±2	3.74'
A. sturio	Atlantic (Baltic) sturgeon	Baltic, Eastern North Atlantic, Mediterranean, and Black seas (all countries of the area)	121 ±3	(3.6)*
A. transmontanus	While sturgeon	Rivers and Pacific coast of North America from the Gulf of Alaska to Baja California (USA and Canada)	271 ±2.5	9.56'
Huso dauricus	Kaluga sturgeon	Amur River system (Russia, China)	120"	3.74-3.81'
H. huso	Beluga or giant sturgeon	Caspian, Black, and Adriatic Seas and rivers entering into them (all countries of the area)	118±2	2.42-2.45' (3.6)»
Pseudo-scaph irhynch us fedtschenkoi	Syr-Dar shovel nose sturgeon	Syr-Darya River (Kazakhstan); possibly extinct	Unknown	Unknown
P. hermanni	Small Amu-Dar shovclnose sturgeon	Amu-Darya River (Uzbekistan, Turkmenistan)	Unknown	Unknown
P. kaufmanni	Large Amu-Dar shovclnose sturgeon	Amu-Darya River (Turkmenistan, Uzbekistan and Tadjikistan)	120"	3.46-3.48'
Scaphirhynchus albus	Pallid sturgeon	Missouri and Mississippi River basins (USA)	Unknown	Unknown
S. platorhynchus	Shovelnose sturgeon	Missouri and Mississippi River basins (USA)	112 ± 2	4.73'
S. suttkusi	Alabama sturgeon	Mobil basin in Alabama and Mississippi (USA)	Unknown	Unknown

"Data from the reviews by Birstein et al. (1997) and Fontana et al. (2001). For *Acipenserfulvescens,* see Fontana et al. (2004).

*Chromosome number determined on the basis of the DNA content.

[c]Only the data determined by flow cytometry method are given. Apparently, the difference in the amount for *P. spathula* given in different papers is a result of different control species used in different studies.

"Birstein et al. (1993)

′ Blacklidge and Bidwell (1993)

i Tierschetal. (1989).

«Zhang etal. (1999).

*Determined by microdensitometry of Feulgen-stained nuclei: for *H. huso, A. naccarii* and *A. sturio,* by Fontana (1976); for/), *brevirosirum* and *A. oxyrinchus,* by Hardie and Hebert (2003).

Table 2. The number of nucleolar organizer regions (NORs) and of the 5S rRNA loci sites per karyotype (KT) and the number of nucleoli per nuclei of erythrocytes in several sturgeon species

Species	Chromosome Number	NORs per KT"		Number of Nuclcoli per Nucleus"		5S loci sites per *KV*
		Number	Location	Total	Modal	
Group 1 (120 chromosomes)						
A. rulhcnus	118±2	6	2smMc, 2A; a11 tcl	1-6	2-3	*2*
A. stellalus	118=t2	6-8	6 mid-Mc. tcl; 2 sm	1-6	2-3	*2*
A. sturio	121 ±3	6-8	6 mid-Mc, tel; 1 sm			*2*
Huso huso	118±2	6	2 A (micro)	1-6	2-3	2
Group 2 (240 chromosomes)						
A. baerii	249 ±5	10-12	4 sm Me, 4 sm micro, all tcl	ND	ND	4
A. juhescens	262 ±6	12		ND ND		4
A.gueldenstaedti i	250 ±8	13	1 large Me. 4 mid-Mc. 4 sm Me, 4 sm A	2-12	6-8	ND
A. naccarii	246 ±8	10-12	2-4 sm Me, 4 sm A, all tcl	ND	ND	ND
A.transmontanus	272 ± 3	10-12	1 sm Me; 5 mid-sized; micro	ND	ND	4

[a] From Fontana et al. (2001, 2003, and 2004) Data obtained by various methods of differential staining of chromosomes. Abbreviations: Me, metacentric; A, acrocentric; mid, medium-sised; sm, small; tel, telomeric location; micro, microchromosome.

[h] Data from Bistein and Vasiliev (1987). Obtained using the AgAs-staining of chromosome slides. ND, not determined.

Table 3. Single copy class in the genomic DNA of the lancelet and various fishes determined from the DNA renaturation analysis

Species	N	Cpg	Single Copy Component[1]	
Amphioxi			% of genome	PS
Branchoistotna lanceolatuw	19	0.39 or 0.60	69	0.28-0.41
Acipenseriformes				
Acipcnscr ruthenus	59	1.87	45	0.84
Lepisosteiformes				
Lepisosteus osseus	28	1.10	42	0.46
Teleostei				
Clupcidac				
Clupca harengus	26	1.0	38	0.44
Salmonidac (ancestral 4n)				
Corcgoninac				
Coregonus lavaretus	40	3.2	24	0.84
Salmoninac				
Oncorhynchus kcla	37	3.5	33	1.16
O. kisutch	30	3.0	40	1.25

Salma trutta	39-40	1.2	42	0.50
Salvelinus fontinalis	42	3.2	21	0.67
Thymallinac				
Thvmallus Ihymallus	51	2.2	23	0.54
Cyprinidac				
Abramis brama	26	1.3	50	0.63
Leuciscus cephalus	25	1.4	42	0.66
Puntius (Barbus) tetrazonu	25	0.73	57	0.51
Rutilus rutilus	25	1.02		
Barbus barbus, 4n	50	1.8	53.5	1.05
Carassius auratus, 4n	50	1.7	60	1.01
Cyprinus carpio, 4n	50	1.9	49	111
Cobitidac				
Misgumus fossilis, 4n	50	1.4	50	0.70
Tctraodontidac				
Fugu (Takifugu) rubripcs	22	0.42	79	0.33
Ti'tiaoctan ni^iwirklis	21	0.39 or 0.50	-	-
Diodontidac				
Diodon hyslrix	23 (?)	0.82	71	0.58

"Main references in Birstein (1987), Table 24, pp. 134-135. For *Fugu rubripes*and *Diodon hystrix,* the data from Neafsey and Palumbi (2003), and C for *Tetraodon nigroviridis,* from *Ibid and* Hardie and Hebert (2003).

Table 4. Two possible scenarios of the main evolutionary events in Acipenseriformes

Period	Million of years ago (mya) or years ago (ya)	Evolutionary event	Reference
1. A traditional view (paleontology data)			
Jurassic	200-175	Origin of Acipcnscriformcs	Grande and Bemis, 1991; Jin, 1995
Jurassic	200-135	Divergence of the Polyodontidae and Acipcnscridae	Yakovlcv, 1977; Grande andBcmis, 1991; Jin, 1995
Middle-Late Jurassic	175-135	Divergence of the Scaphirhynchinac and Acipcnscrinae	Wilimovsky, 1956; Grande andBcmis, 1991, 1996
Middle Jurassic	175-155	Origin of *Asiacipenser*	Nesov et al., 1990
Upper Cretaceous	95-65	Origin and divergence *ot'Acipenser*	Berg, 1948a; Wilimovsky, 1956; Ncsov and Kaznyshkin, 1983
Upper Cretiiccous	-95	Origin of the *A. sturio* line	Ncsov and Kaznyshkin, 1983
2. An alternative	iew	and molecular data)	
Jurassic	200-175	Origin of Aeipenscriformcs	Grande and Bcmis, 1991; Jin, 1995
Jurassic	200-135	Divergence of the Polyodontidae and Acipenscridac	Yakovlcv, 1977; Grande and Bemis, 1991; Jin, 1995
Middle Jurassic	175-155	Origin *of Asiacipenser*	Ncsov ctal., 1990
Upper Cretaceous	95-65	Origin and divergence of *Acipenser,* including the appearance of the *A. sturio* lineage and other main lineages (Fig. 2)	Berg, 1948a; Wilimovsky, 1956; Ncsov and Kaznvshkin, 1983
Upper Cretaceous	95-65	Origin *oi Protoscaphirhynchus*	Wilimovsky, 1956

Lower Eocene	5(M0	Origin *of A. fuhescent* and *A. brevirostrum* lineages from Asian ancestors	This paper
Middle-Late Pliocene	15-1.5	Formation and evolution of the Ponto-Caspian group of extant species	This paper
Early-Late Pliocene	5.5-3.5	Divergence of *Acepenser stellatus* and *Pseudoscaphirhynchus,* evolution of *Pseudoscaphirhynchus*	This paper
Middle-Late Pleistocene	1.10-0.07	Formation and diversification of the A *gue/denstaedtii-A. naccarii-A. bacrii* complex	This paper
Holoccnc	3,000 ya	Colonization the Baltic Sea by *A. sturio*	Ludwig ctal., 2002
Holoccnc	1.200-800 ya	Replacement of *A. sturio* by *A. oxvrinchus* in the Baltic Sea	Ludwig c t a l. 2002

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