ACTA ICHTHYOLOGICA ET PISCATORIA (2014) 44 (2): 111–116 DOI: 10.3750/AIP2014.44.2.04

THE KARYOTYPE OF THE AMU DARYA STURGEON, *PSEUDOSCAPHIRHYNCHUS KAUFMANNI* (ACTINOPTERYGII: ACIPENSERIFORMES: ACIPENSERIDAE)

Konstantin V. KOVALEV [1], Dmitry A. BALASHOV [1], Alexey L. CHERNIAK [2], Elena B. LEBEDEVA [3], Ekaterina D. VASIL'EVA [4*], and Victor P. VASIL'EV [3]

[1] *All-Russia Institute of Freshwater Fisheries, Rybmoe, Moskovskaya oblast, Russia*

[2] *Moscow Zoo, Moscow, Russia*

[3] *Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia*

[4] *Zoological Museum, Moscow State University, Moscow, Russia*

Kovalev K.V., Balashov D.A., Cherniak A.L., Lebedeva E.B., Vasil'eva E.D., Vasil'ev V.P. 2014. The karyotype of the Amu Darya sturgeon, *Pseudoscaphirhynchus kaufmanni* (Actinopterygii: Acipenseriformes: Acipenseridae). Acta Ichthyol. Piscat. 44 (2): 111–116.

Background. Karyological studies of acipenserid fishes are of great importance because they present the only direct method to evaluate their ploidy levels for further research on polyploid evolution in these fishes. They are also important for prediction of the results of interspecific hybridizations in sturgeon aquaculture. None of the species of the genus *Pseudoscaphirhynchus* has hitherto been studied karyologically. The aim of this paper was to present the first data on the karyotype of the dwarf form of *Pseudoscaphirhynchus kaufmanni* (Kessler, 1877).
Materials and methods. Three females of the dwarf form of *Pseudoscaphirhynchus kaufmanni* of the total body length 19–23 cm were caught in the Vakhsh River (Amu Darya River drainage), Tadzhikistan, in 2012. The chromosome slides were prepared by using previously published karyological method of Vasil'ev and Sokolov. Totally, 14 metaphase plates were analyzed.
Results. The karyotype of *Pseudoscaphirhynchus kaufmanni* consists of 118–120 chromosomes and includes about 18–20 large bi-armed chromosomes, about 32–34 small bi-armed chromosomes, from one to two pairs of large acrocentrics, and about 64 small acrocentrics or microchromosomes.
Conclusion. The karyological study revealed that *Pseudoscaphirhynchus kaufmanni* belongs to low-chromosome acipenserid group with about 120 chromosomes. Its karyotype demonstrates noticeable differences from the karyotype of the shovelnose sturgeon, *Scaphirhynchus platorhynchus* (Rafinesque, 1820), in the number of large acrocentrics, thereby, karyological data confirms polyphyletic origin of the subfamily Scaphirhynchinae (or tribe Scaphirhynchini).

Keywords: acipenserids, karyology, ploidy levels, polyphyly, shovelnose sturgeons

INTRODUCTION

The order Acipenseriformes is an ancient group of vertebrate animals represented by two fossil families: Peipiaosteidae, extending back to the Upper Jurassic, and Chondrosteidae, dating from the Lower Jurassic, and extant representatives in two families: Acipenseridae and Polyodontidae (see Findeis 1997, Nelson 2006). Both extant families have fossil records with Mesozoic dating. Most primitive and the oldest paddlefishes (Polyodontidae) are known from the Lower Cretaceous of China (*Protopsephurus*) and North America (*Paleopsephurus*) (Grande and Bemis 1991); the only two living freshwater species represent two distinct phylogenetic lineages and genera (Hilton 2004): *Polyodon spathula* (Walbaum, 1792) in United States and *Psephurus gladius* (Martens, 1862) in China. Both the oldest sturgeon (Acipenseridae) species dated back to the Late Cretaceous in Montana are treated as taxa with an uncertain position, namely plesion *Protoscaphirhynchus* Wilimovsky, 1956, *insertae sedis,* and plesion *Priscosturion* Grande et Hilton, 2009, *sedis mutabilis* (see Hilton et al. 2011). The phylogenetic relations and taxonomic states of recent 25 sturgeon species also are the subject of a long-term discussion.

Usually, living Acipenserid fish are subdivided into two subfamilies. Some authors (Findeis 1997, Nelson 2006)

* Correspondence: Dr Ekaterina D. Vasil'eva (Екатерина Васильева), Зоологический музей, Московский государственный университет, Большая Никитская 6, Moskva, 125009 Russia, phone: +7 495 629-49-06, fax: +7 495 629-48-25, e-mail: (EDV) *vas_katerina@mail.ru*, (KVK) *silur5@mail.ru*, (DAB) *balashofff@gmail.com*, (ChAL) *alexeycherniak@yandex.ru*, (EBL) *lebedester@gmail.com*, (VPV) *vas_katerina@mail.ru*.

recognize two species, namely *Huso huso* (Linnaeus, 1758) and *Huso dauricus* (Georgi, 1775), as a separate subfamily Husinae. (The recent taxonomic revision—Vasil'eva et al. 2009—proved, by using recent molecular genetic, karyological, and morphological evidences, that *Huso* Brandt et Ratzeburg, 1833 is not valid genus, but a junior synonymof *Acipenser* Linnaeus, 1758). The remaining species from the subfamily Acipenserinae they divide into two tribes—Acipenserini, and Scaphirhynchini—the latter tribe including genera *Scaphirhynchus* and *Pseudoscaphirhynchus* (see Grande and Bemis 1996). Another taxonomic hypothesis accepts Scaphirhynchinae as a separate subfamily opposed to all other Acipenserids (Berg 1905, Sokolov and Berdichevskiĭ 1989). Both of these taxonomic concepts do not agree with recent molecular data (Ludwig et al. 2001, Dillman et al. 2007, Krieger et al. 2008).

The genus *Scaphirhynchus* includes three species occurring in the freshwaters of North America: *Scaphirhynchus albus* (Forbes et Richardson, 1905), *Scaphirhynchus platorhynchus* (Rafinesque, 1820), and *Scaphirhynchus suttkusi* Williams et Clemmer, 1991. Three species of the genus *Pseudoscaphirhynchus* used to inhabit rivers of the Aral Sea basin in Central Asia. The endemic Syr Darya shovelnose sturgeon, *Pseudoscaphirhynchus fedtschenkoi* (Kessler, 1872), has not been recorded since the 1960s (Birstein 1997) and most probably is extinct. The second species, the small Amu Darya shovelnose sturgeon *Pseudoscaphirhynchus hermanni* (Kessler, 1877), endemic in the middle and lower reaches of the Amu Darya drainage, is a very rare, critically endangered fish. Only the third species, the large Amu Darya shovelnose sturgeon, *Pseudoscaphirhynchus kaufmanni* (Kessler, 1877), despite of its disappearance from the most part of historic area, is still recorded in the Vakhsh River and in the middle reaches of the Amu Darya River (Birstein 1997). *Pseudoscaphirhynchus kaufmanni* is represented by two forms with unclear taxonomic relations, namely the common- and the dwarf forms (Sagitov 1969). In the early 1990s, the dwarf form predominated in the population (Birstein 1997).

The comparative karyological studies of Acipenserid fishes are of great importance for many reasons. First of all, the karyological studies in Acipenseridae revealed three ploidy levels in these fishes (Fontana et al. 2008a, Vasil'ev 2009, Vasil'ev et al. 2010), treated as diploid–tetraploid–hexaploid or tetraploid–octoploid–12-ploid (Vasil'ev 2009). Therefore—the polyploidization events in different Acipenserid evolutionary branches as well as the phylogenetic relations between groups with different ploidy levels—call for further studies. Moreover, the knowledge on acipenserid karyotypes is essential in sturgeon aquaculture, as a tool for predicting fertile artificial hybrid lines, because their fertility depends on the ploidy level of the hybridized original parental species. However, the karyotypes of *Pseudoscaphirhynchus* species have never been studied. It should be mentioned that Birstein et al. (1993) presented data on the nuclear DNA content in *P. kaufmanni*, but this method, as well as the microsatellite analyses—in terms of ploidy level evaluation—are considered indirect (and therefore less accurate) comparative methods. Consequently, they often lead to erroneous conclusions about the ploidy state in some sturgeon species. For example, Ludwig et al. (2001) erroneously estimated octoploid level for *Acipenser mikadoi* Hilgendorf, 1892 and diploid level for *Acipenser dauricus* Georgi, 1775 by the analysis of microsatellite allelic distributions. Thus, they repeat the same mistaken conclusions obtained by Birstein et al. (1993) based on the nuclear DNA content values. In contrast to these inferences, the direct karyological study at first proved about 250 chromosome numbers (tetraploid ploidy level) both for *A. mikadoi* and *A. dauricus* (Vasil'ev et al. 2009).

Therefore, the main goal of this research was to evaluate the ploidy level of *Pseudoscaphirhynchus kaufmanni* by direct karyological study.

MATERIALS AND METHODS

The work was carried out within the Program of EAR-AZA (Euro Asian Regional Association of Zoos and Aquariums) entitled "Conservation of the rarest sturgeons of Eurasia" (http://earaza.ru/?p=449). In this Program, we develop methods for artificial propagation of the Amu Darya shovelnose sturgeon, *Pseudoscaphirhynchus kaufmanni*, which is a protected species in Tadzhikistan.

Three specimens used in this study were caught in the Vakhsh River (tributary of the Amu Darya River), Tadzhikistan in April 2012 under the permission of the Tadzhikistan Government. These females, with total body length of 19–23 cm and weighing 50–75 g, were identified as the dwarf form of *Pseudoscaphirhynchus kaufmanni* because they possessed its species-specific characters, namely the tail filament and developed snout thorns (Berg 1948). All females were injected with 0.5–0.75 mL colchicine solution (0.5%–0.6%) depending on their weight. After 4.5–10.5 h cells of the head lymphoid organ were used for chromosome slide preparing by using previously published karyological method (Vasil'ev and Sokolov 1980). The study was conducted by using anesthesia of fishes with a solution of MS-222. Metaphase chromosomes stained in 4% Giemsa solution in phosphate buffer (pH 6.8) were counted with PC software Quick Photo Micro. For karyological description we selected metaphase plates in which the chromosome number can be count accurate to two chromosomes. Totally, 14 metaphase plates were detailed analyzed: five, six, and three plates, respectively, from each female. These numbers are suitable for the primary karyotype description. Chromosome morphology in chromosomes with large and medium sizes was determined according to Levan et al. (1964). The classification of microchromosomes was not possible. Karyotype was arranged using PC software and Adobe Photoshop®CS5.

RESULTS AND DISCUSSION

The karyotype of the large Amu Darya shovelnose sturgeon, *Pseudoscaphirhynchus kaufmanni*, consists of 118–120 chromosomes and includes about 18–20 large bi-armed chromosomes, about 32–34 small bi-armed chromosomes, one–two pairs of large acrocentrics (26th–27th pairs) and about 64 small acrocentrics or microchromosomes (Fig. 1). Among large metacentrics, the smallest last (8th–10th pairs) are about equal or longer than the arm of the first pair of metacentrics, while, the largest first small metacentrics (11th–12th pairs) are distinctly shorter than the arm of the first pair of large metacentrics. The large acrocentrics have their length similar to the length of the arm of chromosomes from the 10th pair of metacentrics.

To date, 18 species from the genus *Acipenser* are karyologically studied, and the only non-karyotyped species is *Acipenser dabryanus* Duméril, 1869. The karyotype of *Scaphirhynchus platorhynchus* is also described (Ohno et al. 1969). All karyotyped Acipenserids are divided into three discrete groups. The first group includes 120-chromosome species (Table 1). The second group combines sturgeon species with 250–270 chromosomes, which are apparently represented by two subgroups with different karyotypes, namely:

- European species (*Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833; *Acipenser persicus* Borodin, 1897; and *Acipenser naccarii* Bonaparte, 1836) and Siberian sturgeon, *Acipenser baerii* Brandt, 1869, with about 250 chromosomes;
- Species distributed in America and Eastern Asia (*Acipenser transmontanus* Richardson, 1836; *Acipenser fulvescens* Rafinesque, 1817; *Acipenser medirostris* Ayres, 1854; *Acipenser schrenckii* Brandt, 1869; *Acipenser sinensis* Gray, 1835; *A. mikadoi*; and *A. dauricus*) with about 264–270 chromosomes.

It should be emphasized that the grouping of 250–270-chromosome species correlates with the separation of Atlantic and Pacific clades of Acipenserids in phylogenetic

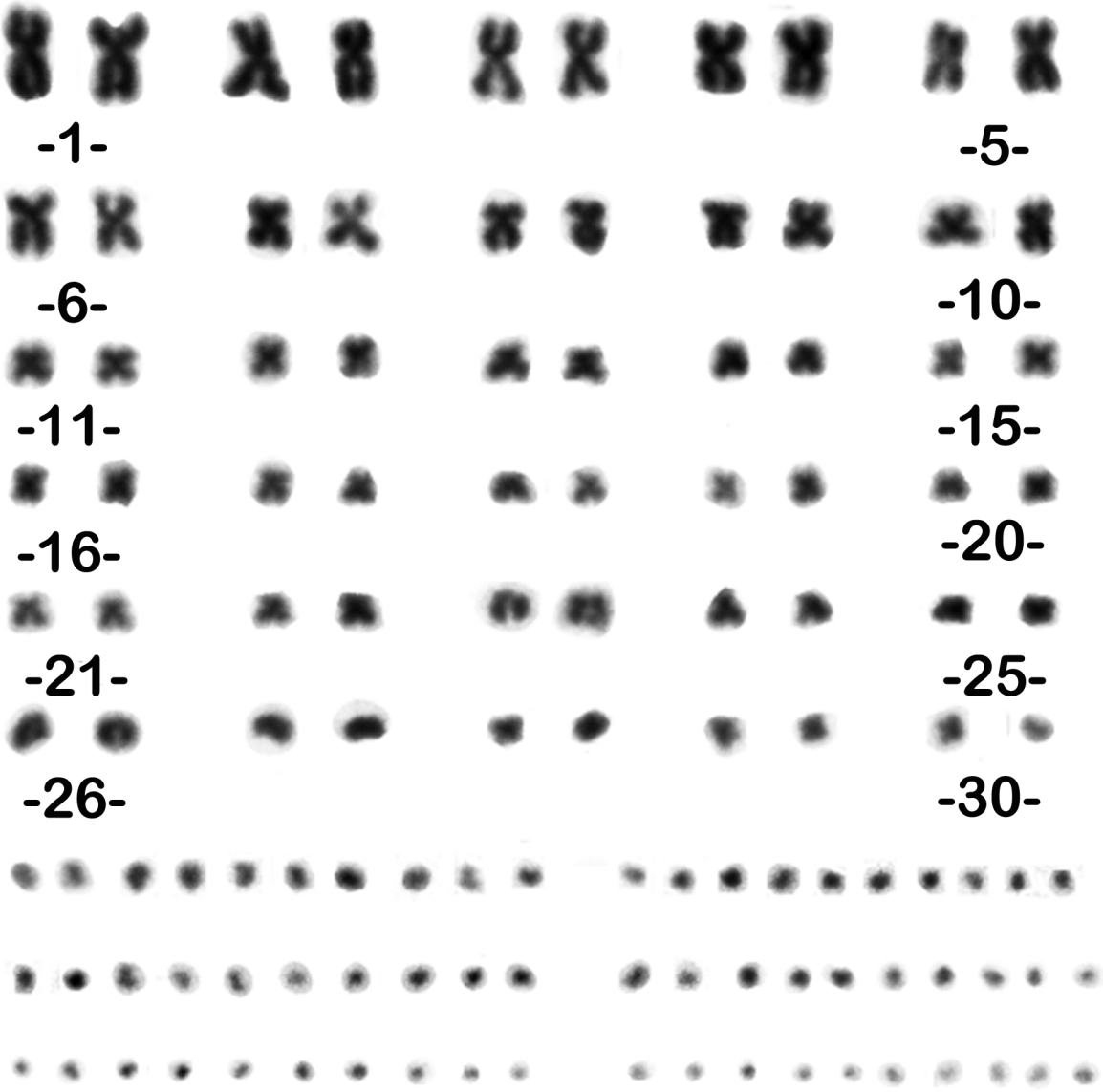

Fig. 1. The karyotype of the Amu Darya sturgeon, *Pseudoscaphirhynchus kaufmanni*; 1–30 = the numeration of the first 30 pairs of the largest chromosomes

Table 1

Karyological characters of the low-chromosome (2n~120) acipenseriform species

Species	2*n*	LB	SB	LA	*M*	NF	Reference
Polyodon spathula	120	18–20	20–22	8	~72	~160	Dingerkus and Howell 1976
Scaphirhynchus platorhynchus	~112	18–20	30–32	8–10	~52	~162	Ohno et al. 1969
Pseudoscaphirhynchus kaufmanni	~118–120	18–20	32–34	2–4	~64	~172	Presently reported study
Acipenser sturio	~121	20–22	48–50	2	~48	~191	Fontana and Colombo 1974
Acipenser nudiventris	~118	16–18	42–44	2	~56	~178	Sokolov and Vasil'ev 1989
Acipenser ruthenus	~118	18–20	36–38	2	~58	~174	Vasil'ev 1985
							Ráb 1986
							Birstein and Vasiliev 1987
Acipenser stellatus	~118	20–22	34–36	4	~58	~174	Vasil'ev 1985
				4–6			Birstein and Vasiliev 1987
Acipenser oxyrinchus	~121	24	54	0–2	~41	~199	Fontana et al. 2008b
Acipenser huso	~118	16–18	44–66	2	~36–50	~180–202	Fontana et al. 1998
							Birstein and Vasiliev 1987

2*n* = chromosome number, LB = number of large bi-armed chromosomes, SB = number of small bi-armed chromosomes, LA = number of large acrocentric chromosomes, *M* = number of microchromosomes, NF = approximate number of chromosome arms (all microchromosomes are classified as acrocentrics); Note: the reported differences in microchromosome numbers in the karyotype of *Acipenser huso* and the increased number of chromosome arms (NF = 202) were caused by very weak chromosome condensation in the karyogram, presented by Fontana et al. (1998); this results in the possibility to define morphology of some microchromosomes.

trees based on molecular genetic data (Ludwig et al. 2001, Krieger et al. 2008). The third group includes the only species *Acipenser brevirostrum* Lesueur, 1818 with 370–372 chromosomes (Kim et al. 2005, Fontana et al. 2008a). According to karyological data obtained in the presently reported study, *Pseudoscaphirhynchus kaufmanni* belongs to the first Acipenserid group of low-chromosome species with about 120 chromosomes.

The detailed analysis of chromosome morphology in the metaphase plates with approximately similar chromosome condensation, demonstrates intraspecific variability in the numbers of different types of chromosomes in low-chromosome species (Table 1). Although slight differences in numbers of large metacentrics can be related to the classification accuracy of these chromosomes in different species, the most noticeable differences are observed in the numbers of small and medium bi-armed chromosomes, as well as large acrocentrics. Exactly, *Polyodon spathula* and *Scaphirhynchus platorhynchus* both have 8–10 large acrocentrics, whereas the other 120-chromosome species have only 2–6 large acrocentrics (Table 1). *Pseudoscaphirhynchus kaufmanni* has 2–4 large acrocentrics (Table 1; Fig. 1); thereby, karyological data does not support close relation between *Scaphirhynchus* and *Pseudoscaphirhynchus* postulated by morphological studies and resulted in their accepted taxonomic integration in the same tribe or subfamily. The same results were obtained by molecular genetic researches, not supported sister relation between *Scaphirhynchus* and *Pseudoscaphirhynchus*, but demonstrated *Pseudoscaphirhynchus* as a sister taxon for the genus *Acipenser* (see Birstein et al. 1997) or for some of *Acipenser* species (Dillman et al. 2007, Krieger et al. 2008). Thus, monophyly of the subfamily Scaphirhynchinae, or tribe Scaphirhynchini, has been consistently rejected by all phylogenetic reconstruction methodologies with the molecular character set, while monophyly of both genera (*Scaphirhynchus* and *Pseudoscaphirhynchus*) was firmly supported (Dillman et al. 2007).

In a concluding remark, we would like to state that our data on the karyotype structure of *Pseudoscaphirhynchus kaufmanni* confirm polyphyletic origin for the subfamily Scaphirhynchinae (or tribe Scaphirhynchini) previously revealed by molecular genetic studies.

ACKNOWLEDGEMENTS

This study was partially supported by the Russian Foundation for Basic Researches, project no. 12-04-01012-a and the Program "Biological Diversity" of the Presidium of the Russian Academy of Sciences, subprogram "Gene Pools and Genetic Diversity".

REFERENCES

Berg L.S. 1905. Ryby Turkestana. Naučnye rezul'taty Aral'skoj ekspeditsii. [Fishes of Turkestan. Scientific results of the Aral expedition.] Izvestiâ Turkestanskogo otdeleniâ Russkogo geografičeskogo obŝestva **4**: 1–261. [In Russian.]

Berg L.S. 1948. Ryby presnyh vod SSSR i sopredel'nyh stran. [Fishes of fresh waters of the USSR and adjacent countries.] Part 1. Izdatel'stvo Akademii Nauk SSSR, Moskva–Leningrad. [In Russian.]

Birstein V.J. 1997. Threatened fishes of the world: *Pseudoscaphirhynchus* spp. (Acipenseridae). Environmental Biology of Fishes **48** (1–4): 381–383. DOI: 10.1023/A:1007377317423

Birstein V.J., Hanner R., DeSalle R. 1997. Phylogeny of the Acipenseriformes: cytogenetic and molecular approaches. Environmental Biology of Fishes **48** (1–4): 127–155. DOI: 10.1023/A:1007366100353

Birstein V.J., Poletaev A.I., Goncharov B.F. 1993. DNA content in Eurasian sturgeon species determined by flow cytometry. Cytometry **14** (4): 377–383. DOI: 10.1002/cyto.990140406

Birstein V.J., Vasiliev V.P. 1987. Tetraploid-octoploid relationships and karyological evolution in the order Acipenseriformes (Pisces): karyotypes, nucleoli, and nucleolus-organizer regions in four acipenserid species. Genetica **73** (1): 3–12. DOI: 10.1007/BF00126973

Dillman C.B., Wood R.M., Kuhajda B.R., Ray J.M., Salnikov V.B., Mayden R.L. 2007. Molecular systematics of Scaphirhynchinae: an assessment of North American and Central Asian freshwater sturgeon species. Journal of Applied Ichthyology **23** (4): 290–296. DOI: 10.1111/j.1439-0426.2007.00919.x

Dingerkus G., Howell W.M. 1976. Karyotypic analysis and evidence of tetraploidy in the North American paddlefish, *Polyodon spathula*. Science **194** (4267): 842–844. DOI: 10.1126/science.982045

Findeis E.K. 1997. Osteology and phylogenetic interrelationships of sturgeons (Acipenseridae). Environmental Biology of Fishes **48** (1–4): 73–126. DOI: 10.1023/A:1007372832213

Fontana F., Colombo G. 1974. The chromosomes of Italian sturgeons. Experientia **30** (7): 739–742. DOI: 10.1007/BF01924158

Fontana F., Congiu L., Mudrak V.A., Quattro J.M., Smith T.I.J., Ware K., Doroshov S.I. 2008a. Evidence of hexaploid karyotype in shortnose sturgeon. Genome **51** (2): 113–119. DOI: 10.1139/g07-112

Fontana F., Lanfredi M., Kirschbaum F., Garrido-Ramos M.A., Robles F., Forlani A., Congiu L. 2008b. Comparison of karyotypes of *Acipenser oxyrinchus* and *A. sturio* by chromosome banding and fluorescent in situ hybridisation. Genetica **132** (3): 281–286. DOI: 10.1007/s10709-007-9171-4

Fontana F., Tagliavini J., Congiu L., Lanfredi M., Chicca M., Laurente C., Rossi R. 1998. Karyotypic characterization of the great sturgeon, *Huso huso*, by multiple staining techniques and fluorescent in situ hybridization. Marine Biology **132** (3): 495–501. DOI: 10.1007/s002270050415

Grande L., Bemis W.E. 1991. Osteology and phylogenetic relationships of fossil and recent paddlefishes (Polyodontidae) with comments on the interrelationships of Acipenseriformes. Journal of Vertebrate Paleontology **11** (Suppl. 1): 1–121. DOI: 10.1080/02724634.1991.10011424

Grande L., Bemis W.E. 1996. Interrelationships of Acipenseriformes, with comments on 'Chondrostei'. Pp. 85–115. *In*: Stiassny M.L.J., Parenty L.R., Johnson G.D. (eds.) Interrelationships of Fishes. Academic Press, San Diego, California, USA.

Hilton E.J. 2004. The caudal skeleton of Acipenseriformes (Actinopterygii: Chondrostei): recent advances and new observations. Pp. 599–617. *In*: Arratia G., Wilson M.V.H., Cloutier R. (eds.) Recent advances in the origin and early radiation of vertebrates. Verlag Dr. Pfeil, Munich, Germany.

Hilton E.J., Grande L., Bemis W.E. 2011. Skeletal anatomy of the shortnose sturgeon, *Acipenser brevirostrum* Lesueur, 1818, and the systematics of sturgeons (Acipenseriformes, Acipenseridae). Fieldiana Life and Earth Sciences **2011** (3): 1–168. DOI: 10.3158/2158-5520-3.1.1

Kim D.S., Nam Y.K., Noh J.K., Park C.H., Chapman F.A. 2005. Karyotype of North American shortnose sturgeon *Acipenser brevirostrum* with the highest chromosome number in the Acipenseriformes. Ichthyological Research **52** (1): 94–97. DOI: 10.1007/s10228-004-0257-z

Krieger J., Hett A.K., Fuerst P.A., Artyukhin E., Ludwig A. 2008. The molecular phylogeny of the order Acipenseriformes revisited. Journal of Applied Ichthyology **24** (Suppl. s1): 36–45. DOI: 10.1111/j.1439-0426.2008.01088.x

Levan A., Fredga K., Sandberg A.A. 1964. Nomenclature for centromeric position chromosomes. Hereditas **52** (2): 201–220. DOI: 10.1111/j.1601-5223.1964.tb01953.x

Ludwig A., Belfiore N.M., Pitra C., Svirsky V., Jenneckens I. 2001. Genome duplication events and functional reduction of ploidy levels in sturgeon (*Acipenser*, *Huso* and *Scaphirhynchus*). Genetics **158** (3): 1203–1215.

Nelson J.S. 2006. Fishes of the world. 4th edn. John Wiley and Sons, Hoboken, NJ, USA.

Ohno S., Muramoto J., Stenius C., Christian L., Kitterell W.A., Atkin N.B. 1969. Microchromosomes in holocephalian, chondrostean and holostean fishes. Chromosoma **26** (1): 35–40. DOI: 10.1007/BF00319498

Ráb P. 1986. A note on the karyotype on the sterlet, *Acipenser ruthenus* (Pisces, Acipenseridae). Folia Zoologica **35** (1): 73–78.

Sagitov N.I. 1969. O karlikovoj forme bol'šogo amudar'inskogo lželopatonosa. [On the dwarf form of the large Amu-Darya shovelnose sturgeon.] Naučnye Doklady Vysšej Školy, Biologičeskie nauki **6**: 12–15 [In Russian.]

Sokolov L.I., Berdichevskiĭ L.S. 1989. Acipenseridae Bonaparte, 1831. Pp. 150–153. *In*: Holčik J. (ed.) The freshwater fishes of Europe. Vol. 1. Part 2. General introduction to fishes. Acipenseriformes. AULA-Verlag, Wiesbaden, Germany.

Sokolov L.I., Vasil'ev V.P. 1989. *Acipenser nudiventris* Lovetsky, 1928. Pp. 206–226. *In*: Holčik J. (ed.) The Freshwater Fishes of Europe. Vol. 1. Part 2. General Introduction to Fishes. Acipenseriformes. AULA-Verlag, Wiesbaden, Germany.

Vasil'ev V.P. 1985. Évoljutsionnaâ kariologiâ ryb. [Evolutionary Karyology of Fishes.] Nauka, Moskva [In Russian.]

Vasil'ev V.P. 2009. Mechanisms of polyploid evolution in fish: polyploidy in sturgeons. Pp. 97–117. *In*: Carmona R., Domezain A., García-Gallego M., Hernando J.A., Rodríguez F., Ruiz-Rejón M. (eds.) Biology, conservation and sustainable development of sturgeons. Fish and Fisheries Series. Vol. 29. Springer, Netherlands. DOI: 10.1007/978-1-4020-8437-9_6

Vasil'ev V.P., Sokolov L.I. 1980. Metod izučeniâ kariotipov hrâŝčevyh ganoidov (Chondrostei). [The method to study

the karyotypes in Chondrostei.] Citologiâ **22** (9): 1106–1109 [In Russian.].

Vasil'ev V.P., Vasil'eva E.D., Shedko S.V., Novomodny G.V. 2009. Ploidy levels in the kaluga, *Huso dauricus* and Sakhalin sturgeon, *Acipenser mikadoi* (Acipenseridae, Pisces). Doklady Biological Sciences **426** (1): 228–231. DOI: 10.1134/S0012496609030119

Vasil'ev V.P., Vasil'eva E.D., Shedko S.V., Novomodny G.V. 2010. How many times has polyploidization occurred during Acipenserid evolution? New data on the karyotypes of sturgeons (Acipenseridae, Actinopterygii) from the Russian Far East. Journal of Ichthyology **50** (10): 950–959. DOI: 10.1134/S0032945210100048

Vasil'eva E.D., Vasil'ev V.P., Shedko S.V., Novomodny G.V. 2009. The revision of the validity of genus *Huso* (Acipenseridae) based on recent morphological and genetic data with particular reference to the kaluga *H. dauricus.* Journal of Ichthyology **49** (10): 861–867. DOI: 10.1134/S0032945209100038

Received: 18 February 2014
Accepted: 29 April 2014
Published electronically: 30 June 2014