Interspecific hybridization in natural sturgeon populations of the Eastern Black Sea: the consequence of drastic population decline?

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Received: 10 February 2021 / Accepted: 14 October 2021 / Published online: 30 October 2021
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Abstract
The eastern part of the Black Sea and its tributaries are suitable habitats for several sturgeon species, among which Acipenser gueldenstaedtii, A. stellatus, A. nuditentris, A. persicus, A. sturio, and H. huso are well documented. However, different threats have led these species to a dramatic decline, all of them are currently listed as Critically Endangered, and some Locally Extinct, in that area. We tested 94 wild sturgeon samples from the Black Sea and Rioni River by analyzing the mitochondrial Control Region and nuclear markers for hybrid identification. The data analyses (1) assessed mitochondrial diversity among samples, (2) identified their species, as well as (3) indicated instances of hybridization. The data collected, besides confirming a sharp decrease of catches of Beluga and Stellate sturgeon in recent years, also revealed four juvenile hybrids between Russian and Stellate sturgeon, providing the first evidence of natural interspecific hybridization in the Rioni. The present communication raises concerns about the status of sturgeon species in this area and underlines the urgent need for conservation programs to restore self-sustaining populations.

Keywords Sturgeons · Interspecific hybrids · Acipenser gueldenstaedtii · Acipenser stellatus · Rioni · Black Sea

Introduction
Sturgeons are among the most endangered species groups in the world according to the International Union for Conservation of Nature (IUCN 2010), with some of the most imperiled species distributed in the Palearctic region. The Eastern part of the Black Sea and a major tributary in the Caucasus, the Rioni River, are known to have hosted in historical times at least five sturgeon species. The Russian sturgeon (Acipenser gueldenstaedtii), the Stellate sturgeon (A. stellatus), the Beluga sturgeon (Huso huso), the European sturgeon (A. sturio), and the Ship sturgeon (A. nuditentris) (Variadilis et al. 1998; Guchmanidze 2009). All of these are listed by IUCN as Critically Endangered, with European and Ship sturgeons also believed to be Locally Extinct, with wild populations considered to be extirpated from the Black Sea basin (Gessner et al. 2010; Mugue et al. 2016).

The population decline is mainly caused by habitat degradation, including river damming and consequent high sediment flushing, overfishing, and pollution. Accurate historical or present assessments of population sizes are not available, but there are indications that sturgeon populations in the region have been in steep decline since the early 20th century (Beridze et al. 2021). Historically, the Rioni River in Georgia is known as one of the main sites for sturgeon spawning in this area (IUCN, 2010, www.iucnredlist.org) and it currently is the only remaining functional sturgeon
spawning river of the Eastern Black Sea. This was confirmed by monitoring research on sturgeon recruitment in the Rioni conducted by Fauna & Flora International (FFI) between 2018 and 2020. Intensive field surveys, annually held from March until October, led to the discovery of multiple juvenile sturgeon specimens of various species. In addition, data collection with associated anglers on the Rioni led to the discovery of eight specimens of *A. nudiventris* in 2020. Their possible origin from an ongoing captive breeding program in Krasnodar (River Kuban) has been excluded, supporting the hypothesis of a relict reproductively active population of *A. nudiventris* in the Rioni River (Beridze et al. 2021).

This study reports the results of the genetic characterization of sturgeons collected within the first three years of these monitoring activities. Monitoring is ongoing, with the aim of evaluating the state of sturgeon populations of this area and verifying abundance of the different species, the purity of the animals and whether the sampled individuals come from restocking activities or if they result from natural reproduction.

The collected data has unexpectedly indicated the occurrence of interspecific hybrids between two species of sturgeon historically known to reproduce in the Rioni River. The occurrence of interspecific hybridization events is discussed in light of implications for the conservation of natural sturgeon populations.

**Materials and methods**

Between 2018 and 2020, a sturgeon sampling campaign was carried out in the Georgian part of the Black Sea (BS) and Rioni River (RR = Rioni River; RM = Rioni mouth) (Fig. 1A) by FFI. A total of 94 tissue samples were collected (Table 1); captured animals, mostly juveniles, were immediately released after sampling.
Table 1 Summary of the results from mitochondrial control region and diagnostic nuclear markers which specifically identify A. stellatus (ste), A. gueldenstaedtii (gue) and H. huso (hus)

| Year | SampleID | mtDNA (present study) | Match with previously known haplotypes | Nuclear markers |
|------|----------|------------------------|----------------------------------------|-----------------|
| 2018 | Ac91_BS  | ste_Hap1               | STE-HAP21                               | AS              |
|      | Ac86_RR  | ste_Hap2               | STE-HAP108                              | CS              |
|      | Ac89_BS  | ste_Hap3               | /                                       | /               |
|      | Ac92_BS, Ac105_BS | ste_Hap4       | /                                       | /               |
|      | Ac94_RM, Ac114_RM | ste_Hap5    | STE-HAP83                               | AS              |
| 2019 | Ac137_RM | ste_Hap5               | STE-HAP83                               | AS              |
|      | Ac101_BS | ste_Hap6               | AF168525*                               | /               |
|      | Ac115_RM | ste_Hap7               | STE-HAP62                               | AS              |
|      | Ac123_BS | ste_Hap8               | AF168528*                               | /               |
|      | Ac139_BS | ste_Hap9               | AF168535*                               | /               |
|      | Ac157_BS | ste_Hap10              | STE-HAP15                                | CS/UR           |
|      | Ac156_BS | ste_Hap11              | STE-HAP64                                | DR/UR           |
| 2020 | Ac192_BS | ste_Hap12              | STE-HAP8                                | AS              |

|      | Ac70_BS, Ac76_BS | gue_Hap1 | /                           | /               |
|      | Ac93_RM, Ac95_RM | gue_Hap2 | GUE_HAP137                 | BS              |
|      | Ac96_RM          | gue_Hap3 | /                           | /               |
|      | Ac112_RR         | gue_Hap4 | /                           | /               |
|      | Ac113_RR         | gue_Hap5 | HQ7304710*                 | GUE_HAP195      | BS/CS           |
| 2019 | Ac125_BS         | gue_Hap4 | /                           | /               |
|      | Ac120_BS         | gue_Hap5 | HQ7304710*                 | GUE_HAP195      | BS/CS           |
|      | Ac116_RM, Ac136_RM | gue_Hap6 | GUE_HAP101                 | AS/BS           |
|      | Ac118_RR, Ac144_RM, Ac145_RM | gue_Hap7 | /                           | /               |
|      | Ac119_BS         | gue_Hap8 | GUE_HAP2                  | BS/CS           |
|      | Ac121_BS, Ac135_RM, Ac143_RM, Ac146_RM, Ac147_RM, Ac148_RM | gue_Hap9 | AF238725*                | GUE_HAP11      | AS/BS           |
|      | Ac127_BS         | gue_Hap10 | GUE_HAP140                 | DR              |
|      | Ac128_BS, Ac140_RM | gue_Hap10 | GUE_HAP140                 | DR              |
|      | Ac133_BS         | gue_Hap11 | GUE_HAP135                | BS              |
|      | Ac150_RM         | gue_Hap11 | GUE_HAP135                | BS              |
| 2020 | Ac198_RM, Ac200_RM, Ac203_RM, Ac205_RM | gue_Hap2 | GUE_HAP137                 | BS              |
|      | Ac196_RM         | gue_Hap4 | /                           | /               |
|      | Ac189_BS, Ac194_BS, Ac210_BS, Ac201_RM | gue_Hap5 | HQ7304710*                 | GUE_HAP195      | BS/CS           |
|      | Ac199_BS         | gue_Hap6 | GUE_HAP101                 | AS/BS           |
|      | Ac1371_RM, Ac182_RM, Ac185_RM, Ac188_RM | gue_Hap7 | /                           | /               |
|      | Ac204_BS, Ac209_BS, Ac165_RM, Ac161_RM, Ac163_RM, Ac168_RM, Ac172_RM, Ac174_RM, Ac177_RM, Ac179_RM, Ac181_RM, Ac183_RM, Ac187_RM, Ac202_RM, Ac208_RM | gue_Hap9 | AF238725*                 | GUE_HAP11      | AS/BS           |
|      | Ac160_RR         | gue_Hap9 | AF238725*                 | GUE_HAP11      | AS/BS           |
|      | Ac197_RM         | gue_Hap10 | GUE_HAP140                | DR              |
|      | Ac175_BS         | gue_Hap11 | GUE_HAP135                | BS              |
|      | Ac162_RM, Ac164_RM, Ac173_RM | gue_Hap11 | GUE_HAP135                | BS              |
|      | Ac167_RM         | gue_Hap12 | GUE_HAP140                | DR/UR           |
|      | Ac169_RM         | gue_Hap13 | /                           | /               |
|      | Ac170_RM, Ac176_RM, Ac180_RM, Ac186_RM, Ac192_RM | gue_Hap14 | GUE_HAP185                | CS              |
|      | Ac207_RM         | gue_Hap15 | /                           | /               |
|      | Ac212_BS         | gue_Hap16 | GUE_HAP142                | DR/CS           |
| 2018 | Ac72_BS, Ac90_BS | hus_Hap1 | HUS_HAP90                  | DR/CS           |
|      | Ac73_BS          | hus_Hap2 | HUS_HAP73                  | DR              |
|      | Ac74_BS          | hus_Hap3 | /                           | /               |
| 2019 | Ac98_BS, Ac99_BS | hus_Hap4 | /                           | /               |
|      | Ac100_BS         | hus_Hap5 | HUS_HAP12                  | AS/BS/CS        |
|      | Ac102_BS         | hus_Hap6 | /                           | /               |
|      | Ac104_BS         | hus_Hap7 | HUS_HAP75                  | DR              |
Samples are sorted by collecting year and by haplotype. Any correspondence with known haplotypes and their distribution is also reported. Codes in italics indicate haplotypes detected in the Russian sturgeon broodstocks. For each sample, the amplification of diagnostic nuclear markers is shown in grey-filled cells. Amplification of nuclear markers from four individuals failed, indicated in the table by the empty cells.

| Maternal species | N  | Nh | Ps | h       | π     |
|------------------|----|----|----|---------|-------|
| A. stellatus     | 15 | 12 | 52 | 0.962 ± 0.040 | 0.019 ± 0.010 |
| A. gueldenstaedtii | 70 | 16 | 87 | 0.867 ± 0.029 | 0.023 ± 0.011 |
| H. huso         | 9  | 7  | 36 | 0.944 ± 0.070 | 0.017 ± 0.009 |

N number of individuals, Nh number of haplotypes, Ps Polymorphic sites, h haplotype diversity, π nucleotide diversity
origin. It is worth noting that, for one of these animals, an informal identification as *A. stellatus* was provided before release by the FFI team members who collected it. The clear discordance between this *a priori* morphological classification and the haplotype sequence likewise indicates hybrid origin of that individual. Moreover, even though morphological indications for the other three detected hybrids were not provided, the *A. stellatus* diagnostic marker has never given false positive amplification in 11 other sturgeon species, including amongst 41 specimens of *A. gueldenstaedtii* from outside of the Black Sea (Boscari et al. 2014), strongly corroborating the reliability of this result. Given the underrepresentation of reference Russian sturgeons from the Black Sea, we cannot exclude that the allele frequency differs across locations such that *A. gueldenstaedtii* in the Black Sea might naturally carry the *A. stellatus*-diagnostic allele. However, commercial controls for species purity we routinely performed also included many *A. gueldenstaedtii* caviar samples from the Black Sea which have never shown the *A. stellatus* allele (data not shown).

The four haplotypes of the hybrid animals were not detected in the Russian sturgeon broodstocks used to generate juveniles for restocking in North Eastern Black Sea (Nikolai Mugue, pers. comm), suggesting that the hybrids found in this study were likely the offspring of wild breeders. Additionally, the four putative hybrids exhibited different haplotypes, indicating that each had a different *A. gueldenstaedtii* mother, and that hybridization involved at least four females. Sturgeons are known for their ability to hybridize in captivity and several species combinations have been generated in aquaculture for production purposes (Boscari et al. 2014). To our knowledge however, hybrids between *A. gueldenstaedtii* and *A. stellatus* are not used in aquaculture, and this species combination is not produced. Furthermore, no hatchery producing *A. stellatus*, either as pure species or as hybrids, is present in the area, excluding the possibility that the detected hybrids represent accidental escapees from aquaculture plants. This is not the first evidence of interspecific hybridization in nature between sturgeon species; for example, natural interspecific hybrids between *A. ruthenus* and *A. baerii* were found in the Danube River following careless release of the allochthonous *A. baerii* (Ludwig 2009). In this instance, it appears that hybridization occurs between indigenous species.

Although, a certain rate of hybridization may have always occurred, the present low density of populations (in particular of the Stellate sturgeon) might increase this phenomenon. Population decline may in fact promote interspecific hybridization due to the scarcity of conspecific mates. This phenomenon, known as Hubb’s ‘desperation’ hypothesis (Hubbs 1955), adds the urgent need for sturgeon conservation measures in the Eastern Black Sea and Rioni drainage. Additional concerns are raised by the possible impact that the presence of interspecific hybrids might have on the already seriously compromised natural populations (Havelka et al. 2011). In our specific case, the two parental species have respectively about 240 (Russian sturgeon) and 120 chromosomes (Stellate sturgeon) and the resulting hybrids, having an intermediate chromosomal set, are expected to be sterile (Birstein 2002; Linhartová et al. 2018). However, the sterile condition does not prevent the animals from taking part to spawning as adults, competing with breeders of pure species with adverse effects on their reproductive success (Arcella et al. 2014; Fjelldal et al. 2014). Furthermore, a relict population of Ship sturgeon (*A. nudiventris*), whose chromosomal set is compatible with the Stellate sturgeon (*A. stellatus*), also inhabits the Rioni River (Beridze et al. 2021). An interspecific admixture between these species would result in fertile hybrid which might backcross with the parental species, potentially compromising their genetic integrity. This would be particularly harmful for the Ship sturgeon, on the verge of being classified as extinct in the wild, and for which a last spawning site of the entire distribution area was recently recorded in the River Rioni (Beridze et al. 2021).

**Acknowledgements** We would like to acknowledge the contributions of Fauna & Flora International’s sturgeon conservation team and their associated fishers who collected the samples used in this project. We thank Dr. Nikolai Mugue, Dr. Natalia Nebesikhina and Viktoria Scherbakova for comparing detected haplotypes with Russian database of captive broodstocks.

**Authors’ contributions** TB and EB equally contributed to this study. FS, CA substantially contributed to the design of the work. TB and EB performed all the experiments and data analyses. TB, EB and LC contribute to results interpretation and wrote the manuscript. All the authors critically revised the manuscript and accepted the final version for publication.

**Funding** This work was financially supported by U.S. Fish and Wildlife Service with Federal Grant number F19AP00450 to Fauna & Flora International, by the UK Government through the Illegal Wildlife Trade Challenge Fund, grant number IWT082 to Fauna & Flora International, and by Shota Rustaveli National Science Foundation (SRNSF) [PHDF_19_532, Molecular taxonomy, phylogeny, and population genetic structure of protected sturgeon species in Georgia].

**Availability of data and material** Accession numbers available upon acceptance.

**Code availability** Not applicable.

**Declarations**

**Conflicts of interest/Competing interests** No conflict of interest.

**Ethical approval** Not applicable.

**Consent to participate** All authors have given consent to participate.

**Consent for publication** All authors have given consent to publication.
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