diversity

MDPI

Article

Genetic Evidence for the Presence of Wild-Caught Sturgeons in Commercial Markets in Georgia

Tamar Beridze [1,2,*,†], Shannon L. White [3,†], David C. Kazyak [3], Levan Ninua [1], Dewayne Fox [4], Arun Sethuraman [5], Tamari Edisherashvili [1], Bianca Roberts [2], Mikheil Potskhishvili [2], Michelle Klailova [2] and Cort Anderson [1]

1 Faculty of Natural Sciences and Medicine, Ilia State University, Tbilisi 0162, Georgia; levan.ninua@iliauni.edu.ge (L.N.); tamari.edisherashvili.1@iliauni.edu.ge (T.E.); cort.anderson@iliauni.edu.ge (C.A.)
2 Fauna & Flora, Caucasus Programme, Tbilisi 0179, Georgia; bianca.roberts@fauna-flora.org (B.R.); mikheil.potskhishvili@fauna-flora.org (M.P.); michelle.klailova@fauna-flora.org (M.K.)
3 U.S. Geological Survey Eastern Ecological Science Center, 11649 Leetown Road, Kearneysville, WV 25430, USA; slwhite@usgs.gov (S.L.W.); dkazyak@usgs.gov (D.C.K.)
4 Department of Agriculture and Natural Resources, Delaware State University, Dover, DE 19901, USA; dfox@desu.edu
5 Department of Biology, San Diego State University, San Diego, CA 92182, USA; asethuraman@sdsu.edu
* Correspondence: tamar.beridze.3@iliauni.edu.ge
† These authors contributed equally to this work.

Abstract: Sturgeons (Family: Acipenseridae) are among the most endangered taxa worldwide. Significant resources have been invested into the conservation of global sturgeon populations, including the development of commercial aquaculture programs. These programs are intended to improve conservation outcomes by reducing the harvest of wild populations while still meeting commercial demand for sturgeon products. However, there is growing concern that commercial aquaculture programs may contribute to wild population declines through continued, illegal harvest and the escape and/or release of captive individuals into wild environments. These concerns may be particularly acute in the country of Georgia which, despite its small territory and altered landscape, is a globally significant hotspot for sturgeon diversity. In order to understand the potential threat of captive culture on wild sturgeon populations in Georgia, we used mitochondrial DNA sequencing and microsatellite analyses to identify the species and origin of sturgeons encountered in commercial settings. Microsatellite analyses showed significant differentiation between wild and commercial Russian sturgeon populations and highlighted the potential for wild-caught individuals to be present in coastal markets in Georgia. The analyses of mitochondrial haplotypes also suggested that commercial markets may contain sturgeon species that are not native to the region. Overall, our results suggest that wild sturgeon populations may still be exploited to support captive aquaculture programs and commercial sales.

Keywords: sturgeon; commercial aquaculture; conservation; Georgia; illegal trade

Citation: Beridze, T.; White, S.L.; Kazyak, D.C.; Ninua, L.; Fox, D.; Sethuraman, A.; Edisherashvili, T.; Roberts, B.; Potskhishvili, M.; Klailova, M.; et al. Genetic Evidence for the Presence of Wild-Caught Sturgeons in Commercial Markets in Georgia. *Diversity* **2024**, *16*, 274. https://doi.org/10.3390/d16050274

Academic Editor: Luc Legal

Received: 1 December 2023
Revised: 22 April 2024
Accepted: 25 April 2024
Published: 4 May 2024

1. Introduction

Sturgeons (Family: Acipenseridae) are among the oldest living animal taxa with evolutionary records dating species as far back as 200 million years ago [1,2]. Although populations were once widely distributed and abundant in the northern hemisphere, sturgeon populations have been declining over the past century, with most extant species now considered to be at risk of extinction by the International Union for the Conservation of Nature (IUCN) [3]. Overharvest is one of the major factors contributing to precipitous population declines, with 14 of 25 extant species being recognized as commercially important for caviar and meat [4]. Over the past century, demand for sturgeon products has

risen, with average landings reaching 28,900 mt (metric tons) between 1976 and 1983 [5]. During this time, 90% of sturgeon harvest occurred in the Soviet Union, with most landings concentrated in the Caspian and Black seas [5,6].

In response to global population decline, sturgeon commercial aquaculture programs began rising in popularity in Russia in 1970. By the 2000s, sturgeon aquaculture had also developed in France, Germany, Italy, Hungary, the USA, and China. Programs are still under development today [5,7], with expansion into new continents such as South America and Australia [8]. Although commercial sturgeon aquaculture is intended to support the recovery of sturgeon populations through the reduced harvest of wild individuals and is a legal, controlled, and regulated activity under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (2021), the propagation and trade of captive sturgeons remains a significant threat to wild populations [9]. For example, there have been reported cases where wild sturgeons have been provided to fish markets for direct sale or used to increase captive production [10]. Pilot poaching monitoring has also shown that 23 wild sturgeons from three different species (Russian sturgeon [(*Acipenser gueldenstaedtii*), stellate sturgeon (*Acipenser stellatus*), and beluga sturgeon (*Huso huso*)) were harvested from December 2017 to July 2018 in the eastern Black Sea [11]. Overall, these reports suggest that illegally harvested wild sturgeons are likely being sold in commercial markets under the guise of commercial production. However, because it is largely impossible to visually differentiate the origin of wild and captive individuals, these reports are largely anecdotal evidence, and the extent to which wild sturgeons are being sold in commercial settings remains unclear.

In addition to illegal sale, the escape or release of captive individuals may pose a threat to wild populations through genetic admixture, interspecific hybridization, resource competition, and the introduction of parasites and pathogens [9,12]. This is of particular concern with regard to the release of interspecific hybrids, which are being commercially grown to optimize growth and early maturation [13–15]. If released, hybrid sturgeons may readily outcompete native species and cause rapid population decline or extirpation [16].

Despite its relatively small size, the country of Georgia is a global diversity hotspot for sturgeons. The Rioni River, a tributary to the eastern Black Sea, historically supported spawning populations of at least five, and potentially six, species of sturgeon, including Russian sturgeon, stellate sturgeon, beluga sturgeon, European sturgeon (*A. sturio*), ship sturgeon (*A. nudiventris*), and Colchic sturgeon (*A. colchicus*, the taxonomic status of which is not clear) [17,18]. Overfishing and habitat degradation greatly reduced sturgeon abundance in the Rioni River, with landings decreasing from 100 mt in the 1930s to just 12 mt tons in the 1960s [17], ultimately leading to a harvest moratorium in 1967 that continues today. Despite the cessation of legal harvest, contemporary populations remain threatened, with evidence that only three species (Russian sturgeon, ship sturgeon, and stellate sturgeon) still spawn in the Rioni River [18]. Further, only adult beluga sturgeon have been found in the eastern coast of the Black Sea, suggesting limited juvenile recruitment [19]. Of the aforementioned species, four (Russian sturgeon, ship sturgeon, stellate sturgeon, and beluga sturgeon) are listed as critically endangered by the IUCN, with the European sturgeon considered extirpated in the Black Sea.

The extent to which captive sturgeon facilities may threaten wild populations in the Rioni River is presently unclear. However, according to surveys carried out by the Fauna & Flora Caucasus Programme, there are currently four fish farms rearing sturgeons within the Rioni River watershed in western Georgia, including one farm located close to the Tekhuri River which is a tributary to the Rioni River [20]. Those surveys suggest that sturgeons in these farms are mostly Russian and Siberian sturgeons (*A. baerii*). Notably, Siberian sturgeon are non-native to the Black Sea and originated from broodstock sourced from Armenia. Studies have shown that Siberian sturgeon readily hybridize with native sturgeons in captive and wild environments [16], highlighting the potential for hybridization to negatively impact wild sturgeon populations. One obstacle for understanding the threat that captive facilities may pose on wild populations is that the hybridization and trans-

portation of individuals among regions has made it difficult to readily identify the source, and even species, of individuals that are sold in markets. However, genetic monitoring may present a viable tool for addressing these questions and can assist in monitoring the effect of aquaculture on wild populations. In particular, because acipenserids are philopatric [6,21], populations tend to show a high degree of genetic differentiation among geographical regions, river systems, and even commercial facilities [22–25]. Therefore, it may be possible to use genetic tools to discern the natal origin of individuals and ultimately determine whether wild individuals are present in commercial facilities or markets.

In this study, we used complementary genetic markers to understand the species and source of sturgeons sold in commercial fish markets and farms, including three coastal markets and one inland market in Tbilisi and one aquaculture facility in Georgia. Our first objective was to use mitochondrial DNA sequencing analysis to identify the species present in commercial environments. We then used microsatellite analyses to ascertain the likely source (wild vs. captive) of sturgeons sold in Georgian markets. The results of this study provide early insights into the potential threats that commercial captive culture may pose on wild sturgeon populations in the Rioni River.

2. Materials and Methods

2.1. Sample Collection

We collected fin clips from presumptive captive sturgeons that were being commercially sold in three coastal fish markets in Batumi, Poti, and Tskaltsminda one inland fish market in Tbilisi, and from a sturgeon aquaculture facility in Georgia, from January 2016 to December 2019 (Figure 1). Importantly, we attempted to include samples from additional aquaculture farms in our analyses, but were unable to collect tissue samples from those locations. However, because all fish farms reportedly source sturgeons from Armenia, which itself is landlocked with no native sturgeon populations, we anticipated the genetic composition of fish from all commercial aquaculture facilities to be similar. In total, 72 tissue samples were collected from individuals of reported captive origin (Table 1).

Table 1. Number of individuals from each species that were sampled from wild and commercial environments. Numbers represent sample size used for microsatellite analyses, of which a subset (shown in parentheses) was used for mitochondrial DNA control region sequencing analyses. When possible, species identification was made using mitochondrial analyses; otherwise, it was inferred from morphological characteristics.

Species	Wild-Caught			Commercial		
	Rioni River	Rioni River Mouth	Black Sea	Coastal Market	Tbilisi Market	Aquaculture
Russian sturgeon	6	40 (34)	9 (6)	21 (18)	43 (45)	2 (1)
Ship sturgeon	4	2	-	-	-	-
Stellate sturgeon	1	5 (4)	9 (6)	2	-	-
Sterlet sturgeon	-	-	-	3	-	-
Beluga sturgeon	-	-	8 (4)	1	-	-
Total for each group	11	47 (40)	26 (16)	27 (24)	43 (45)	2 (1)
Total		84 (67)			72 (70)	

We also analyzed tissue samples from 84 wild sturgeons captured in the Rioni River and the Black Sea collected by the Fauna & Flora Caucasus Programme Sturgeon Conservation Team from August 2018 to December 2020 (Figure 1 and Table 1). All wild-caught individuals were immediately released after tissue collection.

In total, 156 samples were collected from captive and wild origin. DNA extraction was carried out using QIAamp Blood & Tissue Mini Kit, according to the manufacturer's protocol (QIAGEN, Hilden, Germany).

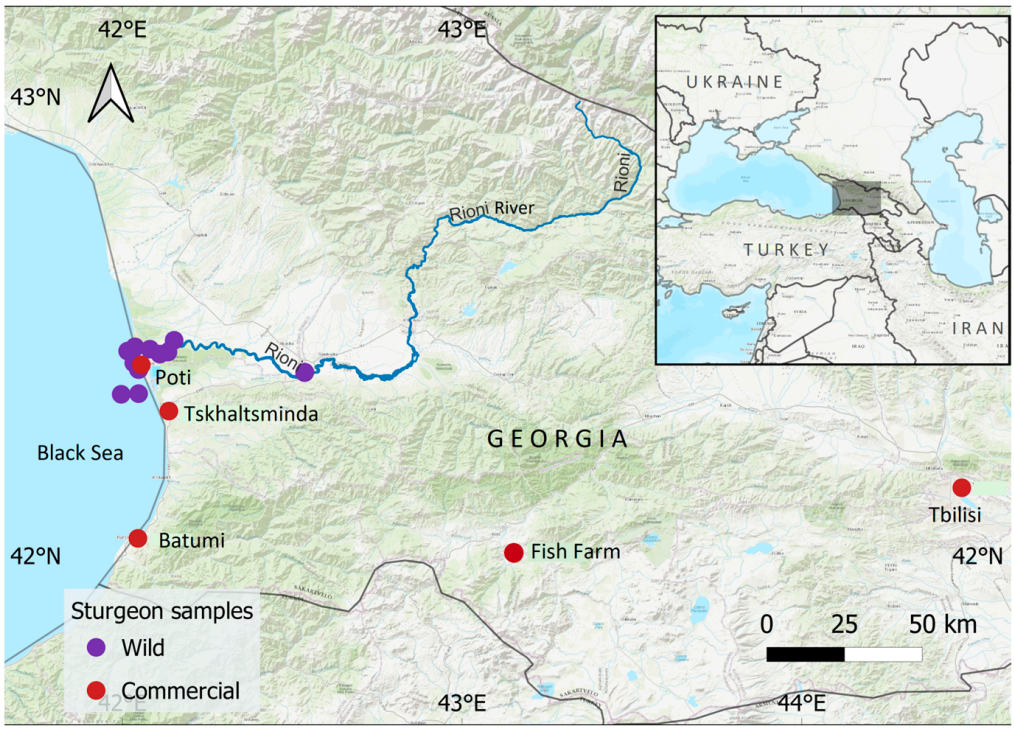

Figure 1. Locations where sturgeon tissue samples were collected from presumed captive (red) and wild (purple) populations. Captive populations included three coastal fish markets in Batumi, Poti, and Tskaltsminda one inland fish market in Tbilisi, and an aquaculture facility. Wild populations were sampled from the Black Sea, Rioni River, and the mouth of the Rioni River.

2.2. Mitochondrial Analysis

We sequenced a 716 bp fragment of the mitochondrial control region to determine the matriarchal lineage of Georgian sturgeon from wild and captive individuals sold either in the market or reared in an aquaculture facility [26]. PCR was performed in the volume of 20 µL with 0.25 µM of each primer, 0.1 mM of dNTP's, 1× buffer, 0.1 U/µL Taq DNA polymerase (OxGEn and Promega), and approximately 80 ng/µL DNA template for each sample. We used the primer pairs Acipenser Pro1-F: 5′-CACCCTTAACTCCCAAAGC-3′ and Acipenser Phe1-R: 5′-CCCATCTTAACATCTTCAGT-3′ [25], with the following conditions: 94 °C—2 m; 94 °C—45 s, 56 °C—45 s, 72 °C—45 s for 33 times; 72 °C—5 m. All samples were sequenced on a 3730xl DNA Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) at Macrogen Europe B.V. (Amsterdam, The Netherlands).

We used MEGA 11 [27] for multiple sequence alignment using the program ClustalW and phylogenetic tree reconstruction. Trees were constructed using the maximum likelihood statistical method with the Tamura 3-parameter model, with rates of change between sites following a gamma distribution. The evolutionary model (Tamura 3-parameter) was selected in MEGA 11 as the best fit for the available sequence data. Trees were examined to assess phylogenetic relationships between wild-caught and commercial sturgeons and DNA sequences from sturgeons available from GeneBank. In this analysis, we specifically expected that haplotypes will reflect an individual's provenance. That is, if all sturgeons sold in the market have a captive lineage, then sturgeon samples from a market or aquaculture facility should cluster together and be distinct from samples collected from wild populations. However, if wild individuals are present in the market, then market samples will represent a mixture of both captive and wild individuals. Phylogenetic haplotype relationships and distance between wild and commercially sold specimens were investigated using the NETWORK 10.2.0.0 Median-Joining method [28].

2.3. Microsatellite Analysis

We genotyped tissues samples at four microsatellite markers including *Afug41*, *An20*, *Aox45*, and *AoxD165* [29–32]. PCRs were performed in 10 µL reactions containing 0.25 µM of each primer, forward primers labeled at 5′ end with either VIC or 6-FAM dye, 2.5 Mm $MgCl_2$, 0.1 Mm of dNTPs, 1× GoTaq Buffer, (Promega, Ph 8.5, 50 mMTris-HCl, 50 Mm NaCl), and 0.1 µL of Promega Go Taq polymerase (5 U/µL, 1 unit/reaction), 50 ng of template DNA, and sterile water. Thermal conditions were as follows: 95 °C—5 min; 95 °C—25 s, 53 °C—25 s, 72 °C—40 s for 34 times; 72 °C—10 min. The PCR reactions were analyzed using 3130xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Genotyping Software GeneMapper 5 (Thermo Fisher Scientific, Waltham, MA, USA) was used for allele calls. Tetraploids were assessed based on individual genotypes showing more than two alleles per marker.

We performed a hierarchical STRUCTURE analysis to visualize patterns of differentiation within and among species, and to identify possible patterns of hybridization among groups [33,34]. Others have shown that STRUCTURE can reliably recover patterns of differentiation in mixed-ploidy groups [35]. Therefore, the first level of our analysis included all five species, with two alleles in diploid species coded as missing. Based on results from this analysis, we grouped collections with similar patterns of differentiation and then performed another STRUCTURE analysis on each of those groups independently. We continued this iterative process until each species was represented as a unique genetic cluster or there was no evidence of further genetic substructuring within the group. For all analyses, we ran STRUCTURE v.2.3.4 with a recessive alleles model assuming admixture, correlated allele frequencies, and allelic ambiguity [34]. For each level of the analysis, we retained 200,000 repetitions after a burn-in of 200,000 steps and ran 10 replicates for each value of K = 1 to G + 1 (where K was the number of genetic clusters and G was the number of groups in each level of the STRUCTURE analysis). The results from STRUCTURE were visualized using STRUCTURESelector [36], with appropriate values for K selected using the ΔK method [37].

3. Results

3.1. Mitochondrial DNA Analysis

Mitochondrial control region sequences were obtained for 137 out of the 156 collected sturgeon samples of captive and wild origin (Table 1). Based on the mitochondrial DNA sequencing analysis, we identified four sturgeon species in the market and aquaculture samples (Table 1). The majority of samples (approximately 92%) were identified as Russian sturgeon, including one Russian sturgeon sampled from the aquaculture facility. The remaining samples were identified as stellate sturgeon (n = 2), sterlet sturgeon (*A. ruthenus*) (n = 3), and beluga sturgeon (n = 1). Among the wild specimens, four species were identified based on mitochondrial DNA sequencing. The majority of these individuals (approximately 70%) were identified as Russian sturgeon. The three other taxa were observed less frequently and included stellate sturgeon (n = 11), ship sturgeon (n = 6), and beluga sturgeon (n = 4). Notably, all ship sturgeon were captured from the Rioni River and the mouth of the Rioni River, whereas all beluga sturgeon were captured in the Black Sea (Table 1).

On the phylogenetic tree and network analysis (Figures 2 and 3), ship sturgeon, stellate sturgeon, beluga sturgeon, and sterlet sturgeon grouped into separate species groups. There was a separation between wild and commercial Russian sturgeon haplotypes. However, there were some market samples that were grouped with wild samples, potentially representing wild haplotypes present in fish markets.

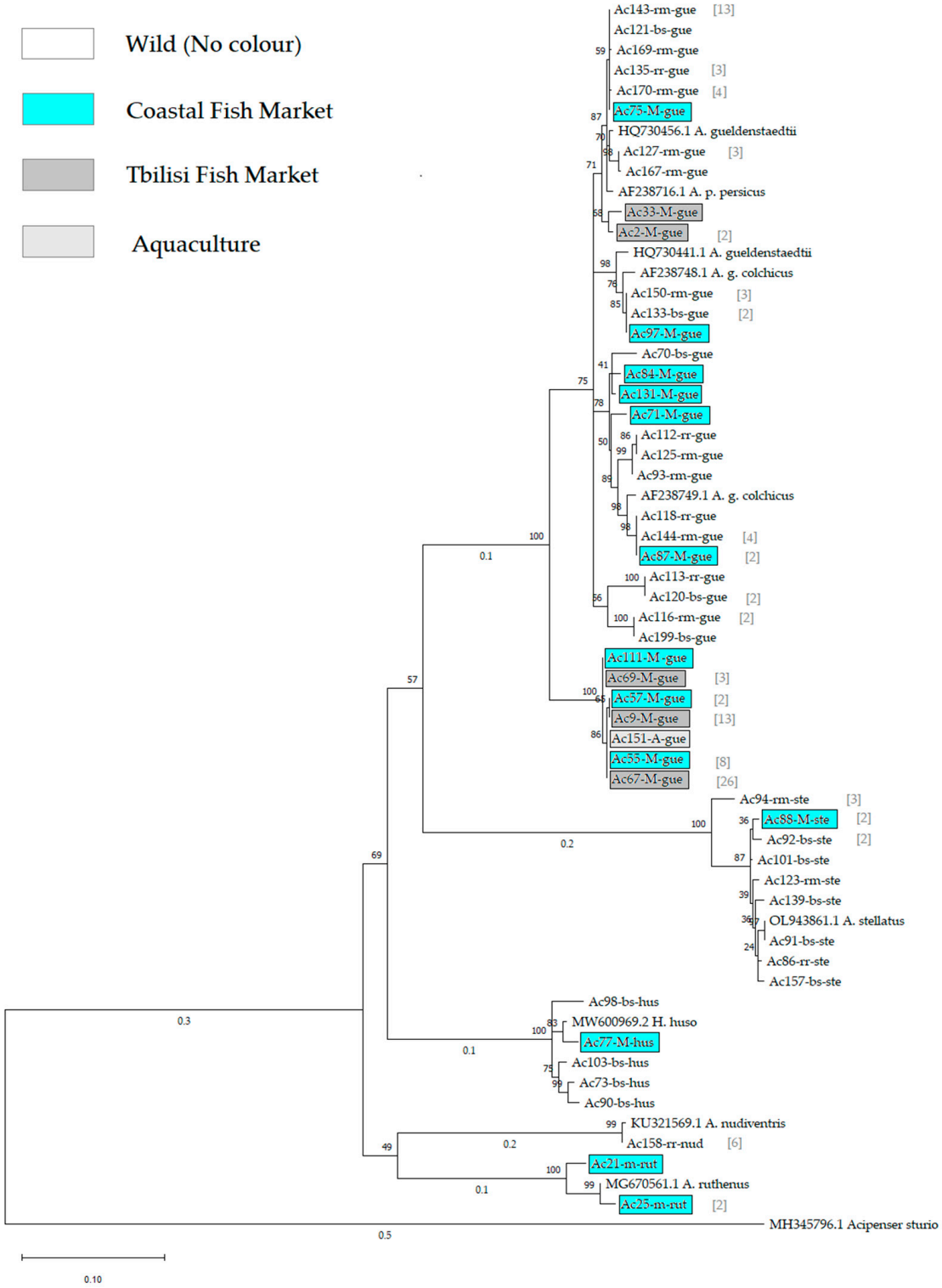

Figure 2. Phylogenetic tree of sturgeons sampled from the wild (bs—Black Sea; rm—Rioni River Mouth; rr—Rioni River), commercial environments (M—Coastal and inland markets; A—Aquaculture), and haplotypes from GenBank. Trees were constructed using the maximum likelihood method with the Tamura 3-parameter model, with rates of change between sites following a gamma distribution (G) and 100 bootstrapped replicates per tree. Species codes: gue-Russian sturgeon; ste—stellate sturgeon; nud-ship sturgeon; rut-sterlet sturgeon; hus—beluga sturgeon. Numbers above the branches show bootstrap support; numbers below indicate branch lengths. Haplotypes with numbers in square brackets show the number of samples with the same haplotype, whereas haplotypes without a number represent those where a single haplotype was analyzed in the sample.

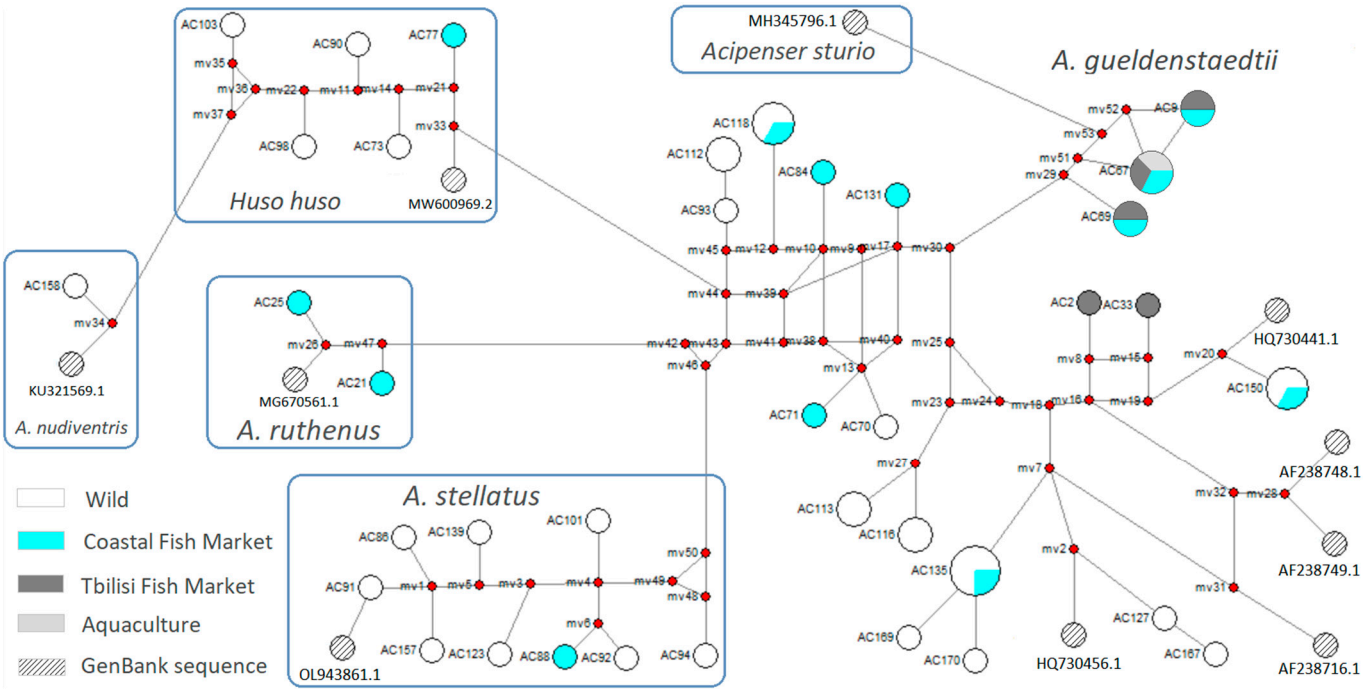

Figure 3. Network analysis of wild sturgeon samples from the Rioni River, the mouth of the Rioni River, and the eastern Black Sea (white), and commercial samples from coastal fish markets (blue), Tbilisi fish market (dark gray), and aquaculture (light gray). NCBI GenBank sequence accession numbers are indicated. Each circle represents a single haplotype and the colors represent the origin of the sample(s).

3.2. Microsatellite Analysis

The first level of the hierarchical STRUCTURE analysis supported K = 3 groups, which separated wild-caught and commercial Russian sturgeon from the other four species in our analysis. A separate STRUCTURE analysis on only the Russian sturgeon generally differentiated wild from commercial individuals. However, this analysis also suggested that several individuals from the markets may have originated from a wild source (Figure 4). In particular, 14 of 64 Russian sturgeon sampled from coastal and inland markets were assigned to the wild-caught cluster with a q-score of at least 0.25. This highlights the potential for a large proportion of commercial Russian sturgeon in those markets to either be of direct wild descent or be a first- or second-generation hybrid with a captive individual.

Additional STRUCTURE analyses on the remaining four species were able to differentiate ship sturgeon and beluga sturgeon, but could not discriminate between stellate sturgeon and sterlet sturgeon. In addition, the analysis could not distinguish between wild and market individuals of these species (Figure 4). Notably, individuals from the market that clustered with the wild-caught collection also grouped to wild haplotypes on the phylogenetic tree.

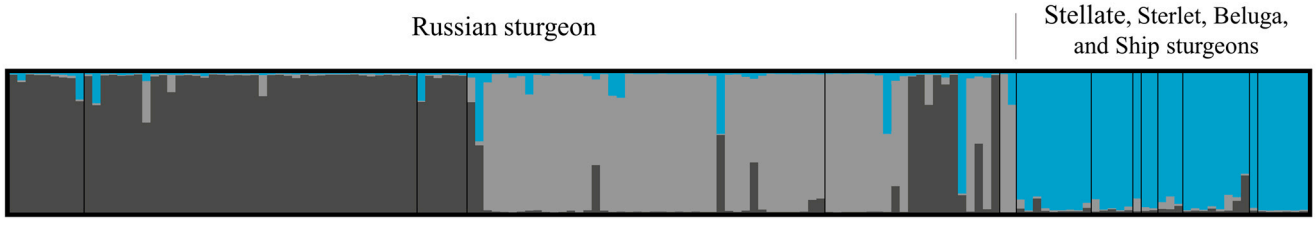

Figure 4. *Cont.*

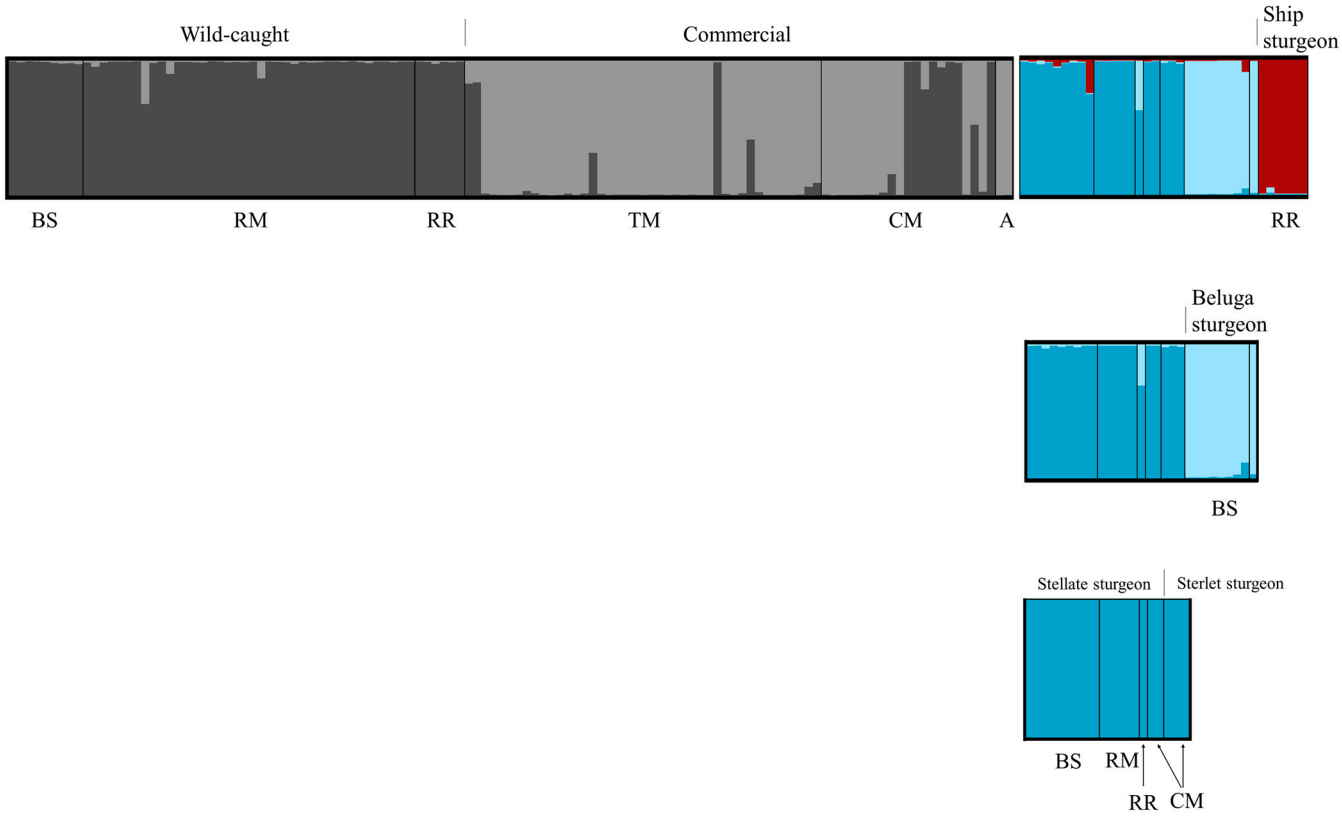

Figure 4. Results of hierarchical STRUCTURE analysis of wild-caught (BS—Black Sea; RM—Rioni River Mouth; RR—Rioni River) and commercial (TM—Tbilisi market; CM—Coastal market; A—Aquaculture) Russian sturgeon, ship sturgeon, stellate sturgeon, sterlet sturgeon, and beluga sturgeon.

4. Discussion

Identifying the species and provenance of sturgeons in aquaculture facilities and commercial markets can be challenging due to species' similar morphologies and the mixed ancestry of many captive populations [38,39]. Through molecular analyses, we typified the species and origin of sturgeons encountered in wild, market, and aquaculture environments in Georgia. We detected Russian sturgeon, stellate sturgeon, sterlet sturgeon, and beluga sturgeon in market and aquaculture facilities. In wild environments, we detected Russian sturgeon, stellate sturgeon, ship sturgeon, and beluga sturgeon, with beluga sturgeon only being detected in the Black Sea and ship sturgeon only detected in the Rioni River. Russian sturgeon is the more abundant species in fish markets and aquaculture stocks, and our findings suggest that captive Russian sturgeon populations are genetically distinct from wild, native Russian sturgeon population. The genetic distinctiveness allowed us to determine that, based on both mitochondrial (Figure 2) and microsatellite analyses (Figure 4), wild-caught Russian sturgeon may either be directly sold in coastal markets or used as broodstock to support captive culture. Taken together, these results suggest commercial aquaculture may present a cryptic, yet significant threat to the conservation of wild sturgeon populations in Georgia as wild specimens of Russian sturgeon, beluga sturgeon, and sterlet sturgeon appear to be available in market environments. The continued illegal harvest of wild populations has the potential to contribute to demographic declines and reduce conservation efficacy.

The largely unregulated nature of Georgian public markets, combined with the sale of largely unidentifiable sturgeon flesh, can make it difficult to accurately identify the species and source of sturgeons available in market environments. Processed sturgeon parts generally lack distinguishing morphological characteristics [10,40], making it nearly impossible to use morphometrics or meristics for species identification. While genetic assays could

aid in species recognition, the high complexity of sturgeon genomes has generally limited broad application of genetic tools for the monitoring of sturgeon trade. Recent studies have increased the number and types of genetic markers that can be used for species and hybrid identification, and today nearly all sturgeon species can be identified with high certainty [13,15,38,41,42]. However, several species remain problematic, including Russian and Siberian sturgeon. These two species can be differentiated from other sturgeon species, but it can be difficult to distinguish between the two species or detect hybrid individuals [42,43]. This can present a challenge for monitoring commercial trade, as both species are regularly used in commercial propagation, and Russian x Siberian sturgeon hybrids are commonly reared in aquaculture [5]. Moreover, sturgeons are characterized by any ability to hybridize with other sturgeon species and produce sterile and fertile offspring [15,44,45]. This high rate of hybridization can reduce the reliability of morphology for species identification, and ultimately highlights the utility of more complex methods such genetic tools or isotope analyses for monitoring sturgeon trade [38].

Based on the maternal lineage analysis of commercial specimens, market samples in our analyses were predominantly Russian sturgeon. This is not surprising, as Russian sturgeonare of high commercial value relative to many other sturgeon species [5,7,38]. Moreover, our phylogenetic tree and network analysis show that most of the market samples that maternally identified as Russian sturgeon grouped into three haplotypes (Ac67, Ac69, and Ac9), which are grouped separately from the wild-caught Russian sturgeons that were sampled from the Rioni River, the mouth of the Rioni River, and the Black Sea (Figure 2). Overall, this suggests that most Russian sturgeon that we sampled from markets did appear to be of captive origin. However, several coastal market samples (Ac71, Ac75, Ac84, Ac87, Ac97, and Ac131) grouped with wild-caught specimen haplotypes, which suggest that wild Russian sturgeon may also occasionally be sold in markets. This finding was supported by the microsatellite genetic analysis, which revealed a clear differentiation between wild and commercial Russian sturgeon and highlighted the presence of some individuals in market samples that clustered most strongly with the wild-caught individuals.

Although the source of wild-caught individuals in the market is unknown, reports of sturgeon poaching in the Rioni River are not uncommon, and sturgeon poaching equipment has been found and confiscated along the river [11,45]. Given that some individuals in STRUCTURE analyses have intermediate q-scores, it is also possible that wild-caught Russian sturgeon are being used as broodstock for fish farms, as has been reported for stellate sturgeon in western Georgia [20]. However, the lack of significant admixture and strong differentiation between wild and captive populations lends limited support to the on-going, large-scale use of wild individuals in commercial propagation.

In addition to Russian sturgeon, we also detected three additional species in Georgian fish markets, namely, stellate sturgeon (n = 2), sterlet sturgeon (n = 3), and beluga sturgeon (n = 1). Due to limited power from low sample sizes and limited microsatellite markers, our analyses could not distinguish between wild and market individuals for the aforementioned three. Nonetheless, their presence in market samples is still of interest. For example, while sterlet sturgeon is not native to Georgia, it is frequently reared on commercial farms [5,42], and so it was not surprising to find this species in commercial settings. Conversely, critically endangered populations of stellate and beluga sturgeon are native to Georgia and the eastern Black Sea; however, their origin in market environments is presently unclear. Recent surveys of Georgian sturgeon farms suggested that none of the nearby commercial facilities rear these two species, and only a single farm had a one stellate sturgeon that was originally captured in the Black Sea as bycatch [20]. Thus, further investigation of the source of these species that were being sold in commercial settings appears warranted.

Although captive sturgeon propagation has been promoted as a means to reduce pressure on wild populations, our findings suggest that without careful monitoring and enforcement, captive sturgeon propagation could contribute to further erosion of critically endangered populations. This may be of particular concern for Russian sturgeon, which appears to be numerically dominant in the fish markets that we surveyed. Our results sug-

gest that wild and aquaculture stocks may not be fully reproductively isolated. Therefore, the release or escape of non-native Russian sturgeon stocks into the Rioni River system might result in admixture with native fish, leading to the erosion of native diversity and compromising the adaptive potential of the native populations [16]. In addition, admixture between wild and captive Russian sturgeon populations could make it more difficult to apply genetics tools to identify the illegal trade of sturgeon and sturgeon products [38].

The genetic characterization and population genetic studies of extant wild sturgeon populations and commercial stocks are essential for the future monitoring of natural populations and commercial markets, including studies to determine the natal origin of commercial sturgeons in Georgian markets. In addition, studies of local, natural population structure may help identify genetically distinct populations and determine the spatial scale in which conservation actions should be applied [24]. On-going monitoring efforts may also be important for the early detection of non-native species introductions and the expansion of inter- and intra-specific hybridization in wild and captive populations.

Future analysis and monitoring efforts may benefit from the development and use of more advanced genetic and genomic tools. Inferences in this study were made from a modest number of microsatellite loci, which was necessary as genetic markers remain underdeveloped for many sturgeon species. However, the high ploidy levels can make analyses possible with relatively few loci. For example, Russian sturgeon is a polyploid species [46,47], and because polyploid species have more alleles per locus and a higher mutation rate than diploid species, differences between populations may accumulate more rapidly and enable analyses with relatively few loci [48]. However, future studies could explore the use of stable isotope analysis, which may be a useful and reliable tool for identifying individual natal origin (e.g., farmed/wild; [38]). For example, using stable isotope analyses, Avigliano et al., 2023 [49] were able to determine the source of introduced Siberian and Russian sturgeon in South American waterways. However, stable isotope analyses may still require the use of genetic methods to avoid species mis-identification. For example, using stable isotopes, a captive American paddlefish (*Polyodon spathula*) in Ukraine that was fed a diet of wild forage and was later assigned as native individual [38]. Combining geochemical and genetic analyses for source stock identification may be particularly important when determining the natal origins of Georgian Russian sturgeon, as it is the main species available in markets and the most widely caught sturgeon in Georgia.

Our study highlights the utility of molecular tools for assessing the species and provenance of sturgeon in wild, aquaculture, and market environments, and documents the potential, continued threat that commercial propagation may have on the conservation of native sturgeons in Georgia. Our work also underscores the challenges of trying to identify the origin of critically imperiled sturgeon species, where very low sample sizes offer limited statistical power to resolve potential differences. Collectively, these findings highlight the importance of developing genetic baselines for wild and farm-reared sturgeons in order to enable future assessments of the provenance of sturgeons. Future studies may be warranted to better understand wild sturgeon genetic diversity in the Rioni River and the eastern Black Sea. For example, the genetic characterization of wild and commercial sturgeon populations could help understand the natural population genetic diversity and differentiation, and ultimately improve the ability to monitor fish markets for illegal harvest and trade.

Author Contributions: Conceptualization, T.B., S.L.W., D.C.K., L.N. and C.A.; methodology, T.B.; software, S.L.W.; validation, T.B. and S.L.W.; formal analysis, T.B. and S.L.W.; investigation, T.B., S.L.W., D.C.K. and C.A.; resources, M.P., M.K. and C.A.; data curation, T.B. and T.E.; writing—original draft preparation, T.B., S.L.W. and D.C.K.; writing—review and editing, T.B., S.L.W., D.C.K., L.N., D.F., A.S., T.E., M.P., B.R., M.K. and C.A.; visualization, T.B., S.L.W. and D.C.K.; supervision, A.S., C.A. and D.F.; project administration, T.B., M.P., B.R., M.K. and C.A.; funding acquisition T.B., M.K. and C.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the UK Government through the Illegal Wildlife Trade Challenge Fund (IWTCF)–IWT082, the United States Fish and Wildlife Service (USFWS)–F19AP00450.

(FPDF-19-532), the Shota Rustaveli National Science Foundation of Georgia (SRNSFG), the Molecular-genetic Laboratory of Ilia State University, and a Matthew Spencer Individual Donation 417-IND through Fauna & Flora Caucasus Programme.

Institutional Review Board Statement: All sampling and handling in our study was conducted under permits reviewed and approved by the Ministry of Environmental Protection and Agriculture of Georgia (Permit Number: N 3216/01; N 8479/01; N 3193/01; N1885/01), following the rules and requirements. Samples for genetic analysis were taken non-invasively from live fish or from fish carcasses, no animals were killed or injured in the course of this research.

Data Availability Statement: See GenBank accession numbers PP194356—PP194365; PP716099-PP716103 for mitochondrial DNA haplotype sequences.

Acknowledgments: We acknowledge the Fauna & Flora Caucasus Programme sturgeon conservation team, especially Conservation Officer Janeli Rogava and local fishers for supporting the sturgeon conservation program, former Fauna & Flora Caucasus Programme sub-regional manager Fleur Scheele for supporting the research, Ilia State University Zurab Javakhishvili for helpful discussions, and associate researcher Vasil Metreveli for technical support. Finally, we thank Andrea Schreier for comments on an earlier draft. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. We acknowledge Matthew Spencer for cycling across Eurasia for sturgeon and for raising funds on his journey to support sturgeon conservation for the Fauna & Flora Caucasus Programme.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Bemis, W.E.; Kynard, B. Sturgeon Rivers: An Introduction to Acipenseriform Biogeography and Life History. In *Sturgeon Biodiversity and Conservation*; Birstein, V.J., Waldman, J.R., Bemis, W.E., Eds.; Developments in Environmental Biology of Fishes; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2002; Volume 17, pp. 167–183, ISBN 978-0-7923-4517-6.
2. Peng, Z.; Ludwig, A.; Wang, D.; Diogo, R.; Wei, Q.; He, S. Age and Biogeography of Major Clades in Sturgeons and Paddlefishes (Pisces: *Acipenseriformes*). *Mol. Phylogenetics Evol.* **2007**, *42*, 854–862. [CrossRef]
3. Congiu, L.; Striebel-Greiter, B.; Gessner, J.; Boscari, E.; Boner, M.; Jahrl, J.; Dalle Palle, S.; Ludwig, A. Identification and Tracking of Sturgeons and Paddlefish Products in Trade: Implications for Trade Control and Biodiversity Management. *Aquaculture* **2023**, *574*, 739708. [CrossRef]
4. Ludwig, A. Identification of Acipenseriformes Species in Trade. *J. Appl. Ichthyol.* **2008**, *24*, 2–19. [CrossRef]
5. Bronzi, P.; Rosenthal, H.; Gessner, J. Global Sturgeon Aquaculture Production: An Overview. *J. Appl. Ichthyol.* **2011**, *27*, 169–175. [CrossRef]
6. Banarescu, P.; Holcik, J. Acipenseriformes. In *The Freshwater Fishes of Europe*; Aula Verlag: Wiesbaden, Germany, 1989; Volume 1.
7. Bronzi, P.; Chebanov, M.; Michaels, J.T.; Wei, Q.; Rosenthal, H.; Gessner, J. Sturgeon Meat and Caviar Production: Global Update 2017. *J. Appl. Ichthyol.* **2019**, *35*, 257–266. [CrossRef]
8. *Import of Live Sturgeon for Aquaculture*; Department of Agriculture, Fisheries and Forestry DAFF: Canberra, Australia, 2023.
9. White, S.L.; Fox, D.A.; Beridze, T.; Bolden, S.K.; Johnson, R.L.; Savoy, T.F.; Scheele, F.; Schreier, A.D.; Kazyak, D.C. Decades of Global Sturgeon Conservation Efforts Are Threatened by an Expanding Captive Culture Industry. *Fisheries* **2023**, *48*, 54–61. [CrossRef]
10. Jahrl, J.; Boner, M.; Striebel, B.; Ludwig, A. *Evidence for Trafficking of Critically Endangered Sturgeon in the Lower Danube Region*; WWF Austria & WWF CEE: Vienna, Austria, 2021.
11. Ninua, L. *Baseline Study of Sturgeon Catch and Illegal Trade in Georgia*; Fauna & Flora Caucasus Programme: Tbilisi, Georgia, 2018.
12. Ludwig, A. A Sturgeon View on Conservation Genetics. *Eur. J. Wildl. Res.* **2006**, *52*, 3–8. [CrossRef]
13. Boscari, E.; Barmintseva, A.; Pujolar, J.M.; Doukakis, P.; Mugue, N.; Congiu, L. Species and Hybrid Identification of Sturgeon Caviar: A New Molecular Approach to Detect Illegal Trade. *Mol. Ecol. Resour.* **2014**, *14*, 489–498. [CrossRef]
14. Boscari, E.; Vitulo, N.; Ludwig, A.; Caruso, C.; Mugue, N.S.; Suciu, R.; Onara, D.F.; Papetti, C.; Marino, I.A.M.; Zane, L.; et al. Fast Genetic Identification of the Beluga Sturgeon and Its Sought-after Caviar to Stem Illegal Trade. *Food Control* **2017**, *75*, 145–152. [CrossRef]
15. Havelka, M.; Bytyutskyy, D.; Symonová, R.; Ráb, P.; Flajšhans, M. The Second Highest Chromosome Count among Vertebrates Is Observed in Cultured Sturgeon and Is Associated with Genome Plasticity. *Genet. Sel. Evol.* **2016**, *48*, 12. [CrossRef]
16. Ludwig, A.; Lippold, S.; Debus, L.; Reinartz, R. First Evidence of Hybridization between Endangered Sterlets (*Acipenser ruthenus*) and Exotic Siberian Sturgeons (*Acipenser baerii*) in the Danube River. *Biol. Invasions* **2009**, *11*, 753–760. [CrossRef]
17. Ninua, N.; Guchmanidze, A. *Sturgeons of Georgia*; Georgian National Museum: Tbilisi, Georgia, 2013; ISBN 978-9941-9311-2-3.
18. Zarkua, Z.; Bolkvadze, I.; Khintibidze, M.; Gogotishvili, M.; Variadilis, L. *The Current State of the Georgian Black Sea Sturgeons*; ASEN2; Springer: Dordrecht, The Netherlands, 1998; Volume 46.

19. Beridze, T.; Boscari, E.; Scheele, F.; Edisherashvili, T.; Anderson, C.; Congiu, L. Interspecific Hybridization in Natural Sturgeon Populations of the Eastern Black Sea: The Consequence of Drastic Population Decline? *Conserv. Genet.* **2022**, *23*, 211–216. [CrossRef]
20. Rogava, J.; Potskhishvili, M.; Seskuria, G. *Report on the Sturgeon Farms in Georgia*; Fauna & Flora Caucasus Programme: Tbilisi, Georgia, 2020.
21. Friedrich, T.; Gessner, J.; Reinartz, R.; Greiter, S.B. *Pan-European Action Plan for Sturgeons, Convention on the Conservation of European Wildlife and Natural Habitats*; World Sturgeon Conservation Society and WWF: Strasbourg, France, 2018.
22. Fox, D.A.; Hightower, J.E.; Parauka, F.M. Gulf Sturgeon Spawning Migration and Habitat in the Choctawhatchee River System, Alabama–Florida. *Trans. Am. Fish. Soc.* **2000**, *129*, 811–826. [CrossRef]
23. Kazyak, D.C.; White, S.L.; Lubinski, B.A.; Johnson, R.; Eackles, M. Stock Composition of Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*) Encountered in Marine and Estuarine Environments on the U.S. Atlantic Coast. *Conserv. Genet.* **2021**, *22*, 767–781. [CrossRef]
24. Laikre, L.; Palm, S.; Ryman, N. Genetic Population Structure of Fishes: Implications for Coastal Zone Management. *AMBIO A J. Hum. Environ.* **2005**, *34*, 111–119. [CrossRef]
25. Schreier, A.D.; Mahardja, B.; May, B. Hierarchical Patterns of Population Structure in the Endangered Fraser River White Sturgeon (*Acipenser transmontanus*) and Implications for Conservation. *Can. J. Fish. Aquat. Sci.* **2012**, *69*, 1968–1980. [CrossRef]
26. Congiu, L.; Pujolar, J.M.; Forlani, A.; Cenadelli, S.; Dupanloup, I.; Barbisan, F.; Galli, A.; Fontana, F. Managing Polyploidy in Ex Situ Conservation Genetics: The Case of the Critically Endangered Adriatic Sturgeon (*Acipenser naccarii*). *PLoS ONE* **2011**, *6*, e18249. [CrossRef] [PubMed]
27. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
28. Bandelt, H.J.; Forster, P.; Rohl, A. Median-Joining Networks for Inferring Intraspecific Phylogenies. *Mol. Biol. Evol.* **1999**, *16*, 37–48. [CrossRef]
29. Henderson-Arzapalo, A.; King, T.L. Novel Microsatellite Markers for Atlantic Sturgeon (*Acipenser oxyrinchus*) Population Delineation and Broodstock Management. *Mol. Ecol. Notes* **2002**, *2*, 437–439. [CrossRef]
30. King, T.L.; Lubinski, B.A.; Spidle, A.P. Microsatellite DNA Variation Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*) and Cross-Species Amplification the Acipenseridae. *Conserv. Genet.* **2001**, *2*, 103–119. [CrossRef]
31. Welsh, A.B.; Blumberg, M.; May, B. Identification of Microsatellite Loci in Lake Sturgeon, *Acipenser fulvescens*, and Their Variability in Green Sturgeon, *A. medirostris*. *Mol. Ecol. Notes* **2002**, *3*, 47–55. [CrossRef]
32. Zane, L.; Patarnello, T.; Ludwig, A.; Fontana, F.; Congiu, L. Isolation and Characterization of Microsatellites in the Adriatic Sturgeon (*Acipenser naccarii*). *Mol. Ecol. Notes* **2002**, *2*, 586–588. [CrossRef]
33. Janes, J.K.; Miller, J.M.; Dupuis, J.R.; Malenfant, R.M.; Gorrell, J.C.; Cullingham, C.I.; Andrew, R.L. The $K = 2$ Conundrum. *Mol. Ecol.* **2017**, *26*, 3594–3602. [CrossRef] [PubMed]
34. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
35. Stift, M.; Kolář, F.; Meirmans, P.G. Structure Is More Robust than Other Clustering Methods in Simulated Mixed-Ploidy Populations. *Heredity* **2019**, *123*, 429–441. [CrossRef] [PubMed]
36. Li, Y.-L.; Liu, J.-X. StructureSelector: A Web-Based Software to Select and Visualize the Optimal Number of Clusters Using Multiple Methods. *Mol. Ecol. Resour.* **2018**, *18*, 176–177. [CrossRef] [PubMed]
37. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the Number of Clusters of Individuals Using the Software Structure: A Simulation Study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef]
38. Ludwig, A.; Congiu, L.; Gessner, J.; Boscari, E.; Striebel-Greiter, B.; Boner, M.; Jahrl, J. Identification of Species and Hybrids, Source and Geographical Origin of Sturgeon and Paddlefish (*Acipenseriformes* spp.) Specimens and Products in Trade. In Proceedings of the Addendum to Identification and Traceability of Sturgeons and Paddlefish (Acipenseriformes spp.), CITES—Thirty-First Meeting of the Animals Committee, Online, 31 May–22 June 2021.
39. Ludwig, A.; Lieckfeldt, D.; Jahrl, J. Mislabeled and Counterfeit Sturgeon Caviar from Bulgaria and Romania. *J. Appl. Ichthyol.* **2015**, *31*, 587–591. [CrossRef]
40. Barmintseva, A.E.; Mugue, N.S. The Use of Microsatellite Loci for Identification of Sturgeon Species (*Acipenseridae*) and Hybrid Forms. *Russ. J. Genet.* **2013**, *49*, 950–961. [CrossRef]
41. Reinartz, R.; Lippold, S.; Lieckfeldt, D.; Ludwig, A. Population Genetic Analyses of *Acipenser ruthenus* as a Prerequisite for the Conservation of the Uppermost Danube Population: Genotype Structure of the Danube Sterlet. *J. Appl. Ichthyol.* **2011**, *27*, 477–483. [CrossRef]
42. Havelka, M.; Boscari, E.; Sergeev, A.; Mugue, N.; Congiu, L.; Arai, K. A New Marker, Isolated by ddRAD Sequencing, Detects Siberian and Russian Sturgeon in Hybrids. *Anim. Genet.* **2019**, *50*, 115–116. [CrossRef] [PubMed]
43. Káldy, J.; Mozsár, A.; Fazekas, G.; Farkas, M.; Fazekas, D.L.; Fazekas, G.L.; Goda, K.; Gyöngy, Z.; Kovács, B.; Semmens, K.; et al. Hybridization of Russian Sturgeon (*Acipenser gueldenstaedtii*, Brandt and Ratzeberg, 1833) and American Paddlefish (*Polyodon spathula*, Walbaum 1792) and Evaluation of Their Progeny. *Genes* **2020**, *11*, 753. [CrossRef] [PubMed]
44. Linhartová, Z.; Havelka, M.; Pšenička, M.; Flajšhans, M. Interspecific Hybridization of Sturgeon Species Affects Differently Their Gonadal Development. *Czech J. Anim. Sci.* **2018**, *63*, 1–10. [CrossRef]

45. Suciu, R.; Molnar, P.; Kynard, B. *Rioni River, Georgia Current Status of Sturgeon, and Implications for Future Development in the Catchment*; Fauna & Flora Caucasus Programme: Tbilisi, Georgia, 2021; pp. 1–132.
46. Lanfredi, M.; Congiu, L.; Garrido-Ramos, M.A.; de la Herrán, R.; Leis, M.; Chicca, M.; Rossi, R.; Tagliavini, J.; Ruiz Rejón, C.; Ruiz Rejón, M.; et al. Chromosomal Location and Evolution of a Satellite DNA Family in Seven Sturgeon Species. *Chromosome Res.* **2001**, *9*, 47–52. [CrossRef] [PubMed]
47. Ludwig, A.; Debus, L.; Jenneckens, I. A Molecular Approach to Control the International Trade in Black Caviar. *Int. Rev. Hydrobiol.* **2002**, *87*, 661–674. [CrossRef]
48. Meirmans, P.G.; Liu, S.; van Tienderen, P.H. The Analysis of Polyploid Genetic Data. *J. Hered.* **2018**, *109*, 283–296. [CrossRef]
49. Avigliano, E.; Leisen, M.; Duquenoy, C.; Liotta, J.; Volpedo, A. Siberian and Russian Sturgeon Natal Origin in South America: Fish Farm or Established Population? *Austral Ecol.* **2023**, *48*, 1121–1131. [CrossRef]