INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE
ISSN: 2067-533X Volume 17, Issue 1, 2026: 383-390 www.ijcs.ro
DOI: 10. 36868/IJCS.2026.01.23

A RELIABLE METHOD FOR THE TOTAL EXTRACTION OF DNA FROM STURGEON FINS PRESERVED IN ETHANOL

György DEÁK[1,2], Monica MATEI[1], Mădălina BOBOC[1], Raluca PRANGATE[1,2,*]

[1] National Institute for Research and Development in Environmental Protection, Splaiul Independenţei 294, Bucharest, Romania, 060031, e-mail: incdpm@incdpm.ro
[2] Doctoral School of Biotechnical Systems Engineering, National University of Science and Technology POLITEHNICA of Bucharest, Splaiul Independenței 313, Bucharest, 060042 Romania

Abstract

The global decline in wild sturgeon populations has led to the implementation of policies to conserve and protect this species. Sturgeon conservation is a major concern today, and actions must be taken in this direction. When discussing this topic, it is important to understand how species adapt to survive in different environments. Studying their DNA is the most accurate method for determining evolutionary relationships among sturgeon species. Molecular genetics focuses on the genetic information contained within DNA molecules that is responsible for the development, functioning, and diversity of all living organisms. Genomic DNA extraction is a fundamental molecular biology technique used in studies of genetic diversity and the phylogenetic evolution of species. In this work, we propose an effective method for extracting DNA from sturgeon fin fragments that were preserved in ethanol and stored in the refrigerator for an extended period of time.

Keywords*: Conservation; DNA extraction; Fin fragments; Long-term storage; Sturgeon*

Introduction

Sturgeons, considered living fossils, are located at the phylogenetic base of fish and are of great economic and biological importance [1], [2], [3]. They evolved 250 million years ago during the Jurassic period, and they belong to the Chordata phylum, the Osteichthyes superclass, the Actinopterygii class, the *Acipenseriformes* order, and the *Acipenseridae* family [1], [4]. According to the International Union for Nature Conservation (IUCN), sturgeons are a critically endangered species [5]-[8]. Sturgeons are found across Europe, Asia, and North America, inhabiting rivers and seas such as the Lower Danube, the Caspian Sea, and the Yangtze River [9]. The *Acipenseridae* family consists of 25 species of sturgeon, six of which are native to the Danube basin: *Acipenser stellatus, Acipenser sturio*, *Acipenser ruthenus*, *Acipenser nudiventris*, *Acipenser gueldenstaedtii,* and *Huso huso* [3], [10], [11]. Recent data suggest that *Acipenser sturio* and *Acipenser nudiventris* are extinct in the Lower Danube and the Black Sea [8], [12].

Natural disasters, alongside anthropogenic factors such as deforestation, fires, water and air pollution, overfishing, and illegal fishing, are leading to ecological degradation, as evidenced by the decline in global fish populations [13], [14], [15]. This is also due to the disruption of historical migration routes to the Middle and Upper Danube sectors resulting from hydro-technical works and dredging operations [16], [17], [18]. These worrying effects emphasize the importance of developing methods and taking action to ensure the conservation and perpetuation of species in the

* Corresponding author: raluca.prangate@incdpm.org

future [19], [20]. The global management and conservation of declining wildlife populations and biodiversity is a crucial concern today. Since most sturgeon species are considered critically endangered, it is necessary to develop a strategic approach to protect genetic diversity and help restock [7]. Biology conservation based on DNA studies provides important information about the ability to adapt [21]. It also provides information about the diversity of species facing extinction [22], [23]. In this context, the scientific literature presents different methods of storing tissue samples from fish. These include fins, gametes (eggs and sperm), spermatozoa (sperm cells), ovarian cells, and gonads (ovaries and testes). The preservation of various fish samples is achieved through the use of the following methods: cryopreservation, storage at temperatures of -80°C, and formaldehyde or ethanol [24], [25]. An essential step in researching the molecular biology of species is the correct extraction of DNA, which can later be used for various applications [26]. The extraction protocol, which was first discovered by Friedrich Miescher in 1869, has undergone numerous modifications over time [27], [28].

This study aimed to develop a reliable DNA extraction technique using sturgeon fin tissues that were refrigerated for an extended period of time. The protocol adapted for tissue samples preserved in an ethanol solution at refrigeration temperatures for approximately ten years is summarized, evaluating the obtained DNA yield and discussing the importance of this method in species identification and conservation monitoring.

Materials and Methods

Collection and preservation of sturgeon tissue samples

The collection of samples was conducted as part of the *in situ* monitoring activities that were conducted on sturgeons [29]. The sampling location is the Lower Danube area in Romania. A veterinarian performed a non-invasive sampling procedure on the sturgeons, harvesting a portion of up to 1 cm² of their fins. Throughout the sample collection period, staff wore protective gloves and used sterile equipment. Once sampling was complete, the sturgeons were released back into the water. The samples were placed in containers with ethanol, labeled, and kept at refrigeration temperature during transportation, and upon arrival at the laboratory, they were stored under refrigerated conditions.

DNA isolation

DNA extraction is the process of isolating DNA from a cell or organism and separating it from other cellular components, such as proteins and lipids [30], [31], [32]. A major concern in molecular biology was the challenge of extracting DNA from different types of biological samples [33]. The process of molecular genetic techniques begins with the extraction of biomolecules, with a focus on genomic DNA [26], [34]. A variety of sources can be used to extract DNA, ranging from clinical samples to water, soil, and plant samples, as well as tissues from vertebrate and invertebrate organisms.

Selecting an effective extraction protocol is important because the quality and quantity of obtained DNA can directly affect subsequent applications. The QIAamp DNA Investigator kit was used for DNA extraction, and in accordance with the manufacturer's guidelines [35], the current protocol was adapted for the total extraction of DNA from tissue samples from sturgeon fins preserved in ethanol and stored at refrigeration temperatures for approximately ten years. We used QIAamp MinElute® Columns for the extraction of DNA from sturgeon fins. The reagents required for the isolation process included collection tubes, 96-100% ethanol, Buffer ATL, Buffer ATE, Buffer AL, Proteinase K, Buffer AW1, and Buffer AW2. The reagents were stored according to the manufacturer's instructions [35]. When handling reagents and conducting laboratory activities, it is important to prevent contamination of biological samples. The process of nucleic acid isolation was initially performed manually, and then adapted to automatic isolation using QIAcube® Connect (QIAGEN, Germany). The working stages of DNA extraction can be

divided into four major phases. The first stage of the process involves lysing the sample in the presence of proteinase K. In the second stage, DNA binds to the membrane and is separated from contaminants. In the third stage, the contaminants are washed away, and in the final stage, pure DNA is removed from the membrane. The working protocol began with the fragmentation of the biological material, sturgeon fins, into small pieces, which were then transferred into 1.5 ml tubes. Then, 300 µL of ATL buffer and 20 µL of proteinase K were added, followed by vortexing for 10 seconds. The tube was then inserted into a thermomixer preheated to 56°C until the sample was dissolved. A 300 µL AL buffer solution was added, followed by vortexing for 10 seconds. Also, the tube was inserted into the thermomixer preheated to a temperature of 70°C for 10 minutes. The supernatant was transferred to a 1.5 ml tube, to which 150 µl of 90-100% ethanol was added, followed by vortexing for 15 seconds. After homogenization, the supernatant was transferred to a QIAamp MinElute column. This was centrifuged for one minute, with repeated centrifugation to ensure the total lysate passed through the filter membrane. The QIAamp MinElute column was then inserted into a 2 ml collection tube, to which 500 µl AW1 buffer was added, followed by a one-minute spin. It was then added to the QIAamp MinElute 700 µl AW2 buffer columns, which were then centrifuged for one minute. After transferring the QIAamp MinElute column to a new tube, 700 µL of 96–100% ethanol was added, followed by centrifugation. The membrane was dried by centrifugation for three minutes. Then, QIAamp MinElute was inserted into a new 1.5 ml microcentrifuge tube and incubated with the lid open at 15–25°C for ten minutes. After incubation, 100 µl of the previously prepared ATE buffer was added to ensure complete elution of the bound DNA. All samples were processed in duplicate. For each sample, 100 µL of eluate was obtained. The buffer solutions required for the protocol were prepared in advance. The DNA that was extracted was then stored at -80°C for future analysis. The steps of the extraction protocol used for the complete extraction of DNA from sturgeon fins that have been preserved in ethanol are shown in Figure 1. The efficiency of the protocol depends on the quality of the reagents and the equipment available in the molecular biology laboratory. Figure 2 shows a collage of the equipment used in the extraction protocol.

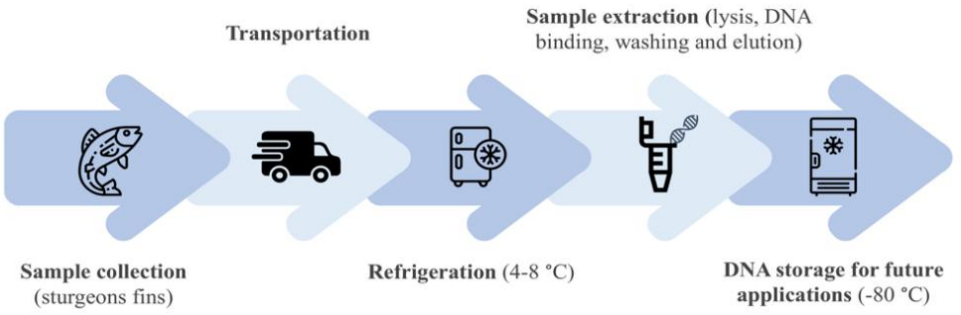

Fig. 1. Workflow used for the total extraction of DNA from sturgeon fin samples

DNA quantification

Evaluation of DNA qualities, including the assessment of its purity, concentration, and quality control, was performed by spectrophotometric analyses. For this purpose, we used the Qubit 4 fluorometer (Thermo Fisher Scientific, USA), which enabled us to measure the concentration of DNA quickly and accurately. It enables the quick and accurate quantification of DNA from a sample size of 1–20 µL. Each DNA sample was eluted in 100 µL, from which a 1:10 dilution was prepared using 1 µL of eluate and 9 µL of nuclease-free water. Our diluted samples were then quantified.

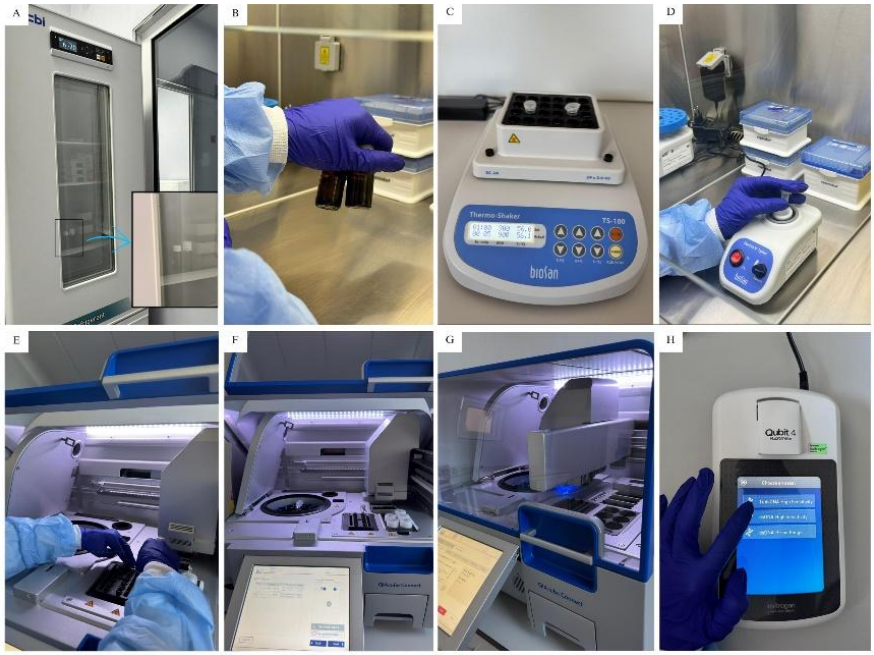

Fig. 2. Equipment used for DNA extraction, where: A. Tissue samples stored in the refrigerator; B. Two fin samples selected for DNA extraction; C. Tubes placed in a thermal shaker; D. Vortexing stage; E. Preparing the QIAcube Connect for automatic extraction; F. The QIAcube Connect system is prepared for analysis; G. UV decontamination of equipment after DNA extraction; H. DNA quantification

Results and discussion

Significant advances in extraction techniques have been made since Friedrich Miescher's groundbreaking work in 1869 [36]. These methods have become more reliable, being fast and easy to perform, with higher yield in terms of quality and quantity of DNA [37], [38]. The isolation of nucleic acids is an important step in biology, as they are vital for obtaining genetic information in various areas of activity, including identifying sturgeon species for conservation purposes. Storing biological material in ethanol under refrigerated conditions for an extended period of time can affect the integrity of the DNA. However, using the earlier-presented protocol, we were able to obtain the favorable results. The measured DNA concentrations ranged from 1.53 ng/µL to 8.53 ng/µL for the sturgeon fin tissue samples. These concentrations obtained from the DNA extraction process confirm the efficiency of the protocol described in our paper.

A variety of DNA extraction protocols and quantification techniques have been applied in numerous studies. In a study conducted by Arciuch-Rutkowska in 2024, the quantity and purity of DNA samples were determined from young hybrid sturgeons (*Acipenser gueldenstaedtii* × *Acipenser baerii*) using a Nanodrop spectrophotometer (Thermo Scientific Nanodrop One, Waltham, MA, USA) [13]. Also, the study conducted by Grunwald in 2002 showed a variation in DNA concentrations extracted using spectrophotometric analysis at 260 and 280 nm [39]. Bruijns's 2022 study used a Qubit 2.0 fluorometer to quantify DNA samples from salmon and herring, obtaining values between 0.6 ± 0.0 ng/µL (herring) and 1.0 ± 0.1 ng/µL (salmon). Since the study compared spectrophotometric and fluorometric quantification methods, the researchers concluded that the spectrophotometric method tends to overestimate DNA concentration compared to fluorometric methods [40]. Jafroudi's 2023 study on the identification and spread of *Acipenser persicus*, *Acipenser ruthenus,* and *Acipenser baerii* in the Caspian Sea revealed variations in DNA concentration extracted from fine tissues, ranging from 150 ng/µL to 370 ng/µL. The concentration of the DNA samples was verified using a spectrophotometric analysis made with Thermo Fisher,

Nanodrop1000 [41]. The study, conducted by Shuttleworth in 2022, compared different methods of DNA extraction from the scales of teleost fish. The concentrations obtained from the extraction varied between 29.66 ng/μL and 81.73 ng/μL [23]. Also, the concentration of DNA in water samples collected from various aquatic areas has been studied in order to determine the diversity of fish species present in those areas. For example, water samples were collected in Lake Mondsee by Rund et al. (2024) and isolated. For quantification of the DNA content, the Qubit 3.0 fluorometer was used according to the Broad-range protocol Assay Kit (Invitrogen, Thermo Fisher Scientific, Schwerte, Germany). The DNA eluate concentrations, in this study, varied from 0.4 pg/μL to 36.6 pg/μL, with a mean concentration of 10.8 ± 8.5 pg/μL [42].

The quantity and quality of the DNA isolated from non-invasive samples, such as sturgeon fins, are of great interest for future genetic diversity studies for conservation purposes. The DNA isolated from sturgeon fins using the above-described protocol can be used for a wide range of downstream applications. These include performing polymerase chain reaction (PCR) on molecular markers such as amplified fragment length polymorphism (AFLP), single-nucleotide polymorphism (SNP), and restriction fragment length polymorphism (RFLP), as well as DNA sequencing [43].

Conclusions

The DNA extraction and purification method proposed in this study proved to be effective for obtaining high-quality genetic material from ethanol-preserved sturgeon fin samples stored under refrigeration. The protocol has been demonstrated to be suitable for long-term stored tissues and can support various downstream molecular analyses. Its successful implementation provides an important tool for non-invasive genetic monitoring and contributes to future research focused on the conservation of endangered sturgeon species in the Danube River. Reliable DNA data allows researchers to evaluate population structure, inbreeding, and gene flow in endangered sturgeon species. Collecting genetic data helps implement breeding programs and habitat protection. Identifying changes in the sturgeon population helps implement improved conservation practices, such as restocking programs, in affected ecosystems. Therefore, since sturgeon samples may originate from various sources, it is essential to have a reliable extraction method enabling molecular analyses.

References

[1] Q. Liu and T. Naganuma, "Metabolomics in sturgeon research: a mini-review," *Fish Physiology and Biochemistry*, vol. 50, 2024, pp. 1895–1910.

[2] M. Raischi, L. Oprea, G. Deak, M. Boboc, M. Matei, and N. Raischi, "Investigation of sturgeon migration routes using the most adequate monitoring techniques in difficult hydrological conditions of the Danube River," *Journal of Environmental Protection and Ecology*, vol. 18, 2017, pp. 142–157.

[3] M. Raischi, G. Deák, L. Oprea, N. Raischi, and T. Danalache, "Age and growth determination of Lower Danube sturgeon species Huso huso and Acipenser stellatus," *IOP Conference Series: Earth and Environmental Science*, vol. 616, 2020, p. 012032.

[4] S. S. O. Hung, "Recent advances in sturgeon nutrition," *Animal Nutrition*, vol. 3, 2017, pp. 191–204.

[5] W. G. Anderson, A. Schreier, and J. A. Crossman, "Chapter 2 - Conservation Aquaculture—A Sturgeon Story," in *Fish Physiology*, N. A. Fangue, S. J. Cooke, A. P. Farrell, C. J. Brauner, and E. J. Eliason, Eds., vol. 39. Academic Press, 2022, pp. 39–109.

[6] G. Deák, E. Holban, I. Sadîca, and A. Jawdhari, "Sturgeon Parasites: A Review of Their Diversity and Distribution," *Diversity*, vol. 16, 2024, p. 163.

[7] L. Gao, R. Franěk, and M. Pšenička, "Cryopreservation of sturgeon egg mitochondria and their replacement in germline: A novel strategy for maternal genetic preservation in sturgeons," *Theriogenology*, vol. 240, 2025, p. 117414.
[8] IUCN, "The IUCN Red List of Threatened Species. Version 2025-1," 2025.
[9] W. E. Bemis and B. Kynard, "Sturgeon rivers: an introduction to acipenseriform biogeography and life history," *Environmental Biology of Fishes*, vol. 48, 1997, pp. 167–183.
[10] G. Deák, R. Matache, R. Prangate, G. Dumitrescu, E. Holban, L. Lupea, N. R. Norlia, and M. Ibrahim, "Risk of contamination of sturgeon species along the Lower Danube with AcIV-E virus from sturgeons raised in aquaculture systems," *IOP Conference Series: Earth and Environmental Science*, vol. 1216, 2023, p. 012012.
[11] M. Raischi, H. György Deák, L. Oprea, N. Raischi, T. Dănălache, and S. Matei, "The impact of anthropogenic pressures on sturgeon migration in the Lower Danube," *IOP Conference Series: Earth and Environmental Science*, vol. 616, 2020, p. 012030.
[12] M. C. Raischi, L. Oprea, G. Deak, S. Zamfir, M. Ilie, and N. Raischi, "Impact of the Lower Danube hydrotechnical works on sturgeons' migration," *International Journal of Environmental Science*, vol. 1, 2016, pp. 213–216.
[13] M. Arciuch-Rutkowska, J. Nowosad, M. K. Łuczyński, S. M. Hussain, and D. Kucharczyk, "Next-Generation Sequencing to Determine Changes in the Intestinal Microbiome of Juvenile Sturgeon Hybrid (Acipenser gueldenstaedtii♀ × Acipenser baerii♂) Resulting from Sodium Butyrate, B-Glucan and Vitamin Supplementation," *Genes*, vol. 15, 2024, p. 1276.
[14] N. Rivers, J. Daly, R. Jones, and P. Temple-Smith, "Cryopreservation of testicular tissue from Murray River Rainbowfish, Melanotaenia fluviatilis," *Scientific Reports*, vol. 10, 2020, p. 19355.
[15] G. Deak, B. Alin Marius, B. P. Iustina, and M. Tudor, "Research on sturgeon migration behaviour using a new monitoring, control and alarming system," *Journal of Environmental Protection and Ecology*, vol. 15, 2014, pp. 944–953.
[16] G. Deák, I. Sadîca, G. Tudor, M. Matei, E. Holban, and S. Matei, "Innovative bypass solution for iron gates to reconnect the historical migration routes of wild anadromous sturgeon species, in order to improve their conservation status," *International Journal of Conservation Science*, vol. 15, 2024, pp. 1479–1488.
[17] G. Deak, B. Alin Marius, D. Tiberius, and M. Tudor, "Use of acoustic telemetry for providing an insight into sturgeons' behaviour and migration routes on lower Danube," *Journal of Environmental Protection and Ecology*, vol. 15, 2014, pp. 954–964.
[18] T. M. Danalache, A. M. Badilita, G. Deák, E. Holban, I. Popescu, A. Daescu, M. C. Raischi, G. Ghita, C. G. Nicolae, and S. Diaconescu, "Assessment of Bastroe Channel possible impact on Lower Danube sturgeon migration," *AACL Bioflux*, vol. 10, 2017.
[19] N. Rivers, J. Daly, and P. Temple-Smith, "New directions in assisted breeding techniques for fish conservation," *Reproduction, Fertility and Development*, vol. 32, 2020, pp. 807–821.
[20] E. Holban, G. Deak, M. Razvan, D. Tiberius, M. Matei, M. Boboc, M. Raischi, I. Gheorghe, Á. Keresztesi, and F. Kilar, "Identification of sturgeon behavior in different hydromorphodynamic conditions resulting from the implementation of hydrotechnical arrangements," *International Journal of Conservation Science*, vol. 13, 2022, pp. 743–750.
[21] V. Ulyanov, I. Beishova, T. Ulyanova, A. Sidarova, N. Ginayatov, A. Kovalchuk, G. Chuzhebaeva#, G. Chuzhebaeva, and B. Sariye, "Genetic health and diversity assessment of Sturgeon species in Kazakhstan's aquaculture and natural habitats," *German Journal of Veterinary Research*, vol. 4, 2024, pp. 127–138.
[22] C. Moritz, "Strategies to Protect Biological Diversity and the Evolutionary Processes That Sustain It," *Systematic Biology*, vol. 51, 2002, pp. 238–254.
[23] L. Shuttleworth and C. J. Oosthuizen, "Comparing DNA yield from fish scales following different extraction protocols," *Scientific Reports*, vol. 12, 2022, p. 2836.

[24] M. M. Hagedorn, J. P. Daly, V. L. Carter, K. S. Cole, Z. Jaafar, C. V. A. Lager, and L. R. Parenti, "Cryopreservation of Fish Spermatogonial Cells: The Future of Natural History Collections," *Scientific Reports*, vol. 8, 2018.
[25] G. T. Kwan, B. W. Frable, A. R. Thompson, and M. Tresguerres, "Optimizing immunostaining of archival fish samples to enhance museum collection potential," *Acta Histochemica*, vol. 124, 2022, p. 151952.
[26] P. Lenka, N. Singh, D. Ghosh, V. Mahato, and S. Ghosh, "A highly cost-effective, eco-friendly tissue lysis and extraction method for faster DNA isolation from fish fin," *PLoS ONE*, vol. 20, 2025, p. e0318708.
[27] P. J. Shetty, "The Evolution of DNA Extraction Methods," *American Journal of Biomedical Science and Research*, vol. 8, 2020, pp. 39–45.
[28] E. Lamm, O. Harman, and S. J. Veigl, "Before Watson and Crick in 1953 Came Friedrich Miescher in 1869," *Genetics*, vol. 215, 2020, pp. 291–296.
[29] M. C. Raischi, L. Oprea, G. Deak, A. Badilita, and M. Tudor, "Comparative Study on the Use of New Sturgeon Migration Monitoring Systems on the Lower Danube," *Environmental Engineering and Management Journal*, vol. 15, 2016, pp. 1081–1085.
[30] A. Dhaliwal, "DNA Extraction and Purification," *Mater Methods*, vol. 3, 2013.
[31] N. Gupta, "DNA extraction and polymerase chain reaction," *Journal of Cytology*, vol. 36, 2019, p. 116.
[32] R. Kobun, Ed., "Chapter 6 - Extraction and Analytical Methods for the Identification of Parasites in Food," in *Advanced Food Analysis Tools*. Academic Press, 2021, pp. 101–125.
[33] S. C. Tan and B. C. Yiap, "DNA, RNA, and Protein Extraction: The Past and The Present," *Journal of Biomedicine and Biotechnology*, 2009, p. 574398.
[34] N. Ali, R. D. C. P. Rampazzo, A. D. T. Costa, and M. A. Krieger, "Current Nucleic Acid Extraction Methods and Their Implications to Point-of-Care Diagnostics," *BioMed Research International*, 2017, pp. 1–13.
[35] QIAamp® DNA Investigator Handbook, Qiagen, Hilden, Germany, 2020, pp. 35-38.
[36] R. Dahm, "Friedrich Miescher and the discovery of DNA," *Developmental Biology*, vol. 278, 2005, pp. 274–288.
[37] C. Barbosa, S. Nogueira, M. Gadanho, and S. Chaves, "Chapter 7 - DNA Extraction: Finding the Most Suitable Method," in *Molecular Microbial Diagnostic Methods*, N. Cook, M. D'Agostino, and K. C. Thompson, Eds. Academic Press, San Diego, 2016, pp. 135–154.
[38] E. Martinez-Espin, L. J. Martinez-Gonzalez, J. C. Alvarez, R. K. Roby, and J. A. Lorente, "Forensic Strategies Used for DNA Extraction of Ancient and Degraded Museum Sturgeon Specimens," in *Biology, Conservation and Sustainable Development of Sturgeons*, R. Carmona, A. Domezain, M. García-Gallego, J. A. Hernando, F. Rodríguez, and M. Ruiz-Rejón, Eds. Springer Netherlands, Dordrecht, 2009, pp. 85–96.
[39] C. Grunwald, J. Stabile, J. R. Waldman, R. Gross, and I. Wirgin, "Population genetics of shortnose sturgeon Acipenser brevirostrum based on mitochondrial DNA control region sequences," *Molecular Ecology*, vol. 11, 2002, pp. 1885–1898.
[40] B. Bruijns, T. Hoekema, L. Oomens, R. Tiggelaar, and H. Gardeniers, "Performance of Spectrophotometric and Fluorometric DNA Quantification Methods," *Analytica*, vol. 3, no. 3, 2022.
[41] H. Torabi Jafroudi, S. Jamshidi, S. Talesh Sasani, and A. Bani, "Molecular Identification of Residual DNA Separated from the Persian Sturgeon (Acipenser persicus) for Modeling eDNA Evaluation in Aquatic Ecosystem," *Journal of Genetic Resources*, vol. 9, 2023, pp. 103–110.
[42] H. Rund, J. Wanzenböck, S. Dobrovolny, and R. Kurmayer, "Relating target fish DNA concentration to community composition analysis in freshwater fish via metabarcoding," *Science of The Total Environment*, vol. 927, 2024, p. 172281.

[43] A. Dudu, S. E. Georgescu, and M. Costache, “Evaluation of genetic diversity in fish using molecular markers,” *Molecular Approaches to Genetic Diversity*, InTech, Edited by Mahmut Caliskan, Guul Cevahir Oz, I. Halil Kavakli, and Birguul Ozcan, 2015.

Received: September 02, 2025
Accepted: February 24, 2026