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Bridging Critical Gaps in Chelonian Conservation via Environmental DNA (eDNA) Monitoring

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Abstract

Background: The habitats of Chelonians are being rapidly damaged by habitat alteration, wetland ecosystems conversion, riverization projects, or illegal poaching worldwide. Traditional types of monitoring have a low-level of ability to identify rare, cryptic, or sparsely distributed turtle species, especially in complex aquatic systems. Environmental DNA (eDNA) has become a promising non-invasive molecular method, which can address the conservation challenges mentioned above because it allows to sense the presence of species on a very sensitive level and monitor all the biodiversity.

Objectives: This systematic review aims to address knowledge gaps with great importance by assessing the use of eDNA in the conservation of Chelonians between 2015 and 2025. The review focuses on appropriate sampling methods, molecular procedures, specifications of detection, research in conservation enforcement, and identification of research priorities in the future.

Methods: Due to the PRISMA guidelines, 50 empirical studies were chosen in Scopus through applications of specific search terms. Data extraction involved sampling matrices, molecular workflows, and detection results and conservation implications. Narrative synthesis was subsequently carried out to determine patterns of methodology and new research trends.

Results: The literature review indicates that the prevailing method of eDNA detection is water-based, which demonstrates a significant aquatic bias. Although, metabarcoding workflows are less commonly used, targeted assays and quantitative PCR (qPCR) methods are more frequently used. Genetic studies have also been used in taxonomy, population genetics, and illegal wildlife trade. Some of the factors that influence detectability include eDNA persistence, optimisation of assays, and coordinated strategies in monitoring. The trends in publication suggest that it has been growing at a fast rate since 2023, with much input coming in the regions of North America, Europe and Asia.

Conclusions: Though the eDNA has significantly enhanced the monitoring of the chelonians, there are still methodological limitations and gaps in research. The expansion of sampling methods, the intensification of community-scale sequencing, and the integration of molecular-based tools into conservation enforcement schemes are inevitable to augment the long-term conservation systems of Chelonians.

Keywords: *Chelonian conservation; Environmental DNA; Genetic monitoring; Illegal wildlife trade; Molecular ecology; PRISMA systematic review; Turtle biodiversity*

1. Introduction

Many freshwater fishes, invertebrates, and mossy vegetation are declining rapidly and extensively, and this is thought to be primarily caused by artificial riverbank alteration for flood- and bank-erosion control measures like riprapping, vegetation removal, and deepening river bottoms [1, 2]. Consequently, numerous conservation strategies, including the restoration of natural waterways, the dismantling of dams or weirs obstructing upstream and downstream migration, and the enhancement of fish passage between rivers and adjacent shallow marshes for spawning and foraging, have been proposed and executed [3, 4; 36-38].

The conversion of natural wetlands for agricultural and industrial purposes, along with modifications to lakes and riverbanks for flood and erosion management, poses significant threats to global freshwater turtle populations [5, 6]. Turtles inhabiting river systems at the urban-rural interface in Japan are endangered by ex situ flood control measures in midstream regions, as the expansion of housing and urban development in upstream areas, coupled with the consequent rise in surface runoff, leads to flooding in downstream rural and urban environments [7, 40]. In regions adjacent to Tokyo and other major Japanese cities, swift urbanisation of upstream landscapes has resulted in modifications to rivers for flood management in numerous rural areas. Nonetheless, no empirical research has quantitatively evidenced the effects of artificial river modification on freshwater turtle populations. Consequently, it is imperative to quantitatively record the actual risks to freshwater turtle populations in Japan and globally. The Swinhoe's (or Yangtze) gigantic softshell turtle (Rafetus swinhoei) is regarded as the most threatened tortoise species globally [8, 9]. Rafetus swinhoei was formerly found in China (Red and Yangtze river systems) and northern Vietnam (Red River and its tributaries), however its previous range is little documented and poorly delineated [10, 39]. In this range, *R. swinhoei* occupied extensive rivers, tributaries, and floodplain lakes and marshes. The synergistic effects of chronic over-exploitation for local use, wetland degradation, hydropower dam construction, pervasive water contamination, and the alteration, sedimentation, and inundation of riverside sandbanks—vital nesting habitats—have driven R. swinhoei to the verge of extinction [11, 12, 40-42]. The global population, encompassing both captive and verified individuals (one male in the Suzhou Zoo, China, and another of indeterminate sex in Xuan Khanh Lake, Vietnam), for which species confirmation relied on eDNA analysis—Dr. Caren Goldberg from Washington State University authenticated a large softshell turtle species captured in a photograph, though it could not be identified from the image (TEEM pers comm.)—alongside unverified sightings of wild turtles, is estimated to comprise fewer than 20 adults. This amount may be an overestimate, considering the majority of wild R. swinhoei reports remain unconfirmed [13, 43, 44]. The conservation status of R. swinhoei is critical, particularly due to the loss of three adult turtles in the past eight years and the probable isolation of remaining individuals, which may exist as solitary specimens or, at most, in small groups within artificial lakes or dammed river segments devoid of natural nesting sites. Recent turtle fatalities comprise a turtle from Hoan Kiem Lake that perished in 2016 (Vietnam) [14, 45-48], a captive female that succumbed in 2019 (China), and most recently, the last known female in existence, discovered deceased in Dong Mo Lake in northern Vietnam in April 2023 [15].

Environmental DNA (eDNA) is genetic material that is released by organisms into water bodies by excretion, secretion, shedding, reproduction and decomposition, which is only persisted in water on a limited scale before it degrades through physical, chemical and biological processes. The cycle of release, transport, persistence and degradation of eDNA in aquatic systems enables it to signal recent or current presence of species and not historical presence. This advantage renders eDNA an effective, non-invasive, instrument of aquatic biodiversity signaling, detecting species, and occupancy modelling, specifically uncommon, cryptic, or low-density species that are hard to survey through conventional techniques. eDNA methods are cheaper and quicker than traditional aquatic surveys and are sensitive, particularly in a big and complicated environment. Moreover, eDNA is increasingly applicable to the study of illegal wildlife trade among aquatic species because it can identify the presence of taxa are prohibited or, at the very least, trafficked in water bodies that are related to harvesting or transportation processes, despite a lack of visual individuals, which supports advanced conservation enforcement and management interventions [16-19].

Across studies from 2015–2025, eDNA has emerged as a valuable but still underutilized tool in reptile and chelonian research. Reviews such as Nordstrom et al. (2022) demonstrate that current applications are heavily biased toward aquatic systems and a few reptilian orders [20], with major methodological constraints including low DNA shedding in keratinized species. Field applications, ranging from density-based detection in ponds [21] to targeted sea turtle assays [22, 49] show promise but require optimized primers, sampling depth, and inhibition control. Innovative integrations, such as community-driven conservation supported by eDNA evidence and viral-shedding monitoring in marine turtles, highlight expanding applications. Collectively, these studies underscore rapid technological advances and growing potential for eDNA to address long-standing challenges in reptile ecology, disease surveillance, and conservation management [23, 50].

2. Research Methodology

2.1. **Study Design:** This study has adopted a systematic review design to assess how eDNA has been used in chelonian conservation between 2015 and 2025. Literature review was performed according to the PRISMA guidelines to ensure the review is transparent and reproducible. The main objective of this design is to systematically collect, sift, and synopsize all studies that have used eDNA for the detection, monitoring, or management of Testudines. The included studies were critically evaluated, categorized, and interpreted in a systematic manner to come up with an integrated overview of current practices, developments, and gaps related to chelonian eDNA monitoring.

2.2. **Data Source and Search Strategy:** A comprehensive search was undertaken using the Scopus database, which offers extensive coverage of peer-reviewed literature. Three targeted search queries were used to capture the full scope of chelonian-related eDNA research:

- "Turtle eDNA"
- "Testudines environmental DNA"
- "Illegal trade turtle genetics/eDNA"

These queries were searched within Title–Abstract–Keywords fields to maximize relevance. The search period was restricted to 2015–2025, a timeframe chosen to reflect the rapid growth and technological refinement of eDNA monitoring. Subject area filters, Environmental Science and Biochemistry, Genetics and Molecular Biology were applied to ensure scientific relevance. Only studies published in English were considered.

2.3. Inclusion and Exclusion Criteria

2.3.1. Inclusion Criteria

- Empirical research articles applying eDNA methods to turtles, tortoises, or Testudines habitats
- Publications dated 2015 to 2025
- Articles written in English and indexed in Scopus
- Full-text available through open access or academic access
- Studies involving detection, quantification, habitat monitoring, or genetic surveillance using eDNA

2.3.2. Exclusion Criteria

- Review articles, book chapters, editorials, conference proceedings
- Studies not involving chelonians or not using eDNA techniques
- Articles lacking full-text access
- Duplicate, incomplete, or unpublished works

2.4. Screening and Selection Process

The screening process followed a structured, multi-stage approach:

- The combined search queries yielded 108 initial records.
- Applying subject area and document-type filters reduced this to 79 research articles.
- Limiting the results to open-access or accessible full-text studies refined the dataset to 50 articles.
- Titles, abstracts, and full texts of these papers were screened for relevance to chelonian-focused eDNA applications.
- All 50 studies met the inclusion criteria and were retained for detailed analysis.

This process ensured that only empirically grounded and methodologically relevant studies were included.

2.5. Data Extraction and Synthesis

Data extraction was conducted manually and systematically. The selected studies were reviewed to obtain essential information regarding:

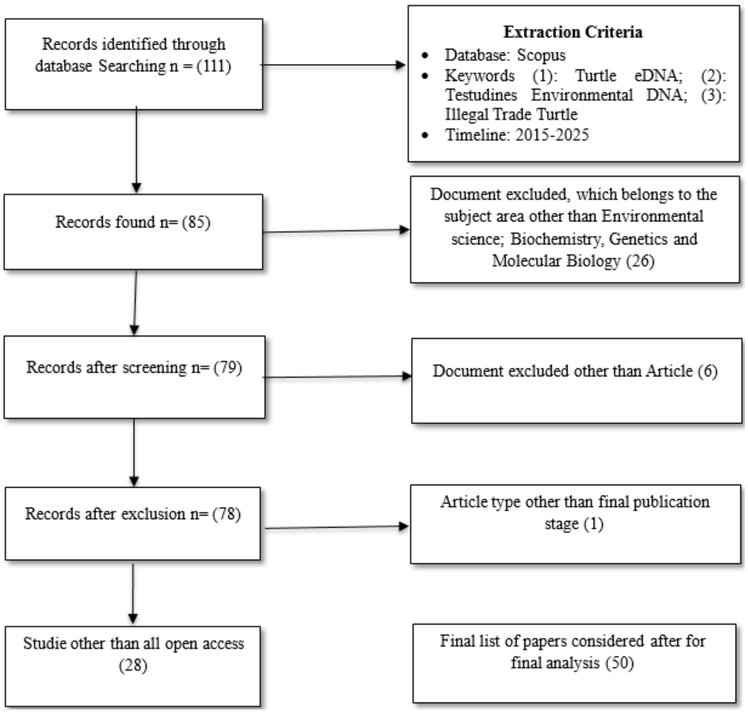
- Target species or chelonian groups
- Type of eDNA sampled (water, sediment, viral shedding, aquaria, field habitats)
- Sampling strategies and environmental conditions
- Molecular approaches used (qPCR, metabarcoding, primer design)
- Detection success, sensitivity, and reported limitations
- Stated conservation outcomes or management implications

After extraction, a narrative synthesis was carried out to integrate findings across studies, identify methodological patterns, compare detection efficiencies, and highlight knowledge gaps within chelonian eDNA research. This approach allowed for a structured yet flexible interpretation of diverse study designs.

2.6. Research Questions

The systematic review was guided by the following research questions:

- Which sampling strategies and molecular techniques have been most effective for eDNA-based chelonian detection?
- What biological, environmental, or methodological factors influence eDNA detectability and reliability in chelonian habitats?
- How has environmental DNA (eDNA) been utilized to support conservation enforcement, particularly in detecting illegal trade and exploitation pressures affecting chelonian species?
- What gaps, limitations, and future research needs exist to strengthen the role of eDNA in chelonian conservation strategies?



Flow chart 1. PRISMA flow diagram for the study.

This is a chart that describes the process from identifying, screening to including all 50 empirical studies reviewed in this study according to preferred reporting items for systematic reviews and Meta-Analyses (PRISMA) guidelines.

3. Results

Table 1 presents the annual distribution of publications that will be a part of the systematic review between 2016 and 2025. The initial period of the research (2016-2017) shows a moderate performance with four and three publications respectively, which is slightly decreased in the year 2018 and 2019, with five documents each. There is a noticeable increase in 2020 with six publications, which shows that the study of chelonian molecular and environmental DNA is gaining increased attention. This will be followed by a one-year decrease in 2021 and an obvious decrease in 2022 when only a single study was noted, implying a temporary diminishing of the level of publication or change of the research direction. But it is soon again a booming area and the number of publications in 2023 and 2024 is five and seven respectively. The research activity is the most intensive in 2025 (eleven documents), and it indicates a high recent increase in research interest. Altogether, the temporal trend shows a developing and swiftly growing area of research, with the most recent years of the dataset having an upswing.

Table 1. Annual distribution of peer-reviewed publications.

Year	Documents
2016	4
2017	3
2018	5
2019	5
2020	6
2021	3
2022	1
2023	5
2024	7
2025	11

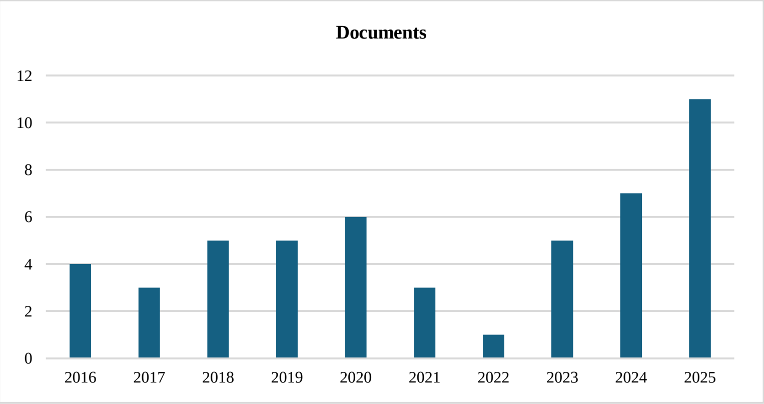


Figure 1. Temporal growth trends of chelonian-focused eDNA research publications (2016-2025)

The bar chart illustrates the growth of chelonian eDNA research output over time, with significant growth beginning in 2023 and peaking in 2025. The publications are distributed in various journals as outlined in Table 2 with the main ones presenting the main outlets of chelonian molecular and environmental DNA research. The journal that is in the top positions with eight documents is environmental DNA which reflects the fact that the sphere is closely related to the development of molecular ecology and monitoring based on eDNA. Ecology and Evolution is then followed by four publications, which represent the ecological and evolutionary environment of numerous researches. Additional evidence of this interdisciplinarity of the research is presented in journals like PeerJ and Diversity each containing three documents that cover the full range of biodiversity science and open-access ecological studies. A number of journals derive two publications each, such as the Frontiers in Marine Science, Zoological Research, Genome, and Genetics and Molecular research, which implies an equal representation of the theme of marine biology, genetics, and zoological research. The rest of the journals include Science of the Total Environment, Mitochondrial DNA Part B: Resources, Animal Biodiversity and Conservation, Proceedings of the Royal Society B, Genes, Ecotoxicology and Environmental Safety and Endangered Species

Research each hold a single document meaning that they are dedicated to specific fields of science. On the whole, the journal distribution points to a multidisciplinary publication environment as well as a very powerful focus on environmental DNA and ecological genetics.

Table 2. Most productive journals in the field.

Journal	Documents
Environmental DNA	8
Ecology and Evolution	4
PeerJ	3
Diversity	3
Frontiers in Marine Science	2
Zoological Research	2
Genome	2
Genetics and Molecular Research	2
Science of the Total Environment	1
Mitochondrial DNA Part B: Resources	1
Animal Biodiversity and Conservation	1
Proceedings of the Royal Society B	1
Genes	1
Ecotoxicology and Environmental Safety	1
Endangered Species Research	1

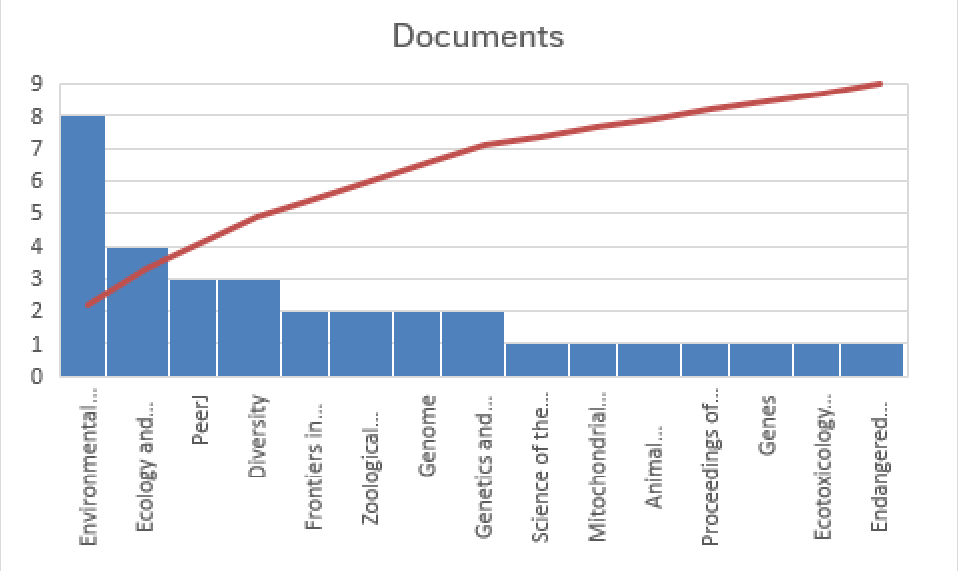


Figure 2. Publication frequency of top academic journals for molecular ecology and edna monitoring research.

The graph shows that the top publishers for eDNA research are primarily ecology and evolution and environmental DNA.

The number of publications by the publications in table 3 shows that there is a wide variety of different publishing houses in the field of chelonian molecular and environmental DNA research. Members of the Wiley group of publishers, such as John Wiley and Sons Inc (five documents), John Wiley and Sons Ltd (four documents) and Blackwell Publishing Inc. (three documents) are the largest contributors of publications, which implies that most of the studies are published in highly established journals in ecological and evolutionary sciences. Two documents each are covered by a number of other major academic publishers, such as Elsevier Ltd, Elsevier B.V., Taylor and Francis Ltd., Springer Science and Business Media, Frontiers Media SA, and MDPI AG, which represent a wide range of interdisciplinary coverage in the environmental science, genetics, and biodiversity research publications. Further publications by Canadian Science Publishing, Science Press, Fundacao de Pesquisas Cientificas and PeerJ Inc. demonstrate the international and open nature of the discipline. Lastly, an individual publication by BioMed Central Ltd and the Public Library of Science (PLOS) proves that even research is published in specialised open-access journals. On the whole, the publisher distribution shows that chelonian eDNA studies are largely being broadcast in the traditional academic publishers as well as in open-access journals, highlighting its international and multi-disciplinary scope.

Table 3. Leading academic publishers of chelonian eDNA research.

Publisher	Documents
John Wiley and Sons Inc	5
John Wiley and Sons Ltd	4
Blackwell Publishing Inc.	3
Elsevier Ltd	2
Canadian Science Publishing	2
Elsevier B.V.	2
Science Press	2
Taylor & Francis Ltd.	2
MDPI AG	2
Fundacao de Pesquisas Cientificas	2
PeerJ Inc.	2
Springer Science and Business Media	2
Frontiers Media SA	2
BioMed Central Ltd	1
Public Library of Science (PLOS)	1

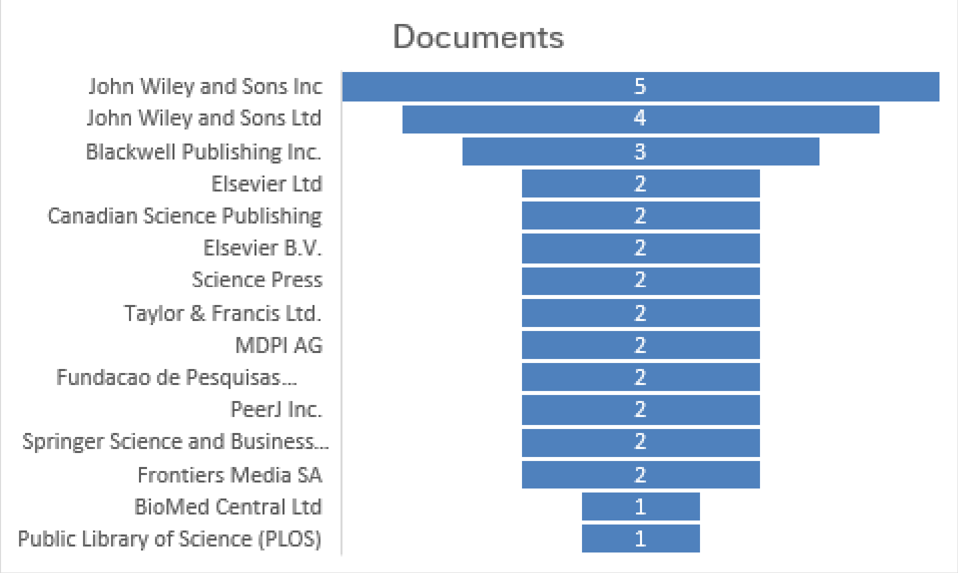


Figure 3. Academic publisher's distribution of publications.

This fig shows how much major publishing houses have contributed, showing a balance between traditional academic publishers (like Wiley and Elsevier) and open-access platforms.

Table 4 gives an excellent summary of the geographical distribution of publications on chelonian molecular and environmental DNA studies and the contribution made to the field is more than international. The number of documents in the United States is 42, which speaks of a high level of concentration of research activity and institutional presence. Australia is the next country with 18 publications that affirm its leading contribution to the observation and conservation of aquatic biodiversity. There are also 16 documents in Italy and Vietnam, which implies that both countries are actively cooperating and are interested in turtle conservation and molecular ecology. There is also a high level of research output in European and Asian nations, e.g., Germany (12), China (10) and Canada (9) and they show the global spread of eDNA methodologies. Others contribute less but significantly with France (8), Malaysia (7) and the United Kingdom (6) and Thailand (5), Brazil, Japan and the Netherlands (each 4) and Mexico (3). In general, the distribution demonstrates that the research on chelonian eDNA is distributed across the world with the strong leadership of North America and substantial input of Europe, Asia, and Oceania, which explains the global nature of conservation genetics and environmental monitoring research.

Table 4. Documents by country, Geographical distribution of research contributions.

Country	Documents
United States	42
Australia	18
Italy	16
Viet Nam	16
Germany	12
China	10
Canada	9
France	8
Malaysia	7
United Kingdom	6
Thailand	5
Brazil	4
Japan	4
Netherlands	4
Mexico	3

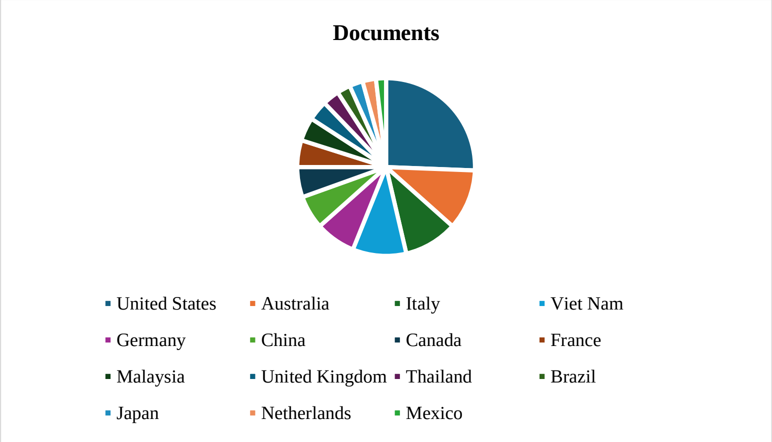


Figure 4. The output of research by country and where it is located.

A global map or bar chart showing where research is happening, with the United States and Australia at the top for chelonian eDNA studies. The main aspects of the environmental DNA sampling and monitoring plans that were used in chelonian studies are summarized in Table 5, with a heavy focus on water-based detection and methodological development. The usefulness of molecular surveillance in establishing the existence of endangered freshwater turtles in the wild is demonstrated by a study by Field-based water eDNA detection, e.g., Osathanunkul et al. (2025) [23]. As–Singkily et al. (2025) [24] also find that applicability assessments underpin the eDNA methods in monitoring marine turtles and presents marine turtle monitoring as predominantly dominated by aquatic sampling. The rapid development and validation of assays reported by Rishan et al. (2025) [25] and the continuous improvement of the detection speed and accuracy of the assays are well-timed activities aimed at increasing the reliability and the ecological interpretation of the information through the use of integrated monitoring strategies combining traditional surveys with eDNA methods, as described by Fasola et al. (2025) [26]. Mesocosm experiments are controlled (Meyer et al., 2025) [27] to proved the ecological detection frameworks, and portable field systems created by Seimon et al. (2024) [28] point at the new direction of real-time and in-situ biodiversity monitoring. Overall, the table indicates that research on chelonian eDNA is focusing on the targeted detection in water bodies and is backed by technological factors that aim to enhance sensitivity, efficiency, and pragmatic conservation procedures.

Table 5. eDNA sampling and monitoring approaches

Technique / Approach	Species / Focus	Key Results	Source/Study
Field water eDNA detection	Endangered freshwater turtles	Confirmed species presence using eDNA monitoring	Osathanunkul et al., 2025 [23]
Applicability testing of eDNA sampling	Marine turtle monitoring	Demonstrated effectiveness of water-based detection	As-Singkily et al., 2025 [24]
Rapid eDNA assay development	Chelonian detection workflows	Developed fast targeted assay for monitoring	Rishan et al., 2025 [25]
Integrated traditional + eDNA monitoring	Marine turtles	Improved detection reliability using combined methods	Fasola et al., 2025 [26]
Mesocosm validation experiments	Turtle ecology systems	Tested ecological detection frameworks	Meyer et al., 2025 [27]
Portable field eDNA workflow	Conservation monitoring	Demonstrated real-time biodiversity detection	Seimon et al., 2024 [28]

Table 6 is used to point out various genetic and molecular methodologies used in chelonian studies outside environmental DNA studies, which are extremely crucial in conservation, taxonomy, and wildlife trade studies. The genetic background of the species, including Yilmaz et al. (2023) [29], is studied using population genetics, which can aid in the conservation planning and management of the endangered species. On the same note, Ngo et al. (2023) [30] uncover the trends in genetic diversity of turtle species put at risk of extinction due to lack of genetic data, and the importance of molecular data as a population viability measure. Genetic-based taxonomic revisions such as Liang et al. (2025) [31] define the species boundaries making the conservation evaluation more accurate. The study of the evolutionary aspects of Asian box turtles through cytogenetics (Clemente et al., 2021) [32] is a source that helps to see the scope of the use of molecular tools in the field. DNA barcoding research, including the work by Kundu et al. (2018) [33], allows specific species to be identified in the pet trade, and more generalized genetic efforts of the trade networks of turtles in the world reveal the potential of molecular techniques to influence conservation management and policymaking (Miller et al., 2019) [34]. In sum, the table indicates the inclusion of genetics in the study of the chelonians, which can be used not only to monitor the ecological conditions but also to manage the endangered species.

Table 6. Genetic and molecular techniques used

Technique	Species / Focus	Key Results	Source/Study
Population genetics analysis	*Testudo hermanni*	Identified genetic structure for conservation planning	Yilmaz et al., 2023 [29]
Genetic diversity assessment	Critically endangered turtles	Revealed conservation-relevant diversity patterns	Ngo et al., 2023 [30]
Taxonomic revision using genetics	Endangered turtle taxa	Clarified species boundaries and classification	Liang et al., 2025 [31]
Cytogenetic analysis	Asian box turtles	Provided chromosomal evolutionary insights	Clemente et al., 2021 [32]
DNA barcoding identification	Pet trade turtles	Identified multiple traded species	Kundu et al., 2018 [33]
Global trade genetics study	Turtle trade networks	Demonstrated role of genetics in trade monitoring	Miller et al., 2019 [34]

Table 7 outlines the key determinants of the literature that exists on the reliability of environmental DNA (eDNA) detection in chelonian research focusing on ecological and methodological aspects. The environmental DNA fate processes analyzed by Nelson et al. (2025) [35] explain that the habitat conditions and DNA survival in aquatic environments directly affect the success of detection. The example of integrated monitoring strategies such as Fasola et al. (2025) [26] shows that the combination of traditional survey methods and eDNA sampling can result in better detection and reduced uncertainty. Additional methodological improvements such as assay optimisation and validation as reported by Rishan et al. (2025) [25] further improve the sensitivity and reliability, by optimising detection processes. Also, field-based systems portable designed by Seimon et al. (2024) [28] testify that designing standardised methodology and equipment is the key factor that contributes to the uniformity and correctness of the results given under different monitoring conditions. On the whole, it is possible to note that the effective eDNA detection depends on the alliance of the environmental factors, effective assay design, and integrated monitoring strategies.

Table 7. Factors influencing eDNA detection reliability

Factor Theme	Species / Study Focus	Key Finding	Source/Study
Environmental DNA fate	Field monitoring systems	Detection affected by habitat and signal persistence	Nelson et al., 2025 [35]
Integrated monitoring design	Marine turtle surveys	Combined surveys increase detection success	Fasola et al., 2025 [26]
Assay optimisation	Rapid detection workflows	Validation improves accuracy	Rishan et al., 2025 [25]
Portable workflow standardisation	Field biodiversity monitoring	Method design influences detectability	Seimon et al., 2024 [28]

Table 8 is a summary of how molecular and genetic methods have been used to tackle the subject of illegal trade and conservation enforcement in Chelonian research. DNA barcoding analyses, including Kundu et.al. (2018) [33], are essential in authentication of species identity in the pet trade to enable the identification of mislabeled or contraband identities of animals in the trade. When Miller et al. (2019) [34] conducted broader genetic studies of global turtle trade networks, it showed the patterns of exploitation and identified threats of conservation in the field of international wildlife trafficking. The genetic population of studies, such as Yilmaz et al. (2023) [29], has an indirect contribution to the enforcement by providing evidence on population structure and units of population management that can be used to inform the conservation policy. Also, taxonomic genetics can help Liang et al. (2025) [31] to identify species on a species level, which in turn enhances the legal framework and aids with proper classification of the endangered species. On the whole, the table shows that genetic tools are essential regarding tracking trade activities, improving the identification of species, and assisting in the implementation of conservation decisions.

Table 8. Genetic tools applied to illegal trade and enforcement

Enforcement Technique	Species / Trade Context	Key Results	Source/Study
DNA barcoding	Pet trade turtles	Verified species identity in trade chains	Kundu et al., 2018 [33]
Trade network genetic analysis	Global turtle trade	Revealed conservation threats linked to trade	Miller et al., 2019 [34]
Population genetics	Threatened turtle populations	Supported conservation management decisions	Yilmaz et al., 2023 [29]
Taxonomic genetics	Critically endangered turtles	Improved species-level legal identification	Liang et al., 2025 [31]

Table 7 shows some of the main gaps in research identified during the systematic analysis of the studies used. There is a strong aquatic bias with most studies focusing on water-based eDNA detection and little attempt being made to use different sampling matrices like sediment or land. The use of metabarcoding and community level sequencing methods is still comparatively low, with targeted assays and species-specific detection workflows still prevailing. The problem of detectability is widely documented, and environmental conditions as well as the process of DNA degradation affect the reliability of monitoring in the studies. In addition, it seems that enforcement-oriented research is not well-represented compared to ecological monitoring research, indicating that future studies should incorporate molecular instruments into wildlife trade research and conservation law enforcement. Together, these gaps can point to valuable paths of future Chelonian molecular studies and also enhance conservation.

Table 9. Evidence gaps identified from systematic review

Gap Theme	Evidence from Results
Aquatic bias in sampling	Majority of studies rely on water-based eDNA detection
Limited metabarcoding adoption	Targeted assays dominate molecular workflows
Detectability challenges	Environmental conditions repeatedly discussed as limitations
Enforcement research underrepresented	Fewer studies focused on illegal trade compared with monitoring

4. Discussion

The evidence in this systematic review indicates that the environmental DNA (eDNA) monitoring has increasingly become a crucial methodological tool in the research of chelonian species, which by definition is a hard-to-detect species in terms of traditional survey methods. Water based sampling techniques are currently guiding research trends which highlights the strong relationship that exists between turtles and water bodies. Empirical research, including that of Osathanunkul et al. (2025) and As-Singkily et al. (2025) supports the idea that endangered freshwater and marine turtles can be reliably detected using targeted eDNA detection. Combined surveillance systems which combine conventional in the field surveys with molecular diagnostics have improved detection efficacies and enabled more sophisticated ecological interpretation. Monitoring efficiency has further been enhanced by the development of rapid assays with the level of technology in the field being significantly enhanced. However, the continued dependence on species-specific detection indicates a methodological bias to specific monitoring, thus potentially limiting the general information of a larger ecosystem. The rise in the number of publications since 2023 suggests a rapid growth and the increased interest of scientists in the application of chelonian eDNA.It has been reported several times that environmental and methodological variables are important determinants of eDNA detectability and reliability. eDNA fate processes such as degradation and transportation have a direct impact on monitoring aquatic system outcomes as highlighted by Nelson et al. (2025) [35]. The combination of traditional and molecular surveys explained by Fasola et al. (2025) [26] by integrated monitoring strategies demonstrates that this combination will increase the rate of detection and alleviate uncertainty. Another factor that increases sensitivity and methodological consistency is assay optimisation and validation, such as the example of Rishan et al. (2025) [25]. The portable field workflow that is introduced by Seimon et al. (2024) [28] is an indication of a shift to real-time biodiversity monitoring. In addition to eDNA genetic methods provide useful information on taxonomy, evolutionary history and conservation planning. The studies of genetic diversity and cytogenetics emphasize the importance of the use of molecular tools in various areas of investigation. Although these methodological improvements have been made, standardisation of protocols is a daunting task, which has to be continued to enhance its reproducibility even in the context of varied geographical environment.

The use of genetic tools in the illegal trade in wildlife is a relatively novel, but relatively understudied, topic in chelonian research. Similar to the work by Kundu et al. (2018) [33], DNA barcoding studies emphasise the ability of molecular methods to detect mislabelled species in the pet trade to facilitate the process of conservation enforcement. The trade-network analyses by Miller et al. (2019) [34] provide evidence of how genetic data could be used to reveal the patterns of exploitation and direct the development of policies. The population-genetic and taxonomic studies also support the species-level identification and management plans. Nevertheless, the studies that emphasize the monitoring are still predominant in the literature, which demonstrates the lack of ecological research and enforcement practices. The spatial dispersion of literature is characterized by the high level of international cooperation led by the United States and Australia, which provides evidence of the universal nature of chelonian preservation issues. The fact thats there are various journals and publishers also supports the interdisciplinary character of the field. Research gaps including aquatic bias and a lack of metabarcoding uptake will be critical aspects that will need attention to ensure future conservation strategies advance.

5. Conclusion

This review highlights the transformative nature of environmental DNA (eDNA) as an approach of non-invasive technique to monitor endangered chelonian species in aquatic environment. The effectiveness of the extant methods is evidenced in the prevalence of water-based sampling and focused analyses, but at the same time is a limitation of ecological scope. Essential complementary genetic analysis sharpens the definition of taxonomy, clarifies population structure, and aids in conservation implementation. The recent increase of publications indicates the more rapid development of technology and increased attention to the topic of molecular monitoring in conservation science. However, longstanding concerns related to detectability, methodological bias and inadequate involvement in enforcement structures suggest that the field is still in a state of flux. The strong global cooperation has enabled the growth of knowledge, but inequality in the scope of studies across regions is glaring. It is urgently necessary to address these limitations in order to enhance the precision of monitoring and increase the conservation outcomes. Overall, eDNA is an effective but developing instrument in the conservation of chelonians.

The area of most need in future studies is the diversification of sampling matrices, which must exclude aqueous environments with the goal of alleviating the bias of aquatic habitats and enhancing detectability in more terrestrial or sediment-derived environments. Increased use of metabarcoding and high-throughput sequencing would allow conducting more detailed biodiversity surveys instead of limiting it to species-specific detection. Incorporation of molecular surveillance into the system of wildlife trade enforcement would enhance conservation policy and management action. The development of portable technologies in the field and real-time analysis pipelines is a promising opportunity of timely biodiversity measurement and adaptive protection measures. The standardized protocols and cross-regional partnerships are essential to improve the comparability of the data and to guarantee the methodological consistency. Expanding research to include underrepresented species and geographies will solve conservation shortfalls across the globe. Combining ecological surveillance and genomic technologies would potentially provide new knowledge on the resilience of populations and evolutionary processes. Together, these potential opportunities can help fill important gaps in the conservation of chelonians with the implementation of new eDNA-based monitoring techniques.

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