

# Application of Next-Generation Sequencing Technology in Southeast Asia: A Practical Framework for Advancing Wildlife Conservation

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**Abstract.** Next-generation sequencing (NGS) has transformed genomics, presenting significant potential for advancing wildlife conservation. NGS technology offers opportunities and challenges for addressing scientific questions in wildlife management. Unfortunately, there is a lack of comprehensive records on NGS implementation in Southeast Asia, particularly concerning wildlife conservation. To address this gap, we analyzed NGS studies focused on wildlife monitoring in Southeast Asia and introduced a practical framework for implementing NGS technologies in global wildlife conservation, especially in Southeast Asia. We systematically reviewed NGS studies in wildlife monitoring from the SCOPUS database. We identified 137 relevant publications from 11 countries, with Malaysia contributing 36% of the studies. The included studies were categorized into five themes: species identification, dietary assessment, health monitoring, taxonomic resolution, and whole-genome sequencing, with the majority focusing on 38 publications related to wildlife health. The framework developed in this study help the researchers and conservation practitioners with insights on NGS technology application in conservation while also addressing the benefits, limitations, and ethical considerations associated with NGS use. This review offers a brief overview

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of NGS usage and provides guidelines for embracing NGS as a valuable tool for effective wildlife conservation strategies in our rapidly changing world.

## 1 Introduction

Next-generation sequencing (NGS) technologies, introduced in 2005, have revolutionized biological science after first-generation sequencing or Sanger sequencing was dominant for three decades. NGS successfully broke the limitations of the first generation, especially in the number of samples reads. The advances in sequencing led to large-scale and broad-scope biosystematics projects to be applied for various applications including the Barcode of Life initiative [1]. The emergence of DNA sequencing has led to several commercially available high-throughput sequencing (HTS) platforms based on different chemistries and detection techniques to be used in various fields, including environmental studies. With its high-throughput capacity and cost-effectiveness, the number of studies related to environment scope has been increasing because it can potentially combat many of the challenges associated with biodiversity monitoring [2]. The power of NGS to sequence environmental DNA (eDNA) can lead to overcoming many issues as eDNA is a genetic material of the organism living in the habitat can be found and collected easily [3]. Biodiversity monitoring can be more feasible because the eDNA enables practical detection and classification of species as it can originate from various sources such as skin, mucus, saliva, sperm, secretions, eggs, feces, urine, blood, roots, leaves, fruit, pollen, and decaying remain of larger organisms [4]. Consequently, the acceptance of detecting eDNA can support ex-situ and in-situ conservation by harnessing leading-edge technology in overcome the limitation using the traditional monitoring methods [5, 6].

Additionally, the application of NGS should be widespread, including in Southeast Asia consisting of Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, the Philippines, Singapore, Thailand, Timor-Leste, and Vietnam [7]. This region is recognized as a global hotspot of biodiversity and endemism [8]. However, it also faces some of the highest deforestation rates, stemming from mining, construction, and human development, which pose a global threat to biodiversity, as reported by Hughes [8]. Hence, the protection and conservation of biodiversity in Southeast Asia is required as some species is now on the verge of extinction. As a powerful tool, NGS can enhance our understanding across various applications, including species identification, health monitoring, dietary assessment, and genomic studies [9,10]. The ability of NGS to trace rare and threatened species across a wide range of taxonomic groups from various habitat and reveal information of entire ecosystems can help to avoid the loss of significant portion especially for endemic species [7, 11,12].

Furthermore, with the development of NGS, this technology can gradually be applied to various taxa, including terrestrial and aquatic populations. Hence, there is a growing need to acknowledge the advantages of NGS which are relevant for the authorities to consider it as part of wildlife monitoring. The efforts can guide for monitoring, importantly caused by the alteration in adapting the surrounding environment from anthropogenic disturbance that affect the host health of wildlife [13,14]. In this study, we aimed to analyze the available research focusing on wildlife monitoring in Southeast Asia using the NGS platform. We also presented an outline of practical NGS-based framework as a standard protocol and include the application in various aspects to provide insight for future wildlife management. Thus, the findings of the study should be further explored especially for Southeast Asia to fill the gaps in understanding the biodiversity loss that will enable more effective and efficient planning to monitor ex-situ and in-situ conservation moving forward. Notably, it is proven that the NGS approach can be applied in conservation management, which provides the

evidence required for program assessment, implementation, and adaptive management, particularly in Southeast Asia [15].

## **2 Methodology**

### **2.1 Literature review and selection criteria**

In this study, we conducted a systematic review of scientific articles related to NGS in Southeast Asia to compile relevant literature [16]. The first stage, the literature search was conducted with a comprehensive search across online indexed manuscripts using the SCOPUS database. Several search strings were used involving 11 countries from Southeast Asia (Malaysia, Indonesia, Thailand, Singapore, Brunei, Laos, Myanmar, Timor-Leste, Philippines, Vietnam, Cambodia) as the main keyword and combined with additional keywords such as “Next generation sequencing”, “metabarcoding”, “metagenomic and “mitogenome”. We screened titles, abstracts, and keywords to identify relevant articles. Next, the article selection was done and we meticulously examined the selected articles related to wildlife to determine their suitability for inclusion in our review. Conference abstracts and articles lacking original data (e.g. review papers, editorials, and commentaries) were excluded from the review. Only studies that performed next-generation sequencing focusing on birds, mammals, fishes, reptiles, and amphibians monitoring were included. All the articles published between 2013 and 2023, where we performed a literature search during September-October 2023.

### **2.2 Thematic analysis**

Extracted data from the scoping review were downloaded as Excel spreadsheets (Microsoft Corporation, Redmond, WA, USA) for thematic analysis. All relevant articles about wildlife monitoring using NGS were reviewed to ensure the consistent interpretation of key content according to a similar concept [17]. For all included publications, we assessed primary research topics for publications based on five categories: (i) species identification, (ii) dietary assessment, (iii) health monitoring including gut microbiomes and disease (iv) taxonomic study consisted of the resolving of relationship between population and species and (v) whole-genome sequencing. Then, the articles were classified based on the research objectives, which can help fill the gaps on wildlife monitoring in Southeast Asia. Our ultimate goal was to identify common themes and protocols in the selected articles and synthesize this information into a conceptual framework for the next-generation wildlife monitoring using eDNA detection and NGS.

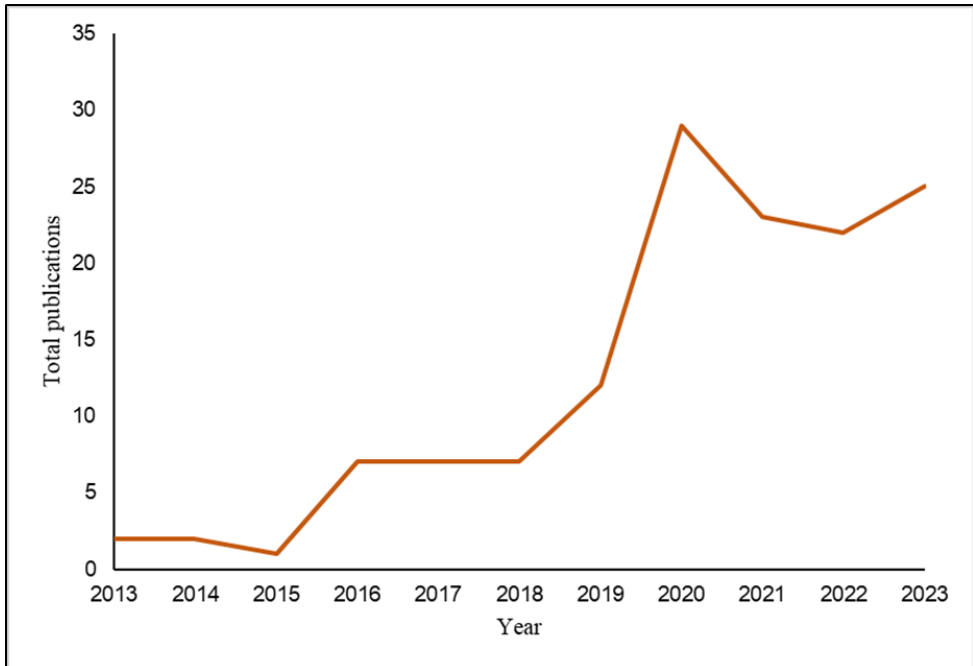
## **3 Result and Discussion**

A total of 1,102 journal publications and conference proceedings were identified during our search in Southeast Asia, with the addition of four keywords (see Table 1). We performed the first screening to select suitable peer-reviewed articles pertaining to wildlife monitoring. We excluded 965 publications that did not meet the inclusion criteria and those that were repetitions for each country. As a result, only 137 relevant peer-reviewed articles, which contained information related to wildlife monitoring utilizing NGS as a conservation tool, were chosen. The most publications related to the implementation of NGS technology are from Malaysia (49/137, 36%), followed by Indonesia (22%) and Thailand (12%). Throughout the searching, no suitable peer-reviewed publication found in Timor-Leste. The trend rates of publication of application NGS in wildlife monitoring are increasing up to 2020

but slightly decreased in 2021 and 2022. Positively, in 2023, the number of publications is increasing as shown in Figure 1. Before 2020, only 38 papers met our selection criteria, representing 28% of the total publications. However, from 2020 to 2023, the proportion of publications in selected journals increased to 73% of the total studies (Figure 1).

**Table 1.** Total number of studies conducted throughout Southeast Asia between 2013 to 2023 in bibliographic search.

Country	All published articles	Suitable peer-reviewed articles
Malaysia	206	49
Indonesia	203	30
Thailand	224	17
Singapore	104	4
Brunei	5	2
Myanmar	45	8
Vietnam	162	10
Laos	21	5
Philippines	81	7
Cambodia	49	5
Timor-Leste	2	0
<b>Total</b>	<b>1102</b>	<b>137</b>



**Fig. 1.** NGS application rate in wildlife monitoring publications from 2013 to 2023.

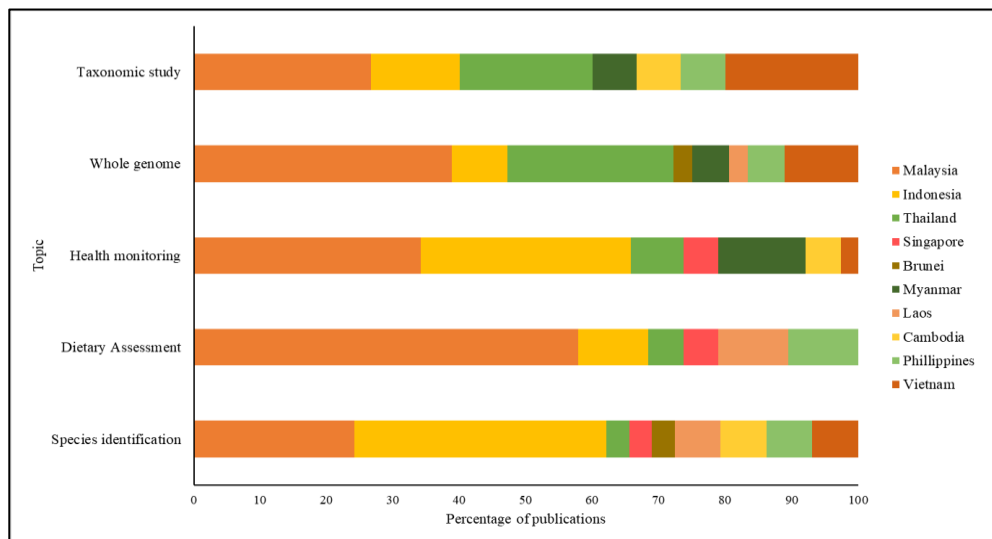
### 3.1 Thematic categories of publications

We classified the 137 suitable peer-reviewed articles into five thematic categories as specified in the study. The majority of these studies, comprising 27.7%, were focused on wildlife health monitoring, representing a significantly higher proportion than other topics. Following closely, the whole-genome study accounted for 26.2%, species identification for 21.2%, dietary assessment for 13.9%, and taxonomic resolution study for 10.9% as stated in Table 2.

**Table 2.** The number of publications according to the related topics in wildlife monitoring.

Topic	Number of related articles
Species identification	29
Dietary assessment	19
Health monitoring	38
Whole genome sequencing	36
Taxonomic study	15

To assess the percentage contribution of each Southeast Asia country to these topics, we treated each country listed in the publications separately. The majority of health monitoring studies in Southeast Asia were conducted in Malaysia (13/137, 9.5%), followed by Indonesia (12/137, 8.7%), Myanmar (5/137, 3.6%), Thailand (3/137, 2.2%), and Singapore and Cambodia (1.5%) each, with Vietnam (0.7%) showing the least representation (Figure 2). For dietary assessment, whole-genome study, and taxonomic resolution, Malaysia had a considerably higher proportion, with 8.0%, 10.2%, and 2.9%, respectively, compared to the other nine countries. Regarding species identification, Indonesia led with 8.0%, as indicated in Figure 2. The classification based on the categories shows only three countries (Malaysia, Indonesia and Thailand) fulfilled the five topics according to the analysis based on the SCOPUS database.



**Fig. 2.** An overview of the availability of 137 publications based on the countries.

### 3.2 Practical framework of application NGS

Based on the findings from the literature searched, the practical framework of NGS-based technology for wildlife is drafted, which can offer to guide: 1) design, 2) validation, 3) analysis of the sequence data, and 4) filling gap in wildlife monitoring. This framework is intended for implementation in Southeast Asia countries. It can be a valuable reference for establishing guiding principles and recommendations in wildlife management, fisheries management, terrestrial protected areas, and forestry in Southeast Asia. Collaboration with government agencies and universities is essential to ensure the framework's objectives align with environmental governance. Bennett and Satterfield emphasized the critical role of government involvement in effective environmental management and conservation actions [18]. Therefore, more precise conceptualizations are needed to advance the usage of NGS technology in research. The practical framework provides a comprehensive reference comprising objectives and attributes to guide future research (Figure 3).

Additionally, the framework for wildlife monitoring using NGS technologies can enhance future conservation plans managed by government agencies. Strengthening scientific knowledge is imperative for informed decision-making in conservation planning. This will facilitate the inclusion of NGS technologies in each country's conservation plan, ultimately improving planning strategies and enhancing the ability to fulfil obligations under the Convention on Biological Diversity (CBD) and other multilateral environmental agreements such as the United Nations Sustainable Development Goals and the Ramsar Convention on Wetlands, governments and civil society organizations are increasing investment in conservation in Southeast Asia [11]. Collaboration with government sectors is crucial for successfully implementing this molecular approach. This framework indirectly contributes to more effective wildlife conservation and the preservation of the rich and diverse plant life in Southeast Asia forests. Consequently, it can promote the plant identification for herbivorous as an ample food source and connecting fragmented forest areas to facilitate species movement for herbivore.

The findings show that health monitoring is the dominant topic compared to others. Health issue of wildlife is crucial as it is related to the zoonotic disease even some of wild animals considered as a reservoir. NGS technology can help detect specific viruses and bacteria in wildlife in order to identify Emerging and Re-Emerging Infectious Disease (EID/REID) where it can potentially affect the human population [19, 20]. Gut microbiome plays an important role in determining the health condition of an organism as it influences digestion control, immune system and also acts as a "second brain" [21]. Hence, the information regarding the surrounding environment is required as the composition of gut microbiomes in host species will differ due to altered habitats to maintain normal physiological processes in animals [14, 22]. It also helps in preventing the emergence of new coronaviruses in Southeast Asia where few species have potential as a carrier of zoonotic disease on bats (*Chiroptera brachjatis*, *Rhinolophus shameli*) [19, 23] and Malayan pangolin (*Manis javanicus*) [24].

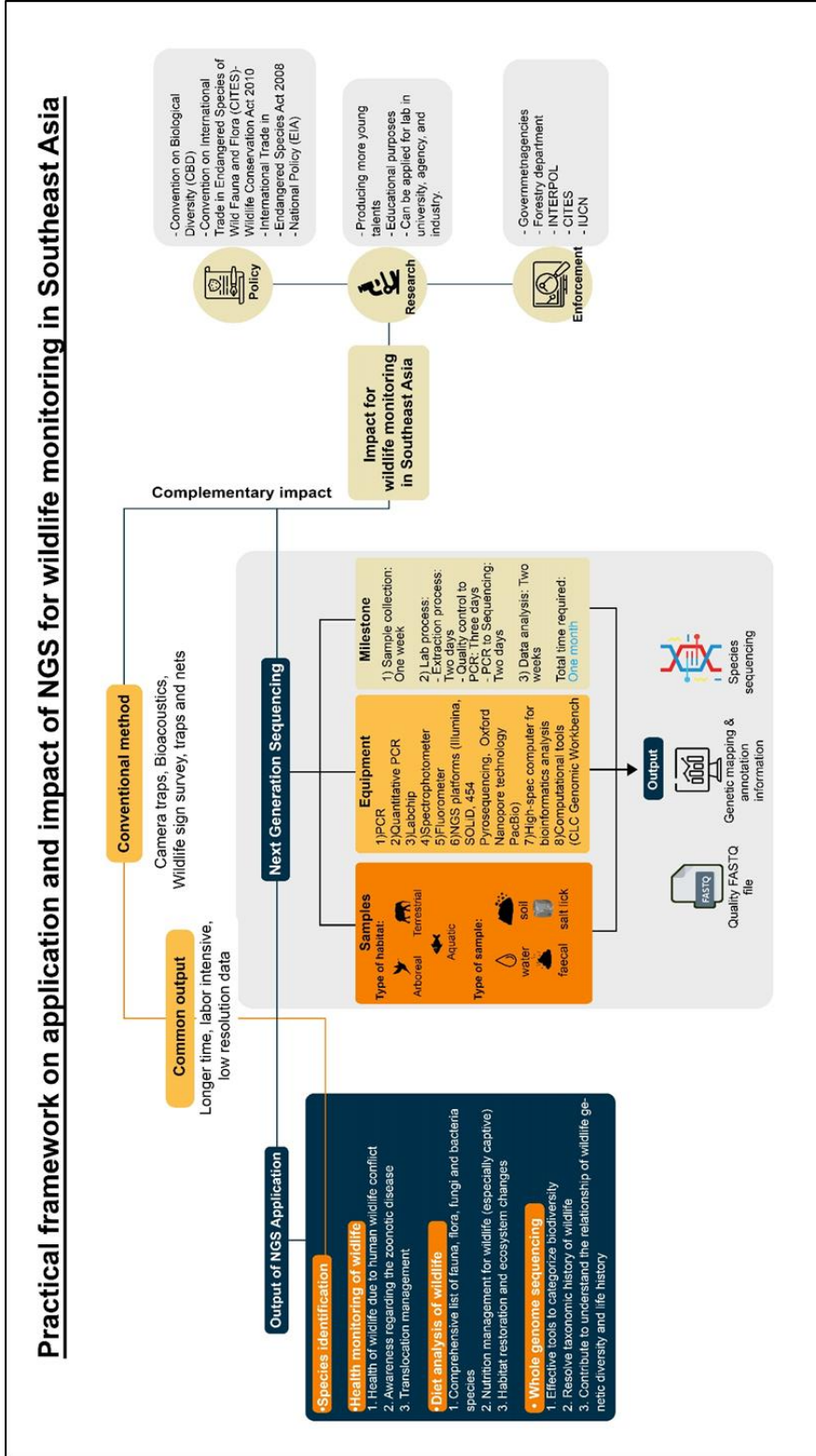
Besides, the effectiveness of NGS technology in determining the complex dietary of wildlife also lead to the increasing pattern of study in Southeast Asia. It proves the study on diet analysis can contribute to various perspectives, including prey taxa composition and structure, trophic interaction that provide valuable information for understanding animal ecology, evolution and conservation [25-27]. In 2016, Srivathsan et al. conducted shotgun sequencing that allows the simultaneous assessment of diet, population genetics and gut parasites from endangered populations (*Presbytis femoralis*) [28]. The changes of environment can alter the food intake by wildlife, especially for captive wildlife. The schedule diet intake is needed for nutritional requirements and food mechanical properties when rearing wildlife to adulthood for reintroduction [29]. This is proved in dietary assessment of Asian elephant (*Elephas maximus*) in captive population [30, 31]. At the same

time, the application of the technology can improve the health as dietary intake is difficult to be measured using traditional diet analysis [32]. The data from the study can use as a baseline data in monitor wildlife using NGS technology that can improve husbandry and health of wildlife [33]. By having the comprehensive result from dietary composition of wildlife, an appropriate home-building-based habitat management can be performed especially in conserving the endangered species [34, 35].

Based on the number research results that focused on whole genome sequencing (WGS) mentioned shows that the genomics is the effective tools to characterize biodiversity because it provides the most comprehensive data of organism [36]. The information from WGS provide insights especially for Southeast Asia in phylogenetic relationships, evolutionary history, extinct and cryptic taxa, and ancestral intraspecific genetic diversity and structure that can guide wildlife conservation and restoration [36-38]. Furthermore, the findings of the study also show the applicability of NGS in species identification which can be an alternative way to obtain the result in a shorter time due to the acceleration of biodiversity loss [39]. Thus, the issue of illegal wildlife trade and mislabelling species can combat the challenges in biodiversity loss [40]. With the sensitivity of NGS technology to sequence challenging specimens such as museum specimen or non-invasive samples via metabarcoding, it can potentially increase the accuracy and overcome the limitation by Sanger sequencing [40- 42]. This is also supported where genetic non-invasive sample can provide a valuable information for population monitoring and allow the inferences of population density and key behavioural traits which is cost-effectiveness for wildlife study [43-45].

While numerous advantages can be gained from the utilization of NGS platforms, as outlined in this study, it is imperative to address and control error factors for a more extensive and in-depth adoption of NGS-based technologies. Building a local reference database is needed to reduce inaccuracies in species classification to help read an enormous sequence produced by NGS technologies [25]. It is helpful to build the extensive local reference database to design a universal primer, especially for wildlife in Southeast Asia [46]. Then, the optimization of PCR protocols and the improvement of primer efficiency is required to minimize the bias reading according to the targeted species [10, 47]. To fully implement genomic techniques in practical conservation, the funding is crucial [48]. Developing countries have limited funding when it comes to effectively managing wildlife conservation through NGS technology in Southeast Asia.

With the help of the drafted practical framework, wildlife conservation in Southeast Asia can be done by referring Figure 3 as a guideline or standard protocol in using a genetic tool. The framework for applying NGS technology in wildlife conservation is based on fundamental principles and concepts that guide the design, implementation, and interpretation of studies aimed at tracking changes in wildlife populations, habitats, and ecosystems over time [49]. By having a practical framework, the overview of how NGS works can be seen but still needs improvement in developing analytical pipelines and establishing reference databases. This is to overcome the biased results which can give inaccurate assessment that will interfere the application [25]. Consequently, this framework is need in bridging ecological theory with conservation practice, designed to assist researchers and managers in better understanding and addressing the complex interactions among wildlife, their habitats and human activities.



**Fig. 3.** The practical framework with steps for applying NGS technology. All the steps involved the elements needed for NGS and the impacts of NGS in wildlife monitoring.



## 4 Conclusion

Overall, the usage of NGS technology in biodiversity assessment of Southeast Asia shows an increasing publication where Malaysia has the highest number of studies implement the technology. This finding indirectly contributes to developing wildlife conservation intervention useful for future references by authorities and stakeholders. The practical framework summarized in this study could be a valuable for researchers to explore and improve the implementation of NGS in wildlife aspects for better ex-situ and in-situ conservation to prevent any extinction of species.

## 5 Acknowledgment

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