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A Review on Next-Generation Wildlife Monitoring using Environmental DNA (eDNA) Detection and Next-Generation Sequencing in Malaysia

(Kajian Pemantauan Hidupan Liar Generasi Masa Hadapan menggunakan Pengesanan DNA (eDNA) Persekitaran dan Penjujukan Generasi Masa Hadapan di Malaysia)

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ABSTRACT

The use of environmental DNA (eDNA) as the genetic tool to monitor biodiversity has been increasing across the world, including Malaysia. Over a decade, the technique has become widely used in wildlife conservation with the technologies called next-generation sequencing (NGS). Unfortunately, as one of the top megadiverse countries, Malaysia is still behind in this field since eDNA methods outperform traditional surveys. Hence, in this study, we analyzed the paper related to eDNA studies in Malaysia, which focused on wildlife monitoring. We conducted a systematic bibliographic search and quantitative review of studies published before June 2021 from Google Scholar and Scopus database. Forty published eDNA studies were found, and each paper was classified based on five themes (species identification, diet assessment, health monitoring, resolve taxonomic, whole-genome sequencing) according to the study analysis. This study aims to identify gaps in eDNA in Malaysia, which can later be useful for future conservation actions and development by applying technology in wildlife monitoring.

Keywords: Biodiversity; conservation; environmental DNA; eDNA; next-generation sequencing; NGS

ABSTRAK

Penggunaan DNA persekitaran (eDNA) sebagai alat genetik untuk memantau kepelbagaian biologi telah pun meningkat di seluruh dunia termasuk Malaysia. Setelah sedekad, teknik ini telah digunakan secara meluas dalam pemuliharaan hidupan liar bersama dengan teknologi yang dikenali sebagai penjujukan generasi masa hadapan (NGS). Walau bagaimanapun, Malaysia yang merupakan salah sebuah negara di dunia yang mempunyai kekayaan kepelbagaian biologi yang tinggi masih lagi ketinggalan dalam bidang ini kerana kaedah eDNA sebenarnya mampu mengatasi kaedah tradisi. Dalam ulasan ini, kami telah menganalisis kajian yang berkaitan dengan eDNA di Malaysia yang memberi tumpuan kepada pemantauan hidupan liar. Kami telah menjalankan pencarian secara sistematik biblografi dan juga secara kuantitatif berdasarkan kajian lepas yang telah diterbitkan sehingga Jun 2021 menggunakan Google Scholar dan pangkalan data Scopus. Terdapat 40 kajian eDNA yang telah diterbitkan dan dikelaskan berdasarkan lima tema (pengenalan spesies, penilaian pemakanan, pemantauan kesihatan, penyelesaian taksonomi dan penjujukan keseluruhan genom) mengikut analisis kajian. Tujuan ulasan ini adalah untuk mengenal pasti jurang kajian eDNA di Malaysia yang dapat membantu untuk aktiviti pemuliharaan dan pembangunan masa hadapan dengan menggunakan teknologi yang terkini dalam pemantauan hidupan liar.

Kata kunci: DNA persekitaran; eDNA; kepelbagaian biologi; konservasi; NGS; penjujukan generasi masa hadapan

INTRODUCTION

As one of the top megadiverse countries, Malaysia plays an important role in the conservation of thousands of floras and faunas, which can be found throughout the tropical rainforest that had evolved over 130 million years. Statistics from Forestry Department of Peninsular Malaysia reported the total forested area is around 5.73 million hectares which is around 40% (FDPM 2020),

serving as habitat for high biological diversity. According to the Sixth National Report of Convention on Biological Diversity in 2019, Malaysia is home to an estimated of 306 species of mammals, 742 species of birds, 567 species of reptiles, 242 species of amphibians, and more than 449 species of freshwater fish. Wildlife monitoring in Malaysia is largely depends on conventional methods such as trapping and observations to gather species information (Mohd-Azlan et al. 2019; Nasron et al. 2019). Furthermore, method like camera trapping requires a long temporal and spatial coverage and is considered expensive in the long run (Burton et al. 2015). New innovations and technologies give a positive impact toward biodiversity, especially on improvement of the ability to explore at ecosystem-level process, conservation dynamics, and also the sensitivity for detecting rare species or difficult to sample taxa (Bohmann et al. 2014).

Habitat loss, fragmentations, and anthropogenic factors have become the prime menaces for biodiversity declination across the world. Therefore, an endeavor to conserve wildlife by earliest researchers through conventional method have faced several limitations in order to complete the research. Apart from harvesting greater workforce and time during direct monitoring, the utilization of morphology as sole reference for species identification had led to difficulties in conservation management (Bohmann et al. 2014). This is because many juvenile organisms or cryptic species are hardly defined due to the lack of taxonomist expertise for certain wildlife groups (Thomsen & Willerslev 2015). The basis of any conservation effort predominantly depends upon a valid information especially on knowledge and description of a species (Mace 2004). Any minute mistake in the identification process of species of interest might cause one loss in term of money, effort, and time.

Sanger DNA sequencing as a novel technique for species identification has been introduced in 1977 and has been used in wildlife monitoring (Sanger, Nicklen & Coulson 1977). Over the period, second-generation sequencing, generally called as next-generation sequencing (NGS), was established and evolved rapidly with various new platforms released, such as Ion Torrent, Illumina, and Roche. The improvised platforms led to the emergence of environmental studies, since they are more practical in analyzing larger amounts of organism and are cost effective (Ambardar et al. 2016). Recently, the application of environmental DNA or eDNA became popular in covering species identification in an ecosystem through NGS technologies (Drinkwater et al. 2020; Ruppert, Kline & Rahman 2019). eDNA is convenient since DNA is easily obtained from various sources such as the skin, mucous, saliva, sperm, secretions, eggs, feces, urine, blood, roots, leaves, fruit, pollen, and rotting bodies of larger organisms (Ruppert, Kline & Rahman 2019). While DNA is easily degraded due to several factors, eDNA allows a genetic material to be extracted when the species had an interaction with the environment by expelling its DNA to their surrounding (Thomsen & Willerslev 2015). Ficetola et al. (2008) were the first to demonstrate the usefulness of eDNA in detecting the presence of an aquatic vertebrate in freshwater. Over time, eDNA has been used as an alternative platform by most researchers in identifying invasive, rare, or endangered species, tracking biodiversity, detecting pathogens, and restoring of diets and ancient communities (Bohman et al. 2014).

However, compared to other countries, the application of environmental DNA and NGS technology in Malaysia is still new in wildlife monitoring. Acknowledging the advantages of these approach that could assist in various sectoral of wildlife monitoring and conservation, it is timely for the relevant authorities and stakeholders to consider as an alternative method. Yet, it is critical to review the intensity and application of these technologies in Malaysian wildlife monitoring context. The NGS technology in Malaysia is still considered new in wildlife monitoring. The gap of the technology can be seen as the study related to the NGS is still limited and need to revolutionize for rapid assessment due to the acceleration in habitat destruction especially in Malaysia. In this review, we intended by analyzing eDNA studies focusing on wildlife monitoring and addressed the most eDNA application used in Malaysia, which gave insight for future wildlife conservation. Thus, the outcome will be beneficial in viewing the advantages of NGS, which can help future researchers in providing aid in wildlife conservation and management.

Materials and Methods

In this study, a systematic review of scientific articles related to eDNA was conducted to collect relevant studies. Firstly, indexed published manuscripts of eDNA which focused on wildlife monitoring in Malaysia were collected based on title, abstract, and keywords using Scopus database and Google Scholar search in June 2021. Several search strings were used, with ‘Malaysia’ as the main keyword and combined with either of additional keywords such as ‘environmental DNA’, ‘eDNA’, ‘next-generation sequencing’, ‘NGS’, ‘metabarcoding’, ‘metagenome’, and ‘metagenomic’.

In addition, the keywords 'wildlife' and 'wildlife monitoring' are included in data mining to determine the acceptable papers that can be used in this review paper.

Our approach consists of four stages: searching and collecting available online indexed manuscript, selecting article for detail reading, identifying repetitive themes from selected articles, and synthesizing themes to form a conceptual framework of the next-generation wildlife monitoring using environmental DNA detection and NGS (Tawfik et al. 2019). Classification of articles was based on five categories: (i) species identification, (ii) diet assessment, (iii) health monitoring, (iv) resolved taxonomy, and (v) whole-genome sequencing. The articles were classified based on the objectives of the research, which can help to fill the gaps on wildlife monitoring in Malaysia.

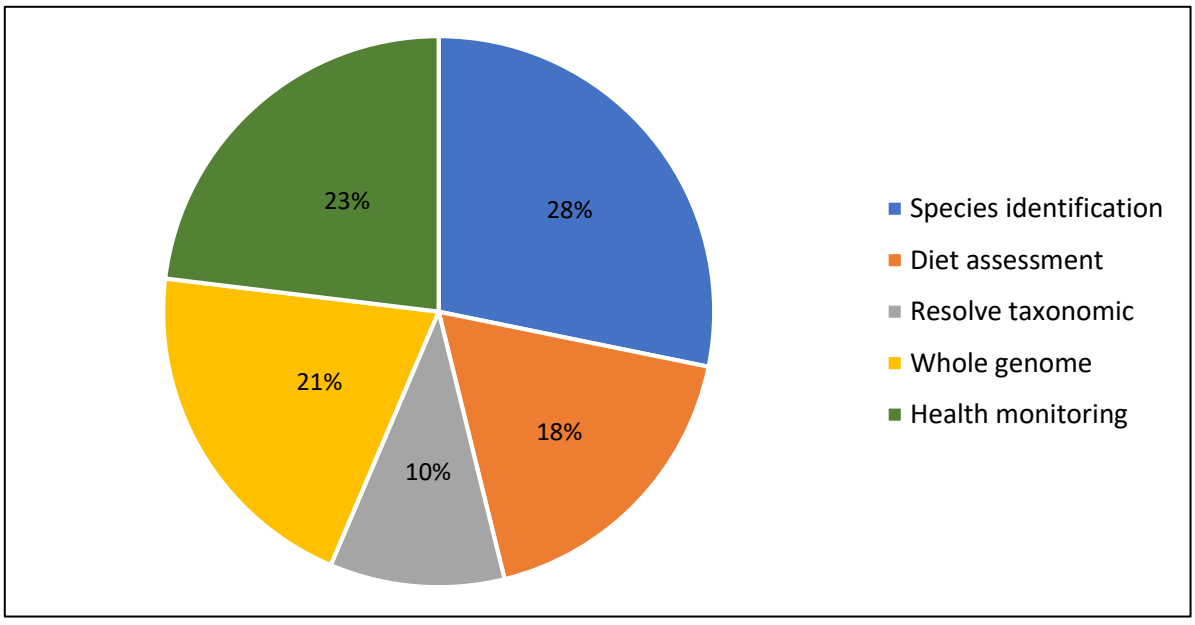
RESULTS

The findings based on articles from Scopus database and Google Scholar searches resulted into 40 non-repetitive articles associated with wildlife monitoring using environmental detection in Malaysia (Table 1). Of the 40 studies analyzed, species identification using technology NGS was the most frequent, accounting to 28%, followed by studies related with health issue of wildlife with 23% and whole-genome sequencing with 21% (Figure 1). An increasing trend of publications per year can be seen starting from 2015 to 2020. In the year 2021, as of June, six articles were successfully retrieved (Figure 2). The most attention using NGS technology based on searching articles is related to species identification of wildlife in Malaysia, which is represented by 11 articles, followed by health issue and whole-genome sequencing of wildlife. Table 2 presents the findings of eDNA studies using NGS.

TABLE 1. Summary on research articles assessed focusing on five main themes

Themes	Total articles
Species identification	11
Diet assessment	5
Health monitoring	10
Resolve taxonomic	4
Whole genome	10

TABLE 2. Summary of previously published research on wildlife monitoring by using eDNA approach conducted in Malaysia



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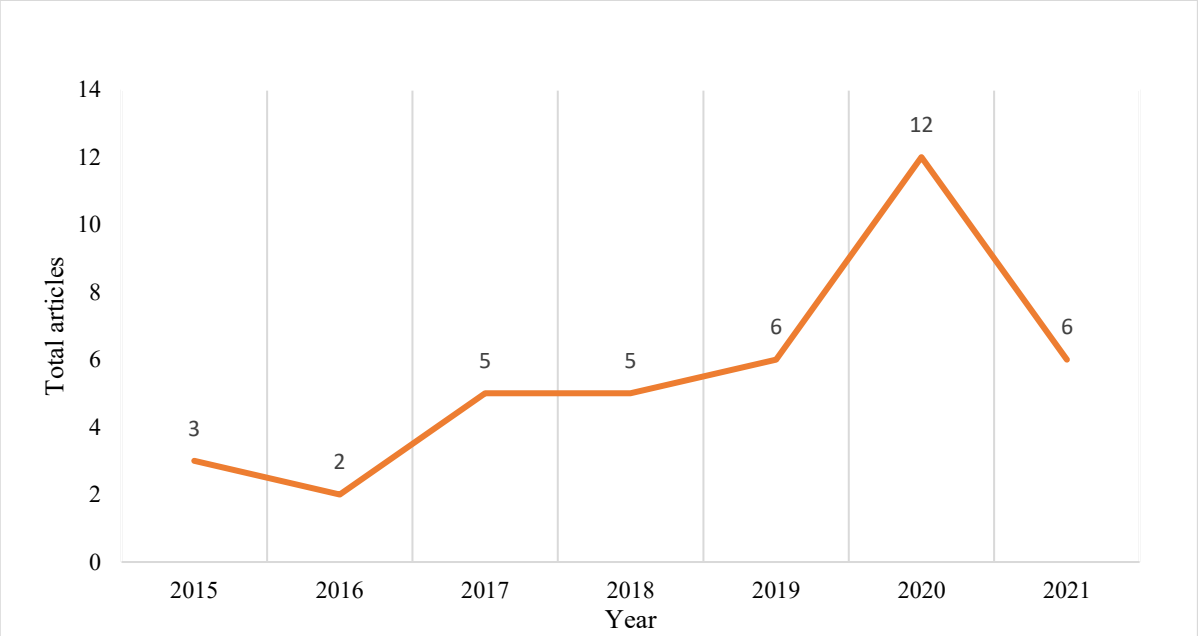

FIGURE 2. Total articles related to NGS technology used in wildlife monitoring in Malaysia

Journals	Source of DNA	Targeted wildlife	Platform	Locus	References
Species identification					
Leech blood-meal invertebrate-derived DNA reveals differences in Bornean mammal diversity across habitats	Invertebrate-derived DNA (iDNA) using blood-feeding leeches	Mammals in Malaysian Borneo	Illumina Miseq	16s rRNA	Drinkwater et al. (2020)
Tracking the southern river terrapin (*Batagur affinis*) through environmental DNA: prospects and challenges	Water	Reptiles in Malay Peninsular	Sanger sequencing	Cytb	Wilson et al. (2017)
Tropical-forest mammals as detected by environmental DNA at natural saltlicks in Borneo	Water	Mammals in Malaysian Borneo	Illumina Miseq	12S rRNA	Ishige et al. (2017)
Field calibration of blowfly-derived DNA against traditional methods for assessing mammal diversity in tropical forests	Blowfly derived DNA	Mammals in Malay Peninsular	Illumina Miseq	COI	Lee at al. (2016)
Diet assessment					

Diet composition of the wild stump-tailed macaque (*Macaca arctoides*) in Perlis State Park, Peninsular Malaysia, using a chloroplast trnL DNA metabarcoding approach: A preliminary study	Fecal	Mammals in Malay Peninsular	Illumina Miniseq	trnL	Osman et al. (2020)
Diet profiling of house-farm swiftlets (Aves, Apodidae, *Aerodramus* sp.) in three landscapes in Perak, Malaysia, using high-throughput sequencing	Fecal	Aves in Malay Peninsular	Illumina Miseq	COI	Chan, Tan and Goh (2019)
Assessing diet of the Rufous-Winged Philentoma (*Philentoma pyrhoptera*) in lowland tropical forest using next-generation sequencing	Fecal	Aves in Malay Peninsular	Illumina Miseq	COI	Mansor, Nor & Ramli (2018)
Elucidating the diet and foraging ecology of the island flying fox (*Pteropus hypomelanus*) in Peninsular Malaysia through Illumina next-generation sequencing	Fecal	Mammals in Malay Peninsular	Illumina Miseq	rbcL	Aziz et al. (2017)
Health monitoring					
Diversity, relative abundance, and functional genes of intestinal microbiota of Tiger Grouper (*Epinephelus fuscoguttatus*) and Asian Seabass (*Lates calcarifer*) reared in a semi-closed hatchery in dry and wet seasons	Gut	Fish in Malay Peninsular	Illumina Miseq	16S rRNA	Sutra et al. (2021)
Comparative study of gut microbiota in wild and captive Malaysian Mahseer (*Tor tambroides*)	Gut	Fish in Malay Peninsular	Illumina Miseq	16S rRNA	Tan & Tan (2021)
Microbiome analysis of Pacific white shrimp gut and rearing water from Malaysia and Vietnam: implications for aquaculture research and management	Water and gut	Arthropod in Malay Peninsular	Illumina Miseq	16S rRNA	Zoqratt et al. (2018)
Resolve taxonomic					

Phenotypic variation and polymorphism confirmed among white-bellied swiftlets of the Collocalia esculenta group (Apodidae, Collocaliini) by mitochondrial and nuclear DNA evidence	Feather	Aves in Malay Peninsular	Illumina Miseq	Cytb, ND2, Fib gene	Davies et al. (2020)
Deep genetic differentiation between two morphologically similar species of wolf herrings (Teleostei, Clupeoidei, Chirocentridae)	Tissue	Fish in Malay Peninsular	Illumina Hiseq	COI, 12S,16S	Lavoué et al. (2019)
Whole genome sequencing					
The first complete mitochondrial genome data of Hippocampus kuda originating from Malaysia	Tissue	Fish in Malay Peninsular	BGISEQ	-	Jahari et al. (2021)
The complete mitochondrial genome of Malayan Gaur (Bos gaurus hubbacki) from Peninsular Malaysia	Tissue	Mammals in Malay Peninsular	Illumina Miseq	-	Rosli et al. (2019)

Species Identification

Brandon-Mong et al. (2015) conducted the first study that applied NGS in species detection using Illumina MiSeq in order to identify species from arthropods. This study has proven the ability of NGS to identify the effectiveness of using eDNA samples as the genetic sources for species identification. Drinkwater et al. (2020, 2019) and Lee et al. (2016) successfully proven the effectiveness from subdisciplines of eDNA which is blowfly derived DNA and invertebrate derived DNA as the alternative monitoring of mammals. The study from Lee et al. (2016) also successfully proposed the new DNA mini-barcode from COI region that can be implemented for mammals from Peninsular Malaysia. Additionally, Ishige et al. (2017) applied eDNA metabarcoding approach to natural saltlicks in tropical forests and successfully detected endangered mammals such as Bornean orangutan (*Pongo pygmaues*) and Asian elephant (*Elephas maximus*). While Evans (2019) was the first to study the freshwater fish using eDNA approach in identifying the native, endemic and rare species which is crucial for fisheries, aquaculture, the ornamental trade and pest-control. Overall, the usage of eDNA samples is more convenient for species identification especially in accessing the wildlife that is difficult to observe directly.

Therefore, further study is needed to identify the wildlife in Malaysia since the country is one of the megadiverse countries. The systematic review also found that gaps still exist, especially in identifying invasive species which is critical in ecosystems of Malaysia. Furthermore, the ability of NGS to identify more than one species at a time can help in terms of time saving compared with using eDNA. In the future, the development of a primer can be increased together with the reference databases for wildlife in Malaysia to ensure that the action for wildlife conservation management can be done before species become extinct.

Health and zoological study

In Malaysia, fish is the most studied group of wildlife in the health aspect. One of the recent studies was done by Sutra et al. (2021), where they studied the intestinal

microbiota of tiger grouper (*Epinephelus fuscoguttatus*) and Asian sea bass (*Lates calcarifer*). Data from other studies conducted by Nurul et al. (2020) and Okomoda et al. (2020) also focused on microbiota composition on wild and captive bluestreak cleaner wrasse (*Labroides dimidiatus*). Although relatively low in number, studies on health and zoological related on mammals were conducted in Malaysia. For instance, study by Mohd-Yusof et al. (2022) reported a metagenomic analysis of gut microbiome from the island flying fox (*Pteropus hypomelanus*) as well as and noninvasive surveys of mammalian viruses using environmental DNA (Alfano et al. 2021).

Compared to the study of wildlife's health in other countries, Malaysia has less studies related to wildlife health. In Canada, a study has been done, where eDNA were used to detect high-risk freshwater fishes (Roy et al. 2018) and to monitor human and animal pathogens from aquatic sources (Farrel et al. 2021). Future research in Malaysia should focus on each group of animals and not to be biased on a certain group of animals, and the use of eDNA and NGS should be utilized more in Malaysia especially for research relating to wildlife animals so that more information about wildlife animals can be used by future researchers which can contribute in wildlife conservation and management.

Dietary assessment of wildlife

The application of eDNA or molecular approach in general in the diet analysis of wildlife offers an easier and practical alternative, especially for species living in remote and less accessible areas (Quéméré et al. 2013). Through bibliographical searches, only five related studies on diet using eDNA were successfully retrieved, published between 2017 and 2020. All of the studies focused on terrestrial vertebrate species, started with flying fox (*P. hypomelanus*) (Aziz et al. 2017), birds (Chan, Tan & Goh 2019; Mansor, Nor & Ramli 2018; Mansor et al. 2020), and the latest one was on primate focusing on *Macaca arctoides* (Osman et al. 2020). The targeted gene region, for instance, cytochrome oxidase I (COI), was largely used to investigate among insectivore avian species such as *Hirundo rustica*, *Philentoma pyrhoptera*, and *Aerodramus* sp. (Chan, Tan & Goh 2019; Mansor, Nor & Ramli 2018; Mansor et al. 2020). Hebert, Cywinska and Ball (2003) mentioned that COI is frequently used for arthropod detection, and in DNA metabarcoding and this gene be employed to further elucidate the insectivory feeding behaviour by primates (Hamad et al. 2014; Lunt et al. 1996).

On the other hand, for herbivore and omnivore mammals, an intron region of a chloroplast tRNA gene (trnL) and ribulose bisphosphate carboxylase large chain precursor (rbcL) were frequently used in studies conducted in Malaysia (Aziz et al. 2017; Osman et al. 2020). Furthermore, trnL intron is known to be one of the most common molecular markers used for diet analysis, which include various kinds of floral such as wetland plants (Quandt & Stech 2005). High usage of trnL is mainly due to its property of having large sequence divergence for various taxonomic levels (Mallot, Garver & Malhi 2018). Nevertheless, these common markers are prone to restrictions, where plants were classified only to the genus level mainly due to ambiguity which could lead to misidentification (Bradley et al. 2007; Hibert et al. 2013; Quéméré et al. 2013; Srivathsan et al. 2016; Valentini et al. 2009).

After interpreting the objectives of the selected studies carried out in Malaysia through eDNA approach, specifically on their dietary study, it can be summarized that generally the studies present the first dietary information through molecular approach to complement the previous dietary studies. Most of the previous studies relied on morphological identification and remnants obtained from the faucal samples to identify the diet of a particular species (Chan, Tan & Goh 2019). Especially among avian species, it would even be harder to observe and detect insect species in their diet since they practiced aerial snatching to catch and eat insects (Chan, Tan & Goh 2019; Mansor, Nor & Ramli 2018). Due to the incomplete taxonomic level data of their diet (particularly on insects), some information may be missed when soft-bodied insects were fully broken down (Chan, Tan & Goh 2019; Mansor, Nor & Ramli 2018). Meanwhile, for volant mammals such as flying fox, a similar hindrance was observed using the traditional method (either capturing the individuals or by collecting the faucal samples), where physical identification of its diets relied heavily on experts, availability of reference collection, as well as expensive equipment (Aziz et al. 2017).

Resolving taxonomic

Through bibliographical searches, only four related studies used NGS technologies in phylogenetic relationship which successfully resolved the taxonomy of wildlife groups published between 2019 and 2021. The study from Lavoué et al. (2019) and Lim et al. (2021) used a few markers of mtDNA to examine genetic differentiation by reconstructing phylogenetic relation in fish group. Chan, Tan and Goh (2020) and Davies et al. (2020) focused on Aves group to compare the taxonomy

using the Illumina platform. Therefore, previous studies showed that the application of wildlife monitoring using NGS in Malaysia can be widely used to resolve other taxonomies since NGS has the ability to individually sequence each while polymerase chain reaction (PCR) amplicon at a certain depth even from small amount of DNA in a short time (Tillmar et al. 2013).

Whole-genome sequencing

Whole-genome sequencing is one of the studies used NGS technologies since the platform provides an effective development for whole mitochondrial genome and gives insight into population processes and the evolutionary history of species (Castañeda-Rico et al. 2020). Through bibliographical searches, ten studies used NGS to sequence the complete genome of wildlife in Malaysia from 2015 to 2021. The study by Liedigk et al. (2015) focused on complete mitochondrial genome of long-tailed macaque (*Macaca fascicularis fascicularis*). A few studies developed a phylogenetic tree in order to identify the differences between groups of wildlife, which can result to genetic variability, as shown by the study of Jahari et al. (2021) on fruit bat, Rosli et al. (2019) on Malaya gaur, and Chung et al. (2020) on small ray-finned fish.

DISCUSSION

TECHNOLOGIES IN DNA SEQUENCING

A powerful technique is required to amplify eDNA and to ensure the molecular analysis can run successfully. PCR and subsequent DNA sequencing are the most approachable methods that have been used in molecular research (Thomsen & Willerslev 2015). Besides, PCR is the enzymatic process that can amplify the specific region of DNA by repeated replication to yield several million copies of a particular sequence (Taberlet, Waits & Luikart 1999). Even when low quantity of DNA was used, a repetition of up to 50 cycles by PCR can amplify DNA in a shorter time compared to other amplification methods for targeting DNA molecules (Lalam 2006). The probability of correct genotype is almost at 99% confidence level (Taberlet, Waits & Luikart 1999). Usually, researchers or scientists use a nanogram to microgram of genomic DNA as a template to run PCR, which corresponds to around 300-300,000 copies of unique sequence. Even though PCR can amplify DNA in a short time, the process is extremely sensitive, where high risk of contamination might occur between the extracts or PCR products and may carry over, thus producing false alleles (Taberlet, Waits & Luikart 1999).

Thus, the amplification of eDNA can be done with single-species approach using specific primers by qPCR or multiple species (multiple taxon) approach using generic primers for a given focal group of organisms (Thomsen & Willerslev 2015). These two approaches can certainly overcome the traditional DNA sequencing method by Sanger, Nicklen and Coulson (1977), which only sequences specimens individually and is insufficient to be used for environmental samples, especially in large-scale studies. Although conventional DNA has provided the most efficient method for the development of large DNA barcode reference, it still does not have the ability to read DNA of thousands of samples in a time. Therefore, DNA metabarcoding was introduced with a low costing to be used for environmental study.

In order to amplify DNA, qPCR or real-time PCR (RT-PCR) has become a powerful molecular tool especially for quantifying organism that is directly extracted from the environment. According to Prévost-Bouré et al. (2011), qPCR is an accurate and culture-independent assay which can differentiate taxonomic levels by targeting different regions in the genome. Smith and Osborn (2009) successfully measured the total bacterial abundance involved in the nitrogen cycle in soils using qPCR. Therefore, qPCR will only be compatible with these two approaches where an internal competitor for each target sequence is used for normalization or quantitative comparative PCR using a normalization gene contained within the sample (Heid et al. 1996). With agarose gel, qPCR is geared accurately in quantifying exponential amplification of DNA compared with traditional PCR (Zhong et al. 2011). For soil fungus studies, the specificity of the primer set is needed to encounter the limitation related with length of targeted region for reproduction and accuracy in ecological studies (Lueders et al. 2004).

Furthermore, several researchers used qPCR in eDNA as a quantitative monitoring tool for the efficiency of conservation within a management framework, especially for a large natural environment area (Lacoursière-Roussel et al. 2016). For instance, Lacoursière-Roussel et al. (2016) used species-specific primer in qPCR in the detection and quantification of wood turtle populations in natural habitats. The comparison between single-species and multispecies approaches showed that qPCR can detect the presence of wood turtle in five rivers, while the eDNA metabarcoding

approach can detect the presence of wood turtle in only one out of five rivers. Thus, qPCR is more sensitive because it can amplify shorter DNA fragments which is 71 bp DNA sequence, while eDNA metabarcoding analyses have 167 bp sequence (Lacoursière-Roussel et al. 2016). Moreover, qPCR can detect more highly degraded organic matter which can improve its ability (Barnes et al. 2014) especially for species-specific studies.

On the other hand, becoming a widespread molecular biology technique, multiplex PCR is used for amplification of multiple targets in a single PCR research. According to Edwards and Gibbs (1994), multiplex PCR can be a two-amplicon system or can amplify 13 or more separate regions of DNA. Therefore, the design of primers is very crucial to ensure its specificity, as well as the melting temperature of primers for routine PCR. Primers with similar annealing temperatures are usually chosen for conventional multiplex PCR. Furthermore, DNA-based assays to amplify multiple species and gene-specific products can be developed in a single reaction tube called multiplex PCR. In addition, multiplex PCR can be performed instead of conventional PCR as it is more applicable to real-time technology wherein the actual quantity of each target DNA is measured by the fluorescence intensity of gene-specific probes known as molecular beacons (Manning & La Claire II 2010).

Before NGS was introduced, Sanger sequencing is used for DNA sequencing and can recover up to 1 kb of sequence data from a single specimen at a time. Over time, high-throughput sequencing devices have been introduced based on different chemistry and detection techniques. As for now, fast advancing technology such as NGS is used as the platform to recover DNA sequence data directly from environmental samples. Thus, DNA metabarcoding can be done in this platform. According to Shokralla et al. (2012), NGS can make biodiversity surveys possible for limited cost and minimal efforts. With thousands of samples, NGS can compare the obtained sequences to a growing standard reference library of known organisms, and taxa present in an environmental sample can be identified with high confidence. Furthermore, time and space by annotating and clustering DNA sequences using a combination of assignment and phylogenetic technique can be determined with advanced computational methods (Hajibabaei et al. 2011). When running PCR for NGS, the primer can be tagged with short nucleotide sequences to uniquely identify their source in a process commonly referred to as multiplexing. Figure 3 shows the framework from detection of eDNA and flow to amplify DNA for species identification. However, these tags can have potential bias results particularly when located on the 5' end, so they also require rigorous testing before implementation (Binladen et al. 2007).

PLATFORMS IN NEXT-GENERATION SEQUENCING (NGS) TECHNOLOGY

The revolution of NGS technology can be classified into two main categories: (i) PCR-based technologies that have four main commercially platforms, i.e., Roche 454 Genome Sequencer (Roche Diagnostics Corp., Branford, CT, USA), HiSeq 2000 (Illumina Inc., San Diego, CA, USA), AB SOLiD™ System (Life Technologies Corp., Carlsbad, CA, USA), and Ion Personal (Life Technologies, South San Francisco, CA, USA), and (ii) non-PCR-based technologies that do not include amplification step prior to sequencing called 'single molecule' sequencing (SMS) technologies (Shokralla et al. 2012). In order to use NGS, the right platform is needed due to rapid advancement which can give difficulties for users to use for their ecological purposes. Although the sequencing step has several platforms available, Illumina sequencing currently outperforms other NGS platforms in terms of depth and accuracy (Deiner et al. 2017). In addition, Illumina sequencing that has been introduced since 2007 also has high capacity and become a workhorse for whole-genome resequencing application (Shokralla et al. 2012). Moreover, the major advantage of Illumina and SOLiD platforms is that both can perform nucleotide detection in a time. Thus, it is important to note that diversity has been found to increase with sequencing depth before reaching a plateau, so theoretically a maximum sequencing depth could be determined for individual studies (Alberdi et al. 2018).

Although NGS can read several thousand sequences, the disadvantage of bias can still happen when conducting the amplification. The PCR bias in the amplification step was identified before the invention of NGS (Schloss, Gevers & Westcott 2011) and it can be reduced in controlling annealing temperature of any primer set (Shokralla et al. 2012). Previously, Ishii and Fukui (2001) were able to lower the temperature when specific amplification is achieved to control PCR bias. Moreover, PCR bias can be reduced by keeping the number of replication low (Suzuki & Giovannoni 1996) and setting the fastest ramping rate from the denaturation step to the annealing step using PCR cyclers, which may even increase heteroduplex formation when PCR reaches the plateau phase (Kurata et al. 2004). Thus,

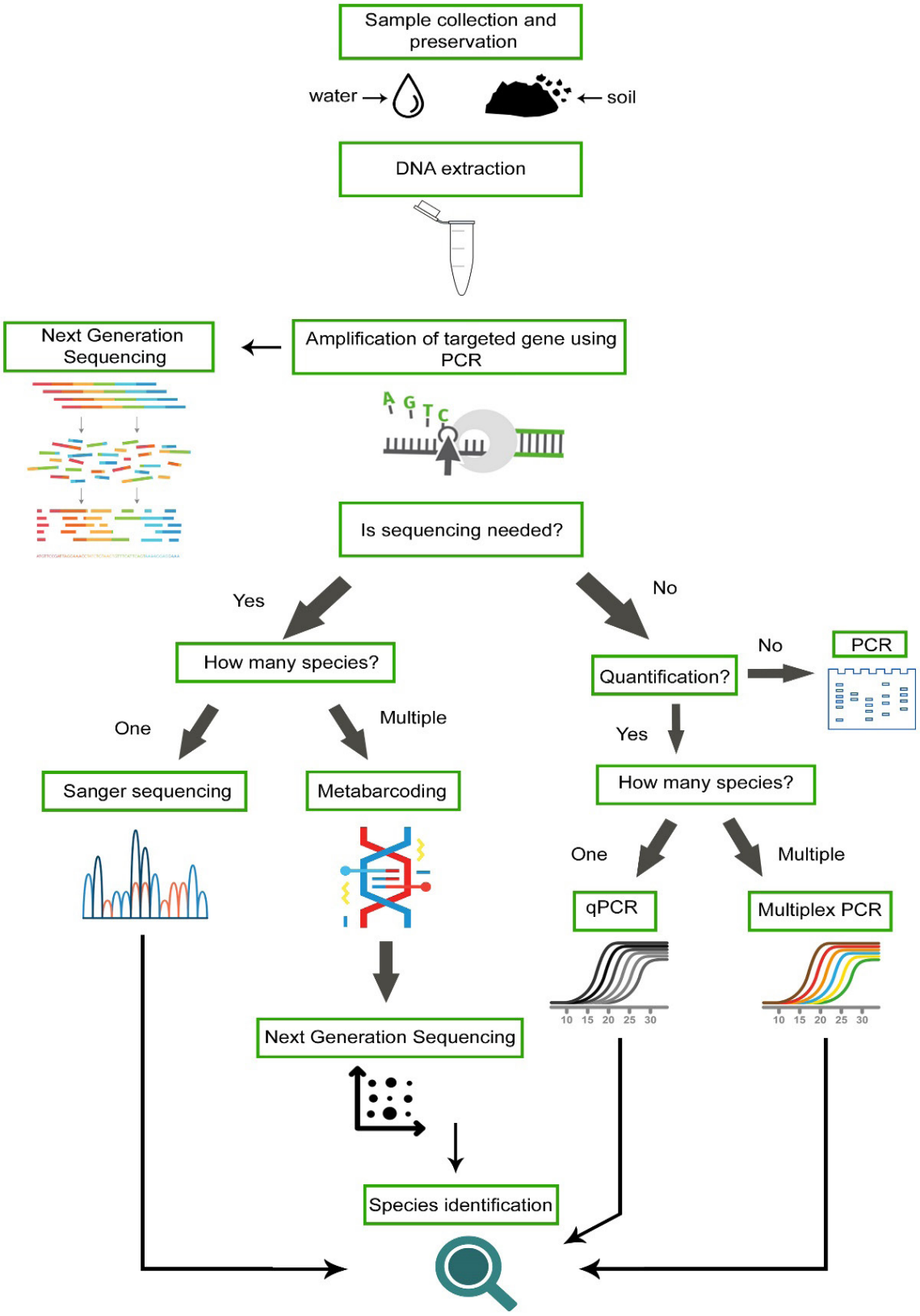

FIGURE 3. The framework of how eDNA can be amplified for species identification depending on the study

many studies suggested that wise primer selection, high template concentrations, and mixed replicate reaction preparations also help many studies reported to reduce PCR bias.

The 454-genome sequencer was introduced in 2005 and became the first NGS technology. The read lengths of 454 sequencer are longer, i.e., 400 bases, compared to Illumina, which can only be 36 bases. Using real-time sequencing-by-synthesis pyrosequencing technology means that each nucleotide incorporated by DNA polymerase results in the release of a pyrophosphate molecule (Shokralla et al. 2012). Using 454 sequencers will initiate a series of downstream reactions to produce light due to the action of enzyme luciferase, luciferin, and ATP sulfurylase. The light produced by the luciferase reaction, which is called chemiluminescent event, will be detected and measured by an avalanche photodiode, photomultiplier tube, or with charge-coupled device camera (Ambardar et al. 2016). However, 454 genome is being phased out now due to its high cost compared with other high-throughput sequencing technologies.

Illumina sequencer was introduced in 2007 after 454 genome sequencer has high capacity in genomic projects. The Illumina sequencer has three versions of sequencers available for commercialization, i.e., HiSeq 2000, HiSeq 1000, and Genome Analyzer IIx, with sequencing outputs of up to 600, 300, and 95 Gb. The platform utilizes a sequencing-by-synthesis approach whereby DNA fragments will attach to the surface of the reaction chamber by bridge PCR followed by sequencing by reversible termination using reversible terminator (RT) nucleotide (Ambardar et al. 2016). There are eight lanes of flow cell for each cluster as a result of performing multiple cycles of sequencing chemistry (Shokralla et al. 2012).

Applied Biosystems Solid Sequencer (SOLiD) sequencer is different with Illumina and 454-genome sequencers, and it was commercialized in 2006 by Life Technologies. SOLiD uses a sequencing-by-oligo technology because the platform does not utilize DNA polymerase to incorporate nucleotides. The process involving immobilized complementary oligos on the surface of 1-mm magnetic beads and the beads will amplify through emulsion PCR, and a universal sequencing primer is complementary to start the ligation-based process that starts to a library frame (Shokralla et al. 2012). However, SOLiD can lead to a very short sequencing read.

In 2010, Life Technologies introduced Ion Torrent as a post light sequencing technology. Ion Torrent relies on real-time detection of hydrogen ion concentration and releases it as byproducts to incorporate nucleotide into a DNA strand through the polymerase action (Shokralla et al. 2012). The realization of hydrogen ion is to detect cluster located above a semiconductor transistor which can detect the changes of pH in a solution. Thus, hydrogen ion is detected by the semiconductor which also has similarity with pyrosequencing. Referring to the previous studies and reviews regarding sequencing technologies and their mechanism, Table 3 presents a summary of each NGS platform with their characteristics as shown by Kchouk, Gibrat and Elloumi (2017) with some modifications and additions.

RECOMMENDATIONS OF WILDLIFE MONITORING FOR MALAYSIA

Although eDNA is already proven to be a promising approach for the study of ecosystem, it still has several glitches that need to be considered. According to Ruppert, Kline and Rahman (2019), eDNA in the environment can be degraded, which can limit the scope of studies. Habitually, only small segments of eDNA can sustain especially in warm or tropical regions. Furthermore, the biggest challenge in eDNA study is the occurrence of contamination. The risk of contamination can possibly cause false result (Thomsen & Willerslev 2015). The contamination may occur during the sampling session or in the lab. Billions of DNA copies can be generated because of frequent use of PCR in eDNA. Thus, a strict clean-lab protocol using decontamination procedures and physical separation of labs for pre- and post-PCR work will significantly limit the risk of contamination (Willerslev & Cooper 2005). In the future, research in Malaysia is recommended to develop a proper framework of eDNA fieldwork in order to increase DNA concentration.

Besides that, humic acids or humic substances coextracted with DNA can strongly inhibit enzyme such as Taq polymerase to sue in PCR reactions to amplify DNA (Matheson et al. 2010). This might lead to false-negative results, especially for the study using soil samples and water samples contaminated with sediment particles (McKee, Spear & Pierson 2015). The identification of DNA sequences depends on the reliability of the reference DNA sequence database (Thomsen & Willerslerv 2015). COI is mostly used for invertebrates and fish, while other rRNA markers (12S, 16S, and 18S) are also used across other taxa in eDNA study (Deagle et al. 2014; Othman et al. 2021). Therefore, future DNA reference database may focus on other locus of mtDNA

TABLE 3. Summarisation of NGS platforms by Kchouk, Gibrat and Elloumi (2017) with some modifications

Platforms	Year	Approach type	Read type	Reads per run	Error type	Error rate (%)	Notes
Roche 454 Genome Sequencer	2005	Sequencing-by-synthesis	SE, PE	100-100M	Indel	1%	• Give the longest reads for *de novo* sequencing (Ergüner, Üstek & Sağıroğlu 2015) • High cost • High error rate in homopolymer region (Luo et al. 2012)
Applied Biosystems Solid Sequencer	2006	Sequencing-by-oligo	SE	3B-6B	Mismatch	-0.1%	• Lead to very short sequencing reads (Ergüner, Üstek & Sağıroğlu 2015)
Illumina Sequencer	2007	Sequencing-by-synthesis	SE, PE	25M-6B	Mismatch	MiniSeq- 1% MiSeq- 0.1% HiSeq- 0.1% HiSeq X- 0.1% NextSeq- 1%	• HiSeq provide highest throughput than IonTorrent and GS FLX+ Roche (Ergüner, Üstek & Sağıroğlu 2015) • High accuracy (Ergüner, Üstek & Sağıroğlu 2015) • Short read length for *de novo* sequencing (Ergüner, Üstek & Sağıroğlu 2015)
Ion Torrent	2010	Detection of hydrogen ion concentration	SE	400,000-80M	Indel	1%	• Fastest among HiSeq and GS FLX+ Roche (Ergüner, Üstek & Sağıroğlu 2015)

or even nuclear genomes for much wider applications in the country. Establishmentof a comprehensive database on DNA references for wildlife in Malaysia is vital in identifying the generated output or sequences of DNA up to species level. Difference in biogeographical of flora and fauna in Malaysia leads to variation in genetic structure and by developing our own reference database, we could increase the accuracy and precision in species classification. Despite that, the data gained through all of these researches will definitely complement and may validate the existing data especially at lower taxonomic level (genus and species) (Aziz et al. 2017; Chan, Tan & Goh 2019; Mansor, Nor & Ramli 2018; Pompanon et al. 2012). The comprehensive records may also provide better information and understanding of the species that may eventually become an indicator of the ecosystem as well as for their conservation management plan (Aziz et al. 2017; Osman et al. 2020). For example, the study by Chan, Tan and Goh (2019) may provide information on extensive freshwater bodies in the urban landscape through list of insects consume by the house farm swiftlets.

Although DNA metabarcoding is powerful, it still has disadvantages in terms of primers used. The problem of the primer will lead to bias results for

certain sequences (species), making it less efficient than others during amplification (Deagle et al. 2014). The unavailability of universal primer for amplification and direct sequencing of targeted region or whole genome resulted in longer time to produce a complete PCR product. The sequences able to amplify at a wide variety of DNA templates and maximize the taxonomic coverage of a primer set to ensure all potential target species are amplified (Liu et al. 2021). Thus, to increase the PCR efficiency, a specific blocking oligonucleotide is needed to improve the sensitivity of species detection as suggested by Liu et al. (2021). According to Othman et al. (2021), COI marker is ideally suited to be utilized as universal marker in metabarcoding to detect targeted species as it is widely used in eDNA metabarcoding. By having the extensive local reference database, it is useful to design universal primer especially for wildlife in Malaysia (Thomsen & Willerslev 2015).

Besides that, most NGS technologies are well established in developed countries, but the usage is limited in developing countries like Malaysia. According to Helmy et al. (2016), developing countries have limited funding, lacked in skilled personal, and have limited access to tools in genomic database. Researchers in Malaysia seek for service providers in both local and foreign companies to access the NGS technologies. Only few local companies offer NGS services, and most of the platforms used are Illumina (MiSeq and HiSeq). However, with vast exposure to the advantages of NGS technologies, the trend of research in Malaysia is expected to improve. The application of NGS in Malaysia is anticipated to progress steadily in the coming years, especially in biodiversity monitoring. With the frequent reduction in research funding, NGS technologies will surely assist researchers in biodiversity monitoring. With a concrete references database, it is expected that NGS technologies in wildlife monitoring could be practical and effective in observing the changes at the species, population and ecosystem levels.

The findings from the NGS study can be helpful for stakeholders in Malaysia, especially the Department of Wildlife and National Park (DWNP), Forestry Department, and other agencies in implementing the conservation plan through the genetic information. The investigation using the metabarcoding approach can increase the understanding of the complex ecosystem in Malaysia, which is helpful to use in facing the amidst of declining biodiversity problems.

Conclusions

eDNA studies in Malaysia show increasing trends of publication starting from 2015 where the highest studies focus on species identification using NGS. However, more findings are still needed to be applied in Malaysia for researchers in the future to use eDNA samples and framework confidently to discover and monitor wildlife and save them from extinction. We conclude that wildlife monitoring using NGS technologies can be implemented for rapid assessment of biological diversity to be used in management and conservation action plan to ensure we are on track with the advancement of technologies.

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References

Alberdi, A., Aizpurua, O., Gilbert, M.T.P. & Bohmann, K. 2018. Scrutinizing key steps for reliable metabarcoding of environmental samples. *Meth Ecology and Evolution* 9: 134-147.

Alfano, N., Dayaram, A., Axtner, J., Tsangaras, K., Kampmann, M-L., Mohamed, A., Wong, S.T., Gilbert, M.T.P., Wilting, A. & Greenwood, A.D. 2021. Non-invasive surveys of mammalian viruses using environmental DNA. *Methods in Ecology and Evolution* 12: 1941-1952.

Ambardar, S., Gupta, R., Trakroo, D., Lal, R. & Vakhlu, J. 2016. High throughput sequencing: An overview of sequencing chemistry. *Indian Journal Microbiology* 56(4): 394-404.

Aziz, S.A., Clements, G.R., Peng, L.Y., Campos-Arceiz, A., McConkey, K.R., Forget, P. & Gan, H.M. 2017. Elucidating the diet of the island flying fox (*Pteropus hypomelanus*) in Peninsular Malaysia through illumina next-generation sequencing. *PeerJ* 5: e3176.

Barnes, M.A., Turner, C.R., Jerde, C.L., Renshaw, M.A., Chadderton, W.L. & Lodge, D.M. 2014. Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science and Technology* 48: 1819-1827.

Binladen, J., Gilbert, M.T.P., Bollback, J.P., Panitz, F., Bendixen, C., Nielsen, R. & Willerslev, E. 2007. The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing. *PLoS ONE* 2: 197.

Bohmann, K., Evans, A., Gilbert, M.T., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W. & de Bruyn, M. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution* 29(6): 358-367.

Bradley, B.J., Stiller, M., Doran-Sheehy, D.M., Harris, T., Chapman, C.A., Vigilant, L. & Pionar, H. 2007. Plant DNA sequences from feces: Potential means for assessing diets of wild primates. *American Journal of Primatology* 69: 1-7.

Brandon-Mong, G.J., Gan, H.M., Sing, K.W., Lee, P.S., Lim, P.E. & Wilson, J.J. 2015. DNA metabarcoding of insects and allies: An evaluation of primers and pipelines. *Bulletin of Entomological Research* 105(6): 717-727.

Burton, A.C., Neilson, E., Moreira, D., Ladle, A., Steenweg, R., Fisher, J.T., Bayne, E. & Boutin, S. 2015. Wildlife camera trapping: a review and recommendations for linking surveys to ecological processes. *Journal of Applied Ecology* 52: 675-685.

Castañeda-Rico, S., León-Paniagua, L., Edwards, C.W. & Maldonado, J.E. 2020. Ancient DNA from museum specimens and next generation sequencing help resolve the controversial evolutionary history of the critically endangered puebla deer mouse. *Frontiers in Ecology and Evolution* 8: 94.

Chan, K.S., Tan, J. & Goh, W.L. 2019. Diet profiling of house-farm swiftlets (*Aves, Apodidae, Aerodramus* sp.) in three landscapes in Perak, Malaysia, using high-throughput sequencing. *Tropical Ecology* 60(3): 379-388.

Chan, K.S., Tan, J. & Goh, W.L. 2020. Taxonomic assignment of dietary arthropods in Malaysian swiftlets (*Aerodramus* sp.) based on DNA metabarcoding. *Journal of Wildlife and National Parks* 35: 73-91.

Chung, H.H., Kamar, C.K.A., Lim, L.W.K., Liao, Y., Lam, T.T. & Chong, Y.L. 2020. Sequencing and characterization of complete mitogenome DNA for *Rasbora hobelmani* (*Cyprinidae*) with phylogenetic consideration. *Journal of Ichthyology* 60(1): 90-98.

Davies, S.E., Goh, W.L., Ball, S.P., Siew, W.S. & Tarburton, M. 2020. Phenotypic variation and polymorphism confirmed among white-bellied swiftlets of the *Collocalia esculenta* group (Apodidae, Collocaliini) by mitochondrial and nuclear DNA evidence. *Bulletin of the British Ornithologists' Club* 140(4): 373-386.

Deagle, B.E., Jarman, S.N., Coissac, E. & Taberlet, P. 2014. DNA metabarcoding and the cytochrome c oxidase subunit I marker: Not a perfect match. *Biology Letters* 10: 20140562.

Deiner, K., Bik, H.M., Elvira, M., Seymour, M., Creer, S., Bista, I., Lodge, D.M., de-Vere, N., Pfrender, M.E. & Bernatchez, L. 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology* 26: 5872-5895.

Drinkwater, R., Schnell, I.B., Bohmann, K., Bernard, H., Veron, G., Clare, E., Gilbert, M.T.P. & Rossiter, S.J. 2019. Using metabarcoding to compare the suitability of two blood-feeding leech species for sampling mammalian diversity in North Borneo. *Molecular Ecology Resources* 19(1): 105-117.

Drinkwater, R., Jucker, T., Potter, J.H., Swinfield, T., Coomes, D.A., Slade, E.M., Gilbert, M.T.P., Lewis, O.T., Bernard, H., Struebig, M.J. & Clare, E.L. 2020. Leech blood-meal invertebrate-derived DNA reveals differences in Bornean mammal diversity across habitats. *Molecular Ecology* 30(13): 3299-3312.

Edwards, M.C. & Gibbs, R.A. 1994. Multiplex PCR: Advantages, development and applications. *Genome Research* 3: 65-75.

Ergüner, B., Üstek, D. & Sağıroğlu, M.S. 2015. Performance comparison of next generation sequencing platforms. *37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*. pp. 6453-6456.

Evans, A.R. 2019. Exploring the biodiversity of the lakes of the Malay Archipelago using environmental DNA metabarcoding. PhD Dissertation (Unpublished).

Farrell, J.A., Whitmore, L. & Duffy, D.J. 2021. The promise and pitfalls of environmental DNA and RNA approaches for the monitoring of human and animal pathogens from aquatic sources. *Bioscience* 71(6): 609-625.

Ficetola, G.F., Miaud, C., Pompanon, F. & Taberlet, P. 2008. Species detection using environmental DNA from water samples. *Biology Letters* 4(4): 423-425.

Forestry Department Peninsular Malaysia (FDPM). 2020. https://www.forestry.gov.my/en/2016-06-07-02-53-46/2016-06-07-03-12-29

Hamad, I., Delaporte, E., Raoult, D. & Bittar, F. 2014. Detection of termites and other insects consumed by African Great Apes using molecular fecal analysis. *Scientific Reports* 4: 4478.

Hebert, P.D., Cywinska, A. & Ball, S.L. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270: 313-321.

Heid, C.A., Stevens, J., Livak, K.J. & Williams, P. 1996. Real time quantitative PCR. *Genome Research* 6: 986-994.

Helmy, M., Awad, M. & Mosa, K.A. 2016. Limited resources of genome sequencing in developing countries: Challenges and solutions. *Applied & Translational Genomics* 9: 15-19.

Hibert, F., Taberlet, P., Chave, J., Scotti-Saintagne, C., Sabatier, D. & Richard-Hansen, C. 2013. Unveiling the diet of elusive rainforest herbivores in next generation sequencing era? The tapir as a case study. *PLoS ONE* 8(4): e60799.

Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G.A.C. & Baird, D.J. 2011. Environmental barcoding: A next-generation sequencing approach for biomonitoring applications using river benthos. *PLoS ONE* 6: e17497.

Ishige, T., Miya, M., Ushio, M., Sado, T., Ushioda, M., Maebashi, K., Yonechi, R., Lagan, P. & Matsubayashi, H. 2017. Tropical-forest mammals as detected by environmental DNA at natural saltlicks in Borneo. *Biological Conservation* 210: 281-285.

Ishii, K. & Fukui, M. 2001. Optimization of annealing temperature to reduce bias caused by a primer mismatch in multitemplate PCR. *Applied and Environmental Microbiology* 67(8): 3753-3755.

Jahari, P.N.S., Mohd Azman, S., Munian, K., Zakaria, N.A., Omar, M.S.S., Richter, S.R. & Mohd Salleh, F. 2021. The first mitochondrial genome data of an old-world fruit bat, *Cynopterus sphinx* from Malaysia. *Mitochondrial DNA Part B* 6(1): 53-55.

Kchouk, M., Gibrat, J. & Elloumi, M. 2017. Generations of sequencing technologies: From first to next generation. *Biology and Medicine* 9(3): 1000395.

Kurata, S., Kanagawa, T., Magariyama, Y., Takatsu, K., Yamada, K., Yokomaku, T. & Kamagata, Y. 2004. Re-evaluation and reduction of a PCR bias caused by reannealing of templates. *Applied and Environmental Microbiology* 70: 7545-7549.

Lacoursière-Roussel, A., Dubois, Y., Normandeau, E. & Bernatchez, L. 2016. Improving herpetological surveys in eastern North America using the environmental DNA method. *Genome* 59: 991-1007.

Lalam, M. 2006. Estimation of the reaction efficiency in polymerase chain reaction. *Journal of Theoretical Biology* 242: 947-953.

Lavoué, S., Bertrand, J.A., Shen, K.N., Ratmuangkhwang, S., Sado, T., Miya, M. & Siti Azizah, M.N. 2019. Deep genetic differentiation between two morphologically similar species of wolf herrings (Teleostei, Clupeoidei, Chirocentridae). *Journal of Applied Ichthyology* 35(3): 693-700.

Lee, P.S., Gan, H.M., Clements, G.R. & Wilson, J.J. 2016. Field calibration of blowfly-derived DNA against traditional methods for assessing mammal diversity in tropical forests. *Genome* 59(11): 1008-1022.

Liedigk, R., Kolleck, J., Böker, K.O., Meijaard, E., Md-Zain, B.M., Abdul-Latiff, M.A.B., Ampeng, A., Lakim, M., Abdul-Patah, P., Tosi, A.J. & Brameier, M. 2015. Mitogenomic phylogeny of the common long-tailed macaque (*Macaca fascicularis fascicularis*). *BMC Genomics* 16(1): 1-11.

Lim, L.W.K., Chung, H.H., Lau, M.M.L., Aziz, F. & Gan, H.M. 2021. Improving the phylogenetic resolution of Malaysian and Javan mahseer (Cyprinidae), *Tor tambroides* and *Tor tambra*: Whole mitogenomes sequencing, phylogeny and potential mitogenome markers. *Gene* 791: 145708.

Liu, G., Zhang, S., Zhao, X., Li, C. & Gong, M. 2021. Advances and limitations of next generation sequencing in animal diet analysis. *Genes* 12(12): 1854.

Lueders, T., Wagner, B., Claus, P. & Friedrich, M.W. 2004. Stable isotope probing of rRNA and DNA reveals a dynamic methylotroph community and trophic interactions with fungi and protozoa in oxic rice field soil. *Environmental Microbiology* 6: 60-72.

Lunt, D.H., Zhang, D.X., Szymura, J.M. & Hewitt, G.M. 1996. The insect cytochrome oxidase I gene: Evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology* 5(3): 153-165.

Luo, C., Tsementzi, D., Kyrpides, N., Read, T. & Konstantinidis, K.T. 2012. Direct comparisons of Illumina vs. Roche 454 sequencing technologies on the same microbial community DNA sample. *PLoS ONE* 7(2): e30087.

Mace, G.M. 2004. The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359(1444): 711-719.

Mallot, E.K., Garver, P.A. & Malhi, R.S. 2018. *trnL* outperforms *rbcL* as a DNA metabarcoding marker when compared with the observed plant component of the diet of wild white-faces capuchins (*Cebus capucinus*, Primates). *PLoS ONE* 13(6): e0199556.

Manning, S.R. & La Claire II., J.W. 2010. Multiplex PCR methods for the species -specific detection and quantification of *Prymnesium parvum* (Haptophyta). *Journal of Applied Phycology* 22: 587-597.

Mansor, M.S., Halim, M.R.A., Abdullah, N.A., Ramli, R. & Cranbrook, E. 2020. Barn Swallows *Hirundo rustica* in Peninsular Malaysia: Urban winter roost counts after 50 years, and dietary segregation from house-farmed swiftlets *Aerodramus* sp. *Raffles Bulletin of Zoology* 68: 238-248.

Mansor, M.S., Nor, S.M. & Ramli, R. 2018. Assessing diet of the Rufous-winged Philentoma (*Philentoma pyrhoptera*) in lowland tropical forest using next-generation sequencing. *Sains Malaysiana* 47(5): 1045-1050.

Matheson, C.D., Gurney, C., Esau, N. & Letho, R. 2010. Assessing PCR inhibition from humic substances. *The Open Enzyme Inhibition Journal* 3: 38-45.

Mckee, A.M., Spear, S.F. & Pierson, T.W. 2015. The effect of dilution and the use of a post-extraction nucleic acid purification column on the accuracy, precision, and inhibition of environmental DNA samples. *Biological Conservation* 183: 70-76.

Miyamoto, D.T., Zheng, Y., Wittner, B.S., Lee, R.J., Zhu, H., Broderick, K.T., Desai, R., Fox, D.B., Brannigan, B.W., Trautwein, J., Arora, K.S., Desai, N., Dahl, D.M., Sequist, L.V., Smith, M.R., Kapur, R., Wu, C.L., Shioda, T., Ramaswamy, S., Ting, D.T., Toner, M., Maheswaran, S. & Haber, D.A. 2015. RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. *Science* 349(6254): 1351-1356.

Mohd-Azlan, J., Yi, M.C.K., Lip, B. & Hon, J. 2019. Camera trapping of wildlife in the newly established Baleh National Park, Sarawak. *Journal of Sustainability Science Management* 14(4): 38-51.

Mohd-Yusof, N.S., Abdul-Latiff, M.A.B., Mohd-Ridwan, A.R., Badrulisham, A.S., Othman, N., Yaakop, S., Md-Nor, S. & Md-Zain, B.M. 2022. First report on metagenomic analysis of gut microbiome in Island Flying Fox (*Pteropus hypomelanus*) revealing latitudinal correlation as opposed to host phylogeny in island populations of Malaysia. *Biodiversity Data Journal* 10: e69631.

Nasron, A.G., Amir, A.M.A.H., Adam, L., Hasnan, M.S.H., Yazi, M.F., Patah, P.A., Rozi, M.S.F.M., Rasid, A.F.FA. & Cheng, T.C. 2019. Wildlife monitoring at Labis Timur Ecological Corridor (CFS2:PL1) in Johor, Malaysia. *Journal of Wildlife and Parks* 34: 9-22.

Nurul, A.A.N., Danish-Daniel, A.M., Okomoda, V.T. & Asma, N.A. 2020. Microbiota composition of captive bluestreak cleaner wrasse *Labroides dimidiatus* (Valenciennes, 1839). *Applied Microbiology and Biotechnology* 104(17): 7391-7407.

Okomoda, V.T., Nurul, A.N.A., Danish-Daniel, A.M., Oladimeji, A.S., Abol-Munafi, A.B., Alabi, K.I. & Nur, A.A. 2020. Microbiota composition data for wild and captive bluestreak cleaner wrasse *Labroides dimidiatus* (Valenciennes, 1839). *Data in Brief* 32: 106120.

Osman, N.A., Abdul-Latiff, M.A.B., Mohd-Ridwan, A.R., Yaakop, S., Nor, S.M. & Md-Zain, B.M. 2020. Diet composition of the wild stump-tailed macaque (*Macaca arctoides*) in Perlis State Park, Peninsular Malaysia, using a chloroplast tRNL DNA metabarcoding approach: A preliminary study. *Animals* 10: 2215.

Othman, N., Haris, H., Fatin, Z., Najmuddin, M.F., Sariyati, N.H., Md-Zain, B.M. & Abdul-Latiff, M.A.B. 2021. A review on environmental DNA (eDNA) metabarcoding markers for wildlife monitoring research. *IOP Conf. Series: Earth and Environmental Science* 736: 012054.

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N. & Taberlet, P. 2012. Who is eating what: Diet assessment using next generation sequencing? *Molecular Ecology* 21: 1931-1950.

Prévost-Bouré, N.C., Christen, R., Dequiedt, S., Mougel, C., Lelièvre, M., Jolivet, Shahbazkia, H.R., Guillou, L., Arrouays, D. & Ranjard, L. 2011. Validation and application of a PCR primer set to quantify fungal communities in the soil environment by real-time quantitative PCR. *PLoS ONE* 6(9): e24166.

Quandt, D. & Stech, M. 2005 Molecular evolution of the *trn*L_{UAA} intron in bryophytes. *Molecular Phylogenetics and Evolutions* 36(3): 429-443.

Quéméré, E., Hibert, F., Miquel, C., Lhuillier, E., Rasolondraibe, E., Champeau, J., Rabarivola, C., Nusbaumer, L., Chatelain, C., Gautier, L., Ranirison, P., Crouau-Roy, B., Taberlet, P. & Chikhi, L. 2013. A DNA metabarcoding study of a primate dietary diversity and plasticity across its entire fragmented range. *PLoS ONE* 8(3): e58971.

Rosli, N., Sitam, F.T., Rovie-Ryan, J.J., Gan, H.M., Lee, Y.P., Ithnin, H., Gani, M., Razak, M.F.A.A., Md-Zain, B.M. & Abdullah, M.T. 2019. The complete mitochondrial genome of Malayan Gaur (*Bos gaurus hubbacki*) from Peninsular Malaysia. *Mitochondrial DNA Part B* 4(2): 2535-2536.

Roy, M., Belliveau, V., Mandrak, N.E. & Gagné, N. 2018. Development of environmental DNA (eDNA) methods for detecting high-risk freshwater fishes in live trade in Canada. *Biology Invasions* 20: 299-314.

Ruppert, K.M., Kline, R.J. & Rahman, M.S. 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation* 17: e00547.

Sanger, F., Nicklen, S. & Coulson, A.R. 1977. DNA sequencing with chain terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* 74: 5463-5467.

Schloss, P.D., Gevers, D. & Westcott, S.L. 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS ONE* 6(12): e27310.

Shokralla, S., Spall, J.L., Gibson, J.F. & Hajibabaei, M. 2012. Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology* 21: 1794-1805.

Smith, C.J. & Osborn, A.M. 2009. Advantages and limitations of quantitative PCR (q-PCR) based approaches in microbial ecology. *FEMS Microbiology Ecology* 67: 6-20.

Srivathsan, A., Ang, A., Vogler, A.P. & Meier, R. 2016. Fecal metagenomics for the simultaneous assessment of diet, parasites, and population genetics of an understudied primate. *Frontiers in Zoology* 13: 17.

Sutra, J., Saadu, H., Hashim, A.M., Saad, M.Z., Yasin, I.S.M. & Amal, M.N.A. 2021. Diversity, relative abundance, and functional genes of intestinal microbiota of tiger grouper (*Epinephelus fuscoguttatus*) and Asian seabass (*Lates calcarifer*) reared in a semi-closed hatchery in dry and wet seasons. *Pertanika Journal of Tropical Agricultural Science* 44(2): JTAS-2178-2020.

Suzuki, M.T. & Giovannoni, S.J. 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Applied and Environmental Microbiology* 62: 625-630.

Taberlet, P., Waits, L.P. & Luikart, G. 1999. Noninvasive genetic sampling: Look before you leap. *Trends Ecology Evolution* 14: 323-327.

Tan, C.H. & Tan, K.Y. 2021. *De novo* venom-gland transcriptomics of spine-bellied sea snake (*Hydrophis curtus*) from Penang, Malaysia - Next-generation sequencing, functional annotation and toxinological correlation. *Toxins* 13(2): 127.

Tawfik, G.M., Dila, K.A.S., Mohamed, M.Y.F., Tam, D.N.H., Kien, N.D., Ahmed, A.M. & Huy, N.T. 2019. A step by step guide for conducting a systematic review and meta-analysis with simulation data. *Tropical Medicine and Health* 47: 46.

Thomsen, P.F. & Willerslev, E. 2015. Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation* 183: 4-18.

Tillmar, A.O., Dell'Amico, B., Welander, J. & Holmlund, G. 2013. A universal method for species identification of mammals utilizing next generation sequencing for the analysis of DNA mixtures. *PLoS ONE* 8(12): e83761.

Valentini, A., Miquel, C., Nawaz, M.A., Bellemain, E., Coissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti, G., Wincker, P., Swenson, J.E. & Taberlet, P. 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: The *trn*L approach. *Molecular Ecology Resources* 9: 51-60.

Willerslev, E. & Cooper, A. 2005. Review paper. Ancient DNA. *Proceedings of the Royal Society B* 272: 3-16.

Wilson, J., Sing, K., Chen, P. & Zieritz, A. 2017. Tracking the southern river terrapin (*Batagur affinis*) through environmental DNA: Prospects and challenges. *Mitochondrial DNA Part A* 29(6): 862-866.

Zhong, Q., Bhattacharya, S., Kotsopoulos, S., Olson, J., Taly, V., Griffiths, A.D., Link, D.R. & Larson, J.W. 2011. Multiplex digital PCR: Breaking the one target per color barrier of quantitative PCR. *Lab Chip* 11: 2167.

Zoqratt, M.Z.H.M., Eng, W.W.H., Thai, B.T., Austin, C.M. & Gan, H.M. 2018. Microbiome analysis of Pacific white shrimp gut and rearing water from Malaysia and Vietnam: Implications for aquaculture research and management. *PeerJ* 6: e5826.

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