Forestry Commission

Research Note

Using DNA barcoding and metabarcoding to detect species and improve forest biodiversity monitoring

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Growing threats to biodiversity from pressure of land use, climate change, and invasive pests and diseases highlight the importance of obtaining accurate baseline measurements of current forest biodiversity, as well as improved monitoring to detect early signals of change. Developments in molecular techniques have advanced to the stage that there are now practical methods available that help reduce the costs and overcome the practical difficulties that restrict the breadth, speed and repeatability of species identification. This Research Note provides an overview of two molecular techniques, DNA barcoding and DNA metabarcoding, and the scope to use them in forest biodiversity surveys in support of a broad range of management and conservation objectives. DNA barcoding is widely used for the unambiguous identification of single species based on DNA extracted either directly from the organism itself, or from environmental DNA via hair, droppings and other cellular debris left in the environment it inhabits. DNA metabarcoding enables the identification of multiple species present in a single sample in a timely and cost-efficient manner. This creates opportunities to explore species interactions, to identify species within a community that may pose a biosecurity risk, and to investigate multiple species responses to environmental change arising naturally or through management interventions.

Introduction

This Research Note describes DNA barcoding and metabarcoding and the power of these two methods to identify species across different taxonomic groups, along with commentaries on their current limitations. Examples are provided to demonstrate how DNA barcoding and metabarcoding can help address species detection and identification needs in the forestry sector, including monitoring and assessment of forest biodiversity for conservation and invasive species management. Box 1 provides definitions of the molecular genetic terms used.

The importance of biodiversity monitoring and its challenges

UK forests are highly valued for the suite of ecosystem goods and services they provide (Quine *et al*., 2011). One of these key ecosystem services is the level of biodiversity present within a forest, which is regarded as essential in conferring resilience, defined as the natural ability of a forest to survive and recover from environmental change (Thompson *et al*., 2009). A major international mandate to protect biodiversity was established by the Convention on Biological Diversity (CBD) in 1992, which was the first treaty to provide a legal framework for biodiversity conservation. Three objectives and elements of the CBD programme of particular relevance to forest management in the UK are: (1) the maintenance of natural ecological processes in managed forests; (2) mitigation of the impacts of threatening processes such as climate change, invasive species and pollution on forest biodiversity (including tree species in their own right); and (3) protection, restoration and enhancement of forest biodiversity through the conservation of habitats and priority species (Forestry Commission, 2011).

Increasing threats from climate change and invasive pests and diseases highlight the importance of obtaining both accurate baseline measurements of forest biodiversity and improved monitoring to detect early signals of change. Biodiversity data are also needed to identify woodlands of the greatest conservation interest, and to assess the effectiveness of forest policy measures designed to protect and enhance forest biodiversity and associated woodland ecosystem functioning (e.g. alternative silvicultural systems). However, biodiversity is broad, multidimensional and multiscale in character, making it challenging to monitor changes across space and time (Puumalainen, Kennedy and Folving, 2003; Boutin *et al*., 2009). Despite agreement among experts of the comparative value of 'actual' compared to 'inferred' assessments of biodiversity (Chirici *et al*., 2012), to record biodiversity comprehensively, even at the smallest spatial scales, is often a prohibitively

Box 1 Basic definitions of molecular genetic terms

Chromosome A threadlike strand of DNA bonded to various proteins present in the cell nucleus of eukaryotes that carries the genes in a linear order.

Diploid cell A cell which contains two complete sets of chromosomes (2n).

DNA (deoxyribonucleic acid) The molecule that contains the genetic code of an organism.

eDNA (environmental DNA) DNA from a range of environmental samples such as soil, water or air rather than that directly sampled from an individual organism. Common sources of eDNA include faeces, mucus, shed skin, hair and carcasses (including bone and teeth).

Gene A unit of hereditary information that occupies a fixed position (locus) on a chromosome. Each gene contains a particular set of instructions, usually coding for a particular protein or for a particular function.

Genome The complete complement of DNA, including both organellar and nuclear genes of an organism.

Next Generation Sequencing (NGS) Recent advances in DNA sequencing that make it possible to sequence millions of DNA fragments in parallel rapidly and inexpensively.

Nuclear genome DNA contained within the nucleus of organisms whose cells possess a nucleus.

Organellar DNA genome DNA contained in organelles located in the cytoplasm of cells which possess nuclei. Unlike nuclear DNA, which is present as linear molecules inside the chromosomes, organellar DNA is present as circular molecules. There are generally only two copies of the nuclear DNA per cells, whereas there are many chloroplasts and mitochondria per cell.

Operational Taxonomic Unit (OTU) This unit represents each unique sequence of DNA that is generated in a given study and is often used as a pragmatic proxy for a species.

Polymerase Chain Reaction (PCR) A laboratory technique used to make multiple accurate copies of a precise sequence of target DNA from a single or a mixture of individuals.

Primer/universal primer A short single stranded sequence of DNA that sticks to the area that flanks the target area to initiate PCR. Universal primers work across a broad range of organisms.

expensive and challenging task. The most common unit of taxonomic enquiry is that of the species; but even at this level biodiversity monitoring encounters numerous challenges which include:

- the difficulty and expense of collecting representative samples of species (e.g. trapping rare or elusive species);
- a shortage of taxonomic expertise to identify specimens correctly from their morphology;
- slow processing of often very large numbers of specimens, resulting in high related costs;
- difficulties in identifying species due to poor quality samples, or juvenile life stages that prevent the use of taxonomic keys.

Therefore, monitoring has tended to focus on: (1) a restricted number of species considered to be at risk of extinction; (2) species that are relatively easy to sample and taxonomically unambiguous and therefore easier to identify; and/or (3) surrogate measures of biodiversity or 'biodiversity indicators' which comprise forest attributes (e.g. woodland structural complexity) or key 'indicator' taxa (e.g. hoverflies) that convey information about the wider state of the biological community and can be assessed relatively quickly and inexpensively by forest managers (Ferris and Humphrey, 1999; Noss, 1999; Coote *et al*., 2013; Barsoum *et al*., 2015) (Figure 1). However, recent research has revealed limited evidence of the universal applicability of many commonly used biodiversity indicators for forest ecosystems (Gossner *et al*., 2014; Gao, Nielsen and Hedblom, 2015; Sabatini *et al*., 2016). For example, only six out of 83 biodiversity indicators were supported by strong evidence of correlations between the biodiversity indicator (e.g. volume of deadwood) and measured levels of associated biodiversity (e.g. wood-dwelling fungal and beetle species richness) (Gao, Nielsen and Hedblom, 2015).

Biodiversity monitoring advances provided by DNA barcoding and DNA metabarcoding

DNA barcoding and metabarcoding

The term barcoding originates from an analogy with supermarket barcode labels which use the small differences in the black stripes of the product code to distinguish between different items. DNA barcoding utilises small differences in a very short genetic sequence from a standard part of the genome to distinguish between species; two species may look very similar to the untrained eye but their DNA barcodes are unambiguously distinct from each other. For both DNA barcoding and DNA metabarcoding, a technique called the Polymerase Chain Reaction (PCR) is used to amplify suitable quantities of DNA from a specific part of the genome to enable further analysis. The precise region of DNA that is chosen for amplification depends on each particular organism, and the necessity of selecting a region that contains sufficient variation to enable organisms to be uniquely identified at the species scale.

For the identification of animal species, amplified regions of the genome are typically matched with DNA sequences on the Barcode of Life Database (BOLD), an online platform designed to curate and store DNA barcode information (http://boldsystems.org/). Since its creation in 2005, BOLD has developed quickly and in October 2017 it contained over 5.8 million barcodes from a global range of species. The barcodes originate from multiple taxonomic groups including 181 thousand animal species, 66 thousand plant species, and 21 thousand fungi species. In BOLD, each entry is accompanied by detailed information including sample location, a voucher specimen and the DNA sequence trace file, which ensures high

Figure 1 Widely recognised taxa used as biodiversity indicators include birds and butterflies to assess national trends in habitat condition (Defra, 2017). For example, (a) the common cross-bill (*Loxia curvirostra*) and (b) the small pearl-bordered fritillary (*Boloria selene*). The conservation status of the pine marten (*Martes martes*) (c) requires regular monitoring as a protected species.

standards of taxonomic identification are adhered to when linking a derived molecular sequence to a particular species. Other freely accessible DNA databases such as GenBank are often used for identification of plants and fungi. However, these were established much earlier when initial species identifications were not so thoroughly quality assured, and therefore care is required to ensure that the correct species identity is attached to the DNA sequence when using these older databases.

DNA barcoding method and applications

DNA barcoding is an approach used to confirm the presence of a single target species using molecular methods: the question, "Is species X present in my forest?" can be answered without actually seeing the organism itself. This can be particularly useful for confirming the presence of a rare and difficult-to-capture species, for juvenile stages which have not yet developed the key identifiable taxonomic characteristics of an adult, and for differentiating between specimens which are genetically distinct but belong to morphologically indistinguishable species. DNA barcoding involves DNA extraction and PCR amplification. The sequence that is generated is compared to sequences in a DNA database to check for a match from a specimen which has been identified by a taxonomic expert (Figure 2a). Advances in commercially available DNA extraction kits have contributed to the success of barcoding by optimising the quantity and quality of DNA that can be extracted from samples which have degraded or contain only small quantities of DNA. DNA extracted from samples collected in the environment (e.g. from hair, feathers, droppings, soil and water samples) is known as environmental DNA (eDNA); eDNA enables the detection of organisms that can cause tree and wildlife diseases as well as verifying the presence of reclusive or nocturnal animals which would otherwise be difficult to detect (Boxes 2 and 3, Table 1).

DNA metabarcoding method and applications

A pivotal breakthrough for species diversity monitoring was the advent of Next Generation Sequencing (NGS) and the subsequent development of a method known as DNA metabarcoding which allowed the much broader question, "How many species are present in my sample and what are they?" to be answered. NGS methods (also known as high throughput sequencing) have revolutionised barcoding methodology by enabling large numbers of high quality DNA sequence reads to be obtained relatively inexpensively from environmental samples in a single sequencing run. The DNA metabarcoding approach can be applied to determine the composition of multiple species samples such as those derived from collections of arthropods in pitfall traps, bacterial or fungal endophytes in leaf tissue, pathogen spores trapped from the air, organisms in water samples, or invertebrate, bacterial and fungal communities in soil samples. Figure 2 provides an illustration of how the sample processing methodology differs between DNA barcoding and DNA metabarcoding when applied to the same four traps containing arthropod samples.

DNA metabarcoding has the potential to: (1) investigate multiple species responses to environmental change (e.g. forest management interventions); (2) simultaneously monitor occurrences of multiple species for conservation or invasive species management; and (3) explore species interactions, including uncovering details of ecosystem service provision by various woodland species (Table 1; Box 4). Another advantage of the DNA metabarcoding approach is that previously undescribed species (sequences with no corresponding matches in databases) can be detected, enabling follow-up sampling to obtain voucher specimens and establish their taxonomic status.

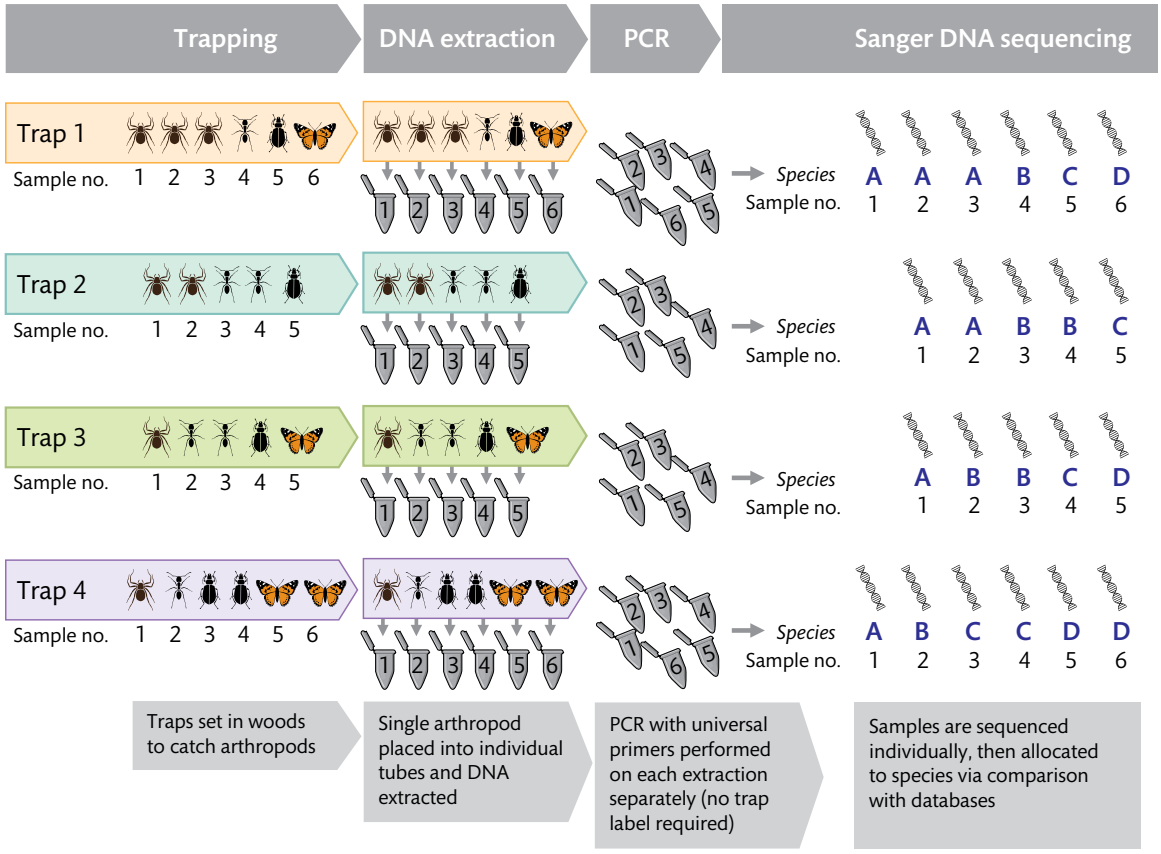
As with DNA barcoding, total DNA is extracted from an environmental sample, creating a 'DNA soup' intended to be

Box 2 Example of the application of eDNA in the detection of a rare species

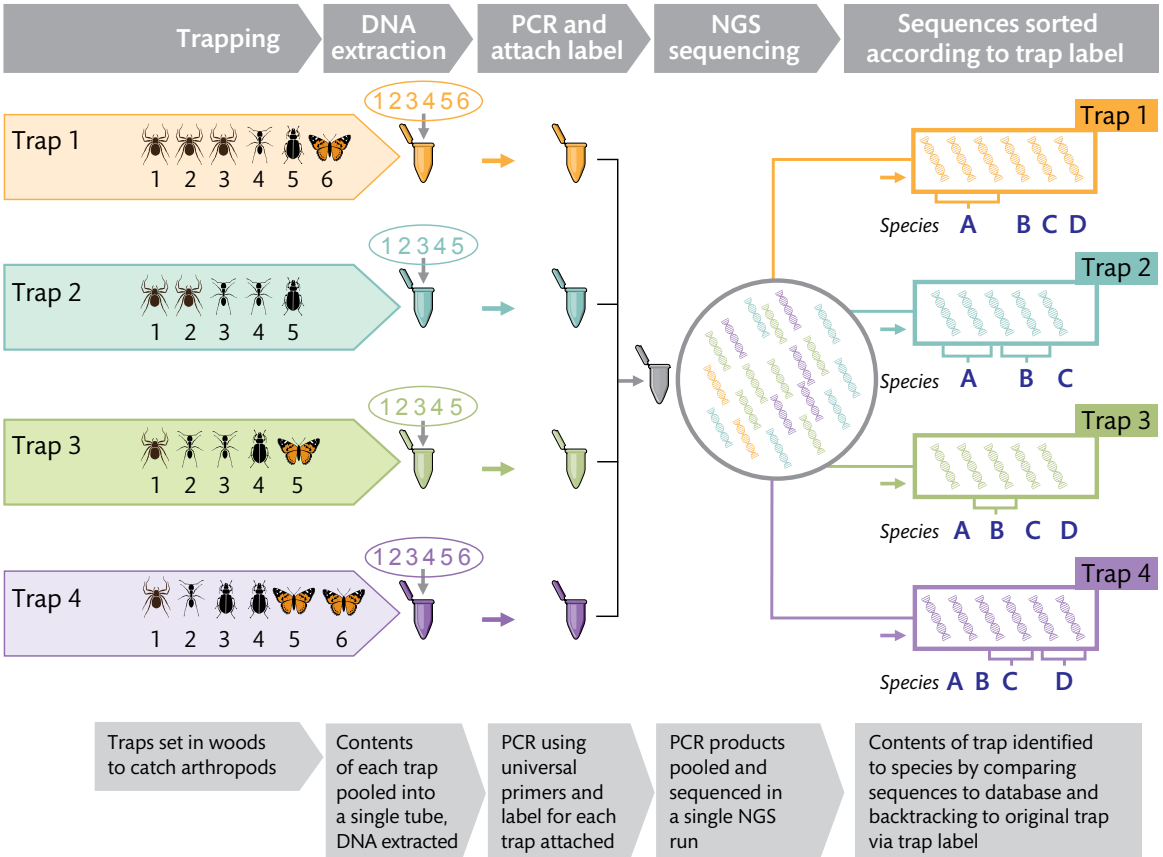
One of the most widely publicised applications of eDNA relates to the confirmation, accepted by Natural England, of the presence of great crested newts (*Triturus cristatus*) from the amplification of DNA samples in pond water. The great crested newt is a priority species, having suffered significant population declines due, in part, to large-scale habitat loss, including the loss of breeding ponds. The eDNA approach has been shown to be more effective at detecting newts than individual 'traditional' survey methods (e.g. torch counts, bottle trapping and egg searches) over the course of the survey season (Biggs *et al.*, 2015), significantly improving species monitoring for more effective conservation and management planning.

Figure 2 Diagram demonstrating how the sample processing methodology differs between (a) DNA barcoding and (b) DNA metabarcoding when applied to the same four traps containing arthropod samples. Individual samples requiring separate processing for target species identification via DNA extraction, PCR and sequencing in (a) can be pooled for the same steps in (b) for multiple species identification, thus greatly reducing the cost and manual processing effort per trap. Note that in this example arthropods are the target organisms for trapping and species identification, but the same techniques can be applied to other organisms of interest.

a) DNA barcoding



b) DNA metabarcoding



Box 3 Applications of DNA barcoding by Forest Research

Forest Research offers DNA barcoding as a service and has experience in applying the technique to address conservation management and biosecurity issues. For example, DNA barcoding has been used by Forest Research ecologists to identify the species of bat occupying winter roosts from droppings collected in the summer when the roosts are unoccupied. This can help bypass the need for bat detection survey work and thus speeds up the processing of planning applications. Forest ecologists have also used the technique to confirm the presence of and the effects of woodland management on a number of endangered forest species (e.g. pine marten, *Martes martes*; black grouse, *Tetrao tetrix*) using faecal or hair samples. DNA barcoding is also routinely used by Forest Research entomologists to identify potentially damaging pests intercepted in imported timber and wood products; it has also been used to investigate the relationship between the Scottish pine tree lappet moth population and European populations (Moore *et al*., 2017). In addition, Forest Research pathologists use the method to confirm the presence of a range of disease-causing organisms in trees; examples include the use of DNA barcoding to detect the bacterial causal agent of horse chestnut bleeding canker, *Pseudomonas syringae* pv. *aesculi* (Green *et al*., 2009); the fungal causal agent of ash dieback, *Hymenoscyphus fraxineus*, and its close relative, the non-pathogen *H. albidus* (King and Webber, 2016); *Dothistroma* needle blight of pines (Mullett *et al*., 2016); and various quarantine-regulated Phytophthoras, including *Phytophthora ramorum*, *P. austrocedri*, *P. kernoviae* and *P. lateralis* (Mulholland *et al*., 2013; Elliot *et al*., 2015). Some of these examples involve a technique known as real-time PCR which allows pathologists to obtain a quantitative measure of the presence of particular pathogens.

Pine tree lappet moth (*Dendrolimus pini*).

Junipers dying due to root infection by *Phytophthora austrocedri*.

representative of all the species present. A specific short region of the genome is amplified using PCR based on universal primers that work across a broad range of taxa in the target organisms. For a given sample, the mass-amplified short fragments of DNA are given a unique chemical label which allows the amplified samples to be pooled and analysed in a single sequencing run. Using the unique sample labels, bioinformatic software assigns the sequences back to the original sample from which they were generated, and a list of unique sequences or Operational Taxonomic Units (OTUs) per sample is produced. Individual OTUs are then compared against existing DNA databases in order to identify the organisms they most closely match. Metabarcoding data should only be used to indicate the presence of a particular species and not to imply its relative abundance. This is because the amount of DNA that is extracted and amplified per individual is not the same across all species; those which consist of physically larger individuals are expected to yield more DNA than those made up of smaller individuals. In addition, it may be the case that the DNA of certain species could be preferentially amplified by the universal primers so that they participate more effectively in the PCR process and produce more reads than those whose DNA is amplified less efficiently. Therefore, the metabarcoding method yields qualitative rather than quantitative data, that is, it provides presence/absence data for each OTU or species in a given sample.

Where there is a restricted number of candidates in a study, for instance, fish species in a lake (Hänfling *et al*., 2016) or *Phytophthora* species in a soil sample (i.e. Figure 3), it is possible to develop a specifically tailored database of the sequences of all possible target organisms likely to be present; where the constituent species are known, it is also feasible to set up control samples and vary their relative concentrations to test whether the number of OTUs could be used as an indication of species relative abundance (Hänfling *et al*., 2016). Test controls

Box 4 Applications of DNA metabarcoding by Forest Research

Forest Research is developing in-house expertise in the methods required for DNA metabarcoding and has been involved in numerous tree health and forest biodiversity conservation projects in which metabarcoding approaches have been applied. For example, forest entomologists have used DNA metabarcoding to identify fungal communities associated with bark beetles as potential vectors of disease (Miller *et al*., 2016). Another project, PROTREE, is examining the foliar endophytes present on different provenances of Scots pine grown in common garden trials to determine the relative importance of site, provenance and family effect on the composition of the needle endophyte community. Forest Research pathologists are investigating *Phytophthora* species richness and distribution in several projects comparing, for example, highly disturbed and less disturbed forest sites in Britain, water samples versus soil samples, and UK plant nurseries operating a range of management practices. One project found over 30 *Phytophthora* species including quarantine-regulated species and a number of putative novel species in soils across 14 public parks, gardens and other amenity woodlands in Scotland (Green, Henricot and Hedley, 2017).

FR ecologists have primarily applied DNA metabarcoding to investigate forest arthropod species responses to environmental change and forest management practice; examples include understanding: (1) the influence of a diversification of forest stands on arthropods of the forest field layer in Thetford Forest, Norfolk (Barsoum *et al*., 2018); and (2) impacts of a collapse in beech-dominated stands on arthropod assemblages of the forest floor in the New Forest, Hampshire (Evans *et al*., 2017).

Other methods not described here are used to identify the sex of a sample. In addition, if the quality of DNA permits, it is possible to distinguish between individuals using markers such as microsatellites. In Forest Research, these methods are applied to identify pine marten, black poplar and aspen individuals, and in the case of pine marten and aspen, also their gender.

of known composition can be constructed to check that all constituent species provide OTUs at the end of the sequencing process. By contrast, for studies where the number of candidate species is much greater and for which the development of a tailored DNA sequence database is not feasible, it is necessary to rely on pre-existing databases such as BOLD for species identification of OTUs. The likelihood of the candidate being present in these databases depends largely on the particular species of interest and its country of origin. This is because of an imbalance in the breadth of taxonomic identification and cataloguing achieved to date in different countries, which is often a reflection of species richness, availability of taxonomic expertise, and available funding for research and species census work. For example, a DNA metabarcoding analysis of arthropods captured in malaise traps from a study site in China only managed to identify about 15% of all generated OTUs to species level, whereas a similar study based on malaise trap sampling in a forest in Thetford (UK) (Figure 4) identified approximately 73% of all OTUs to species level (Yu *et al*., 2012; Barsoum *et al*., 2018).

Figure 3 Soil sample used to detect the presence of *Phytophthora* species using DNA metabarcoding.

Figure 4 Malaise trap used to capture flying arthropods in a Scots pine plantation (Thetford Forest, Norfolk).

Table 1 Examples demonstrating the application potential of DNA barcoding and DNA metabarcoding in forest biodiversity monitoring and assessment.

Species identification/ detection needs in forests	Real-world examples	Location of study	Sample type	Reference
DNA BARCODING - Target species identification				
Rare species detection	Blood-sucking leech diet as a way to detect rare deer species Truong Son muntjac (*Muntiacus truongsonensis*).	Vietnam	Bloodmeal from leeches	Schnell *et al.* (2012)
Tree disease detection	Detection of the fungus *Hymenoscyphus fraxineus* (causal agent of ash dieback) in ash plantations and mixed forest.	Belgium	Spores in air using a spore trap	Chandelier *et al.* (2014)
Wildlife disease detection and host-vector relationships	Detection of the chytrid fungus *Batrachochytrium dendrobatidis*, likely to be a primary cause of amphibian population declines.	Spain	<1 l water	Walker *et al.* (2007)
	Patterns of tick infestation as important vectors of disease in vertebrate hosts (e.g. deer).	Canada	Bloodmeal from ticks	Gariepy *et al.* (2012)
Invasive species detection	Detection of the invasive American bullfrog (*Rana catesbeiana*) in wetland habitats (e.g. woodland ponds, streams).	France	15 ml water	Ficetola *et al.* (2008)
Hybrid detection	Discrimination of European Black Poplar (*Populus nigra*) from American Black Poplar (*P. deltoides*) and Hybrid Poplars (*P. x canadensis*).	Switzerland	Buds, leaves and cambium from wood cores	Holderegger *et al.* (2005)
Species identification/ delineation where taxonomic identification is unavailable or poorly described	Measures of species richness among poorly known tropical forest flora.	Australia	Leaf tissue	Costion *et al.* (2011)
	Assessing the degree of genetic divergence between different barn owl (*Tyto alba*) populations across Europe and the Americas to facilitate species delineation.	UK	Blood and tissue	Nijman and Aliabadian (2013)
	Disentangling species identification among cryptic butterfly species.	Costa Rica	Single caterpillar leg	Hebert *et al.* (2004)
METABARCODING - Multiple species detection				
Distribution and diversity of multiple pests/pathogens in the environment	Distribution and diversity of *Phytophthora* within native asymptomatic vegetation communities at a near continental scale.	Australia	Soil	Burgess *et al.* (2017)
	Distribution and diversity of *Phytophthora* in natural forests, plantations and aquatic environments.	Spain	Soil and water	Català, Pérez-Sierra and Abad-Campos (2015)
	Repeat sampling to describe the *Phytophthora* community in two sweet chestnut (*Castanea sativa*) forested areas.	Italy	Soil	Vannini *et al.* (2013)
Biodiversity associations by woodland type/ management intervention e.g. effects of tree species identity, tree species diversity, developmental stage of woodland, forest management interventions that change structure/ composition.	Comparison of earthworm species richness in ancient beech coppice, 50–60 yr old spruce plantation and pasture in pre-alpine region.	France	Soil	Pansu *et al.* (2015)
	Comparison of soil microorganisms (ectomycorrhizal fungi, plant pathogens, saprotrophs) and meiofauna (soil protists, collembola, nematoda) in monoculture and mixed species treatments of *Pinus sylvestris, Picea abies, Larix sibirica, Betula pendula* and *Alnus glutinosa*.	Estonia and Finland	Soil	Tedersoo *et al.* (2016)
	Arthropod, nematode, annelid, metazoa communities in open canopy forest, acacia plantations, protected forest.	China	100 g of soil and arthropods captured in malaise traps, Winkler litter traps and by canopy fogging	Yang *et al.* (2014)

Table 1 (continued).

Species identification/ detection needs in forests	Real-world examples	Location of study	Sample type	Reference
	Comparison of flying arthropod species richness in unlogged forest, selectively logged forest and oil palm plantations.	Borneo, Malaysia	Malaise traps	Ji *et al.* (2013)
Monitoring species presence/ absence in multiple taxonomic groups for conservation management	Presence of threatened freshwater fauna including fish, amphibians, arthropods and custaceans in ponds, streams and lakes.	Denmark, Sweden, Germany, Poland and Estonia	15 ml water	Thomsen *et al.* (2011)
	Detection of freshwater fish species in lakes.	Cumbria, UK	2 l water	Hänfling *et al.* (2016)
	Monitoring of amphibians and bony fish in freshwater habitats.	France, Holland	Water	Valentini *et al.* (2016)
	Inventory of vegetation species present in a pristine lowland tropical rainforest.	French Guiana	Soil	Yoccoz *et al.* (2012)
	Blood-sucking fly diet as a way to inventory mammalian diversity in tropical rainforests, including endangered species.	Ivory Coast	Bloodmeal from biting flies	Calvignac-Spencer *et al.* (2013)
Understanding multiple species interactions (e.g. food webs, host-vector-pathogen relationships)	Quantifying parasitism rates in the invasive Lepidopteran (*Thaumetopoea processionea*), the oak processionary moth (OPM).	Richmond Park, London, UK	OPM caterpillars and pupae	Kitson *et al.* (2016)
	Browsing patterns and intensity by ungulate species; i.e. moose (*Alces alces*), red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*).	Sweden	Ungulate saliva collected from twigs	Nichols *et al.* (2012)
	Diversity of prey consumed by the insectivorous bat species *Myotis nattereri*.	Hampshire, UK	Bat droppings	Hope *et al.* (2014)
	Diversity of pest species consumed by birds in orchards.	Australia	Bird droppings	Crisol-Martínez *et al.* (2016)
	Flowering plants important for honey production.	Sweden	1 ml of honey	Schnell *et al.* (2010)
Monitoring community responses to environmental change (e.g. pollution, loss of tree species, drought, climate)	Moth species richness along a climate gradient in a subtropical forest, i.e. altitudes of 2000, 2200, 2400 and 2600 m above sea level, and at two strata (canopy, ground).	Yunnan, China	Moths captured via light-traps	Ji *et al.* (2013)
	Assessment of a needle endophyte community on Scots pine along a latitudinal gradient.	Sweden	Scots pine needles	Millberg, Boberg and Stenlid (2015)

DNA metabarcoding stipulations

There are a number of stipulations concerning the DNA metabarcoding methodology. Appropriate sampling and storage strategies are paramount in achieving realistic representations of species richness. Care is necessary to guard against cross-contamination since this technique will detect minute quantities of DNA. Appropriate storage of samples to avoid degradation of DNA is also critical. DNA metabarcoding projects demand bioinformatics skills and generate large quantities of DNA sequence data which are time-consuming to analyse and interpret. However, considerable investment in the development of analytical pipelines (a set of processing steps needed to transfer raw sequence data into interpretable taxonomic units) should improve efficiency via more automated quality assurance testing, construction of OTU lists, and searching of existing databases to allocate species identifications to individual OTUs. The availability of analytical pipelines in the public domain is already reducing the costs associated with undertaking DNA metabarcoding projects, as

well as making DNA metabarcoding approaches more accessible to molecular biologists who may not have the bioinformatics expertise required to handle large DNA datasets.

Target regions of DNA for identifying animals, plants or fungi using DNA barcoding and metabarcoding

Short sequences located in the organellar DNA genomes of mitochondria and chloroplasts make good targets for barcoding and metabarcoding for two reasons. Firstly, in contrast to the nuclear genome which is arranged as long delicate chromosomes, the organellar genomes are much shorter and are circular in structure, which makes them more robust to degradation after sampling and storage. Secondly, unlike the nuclear genome in which each chromosome is only present as two copies per diploid cell, mitochondria and chloroplasts occur in far greater numbers per cell, giving multiple targets for the PCR reaction. This is important if field-collected samples have begun to degrade prior to collection or if only small amounts of tissue sample are available. Target regions of DNA for identification of animals, plants or fungi are not the same and in some cases more than one gene region is required for species identification.

Animal identification

The gene region that is being used as the standard barcode for almost all animal groups is a region in the mitochondrial cytochrome c oxidase 1 gene (COI). COI contains sufficient variation between species to facilitate species discrimination while having little within-species variation (Ratnasingham and Hebert, 2007). It also has the key attribute of having regions of flanking DNA that are stable so universal primers can be used successfully across a broad range of species.

Plant identification

COI is not an effective barcode region in plants because it evolves too slowly and therefore is not sufficiently variable to resolve plant sample identification to the species level. Instead, two gene regions in the chloroplast genome, matK and rbcL, have been approved by the CBOL Plant Working Group (2009) as the barcode regions appropriate for plants. However, other gene regions are often also required to arrive at an unambiguous species identification. Therefore, identification of plant species via DNA barcoding is generally less effective and more labour-intensive than for animal identification (Hollingsworth, Graham and Little, 2011). For example, a recent initiative to provide a DNA barcode for the flowering plants and conifers in Wales based on verified herbarium specimens succeeded in discriminating over 98% of the genera but only 69–75% of the species using these two gene regions (de Vere *et al*., 2012).

Fungal identification

For fungal identification, the International Fungal Barcoding Consortium has formally recommended that the Internal Transcribed Spacer (ITS) region is used as the primary fungal barcode since it has been found to provide the best species discrimination (Schoch *et al*., 2012). Although the ITS region occurs in the nucleus, it is unusual in that it is present several times in different areas of this genome and occurs in tandem repeats containing many ITS copies. This is one of the reasons for the ITS being universally popular in barcoding: it will amplify more readily due to the high number of copies within a single genome. However, one drawback to using the ITS region is that the multiple copies present within an individual can exhibit sequence variation due to natural mutations. Consequently, efforts are underway to address this issue by developing reference DNA sequence databases that include known within-species ITS sequence variation. Efforts are also focused on looking for alternative fungal barcodes, for example, COX1, COX2 or single-copy nuclear genes. Although only approximately a hundred thousand of the estimated 1.5 million fungal species have been described to date, with only about half of these species represented in publicly accessible DNA sequence databases, the process of DNA barcoding is contributing significantly to understanding of fungal taxonomy. Examples of the utility of DNA information include an individual fungal species exhibiting various developmental stages that had previously been split into separate species based on morphology alone; and where multiple cryptic species exist which had previously been morphologically classified as a single species (Reignoux, Green and Ennos, 2014; Xu, 2016).

Conclusions and future developments

Technical advances in molecular ecology are moving quickly, offering increasing potential to catalogue and monitor biodiversity rapidly, accurately and cost-effectively. For instance, the launch in 2014 of the highly portable minION by Oxford Nanopore offers the scope to identify species directly in the field from a small sample of extracted DNA (Parker *et al*., 2017). However, because this method is non-discriminatory in the section of an organism's genome that is used for species identification (that is, no specific DNA region is targeted as in DNA barcoding and DNA metabarcoding), to date the minION

has only been able to reliably identify species that have small genomes such as those of bacteria, yeast and certain higher plant species (e.g. mouse-ear cress (*Arabidopsis thaliana*)) (Hayden, 2015; Parker *et al*., 2017). For the identification of species with large genomes, a laboratory-based approach is still required, and DNA barcoding and DNA metabarcoding offer operational tools that are increasingly recognised as flexible and valuable in the identification of species present in forests. For minute or degraded environmental samples, very short regions of target DNA fragments known as mini-barcodes are additionally applicable to an increasing range of taxa for accurate species identification. Therefore, where species identification has previously proven difficult from certain environmental samples (e.g. degraded DNA in owl pellets preventing the identification of species consumed by owls), in many cases the development of mini-barcodes has helped to resolve these difficulties (Galan, Page and Cosson, 2012; Hajibabaei and McKenna, 2012; Guimaraes *et al*., 2016). Detailed studies have shown that if consistent extraction, amplification and bioinformatics methods are applied, then they can offer a more comprehensive, repeatable and spatially consistent identification of species than morphology-based identification using taxonomic keys (Hajibabaei *et al*., 2016; Deiner *et al*., 2017).

As standardised and optimised methods of DNA extraction and bioinformatic analysis become available, DNA-based detection of species will: (1) provide a powerful tool in biodiversity monitoring to enable a better understanding of how management practices or changes to the environment can affect species biodiversity; and (2) become increasingly useful for large-scale ecosystem monitoring, enabling detection and response at an early stage to significant spatial and/or temporal shifts in species distributions, including invasive pests and diseases. Progress relies on good communication between molecular biologists and field ecologists, pathologists and entomologists so that the potential of these new developments are exploited fully to improve forest biodiversity monitoring and assessment.

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