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REVIEW

Barcoding the food chain: from Sanger to high-throughput sequencing[1]

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Abstract: Society faces the complex challenge of supporting biodiversity and ecosystem functioning, while ensuring food security by providing safe traceable food through an ever-more-complex global food chain. The increase in human mobility brings the added threat of pests, parasites, and invaders that further complicate our agro-industrial efforts. DNA barcoding technologies allow researchers to identify both individual species, and, when combined with universal primers and high-throughput sequencing techniques, the diversity within mixed samples (metabarcoding). These tools are already being employed to detect market substitutions, trace pests through the forensic evaluation of trace "environmental DNA", and to track parasitic infections in livestock. The potential of DNA barcoding to contribute to increased security of the food chain is clear, but challenges remain in regulation and the need for validation of experimental analysis. Here, we present an overview of the current uses and challenges of applied DNA barcoding in agriculture, from agro-ecosystems within farmland to the kitchen table.

Key words: DNA barcoding, metabarcoding, agriculture, agro-ecosystems, food.

Résumé : La société doit faire face au défi complexe de supporter la biodiversité et le bon fonctionnement des écosystèmes, tout en assurant la sécurité alimentaire et en produisant une nourriture sécuritaire et traçable par le biais d'une chaine alimentaire globale de plus en plus complexe. L'accroissement de la mobilité des populations entraîne des menaces nouvelles en matière d'organismes nuisibles, de parasites et d'espèces envahissantes qui compliquent davantage les efforts agroalimentaires. Les technologies de codage à barres de l'ADN permettent aux chercheurs d'identifier à la fois des espèces individuelles et, lorsque combinées avec des amorces universelles et des techniques de séquençage à haut débit, la diversité présente au sein d'échantillons mixtes (métacodage à barres). Ces outils sont déjà employés pour détecter des substitutions de produits sur le marché, suivre des organismes nuisibles via l'analyse de traces d'« ADN environnemental » et de suivre les infections de parasites au sein du bétail. Le potentiel du codage à barres de l'ADN à contribuer à une sécurité accrue de la chaine alimentaire est évidente, mais des défis demeurent en ce qui a trait à la réglementation et à la nécessité de valider les analyses expérimentales. Dans cette synthèse, les auteurs présentent une vue d'ensemble de usages actuels et des défis liés à l'application du codage à barres en agriculture, des agro-écosystèmes des terres agricoles jusqu'à la table. [Traduit par la Rédaction]

Mots-clés : codage à barres de l'ADN, métacodage à barres, agriculture, agro-écosystèmes, nourriture.

The challenge of feeding 9–10 billion (bn) people by 2050 means that increasing agricultural capacity and efficiency is of great importance, but significant food security challenges are posed by anthropogenic pressures, global climate change, and the resulting environmental and biotic changes such as the movement of pest population ranges (Godfray et al. 2010; Godfray 2011). It is necessary to employ the latest techniques to maximise yields and consumer safety, whilst also farming sustainably in agro-ecosystems which are increasingly intensified. Genetic and genomic techniques have been very useful for plant and animal breeders to maximise fitness and create genetically modified organisms (Herdt 2006; Zivy et al. 2015) that are capable of wider climate tolerances and pest resistance. Research into new molecular techniques and their applications are increasing at a rapid pace. Over the last 10 years there has been an explosion in the use of DNA barcoding as a tool for eukaryote identification (Hebert et al. 2003), with many papers cited by this review published in the preceding two or three years.

DNA barcodes are small, generally species-specific fragments of DNA. Standard regions include a 658-bp

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region of the cytochrome *c* oxidase subunit 1 (COI) mitochondrial gene in animals (Hebert et al. 2003) or other regions, for example, ~800 bp of *mat*K and ~600 bp of *rbc*L in plants (CBOL Plant Working Group 2009) or the internal transcribed spacers of nuclear ribosomal DNA in plants and fungi (Schoch et al. 2012). These are extracted, PCR amplified, and sequenced, using Sanger sequencing or, increasingly, high-throughput sequencing (HTS). These sequences can be used to identify and differentiate existing species when matched against existing databases, for example, GenBank, the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007), and integrated into the identification and species delineation of new taxa (Hubert and Hanner 2015). Known as metabarcoding, primers with broad binding affinity combined with high-throughput sequencing allow the parallel sequencing of thousands of barcodes to identify multiple species in a mixed sample (Pompanon et al. 2012; Taberlet et al. 2012*b*). Although we focus on the utility of DNA barcoding and metabarcoding for agriculture in this review, we also discuss other molecular methods of identification, such as real-time PCR, in our perspectives on developing techniques.

The use of DNA barcoding for the identification of species has the potential for an extensive range of applications, particularly in monitoring and product validation (Bohmann et al. 2014; Adamowicz and Steinke 2015; Kress et al. 2015). Agri-food supply chains are becoming elongated as they are globalized, which exposes consumers to the risks of greater potential for contamination, misidentification, and even food fraud (Lee et al. 2012). Although the application of molecular techniques has been reviewed for certain aspects of the food industry and the ecosystems on which it depends (e.g., food fraud (Clark 2015), host–parasitoid interactions (Hrcek and Godfray 2015)), a review of the applications of DNA barcoding throughout the entire food chain from farm to fork, with a particular emphasis on the use of the new technology of metabarcoding, is lacking. In this article, we will review the potential applications of DNA barcoding in agriculture, from monitoring communities under threat in agro-ecosystems, ensuring food security against attack from pests and parasites, to providing safe and traceable food to the kitchen table (Fig. 1). We will highlight issues with the implementation of DNA barcoding as a tool in industry, based on problems faced by academic studies, and propose future directions for its use.

Examining agricultural landscapes as ecosystems

While the identification of individuals will remain important, DNA barcoding is moving towards the identification of whole communities (Cristescu 2014; Gibson et al. 2015), known as metabarcoding (Taberlet et al. 2012*a*). Agro-ecosystems are one such community, with species diversity linked to the provision of ecosystem services (Balvanera et al. 2006) and negatively correlated with farming intensity (Kleijn et al. 2009). In metabarcoding, bulk samples are collected en masse, combined, mass-amplified, sequenced with high-throughput sequencing, and subjected to bioinformatic analysis (Shokralla et al. 2015). It is a molecular approach to examine species diversity and trophic interactions, which could be developed for repeatable measures of biodiversity on large spatial scales.

Specifically within agricultural landscapes, schemes exist to increase biodiversity and support ecosystem services, some of which form part of international goals such as the EU's Common Agricultural Policy (CAP) "greening" component, which links subsidies to environmentally friendly farming practices (EUR-Lex-52011PC0625 2011), or on a smaller local scale such as conservation agriculture interventions. Monitoring is a key component of any scheme to evaluate whether levels of biodiversity are being supported or increased (Targetti et al. 2014). Although crucial, monitoring using the existing methods of trapping and visual assessments is often time-consuming and expensive (Ji et al. 2013; Targetti et al. 2014). Metabarcoding has been proposed as a fast, cheap, and auditable way to monitor biodiversity, which is less reliant on taxonomic expertise that is often expensive and unevenly distributed around the globe (Ji et al. 2013; Gibson et al. 2015; Syaripuddin et al. 2015). If implemented, it has been suggested that metabarcoding will allow us to move from monitoring indicator species, the use of which is sometimes problematic (Moonen and Bàrberi 2008), to taking measures of whole biodiversity. Payments in "greening" schemes of agricultural settings could even be made contingent on biodiversity outcomes with this information (Ji et al. 2013).

Environmental DNA (eDNA) will be useful for monitoring species diversity in water or soil resources within agro-ecosystems. eDNA sampling involves the extraction of exogenous DNA without first isolating target organisms for sequencing (Taberlet et al. 2012*a*; Yang et al. 2014). Methods development is ongoing, but research effort has concentrated on monitoring fish and aquatic invertebrate communities from DNA extracted from filtered water (Lodge et al. 2012; Thomsen et al. 2012). In the context of agro-ecosystems, these techniques could supplement studies of the ecological impacts of agriculture on species diversity, such as the impacts of run-off into adjacent waterways. eDNA could similarly be extracted from soil to monitor the diversity of soil-dwelling invertebrates, fungi and bacteria (Orgiazzi et al. 2015), which are all affected by ongoing agricultural activity (Caldwell et al. 2015), but are also linked to ecosystem functioning (Bender et al. 2016).

Several regulatory and industrial bodies support biodiversity assessments with eDNA assays in conjunction with metabarcoding, assisted by the rapid sampling protocols and apparently high detection rates relative to traditional sampling methods (Biggs et al. 2015). Farmers or volunteer citizen scientists could easily play a role in the

Fig. 1. Many components of our diet are produced by a food supply chain that is increasingly lengthened and globalised. At the same time we must ensure adequate levels of safe food for an expanding human population. DNA barcoding is being applied as a tool for species identification and biodiversity assessment at many stages of this food chain.

Metabarcoding and high-throughput sequencing techniques are helping scientists to barcode **entire communities** such as agro-ecosystems.

This could feed into schemes to monitor agricultural biodiversity such as the CAP greening schemes. **Citizen scientists and farmers** could be involved here.

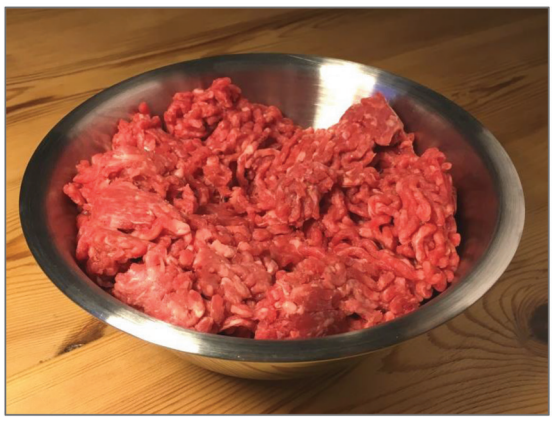
Molecular gut contents analysis has increased our understanding of **trophic interactions**.

DNA barcoding and real-time PCR assays **identify insect pests** where morphological identification is difficult. Traces of DNA from frass, eggs and pupal cases can be tested in these assays.

Barcoding is used to monitor the spread of agriculturally important **invasive species**. Additionally, real-time PCR assays could be developed to provide rapid identification of pests at **import/export** points.

Livestock parasites can be identified non-invasively using barcoding, and disease transmission pathways can be confirmed.

This could provide more up-to-date information than ELISA-based testing where antibodies remain in the system post-infection.



Barcoding has **uncovered mislabelling** in meat, fish and herbal supplement products. As well as misinforming the consumer, mislabelling incidences can result in toxicity, allergies or drug-plant interactions.

Retailers can perform their own independent checks of their supply chains. Certification **can influence consumer purchasing behaviour.**

collection of eDNA samples such as water or soil, which might also facilitate citizen scientist monitoring of species which are not charismatic or easily identifiable (Janzen et al. 2005; Adamowicz and Steinke 2015; Ugochukwu et al. 2015). Citizen scientist data from morphological identifications is already feeding into policy reports (Geijzendorffer et al. 2016), which could usefully reduce the considerable amount of person-hours required for monitoring. Yet researchers have highlighted issues with the use of eDNA, which necessitates knowledge of how eDNA is released from the originating organism. eDNA accumulation and degradation rates need to be quantified along with assay sensitivity. Mechanical processes, animal behaviour, and weather conditions are known to influence the degradation of DNA (Andersen et al. 2012; Pilliod et al. 2014), and the extent to which eDNA disperses throughout the environment must be reliably quantified if the presence of a species is to be inferred (Goldberg et al. 2015). eDNA originates from dead individuals as well as live ones, although repeated measurements over time will give an idea as to the persistence of DNA within a given environment, which could indicate whether an individual is alive (Ardura et al. 2015). An alternative to this may involve the use of environmental RNA (eRNA), which is generally less stable than DNA and therefore is more likely to indicate ongoing gene transcription (Barnes and Turner 2016).

Characterising trophic interactions

DNA barcoding allows researchers to examine trophic interactions within agro-ecosystems (Clare 2014), contributing, for example, to our understanding of how farmland predators regulate pest species (Piñol et al. 2014; Furlong 2015). Traditionally, understanding predator–prey interactions required morphological examination of gut contents, faeces, and regurgitated pellets, with poor and taxonomically biased resolution (Sheppard and Harwood 2005). When gut contents contain liquids such as blood, haemolymph, or sap, species-specific interactions are very difficult to determine without molecular analysis. By examining gut contents or faeces with DNA barcoding (sometimes termed molecular scatology), we can examine trophic interactions in a non-invasive way which also has the potential for higher taxonomic resolution (King et al. 2015). This high-resolution analysis of interactions holds promise for improving our understanding of entire interaction networks (Roslin and Majaneva 2016) and, when applied to vulnerable farmland predators (e.g., aerial insectivores), could provide valuable information on subtle differences in how species use habitats set aside for conservation.

Some trophic interactions will be directly linked to ecosystem services such as pollination or biocontrol of weed seeds and invertebrate pests (Kearns et al. 1998; Klein et al. 2007; Bohan et al. 2011). For example, the faeces of 108 avian species were subject to DNA barcoding to determine the main predators of an economically important invertebrate pest of coffee (Karp et al. 2014). This information can be used to develop strategies to attract and retain target predators that are important in conservation biocontrol. The production of fruits, grains, and nuts is reliant on the essential ecosystem service of pollination. Metabarcoding can identify thousands of plant–pollinator interactions from samples of pollen from bees, presenting a genuine advantage over traditional pollen analysis using light microscopy, which is time consuming and often yields only coarse taxonomic resolution (Wilson et al. 2010; Clare et al. 2013; Sickel et al. 2015; Bell et al. 2016). One study recently identified 650 plant taxa (95% to species level) using the ITS2 region of 384 mixed pollen samples collected from bees (Sickel et al. 2015).

Another important ecosystem service is invertebrate pest control provided by parasitoids. DNA barcoding has revealed host–parasitoid dynamics, showing that host–parasitoid specificity is underestimated (Smith et al. 2006, 2007; Moreno-Ripoll et al. 2012; Alex Smith et al. 2013). Labour-intensive rearing traditionally revealed links between hosts and parasitoids, whereas molecular methods can be used to barcode parasitoid gut contents or traces of parasitoid DNA on hosts (Hrcek et al. 2011). This research provided evidence that there are many more parasitoid species than previously realised: morphological identification is difficult (Hrcek and Godfray 2015), and DNA barcoding suggests that generalist parasitoids are actually several host-specific cryptic species in some cases (Smith et al. 2006; Zhang et al. 2011). The addition of cryptic taxa in ecological networks has the potential to change our understanding of their structure and function (Wirta et al. 2014; Hrcek and Godfray 2015), for example, by altering the number and identities of trophic links (Hrcek et al. 2011), as well as our understanding of the role of parasitoids in the biocontrol of pests.

Identification of agricultural pests

Crop damage from pests causes significant revenue loss and threatens food security (Godfray et al. 2010). Annual costs of damage from insect pests are estimated at $12bn in Brazil (Oliveira et al. 2013), and $14.4bn in the USA (Pimentel et al. 2005). The best predictor of the number of invasive alien species within a country is its degree of international trade, indicating that damage from invasive pests is likely to increase as a result of the globalised agri-food supply chain (Westphal et al. 2008). The identification of invertebrate and weed pests will be important both in ports and in a domestic scenario to record and control invasions, where DNA barcoding is facilitating the rapid and accurate identification and tracking of agricultural pests (Scheffer et al. 2006; Ball and Armstrong 2008; Floyd et al. 2010; Comtet et al. 2015; Tyagi et al. 2015).

The morphological identification of invertebrate pests can be problematic. Accurate identification may require highly skilled taxonomic expertise such as the dissection of male genitalia for identification (e.g., Pauly et al. 2015), which can be complex and time consuming. Sometimes only immature life stages are collected, which lack distinguishing characteristics, or do not retain colouration in storage media such as ethanol. One option is to rear the stages to adulthood, but again this requires both space and time for development, and specialist resources such as temperature-controlled rooms and technicians. There are now multiple examples of molecular methods being used to identify invertebrates at different life stages, for example, the immature stages of syrphid and *Bradysia* (pests of greenhouse crops) fly species (Gomez-Polo et al. 2014; Shin et al. 2015).

Species leave traces of their presence in the environment, which can also be investigated with DNA barcoding, even after the individual is no longer present in the area. Trace DNA can exist for a significant amount of time; for example, spider and prey DNA was detected from the webs of spiders 88 days after the removal of the organisms (Xu et al. 2015). The detection of trace amounts of DNA could be very useful in monitoring and preventing the spread of pests; for example, trace amounts of host and parasitoid DNA (from webs, eggs, frass, or pupal cases) in crops and shipments could be barcoded using HTS and non-specific primers (e.g., Gariepy et al. 2014).

It is important to note that challenges remain for the identification of agricultural pest species with molecular methods. Identifications rely on detailed and accurate reference libraries supporting the analysis, while at the same time invertebrates are still being discovered that are new to science. However, rapid progress is being made, particularly for taxa of special concern. For example, a recent gap analysis comparing barcode sequences in BOLD with a checklist of known arthropod plant pests estimated that 638 of 943 species are currently barcoded (Frewin et al. 2015). This represents a 10% increase in two years, indicating that library coverage is progressing under the Plant Pest Barcoding campaign (Frewin et al. 2015).

Although still faster than rearing and morphological examination, traditional sequencing also involves a time delay and specialised laboratory equipment. This will be critical as inspections and subsequent quarantine are reliant on the rapid identification of pests. One solution is the development of real-time PCR assays (see Text Box 1; Naaum et al. 2012; Islam et al. 2015), which are faster and often portable, making these suitable for scientists in the field or inspection officials surveying for a particular pest of interest. Here, species-specific primers must be developed, or we risk the cross-amplification of closely related species leading to potential false positives. While real-time PCR is a viable solution to tracking targets of identified interest rapidly and with low expense, it lacks the broader scope to identify unexpected pests.

Fig. 2. Major crop pests *Helicoverpa armigera* (left) and *Helicoverpa zea* (right) require genitalia dissection to distinguish between the two which requires time-consuming expertise. Text Box 1 describes the development of a real-time multiplex PCR assay by Gilligan et al. 2015 which uses a single primer pair to identify the moths to species level.

Text Box 1: Hidden *Helicoverpa*: the development of an identification assay for an economically important crop pest

Amongst the most significant agricultural pests in the modern world are the noctuid moths *Helicoverpa armigera* (Old World) and *Helicoverpa zea* (New World). In 2013, the polyphagous *H. armigera* was found to have invaded South America where it had not previously been found, and it was estimated that US crops worth $843m per annum are vulnerable to the expansion of *H. armigera* (Kriticos et al. 2015). The larvae of the two species do not have any characteristics that can be reliably distinguished from each other, and adults can only be separated by dissection of genitalia, making identification time- and resource-intensive (Fig. 2). Gilligan et al. (2015) created a simple real-time PCR probe with 99% accurate identification of both *H. armigera* and *H. zea*, which can be performed in 50 min from isolated DNA. The assay uses a segment of ITS2 which is amplified using just one primer pair. Inconclusive results were generated by 4 out of 452 samples: this error was attributed to a combination of one false negative result (*H. zea*), values outside the established zone for interpretation, and pipetting error. While DNA barcoding has been used to distinguish between these species (Mastrangelo et al. 2014), the lack of a sequencing step in the rapid PCR assessment represents a significant time saving. Real-time PCR assays such as this could be used on larvae and adults intercepted at import/export ports or in domestic settings to track the spread of this invasive pest. It is worth noting that this assay used the ITS2 region rather than the standard animal barcode COI. By using COI in assays, this gives a greater degree of flexibility because inconclusive or negative results can be investigated by sequencing and matching to barcode libraries to identify species.

Identification of agricultural parasites

Livestock disease represents a further significant economic cost to the agricultural industry. In the UK, annual losses of £1.7bn are reported as a result of livestock

disease, with a 17% impact on production (Flint and Woolliams 2008). Many of these livestock diseases result from macro-parasitic infection, such as digeneans, cestodes, and nematodes.

During the treatment of ruminants, parasitic load and species must first be identified, usually by faecal egg counts or ELISA testing. Eggs cannot usually be differentiated morphologically, and reliance on faecal egg counts only identifies the presence of reproductively mature adult parasites (Roeber et al. 2013; Budischak et al. 2015). Eggs can be hatched in faecal cultures and reared until the infective stage, but again larval identification is difficult and usually requires taxonomic expertise. It is also difficult to determine whether a parasitic infection is ongoing or has been cleared by treatment or the immune system, as eggs are not shed consistently. This is also a disadvantage of ELISA testing, as a time-lag exists between initial infection and the production of antibodies, which also persist in the body for a short time after the infection has been cleared (Roeber et al. 2013). Some gastrointestinal nematodes are serologically cross-reactive, which can lead to false positives when using ELISA for species-level identifications (Johnson et al. 1996; Eysker and Ploeger 2000).

DNA barcoding could facilitate species-level identifications of parasites (Elsasser et al. 2009) and ongoing monitoring of infection in livestock. Barcoding can provide taxonomic resolution to species level of eggs or larvae. If parasites also release traces of DNA in addition to eggs, this could be tested non-invasively using DNA barcoding of the faeces. In this way, researchers could identify infection even if eggs were not released consistently or in cases where antibodies persist despite the infection being cleared. Of course, one problem is the persistence of trace DNA post-infection. Work still has to be done to test the quantity of trace DNA that can be detected, the accumulation rate and persistence of that DNA, and whether this is released at a consistent rate which can definitively confirm the presence of a parasitic load.

More broadly, the development of parasite barcoding assays could be used to confirm disease transmission pathways. For example, the protozoan parasite *Neospora* infects cattle but also has hosts in foxes, domestic dogs, and coyotes (McAllister et al. 1998; Gondim et al. 2004). By barcoding the faeces of these animals, it can be confirmed if transmission is through these routes and appropriate animal health measures taken (e.g., enforcing removal of pet dog faeces on farmland in the case of *Neospora*). Molecular methods have also been used alongside morphology to link the adult and immature stages of a parasite's life cycle, which can occur in different hosts (Alcántar-Escalera et al. 2013).

Very little parasite diversity is currently barcoded—only an estimated 3.42% of Nematoda and 3.02% of Platyhelminthes (Kvist 2013), although this percentage increases when considering medically important vectors of human disease (43% of 1403 species) (Ondrejicka et al. 2014). Barcoding campaigns increase the completeness of reference libraries, which in turn aids primer development. For example, the European Union 7th Framework project QBOL: "Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health" aimed to establish barcodes for all European quarantine organisms (Kiewnick et al. 2011).

Multiplex PCR assays have been described that can rapidly distinguish between species of agriculturally important parasites, for example, species of *Eimeria*, the cause of coccidiosis (Fernandez et al. 2003; You 2014). These assays have been utilised in commercial poultry flocks (Ogedengbe et al. 2011), where it is relatively cost effective because tests for seven species of *Eimeria* can be carried out as a single PCR reaction. Schwarz et al. (2009) examined the relationship between the genetic diversity of *Eimeria* infection with flock performance (measured as cost-per-mass produced), and found that more pathogenic species were associated with lower performance farms. DNA barcoding of some parasitic taxa has been problematic; for example, high nucleotide diversity in the COI region of platyhelminths made it difficult to design primers to amplify the entire phylum (Moszczynska et al. 2009). Three new degenerate primers have been tested that achieve 100% sequencing success, in the 6 orders of cestodes and 23 families of digeneans tested. Other flatworm groups were excluded from the design due to high levels of variation in initial sequence alignments (Van Steenkiste et al. 2015).

Preventing the mislabelling of food products

The uses of DNA barcoding as an identification tool extend to the final stages of the agricultural supply chain—the finished products that are distributed to retailers, wholesalers, and consumers. DNA barcoding has revealed the accidental or intentional mislabelling of food products, including additions of species not found on the product label or entire substitutions of animal or plant species. This contentious subject has received high-profile media coverage in recent years, resulting in the withdrawal of affected products from shelves (Mariani et al. 2014). Mislabelling appears to be widespread amongst processed food products, with rates as high as 41% of 236 fish products purchased from Canadian retailers (Hanner et al. 2011) and 18% of 149 fish purchased from South African restaurants and retailers (Cawthorn et al. 2015). Another study of 48 samples originating from ground meat products in California showed that 10 were mislabelled, of which nine contained additional undeclared species other than the specified animal and one was a complete species substitution (Kane and Hellberg 2015). The substitution of products is not limited to the animal supply chain. Recent investigations of herbal products found that 59% of commercial products

contained species not listed as ingredients (Newmaster et al. 2013).

Mislabelling can lead to serious problems for consumers and industry. Consumer safety is put at risk by allergens, interactions with medication or supplements (in the case of mislabelled herb or plant products), and toxicity (Clark 2015). A lack of confidence in industry can result in behaviour change and damage to the revenue and reputation of suppliers, with trickle-down effects to retailers, restaurants, and other food outlets (Barnett et al. 2016). Retailers and distributors may try to mitigate this by entering voluntarily certification schemes, such as the Marine Stewardship Council chain of custody certification, which traces seafood to their fishery of origin through every stage of processing (Marine Stewardship Council Get Certified! Chain of Custody n.d.).

Different factors in the agri-food supply chain contribute to accidental or intentional substitutions. Some related species share physical characteristics; for example, fillets of fish are difficult to distinguish as identifying features have been removed. Some products are composed of the output from many different suppliers; for example, minor crops are often grown by many different small-scale farmers and subsequently combined, resulting in increased potential for contamination in the supply chain (Galimberti et al. 2014). Strong manufacturing processes (mincing, blending, drying, and (or) reconstituting) involved in creating heavily processed products also disguise physical characteristics. The common name problem also complicates this process as food products from different geographic regions may be sold under common names that lack scientific validity, or have multiple uses, and thus substitutions may be inadvertent (Wong and Hanner 2008).

With well-defined reference libraries, DNA barcoding and metabarcoding can identify products to species level despite strong processing or degradation and is likely to play a major role in discovering incorrectly marketed products and blends in the future. DNA barcoding has specific advantages over protein analysis that might have previously been used to examine processed fish or meat, as it can be used on cooked samples in which proteins are distorted or degraded (Bossier 1999). However, only short fragments can be sequenced from highly degraded material, which will complicate the identification of closely related species. Using HTS techniques and mini-primers to amplify multiple short (100–300 bp) fragments can expedite this. Furthermore, issues remain around the use of sensitive molecular methods for the interpretation of the contents of commercial plant products, given the potential for contamination during the acquisition or manufacturing process, shared soil environments, or mycorrhizal relationships during the growth of plants (Ivanova et al. 2015, 2016). Finally, a large portion of food frauds do not involve the addition or substitution of species (e.g., product dilution, substituting farmed for wild fish). DNA barcoding is not always appropriate in these scenarios which may require other techniques such as multi-element analysis or gas chromatography (Ballin 2010; Drivelos and Georgiou 2012). For example, it is generally difficult to determine the geographic provenance of a sample with DNA barcoding, which could be useful for uncovering illegal fishing or protected designation of origin substitutions, although some populations do contain distinct COI sequences (Bogdanowicz et al. 2000). Inferring data on biomass from copy numbers of sequences is still contentious (Deagle et al. 2013) and the subject of system-specific research (Saitoh et al. 2016), so frauds such as product dilution will be difficult to detect.

Regulatory bodies with responsibility for food safety, such as the US Food and Drug Administration (USA), are beginning to implement DNA barcoding to identify product substitutions (Handy et al. 2011). Consumer demand for greater food traceability after recent food fraud scandals will also be a driver for product validation (Barnett et al. 2016). Due to high consumer interest and media coverage, it is likely that DNA barcoding can be used to engage citizen scientists in food safety research; for example, in sample collection as used by Naaum and Hanner (2015). Issues remain, however, around the affordability and accessibility of DNA barcoding in developing countries. Data from South Africa suggests that areas with higher proportions of low-income groups experience a higher incidence of food misrepresentation, possibly due to consumer focus on cost savings rather than food traceability (Cawthorn et al. 2015), or a less strict regulation of domestic supply chains. Developing countries also face unique challenges around species identification in the food chain which could be assisted by the use of DNA barcoding; for example, unidentified meat sources in bushmeat markets can pose risks from zoonotic diseases or illegal hunting that threatens endangered species (Wolfe et al. 2004; Minhós et al. 2013). There is some scope for resource matching and sharing between countries with significant biodiversity and those with significant infrastructure, but this has been targeted more at the library-building component of DNA barcoding. As global food chains expand through trade, such resource matching may be mutually advantageous.

Issues and implementation

DNA barcoding has significant advantages and is likely to play a major part in sample identification in the future, either alone or to complement existing methods. Sequencing mixed samples in large quantities is cheaper than ever using HTS techniques when compared with traditional Sanger sequencing (Shokralla et al. 2014), with recent estimates of cost at $20 per metabarcoded sample (materials cost only, Sickel et al. 2015). While the initial generation of reference barcodes requires bidirectional sequencing of the highest quality, subsequent

studies could save costs with unidirectional sequencing or qPCR, which would be sufficient for species detection. The approaches differentiate between the high-quality sequences required for reference library generation and relatively low quality but less expensive approaches required to confirm the presence of a particular species. Degraded samples can be used in DNA barcoding which might not be possible in morphological analyses; samples can be collected as raw, cooked, frozen, mixed, or preserved in relatively cheap kinds of medium such as ethanol. Procedures developed in ancient DNA laboratories mean that even very small amounts of degraded DNA can be used in combination with HTS techniques, whereas reasonably high-quality DNA is needed for Sanger sequencing. Identification by third parties can be auditable, and a subjective element of identification is removed due to its reliance on a reference library (Clark 2015), once these libraries are built and validated to obtain accurate identifications. Open access tools will increase reproducibility of barcode data further—bioinformatics scripts can be uploaded to github, and data can be stored in online repositories such as EBI, NCBI, DataDryad, or Figshare, although long-term accessible storage space for large datasets is under pressure.

Some common issues remain across DNA barcoding and metabarcoding studies. Choice of methods can be a contentious and confusing process when designing a metabarcoding study. Considerable laboratory expertise and resources may be required; for example, some eDNA protocols have advocated the use of positive and negative air pressure rooms and whole-room irradiation to restrict contamination (Biggs et al. 2015). All studies must make extensive use of both field and laboratory controls to monitor contamination. The inclusion of mock communities alongside mixtures of unknown samples should be an important part of ensuring that laboratory and bioinformatics methods development are fit for purpose, for example, resulting in the construction of reliable estimates of Molecular Operational Taxonomic Unit (MOTU) diversity (Clare et al. 2016). Finally, amplicon sequencing is problematic, due to taxonomic bias and the potential amplification of contaminants, but PCR-free sequencing is on the horizon (Liu et al. 2016).

Once methods have been selected, interpreting relative and absolute abundance of organisms from DNA barcode reads remains contentious. Some studies have shown that eDNA extracted from water is a good indicator of relative species abundance in aquatic systems (Lodge et al. 2012; Thomsen et al. 2012; Pilliod et al. 2014), and while this provides a good starting point, it remains a thorny issue for terrestrial systems (Saitoh et al. 2016), for invertebrate species (due to primer bias), or when degraded DNA is used (King et al. 2008). One solution is to model occupancy (species-level) or spatial mark-recapture (individual-level) data, although careful experimental designs are needed before data collection can begin (Schnell et al. 2015). Finally, barcoding techniques can always be used in parallel with a subsample of traditional trapping methods and morphological analysis of samples (Furlong 2015).

Standardisation across laboratories is an issue when assays are used as a management tool (e.g., quantifying food fraud) or to inform policy relating to biodiversity monitoring (Griffiths et al. 2014). In addition to molecular laboratory techniques, metabarcoding datasets also require the development of bioinformatics pipelines. Programmes and data cleaning steps vary between research groups; the different clustering methods and data cleaning steps can considerably change the estimate of MOTU diversity (Brandon-Mong et al. 2015; Clare et al. 2016). Analysis of how bioinformatics pipelines affect ecological conclusions will be valuable, but as yet they are rare (although see Clare et al. 2016; Roslin and Majaneva 2016).

Text box 2: Outstanding R&D questions for the use of barcoding methods in agriculture

Agro-ecosystems

- How can we use mock communities and bioinformatics pipelines validated against ecological systems to produce reliable estimates of MOTU?
- Can eDNA and eRNA be used as a reliable indicator of the presence and diversity of living organisms? What controls the rates of eDNA and eRNA production, dissemination throughout the environment, and degradation?
- Development of PCR-free techniques to remove problems with taxa-specific amplification bias and the amplification of contaminants. Investigate the relationship between the products of PCR-free sequencing and species biomass/abundance.
- Development of genome skimming techniques using shallow-pass shotgun sequencing for DNA-based mark-recapture studies, for example, mammals/birds in agricultural landscapes, and subsequent estimates of population size.

Pests and parasites

- Is eDNA produced by parasites in faeces and is it a reliable indicator of infection status? How does it compare to ELISA and egg counting techniques?
- Continued library building through barcoding drives for reference databases of understudied taxa, many of which are pest and parasite species.

Food fraud

- Development of fast, cheap assays for the high-throughput sampling of products. A high degree of accuracy will be required to ensure consumer safety and the reputation of industry.
- Integration of DNA barcoding into designation of origin fraud or wild-farmed fraud, possibly through

sequencing larger areas than the standard DNA barcode ("ultrabarcoding" or genome skimming). Implementation will be context-dependent on the study system.

Conclusions and future directions

As with other genomics technologies, barcoding data will become more accessible in the future. As sequencing costs and the person-hours required drop, the assays themselves will become quicker and cheaper to perform. The rise of portable handheld sequencers will allow very fast in-situ sequencing (Hayden 2015), which could be used to perform pest identifications at ports or to assess biodiversity in real-time. With the shift from Sanger sequencing to HTS techniques, more sequence information can be collected. The development of primers for mini-barcodes means that even information from degraded samples can be recovered where this was not previously possible. Finally, it is likely that ease-of-use and decreasing costs will put DNA barcoding into the hands of farmers and citizen scientists (Adamowicz and Steinke 2015).

The agricultural industry faces the twin challenges of supporting biodiversity and ecosystem functioning, while providing safe traceable food to feed the world. Supply chains are further threatened by inefficiencies that result from waste and loss of harvest due to pests and parasites. Barcoding technology allows researchers to identify both individual species and the diversity of mixed samples, which we have argued will have diverse applications in agro-ecosystems and the agri-food supply chain. It will require academics and private laboratories at the forefront of DNA barcoding to work collaboratively alongside agro-industry, from individual farmers to large-scale companies and regulators.

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References

Adamowicz, S.J., and Steinke, D. 2015. Increasing global participation in genetics research through DNA barcoding. Genome, **58**(12): 519–526. doi:10.1139/gen-2015-0130. PMID:26642251.

Alcántar-Escalera, F.J., García-Varela, M., Vázquez-Domínguez, E., and Pérez-Ponce de León, G. 2013. Using DNA barcoding to link cystacanths and adults of the acanthocephalan *Polymorphus brevis* in central Mexico. Mol. Ecol. Resour. **13**(6): 1116–1124. doi:10.1111/1755-0998.12090. PMID:23480472.

Alex Smith, M., Fernández-Triana, J.L., Eveleigh, E., Gómez, J., Guclu, C., Hallwachs, W., et al. 2013. DNA barcoding and the taxonomy of Microgastrinae wasps (Hymenoptera, Braconidae): impacts after 8 years and nearly 20 000 sequences. Mol. Ecol. Resour. **13**(2): 168–176. doi:10.1111/1755-0998.12038. PMID:23228011.

Andersen, K., Bird, K.L., Rasmussen, M., Haile, J., Breuning-Madsen, H., Kjaer, K.H., et al. 2012. Meta-barcoding of "dirt" DNA from soil reflects vertebrate biodiversity. Mol. Ecol. **21**(8): 1966–1979. doi:10.1111/j.1365-294X.2011.05261.x. PMID:21917035.

Ardura, A., Zaiko, A., Martinez, J.L., Samuiloviene, A., Borrell, Y., and Garcia-Vazquez, E. 2015. Environmental DNA evidence of transfer of North Sea molluscs across tropical waters through ballast water. J. Mollusc. Stud. **81**: 495–501. doi:10.1093/mollus/eyv022.

Ball, S.L., and Armstrong, K.F. 2008. Rapid, one-step DNA extraction for insect pest identification by using DNA barcodes. J. Econ. Entomol. **101**(2): 523–532. doi:10.1093/jee/101.2.523. PMID:18459420.

Ballin, N.Z. 2010. Authentication of meat and meat products. Meat Sci. **86**(3): 577–587. doi:10.1016/j.meatsci.2010.06.001. PMID:20685045.

Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D., and Schmid, B. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. **9**(10): 1146–1156. doi:10.1111/j.1461-0248.2006.00963.x. PMID:16972878.

Barnes, M.A., and Turner, C.R. 2016. The ecology of environmental DNA and implications for conservation genetics. Conserv. Genet. **17**: 1–17. doi:10.1007/s10592-015-0775-4.

Barnett, J., Begen, F., Howes, S., Regan, A., McConnon, A., Marcu, A., et al. 2016. Consumers' confidence, reflections and response strategies following the horsemeat incident. Food Control, **59**: 721–730. doi:10.1016/j.foodcont.2015.06.021.

Bell, K.L., de Vere, N., Keller, A., Richardson, R.T., Gous, A., Burgess, K.S., and Brosi, B.J. 2016. Pollen DNA barcoding: current applications and future prospects. Genome, **59**(9): 629–640. doi:10.1139/gen-2015-0200.

Bender, S.F., Wagg, C., and van der Heijden, M.G.A. 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol. Evol. **31**(6): 440–452. doi:10.1016/j.tree.2016.02.016. PMID:26993667.

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R.A., et al. 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). Biol. Conserv. **183**: 19–28. doi:10.1016/j.biocon.2014.11.029.

Bogdanowicz, S.M., Schaefer, P.W., and Harrison, R.G. 2000. Mitochondrial DNA variation among worldwide populations of gypsy moths, *Lymantria dispar*. Mol. Phylogenet. Evol. **15**(3): 487–495. doi:10.1006/mpev.1999.0744. PMID:10860656.

Bohan, D.A., Boursault, A., Brooks, D.R., and Petit, S. 2011. National-scale regulation of the weed seedbank by carabid predators. J. Appl. Ecol. **48**(4): 888–898. doi:10.1111/j.1365-2664.2011.02008.x.

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., et al. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. Trends Ecol. Evol. **29**(6): 358–367. doi:10.1016/j.tree.2014.04.003. PMID:24821515.

Bossier, P. 1999. Authentication of seafood products by DNA patterns. J. Food Sci. **64**(2): 189–193. doi:10.1111/j.1365-2621.1999.tb15862.x.

Brandon-Mong, G.-J., Gan, H.-M., Sing, K.-W., Lee, P.-S., Lim, P.-E., and Wilson, J.-J. 2015. DNA metabarcoding of insects and allies: an evaluation of primers and pipelines. Bull. Entomol.

Res. **105**(6): 717–727. doi:10.1017/S0007485315000681. PMID: 26344799.

Budischak, S.A., Hoberg, E.P., Abrams, A., Jolles, A.E., and Ezenwa, V.O. 2015. A combined parasitological molecular approach for noninvasive characterization of parasitic nematode communities in wild hosts. Mol. Ecol. Resour. **15**(5): 1112–1119. doi:10.1111/1755-0998.12382. PMID:25644900.

Caldwell, A.C., Silva, L.C.F., Da Silva, C.C., and Ouverney, C.C. 2015. Prokaryotic diversity in the rhizosphere of organic, intensive, and transitional coffee farms in Brazil. PLoS ONE, **10**(6): 1–17. doi:10.1371/journal.pone.0106355.

Cawthorn, D.M., Duncan, J., Kastern, C., Francis, J., and Hoffman, L.C. 2015. Fish species substitution and misnaming in South Africa: an economic, safety and sustainability conundrum revisited. Food Chem. **185**: 165–181. doi:10.1016/j.foodchem.2015.03.113. PMID:25952855.

CBOL Plant Working Group. 2009. A DNA barcode for land plants. Proc. Natl. Acad. Sci. U.S.A. **106**: 12794–12797. doi:10.1073/pnas.0905845106. PMID:19666622.

Clare, E.L. 2014. Molecular detection of trophic interactions: emerging trends, distinct advantages, significant considerations and conservation applications. Evol. Appl. **7**: 1144–1157. doi:10.1111/eva.12225. PMID:25553074.

Clare, E.L., Schiestl, F.P., Leitch, A.R., and Chittka, L. 2013. The promise of genomics in the study of plant–pollinator interactions. Genome Biol. **14**(6): 207. doi:10.1186/gb-2013-14-6-207. PMID:23796166.

Clare, E.L., Chain, F.J.J., Littlefair, J.E., and Cristescu, M.E. 2016. The effects of parameter choice on defining molecular operational taxonomic units and resulting ecological analyses of metabarcoding data. Genome, **59**. [Online ahead of print.] doi:10.1139/gen-2015-0184.

Clark, L.F. 2015. The current status of DNA barcoding technology for species identification in fish value chains. Food Pol. **54**: 85–94. doi:10.1016/j.foodpol.2015.05.005.

Comtet, T., Sandionigi, A., Viard, F., and Casiraghi, M. 2015. DNA (meta)barcoding of biological invasions: a powerful tool to elucidate invasion processes and help managing aliens. Biol. Invasions, **17**(3): 905–922. doi:10.1007/s10530-015-0854-y.

Cristescu, M.E. 2014. From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. Trends Ecol. Evol. **29**(10): 566–571. doi:10.1016/j.tree.2014.08.001. PMID: 25175416.

Deagle, B.E., Thomas, A.C., Shaffer, A.K., Trites, A.W., and Jarman, S.N. 2013. Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? Mol. Ecol. Resour. **13**(4): 620–633. doi:10.1111/1755-0998.12103. PMID:23590207.

Drivelos, S.A., and Georgiou, C.A. 2012. Multi-element and multi-isotope-ratio analysis to determine the geographical origin of foods in the European Union. Trends Anal. Chem. **40**: 38–51. doi:10.1016/j.trac.2012.08.003.

Elsasser, S.C., Floyd, R., Hebert, P.D.N., and Schulte-Hostedde, A.I. 2009. Species identification of North American guinea worms (Nematoda: Dracunculus) with DNA barcoding. Mol. Ecol. Resour. **9**(3): 707–712. doi:10.1111/j.1755-0998.2008.02393.x. PMID:21564728.

EUR-Lex-52011PC0625. 2011. Proposal for a regulation of the European parliament and of the council establishing rules for direct payments to farmers under support schemes within the framework of the common agricultural policy. Available from http://eur-lex.europa.eu/legal-content/EN/NOT/?uri=CELEX:52011PC0625.

Eysker, M., and Ploeger, H.W. 2000. Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. Parasitology, **120**: S109–S119. doi:10.1017/S0031182099005752. PMID:10874714.

Fernandez, S., Pagotto, A.H., Furtado, M.M., Katsuyama, A.M., Madeira, A.M.B.N., and Gruber, A. 2003. A multiplex PCR assay for the simultaneous detection and discrimination of the seven *Eimeria* species that infect domestic fowl. Parasitology, **127**: 317–325. doi:10.1017/S0031182003003883. PMID:14636018.

Flint, A.P.F., and Woolliams, J.A. 2008. Precision animal breeding. Philos. Trans. R. Soc. B Biol. Sci. **363**: 573–590. doi:10.1098/rstb.2007.2171.

Floyd, R., Lima, J., de Waard, J., Humble, L., and Hanner, R. 2010. Common goals: Policy implications of DNA barcoding as a protocol for identification of arthropod pests. Biol. Invasions, **12**(9): 2947–2954. doi:10.1007/s10530-010-9709-8.

Frewin, A., Scott-Dupree, C., and Hanner, R.H. 2015. Plant Pest Barcoding Campaign update. *In* Scientific abstracts from the 6th International Barcode of Life Conference. Genome, **58**(5): 217. doi:10.1139/gen-2015-0087.

Furlong, M.J. 2015. Knowing your enemies: integrating molecular and ecological methods to assess the impact of arthropod predators on crop pests. Insect Sci. **22**: 6–19. doi:10.1111/1744-7917.12157. PMID:25081301.

Galimberti, A., Labra, M., Sandionigi, A., Bruno, A., Mezzasalma, V., and De Mattia, F. 2014. DNA barcoding for minor crops and food traceability. Adv. Agric. **2014**: 1–8. doi:10.1155/2014/831875.

Gariepy, T.D., Haye, T., and Zhang, J. 2014. A molecular diagnostic tool for the preliminary assessment of host-parasitoid associations in biological control programmes for a new invasive pest. Mol. Ecol. **23**(15): 3912–3924. doi:10.1111/mec.12515. PMID:24102670.

Geijzendorffer, I.R., Targetti, S., Schneider, M.K., Brus, D.J., Jeanneret, P., Jongman, R.H.G., et al. 2016. How much would it cost to monitor farmland biodiversity in Europe? J. Appl. Ecol. **53**(1): 140–149. doi:10.1111/1365-2664.12552.

Gibson, J.F., Shokralla, S., Curry, C., Baird, D.J., Monk, W.A., King, I., and Hajibabaei, M. 2015. Large-scale biomonitoring of remote and threatened ecosystems via high-throughput sequencing. PLoS ONE, **10**(10): e0138432. doi:10.1371/journal.pone.0138432. PMID:26488407.

Gilligan, T.M., Tembrock, L.R., Farris, R.E., Barr, N.B., van der Straten, M.J., van de Vossenberg, B.T.L.H., and Metz-Verschure, E. 2015. A multiplex real-time PCR assay to diagnose and separate *Helicoverpa armigera* and *H. zea* (Lepidoptera: Noctuidae) in the New World. PLoS ONE, **10**(11): e0142912. doi:10.1371/journal.pone.0142912.

Godfray, H.C.J. 2011. Food and biodiversity. Science, **333**: 1231–1232. doi:10.1126/science.1211815. PMID:21885765.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., et al. 2010. The challenge of feeding 9 billion people. Science, **327**(5967): 812–818. doi:10.1126/science.1185383. PMID:20110467.

Goldberg, C.S., Strickler, K.M., and Pilliod, D.S. 2015. Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. Biol. Conserv. **183**: 1–3. doi:10.1016/j.biocon.2014.11.040.

Gomez-Polo, P., Traugott, M., Alomar, O., Castane, C., Rojo, S., and Agusti, N. 2014. Identification of the most common predatory hoverflies of Mediterranean vegetable crops and their parasitism using multiplex PCR. J. Pest Sci. **87**: 371–378. doi:10.1007/s10340-013-0550-6.

Gondim, L.F.P., McAllister, M.M., Pitt, W.C., and Zemlicka, D.E. 2004. Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. Int. J. Parasitol. **34**(2): 159–161. doi:10.1016/j.ijpara.2004.01.001. PMID:15037103.

Griffiths, A.M., Sotelo, C.G., Mendes, R., Pérez-Martín, R.I., Schröder, U., Shorten, M., et al. 2014. Current methods for seafood authenticity testing in Europe: Is there a need for harmonisation? Food Control, **45**: 95–100. doi:10.1016/j.foodcont.2014.04.020.

Handy, S.M., Deeds, J.R., Ivanova, N.V., Hebert, P.D.N.,

Hanner, R.H., Ormos, A., et al. 2011. A single-laboratory validated method for the generation of DNA barcodes for the identification of fish for regulatory compliance. J. AOAC Int. **94**(1): 201–211. PMID:21391497.

Hanner, R., Becker, S., Ivanova, N.V., and Steinke, D. 2011. FISH-BOL and seafood identification: geographically dispersed case studies reveal systemic market substitution across Canada. Mitochondrial DNA, **22**(s1): 106–122. doi:10.3109/19401736.2011.588217. PMID:21980986.

Hayden, E.C. 2015. Pint-sized DNA sequencer impresses first users. Nature, **521**: 15–16. doi:10.1038/521015a. PMID:25951262.

Hebert, P.D.N., Cywinska, A., Ball, S.L., and deWaard, J.R. 2003. Biological identifications through DNA barcodes. Proc. R. Soc. B Biol. Sci. **270**(1512): 313–321. doi:10.1098/rspb.2002.2218.

Herdt, R.W. 2006. Biotechnology in agriculture. Annu. Rev. Environ. Resour. **31**: 265–295. doi:10.1146/annurev.energy.31.031405.091314.

Hrcek, J., and Godfray, H.C.J. 2015. What do molecular methods bring to host–parasitoid food webs? Trends Parasitol. **31**(1): 30–35. doi:10.1016/j.pt.2014.10.008. PMID:25467127.

Hrcek, J., Miller, S.E., Quicke, D.L.J., and Smith, M.A. 2011. Molecular detection of trophic links in a complex insect host–parasitoid food web. Mol. Ecol. Resour. **11**(5): 786–794. doi:10.1111/j.1755-0998.2011.03016.x. PMID:21535428.

Hubert, N., and Hanner, R. 2015. DNA Barcoding, species delineation and taxonomy: a historical perspective. DNA Barcodes, **3**: 44–58. doi:10.1515/dna-2015-0006.

Islam, M.S., Barr, N.B., Braswell, W.E., Martinez, M., Ledezma, L.A., Molongoski, J., et al. 2015. A multiplex real-time PCR assay for screening gypsy moths (Lepidoptera: Erebidae) in the United States for evidence of an Asian genotype. J. Econ. Entomol. **108**(5): 2450–2457. doi:10.1093/jee/tov212. PMID:26453734.

Ivanova, N., Kuzmina, M., and Braukmann, T. 2015. Pandora's Box in a pill—unveiling the composition of herbal supplements. *In* Scientific abstracts from the 6th International Barcode of Life Conference. Genome, **58**(5): 231. doi:10.1139/gen-2015-0087.

Ivanova, N.V., Kuzmina, M.L., Braukmann, T.W.A., Borisenko, A.V., and Zakharov, E.V. 2016. Authentication of herbal supplements using next-generation sequencing. PLoS ONE, **11**(5): e0156426. doi:10.1371/journal.pone.0156426. PMID:27227830.

Janzen, D.H., Hajibabaei, M., Burns, J.M., Hallwachs, W., Remigio, E., and Hebert, P.D.N. 2005. Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. Philos. Trans. R. Soc. B Biol. Sci. **360**(1462): 1835–1845. doi:10.1098/rstb.2005.1715.

Ji, Y., Ashton, L., Pedley, S.M., Edwards, D.P., Tang, Y., Nakamura, A., et al. 2013. Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecol. Lett. **16**(10): 1245–1257. doi:10.1111/ele.12162. PMID:23910579.

Johnson, M.J., Behnke, J.M., and Coles, G.C. 1996. Detection of gastrointestinal nematodes by a coproantigen capture ELISA. Res. Vet. Sci. **60**: 7–12. doi:10.1016/S0034-5288(96)90122-8. PMID:8745247.

Kane, D.E., and Hellberg, R.S. 2015. Identification of species in ground meat products sold on the U.S. commercial market using DNA-based methods. Food Control, **59**: 158–163. doi:10.1016/j.foodcont.2015.05.020.

Karp, D.S., Judson, S., Daily, G.C., and Hadly, E.A. 2014. Molecular diagnosis of bird-mediated pest consumption in tropical farmland. Springerplus, **3**: 630. doi:10.1186/2193-1801-3-630. PMID:25392800.

Kearns, C.A., Inouye, D.W., and Waser, N.M. 1998. Endangered mutualisms: the conservation of plant–pollinator interactions. Annu. Rev. Ecol. Syst. **29**: 83–112. doi:10.1146/annurev.ecolsys.29.1.83.

Kiewnick, S., Holterman, M., Helder, H., and Frey, J. 2011. QBOL—Barcoding as a new tool for identification of quarantine nematodes and their close relatives. Phytopathology, **101**(6): S90.

King, R.A., Read, D.S., Traugott, M., and Symondson, W.O.C. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. **17**(4): 947–963. doi:10.1111/j.1365-294X.2007.03613.x. PMID:18208490.

King, R.A., Symondson, W.O.C., and Thomas, R.J. 2015. Molecular analysis of faecal samples from birds to identify potential crop pests and useful biocontrol agents in natural areas. Bull. Entomol. Res. **105**(3): 261–272. doi:10.1017/S0007485314000935. PMID:25572526.

Kleijn, D., Kohler, F., Báldi, A., Batáry, P., Concepción, E.D., Clough, Y., et al. 2009. On the relationship between farmland biodiversity and land-use intensity in Europe. Proc. R. Soc. B Biol. Sci. **276**(1658): 903–909. doi:10.1098/rspb.2008.1509.

Klein, A.-M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., and Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. **274**(1608): 303–313. doi:10.1098/rspb.2006.3721.

Kress, W.J., García-Robledo, C., Uriarte, M., and Erickson, D.L. 2015. DNA barcodes for ecology, evolution, and conservation. Trends Ecol. Evol. **30**(1): 25–35. doi:10.1016/j.tree.2014.10.008. PMID:25468359.

Kriticos, D.J., Ota, N., Hutchison, W.D., Beddow, J., Walsh, T., Tay, W.T., et al. 2015. The potential distribution of invading *Helicoverpa armigera* in North America: is it just a matter of time? PLoS ONE, **10**(3): e0119618. doi:10.1371/journal.pone.0119618. PMID:25786260.

Kvist, S. 2013. Barcoding in the dark?: A critical view of the sufficiency of zoological DNA barcoding databases and a plea for broader integration of taxonomic knowledge. Mol. Phylogenet. Evol. **69**: 39–45. doi:10.1016/j.ympev.2013.05.012. PMID:23721749.

Lee, J., Gereffi, G., and Beauvais, J. 2012. Global value chains and agrifood standards: challenges and possibilities for smallholders in developing countries. Proc. Natl. Acad. Sci. **109**(31): 12326–12331. doi:10.1073/pnas.0913714108. PMID:21149723.

Liu, S., Wang, X., Xie, L., Tan, M., Li, Z., Su, X., et al. 2016. Mitochondrial capture enriches mito-DNA 100 folds enabling PCR-free mitogenomics biodiversity analysis. Mol. Ecol. Resour. **16**: 470–479. doi:10.1111/1755-0998.12472. PMID:26425990.

Lodge, D.M., Turner, C.R., Jerde, C.L., Barnes, M.A., Chadderton, L., Egan, S.P., et al. 2012. Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA. Mol. Ecol. **21**(11): 2555–2558. doi:10.1111/j.1365-294X.2012.05600.x. PMID:22624944.

Mariani, S., Ellis, J., O'Reilly, A., Bréchon, A.L., Sacchi, C., and Miller, D.D. 2014. Mass media influence and the regulation of illegal practices in the seafood market. Conserv. Lett. **7**(5): 478–483. doi:10.1111/conl.12085.

Marine Stewardship Council Get Certified! Chain of Custody. (n.d.). [Online.] Available from https://www.msc.org/get-certified/supply-chain [accessed 11 May 2016].

Mastrangelo, T., Paulo, D.F., Bergamo, L.W., Morais, E.G.F., Silva, M., Bezerra-Silva, G., and Azeredo-Espin, A.M.L. 2014. Detection and genetic diversity of a heliothine invader (Lepidoptera: Noctuidae) from north and northeast of Brazil. J. Econ. Entomol. **107**(3): 970–980. doi:10.1603/EC13403. PMID:25026655.

McAllister, M.M., Dubey, J.P., Lindsay, D.S., Jolley, W.R., Wills, R.A., and McGuire, A.M. 1998. Dogs are definitive hosts of *Neospora caninum*. Int. J. Parasitol. **28**: 1473–1478. doi:10.1016/S0020-7519(98)00138-6. PMID:9770635.

Minhós, T., Wallace, E., Ferreira da Silva, M.J., Sá, R.M., Carmo, M., Barata, A., and Bruford, M.W. 2013. DNA identification of primate bushmeat from urban markets in Guinea-

Bissau and its implications for conservation. Biol. Conserv. **167**: 43–49. doi:10.1016/j.biocon.2013.07.018.

Moonen, A.C., and Bàrberi, P. 2008. Functional biodiversity: an agroecosystem approach. Agric. Ecosyst. Environ. **127**: 7–21. doi:10.1016/j.agee.2008.02.013.

Moreno-Ripoll, R., Gabarra, R., Symondson, W.O.C., King, R.A., and Agustí, N. 2012. Trophic relationships between predators, whiteflies and their parasitoids in tomato greenhouses: a molecular approach. Bull. Entomol. Res. **102**: 415–423. doi:10.1017/S0007485311000836. PMID:22314013.

Moszczynska, A., Locke, S.A., McLaughlin, J.D., Marcogliese, D.J., and Crease, T.J. 2009. Development of primers for the mitochondrial cytochrome *c* oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. Mol. Ecol. Resour. **9**: 75–82. doi:10.1111/j.1755-0998.2009.02634.x. PMID:21564967.

Naaum, A.M., and Hanner, R. 2015. Community engagement in seafood identification using DNA barcoding reveals market substitution in Canadian seafood. DNA Barcodes, **3**: 74–79. doi:10.1515/dna-2015-0009.

Naaum, A.M., Foottit, R.G., Maw, H.E.L., and Hanner, R. 2012. Differentiation between *Aphis pomi* and *Aphis spiraecola* using multiplex real-time PCR based on DNA barcode sequences. J. Appl. Entomol. **136**(9): 704–710. doi:10.1111/j.1439-0418.2012.01706.x.

Newmaster, S.G., Grguric, M., Shanmughanandhan, D., Ramalingam, S., and Ragupathy, S. 2013. DNA barcoding detects contamination and substitution in North American herbal products. BMC Med. **11**: 222. doi:10.1186/1741-7015-11-222. PMID:24120035.

Ogedengbe, J.D., Hunter, D.B., and Barta, J.R. 2011. Molecular identification of *Eimeria* species infecting market-age meat chickens in commercial flocks in Ontario. Vet. Parasitol. **178**: 350–354. doi:10.1016/j.vetpar.2011.01.009. PMID:21295915.

Oliveira, C.M., Auad, A.M., Mendes, S.M., and Frizzas, M.R. 2013. Economic impact of exotic insect pests in Brazilian agriculture. J. Appl. Entomol. **137**(1–2): 1–15. doi:10.1111/jen.12018.

Ondrejicka, D.A., Locke, S.A., Morey, K., Borisenko, A.V., and Hanner, R.H. 2014. Status and prospects of DNA barcoding in medically important parasites and vectors. Trends Parasitol. **30**(12): 582–591. doi:10.1016/j.pt.2014.09.003. PMID:25447202.

Orgiazzi, A., Dunbar, M.B., Panagos, P., de Groot, G.A., and Lemanceau, P. 2015. Soil biodiversity and DNA barcodes: opportunities and challenges. Soil Biol. Biochem. **80**: 244–250. doi:10.1016/j.soilbio.2014.10.014.

Pauly, A., Devalez, J., Sonet, G., Nagy, Z.T., and Boevé, J. 2015. DNA barcoding and male genital morphology reveal five new cryptic species in the West Palearctic bee *Seladonia smaragdula* (Vachal, 1895) (Hymenoptera: Apoidea: Halictidae). Zootaxa, **4034**(2): 257–290. doi:10.11646/zootaxa.4034.2.2. PMID:26624441.

Pilliod, D.S., Goldberg, C.S., Arkle, R.S., and Waits, L.P. 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. Mol. Ecol. Resour. **14**: 109–116. doi:10.1111/1755-0998.12159. PMID:24034561.

Pimentel, D., Zuniga, R., and Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. **52**: 273–288. doi:10.1016/j.ecolecon.2004.10.002.

Piñol, J., San Andrés, V., Clare, E.L., Mir, G., and Symondson, W.O.C. 2014. A pragmatic approach to the analysis of diets of generalist predators: the use of next-generation sequencing with no blocking probes. Mol. Ecol. Resour. **14**: 18–26. doi:10.1111/1755-0998.12156. PMID:23957910.

Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., and Taberlet, P. 2012. Who is eating what: diet assessment using next generation sequencing. Mol. Ecol. **21**(8): 1931–1950. doi:10.1111/j.1365-294X.2011.05403.x. PMID:22171763.

Ratnasingham, S., and Hebert, P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Mol. Ecol. Notes, **7**: 355–364. doi:10.1111/j.1471-8286.2006.01678.x.

Roeber, F., Jex, A.R., and Gasser, R.B. 2013. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance—an Australian perspective. Parasit. Vectors, **6**(1): 153. doi:10.1186/1756-3305-6-153.

Roslin, T., and Majaneva, S. 2016. The use of DNA barcodes in food web construction—terrestrial and aquatic ecologists unite! Genome, **59**(9): 603–628. doi:10.1139/gen-2015-0229.

Saitoh, S., Aoyama, H., Fujii, S., Sunagawa, H., Nagahama, H., Akutsu, M., et al. 2016. A quantitative protocol for DNA metabarcoding of springtails (Collembola). Genome, **59**(9) 705–723. doi:10.1139/gen-2015-0228.

Scheffer, S.J., Lewis, M.L., and Joshi, R.C. 2006. DNA barcoding applied to invasive leafminers (Diptera: Agromyzidae) in the Philippines. Ann. Entomol. Soc. Am. **99**(2): 204–210. doi:10.1603/0013-8746(2006)099[0204:DBATIL]2.0.CO;2.

Schnell, I.B., Sollmann, R., Calvignac-Spencer, S., Siddall, M.E., Yu, D.W., Wilting, A., and Gilbert, M.T.P. 2015. iDNA from terrestrial haematophagous leeches as a wildlife surveying and monitoring tool—prospects, pitfalls and avenues to be developed. Front. Zool. **12**(1): 24. doi:10.1186/s12983-015-0115-z. PMID:26430464.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., et al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc. Natl. Acad. Sci. U.S.A. **109**(16): 6241–6246. doi:10.1073/pnas.1117018109. PMID:22454494.

Schwarz, R.S., Jenkins, M.C., Klopp, S., and Miska, K.B. 2009. Genomic analysis of *Eimeria* spp. populations in relation to performance levels of broiler chicken farms in Arkansas and North Carolina. J. Parasitol. **95**(4): 871–880. doi:10.1645/GE-1898.1. PMID:20049993.

Sheppard, S.K., and Harwood, J.D. 2005. Advances in molecular ecology: tracking trophic links through predator–prey foodwebs. Funct. Ecol. **19**(5): 751–762. doi:10.1111/j.1365-2435.2005.01041.x.

Shin, S., Jung, S., Heller, K., Menzel, F., Hong, T.K., Shin, J.S., et al. 2015. DNA barcoding of *Bradysia* (Diptera: Sciaridae) for detection of the immature stages on agricultural crops. J. Appl. Entomol. **139**(8): 638–645. doi:10.1111/jen.12198.

Shokralla, S., Gibson, J.F., Nikbakht, H., Janzen, D.H., Hallwachs, W., and Hajibabaei, M. 2014. Next-generation DNA barcoding: using next-generation sequencing to enhance and accelerate DNA barcode capture from single specimens. Mol. Ecol. Resour. **14**(5): 892–901. doi:10.1111/1755-0998.12236. PMID:24641208.

Shokralla, S., Porter, T.M., Gibson, J.F., Dobosz, R., Janzen, D.H., Hallwachs, W., et al. 2015. Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform. Sci. Rep. **5**: 9687. doi:10.1038/srep09687. PMID:25884109.

Sickel, W., Ankenbrand, M.J., Grimmer, G., Holzschuh, A., Härtel, S., Lanzen, J., et al. 2015. Increased efficiency in identifying mixed pollen samples by meta-barcoding with a dual-indexing approach. BMC Ecol. **15**(1): 20. doi:10.1186/s12898-015-0051-y. PMID:26194794.

Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W., and Hebert, P.D.N. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). Proc. Natl. Acad. Sci. U.S.A. **103**(10): 3657–3662. doi:10.1073/pnas.0511318103. PMID:16505365.

Smith, M.A., Wood, D.M., Janzen, D.H., Hallwachs, W., and Hebert, P.D.N. 2007. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Ta-

chinidae) are not all generalists. Proc. Natl. Acad. Sci. U.S.A. **104**(12): 4967–4972. doi:10.1073/pnas.0700050104. PMID: 17360352.

Syaripuddin, K., Sing, K., and Wilson, J. 2015. Comparison of butterflies, bats and beetles as bioindicators based on four key criteria and DNA barcodes. Trop. Conserv. Sci. **8**(1): 138–149.

Taberlet, P., Coissac, E., Hajibabaei, M., and Rieseberg, L.H. 2012*a*. Environmental DNA. Mol. Ecol. **21**(8): 1789–1793. doi: 10.1111/j.1365-294X.2012.05542.x. PMID:22486819.

Taberlet, P.P., Coissac, E., Pompanon, F.F., Brochmann, C., and Willerslev, E. 2012*b*. Towards next-generation biodiversity assessment using DNA metabarcoding. Mol. Ecol. **21**(8): 2045–2050. doi:10.1111/j.1365-294X.2012.05470.x. PMID:22486824.

Targetti, S., Herzog, F., Geijzendorffer, I.R., Wolfrum, S., Arndorfer, M., Balàzs, K., et al. 2014. Estimating the cost of different strategies for measuring farmland biodiversity: evidence from a Europe-wide field evaluation. Ecol. Indic. **45**: 434–443. doi:10.1016/j.ecolind.2014.04.050.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., et al. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Mol. Ecol. **21**(11): 2565–2573. doi:10.1111/j.1365-294X.2011.05418.x. PMID:22151771.

Tyagi, K., Kumar, V., Singha, D., and Chakraborty, R. 2015. Morphological and DNA barcoding evidence for invasive pest thrips, *Thrips parvispinus* (Thripidae: Thysanoptera), newly recorded from India. J. Insect Sci. **15**(1): 105. doi:10.1093/jisesa/iev087. PMID:26198870.

Ugochukwu, A.I., Hobbs, J.E., Phillips, P.W.B., and Gray, R. 2015. An economic analysis of private incentives to adopt DNA barcoding technology for fish species authentication in Canada. Genome, **58**(12): 559–567. doi:10.1139/gen-2015-0033. PMID: 26577715.

Van Steenkiste, N., Locke, S.A., Castelin, M., Marcogliese, D.J., and Abbott, C.L. 2015. New primers for DNA barcoding of digeneans and cestodes (Platyhelminthes). Mol. Ecol. Resour. **15**: 945–952. doi:10.1111/1755-0998.12358. PMID:25490869.

Westphal, M.I., Browne, M., MacKinnon, K., and Noble, I. 2008. The link between international trade and the global distribution of invasive alien species. Biol. Invasions, **10**(4): 391–398. doi:10.1007/s10530-007-9138-5.

Wilson, E.E., Sidhu, C.S., Levan, K.E., and Holway, D.A. 2010. Pollen foraging behaviour of solitary Hawaiian bees revealed through molecular pollen analysis. Mol. Ecol. **19**(21): 4823–4829. doi:10.1111/j.1365-294X.2010.04849.x. PMID:20958818.

Wirta, H.K., Hebert, P.D.N., Kaartinen, R., Prosser, S.W., Várkonyi, G., Roslin, T., et al. 2014. Complementary molecular information changes our perception of food web structure. Proc. Natl. Acad. Sci. **111**(5): 1885–1890. doi:10.1073/pnas.1316990111. PMID:24449902.

Wolfe, N.D., Switzer, W.M., Carr, J.K., Bhullar, V.B., Shanmugam, V., Tamoufe, U., et al. 2004. Naturally acquired simian retrovirus infections in central African hunters. Lancet, **363**(9413): 932–937. doi:10.1016/S0140-6736(04)15787-5. PMID: 15043960.

Wong, E.H.-K., and Hanner, R.H. 2008. DNA barcoding detects market substitution in North American seafood. Food Res. Int. **41**(8): 828–837. doi:10.1016/j.foodres.2008.07.005.

Xu, C.C.Y., Yen, I.J., Bowman, D., and Turner, C.R. 2015. Spider web DNA: a new spin on noninvasive genetics of predator and prey. PLoS ONE, **10**(11): e0142503. doi:10.1371/journal.pone.0142503. PMID:26606730.

Yang, C., Wang, X., Miller, J.A., de Blécourt, M., Ji, Y., Yang, C., et al. 2014. Using metabarcoding to ask if easily collected soil and leaf-litter samples can be used as a general biodiversity indicator. Ecol. Indic. **46**: 379–389. doi:10.1016/j.ecolind.2014.06.028.

You, M.J. 2014. Detection of four important *Eimeria* species by multiplex PCR in a single assay. Parasitol. Int. **63**(3): 527–532. doi:10.1016/j.parint.2014.01.006. PMID:24495953.

Zhang, Y.-Z., Si, S.-l., Zheng, J.-T., Li, H.-L., Fang, Y., Zhu, C.-D., and Vogler, A.P. 2011. DNA barcoding of endoparasitoid wasps in the genus *Anicetus* reveals high levels of host specificity (Hymenoptera: Encyrtidae). Biol. Control, **58**(3): 182–191. doi:10.1016/j.biocontrol.2011.05.006.

Zivy, M., Wienkoop, S., Renaut, J., Pinheiro, C., Goulas, E., and Carpentier, S. 2015. The quest for tolerant varieties: the importance of integrating "omics" techniques to phenotyping. Front. Plant Sci. **6**: 448. doi:10.3389/fpls.2015.00448. PMID: 26217344.