



Submissions for Diagnostic Protocols

I. General information

Submission number	2023-025
Title of Proposal	DNA BARCODING AS AN IDENTIFICATION TOOL FOR REGULATED PESTS
Submitted by (Country or Organization)	IPPC Contracting Party
IPPC Official Contact Point or RPPO	Kenya
Supported by	Kenya

2. Contact information

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3. Summary of proposal

Summary of justification for the proposal	<p>Global trade of plant and plant products increases the opportunity and incidence of pest establishment to new areas and or non-native areas around the world (Chapman et al., 2017). Spread and establishment of plant pest globally is mitigated by implementation of phytosanitary measures. Accurate identifications of pests to the lowest possible taxonomic level provide critical information for these pest risk assessments , which in turn inform regulatory decisions (Kennedy et al., 2022). DNA barcoding, a rapid, accurate, and standardized method for species level identification, using short DNA sequences otherwise known as barcodes (Hebert et al., 2003) offers a solution for NPPOs in this endeavour. The method facilitates species identification and discoveries of new pest (Lebonah et al., 2014) and can utilize only a minute tissue sample from a plant, pathogen and insects for identification and in cases where the sample is processed or degraded or when it consists of only non-characteristics parts of the organism (Mwaura et al., 2023). Inconsistent or erroneous identifications can have profound consequences for global agriculture, ecosystems and trade. DNA barcoding protocols for the</p>
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	identification of regulated pest including arthropods, bacteria, fungi, invasive plant species, nematodes and phytoplasmas have been developed and are currently in use in various jurisdictions (EPPO, 2021). Phytosanitary decision making supported by accurate diagnosis help plant protection authorities strike a balance between facilitating trade and protecting agriculture and the environment from pest invasions.
Proposed priority	I (high)
Comments	The application of DNA barcoding will revolutionize pest management, enhancing precision and enabling timely responses to safeguard our environment and food security.

4. Literature review

Literature review	<p>Global trade is important in enhancing development and economic growth; however it is also a gateway for many undesirable, invasive pest species which can adversely affect the environment, biodiversity and social welfare (Youm et al., 2011). Globally, losses associated with international trade, especially on the cost of pest management interventions runs to in billions of dollars (Perrings et al., 2002). Identification of pests intercepted during inspections of imported plant and products is one of the global first line defenses against the movement and establishment of new pest (Kennedy et al., 2022). Plant protection organization around the world require a robust, quick and accurate technique in identification of pest and DNA barcoding has proofed to be the method of choice. DNA barcoding is the identification to species level using standardized DNA fragments that are universal following established protocols (Newmaster et al., 2013) Since the inception of DNA barcoding (Herbert et al., 2003), it has become widely used as a taxonomic tool (DeSalle and Goldstein, 2019), especially useful in identification of species when accurate morphological information and taxonomic expertise are limiting factors (Ahrens et al., 2007; Valentini et al., 2009). DNA barcoding protocols for the identification of regulated pest including arthropods, bacteria, fungi, invasive plant species, nematodes and phytoplasmas have been developed and are currently in use (EPPO, 2021). The protocol has been utilized in identification of invasive species in various jurisdictions including apple snail in Kenya (Buddie et al., 2021), false codling Moth in Nigeria (Onah et al., 2016), similarly the method has been utilized in identification of invasive weeds; <i>Nassella trichotoma</i> (Poaceae: Stipeae) in Australia (Wang et al., 2022), and serpentine leaf miner <i>Liriomyza huidobrensis</i> in Australia (Mulholland et al., 2022). Differentiation of species of cyst nematodes was undertaken using the barcodes of <i>cox1</i>, <i>18S</i>, <i>ITS</i> and <i>28S</i> in Australia. In South Africa, DNA barcoding has been as a quick and accurate identification method for <i>Cerambycidae</i> species in sugarcane in order to track their potential abundance and spread marion et al., 2021. The method has been utilized successfully as a tool for</p>
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	invertebrate pest bio-monitoring (Wu et al. (2017), and has aided identification of invasive north American bull frog (ficetola et al., 2008). This method is fast, accurate and reliable ,its provides critical information to the lowest taxonomic level of pest which aid enhancing in phytosanitary decisions making by plant protection organization.
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5. Criteria for prioritization of Diagnostic Protocols

Criteria	Information provided by Submitter
1. Need for international harmonization of the diagnostic techniques for the pest (e.g. due to difficulties in diagnosis or disputes on methodology)	The proposed diagnostic protocol addresses the critical need for international harmonization due to existing difficulties in pest diagnosis or disputes over methodology. DNA barcoding offers a standardized and universally applicable approach, ensuring consistent pest identification across borders (Hebert et al., 2003). DNA barcoding has been used to differentiate between species for the vast majority of regulated arthropods, bacteria, fungi, invasive plant species, nematodes, and phytoplasmas as it acts as a tool that uses the divergence rate of DNA barcodes (Hebert et al. 2003). It also effectively supports rapid, accurate identification of individuals at the species and subspecies level. Due to its extensive application, it is feasible to identify an individual as belonging to a species regardless of its phenotypic, developmental stage, or the condition of the specimen that was obtained (Armstrong et al., 2005). Accurate identifications of pests to the lowest possible taxonomic level provide critical information for these pest risk assessments , which in turn inform regulatory decisions (Kennedy et al., 2022). Inconsistent or erroneous identifications can have profound consequences for global agriculture, ecosystems and trade.
2. The relevance of the diagnosis to the protection of plants including measures to limit the impact of the pest.	Pests' diagnosis through barcoding directly contributes to plant protection efforts by enabling the accurate and rapid identification of regulated pests to the species level (Hebert et al., 2003). It's a transformative tool in taxonomy and ecology, revolutionizing the way scientists identify and classify species (DeSalle and Golstein, 2019). Barcoding addresses the limitations of the traditional morphological identification methods where external characteristics are not distinct. The protocol uses a standardized barcode to identify species hence its accurate identification is pivotal in implementing measures to limit the impact of pests on crops, forests, and natural environments (Hebert et al., 2003).The method can also be used in cases of the international dispute management (Chen et al., 2014). Misidentification can result in ineffective control measures, leading to economic losses and ecological damage (Hebert et al.,2003).
3. Importance of the plants protected on the global level (e.g. relevant to many countries or of	The proposed protocol is relevant to all plant species. Countries regulate plant pest in order to prevent introduction and spread into their territories and hold major economic and ecological significance. DNA barcoding ensures the safeguarding of these vital plant resources on a global scale.

major importance to a few countries).	
4. Volume / importance of trade of the commodity that is subjected to the diagnostic procedures (e.g. relevant to many countries or of major importance to a few countries).	The protocol addresses pests associated with commodities traded globally. DNA barcoding is an extensive application, it is feasible to identify an individual as belonging to a species regardless of its phenotypic, developmental stage, or the condition of the specimen that was obtained (Armstrong et al., 2005). The method is used to identify vast majority of regulated arthropods, bacteria, fungi, invasive plant species, nematodes, and phytoplasmas (Hebert et al. 2003) to the subspecies level. Pest infestations in traded commodities can lead to the spread of invasive species, impacting international trade and food security. DNA barcoding can facilitate efficient border inspections and help prevent the inadvertent movement of pests through trade.
5. Other criteria for topics as determined by CPM that are relevant to determining priorities	The proposal aligns with additional criteria outlined by the Commission on Phytosanitary Measures (CPM) that are relevant to determining priorities. The criteria includes considerations relevance to emerging pest threats, the availability of resources, and the potential for the protocol to have a wide-reaching impact on phytosanitary measures globally. This protocol is instrumental in early and rapid detection of emerging pest regardless of phenotypic, developmental stage, or the condition and size of the sample. This is a cutting edge technology, which is accurate , reliable and amenable to phytosanitary decision making in facilitating trade.
6. The balance between pests of importance in different climatic zones (temperate, tropics etc) and commodity classes.	This is a protocol for detection and diagnosis of regulated pests including arthropods, bacteria, fungi, invasive plant species, nematodes and phytoplasma.
7. Number of labs undertaking the diagnosis.	The prioritization of this protocol takes into account the number of laboratories and institutions engaged in the diagnosis of the targeted pests. DNA barcoding offers a versatile and accessible approach that can be implemented by a wide range of laboratories globally. Its applicability to diverse pest species and adaptability to various laboratory settings enhance the feasibility of widespread adoption. Different laboratories globally undertake pest diagnosis by use DNA barcoding and several protocols have been developed and verified for use. The laboratories are in Africa (Kenya, Egypt, South Africa, Morocco, Zambia amongst others), Asia (India, South Korea, Japan, Vietnam amongst others) Central and South America, Australia, New Zealand and EU (EU member states).
8. Feasibility of production of a protocol, including availability of knowledge and expertise.	The feasibility of producing and implementing the protocol is assessed, considering factors such as the availability of knowledge and expertise in the field of DNA barcoding. The protocol leverages existing scientific knowledge and expertise in molecular biology and genetics. DNA barcoding techniques are well-documented, and there is a substantial body of

	<p>literature supporting their application. This, along with the availability of specialized equipment and resources in many laboratories, enhances the feasibility of protocol development and implementation. Additionally, the protocol can benefit from established networks and databases, such as the Barcode of Life Data Systems (BOLD), which facilitate data sharing and support expertise development (Ratnasingham et al., 2007). There is potential for collaboration and knowledge sharing among labs that are already using the procedure, which can expedite the development and dissemination of the diagnostic protocol. The prioritization of this proposal is in line with the agreements made by CPM-7 (2012) and CPM-11 (2016), emphasizing the importance of addressing pest identification challenges and harmonizing diagnostic techniques to protect global agriculture and ecosystems effectively. DNA barcoding represents a cutting-edge approach to meet these criteria, ensuring that the protocol has a substantial and positive global impact.</p>
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