

DNA barcodes for bio-surveillance: regulated and economically important arthropod plant pests¹

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Abstract: Many of the arthropod species that are important pests of agriculture and forestry are impossible to discriminate morphologically throughout all of their life stages. Some cannot be differentiated at any life stage. Over the past decade, DNA barcoding has gained increasing adoption as a tool to both identify known species and to reveal cryptic taxa. Although there has not been a focused effort to develop a barcode library for them, reference sequences are now available for 77% of the 409 species of arthropods documented on major pest databases. Aside from developing the reference library needed to guide specimen identifications, past barcode studies have revealed that a significant fraction of arthropod pests are a complex of allied taxa. Because of their importance as pests and disease vectors impacting global agriculture and forestry, DNA barcode results on these arthropods have significant implications for quarantine detection, regulation, and management. The current review discusses these implications in light of the presence of cryptic species in plant pests exposed by DNA barcoding.

Key words: species identification, cryptic taxa, invasive species, quarantine, pest management.

Résumé : Plusieurs des espèces d'arthropodes qui constituent d'importants ravageurs en agriculture en foresterie sont impossibles à distinguer sur la base morphologique au cours de certains stades de vie. Il est impossible d'en distinguer certains à tous les stades. Au cours de la dernière décennie, l'adoption du codage à barres de l'ADN s'est accrue tant pour l'identification des espèces connues que cryptiques. Bien qu'il n'y ait pas eu d'effort visant à développer une banque de références pour ces espèces, des séquences de référence sont présentement disponibles pour 77 % des 409 espèces d'arthropodes cataloguées parmi les bases de données de ravageurs importants. Outre le développement de la banque de références nécessaire à l'identification des spécimens, les études antérieures ont révélé qu'une portion significative des arthropodes nuisibles sont composés d'un ensemble de taxons apparentés. En raison de leur importance en tant que ravageurs ou vecteurs de maladies ayant un impact global en agriculture et en foresterie, les résultats du codage à barres de ces arthropodes ont des implications importantes en matière de détection, de réglementation et de gestion de quarantaines. Dans cette synthèse, les auteurs discutent des implications à la lumière de la présence d'espèces cryptiques, au sein de ravageurs de plantes, mises au jour grâce au codage à barres de l'ADN. [Traduit par la Rédaction]

Mots-clés : identification d'espèces, taxons cryptiques, espèces envahissantes, quarantaine, gestion des espèces nuisibles.

Introduction

Thousands of arthropod species either directly attack economically important plants or transmit viral and bacterial diseases, imposing massive costs on agriculture and forestry. For example, the widely distributed diamondback moth, *Plutella xylostella*, causes \$US4B–5B in damage to crops each year (Zalucki et al. 2012). The economic losses due to arthropods are increasing as new species gain pest status, often following human-mediated range expansions that, in the absence of natu-

ral enemies (Liebhold et al. 2013), can lead to population explosions that move a species from non-pest in its native range to pest in newly colonized areas (Aukema et al. 2011). The number of alien species continues to rise with arthropods representing the dominant component of this cross-border traffic, for example, in Europe, as documented by the European Environment Agency (<http://www.eea.europa.eu/>). Five major databases track the most important arthropod pests and disease vectors of agriculture and forestry; they are maintained by the

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Canadian Food Inspection Agency (CFIA) (www.inspection.gc.ca), the European and Mediterranean Plant Protection Organization (EPPO) (www.eppo.int), the Global Invasive Species Database (GISD) (<http://www.iucngisd.org>), the National Agricultural Pest Information System (NAPIS) (<https://napis.ceris.purdue.edu>), and the United States Department of Agriculture – Animal and Plant Health Inspection Service (USDA–APHIS) (www.aphis.usda.gov) (accessed 3–5 January 2016). These databases, which include regulated, invasive, and economically important plant pests with a focus on species important in Europe and North America, have been used to compile unified pest lists (Frewin et al. 2013). Since significant progress on DNA barcoding of plant pests has also been made in these regions, the current article draws the core information from these five sources as a basis for a discussion of cryptic species and their implications for DNA barcode applications. All but 21 of the 409 arthropod pest species on these lists belong to five insect orders (Coleoptera, Diptera, Hemiptera, Lepidoptera, Thysanoptera) (supplementary data, Table S1²). An additional 36 cryptic pest species reported in literature but not included in the five databases are considered in this article (Table S1²). Taxonomic difficulties continue to constrain the regulation of pest arthropods as indicated by the fact that 18 of the entries on these lists are only identified to a genus (Table S1²), while some with a species designation are known to be a species complex (Mally et al. 2015). These taxonomic complexities have provoked growing interest in the adoption of DNA-based approaches for species recognition, creating interesting challenges for traditional approaches to regulatory compliance (Jörger and Schrödl 2013).

The balance of this article begins by considering the effectiveness of DNA barcoding in identifying arthropods and in revealing cryptic species. It then proceeds to consider past studies of cryptic diversity in pest arthropods, before concluding with a discussion of the implications of DNA barcoding for pest regulation, management, and quarantine.

Effectiveness of DNA barcodes for arthropod identification

The efficacy of DNA barcoding (Hebert et al. 2003a) for specimen identification and species discovery has stimulated an international research program that has generated a barcode reference library for more than 250 000 described species of animals, fungi, and plants, data which resides in BOLD, the Barcode of Life Data System (Ratnasingham and Hebert 2007). Each of the 4.5 million animal records on BOLD has been assigned to a BIN, a Barcode Index Number (Ratnasingham and Hebert 2013), generating 452 000 BINs, predominantly insects

(315 000) (accessed 21 April 2016). Because BINs show strong congruence with species designated through past morphological study (Pentinsaari et al. 2014; Zahiri et al. 2014; Mutanen et al. 2015), they can be viewed as species proxies (Telfer et al. 2015) to facilitate the assessment of large-scale biodiversity patterns (Blagojev et al. 2016) and the detection of cryptic species complexes (Kekkonen and Hebert 2014; Ashfaq et al. 2015). The use of barcode data to identify species (Rakauskas and Basilova 2013) as opposed to BINs requires the species in question be represented in the barcode reference library (Ekrem et al. 2007). Because the barcode reference library now includes records for many agricultural and forestry pests (Ashfaq et al. 2014; Footitt et al. 2014; Raupach et al. 2014), newly encountered specimens of these species can be identified through barcode analysis. As well, the extension of DNA barcoding protocols onto next-generation sequencing (NGS) platforms (Shokralla et al. 2014) is enabling metabarcoding studies that permit large-scale assessments of species composition (Taberlet et al. 2012), an approach of high importance in monitoring plant pests.

DNA barcoding has a strong track record in delivering species-level identifications for the five insect orders with the most pest species—Coleoptera (Woodcock et al. 2013; Rougerie et al. 2015), Diptera (Nagy et al. 2013; Smit et al. 2013), Hemiptera (Park et al. 2011b; Raupach et al. 2014), Lepidoptera (Janzen et al. 2005; Ashfaq et al. 2013), and Thysanoptera (Rebijith et al. 2014; Iftikhar et al. 2016). For example, 92.2% of 3514 species of European beetles were assigned to a distinct BIN that coincided with a known morphological species, while most of the other species were assigned to two or three BINs, suggesting they represent cryptic species complexes (Hendrich et al. 2015). Work on Diptera has similarly validated the effectiveness of DNA barcoding for the identification of species in this order, including some of the most important agricultural pests, such as fruit flies (Virgilio et al. 2012) and leafminers (Amin et al. 2014). Another study that examined 1849 species of Canadian Hemiptera assigned these species to 1867 BINs with high correspondence between species and BINs, but also revealed 27 species with high divergences suggestive of cryptic species complexes (Gwiazdowski et al. 2015). Lepidoptera, the most intensively studied order, is represented by more than 100 000 BINs on BOLD. Work on this group has demonstrated the efficacy of DNA barcoding in identifying known species (Wilson et al. 2013; Dinca et al. 2015) and in revealing cryptic species complexes (Burns et al. 2008; Huemer et al. 2014a). It has also shown that there is very limited geographic variation in barcode sequences in populations of most species, so increased geographic scale does not reduce the success of species identification

²Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/gen-2016-0024>.

Table 1. Progress in the development of a DNA barcode reference library for regulated arthropod plant pests based on records in BOLD, the Barcode of Life Data Systems (www.boldsystems.org), on 22 December 2015.

Order	No. of pest species ^a	No. of species with barcodes on BOLD (with single record)	No. of species in a single BIN ^b	No. of species with more than one BIN ^b	No. of species without a BIN ^c
Acari	8	4	1	1	2
Coleoptera	162	116 (17)	61	29	9
Diptera	42	38 (2)	24	11	1
Hemiptera	59	38 (4)	20	8	6
Hymenoptera	10	7 (1)	3	3	0
Lepidoptera	115	101 (10)	68	23	0
Orthoptera	3	2	0	2	0
Thysanoptera	10	8	1	7	0
Total	409	314 (34)	178	84	18

^aSources: CFIA, EPPO (A1 & A2 lists), NAPIS, USDA-APHIS, and Global Invasive Species Database (100 of the world's worst invasive alien species).

^bOnly species with multiple records are included. Additional information is available in the supplementary data, Table S1².

^cSequences with <500 bp, >1% ambiguous bases, or with stop codons are not assigned a BIN.

(Lukhtanov et al. 2009; Huemer et al. 2014b, Candek and Kuntner 2015). However, in certain groups, such as diving beetles of the tribe Agabini (Bergsten et al. 2012), the correlation between genetic and geographic distances is strong enough to impact the success of species identification unless sampling of each species is fairly comprehensive. Studies on Lepidoptera have further shown the way in which DNA barcoding can challenge current taxonomic boundaries, can shift understanding of distributions (Rougerie et al. 2014), and can expose inconsistent usage of species names (Mutanen et al. 2012). Finally, work on Lepidoptera has confirmed that BINs are a strong proxy for species. For example, 93% of 215 species of European geometrid moths showed a perfect correspondence between BINs and known species (Hausmann et al. 2013).

The effectiveness of DNA barcoding in species recognition has also been established for Acari, the other major group of arthropods with important plant pests (Young et al. 2012). For example, it allowed resolution of the species composition of spider mites in agricultural settings in Vietnam (Hinamoto et al. 2007) and Europe (Ros and Breeuwer 2007). These studies have stimulated the development of barcode reference libraries, and they have also revealed overlooked species in this group of pests (Matsuda et al. 2013).

A search on BOLD (22 December 2015) revealed sequence coverage for 314 (269 public) of the 409 species (77%) of plant arthropod pests (Table 1). Although sequences and BIN assignments for 45 species on BOLD were private, they were included as taxa with coverage. Coverage for species of Diptera (90%) and Lepidoptera (87%) was higher than that for Coleoptera (71%) and Hemiptera (64%). Table 2 details the number of barcode records, BINs, and intraspecific divergence values for the pest species with five or more sequences on BOLD. All but 18 of these 314 species possessed at least one barcode-compliant (>500 bp, <1% ambiguous bases, no stop codon or contamination) record and 1/3 of the species (61/178)

represented by five or more records showed a BIN split, suggesting that they represent a species complex (Table 2; Table S1²). However, the possibility that some of these cases may reflect misidentifications or introgression (Harrison and Larson 2014) cannot be ruled out without detailed study on a case-by-case basis.

Incomplete resolution or flawed taxonomy?

DNA barcoding has failed to separate some congeneric species of important pests such as the tephritid fruit flies *Bactrocera occipitalis* and *B. philippinensis* (Sumalde et al. 2013). Another study which examined 193 tephritid species failed to discriminate six species in the *B. dorsalis* complex (*B. dorsalis*, *B. occipitalis*, *B. carambolae*, *B. papaya*, *B. invadens*, *B. philippinensis*) (Frey et al. 2013; Jiang et al. 2014). This discrepancy between taxonomic assignments and barcode results (Hendrichs et al. 2015) provoked a reevaluation of their taxonomy, work which suggested that at least some of the taxa involved are conspecific (Khamis et al. 2012; Schutze et al. 2012). In fact, a subsequent taxonomic revision synonymized *B. invadens* and *B. papaya* with *B. dorsalis* (Schutze et al. 2015). There are many other cases where pest species on different plants have been assigned to separate species when they likely represent just one taxon. For example, populations of the “mango mealybug” on mango trees in Pakistan were identified to *Drosicha mangiferae* (Latif 1949), while those on forest trees were assigned to *D. stebbingi* (Gul et al. 1997). A barcode study (Ashfaq et al. 2011), supported by results from three other gene regions, did not reveal any genetic divergence between mealybugs on mango and six other fruit and forest trees, supporting an earlier suggestion of their synonymy (Latif 1949).

Cryptic species

Although the definition of cryptic species is fluid (Jörger and Schrödl 2013), it is generally accepted that lineages with deep mitochondrial divergence without obvious morphological differences are strong candidates

Table 2. The number of Barcode Index Numbers (BINs) and maximum intraspecific sequence divergences (K2P) at COI for arthropod plant pests based on data in BOLD, the Barcode of Life Data Systems (www.boldsystems.org).

Order	Common name	Species	Barcode records	No. of BINs	Max. intraspecific divergence (%)
Acari	Red spider mite	<i>Tetranychus evansi</i>	6	2	3.0
Coleoptera	Oak splendour beetle	<i>Agrilus biguttatus</i>	7	2	22.0
	Citrus longhorned beetle	<i>Anoplophora chinensis</i>	33	3	4.6
	Boll weevil	<i>Anthonomus grandis</i>	119	3	7.8
	Strawberry bud weevil	<i>Anthonomus signatus</i>	12	3	4.3
	Spruce beetle	<i>Dendroctonus rufipennis</i>	10	4	5.5
	Northern corn rootworm	<i>Diabrotica barberi</i>	32	3	4.4
	Diaprepes root weevil	<i>Diaprepes abbreviatus</i>	79	13	10.9
	Lesser spruce shoot beetle	<i>Hylurgops palliatus</i>	21	4	5.5
	Alfalfa weevil	<i>Hypera postica</i>	16	2	5.6
	Six-toothed bark beetle	<i>Ips sexdentatus</i>	6	2	12.1
	European spruce bark beetle	<i>Ips typographus</i>	15	2	3.0
	White clover weevil	<i>Ischnopterapion virens</i>	10	2	4.6
	Sugarbeet wireworm	<i>Limonius californicus</i>	81	4	5.7
	Small white-marmorated longhorned beetle	<i>Monochamus sutor</i>	15	2	5.1
	Alfalfa snout beetle	<i>Otiorhynchus ligustici</i>	6	2	4.9
	Cereal leaf beetle	<i>Oulema melanopus</i>	16	3	8.7
	Six-toothed spruce bark beetle	<i>Pityogenes chalcographus</i>	14	4	8.7
	European oak bark beetle	<i>Scolytus intricatus</i>	17	6	11.5
	European spruce longhorn beetle	<i>Tetropium castaneum</i>	12	5	27.0
	Asian ambrosia beetle	<i>Xylosandrus crassiusculus</i>	5	3	11.0
Diptera	Guava fruit fly	<i>Anastrepha striata</i>	18	2	4.2
	Carambola fruit fly	<i>Bactrocera carambolae</i>	109	2	15.8
	Oriental fruit fly	<i>Bactrocera dorsalis</i>	645	5	10.4
	Asian fruit fly	<i>Bactrocera invadens</i>	230	2	8.4
	Olive fruit fly	<i>Bactrocera oleae</i>	107	3	8.6
	Peach fruit fly	<i>Bactrocera zonata</i>	42	4	10.6
	Vegetable leafminer	<i>Liriomyza sativae</i>	38	2	11.8
	American serpentine leafminer	<i>Liriomyza trifolii</i>	12	2	14.1
	Apple maggot	<i>Rhagoletis pomonella</i>	23	2	6.0
	European crane fly	<i>Tipula paludosa</i>	129	2	12.2
Hemiptera	Hemlock woolly adelgid	<i>Adelges tsugae</i>	150	4	8.3
	Silverleaf whitefly	<i>Bemisia tabaci</i>	918	16	20.0
	Green scale	<i>Coccus viridis</i>	5	2	16.8
	Brown marmorated stink bug	<i>Halyomorpha halys</i>	37	3	17.5
	Vine mealybug	<i>Planococcus ficus</i>	6	2	4.0
Hymenoptera	Oriental chestnut gall wasp	<i>Dryocosmus kuriphilus</i>	7	2	2.3
	Green alder sawfly	<i>Monsoma pulveratum</i>	18	2	3.8
Lepidoptera	Summer fruit tortrix moth	<i>Adoxophyes orana</i>	23	3	6.7
	Asiatic rice borer	<i>Chilo suppressalis</i>	57	3	18.9
	Yellow peach moth	<i>Conogethes punctiferalis</i>	202	3	8.9
	Mexican rice borer	<i>Eoreuma loftini</i>	8	2	3.1
	Oriental fruit moth	<i>Grapholita molesta</i>	56	2	6.2
	Old World bollworm	<i>Helicoverpa armigera</i>	433	2	3.2
	Hemlock looper	<i>Lambdina fiscellaria</i>	570	3	3.7
	Pea blue	<i>Lampides boeticus</i>	126	2	8.8
	Soybean pod borer	<i>Leguminivora glycinivorella</i>	17	2	3.7
	Brinjal borer	<i>Leucinodes orbonalis</i>	159	6	8.0
	Pear leaf blister moth	<i>Leucoptera malifoliella</i>	5	2	3.6
	Rosy moth	<i>Lymantria mathura</i>	41	2	9.2
	Soybean pod borer	<i>Maruca vitrata</i>	633	4	9.2
	Bruce spanworm	<i>Operophtera bruceata</i>	353	2	5.8

Table 2 (concluded).

Order	Common name	Species	Barcode records	No. of BINs	Max. intraspecific divergence (%)
	Lime swallowtail	<i>Papilio demoleus</i>	33	3	11.0
	Tufted apple bud moth	<i>Platynota idaeusalis</i>	242	2	3.7
	Fall armyworm	<i>Spodoptera frugiperda</i>	6	2	1.7
	Oak processionary moth	<i>Thaumetopoea processionea</i>	7	2	1.9
Orthoptera	Mormon cricket	<i>Anabrus simplex</i>	72	3	11.3
Thysanoptera	European flower thrips	<i>Frankliniella intonsa</i>	27	2	3.4
	Western flower thrips	<i>Frankliniella occidentalis</i>	457	4	3.9
	Chili Thrips	<i>Scirtothrips dorsalis</i>	233	12	18.0
	Melon thrips	<i>Thrips palmi</i>	212	2	13.0

Note: Species with at least five publicly available sequences on BOLD were included in the analysis. Additional information is provided in the supplementary data, Table S1².

(Rivera and Currie 2009). Although divergence patterns in mitochondrial and nuclear genes are sometimes discordant (Humphries et al. 2011), surveys of mitochondrial diversity provide quick insight into species boundaries (Mutanen et al. 2015). Lineages showing sequence divergence, often termed molecular operational taxonomic units (MOTUs) (Blaxter et al. 2005), have been discriminated by analyzing sequence variation using distance analysis (Schloss and Handelsman 2005), neighbor-joining clustering (Saitou and Nei 1987), phylogenetic trees (Zhang et al. 2013), Bayesian inference (Yang and Rannala 1997), coalescence-based approach (Pons et al. 2006; Fujisawa and Barraclough 2013), barcode-gap analysis (Puillandre et al. 2012), and BINs (Ratnasingham and Hebert 2013). However, there is a growing trend to integrate multiple methods (Ashfaq et al. 2015; Kekkonen et al. 2015; Blagoev et al. 2016) and multiple markers (Jörger et al. 2012; Mrinalini et al. 2015) to assess MOTU diversity. The results from such analyses have frequently been helpful in resolving taxonomic problems and in estimating species richness and abundance (Unterseher et al. 2011; Mutanen et al. 2013; Stahlhut et al. 2013). However, the use of a single, standardized, marker has the advantage of simplifying molecular identification (Savolainen et al. 2005), a valuable feature for pest managers and regulators in field applications (Armstrong and Ball 2005). Reflecting the ease of recovering sequence information, and access to refined analytical tools, the discovery of new cryptic species is now commonplace in many animal groups.

Cryptic species have important implications for taxonomic, evolutionary, and biodiversity studies, but their presence in pest taxa also has economic and regulatory implications (Piffaretti et al. 2013; Rebijith et al. 2014). Several studies have considered the impact of cryptic species on pest management (Frewin et al. 2014; Ovalle et al. 2014), biological control strategies (Derocles et al. 2015), the detection of invasive species (Blackett et al. 2015; Li et al. 2015), and quarantine inspections (Kang et al. 2015). The most obvious implications of DNA barcoding relate to its capacity to discriminate the members

of cryptic species complexes (Robe et al. 2013). Systems that employ sequence matches to identify specimens are already a reality (MacLeod et al. 2010), allowing the automated detection of regulated species (Lammers et al. 2014). However, in large-scale metabarcoding, standardization of metadata and taxonomic identifications still require consensus (Tedersoo et al. 2015), factors that currently limit the application of this approach in a regulatory context. This gap could be addressed through community action, perhaps via the International Barcode of Life (iBOL) (<http://ibol.org>) or the Genomics Standards Consortium (<http://gensc.org/>) model (Yilmaz et al. 2011).

Revealing cryptic species in pest arthropods

The effectiveness of DNA barcoding in revealing overlooked species in global pests like bark beetles, leafminers, gall midges, mealybugs, wireworms, seed borers, gypsy moths, armyworms, and thrips is well documented (Table 3). For example, *Liriomyza langei* (Diptera: Agromyzidae), a leafminer pest native to California, is morphologically indistinguishable from the invasive *L. huidobrensis*. However, the two species are easily discriminated by barcoding, a factor that enabled the analysis of their distributions (Scheffer et al. 2014). Similarly, the larvae of *Camptomyia corticalis* and *C. heterobia*, which inflict severe damage on the production of shiitake mushrooms, are easily separated by their 10.3% barcode divergence (Shin et al. 2013). Aphids are globally important pests and major vectors of many plant diseases, but they are challenging to identify because most of the 3000 species in this family possess striking phenotypic plasticity and life-stage diversity. DNA barcoding has proven an effective tool for species discrimination because intraspecific distance is low in most species, averaging just 0.2%, while congeneric distances are high (Footitt et al. 2008). However, some species, such as *Neomyzus circumflexus*, have higher divergences (>3%), suggesting they represent a complex (Table 3). Tussock moths (Lepidoptera: Lymantriidae) are worldwide pests that have been well-studied taxonomically. Nonetheless, DNA barcode studies revealed

Table 3. Arthropod plant pests reported as representing a cryptic species complex following DNA barcode analysis.

Order	Common name	Species	Max. divergence (%)	Nj/phylogenetic clades	Reference
Acari	Kanzawa spider mite	<i>Tetranychus kanzawai</i>	4.7 ^a	2	Matsuda et al. 2013
	N/A	<i>Tetranychus parakanzawai</i>	5.1 ^a	2	Matsuda et al. 2013
Coleoptera	Bark beetle	<i>Crypturgus subcribrosus</i>	6.7 ^a	2	Jordal and Kambestad 2014
	Bark beetle	<i>Crypturgus hispidulus</i>	6.8 ^a	2	Jordal and Kambestad 2014
	Hairy spruce bark beetle	<i>Dryocoetes autographus</i>	9.8 ^a	3	Jordal and Kambestad 2014
	Bark beetle	<i>Dryocoetes hectographus</i>	5.2 ^a	3	Jordal and Kambestad 2014
	Lesser spruce shoot beetle	<i>Hylurgops palliatus</i>	5.7 ^a	3	Jordal and Kambestad 2014
	Engraver beetle	<i>Ips acuminatus</i>	9.7 ^a	2	Jordal and Kambestad 2014
	Bark beetle	<i>Orthotomicus suturalis</i>	5.6 ^a	2	Jordal and Kambestad 2014
	Striped Ambrosia Beetle	<i>Trypodendron lineatum</i>	4.6 ^a	2	Jordal and Kambestad 2014
	Fruit-tree pinhole borer	<i>Xyleborinus saxeseni</i>	12.5 ^a	2	Jordal and Kambestad 2014
	Ambrosia beetle	<i>Xyleborus affinis</i>	20.1 ^b	3	Chang et al. 2014
	Wireworm	<i>Hypnoidus bicolor</i>	3.0 ^a	3	Etzler et al. 2014
	Yellow toadflax stem weevil	<i>Mecinus janthinus</i>	2.4 ^a	2	TošEvski et al. 2011
	Coffee berry borer	<i>Hypothenemus hampei</i>	11.8 ^a	4	Gauthier 2010
	Coconut leaf beetle	<i>Brontispa longissima</i>	3.0 ^b	2	Takano et al. 2011
	Root weevil	<i>Diaprepes abbreviatus</i>	14.9 ^a	3	Ascunce et al. 2008
Diptera	Hessian fly	<i>Mayetiola destructor</i>	3.0 ^b	2	Johnson et al. 2012
	African fig fly	<i>Zaprionus indianus</i>	11.2 ^a	2	Yassin et al. 2008
	Blueberry gall midge	<i>Dasineura oxycoccana</i>	10.7 ^c	2	Mathur et al. 2012
	Glasshouse Striped Sciarid	<i>Bradysia ocellaris</i>	2.4 ^b	2	Shin et al. 2015
Hemiptera	Citrus mealybug	<i>Planococcus citri</i>	NA	2	Abd-Rabou et al. 2012
	Solenopsis mealybug	<i>Phenacoccus solenopsis</i>	3.0 ^b	2	Dong et al. 2009
	Apple mealybug	<i>Phenacoccus aceris</i>	5.6 ^b	2	Park et al. 2011a
	Matsumoto mealybug	<i>Crisicoccus matsumotoi</i>	4.0 ^b	2	Park et al. 2011a
	Cyanotis scale	<i>Aspidiotus excises</i>	4.6 ^b	2	Park et al. 2011a
	Silverleaf whitefly	<i>Bemisia tabaci</i>	20 ^b	15	Ashfaq et al. 2014
	Mottled arum aphid	<i>Neomyzus circumflexus</i>	3.1 ^b	2	Footitt et al. 2008
	Leaf curl plum aphid	<i>Brachycaudus helichrysi</i>	2.5 ^b	2	Rebijith et al. 2013
	Cabbage aphid	<i>Brevicoryne brassicae</i>	3.8 ^b	2	Rebijith et al. 2013
	Asian sowthistle aphid	<i>Hyperomyzus carduellinus</i>	3.0 ^b	2	Rebijith et al. 2013
	Aphid	<i>Toxoptera aurantii</i>	3.1 ^b	2	Wang and Qiao 2009
Bronze bug	<i>Thaumastocoris peregrinus</i>	8.7 ^b	3	Nadel et al. 2010	
Lepidoptera	Grapewine leafminer	<i>Antispila hydrangaeella</i>	5.2 ^b	2	Van Nieukerken et al. 2012
	Durian fruit borer	<i>Conogethes punctiferalis</i>	5.8 ^b	2	Shashank et al. 2014
	Leaf worm	<i>Copitarsia decolora</i>	4.2 ^a	2	Simmons and Scheffer 2004
	Asian gypsy moth	<i>Lymantria dispar</i>	3.1 ^b	2	Ball and Armstrong 2006
	Pink gypsy moth	<i>Lymantria mathura</i>	4.7 ^b	2	Ball and Armstrong 2006
		<i>Lymantria sinica</i>	2.9 ^b	2	deWaard et al. 2010
	Beet armyworm	<i>Spodoptera exigua</i>	NA	2	Dumas et al. 2015
	Paddy armyworm	<i>Spodoptera mauritia</i>	NA	2	Dumas et al. 2015
Thysanoptera	Western flower thrips	<i>Frankliniella occidentalis</i>	4.4 ^b	2	Rugman-Jones et al. 2010
	Melon thrips	<i>Thrips palmi</i>	19.9 ^b	4	Rebijith et al. 2014; Kadirvel et al. 2013; Iftikhar et al. 2016
	Onion thrips	<i>Thrips tabaci</i>	10.4 ^b	3	Rebijith et al. 2014; Kadirvel et al. 2013; Iftikhar et al. 2016
	Chili thrips	<i>Scirtothrips dorsalis</i>	20.8 ^b	11	Dickey et al. 2015; Iftikhar et al. 2016
	Black flower thrips	<i>Haplothrips reuteri</i>	3.7 ^b	2	Iftikhar et al. 2016

Note: The citations were collected by literature survey on the Web of Science with search terms "DNA barcoding pest cryptic" (accessed 12 January 2016). The citations were further restricted by only considering studies explicitly using COI-5' and reporting cryptic species. Species also included in the five pest databases (Table S1²) are bold faced. N/A, information not available.

^ap-distances.

^bK2P distances.

^cGTRpG.

deep intraspecific divergences in *Lymantria dispar* (3.1%), *L. mathura* (4.7%), and *L. sinica* (2.9%) suggesting possible overlooked species (Table 3). Similarly, DNA barcode results have generally indicated congruence with morphologically recognized species of thrips (Qiao et al. 2012), but cryptic diversity is common with cases of high intraspecific divergence and deep genetic splits in several pest species (Tables 2, 3).

Practical implications of DNA barcoding

Pest regulation and management

DNA barcoding is gaining broad application in integrated pest management (IPM) (Etzler et al. 2014) and bio-surveillance (Jones et al. 2013) programs as the standard method for species identification by “matching unknown against the known” (Armstrong 2010). This is evidenced by the development of barcode-based kits for the identification of quarantine pests (Ye et al. 2015). IPM requires the correct identification of target species and monitoring of its effectiveness often involves diverse stakeholders including farmers, crop pest regulators, and quarantine agents. As long as “query” specimens show close sequence similarity to species in the reference library, match-based identifications are effective (Hebert et al. 2003b). Although most morphological species show congruence with BINs (Pentinsaari et al. 2014; Zahiri et al. 2014; Table 1), inconsistencies between morphologically recognized species and the genetic clusters discriminated by BINs are not infrequent. Such discrepancies complicate the identification of their component taxa with implications for pest regulation and management. For example, the whitefly, *Bemisia tabaci*, is now known to be a complex of at least 34 species (Lee et al. 2013) that differ in their invasiveness (De Barro and Ahmed 2011), their capacity to transmit plant pathogens (Chowda-Reddy et al. 2012), and their regional pest status (Ashfaq et al. 2014). Likewise, two pest thrips, *Scirtothrips dorsalis* and *Thrips tabaci*, are species complexes whose members vary in their invasiveness and viral-transmission ability (Jacobson et al. 2013; Dickey et al. 2015). Similarly, root weevil, *Diaprepes abbreviatus*, is a species complex (Ascunce et al. 2008) with 13 BINs that show differing geographic distributions. Any effort to regulate such species complexes as a single entity is inappropriate because it can complicate regulatory compliance and compromise management and control efforts by raising concerns when non-invasive or non-vector species are treated as pests. Although many regulated pest species with barcode records are assigned to a single BIN, about one third on BOLD show a BIN split (Table 1). For example, *Leucinodes orbonalis*, a quarantine pest, is represented by six BINs, with diverse geographic origins. The cross-border movements of species with BIN diversity raise complexities that will often create ambiguity in species matches requiring revisions to quarantine regulations. Conversely, there are cases where different species lack

barcode divergence, preventing their discrimination. The most dramatic case involves 20 species in the fruit fly genus *Bactrocera* that share the same BIN. While some of these taxa have recently been synonymized (Schutze et al. 2015) and other cases may reflect misidentifications, it is likely that other cases reflect closely allied species that hybridize. Such cases require more detailed investigation to ascertain if other genetic markers allow their discrimination or reveal that they are actually synonyms.

Since DNA barcoding reliably identifies both immatures and adults (Shin et al. 2013), and can differentiate introduced and native pests (Chown et al. 2008), it has been used to help manage species complexes in agricultural systems (Li et al. 2011; Frewin et al. 2014). Such applications are important because insecticide resistance can vary between closely related species, and even between genotypes of the same species (van Toor et al. 2008). For example, two cryptic whiteflies in the *B. tabaci* complex, *Mediterranean* and *Middle East-Asia Minor 1* (MEAM1), differ in their susceptibility to insecticides. A study on the response of mixed populations of these species showed that the *Mediterranean* species prevails under insecticide-based management, but it is displaced by MEAM1 under biological control (Frewin et al. 2014).

Biological control is widely viewed as the optimal pest management strategy, but incorrect identification of either the pest species or its control agent due to cryptic diversity can lead to unpredictable outcomes (Van Lenteren et al. 2003). Because of its capacity to determine host-parasitoid associations (Erlandson et al. 2003), DNA barcoding has been employed to gain a deeper understanding of biological control using natural enemies. For example, Davis et al. (2011) differentiated immatures of *Laricobius rubidus*, a native biological control agent of hemlock woolly adelgid, *Adelges tsugae* (complex of four BINs) from the closely related *L. nigrinus* introduced from Japan, while Derocles et al. (2015) examined interactions between a leafminer and its parasitoids to advance biological control. Several studies have revealed unexpected diversity and interactions linked to cryptic diversity. The outcomes of biological control rapidly gain unpredictability (Roderick et al. 2012) when both the control agent and the target are cryptic species complexes, as, for example, in the case of the hymenopteran wasp, *Trichogramma japonicum* (3 BINs), which is employed as a control agent for the brinjal borer, *L. orbonalis* (6 BINs).

Quarantine of invasive alien species

Expansions in the geographic range of insect pests often require a rapid response (Adrión et al. 2014), and DNA barcoding can provide insights beyond those obtained through morphological analysis. For example, aside from enabling the early detection of invasive species (Onah et al. 2015), DNA barcoding can reveal source regions (Bellis et al. 2015) and introduction patterns (Blacket et al. 2015). Nagoshi et al. (2011) used it to moni-

tor introductions of the invasive armyworm species, *Spodoptera litura* and *S. littoralis*, into Florida, while Tyagi et al. (2015) and Wei et al. (2010) detected the first invasions of *Thrips parvispinus* and *Echinothrips americanus* in India and China, respectively. In a similar fashion, DNA barcoding differentiated *Heliothis armigera* from the native *H. zea*, revealing the spread of the former species after its introduction into Brazil (Mastrangelo et al. 2014). More recently, DNA barcoding led to the recognition of a buprestid beetle, *Agrilus ribesi*, whose introduction to North America had been overlooked for a century (Jendek et al. 2015). Barcoding has also been used to detect invasive tephritid fruit flies in Nigeria (Onah et al. 2015), leafminers in Papua New Guinea (Blackett et al. 2015) and Italy (Bernardo et al. 2015), a stink bug (*Halyomorpha halys*) in Europe (Cesari et al. 2015), and a cotton bug, *Oxycarenus hyalinipennis*, in Florida (Nagoshi et al. 2012). Based on its effectiveness in species identification, the Quarantine Barcode of Life project (www.qbol.org) worked towards establishing DNA barcoding as the core technology for identifying quarantine organisms in support of plant health, and to gain its acceptance by the pest regulators as the standard method for the identification of plant pests (van de Vossen et al. 2013). Since DNA barcoding meets the minimum standards (Floyd et al. 2010) set under the International Standards for Phytosanitary Measures (ISPM) No. 27, “Diagnostic Protocols for Regulated Pests” and has been validated for use in regulatory sciences (Jones et al. 2013), it is positioned for adoption as the preferred diagnostic tool for species for quarantine and regulation. For example, USDA–Agriculture Research Service is using DNA barcodes to monitor pest and invasive arthropods (<http://agresearchmag.ars.usda.gov/AR/archive/2012/Apr/insects0412.pdf>). However, the incorporation of this technology into the routine workflows of regulatory agencies is still under development. There is a particular need to develop well-parameterized reference datasets on important pest species for the countries of origin. Because nearly 75% of all BINs on BOLD, in general, derive from North America and Europe, with relatively low coverage for regions that host many potentially important global pests, there is a need for aggressive efforts for further parameterization of the barcode reference library. However, even before this task is complete, DNA barcode analysis makes it possible to signal the detection of any newly encountered species, providing a stimulus for its further investigation.

Conclusions

The increased global trade in plants and plant products has dramatically increased the risk of introducing novel pest species, creating the potential for massive economic losses to agriculture and forestry. For example, loss linked to the introduction of the emerald ash borer into North America has been estimated at tens of billions of dollars (Kovacs et al 2010; Herms and McCullough

2014). Because of its capability to support large-scale bio-surveillance programs, DNA barcoding can be an effective tool for intercepting invasive species at their points of introduction, and it can also enable their early detection in natural environments. While species-specific primer sets can be a valuable tool for the quarantine detection of a particular species (Yeh et al. 2014), there is a critical need to gain a deeper understanding of diversity in many pest species as nearly one third of these taxa on BOLD (Table 1) appear to represent a species complex. High throughput sequencing platforms represent a powerful technology that will enable the use of DNA barcoding in large-scale bio-surveillance programs that can target both known pest species and new arrivals (Pochon et al. 2013). Given the complexities in morphological discrimination of many cryptic taxa, there is an obvious need to adopt DNA-based systems of nomenclature for pest regulation and quarantine (Cook et al. 2010).

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