The Application of DNA Barcoding to Enhance Integrated Pest Management

by

Andrew Jordan Frewin

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ABSTRACT

The Application of DNA Barcoding to Enhance Integrated Pest Management

Andrew Jordan Frewin
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Co-Advisors:
Dr. Hanner
Dr. Scott-Dupree

Integrated pest management (IPM) is currently the dominant pest management paradigm globally. Key to the success of IPM is tailoring management actions to the particular suite of pests and beneficial species present in a given system. As such, the ability to accurately identify pests and beneficials to the level of species is critically important to IPM research and application. DNA barcoding is a genetic method for specimen identification that aims to extend taxonomic expertise to new user communities, and new specimen categories (e.g., previously unidentifiable life-stages). In this thesis, I extend the use of DNA barcoding as a diagnostic method for application in the sphere of IPM. Frist, I examine the application of barcoding to regulatory IPM activities and present a summary of currently available DNA barcode data on a newly generated list of globally important pest species. Second, I explore the ability of DNA barcoding to contribute to the identification of Lepidoptera intercepted during border inspection programs. In this case, DNA barcoding was able to increase the number of specimens identified to the level of species compared to traditional morphological approaches. Third, I use DNA barcoding as a diagnostic tool for applied IPM research and on-farm decision making, to investigate demographic trends in a mixed cryptic-species infestations of the whitefly *Bemisia tabaci*. I then demonstrated how commonly-implemented greenhouse pest monitoring activities can provide specimens suitable for

DNA barcode based identification, thereby providing a feasible way to incorporate DNA barcoding into greenhouse IPM. Fourth, I demonstrate the need for a standardized diagnostic tool for arthropods in the context of biological control, an important component of the IPM paradigm.

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LIST OF ACRONYMS AND ABBREVIATIONS

AFLPs – Amplified Fragment Length Polymorphisms

BIN – Barcode Index Number

BOLD – Barcode of Life Data Systems

BOLD-IDS – Barcode of Life Data Systems – Identification System

ca. – Approximately

CABI – Centre for Agriculture and Biosciences International

CFIA – Canadian Food Inspection Agency

COI – Cytochrome oxidase subunit I

COL – Catalogue of Life

DNA – Deoxyribonucleic acid

DDBJ – DNA Data Bank of Japan

EMBL – European Molecular Biology Laboratory

EPPO – European Plant Protection Organization

GBIF – Global Biodiversity Information Facility

iBOL – International Barcode of Life

IGR – Insect Growth Regulator

INSDC – International Nucleotide Sequence Database Collaboration

IPM – Integrated Pest Management

IPPC – International Plant Protection Convention

ISPM – International Standards for Phytosanitary Measures

ITS1 – Internal transcribed spacer 1

K2P – Kimura two parameter model

MEAM1 – Middle East-Asia Minor 1

NAPIS – National Agricultural Pest Information System

NPPO – National Plant Protection Organization

OTU – Operational Taxonomic Unit

PCR – Polymerase Chain Reaction

qPCR – Quantitative Polymerase Chain Reaction

USDA – U.S. Department of Agriculture

USDA-APHIS – U.S. Department of Agriculture - Animal and Plant Health Inspection Service

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Chapter 1

GENERAL INTRODUCTION

Integrated pest management (IPM) is one of the most important concepts in modern agricultural production. Although many of the tenets of IPM can be traced as far back as the 1800s, the synthesis of what is now recognized as IPM only emerged in the 1970s (Kogan 1998). Since then, at least 65 working definitions of IPM have been proposed. After a review of these definitions, Kogan (1998) proposed the following, which will be adopted for the purpose of my thesis:

> "IPM is a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment".

IPM is a key departure from the previous pest management paradigm, which relied primarily or exclusively on inexpensive synthetic pesticides applied on fixed calendar dates. As IPM aims to incorporate multiple complementary control tactics with explicit consideration for the potential negative impacts of these control measures, IPM is often regarded as a key component of sustainable agricultural (Maredia et al. 2003, Birch et al. 2011). Also, IPM involves the recognition that optimal pest control tactics are targeted at the specific pest species present in a system, not at entire pest groups (e.g., aphids, or grubs), as species-specific control tactics are likely to be the most effective, and have fewer non-target effects. Although IPM can be applied for the management of weeds (Swanton and Weise 1991), pathogens and diseases (Curtis 2009), the focus of my thesis is arthropod IPM.

Integrated pest management is implemented at various geographic and socioeconomic scales (Kogan 1998), the most familiar being on-farm. On-farm IPM is enacted to manage pests within and between cropping cycles for one or a relatively few number of crops. More complex IPM systems may be implemented within regional production systems encompassing multiple farms and crops. Regional or landscape level IPM is often directed and organized by grower cooperatives, agricultural extension groups, and governments. These regional approaches are particularly well-suited to address pest issues that threaten the environment (e.g., protected parks or reserves, or areas of conservation significance) or the viability of an entire agricultural sector (Brewer and Goodell 2012). In regional IPM, governments can play an important role by enacting biosecurity policies which reduce the potential for pest organisms (e.g., invasive species) to establish and/or expand their range within their jurisdiction (Paini et al. 2010), thereby safeguarding the entire agricultural projection system. Under this paradigm, invasive species legislation, border quarantine and biosecurity can be viewed as components of IPM.

As a decision support system, successful implementation of IPM at all scales hinges on the accurate identification of pest and beneficial arthropods to the level of species. For IPM accurate identifications are essential for the effective communication of biological data (e.g., phenology, host-range, susceptibility to management tactics) both within the research community and in an extension capacity to growers. For example, pest species which are closely related and/or superficially similar in appearance may vary in their phenology and susceptibility to agrochemicals (Muñiz and Nombela 2001, Lowery et al. 2005, Lowery et al. 2006, Dennehy et al. 2010) such that species-specific management tactics which target one species may be completely ineffective on the other. Therefore, from an on-farm perspective identifications are necessary in order to implement these species-specific pest management tactics of IPM. Furthermore, the identification of novel invasive pests on-farm is an essential part of regional biosecurity programs. In this context invasive species need to be identified against

the background of native diversity, a potentially challenging task given the magnitude of arthropod biodiversity (Zhang 2013). Finally, at the regional level, governments need to be able to accurately and quickly identify invasive arthropods to enact and enforce biosecurity policies and programs, as well as fulfill international obligations and treaties (i.e., reporting requirements for International Plant Protection Convention (IPPC) signatories) (MacLeod et al. 2010).

Although critical to the success of IPM, species-level identification of arthropods is hindered by a series of well-recognized taxonomic challenges. Primary among these is termed simply the “taxonomic impediment”, referring to the dwindling taxonomic workforce and expertise in recent years (Hopkins and Freckleton 2002, Wheeler et al. 2004, Wheeler 2014). Other challenges include the lack or inaccessibility of taxonomic resources (including both usability and actual physical/digital access), the sheer magnitude of mostly undescribed arthropod biodiversity (Stork 1993, Hawksworth and Kalin-Arroyo 1995, Costello et al. 2012), the lack of knowledge regarding the distribution of described species, the lack of descriptions and/or diagnostic characters for particular life-stages and/or sexes of otherwise described species, the structural diversity and variety of characters used in arthropod taxonomy, and the use of internal (e.g., genital morphology) or otherwise difficult to observe diagnostic characters. Unfortunately, these impediments affect the entire spectrum of IPM applications, including on-farm pest management, regulatory biosecurity, and basic and applied IPM research.

A potential solution to these challenges is to implement DNA barcoding for species identification in both IPM research and application. In brief, DNA barcoding is a standardized DNA based specimen identification technique, which can be applied broadly across metazoan life (Hebert et al. 2003b, Hebert et al. 2003a). Identifications are made by comparing DNA barcodes (~650bp of the 5’ region of the mitochondrial encoded gene cytochrome c oxidase subunit I) generated from ‘query’ specimens to a DNA barcode reference library consisting of barcodes generated from previously identified specimens. A number of different criteria and

methods have been developed to make these comparisons (see chapter 2); however, in most cases DNA barcode-based identifications hinge on the observation that within the DNA barcode, the level of intra-specific genetic divergence is less than the inter-specific divergence for the vast majority of species studied to date (Hebert et al. 2003b, Hebert et al. 2003a). Therefore, measures of genetic distance can be used to identify query specimens when comparing them to a reference library. In the simplest cases, the smallest genetic distances less than a predetermined threshold (typically 2-3%) between a 'query' sequence and individual specimens in the reference library are considered match and identification can be inferred (Brown et al. 2012, Collins et al. 2012b).

One of the primary goals of DNA barcoding is to extend taxonomic knowledge and capacity to non-taxonomists, thereby reducing the negative impact of the taxonomic impediment on science and society (Hebert et al. 2003b). This is accomplished through collaborations with the taxonomic community in the construction of DNA barcode reference libraries (Kerr et al. 2007, Ward et al. 2009, Park et al. 2011a, Rougerie et al. 2014). In terms of its applications to IPM, this has the potential to allow users the ability to access and identify the diverse array of pest and beneficial arthropods important to plant production (Ball and Armstrong 2006, Foottit et al. 2008, Park et al. 2011b, Magnacca and Brown 2012, Nzeduru et al. 2012).

Like other molecular identification methods, DNA barcoding can overcome many of the taxonomic impediments which concern the status of the physical specimen. For example molecular methods can be applied to any life-stage, sex, or even fragmentary specimens, thereby negating the fact that diagnostic characters for some species are life-stage or sex-specific. However, DNA barcoding goes farther than other molecular identification methods (e.g., AFLPs, qPCR, esterase zymograms) in a number of important ways, increasing its utility and usability. First, DNA barcoding is a 'biodiversity orientated' approach to specimen identification, meaning that it can be applied to any biological material, and only superficial

knowledge of the classification of the specimen (e.g., phylum or order) may be required to successfully barcode the specimen. Specifically, this knowledge is required to select appropriate primers for PCR. In contrast, traditional molecular diagnostics require *a priori* knowledge of the classification of the organism, as these types of diagnostics are designed to differentiate a small number of closely related species (Rugman-Jones et al. 2006, deWaard et al. 2010b). In other words, traditional diagnostics need to be designed for each taxon in question, whereas DNA barcoding can be applied to virtually any specimen.

Secondly, DNA barcoding is sequence-based, and in contrast to the outputs from many other molecular diagnostics (i.e., AFLPs, qPCR, esterase zymograms, etc.), sequence data is relatively easy to share and distribute digitally and existing web-based platforms (Benson et al. 2005, Ratnasingham and Hebert 2007, Liu et al. 2013). This facilitates transparency as the data generated in the process of barcoding (e.g., the sequence data and associated electropherogram trace files, specimen metadata and photos) can be reanalysed, scrutinized, and together all of these data can serve as a digital specimen voucher. Furthermore, DNA barcoding is supported by the online bioinformatics platform The Barcode of Life Data Systems (BOLD, www.boldsystems.org, Ratnasingham and Hebert 2007), which directly facilitates data sharing and transparency. This platform houses the DNA barcode reference library, the construction of which is being directed by the International Barcode of Life Project, and contributed to by numerous other independent scientists and research groups. Furthermore, BOLD also provides a number of analytical and data visualisation tools for DNA barcode data.

Thirdly, inherent in the process of DNA barcoding, is the explicit association between a physical specimen voucher, specimen metadata and derived sequence data and genetic materials (e.g., DNA extracts, and PCR products). These associations are made available by linking data elements via a specimen-record on BOLD. These elements are summarized in the DNA barcode data-standard (Hanner 2009), which meets the requirements for diagnostic

methods for regulated pests set forth by the IPPC (Floyd et al. 2010). For these reasons, it is justifiable to apply DNA barcoding to address the diagnostic challenges in agriculture such as those faced by IPM.

The objective of my research was to extend and develop the use of DNA barcoding to address species identification challenges in the sphere of IPM.

Mirroring the actions taken by IPM in dealing with the arrival of a novel invasive pest species, the thesis will begin by exploring the applications of DNA barcoding for regulatory biosecurity, and then proceed to on-farm applications of DNA barcoding. Chapter 2 [DNA BARCODING FOR PLANT PROTECTION] comprises a review of DNA barcoding and its applications to IPM of regulated and non-regulated pest species. In this chapter I also synthesize a novel a list of 1044 global agricultural pest species compiled from various sources, and summarize the available DNA barcode data for these species, highlighting species coverage and taxonomic gaps to be addressed in the use of barcoding for IPM.

Chapter 3 [DNA BARCODING TO IMPROVE IDENTIFICATION OF BORDER INTERCEPTED SPECIMENS OF POTENTIAL PEST ARTHROPODS] continues on the regulatory theme in two sections. In the first section I examine the ability of DNA barcoding to assist in the identification of pest interceptions in the context of border inspection programs. To accomplish this, I examine the concordance between morphological and DNA barcode-based identification of a set of intercepted Lepidoptera specimens collected by the U.S. Department of Agriculture (USDA). I continue by generating reference DNA barcodes for a number of Tortricidae that are suspected to be encountered during border interception.

In chapter 4 [CONTRIBUTIONS TO IPM OF THE *BEMISIA TABACI* CRYPTIC SPECIES COMPLEX], I address the application of DNA barcoding for on-farm decision making and IPM research. First, I use DNA barcoding as a research tool to facilitate demographic

analysis of infestations of mixtures of cryptic species from the *Bemisia tabaci* complex. I then develop molecular diagnostic for *B. tabaci* cryptic species identification use in the field by greenhouse extension officials or pest managers.

In Chapter 5 [INCORPORATING DNA BARCODING INTO GREENHOUSE IPM: USING PLANT WASHING AND STICKY CARDS FOR SPECIMEN COLLECTION], I continue to examine applications of DNA barcoding for on-farm decision making. I examine the suitability of arthropod specimens collected from sticky cards and plant washing to be DNA barcoded. Sticky cards are a commonly used pest monitoring tool for greenhouses, whereas washing plants is a greenhouse phytosanitary technique, demonstrating both methods can be adapted to barcode workflows.

In Chapter 6 [DNA BARCODING AS A STANDARDIZED DIAGNOSTIC FOR THE IDENTIFICATION OF RESEARCH ARTHROPODS IN THE BIOLOGICAL CONTROL LITERATURE], I examine the biological control literature, and develop a case for using DNA barcoding as a standardized identification method for research involving arthropods and as a quality control tool for use in biological control agent propagation. First I demonstrate a data deficiency in peer-reviewed biological control literature relating to the identification of research arthropods and their associated specimen metadata. Then, using newly generated DNA barcode data on commercially available biological control agents and publically available barcode data on species researched in this body of literature, I illustrate potential cases of misidentified specimens or unrecognized cryptic species. Together this supports the use of routine molecular diagnostics in biological control research and its application.

Finally, in Chapter 7, I provide a summary of the key themes of my research, highlight some of the challenges for adopting DNA Barcoding in IPM, and discuss areas of future research.

Chapter 2

DNA BARCODING FOR PLANT PROTECTION

This chapter consists of work completed primarily by Frewin at the University of Guelph from: Frewin, A., C. Scott-Dupree, and R. Hanner. 2013. DNA barcoding for plant protection: applications and summary of available data for arthropod pests. CAB Reviews 8: 1-13.

2.0 Abstract

Over the last decade DNA Barcoding – the use of short, standardized DNA sequences for species identification – has emerged as an important tool to study biodiversity. However, given the challenge and importance of accurately identifying plant pests I argue for the use of DNA barcoding as a tool to aid phytosanitary regulation and IPM. The rigorous data quality standard of DNA barcoding complements both of these tasks. In this review I describe the current and potential applications of DNA barcoding for plant pest management and discuss some analytical methodologies and tools used for DNA barcoding research. In addition a new global pest checklist of 1044 plant pests is compiled from six international pest lists. The DNA barcode coverage for this new global pest list is then assessed by querying specimen records for these species on BOLD. In total 53.7% of the species on this pest list have representative DNA barcodes on BOLD, therefore while many species have at some representation much work remains, before DNA barcoding can be used broadly for the identification of agriculturally significant organisms.

2.1 Introduction

Accurate and timely identification of pest and beneficial arthropods is an essential component of plant production (e.g., agriculture, silviculture, and horticulture), from the pest management decisions of individual growers to international phytosanitary regulation. For plant producers, accurate species-level identifications of both beneficial and pest organisms are required to implement effective IPM techniques necessary to prevent crop losses. For governments, species identifications are required to safeguard domestic plant production from invasive pests through the implementation of quarantine and eradication programs, and to fulfill international obligations under the International Plant Protection Convention (IPPC; FAO 2011). For agricultural researchers, accurate identifications are required for effective scientific communication, to prevent erroneous data from contaminating research literature, to facilitate agricultural extension research, and for communication between growers and governments. However even for experts, identifying specimens to the level of species is challenged by the volume, diversity, cryptic lifestyles and morphologically distinct life-stages of arthropods; for non-experts it is further constrained by the lack of access to taxonomic expertise.

Traditionally species are identified using taxonomic keys that are based on the presence/absence of presumed diagnostic morphological characters derived from extensive and time-consuming comparative studies. As a result, only expert taxonomists are capable of critically identifying the majority of known species on earth, and few individual experts are capable of identifying >0.01% of species known to science (Hawksworth and Kalin-Arroyo 1995). Furthermore, in recent years taxonomy as a science is considered to be in decline, a situation referred to as the “taxonomic impediment” (Hopkins and Freckleton 2002, Hebert et al. 2003a). As a consequence, alternative identification techniques which can be implemented by non -experts are needed to meet the demand for arthropod species identifications required by

government, academic and public stakeholders; who as a result of globalization and climate change are increasingly concerned about the potential threats agricultural pests pose to plant production (Hopkins and Freckleton 2002, Magarey et al. 2009, Dehnen-Schmutz et al. 2010, Legreve and Duveiller 2010, MacLeod et al. 2010, Liebhold et al. 2012).

Characterized by rapid conceptual and technological advances, genetic data has played an increasingly important role in taxonomy over the last several decades. Contemporary molecular systematic studies provide taxonomists with a wealth of data that enable a far better assessment and understanding of arthropod diversity than was previously possible with traditional approaches based solely on morphology. As such, they play an increasingly important role in the recognition, identification and classification of species. DNA sequence data in particular has been used successfully to identify bacteria and other micro-organisms since the early 1980s (Busse et al. 1996). Introduced as tool for species identification and discovery nearly a decade ago, DNA barcoding (Hebert et al. 2003b, Hebert et al. 2003a) relies on the use of short, standardized gene sequences to discriminate eukaryotic species. Subsequent studies aimed at creating barcode profiles for known species build capacity for the identification of unknowns using DNA sequences. DNA barcoding thus represents a repeatable and scalable method which offers the potential for automating species identification. By extending taxonomic knowledge to non-experts, it fills an important bio-security gap left by the dwindling number of taxonomists, and meets the diagnostic standards set forth by the International Plant Protection Convention (IPPC; e.g. see: Floyd et al. 2010). However, further implementation of DNA barcoding for biosecurity and pest management hinges on the continued development of DNA barcode reference libraries (used for the identification of unknowns), and the standardization of DNA barcode species identification methods. The following review places DNA barcoding and its potential uses for plant production in a conceptual framework, providing a summary of DNA

barcode data currently available for the DNA-based identification of various plant pests with an emphases on arthropods.

2.1.1 DNA Barcoding

In 2003, Hebert *et al*. (2003a) proposed 'DNA Barcoding', a technique to identify all metazoan life using a short, standardized fragment of DNA. The region chosen for animals was a 648bp fragment of the mitochondrial encoded cytochrome c oxidase subunit I (COI) gene. This gene region was chosen because it is phylogenetically informative (at the level of species), and for a number of practical reasons. First, the mitochondrial genome lacks introns and experiences a low rate of recombination which simplifies sequence alignment and analysis. Second, highly conserved flanking regions of the 5' end of COI facilitate PCR amplification using universal primers capable of amplifying this gene region from most animal phyla (Ivanova et al. 2007, Kerr et al. 2009). Third, COI has been successful in resolving closely related species due to a relatively high rate of molecular evolution of the mitochondrial genome. And finally, the COI gene (in common with other mitochondrial genes) exists as multiple copies per cell making it easier to amplify from small or degraded samples.

Species are identified by comparing DNA barcode sequences from unidentified specimens to those of reference sequences generated from taxonomically validated specimens (Hebert et al. 2003b). Reference sequences must be associated with a voucher specimen, and include provenance data (species name, collection records, who identified the specimen), documentation of laboratory methods used (e.g., list of PCR primers used, retention of electropherogram sequence profiles), and have a minimum sequence length of 500 bp (Hanner 2009, Floyd et al. 2010). This level of transparency sets DNA barcoding apart from earlier

molecular approaches for species recognition as individual specimens and raw sequence data used to generate reference barcode sequences can be re-examined if needed. This is particularly advantageous for regulatory applications, and the typical workflow for DNA barcoding (reviewed in Floyd et al. 2010) meets the standards for pest diagnostics set forth by the IPPCs International Standards for Phytosanitary Measures (ISPM) No. 27 (FAO. 2006, Floyd et al. 2010).

The utility of DNA barcoding lies in the observation that most species possess unique DNA barcode sequence clusters or "haplogroups"; where measures of intra-specific sequence divergence are less than the inter-specific sequence divergences observed among congeneric species, often referred to as a 'barcode gap' (Meier et al. 2008). This gap has been observed for the vast majority of animal species subjected to DNA barcoding including birds (Kerr et al. 2007, Kerr et al. 2009), fish (Hubert et al. 2008), mammals (Clare et al. 2007), and many agriculturally-important arthropods [Aphididae (Foottit et al. 2008, Foottit et al. 2009b, Lee et al. 2011b), Apoidae (Sheffield et al. 2009), Aphidiinae (Derocles et al. 2011), Carabidae (Raupach et al. 2010), Heteroptera (Jung et al. 2011, Park et al. 2011a), Lepidoptera (Hajibabaei et al. 2006, deWaard et al. 2009, Hebert et al. 2009, deWaard et al. 2010a, deWaard et al. 2010b, deWaard et al. 2011), Pseudococcidae and Diaspididae (Park et al. 2011b), and Tephritidae (Barr et al. 2012)].

However, because evolution is an ongoing process, some young morphologically distinct species may have overlapping or identical DNA barcodes (Roe and Sperling 2007, Schmidt and Sperling 2008, Derocles et al. 2011, deWaard et al. 2011, Barr et al. 2012) as a result of incomplete lineage sorting or mitochondrial introgression via hybridization. To date, few agriculturally-important species are known to share DNA barcode sequences with congeners, some exceptions being the Mediterranean fruitfly-*Ceratitis capitata* (Weidemann) and its sister taxon *Ceratitis caetrata* Munro (Barr et al. 2012), balsam woolly adelgid-*Adelges piceae*

(Ratzeburg) and *Adelges nordmannianae* (Eckstein) (Foottit et al. 2009b), and the aphid parasitoids-*Aphidius ervi* Haliday and *Aphidius microlophii* Pennachio & Tremblay (Derocles et al. 2011).

The potential for species to share DNA barcodes is often cited as a critical impediment for the application of DNA barcoding to species identification (Wiemers and Fiedler 2007, Schmidt and Sperling 2008). However, this complication can be easily overcome by flagging these species groups as requiring discrimination using a suite of secondary molecular markers or other independent identification techniques. For example, *C. capitata* and *C. caetrata* can be differentiated from other *Ceratitis* spp. using DNA barcoding, and then differentiated from each other using ITS1 (Barr and McPheron 2006). Similar nested workflows have already been adopted for the identification of regulated pests, where species are identified using morphological characters to a particular taxonomic level then subjected to species-specific molecular tests (Bogdanowicz et al. 1993, EPPO 2005). In this way barcoding can serve as a standardized framework for analysis of regulated pests.

2.1.2 Identification Tools and Resources

The Barcode of Life Data Systems (BOLD; www.boldsystems.org) is a publicly accessible, online workbench for the analysis, management and archiving of barcode data. The BOLD platform provides standard tools to evaluate the performance of DNA barcode data sets and also includes an “ID engine” to identify unknowns based on matching their barcode sequence to the reference library (Ratnasingham and Hebert 2007). The BOLD platform also harvests sequence records from the International Nucleotide Sequence Database Collaboration (INSDC; which is comprised of the DNA Data Bank of Japan (DDBJ), the European Molecular

Biology Laboratory (EMBL) and GenBank). It contains over 1.8 million sequences at the time of this writing, and is increasing at a rate of about 500K sequences annually. BOLD also contains extensive metadata (e.g., specimen images, collection location, and date) associated with individual specimens records. This metadata is extremely useful in validating and vetting reference libraries, and may play a role in the future for spatial-temporal source tracking of emerging and invasive pests (Lees et al. 2011; and see Cameraria ohridella records in BOLD). While the INSDC databases form an "archival record" for published sequences, they are known to include errors and do not contain the same level of specimen/sequence metadata presented in BOLD (e.g., specimen photos, electropherogram trace files) although, recent data-standard agreements ensure that sequences tagged with the keyword 'BARCODE' in INSDC meet the DNA barcode reference sequence data standards (Hanner 2009). Moreover, only BOLD provides an annotation framework that supports tagging and commenting on records and their components (i.e., taxonomy, images, and sequences), allowing for community-based validation of barcode data. BOLD also provides an interim taxonomic system of Barcode Index Numbers (BINs) to facilitate recognition of un-named taxa (Ratnasingham and Hebert 2013). These features distinguish BOLD from the INSDC, which is why BOLD was selected as the standard repository of DNA barcode data for the International Barcode of Life Project (iBOL), the largest biodiversity genomics initiative ever undertaken which aims to create a digital identification system for all eukaryotic life (see: iBOL.org). Importantly, BOLD provides automated support for depositing BARCODE compliant sequence data into the INSDC when researchers prepare their data sets for publication. It also provides data feeds to various barcode campaign websites (e.g., PlantPestBarcoding.org; see discussion for details).

In addition to BOLD, a number of stand-alone and web-based software packages are available to analyze DNA barcode data (DasGupa et al. 2005, Sarkar et al. 2008, Singer and Hajibabaei 2009, Brown et al. 2012). Notability, Q-bank (http://www.q-bank.eu/), a product of the

7th Framework Program of the European Union's Quarantine Barcode of Life (QBOL) initiative (Bonants et al. 2010), provides a web-based identification engine for a number of plant pests, including arthropods. Additionally, a number of stand-alone and web-based statistical and phylogenetic software packages can be applied to analyze DNA barcode data (examples reviewed by Casiraghi et al. 2010).

2.1.3 Identification Methods

For plant production the most common application of DNA barcoding will be identifying unknown specimens to either inform local management or plant pest regulatory actions. Currently, there are a number of methods available to infer species identity with DNA barcode data. However the most commonly used are genetic distance-based methods (e.g., K2P and p-distance) (Collins et al. 2012a). To identify unknowns with this method, distance between the unknown and reference sequences are calculated. This measure is then used to infer identity of unknown specimens, where the least divergence between an unknown sequence and a reference sequence represents a possible match. Once distance measures are calculated, a number of techniques can be applied to infer species identification including tree-based approaches such as neighbour joining (Hebert et al. 2003b), best match (Austerlitz et al. 2009), best close match (Meier et al. 2008) and BOLD-IDS (Ratnasingham and Hebert 2007). The strength of distance-based methods for species identification lies in their flexibility, ease of use, scalability, and their relative robustness versus other identification methodologies (Collins et al. 2012b).

Character-based methods represent another class of DNA barcode identification techniques (DasGupa et al. 2005, Sarkar et al. 2008, Bertolazzi et al. 2009, Wong et al. 2009).

This approach uses species-specific diagnostic nucleotide profiles to identify species. As such, character-based methods are more similar to traditional morphological species identification methods and have been promoted as visually meaningful, analogous to commercial product identification barcodes (Lowenstein et al. 2009). While this approach has a number of strengths, including its adherence to contemporary taxonomic practices, to date character based methods have not been employed as widely as phenetic methods, in part because they do not scale well.

Additionally, numerous computationally-complex, phylogenetic methods, including maximum parsimony (Ekrem et al. 2007), general mixed Yule-coalescent (Derocles et al. 2011, Collins et al. 2012b), minimum distance and fuzzy set approach (Zhang et al. 2012b), machine learning (Zhang et al. 2012a) and Bayesian methods (Lou and Golding 2010) have been used to identify unknowns using DNA barcode data. The methodologies and assumptions among this group of techniques are highly variable. Moreover, run times for some algorithms can be in the order of hours to days for even modest data sets and because these methods are significantly more challenging to implement and interpret they are beyond the scope of this review.

There is no consensus on which method(s) is optimal for species assignment, however, when simple distance based methods are directly compared with computationally intensive methods under realistic circumstances they perform equally well (Austerlitz et al. 2009, Collins et al. 2012b, van Velzen et al. 2012, Zhang et al. 2012a, Zhang et al. 2012b) in all but the most complex cases (Boykin et al. 2011). However, for recently diverged species with low interspecific variation, character based methods may perform better than distance or tree-based methods (van Velzen et al. 2012). Similarly Bayesian methods work well for datasets where there is low interspecific divergence (Lou and Golding 2010). Bayesian methods also provide a statistical framework (Nielsen and Matz 2006) for species identification which may be preferential for some regulatory applications. However, methods based on genetic distance

analyses have been successfully applied to identify high-risk agricultural pests in a regulatory context (Armstrong and Ball 2005, Armstrong 2010).

It is unlikely that any one method of species assignment will be optimal for all data sets and lines of inquiry (Casiraghi et al. 2010), therefore consensus approaches using multiple methods may be beneficial for applications of DNA barcoding (Boykin et al. 2011). However, the need for rapid identification may preclude the use of complex phylogenetic based methods for large data sets. The challenge for DNA barcoding as it matures as a biosecurity tool is to develop best practices for the selection and application of species identification methods in particular contexts in order to harmonize data interpretation among users. This discussion is currently underway among academics (Nielsen and Matz 2006, Boykin et al. 2011, Srivathsan and Meier 2011, Virgilio et al. 2012), but the relative merits of various techniques will need to be tested within a regulatory context as well. Because most methods provide similar results, it is perhaps of equal or greater importance to consider characteristics of the reference data to assess its "fitness-of-use" for regulatory applications. Chief among these is the extent of barcode coverage for the taxa in question, which is a major objective of this review.

2.2 Direct Applications of DNA Barcoding for Plant Production

2.2.1 Regulatory Biosecurity

One of the biggest threats to plant production is the spread of invasive pests within and between national boundaries. To prevent this, national plant protection organizations must be diligent with respect to their pest monitoring programs, which are challenged by both the volume of material requiring inspection (e.g., cargo at ports of entry) and the taxonomic breadth of intercepted organisms. For example, in a survey of interceptions of non-indigenous arthropods

entering the United States over a 17 year period, only 40% of the 565,046 specimens were identified to species (McCullough et al. 2006). It is unclear whether the species identification rate is constrained by lack of time and funding, trained professionals, and/or taxonomist knowledge. Furthermore, the identification accuracy for this border interception data set is unknown. Published rates of morphological species identification accuracy are scarce for arthropods; however, error rates between 7 and 33% at the genus level have been observed for regionally constrained samples of benthic arthropods (Stribling et al. 2008), suggesting this would be a conservative estimate for intercepted pests associated with internationally traded commodities.

It is clear that improvements in current monitoring programs are warranted, and that many operational efficiencies could be gained by the implementation of DNA barcoding. For example, laboratory protocols for DNA barcoding are highly scalable (Hajibabaei et al. 2005, Ivanova et al. 2009), which could accommodate the increasing volume of border interceptions. DNA barcoding also reduces the need for numerous species-specific molecular tests (Armstrong 2010). For example, 17 different molecular methods are currently available for identifying various species of economically important Thysanoptera (Mehle and Trdan 2012), all could potentially be replaced by DNA barcoding. Additionally, DNA barcoding provides more taxonomic information than most alternative molecular species diagnostics which have a binary output, and furthermore may reduce the number of false positives generated by some species-specific tests (deWaard et al. 2010a).

To date DNA barcoding has shown success in identifying and discriminating a number of arthropods of regulatory importance including species of *Liriomyza* (Agromyzidae) (Scheffer et al. 2006), Lymantriidae (Armstrong and Ball 2005, Ball and Armstrong 2006, deWaard et al. 2010b), *Spodoptera* (Noctuidae) (Nagoshi et al. 2011), Tephritidae (Armstrong and Ball 2005, Ball and Armstrong 2006), Thysanoptera (Glover et al. 2010, Qiao et al. 2012) and siricid wood

wasps (Wilson and Schiff 2010, Schiff et al. 2012). It is notable that DNA barcoding has been employed in New Zealand since 2005 to identify border-intercepted immature life stages of Tephritidae, and Lymantriidae (Armstrong 2010).

Perceptively, DNA barcoding was included as a molecular identification technique for potential inclusion in diagnostic protocols for regulated pests by the IPPC (FAO. 2006). Therefore, it is likely to play a role in international biosecurity as additional diagnostic protocols for regulated pests are published under the IPPC ISPM No. 27 (FAO. 2006). While the pace of publication for internationally accepted diagnostic protocols of any kind remains alarmingly slow, broad methodological adoption of barcoding could help expedite the process. However, the future of DNA barcoding as a biosecurity tool will largely depend on the continued development of reference libraries for pest taxa and more critical resolution of sequence data as it relates to previously established species boundaries (Armstrong 2010, Boykin et al. 2011). This will be particularly important in cases where DNA barcode data suggests the presence of additional taxa not previously recognized by classical taxonomy, where descriptions can take decades (Fontaine et al. 2012).

2.2.2 Integrated Pest Management Decision Making

Another potential application of DNA barcoding for agriculture is to aid in IPM decision-making, which hinges on the ability to properly identify pest and beneficial organisms. This is because the biological traits (e.g., phenology or pesticide susceptibility) exploited by IPM tactics to prevent pest establishment and manage pest populations are often species specific. For example, *Aphis pomi* and *Aphis spiraecola* are sympatric pests of apples in the American Midwest, and can be difficult to discriminate morphologically (Foottit et al. 2009a, Naaum et al.

2012). Management of these pests can involve application of pesticides and/or horticultural oils yet these species vary significantly in their susceptibility to pesticides (Lowery et al. 2005, Lowery et al. 2006). Furthermore, both species use a different overwintering host, which can affect both the selection and timing of management practices. DNA barcoding readily differentiates these two pests (Foottit et al. 2009a), and therefore could be easily incorporated into IPM programs for this particular pest complex.

Another example is *Liriomyza* spp. leafminers, a group consisting of at least 376 species. Four of these are significant global pests and can be difficult to diagnose with morphological characters (EPPO 2005). They differ in their fecundity and susceptibility to various pesticides and biological control agents (Reitz and Trumble 2002, Gao et al. 2011, Gao et al. 2012). These biological differences are speculated to have led to the displacement of *Liriomyza sativae* (Blanchard) by *Liriomyza trifolii* (Burgess) in China and California, and of *L. trifolii* by *L. sativae* in Japan (Gao et al. 2011). Given the potential instability in these pest complexes as a result of common pest management practices, it seems that DNA barcoding could assist management decisions, as DNA barcoding readily identifies the four most common *Liriomyza* pest species (Scheffer et al. 2006). In practice, DNA barcoding will be highly useful in the context of IPM when morphologically similar pest species have different economic action/injury thresholds, phenology (i.e., number of generations, overwintering host), and/or susceptibility to specific management practices (i.e., pesticide resistance, or susceptibility to a biological control agent).

2.2.3 Integrative Taxonomy of Pest And Beneficial Organisms

DNA barcoding has shown great potential for illuminating cases of cryptic diversity (Hebert et al. 2004, Mutanen et al. 2012a) and assisting in taxonomic revisions (Schiff et al.

2012). Although the concept of defining species based solely on evidence from DNA barcode data is highly controversial, the use of DNA barcoding to complement traditional morphological taxonomy is widely accepted (Sheffield et al. 2009, Chesters et al. 2012, Forbes et al. 2012, Schiff et al. 2012, van Nieukerken et al. 2012). As a result, DNA barcoding is well positioned to aid in the integrative taxonomy of pest and beneficial organisms.

To date, DNA barcoding has helped form species hypotheses for a number of pest taxa, which may eventually lead to better management of particular pests. For example, the western flower thrips, *Frankliniella occidentalis* (Pergande), is proposed to consist of two sympatric species in both California and New Zealand (Rugman-Jones et al. 2010) based partly on DNA barcode data. This division may account for at least some of the phenotypic and behavioral plasticity observed in *F. occidentalis*, a species with 18 recognized synonyms (Hoddle et al. 2012). Similarly, the species status of *Bemisia tabaci* is again being brought into question on the basis of strong evidence from mating studies and DNA barcode data (De Barro et al. 2011). It has been proposed that *B. tabaci* consists of 24 cryptic species, most of which correspond to previously identified biotypes (De Barro et al. 2011), some of which differ in traits influencing their management, such as pesticide susceptibility and ability to transmit plant viruses. Notably, DNA barcoding successfully distinguishes *B. tabaci* biotypes of significance to plant production (Shatters et al. 2009). It has also helped identify two cryptic species within the fruit pest- *Zaprionus indianus* (Drosophilidae) (Yassin et al. 2008). In addition to behavioral and morphological data, a single fixed nucleotide difference within the DNA barcode region helped facilitate the description of a cryptic species of soybean aphid parasitoid- *Binodoxys koreanus* (Braconidae) from Korea (Desneux et al. 2009). Barcoding also played an important role in the identification of *Antispila oinophylla* (Heliozelidae) a species of leaf mining moths invasive in Italy, originating from North America (van Nieukerken et al. 2012).

Although a number of analytical techniques are available to investigate the presence of cryptic species, they are typically only applied in the context of testing an established hypothesis. Because barcoding can aid the identification of known species and flag the presence of potential new ones, it can be used to test existing species hypotheses and in the process, it often generates new taxonomic hypotheses for testing within an integrative taxonomic framework (van Nieukerken et al. 2012). As a tool of integrative taxonomy, DNA barcoding also aids in the interpretation of morphological variation, helping to expedite the descriptive taxonomic process (Yang et al. 2012).

2.3 DNA Barcode Coverage for Pest Species

The adoption of DNA barcoding as a bio-surveillance tool is largely dependent on the presence of reference sequences for each pest organism. To assess the utility of current DNA barcode reference libraries for agricultural applications, I established a unique new global pest list by integrating all members of the phyla Arthropoda, Mollusca, and Nematoda and from the following pest lists:

- CABI - Distribution Maps of Plant Pests (retrieved May 20th, 2012): http://www.cabi.org/dmpp/
- CFIA - Pests Regulated by Canada, (retrieved May 20th, 2012) http://www.inspection.gc.ca/english/plaveg/protect/listpespare.shtml
- EPPO - A1 List Of Pests Recommended For Regulation As Quarantine Pests - September 2011 (retrieved May 20th, 2012): http://www.eppo.int/QUARANTINE/quarantine.htm

- EPPO - A2 List Of Pests Recommended For Regulation As Quarantine Pests - September 2011 (retrieved May 20th, 2012): http://www.eppo.int/QUARANTINE/quarantine.htm
- NAPIS - Pest Tracker - National Agricultural Pest Information System (retrieved, May 28th, 2012): http://pest.ceris.purdue.edu/index.php
- USDA-APHIS Regulated Pest List (retrieved May 20th, 2012): http://www.aphis.usda.gov/import_export/plants/plant_imports/regulated_pest_list.shtml

Each species name and author were validated for all unique pest list entries using the Catalogue of Life: 30th May 2012 (www.catalogueoflife.org) (Bisby et al. 2012). If absent, species names or ranks were queried against the Global Biodiversity Information Facility (GBIF) Checklist Bank at (http://www.gbif.org/species). In cases where the species name was not present in either database then author information from the source which the query originated was used, and noted (Appendix 1). Classification follows Catalogue of Life: 30th May 2012 (Bisby et al. 2012), and BOLD (Ratnasingham and Hebert 2007). The estimated number of species for each genus was determined using Catalogue of Life by summing the number of accepted species names (not including sub-species) in each genus. Species names were queried then against BOLD (Ratnasingham and Hebert 2007) to determine the number of currently available DNA barcode sequences, and the number of publically available sequences on BOLD. Of the publically available sequences, the number of sequences which were harvested from INSDC was also recorded. All genera were subsequently queried against BOLD to determine the number of included species with DNA barcode sequences to provide an estimate of generic-level coverage.

2.3.1 Summary of Pest List DNA Barcode Data

Examination of the six pest lists yielded 1044 unique species level entries derived from 22 orders, 145 families, and 619 genera (Table 2.1: Appendix 1), in addition to 29 regulated genera (Table 2.2: Appendix 1). A total of 25 entries on the six pest lists were listed under synonyms according to the COL and GBIF databases. The composite pest list is dominated by insects (86%: 897/1044), followed by nematodes (6%: 63/1044), mites (4.5%: 47/1044) and mollusks (3.4%: 35/1044). Coleopteran species represent the largest proportion of entries at 24.7% (258/1044), followed by Hemiptera (23.1%: 241/1044), Lepidoptera (22.8%: 238/1044), and Diptera (7.8%: 81/1044). All other orders represented less than <5% of total entries. More than half (53.5%: 559/1044) of the species level entries on the composite pest list had at least one barcode sequence. Of this group, 28.4% (297/1044) had between 1-10 DNA barcodes per species, the remainder, 25.1% (262/1044) had >10 per species. However, of the 559 species with DNA barcodes only 334 are currently publically available, and all records for 171 of these species were harvested from INSDC. Of the most representative orders, Lepidoptera had the best species-level barcode coverage, with 76.9% (183/238)with at least 1 barcode record and 55.0% (131/238) with >10, followed by Diptera with 67.9% (55/81) and 33.3% (27/81), Hemiptera with 48.5% (117/241), and 19.5% (47/241), and Coleoptera 46.9% (121/258) and 10.9% (28/258), respectively. The lowest coverage was for the following orders Trombidiformes, Triplonchida and Tylenchida. Of the 561 species with DNA barcode data, 356 have at least 1 public record and 186 of those species are only represented by sequences harvested from INSDC Genbank.

Table 2.1: Taxonomic and DNA barcode coverage of six pest lists summarized to order.

Phylum: Class Order	Spp. on Global Pest List[1]		DNA Barcode Coverage: Spp. with 1-10 Barcodes[2]		DNA Barcode Coverage: Spp. with >10 Barcodes[2]		Public Data: Spp. with >=1 Public Record	Public Data: Spp. only represented by INSDC data
Arthropoda: Arachnida								
Mesostigmata	4	(0.4)	1	(25.0)	2	(50.0)	3	2
Trombidiformes	43	(4.1)	3	(7.0)	3	(7.0)	7	7
Arthropoda: Collembola								
Symphypleona	2	(0.2)	1	(50.0)	0	(0.0)	1	0
Arthropoda: Insecta								
Coleoptera	258	(24.7)	93	(36.0)	28	(10.9)	68	50
Dermaptera	1	(0.1)	1	(100.0)	0	(0.0)	0	0
Diptera	81	(7.8)	28	(34.6)	27	(33.3)	51	26
Hemiptera	241	(23.1)	69	(28.6)	48	(19.9)	68	37
Hymenoptera	39	(3.7)	20	(51.3)	8	(20.5)	17	9
Isoptera	3	(0.3)	3	(100.0)	0	(0.0)	0	0
Lepidoptera	238	(22.8)	52	(21.8)	131	(55.0)	105	26
Orthoptera	11	(1.1)	6	(54.5)	0	(0.0)	2	2
Thysanoptera	25	(2.4)	6	(24.0)	9	(36.0)	12	12
Sub-total	946		283		256		334	171
Mollusca: Bivalvia								
Veneroida	2	(0.2)	1	(50.0)	1	(50.0)	2	1
Mollusca: Gastropoda								
Architaenioglossa	4	(0.4)	2	(50.0)	2	(50.0)	4	4
Littorinimorpha	1	(0.1)	0	(0.0)	1	(100.0)	1	1
Pulmonata	4	(0.4)	1	(25.0)	0	(0.0)	1	1
Stylommatophora	22	(2.1)	5	(22.7)	2	(9.1)	7	7
Systellommatophora	2	(0.2)	0	(0.0)	0	(0.0)	0	0
Sub-total	35		9		6		15	14
Nematoda: Adenophorea								
Dorylaimida	8	(0.8)	1	(12.5)	0	(0.0)	1	0
Triplonchida	9	(0.9)	0	(0.0)	0	(0.0)	0	0
Nematoda: Secernentea								
Aphelenchida	4	(0.4)	3	(75.0)	0	(0.0)	3	3
Tylenchida	42	(4.0)	1	(2.4)	0	(0.0)	1	1
Sub-total	63		6		0	(0.0)	5	5
Total	1044		297	(28.4)	262	(25.1)	356	186

[1] Number in parentheses represents the number of species within an order as a percentage of the total number of species from all orders.

[2] Number in parentheses represent the number of species with DNA barcodes as a percentage within an Order in a given category.

Table 2.2: Summary of pests listed at the generic level from six pest lists, the estimated number of species in each genus, number of species represented in BOLD, and number of species with species with DNA barcodes.

Phylum: Class: Order: Family	Genus	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	Num. of Spp. in COL[1]	Num. of Spp. in BOLD	Num. of Spp. with Barcodes
Arthropoda: Insecta										
Coleoptera: Brachyceridae	*Brachycerus*	-	-	-	+	-	-	601	0	0
Coleoptera: Cerambycidae	*Anoplophora*	-	-	-	-	+	+	42	9	8
Coleoptera: Cerambycidae	*Monochamus*	-	-	-	-	+	+	137	17	17
Coleoptera: Cerambycidae	*Xylotrechus*	-	-	-	-	+	-	204	15	15
Coleoptera: Curculionidae	*Conotrachelus*	-	-	-	+	-	-	854	31	22
Coleoptera: Curculionidae	*Xyleborus*	-	-	-	+	+	-	1496	58	9
Coleoptera: Dryophthoridae	*Metamasius*	-	-	-	+	-	-	67	27	15
Coleoptera: Scarabidae	*Adoretus*	-	-	-	+	-	-	464	4	0
Coleoptera: Scarabidae	*Phyllophaga*	-	-	-	+	-	-	827	205	123
Coleoptera: Scarabidae	*Popillia*	-	-	-	-	+	-	320	1	1
Diptera: Tephritidae	*Ceratitis*	-	-	-	+	-	-	184	57	53
Diptera: Tephritidae	*Pterandrus*	-	-	-	+	-	-	0	0	0
Hymenoptera: Chrysididae	*Chrysis*	-	-	-	+	-	-	8	79	57
Hymenoptera: Megachilidae	*Coelioxys*	-	-	-	+	-	-	500	111	91
Lepidoptera: Noctuidae	*Copitarsia*	-	-	-	-	+	-	8	5	5
Lepidoptera: Pyralidae	*Dioryctria*	-	-	-	-	+	-	66	37	36
Lepidoptera: Tortricidae	*Laspeyresia*	-	-	-	+	-	-	0	8	5
Lepidoptera: Tortricidae	*Proeulia*	-	-	-	+	-	-	23	0	0
Mollusca: Gastropoda										
Stylommatophora: Hygromiidae	*Cochlicella*	-	-	-	-	+	-	0	2	2
Stylommatophora: Helicidae	*Helix*	-	-	-	-	-	+	2	3	3
Stylommatophora: Hygromiidae	*Monacha*	-	-	-	-	+	-	1	4	4
Systellommatophora: Veronicellidae		-	-	-	-	+	-	10	5	4
Systellommatophora: Veronicellidae	*Veronicella*	-	-	-	-	+	-	1	0	0
Nematoda: Adenophorea										
Dorylaimida: Longidoridae	*Longidorus*	-	-	-	-	-	+	4	3	0
Triplonchida: Trichodoridae	*Paratrichodorus*	-	-	-	-	+	-	5	0	0
Triplonchida: Trichodoridae	*Trichodorus*	-	-	-	-	-	+	1	0	0
Dorylaimida: Longidoridae	*Xiphinema*	-	-	-	-	+	+	4	4	3
Nematoda: Secernentea										
Tylenchida: Tylenchidae	*Anguina*	-	-	-	-	+	-	5	0	0
Tylenchida: Heteroderidae	*Meloidogyne*	-	-	-	-	+	-	6	0	0
Tylenchida: Hoplolaimidae	*Pratylenchus*	-	-	-	-	+	-	5	0	0

[1] (COL) Catalogue of Life: 30th May 2012 (www.catalogueoflife.org)

Of the 29 regulated genera in the composite pest list, 19 have species with barcodes (Table 2.2). However, the species coverage within each genus was relatively low, ranging from 0.00% to 75.0%, (avg. 14.5%), when excluding the three entries where COL listed 0 species in those genera, or when BOLD had more entries than COL (Hymenoptera: Chrysididae: *Chrysis*). Species coverage within each genus for the entire composite pest list (Appendix 1) ranged from 0.00% to 100% (avg. 24.5%), when excluding entries where COL listed 0 species in a given genus, or when BOLD had listed more species than COL.

The percentage of pest species with at least one DNA barcode for each pest listed was highest for, CFIA (67.9%, 55/81), followed by EPPO A1 (62.8%, 59/94), APHIS (59.6%, 53/89), NAPIS (59.5%, 201/338), CABI (58.4%, 419/718), and EPPO A2 (53.8%, 35/65) (Table 2.3).

Table 2.3: Number of species and percent DNA barcode coverage for various categories of public pest lists.

		DNA Barcode Coverage				Public Data	
Pest list	Spp. on Global Pest List	Spp. with 1-10 Barcodes		Spp. with >10 Barcodes		Spp. with >=1 Public Record	Spp. only represented by INSDC data
CABI	718	225	(31.3)	194	(27.0)	263	144
EPPO-A1	94	30	(31.9)	29	(30.9)	51	31
EPPO-A2	65	20	(30.8)	15	(23.1)	25	18
APHIS	89	25	(28.1)	28	(31.5)	34	20
NAPIS	338	93	(27.5)	108	(32.0)	140	73
CFIA	81	26	(32.1)	29	(35.8)	36	19

2.4 Discussion

Since DNA barcoding was first proposed (Hebert et al. 2003b, Hebert et al. 2003a), associated research has primarily focused on answering ecological (Fernandez-Triana et al. 2011) and taxonomic questions (Forbes et al. 2012, Mutanen et al. 2012b). To date there have been relatively few DNA barcoding projects explicitly dedicated to agricultural pest taxa, with

some notable exceptions (Armstrong and Ball 2005, Ball and Armstrong 2006, Foottit et al. 2008, Bonants et al. 2010, deWaard et al. 2010b). However, DNA barcodes have been generated for 53.7% (561/1044) of species present on the current composite pest list. Equally encouraging is the fact that more than half (25.1%, 262/1044) of the species which have been barcoded are represented by >10 DNA barcode records. Given this current level of DNA barcode coverage for plant pests, it would seem appropriate to use DNA barcoding as a species-specific diagnostic protocol and as a general identification tool for both regulatory biosecurity and IPM decision making. However, the ability of barcoding to contribute to pest monitoring programs varies substantially among taxa. Not surprisingly, Lepidoptera have the best coverage of any order in the current composite pest list, owing to the exhaustive Lepidoptera DNA barcode library building campaigns (Hebert et al. 2009, International Barcode of Life Project 2009). In comparison, Coleoptera, which comprises the largest order on the current composite pest list, and comparable low number of species represented by >10 DNA barcodes in BOLD. Hemiptera has received substantial attention, particularly for Aphididae, Adelgidae, and Heteroptera (Foottit et al. 2008, Foottit et al. 2009b, Lee et al. 2011b, Park et al. 2011a, Park et al. 2011b), but there are a large number of species without barcodes in the families Cicadellidae, Diaspididae, and Pseudococcidae. Despite the overall coverage for the order Lepidoptera, there are 17 species of Tortricidae that remain absent. Also troubling is the lack of DNA barcodes for Eriophyidae, Tetranychidae, and Thysanoptera. Thysanoptera is a particularly important global pest order that has had substantial resources dedicated to construction of traditional identification tools (Mehle and Trdan 2012) and should be the focus of future barcode reference library building projects.

This patchy sequence coverage illustrates the need for dedicated DNA barcode projects for pest taxa, particularly for families with low coverage. Further sampling of pest taxa is needed to better capture the phylogeographic and /or cryptic variation within these species. As such,

taxonomic experts working on these pest groups should be encouraged to contribute to the construction of the barcode reference library. To facilitate collaboration among the biologists, taxonomists, and regulators who will use the data, I (along with colleagues, see website for details) created a web-portal (plantpestbarcoding.org) to track the growth of barcode coverage for pest species in BOLD in real time. The objective of this web-portal is to consolidate available information about barcode coverage for pest arthropods, providing a "gap analysis" needed to support efficient barcode reference library construction for uncharacterized pest taxa, and, most importantly, to provide a resource for those wishing to use DNA barcode as a bio-monitoring tool.

2.5 Conclusions

DNA barcoding has great potential to assist plant production and phytosanitary regulation by facilitating pest identifications. Although current DNA barcoding reference libraries for pest taxa are incomplete they do contain a large proportion of pest species of global concern, which augurs well for the future of barcoding as a tool for pest identification. I created a web-portal to track the DNA barcode library coverage of pest species (plantpestbarcoding.org). I encourage further reference library construction for pest species globally and the development of more IPPC protocols using barcoding in order to increase efficiency and volume of identifications related to pest interceptions, thereby safeguarding plant production efforts on a global scale.

Chapter 3

DNA BARCODING TO IMPROVE IDENTIFICATION OF BORDER-INTERCEPTED SPECIMENS OF POTENTIAL PEST ARTHROPODS

3.0 Abstract

A key component of national and regional integrated pest management (IPM) is border inspection programs, which are responsible for detecting and identifying invasive pest arthropods in transit. These programs inform biosecurity actions (e.g., destruction or disinfestation of infested shipments) and biosecurity policies (e.g., refining pathway analysis for particular pests) that vary depending on the species of the intercepted specimens. Unfortunately, the detection targets for most pest arthropods are the immature life stages, which in many cases are more difficult to identify than their adult counterparts. This inability to identify intercepted specimens to the level of species reduces the capacity of border inspection programs to inform biosecurity actions and policies thereby jeopardizing national IPM programs and threatening entire agricultural sectors. A potential solution is to use DNA barcoding to assist in the identification of border intercepted specimens. To explore the suitability of DNA barcoding for use as an identification technique for border inspection programs, I examined the discordance between a set of intercepted Lepidoptera, identified morphologically and via DNA barcoding, using Barcode of Life Data Systems Identification Systems (BOLD-IDS), Barcode Index Number (BIN) system, and current DNA barcoding reference libraries. Although current barcode reference libraries were not able to identify all of the intercepted specimens to the level of species, DNA barcoding did provide more taxonomic information for most specimens when compared with morphological methods. Furthermore, employing the BIN system provided a method to assign intercepted specimens a species-like identifier, which in the absence of binomial names may facilitate communication and research regarding unidentifiable specimens.

As a result, these data support the use of DNA barcoding to assist in the identification of border interceptions. Finally, to enhance the identification success rate of DNA barcoding when applied to intercepted Lepidoptera, I generated reference barcodes for 25 species of Tortricidae that are anticipated to be found in interception pathways but for which no reference barcodes previously existed.

3.1 Ability of DNA Barcoding to Identify Border-Intercepted Lepidoptera

3.1.1 Introduction

Invasive agricultural and environmental insect pests are a major threat to global food security and the economy. The direct damage caused by and management of invasive insect pests costs the global economy billions of dollars each year (Pimentel et al. 1992, Pimentel et al. 2005, Oerke 2006, Oliveira et al. 2014). Furthermore, incursions of invasive pests may disrupt international trade and market access, jeopardizing the financial security and personal wellbeing of individuals and communities relying on the production of the affected commodities. In addition, invasive pests have the capacity to cause long-term and potentially irreversible environmental damage, reducing the quality of ecosystems on a landscape scale and further impacting conservation plans for native species (Caffrey et al. 2014). Unfortunately, as a result of globalization and climate change, it is generally expected that the frequency of pest invasions will increase in the coming decades (Work et al. 2005, Westphal et al. 2008, Navia et al. 2010, Lenda et al. 2014).

In order to prevent the establishment and spread of invasive species, National Plant Protection Organisations (NPPOs) enact various biosecurity policies and procedures. One such procedure is border inspection programs, which aim to detect and identify invasive pests in

transit associated with a particular commodity. The detections made during border inspection programs are meant to inform policies (e.g., import restrictions and guidelines) and/or trigger biosecurity actions (e.g., trade sanctions, commodity decontamination, and/or quarantine). Furthermore, data gathered from inspection programs can be used to improve pathway analysis for invasive species, thereby increasing the ability of regulators to manage pest invasions. However, in some cases only 10% of established invasives are intercepted prior to their establishment (Kenis et al. 2007), demonstrating a clear need to improve biosecurity programs. Currently, strategies to advance pathway targeting and specimen detection have been developed for numerous commodity and trade-linkage scenarios both pre- and post-invasion (Jeger et al. 2007, Harwood et al. 2009, Moslonka-Lefebvre et al. 2011, Vanninen et al. 2011, Whittle et al. 2013). Ultimately, however, the effectiveness of these programs will not only rely on the ability of NPPOs to detect potential invasives in transit, but it also hinges on the NPPO's ability to identify intercepted specimens to relevant taxonomic ranks (e.g., species).

The taxonomic impediments that challenge the identification of intercepted arthropods are not unique, but rather are shared by the larger research community and stem from the heavy or exclusive reliance on morphologically-based taxon identification. Although critical evaluations on the reproducibility of morphologically-based taxon assignments are limited, a few general conclusions can be made from the available literature. First is the potential for subjective interpretation of morphological characters, which may result in inconsistent taxonomic assignment of specimens by different investigators. Second, discordance between identifications performed by different investigators increases at lower taxonomic ranks (Sweeney et al. 2011, Ko et al. 2013). Third, due to the variety of characters used to diagnose taxa, some taxa are simply more difficult to identify than others. Further complicating matters is that character suites used for identification of many species only may be present on a particular life stage (e.g., adults) and/or sex, rendering the others unidentifiable. For invasive arthropods

this is particularly significant since the interception targets for most species are immature life stages, yet the majority of arthropod taxonomy is based on adult morphology.

While the preceding impediments are concerned with described species, what may present a greater challenge is that the majority of insect biodiversity remains undescribed (Stork 1993, Mora et al. 2011). Conservative estimates place global insect biodiversity between 5 and 15 million species, of which approximately 1 million have been named (Stork 1993). While the overwhelming majority of these undescribed species are unlikely to pose a significant threat to agriculture or the environment, accurately identifying individuals of species that pose a threat against this background diversity is certainly challenging, particularly in a time-sensitive manner.

The potential outcome of these factors is that many intercepted specimens may only be identifiable to taxonomic ranks above the level of species. This is clearly illustrated by an interception dataset ranging from 1984 to 2000 containing over 565,046 specimens, of which only 40% were identified to the level of species (McCullough et al. 2006). This low species identification rate is important for two reasons. First, is that the vast majority of invasives are typically managed and/or regulated at the species level, and therefore biosecurity actions may only be implemented after a species-level identification of a regulated pest. Second, the ability to access life history data associated with a given species and/or its close relatives may have important predictive power (Worner and Gevrey 2006), particularly in cases of repeated interceptions of species which are not currently classified ‘invasive’. For example, it is possible that lesser known or otherwise innocuous species, once free of their natural enemies, may become economically important in novel environments (Brasier 2008). However, this is entirely dependent on a given species’ biology and life history, information that is only accessible once specimens have been appropriately identified. Therefore, in the absence of species names or other unique identifiers, interception datasets have a reduced capacity to contribute to biosecurity actions.

A potential solution to many of these species identification challenges is the integration of DNA barcoding into border interception programs. In brief DNA barcoding is a standardized molecular specimen identification methodology (Hebert et al. 2003b), with numerous biosecurity applications (Ball and Armstrong 2006, Armstrong 2010, Bonants et al. 2010, deWaard et al. 2010b, Frewin et al. 2013, Wilson et al. 2013, Serrao et al. 2014). DNA barcoding also has been used extensively in research to identify immature life stages of animals for which morphological diagnosis is difficult or impossible (Wilson and Schiff 2010, Sweeney et al. 2011, Ko et al. 2013, Brabrand et al. 2014, Mastrangelo et al. 2014, Pramual and Wongpakam 2014). Furthermore, DNA barcoding as it is currently practised is supported by a bio-informatics platform the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007), which has recently implemented a system to generate a persistent interim taxonomic framework called the Barcode Index Number (BIN) system, for unidentified specimens based on their DNA barcode sequence data (Ratnasingham and Hebert 2013). The BINs framework has been shown to approximate current species level classifications for a number of arthropods groups, supporting its use as a method to assign unidentified specimens to operational taxonomic units (OTUs) (Ratnasingham and Hebert 2013, Zahiri et al. 2014). By enhancing NPPOs' ability to identify immature arthropods while simultaneously providing a mechanism to provide a species-level-interim taxonomy for morphologically unidentifiable specimens, DNA barcoding is an attractive solution for improving the identifications made as part of border inspection programs.

The potential for DNA barcoding to aid in the identification of border-intercepted specimens can be measured by its ability to provide taxonomic resolution equivalent to or better than that achieved by traditional methods. In the strict sense, this refers to the number of specimens that can be identified to species. In a broader sense, this may also include the number of specimens that it can group into an interim taxonomic framework (e.g., BINs). Furthermore, when a molecular diagnostic is applied as a complementary approach to

specimen identification, its value can also be evaluated by its ability to highlight potential misidentification. As a test case to explore the ability of DNA barcoding and the BINs framework to assist in the identification of intercepted insect specimens, I examined a set of predominantly immature Lepidoptera, primarily of the Gelechioidea and Tortricidae, intercepted by USDA-APHIS-PPQ inspectors and identified by USDA Systematic Entomology Lab. Both of these taxa are extremely diverse and contain numerous regulated and economically important species that are well represented on BOLD (Frewin et al. 2013). Tortricidae contains ca. 10,350 species (Gilligan et al. 2012), of which ca. 3,800 species presently are represented on BOLD. Gelechioidea, one of the largest super-families of Lepidoptera, contains ca. 18,480 species across 21 families (Zhang 2011), and it is believed that the majority of species are yet to be described (Hodges 1999). Currently, 7,900 Gelechioidea species are represented on BOLD. The objective of this test case is to examine the concordance between morphological- and DNA barcode-based identifications, and to develop a framework to use DNA barcoding and the BINs system to assist in the identification of border intercepted specimens. I generated DNA barcodes from 201 border-intercepted Lepidoptera and employed the BOLD platform to identify and characterize the diversity of these specimens.

3.1.2 Methods

A set of 241 Lepidoptera intercepted at US ports of entry was compiled and identified with currently available morphological resources by John W. Brown (JWB). This set consisted mainly of larvae; however, there were a few adult specimens. Complete specimen data for these individuals can be found on BOLD under project ITLP.

For adult specimens a single hind leg was used as a source of DNA. For immature specimens (larvae or pupae) > 5mm in length, DNA was obtained from a 2-3 mm^2 piece of tissue removed from the lateral side of the specimen with flame-sterilized forceps and scissors. DNA was extracted from whole immature specimens (larvae or pupae) < 5mm in length, many of which were destroyed in the extraction process. DNA was extracted using the alkaline lysis DNA extraction XytXtract Insect (ANDE) (Xytogen; Perth, Australia) kit using manufacturer recommended protocols (Castalanelli et al. 2010). DNA extracts were stored at -20°C prior to analysis.

All PCR reactions were conducted in a total volume of 12.6µL, which included 6.25µL of 10% trehalose, 2.00µL of water, 1.25µL of 10X PCR Buffer (Life Technologies™), 0.625µL $MgCl_2$ (50 mM), 0.25µL of dNTP (10 mM), 0.0625µL of Platinum® Taq (Life Technologies™), 0.10µL total volume of each forward and reverse primers, and 2.00µL of DNA template (Ivanova et al. 2009). All DNA extracts were subjected to PCR with the primer pair LepF1(5'-ATT CAA CCA ATC ATA AAG ATA TTG G-3')/LepR1(5'-TAA ACT TCT GGA TGT CCA AAA AAT CA-3'). Extracts that failed to amplify were failure tracked with the primers mLepF1(5'-GCT TTC CCA CGA ATA AAT AAT A-3')/LepR1, and LepF1/mLepR1(5'- CCT GTT CCA GCT CCA TTT TC-3'). The following cycling conditions were used for all primer pairs: an initial denaturation at 94°C for 1min, followed by 5 cycles of 94°C for 40s, 45°C for 40s, 72°C for 1min, followed by 35 cycles of 94°C for 40s, 51°C for 40s, 72°C for 1min, and a final extension at 72°C for 5min (Hajibabaei et al. 2006). PCR products were visualized on a 2% agarose gel pre-stained with SYBR® Safe DNA gel stain (Life Technologies™). PCR products were sent to the Advanced Analysis Centre at the University of Guelph (Guelph, Ontario, Canada) for sequencing. Sequencing was performed on an Applied Biosystems® 3730 DNA Analyzer. Specimen metadata, photographs, trace-files, and DNA barcode sequence were deposited in BOLD project ITLP. All specimens with sequences ≥407bp were assigned a BIN by the BINs algorithm on BOLD.

3.1.2.1 Data Analysis

All specimens from which a sequence was generated were identified using BOLD-IDS with the *Species Level Barcode Record* option. The identification outcome for each specimen was assigned to one of the following categories: a species-level match (ID), match to specimens with interim taxonomy or manuscript name (ID-interim), match to multiple species (Multi), match to multiple specimens with interim taxonomy or manuscript name (Multi-interim) or no species level match (NoMatch). For specimens in the ID-interim, Multi, Multi-interim, and NoMatch category, taxon assignments were at the level of genus and were obtained from the Identification Summary box in the BOLD-IDS output, where the lowest taxonomic rank with >98% probability of placement was used.

For each specimen, morphological taxon assignments were directly compared with those inferred from BOLD (as above) at each at the following ranks: superfamily, family, subfamily, tribe (only for the Tortricidae), genus, and species, and recorded as either concordant or discordant. In addition, the identification method that generated more taxonomic information for each specimen was recorded.

Each unique BIN was assessed for taxonomic discordance by manually accessing each individual BIN page on BOLD. Each BIN was assigned to one of the following categories: Singleton (a BIN with only a single specimen), concordant (a BIN with no conflicting taxonomic assignments, including specimens listed under a synonym, and/or manuscript/interim names listed alongside valid names), or discordant (a BIN with conflicting taxonomic assignments).

3.1.3 Results

Of the 241 specimens examined, full length DNA barcodes (≥648bp) were obtained for 188 individuals. A single primer pair LepF1/LepR1 was used to generate barcodes for 173 individuals, while the remaining individuals were generated with a combination of mLepF1/LepR1 and LepF1/mLepR1. DNA Barcode fragments (≤407bp) were obtained from an additional 13 individuals, bringing the total number of specimens with sequence data to 201.

Using the *Species Level Barcode Record* option BOLD-IDS assigned a species level identification (ID) for 108 specimens, and 9 additional specimens were matched to single species with interim taxonomy or manuscript name (ID-interim). A further 18 specimens were individually matched to multiple species (Multi), and 4 specimens were individually matched to multiple species with manuscript names (Multi-interim). Finally, 63 specimens from the intercepted dataset were not assigned species level matches using BOLD-IDS with the *Species Level Barcode Record* option. The 195 specimens with sequences ≥407bp were assigned to 93 unique BINS by BOLD. In total 31 specimens were assigned to a singleton BIN, 119 to concordant BINs and 44 to discordant BINs. Of the 93 unique BINs in this project, 31 were singleton, 43 concordant, and 19 were discordant.

Compared to morphological identification, BOLD provided a more precise identification (i.e., lower level taxon assignment) for 39.8% (80/201) of specimens, the majority of which were identified to the level of genus or species (Figure 3.1.1.A, Table 3.1.1). Morphology provided a more precise identification for 15.9% (32/201) of specimens, while the remaining 89 specimens received the same level of identification from both methods. The concordance between morphological- and BOLD-based identifications was 73.1% (146/201) for all specimens at the lowest rank which a direct comparison could be made (Figure 3.1.1.B, Table 3.1.1). Instances of discordance occurred at all ranks but were most common at the level of species.

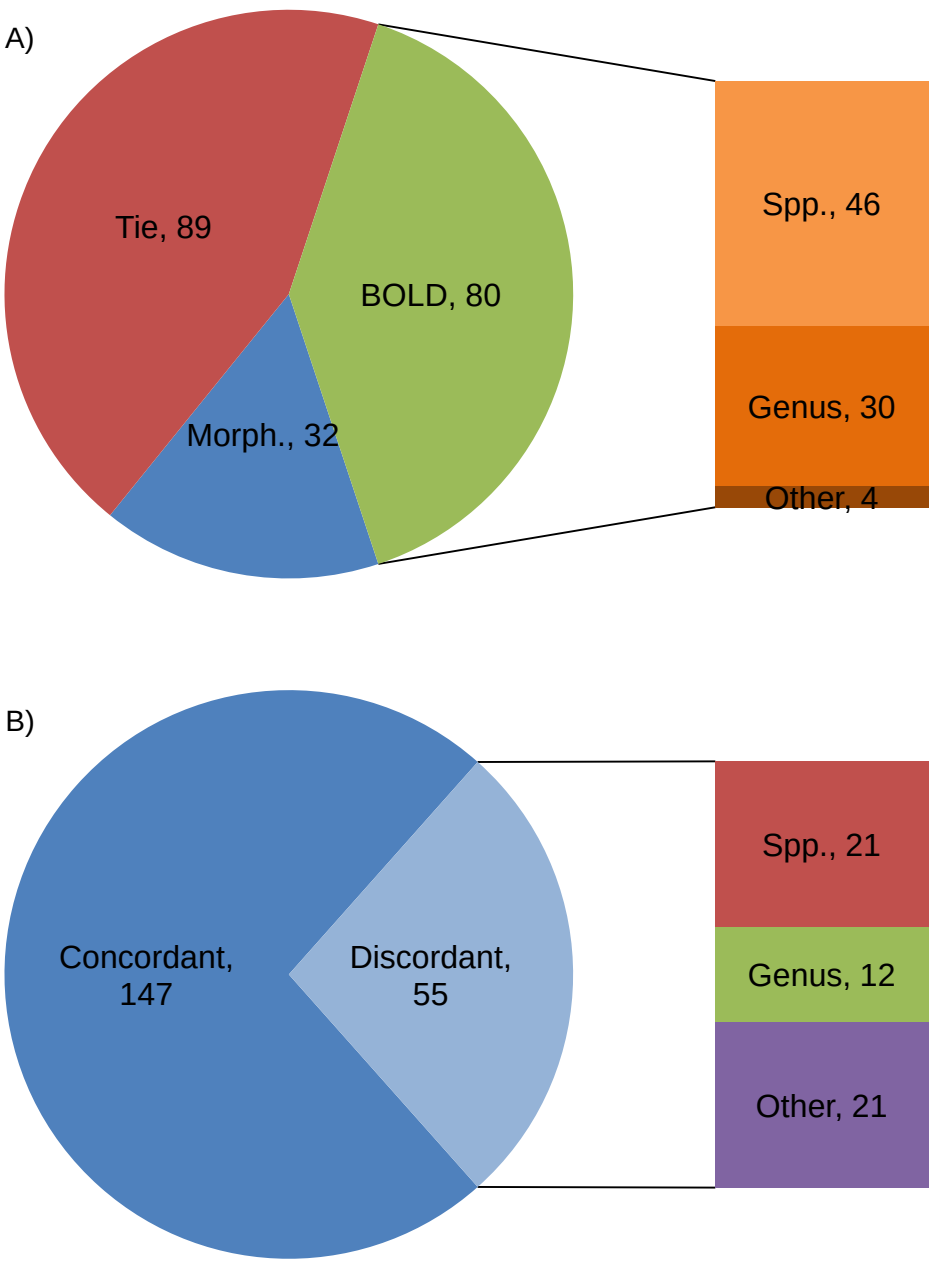

Figure 3.1.1: A) The identification method BOLD vs. morphology which yielded the most taxonomic information for each intercepted specimen from which yielded sequence data. Bar graph breakout: shows the ranks which specimens were identified to. B): Concordance between BOLD and morphology taxon-assignments for intercepted specimens. Bar graph breakout shows the rank at which discordance occurred.

Table 3.1.1: Summary of concordance between morphology- and DNA barcode-based identification methods at various taxonomic ranks and the method that produced the most taxonomic information.

	Concordance				Information Content			
	Concordant		Discordant		BOLD		Morphology	
Overall	147 n=201	(73.13%)	54	(26.87%)	80 n=112	(71.43%)	32	(28.57%)
Order	201 n=201	(100%)	0	(0%)	0 n=0	-	0	-
Superfamily	189 n=192	(98.44%)	3	(1.56%)	2 n=8	(25.%)	6	(75.%)
Family	169 n=187	(90.37%)	18	(9.63%)	7 n=13	(53.85%)	6	(46.15%)
Sub-family	143 n=147	(97.28%)	4	(2.72%)	31 n=35	(88.57%)	4	(11.43%)
Tribe*	112 n=125	(89.6%)	13	(10.4%)	9 n=15	(60.00%)	6	(40.00%)
Genus	111 n=128	(86.72%)	17	(13.28%)	49 n=56	(87.5%)	7	(12.5%)
Spp.	41 n=62	(66.13%)	21	(33.87%)	46 n=67	(68.66%)	21	(31.34%)

*Comparison at the level of tribe were only for members of the Tortricidae.

3.1.4 Discussion

The success of a molecular approach for the identification of border intercepted specimens hinge on its ability to provide taxonomic resolution equivalent to or better than that achieved by traditional methods. In this context, molecular identification can be evaluated by the number of specimens it can identify, particularly to taxonomic levels that are relevant to inform biosecurity decisions, primarily species. In this dataset, DNA barcoding (facilitated by BOLD-IDS and BIN) was able to provide species level identifications for 53.7% (108/201) of specimens, while tentative identifications (matches to multiple species, and/or specimens with interim

taxonomy) were made for a further 12.9% (26/201), based on the state of current DNA barcode reference libraries in BOLD. Compared to morphological methods, DNA barcoding was able to increase the number of species level identifications by 34.6% and provided a more precise (e.g., lower taxonomic) identification for 40.8% of specimens that yielded DNA sequences.

In this study DNA barcoding was able to complement morphological identifications by increasing the number of specimens that received a species level identification. However, some specimens remained unidentified at the level of species due to the lack of corresponding reference sequences on BOLD. While it would be ideal if all specimens in a given any sample could be identified, this goal is unrealistic for the foreseeable future given the magnitude arthropod biodiversity. However, it is worth noting that the bioinformatic re-examination of barcode data is a trivial task, especially compared to a morphological specimen-based approach. Therefore, as reference barcode sequences continue to be generated it should be possible to retroactively infer specimen identifications even years later, thereby increasing data content and hence the value of interception database that have integrated DNA barcode based identification. But, regardless of the current completeness of barcode reference libraries, all specimens with sequence lengths of >407bp, including those specimens that did not receive a species level identification, were assigned to a BIN. Due to their persistence on BOLD, BIN assignments may be used as an interim taxonomic framework to facilitate the communication and analysis of such these specimens. Under this paradigm 98% of the specimens for which sequence data was generated in the dataset were “identified”, either with a Linnaean binomial name and an associated BIN, or strictly a BIN number.

When applied as a complementary identification technique DNA barcoding has particular value in highlighting potential misidentifications. In this dataset discordance between morphological and DNA barcode inferred identifications was observed for 27% of specimens, 40% of which occurred at the species level (Figure 3.1.1.B). Discordance between identification

methods may reflect a misidentification by either method, unrecognized diversity within a particular species, or an insufficient number of reference barcode sequences (Table 3.1.2).

Discordance between identifications at the level of species occurred for 21 specimens (Table 3.1.2). While some of these cases likely represent a genuine misidentification by either or both methods, many are difficult to evaluate due to the lack of public barcode records for one or both of the proposed species. For example, specimen ITLP286-13 was identified as *Cacoecimorpha pronubana* by morphology and *Acleris variegana* by BOLD-IDS. However at the time of writing neither of these species have public records in BOLD, as a result it is impossible to evaluate, the data quality of the reference sequence and the corresponding barcode based specimen identification. Other similar examples exist among specimens concordant between identification methods (e.g., ITLP092-13: *Clepsis spectrana*), and specimens only identifiable with BOLD-IDS (e.g., ITLP048-13: *Lorita scarificata*). This illustrates the need for caution when using BOLD-IDS with *Species Level Barcode Record* or the *Full database* option, as data used to infer identifications may not be public and therefore unavailable for scrutiny. Although data present on BOLD is partitioned into public and non-public, it is important to note that it is possible to contact data owners so that non-public data can be made accessible to researchers (refer to www.boldsystems.org). In all cases DNA barcode data used to infer taxon assignment should be evaluated by a content expert prior to accepting the DNA barcode-based identification. As barcode data on BOLD are associated with voucher specimens and metadata (Hanner 2009), this evaluation is possible when specimen records are complete and publically accessible. However, this may not be possible for other DNA sequence databases such as INDSC Genbank (Benson et al. 2005), which, historically have not supported sequence–specimen metadata associations. Therefore it is important to assess the level of data quality of individual specimen. As a result the adoption systems to evaluate and/or rank the quality of specimen

Table 3.1.2: Summary of Discordance between Morphological and DNA Barcode Identifications. DNA Barcode identifications were conducted using BOLD-IDS on www.boldsystems.org.

Morphological-ID	BOLD-IDS	Specimens	BIN	Notes
Cydia splendana	*Cydia* sp.	ITLP015-13 ITLP033-13 ITLP096-13 ITLP227-13 ITLP330-13	BOLD:ACJ4323	Records for *C. splendana* fall in two marginally divergent BINS (BOLD:ACJ4322, and BOLD:AAC0640), neither of which contain our specimens. At the time of writing no records from BOLD: ACJ4322 were public. Distances between all three BINS is low <2% (as reported on the BIN page). This may reflect inefficient sampling of these taxa, which has prematurely spilt this species into multiple BINS, or alternatively unrecognized diversity within *C. splendana*. Although *C. splendana* is native and widespread in Europe and not known in the Americas, our specimens most closely match a specimen collected in New Jersey, USA, in or near the New Jersey International Airport (GBMIN12105-13).
Talponia prob. *batesi*	No Match	ITLP305-13	BOLD:ACJ4349	Records for *T. batesi* falls in BIN (BOLD:ACH1934). Divergence between specimen ITLP305-13 and *T. batesi* (BOLD:ACH1934) is 9.2%. This suggests that this specimen is not *T. batesi.*
Phereoeaca uterella	No Match	ITLP342-13 ITLP296-13	BOLD:ACJ4290	Records for *P. uterella* falls in to BIN (BOLD:AAH8518). Mean distance between these specimen and *P. uterella* (BOLD:AAH8518) is 6.5%. This suggests that these specimens are not *P. uterella*.
Gymnandrosoma aurantianum	*Olethreut01 phillipsEPR14*	ITLP005-13	BOLD:ABV2351	*G. aurantianum* falls in BIN (BOLD:AAA4028). Unfortunately the sequence for specimen ITLP005-13 is only 407bp. However, the distance between it and *G. aurantianum* (BOLD:AAA4028), is 4.6%. This suggest this specimen is not *G. aurantianum.* BOLD:ABV2351 contains three vouchered adult specimens collected in central and south America.

Morphological-ID	BOLD-IDS	Specimens	BIN	Notes
Gymnandrosoma aurantianum	No match	ITLP025-13	BOLD:ACJ4676	*G. aurantianum* falls in BIN (BOLD:AAA4028).
		ITLP071-13		Mean distance between BOLD:ACJ4676 and *G. aurantianum* BIN (BOLD:AAA4028) is 2.0%. This may reflect inefficient sampling of these taxa ,which has prematurely spilt this species into multiple BINS, or unrecognized diversity within *G. aurantianum*.
		ITLP072-13		
		ITLP073-13		
		ITLP241-13		
		ITLP242-13		
		ITLP328-13		
		ITLP343-13		
Gymnandrosoma aurantianum	*Gymnandrosoma leucothorax*	ITLP056-13	BOLD:ACH2052	*G. aurantianum* falls in BIN (BOLD:AAA4028).
		ITLP057-13		Mean distance between these intercepted specimens and *G. leucothorax* (BOLD:ACH2052) is 0.1%.
				Mean distance between these specimens and *G. aurantianum* (BOLD:AAA4028) is 9.0%. This suggests these intercepted specimen are not *G. aurantianum*
Rudenia leguminana	*Galleria mellonella*	ITLP319-13	BOLD:AAA0965	*Rudenia leguminana* falls in BIN (BOLD:ABZ6333), and BIN (BOLD:ACF0688)
				Galleria mellonella falls in BIN (BOLD:AAA0965).
				Mean distance between ITLP319-13 and *Rudenia leguminana* (BOLD:ABZ6333) + BIN (BOLD:ACF0688) is 15.3%.
				This suggests that this specimen has been misidentified.

Morphological-ID	BOLD-IDS	Specimens	BIN	Notes
Cacoecimorpha pronubana	*Acleris variegana*	ITLP286-13	BOLD:AAB2294	All records for *C. pronubana* are private. *A. variegana* falls in BIN (BOLD:AAB2294) and BIN (BOLD:ACE3007) Mean distance between ITLP286-13and *A. variegana* BIN (BOLD:AAB2294) is <1%. Together this suggest this specimen is not *Cacoecimorpha pronubana.*
Thaumatotibia leucotreta	*Lobesia vanillana*	ITLP052-13 ITLP053-13	BOLD:ABV8007	*T. leucotreta* falls in BIN (BOLD:AAE7729). *L. vanillana* falls in BIN (BOLD:ABV8007) Mean distance between these specimens and *T. leucotreta* is 11.1%. This suggests that these specimens have been mis-identified and are *Lobesia vanillana*.
Cydia fabivora	*Thaumatotibia hematoma*	ITLP002-13	BOLD:ABA8564	All records for *C. fabivora* falls are private *T. hemitoma* falls in BIN(BOLD:ABA8564), no members of *Cydia* are found in BOLD:ABA8564.

records (Costa et al. 2012) will be a necessary prerequisite to the widespread adaptation of DNA barcoding for regulatory applications.

While evaluating the discordance between identification methods can help improve the quality of data entering pathway analysis by highlighting errors, such exercises have other biosecurity applications. For example, DNA barcoding provides an objective framework to evaluate the accuracy of morphological-based identifications (Ko et al. 2013), which could be used as a job training tool for taxonomists and para-taxonomists. Professional development normally occurs during training seminars and workshops. However, few opportunities exist for on the job assessments, particularly for senior staff or content experts, who in many cases may be the institutional authority. Periodically employing DNA barcoding as a complementary specimen identification technique would allow these staff to evaluate their performance by highlighting potential mis-identifications or previously unrecognized diversity (Brown et al. 2011, Rougerie et al. 2014) for their review, leading to the improvement of both traditional and DNA barcode based identification resources.

In this dataset intercepted specimens that were not identifiable by BOLD-IDS fell into 53 unique BINS. Of these, 39 BINS were new to BOLD. This is surprising considering that the scope of this project pales in comparison to many of the large scale barcoding initiatives based in Central and South America (Janzen et al. 2005), the likely origin of many of the specimens in the intercepted dataset. In the long term, this exemplifies the need for the further assembly of DNA barcode reference libraries of insects globally. This also highlights the necessity of nations that are connected by land or trade to collaborate on building barcoding libraries of their native fauna for use in biosecurity applications (Vernooy et al. 2010). To that end, it is interesting to note that 11 of the BINs contained specimen records for unidentified vouchered adult specimens collected as part of ecological and biodiversity projects. Linkage between these specimens, the intercepted larvae, and vouchered adults would not be possible without the

metadata (voucher accession numbers, and storing institutions) associated with these specimens. Furthermore, the existence of these vouchered specimens provides a path for obtaining names for the intercepted larvae, (e.g., examining the vouchered adults in a morphological context) if indeed they are described species. Alternatively, if undescribed, this demonstrates the potential of this methodology to contribute to the process of integrative taxonomy now that specimens of two life-stages have been collected (Padial et al. 2010). It also provides a framework for targeted DNA barcode reference library construction by focusing on species that represent an actual biosecurity threat because they have been intercepted at international borders. Regardless of their potential importance as pest or invasive organisms, the fact that they have been or are being intercepted at national borders will require that they be successfully identified in the future. It should be expected that cases similar to those observed here will occur as the number of and scope of biodiversity-focused barcoding projects increase (Janzen et al. 2005, Janzen et al. 2012, Smith et al. 2012a). However, with the advent of environmental-barcoding (sometimes referred meta-barcoding) and next-generation sequencing technologies, there is a trend towards using homogenized environment samples for biodiversity assessments (Hajibabaei et al. 2011, Ji et al. 2013, Shokralla et al. 2014). Using this approach the linkage between an individual specimen and its DNA barcode is broken, rendering material unsuitable for integrative taxonomy or DNA barcode reference library construction. As a result the value of specimen oriented approaches to biodiversity surveys should not be underestimated, due to their ability to contribute to both integrative taxonomy and biosecurity.

The inability for DNA barcoding to differentiate species that share similar or identical barcode haplotypes is often cited as a serious impediment to the application of DNA barcoding (Meier et al. 2006, Wiemers and Fiedler 2007). This can be overcome by designing and applying additional species-specific molecular diagnostics based on alternative genetic markers which differentiate species in these groups. However, in most applied scenarios I would expect

this to be unnecessary. For example, for border interceptions, additional diagnostics would only be required if a specimen was assigned to a group which also contained a regulated or actionable species. Nonetheless, an added benefit of employing DNA barcoding as a first pass to identify specimens, may be to reduce the number of false positives generated by species-specific diagnostics by ensuring that only appropriate specimens (i.e., belonging to the group of species for which the diagnostic was designed for) are tested (deWaard et al. 2010b).

Although there are methods that can address the issue of barcode sharing and low-interspecific divergence, this does highlight one of the one of the central challenges to working with DNA barcoding libraries: current DNA barcode libraries are imperfect. In other words, when two species share DNA barcode profiles or are placed in the same BIN, it may reflect biological reality or that some of the specimens were misidentified. This issue will likely be addressed over time if DNA barcode libraries are curated and annotated, but in the meantime, this will present a continuing challenge to those using DNA barcode libraries. Finally, given that the process of generating DNA barcode data can be almost entirely automated, the temptation may arise to fully automate the assignment of names to these specimens as well. However, at this time I do not support this approach, given the imperfect state of barcode libraries and the lack of consensus on metrics used to assign names to specimens using sequence data. Rather I endorse the use of DNA barcoding as a tool like any other taxonomic resource to help inform the identifications made by a content expert.

Unfortunately, 16% (38/239) of intercepted specimens in this dataset did not yield sequence data, which was surprising given the majority of samples, were less than 1 year old at the time of analysis and presumably experienced similar storage conditions. Considering that the primers employed in this study have been used extensively for barcoding of Lepidoptera (Janzen et al. 2005, Hebert et al. 2009, deWaard et al. 2011, Janzen et al. 2012, Hebert et al. 2013) it seems likely that failure to amplify sequences from these specimens was a result of

DNA quality rather than primer selection. This suggestion is supported by the fact that only low molecular weight DNA was observed from the majority of these samples when visualized on an agarose-gel (data not shown). A probable explanation is that the final ethanol concentration of the preservative used with these samples was too low, resulting from the use of<95% ethanol or an improper tissue-volume to ethanol-volume ratio when initially storing the specimen, leading to the degradation of DNA. Alternatively, storage temperature of some of the samples may have affected DNA quality. Therefore I recommend that all border intercepted arthropods destined for molecular analysis be preserved in 95% ethanol with a tissue-volume to ethanol volume-ratio no more than 1:10 (Prendini et al. 2002, Vink et al. 2005), and if appropriate facilities exist, specimens should be stored at ≤-4ºC. Additional recommendations for specimen and tissue storage can be found in Prendini et al. (2002).

To facilitate the use of BINs as an interim taxonomic framework for specimens unidentifiable at the level of species, a standardized nomenclature for communicating BIN defined taxa should be employed. Rather than simply using the BIN number, which by itself conveys no taxonomic information, I advocate (with minor adjustments) the candidate species naming convention described by Padial *et al*. (2010). In brief a candidate species name consists of the binomial species name of the most closely related nominal species. This is followed by a 'Ca' in square brackets, designating the current entity as a candidate species, a numerical code to account for the potential of more than one candidate species for each binomial name, and ending, if applicable, with either a citation of the article where this candidate species was first proposed, and/or specimen accession number (e.g., GenBank, or BOLD accession number). Under this framework I would recommend using the BIN number in place of the numerical code following Ca (e.g., *Rudina* sp. [Ca.BOLD:XXXXX]). Also, in order to limit the potential for confusion with other accession numbers (e.g., GenBank), the BIN number should be written in full including the '*BOLD:*' preface to unambiguously reflect the fact that the number represents a

BIN that can be retrieved using Barcode of Life Data Systems. Furthermore, for specimens that have been only examined by DNA I suggest using higher taxonomic ranks for the binomial in place of the '*most closely related nominal species*' in order to avoid spurious association caused by a lack of appropriate and closely related reference material (e.g., large genetic distances between the *candidate species* and its close relatively represented by sequence data). Evidence suggests that phylogenetic signal present in the DNA barcode region is capable of assigning specimens to higher order taxa (Wilson et al. 2011, Zahiri et al. 2014), and 'Identification Summary' box presented on the BOLD-IDS output page could be used for this purpose.

The application of DNA barcoding has the potential to increase the information content of interception datasets by increasing the taxonomic resolution of individual specimen records, highlighting cases of potential misidentification, and contributing to the process of integrative taxonomy. Together this makes a strong argument for application of these technologies for the identification of intercepted arthropods, and this project demonstrated on a small scale how this could be accomplished by leveraging an existing an bio-informatics platform. Of all arthropods, the Lepidoteran DNA barcode reference libraries are the most well developed and are well suited as target taxa for the implementation of the described techniques, as it is likely the taxa which the highest rate of species level matches of intercepted material could be made. However, despite the relatively advanced development of Lepidotera barcode libraries, the overall ID rate for this dataset is only 53%, stressing the need for further library development particularly for bio-diverse countries and taxa. Library development needs to include not only catalogued biodiversity from museum collections but also material obtained from biodiversity surveys. Material collected from biodiversity and ecological surveys are in a unique position to catalogue un-described species (with BINs) as well fill out the species distributions and haplotype diversity for species represented in barcode reference libraries from only museum

specimens. To this end, cataloguing DNA barcoding data by archiving query sequences along with associated metadata, as was done for this study, will also help flesh out reference libraries, as illustrated by number of new BINs added to BOLD herein. As such it is encouraged that all query sequences be archived in publically accessible libraries regardless of the reason they were initially generated. Finally by using clustering algorithms such as BINs, the task of organizing otherwise unidentifiable material into species-like operational taxonomic units (OTUs) is entirely possible on a large scale. Furthermore, harmonizing these OTUs in a central database (e.g., BOLD) greatly facilitates the use of OTUs as interim taxonomy accessible by multiple working groups and researchers globally.

3.2 Contributions towards a DNA Barcoding Library of the Tortricidae: A Valuable Diagnostic Tool for the Research and Regulatory Community

3.2.1 Introduction

The spread of invasive arthropods poses a serious economic threat to agriculture and the environment. A critical intervention to prevent the establishment of invasive pests is border inspection programs. Through these programs, intercepted arthropods are identified and the taxonomic data collected are used to inform pathway analysis for invasive and non-native species. Unfortunately, the interception targets for many invasive arthropods are the immature life stages, which for many arthropods are undescribed. This presents an ideal opportunity to employ DNA barcoding (i.e., a standardized molecular diagnostic) to assist in the identification of specimens and associate immature life stages with their adult counterparts. However, to achieve this species that are known or expected to be present in interception pathways must first be represented in DNA barcode reference libraries.

As originally conceived, DNA barcoding was in part a tool to help liberate and extend taxonomic knowledge and in part a diagnostic tool for a larger research and regulatory community (Hebert et al. 2003b, Hebert et al. 2003a). The ability of DNA barcoding to meet this objective is predicated on the well-populated reference libraries containing barcode sequences generated from previously authoritatively identified specimens. For practical reasons, DNA barcode libraries are typically constructed with a taxonomic (Foottit et al. 2008, Foottit et al. 2009b) and/or geographic scope (Kerr et al. 2007). Unfortunately, building DNA barcode libraries in this fashion may not always serve the end-user community, who may have specific identification needs and challenges, for example those who need to diagnose regulated environmental and agricultural pests (Bonants et al. 2010). Targeted library building projects that focus on the diagnostics needs of particular research communities can complement

traditional library building projects. As such, small but targeted library building projects, if put in the context of available data, may be able to contribute to user-uptake of DNA barcoding as a diagnostic tool. The purpose of this manuscript was to provide the rationale and metadata for one such project, targeting a number of moths from various genera of the economically-important family Tortricidae. Twenty-nine species of Tortricidae which are anticipated to be present in interception pathways, but for which no reference barcodes sequences were available at the time were sampled and DNA barcodes were generated for 28 of these.

3.2.2 Methods

A list of Tortricidae species likely to be intercepted at international borders but lacking specimen records and/or sequence data in Barcode of Life Data Systems (BOLD: www.boldsystems.org) (Ratnasingham and Hebert 2007), and with representative material within the U.S. National Entomological Collection (USNM) collection, was compiled by John W. Brown (JWB) and Andrew J. Frewin (AJF). The final list contained 29 species from which 95 individuals were sampled with a maximum of 7 individuals per species from the USNM collections. Only material collected after 1980 was included to increase the probability of successfully recovering and amplifying barcode sequences (Hebert et al. 2013). Complete specimen data for these individuals can be found on BOLD under project ITLPA

A single hind leg was used as a source of DNA. DNA was extracted using a commercial alkaline lysis extraction (XytXtract Insect (ANDE), Xytogen; Perth, Australia) using manufacturer recommended protocols (Castalanelli et al. 2010). DNA extracts were stored at -20°C prior to analysis.

All PCR reactions were conducted in a total volume of 12.63µL which included, 6.25µL of 10% trehalose, 2.00µL of water, 1.25µL of 10X PCR Buffer (Life Technologies™), 0.625µL $MgCl_2$ (50 mM), 0.25µL of dNTP (10 mM), 0.0625µL of Platinum® Taq (Life Technologies™), 0.10µL total volume of each forward and reverse primers, and 2.00µL of DNA template (Ivanova et al. 2009). All DNA extracts were subjected to PCR with the following primer pair LepF1 (5'-ATT CAA CCA ATC ATA AAG ATA TTG G-3')/LepR1 (5'-TAA ACT TCT GGA TGT CCA AAA AAT CA-3'). Extracts which failed to produce a product were failure tracked with the primers mLepF1 (5'-GCT TTC CCA CGA ATA AAT AAT A-3')/LepR1, and LepF1/mLepR1 (5'- CCT GTT CCA GCT CCA TTT TC-3'). The following cycling conditions were used for all primer-pairs, an initial denaturation at 94°C for 1min, followed by 5 cycles of 94°C for 40s, 45°C for 40s, 72°C for 1min, followed by 35 cycles of 94°C for 40s, 51°C for 40s, 72°C for 1min then a final extension at 72°C for 5min (Hajibabaei et al. 2006). PCR products were visualized on a 2% agarose gel pre-stained with SYBR® Safe DNA gel stain (Life Technologies™). PCR products were sequenced at the Advanced Analysis Centre at the University of Guelph (Guelph, Ontario, Canada), on a Applied Biosystems® 3730 DNA Analyzer. Specimen metadata, photographs, trace-files, and DNA barcode sequences were deposited in BOLD project ITLP. All pairwise genetic distances were calculated using the K2P method using BOLD (Ratnasingham and Hebert 2007). All specimens with sequences ≥407bp were assigned a BIN number OTU by the BINs algorithm on BOLD (Ratnasingham and Hebert 2013).

3.2.3 Results

Of the 95 specimens, full length DNA barcodes (≥648bp) were obtained from 65 individuals representing 25 species. Three additional species were represented only by short sequences (=<307bp). Specimens of *Cydia araucariae* (Pastrana) (Tortricidae: Olethreutinae:

Grapholitini) failed to yield sequences. Four species were represented by a single specimen, while the remainder had between two and seven specimens. The mean K2P sequence divergence between species was ~10 times greater (x=12.10%, SD=1.92%, range=0.21-19.20%) than within species variation (x=1.03%, SD=2.08%, range=0-8.75%). Max within species divergence was greater than typically observed (i.e., >2-3%) for both *Cryptaspasma montana* (x=9.7%) and *Cydia motrix* (x=3.46%). All BINS were concordant at the level of species, both within this dataset and with all public data. A full data summary can be found in Table 3.2.1.

3.2.4 Discussion

In general the intra-specific divergence in this dataset was low with two exceptions. For *C. motrix* the two specimens collected within the city limits of Curibita, Brazil, were 3.46% divergent from specimens collected ca. 650 km inland from Itapua, Paraguay. This level of divergence may indicate the presence of a cryptic species or may reflect sequence variation across the species range (Bergsten et al. 2012). While proximate, these two regions have different climates, which may support either of these hypotheses. High intraspecific divergence was also observed in *C. montana*: all three specimens were collected in Costa Rica; however, specimens diverged by 9.74%. This level of divergence would seem to indicate these specimens represent different species. Thus, specimens of both of these species will need to be re-examined in the future, preferably in the context of additional congeneric species.

Although barcode sharing and/or low-interspecific divergence is not uncommon among species of Lepidoptera (deWaard et al. 2011, Hausmann et al. 2013, Zahiri et al. 2014), neither was observed for the specimens examined here, as indicated by the generally high distance to

nearest neighbour and concordant BINs across the dataset. However, this may simply reflect the lack of Tortricidae specimen records from the Neotropics, where the type localities for the majority of species examined here are located (Table 3.1). Therefore, I encourage conservative applications of these data for species identification purposes until a time when more species of Tortricidae from this ecozone are barcoded. However, in the short term these data may be useful for assigning tentative identifications to intercepted larvae, which otherwise may be difficult or impossible to identify to ranks below the level of tribe.

The benefits of including name bearing type specimens in DNA barcoding reference libraries is well recognized (Wilson et al. 2010, Puillandre et al. 2011, Strutzenberger et al. 2012), and it is now common to see DNA barcoding sequences generated from types accompanying new species descriptions (Blagoev and Dondale 2014, Brown et al. 2014). Unfortunately, it will be impossible to generate DNA barcodes from the vast majority of type specimens due to their age, preservation status, and value which may prohibit destructive sampling (however see, Andersen and Mills 2012, Strutzenberger et al. 2012). A practical substitute for DNA barcodes generated from name-bearing types would be those generated from specimens collected from the type localities (e.g., topotypes). For example, sequence data generated from topotypes may help resolve nomenclatural conflicts which arise when geographically partitioned cryptic diversity within a species is encountered (Tay et al. 2012). As such efforts should be made to include and appropriately label specimens collected from type localities in DNA barcode reference libraries. However, the precision of type localities are highly variable, whether or not an individual specimen can be considered a topotype should be left to content experts. To help address this issue the type localities of all species considered in this manuscript are provided in Table 3.2.1.

Table 3.2.1: Summary of Tortricidae specimens with sequence data.

Taxa	# of Individuals	BIN	Max intraspecific divergence %	Sampling Locations	Type Locality
Olethreutinae: Eucosmini					
Crocidosema accessa	1	ACH1978	-	United States, TX	Panama, Trinidad River
Crocidosema lantana	2	AAH5763	0.46	Jamaica	Oahu, Tantalus
Crocidosema longipalpana	3	ACH1993	0.61	United States, FL	Puerto Rico
Olethreutinae: Grapholitini					
Cryptophlebia illepida	6	ACH2132	0.50	United States, HI	Hawaiian Islands
Cydia fabivora	2	ACH2002	0.31	Ecuador, Los Rios	Colombia, Honda
Cydia kurokoi	2	ACH2078	0	Japan, Kanto	Japan, Kyushu, Fukuoka, Mt.Hikosan
Cydia motrix	3	ACH2003	3.47	Brazil Paraguay, Itapua	Uruguay
Cydia tonosticha	3	AAH5464	0	Brazil	Brazil, Amazonas, Parintins, Teff
Gymnandrosoma leucothorax[1]	1	ACH2108	-	Dominican Republic	Cuba, Santiago, Turguino
Talponia batesi	2	ACH1934	0.93	Brazil Venezuela, Zulia	Guatemala, Antigua
Olethreutinae: Microcorsini					
Cryptaspasma bipenicilla[1]	2	ACH2101	1.39	Dominican Republic, Pedernales United States, TX	USA, Florida, Putnam Co., Palatka.
Cryptaspasma montana[2]	3	-	9.74	Costa Rica Costa Rica, San Jose	Costa Rica, Cerro de la Muerte
Cryptaspasma perseana[1]	3	AAB0277	0	Guatemala, Sacatepéquez	Guatemala, Sacatepéquez (Gilligan et al. 2011)
Cryptaspasma sp.[2]	1	-	-	Costa Rica, Cartago	-
Cryptaspasma sp.	2	ACH2154	1.24	Costa Rica, San Jose	-

Taxa	# of Individuals	BIN	Max intraspecific divergence %	Sampling Locations	Type Locality
Olethreutinae: Olethreutini					
Episimus unguiculus	7	ACH1915	0.62	Argentina, Misiones Brazil, Rio Grande do Sul Brazil, Santa Catarina Paraguay	Argentina, Tigre
Lobesia botrana	2	ACH2178	0	Greece, Crete	Austria, Vienna
Tortricinae: Archipini					
Archips fuscocupreanus	2	AAD6614	1.08	United States, Washington	Japan, Kyushu, Satsuma
Argyrotaenia sphaleropa	3	ACH2098	0.46	Brazil	Bolivia, Sapago
Clepsis abscisana	1	ACH1873	-	Colombia	Colombia, Barroblanco
Tortricinae: Euliini					
Accuminulia buscki[1,2]	2	-	0	Chile, Coquimbo	Chile, Santiago Province
Bonagota cranaodes	5	AAI4759	0.31	Argentina, Buenos Aires Brazil, Rio Grande do Sul	Argentina, Buenos Aires
Proeulia chrysopteris	2	ACH1881	0.61	Chile, Araucania	Chile
Proeulia triquetra	2	ACH2098	0.46	Chile	Chile, Maule, Cauquenes Province
Seticosta rubicola	3	ACH1997	0.77	Costa Rica, San Jose	Costa Rica, Cartago Province, Parque Nacional Tapant
Tortricinae: Sparganothini					
Platynota meridionalis	3	ACH2108	0	Argentina, Burenos Aires	Argentina, Buenos Aires(Brown 2006)
Platynota subtinae[2]	1	-	-	Venezuela, Guarico	Venezuela, Guarico (Brown 2006)
Platynota xylophaea	3	ACH2139	0	Argentina, Burenos Aires	Argentina, Tucumn
Tortricinae: Tortricini					
Acleris undulana	6	ACH1926	0.46	Turkey	Turkey, Zeitun

Intra-specific divergence calculated using the K2P method. Type localities retrieved from (Gilligan et al. 2012), unless otherwise noted. [1]Specimens include a paratype, [2]Only sequences <407bp were generated for these specimens.

3.2.5 Conclusions

DNA barcodes were generated for 28 species of Tortricidae suspected to be detected in interception pathways. Developing barcoding libraries of tortricids (and other economically important moths) are imperative in order to develop diagnostic tools that are beneficial to the larger research and regulatory community. Unfortunately, due to species level diversity completing DNA barcode libraries for the Tortricidae (and other taxa) will be challenging. However, from a diagnostic perspective there is particular value in having economically important species (or in our case species likely to be detected in interception pathways) represented in barcode reference libraries (Virgilio et al. 2012). Therefore in the short term I would encourage others to target species which meet these criteria when building barcode libraries. Although, until reference libraries are near complete cautious evaluation of identifications inferred from incomplete libraries will be required.

Chapter 4

CONTRIBUTIONS TO IPM OF THE *BEMISIA TABACI* CRYPTIC-SPECIES COMPLEX

A portion of this chapter (Section 4.1) consists of work completed primarily by Frewin at the University of Guelph from: Frewin, A. J., C. Scott-Dupree, G. Murphy, and R. Hanner. 2014. Demographic trends in mixed *Bemisia tabaci* (Hemiptera: Aleyrodidae) cryptic species populations in commercial poinsettia under biological control- and insecticide-based management. Journal of Economic Entomology 107: 1150-1155.

4.0 Abstract

Bemisia tabaci is an economically important pest of agriculture globally. *Bemisia tabaci* is a cryptic species complex, and often occurs in mixed species infestations. Although the different *B. tabaci* cryptic species are morphologically indistinguishable, they vary significantly in their tolerance to insecticides, which complicates management of mixed populations. Of particular importance to Ontario greenhouse horticulture are the cryptic species *Mediterranean* and *Middle East-Asia Minor 1*. To improve the management of these species, I examined the demographic trends of mixed species infestations under biological control- and insecticide-based management programs in commercial production systems. I identified *B. tabaci* cryptic species with DNA barcoding and other molecular diagnostics. My data suggests that biological control based management promotes displacement of the insecticide tolerant *Mediterranean* species in mixed species infestations under the observed conditions, whereas under insecticide based management, the insecticide tolerant *Mediterranean* species persists. This displacement is presumed to be a result of asymmetric mating interference between the *Mediterranean* and *Middle East-Asia Minor 1,* which is frequently observed under laboratory conditions, but inferred

here for the first time in a commercial greenhouse. Therefore, I suggest that biological control-based management of *B. tabaci* is the preferred management technique for mixed species infestations when reducing the proportion of *Mediterranean* individuals is a specific management objective. To complement these recommendations I make two contributions towards the diagnostics for these *Bemisia* cryptic species. First, I generated full length COI sequences for *Mediterranean* and *Middle East-Asia Minor 1*, which explicitly link the two separate DNA markers used for *B. tabaci* cryptic species identification in the published literature. Second, I adapted a previously published molecular diagnostic for *Bemisia* cryptic species identification for deployment in the field.

4.1 Demographic Trends in Mixed *Bemisia tabaci* (Hemiptera: Aleyrodidae) Cryptic Species Populations in Commercial Poinsettia Under Biological Control- and Insecticide- based Management

4.1.1 Introduction

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is a small phloem feeding insect which predominantly feeds on herbaceous plants, although its host range includes upwards of 600 species (Oliveira et al. 2001). *Bemisia tabaci* has a global distribution (Dinsdale et al. 2010), and throughout much of its range it is a significant pest of ornamentals, vegetables, legumes, and cotton (Oliveira et al. 2001). In many crops, plant damage is caused by direct feeding, transmission of plant viruses (Jones 2003), or growth of sooty-mold fungus as a result of the secretion of honeydew. However, for ornamentals such as poinsettia (*Euphorbia pulcherrima*), which are marketed for their aesthetic value, the presence of any life-stage (larva, pre-pupa, pupa, adult or exuvia) on the plants may result in entire consignments being rejected by buyers/wholesalers, causing significant financial loss for the grower. These market

conditions present an important management challenge for poinsettia producers as *B. tabaci* densities must be reduced to levels typically lower than non-ornamental commodities.

It is now widely recognized that *B. tabaci* is a cryptic species-complex consisting of at least 24 morphologically indistinguishable, yet behaviorally and physiologically distinct species (Boykin et al. 2007, Dinsdale et al. 2010, De Barro et al. 2011, Liu et al. 2012, McKenzie et al. 2012, Tay et al. 2012). To account for this variation, a convention of assigning a unique 'biotype' designation to distinct *B. tabaci* populations was adopted, of which 36 biotypes are reported in the literature (De Barro et al. 2011). However, for various reasons, including the taxonomic appropriateness of the term "biotype", the biotype-naming convention for *B. tabaci* has been largely replaced by a classification system which represents the presumed phylogeographic origins of the proposed cryptic species (Dinsdale et al. 2010, Tay et al. 2012). This classification system is based on a partial cytochrome c oxidase subunit I (COI) sequences derived from the 5'-region of the gene. For the purposes of this manuscript, I follow this phylogeographic naming convention. Of the 24 putative cryptic-species, *Mediterranean* (formerly *'biotype Q'*), and *Middle East-Asia Minor 1* (MEAM1: formerly '*biotype B*', or *Bemisia argentifolii* Bellows & Perring) are the most commonly encountered in the pest management literature (Liu et al. 2007, McKenzie et al. 2012) and often infest ornamental crops in North America including poinsettia. Both of these species have experienced significant range expansions as a result of international trade (Cheek and Macdonald 1994, Dalton 2006). A third cryptic-species, *New World* (formerly, '*biotype A*, *C*, *D*, *F*, *Jatropha*, *N*, *R*, *Sida*) is present in North America, but it rarely infests crop plants and is generally not considered economically significant (Dinsdale et al. 2010, McKenzie et al. 2012). Of practical importance is the fact that *Mediterranean* is more resistant to, and likely has a greater capacity to evolve resistance to various insecticides than other *B. tabaci* cryptic species, in particular MEAM1 (Dennehy et al. 2010, Li et al. 2012). Also, both the *Mediterranean* and the MEAM1 cryptic species frequently occur on the same crop (McKenzie et al. 2012).

Together this can present a significant pest management challenge for growers due to the uncertainty surrounding the identity and hence resistance status of their *B. tabaci* infestations.

In Ontario, Canada (where this study was conducted) poinsettia crops are produced from imported vegetative cuttings. *Bemisia tabaci* is not known to overwinter outdoors in Ontario, and thus infestations in poinsettia result from the importation of infested vegetative cuttings. In Ontario, typical *B. tabaci* management programs are based on either insecticides or biological control. Insecticides registered for control of whitefly in poinsettia in Canada include neonicotinoids (imidacloprid and acetamiprid), insect growth regulators (IGR) (pyriproxifen and kinoprene), organophosphorates (dichlorvos, malathion, chlorpyrifos, acephate and naled), pyrethroids (permethrin), as well as endosulfan, flonicamid, spiromesifen, spirotetramat, pyridaben, pymetrozine, insecticidal soap, and mineral oil. Insecticide-based management programs are initiated in August (at the earliest) and conclude in October-November prior to shipping of the finished crop. Of these classes of insecticides it has been demonstrated that *Mediterranean* is more tolerant to IGR, neonicotinoids and pyrethroids than MEAM1 (Horowitz et al. 2005, Dennehy et al. 2010). Alternatively, many growers use one or more commercially available biological control agents: *Eretmocerus eremicus* Rose & Zolnerowich, *Eretmocerus mundus* Mercet, *Encarsia formosa* Gahan, *Amblyseius* (=*Typhlodromips*) *swirskii* (Athias-Henriot) and *Delphastus catalinae* (Horn). Under both biological control and insecticide-based management schemes, it may be necessary to apply a 'clean-up' application of a foliar insecticide towards the end of the season to ensure the marketability of the crop.

In mixed laboratory populations it has been shown that MEAM1 will displace *Mediterranean* via asymmetric mating interference (Pascual and Callejas 2004, Crowder et al. 2010). Similarly MEAM1 has displaced other *B. tabaci* cryptic-species on a landscape scale in both China and Australia (Liu et al. 2007). However in the presence of insecticide selection pressures, *Mediterranean* will persist in mixed populations (Horowitz et al. 2005, Crowder et al.

2010). Therefore if season long application of insecticides promotes the proliferation or maintenance of *Mediterranean* individuals in mixed *Bemisia* populations, it could limit the utility of late season 'clean-up' insecticide applications, jeopardizing the marketability of the crop. At this point little is known about the population dynamics of mixed populations under biological control compared with insecticide-based management regimes in commercial greenhouses. Therefore, understanding how the composition of mixed populations of *Mediterranean* and MEAM1 change under realistic greenhouse-production conditions could help improve *B. tabaci* management. Results from a *B. tabaci* survey from commercial poinsettia greenhouses using either biological control or insecticide-based *B. tabaci* management programs in Ontario, Canada are reported and the potential management implications of the observed trends are discussed. Furthermore I generate full COI sequences for both *Mediterranean* and MEAM1 collected in Ontario, to bridge the gap between the 5'-COI marker used for the current classification of *B. tabaci* cryptic species and the marker used for DNA barcoding.

4.1.2 Methods

4.1.2.1 *Bemisia tabaci* survey:

Bemisia tabaci were hand collected from six commercial poinsettia greenhouses throughout the Niagara Peninsula in Ontario, Canada, during the 2012 production season. Three of the greenhouses used biological control as their primary strategy for *Bemisia* management, and, although programs varied between greenhouses, all three relied heavily on *E. eremicus* and *E. formosa*. The remaining three greenhouses exclusively used insecticides for *B. tabaci* management. These programs varied by greenhouse but included neonicotinoids, IGR (primarily pyriproxifen) and pyridaben. Insecticide applications were initiated between August 7th and 22nd.

Two different surveys were carried out during the season. In the first, each greenhouse was regularly monitored during the poinsettia production cycle from July 5th to November 21st, by inspecting approximately 10% of the crop during each visit. The data collected from these greenhouses comprised the seasonal data set. For the seasonal data set, the proportion of *Mediterranean* individuals in each greenhouse collection event was arcsine square-root transformed to normalize the data and subjected to analysis of variance in R version 2.14.1 (R Development Core Team 2011). Variance was partitioned by greenhouse management method (i.e., biological control- or insecticide-based), week of sampling and the greenhouse sampled.

The second survey was conducted on December 12th and consisted of one time collections from seven additional greenhouses from the Niagara Peninsula, two of which exclusively used insecticide-based *Bemisia* management, and five used biological control-based *Bemisia* management. These data comprised the end of season survey.

During surveys, plants were individually inspected and whiteflies were aspirated directly into 95% ethanol. The majority of individuals collected were adults; however, on occasion nymphs/pupae were collected if they were the only life stage present on a given plant. If multiple individuals were collected from a single plant they were combined in a single collection vial. Whitefly samples were stored at -20°C prior to DNA extraction. DNA was extracted from whole specimens with XytXtract Insect (ANDE) (Xytogen; Perth, Australia) DNA extraction kits using manufacturer recommended protocols (Castalanelli et al. 2010). When possible, whitefly specimens were retained and stored individually in 95% ethanol. DNA extracts were stored at -20°C for a maximum of three days prior to analysis.

4.1.2.2 Cryptic-species identification

The cryptic species' identity of *B. tabaci* individuals was determined using a method developed by Shatters et al. (2009). DNA extracts from unidentified *B. tabaci* specimens were subjected to a diagnostic multiplex PCR amplification containing three sets of primers designed to amplify fragments of different lengths from each of the 'biotypes' or cryptic species: 303bp from *Mediterranean*, 405bp from *New World*, and 478bp from *MEAM1*. Fragments were then visualized on a 2% agarose gel pre-stained with SYBR® Safe DNA gel stain (Life Technologies™).

4.1.2.3 COI Sequence Bridging

Near complete 1466bp cytochrome c oxidase subunit I (COI) sequences were generated for 6 *Mediterranean* and 6 MEAM1 individuals, randomly selected from all collections. This sequence encompasses both the 5'-COI marker used for *B. tabaci* cryptic species classification and the DNA barcoding region. Pairwise distances were computed between each haplotype and a consensus sequence for each *B. tabaci* cryptic species (Dinsdale et al. 2010) in MEGA5 (Tamura et al. 2011) to verify cryptic-species designation. All PCR reactions included 6.25µL of 10% trehalose, 2.00µL of water, 1.25µL of 10X PCR Buffer (Life Technologies™), 0.625µL $MgCl_2$ (50 mM), 0.25µL of dNTP (10 mM), 0.0625µL of Platinum® Taq (Life Technologies™), 0.10µL total volume of each forward and reverse primers, and 2.00µL of DNA template (Ivanova et al. 2009). A complete list of primers and PCR conditions can be found in Table 4.1.1.

Table 4.1.1: Primers and PCR cycling conditions used in this study.

Primer mixture:	Sequence 5' – 3'	Cycling Conditions	Source
Biotype			
BioB-L:	CTAGGGTTTATTGTTTGAGGTCATCATATATTC	94°C for 2 min 35 cycles of, 94°C for 30s, 64°C for 1 min, 72°C for 1 min 72°C for 10 min	Shatters et al. (2009)
BioB-R:	AATATCGACGAGGCATTCCCCCT		
New World-L:	TACTGTTGRAATAGATGTTGACACTCGGG		
New World-R:	GGAAAAAATGTAAGRTTTACTCCCWCAAATATT		
BioQ-L:	CTTGGTAACTCTTCTGTAGATGTGTGTT		
BioQ-R:	CCTTCCCGCAGAAGAAATTTTGTTC		
COI(42-699*)		94°C for 1 min 5 cycles of, 94°C for 40s, 45°C for 40sec, 72°C for 1 min 35 cycles of, 94°C for 40sec, 51°C for 40sec, 72°C for 1 min 72°C for 10 min	This Study
WF-F:	ATTCAACCAATCAYAARGATATYGG		
WF-R:	TAAACTTCTGGATGHCCAAARAAYCA		
COI(581-1003*)		94°C for 2 min 35 cycles of, 94°C for 30s, 57.3°C for 1 min, 72°C for 1 min 72°C for 10 min	This Study
BtabINT-F:	GATTTCTCTYCCTGTTCTTGCA		
BtabINT-R:	TCCTGTAAATCAAAGGCCAAGRG		
COI(801-1537*)		94°C 2 min 35 cycles of, 94°C for 30s, 46°C for 1 min, 72°C for 1 min 72°C for 10 min	Shatters et al. (2009)
Btab-Uni-L:	GAGGCTGRAAAATTARAAGTATTTGG		
Btab-Uni-R:	CTTAAATTTACTGCACTTTCTGCCAYATTAG		

*Binding sites on GenBank Accession AY521259 *Bemisia tabaci* (New World biotype) complete mitochondrial genome

4.1.3 Results

In total, 632 *B. tabaci* were collected from 319 poinsettia plants derived from 43 separate greenhouse collection events (Table 4.1.2) that included both seasonal and end of year sampling. The vast majority, 89% (n=564) of individuals were identified as MEAM1; the remainder were identified as *Mediterranean,* no New World individuals were found. Of the 171 plants from which multiple whiteflies were collected, only one harboured individuals of both MEAM1 and Mediterranean; this detection was made in a greenhouse with biological control-based *Bemisia* management as part of the end of season survey on December 12th. Individual greenhouse collections were predominantly MEAM1 (60% of greenhouse visits), followed by mixed MEAM1-*Mediterranean* (28%), and *Mediterranean* (12%). The majority (81%) of all *Mediterranean* individuals found in this survey were in greenhouses using insecticide-based *Bemisia* management programs.

Table 4.1.2: Summary of *Bemisia tabaci* cryptic-species surveys conducted in Ontario, Canada in commercial poinsettia greenhouses.

Dataset / Greenhouse Management	# of Greenhouses	# of *B. tabaci*	# of MEAM1		# of Mediterranean	
Seasonal Data Set	6	512	453	(88%)	59	(12%)
Biological control	3	366	353	(96%)	13	(4%)
Insecticide	3	146	100	(68%)	46	(32%)
End of Season Survey	7	119	111	(93%)	8	(7%)
Biological control	5	96	95	(99%)	1	(1%)
Insecticide	2	23	16	(70%)	7	(30%)
Total	11	632	564	(89%)	67	(11%)
Biological control	8	462	448	(97%)	14	(3%)
Insecticide	5	170	116	(68%)	54	(32%)

4.1.3.1 Seasonal Data set

The seasonal data set consisted of 512 individuals collected during 36 greenhouse visits. The majority (88%) of individuals were identified as MEAM1. The proportion of *Mediterranean* individuals in biological control-managed greenhouses decreased from 17% on July 5th to 0% on November 21st. In the insecticide-managed greenhouses, the proportion of *Mediterranean* individuals was 50% on July 5th and 36% on November 21st (Figure 4.1.1). *Mediterranean* individuals were detected in 2 of the 3 biological control- and all of the insecticide-managed greenhouses on the first sampling date on July 5th. After July 29th no *Mediterranean* individuals were detected in any of the biological control-managed greenhouses. In the insecticide- management greenhouses, *Mediterranean* individuals were found in two of the greenhouses for the duration of the survey, but were not detected in the third after July 5th. Greenhouse management method had a significant effect on the proportion of *Mediterranean* individuals in sampled populations (F= 18.10, df=1,32, P <0.05), whereas sampling week, and the greenhouse did not.

4.1.3.2 End of season survey

In total 119 whiteflies were collected from the end of season survey on December 12th. The majority (93%, n=111) of individuals were MEAM1, with the remainder being *Mediterranean*. Collections from the five biological control- managed greenhouses were comprised predominantly of MEAM1, with the exception of a single *Mediterranean* individual. Collections from one of the insecticide-managed greenhouses was comprised entirely of *Mediterranean* (n=8) individuals, while the other was entirely of MEAM1 (n=14).

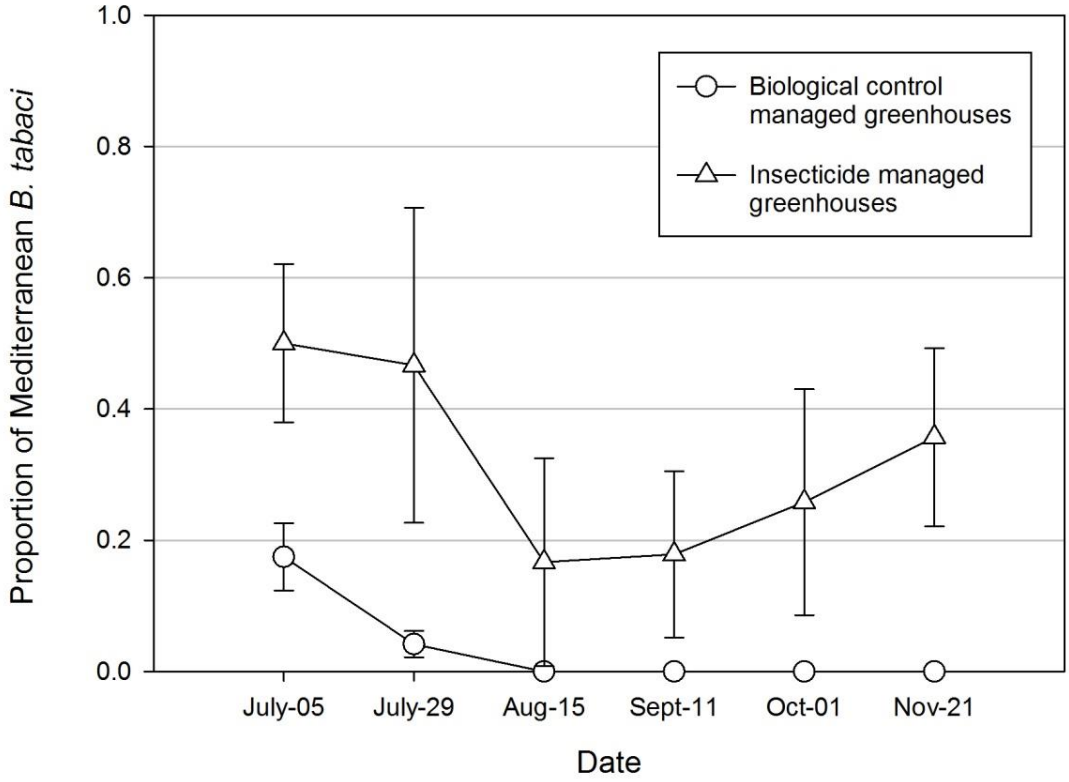

Figure 4.1.1: Proportion ± SE of *Mediterranean B. tabaci* detected in six commercial poinsettia greenhouses surveyed between July 5th and November 22nd 2012. Three greenhouses used biological control based management, and three used insecticide based management.

4.1.3.3 COI Sequencing

Two COI haplotypes were identified, one corresponding to MEAM1 and the other to *Mediterranean*. Identifications inferred from the fragment length assay (from Shatters et al. 2009) and the consensus sequence approach (from Dinsdale et al. 2010) were in complete agreement. That is, individuals identified as MEAM1 and *Mediterranean* via the fragment length assay were identical (i.e., 0.00% divergent) to their respective consensus sequence (Table 4.1.3). Sequence, trace-file and specimen images for these specimens can be found in BOLD

Table 4.1.3: Distance summary of unique haplotypes of Ontario *Bemisia tabaci* specimens, and consensus sequences of *B. tabaci* cryptic species from Dinsdale et al. (2010). Distances calculated are P-distance, the number of base differences per site expressed as a percent. Analyses were conducted in MEGA5 (Tamura et al. 2011).

Bemisia cryptic species name	Ontario Haplotype 1	Ontario Haplotype 2
Mediterranean	0.026	**0.000**
Middle East-Asia Minor 1	**0.000**	0.026
Middle East-Asia Minor 2	0.022	0.018
Indian Ocean	0.050	0.046
Asia I	0.116	0.116
Australia/Indonesia	0.118	0.116
Australia	0.122	0.133
China 1	0.113	0.118
China 2	0.107	0.115
Asia II 1	0.120	0.120
Asia II 2	0.079	0.087
Asia II 3	0.118	0.115
Asia II 4	0.113	0.118
Asia II 5	0.124	0.124
Asia II 6	0.126	0.124
Asia II 7	0.116	0.115
Asia II 8	0.116	0.116
Italy	0.113	0.116
SubSaharan Africa 1	0.124	0.129
SubSaharan Africa 2	0.128	0.133
SubSaharan Africa 3	0.128	0.131
SubSaharan Africa 4	0.131	0.133
New World	0.115	0.118
Uganda	0.122	0.133

project BTB, BOLD Process-ID: BTB004-13 to BTB0015-13. GenBank accession numbers for these specimens are KJ591609 to KJ591620. All sequences derived from Mediterranean individuals were assigned to Barcode Index Number (BIN) BOLD:AAG4846, and MEAM1 to BOLD:AAT8875 by the Barcode of Life Data Systems (Ratnasingham and Hebert 2007, 2013).

4.1.4 Discussion

Our data suggest that MEAM1 is capable of displacing *Mediterranean* populations in a greenhouse environment, similar to observations in both laboratory populations and in the field (Liu et al. 2007, Crowder et al. 2010, Dinsdale et al. 2012, Luan et al. 2013). This trend is evident in the biological control-managed greenhouses as indicated by our inability to detect any *Mediterranean* individuals after July 29th in the seasonal data set. This is supported by observations made at the end of season survey, where only a single *Mediterranean* individual was found. Our data also suggest that MEAM1 displaced *Mediterranean* populations in the insecticide-managed greenhouses until the start of insecticide programs in August. Prior to the initiation of insecticide based management programs the proportion of *Mediterranean* individuals decreased; however, after insecticide-based management programs began, the proportion of *Mediterranean* individuals increased (Figure 4.1.1). This increase likely occurred due to insecticide applications selecting for *Mediterranean* individuals in those greenhouses, demonstrating the potential for pest resurgence due to insecticide resistance. Overall our data supports the predictions made by Crowder et al. (2010) regarding the competitive exclusion of *B. tabaci* cryptic species in the presence and absence of insecticides. Together, these observations emphasize the importance of periodically determining the composition of *Bemisia* populations to inform management decisions, particularly insecticide active ingredient selection in the context of a resistance management program in mixed species infestations.

Our results suggest that mixed species infestations in commercial greenhouses can revert to MEAM1 under biological control-based management. Therefore biological control may be the preferred management technique if reduction of *Mediterranean* individuals is an important pest management objective, which may be the case in some ornamental production systems (e.g., poinsettia). The decline in *Mediterranean* individuals in biological control-based management greenhouses was likely a result of asymmetric mating interference between MEAM1 and *Mediterranean* individuals (Crowder et al. 2010). This would occur more rapidly in the biological control greenhouses due to the greater proportion of MEAM1 individuals compared with the insecticide managed greenhouses (Figure 4.1.1). Alternatively, it's possible that the biological control agents employed by growers may preferentially parasitize or prey upon the *Mediterranean* individuals. However, to our knowledge no comparable data exist on the host preferences or functional response of parasitoids against *B. tabaci* cryptic species. The only example found in the literature dealt with *Encarsia sophia* (Girault & Dodd), a parasitoid with a cosmopolitan distribution, which showed a preference for MEAM1 (Wang et al. 2011). Given the economic importance of *B. tabaci,* I encourage more studies to determine if any host preferences exist for the commercially available *B. tabaci* biological control agents.

To date the majority of DNA sequence data generated for *B. tabaci* has been for the 3' region of the COI gene (Frohlich et al. 1999, Boykin et al. 2007, Shatters et al. 2009, Dinsdale et al. 2010, Ahmed et al. 2012). Information gleaned from these data was used to described the genetic variation within the *B. tabaci* cryptic-species complex (Frohlich et al. 1999, Shatters et al. 2009, Dinsdale et al. 2010, De Barro et al. 2011), and has been employed by the current authors and others (Ahmed et al. 2012) to identify *B. tabaci* cryptic species. However, a 650bp fragment of the 5' region of this gene (also known as the DNA barcode) has been widely adopted for use as a genetic marker for the identification of animals, including pest insects, in both an applied and research context (Hebert et al. 2003b, Armstrong 2010, Bonants et al.

2010, Frewin et al. 2013). At the time of writing this article, DNA barcodes have been generated for 138,537 insect species (refer to BOLD: www.boldsystems.org, Ratnasingham and Hebert 2007). Given the widespread adoption of DNA barcoding as a tool for species identification, I would encourage researchers generating COI sequence data for *B. tabaci* to consider including both the 5' and 3' regions, and to follow DNA barcoding metadata standards (Hanner 2009). This will ensure that data collected with one marker can be unambiguously linked to data collected from the other and that the data will be useful to the largest community possible.

Our study suggests that MEAM1 is capable of displacing *Mediterranean* in greenhouses in a single growing season, which encompasses approximately 5-6 generations of *B. tabaci*. This knowledge may help growers to more efficiently manage mixed *B. tabaci* populations. For example, the ability of MEAM1 *B. tabaci* to naturally displace *Mediterranean* under biological control-based management may increase the efficacy of 'clean-up' insecticide applications when necessary, which can be important for ornamental crops. This data also supports the use of biological control-based *B. tabaci* management as it can both effectively reduce *B. tabaci* populations below economic levels while allowing MEAM1 to displace *Mediterranean*. Conversely, repeated use of insecticides may promote the persistence of the *Mediterranean* cryptic species.

4.2 A Gel-free PCR Based Diagnostic Method for In-Field Differentiation of MEAM1 and Mediterranean *Bemisia tabaci* Cryptic Species

4.2.1 Introduction

Bemisia tabaci is an economically significant pest of food and horticultural crops, with a host range in excess of 600 plant species (Oliveira et al. 2001, Simmons et al. 2008). As a result of international trade this pest now has a near global distribution (Cheek and Macdonald 1994, Dalton 2006) and is recognized as one of the world's 100 most invasive species (Global Invasive Species Database, http://www.issg.org/worst100_species.html).

As early as the 1990s it was suspected that *B. tabaci* may in fact be a cryptic species complex based on behavioural, genetic, and physiological differences among populations (Perring et al. 1993). However, consensus on the rank of these proposed taxa was not universal (Bedford et al. 1994, Bellows et al. 1994, Brown et al. 1995). As a result, a convention of assigning unique "biotype" designations to behaviourally and/or physiologically distinct *B. tabaci* populations was adopted, of which at least 36 are described in the literature (Dinsdale et al. 2010, De Barro et al. 2011). Due to mounting genetic and physiological evidence, it is now widely accepted that *B. tabaci* is a cryptic-species complex, consisting of at least 24 members (Frohlich et al. 1999, Boykin et al. 2007, Dinsdale et al. 2010, Elbaz et al. 2010, De Barro et al. 2011, Luan and Liu 2012, Saleh et al. 2012, Lee et al. 2013, Luan et al. 2013). As a result, the biotype classification system for *B. tabaci* has been largely replaced with a naming convention based on presumed phylogeographic origins of the *B. tabaci* cryptic species (Dinsdale et al. 2010). For the purposes of this manuscript I follow this naming convention but also cross-reference it with biotype nomenclature for clarity.

Of practical importance to IPM specialists is that members of the *B. tabaci* cryptic-species complex vary in their capacity to transmit plant viruses (Bedford et al. 1994), developmental rates (Muñiz and Nombela 2001, Qiu et al. 2011), susceptibility to parasitism (Wang et al. 2011), and, most importantly, insecticide resistance profiles (Horowitz et al. 2005, Dennehy et al. 2010, Li et al. 2012). For example, two of the most commonly encountered species in North America and Europe are *Middle East Minor 1*(MEAM1: formerly 'Biotype B', *B. argentifolii*) and *Mediterranean* (formerly 'Biotype Q', *B. tabaci*, see Tay et al. 2012) species. While *Mediterranean* generally is more resistant to insecticides than MEAM1 (Horowitz et al. 2005), from a greenhouse management perspective biological control appears equally effective against both species. Further complicating matters is that in greenhouses, MEAM1 and *Mediterranean* often occur in mixed-species infestations, the proportion of which may change over time in response to insecticide selection pressure and/or asymmetric mating interference (Crowder et al. 2010, Frewin et al. 2014). Thus knowledge of which *B. tabaci* cryptic specie(s) comprise an infestation may help inform appropriate and effective pest management decisions. However, identification of these species is only possible with molecular diagnostics, and despite the need for such tools, currently there are limited options for field diagnostics for *Bemisia* cryptic species.

Over the last two decades there have been a number of molecular diagnostic tests developed that are capable of discriminating among various members of the *B. tabaci* species complex including esterase zymogram analysis (Byrne and Devonshire 1991), PCR amplification and size determination of microsatellite markers (De Barro et al. 2003), comparison of random amplified polymorphic DNA (RAPD) profiles (De Barro and Driver 1997), and PCR amplification and sequence comparison of mitochondrial gene fragments (Shatters et al. 2009), including DNA Barcoding (Frewin et al. submitted). However, due to the complexity, cost, and time required to conduct these diagnostics; they are not typically implemented in-field.

A more practical option for in-field diagnostics may be real-time PCR (Jones et al. 2008), as it does not require gel electrophoresis or sequencing. However, portable real-time PCR machines are still relatively expensive and scarce compared to portable (or at least easily-moveable) thermal cyclers required for conventional PCR. Another practical option is the loop-mediated isothermal amplification (LAMP) assay (Hsieh et al. 2012), which can be performed using an iso-thermal hot-block or conventional thermal cycler. However, crop scouts and extension workers (the intended user-group for these diagnostics) may be dissuaded from adopting this approach as iso-thermal amplification has currently been incorporated into fewer agricultural pest diagnostics than conventional PCR and requires its own suite of specialized reagents.

This paper describes a simple and quick PCR based assay to differentiate *Mediterranean* and MEAM1 cryptic-species of *B. tabaci* that can be conducted in less than 1 hour using reagents with no special disposal requirements. To accomplish this, I used previously published, and MEAM1 and *Mediterranean*-specific primers (Shatters et al. 2009) with a high fidelity polymerase to reduce PCR cycling time. The resulting PCR products can then be directly visualized with fluorescent nucleic acid stain and a portable/handheld UV light.

4.2.2 Methods

4.2.2.1 Bemisia Collection and Cryptic species Identification

All *B. tabaci* specimens for this study were collected from commercial poinsettia greenhouses in the Niagara Region of Ontario, Canada during the summer and fall of 2012 as part of a *B. tabaci* survey (Frewin et al. 2014). DNA was extracted from whole specimens without maceration using a commercial alkaline lysis extraction kit [XytXtract Insect (ANDE) DNA extraction kit (Xytogen; Perth, Australia)] following manufacturer recommended protocols (Castalanelli et al. 2010). However, similar results could be produced using alkaline lysis

procedures described by (Ivanova et al. 2009)(Data not shown). All DNA extracts were stored at -20°C prior to analysis. The cryptic-species identity of these specimens was determined using a method developed by Shatters et al. (2009). In brief, DNA extracts were subjected to PCR using primers designed to amplify products of different lengths from MEAM1 and *Mediterranean* which were differentiated using gel electrophoresis. The PCR reaction mix for this assay consisted of 6.25µL of 10% trehalose, 2.00µL of water, 1.25µL of 10X PCR Buffer (Life Technologies™), 0.625µL $MgCl_2$ (50 mM), 0.25µL of dNTP (10 mM), 0.0625µL of Platinum® Taq (Life Technologies™), 0.10µL total volume of each forward and reverse primers, and 2.00µL of DNA template (Ivanova et al. 2009). The primers were as follows: MEAM1: BioB-L (5'-CTA GGG TTT ATT GTT TGA GGT CAT CAT ATA TTC-3'), BioB-R (5'-AAT ATC GAC GAG GCA TTC CCC CT-3'), and *Mediterranean*: BioQ-L (5-CTT GGT AAC TCT TCT GTA GAT GTG TGT T-3), BioQ-R (5-CCT TCC CGC AGA AGA AAT TTT GTT C-3) (Shatters et al. 2009). Cycling conditions were as described in Shatters et al. (2009). PCR products were then visualized on a 2% agarose gel pre-stained with SYBR® Safe DNA gel stain (Life Technologies™).

4.2.2.2 Gel-free Rapid Assay

4.2.2.2.1 Dilution series

DNA extracts derived from 44 individuals identified as MEAM1 and 44 from *Mediterranean* were randomly selected from all individuals collected and analysed as part of the larger survey (Frewin et al. 2014). Concentrations of these DNA extracts were determined using Qubit® dsDNA HS Assay (Invitrogen™, Life Technologies™, Thermo Fisher Scientific, Inc.). A 10X, 100X, 1000X dilution of each DNA extract was prepared. Each individual *B. tabaci* DNA extract and dilution was subjected to two PCRs, one containing the MEAM1- and one containing

Mediterranean-specific primers listed above. PCR reaction mixture consisted of 13.4µL of water, 4.00µL of 5X Phire® Reaction Buffer (Thermo Fisher Scientific Inc.), 0.40µL of dNTPs (10 mM), 0.04µL of Phire® Hot Start II DNA Polymerase, 0.4µL of each forward and reverse primers (10 mM), and 1.0µL of DNA template. Cycling conditions were as follows: an initial denaturation at 98°C for 30s, followed by 35 cycles of 94°C for 5s, 64.5°C for 5s, 72°C for 10s, then a final extension at 72°C for 1 min. After the PCR cycling was competed, 1µL of a (1:100) dilution of GelGreen™ (Biotium, Inc., Hayward, CA) in ddH_20, was added to each PCR reaction, and shaken by hand to mix. Results (i.e., presence or absence of fluorescence) were then visualized directly using portable UV light in an Alphaimager® HP (ProteinSimple, Santa Clara, CA).

4.2.2.2.2 Test series

DNA extracts from an additional 24 previously unidentified *B. tabaci* samples were used as 'unknowns' to test our gel-free assay. Following these tests, the cryptic-species identity of these individuals was determined using methods developed by Shatters et al. (2009). The test series was setup in 8-well PCR strips as pictured in Figure 4.2.1. Each 8-well strip contained a positive and negative control for both MEAM1 and *Mediterranean*. Four wells in each strip were set aside to test two unknown samples. One well for each unknown contained *Mediterranean*-specific primers and the other contained MEAM1-specific primers. PCR reagents and reaction conditions were identical to those of the dilution series.

4.2.3 Results

Fluorescence was observed for all DNA extracts and 10X dilutions for *Mediterranean*-individuals subjected to *Mediterranean*-specific primers and MEAM1-individuals subjected to MEAM1 primers using the Alpha-imager and portable UV light in both the dilution and test series (Figures 4.2.1 and 4.2.2). Fluorescence was observed in 41% and 1% of 100X and 1000X dilutions, respectively, of *Mediterranean*- and MEAM1-individuals subjected to their respective primers. Identity of samples was entirely consistent between methods (i.e., gel free in-tube fluorescence assay and method by Shatters et al. 2009) for both the dilution and test series. No visible fluorescence (i.e., no cross reactivity) was observed for DNA extracts or dilutions of *Mediterranean*-individuals subjected to MEAM1- specific primers or MEAM1-individuals subjected to *Mediterranean*-specific primers. The average concentration of DNA extracts was 1.7ng/µL (range: 0.8 to 4.2 ng/µL).

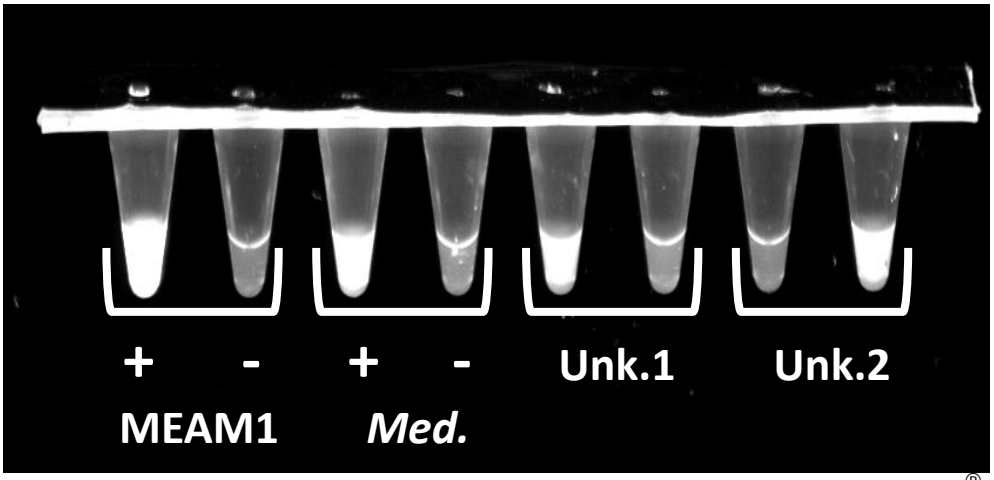

Figure 4.2.1: A typical test series as visualized with an Alphaimager® HP. The first four wells are designated for positive and negative controls of MEAM1 and *Mediterranean* (*Med.*). The last four wells are designated for two unknown specimens whose DNA is subjected to MEAM1 (well 5,7)- and *Mediterranean* (well 6,8)- specific primers. In this case Unknown 1(Unk.1) is identified as MEAM1, whereas Unknown2 (Unk.2) is identified as *Mediterranean* as indicated by the observed fluorescence.

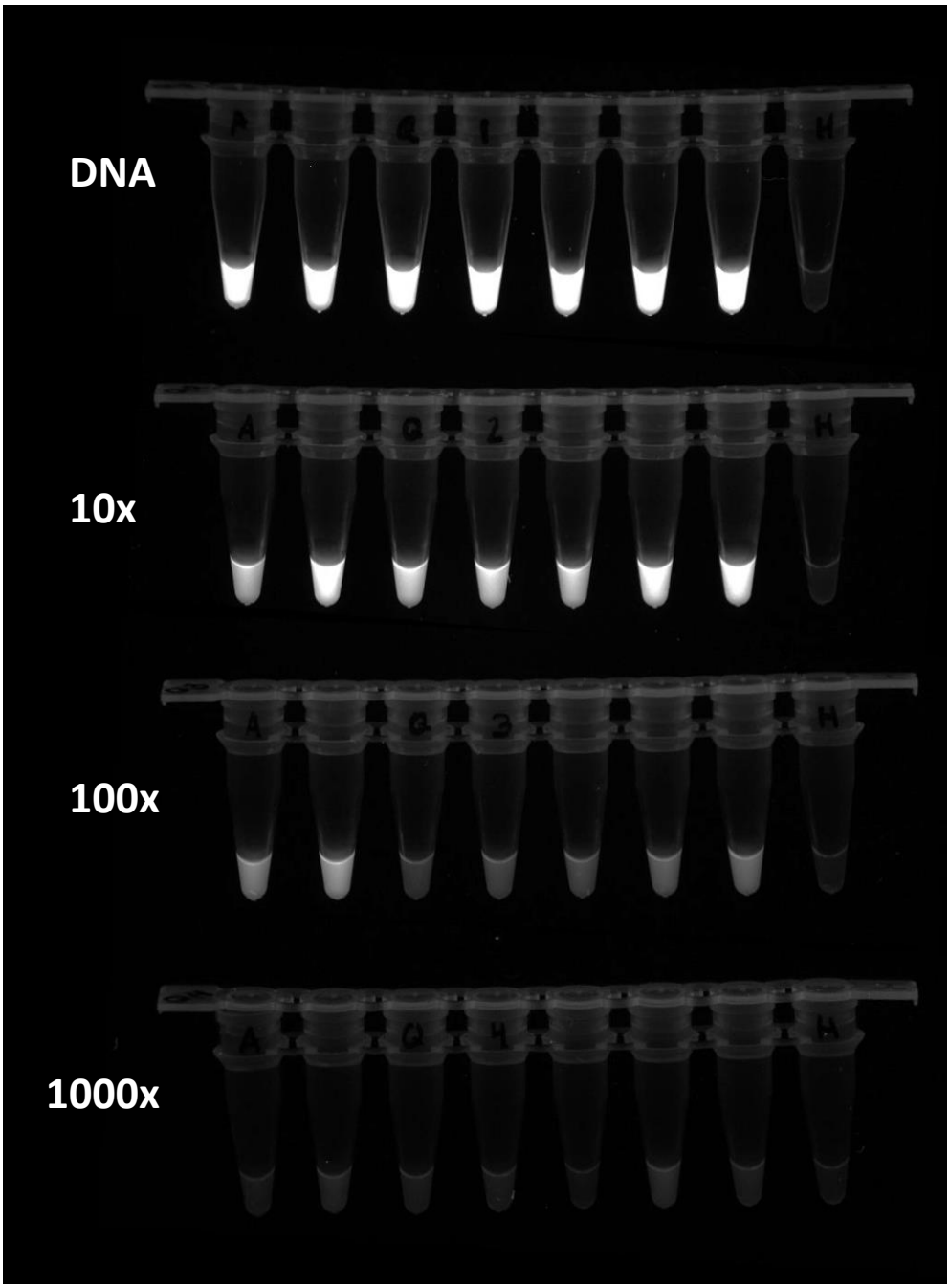

Figure 4.2.2: A typical dilution series of DNA derived from *Mediterranean* specimens subjected to PCR with *Mediterranean* specific primers, visualized with an Alphaimager®. The top row pictures undiluted DNA extracts, followed by the 10X, 100X, and the 1000X dilutions. The last well in each 8-well strip contains a negative control (PCR reagents and primers without DNA).

4.2.4 Discussion

The relatively simple PCR based assay described here is capable of discriminating *Mediterranean* and MEAM1 members of the *B. tabaci* species complex in less than 1 h (20 min incubation for DNA extraction, 23 min for PCR, plus 10-15 min for setup and observation of results). All reagents used in this assay are classified as nonhazardous and non-mutagenic, and thus can be easily and safely used and disposed of outside of a laboratory setting. The current reagent cost for this assay is <$1US per reaction. The cost, time required, and the few pieces of equipment (e.g., a thermal cycler, pipette(s), a UV light source) required to complete this assay, make it ideal for deployment in the field by consultants or extension workers as a tool to assist the IPM decision making process for *Mediterranean* and MEAM1 members of the *B. tabaci* species complex.

Results from this assay are likely to be most useful in helping inform insecticide active ingredient selection, given the differences in insecticide tolerance between *Mediterranean* and MEAM1. However, prior to adopting this assay, consideration must be given to the sampling and analysis effort (i.e., *B. tabaci* sampling protocol and number of samples that should be analysed). Obviously, the more samples that are analysed (assuming the sampling is random) the more accurate the estimates of species composition will be. However, it is an open question as to what level of accuracy is needed to inform the majority of real-world management decisions, and what the optimal management choices for mixed-species infestations are. Rather than speculate, these considerations are left to individual users who are better equipped to incorporate their knowledge related to their particular cropping systems and available pest management strategies.

Although a limit of detection for this assay was not explicitly determined, the dilution series suggests it is less than 0.17 ng/µL (the concentration of the 10X dilutions), which was the

highest dilution factor in which all samples expected to fluoresce clearly did so. Considering that the lowest concentration of the DNA extracts was 0.8ng/µL, four times greater than this conservative 'limit of detection' of 0.17 ng/µL, I would not expect this assay to fail due to low DNA yields, particularly when analysing newly collected specimens. Although measures could be taken to increase DNA yields such as macerating specimens prior to DNA extraction, this step was intentionally avoided to minimize the number of in-field analytical steps and thus reduce the potential to introduce error. As macerating specimens in-field requires a sterile tool for each individual sample, ideally this would be avoided to reduce the potential for cross-contamination between samples. It is also worth noting that PCR reagents including primers can be pre-mixed and/or lyophilized ahead of time, further reducing the number of analytical steps conducted outside of a controlled laboratory environment.

No cross reactivity was observed in this assay using the listed cycling conditions. However, in the design phase of this assay cross-reactivity was observed at annealing temperatures <64°C for MEAM1 primers and *Mediterranean* specimens. I advise caution if attempting to conduct this assay with different reaction buffers as they may alter primer annealing properties. When the primers used in this assay were originally designed and tested they showed no cross reactivity with other whitefly or *B. tabaci* cryptic species (Shatters et al. 2009). Although additional species were not tested here, cross reactivity is not expected since this was taken into consideration during the primer design process (Shatters et al. 2009). However, caution is advise when applying this test for diagnostics in areas where *B. tabaci* cryptic-species other than MEAM1 and *Mediterranean* are likely to be frequently encountered (i.e., outside of Europe and North America), and in these cases would suggest confirmation of *B. tabaci* cryptic-species by DNA sequencing using one of a number of available primer sets/reaction conditions (Shatters et al. 2009, Dinsdale et al. 2010, Frewin et al. 2014).

Differences in the insecticide resistance profiles of MEAM1 and *Mediterranean B. tabaci* have the potential to influence management programs for this pest complex. However, until now a lack of field diagnostics has potentially prevented growers from tailoring their IPM programs to their specific *B. tabaci* population composition. The ability to diagnose *Mediterranean* and MEAM1 in-field will not only improve management of these pests but also assist in insecticide resistance management programs. Typically insecticide resistance observed in *B. tabaci* populations has been attributed to the presence of *Mediterranean* cryptic species; however, the ability to determine if MEAM1 is developing resistance will be important for the long term management of these pests, and this is only possible if the composition of *B. tabaci* populations is known. The assay described in this manuscript can facilitate the identification of *Mediterranean* and MEAM1 *B. tabaci* in-field by extension entomologists and crop scouts without specialist knowledge of molecular methods, and can completed in less than 1 h.

Chapter 5

INCORPORATING DNA BARCODING INTO GREENHOUSE IPM: USING PLANT WASHING AND STICKY CARDS FOR SPECIMEN COLLECTION

5.0 Abstract

It is important to tailor IPM programs to the particular suite of pest and beneficial arthropods present in a production system in order to maximize the efficacy of any individual management tactic. Therefore, pests and beneficial arthropods must be identified accurately. Unfortunately many arthropods of economic significance in greenhouse production can be difficult to detect due to their cryptic lifestyles, and to identify due to their size. A potential solution to these challenges is to employ DNA barcoding to assist in the identification of greenhouse arthropods. However, in this context it would be advantageous to use existing greenhouse monitoring and phytosanitary techniques to collect specimens for DNA barcode-based identification. Therefore, the suitability of DNA barcoding for the identification of arthropod specimens collected using plant washing and sticky cards to be DNA barcoded is examined. First, plant washings are conducted on a large number of imported un-rooted vegetative cuttings of ornamental plant species. Only two specimens were found on this material, neither of which could be barcoded. Second, sticky cards and plant material known to be infested with various arthropods were obtained from local greenhouses. In this case, the majority of specimens isolated from plant washings of infested plant material and specimens retrieved from 2 week old sticky cards yielded DNA suitable for DNA barcode based identification. In general, arthropod specimens obtained from plant washing and sticky cards can be successfully DNA barcoded, and therefore this may be a useful technique to increase the arthropod pest diagnostic capacity of greenhouse pest managers.

5.1 Introduction

Due to the importance of tailoring integrated pest management (IPM) programs to the actual pest species present in a cropping system, the accurate identification of pest arthropods is a critical component of all IPM programs. However, as a result of their diversity, size and cryptic lifestyles, pest arthropods can be difficult to identify to the level of species which may be a barrier to their successful management. Moreover, failure to promptly recognized novel invasive pests at the farm-level may significantly jeopardize eradication programs and threaten regional agriculture systems. This can be overcome by using molecular species diagnostics such as DNA barcoding (Hebert et al. 2003a) to identify specimens of pest and beneficial organisms, providing necessary information for on-farm pest management decisions, and assist regulatory biosecurity programs (Armstrong 2010, Floyd et al. 2010, Frewin et al. 2013). However, to facilitate the adoption of DNA barcoding for on-farm decision making, it should be incorporated into well-established pest management techniques. Two such methods implemented in greenhouses are plant washings, a phytosanitary procedure, and sticky cards, a technique for monitoring arthropod pests.

In agriculture pest specific IPM and the threat of invasive species are critical factors influencing greenhouse production. Greenhouse plant production is characterized as highly susceptible to invasive pests (Dehnen-Schmutz et al. 2010), as greenhouses provide stable environmental condition which allow pests from warmer climates to colonizes temperate regions year-round. Furthermore, the interconnectivity of the greenhouse ornamental industry in which propagative plant material is shipped between suppliers, distributors and propagators both domestically and internationally, can result in the rapid spread of hitch-hiking pests from one region to another and has likely contributed to the spread of many pests (e.g., *Bemisia tabaci* (Bedford et al. 1993, as cited by Brown et al. 1995), and *Frankliniella occidentalis* (Nickle

2004)). Therefore diligent monitoring efforts both by greenhouse producers and extension officials are required to curb the introduction and spread of invasive species.

Plant washing is a simple technique which has both research and on-farm applications. For example, in research it has been used to estimate the number of arthropods on plant material for studies on population dynamics (Shipp and Zariffa 1991, Parajulee et al. 2006). This process involves submerging plant material in a liquid media, agitating it to dislodge and immobilize any arthropods, and then decanting the liquid over a filter to collect these arthropods which can then be quantified. This technique aims to provide whole plant counts of arthropods difficult to achieve with other methods (Shipp and Zariffa 1991). On-farm, plant washings have been developed as a phytosanitary technique for greenhouse horticulture (Haviland et al. 2005, Buitenhuis et al. 2014). In this case, prior to entering the greenhouse propagative plant material is submerged in water which may be heated or treated with a (bio)-pesticide in order to reduce the potential of pest populations entering the greenhouse.

Another widely adopted greenhouse IPM technique is the use of sticky cards to detect the presence of pests, information which can then be used to inform management decisions such as pesticide applications or biological control agent release rate. Unfortunately, at times it can be extremely difficult to identify specimens to the level of species while attached to sticky cards as characters may become obscured or damaged by the glue. However, solvents such as orange oil (Marshall et al. 2010) can be used to remove specimens from sticky cards so that morphological characters can be observed.

Both of these techniques are extremely efficient at obtaining specimens, however, the challenge of identifying the specimens to the level of species remains. Identifying even common pest arthropods can be challenging due to the difficultly inherent in morphological species diagnoses, such as lack of diagnostic characters on particular life-stages, lack of accessible

taxonomic expertise and/or training, the presence of cryptic diversity within pest taxa, and the subjective interruption of morphological characters. To overcome these challenges the potential of using DNA barcoding to identify arthropods collected from propagative plant material using plant washings, and those obtained from greenhouse sticky cards are investigated. The objective of this work is to provide a methodology for plant washing and sticky cards to obtain arthropod specimens which are amendable to DNA barcoding, with the intension to ultimately use these techniques to assist in the identification of arthropods in an agricultural setting.

5.3 Methods

5.3.1 Plant Washings - Plant Sources

Plant material infested with arthropods was collected from greenhouses in Guelph and the Niagara Peninsula, both in Ontario, Canada. This included poinsettia (*Euphorbia pulcherrima*) infested with *B. tabaci*, eggplant (*Solanum melongena*) with spider mites (*Tetranychus* sp.), and chrysanthemums (*Chrysanthemum* sp.) with the biological control agent *Amblyseius swirskii.* To test the ability for this method to detect arthropods in commercial propagative plant material, ~4000 un-rooted ornamental plant cuttings were purchased from local brokers originating from various international sources and included New Guinea Impatiens (*Impatiens hawker*), Double Impatiens (*Impatiens* sp.), *Calibrachoa* sp., and *Verbena* sp.

5.3.2 Plant Washing Procedure

Plant material was submerged in a collection fluid (specified below), in 2L beakers and agitated by hand for 30s. After this the plant material was removed from the collection fluid and discarded. Unrooted cuttings were subjected to the plant washing procedure using 70% ethanol as a collection fluid. The chrysanthemums harboring *A. swirskii* where washed using a 0.1 % Triton X- 15 solution (Shipp and Wang 2003). Poinsettia with *B. tabaci*, and eggplant with *Tetranychus* sp. were washed with either 70% ethanol or water. Collection fluid containing plant debris was then decanted through a 10cm Whatman™ number 4 filter paper in Büchner funnel. Filter papers with plant debris were transferred individually to 10cm petri dishes. Petri dishes were then sealed with Parafilm M, and stored at -20°C for a maximum of 4 days until samples could be examined. Filter papers were inspected with a stereo-microscope and arthropod specimens were individually transferred to 1.5mL centrifuge tubes with flame sterilized forceps.

5.3.3 Sticky Cards Procedure

Yellow sticky cards were obtained from Koppert®, and placed in a greenhouse on the University of Guelph campus. Sticky cards were removed from greenhouses after 14d. In commercial greenhouses sticky cards are normally replaced on a weekly or bi-weekly basis. Sticky cards were immersed in bath of orange oil (New Directions Aromatics, Mississauga, Ontario) in a 5cm deep metal tray for ~5 mins. The sticky card was gently agitated with forceps while submerged until specimens floated free from the card. Occasionally specimens needed to be removed from the card with a fine paint brush while still submerged in the orange oil. Specimens were retrieved from the orange oil bath with forceps. Specimens were cleaned by transferring them through a series of three containers of 95% ethanol, in each of which they

were submerged for ca. 5-10sec. Finally, they were placed individually in 1.5 mL centrifuge tubes with 95% ethanol and stored at 4°C for up to 48h prior to analysis. In total 24 specimens were randomly sampled from sticky cards and subjected to DNA barcode analysis.

5.3.4 DNA Barcoding

Arthropods specimens obtained from plant washings were extracted from whole specimens using the QIAGEN DNeasy Blood & Tissue Kit (QIAGEN Inc., Valencia, CA) as per manufacture protocols, with one modification. The volume of the final AE elution buffer was reduced to 80µl from 200µl. DNA from whole specimens obtained from sticky cards were extracted using a commercial alkaline lysis DNA extraction kit Insect (ANDE) DNA extraction kit (Xytogen, Perth, Australia) using manufacture protocols (Castalanelli et al. 2010). Specimens subjected to QIAGEN DNA extraction were destroyed in the extraction process, whereas specimens subjected to Xytogen DNA extraction remained intact and where stored in 95% ethanol. DNA extracts from all specimens were stored at -20°C prior to analysis.

PCR amplification of DNA barcode sequences were performed with the follow primer pairs, Lep-F1 (5'- ATT CAA CCA ATC ATA AAG ATA TTG G-3') and Lep-R1 5'- (TAA ACT TCT GGA TGT CCA AAA AAT CA-3') (Hebert et al. 2004), or LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3)' and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994). *Bemisia tabaci* specimens where amplified with the following primer pair WF-F (5'-ATT CAA CCA ATC AYA ARG ATA TYG G-3') and WF-R (5'-TAA ACT TCT GGA TGH CCA AAR AAY CA-3') (Frewin et al. 2014). PCR reactions were performed in total volume of 12.63µL, containing 0.06µL (0.3U) Platinum *Taq* DNA polymerase (Invitrogen™, Life Technologies Corporation, Burlington, ON).), 1.25µL 10X reaction buffer, 0.625µL (50mM)

$MgCl_2$, 0.1µL (10µM) of each primer, 0.0625 (50µM) of dNTPs, 6.25µL 10% trehalose, and 2µL of template DNA. Cycling conditions for all primers pairs were as follows an initial denaturation at 94°C for 1min, followed by 5 cycles of 94°C for 40s, 45°C for 40s, 72°C for 1min, followed by 35 cycles of 94°C for 40s, 51°C for 40s, 72°C for 1min then a final extension at 72°C for 5 min.

All barcoded specimens were identified using the species identification engine (BOLD-IDS) on the Barcode of Life Data Systems (BOLD: Ratnasingham and Hebert 2007). The cryptic-species of *B. tabaci* were determined by comparison with *Middle East Asia-Minor 1* (MEAM1) and *Mediterranean* sequences generated in Chapter 4.

5.4 Results

All arthropod specimens collected from plant washings subjected to DNA Barcoding are summarized in Table 5.1. The vast majority of individuals collected with plant washing technique yielded amplifiable DNA suitable for DNA barcode analysis, regardless of collection fluid (Table 5.1). All spider mite specimens collected from eggplant were identified as *Tetranychus urticae*. No reference sequences for *A. swirskii* were available at the time this work was conducted therefore the DNA barcode identification of specimens presumed to be *A. swirskii* could not be made. These specimens were identified by BOLD as Phytoseiidae. In addition to the *A. swirskii* two adult and one larval dipteran where collected from chrysanthemums. The sequences generated from these specimens were identical to each other and identified as *Bradysia difformis* on BOLD. The *B. tabaci* specimens were identified as *B. tabaci*- Middle East Asia Minor 1 (MEAM1, = '*Biotype B*').

Table 5.1: Summary of specimens collected using plant washings subjected to DNA barcoding.

	Number sampled	Number barcoded	Extraction Fluid	DNA Barcode Identification[1]
Unrooted Cuttings				
Adult fly	1	0	70% Ethanol	N/A
Acari	1	0	70% Ethanol	N/A
Poinsettia				
Whitefly pupae	11	11	Water	*B. tabaci* (MEAM1/Biotype B)
Whitefly adult	1	1	Water	*B. tabaci* (MEAM1/Biotype B)
Whitefly pupae	10	10	70% Ethanol	*B. tabaci* (MEAM1/Biotype B)
Whitefly adult	1	1	70% Ethanol	*B. tabaci* (MEAM1/Biotype B)
Eggplant				
Spider mite	3	3	Water	*Tetranychus urticae*
Spider mite	3	3	70% Ethanol	*Tetranychus urticae*
Chrysanthemums				
Adult fly	2	2	0.1 % Triton X-15	*Bradysia difformis*
Larval dipteran	1	1	0.1 % Triton X-15	*Bradysia difformis*
Predatory mite	5	5	0.1 % Triton X-15	Phytoseiidae

[1]Identification conducted using BOLD-IDS and all public data on BOLD.

Un-rooted cuttings produced only two arthropod specimens, an adult fly (Diptera), and a mite (Acari). Unfortunately, DNA barcode sequences could not be amplified from these templates, and since both specimens were destroyed in the extraction process no further attempt could be made to determine their identity.

Arthropods specimens collected from sticky cards subjected to DNA Barcoding are summarized in Table 5.2. In total 87.5% of the specimens were successfully barcoded.

5.5 Discussion

Small arthropods can be difficult to detect in complex plant material, and challenging to identify to species. Despite their difficulty, these tasks are critically important for effective IPM and developing methods which can simplify these tasks will be benefit producers, researchers and extension workers. The methods described here can potentially overcome both of these challenges by allowing the user to easily collect specimens which are suitable for DNA barcode based species identification. For the plant washings regardless of the collection fluid used (water, 70% ethanol, 0.1% Triton X-15) the vast majority of specimens obtained yielded DNA suitable for DNA barcode based identification. Similarly, the majority of specimens eluted from sticky cards with orange oil solvent up to 14 days after collection were found suitable for DNA barcode based identification.

Given that both of these techniques yield material suitable for DNA barcode based species identification, they appear well suited for incorporation into various IPM programs. Both of these methods may have direct applications for ornamental poinsettia production. For example, a principle pest of greenhouse poinsettia is *B. tabaci*. In temperate climates this pest does not overwinter in the environment, instead it enters greenhouses on infested vegetative plant material (e.g., un-rooted cuttings). Plant washing procedures are being developed to sanitize un-rooted cuttings as they enter the greenhouse.

Table 5.2: Summary of specimens collected from sticky cards subjected to DNA barcoding.

Morpho-group	Number Sampled	Number Barcoded	DNA barcode Identification[1]
Fungus Gnat	21	18	*Bradysia difformis*
Hymenoptera	1	1	Bethylidae sp.
Diptera-Brachycera	2	2	*Coenosia attenuata*

[1]Identification conducted using BOLD-IDS and all public data on BOLD.

While this method is likely to reduce the starting populations of *B. tabaci* entering greenhouses it is unlikely to eliminate them completely. Interestingly, various members of the *B. tabaci* - species complex are known to possess different physiological attributes (e.g., development rate, insecticide tolerance) which may affect their management (Dennehy et al. 2010). Therefore plant-washing coupled with DNA barcode based species identification seems particularly well suited to serve as a phytosanitary technique which can also inform management decisions.

It is encouraging that the imported un-rooted cuttings used for this study were virtually free of arthropods, as it suggests that the phytosanitary practices employed (at least by these producers/exporters) are effective. However, it has been well documented that propagative plant material is an important pathway for introduction and movement of pests (Dehnen-Schmutz et al. 2010, Saccaggi and Pieterse 2013). It should be expected that if sampling effort was increased additional specimens would be detected, given that the effectiveness of plant washings for detecting arthropods at low density has been well established (Shipp and Zariffa 1991, Shipp and Wang 2003). However, because I was unable to detect arthropods on this material I cannot comment on the effectiveness of plant washings as a biosecurity tool to detect arthropods from at low densities.

Sticky cards are likely the most common pest monitoring technique employed by greenhouse producers and there are many opportunities to couple DNA barcoding and sticky card collections for agricultural research and IPM decision making. In particular, this will be useful for any pest management scenario in which easily confused or misidentified pests may require unique management actions, such as the apple aphids (*Aphis pomi* De Geer, and *Aphis spiraecola* Patch) (Hemiptera: Aphididae: Aphidinae) (Lowery et al. 2005, Foottit et al. 2009a) and *Bemisia tabaci* (Frewin et al. 2014), and may be extended to identifying cryptic natural enemies (Forbes et al. 2012). Typically sticky cards are replaced on a weekly or bi-weekly basis, it was found that specimens eluted from 2 week old sticky cards yielded DNA suitable for DNA barcoding analysis. However, some adverse environmental factors, such as high-heat, -UV, or -humidity may reduce DNA quality of specimens (Prendini et al. 2002), therefore I recommend exercising caution when applying this method under these conditions, and would recommend further validation.

In conclusion, it would appear that arthropod specimens collected from plant washings and sticky cards and processed using methods described here yield DNA suitable for DNA barcode based specimen identification in most cases. As such these techniques may be useful tool for greenhouse IPM, incorporating DNA barcoding into pest identification workflows.

Chapter 6

DNA BARCODING AS A STANDARDIZED DIAGNOSTIC FOR THE IDENTIFICATION OF RESEARCH ARTHROPODS IN THE BIOLOGICAL CONTROL LITERATURE

6.0 Abstract

Biological control is an increasingly important component of IPM programs. Successful implementation of biological control hinges on extensive knowledge of the biology and life history of biological control agents and their interactions with the pests they are intended to control. These life history and behavioural traits are species specific, and therefore it is important that research arthropods are identified accurately. To this end, standardized molecular diagnostics such as DNA barcoding may be useful for the routine identification of arthropods in biological control research. I surveyed 4 years' worth of biological control literature from two peer-reviewed journals to determine how research arthropods are being identified and documented. My survey revealed that biological control researchers often do not report the method used to identify their study species, specimen metadata, and/or voucher status. I also examined public DNA sequence data for species researched in this literature and discovered genetic patterns suggestive of either specimen misidentification or the presence of cryptic taxa. Finally, DNA barcodes are generated for a number of commercially-available biological control agents and discover genetic patterns that may reflect the presence of cryptic or mislabelled specimens. Together, these data reinforce the importance of properly identifying research arthropods in a documentable fashion in biological control research. To accomplish this, DNA barcoding as a standardized diagnostic for the routine identification of biological control agents is recommended.

6.1 Introduction

Biological control refers to the use of predators, parasitoids, pathogens, or herbivores (biological control agents) to manage populations of a pest. In the last 30 years, biological control has become a critical component of integrated pest management (IPM) programs globally (van Lenteren 2000, Cock et al. 2010, Birch et al. 2011) and it has been successfully implemented for the management of both agricultural and environmental pests as well as human and livestock disease vectors (Curtis 2009). Biological control programs are very species specific; that is, the specific pest(s) targeted, conditions under which optimal reproduction occurs, and nature of interactions with other arthropods are typically unique to each species of biological control agent. Thus, successful biological control programs depend on rigorous research into the biology, life history, and behaviour of biological control agents and the pests they are intended to control.

Given the species-specific focus of biological control, it is critically important that research organisms in this field are identified accurately using a documented methodology and accompanied by some form of specimen voucher. A specimen voucher refers to permanent reference material which can be used to corroborate that the research was conducted on a given species (Yoshimoto 1978). The retention of voucher specimens is important if it is ever suspected that the organisms employed in a particular piece of research were misidentified.

Misidentification of research organisms is likely to lead to the publication of erroneous life history data (Noyes 1994, Gibson et al. 2005, Bin et al. 2012), the consequences of which may include unnecessary replication of research, the pursuit of unproductive research objectives, or ultimately the failure of IPM programs. In fact numerous instances of misidentification of research animals (and derived tissues or cell-cultures) have been documented in the broader scientific community in recent years to the detriment of their

respective fields, and serve as a cautionary reminder of the implications of lax taxonomic practices in the applied sciences (Bely and Weisblat 2006, Hughes et al. 2007, Locke and Coates 2008, Du Preez et al. 2009, Anastasio et al. 2011, Lee et al. 2011a, Botero-Castro et al. 2014, Runnel et al. 2014).

Unfortunately, the application of names to specimens, regardless of the diagnostic methodology employed (i.e., morphology or molecular) can be a challenging task, particularly for hyper-diverse groups such as arthropods. Many factors influence the ability to identify specimens to the level of species, including a lack of taxonomic expertise and resources, taxonomic instability (Hawksworth 1992), the 'Linnaean shortfall' (i.e., the fact that most species on earth have not been formally described) (Lomolino and Heaney 2004, Mora et al. 2011, Rougerie et al. 2014), rapidly changing patterns of biodiversity as a result of globalization and climate change (Navia et al. 2010), a history of erroneous name usage (Gibson et al. 2005, Brown 2006), cryptic-diversity (Haruyama et al. 2008, Desneux et al. 2009, Williams et al. 2012), and the subjective interpretation of morphological characters (Ko et al. 2013).

A potential solution to the challenge of identifying research organisms is employing a standardized molecular diagnostic technique such as 'DNA barcoding' (Hebert et al. 2003a). Currently, DNA barcoding as a diagnostic technique has been adopted by a number of different disciplines (Cooper et al. 2007, Armstrong 2010, Bonants et al. 2010, Handy et al. 2011, Hanner et al. 2011). In contrast with traditional molecular diagnostics, DNA barcoding is not species-specific, but rather 'biodiversity-oriented', meaning that it can be broadly applied across taxa while requiring only a superficial knowledge of the classification of the query organism (i.e., in most cases the user only requires knowledge of the class or order of the un-identified organism to choose appropriate PCR primers). As such, DNA barcoding is broadly applicable as a standardized diagnostic methodology for biological control research, which typically deals with organisms from a wide variety of taxa. Furthermore, DNA barcoding is supported by the freely

accessible online bio-informatics platform Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007), which is capable of storing, organizing and visualizing specimen metadata and associated DNA sequences. Together this can provide the evidentiary basis for subsequent research, by allowing open access to this data in a digitally accessible format.

The ability of DNA barcoding to contribute to identifications is highly dependent on the extent to which DNA Barcode reference libraries contain representative individuals from the 'query' species to be identified (Ekrem et al. 2007). And currently, that is one of the shortcomings of DNA barcoding that reference libraries are incompletely populated, so that inferring identifications for unidentified specimens is never certain. However recent advances in the assignment of specimens to operational taxonomic units (OTUs) under the Barcode Index Number system (BINs) framework partly addresses this shortfall (Ratnasingham and Hebert 2013). In brief, the BIN algorithm assigns specimens to persistent OTUs based on their DNA barcode sequence. A number of studies have demonstrated the ability of the BIN algorithm to approximate current classification in a number of datasets and taxa (Hausmann et al. 2013, Ratnasingham and Hebert 2013, Zahiri et al. 2014). Therefore, in the absence of associated reference sequences, specimens can still be assigned a persistent identifier which can be used as interim taxonomy, providing a suitable framework for current and future comparison of specimens. Therefore, the BIN algorithm can facilitate the adoption of DNA barcoding for routine identification of research organisms while authoritative reference libraries continue to be built.

The objective of this research was to demonstrate the need for improved practices regarding the publication of name-associations in the field of biological control research. First, the publishing practices in the biological control literature as they relate to the documentation of identification methods and provenance data associated with arthropods used are reviewed. Second, currently available DNA barcode data on arthropod species found in the biological control literature is summarized. Third, a DNA barcode library of biological control agents sold

commercially in Canada is generated, to serve as baseline genetic resource for researchers working with these organisms.

6.2 Methods

6.2.1 Literature Review

All articles published between 2008-2012 from the journals BioControl: Journal of the International Organization for Biological Control (Springer) and Biological Control (Elsevier B.V) were screened for inclusion in this review. Only research articles that published experimental or observational data on arthropods were included; therefore all reviews, letters, modeling based manuscripts, and articles describing the development of an identification resource were excluded.

All arthropod taxa used in or observed as part of the published research were recorded and hereafter referred to as a literature review taxon list. Furthermore, each research paper was evaluated on three criteria: the origin of, the method used to identify, and voucher status of experimental arthropod(s). The origin of arthropods was sub-categorized as collected (or observed) from the environment or obtained from a commercial (e.g., for-profit distributor/producer of biological control agents) or non-commercial (e.g., a university or government research lab) supplier. If an article failed to specify the origin of their experimental arthropods, it was assumed that they were obtained from the environment. For arthropods obtained or observed in the environment, the precision of collection locality data was recorded as follows: no locality data, approximate locality data (e.g., country, region, municipality), or specific locality data (e.g., GPS coordinates, city/town, name of research institute/park/farm). The methodology used to determine the identity of experimental arthropods was categorized as

follows: no identification method stated, taxonomic resource (e.g., field guide, taxonomic key, or species descriptions), molecular diagnostic (including all DNA based methods and other bio-chemical markers), or consultation with a taxonomist (this included all circumstances where any individual(s) were attributed with identifying specimens related to the research). If an experimental arthropod was obtained from an insectary and not independently identified, it was considered that no identification method was provided. In some cases a research article provided an inconsistent documentation of metadata or vouchering when using multiple experimental arthropods. For example, exact collection data was provided for only one of the experimental arthropods or the identification methodology for one of the experimental arthropods was stated while another was not. Initial review of this literature suggested that such inconsistent reporting was rare, and therefore to simplify data organization and interpretation, only the best practice from each manuscript was recorded, while recognizing that all results represent a best-case scenario. Finally, to determine if there were any differences between journals and years in the number of papers that stated an identification method and provided vouchered specimen and collection locality metadata, a chi-squared test of independence was performed in R v.3.0.2 (R Core Team 2013) using the ‘vcd’ package (Meyer et al. 2014). Mosaic plots generated by the 'vcd' package were used to visualize the data.

6.2.2 Literature Review Taxa List Evaluation

All taxon names from the literature review taxa list were validated using a two-step process. First, taxon names were queried against Catalogue of Life (COL) (Bisby et al. 2012). Names not present in COL were queried against the taxonomy backbone of the Global Biodiversity Information Facility (www.gbif.org). If a name was not present in either database, no further effort was made to validate it. Classification for all taxa was retrieved from these

databases. Both name validation and classification queries were conducted using the 'Taxize' package (Chamberlain and Szocs 2013) in R v3.0.2 (R Core Team 2013). If a taxon was not present in either of these databases, classification was retrieved from the source article.

In order to generate summaries of available DNA barcode data for these species, all public specimen records on BOLD species on the 'literature review species' list were imported into a BOLD dataset DS-BCAV, and a BINs discordance report was generated in October, 2014. The concordance of species names and BINs were all examined by placing each species in one of 4 categories (Match, Split, Merge, Mixture) (Ratnasingham and Hebert 2013). In brief, species for which all specimens fell into a single barcode cluster (BIN) with no members of another species were assigned to the 'Match' category. For species for with specimens that fell into multiple BINs private to that species, were assigned to the 'Split' category. Species which fell into a single BIN which also contained specimens of another species were assigned to the 'Merge' category. Finally, any species for which the specimens' concordance pattern included both Spilt and Merges were assigned to the 'Mixture' category.

6.2.3 DNA Barcoding

Biological control agents were purchased from commercial suppliers. Total DNA was extracted from either whole specimens or leg tissue using the DNeasy Tissue Kit (Qiagen Corp.) following the manufacturer's protocol with one modification: the volume of AE buffer used in the final column elution was reduced from 200 µl to 50 µl to account for the small tissue volume of biological control agents samples. DNA extracts from all specimens were subjected to PCR with standard barcoding primers (refer to individual specimen pages on BOLD for details) with recommended PCR cycling conditions (Ivanova et al. 2009). PCR products were sequenced at

the Genomics Facility (Advanced Analysis Centre, University of Guelph) or the Canadian Center for DNA Barcoding (University of Guelph). DNA sequences were assembled in Sequencher 4.9 (Gene Codes Corporation, Michigan) and manually aligned in MEGA6 (Tamura et al. 2013). After observing an unusually high intra-specific divergence in barcode sequences (see Results and Discussion), ITS1 sequences were generated for specimens of *Diglyphus isaea* following the methods of Sha *et al*. (2006). These individuals were also screened for *Wolbachia* infection using a PCR-based assay described by (Braig et al. 1998). Attempts were made to sequence ITS1 from *Chysoperla carnea* but failed (data not shown). All specimen meta- and sequence-data were uploaded to BOLD under the publicly accessible project CBCAS. Neighbor-joining trees were generated in MEGA6 (Tamura et al. 2013), and evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980).

6.3 Results

6.3.1 Literature Review

A total of 878 articles were reviewed for inclusion in this review, and of these, 511 met all criteria for inclusion. No consistent differences were found within or between journals within or between years, in terms of the number of papers that vouchered research organisms or provided specimen identification and locality metadata (Figure 6.1, Figure 6.2). Therefore data presented is summarized across journals and years. Of these research papers, 345 used arthropods exclusively collected from the environment, 76 used arthropods obtained exclusively from commercial or non-commercial insectaries, and 90 used arthropods from both. Regardless of the source , the vast majority of research papers did not explain how they identified their

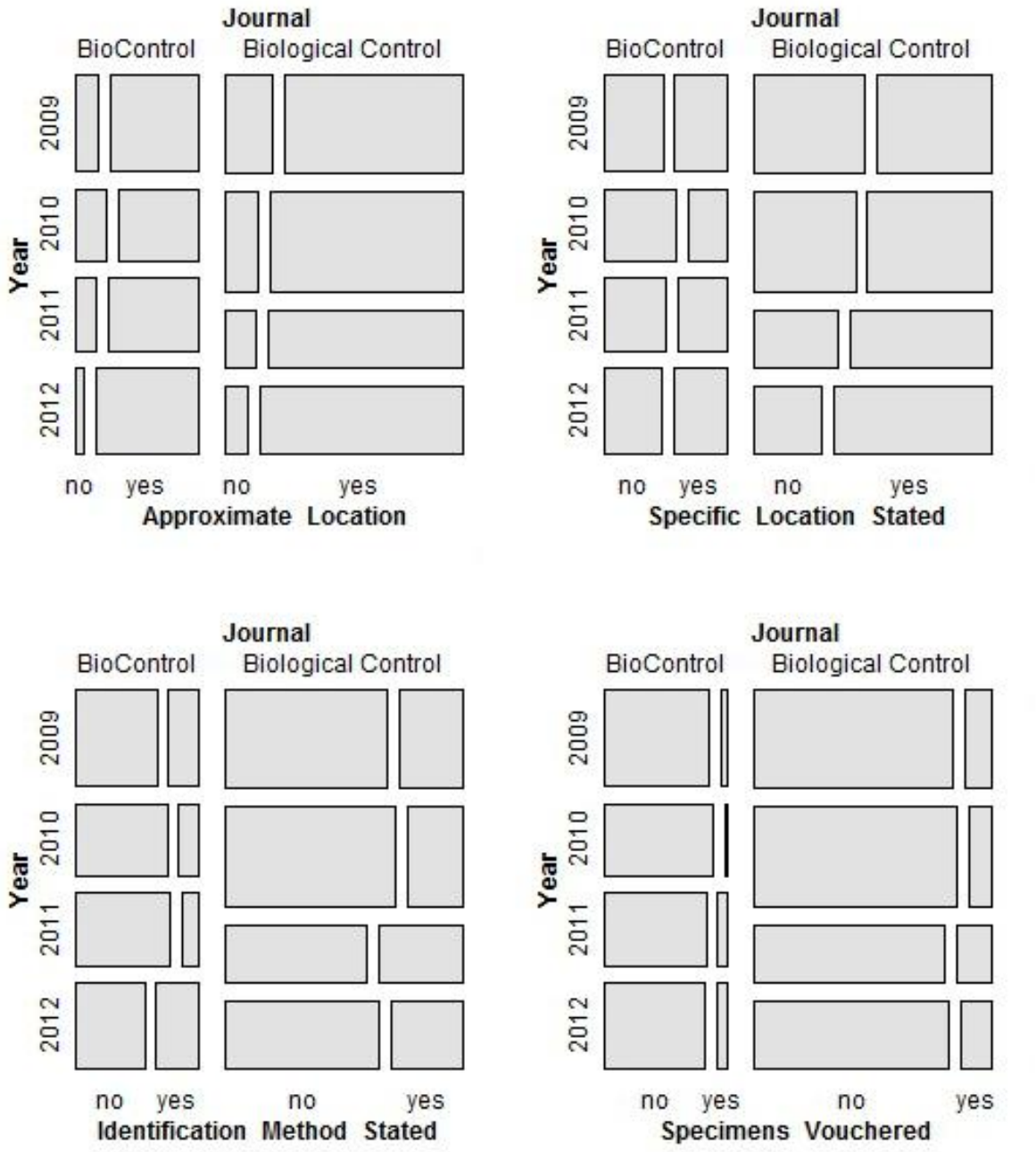

Figure 6.1: Mosaic plots depicting chi-squared test for independence of journal articles that provided approximate collection locations (A: χ^2= 13.71, df = 10, p= 0.1866), specific collection locations (B: χ^2= 17.947, df = 10, p= 0.05587), identification method stated (C: χ^2= 14.607, df= 10, p= 0.147), and voucher status (D: χ^2= 9.108, df= 10, p= 0.5219) of research arthropods that were collected from the environment. Data were collected from the journals BioControl and Biological Control between 2009 and 2012.

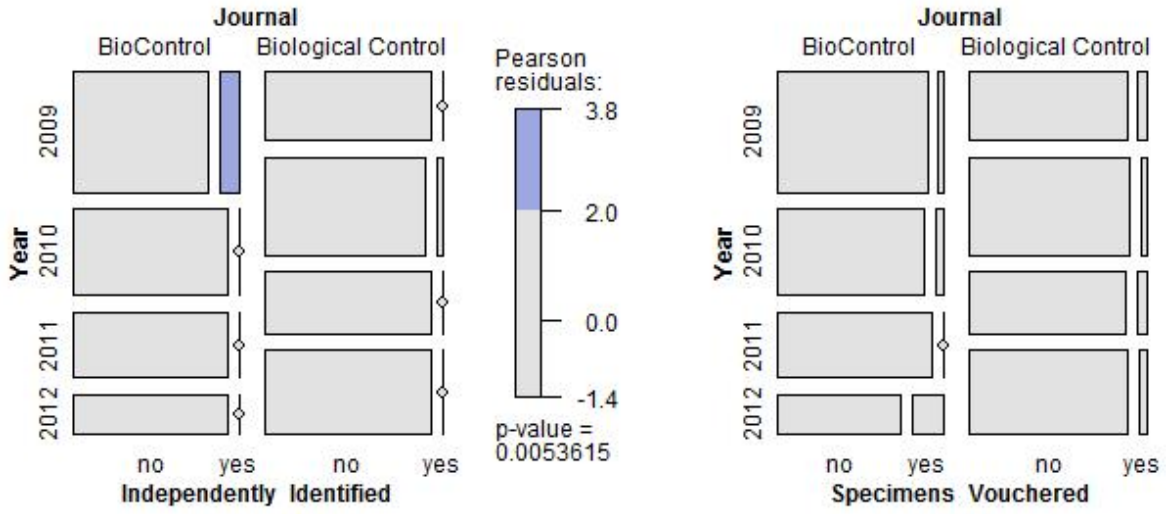

Figure 6.2: Mosaic plots depicting chi-squared test for independence of journal articles that provided metadata related to independent identification method stated (A: χ^2= 24.992, df= 10, p= 0.005361), and voucher status (B: χ^2= 12.721, df= 10, p= 0.2397) of research arthropods which were obtained from insectaries. Data were collected from the journals BioControl and Biological Control between 2009 and 2012.

research organisms (Table 6.1). Similarly the majority of researchers did not document the deposition of voucher specimens of their research organisms (Table 6.1). Furthermore, for 435 instances where experimental arthropods were collected from the environment, only 46.2% (n=234) of research papers provided the specific collection location, while 29.9% (n=130) papers provide an approximate collection location and 16.3% (n=71) papers did not specify a collection location.

Table 6.1: Identification methods employed and voucher status of arthropods used or observed as part of biological control research. Arthropods were obtained from the environment or a commercial insectary

	Arthropods collected from or observed in the environment		Arthropods obtained from a insectary	
n	435		166	
Identification method stated	122 (17)*	28.05%	5	3.01%
Taxonomic Key or Guide	47	10.80%	1	0.60%
Molecular Diagnostic	33	7.59%	2	1.20%
Taxonomist Consultation	59	13.56%	2	1.20%
Where specimens vouchered	46	10.57%	8	4.82%

*Parenthesis indicates the number of research papers in which 2 identification methods were employed. No paper employed all three identification types.

6.3.2 Literature Review Taxa Evaluations

The literature review taxon list contained a total of 1188 unique taxa prior to validation. A complete list of all taxa found in the literature review can be found in Appendix 2. Arthropods were identified to various ranks in different manuscripts, but the most common rank was species (n=999), followed by genus (n=109), family (n=65), order (n=9), subfamily (n=3), and tribe (n=3). The majority (n=1085) of these taxa could be validated using either the COL or GBIF database (Appendix 2). Of the 999 species level taxa, 24 were found to be synonyms according to either the COL or GBIF database. The accepted name of 4 of these species was already on the species list, bringing the total of unique species to 995. The majority of these species belonged to the orders Coleoptera (29%, n=288), Hymenoptera (28%, n=278), and Hemiptera (15%, n=147). All other orders individually contained <10% of the total number of species (Figure 6.3, Appendix 2). The vast majority (75%) of unique species names occurred only once in the dataset (Figure 6.4, Appendix 2).

Of the 995 species, 474 species had public specimen records with sequence data on BOLD, representing a total of 16,717 specimen records. The majority of these species (54%, n=257) were represented by between 2 and 20 specimen records, while 14% (n=66) were represented by a single specimen record (Figure 6.5, Appendix 2). The remaining 32% (n=151) species were all represented by more than 20 specimen records. The majority of specimen records (64%, 10,639) for these species present in BOLD originated from NCBI-Genbank (Figure 6.5, Appendix 2).

In total, 429 species, which included the majority of specimen records (n=14045) in this dataset, were assigned a BIN number. The remaining specimens were not assigned a BIN number, because the associated sequence data was not of sufficient length (i.e., <500bp in length, Ratnasingham and Hebert 2013) to be included by the BIN algorithm. Specimens in this dataset were assigned to 661 unique BINs, which represents 232 more BINS than named species. Of these BINs, 66 were singletons, 349 were concordant, and 254 were discordant. Most discordant BINs were discordant at the level of species (n= 159), followed by genus (n= 50), family (n= 24), order (n= 16), class (n= 3), and phylum (n= 2).

When including singletons all individuals of most species (n=305) were assigned to a single BIN (e.g., Match or Merge), individuals from 124 species were assigned to multiple BINs (Table 6.2). When singletons were removed from the analysis, the number of species that were assigned to a BIN was 414, and the number of species for which all individuals were assigned to a single BIN (e.g., Match or Merge) was 309. Once singletons were removed, the number species assigned to multiple BINS (e.g., Split or Mix) was 105.

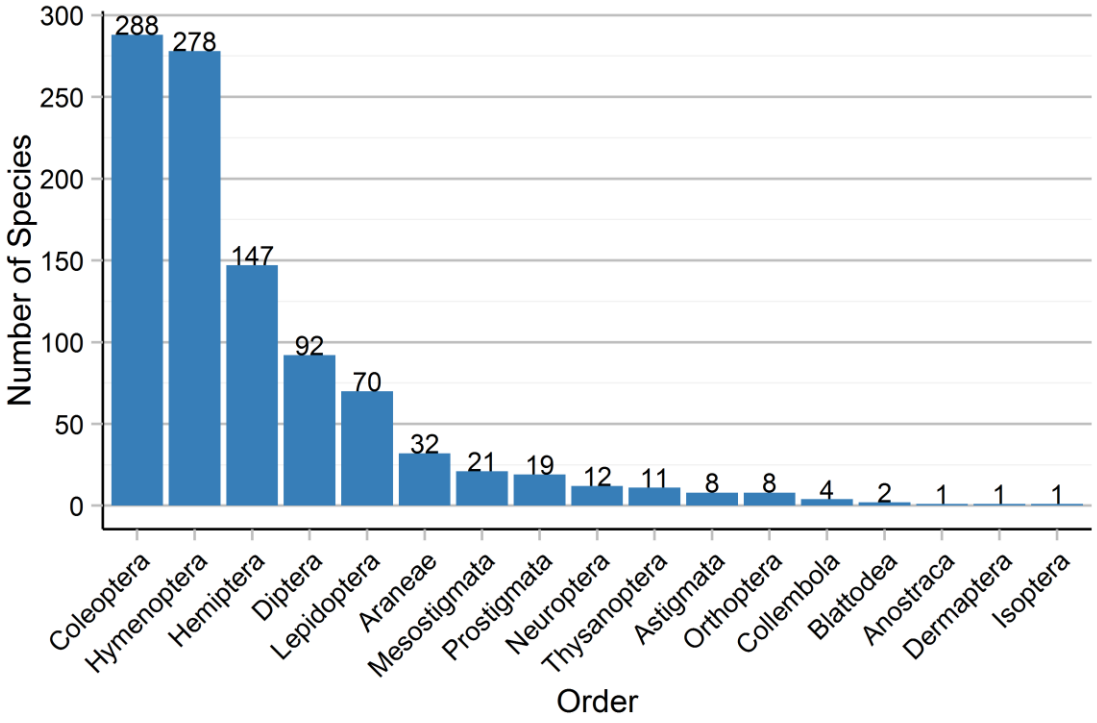

Figure 6.3: The order-level classification of the 995 unique species level arthropod taxa found in the survey of biological control journals.

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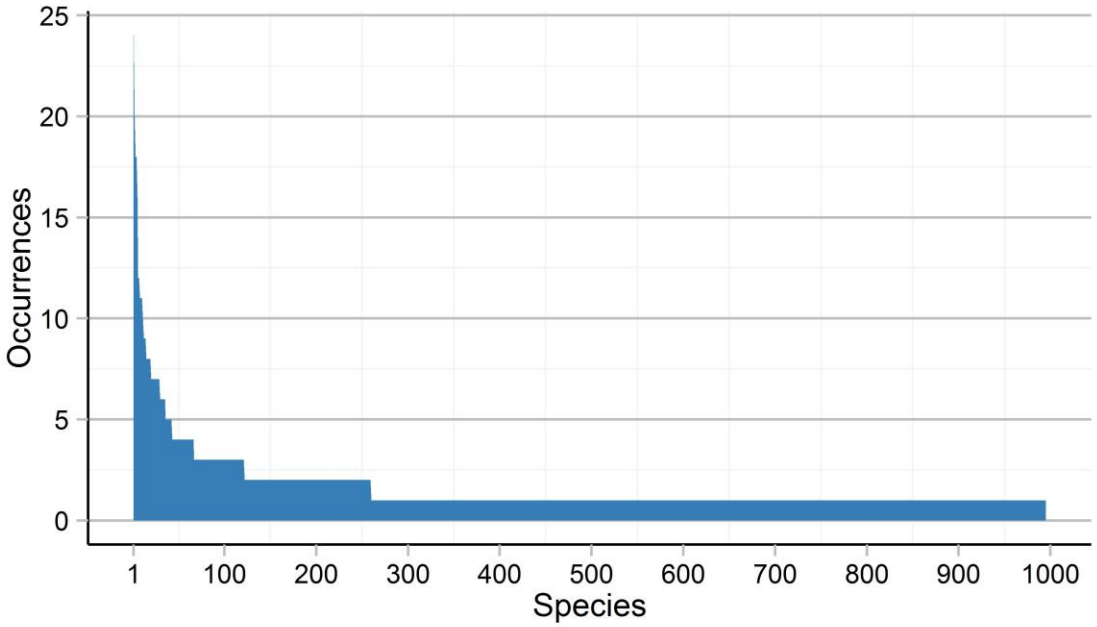

Figure 6.4: A frequency distribution of the number of occurrences by manuscript of each species level taxa in the review of biological control literature. This includes the 1001 unique species level taxa.

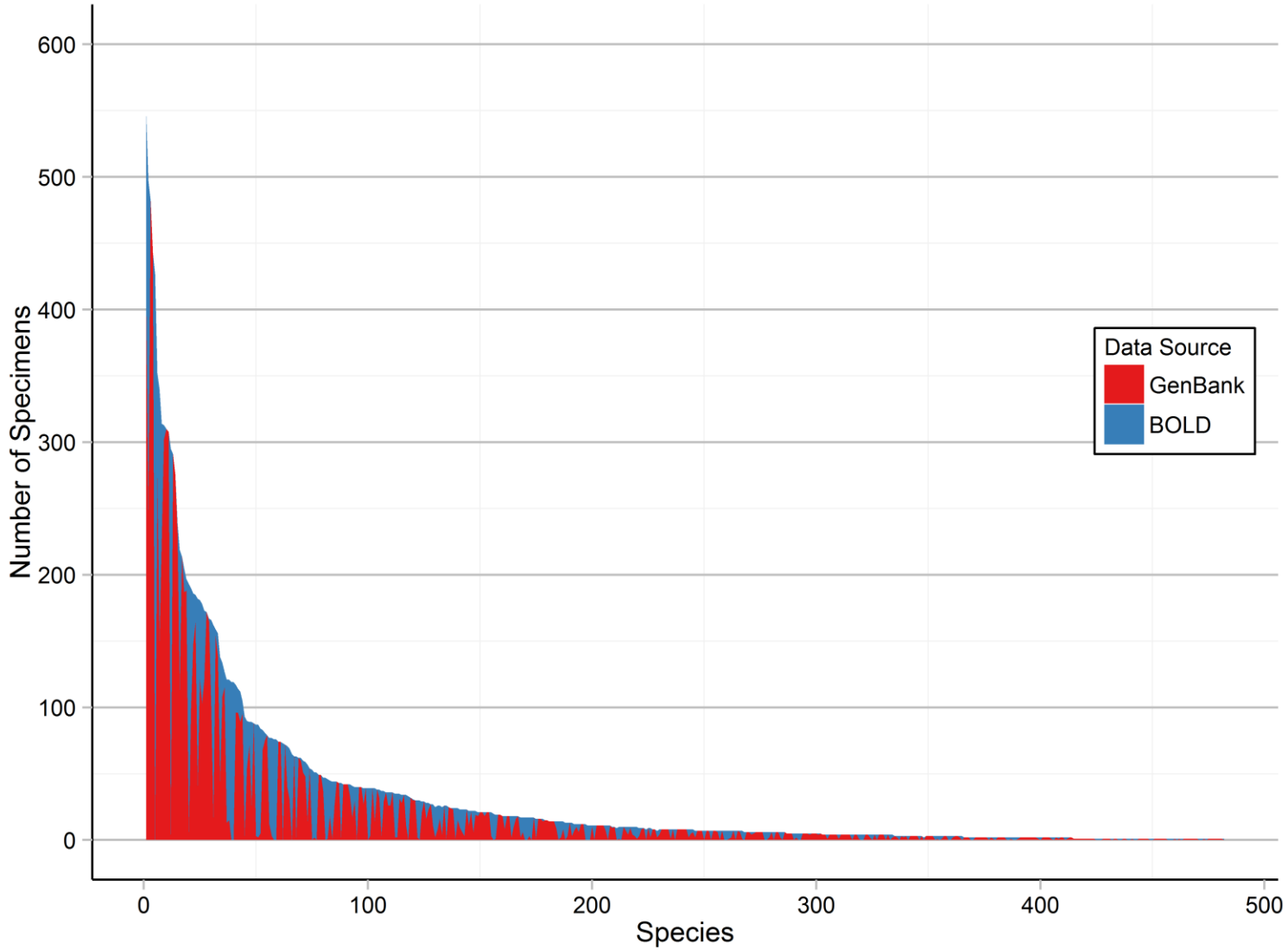

Figure 6.5: Number of specimen records in BOLD per species colour coded by data source. Data was obtained from BOLD (blue) or NCBI GenBank (red).

Table 6.2: Summary of Barcode Index Number (BIN) assignments for species from the literature review species list that had publically available DNA barcode data on the BOLD, including and excluding singleton.

With Singletons

		BIN Category for each taxa			
Number of Species	Number of BINS	Match	Spilt	Merge	Mix
305	1	154	-	151	-
72	2	-	30	-	42
22	3	-	6	-	16
11	4	-	5	-	6
9	5	-	2	-	7
5	6	-	2	-	3
-	7	-	-	-	-
2	8	-	0	-	2
-	9	-	-	-	-
-	10	-	-	-	-
-	11	-	-	-	-
1	12	-	0	-	1
1	13	-	1	-	0
1	14	-	1	-	0
429		154	47	151	77

Without Singletons

		BIN Category for each taxa			
Number of Species	Number of BINS	Match	Spilt	Merge	Mix
309	1	149	-	160	-
64	2	-	26	-	38
19	3	-	5	-	14
9	4	-	2	-	7
6	5	-	2	-	4
2	6	-	0	-	2
2	7	-	0	-	2
-	8	-	-	-	-
-	9	-	-	-	-
-	10	-	-	-	-
1	11	-	0	-	1
1	12	-	1	-	0
-	13	-	-	-	-
1	14	-	1	-	0
414		149	37	160	68

6.3.3 Barcoding and Molecular Analysis

In total, barcode sequences were generated for 213 individuals of 29 species from 4 suppliers in Canada. Five species were represented by a single specimen, while the remainder had between 2 and 24 specimens. A neighbour-joining phenogram of pairwise sequence divergence data (Figure 6.6) provides a visual depiction of the intra- and interspecific variation in barcode sequences. The mean sequence divergence within species was low on average (mean = 0.5%, range: 0 - 4.5%). However, mean sequence divergence within specimens labelled as *D. isaea* was greater than typically observed within insects, with a mean of 2.2% (range: 0 - 4.5%). Additionally, the distribution of COI haplotypes was not concordant with the organisms' supplier (Figure 6.6.B). All ITS1 sequences for *D. isaea* were identical, so no further analysis of ITS1 was conducted. Finally the *Wolbachia* PCR-assay was negative for all *D. isaea* individuals tested. The sequence divergence within labelled *C. carnea* also was greater than expected (mean: 1.4%, range: 0 – 3.2%) Interestingly, COI sequences generated for *C. carnea* fell into two clusters concordant with their supplier (Figure 6.6.C). Finally, sequence divergence within labelled *Neoseiulus* (=*Amblyseius) cucumeris* was greater than expected (mean: 1.1%, range: 0 – 2.9%), and, as with *D. isaea*, clusters were not concordant with supplier (Figure 6.6.D).

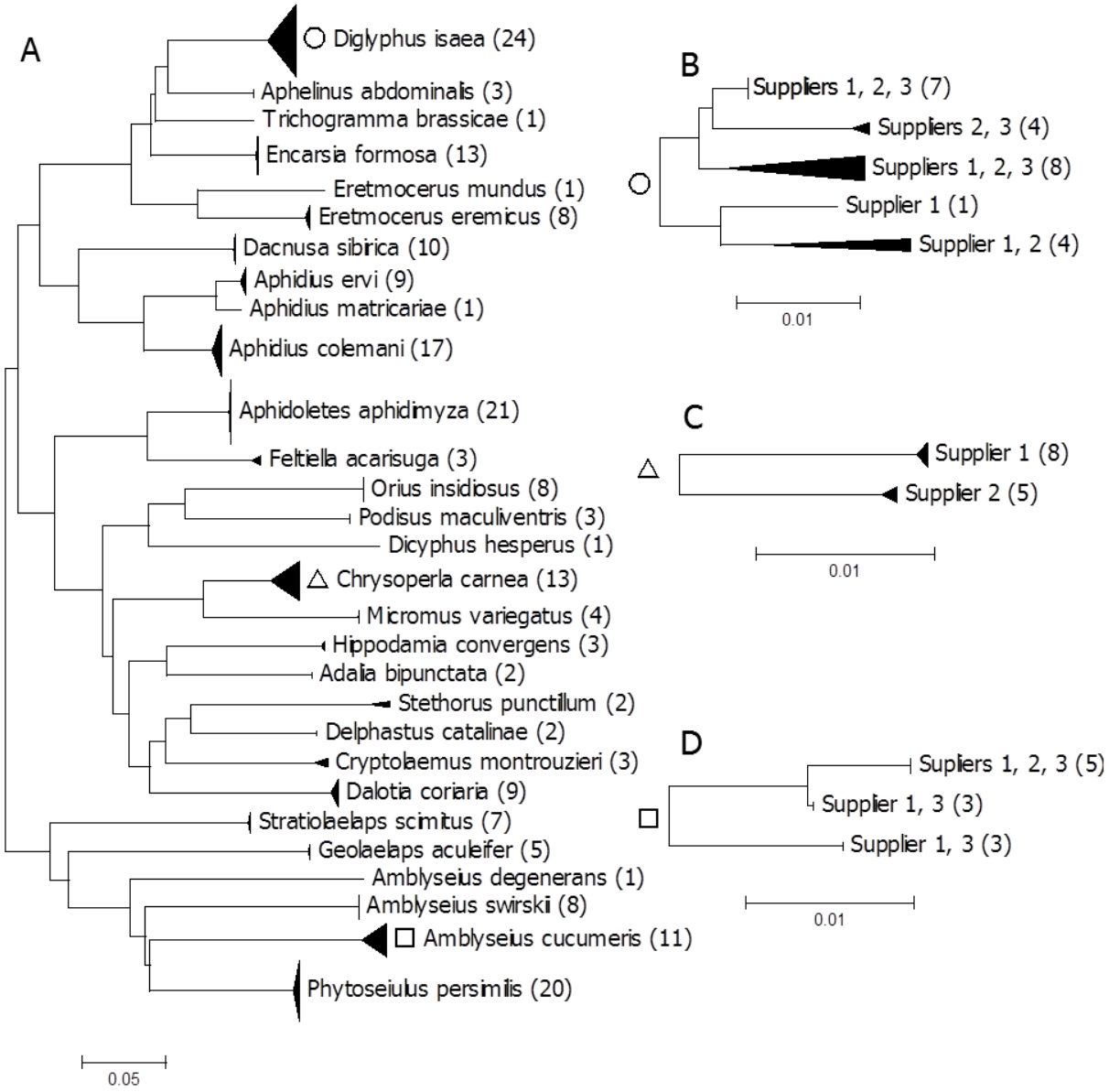

Figure 6.6: A neighbor-joining phenogram for commercially available biological control agents in Canada: entire data set (A), sub-tree for labelled, *Diglyphus isaea* (B), *Chrysoperla carnea* (C), and *Amblyseius cucumeris* (D). The number in parenthesis is the number of specimens sequenced for each taxa. Supplier number was randomized between figures C-D.

6.4 Discussion

6.4.1 Literature Review

This review revealed that the vast majority of biological control research papers failed to provide important metadata related to their research organisms, including how they were identified, where they were collected, and if voucher specimens were retained. This lack of documentation concerning experimental organisms is certainly not unique to the biological control literature, as similar oversights have been documented in other areas of experimental biology (for example see Ruedas et al. 2000, Pleijel et al. 2008). Of particular importance is that the majority of research papers failed to describe how they identified their research organisms. This lack of transparency is particularly troubling as the identification of arthropods is not a trivial process due to their structural and species-level diversity. Therefore an explanation on how the identity of a research organism was determined seems fully appropriate and should be a necessary component all manuscripts. While stating how research arthropods were identified by no means ensures that they were identified accurately, it does provide proof of due-diligence and affords the opportunity for the methodology to be scrutinized. For example, taxonomic keys and guides may be updated or revised, and as a result, researchers may be consulting out of date material (Godfrey and Stehr 1985, Schiff et al. 2012). Furthermore, molecular diagnostics may generate false positives and/or false negatives when protocols are not followed appropriately (e.g., inappropriate controls, incorrect *a priori* taxonomic assignment) (Armstrong and Ball 2005, Ball and Armstrong 2006, deWaard et al. 2010b). Therefore, it is clear that without reference to the identification methods employed by researchers, it is impossible to determine if appropriate identification resources were consulted or diagnostic procedures followed.

The lack of documentation regarding identification methodologies suggests that many biological control research organisms were not robustly identified. This assertion may be supported by the fact that research organisms obtained from insectaries were independently identified at a rate less than those obtained from the field, perhaps because the colonies from which these individuals originated had already been "identified" by the personnel who maintained them. However, congeneric and/or cryptic-species can contaminate laboratory colonies (Desneux et al. 2009), and the misidentification and contamination of commercially available biological material in other branches of science is common (Bely and Weisblat 2006, Hughes et al. 2007, Du Preez et al. 2009, Korch et al. 2012). As a result, taking independent measures to verify the identity of commercially-reared arthropods used for research seems thoroughly justifiable, especially in light of our results concerning commercial biological control agents (see below).

Alternatively, it is possible that appropriate steps are being taken to identify research organisms but these steps are not documented in the associated manuscript. In some cases, this information may exist in a previously published work by individual authors but reference to the information is omitted, perhaps in order to minimize self-citation. Although this possibility was not investigated further, I contend that individual manuscripts should stand alone. Therefore if the identification methodology for a particular cohort of research organisms has been published previously then it should be explicitly stated and referenced. To this end it was frequently observed that manuscripts thanked a taxonomist (or technician) for their identification services in the acknowledgements section rather than the methods (data not shown). While the acknowledgments section of a manuscript is an entirely appropriate place to thank non-authors for their contributions to the published work, given the importance of specimen identification, this information also should be clearly indicated in the methods.

Another important issue related to the identification of research organisms is collection metadata, in particular locality data. Regional differences in insect biology are well documented, particularly for traits such as insecticide resistance (Gbaye et al. 2012), phenology (Dunbar and Gassmann 2013), morphology and melanisation (Brakefield and de Jong 2011, Perrard et al. 2014), host and climate adaptation (Arakaki et al. 1997, Henry et al. 2010), and other life-history traits (Vuarin et al. 2012). Furthermore, insect population genetics studies clearly demonstrate the potential for fine scale genetic structure in relation to landscape features (Cao et al. 2012, Diome et al. 2013, Foley et al. 2013). While the broader implications of genetic structure within and between insect populations may not be as apparent as those of populations with varying levels of insecticide tolerance, any population level genetic trait certainly has the potential to influence experimental outcomes. Despite these well-known phenomena, a surprisingly large proportion of papers failed to provide collection locality data (18%) or provided only approximate locality data (37%) for their experimental organisms. This is particularly puzzling considering the ease with which this information can be collected and conveyed. For example, stating the municipalities or towns or cities where research organisms were collected requires a single brief sentence. Alternatively, for more complicated collecting schemes, locality data could be provided as online supplementary data files, or catalogued in web platforms such as the Global Biodiversity Information Facility (www.gbif.org). Furthermore, with the advent of free web-based geospatial software such as Google Earth (http://www.google.com/earth/), obtaining geospatial co-ordinates for collection sites is simplified and no longer requires a personal satellite navigation system. While it is understandable that some authors may be hesitant to offend collaborators and/or land-owners when reporting the presence of pest species, occurrence data for common and non-regulated pests species is often documented in extension literature which should make the disclosure of locality data a non-issue. Although, given the potential sensitivities regarding pests, special consideration at the authors' discretion should be given to the publication of locality data for pest organisms. However, similar arguments cannot be made

for beneficial species for which locality data should always be provided. Finally, while recognizing that interpreting the results of any individual experiment in the context of population genetics may be impossible due to the paucity of comparable studies, the small effort required to provide this data in the first place can be justified by the potential benefits. Moreover, routinely providing digitally accessible (i.e., GPS) geo-spatial collection and occurrence data as a component of all manuscripts working with biological material has other benefits. A significant challenge facing biodiversity sciences is the lack of distribution and occurrence data for many species, termed the 'Wallacean shortfall' (Lomolino and Heaney 2004). In the last decade significant resources have been mobilized to address this issue (Nelson et al. 2012). Considering the breadth of biodiversity encountered in this biological control literature review (995 species over 4 years), it would seem that publishing occurrence data for pest and beneficial species has the potential to significantly contribute to these and other biodiversity mapping projects (Robertson et al. 2014), which have tremendous potential to improve the management of natural and agro-ecosystems (Worner and Gevrey 2006, Sutherst 2014). Therefore, providing collection data for research organisms in a digitally accessible format will, when aggregated, have significant value to the scientific community as a whole, while also supporting reproducible research within the biological control research community.

Another observation from the literature is that relatively few papers explicitly stated whether they retained voucher specimens of their research organisms. The importance of voucher specimens is obvious for works of descriptive or revisionary taxonomy for which the individual specimens form the evidentiary foundation of the work and are likely to require re-examination at a later date to facilitate subsequent taxonomic research. However, the importance of retaining voucher specimens used for research unrelated to taxonomy is less obvious. While the voucher does provide the evidential basis for that particular research project, how likely is it that these specimens associated with applied biology will be re-examined by a

taxonomist in this context? And does the likelihood of this justify the cost associated with the storage and curation of these specimens in a permanent collection? And are permanent collections willing to accept material associated with routine research?

An alternative approach would be to provide digital vouchers of the specimens in the form of digital macro-photographs and/or DNA sequence data. Digital vouchers (or e-Vouchers, Monk and Baker 2001) would simplify their re-examination, although in a limited capacity compared to a physical voucher. Digital vouchers can also be supplemented with molecular or tissue vouchers of specimens in lieu of traditional physical voucher specimens. This would be particularly advantageous as it would facilitate growing interest in studying the genetics of pest and beneficial organisms (Evans et al. 2013, i5K Consortium 2013). Furthermore, a growing interest and implementation of genetic data in natural science research means that storage facilities for molecular and tissue vouchers are likely to be more numerous and potentially more accessible in coming years. Regardless, there are clear advantages in terms of data accessibility, usability, and research transparency to using molecular vouchers in tandem with physical vouchers for routine applied research.

6.4.2. Taxa Evaluations

A large number of unique taxa were investigated by manuscripts surveyed for the literature review, which included 995 unique species. Although I am not aware of any other survey of the taxonomic breadth of a scientific discipline to compare our numbers with, the numbers gathered here appear high for a discipline which in most cases does not seem to regard itself as a biodiversity science in the traditional sense (compared to ecology or systematics). Regardless, it is important to note that the majority of species encountered in this

literature review appeared only once (Figure 6.2). Therefore, developing identification resources (i.e., species-specific molecular diagnostics or morphological identification resources) to aid in the identification of these taxa is likely cost prohibitive given the large number of species and the potential limited usage of the resource by this research community. Alternatively, this supports adopting DNA barcoding as a standardized molecular diagnostic for identification of these organisms. Developing DNA barcode reference libraries for these species is likely less expensive and will have more end-use applications.

Currently, less than half of the species level taxa on this list have representative DNA barcode data in BOLD. While it is encouraging that a large proportion of these species have representative reference sequences, it is important to recognize the need for continued development of barcode reference libraries. As such the summary of species occurring in the biological control literature presented here can serve as a reference for continued development of DNA reference libraries which target species of interest to a particular end-user group, in this case the biological control research community. Although most DNA barcode reference library construction projects are focused on capturing the diversity of particular taxa and/or geographic regions, similar end-user focused library building projects have been adopted for arthropods (Bonants et al. 2010) and other taxa (Deeds et al. 2014) of regulatory significance.

Individual specimen records from 124 species in the review were assigned to multiple BINs, and in total this accounted for 29% of the unique species. Unfortunately it is difficult to contextualize this finding due to the lack of similar analysis in the literature. Typically the BIN algorithm has been used to analyze geographically restricted and higher-order monophyletic lineages (Hausmann et al. 2013, Ratnasingham and Hebert 2013, Zahiri et al. 2014), whereas our dataset consists of organisms from 17 orders and individuals collected globally. In the examples the number of named species that are assigned to multiple BINS is typically lower than that observed here. However, the purpose of most of these studies was to evaluate taxon

concepts by using sequence data generated explicitly to meet that objective. Therefore, it would be expected that some proportion of BIN discordances were resolved prior to publication, facilitated by the fact that all sequence data for these projects was linked to a digital specimens record and a physical voucher specimen. Furthermore, in these cases sequences lacking provenance data are excluded. This is not the case with our dataset, which represents solely a summary of all data currently available on BOLD (many of which originated from NCBI Genbank) and has been generated by numerous researchers for various purposes. Hence, the difference in the proportion of named species assigned to multiple BIN numbers may in part reflect the level of data curation (i.e., errors in the dataset), rather than some biological phenomena, where the curated dataset would be expected to have fewer conflicts which arose through human error (e.g., misapplied names or sample mix ups) . For example, BINs that are discordant at the level of family or higher likely reflect curation oversights rather than biology (e.g., introgression or incomplete lineages sorting). In our case, this is supported by the fact that the vast majority of sequences herein originated from NCBI Genbank, an archival database that has historically not supported specimen-level metadata, which is necessary to effectively resolve taxonomic conflicts concerning individual data records.

Nonetheless, individuals from a large proportion of named species in this dataset were assigned to multiple BINs and a large proportion of individuals from this dataset were assigned to BINs that were discordant at the level of species or genus. There are three possible explanations for these observations. First it is possible that DNA barcoding and BINs are unable to recover the currently accepted taxonomy for these particular species/individuals. While most large scale DNA barcoding studies illustrate examples of evolutionarily young and/or sibling species that cannot be differentiated based on their DNA barcoding sequence, these cases typically constitute a small proportion of total species considered. A number of biological mechanisms may account for this observation and include incomplete lineage sorting (Raupach

et al. 2010, Astrin et al. 2012), or uni- or bi-directional mitochondrial introgression (Schmidt and Sperling 2008), heteroplasmy (Magnacca and Brown 2010), or simply a lack of DNA barcode variation (Boykin et al. 2014). More recently, it also has been shown that intra-specific genetic variation of the DNA barcode sometimes increases with geographic distance between specimens, which may influence BIN assignment and barcode based species diagnostics (Bergsten et al. 2012, Hausmann et al. 2013, Zahiri et al. 2014). While this phenomenon can likely be attributed to any one of the biological mechanisms previously mentioned, it emphasizes the importance of specimen metadata (i.e., locality/collection data), which can be used to contextualize named species-BIN associations on a specimen by specimen basis.

A second possible explanation for the discordant BINs and multi-BIN species in our dataset is that these particular taxa harbor a number of unrecognized cryptic species. DNA barcoding, like many molecular methods for species discrimination, is particularly well-suited to detecting cryptic species (Hebert et al. 2004, Rugman-Jones et al. 2010, Skoracka et al. 2013, Shashank et al. 2014). In fact many named-species in this dataset for which individuals were assigned to multiple BINs are known or suspected to represent cryptic species complexes, including *Bemisia tabaci* (Dinsdale et al. 2010, De Barro et al. 2011), *Scirtothrips dorsalis* (Hoddle et al. 2008), and for these examples the BIN assignments reflects current understanding of the cryptic-taxa (Ashfaq et al. 2014, Frewin et al. 2014). Finally, a third possibility for discordances between BINs and named taxa in our data set is simply that names for these species are being misapplied.

Overall it is likely that all of these mechanisms underpin various instances of discordance and multi-BIN species within our data set. Unfortunately, due to the lack of metadata associated with most of the specimen records (primarily those originating from NCBI-GenBank), it may be extremely challenging or impossible to investigate these cases further. However, elucidating these discrepancies in detail is not the objective of this work. Rather the

goal is to illustrate the complexity involved in identifying and classifying arthropods of agricultural significance, and, judging from this data, it is clear that there is taxonomic uncertainty and ambiguity surrounding a number of taxa investigated in the biological control literature. Some of these BIN-species conflicts (e.g., BIN merges and splits in Table 6.2) likely represent, cryptic species complexes and introgression with congeneric species, while others represent the misapplication of names. These findings suggest that efforts should be made to increase the taxonomic certainty around the applications of names in the biological control literature.

6.4.3 Biological Control Agent DNA Barcoding

In the biological control agent library, three cases of unusually high intraspecific divergence were uncovered in the taxa labelled as *D. isaea*, *C. carnea,* and *A. cucumeris*. High intraspecific divergence in labeled *D. isaea* was not entirely surprising given that this species is a suspected cryptic species complex (Sha et al. 2006, Sha et al. 2007). Oddly though COI divergence was not concordant with supplier and multiple haplotypes for this species were found among individuals from all suppliers. One plausible explanation for this pattern would be infection with *Wolbachia*. Cytoplasmic incompatibility induced by *Wolbachia* (or another facultative endosymbiont) can, in some cases increase diversity within a species, similar to the pattern seen here (Smith et al. 2012b). Despite the fact that *Wolbachia* infections have been reported in *D. isaea* (Zchori-Fein and Perlman 2004), all individuals tested were negative for *Wolbachia* infection. This would seem to suggest that high COI divergence is natural in this species or source populations of these colonies. However, it cannot be ruled out that individuals from divergent allopatric populations were used to establish these colonies, thereby increasing the observed diversity. A similar pattern was observed for *A. cucumeris,* with intraspecific

divergence higher than typically observed within a species and the divergence was not concordant with supplier. Again this divergence may reflect natural variation in the species or may be an artifact of mitochondrial introgression or outbreeding between lineages. Finally, the most interesting case is that of *C. carnea* for which divergence was entirely concordant with supplier. Unfortunately, the paucity of *Chysoperla* reference DNA barcode sequences restricts further inferences of these data. However, this level of sequence divergence may indicate these individuals are different species or divergent lineages of the same species. It is important to note that species boundaries in the *Chysoperla* are challenged by the lack of diagnostic characters on both the adult and immature forms, and in many cases behavioral characters are useful and/or essential for differentiating species (Henry et al. 1999, Haruyama et al. 2008). As such this makes this group particularly well suited to characterization with molecular markers, such as the DNA barcode.

These results indicate that there is high DNA barcode sequence variation within commercially available lines of some biological control agents in Canada. A numbers of factors may explain uncharacteristically high (>2-3%) intra-specific barcode divergence in natural-populations, including the presence of cryptic-species, mitochondrial introgression, or cytoplasmic incompatibility induced by endosymbionts. However, commercial colonies of biological control agents are not natural-populations, and the high divergence observed may be simply an artifact of rearing. For example, intra-specific divergences of ≥2-3% may be observed between allopatric populations of some species (Bergsten et al. 2012, Zahiri et al. 2014). It is possible that individuals from divergent allopatric populations were brought together in the lab and interbred, thereby producing the observed pattern. The sequence divergence observed here could be explained by any of these phenomena, or alternatively this may simply reflect natural variation within the source populations of these colonies. At this time the biological implications of these genetic patterns are unknown. However, their presence raises some

interesting questions, the most of practical of which is, are some biological control agent products (e.g., *C. carnea*) being mislabelled? But perhaps more interesting is the possibility that diversity represents outbreeding between lineages within a single species (e.g., *D. isaea*), and that desirable biological traits may be associated with particular lineages which can now be identified and used to improve the efficacy of biological control programs. To explore this possibility, biological data will need to be collected on these lineages, preferably from individuals recently collected from the wild, and compared with lab reared individuals. This also illustrates the difficulty in explaining patterns in genetic data generated from individuals of unknown provenance. This mirrors the situation commonly encountered in the phylogenetic community in which publically available genetic data is unaccompanied by specimen metadata, which has the result of confusing and obscuring data interpretation (Ruedas et al. 2000).

6.5 Conclusions

Our review of biological control literature suggests that publishing practices regarding identification methods of research organisms could be improved. Often little or no explanation was given as to how research organisms were identified, despite that: a) identifying specimens can be challenging for diverse groups such as the arthropods, and b) specimen identification is critically important for communicating and applying research in species-specific fields such as biological control. Unfortunately, data crucial for uncovering and resolving instances of misapplied names, including provenance data and the status (and/or existence) of specimen vouchers, are also often omitted from manuscripts. Furthermore, the analysis of publically available sequence data for species investigated in the biological control literature and a survey of commercially available biological control agents reveals a number of irregularities, consistent with either mis-applied names (i.e., mis-identifications), presence of cryptic diversity,

mitochondrial introgression, and/or hybridization. These results cast doubt on the repeatability of the science in the reviewed biological control-oriented manuscripts. Together these finding emphasizes the importance of employing repeatable and documentable methods for the identification of research organisms in the biological control literature.

A potential solution to these deficiencies is the adoption of DNA barcoding supported by the bioinformatics platform BOLD as a primary or secondary (i.e., confirmatory) identification methods for research organisms in the biological control literature. For example, by publishing sequence data for research organism and explicitly referencing the sequence data (i.e., the accession numbers for individual specimens or datasets) used to infer taxonomic assignments the process of identifying biological material becomes increasingly transparent and reproducible. Furthermore by associating research organism metadata such as locality, voucher status, specimen images, and analytical procedures on BOLD, this fully addresses the metadata concerns revealed in the literature review, and contributes to the reproducibility of the research.

Under this framework, generating and depositing DNA barcode sequence data along with associated specimen metadata for a select number of individuals from each cohort of research organisms, and then referring to these specimen records in all associated manuscripts is recommended. Using this approach, researchers should be able to detect potential mis-identifications and detect (using BINs) cryptic lineages within named species. Furthermore, linking research papers to digital specimen records may also enable biological data to be quickly contextualized under newly established taxon concepts in the event of taxonomic revisions and new descriptions.

The success of DNA barcoding as an identification technique is dependent on the taxonomic breadth and depth of the reference libraries used to infer identifications (Ekrem et al. 2007, Virgilio et al. 2012). The survey of species considered in the biological control literature

indicates that reference sequences for many species are missing, and furthermore much of the current data lacks provenance and comprehensive specimen metadata. While this may be discouraging, there are two reasons to believe these issues are currently and will continue to be addressed by the research community. First, in recent years there has been a growing recognition of the importance of DNA sequence – specimen metadata associations (Ruedas et al. 2000, Field et al. 2008, Yilmaz et al. 2011, Botero-Castro et al. 2014). As such it should be expected that as DNA sequence metadata standards become common place that most newly generated barcode data will be associated with appropriate metadata, and thus fit-for-use in diagnostic applications. Second, campaigns and projects to generate DNA barcode reference libraries for all life are ongoing thanks to the efforts of IBOL, but also in part to many smaller scale initiatives. DNA barcode library construction for particular taxa and geographic regions has been accelerated due to the research community's realization of its diagnostic and research applications. For example, extensive barcoding of fish was likely stimulated by the realization of its applications to food fraud and market substitution (Wong and Hanner 2008, Hanner et al. 2011, Deeds et al. 2014). Similarly DNA barcoding libraries of pest arthropods of regulatory significance has received much attention due to the need for rapid pests diagnostics (Foottit et al. 2008, Foottit et al. 2009b, Bonants et al. 2010, Lee et al. 2011b, Blacket et al. 2012, Lee et al. 2012, Smit et al. 2013, van de Vossenberg et al. 2013, Miller et al. 2014). As a result a database for this group of arthropods has grown and is being curated in the process. If the idea of using DNA barcoding as a standardized diagnostic method for biological control agents gains momentum, the research community will then have justification for the continued building and curating of barcode libraries with this goal in mind. To some extent this is already happening as examples of the application of DNA barcoding for biological control and related agricultural and environmental research are beginning to appear in the scientific literature (Forbes et al. 2012, Nzeduru et al. 2012, Pina et al. 2012, Hrcek et al. 2013, Ashfaq et al. 2014); however, increased involvement of the biological control research community will certainly expedite this process. In

the short term this may be as simple as donating reference specimens to research groups undertaking library building campaigns.

In summary, given the important contribution of biological control to the management of both natural and agro-ecosystems, it is recommended that that researchers in this field reflect upon how they determine the identity of their research organisms and how this information is communicated in publications. Ultimately there may be numerous ways to address this issue; however, due to its transparency, supported metadata associations, and digital-accessibility the use of DNA barcoding is recommended.

Chapter 7

GENERAL CONCLUSIONS

IPM will play an increasingly important role in agricultural production, due to ongoing and emerging threats posed by a variety of pest arthropods. Underpinning the successful implementation of IPM at all scales is the accurate identification of pest and beneficial arthropods. As such there is a need for increased diagnostic capacity at all levels of IPM, including regulatory (Chapter 3), researcher (Chapter 5) and on-farm pest management (Chapter 4). As my work demonstrates, DNA barcoding has great potential to enhance and improve IPM by improving the identification of both pest and beneficial arthropods. Until the advent of DNA barcoding, there was no single molecular diagnostic method capable of effectively addressing the magnitude of arthropod identification challenges across the field of IPM. However, the application of a 'universal' diagnostic tool such as DNA barcoding also presents its own series of challenges that must be overcome in order for it to become more broadly integrated into IPM. I suspect that the majority of these challenges will be of primary concern to the research and regulatory communities and therefore the majority of the following discussion will focus on those areas.

Ultimately, the utility of DNA barcoding as an identification tool hinges on the robustness of DNA barcode reference libraries (e.g., BOLD). In this sense the robustness of the libraries has two major components: completeness (i.e., the number of species, and number of specimens representing those species) and level of curation (i.e., how complete is the metadata for each specimen).

Unfortunately for arthropods, completing the reference library will be extremely difficult due to the magnitude of arthropod biodiversity. Conservative estimates place insect biodiversity

around 10 million species but other estimates exceed 100 million (Mora et al. 2011). Regardless of the estimated number of species, the number of described species barely exceeds 1 million (Zhang 2011, Zhang 2013). The large disparity between the number of named and estimated species presents a challenge for the application of DNA barcoding as currently practiced, in which DNA barcoding is primarily an extension of traditional taxonomy (e.g., species require descriptions supported by morphological taxonomy, specimens of these species can then be barcoded at which point barcoding can support the identification of these species). In the short term this problem can be addressed if BINS or a similar framework (Marakeby et al. 2014) gains acceptance as an interim taxonomic system. Nonetheless, the incompleteness of reference libraries will present a direct challenge when DNA barcoding is used for IPM activities, because specimens will continued to be encountered for which no corresponding reference sequence exists in reference library, as illustrated in the Chapter 3. As such, it will be important to develop tools and strategies for working with incomplete DNA barcode libraries to identify unknown specimens (Ekrem et al. 2007, Virgilio et al. 2012, Kvist 2013), and will also further developing methods to assigning specimens to higher taxonomic ranks (Wilson et al. 2011) when species level identifications are not possible.

Another important factor that contributes to the overall completeness of a barcode library is haplotype and geographic sampling within a species. DNA barcode haplotypes (e.g., unique sequences) vary within a species and this variation may exist both between individuals and between populations. Therefore it is important that species are adequately sampled across their ranges in order to encompass the full range of haplotypic variation. Better representation of the haplotype diversity within a species will likely increase the user's ability to identify specimens of those species, particularly in cases where within-species variation exceeds values typically encountered for related taxa (Bergsten et al. 2012, Zahiri et al. 2014), or share haplotypes with congenic species over parts of their range (Schmidt and Sperling 2008).

Although near complete haplotype sampling may be impractical for the majority of species, it may be justifiable for pests and beneficial species due to their economic importance. Near complete haplotype sampling may be difficult to conduct, however sampling efforts can be guided by rarefaction curves (Fischer et al. 2014), such that curves metrics could be used as a measure of DNA barcode library completeness. For example, rarefaction curves at or near their asymptote would imply confidence the library represents the true haplotype diversity of that species, where curves in their linear phase would imply that more sampling is needed.

Beyond species-level representation another important factor is the level of curation of DNA barcode libraries. As originally conceived DNA barcoding was developed as a tool to both extend taxonomic expertise and facilitate the description of biodiversity. To achieve this objective a metadata standard was implemented by the DNA barcode community (Hanner 2009) to ensure that DNA barcode data was fit-for-use in molecular diagnostics applications. Although the importance of sequence-specimen metadata is widely accepted across scientific communities (Yilmaz et al. 2011, Botero-Castro et al. 2014), and metadata standards for DNA barcode data do exist, some have suggested the metadata connected to many specimens on BOLD is lacking (Kwong et al. 2012). Recognizing that BOLD is a public database that contains specimen records of variable quality including data from Genbank, illustrates the need for an increased level of curation and annotation of specimen's records. For example, annotating specimen records and clearly indicating that they have or have not be examined by a qualified taxonomist. In the case of multi-species BINs, are these groupings novel or have they been suspected to be synonymies or hybrid complexes? Providing annotation that more clearly links specimen records, species and BIN pages with available taxonomic information will greatly facilitate the use of DNA barcoding via BOLD as a diagnostic tool.

Another pressing challenge to implementing DNA barcoding into IPM, particularly for regulatory scenarios, is establishing widely accepted criteria for a DNA barcode based

identification. A survey of the literature reveals a number of different criteria and metrics available (Zou et al. 2011, Brown et al. 2012, Collins et al. 2012a, Puillandre et al. 2012). Yet despite more than 10 years of DNA Barcode research, there is still no consensus on which methods or metrics should be used to infer identifications using DNA barcode data. Computationally-complex, phylogenetic based methods may be attractive due to their scientific underpinning or statistical support. However in some cases these advanced methods have been shown to perform equally or only marginally better than simple distance based methods (Virgilio et al. 2010, Virgilio et al. 2012, Ratnasingham and Hebert 2013), with the caveat that they are time consuming to conduct and may be inaccessible to non-specialist users. The majority of the barcoding community seems to have casually adopted the use of a 2-3% distance heuristic for specimen identification for research purposes. However, this approach is less suitable for regulatory applications, since there is no universal distance threshold that can be used for all species and calculating optimal distance thresholds (Brown et al. 2012, Collins et al. 2012a) for various taxa will only be possible once the barcode library is complete. Regardless, there are numerous methods that could be employed and little consensus on which methods should be used for various applications; this should be an area of future research.

Despite these challenges, DNA barcoding has a number of strengths which will benefit IPM. One of its greatest strengths is that it is universally applicable. Under current biosecurity regulatory protocols, an arthropod must be identified as a threat before diagnostic tools for its identification are developed or located. This is known as a “black-list” approach to regulation. However, this may not be practical as many pest arthropods only become pests in the absence of their natural enemies, which occurs during range expansions. Therefore, current regulatory approaches that focus on managing a black-list of established pest organisms will always be playing catch up to newly invading species and emerging pests. The black-list approach is predicated by the previous generation of diagnostics tools (e.g. morphological keys, individual

experts, and species-specific molecular diagnostics), which are not scalable with the magnitude of arthropod biodiversity. However, DNA barcoding is highly scalable, and if used in regulatory applications, it will proved an increased capacity to determine the actually identity of specimens, rather than its' membership to a particular species on a black-list. At the very least, DNA barcoding will provide more information. In turn, this information will provide regulators with a more complete picture of the influx of non-native species, which may be useful in setting policy directives and developing and refining pathway analysis for invasive species in general. However in specific cases it is also likely that by employing DNA barcoding, regulators will be able to identify non-black-listed species with potential pest traits (e.g. host plants), which may trigger biosecurity actions moving from a black-list approach towards a more predictive framework.

A significant strength of DNA barcoding in the regulatory sphere is the BINs framework. The BIN system functions like a species aggregator, and while not perfect, does provide a reasonable approximation of species level taxonomy in a number of groups (Ratnasingham and Hebert 2013, Ashfaq et al. 2014, Zahiri et al. 2014). Given the magnitude of global arthropod biodiversity, it is highly impractical to create exhaustive regional catalogues for all native and naturalized arthropod species, especially considering the estimated number of undescribed species. However, using species estimates from BINs, this task may be feasible. By creating BIN catalogued inventories of native species globally, regulatory managers will have a greater capacity to determine if a given specimen is "non-native", and therefore take appropriate management actions. Also, the digital nature of DNA barcode data would allow these data to be shared between nations and regions in near real-time. This would give regulators tools similar to the "Google Flu Trends Maps" (http://www.google.org/flutrends/), to identify the locations of detections of non-native species indexed with BIN numbers and GPS coordinates.

DNA barcoding is likely to have an import role to play in enhancing user ability to identify arthropods in a wide range of IPM activities. However, current reference libraries, as demonstrated in Chapters 2, 3, and 6, have large gaps for common pest and beneficial species. This will need to be addressed if DNA barcoding is to become more widely adopted as an identification methodology. As such my data can be used to help target DNA barcoding library construction for taxa of interest to the scientific and IPM communities. Furthermore, in a number of cases DNA barcode data and the BIN algorithm indicate the presence of cryptic taxa within a number of pest (Chapter 2, Appendix 1) and beneficial (Chapter 6, Appendix 2) species. If these named species harbour truly cryptic taxa, as is the case with *Bemisia tabaci*, then molecular diagnostics such as DNA barcoding will be essential for their management. This will be particularly important in cases where cryptic species that occur in close proximity or mixed infestations may have biological traits that influence their management (Chapter 4). At this point in time, developing additional use cases similar to those presented in Chapter 3, 5, and 6, will likely be required prior to the widespread adoption of DNA barcoding into IPM systems. However, my data strongly suggests that DNA barcoding as a diagnostic tool can contribute significantly to IPM research and application, and can be easily integrated within existing monitoring frameworks.

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Appendix 1

Global Pest list with number of records for each species present on BOLD. List compiled from the CABI, EPPO, APHIS, NAPIS and CFIA pest lists see Chapter 2 for details. Number in parentheses next to genera indicate the number of species in BOLD and Catalogue of Life (COL) from that particular genera. Species names marked with; (1) were validated using COL, (2) were validated with GBIF, * was not validated and use spelling and classification from source.

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Arthropoda: Arachnida										
Mesostigmata: Laelapidae										
Tropilaelaps (BOLD: 4 spp. / COL: 0 spp.)										
Tropilaelaps clareae Delfinato & Baker, 1961(2)	-	-	-	+	+	-	-	16	16	16
Mesostigmata: Varroidae										
Euvarroa (BOLD: 0 spp. / COL: 0 spp.)										
Euvarroa sinhai*	-	-	-	+	-	-	-	0	0	0
Varroa (BOLD: 2 spp. / COL: 1 spp.)										
Varroa destructor Anderson & Trueman, 2000(1)	-	-	-	-	+	-	-	35	35	3
Varroa jacobsoni Oudemans, 1904(2)	-	-	-	+	-	-	-	3	3	3
Trombidiformes: Eriophyidae										
Acalitus (BOLD: 0 spp. / COL: 15 spp.)										
Acalitus gossypii*	+	-	-	-	-	-	-	0	0	0
Acaphylla (BOLD: 0 spp. / COL: 0 spp.)										
Acaphylla theae (Watt, 1898)(2)	+	-	-	-	-	-	-	0	0	0
Aceria (BOLD: 2 spp. / COL: 33 spp.)										
Aceria guerreronis*	+	-	-	-	-	-	-	0	0	0
Aceria malherbae Nuzzaci, 1985(2)	-	-	-	-	+	-	-	0	0	0
Aceria sheldoni (Ewing, 1937)(1)	+	-	-	-	-	-	-	0	0	0
Aceria litchii (Keifer, 1943)(2)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Eriophyes litchii	-	-	-	+	-	-	-	0	0	0
Aculops (BOLD: 0 spp. / COL: 9 spp.)										
Aculops fuchsiae*	+	-	+	-	-	-	+	0	0	0
Aculops lycopersici (Massee, 1937)(1)	+	-	-	-	-	-	-	0	0	0
Aculus (BOLD: 1 spp. / COL: 4 spp.)										
Aculus schlechtendali (Nalepa, 1890)(1)	+	-	-	-	-	-	-	0	0	0
Calacarus (BOLD: 0 spp. / COL: 1 spp.)										
Calacarus carinatus (Green, 1890)(1)	+	-	-	-	-	-	-	0	0	0
Cecidophyopsis (BOLD: 0 spp. / COL: 1 spp.)										
Cecidophyopsis ribis (Westwood, 1869)(1)	+	-	-	-	-	-	-	0	0	0
Eriophyes (BOLD: 0 spp. / COL: 0 spp.)										
Eriophyes gossypii*	-	-	-	+	-	-	-	0	0	0
Eriophyes pyri (Pagenstecher, 1857)(2)	+	-	-	-	-	-	-	0	0	0
Phyllocoptruta (BOLD: 0 spp. / COL: 1 spp.)										
Phyllocoptruta oleivora (Ashmead, 1879)(1)	+	-	-	-	-	-	-	0	0	0

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Trombidiformes: Penthaleidae										
Halotydeus (BOLD: 0 spp. / COL: 1 spp.)										
Halotydeus destructor (Tucker, 1925)(1)	+	-	-	-	-	-	-	0	0	0
Trombidiformes: Tarsonemidae										
Acarapis (BOLD: 0 spp. / COL: 3 spp.)										
Acarapis woodi (Rennie, 1921)(2)	-	-	-	+	-	-	-	0	0	0
Steneotarsonemus (BOLD: 0 spp. / COL: 1 spp.)										
Steneotarsonemus spinki Smiley, 1967(2)	-	-	-	-	+	-	-	0	0	0
Polyphagotarsonemus (BOLD: 0 spp. / COL: 1 spp.)										
Polyphagotarsonemus latus (Banks, 1904)(1)	+	-	-	-	-	-	-	0	0	0
Trombidiformes: Tenuipalpidae										
Brevipalpus (BOLD: 0 spp. / COL: 282 spp.)										
Brevipalpus californicus (Banks, 1904)(1)	+	-	-	-	-	-	-	0	0	0
Brevipalpus chilensis Baker, 1949(1)	-	-	-	+	-	-	-	0	0	0
Brevipalpus obovatus Donnadieu, 1875(1)	+	-	-	-	-	-	-	0	0	0
Brevipalpus phoenicis (Geijskes, 1939)(1)	+	-	-	-	-	-	-	0	0	0
Raoiella (BOLD: 2 spp. / COL: 12 spp.)										
Raoiella indica Hirst, 1924(1)	+	-	-	-	+	-	+	17	20	20
Trombidiformes: Tetranychidae										
Amphitetranychus (BOLD: 1 spp. / COL: 3 spp.)										
Amphitetranychus viennensis (Zacher, 1920)(1)	-	-	-	+	-	+	-	0	1	1
Bryobia (BOLD: 0 spp. / COL: 130 spp.)										
Bryobia rubrioculus (Scheuten, 1857)(1)	+	-	-	-	-	-	-	0	0	0
Eotetranychus (BOLD: 0 spp. / COL: 186 spp.)										
Eotetranychus lewisi (McGregor, 1943)(1)	+	-	-	-	-	-	+	0	0	0
Eutetranychus (BOLD: 0 spp. / COL: 33 spp.)										
Eutetranychus orientalis (Klein, 1936)(1)	+	-	+	-	+	-	+	0	0	0
Mononychellus (BOLD: 0 spp. / COL: 30 spp.)										
Mononychellus tanajoa (Bondar, 1938)(1)	-	-	-	+	-	-	-	0	0	0
Oligonychus (BOLD: 1 spp. / COL: 204 spp.)										
Oligonychus calicicola Knihinicki & Flechtmann, 1999(1)	-	-	-	-	+	-	-	0	0	0
Oligonychus coffeae (Nietner, 1861)(1)	+	-	-	-	-	-	-	0	0	0
Oligonychus mangiferus (Rahman & Sapra, 1940)(1)	+	-	-	-	-	-	-	0	0	0
Oligonychus palus Beard, 2008(1)	-	-	-	-	+	-	-	0	0	0
Oligonychus perditus Pritchard & Baker, 1955(1)	+	+	-	-	-	-	+	0	0	0
Oligonychus perseae Tuttle, Baker & Abbatiello, 1976(1)	+	-	-	-	-	-	+	0	0	0
Oligonychus peruvianus (McGregor, 1917)(1)	+	-	-	-	-	-	-	0	0	0
Panonychus (BOLD: 4 spp. / COL: 16 spp.)										
Panonychus citri (McGregor, 1916)(1)	+	-	-	-	-	-	-	3	1	1
Panonychus ulmi (Koch, 1836)(1)	+	-	-	-	-	-	-	3	2	2

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Stigmaeopsis (BOLD: 7 spp. / COL: 7 spp.)										
Stigmaeopsis celarius Banks, 1917(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Schizotetranychus celarius (Banks, 1917)	-	-	-	-	+	-	-	0	0	0
Tetranychus (BOLD: 5 spp. / COL: 148 spp.)										
Tetranychus evansi Baker & Pritchard, 1960(1)	+	-	+	-	-	-	+	0	0	0
Tetranychus kanzawai Kishida, 1927(1)	+	-	-	-	+	-	-	29	30	30
Tetranychus marianae McGregor, 1950(1)	+	-	-	-	-	-	-	0	0	0
Tetranychus truncatus Ehara, 1956(1)	+	-	-	-	-	+	-	3	4	4
Tetranychus urticae Koch, 1836(1)	+	-	-	-	-	-	-	17	33	33
-also listed as Tetranychus cinnabarinus (Boisduval, 1867)	+	-	-	-	-	-	-	0	0	0
Arthropoda: Collembola										
Symphypleona: Sminthuridae										
Sminthurus (BOLD: 2 spp. / COL: 15 spp.)										
Sminthurus viridis (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	2	2	
Sminthurus viridus (Linnaeus)*	-	-	-	+	-	-	-	0	0	0
Arthropoda: Insecta										
Coleoptera: Attelabidae										
Deporaus (BOLD: 3 spp. / COL: 165 spp.)										
Deporaus marginellus (Faust)*	+	-	-	-	-	-	-	0	0	0
Coleoptera: Bostrichidae										
Heterobostrychus (BOLD: 2 spp. / COL: 6 spp.)										
Heterobostrychus aequalis (Waterhouse, 1884)(1)	+	-	-	-	-	-	-	1	0	0
Prostephanus (BOLD: 2 spp. / COL: 5 spp.)										
Prostephanus truncatus (Horn, 1878)(1)	+	-	-	-	-	-	-	0	0	0
Sinoxylon (BOLD: 2 spp. / COL: 52 spp.)										
Sinoxylon unidentatum (Fabricius, 1801)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Sinoxylon conigerum Gerstäcker, 1855	+	-	-	-	-	-	-	1	1	1
Coleoptera: Brachyceridae										
Brachycerus (BOLD: 0 spp. / COL: 601 spp.)	-	-	-	+	-	-	-			
Coleoptera: Brentidae										
Cylas (BOLD: 2 spp. / COL: 9 spp.)										
Cylas brunneus (Fabricius)*	+	-	-	-	-	-	-	0	0	0
Cylas formicarius Olivier(1)	+	-	-	-	-	-	-	13	10	10
Cylas puncticollis Boheman , 1833(1)	+	-	-	-	-	-	-	0	0	0
Ischnopterapion (BOLD: 3 spp. / COL: 0 spp.)										
Ischnopterapion virens (Hbst., 1797)(2)	-	-	-	-	+	-	-	0	0	0
Coleoptera: Buprestidae										
Agrilus (BOLD: 153 spp. / COL: 182 spp.)										
Agrilus anxius Gory, 1841(1)	-	-	-	-	+	-	-	10	0	0
Agrilus biguttatus (F., 1777)(2)	-	-	-	-	+	-	-	1	0	0
Agrilus coxalis auroguttatus Schaeffer,	-	-	-	-	-	-	-	0	0	0

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
1905(1)										
-listed under synonym Agrilus auroguttatus Schaeffer, 1905	-	-	-	-	+	-	-	0	0	0
Agrilus hyperici (Creutzer, 1799)(1)	-	-	-	-	+	-	-	5	0	0
Agrilus planipennis Fairmaire, 1888(1)	+	-	+	-	+	+	+	7	3	3
Agrilus sulcicollis Lacordaire, 1835(1)	-	-	-	-	+	-	-	14	0	0
Melanophila (BOLD: 5 spp. / COL: 5 spp.)										
Melanophila fulvoguttata*	-	-	-	-	+	-	-	12	0	0
Sphenoptera (BOLD: 2 spp. / COL: 1 spp.)										
Sphenoptera gossypii*	+	-	-	-	-	-	-	0	0	0
Coleoptera: Cerambycidae										
Aeolesthes (BOLD: 1 spp. / COL: 31 spp.)										
Aeolesthes sarta (Solsky, 1871)(1)	-	-	+	-	+	-	-	0	0	0
Anoplophora (BOLD: 9 spp. / COL: 42 spp.)	-	-	-	-	+	+	-			
Anoplophora chinensis (Forster, 1771)(1)	+	-	+	-	+	-	+	2	5	5
Anoplophora glabripennis (Motschulsky, 1854)(1)	+	+	-	+	+	+	+	428	430	430
Anoplophora nobilis (Ganglbauer, 1889)(2)	-	-	-	-	-	+	+	1	1	1
Anthores (BOLD: 0 spp. / COL: 1 spp.)										
Anthores leuconotus Pascoe, 1869(2)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Monochamus leuconotus Adlbauer, 1997	+	-	-	-	-	-	-	0	0	0
Apomecyna (BOLD: 3 spp. / COL: 79 spp.)										
Apomecyna binubila Pascoe, 1858(1)	+	-	-	-	-	-	-	0	0	0
Aromia (BOLD: 3 spp. / COL: 3 spp.)										
Aromia bungii (Faldermann, 1835)(1)	-	-	-	-	+	-	-	0	1	1
Batocera (BOLD: 7 spp. / COL: 57 spp.)										
Batocera rufomaculata (Degeer, 1775)(1)	+	-	-	-	-	-	-	0	0	0
Bixadus (BOLD: 0 spp. / COL: 1 spp.)										
Bixadus sierricola (White, 1858)(1)	+	-	-	-	-	-	-	0	0	0
Callidiellum (BOLD: 2 spp. / COL: 6 spp.)										
Callidiellum rufipenne (Motschulsky, 1860)(2)	+	-	-	-	+	-	-	1	0	0
Callidiellum villosulum (Fairmaire, 1899)(1)	-	-	-	-	+	-	-	5	0	0
Callipogon (BOLD: 1 spp. / COL: 8 spp.)										
Callipogon relictus Semenov, 1899(1)	-	-	-	-	-	+	-	0	0	0
Chlorophorus (BOLD: 8 spp. / COL: 234 spp.)										
Chlorophorus annularis (Fabricius, 1787)(1)	-	-	-	-	+	-	-	0	0	0
Chlorophorus strobilicola Champion, 1919(1)	-	-	-	-	+	-	-	0	0	0
Dectes (BOLD: 1 spp. / COL: 3 spp.)										
Dectes texanus LeConte, 1862(1)	-	-	-	-	+	-	-	0	0	0
Hesperophanes (BOLD: 2 spp. / COL: 7 spp.)										
Hesperophanes campestris Faldermann*	-	-	+	-	-	+	+	2	0	0
Hylotrupes (BOLD: 1 spp. / COL: 1 spp.)										
Hylotrupes bajulus (Linné, 1758)(1)	+	-	-	-	-	-	-	1	1	1

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Lagocheirus (BOLD: 11 spp. / COL: 22 spp.)										
Lagocheirus araneiformis (Linné, 1767)(1)	+	-	-	-	-	-	-	4	0	0
Monochamus (BOLD: 17 spp. / COL: 137 spp.)	-	-	-	-	+	+	-			
Monochamus alternatus Hope, 1842(1)	+	-	-	-	+	+	+	5	0	0
Monochamus carolinensis (Olivier, 1792)(1)	+	-	-	-	-	-	+	15	0	0
Monochamus galloprovincialis (Olivier, 1795)(1)	+	-	-	-	-	-	-	2	1	0
Monochamus saltuarius Gebler, 1830(1)	-	-	-	-	+	-	+	1	0	0
Monochamus scutellatus (Say, 1824)(1)	-	-	-	-	+	-	+	44	0	0
Monochamus sutor (Linné, 1758)(1)	+	-	-	-	+	-	-	6	1	1
Phoracantha (BOLD: 3 spp. / COL: 42 spp.)										
Phoracantha recurva Newman, 1840(1)	+	-	-	-	-	-	-	1	0	0
Phoracantha semipunctata (Fabricius, 1775)(1)	+	-	-	-	-	-	-	0	0	0
Prionus (BOLD: 6 spp. / COL: 51 spp.)										
Prionus laticollis (Drury, 1773)(1)	-	-	-	-	+	-	-	0	0	0
Saperda (BOLD: 26 spp. / COL: 45 spp.)										
Saperda calcarata Say, 1824(1)	-	-	-	-	+	-	-	17	0	0
Saperda candida Fabricius, 1787(1)	+	+	-	-	-	-	-	8	0	0
Steirastoma (BOLD: 8 spp. / COL: 19 spp.)										
Steirastoma breve (Sulzer, 1776)(1)	+	-	-	-	-	-	-	0	0	0
Stromatium (BOLD: 1 spp. / COL: 5 spp.)										
Stromatium barbatum (Fabricius, 1775)(1)	+	-	-	-	-	-	-	0	0	0
Tetropium (BOLD: 6 spp. / COL: 27 spp.)										
Tetropium castaneum (Linné, 1758)(1)	-	-	-	-	+	+	-	2	0	0
Tetropium fuscum (Fabricius, 1787)(1)	-	-	-	-	+	+	-	2	0	0
Tetropium gracilicorne Reitter, 1889(1)	-	-	+	-	-	-	+	0	0	0
Trichoferus (BOLD: 1 spp. / COL: 26 spp.)										
Trichoferus campestris (Faldermann, 1835)(1)	-	-	-	-	+	+	-	6	2	2
Xylotrechus (BOLD: 15 spp. / COL: 204 spp.)	-	-	-	-	+	-	-			
Xylotrechus altaicus (Gebler, 1835)(1)	+	-	+	-	-	-	+	0	0	0
Xylotrechus chinensis (Chevrolat, 1852)(1)	-	-	-	-	+	-	-	0	0	0
Xylotrechus colonus (Fabricius, 1775)(1)	-	-	-	-	+	-	-	11	0	0
Xylotrechus javanicus (Castelnau & Gory, 1841)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Xylotrechus quadripes Chevrolat, 1863	-	-	-	-	+	-	-	0	0	0
Xylotrechus namanganensis Heyden*	-	-	+	-	-	-	+	0	0	0
Xylotrechus pyrrhoderus Bates, 1873(1)	-	-	-	-	+	-	-	0	0	0
Xystrocera (BOLD: 1 spp. / COL: 62 spp.)										
Xystrocera globosa (Olivier, 1795)(1)	+	-	-	-	-	-	-	0	0	0
Coleoptera: Chrysomelidae										
Acanthoscelides (BOLD: 8 spp. / COL: 54 spp.)										
Acanthoscelides obtectus (Say, 1831)(1)	+	-	-	-	-	-	-	5	3	3
Alocypha (BOLD: 0 spp. / COL: 0 spp.)										

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Alocypha bimaculata*	+	-	-	-	-	-	-	0	0	0
Aulacophora (BOLD: 4 spp. / COL: 0 spp.)										
Aulacophora indica (Gmelin, 1790)(2)	+	-	-	-	-	-	-	0	2	2
Brontispa (BOLD: 0 spp. / COL: 23 spp.)										
Brontispa longissima (Gestro, 1885)(1)	+	-	-	-	-	-	-	0	0	0
Chaetocnema (BOLD: 30 spp. / COL: 59 spp.)										
Chaetocnema basalis (Baly)*	+	-	-	-	-	-	-	0	0	0
Chaetocnema confinis Crotch, 1873(1)	+	-	-	-	-	-	-	0	0	0
Coelaenomenodera (BOLD: 0 spp. / COL: 42 spp.)										
Coelaenomenodera elaeidis Maulik, 1920(1)	+	-	-	-	-	-	-	0	0	0
Diabrotica (BOLD: 45 spp. / COL: 8 spp.)										
Diabrotica balteata J. L. LeConte, 1865(1)	+	-	-	-	-	-	-	1	3	3
Diabrotica barberi R. Smith and Lawrence, 1967(1)	+	+	-	-	-	-	+	23	16	16
Diabrotica speciosa (Germar)*	+	+	-	-	+	-	+	4	5	5
Diabrotica undecimpunctata Mannerheim, 1843(1)	+	+	-	-	-	-	+	27	7	7
Diabrotica virgifera J. L. LeConte, 1868(1)	+	-	+	-	+	-	+	222	216	216
Dicladispa (BOLD: 2 spp. / COL: 132 spp.)										
Dicladispa armigera (Olivier, 1808)(1)	+	-	-	-	-	-	-	0	0	0
Dicladispa gestroi (Chapuis, 1877)(1)	+	-	-	-	-	-	-	0	0	0
Epitrix (BOLD: 27 spp. / COL: 12 spp.)										
Epitrix cucumeris (Harris, 1851)(1)	-	+	-	-	-	-	+	0	0	0
Epitrix fasciata Blatchley, 1918(1)	+	-	-	-	-	-	-	3	1	1
Epitrix similaris Gentner, 1944(1)	-	-	+	-	-	-	-	0	0	0
Epitrix subcrinita (J. L. LeConte, 1857)(1)	-	+	-	-	-	-	-	0	0	0
Epitrix tuberis Gentner, 1944(1)	+	+	-	-	-	-	+	0	0	0
Exosoma (BOLD: 2 spp. / COL: 0 spp.)										
Exosoma lusitanica Linnaeus*	-	-	-	+	-	-	-	0	0	0
Leptinotarsa (BOLD: 4 spp. / COL: 12 spp.)										
Leptinotarsa decemlineata (Say, 1824)(1)	+	-	+	-	+	+	+	17	14	14
Lilioceris (BOLD: 2 spp. / COL: 1 spp.)										
Lilioceris lilii (Scopoli, 1763)(1)	-	-	-	-	+	-	-	12	0	0
Madurasia (BOLD: 0 spp. / COL: 0 spp.)										
Madurasia obscurella Jacoby*	+	-	-	-	-	-	-	0	0	0
Medythia (BOLD: 0 spp. / COL: 0 spp.)										
Medythia quaterna (Fairmaire)*	+	-	-	-	-	-	-	0	0	0
Odontota (BOLD: 3 spp. / COL: 7 spp.)										
Odontota dorsalis (Thunberg, 1805)(1)	-	-	-	-	+	-	-	0	0	0
Ootheca (BOLD: 0 spp. / COL: 0 spp.)										
Ootheca bennigseni Weise*	+	-	-	-	-	-	-	0	0	0
Ootheca mutabilis Sahlberg*	+	-	-	-	-	-	-	0	0	0
Oulema (BOLD: 12 spp. / COL: 17 spp.)										
Oulema melanopus (Linnaeus, 1758)(1)	+	-	-	-	+	+	-	6	0	0

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Phaedon (BOLD: 8 spp. / COL: 8 spp.)										
Phaedon brassicae Baly, 1874(2)	+	-	-	-	-	-	+	0	0	0
Phyllotreta (BOLD: 19 spp. / COL: 47 spp.)										
Phyllotreta chotanica Duvivier*	+	-	-	-	-	-	-	0	0	0
Phyllotreta cruciferae (Goeze, 1777)(1)	+	-	-	-	-	-	-	3	0	0
Phyllotreta striolata (Fabricius, 1801)(1)	+	-	-	-	-	-	-	4	0	0
Promecotheca (BOLD: 0 spp. / COL: 33 spp.)										
Promecotheca cumingii Baly, 1858(1)	+	-	-	-	-	-	-	0	0	0
Pyrrhalta (BOLD: 4 spp. / COL: 1 spp.)										
Pyrrhalta viburni (Paykull, 1799)(1)	-	-	-	-	+	-	-	7	0	0
Trichispa (BOLD: 0 spp. / COL: 1 spp.)										
Trichispa sericea (Guérin-Méneville in Cuvier, 1844)(1)	+	-	-	-	-	-	-	0	0	0
Typophorus (BOLD: 1 spp. / COL: 1 spp.)										
Typophorus nigritus (Fabricius, 1801)(1)	+	-	-	-	-	-	-	0	0	0
Coleoptera: Coccinellidae										
Chilocorus (BOLD: 12 spp. / COL: 11 spp.)										
Chilocorus kuwanae Silvestri, 1909(1)	-	-	-	-	+	-	-	0	0	0
Chnootriba (BOLD: 1 spp. / COL: 0 spp.)										
Chnootriba similis (Thunberg)*	+	-	-	-	-	-	-	5	0	0
Coleomegilla (BOLD: 2 spp. / COL: 2 spp.)										
Coleomegilla maculata (De Geer, 1775)(1)	-	-	-	-	+	-	-	35	4	3
Epilachna (BOLD: 10 spp. / COL: 3 spp.)										
Epilachna elaterii (Rossi)*	+	-	-	-	-	-	-	0	0	0
Epilachna varivestis Mulsant, 1850(1)	+	-	-	-	+	-	-	0	0	0
Epilachna vigintioctopunctata (Fabricius)*	+	-	-	-	-	-	-	1	0	0
Harmonia (BOLD: 4 spp. / COL: 5 spp.)										
Harmonia axyridis (Pallas, 1773)(1)	+	-	-	-	+	-	-	89	20	15
Sasajiscymnus (BOLD: 0 spp. / COL: 1 spp.)										
Sasajiscymnus tsugae*	-	-	-	-	+	-	-	0	0	0
Stethorus (BOLD: 4 spp. / COL: 7 spp.)										
Stethorus punctum (LeConte, 1852)(1)	-	-	-	-	+	-	-	0	0	0
Coleoptera: Curculionidae										
Alcidodes (BOLD: 1 spp. / COL: 148 spp.)										
Alcidodes dentipes (Olivier)*	+	-	-	-	-	-	-	0	0	0
Anisandrus (BOLD: 8 spp. / COL: 6 spp.)										
Anisandrus dispar (Fabricius, 1792)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Xyleborus dispar (Fabricius, 1792)	-	-	-	-	+	-	-	14	0	0
Anisandrus maiche Stark, V.N. , 1936e(1)	-	-	-	-	+	-	-	1	1	0
Anthonomus (BOLD: 52 spp. / COL: 424 spp.)										
Anthonomus bisignifer Schenkling, S. & Marshall G.A.K. , 1934(1)	+	+	-	-	-	-	+	0	0	0
Anthonomus eugenii Cano, 1894(2)	+	+	-	-	-	-	+	9	8	0
Anthonomus grandis Boheman, 1843(2)	+	+	-	-	+	-	+	0	22	22

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Anthonomus pomorum (L., 1758)(2)	+	-	-	-	-	-	-	1	0	0
Anthonomus quadrigibbus Say, 1831(2)	+	-	-	-	-	-	+	1	0	
Anthonomus signatus Say, 1831(2)	+	+	-	-	-	-	+	1	0	0
Blosyrus (BOLD: 0 spp. / COL: 114 spp.)										
Blosyrus asellus Schoenherr, 1833(2)	-	-	-	-	+	-	-	0	0	0
Bothynoderes (BOLD: 0 spp. / COL: 32 spp.)										
Bothynoderes punctiventris (Germ.)*	+	-	-	-	-	-	-	0	0	0
Cnestus (BOLD: 5 spp. / COL: 23 spp.)										
Cnestus mutilatus (Blandford, 1894)(2)	-	-	-	-	+	-	-	0	0	0
Conotrachelus (BOLD: 31 spp. / COL: 854 spp.)	-	-	-	+	-	-	-			
Conotrachelus aguacatae Barber , 1923(1)	-	-	-	+	-	-	-	0	0	0
Conotrachelus nenuphar Harris , 1841(1)	+	+	-	-	+	-	+	2	0	0
Conotrachelus perseae Barber , 1919(1)	+	-	-	-	-	-	-	0	0	0
Copturus (BOLD: 1 spp. / COL: 194 spp.)										
Copturus aguacatae Kiss.*	-	-	-	+	-	-	-	27	0	0
Crossotarsus (BOLD: 10 spp. / COL: 263 spp.)										
Crossotarsus externedentatus, (Fairmaire, 1849)	+	-	-	-	-	-	-	0	0	0
Cryptorhynchus (BOLD: 2 spp. / COL: 467 spp.)										
Cryptorhynchus mangiferae Dejean(1)	-	-	-	+	-	-	-	0	0	0
Curculio (BOLD: 25 spp. / COL: 1923 spp.)										
Curculio caryae Horn, G.H. , 1873(1)	-	-	-	-	+	-	-	0	0	0
Curculio elephas (Gyllenhal, 1836)(2)	-	-	-	+	+	-	-	1	1	1
Curculio nucum L., 1758(2)	-	-	-	+	-	-	-	0	0	0
Dendroctonus (BOLD: 18 spp. / COL: 46 spp.)										
Dendroctonus adjunctus Blandford , 1897a(1)	+	+	-	-	-	-	+	3	3	3
Dendroctonus brevicomis Leconte , 1876(1)	+	+	-	-	-	-	-	3	2	2
Dendroctonus frontalis Zimmermann , 1868(1)	+	+	-	-	+	-	+	2	2	2
Dendroctonus micans Erichson , 1836(1)	+	-	-	-	+	-	+	2	5	5
Dendroctonus ponderosae Hopkins , 1902c(1)	+	+	-	-	+	-	+	47	46	46
Dendroctonus pseudotsugae Hopkins , 1905c(1)	+	+	-	-	-	-	+	67	63	63
Dendroctonus rufipennis Kirby , 1837(1)	+	+	-	-	-	-	+	6	1	1
Diaprepes (BOLD: 8 spp. / COL: 57 spp.)										
Diaprepes abbreviatus Hustache, A. , 1929(1)	+	-	-	-	+	-	-	8	8	8
Dryocoetes (BOLD: 14 spp. / COL: 133 spp.)										
Dryocoetes confusus Swaine, J.M. , 1912a(1)	-	+	-	-	-	-	+	5	0	0
Elytroteinus (BOLD: 0 spp. / COL: 1 spp.)										
Elytroteinus subtruncatus Marshall , 1920(1)	-	-	-	+	-	-	-	0	0	0
Euscepes (BOLD: 0 spp. / COL: 23 spp.)										
Euscepes postfasciatus (Fairmaire, 1849)(2)	+	-	-	+	-	-	-	0	0	0

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Gnathotrichus (BOLD: 3 spp. / COL: 41 spp.)										
Gnathotrichus sulcatus, (LeConte, 1868)	-	+	-	-	-	-	+	0	0	0
Gonipterus (BOLD: 0 spp. / COL: 41 spp.)										
Gonipterus gibberus Boisduval, J.B.A., 1835(2)	+	+	-	-	-	-	+	0	0	0
Gonipterus scutellatus Gyllenhal, 1833(2)	+	-	+	-	-	-	+	0	0	0
Graphognathus (BOLD: 0 spp. / COL: 2 spp.)										
Graphognathus leucoloma (Boheman, 1840)(2)	+	-	-	-	-	-	-	0	0	0
Heilipus (BOLD: 0 spp. / COL: 272 spp.)										
Heilipus lauri Boheman , 1845(1)	-	-	-	+	-	-	-	0	0	0
Hylastes (BOLD: 12 spp. / COL: 93 spp.)										
Hylastes ater Erichson , 1836(1)	-	-	-	-	-	+	-	1	0	0
Hylobius (BOLD: 9 spp. / COL: 107 spp.)										
Hylobius abietis (L., 1758)(2)	+	-	-	-	+	-	-	5	1	1
Hylobius transversovittus*	-	-	-	-	+	-	-	0	0	0
Hylurgopinus (BOLD: 1 spp. / COL: 1 spp.)										
Hylurgopinus rufipes Swaine, J.M. , 1918a(1)	+	-	-	-	-	-	-	0	0	0
Hylurgops (BOLD: 14 spp. / COL: 44 spp.)										
Hylurgops palliatus (Gyllenhal, 1813)	-	-	-	-	+	-	-	17	16	0
Hylurgus (BOLD: 2 spp. / COL: 43 spp.)										
Hylurgus ligniperda (F., 1792)(2)	-	-	-	-	+	-	-	2	4	2
Hypera (BOLD: 17 spp. / COL: 244 spp.)										
Hypera postica Dejean(1)	+	-	-	-	+	-	-	3	0	0
Hypolixus (BOLD: 1 spp. / COL: 0 spp.)										
Hypolixus truncatulus (Fabricius)*	+	-	-	-	-	-	-	0	0	0
Hypomeces (BOLD: 0 spp. / COL: 33 spp.)										
Hypomeces squamosus (Fabricius, 1792)(2)	+	-	-	-	-	-	-	0	0	0
Hypothenemus (BOLD: 24 spp. / COL: 284 spp.)										
Hypothenemus hampei Wood & Bright , 1992(1)	+	-	-	+	-	-	-	0	0	0
Ips (BOLD: 36 spp. / COL: 232 spp.)										
Ips amitinus (Eichhoff, 1872)	+	-	-	-	-	-	+	0	0	0
Ips calligraphus (Germar, 1824)	+	+	-	-	+	-	+	0	0	0
Ips cembrae (Heer, 1836)	+	-	-	-	-	-	+	0	0	0
Ips confusus (LeConte, 1876)	-	+	-	-	-	-	+	1	0	0
Ips grandicollis (Eichhoff, 1868)	+	+	-	-	-	-	-	3	0	0
Ips hauseri Reitter , 1894a(1)	-	-	+	-	-	-	+	0	0	0
Ips lecontei Swaine, J.M. , 1924c(1)	-	+	-	-	-	-	+	0	0	0
Ips pini (Say, 1836)	+	+	-	-	-	-	+	1	0	0
Ips plastographus Lanier, 1970(2)	-	+	-	-	-	-	+	0	0	0
Ips sexdentatus (Börner)*	+	-	-	-	+	-	+	5	4	0
Ips subelongatus (Motschulsky, 1860)	+	-	+	-	+	-	+	0	0	0
Ips typographus (L., 1758)(2)	+	-	-	-	+	+	+	12	12	1

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Lissorhoptrus (BOLD: 2 spp. / COL: 3 spp.)										
Lissorhoptrus oryzophilus Kuschel, 1952(2)	+	-	-	-	-	-	+	26	21	21
Listroderes (BOLD: 2 spp. / COL: 155 spp.)										
Listroderes costirostris Schönherr, 1826(2)	+	-	-	-	-	-	-	1	0	0
Listroderes subcinctus Boheman, 1842(2)	-	-	-	+	-	-	-	0	0	0
Listronotus (BOLD: 29 spp. / COL: 40 spp.)										
Listronotus bonariensis (Kushcel)*	+	+	-	-	-	-	+	1	7	7
Lixus (BOLD: 12 spp. / COL: 425 spp.)										
Lixus juncii Boheman , 1835(1)	+	-	-	-	-	-	-	0	0	0
Megalometis (BOLD: 0 spp. / COL: 13 spp.)										
Megalometis chilensis*	-	-	-	+	-	-	-	0	0	0
Megaplatypus (BOLD: 2 spp. / COL: 95 spp.)										
Megaplatypus mutatus (Chapuis, 1865)	+	-	+	-	-	-	+	0	0	0
Naupactus (BOLD: 22 spp. / COL: 267 spp.)										
Naupactus leucoloma Boheman, 1840(2)	-	+	-	-	+	-	+	1	1	1
Naupactus xanthographus Sturm, 1826(2)	-	-	-	+	-	+	-	1	0	0
Orchestes (BOLD: 7 spp. / COL: 100 spp.)										
Orchestes alni Redtenbacher, L., 1849(2)	-	-	-	-	+	-	-	0	0	0
Orthotomicus (BOLD: 10 spp. / COL: 22 spp.)										
Orthotomicus erosus (Wollaston, 1857)	-	-	-	-	+	-	-	2	2	0
Otiorhynchus (BOLD: 29 spp. / COL: 380 spp.)										
Otiorhynchus corruptor Gistel , 1848(1)	-	-	-	-	-	+	-	0	0	0
Otiorhynchus cribricollis Gyllenhal , 1834(1)	+	-	-	-	-	-	-	0	0	0
Otiorhynchus dieckmanni Magn., 1979(2)	-	-	-	-	+	-	-	0	0	0
Otiorhynchus ligustici Germar(1)	-	-	-	-	+	+	-	2	0	0
Otiorhynchus sulcatus Germar(1)	+	-	-	-	-	-	-	2	0	0
Pantomorus (BOLD: 9 spp. / COL: 73 spp.)										
Pantomorus cervinus (Boheman, 1840)(2)	+	-	-	-	-	-	-	1	0	0
Pissodes (BOLD: 13 spp. / COL: 127 spp.)										
Pissodes castaneus (Geer, 1775)(2)	+	-	-	-	-	-	-	0	0	0
Pissodes nemorensis Germar, 1824(2)	-	+	-	-	-	-	+	0	0	0
Pissodes strobi (Peck, 1817)(2)	+	+	-	-	-	-	+	1	1	1
Pissodes terminalis Hopping , 1920(1)	+	+	-	-	-	-	+	0	0	0
Pityogenes (BOLD: 15 spp. / COL: 39 spp.)										
Pityogenes chalcographus Bedel , 1888b(1)	-	-	-	-	+	-	-	11	12	0
Pityophthorus (BOLD: 100 spp. / COL: 512 spp.)										
Pityophthorus juglandis Blackman , 1928b(1)	-	-	-	-	+	-	-	3	0	0
Platypus (BOLD: 39 spp. / COL: 1032 spp.)										
Platypus parallelus (Fabricius, 1801)(2)	+	-	-	-	-	-	-	0	0	0
Platypus quercivorus Murayama , 1925b(1)	-	-	-	-	+	-	-	0	0	0
Premnotrypes (BOLD: 0 spp. / COL: 1 spp.)										
Premnotrypes latithorax (Pierce)*	+	+	-	-	-	-	+	0	0	0
Premnotrypes suturicallus Kuschel*	-	+	-	-	-	-	+	0	0	0

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Premnotrypes vorax (Hustache)*	+	+	-	-	-	-	+	0	0	0
Pseudocneorhinus (BOLD: 0 spp. / COL: 0 spp.)										
Pseudocneorhinus bifasciatus*	-	-	-	-	+	-	-	0	0	0
Scolytus (BOLD: 34 spp. / COL: 261 spp.)										
Scolytus intricatus (Ratzeburg, 1837)	-	-	-	-	+	-	-	5	3	0
Scolytus morawitzi Semenov Tjan-Shansky, A.P. , 1902(1)	+	-	+	-	-	-	+	0	0	0
Scolytus multistriatus (Marsham, 1802)	+	-	-	-	-	-	-	9	1	1
Scolytus rugulosus (Mueller, 1818)(2)	+	-	-	-	-	-	-	0	0	0
Scolytus schevyrewi Semenov Tjan-Shansky, A.P. , 1902(1)	+	-	-	-	+	-	+	0	0	0
Scolytus scolytus (Fabricius, 1775)(2)	+	-	-	-	+	-	-	0	0	0
Sitona (BOLD: 24 spp. / COL: 323 spp.)										
Sitona cylindricollis Fåhraeus, O.I. in Schönherr, C.J. , 1840(1)	+	-	-	-	-	-	-	2	0	0
Sitona discoideus Gyllenhal, 1834(2)	+	-	-	-	-	-	-	34	34	34
Sitona hispidulus (Fabricius, 1776)(2)	+	-	-	-	-	-	-	6	0	0
Sitona humeralis Steph., 1831(2)	+	-	-	-	-	-	-	2	0	0
Sternochetus (BOLD: 2 spp. / COL: 1 spp.)										
Sternochetus frigidus*	+	-	-	-	-	-	-	0	0	0
Sternochetus mangiferae (Fabricius, 1775)(2)	+	+	-	+	+	-	+	1	1	0
Tanymecus (BOLD: 4 spp. / COL: 171 spp.)										
Tanymecus dilaticollis Gylh.*	+	-	-	-	-	-	-	0	0	0
Temnoshoita (BOLD: 0 spp. / COL: 0 spp.)										
Temnoshoita nigroplagiata (Quedenfeldt)*	+	-	-	-	-	-	-	0	0	0
Tomicus (BOLD: 4 spp. / COL: 106 spp.)										
Tomicus destruens (Wollaston, 1865)	-	-	-	-	+	-	-	3	3	3
Tomicus minor (Hartig, 1834)	-	-	-	-	+	-	-	3	3	1
Tomicus piniperda (L., 1758)(2)	-	-	-	+	+	+	-	13	50	41
Trypodendron (BOLD: 8 spp. / COL: 36 spp.)										
Trypodendron domesticum (Linnaeus, 1758)(2)	-	-	-	-	+	-	-	20	3	0
Xyleborus (BOLD: 58 spp. / COL: 1496 spp.)	-	-	-	+	+	-	-			
Xyleborus ferrugineus (Fabricius, 1801)	+	-	-	-	-	-	-	0	0	0
Xyleborus fornicatus Eichhoff , 1868c(1)	+	-	-	-	-	-	-	0	0	0
Xyleborus glabratus Eichhoff , 1877a(1)	-	-	-	-	+	-	-	0	0	0
Xyleborus perforans (Wollaston, 1857)	+	-	-	-	-	-	-	0	0	0
Xylosandrus (BOLD: 22 spp. / COL: 55 spp.)										
Xylosandrus compactus (Eichhoff, 1876)	+	-	-	-	-	-	-	0	0	0
Xylosandrus crassiusculus (Motschulsky, 1866)	+	-	-	-	+	-	-	0	0	0
Xylosandrus germanus (Blandford, 1894)	+	-	-	-	-	-	-	4	0	0
Xylosandrus morigerus Reitter , 1913a(1)	+	-	-	-	-	-	-	1	1	1
Xyloterus (BOLD: 3 spp. / COL: 16 spp.)										
Xyloterus lineatus Erichson , 1836(1)	+	-	-	-	-	-	-	1	0	0
Coleoptera: Dermestidae										

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Trogoderma (BOLD: 8 spp. / COL: 18 spp.)										
Trogoderma granarium Everts, 1899(1)	-	-	+	+	+	+	+	4	0	0
Coleoptera: Dryophthoridae										
Cosmopolites (BOLD: 1 spp. / COL: 5 spp.)										
Cosmopolites sordidus Marshall, G.A.K. , 1930(1)	+	-	-	-	-	-	-	4	1	1
Diocalandra (BOLD: 1 spp. / COL: 11 spp.)										
Diocalandra frumenti Marshall, G.A.K. , 1931(1)	+	-	-	-	-	-	+	1	1	1
Diocalandra taitense (Guéer.)*	+	-	-	-	-	-	-	0	0	0
Metamasius (BOLD: 27 spp. / COL: 67 spp.)	-	-	-	+	-	-	-			
Metamasius hemipterus Champion, G.C. , 1910(1)	+	-	+	-	+	-	+	1	1	1
Odoiporus (BOLD: 0 spp. / COL: 7 spp.)										
Odoiporus longicollis Marshall, G.A.K. , 1930(1)	+	-	-	-	-	-	-	0	0	0
Rhabdoscelus (BOLD: 1 spp. / COL: 0 spp.)										
Rhabdoscelus obscurus (Boisduval, 1835)(2)	+	-	-	+	-	-	-	1	1	1
Rhinostomus (BOLD: 2 spp. / COL: 2 spp.)										
Rhinostomus barbirostris Rafinesque(1)	+	-	-	-	-	-	-	6	1	1
Rhynchophorus (BOLD: 5 spp. / COL: 98 spp.)										
Rhynchophorus ferrugineus (Olivier, 1790)(2)	+	-	+	-	+	-	+	0	0	0
Rhynchophorus palmarum (Linnaeus, 1758)(2)	+	+	-	-	+	-	+	4	1	1
Scyphophorus (BOLD: 2 spp. / COL: 8 spp.)										
Scyphophorus acupunctatus Gyllenhal, 1838(2)	+	-	-	-	-	-	-	5	1	1
Sphenophorus (BOLD: 26 spp. / COL: 226 spp.)										
Sphenophorus venatus Schoenherr , 1838(1)	+	-	-	-	-	-	-	5	1	1
Coleoptera: Elateridae										
Agriotes (BOLD: 38 spp. / COL: 2 spp.)										
Agriotes sputator (L., 1758)(2)	-	-	-	-	+	-	-	13	8	0
Agriotes ustulatus (Schall., 1783)(2)	-	-	-	-	+	-	-	5	5	0
Conoderus (BOLD: 28 spp. / COL: 7 spp.)										
Conoderus rufangulus Gyllenhal*	-	-	-	+	-	-	-	0	0	0
Limonius (BOLD: 18 spp. / COL: 0 spp.)										
Limonius californicus (Mannerheim)(2)	-	+	-	-	-	-	-	0	0	0
Melanotus (BOLD: 41 spp. / COL: 1 spp.)										
Melanotus communis E. Horak(2)	+	+	-	-	-	-	+	3	1	1
Coleoptera: Lymexylidae										
Melittomma (BOLD: 0 spp. / COL: 20 spp.)										
Melittomma insulare Fairm.*	+	-	-	-	-	-	-	0	0	0
Coleoptera: Nitidulidae										
Aethina (BOLD: 3 spp. / COL: 0 spp.)										
Aethina tumida*	-	-	-	-	+	-	-	2	5	5
Meligethes (BOLD: 54 spp. / COL: 0 spp.)										

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Meligethes aeneus (Fabricius, 1775)(2)	+	-	-	-	-	-	-	27	15	15
Coleoptera: Scarabidae										
Adoretus (BOLD: 4 spp. / COL: 464 spp.)	-	-	-	+	-	-	-			
Adoretus sinicus Burmeister, 1855(1)	+	-	-	+	-	-	-	0	0	0
Adoretus versutus Harold, 1869(1)	+	-	-	-	-	-	-	0	0	0
Amphimallon (BOLD: 2 spp. / COL: 76 spp.)										
Amphimallon majalis (Razoum)*	+	-	-	-	-	-	-	0	0	0
Amphimallon volgense (Fischer, 1823)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Amphimallon solstitialis Reiche, 1859	+	-	-	-	-	-	-	0	0	0
Anomala (BOLD: 158 spp. / COL: 1192 spp.)										
Anomala orientalis (Waterhouse, 1875)(2)	+	-	-	-	+	-	-	2	0	0
Anomala sulcatula Burmeister, 1844(1)	-	-	-	+	-	-	-	0	0	0
Blitopertha (BOLD: 0 spp. / COL: 11 spp.)										
Blitopertha orientalis (Waterhouse)*	-	+	-	-	-	-	+	0	0	0
Heteronychus (BOLD: 2 spp. / COL: 58 spp.)										
Heteronychus arator (Fabricius, 1775)(1)	+	+	-	-	+	-	+	0	0	0
Holotrichia (BOLD: 1 spp. / COL: 311 spp.)										
Holotrichia mindanaona Brenske, 1893(1)	-	-	-	+	-	-	-	0	0	0
Maladera (BOLD: 9 spp. / COL: 372 spp.)										
Maladera castanea (Arrow, 1913)(1)	+	-	-	-	+	-	-	1	0	0
Melolontha (BOLD: 2 spp. / COL: 67 spp.)										
Melolontha hippocastani Fabricius, 1801(1)	+	-	-	-	-	-	-	5	0	0
Melolontha melolontha (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	2	12	12
Oryctes (BOLD: 2 spp. / COL: 45 spp.)										
Oryctes boas (Fabricius, 1775)(1)	+	-	-	-	-	-	-	0	0	0
Oryctes monoceros (Olivier, 1789)(1)	+	-	-	-	-	-	-	0	0	0
Oryctes rhinoceros Linnaeus, 1758(1)	+	-	-	-	+	-	-	0	0	0
Phyllophaga (BOLD: 205 spp. / COL: 827 spp.)	-	-	-	+	-	-	-			
Phyllophaga smithi (Arrow, 1912)(1)	+	-	-	-	-	-	-	0	0	0
Popillia (BOLD: 1 spp. / COL: 320 spp.)	-	-	-	-	+	-	-			
Popillia japonica Newman, 1838(1)	+	-	+	+	+	+	+	9	0	0
Rhizotrogus (BOLD: 0 spp. / COL: 63 spp.)										
Rhizotrogus majalis*	-	-	-	-	+	-	-	0	0	0
Xylotrupes (BOLD: 1 spp. / COL: 28 spp.)										
Xylotrupes gideon (Linnaeus, 1767)(1)	+	-	-	-	-	-	-	8	0	0
Dermaptera: Forficulidae										
Forficula (BOLD: 5 spp. / COL: 1 spp.)										
Forficula auricularia Linnaeus, 1758(1)	+	-	-	-	-	-	-	5	0	0
Diptera: Drosophilidae										
Drosophila (BOLD: 451 spp. / COL: 1529 spp.)										
Drosophila suzukii (Matsumura, 1931)(1)	-	-	-	-	+	-	-	23	1	1
Diptera: Agromyzidae										
Agromyza (BOLD: 17 spp. / COL: 203 spp.)										

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Agromyza oryzae (Munakata, 1910)(1)	+	-	-	-	-	-	-	0	0	0
Amauromyza (BOLD: 5 spp. / COL: 62 spp.)										
Amauromyza maculosa (Malloch, 1913)(2)	-	+	-	-	-	-	+	0	0	0
-listed under synonym Nemorimyza maculosa*	+	-	-	-	-	-	-	2	1	1
Chromatomyia (BOLD: 22 spp. / COL: 105 spp.)										
Chromatomyia horticola Goureau, 1851(1)	+	-	-	-	-	-	-	1	1	1
Chromatomyia syngenesiae (Hardy, 1849)(1)	+	-	-	-	-	-	-	1	1	1
Liriomyza (BOLD: 19 spp. / COL: 390 spp.)										
Liriomyza bryoniae (Kaltenbach, 1858)(1)	+	-	-	-	-	-	+	2	0	0
Liriomyza huidobrensis Blanchard, 1926(1)	+	-	+	-	-	-	+	14	4	4
Liriomyza sativae Blanchard, 1938(1)	+	-	+	-	-	-	+	10	5	5
Liriomyza trifolii Burgess, 1880(1)	+	-	+	-	-	-	+	9	6	3
Melanagromyza (BOLD: 8 spp. / COL: 367 spp.)										
Melanagromyza obtusa (Malloch, 1914)(1)	+	-	-	-	-	-	-	1	1	1
Ophiomyia (BOLD: 9 spp. / COL: 275 spp.)										
Ophiomyia phaseoli (Tryon, 1951)(1)	+	-	-	-	-	-	-	1	1	1
Phytobia (BOLD: 3 spp. / COL: 94 spp.)										
Phytobia cepae (Her.)*	+	-	-	-	-	-	-	0	0	0
Phytomyza (BOLD: 113 spp. / COL: 578 spp.)										
Phytomyza gymnostoma Loew, 1858(2)	+	-	-	-	-	-	-	1	1	1
Diptera: Anthomyiidae										
Delia (BOLD: 21 spp. / COL: 332 spp.)										
Delia antiqua (Meigen, 1826)(1)	+	-	-	-	-	-	-	355	0	0
Delia coarctata (Fallen, 1825)(1)	+	-	-	-	-	-	-	0	0	0
Delia flavibasis (Stein, 1903)(1)	+	-	-	-	-	-	-	0	0	0
Delia floralis (Fallen, 1824)(1)	+	-	-	-	-	-	-	0	0	0
Delia platura (Meigen, 1826)(1)	+	-	-	-	-	-	-	103	96	0
Delia radicum (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	0	0	0
Diptera: Cecidomyiidae										
Contarinia (BOLD: 4 spp. / COL: 312 spp.)										
Contarinia nasturtii (Kieffer, 1888)(1)	-	-	-	-	+	+	-	2	0	0
Contarinia sorghicola (Coquillett, 1899)(1)	+	-	-	-	-	-	-	0	0	0
Contarinia tritici (Kirby, 1798)(1)	+	-	-	-	-	-	-	0	0	0
Dasineura (BOLD: 6 spp. / COL: 485 spp.)										
Dasineura mali (Kieffer, 1904)(1)	+	-	-	-	-	-	-	1	0	0
Dasineura pyri (Bouche, 1847)(1)	+	-	-	-	-	-	-	0	0	0
Orseolia (BOLD: 0 spp. / COL: 29 spp.)										
Orseolia oryzae (Wood-Mason, 1889)(1)	+	-	-	-	-	-	-	0	0	0
Orseolia oryzivora Harris & Gagne, 1982(1)	+	-	-	-	-	-	-	0	0	0
Rhopalomyia (BOLD: 25 spp. / COL: 220 spp.)										
Rhopalomyia chrysanthemi (Ahlberg, 1939)(1)	+	-	-	-	-	-	-	0	0	0
Mayetiola (BOLD: 1 spp. / COL: 33 spp.)										

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Mayetiola destructor (Say, 1817)(1)	+	-	-	-	-	+	-	3	1	1
Sitodiplosis (BOLD: 0 spp. / COL: 6 spp.)										
Sitodiplosis mosellana (Gehin, 1857)(1)	+	-	-	-	-	-	-	0	0	0
Thecodiplosis (BOLD: 1 spp. / COL: 6 spp.)										
Thecodiplosis japonensis Uchida & Inouye, 1955(1)	+	-	-	-	-	-	-	0	1	1
Diptera: Chloropidae										
Oscinella (BOLD: 14 spp. / COL: 74 spp.)										
Oscinella frit (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	0	0	0
Diptera: Culicidae										
Aedes (BOLD: 122 spp. / COL: 959 spp.)										
Aedes albopictus Skuse, 1894(1)	-	-	-	-	+	-	-	72	26	25
Diptera: Ephydridae										
Hydrellia (BOLD: 20 spp. / COL: 229 spp.)										
Hydrellia wirthi Korytkowski, 1982(1)	-	-	-	-	+	-	-	0	0	0
Diptera: Muscidae										
Atherigona (BOLD: 11 spp. / COL: 265 spp.)										
Atherigona oryzae Malloch, 1925(1)	+	-	-	-	-	-	-	0	0	0
Atherigona soccata Rondani, 1871(1)	+	-	-	-	-	-	-	0	0	0
Diptera: Psilidae										
Psila (BOLD: 4 spp. / COL: 127 spp.)										
Psila rosae (Fabricius, 1794)(2)	+	-	-	-	-	-	-	0	0	0
Diptera: Syrphidae										
Merodon (BOLD: 30 spp. / COL: 151 spp.)										
Merodon equestris (Fabricius, 1794)(1)	+	-	-	-	-	-	-	5	1	1
Diptera: Tephritidae										
Acanthiophilus (BOLD: 3 spp. / COL: 10 spp.)										
Acanthiophilus helianthi (Rossi, 1794)(1)	+	-	-	-	-	-	-	10	1	0
Anastrepha (BOLD: 75 spp. / COL: 206 spp.)										
Anastrepha fraterculus (Wiedemann, 1830)(1)	+	+	-	+	-	-	+	24	8	8
Anastrepha grandis (Macquart, 1846)(1)	+	-	-	+	-	-	-	4	3	3
Anastrepha ludens (Loew, 1873)(1)	+	+	-	+	+	-	+	27	1	1
Anastrepha obliqua (Macquart, 1835)(1)	+	+	-	+	-	-	+	16	7	7
Anastrepha serpentina (Wiedemann, 1830)(1)	+	-	-	+	-	-	-	13	2	2
Anastrepha striata Schiner, 1868(1)	+	-	-	+	-	-	-	31	8	8
Anastrepha suspensa (Loew, 1862)(1)	+	+	-	+	+	-	+	26	5	5
Bactrocera (BOLD: 171 spp. / COL: 562 spp.)										
Bactrocera carambolae Drew & Hancock, 1994(1)	+	-	-	-	-	-	-	12	10	10
Bactrocera caryeae (Kapoor, 1971)(1)	+	-	-	-	-	-	-	1	1	1
Bactrocera correcta (Bezzi, 1916)(1)	+	-	-	-	+	-	-	15	14	4
Bactrocera cucumis (French, 1907)(1)	+	+	-	-	-	-	+	10	1	1
Bactrocera cucurbitae (Coquillett, 1899)(1)	+	+	-	+	+	-	+	113	84	13
Bactrocera dorsalis (Hendel, 1912)(1)	+	+	-	+	+	-	+	34	29	19

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Bactrocera invadens Drew, Tsuruta & White, 2005(1)	+	+	-	-	-	-	+	268	149	1
Bactrocera kandiensis Drew & Hancock, 1994(1)	+	-	-	-	-	-	-	10	11	1
Bactrocera latifrons (Hendel, 1915)(1)	+	-	-	-	+	-	-	20	15	3
Bactrocera minax (Enderlein, 1920)(1)	+	+	-	-	-	-	+	2	0	0
Bactrocera occipitalis (Bezzi, 1919)(1)	+	-	-	-	-	-	-	9	5	4
Bactrocera oleae (Rossi, 1790)(1)	+	-	-	-	+	-	-	23	16	6
Bactrocera papayae Drew & Hancock, 1994(1)	+	-	-	-	-	-	-	17	11	9
Bactrocera philippinensis Drew & Hancock, 1994(1)	+	-	-	-	-	-	-	10	9	8
Bactrocera pyrifoliae Drew & Hancock, 1994(1)	+	-	-	-	-	-	-	0	0	0
Bactrocera tryoni (Froggatt, 1897)(1)	+	+	-	+	-	-	+	13	3	3
Bactrocera tsuneonis (Miyake, 1919)(1)	+	+	-	-	-	-	+	0	0	0
Bactrocera zonata (Saunders, 1842)(1)	+	+	-	-	-	-	+	27	21	7
Ceratitis (BOLD: 57 spp. / COL: 184 spp.)	-	-	-	+	-	-	-			
Ceratitis capitata (Wiedemann, 1824)(1)	+	-	+	+	+	-	+	170	129	108
Ceratitis catoirii Guérin-Méneville, 1843(1)	+	-	-	-	-	-	-	2	2	0
Ceratitis cosyra (Walker, 1849)(1)	+	-	-	-	-	-	+	75	44	4
Ceratitis quinaria (Bezzi, 1918)(1)	+	-	-	-	-	-	+	6	8	0
Ceratitis rosa Karsch, 1887(1)	+	+	-	-	-	-	+	30	29	10
Dacus (BOLD: 81 spp. / COL: 300 spp.)										
Dacus ciliatus Loew, 1862(1)	+	+	-	-	-	-	+	31	34	5
Myiopardalis (BOLD: 1 spp. / COL: 0 spp.)										
Myiopardalis pardalina (Bigot, 1891)(2)	+	-	-	-	-	-	-	0	2	2
Neoceratitis (BOLD: 1 spp. / COL: 7 spp.)										
Neoceratitis cyanescens (Bezzi, 1923)(1)	+	-	-	-	-	-	+	1	1	1
Pterandrus (BOLD: 0 spp. / COL: 0 spp.)	-	-	-	+	-	-	-			
Rhagoletis (BOLD: 20 spp. / COL: 68 spp.)										
Rhagoletis cerasi (Linnaeus, 1758)(1)	+	-	-	-	-	+	-	11	4	2
Rhagoletis cingulata (Loew, 1862)(1)	+	-	+	-	+	-	+	9	9	1
Rhagoletis completa Cresson, 1929(1)	+	-	-	-	-	-	+	0	0	0
Rhagoletis fausta (Osten Sacken, 1877)(1)	+	+	-	-	-	-	+	1	6	1
Rhagoletis indifferens Curran, 1932(1)	+	+	-	-	-	-	+	0	0	0
Rhagoletis mendax Curran, 1932(1)	+	+	-	-	+	+	+	2	2	2
Rhagoletis pomonella (Walsh, 1867)(1)	+	+	-	-	+	+	+	7	16	3
Rhagoletis ribicola Doane, 1898(1)	+	-	-	-	-	-	+	0	0	0
Toxotrypana (BOLD: 0 spp. / COL: 7 spp.)										
Toxotrypana curvicauda Gerstaecker, 1860(1)	-	-	-	+	-	-	-	0	0	0
Diptera: Tipulidae										
Tipula (BOLD: 285 spp. / COL: 2280 spp.)										
Tipula paludosa Meigen, 1830(1)	+	-	-	-	+	-	-	15	0	0
Hemiptera: Aclerdidae										
Aclerda (BOLD: 0 spp. / COL: 48 spp.)										

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Aclerda takahashii Kuwana, 1932(1)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Adelgidae										
Adelges (BOLD: 18 spp. / COL: 50 spp.)										
Adelges piceae(1)	-	-	-	-	-	+	-	14	13	0
-also listed as Dreyfusia piceae Ratzeburg*	-	-	-	-	-	+	-	3	3	3
Adelges tsugae Annand, 1924(1)	-	-	-	-	+	+	-	159	119	0
Hemiptera: Aleyrodidae										
Aleurocanthus (BOLD: 17 spp. / COL: 2 spp.)										
Aleurocanthus spiniferus (Quaintance, 1903)(1)	+	-	+	+	+	-	+	2	0	0
Aleurocanthus woglumi Ashby, 1915(1)	+	+	-	-	-	-	+	0	0	0
Aleurodicus (BOLD: 3 spp. / COL: 1 spp.)										
Aleurodicus destructor Mackie, 1912(2)	+	-	-	-	-	-	-	0	0	0
Aleurodicus dispersus Russell, 1965(1)	+	-	-	-	-	-	-	15	1	1
Aleurothrixus (BOLD: 1 spp. / COL: 2 spp.)										
Aleurothrixus floccosus (Maskell, 1896)(1)	+	-	-	-	-	-	-	3	0	0
Aleurotrachelus (BOLD: 2 spp. / COL: 0 spp.)										
Aleurotrachelus trachoides*	+	-	-	-	-	-	-	0	0	0
Bemisia (BOLD: 6 spp. / COL: 2 spp.)										
Bemisia tabaci (Gennadius, 1889)(1)	+	-	+	-	+	-	+	193	153	153
Dialeurodes (BOLD: 1 spp. / COL: 2 spp.)										
Dialeurodes citri (Ashmead, 1885)(2)	+	-	-	-	-	-	-	0	0	0
Neomaskellia (BOLD: 2 spp. / COL: 0 spp.)										
Neomaskellia bergii (Signoret, 1868)(2)	-	-	-	+	-	-	-	2	0	0
Parabemisia (BOLD: 0 spp. / COL: 1 spp.)										
Parabemisia myricae (Kuwana, 1927)(1)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Alydidae										
Leptocorisa (BOLD: 6 spp. / COL: 0 spp.)										
Leptocorisa acuta (Thunberg, 1783)(2)	+	-	-	+	-	-	-	7	0	0
Leptocorisa oratorius (Fabricius, 1794)(2)	+	-	-	-	-	-	-	1	0	0
Hemiptera: Aphididae										
Acyrthosiphon (BOLD: 11 spp. / COL: 74 spp.)										
Acyrthosiphon kondoi Shinji, 1938(1)	+	-	-	-	-	-	-	7	2	2
Acyrthosiphon pisum (Harris, M., 1776)(1)	+	-	-	-	-	-	-	87	22	13
Aphis (BOLD: 99 spp. / COL: 541 spp.)										
Aphis craccivora Koch, 1854(1)	+	-	-	-	-	-	-	48	20	11
Aphis fabae(1)	+	-	-	-	-	-	-	130	20	11
Aphis glycines Matsumura, 1917(1)	-	-	-	-	+	-	-	91	11	6
Aphis gossypii Glover, 1877(1)	+	-	-	-	-	-	-	157	107	25
Aphis illinoisensis Shimer, 1866(1)	+	-	-	-	-	-	-	8	1	0
Aphis pomi(1)	+	-	-	-	-	-	-	98	76	0
Aphis spiraecola Patch, 1914(1)	+	-	-	-	-	-	-	145	62	5
Aulacorthum (BOLD: 5 spp. / COL: 46 spp.)										
Aulacorthum solani (Kaltenbach, 1843)(1)	+	-	-	-	-	-	-	41	9	5

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Brevicoryne (BOLD: 1 spp. / COL: 9 spp.)										
Brevicoryne brassicae (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	16	6	4
Cerataphis (BOLD: 5 spp. / COL: 8 spp.)										
Cerataphis brasiliensis(1)	+	-	-	-	-	-	-	2	0	0
Cerataphis lataniae(1)	+	-	-	-	-	-	-	1	0	0
Cerataphis orchidearum(1)	+	-	-	-	-	-	-	0	0	0
Diuraphis (BOLD: 4 spp. / COL: 8 spp.)										
Diuraphis noxia(1)	+	-	-	-	+	-	-	7	117	117
Dysaphis (BOLD: 5 spp. / COL: 99 spp.)										
Dysaphis plantaginea(1)	+	-	-	-	-	-	-	6	1	0
Elatobium (BOLD: 1 spp. / COL: 7 spp.)										
Elatobium abietinum(1)	+	-	-	-	-	-	-	5	1	0
Eriosoma (BOLD: 6 spp. / COL: 38 spp.)										
Eriosoma lanigerum(1)	+	-	-	-	-	-	-	15	1	0
Hysteroneura (BOLD: 1 spp. / COL: 1 spp.)										
Hysteroneura setariae (Thomas, 1878)(1)	+	-	-	-	-	-	-	8	0	0
Illinoia (BOLD: 18 spp. / COL: 42 spp.)										
Illinoia liriodendri (Monell, 1879)(1)	+	-	-	-	-	-	-	4	3	0
Lipaphis (BOLD: 1 spp. / COL: 11 spp.)										
Lipaphis erysimi (Kaltenbach, 1843)(1)	+	-	-	-	-	-	-	0	0	0
Macrosiphum (BOLD: 44 spp. / COL: 142 spp.)										
Macrosiphum euphorbiae (Thomas, 1878)(1)	+	-	-	-	-	-	-	136	6	2
Melanaphis (BOLD: 3 spp. / COL: 24 spp.)										
Melanaphis sacchari(1)	+	-	-	-	-	-	-	2	0	0
Metopolophium (BOLD: 1 spp. / COL: 20 spp.)										
Metopolophium dirhodum(1)	+	-	-	-	-	-	-	11	4	4
Myzus (BOLD: 9 spp. / COL: 65 spp.)										
Myzus ascalonicus(1)	+	-	-	-	-	-	-	10	2	0
Myzus ornatus(1)	+	-	-	-	-	-	-	3	2	1
Myzus persicae(1)	+	-	-	-	-	-	-	109	20	10
Nasonovia (BOLD: 12 spp. / COL: 43 spp.)										
Nasonovia ribisnigri(1)	+	-	-	-	-	-	-	33	2	0
Neomyzus circumflexum (Buckton, 1876)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Aulacorthum circumflexum*	+	-	-	-	-	-	-	0	0	0
Neotoxoptera (BOLD: 1 spp. / COL: 8 spp.)										
Neotoxoptera formosana (Takahashi, R., 1921)(1)	+	-	-	-	-	-	-	4	0	0
Pentalonia (BOLD: 2 spp. / COL: 3 spp.)										
Pentalonia nigronervosa(1)	+	-	-	-	-	-	-	37	0	0
Pterochloroides (BOLD: 0 spp. / COL: 1 spp.)										
Pterochloroides persicae (Cholodkovsky, 1899)(1)	+	-	-	-	-	-	-	0	0	0
Rhopalosiphum (BOLD: 13 spp. / COL: 18 spp.)										

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Rhopalosiphum maidis (Fitch, 1856)(1)	+	-	-	-	-	-	-	17	5	4
Rhopalosiphum padi (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	102	11	6
Rhopalosiphum rufiabdominalis(1)	+	-	-	-	-	-	-	2	2	2
Schizaphis (BOLD: 5 spp. / COL: 39 spp.)										
Schizaphis graminum(1)	+	-	-	-	-	-	-	44	39	38
Sitobion (BOLD: 7 spp. / COL: 82 spp.)										
Sitobion avenae (Fabricius, 1775)(1)	+	-	-	-	-	-	-	44	5	4
Sitobion miscanthi (Takahashi, R., 1921)(1)	+	-	-	-	-	-	-	0	0	0
Therioaphis (BOLD: 3 spp. / COL: 25 spp.)										
Therioaphis trifolii (Monell, 1882)(1)	+	-	-	-	-	-	-	120	2	0
Toxoptera (BOLD: 5 spp. / COL: 6 spp.)										
Toxoptera aurantii(1)	+	-	-	-	-	-	-	25	12	6
Toxoptera citricidus(1)	-	-	-	-	-	-	-	4	3	0
-listed under synonym Toxoptera citricida	-	-	+	-	-	-	+	8	3	3
Toxoptera odinae (van der Goot, 1917)(1)	+	-	-	-	-	-	-	9	8	8
Hemiptera: Asterolecaniidae										
Asterodiaspis (BOLD: 3 spp. / COL: 0 spp.)										
Asterodiaspis variolosa (Ratzeburg, 1870)(2)	+	-	-	-	-	-	-	0	0	0
Asterolecanium (BOLD: 1 spp. / COL: 0 spp.)										
Asterolecanium pustulans (Cockerell, 1892)(2)	+	-	-	-	-	-	-	0	0	0
Planchonia (BOLD: 1 spp. / COL: 0 spp.)										
Planchonia stentae*	-	-	-	-	+	-	-	14	0	0
Hemiptera: Blissidae										
Blissus (BOLD: 5 spp. / COL: 17 spp.)										
Blissus leucopterus (Say, 1832)(1)	+	-	-	-	-	-	-	4	0	0
Hemiptera: Cicadellidae										
Amrasca (BOLD: 1 spp. / COL: 15 spp.)										
Amrasca devastans*	+	-	-	-	-	-	-	14	0	0
Cicadella (BOLD: 1 spp. / COL: 50 spp.)										
Cicadella spectra*	+	-	-	-	-	-	-	0	0	0
Cicadulina (BOLD: 1 spp. / COL: 23 spp.)										
Cicadulina mbila Naudé 1924(1)	+	-	-	-	-	-	-	0	0	0
Circulifer (BOLD: 0 spp. / COL: 0 spp.)										
Circulifer opacipennis (Lethierry, 1876)(2)	+	-	-	-	-	-	-	0	0	0
Circulifer tenellus (Baker, 1896)(2)	+	-	-	-	-	-	+	0	0	0
Edwardsiana (BOLD: 2 spp. / COL: 79 spp.)										
Edwardsiana crataegi (Douglas, 1876)(1)	+	-	-	-	-	-	-	0	0	0
Edwardsiana flavescens (Fabricius, 1794)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Empoasca flavescens DeLong, 1931	+	-	-	-	-	-	-	0	0	0
Empoasca (BOLD: 97 spp. / COL: 674 spp.)										
Empoasca fabae (Harris, 1841)(1)	+	-	-	-	-	-	-	0	0	0

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Homalodisca (BOLD: 6 spp. / COL: 18 spp.)										
Homalodisca coagulata*	+	+	-	-	-	-	+	0	0	0
Homalodisca vitripennis Germar, 1821(1)	-	-	-	-	+	-	-	6	3	3
Jacobiasca (BOLD: 0 spp. / COL: 22 spp.)										
Jacobiasca lybica (Bergevin & Zanon, 1922)(1)	+	-	-	-	-	-	-	0	0	0
Jacobiella (BOLD: 0 spp. / COL: 2 spp.)										
Jacobiella facialis (Jacobi, 1912)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Empoasca facialis Bedford, 1923	+	-	-	-	-	-	-	0	0	0
Nephotettix (BOLD: 1 spp. / COL: 8 spp.)										
Nephotettix nigropictus(1)	+	-	-	-	-	-	-	0	0	0
Nephotettix virescens (Distant, 1908)(2)	+	-	-	-	-	-	-	0	0	0
Scaphoideus (BOLD: 33 spp. / COL: 162 spp.)										
Scaphoideus titanus Ball 1932(1)	+	-	-	-	-	-	-	1	0	0
Hemiptera: Cixiidae										
Haplaxius (BOLD: 0 spp. / COL: 5 spp.)										
Haplaxius crudus (Van Duzee, 1907)(1)	-	-	-	-	+	-	-	0	0	0
Hemiptera: Coccidae										
Ceroplastes (BOLD: 5 spp. / COL: 133 spp.)										
Ceroplastes destructor Newstead, 1917(1)	+	-	-	-	+	-	-	5	0	0
Ceroplastes floridensis Comstock, 1881(1)	+	-	-	-	-	-	-	0	0	0
Ceroplastes japonicus Green, 1921(1)	+	-	-	-	+	-	-	0	0	0
Ceroplastes rubens Maskell, 1893(1)	+	-	-	-	-	-	-	1	1	1
Ceroplastes rusci (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	14	0	0
Ceroplastes sinensis Del Guercio, 1900(1)	+	-	-	-	-	-	-	0	0	0
Coccus (BOLD: 267 spp. / COL: 85 spp.)										
Coccus celatus De Lotto, 1960(1)	+	-	-	-	-	-	-	1	1	1
Coccus hesperidum Linnaeus, 1758(2)	+	-	-	-	-	-	-	21	1	1
Coccus pseudomagnoliarum (Kuwana, 1914)(1)	+	-	-	-	-	-	-	1	1	1
Coccus viridis (Green, 1889)(1)	+	-	-	+	-	-	-	1	1	1
Eucalymnatus (BOLD: 0 spp. / COL: 12 spp.)										
Eucalymnatus tessellatus (Signoret, 1873)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Eucalymnatus tesselatus Balachowsky, 1927	+	-	-	-	-	-	-	0	0	0
Parasaissetia (BOLD: 1 spp. / COL: 5 spp.)										
Parasaissetia nigra (Nietner, 1861)(1)	+	-	-	-	-	-	+	21	0	0
Parthenolecanium (BOLD: 2 spp. / COL: 13 spp.)										
Parthenolecanium corni (Bouché, 1844)(2)	+	-	-	-	-	-	-	1	1	1
Parthenolecanium persicae (Fabricius, 1776)(2)	+	-	-	-	-	-	-	0	0	0
Pulvinaria (BOLD: 6 spp. / COL: 135 spp.)										
Pulvinaria polygonata Cockerell, 1905(1)	-	-	-	-	+	-	-	0	0	0
Pulvinaria psidii Maskell, 1893(1)	+	-	-	-	-	-	-	9	1	1
Saissetia (BOLD: 1 spp. / COL: 44 spp.)										

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Saissetia coffeae (Walker, 1852)(1)	+	-	-	-	-	-	-	4	1	1
Saissetia oleae Sanders, 1909(1)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Coreidae										
Anoplocnemis (BOLD: 0 spp. / COL: 0 spp.)										
Anoplocnemis curvipes (Fabricius, 1781)(2)	+	-	-	-	-	-	-	0	0	0
Clavigralla (BOLD: 1 spp. / COL: 0 spp.)										
Clavigralla tomentosicollis Stål, 1855(2)	+	-	-	-	-	-	-	0	0	0
Leptoglossus (BOLD: 8 spp. / COL: 12 spp.)										
Leptoglossus australis*	+	-	-	-	-	-	-	1	0	0
Leptoglossus chilensis (Spinola, 1852)(2)	-	-	-	+	-	-	-	0	0	0
Pseudotheraptus (BOLD: 1 spp. / COL: 0 spp.)										
Pseudotheraptus devastans (Distant, 1917)(2)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Cryptococcidae										
Cryptococcus (BOLD: 2 spp. / COL: 6 spp.)										
Cryptococcus fagisuga Lindinger, 1936(1)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Delphacidae										
Laodelphax (BOLD: 2 spp. / COL: 1 spp.)										
Laodelphax striatellus (Fallén, 1826)(2)	+	-	-	-	-	-	-	32	32	32
Nilaparvata (BOLD: 4 spp. / COL: 15 spp.)										
Nilaparvata lugens (Stål, 1854)(1)	+	-	-	-	-	-	-	138	138	138
Peregrinus (BOLD: 1 spp. / COL: 2 spp.)										
Peregrinus maidis (Ashmead, 1890)(1)	+	-	-	-	-	-	-	0	0	0
Perkinsiella (BOLD: 0 spp. / COL: 25 spp.)										
Perkinsiella saccharicida Kirkaldy, 1903(1)	+	-	-	-	-	-	-	0	0	0
Perkinsiella vastatrix (Breddin, 1896)(1)	+	-	-	-	-	-	-	0	0	0
Saccharosydne (BOLD: 0 spp. / COL: 8 spp.)										
Saccharosydne saccharivora (Westwood, 1833)(1)	+	-	-	-	-	-	-	0	0	0
Sogatella (BOLD: 7 spp. / COL: 20 spp.)										
Sogatella furcifera (Horváth, 1899)(1)	+	-	-	-	-	-	-	1	1	1
Sogatodes (BOLD: 0 spp. / COL: 5 spp.)										
Sogatodes cubanus*	+	-	-	-	-	-	-	0	0	0
Sogatodes orizicola*	+	-	-	-	-	-	-	0	0	0
Tarophagus (BOLD: 0 spp. / COL: 1 spp.)										
Tarophagus proserpina (Kirkaldy, 1907)(1)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Diaspididae										
Aonidiella (BOLD: 2 spp. / COL: 32 spp.)										
Aonidiella aurantii (Maskell, 1879)(1)	+	-	-	-	-	-	-	9	0	0
Aonidiella citrina (Coquillett, 1891)(1)	+	-	-	-	-	-	+	0	0	0
Aonidiella orientalis (Newstead, 1894)(1)	+	-	-	-	-	-	-	0	0	0
Aonidomytilus (BOLD: 0 spp. / COL: 16 spp.)										
Aonidomytilus albus (Cockerell, 1893)(1)	+	-	-	-	-	-	-	0	0	0
Aspidiella (BOLD: 1 spp. / COL: 8 spp.)										
Aspidiella hartii (Cockerell, 1895)(1)	+	-	-	-	-	-	-	1	0	0

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Aspidiotus (BOLD: 4 spp. / COL: 81 spp.)										
Aspidiotus destructor Signoret, 1869(1)	+	-	-	-	-	-	-	5	0	0
Aspidiotus nerii Bouche, 1833(1)	+	-	-	-	-	-	-	16	0	0
Aulacaspis (BOLD: 4 spp. / COL: 89 spp.)										
Aulacaspis madiunensis (Zehntner, 1898)(1)	+	-	-	-	-	-	-	0	0	0
Aulacaspis tegalensis (Zehntner, 1898)(1)	+	-	-	-	-	-	-	0	0	0
Aulacaspis tubercularis Newstead, 1906(1)	+	-	-	-	-	-	-	7	0	0
Aulacaspis yasumatsui Takagi, 1977(1)	+	-	-	-	-	-	+	0	0	0
Chrysomphalus (BOLD: 2 spp. / COL: 16 spp.)										
Chrysomphalus aonidum (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	3	0	0
Chrysomphalus dictyospermi (Morgan, 1889)(1)	+	-	-	-	-	-	-	0	0	0
Diaspidiotus (BOLD: 2 spp. / COL: 88 spp.)										
Diaspidiotus ostreaeformis (Curtis, 1843)(1)	+	-	-	-	-	-	-	0	0	0
Diaspidiotus perniciosus (Comstock, 1881)(1)	-	-	-	-	-	-	-	6	2	0
-listed under synonym Quadraspidiotus perniciosus Ferris, 1938	+	-	+	-	-	-	+	0	0	0
Diaspidiotus pyri (Lichtenstein, 1881)(1)	-	-	-	-	-	+	-	0	0	0
Diaspis (BOLD: 2 spp. / COL: 57 spp.)										
Diaspis bromeliae (Kerner, 1778)(1)	+	-	-	-	-	-	-	1	1	1
Fiorinia (BOLD: 0 spp. / COL: 66 spp.)										
Fiorinia externa Ferris, 1942(1)	-	-	-	-	+	-	-	0	0	0
Furcaspis (BOLD: 1 spp. / COL: 9 spp.)										
Furcaspis oceanica Lindinger, 1909(1)	-	-	-	+	-	-	-	0	0	0
Hemiberlesia (BOLD: 1 spp. / COL: 34 spp.)										
Hemiberlesia lataniae (Signoret, 1869)(1)	+	-	-	-	-	-	-	2	1	1
Hemiberlesia pitysophila Takagi, 1969(1)	+	-	-	-	-	-	-	0	0	0
Hemiberlesia rapax (Comstock, 1881)(1)	+	-	-	-	-	-	-	0	0	0
Howardia (BOLD: 0 spp. / COL: 3 spp.)										
Howardia biclavis (Comstock, 1883)(1)	+	-	-	-	-	-	-	0	0	0
Ischnaspis (BOLD: 0 spp. / COL: 7 spp.)										
Ischnaspis longirostris (Signoret, 1882)(1)	+	-	-	-	-	-	-	0	0	0
Lepidosaphes (BOLD: 9 spp. / COL: 159 spp.)										
Lepidosaphes beckii (Newman, 1869)(1)	+	-	-	-	-	-	-	0	0	0
Lepidosaphes gloverii (Packard, 1869)(1)	+	-	-	-	-	-	-	0	0	0
Lepidosaphes ulmi (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	0	0	0
Lepidosaphes ussuriensis (Borchsenius, 1962)(1)	-	-	+	-	-	-	+	0	0	0
Lopholeucaspis (BOLD: 1 spp. / COL: 6 spp.)										
Lopholeucaspis japonica (Cockerell, 1897)(1)	+	-	+	-	-	-	+	3	0	0
Parlatoria (BOLD: 4 spp. / COL: 68 spp.)										
Parlatoria blanchardi (Targioni Tozzetti, 1892)(1)	+	-	-	-	-	-	-	0	0	0
Parlatoria oleae (Colvée, 1880)(1)	+	-	-	-	-	-	-	0	0	0

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Parlatoria pergandii Comstock, 1881(1)	+	-	-	-	-	-	-	0	0	0
Parlatoria ziziphi (Lucas, 1853)(1)	+	-	-	-	+	-	-	5	0	0
Pinnaspis (BOLD: 1 spp. / COL: 40 spp.)										
Pinnaspis aspidistrae (Signoret, 1869)(2)	+	-	-	-	-	-	-	0	0	0
Pinnaspis buxi (Bouché, 1851)(1)	+	-	-	-	-	-	-	0	0	0
Pseudaonidia (BOLD: 1 spp. / COL: 17 spp.)										
Pseudaonidia trilobitiformis (Green, 1896)(1)	+	-	-	-	-	-	-	0	0	0
Pseudaulacaspis (BOLD: 3 spp. / COL: 65 spp.)										
Pseudaulacaspis cockerelli (Cooley, 1897)(1)	+	-	-	-	-	-	-	17	0	0
Pseudaulacaspis pentagona (Targioni Tozzetti, 1886)(1)	+	-	-	-	+	-	-	12	0	0
Selenaspidus (BOLD: 0 spp. / COL: 29 spp.)										
Selenaspidus articulatus (Morgan, 1889)(1)	+	-	-	-	-	-	-	0	0	0
Unaspis (BOLD: 2 spp. / COL: 18 spp.)										
Unaspis citri (Comstock, 1883)(1)	+	+	-	-	-	-	-	0	0	0
Unaspis euonymi (Comstock, 1881)(1)	+	-	-	-	-	-	-	29	0	0
Unaspis yanonensis (Kuwana, 1923)(1)	+	-	-	-	+	-	-	3	0	0
Hemiptera: Flatidae										
Metcalfa (BOLD: 1 spp. / COL: 5 spp.)										
Metcalfa pruinosa (Say, 1830)(1)	+	-	-	-	-	-	-	3	0	0
Hemiptera: Kerriidae										
Paratachardina (BOLD: 0 spp. / COL: 8 spp.)										
Paratachardina pseudolobata*	-	-	-	-	+	-	-	0	0	0
Hemiptera: Lophopidae										
Pyrilla (BOLD: 2 spp. / COL: 7 spp.)										
Pyrilla perpusilla (Walker, 1851)(1)	+	-	-	-	-	-	-	2	0	0
Hemiptera: Lygaeidae										
Nysius (BOLD: 16 spp. / COL: 39 spp.)										
Nysius huttoni F.B. White, 1878(2)	-	-	-	-	+	-	+	0	0	0
Nysius vinitor Bergroth, 1891(2)	-	-	-	-	-	+	-	0	0	0
Hemiptera: Margarodidae										
Crypticerya (BOLD: 0 spp. / COL: 15 spp.)										
Crypticerya multicicatrices*	-	-	-	-	+	-	-	0	0	0
Icerya (BOLD: 3 spp. / COL: 44 spp.)										
Icerya aegyptiaca (Douglas, 1890)(1)	+	-	-	+	-	-	-	1	1	1
Icerya purchasi(1)	+	-	-	-	+	-	-	6	1	1
Icerya seychellarum (Westwood, 1855)(2)	+	-	-	-	-	-	-	4	0	0
Margarodes (BOLD: 0 spp. / COL: 30 spp.)										
Margarodes prieskaensis (Jakubski, 1965)(1)	-	+	-	-	-	-	+	0	0	0
Margarodes vitis (Philippi, 1884)(1)	-	+	-	-	-	-	+	0	0	0
-listed under synonym Sphaeraspis vitis Jakubski, 1965	+	-	-	-	-	-	-	0	0	0
Margarodes vredendalensis (De, 1983)(1)	-	+	-	-	-	-	+	0	0	0

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Matsucoccus (BOLD: 0 spp. / COL: 40 spp.)										
Matsucoccus feytaudi Ducasse, 1941(1)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Miridae										
Creontiades (BOLD: 0 spp. / COL: 3 spp.)										
Creontiades pallidus (Rambur, 1839)(2)	+	-	-	-	-	-	-	0	0	0
Distantiella (BOLD: 0 spp. / COL: 0 spp.)										
Distantiella theobroma*	+	-	-	-	-	-	-	0	0	0
Eurystylus (BOLD: 0 spp. / COL: 0 spp.)										
Eurystylus oldi*	+	-	-	-	-	-	-	0	0	0
Helopeltis (BOLD: 4 spp. / COL: 0 spp.)										
Helopeltis antonii*	+	-	-	-	-	-	-	0	0	0
Helopeltis bergrothi*	+	-	-	-	-	-	-	0	0	0
Helopeltis bradyi*	+	-	-	-	-	-	-	0	0	0
Helopeltis schoutedeni*	+	-	-	-	-	-	-	6	0	0
Helopeltis theivora*	+	-	-	-	-	-	-	0	0	0
Lygus (BOLD: 48 spp. / COL: 34 spp.)										
Lygus lineolaris (Palisot, 1818)(1)	+	-	-	-	-	-	+	242	22	2
Lygus pratensis (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	5	0	0
Nesidiocoris (BOLD: 0 spp. / COL: 1 spp.)										
Nesidiocoris tenuis (Reuter, 1895)(1)	+	-	-	-	-	-	-	0	0	0
Sahlbergella (BOLD: 0 spp. / COL: 0 spp.)										
Sahlbergella singularis*	+	-	-	-	-	-	-	0	0	0
Taylorilygus (BOLD: 1 spp. / COL: 3 spp.)										
Taylorilygus apicalis (Fieber, 1861)(1)	+	-	-	-	-	-	-	4	0	0
Insignorthezia (BOLD: 0 spp. / COL: 10 spp.)										
Insignorthezia insignis (Browne, 1887)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Orthezia insignis Browne, 1887	+	-	-	-	-	-	-	5	0	0
Hemiptera: Oxycarenidae										
Oxycarenus (BOLD: 5 spp. / COL: 0 spp.)										
Oxycarenus hyalinipennis*	+	-	-	-	+	-	-	25	0	0
Hemiptera: Pentatomidae										
Antestiopsis (BOLD: 0 spp. / COL: 0 spp.)										
Antestiopsis intricata*	+	-	-	-	-	-	-	0	0	0
Antestiopsis orbitalis*	+	-	-	-	-	-	-	0	0	0
Bagrada (BOLD: 0 spp. / COL: 0 spp.)										
Bagrada hilaris*	+	-	-	-	-	-	-	0	0	0
Bathycoelia (BOLD: 0 spp. / COL: 0 spp.)										
Bathycoelia thalassina*	+	-	-	-	-	-	-	0	0	0
Halyomorpha (BOLD: 2 spp. / COL: 0 spp.)										
Halyomorpha halys (Stl, 1855)(2)	-	-	-	-	+	-	-	109	2	2
Nezara (BOLD: 1 spp. / COL: 1 spp.)										
Nezara viridula (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	26	10	10
Tibraca (BOLD: 1 spp. / COL: 0 spp.)										

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Tibraca limbativentris*	-	-	-	-	+	-	-	1	0	0
Hemiptera: Phylloxeridae										
Daktulosphaera (BOLD: 0 spp. / COL: 1 spp.)										
Daktulosphaera vitifoliae (Fitch, 1851)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Daktulosphaira vitifoliae (Fitch, 1851)	-	-	-	-	-	+	-	14	14	14
-listed under synonym Viteus vitifoliae (Fitch, 1851)	+	-	+	-	-	+	+	3	3	3
Hemiptera: Piesmidae										
Piesma (BOLD: 4 spp. / COL: 8 spp.)										
Piesma quadratum Fieber(2)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Plataspidae										
Megacopta (BOLD: 1 spp. / COL: 0 spp.)										
Megacopta cribraria (Fabricius, 1798)(2)	-	-	-	-	+	-	-	2	0	0
Hemiptera: Pseudococcidae										
Antonina (BOLD: 0 spp. / COL: 34 spp.)										
Antonina graminis (Maskell, 1897)(1)	+	-	-	-	-	-	-	0	0	0
Brevennia (BOLD: 0 spp. / COL: 6 spp.)										
Brevennia rehi (Lindinger, 1943)(1)	+	-	-	-	-	-	-	0	0	0
Dysmicoccus (BOLD: 4 spp. / COL: 110 spp.)										
Dysmicoccus boninsis (Kuwana, 1909)(1)	+	-	-	-	-	-	-	0	0	0
Dysmicoccus brevipes (Cockerell, 1893)(1)	+	-	-	-	-	-	-	12	0	0
Exallomochlus (BOLD: 0 spp. / COL: 0 spp.)										
Exallomochlus hispidus*	-	-	-	-	+	-	-	0	0	0
Ferrisia (BOLD: 2 spp. / COL: 10 spp.)										
Ferrisia virgata (Cockerell, 1893)(1)	+	-	-	-	-	-	-	4	2	2
Geococcus (BOLD: 0 spp. / COL: 7 spp.)										
Geococcus coffeae Green, 1933(1)	+	-	-	-	-	-	-	0	0	0
Hypogeococcus (BOLD: 1 spp. / COL: 11 spp.)										
Hypogeococcus pungens Granara de Willink, 1981(1)	-	-	-	-	+	-	-	0	0	0
Maconellicoccus (BOLD: 2 spp. / COL: 8 spp.)										
Maconellicoccus hirsutus (Green, 1908)(1)	+	+	-	-	+	-	+	5	1	1
Nipaecoccus (BOLD: 1 spp. / COL: 43 spp.)										
Nipaecoccus nipae (Maskell, 1893)(1)	+	-	-	-	-	-	-	0	0	0
Nipaecoccus viridis (Newstead, 1894)(1)	+	-	-	-	-	-	-	8	0	0
Paracoccus (BOLD: 2 spp. / COL: 80 spp.)										
Paracoccus marginatus Williams & Granara de Willink, 1992(1)	+	-	-	-	-	-	-	8	0	0
Phenacoccus (BOLD: 7 spp. / COL: 170 spp.)										
Phenacoccus madeirensis Green, 1923(1)	+	-	-	-	-	-	-	10	0	0
Phenacoccus manihoti Matile-Ferrero, 1977(1)	+	-	-	+	-	-	-	4	0	0
Phenacoccus parvus Morrison, 1924(1)	+	-	-	-	-	-	-	1	0	0
Planococcoides (BOLD: 0 spp. / COL: 15 spp.)										
Planococcoides njalensis (Laing, 1929)(1)	+	-	-	-	-	-	-	0	0	0

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Planococcus (BOLD: 6 spp. / COL: 39 spp.)										
Planococcus citri (Risso, 1813)(1)	+	-	-	-	-	-	-	17	1	1
Planococcus ficus (Signoret, 1875)(1)	-	-	-	-	+	-	-	10	1	1
Planococcus kenyae (Le Pelley, 1935)(1)	+	-	-	-	-	-	-	0	0	0
Planococcus lilacinus (Cockerell, 1905)(1)	+	-	-	-	+	-	-	8	1	1
Planococcus minor (Maskell, 1897)(1)	-	-	-	-	+	-	-	11	1	1
Pseudococcus (BOLD: 10 spp. / COL: 161 spp.)										
Pseudococcus calceolariae (Maskell, 1879)(1)	+	-	-	-	-	-	-	7	0	0
Pseudococcus comstocki (Kuwana, 1902)(1)	+	-	-	-	-	-	-	18	0	0
Pseudococcus longispinus (Targioni Tozzetti, 1867)(1)	+	-	-	-	-	-	-	14	0	0
Pseudococcus maritimus (Ehrhorn, 1900)(1)	+	-	-	-	-	-	-	0	0	0
Rastrococcus (BOLD: 0 spp. / COL: 22 spp.)										
Rastrococcus iceryoides (Green, 1908)(1)	+	-	-	-	-	-	-	0	0	0
Rastrococcus invadens Williams, 1986(1)	+	-	-	-	-	-	-	0	0	0
Rhizoecus (BOLD: 0 spp. / COL: 123 spp.)										
Rhizoecus americanus (Hambleton, 1946)(1)	+	-	-	-	-	-	-	0	0	0
Rhizoecus hibisci Kawai & Takagi, 1971(1)	+	+	-	-	-	-	+	0	0	0
Saccharicoccus (BOLD: 0 spp. / COL: 1 spp.)										
Saccharicoccus sacchari (Cockerell, 1895)(1)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Psyllidae										
Acizzia (BOLD: 1 spp. / COL: 42 spp.)										
Acizzia jamatonica (Kuwayama, 1908)(1)	+	-	-	-	-	-	-	0	0	0
Cacopsylla (BOLD: 7 spp. / COL: 168 spp.)										
Cacopsylla mali (Schmidberger, 1836)(1)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Psylla mali (Schmidberger, 1836)	+	-	-	-	-	-	-	0	0	0
Cacopsylla pyri (Linné, 1761)(1)	+	-	-	-	-	-	-	1	1	1
Cacopsylla pyricola (Foerster, 1848)(1)	+	-	-	-	-	-	-	0	1	1
Ctenarytaina (BOLD: 1 spp. / COL: 12 spp.)										
Ctenarytaina spatulata Taylor, 1997(1)	+	-	-	-	-	-	+	0	0	0
Diaphorina (BOLD: 1 spp. / COL: 68 spp.)										
Diaphorina citri Kuwayama, 1908(1)	+	+	-	-	+	-	+	211	210	210
Heteropsylla (BOLD: 0 spp. / COL: 18 spp.)										
Heteropsylla cubana Crawford, 1914(1)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Pyrrhocoridae										
Dysdercus (BOLD: 20 spp. / COL: 9 spp.)										
Dysdercus cingulatus (Fabricius, 1775)(2)	+	-	-	-	-	-	-	3	3	3
Dysdercus fasciatus*	+	-	-	-	-	-	-	0	0	0
Dysdercus koenigii*	+	-	-	-	-	-	-	1	1	1
Dysdercus sidae Montrouzier, 1861(2)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Scutelleridae										

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Eurygaster (BOLD: 3 spp. / COL: 6 spp.)										
Eurygaster austriaca (Schrank, 1776)(2)	+	-	-	-	-	-	-	0	0	0
Eurygaster integriceps*	+	-	-	-	-	-	-	0	0	0
Hemiptera: Tettigometridae										
Hilda (BOLD: 1 spp. / COL: 15 spp.)										
Hilda patruelis (Stål, 1855)(1)	+	-	-	-	-	-	-	0	0	0
Hemiptera: Tingidae										
Corythucha (BOLD: 23 spp. / COL: 50 spp.)										
Corythucha arcuata (Say, 1832)(1)	+	-	-	-	-	-	-	0	0	0
Stephanitis (BOLD: 5 spp. / COL: 5 spp.)										
Stephanitis pyri (Fabricius, 1775)(1)	+	-	-	-	-	-	-	0	0	0
Stephanitis takeyai Drake and Maa, 1955(1)	+	-	-	-	-	-	-	3	3	0
Stephanitis typica (Distant, 1903)(2)	+	-	-	-	-	-	-	0	0	0
Urentius (BOLD: 0 spp. / COL: 0 spp.)										
Urentius hystricellus*	+	-	-	-	-	-	-	0	0	0
Hemiptera: Triozidae										
Trioza (BOLD: 13 spp. / COL: 346 spp.)										
Trioza erytreae (Del Guercio, 1918)(1)	+	+	-	-	+	-	+	0	0	0
Hymenoptera: Apidae										
Apis (BOLD: 17 spp. / COL: 7 spp.)										
Apis mellifera capensis*	-	-	-	+	-	-	-	0	0	0
Apis mellifera european*	-	-	-	-	+	-	-	0	0	0
Apis mellifera scutellata*	-	-	-	+	+	-	-	50	0	0
Hymenoptera: Cephidae										
Cephus (BOLD: 10 spp. / COL: 2 spp.)										
Cephus cinctus Norton(1)	+	-	-	-	-	-	-	7	2	2
Cephus pygmeus (Linnaeus, 1767)(2)	+	-	-	-	-	-	-	13	1	1
Hymenoptera: Chrysididae										
Chrysis (BOLD: 79 spp. / COL: 8 spp.)	-	-	-	+	-	-	-			
Hymenoptera: Cynipidae										
Callirhytis (BOLD: 2 spp. / COL: 2 spp.)										
Callirhytis cornigera*	-	-	-	-	+	-	-	0	0	0
Dryocosmus (BOLD: 7 spp. / COL: 1 spp.)										
Dryocosmus kuriphilus Yasumatsu, 1951(1)	+	-	+	+	-	-	+	1	1	1
Hymenoptera: Diprionidae										
Gilpinia (BOLD: 8 spp. / COL: 1 spp.)										
Gilpinia hercyniae (Hartig)(1)	+	-	-	-	-	-	+	4	0	0
Gilpinia polytoma (Hartig, 1834)(2)	+	-	-	-	-	-	-	3	0	0
Neodiprion (BOLD: 79 spp. / COL: 14 spp.)										
Neodiprion sertifer (Geoffroy)(1)	+	-	-	-	+	-	-	6	4	4
Hymenoptera: Eulophidae										
Leptocybe (BOLD: 8 spp. / COL: 1 spp.)										
Leptocybe invasa Fisher & La Salle,	+	-	-	-	-	-	+	0	0	0

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2004(1)										
Quadrastichus (BOLD: 9 spp. / COL: 46 spp.)										
Quadrastichus erythrinae Kim, 2004(1)	+	-	-	-	+	-	-	1	1	1
Hymenoptera: Eurytomidae										
Bruchophagus (BOLD: 5 spp. / COL: 108 spp.)										
Bruchophagus roddi Gussakovskiy, 1933(1)	+	-	-	-	-	-	-	0	0	0
Hymenoptera: Formicidae										
Anoplolepis (BOLD: 6 spp. / COL: 22 spp.)										
Anoplolepis gracilipes (Smith, 1857)(1)	-	-	-	-	+	-	-	55	25	3
Atta (BOLD: 8 spp. / COL: 17 spp.)										
Atta cephalotes (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	1	1	0
Linepithema (BOLD: 19 spp. / COL: 16 spp.)										
Linepithema humile (Mayr, 1868)(1)	+	-	-	-	+	-	-	74	28	22
Myrmica (BOLD: 116 spp. / COL: 141 spp.)										
Myrmica rubra (Linnaeus, 1758)(1)	-	-	-	-	+	-	-	72	64	61
Nylanderia (BOLD: 94 spp. / COL: 0 spp.)										
Nylanderia fulva*	-	-	-	-	+	-	-	0	0	0
Pogonomyrmex (BOLD: 26 spp. / COL: 60 spp.)										
Pogonomyrmex occidentalis (Cresson, 1865)(1)	-	-	-	-	-	+	-	3	2	2
Solenopsis (BOLD: 127 spp. / COL: 195 spp.)										
Solenopsis geminata (Fabricius, 1804)(1)	+	-	-	-	+	-	-	96	27	0
Solenopsis invicta Buren, 1972(1)	-	-	-	+	+	-	-	6	1	0
Solenopsis richteri Forel, 1909(1)	-	-	-	+	+	-	-	3	0	0
Solenopsis saevissima (Smith, 1855)(1)	+	-	-	-	-	-	-	0	0	0
Wasmannia (BOLD: 5 spp. / COL: 10 spp.)										
Wasmannia auropunctata (Roger, 1863)(1)	-	-	-	-	+	-	-	108	90	90
Hymenoptera: Megachilidae										
Coelioxys (BOLD: 111 spp. / COL: 500 spp.)	-	-	-	+	-	-	-			
Hymenoptera: Mymaridae										
Anaphes (BOLD: 15 spp. / COL: 206 spp.)										
Anaphes flavipes (Forster, 1841)(2)	-	-	-	-	+	-	-	0	0	0
Hymenoptera: Siricidae										
Sirex (BOLD: 19 spp. / COL: 1 spp.)										
Sirex ermak (Semenov, 1921)(2)	-	-	+	-	-	-	+	1	0	0
Sirex noctilio Fabricius, 1773(2)	+	-	-	-	+	+	-	17	1	1
Tremex (BOLD: 3 spp. / COL: 1 spp.)										
Tremex fuscicornis (Fabricius, 1787)(2)	-	-	-	-	+	-	-	3	0	0
Urocerus (BOLD: 8 spp. / COL: 0 spp.)										
Urocerus gigas gigas*	-	-	-	-	+	-	-	3	2	1
Hymenoptera: Tenthredinidae										
Caliroa (BOLD: 17 spp. / COL: 2 spp.)										
Caliroa cerasi (Linnaeus)(1)	+	-	-	-	-	-	-	2	0	0
Caliroa quercuscoccineae (Dyar)(1)	-	-	-	-	+	-	-	0	0	0

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Hoplocampa (BOLD: 15 spp. / COL: 2 spp.)										
Hoplocampa brevis (Klug, 1816)(2)	+	-	-	-	-	-	-	1	0	0
Hoplocampa flava (Linnaeus, 1761)(2)	+	-	-	-	-	-	-	0	0	0
Hoplocampa minuta (Christ, 1791)(2)	+	-	-	-	-	-	-	2	0	0
Hoplocampa testudinea (Klug)(1)	+	-	-	-	-	-	-	3	0	0
Monsoma (BOLD: 1 spp. / COL: 0 spp.)										
Monsoma pulveratum (Retzius, 1783)(2)	-	-	-	-	+	-	-	10	0	0
Pristiphora (BOLD: 74 spp. / COL: 3 spp.)										
Pristiphora erichsonii (Hartig)(1)	+	-	-	-	-	-	-	2	2	1
Hymenoptera: Torymidae										
Megastigmus (BOLD: 18 spp. / COL: 127 spp.)										
Megastigmus spermotrophus Wachtl, 1893(1)	+	-	-	-	-	-	-	0	0	0
Hymenoptera: Trichogrammatidae										
Trichogramma (BOLD: 11 spp. / COL: 173 spp.)										
Trichogramma ostriniae Pang & Chen, 1974(1)	-	-	-	-	+	-	-	1	1	1
Isoptera: Kalotermitidae										
Cryptotermes (BOLD: 2 spp. / COL: 7 spp.)										
Cryptotermes brevis (Walker, 1853)(1)	+	-	-	-	-	-	-	1	0	0
Isoptera: Rhinotermitidae										
Coptotermes (BOLD: 7 spp. / COL: 4 spp.)										
Coptotermes formosanus Shiraki, 1909(1)	+	-	-	-	+	-	-	4	0	0
Reticulitermes (BOLD: 6 spp. / COL: 6 spp.)										
Reticulitermes flavipes (Kollar, 1837)(1)	+	-	-	-	-	-	-	10	0	0
Lepidoptera: Acrolepiidae										
Acrolepiopsis (BOLD: 11 spp. / COL: 0 spp.)										
Acrolepiopsis assectella (Zeller, 1839)(2)	+	-	-	+	+	-	-	27	14	0
Lepidoptera: Carposinidae										
Carposina (BOLD: 22 spp. / COL: 138 spp.)										
Carposina niponensis Walsingham, 1900(1)	-	-	-	+	-	-	+	7	0	0
Carposina sasakii Matsumura, 1900(1)	+	-	+	-	-	+	-	0	0	0
Lepidoptera: Castniidae										
Paysandisia (BOLD: 1 spp. / COL: 1 spp.)										
Paysandisia archon Burmeister, 1880(1)	+	-	+	-	-	-	+	3	1	0
Lepidoptera: Cossidae										
Chilecomadia (BOLD: 6 spp. / COL: 3 spp.)										
Chilecomadia valdiviana Philippi, 1860(1)	-	-	-	-	+	-	-	7	0	0
Dyspessa (BOLD: 4 spp. / COL: 44 spp.)										
Dyspessa ulula Borkhausen, 1790(1)	-	-	-	+	-	-	-	24	1	0
Eulophonotus (BOLD: 0 spp. / COL: 2 spp.)										
Eulophonotus myrmeleon Felder, 1874(1)	+	-	-	-	-	-	-	0	0	0
Zeuzera (BOLD: 8 spp. / COL: 49 spp.)										
Zeuzera coffeae Nietner, 1861(1)	+	-	-	-	-	-	-	3	0	0

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Zeuzera pyrina Linnaeus, 1761(1)	+	-	-	-	-	-	-	15	4	0
Lepidoptera: Crambidae										
Antigastra (BOLD: 1 spp. / COL: 2 spp.)										
Antigastra catalaunalis Duponchel, 1833(1)	+	-	-	-	-	-	-	10	0	0
Chilo (BOLD: 16 spp. / COL: 63 spp.)										
Chilo agamemnon Bleszynski, 1962(1)	+	-	-	-	-	-	-	0	0	0
Chilo auricilius Dudgeon, 1905(2)	+	-	-	-	-	-	-	0	0	0
Chilo infuscatellus Snellen, 1890(1)	+	-	-	-	-	-	-	5	0	0
Chilo partellus Swinhoe, 1885(1)	+	-	-	-	-	-	-	19	0	0
Chilo sacchariphagus Bojer, 1856(1)	+	-	-	-	-	-	-	1	0	0
Chilo suppressalis Walker, 1863(1)	+	-	-	+	+	-	-	36	55	54
Conogethes (BOLD: 20 spp. / COL: 0 spp.)										
Conogethes punctiferalis (Guenee, 1854)(2)	-	-	-	+	+	+	-	68	0	0
Cnaphalocrocis (BOLD: 13 spp. / COL: 37 spp.)										
Cnaphalocrocis medinalis Guenée, 1854(1)	+	-	-	-	-	-	-	122	0	0
Crocidolomia (BOLD: 8 spp. / COL: 7 spp.)										
Crocidolomia binotalis Zeller, 1852(1)	+	-	-	-	+	-	-	10	0	0
Diaphania (BOLD: 88 spp. / COL: 0 spp.)										
Diaphania nitidalis*	+	-	-	-	-	-	-	8	0	0
Diatraea (BOLD: 8 spp. / COL: 61 spp.)										
Diatraea lineolata Walker, 1856(1)	+	-	-	-	-	-	-	0	0	0
Diatraea saccharalis Fabricius, sensu Guenée, 1862(1)	+	-	-	-	-	-	-	3	1	1
Dichocrocis (BOLD: 14 spp. / COL: 80 spp.)										
Dichocrocis punctiferalis Guenée, 1854(1)	-	-	-	-	-	+	-	0	0	0
Duponchelia (BOLD: 1 spp. / COL: 4 spp.)										
Duponchelia fovealis Zeller, 1847(1)	+	-	-	-	+	-	-	35	0	0
Eoreuma (BOLD: 6 spp. / COL: 8 spp.)										
Eoreuma loftini Dyar, 1917(1)	-	-	-	-	+	-	-	5	0	0
Hellula (BOLD: 8 spp. / COL: 11 spp.)										
Hellula phidilealis Walker, 1859(1)	+	-	-	-	-	-	-	13	0	0
Hellula undalis Fabricius, 1781(1)	+	-	-	-	-	-	-	28	0	0
Lamprosema (BOLD: 49 spp. / COL: 261 spp.)										
Lamprosema diemenalis*	+	-	-	-	-	-	-	0	0	0
Leucinodes (BOLD: 5 spp. / COL: 19 spp.)										
Leucinodes orbonalis Guenée, 1854(1)	+	-	-	-	-	-	-	19	0	0
Maruca (BOLD: 15 spp. / COL: 4 spp.)										
Maruca vitrata Fabricius, 1787(1)	+	-	-	+	+	-	-	100	4	0
Nacoleia (BOLD: 27 spp. / COL: 0 spp.)										
Nacoleia octasema*	+	-	-	-	-	-	-	11	0	0
Neoleucinodes (BOLD: 14 spp. / COL: 5 spp.)										
Neoleucinodes elegantalis Guenée, 1854(1)	-	-	-	-	+	-	-	3	0	0
Omphisa (BOLD: 1 spp. / COL: 10 spp.)										

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Omphisa anastomosalis Guenée, 1854(1)	-	-	-	+	-	-	-	0	0	0
Ostrinia (BOLD: 18 spp. / COL: 21 spp.)										
Ostrinia furnacalis Guenée, 1854(1)	+	-	-	-	+	-	-	61	15	15
Ostrinia nubilalis Hübner(1)	+	-	-	-	+	+	-	159	57	46
Parapoynx (BOLD: 27 spp. / COL: 26 spp.)										
Parapoynx stagnalis Zeller, 1852(1)	+	-	-	-	-	-	-	5	0	0
Scirpophaga (BOLD: 13 spp. / COL: 41 spp.)										
Scirpophaga incertulas Walker, 1863(1)	+	-	-	-	-	-	-	23	10	10
Scirpophaga innotata Walker, 1863(1)	+	-	-	-	-	-	-	1	1	1
Spoladea (BOLD: 3 spp. / COL: 2 spp.)										
Spoladea recurvalis Fabricius, 1775(1)	+	-	-	-	-	-	-	203	0	0
Sylepta (BOLD: 3 spp. / COL: 0 spp.)										
Sylepta derogata*	+	-	-	-	-	-	-	0	0	0
Lepidoptera: Elaschistidae										
Stenoma (BOLD: 161 spp. / COL: 681 spp.) Stenoma catenifer Walsingham, 1912(1)	-	-	-	+	-	-	-	2	0	0
Lepidoptera: Erebidae										
Achaea (BOLD: 23 spp. / COL: 0 spp.)										
Achaea janata (Linnaeus)(2)	+	-	-	-	-	-	-	44	0	0
Alabama (BOLD: 1 spp. / COL: 0 spp.)										
Alabama argillacea (Hubner)(2)	+	-	-	-	-	-	-	3	0	0
Anomis (BOLD: 52 spp. / COL: 127 spp.)										
Anomis flava Fabricius, 1775(1)	+	-	-	-	-	-	-	71	2	0
Anomis sabulifera Guenée, 1852(1)	+	-	-	-	-	-	-	1	0	0
Eudocima (BOLD: 31 spp. / COL: 2 spp.)										
Eudocima fullonia (Clerck, 1764)(2)	+	-	-	-	+	-	-	24	0	0
Euproctis (BOLD: 99 spp. / COL: 544 spp.)										
Euproctis chrysorrhoea Linnaeus, 1758(1)	+	-	-	-	-	+	-	45	3	0
Euproctis similis Moore, 1879(1)	+	-	-	-	-	-	-	17	2	0
Hyphantria (BOLD: 1 spp. / COL: 5 spp.)										
Hyphantria cunea Drury, 1770(1)	+	-	-	-	+	-	-	185	69	14
Leucoma (BOLD: 5 spp. / COL: 37 spp.)										
Leucoma salicis Linnaeus, 1758(1)	-	-	-	-	+	-	-	86	3	0
Lymantria (BOLD: 70 spp. / COL: 145 spp.)										
Lymantria dispar asiatica Vunkovskij?, 1926(1)	-	-	-	-	+	-	-	216	30	26
Lymantria dispar Linnaeus, 1758(1)	+	-	-	+	+	+	-	216	30	26
Lymantria mathura Moore, 1865(1)	-	-	+	-	+	+	+	26	4	4
Lymantria monacha Linnaeus, 1758(1)	+	-	-	-	+	+	-	75	35	29
Mocis (BOLD: 22 spp. / COL: 0 spp.)										
Mocis frugalis (Fabricius, 1775)(2)	+	-	-	-	-	-	-	54	0	0
Mocis repanda (Fabricius, 1794)(2)	+	-	-	-	-	-	-	0	0	0

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Orgyia (BOLD: 42 spp. / COL: 43 spp.)										
Orgyia anartoides Walker, 1855(1)	-	-	-	-	-	+	-	0	0	0
Orgyia postica Walker, 1855(1)	+	-	-	-	-	-	-	5	0	0
Orgyia pseudotsugata*	+	+	-	-	-	-	+	155	2	2
Teia (BOLD: 4 spp. / COL: 0 spp.)										
Teia anartoides Walker, 1855(2)	-	-	-	-	-	+	-	17	4	4
Lepidoptera: Euteliidae										
Penicillaria (BOLD: 4 spp. / COL: 14 spp.)										
Penicillaria jocosatrix Guenée, 1852(1)	+	-	-	-	-	-	-	6	0	0
Lepidoptera: Gelechiidae										
Anarsia (BOLD: 37 spp. / COL: 84 spp.)										
Anarsia lineatella Zeller, 1839(1)	+	-	-	-	-	-	-	27	1	0
Pectinophora (BOLD: 3 spp. / COL: 4 spp.)										
Pectinophora gossypiella Saunders, 1843(1)	+	-	-	+	+	-	-	9	0	0
Pectinophora scutigera Holdaway, 1926(1)	+	-	-	+	-	-	-	2	0	0
Phthorimaea (BOLD: 1 spp. / COL: 16 spp.)										
Phthorimaea operculella Zeller, 1873(1)	+	-	-	-	+	-	-	39	7	7
Scrobipalpa (BOLD: 114 spp. / COL: 250 spp.)										
Scrobipalpa heliopa Lower, 1900(1)	+	-	-	-	-	-	-	0	0	0
Scrobipalpa ocellatella Boyd, 1858(1)	+	-	-	-	-	-	-	0	0	0
Tecia (BOLD: 0 spp. / COL: 9 spp.)										
Tecia solanivora Povolny, 1973(1)	+	-	+	-	-	-	+	0	0	0
Tuta (BOLD: 8 spp. / COL: 1 spp.)										
Tuta absoluta (Meyrick, 1917)(2)	+	-	+	-	+	+	+	1	0	0
Lepidoptera: Geometridae										
Erannis (BOLD: 12 spp. / COL: 14 spp.)										
Erannis defoliaria Clerck, 1764(1)	-	-	-	-	+	-	-	53	10	1
Lambdina (BOLD: 14 spp. / COL: 21 spp.)										
Lambdina fiscellaria Guenée, 1858(1)	-	-	-	-	+	-	-	144	29	2
Operophtera (BOLD: 9 spp. / COL: 14 spp.)										
Operophtera bruceata Hulst, 1886(1)	-	-	-	-	+	-	-	146	105	1
Operophtera brumata Linnaeus, 1758(1)	+	-	-	-	+	+	-	125	18	2
Paleacrita (BOLD: 3 spp. / COL: 3 spp.)										
Paleacrita vernata Peck, 1795(1)	-	-	-	-	+	-	-	90	79	0
Lepidoptera: Gracillariidae										
Acrocercops (BOLD: 41 spp. / COL: 336 spp.)										
Acrocercops cramerella Snellen, 1904(2)	+	-	-	-	-	-	-	0	0	0
Caloptilia (BOLD: 108 spp. / COL: 325 spp.)										
Caloptilia azaleella (Brants, 1913)(1)	+	-	-	-	-	-	-	15	0	0
Caloptilia syringella Fabricius, 1794(2)	+	-	-	-	-	-	-	13	6	0
Cameraria (BOLD: 37 spp. / COL: 72 spp.)										
Cameraria ohridella Deschka & Dimic, 1986(1)	+	-	-	-	-	-	-	555	537	0
Conopomorpha (BOLD: 3 spp. / COL: 13 spp.)										

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Conopomorpha cramerella (Snellen, 1904)(1)	-	-	-	+	-	-	-	93	92	92
Phyllocnistis (BOLD: 25 spp. / COL: 90 spp.)										
Phyllocnistis citrella Stainton, 1856(1)	+	-	-	-	-	-	-	23	2	2
Lepidoptera: Hesperiidae										
Erionota (BOLD: 1 spp. / COL: 7 spp.)										
Erionota thrax Linnaeus, 1767(1)	+	-	-	-	-	-	-	1	0	0
Lepidoptera: Hyblaeidae										
Hyblaea (BOLD: 31 spp. / COL: 39 spp.)										
Hyblaea puera Cramer, 1777(1)	+	-	-	-	-	-	-	81	2	0
Lepidoptera: Lasiocampidae										
Dendrolimus (BOLD: 37 spp. / COL: 34 spp.)										
Dendrolimus pini Linnaeus, 1767(1)	-	-	-	-	+	-	-	40	11	0
Dendrolimus spectabilis Butler, 1877(1)	+	-	-	-	-	-	-	2	0	0
Dendrolimus superans Butler, 1877(1)	-	-	-	-	-	-	-	13	6	6
-listed under synonym Dendrolimus sibiricus Tschetverikov, 1908	-	-	+	-	-	-	+	7	6	6
Dendrolimus superans sibiricus (Tschetverikov, 1908)(2)	+	-	-	-	-	-	-	8	0	0
Malacosoma (BOLD: 40 spp. / COL: 25 spp.)										
Malacosoma americanum (Fabricius)(2)	+	+	-	-	-	-	+	0	0	0
Malacosoma disstria Hübner, 1822(1)	+	+	-	-	+	-	+	104	24	0
Malacosoma parallela Staudinger, 1887(1)	-	-	+	-	-	-	+	0	0	0
Malacosoma parallelum*	+	-	-	-	-	-	-	0	0	0
Lepidoptera: Limacodidae										
Darna (BOLD: 3 spp. / COL: 5 spp.)										
Darna pallivitta (Moore, 1877)(2)	-	-	-	-	+	-	-	1	0	0
Parasa (BOLD: 76 spp. / COL: 132 spp.)										
Parasa lepida Cramer, 1779(1)	+	-	-	-	-	-	-	1	0	0
Setora (BOLD: 8 spp. / COL: 7 spp.)										
Setora nitens Walker, 1855(1)	+	-	-	-	-	-	-	1	0	0
Lepidoptera: Lycaenidae										
Cacyreus (BOLD: 4 spp. / COL: 7 spp.)										
Cacyreus marshalli Butler, 1897(1)	+	-	+	-	-	-	+	22	2	2
Lampides (BOLD: 1 spp. / COL: 68 spp.)										
Lampides boeticus Linnaeus, 1767(1)	+	-	-	+	-	-	-	127	64	61
Lepidoptera: Lyonetiidae										
Leucoptera (BOLD: 16 spp. / COL: 75 spp.)										
Leucoptera caffeina Washburn, 1940(1)	+	-	-	-	-	-	-	0	0	0
Leucoptera coma Ghesquière, 1940(1)	+	-	-	-	-	-	-	0	0	0
Leucoptera malifoliella (O. Costa, 1836)(2)	-	-	-	+	+	+	-	4	0	0
Leucoptera meyricki Ghesquière, 1940(1)	+	-	-	-	-	-	-	0	0	0
Perileucoptera (BOLD: 0 spp. / COL: 1 spp.)										
Perileucoptera coffeella Guérin-Méneville, 1842(1)	+	-	-	-	-	-	-	0	0	0
Lepidoptera: Noctuidae										

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Agrotis (BOLD: 79 spp. / COL: 253 spp.)										
Agrotis ipsilon Hufnagel, 1766(1)	+	-	-	-	+	-	-	185	36	1
Agrotis segetum Schiffermüller, 1775(1)	+	-	-	-	-	-	-	46	4	0
Argyrogramma (BOLD: 9 spp. / COL: 6 spp.)										
Argyrogramma signata Fabricius, 1792(1)	+	-	-	-	-	-	-	13	0	0
Autographa (BOLD: 26 spp. / COL: 44 spp.)										
Autographa gamma Linnaeus, 1758(1)	-	-	-	-	+	-	-	42	6	0
Busseola (BOLD: 3 spp. / COL: 16 spp.)										
Busseola fusca Fuller, 1901(1)	+	-	-	-	-	-	-	8	9	9
Chrysodeixis (BOLD: 13 spp. / COL: 22 spp.)										
Chrysodeixis acuta Walker, 1857(1)	+	-	-	-	-	-	-	51	7	0
Chrysodeixis chalcites Esper, 1789(1)	+	-	-	-	+	-	-	23	0	0
Chrysodeixis eriosoma Doubleday, 1843(1)	+	-	-	-	-	-	-	50	0	0
Copitarsia (BOLD: 5 spp. / COL: 8 spp.)	-	-	-	-	+	-	-			
Diparopsis (BOLD: 3 spp. / COL: 4 spp.)										
Diparopsis castanea Hampson, 1902(1)	+	-	-	-	-	-	-	1	0	0
Diparopsis tephragramma Bethune-Baker, 1911(1)	+	-	-	-	-	-	-	0	0	0
Diparopsis watersi Rothschild, 1901(1)	+	-	-	-	-	-	-	0	0	0
Helicoverpa (BOLD: 15 spp. / COL: 20 spp.)										
Helicoverpa armigera Hübner, 1827(1)	+	-	+	-	+	-	+	148	12	2
Helicoverpa assulta Guenée, 1852(1)	+	-	-	-	-	-	-	11	1	1
Helicoverpa punctigera Wallengren, 1860(1)	+	-	-	-	-	-	-	136	2	2
Helicoverpa zea Boddie, 1850(1)	+	+	-	-	-	-	+	113	11	1
Heliothis (BOLD: 29 spp. / COL: 0 spp.)										
Heliothis virescens*	+	-	-	-	-	-	-	36	13	1
Leucania (BOLD: 97 spp. / COL: 219 spp.)										
Leucania venalba Moore, 1867(1)	+	-	-	-	-	-	-	11	0	0
Loxagrotis (BOLD: 0 spp. / COL: 0 spp.)										
Loxagrotis albicosta (Smith)(2)	-	-	-	-	+	-	-	0	0	0
Macronoctua (BOLD: 1 spp. / COL: 2 spp.)										
Macronoctua onusta Grote, 1874(1)	-	-	-	-	+	-	-	8	5	0
Mamestra (BOLD: 12 spp. / COL: 15 spp.)										
Mamestra brassicae Linnaeus, 1758(1)	+	-	-	-	-	-	-	26	6	2
Mythimna (BOLD: 77 spp. / COL: 31 spp.)										
Mythimna loreyi*	+	-	-	-	-	-	-	1	0	0
Mythimna separata (Walker, 1865)(2)	+	-	-	-	-	-	-	4	0	0
Mythimna unipuncta (Haworth, 1809)(2)	+	-	-	-	-	-	-	213	28	1
Papaipema (BOLD: 40 spp. / COL: 58 spp.)										
Papaipema nebris Guenée, 1852(1)	-	-	-	-	+	-	-	13	0	0
Pericyma (BOLD: 5 spp. / COL: 12 spp.)										
Pericyma cruegeri Butler, 1886(1)	-	-	-	-	+	-	-	28	0	0
Phalaenoides (BOLD: 2 spp. / COL: 3 spp.)										
Phalaenoides glycinae Lewin, 1805(1)	-	-	-	-	-	+	-	6	0	0

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Sacadodes (BOLD: 0 spp. / COL: 1 spp.)										
Sacadodes pyralis Dyar, 1912(1)	+	-	-	-	-	-	-	0	0	0
Sesamia (BOLD: 6 spp. / COL: 51 spp.)										
Sesamia calamistis Hampson, 1910(1)	+	-	-	-	-	-	-	5	0	0
Sesamia cretica Lederer, 1857(1)	+	-	-	-	-	+	-	4	0	0
Sesamia inferens Walker, 1856(1)	+	-	-	-	-	-	-	2	0	0
Sesamia nonagrioides Lefèbvre, 1827(1)	+	-	-	-	-	-	-	8	5	5
Spodoptera (BOLD: 28 spp. / COL: 35 spp.)										
Spodoptera cilium Guenée, 1852(1)	+	-	-	-	-	-	-	30	0	0
Spodoptera eridania Stoll, 1781(1)	+	+	-	-	-	-	+	64	6	0
Spodoptera exempta Walker, 1856(1)	+	-	-	-	-	-	-	8	8	7
Spodoptera exigua Hübner, 1803/08(1)	+	-	-	-	-	-	-	269	6	1
Spodoptera frugiperda Smith & Abbot, 1797(1)	+	+	-	-	-	-	+	123	40	6
Spodoptera littoralis Boisduval, 1833(1)	+	-	+	-	+	-	+	20	0	0
Spodoptera litura Fabricius, 1775(1)	+	+	-	-	+	-	+	77	1	0
Spodoptera mauritia Boisduval, 1833(1)	+	-	-	-	-	-	-	161	0	0
Spodoptera ornithogalli Guenée, 1852(1)	+	-	-	-	-	-	-	43	8	2
Thysanoplusia (BOLD: 16 spp. / COL: 5 spp.)										
Thysanoplusia orichalcea Fabricius, 1775(1)	+	-	-	-	-	-	-	54	0	0
Tiracola (BOLD: 4 spp. / COL: 10 spp.)										
Tiracola plagiata Walker, 1857(1)	+	-	-	-	-	-	-	33	0	0
Trichoplusia (BOLD: 9 spp. / COL: 60 spp.)										
Trichoplusia ni Hübner, 1802(1)	+	-	-	-	-	-	-	52	2	0
Xestia (BOLD: 91 spp. / COL: 131 spp.)										
Xestia c-nigrum (Linnaeus, 1758)(2)	+	-	-	-	-	-	-	220	46	0
Lepidoptera: Nolidae										
Earias (BOLD: 24 spp. / COL: 49 spp.)										
Earias biplaga Walker, 1866(1)	+	-	-	-	-	-	-	40	0	0
Earias fabia Stoll, 1781(2)	-	-	-	+	-	-	-	0	0	0
Earias insulana Boisduval, 1833(1)	+	-	-	-	-	-	-	28	0	0
Earias vittella Fabricius, 1794(1)	+	-	-	-	-	-	-	16	0	0
Erschoviella (BOLD: 0 spp. / COL: 1 spp.)										
Erschoviella musculana Erschoff, 1874(1)	+	-	+	-	-	-	+	0	0	0
Lepidoptera: Notodontidae										
Thaumetopoea (BOLD: 8 spp. / COL: 13 spp.)										
Thaumetopoea pityocampa Schiffermüller, 1776(1)	+	-	-	-	-	-	+	8	1	0
Thaumetopoea processionea Linnaeus, 1758(1)	-	-	-	-	+	-	-	3	0	0
Lepidoptera: Oecophoridae										
Nephantis (BOLD: 0 spp. / COL: 1 spp.)										
Nephantis serinopa Meyrick, 1905(1)	+	-	-	-	-	-	-	0	0	0
Lepidoptera: Papilionidae										
Papilio (BOLD: 117 spp. / COL: 194 spp.)										

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Papilio demodocus Esper, 1798(1)	+	-	-	-	-	-	-	23	4	2
Papilio demoleus Linnaeus, 1758(1)	+	-	-	-	+	-	-	16	3	3
Lepidoptera: Pieridae										
Pieris (BOLD: 38 spp. / COL: 22 spp.)										
Pieris brassicae (Linnaeus, 1758)(1)	+	-	-	-	+	-	-	60	17	5
Pieris rapae (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	284	115	99
Lepidoptera: Plutellidae										
Plutella (BOLD: 16 spp. / COL: 43 spp.)										
Plutella xylostella Linnaeus, 1767(1)	+	-	-	-	-	-	-	557	240	190
Lepidoptera: Praydidae										
Prays (BOLD: 15 spp. / COL: 27 spp.)										
Prays citri Millière, 1873(1)	+	-	-	-	-	-	-	4	0	0
Prays endocarpa Meyrick, 1919(1)	+	-	-	+	-	-	-	0	0	0
Prays oleellus*	+	-	-	-	-	-	-	0	0	0
Lepidoptera: Pyralidae										
Acrobasis (BOLD: 47 spp. / COL: 84 spp.)										
Acrobasis nuxvorella Neunzig, 1970(1)	-	-	-	-	+	-	-	6	0	0
Acrobasis pyrivorella*	+	-	-	-	-	+	+	0	0	0
Cactoblastis (BOLD: 2 spp. / COL: 5 spp.)										
Cactoblastis cactorum Berg, 1885(1)	-	-	-	-	+	-	-	57	1	1
Corcyra (BOLD: 1 spp. / COL: 4 spp.)										
Corcyra cephalonica Stainton, 1866(1)	+	-	-	-	-	-	-	3	0	0
Dioryctria (BOLD: 37 spp. / COL: 66 spp.)	-	-	-	-	+	-	-			
Dioryctria abietella Denis & Schiffermüller, 1775(1)	+	-	-	-	-	-	-	31	4	3
Dioryctria zimmermani Grote, 1877(1)	-	-	-	-	+	-	-	1	1	1
Elasmopalpus (BOLD: 1 spp. / COL: 3 spp.)										
Elasmopalpus lignosellus*	+	-	-	-	-	-	-	0	0	0
Eldana (BOLD: 1 spp. / COL: 2 spp.)										
Eldana saccharina Walker, 1865(1)	+	-	-	-	-	-	-	21	21	21
Etiella (BOLD: 9 spp. / COL: 7 spp.)										
Etiella zinckenella Treitschke, 1832(1)	+	-	-	-	-	-	-	51	1	0
Hypsipyla (BOLD: 5 spp. / COL: 13 spp.)										
Hypsipyla grandella Zeller, 1848(1)	+	-	-	-	-	-	-	52	0	0
Hypsipyla robusta Moore, 1886(1)	+	-	-	-	-	-	-	6	1	1
Maliarpha (BOLD: 0 spp. / COL: 6 spp.)										
Maliarpha separatella Ragonot, 1888(1)	+	-	-	-	-	-	-	0	0	0
Mussidia (BOLD: 3 spp. / COL: 8 spp.)										
Mussidia nigrivenella Ragonot, 1888(1)	+	-	-	-	-	-	-	0	0	0
Numonia (BOLD: 0 spp. / COL: 0 spp.)										
Numonia pirivorella*	-	-	+	-	-	-	-	0	0	0
Susumia (BOLD: 0 spp. / COL: 0 spp.)										
Susumia exigua*	+	-	-	-	-	-	-	0	0	0
Lepidoptera: Saturniidae										

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Cricula (BOLD: 65 spp. / COL: 8 spp.)										
Cricula trifenestrata Helfer, 1837(1)	+	-	-	-	-	-	-	93	1	0
Lepidoptera: Sesiidae										
Melittia (BOLD: 38 spp. / COL: 105 spp.)										
Melittia cucurbitae Harris, 1828(1)	-	-	-	-	+	-	-	1	0	0
Synanthedon (BOLD: 104 spp. / COL: 202 spp.)										
Synanthedon myopaeformis (Borkhausen, 1789)(2)	-	-	-	-	+	-	-	19	2	0
Lepidoptera: Sphingidae										
Agrius (BOLD: 5 spp. / COL: 6 spp.)										
Agrius convolvuli Linnaeus, 1758(1)	+	-	-	-	-	-	-	209	13	1
Cephonodes (BOLD: 17 spp. / COL: 17 spp.)										
Cephonodes hylas Linnaeus, 1771(1)	+	-	-	-	-	-	-	11	1	0
Erinnyis (BOLD: 17 spp. / COL: 10 spp.)										
Erinnyis alope Drury, 1773(1)	+	-	-	-	-	-	-	79	76	0
Erinnyis ello Linnaeus, 1758(1)	+	-	-	-	-	-	-	85	37	0
Hyles (BOLD: 57 spp. / COL: 18 spp.)										
Hyles lineata Fabricius, 1775(1)	+	-	-	-	-	-	-	57	9	0
Manduca (BOLD: 100 spp. / COL: 71 spp.)										
Manduca sexta Linnaeus, 1763(1)	+	-	-	-	-	-	-	25	8	2
Lepidoptera: Tineidae										
Opogona (BOLD: 28 spp. / COL: 180 spp.)										
Opogona sacchari (Bojer, 1856)(1)	+	-	+	+	+	-	+	9	0	0
Lepidoptera: Tortricidae										
Acleris (BOLD: 96 spp. / COL: 243 spp.)										
Acleris gloverana (Walsingham)*	+	+	-	-	-	-	+	0	0	0
Acleris variana Fernald, 1886(1)	+	+	-	-	-	-	+	80	0	0
Acropolitis (BOLD: 17 spp. / COL: 0 spp.)										
Acropolitis rudisana Walker*	-	-	-	-	-	+	-	93	0	0
Adoxophyes (BOLD: 32 spp. / COL: 54 spp.)										
Adoxophyes orana Fischer von Röslerstamm, 1834(1)	+	-	-	+	+	+	-	16	0	0
Archips (BOLD: 40 spp. / COL: 126 spp.)										
Archips fuscocupreanus Walsingham, 1900(1)	-	-	-	-	+	-	-	0	0	0
Archips podana Scopoli, 1763(1)	-	-	-	-	+	-	-	29	1	0
Archips xylosteanus*	-	-	-	-	+	-	-	0	0	0
Argyroploce (BOLD: 8 spp. / COL: 160 spp.)										
Argyroploce schistaceana (Sn.)*	+	-	-	-	-	-	-	0	0	0
Argyrotaenia (BOLD: 58 spp. / COL: 50 spp.)										
Argyrotaenia ljungiana Thunberg, 1797(1)	-	-	-	-	-	+	-	19	1	0
Argyrotaenia pulchellana Haworth, 1811(2)	-	-	-	+	-	-	-	0	0	0
Cacoecimorpha (BOLD: 1 spp. / COL: 1 spp.)										
Cacoecimorpha pronubana Hübner, 1800(1)	+	-	+	-	-	+	+	18	0	0
Capua (BOLD: 70 spp. / COL: 156 spp.)										

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Capua tortrix*	-	-	-	+	-	-	-	0	0	0
Choristoneura (BOLD: 20 spp. / COL: 32 spp.)										
Choristoneura conflictana Walker, 1863(1)	+	+	-	-	-	-	+	121	5	0
Choristoneura fumiferana Clemens, 1865(1)	+	+	-	-	-	-	+	202	26	1
Choristoneura occidentalis Freeman, 1967(1)	-	+	-	-	-	-	+	109	23	3
Choristoneura rosaceana Harris, 1841(1)	+	+	-	-	-	-	+	366	49	0
Crocidosema (BOLD: 5 spp. / COL: 11 spp.)										
Crocidosema aporema*	-	-	-	-	+	-	-	0	0	0
Cryptophlebia (BOLD: 19 spp. / COL: 56 spp.)										
Cryptophlebia leucotreta Meyrick, 1913(1)	-	-	-	+	-	-	-	3	0	0
Cryptophlebia ombrodelta Lower, 1898(1)	+	-	-	-	-	-	-	73	0	0
Cydia (BOLD: 77 spp. / COL: 205 spp.)										
Cydia caryana Fitch, 1856(1)	-	-	-	-	+	-	-	81	13	0
Cydia funebrana (Treitsch)*	+	-	-	+	-	+	-	3	0	0
Cydia inopinata Heinrich*	-	-	+	-	-	+	+	0	0	0
Cydia latiferreana Walsingham, 1879(1)	-	-	-	-	-	+	-	104	5	0
Cydia leucostoma (Meyrick, 1912)(2)	+	-	-	-	-	-	-	0	0	0
Cydia molesta (Busck)*	+	-	-	-	-	-	-	0	0	0
Cydia nigricana Fabricius, 1794(1)	+	-	-	-	-	-	-	20	0	0
Cydia packardi (Zeller)*	-	+	-	-	-	-	+	0	0	0
Cydia pomonella Linnaeus, 1758(1)	+	-	-	-	+	+	-	136	28	20
Cydia prunivora (Walsh)*	+	+	-	-	-	-	+	0	0	0
Cydia pyrivora Danilevsky, 1947(1)	+	-	-	-	-	-	-	0	0	0
Cydia splendana Hübner, 1796(1)	-	-	-	+	-	-	-	20	1	0
Enarmonia (BOLD: 1 spp. / COL: 219 spp.)										
Enarmonia formosana Scopoli, 1763(1)	-	-	-	-	+	-	-	16	0	0
Epichoristodes (BOLD: 1 spp. / COL: 14 spp.)										
Epichoristodes acerbella Walker, 1864(1)	+	-	-	-	-	-	-	9	1	1
Epiphyas (BOLD: 37 spp. / COL: 34 spp.)										
Epiphyas postvittana Walker, 1863(1)	+	-	-	+	+	+	-	90	1	1
Eupoecilia (BOLD: 5 spp. / COL: 35 spp.)										
Eupoecilia ambiguella Hübner, 1796(1)	+	-	-	-	+	+	-	11	1	0
Grapholita (BOLD: 58 spp. / COL: 99 spp.)										
Grapholita funebrana Treitschke, 1835(1)	-	-	-	-	+	-	-	20	10	10
Grapholita inopinata Heinrich, 1928(1)	+	-	-	-	-	+	-	0	0	0
Grapholita molesta Busck, 1916(1)	-	-	-	-	+	+	-	49	18	13
Grapholita packardi Zeller*	+	-	-	-	-	-	-	51	8	3
Gypsonoma (BOLD: 18 spp. / COL: 40 spp.)										
Gypsonoma aceriana Duponchel, 1843(1)	-	-	-	-	+	-	-	71	31	31
Hemimene (BOLD: 0 spp. / COL: 0 spp.)										
Hemimene juliana (Curtis)*	-	-	-	+	-	-	-	0	0	0
Homona (BOLD: 15 spp. / COL: 46 spp.)										
Homona coffearia Nietner, 1861(1)	+	-	-	-	-	-	-	24	0	0

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Laspeyresia (BOLD: 8 spp. / COL: 0 spp.)	-	-	-	+	-	-	-			
Leguminivora (BOLD: 5 spp. / COL: 4 spp.)										
Leguminivora glycinivorella Matsumura, 1900(1)	-	-	-	-	+	-	-	0	0	0
Lobesia (BOLD: 35 spp. / COL: 110 spp.)										
Lobesia botrana Denis & Schiffermüller, 1775(1)	+	-	-	+	+	+	-	0	0	0
Pammene (BOLD: 39 spp. / COL: 91 spp.)										
Pammene fasciana Linnaeus, 1761(1)	-	-	-	+	-	-	-	15	1	0
Paralobesia (BOLD: 9 spp. / COL: 18 spp.)										
Paralobesia viteana Clemens, 1860(1)	+	-	-	-	-	-	-	65	0	0
Platynota (BOLD: 39 spp. / COL: 17 spp.)										
Platynota idaeusalis Walker, 1859(1)	-	-	-	-	+	-	-	87	6	0
Proeulia (BOLD: 0 spp. / COL: 23 spp.)	-	-	-	+	-	-	-			
Rhyacionia (BOLD: 20 spp. / COL: 42 spp.)										
Rhyacionia buoliana Schiffermüller, 1775(1)	+	-	-	-	+	-	-	27	2	0
Spilonota (BOLD: 14 spp. / COL: 78 spp.)										
Spilonota ocellana Fabricius, 1775(1)	+	-	-	-	-	-	-	94	1	0
Thaumatotibia (BOLD: 8 spp. / COL: 0 spp.)										
Thaumatotibia leucotreta (Meyrick, 1913)(2)	+	-	-	+	-	+	-	39	1	1
Tortrix (BOLD: 40 spp. / COL: 242 spp.)										
Tortrix viridana Linnaeus, 1758(1)	-	-	-	-	+	-	-	34	4	2
Lepidoptera: Yponomeutidae										
Yponomeuta (BOLD: 25 spp. / COL: 80 spp.)										
Yponomeuta malinellus Zeller, 1838(2)	-	-	-	-	+	+	-	66	9	1
Lepidoptera: Zygaenidae										
Artona (BOLD: 2 spp. / COL: 31 spp.)										
Artona catoxantha Hampson, 1892(1)	+	-	-	-	-	-	-	0	0	0
Orthoptera: Acrididae										
Anacridium (BOLD: 1 spp. / COL: 13 spp.)										
Anacridium melanorhodon (Walker, F., 1870)(1)	+	-	-	-	-	-	-	0	0	0
Catantops (BOLD: 3 spp. / COL: 26 spp.)										
Catantops melanostictus Schaum, 1853(1)	+	-	-	-	-	-	-	0	0	0
Dociostaurus (BOLD: 4 spp. / COL: 26 spp.)										
Dociostaurus maroccanus (Thunberg, 1815)(1)	+	-	-	-	-	-	-	1	1	1
Oxya (BOLD: 5 spp. / COL: 49 spp.)										
Oxya hyla Serville, 1831(1)	+	-	-	-	-	-	-	5	0	0
Oxya japonica (Thunberg, 1815)(1)	+	-	-	-	-	-	-	3	0	0
Trimerotropis (BOLD: 8 spp. / COL: 50 spp.)										
Trimerotropis fratercula McNeill, 1901(1)	-	-	-	-	+	-	-	0	0	0
Valanga (BOLD: 2 spp. / COL: 31 spp.)										
Valanga nigricornis (Burmeister, 1838)(1)	+	-	-	-	-	-	-	5	0	0
Orthoptera: Gryllotalpidae										

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Gryllotalpa (BOLD: 7 spp. / COL: 63 spp.)										
Gryllotalpa africana Beauvois, 1805(1)	+	-	-	-	-	-	-	2	0	0
Scapteriscus (BOLD: 0 spp. / COL: 21 spp.)										
Scapteriscus borellii Giglio-Tos, 1894(1)	-	-	-	-	+	-	-	0	0	0
Orthoptera: Pyrgomorphidae										
Zonocerus (BOLD: 1 spp. / COL: 2 spp.)										
Zonocerus variegatus (Linnaeus, 1758)(1)	+	-	-	-	-	-	-	0	0	0
Orthoptera: Tettigoniidae										
Anabrus (BOLD: 2 spp. / COL: 4 spp.)										
Anabrus simplex Haldeman, 1852(1)	-	-	-	-	+	-	-	6	4	4
Thysanoptera: Phlaeothripidae										
Gynaikothrips (BOLD: 4 spp. / COL: 2 spp.)										
Gynaikothrips ficorum (Marchal, 1908)(1)	+	-	-	-	-	-	-	35	2	2
Haplothrips (BOLD: 16 spp. / COL: 26 spp.)										
Haplothrips chinensis Priesner, 1936(2)	-	-	-	+	-	-	-	0	0	0
Klambothrips (BOLD: 0 spp. / COL: 0 spp.)										
Klambothrips myopori*	-	-	-	-	+	-	-	0	0	0
Thysanoptera: Thripidae										
Chaetanaphothrips (BOLD: 0 spp. / COL: 3 spp.)										
Chaetanaphothrips orchidii (Moulton, 1907)(1)	+	-	-	-	-	-	-	0	0	0
Chaetanaphothrips signipennis (Bagnall, 1914)(1)	+	-	-	-	-	-	-	0	0	0
Frankliniella (BOLD: 15 spp. / COL: 50 spp.)										
Frankliniella intonsa (Trybom, 1895)(1)	+	-	-	-	+	-	-	18	3	3
Frankliniella occidentalis (Pergande, 1895)(1)	+	-	+	-	-	-	+	52	167	167
Frankliniella schultzei (Trybom, 1910)(1)	+	-	-	-	-	-	-	8	1	1
Heliothrips (BOLD: 2 spp. / COL: 1 spp.)										
Heliothrips haemorrhoidalis (Bouché, 1833)(1)	+	-	-	-	-	-	-	4	0	0
Hercinothrips (BOLD: 1 spp. / COL: 2 spp.)										
Hercinothrips bicinctus (Bagnall, 1919)(1)	+	-	-	-	-	-	-	0	0	0
Hercinothrips femoralis (Reuter, 1891)(1)	+	-	-	-	-	-	-	5	0	0
Limothrips (BOLD: 0 spp. / COL: 4 spp.)										
Limothrips cerealium (Haliday, 1836)(1)	+	-	-	-	-	-	-	0	0	0
Rhipiphorothrips (BOLD: 0 spp. / COL: 1 spp.)										
Rhipiphorothrips cruentatus Hood, 1919(2)	+	-	-	-	-	-	-	0	0	0
Scirtothrips (BOLD: 17 spp. / COL: 17 spp.)										
Scirtothrips aurantii Faure, 1929(1)	+	+	-	-	-	-	+	3	2	2
Scirtothrips citri (Moulton, 1909)(1)	+	+	-	-	-	-	+	2	2	2
Scirtothrips dorsalis Hood, 1919(1)	+	-	+	-	+	-	+	24	20	20
Selenothrips (BOLD: 1 spp. / COL: 1 spp.)										
Selenothrips rubrocinctus (Giard, 1901)(1)	+	-	-	-	-	-	-	13	0	0
Stenchaetothrips (BOLD: 1 spp. / COL: 0 spp.)										
Stenchaetothrips biformis (Bagnall,	+	-	-	-	+	-	-	16	0	0

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1913)(2)										
Taeniothrips (BOLD: 2 spp. / COL: 3 spp.)										
Taeniothrips inconsequens (Uzel, 1895)(1)	+	-	-	-	+	-	-	0	1	1
Thrips (BOLD: 20 spp. / COL: 77 spp.)										
Thrips pini (Uzel, 1895)(2)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Taeniothrips laricivorus	+	-	-	-	-	-	-	0	0	0
Thrips hawaiiensis (Morgan, 1913)(1)	+	-	-	-	-	-	-	15	4	4
Thrips nigropilosus Uzel, 1895(1)	+	-	-	-	-	-	-	7	14	14
Thrips palmi Karny, 1925(1)	+	+	-	-	+	-	+	22	52	52
Thrips simplex (Morison, 1930)(1)	+	-	-	-	-	-	-	0	0	0
Thrips tabaci Lindeman, 1889(1)	+	-	-	-	-	-	-	87	62	62
Mollusca: Bivalvia										
Veneroida: Dreissenidae										
Dreissena (BOLD: 7 spp. / COL: 2 spp.)										
Dreissena polymorpha *	-	-	-	-	+	-	-	79	71	71
Dreissena bugensis Andrusov, 1897 (2)	-	-	-	-	-	-	-	8	8	8
-listed under synonym Dreissena rostriformis bugensis Andrusov, 1897	-	-	-	-	+	-	-	0	0	0
Mollusca: Gastropoda										
Architaenioglossa: Ampullariidae										
Pomacea (BOLD: 16 spp. / COL: 6 spp.)										
Pomacea canaliculata (Lamarck, 1828)(1)	-	-	-	-	+	-	-	100	93	93
Pomacea dolioides *	-	-	-	-	+	-	-	6	6	6
Pomacea insularum	-	-	-	-	+	-	-	125	125	125
Architaenioglossa: Viviparidae										
Cipangopaludina (BOLD: 2 spp. / COL: 2 spp.)										
Cipangopaludina chinensis *	-	-	-	-	+	-	-	3	3	3
Littorinimorpha: Hydrobiidae										
Potamopyrgus (BOLD: 4 spp. / COL: 1 spp.)										
Potamopyrgus antipodarum (J. E. Gray, 1853)(1)	-	-	-	-	+	-	-	25	21	21
Pulmonata: Achatinidae										
Achatina (BOLD: 2 spp. / COL: 1 spp.)										
Achatina achatina L.*	-		-	-	+	+	-	0	0	0
Achatina fulica (Férussac, 1821)(1)	-	-	-	-	-	+	-	2	1	1
Limicolaria (BOLD: 0 spp. / COL: 0 spp.)										
Limicolaria aurora *	-	-	-	-	+	-	-	0	0	0
Lissachatina (BOLD: 0 spp. / COL: 0 spp.)										
Lissachatina fulica *	-	-	-	-	+	-	-	0	0	0
Stylommatophora: Achatinidae										
Archachatina (BOLD: 0 spp. / COL: 0 spp.)										
Archachatina degneri Bequaert & Clench*	-	-	-	-	-	+	-	0	0	0
Archachatina marginata *	-	-	-	-	+	-	-	0	0	0
Archachatina purpurea Gmelin*	-	-	-	-	-	+	-	0	0	0

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Archachatina ventricosa Gould*	-	-	-	-	-	+	-	0	0	0
Stylommatophora: Arionidae										
Arion (BOLD: 30 spp. / COL: 10 spp.)										
Arion vulgaris *	-	-	-	-	+	-	-	0	0	0
Stylommatophora: Helicarionidae										
Ovachlamys (BOLD: 1 spp. / COL: 0 spp.)										
Ovachlamys fulgens (Gude, 1900)	-	-	-	-	+	-	-	2	1	1
Stylommatophora: Helicidae										
Cantareus (BOLD: 1 spp. / COL: 0 spp.)										
Cantareus apertus (Born, 1778)(2)	-	-	-	-	+	-	-	0	0	0
Cepaea (BOLD: 3 spp. / COL: 2 spp.)										
Cepaea nemoralis *	-	-	-	-	+	+	-	15	2	2
Cornu (BOLD: 1 spp. / COL: 0 spp.)										
Cornu aspersum (O.F. Müller, 1774)(2)	-	-	-	-	+	+	-	0	0	0
Helix (BOLD: 3 spp. / COL: 2 spp.)	-	-	-	-	-	+	-			
Helix aspersa Muller*	-	-	-	-	-	+	-	1	1	1
Helix pomatia Linnaeus, 1758(1)	-	-	-	-	+	-	-	0	0	0
Otala (BOLD: 2 spp. / COL: 1 spp.)										
Otala lactea (Müller, 1774)(1)	-	-	-	-	+	+	-	0	0	0
Otala vermiculata Müller*	-	-	-	-	-	+	-	0	0	0
Theba (BOLD: 1 spp. / COL: 1 spp.)										
Theba pisana (Müller, 1774)(1)	-	-	-	-	+	+	-	0	0	0
Stylommatophora: Hygromiidae										
Candidula (BOLD: 7 spp. / COL: 1 spp.)										
Candidula intersecta (Poiret, 1801)(1)	-	-	-	-	+	-	-	1	1	1
Cernuella (BOLD: 4 spp. / COL: 1 spp.)										
Cernuella virgata *	-	-	-	-	+	-	-	1	1	1
Cochlicella (BOLD: 2 spp. / COL: 0 spp.)	-	-	-	-	+	-	-			
Hygromia (BOLD: 2 spp. / COL: 0 spp.)										
Hygromia cinctella (Draparnaud, 1801)(2)	-	-	-	-	+	-	-	11	2	2
Monacha (BOLD: 4 spp. / COL: 1 spp.)										
Monacha cantiana *	-	-	-	-	+	-	-	1	1	1
Prietocella (BOLD: 0 spp. / COL: 0 spp.)										
Prietocella barbara (Linnaeus, 1758)(2)	-	-	-	-	+	-	-	0	0	0
Xerolenta (BOLD: 0 spp. / COL: 0 spp.)										
Xerolenta obvia (Menke, 1828)(2)	-	-	-	-	+	-	-	0	0	0
Xerotricha (BOLD: 1 spp. / COL: 0 spp.)										
Xerotricha conspurcata (Draparnaud, 1801)(2)	-	-	-	-	+	-	-	0	0	0
Monacha (BOLD: 4 spp. / COL: 1 spp.)	-	-	-	-	+	-	-			
Monacha cartusiana (Montagu, 1803)(1)	-	-	-	-	+	-	-	0	0	0
Systellommatophora: Veronicellidae	-	-	-	-	+	-	-			
Sarasinula (BOLD: 1 spp. / COL: 1 spp.)										
Sarasinula plebeia (P. Fischer, 1868)(1)	-	-	-	-	+	-	-	0	0	0

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Veronicella (BOLD: 0 spp. / COL: 1 spp.)	-	-	-	-	+	-	-			
Veronicella floridana *	-	-	-	-	+	-	-	0	0	0
Nematoda: Adenophorea										
Dorylaimida: Longidoridae										
Longidorus (BOLD: 3 spp. / COL: 4 spp.)	-	-	-	-	-	+	-			
Longidorus diadecturus Eveleigh & Allen, 1982(2)	-	-	-	-	+	-	+	0	0	0
Xiphinema (BOLD: 4 spp. / COL: 4 spp.)	-	-	-	-	+	+	-			
Xiphinema americanum Cobb, 1913(1)	-	+	-	-	-	-	+	0	0	0
Xiphinema bakeri Williams, 1961(2)	-	-	-	-	+	-	-	0	0	0
Xiphinema bricolense *	-	+	-	-	-	-	+	0	0	0
Xiphinema americanum Cobb, 1913(2)	-	-	-	-	-	-	-	2	2	2
-listed under synonym Xiphinema californicum Lamberti & Bleve-Zacheo, 1979(2)	-	+	-	-	-	-	+	0	0	0
Xiphinema coxi Tarjan, 1964(2)	-	-	-	-	+	-	-	0	0	0
Xiphinema diversicaudatum (Micoletzky, 1927)(1)	-	-	-	-	+	-	+	0	0	0
Xiphinema rivesi Dalmasso, 1969(2)	-	-	+	-	-	-	+	0	0	0
Triplonchida: Trichodoridae										
Paratrichodorus (BOLD: 0 spp. / COL: 5 spp.)	-	-	-	-	+	-	-			
Paratrichodorus allius (Jensen, 1963)(2)	-	-	-	-	+	-	-	0	0	0
Paratrichodorus anemones (Loof, 1965)(2)	-	-	-	-	+	-	+	0	0	0
Paratrichodorus minor (Colbran, 1956)(1)	-	-	-	-	+	-	-	0	0	0
Paratrichodorus nanus (Allen, 1957)(2)	-	-	-	-	+	-	-	0	0	0
Paratrichodorus pachydermus (Seinhorst, 1954)(1)	-	-	-	-	+	-	+	0	0	0
Paratrichodorus teres (Hooper, 1962)(2)	-	-	-	-	+	-	-	0	0	0
Trichodorus (BOLD: 0 spp. / COL: 1 spp.)	-	-	-	-	-	+	-			
Trichodorus obtusus (Cobb, 1913)(2)	-	-	-	-	+	-	-	0	0	0
Trichodorus primitivus (de Man, 1880)(2)	-	-	-	-	+	-	+	0	0	0
Trichodorus viruliferus Hooper, 1963(2)	-	-	-	-	+	-	-	0	0	0
Nematoda: Secernentea										
Aphelenchida: Aphelenchoididae										
Aphelenchoides (BOLD: 4 spp. / COL: 1 spp.)										
Aphelenchoides besseyi Christie, 1942(2)	-	-	+	-	+	-	+	3	3	3
Aphelenchoides fragariae (Ritzema Bos, 1890)(2)	-	-	-	-	+	-	-	0	0	0
Bursaphelenchus (BOLD: 27 spp. / COL: 4 spp.)										
Bursaphelenchus cocophilus (Cobb, 1919)(2)	-	-	-	-	+	-	-	1	1	1
Bursaphelenchus xylophilus (Steiner & Buhrer, 1934)(2)	-	-	+	-	+	-	+	6	4	4
Tylenchida: Anguinidae										
Ditylenchus (BOLD: 0 spp. / COL: 1 spp.)										
Ditylenchus angustus (Butler, 1913)(2)	-	-	-	-	+	-	-	0	0	0
Ditylenchus destructor Thorne, 1945(2)	-	-	-	-	+	+	+	0	0	0
Ditylenchus dipsaci (Kuhn, 1857)(1)	-	-	+	-	+	+	+	0	0	0

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Tylenchida: Belonolaimidae										
Tylenchorhynchus (BOLD: 0 spp. / COL: 2 spp.)										
Tylenchorhynchus ewingii *	-	-	-	-	+	-	-	0	0	0
Tylenchida: Criconematidae										
Macroposthonia (BOLD: 0 spp. / COL: 0 spp.)										
Macroposthonia xenoplax (Raski, 1952)(2)	-	-	-	-	+	-	-	0	0	0
Tylenchida: Heteroderidae										
Globodera (BOLD: 0 spp. / COL: 3 spp.)										
Globodera pallida (Stone, 1973)(1)	-	-	+	+	+	+	+	0	0	0
Globodera rostochiensis (Wollenweber, 1923)(1)	-	-	+	+	+	+	+	0	0	0
Heterodera (BOLD: 0 spp. / COL: 5 spp.)										
Heterodera avenae (1)	-	-	-	-	+	-	+	0	0	0
Heterodera cajani Koshy, 1967(2)	-	-	-	-	+	-	-	0	0	0
Heterodera ciceri Vovlas, Greco & di Vito, 1985(2)	-	-	-	-	+	-	-	0	0	0
Heterodera filipjevi (Madzhidov, 1981)(2)	-	-	-	-	+	-	+	0	0	0
Heterodera glycines (1)	-	-	+	-	+	+	+	0	0	0
Heterodera goettingiana Liebscher, 1982(2)	-	-	-	-	+	-	-	0	0	0
Heterodera latipons Franklin, 1969(2)	-	-	-	-	+	-	+	0	0	0
Heterodera sacchari Luc & Merny, 1963(2)	-	-	-	-	+	-	+	0	0	0
Heterodera zeae Koshy, Swarup & Sethi, 1971(2)	-	-	-	-	+	-	-	0	0	0
Punctodera (BOLD: 0 spp. / COL: 0 spp.)										
Punctodera chalcoensis Stone, Sosa Moss & Mulvey, 1976(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne (BOLD: 0 spp. / COL: 6 spp.)	-	-	-	-	+	-	-			
Meloidogyne arenaria (Neal, 1889)(2)	-	-	-	-	+	-	+	0	0	0
Meloidogyne artiellia Franklin, 1961(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne chitwoodi Golden et al., 1980(2)	-	-	+	-	+	+	+	0	0	0
Meloidogyne citri Zhang, Gao & Weng, 1990(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne coffeicola Lordello & Zamith, 1960(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne donghaiensis *	-	-	-	-	+	-	-	0	0	0
Meloidogyne enterolobii Yang & Eisenback, 1983(2)	-	-	+	-	-	-	+	0	0	0
Meloidogyne fallax Karssen, 1996(2)	-	-	+	-	+	-	+	0	0	0
Meloidogyne fujianensis Pan, 1985(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne hapla Chitwood, 1949(1)	-	-	-	-	+	-	+	0	0	0
Meloidogyne incognita (Kofoid & White, 1919)(2)	-	-	-	-	+	-	+	0	0	0
Meloidogyne indica Whitehead, 1968(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne javanica (Treub, 1885)(2)	-	-	-	-	+	-	+	0	0	0
Meloidogyne jianyangensis Zhu, Lan, Hu, Yang & Wang, 1990(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne kongi Yang, Hu & Xu, 1988(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne mali Itoh, Ohshima &	-	-	-	-	+	-	-	0	0	0

Taxonomic Rank: Genus (Species Number Estimates): Species Name	CABI	EPPO-A1	EPPO-A2	APHIS	NAPIS	CFIA	QBol	Barcode Sequences	Public Sequences	Genbank Records
Ichinohe, 1969(2)										
Meloidogyne mayaguensis Rammah & Hirschmann, 1988(2)	-	-	-	-	+	-	+	0	0	0
Meloidogyne mingnanica Zhang, 1993(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne paranaensis Carneiro, Carneiro, Abrantes, Santos & Almeida, 1996(2)	-	-	-	-	+	-	-	0	0	0
Meloidogyne partityla Kleynhans, 1986(2)	-	-	-	-	+	-	-	0	0	0
Tylenchida: Hoplolaimidae										
Pratylenchus (BOLD: 0 spp. / COL: 5 spp.)	-	-	-	-	+	-	-			
Tylenchida: Pratylenchidae										
Nacobbus (BOLD: 0 spp. / COL: 4 spp.)										
Nacobbus aberrans (1)	-	-	-	-	+	-	+	0	0	0
Radopholus (BOLD: 0 spp. / COL: 3 spp.)										
Radopholus similis (1)	-	+	+	-	+	-	+	2	2	2
Rotylenchulus (BOLD: 1 spp. / COL: 0 spp.)										
Rotylenchulus reniformis Linford & Oliveira, 1940(2)	-	-	-	-	+	-	-	0	0	0
Tylenchida: Tylenchidae										
Anguina (BOLD: 0 spp. / COL: 5 spp.)	-	-	-	-	+	-	-			
Anguina agrostis (Steinbuch, 1799)	-	-	-	-	-	-	-	0	0	0
-listed under synonym Anguina funesta Price, Fisher & Kerr, 1979	-	-	-	-	+	-	-	0	0	0
Anguina tritici (1)	-	-	-	-	+	-	+	0	0	0

Appendix 2

Complete list of taxa from the review of the biological control literature. Classification of taxa and validity of names were retrieved from Catalogue of Life (COL) or Global Biodiversity Information Facility (GBIF). If no source is provided then classification used in source article was adopted. Number of occurrences in the literature review are broken out by specimens obtained from the environment (E) and those obtained from commercial or non-commercial insectaries (I). Number of records on BOLD includes only public records for those species.

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
Arachnida							
Araneae		Araneae	COL	3 (E:3 I:0)	-	-	-
Anyphaenidae							
	Anyphaena						
		Anyphaena accentuata	COL	1 (E:1 I:0)	1	2	0
Araneidae							
	Araneus						
		Araneus diadematus	COL	1 (E:1 I:0)	1	17	2
Araneidae							
	Araniella						
		Araniella cucurbitina	COL	1 (E:1 I:0)	1	1	0
		Araniella opistographa	GBIF	1 (E:1 I:0)	0	0	0
Clubionidae							
	Clubiona	Clubiona	COL	1 (E:1 I:0)	-	-	-
Gnaphosidae							
	Zelotes	Zelotes	COL	1 (E:1 I:0)	-	-	-
Linyphiidae		Linyphiidae	COL	1 (E:1 I:0)	-	-	-
	Bathyphantes	Bathyphantes	COL	1 (E:1 I:0)	-	-	-
	Entelecara						
		Entelecara acuminata	COL	1 (E:1 I:0)	1	13	0
	Erigone	Erigone	COL	1 (E:1 I:0)	-	-	-
		Erigone atra	COL	1 (E:1 I:0)	2	18	4
		Erigone autumnalis	COL	1 (E:1 I:0)	1	17	0
	Eringonidium						
		Eringonidium graminicola	-	1 (E:1 I:0)	0	0	0
	Grammonota						
		Grammonota inornata	COL	1 (E:1 I:0)	0	0	0
	Mermessus						
		Mermessus denticulatus	COL	1 (E:1 I:0)	0	0	0
	Tennesseellum						
		Tennesseellum formicum	-	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Tenuiphantes						
		Tenuiphantes tenuis	GBIF	1 (E:1 I:0)	0	0	0
		--appeared as Lepthyphantes tenuis					
Lycosidae		Lycosidae	COL	1 (E:1 I:0)	-	-	-
	Alopecosa	Alopecosa	COL	1 (E:1 I:0)	-	-	-
	Lycosa						
		Lycosa sinesis	-	1 (E:1 I:0)	0	0	0
	Pardosa	Pardosa	COL	3 (E:3 I:0)	-	-	-
		Pardosa agrestis	COL	1 (E:1 I:0)	0	0	0
		Pardosa astrigera	COL	1 (E:1 I:0)	1	72	72
		Pardosa cribata	-	1 (E:1 I:0)	0	0	0
		Pardosa palustris	COL	1 (E:1 I:0)	2	4	2
		Pardosa prativaga	COL	1 (E:1 I:0)	2	2	1
	Trochosa	Trochosa	COL	1 (E:1 I:0)	-	-	-
Philodromidae							
	Philodromus						
		Philodromus cespitum	COL	3 (E:3 I:0)	4	77	3
		Philodromus dispar	COL	1 (E:1 I:0)	1	11	0
		Philodromus praedatus	COL	1 (E:1 I:0)	1	1	0
Pisauridae							
	Pisaura	Pisaura	COL	1 (E:1 I:0)	-	-	-
Salticidae							
	Heliophanus	Heliophanus	COL	1 (E:1 I:0)	-	-	-
	Phintella						
		Phintella bifurcilinea	COL	1 (E:1 I:0)	0	0	0
		--appeared as Telamonia bifurcilinea					
Tetragnathidae							
	Pachygnatha	Pachygnatha	COL	1 (E:1 I:0)	-	-	-
		Pachygnatha clercki	COL	1 (E:1 I:0)	4	5	4
	Tetragnatha	Tetragnatha	COL	1 (E:1 I:0)	-	-	-
		Tetragnatha extensa	COL	1 (E:1 I:0)	6	27	2
Theridiidae							
	Coleosoma						
		Coleosoma octomacutatum	-	1 (E:1 I:0)	0	0	0
	Enoplognatha						
		Enoplognatha gemina	COL	1 (E:1 I:0)	0	0	0
	Neottiura						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Neottiura bimaculata	COL	1 (E:1 I:0)	2	19	1
	Paidiscura						
		Paidiscura pallens --appeared as Theridion pallens	GBIF	1 (E:1 I:0)	0	0	0
	Theridion						
		Theridion varians	COL	1 (E:1 I:0)	1	10	1
Thomisidae							
	Misumjenops						
		Misumjenops tricuspidatus	-	1 (E:1 I:0)	0	0	0
	Xysticus	Xysticus	COL	1 (E:1 I:0)	-	-	-
		Xysticus cristatus	COL	1 (E:1 I:0)	1	7	1
		Xysticus kochi	COL	1 (E:1 I:0)	0	0	0
Astigmata							
Acaridae	Acarus						
		Acarus siro	GBIF	2 (E: I:2)	0	0	0
	Aleuroglyphus						
		Aleuroglyphus ovatus	GBIF	2 (E: I:2)	0	0	0
	Rhizoglyphus						
		Rhizoglyphus echinopus	COL	1 (E:1 I:0)	0	0	0
	Tyrophagus						
		Tyrophagus putrescentiae	COL	4 (E:1 I:3)	0	0	0
Carpoglyphidae							
	Carpoglyphus						
		Carpoglyphus lactis	COL	1 (E:1 I:0)	1	1	1
Glycyphagidae							
	Caloglyphus						
		Caloglyphus redickorzevi	-	1 (E:0 I:1)	0	0	0
	Lepidoglyphus						
		Lepidoglyphus destructor	GBIF	1 (E:0 I:1)	0	0	0
Hemisarcoptidae							
	Hemisarcoptes						
		Hemisarcoptes coccophagus	COL	1 (E:1 I:0)	0	0	0
Mesostigmata							
Phytoseiidae							
	Amblyseius						
		Amblyseius andersoni	COL	2 (E:2 I:0)	0	0	0
		Amblyseius largoensis	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Amblyseius swirskii	COL	9 (E:2 I:7)	0	0	0
	Anthoseius						
		Anthoseius rhenanus	GBIF	1 (E:1 I:0)	0	0	0
	Euseius						
		Euseius finlandicus	COL	2 (E:2 I:0)	0	0	0
		Euseius scutalis	COL	1 (E:1 I:0)	0	0	0
	Galendromus						
		Galendromus occidentalis	COL	4 (E:1 I:3)	0	0	0
	Iphiseius						
		Iphiseius degenerans	COL	1 (E:1 I:0)	0	0	0
	Kampimodromus						
		Kampimodromus aberrans	COL	1 (E:0 I:1)	0	0	0
	Neoseiulus						
		Neoseiulus baraki	COL	1 (E:1 I:0)	0	0	0
		Neoseiulus barkeri	COL	1 (E:1 I:0)	0	0	0
		Neoseiulus californicus	COL	8 (E:6 I:2)	1	8	8
		Neoseiulus cucumeris	COL	8 (E:3 I:5)	0	0	0
		Neoseiulus paspalivorus	COL	1 (E:1 I:0)	0	0	0
		Neoseiulus womersleyi	COL	1 (E:0 I:1)	0	0	0
	Phytoseiulus						
		Phytoseiulus longipes	COL	1 (E:1 I:0)	0	0	0
		Phytoseiulus persimilis	COL	10 (E:3 I:7)	1	5	5
	Phytoseius						
		Phytoseius plumifer	COL	1 (E:1 I:0)	0	0	0
	Typhlodromus						
		Typhlodromus athiasae	COL	1 (E:1 I:0)	0	0	0
		Typhlodromus pyri	COL	2 (E:2 I:0)	5	29	29
Rhodacaridae							
	Protogamasellopsis						
		Protogamasellopsis posnaniensis	COL	1 (E:1 I:0)	0	0	0
Opiliones		Opiliones	COL	1 (E:1 I:0)	-	-	-
Prostigmata							
Bdellidae							
	Bdellodes						
		Bdellodes lapidaria	COL	1 (E:1 I:0)	0	0	0
Cheyletidae							
	Cheyletus						

Class/Order/Family/Genus	Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Cheyletus malaccensis	GBIF	2 (E:1 I:1)	3	42	42
Eriophyidae						
Aceria						
	Aceria guerreronis	GBIF	2 (E:2 I:0)	0	0	0
	Aceria salsolae	GBIF	1 (E:1 I:0)	0	0	0
Eriophyes						
	Eriophyes chondrillae	-	1 (E:1 I:0)	0	0	0
Penthaleidae						
Halotydeus						
	Halotydeus destructor	COL	1 (E:1 I:0)	0	0	0
Podapolipidae						
Coccipolipus						
	Coccipolipus hippodamiae	GBIF	1 (E:1 I:0)	0	0	0
Tarsonemidae						
Phytonemus						
	Phytonemus pallidus	GBIF	1 (E:1 I:0)	0	0	0
Tenuipalpidae						
Raoiella						
	Raoiella indica	COL	1 (E:1 I:0)	3	21	21
Tetranychidae						
Eotetranychus						
	Eotetranychus willamettei	COL	2 (E:2 I:0)	0	0	0
Oligonychus						
	Oligonychus coffeae	COL	1 (E:1 I:0)	0	2	2
	Oligonychus perseae	COL	1 (E:1 I:0)	2	7	7
Panonychus						
	Panonychus citri	COL	5 (E:5 I:0)	0	4	4
	Panonychus ulmi	COL	1 (E:1 I:0)	1	2	2
Tetranychus						
	Tetranychus evansi	COL	5 (E:5 I:0)	2	6	6
	Tetranychus gloveri	COL	1 (E:1 I:0)	0	0	0
	Tetranychus kanzawai	COL	2 (E:1 I:1)	1	36	36
	Tetranychus pacificus	COL	3 (E:3 I:0)	0	0	0
	Tetranychus urticae --also appears as Tetranychus cinnabarinus	COL	20 (E:14 I:6)	8	40	40
Branchiopoda						
Anostraca						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
Artemiidae							
	Artemia						
		Artemia franciscana	COL	1 (E: I:1)	4	38	38
Entognatha							
Collembola							
Entomobryidae							
	Sinella						
		Sinella curviseta	COL	1 (E:1 I:0)	0	0	0
Isotomidae							
	Folsomia						
		Folsomia candida	COL	1 (E:1 I:0)	2	6	6
	Isotoma						
		Isotoma anglicana	GBIF	1 (E:1 I:0)	5	44	1
Sminthuridae							
	Sminthurus						
		Sminthurus viridis	COL	1 (E:1 I:0)	6	39	39
Insecta							
Blattodea							
Blattidae							
	Periplaneta						
		Periplaneta americana	COL	1 (E:1 I:0)	4	28	23
Ectobiidae							
	Blattella						
		Blattella asahinai	COL	1 (E:1 I:0)	0	0	0
Coleoptera		Coleoptera	COL	1 (E:1 I:0)	-	-	-
Anobiidae							
	Lasioderma						
		Lasioderma serricorne	COL	1 (E:1 I:0)	1	1	1
Anthribidae							
	Euciodes						
		Euciodes suturalis	COL	1 (E:1 I:0)	0	0	0
Apionidae							
	Apion	Apion	COL	1 (E:1 I:0)	-	-	-
	Aspidapion						
		Aspidapion aeneum	GBIF	1 (E:1 I:0)	0	0	0
		Aspidapion radiolus	GBIF	1 (E:1 I:0)	0	0	0
	Protapion						
		Protapion nigritarse	GBIF	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Stenopterapion						
		Stenopterapion tenue	GBIF	1 (E:1 I:0)	0	0	0
	Taeniapion						
		Taeniapion rufulum	GBIF	1 (E:1 I:0)	0	0	0
Attelabidae							
	Euops						
		Euops chinesis	-	1 (E:1 I:0)	0	0	0
Bostrichidae							
	Rhyzopertha						
		Rhyzopertha dominica	COL	1 (E:1 I:0)	1	3	3
Bothrideridae							
	Dastarcus						
		Dastarcus helophoroides	GBIF	1 (E:0 I:1)	0	0	0
Brentidae							
	Malvapion						
		Malvapion malvae	GBIF	1 (E:1 I:0)	0	0	0
	Perapion						
		Perapion violaceum	GBIF	1 (E:1 I:0)	0	0	0
Buprestidae							
	Agrilus						
		Agrilus coxalis	COL	1 (E:1 I:0)	0	0	0
		Agrilus coxalis auroguttatus	COL	1 (E:1 I:0)	0	0	0
		--appeared as Agrilus auroguttatus					
		Agrilus planipennis	COL	7 (E:7 I:0)	0	3	3
	Capnodis						
		Capnodis tenebrionis	GBIF	1 (E:0 I:1)	0	0	0
	Sphenoptera						
		Sphenoptera jugoslavica	COL	1 (E:1 I:0)	1	3	0
Carabidae		Carabidae	COL	4 (E:4 I:0)	-	-	-
	Agonum	Agonum	COL	1 (E:1 I:0)	-	-	-
		Agonum cupripenne	COL	1 (E:1 I:0)	0	0	0
		Agonum dorsale	-	1 (E:1 I:0)	0	0	0
		Agonum muelleri	COL	2 (E:2 I:0)	1	4	4
		Agonum octopunctatum	COL	1 (E:1 I:0)	0	1	1
		Agonum placidum	COL	2 (E:2 I:0)	1	11	0
	Amara	Amara	COL	4 (E:4 I:0)	-	-	-
		Amara aenea	COL	1 (E:1 I:0)	0	0	0
		Amara impunctata	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Amara obesa	COL	1 (E:1 I:0)	1	6	0
	Anchomenus						
		Anchomenus dorsalis	COL	4 (E:4 I:0)	1	7	7
	Anisodactylus						
		Anisodactylus binotatus	COL	1 (E:1 I:0)	1	2	2
		Anisodactylus harrisii	COL	1 (E:1 I:0)	0	0	0
		Anisodactylus ovularis	COL	1 (E:1 I:0)	0	0	0
		Anisodactylus rusticus	COL	1 (E:1 I:0)	1	2	0
		Anisodactylus sanctaecrucis	COL	3 (E:3 I:0)	0	0	0
	Asaphidion						
		Asaphidion flavipes	COL	1 (E:1 I:0)	0	0	0
	Bembidion						
		Bembidion femoratum	COL	2 (E:2 I:0)	0	0	0
		Bembidion guttula	COL	1 (E:1 I:0)	0	0	0
		Bembidion lampros	COL	3 (E:3 I:0)	1	10	10
		Bembidion nitidum	COL	1 (E:1 I:0)	1	1	0
		Bembidion obtusum	COL	2 (E:2 I:0)	1	2	2
		Bembidion properans	COL	1 (E:1 I:0)	1	8	8
		Bembidion quadrimaculatum	COL	3 (E:3 I:0)	1	1	0
		Bembidion rapidum	COL	1 (E:1 I:0)	2	8	2
		Bembidion rupicola	COL	1 (E:1 I:0)	0	0	0
		Bembidion tetracolum	COL	1 (E:1 I:0)	2	9	8
	Brachinus						
		Brachinus ovipennis	COL	1 (E:1 I:0)	0	0	0
	Calathus						
		Calathus fuscipes	COL	1 (E:1 I:0)	1	2	2
		Calathus gregarius	COL	1 (E:1 I:0)	1	3	0
		Calathus melanocephalus	COL	2 (E:2 I:0)	0	0	0
	Calosoma						
		Calosoma chlorostictum chlorostictum --appeared as Calosoma calidum	COL	1 (E:1 I:0)	0	0	0
	Carabus						
		Carabus nemoralis	COL	1 (E:1 I:0)	1	26	8
		Carabus violaceus	COL	1 (E:1 I:0)	1	1	1
	Chlaenius						
		Chlaenius platyderus	COL	1 (E:1 I:0)	0	0	0
		Chlaenius pusillus	COL	2 (E:2 I:0)	0	2	2

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Chlaenius sericeus	COL	1 (E:1 I:0)	0	0	0
		Chlaenius tomentosus	COL	1 (E:1 I:0)	1	10	0
		Chlaenius tricolor	COL	2 (E:2 I:0)	0	0	0
	Cicindela						
		Cicindela punctata	COL	1 (E:1 I:0)	0	0	0
	Clivina	Clivina	COL	1 (E:1 I:0)	-	-	-
		Clivina bipustulata	COL	1 (E:1 I:0)	0	1	1
		Clivina impressefrons	COL	1 (E:1 I:0)	1	1	0
	Cratacanthus						
		Cratacanthus dubius	COL	1 (E:1 I:0)	1	9	0
	Ctenognathus						
		Ctenognathus novaezelandiae	COL	1 (E:1 I:0)	0	0	0
	Cyclotrachelus						
		Cyclotrachelus seximpressus	COL	1 (E:1 I:0)	0	2	2
		Cyclotrachelus sodalis	COL	1 (E:1 I:0)	0	0	0
	Demetrias						
		Demetrias atricapalus	-	1 (E:1 I:0)	0	0	0
	Dicaelus						
		Dicaelus elongatus	COL	1 (E:1 I:0)	1	1	0
	Dyschirius						
		Dyschirius globosus	COL	1 (E:1 I:0)	1	1	0
	Elaphropus						
		Elaphropus anceps	COL	1 (E:1 I:0)	1	2	0
	Galerita						
		Galerita janus	COL	1 (E:1 I:0)	1	2	0
	Geopinus						
		Geopinus incrassatus	COL	1 (E:1 I:0)	0	0	0
	Harpalus						
		Harpalus affinis	COL	2 (E:2 I:0)	1	9	9
		Harpalus caliginosus	COL	2 (E:2 I:0)	1	6	0
		Harpalus eraticus --appeared as Harpalus erraticus	COL				
		Harpalus herbaivagus	-	1 (E:1 I:0)	0	0	0
		Harpalus pensylvanicus	COL	3 (E:3 I:0)	1	8	0
		Harpalus rufipes	COL	1 (E:1 I:0)	1	4	4
		Harpalus rufipes --appeared as Pseudoophonus	GBIF	1 (E:1 I:0)	1	4	4

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		rufipes					
	Lebia	Lebia	COL	1 (E:1 I:0)	-	-	-
		Lebia fuscata	COL	1 (E:1 I:0)	0	0	0
		Lebia grandis	COL	1 (E:1 I:0)	0	4	4
	Leptotrachelus						
		Leptotrachelus dorsalis	COL	1 (E:1 I:0)	1	4	0
	Loricera						
		Loricera pilicornis	COL	1 (E:1 I:0)	3	10	7
	Microlestes						
		Microlestes minutulus	COL	1 (E:1 I:0)	0	0	0
	Nebria						
		Nebria brevicollis	COL	2 (E:2 I:0)	1	2	2
	Notiophilus						
		Notiophilus biguttatus	COL	2 (E:2 I:0)	1	1	1
		Notiophilus palustris	COL	1 (E:1 I:0)	0	0	0
	Poecilus						
		Poecilus chalcites	COL	3 (E:3 I:0)	1	10	9
		Poecilus cupreus	COL	4 (E:4 I:0)	1	8	8
		Poecilus lucublandus	COL	3 (E:3 I:0)	1	13	3
	Pterostichus	Pterostichus	COL	1 (E:1 I:0)	-	-	-
		Pterostichus algidus	COL	1 (E:1 I:0)	1	4	0
		Pterostichus commutabilis	COL	1 (E:1 I:0)	0	0	0
		Pterostichus femoralis	COL	1 (E:1 I:0)	0	0	0
		Pterostichus madidus	COL	1 (E:1 I:0)	1	1	1
		Pterostichus melanarius	COL	6 (E:6 I:0)	1	27	11
		Pterostichus permundus	-	1 (E:1 I:0)	0	0	0
		Pterostichus strenuus	COL	1 (E:1 I:0)	1	1	1
		Pterostichus vernalis	COL	1 (E:1 I:0)	0	0	0
	Scaphinotus						
		Scaphinotus marginatus	COL	1 (E:1 I:0)	4	43	2
	Scarites						
		Scarites quadriceps	COL	1 (E:1 I:0)	0	1	1
		Scarites subterraneus	COL	1 (E:1 I:0)	0	0	0
	Stenolophus						
		Stenolophus comma	COL	1 (E:1 I:0)	1	1	0
		Stenolophus lecontei	COL	1 (E:1 I:0)	0	2	2
		Stenolophus ochropezus	COL	1 (E:1 I:0)	1	3	0
		Stenolophus teutonus	COL	1 (E:1 I:0)	1	5	5

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Stroluphus						
		Stroluphus chropezus	-	1 (E:1 I:0)	0	0	0
	Syntomus						
		Syntomus foveatus	COL	1 (E:1 I:0)	0	0	0
		Syntomus truncatellus	COL	1 (E:1 I:0)	0	0	0
	Synuchus						
		Synuchus impunctatus	COL	1 (E:1 I:0)	1	30	0
	Tetracha						
		Tetracha virginica	COL	1 (E:1 I:0)	0	0	0
	Trechus						
		Trechus quadristriatus	COL	2 (E:2 I:0)	1	3	2
Cerambycidae							
	Anoplophora						
		Anoplophora glabripennis	COL	3 (E: I:3)	1	443	443
	Massicus						
		Massicus raddei	COL	1 (E:0 I:1)	0	1	1
	Monochamus						
		Monochamus alternatus	COL	2 (E:1 I:1)	1	13	13
	Phoracantha						
		Phoracantha recurva	COL	1 (E:0 I:1)	0	0	0
		Phoracantha semipunctata	COL	2 (E:1 I:1)	1	1	1
	Saperda						
		Saperda populnea	COL	1 (E:0 I:1)	0	0	0
	Thyestilla						
		Thyestilla gebleri	COL	1 (E:0 I:1)	0	1	1
Chrysomelidae		Chrysomelidae	COL	1 (E:1 I:0)	-	-	-
	Acalymma						
		Acalymma vittatum	COL	1 (E:1 I:0)	1	7	2
	Agasicles						
		Agasicles hygrophila	COL	2 (E:2 I:0)	0	0	0
	Algarobius						
		Algarobius prosopis	COL	1 (E:1 I:0)	1	1	1
	Aphthona						
		Aphthona cyparissiae	COL	1 (E:1 I:0)	0	0	0
		Aphthona czwalinae	COL	1 (E:1 I:0)	0	0	0
		Aphthona flava	COL	1 (E:1 I:0)	0	0	0
		Aphthona lacertosa	COL	1 (E:1 I:0)	0	0	0
		Aphthona nigriscutis	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Aulacophora						
		Aulacophora foveicollis	-	1 (E:1 I:0)	0	0	0
	Bikasha						
		Bikasha collaris	GBIF	1 (E:1 I:0)	0	0	0
	Callosobruchus						
		Callosobruchus maculatus	COL	4 (E:2 I:2)	1	3	3
	Cassida						
		Cassida rubiginosa	COL	1 (E:1 I:0)	0	0	0
	Chrysolina						
		Chrysolina hyperici	COL	1 (E:1 I:0)	1	2	1
		Chrysolina quadrigemina	COL	1 (E:1 I:0)	0	0	0
	Diabrotica	Diabrotica	COL	1 (E:1 I:0)	-	-	-
		Diabrotica virgifera virgifera	COL	3 (E:1 I:2)	0	0	0
	Dicranosterna						
		Dicranosterna semipunctata	GBIF	2 (E:2 I:0)	0	0	0
	Diorhabda						
		Diorhabda carinata	-	1 (E:1 I:0)	1	8	8
		Diorhabda carinulata	GBIF	1 (E:1 I:0)	1	14	14
		Diorhabda elongata	COL	2 (E:1 I:1)	3	8	8
		Diorhabda sublineata	-	1 (E:1 I:0)	1	4	4
	Eumolpus						
		Eumolpus asclepiadeus	-	1 (E:1 I:0)	0	0	0
	Galerucella						
		Galerucella pusilla	GBIF	1 (E:1 I:0)	1	3	3
	Gallerucida						
		Gallerucida bifasciata	GBIF	1 (E:1 I:0)	0	0	0
	Gastrophysa						
		Gastrophysa viridula	GBIF	1 (E:1 I:0)	0	0	0
	Gonioctena						
		Gonioctena fornicata	GBIF	1 (E:1 I:0)	0	0	0
	Gratiana						
		Gratiana boliviana	COL	2 (E:1 I:1)	0	0	0
		Gratiana graminea	COL	1 (E:0 I:1)	0	0	0
	Leptinotarsa						
		Leptinotarsa decemlineata	COL	6 (E:6 I:0)	1	14	14
	Longitarsus						
		Longitarsus bethae	-	1 (E:1 I:0)	0	0	0
		Longitarsus jacobaeae	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Nesaecrepida						
		Nesaecrepida infuscata	COL	1 (E:1 I:0)	0	0	0
	Ophraella						
		Ophraella communa	COL	1 (E:1 I:0)	1	88	88
	Oulema						
		Oulema melanopus	COL	1 (E:1 I:0)	2	7	0
	Paropsis						
		Paropsis charybdis	GBIF	2 (E:2 I:0)	0	0	0
	Phratora						
		Phratora vulgatissima	GBIF	2 (E:2 I:0)	0	0	0
	Phyllotreta						
		Phyllotreta striolata	COL	1 (E:1 I:0)	2	119	0
	Trachymela						
		Trachymela catenata	GBIF	1 (E:1 I:0)	0	0	0
		Trachymela sloanei	COL	1 (E:1 I:0)	0	0	0
	Xanthogaleruca						
		Xanthogaleruca luteola --appeared as Pyrrhalta luteola	COL	1 (E:1 I:0)	0	0	0
Cleridae							
	Thanasimus						
		Thanasimus dubius	-	1 (E:1 I:0)	1	62	60
Coccinellidae		Coccinellidae	COL	4 (E:4 I:0)	-	-	-
	Adalia						
		Adalia bipunctata	COL	7 (E:5 I:2)	4	21	18
		Adalia decempunctata	GBIF	1 (E:1 I:0)	1	4	4
	Anatis						
		Anatis ocellata	GBIF	1 (E:1 I:0)	1	1	1
	Brachiacantha	Brachiacantha	COL	1 (E:1 I:0)	-	-	-
	Chilocorus						
		Chilocorus bipustulatus	COL	1 (E:1 I:0)	1	1	1
	Coccinella						
		Coccinella novemnotata	COL	1 (E:1 I:0)	1	1	0
		Coccinella septempunctata	COL	12 (E:12 I:0)	2	190	99
		Coccinella transversalis	GBIF	1 (E:1 I:0)	1	6	5
		Coccinella trifasciata	COL	1 (E:1 I:0)	1	17	0
		Coccinella undecimpunctata	COL	1 (E:1 I:0)	1	2	2
	Coleomegilla						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Coleomegilla maculata	COL	9 (E:8 I:1)	1	41	17
		Coleomegilla septempunctata	-	1 (E:1 I:0)	0	0	0
	Cryptognatha						
		Cryptognatha nodiceps	COL	1 (E:1 I:0)	0	0	0
	Cryptolaemus						
		Cryptolaemus montrouzieri	COL	1 (E:1 I:0)	1	6	6
	Curinus						
		Curinus coeruleus	COL	1 (E:1 I:0)	0	1	1
	Cycloneda						
		Cycloneda munda	COL	3 (E:3 I:0)	1	7	0
		Cycloneda sanguinea	COL	1 (E:1 I:0)	0	0	0
	Delphastus						
		Delphastus catalinae	COL	1 (E:0 I:1)	0	0	0
	Diomus	Diomus	COL	2 (E:2 I:0)	-	-	-
		Diomus robert	-	1 (E:1 I:0)	0	0	0
		Diomus sydneyensis	-	1 (E:1 I:0)	0	0	0
	Harmonia	Harmonia	COL	1 (E:1 I:0)	-	-	-
		Harmonia axyridis	COL	24 (E:22 I:2)	1	314	227
		Harmonia quadripunctata	COL	1 (E:1 I:0)	1	1	1
	Hippodamia						
		Hippodamia convergens	COL	9 (E:7 I:2)	1	340	150
		Hippodamia parenthesis	COL	3 (E:3 I:0)	1	89	71
		Hippodamia tredecimpunctata	COL	1 (E:1 I:0)	1	2	0
		Hippodamia variegata	COL	3 (E:3 I:0)	2	17	3
	Hyperaspis	Hyperaspis	COL	1 (E:1 I:0)	-	-	-
	Micraspis						
		Micraspis frenata	GBIF	1 (E:1 I:0)	1	1	1
	Olla						
		Olla v-nigrum	COL	1 (E:1 I:0)	1	1	1
	Propylaea						
		Propylea japonica	-				
		Propylea quatuordecimpunctata	COL		1	10	4
		Propylea dissecta	-	1 (E:1 I:0)	0	0	0
	Psyllobora						
		Psyllobora vigintimaculata	COL	2 (E:2 I:0)	6	427	0
	Rhyzobius						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Rhyzobius lophantae	-	1 (E:1 I:0)	1	2	2
	Rodolia						
		Rodolia cardinalis	COL	2 (E:2 I:0)	1	1	1
	Scymnus						
		Scymnus notescens --appeared as Diomus notescens	COL				
	Stethorus	Stethorus	COL	1 (E:1 I:0)	-	-	-
		Stethorus punctillum	COL	2 (E:0 I:2)	2	8	2
		Stethorus tridens	-	1 (E:1 I:0)	0	0	0
	Tenuisvalvae						
		Tenuisvalvae bisquinquepustulata	-	1 (E:1 I:0)	0	0	0
Curculionidae		Cryptorhynchini	-	1 (E:1 I:0)	-	-	-
Curculionidae		Curculionidae	COL	2 (E:2 I:0)	-	-	-
	Acythopeus						
		Acythopeus curvirostris granulipennis	-	1 (E:1 I:0)	0	0	0
	Anthonomus						
		Anthonomus pomorum	COL	1 (E:1 I:0)	0	0	0
	Aparete						
		Aparete palpebrosa	COL	1 (E:1 I:0)	0	0	0
	Artipus						
		Artipus floridanus	COL	1 (E:1 I:0)	0	0	0
	Aspidiotes						
		Aspidiotes cottyi	-	1 (E:1 I:0)	0	0	0
	Aulacobaris						
		Aulacobaris fallax	GBIF	1 (E:1 I:0)	0	0	0
	Ceutorhynchus						
		Ceutorhynchus fallax	GBIF	1 (E:1 I:0)	0	0	0
		Ceutorhynchus napi	COL	2 (E:2 I:0)	0	0	0
		Ceutorhynchus obstrictus	GBIF	1 (E:1 I:0)	1	6	4
		Ceutorhynchus pallidactylus	GBIF	2 (E:2 I:0)	0	0	0
		Ceutorhynchus scrobicollis	GBIF	1 (E:1 I:0)	0	0	0
	Charagmus						
		Charagmus gressorius	-	1 (E:1 I:0)	0	0	0
		Charagmus griseus	-	1 (E:1 I:0)	0	0	0
	Coelositona						
		Coelositona ocellatus	-	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Coniatus						
		Coniatus repandus	COL	1 (E:1 I:0)	0	0	0
	Coniocleonus						
		Coniocleonus excoriatus	GBIF	1 (E:1 I:0)	0	0	0
	Cyphocleonus						
		Cyphocleonus achates	GBIF	1 (E:1 I:0)	0	0	0
	Dendroctonus						
		Dendroctonus frontalis	COL	1 (E:1 I:0)	0	2	2
	Diaprepes						
		Diaprepes abbreviatus	COL	1 (E:1 I:0)	13	79	79
	Eremobaris						
		Eremobaris picturata	GBIF	1 (E:1 I:0)	0	0	0
	Ethemaia	Ethemaia	COL	1 (E:1 I:0)	-	-	-
		Ethemaia sellata	COL	1 (E:1 I:0)	0	0	0
	Gonipterus						
		Gonipterus scutellatus	COL	1 (E:0 I:1)	0	0	0
	Gronops						
		Gronops luctuosus	COL	1 (E:1 I:0)	0	0	0
	Heydeneonymus						
		Heydeneonymus	COL	1 (E:1 I:0)	-	-	-
	Hypera	Hypera	COL	1 (E:1 I:0)	-	-	-
		Hypera nigrirostris	COL	1 (E:1 I:0)	1	7	0
		Hypera postica	COL	1 (E:1 I:0)	0	0	0
	Hypothenemus						
		Hypothenemus hampei	COL	2 (E:2 I:0)	0	26	26
	Larinus						
		Larinus minutus	COL	1 (E:1 I:0)	0	0	0
		Larinus onopordi	GBIF	1 (E:1 I:0)	0	0	0
	Listroderes						
		Listroderes delaiguei	COL	1 (E:1 I:0)	0	0	0
	Lixus						
		Lixus albomarginatus	COL	1 (E:1 I:0)	0	0	0
		Lixus linearis	COL	1 (E:1 I:0)	0	0	0
		Lixus ulcerosus	GBIF	1 (E:1 I:0)	0	0	0
	Mecinus						
		Mecinus janthinus	COL	1 (E:1 I:0)	0	0	0
	Microlarinus						
		Microlarinus lypriformis	GBIF	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Mogulones						
		Mogulones larvatus	GBIF	1 (E:1 I:0)	0	0	0
	Naupactus						
		Naupactus cervinus	COL	1 (E:1 I:0)	1	18	18
		Naupactus leucoloma	COL	1 (E:1 I:0)	1	74	74
	Otiorhynchus						
		Otiorhynchus sulcatus	COL	2 (E:1 I:1)	0	1	0
	Oxyops						
		Oxyops vitiosa	COL	1 (E:1 I:0)	0	0	0
	Pachnaeus						
		Pachnaeus litus	COL	1 (E:1 I:0)	0	0	0
	Prosayleus	Prosayleus	COL	1 (E:1 I:0)	-	-	-
	Pseudorchestes						
		Pseudorchestes asterici	-	1 (E:1 I:0)	0	0	0
	Rhinocyllus						
		Rhinocyllus conicus	GBIF	1 (E:1 I:0)	0	0	0
	Rhinoncomimus						
		Rhinoncomimus latipes	-	3 (E:2 I:1)	0	0	0
	Rhinusa	Rhinusa	COL	1 (E:1 I:0)	-	-	-
	Sibinia						
		Sibinia arenariae	COL	1 (E:1 I:0)	0	0	0
	Sitona						
		Sitona cachectus	GBIF				
		--appeared as Charagmus cachectus					
		Sitona discoideus	COL	2 (E:2 I:0)	1	34	34
		Sitona intermedius	GBIF				
		--appeared as Charagmus intermedius					
		Sitona lepidus	COL	1 (E:1 I:0)	0	0	0
		Sitona lineatus	COL	2 (E:2 I:0)	1	1	0
		Sitona macularius	COL	1 (E:1 I:0)	0	0	0
		Sitona tenuis	COL	1 (E:1 I:0)	0	0	0
	Smicronyx						
		Smicronyx kiesenwetteri	COL	1 (E:1 I:0)	0	0	0
	Titinia	Titinia	COL	1 (E:1 I:0)	-	-	-
	Tychius	Tychius	COL	1 (E:1 I:0)	-	-	-
		Tychius bicolor	COL	1 (E:1 I:0)	0	0	0
		Tychius depauperatus	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Tychius elongatulus	COL	1 (E:1 I:0)	0	0	0
		Tychius mozabitus	COL	1 (E:1 I:0)	0	0	0
		Tychius striatulus	COL	1 (E:1 I:0)	0	0	0
	Xylosandrus						
		Xylosandrus germanus	COL	1 (E:1 I:0)	2	21	3
Derodontidae							
	Laricobius						
		Laricobius kangdingensis	-	1 (E:1 I:0)	1	1	1
		Laricobius nigrinus	COL	2 (E:2 I:0)	1	308	308
		Laricobius osakensis	-	2 (E:2 I:0)	1	291	291
		Laricobius rubidus	COL	1 (E:1 I:0)	1	276	276
Dryophthoridae							
	Rhynchophorus						
		Rhynchophorus ferrugineus	COL	1 (E:1 I:0)	0	310	310
	Sitophilus	Sitophilus	-	1 (E:1 I:0)	-	-	-
		Sitophilus granarius	COL	1 (E:1 I:0)	1	2	2
		Sitophilus oryzae	COL	2 (E:2 I:0)	1	3	3
		Sitophilus zeamais	GBIF	1 (E:0 I:1)	1	2	1
Erirhinidae							
	Neochetina						
		Neochetina eichhorniae	GBIF	1 (E:1 I:0)	0	0	0
Melolonthidae							
	Phyllophaga						
		Phyllophaga dentex	COL	1 (E:1 I:0)	0	0	0
		Phyllophaga rubella	-	1 (E:1 I:0)	0	0	0
		Phyllophaga vetula	-	1 (E:1 I:0)	0	0	0
Nanophyidae							
	Corimalia						
		Corimalia tamarisci	GBIF	1 (E:1 I:0)	0	0	0
	Nanodiscus						
		Nanodiscus transversus	GBIF	1 (E:1 I:0)	0	0	0
Nitidulidae							
	Aethina						
		Aethina tumida	-	1 (E:1 I:0)	1	5	5
	Meligethes						
		Meligethes aeneus	GBIF	2 (E:2 I:0)	4	5	5
		Meligethes viridescens	GBIF	2 (E:2 I:0)	0	0	0
Rutelidae							

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Anomala						
		Anomala cincta	COL	1 (E:1 I:0)	0	0	0
		Anomala orientalis	GBIF	1 (E:1 I:0)	0	0	0
	Popillia						
		Popillia japonica	COL	1 (E:1 I:0)	1	6	0
Staphylinidae		Staphylinidae	COL	2 (E:2 I:0)	-	-	-
	Aleochara	Aleochara	COL	1 (E:1 I:0)	-	-	-
		Aleochara bilineata	COL	3 (E:2 I:1)	1	3	2
		Aleochara bipustulata	COL	1 (E:1 I:0)	0	1	1
		Aleochara verna	COL	2 (E:2 I:0)	1	51	2
	Conosoma	Conosoma	-	1 (E:1 I:0)	-	-	-
	Dalotia						
		Dalotia coriaria	COL	1 (E:0 I:1)	0	0	0
		--appeared as Atheta coriaria					
	Dinaraea						
		Dinaraea angustula	COL	1 (E:1 I:0)	0	0	0
	Gabriusa	Gabriusa	-	1 (E:1 I:0)	-	-	-
	Lathrobium	Lathrobium	COL	1 (E:1 I:0)	-	-	-
	Leptacinus	Leptacinus	COL	2 (E:2 I:0)	-	-	-
	Mocyta						
		Mocyta fungi	COL	1 (E:1 I:0)	0	0	0
	Mycetoporus						
		Mycetoporus lucidulus	-	1 (E:1 I:0)	0	0	0
	Ocalea	Ocalea	COL	1 (E:1 I:0)	-	-	-
	Oligota						
		Oligota pygmaea	-	1 (E:1 I:0)	0	0	0
	Othius	Othius	COL	1 (E:1 I:0)	-	-	-
	Oxypoda						
		Oxypoda robusticornis	COL	1 (E:1 I:0)	0	0	0
	Oxytelus	Oxytelus	COL	1 (E:1 I:0)	-	-	-
	Paederus	Paederus	COL	1 (E:1 I:0)	-	-	-
	Philhygra	Philhygra	COL	1 (E:1 I:0)	-	-	-
		Philhygra subpolaris	COL	1 (E:1 I:0)	0	0	0
	Philonthus	Philonthus	COL	2 (E:2 I:0)	-	-	-
		Philonthus cognatus	GBIF	1 (E:1 I:0)	1	4	0
		Philonthus occidentalis	-	1 (E:1 I:0)	0	0	0
	Pseudoplandria	Pseudoplandria	COL	1 (E:1 I:0)	-	-	-
	Stenus	Stenus	COL	2 (E:2 I:0)	-	-	-

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Stilicus	Stilicus	-	1 (E:1 I:0)	-	-	-
	Tachinus	Tachinus	COL	3 (E:3 I:0)	-	-	-
	Tachyporus	Tachyporus	COL	1 (E:1 I:0)	-	-	-
		Tachyporus chrysomelinus	COL	1 (E:1 I:0)	1	51	0
		Tachyporus hypnorum	COL	1 (E:1 I:0)	0	0	0
		Tachyporus nitidulus	GBIF	1 (E:1 I:0)	1	47	0
		Tachyporus obtusum	-	1 (E:1 I:0)	0	0	0
	Trogophloeus	Trogophloeus	GBIF	1 (E:1 I:0)	-	-	-
	Xantholinus	Xantholinus	COL	2 (E:2 I:0)	-	-	-
		Aleocharinae	COL	1 (E:1 I:0)	-	-	-
Tenebrionidae							
	Alphitobius						
		Alphitobius diaperinus	COL	1 (E:1 I:0)	0	0	0
	Tenebrio						
		Tenebrio molitor	COL	3 (E:3 I:0)	3	17	17
	Tribolium						
		Tribolium castaneum	COL	1 (E:0 I:1)	2	8	6
		Tribolium confusum	COL	1 (E:1 I:0)	1	3	3
Dermaptera							
Forficulidae							
	Forficula						
		Forficula auricularia	COL	2 (E:2 I:0)	2	2	1
Diptera		Diptera	COL	3 (E:3 I:0)	-	-	-
Agromyzidae	Agromyzidae	Agromyzidiae	COL	1 (E:1 I:0)	0	0	0
	Liriomyza						
		Liriomyza huidobrensis	COL	1 (E:0 I:1)	1	11	11
Anthomyiidae							
	Delia						
		Delia radicum	COL	3 (E:2 I:1)	1	10	1
	Pegomya	Pegomya	COL	1 (E:1 I:0)	-	-	-
Bibionidae		Bibionidae	COL	1 (E:1 I:0)	-	-	-
Calliphoridae							
	Calliphora						
		Calliphora vicina	COL	1 (E:1 I:0)	1	90	54
	Lucilia						
		Lucilia sericata	COL	1 (E:1 I:0)	1	117	96
Cecidomyiidae							
	Aphidoletes	Aphidoletes	COL	1 (E:1 I:0)	-	-	-

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Aphidoletes aphidimyza	COL	1 (E: I:1)	1	7	0
Cecidomyiidae		Cecidomyiidae	COL	1 (E:1 I:0)	-	-	-
	Contarinia						
		Contarinia nasturtii	COL	1 (E:1 I:0)	0	0	0
	Dasineura						
		Dasineura dielsi	COL	1 (E:1 I:0)	0	0	0
		Dasineura mali	COL	1 (E:1 I:0)	0	0	0
	Diadiplosis						
Cecidomyiidae		Diadiplosis coccidarum	-	1 (E:1 I:0)	0	0	0
	Endaphis						
		Endaphis fugitiva	-	1 (E:1 I:0)	1	1	1
	Lestodiplosis						
		Lestodiplosis aonidiellae	COL	1 (E:1 I:0)	0	0	0
	Obolodiplosis						
	Obolodiplosis	Obolodiplosis robiniae	COL	1 (E:1 I:0)	0	5	5
Chironomidae		Chironomidae	COL	1 (E:1 I:0)	-	-	-
Chloropidae		Chloropidae	COL	1 (E:1 I:0)	-	-	-
Conopidae		Conopidae	COL	1 (E:1 I:0)	-	-	-
Culicidae		Culicidae	COL	1 (E:1 I:0)	-	-	-
	Aedes						
		Aedes aegypti	COL	4 (E:2 I:2)	2	81	75
	Anopheles						
		Anopheles subpictus	COL	1 (E:1 I:0)	3	21	21
	Culex						
		Culex bitaeniorhynchus	COL	1 (E:1 I:0)	1	15	15
		Culex pipiens	COL	2 (E:2 I:0)	3	313	302
		Culex quinquefasciatus	COL	2 (E:2 I:0)	1	159	154
	Ochlerotatus						
		Ochlerotatus albifasciatus	-	1 (E:1 I:0)	0	0	0
Dolichopodidae		Dolichopodidae	COL	1 (E:1 I:0)	-	-	-
Drosophilidae							
	Drosophila						
		Drosophila melanogaster	COL	3 (E:3 I:0)	2	239	232
	Scaptomyza	Scaptomyza flava	COL	1 (E:1 I:0)	1	24	1
Ephydridae							
	Scatella						
		Scatella tenuicosta	COL	2 (E:2 I:0)	2	65	0
Fanniidae							

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Fannia						
		Fannia scalaris	COL	1 (E:1 I:0)	0	3	0
Lauxaniidae		Lauxaniidae	COL	1 (E:1 I:0)	-	-	-
Lonchaeidae							
	Lonchaea	Lonchaea	COL	1 (E:1 I:0)	-	-	-
		Lonchaea corticis	COL	1 (E:1 I:0)	0	0	0
Lonchopteridae		Lonchopteridae	COL	1 (E:1 I:0)	-	-	-
Muscidae		Muscidae	COL	1 (E:1 I:0)	-	-	-
	Hydrotaea						
		Hydrotaea aenescens	COL	1 (E:0 I:1)	1	5	0
		Hydrotaea ignava	COL	1 (E:1 I:0)	1	2	1
	Musca						
		Musca autumnalis	COL	1 (E:0 I:1)	1	173	121
		Musca domestica	COL	5 (E:3 I:2)	5	105	95
	Muscina						
		Muscina levida	COL	1 (E:1 I:0)	1	63	2
	Ophyra						
		Ophyra aenescens	-	1 (E:0 I:1)	1	2	2
	Polietes						
		Polietes domitor	COL	1 (E:1 I:0)	0	0	0
	Stomoxys						
	Stomoxys	Stomoxys calcitrans	COL	1 (E:0 I:1)	3	178	103
Phoridae							
	Pseudacteon						
		Pseudacteon curvatus	COL	3 (E:2 I:1)	0	0	0
		Pseudacteon nocens	COL	1 (E:1 I:0)	0	0	0
		Pseudacteon obtusus	COL	4 (E:4 I:0)	0	0	0
		Pseudacteon tricuspis	COL	4 (E:3 I:1)	0	0	0
Piophilidae							
	Stearibia						
	Stearibia	Stearibia nigriceps	GBIF	1 (E:1 I:0)	0	0	0
Pipunculidae		Pipunculidae	COL	1 (E:1 I:0)	-	-	-
Psilidae							
	Psila	Psila rosae	GBIF	1 (E:0 I:1)	0	0	0
Psychodidae							
	Lutzomyia						
		Lutzomyia longipalpis	COL	1 (E:1 I:0)	1	4	4

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
Rhagionidae		Rhagionidae	COL	1 (E:1 I:0)	-	-	-
Sarcophagidae							
	Agria						
		Agria mamillata	COL	1 (E:1 I:0)	0	0	0
Scathophagidae		Scathophagidae	COL	1 (E:1 I:0)	-	-	-
Sciaridae		Sciaridae	COL	1 (E:1 I:0)	-	-	-
	Bradysia						
		Bradysia matogrossensis	COL	1 (E:1 I:0)	0	0	0
Sciomyzidae		Sciomyzidae	COL	1 (E:1 I:0)	-	-	-
Sepsidae		Sepsidae	COL	1 (E:1 I:0)	-	-	-
Sphaeroceridae							
	Spelobia						
		Spelobia luteilabris	COL	1 (E:1 I:0)	2	76	0
Syrphidae		Syrphidae	COL	4 (E:4 I:0)	-	-	-
	Allograpta						
		Allograpta obliqua	COL	4 (E:4 I:0)	1	4	1
	Chrysotoxum	Chrysotoxum	COL	1 (E:1 I:0)	-	-	-
	Episyrphus						
		Episyrphus balteatus	COL	7 (E:4 I:3)	2	84	5
	Eupeodes						
		Eupeodes corollae	COL	1 (E:1 I:0)	1	35	2
		Eupeodes fumipennis	COL	2 (E:2 I:0)	0	1	1
		Eupeodes latilunulatus	COL	1 (E:1 I:0)	0	0	0
		Eupeodes luniger	COL	1 (E:1 I:0)	1	2	0
		Eupeodes nitens	COL	1 (E:1 I:0)	1	3	0
	Melanostoma						
		Melanostoma mellinum	COL	1 (E:1 I:0)	2	39	0
	Ocyptamus						
		Ocyptamus stenogaster	COL	1 (E:1 I:0)	0	0	0
	Paragus						
		Paragus haemorrhous	COL	1 (E:1 I:0)	3	10	4
	Platycheirus						
		Platycheirus scutatus	COL	1 (E:1 I:0)	1	2	0
		Platycheirus stegnus	COL	3 (E:3 I:0)	0	1	0
	Scaeva						
		Scaeva pyrastri	COL	1 (E:1 I:0)	2	10	3
	Sphaerophoria	Sphaerophoria	COL	1 (E:1 I:0)	-	-	-
		Sphaerophoria rueppellii	COL	1 (E:1 I:0)	1	2	1

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Sphaerophoria scripta	COL	2 (E:2 I:0)	1	6	2
		Sphaerophoria sulphuripes	COL	4 (E:4 I:0)	0	0	0
	Syrphus	Syrphus	COL	1 (E:1 I:0)	-	-	-
		Syrphus opinator	COL	1 (E:1 I:0)	0	1	1
		Syrphus ribesii	COL	1 (E:1 I:0)	1	6	0
	Toxomerus						
		Toxomerus marginatus	COL	5 (E:5 I:0)	1	295	4
		Toxomerus occidentalis	COL	1 (E:1 I:0)	0	0	0
Tachinidae		Tachinidae	COL	1 (E:1 I:0)	-	-	-
	Actia						
		Actia interrupta	COL	1 (E:1 I:0)	1	33	0
	Celatoria						
		Celatoria compressa	COL	1 (E:1 I:0)	0	0	0
		Celatoria setosa	COL	1 (E:1 I:0)	0	0	0
	Exorista						
		Exorista larvarum	COL	2 (E:1 I:1)	3	5	2
	Gymnosoma						
		Gymnosoma rotundatum	GBIF	1 (E:1 I:0)	0	0	0
	Nilea						
		Nilea erecta	COL	1 (E:1 I:0)	0	0	0
	Phytomyptera						
		Phytomyptera nigrina	COL	1 (E:1 I:0)	0	0	0
Tephritidae		Tephritidae	COL	1 (E:1 I:0)	-	-	-
	Anastrepha						
		Anastrepha fraterculus	COL	1 (E:1 I:0)	1	49	49
		Anastrepha ludens	COL	5 (E:2 I:3)	1	21	21
		Anastrepha obliqua	COL	1 (E: I:1)	1	43	43
		Anastrepha serpentina	COL	1 (E: I:1)	1	8	8
		Anastrepha striata	COL	1 (E: I:1)	2	18	18
		Anastrepha suspensa	COL	1 (E:1 I:0)	0	2	2
	Bactrocera						
		Bactrocera dorsalis	COL	1 (E:1 I:0)	2	197	188
		Bactrocera invadens	COL	1 (E:1 I:0)	2	162	17
		Bactrocera kirki	COL	1 (E:1 I:0)	1	5	5
		Bactrocera latifrons	COL	1 (E:1 I:0)	1	28	16
		Bactrocera oleae	COL	7 (E:7 I:0)	2	47	37
		Bactrocera tryoni	COL	1 (E:1 I:0)	1	16	16
	Ceratitis						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Ceratitis capitata	COL	12 (E:7 I:5)	2	185	164
		Ceratitis cosyra	COL	2 (E:1 I:1)	8	69	31
		Ceratitis fasciventris	COL	1 (E:0 I:1)	1	26	16
		Ceratitis quinaria	COL	1 (E:1 I:0)	1	12	6
		Ceratitis silvestrii	COL	1 (E:1 I:0)	1	8	4
	Procecidochares						
		Procecidochares utilis	COL	1 (E:1 I:0)	0	0	0
	Rhagoletis						
		Rhagoletis cerasi	COL	1 (E:1 I:0)	1	16	14
		Rhagoletis completa	COL	1 (E:1 I:0)	1	19	19
		Rhagoletis mendax	COL	1 (E:1 I:0)	1	2	2
	Urophora						
		Urophora affinis	COL	1 (E:1 I:0)	1	1	0
		Urophora quadrifasciata	COL	1 (E:1 I:0)	1	4	2
Hemiptera		Hemiptera	COL	2 (E:2 I:0)	-	-	-
Adelgidae							
	Adelges						
		Adelges tsugae	COL	2 (E:2 I:0)	4	119	0
Aleyrodidae							
	Aleurocanthus						
		Aleurocanthus woglumi	COL	1 (E:1 I:0)	0	0	0
	Aleurodicus						
		Aleurodicus dispersus	COL	2 (E:2 I:0)	1	7	7
		Aleurodicus pulvinatus	COL	1 (E:1 I:0)	0	0	0
	Aleurothrixus						
		Aleurothrixus floccosus	COL	1 (E:1 I:0)	0	0	0
	Aleurotrachelus	Aleurotrachelus	COL	1 (E:1 I:0)	-	-	-
		Aleurotrachelus atratusa	-	1 (E:1 I:0)	0	0	0
	Aleyrodes						
		Aleyrodes proletella	COL	3 (E:2 I:1)	0	0	0
	Bemisia						
		Bemisia tabaci --as appears as Bemisia argentifolii	COL	11 (E:7 I:4)	12	214	214
	Parabemisia						
		Parabemisia myricae	COL	1 (E:1 I:0)	0	0	0
	Paraleyrodes						
		Paraleyrodes bondari	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Paraleyrodes urichii	COL	1 (E:1 I:0)	0	0	0
	Tetraleurodes	Tetraleurodes	COL	1 (E:1 I:0)	-	-	-
	Trialeurodes						
		Trialeurodes lauri	COL	1 (E:1 I:0)	0	0	0
		Trialeurodes vaporariorum	COL	4 (E:3 I:1)	1	22	19
		Trialeurodes variabilis	COL	1 (E:1 I:0)	0	0	0
Anthocoridae		Anthocoridae	COL	2 (E:2 I:0)	-	-	-
	Anthocoris						
		Anthocoris nemoralis	COL	2 (E:1 I:1)	1	3	2
		Anthocoris nemorum	COL	2 (E:2 I:0)	1	1	1
	Calliodis						
		Calliodis	COL	1 (E:1 I:0)	-	-	-
	Montandoniola						
		Montandoniola confusa	-	1 (E:1 I:0)	0	0	0
	Orius	Orius	COL	2 (E:2 I:0)	-	-	-
	Orius	Orius albidipennis	GBIF	2 (E:1 I:1)	0	0	0
	Orius	Orius insidiosus	COL	7 (E:4 I:3)	3	14	2
	Orius	Orius laevigatus	GBIF	6 (E:2 I:4)	1	5	5
	Orius	Orius minutes	-	1 (E:1 I:0)	0	0	0
	Wollastoniella						
		Wollastoniella rotunda	-	1 (E:1 I:0)	0	0	0
Aphididae		Aphididae	COL	2 (E:2 I:0)	-	-	-
	Acyrthosiphon						
		Acyrthosiphon pisum	COL	18 (E:14 I:4)	1	57	14
		Amphorophora idaei	COL	1 (E:1 I:0)	0	1	1
	Aphis						
		Aphis craccivora	COL	2 (E:2 I:0)	2	181	121
		Aphis fabae	COL	6 (E:4 I:2)	1	121	13
		Aphis glycines	COL	4 (E:4 I:0)	1	39	27
		Aphis gossypii	COL	3 (E:2 I:1)	1	219	113
		Aphis pomi	COL	1 (E:1 I:0)	2	93	3
		Aphis spiraecola	COL	2 (E:2 I:0)	3	138	44
		Aphis urticata	COL	1 (E:1 I:0)	1	9	3
	Aulacorthum						
		Aulacorthum solani	COL	1 (E:1 I:0)	1	17	6
	Brevicoryne						
		Brevicoryne brassicae	COL	1 (E:1 I:0)	3	22	11

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Chromaphis						
		Chromaphis juglandicola	COL	1 (E:1 I:0)	1	1	0
	Dysaphis						
		Dysaphis plantaginea	COL	3 (E:3 I:0)	1	8	1
	Hyalopterus						
		Hyalopterus pruni	COL	2 (E:2 I:0)	3	205	187
	Lipaphis						
		Lipaphis erysimi	COL	2 (E:2 I:0)	1	3	1
	Macrosiphum						
		Macrosiphum euphorbiae	COL	4 (E:4 I:0)	1	37	3
	Megoura						
		Megoura viciae	COL	1 (E:1 I:0)	1	9	2
	Metopolophium						
		Metopolophium dirhodum	COL	1 (E:1 I:0)	1	14	9
	Microlophium						
		Microlophium carnosum	COL	2 (E:2 I:0)	1	4	1
	Myzus						
		Myzus cerasi	COL	1 (E:1 I:0)	1	26	1
		Myzus persicae	COL	16 (E:14 I:2)	1	44	26
	Nasonovia						
		Nasonovia ribisnigri	COL	4 (E:4 I:0)	2	4	0
	Pentalonia						
		Pentalonia nigronervosa	COL	1 (E:1 I:0)	2	45	18
	Rhopalosiphum						
		Rhopalosiphum padi	COL	2 (E:1 I:1)	2	121	15
	Schizaphis						
		Schizaphis graminum	COL	1 (E:1 I:0)	5	166	101
	Sitobion						
		Sitobion avenae	COL	6 (E:6 I:0)	1	89	26
	Toxoptera						
		Toxoptera aurantii	COL	1 (E:1 I:0)	1	14	8
		Toxoptera citricidus	COL	1 (E:1 I:0)	1	8	5
Berytidae		Berytidae	COL	1 (E:1 I:0)	-	-	-
Blissidae							
	Ischnodemus						
		Ischnodemus variegatus	-	2 (E:2 I:0)	0	0	0
Cicadellidae		Cicadellidae	COL	2 (E:2 I:0)	-	-	-

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
Cicadellidae		Erythroneurini	-	1 (E:1 I:0)	-	-	-
	Alebra						
		Alebra wahlbergi	COL	1 (E:1 I:0)	0	0	0
	Empoasca	Empoasca	GBIF	1 (E:1 I:0)	-	-	-
		Empoasca vitis	COL	1 (E:1 I:0)	1	62	62
	Erythroneura						
		Erythroneura elegantula	COL	1 (E:0 I:1)	0	0	0
	Graphocephala						
		Graphocephala atropunctata	COL	1 (E:0 I:1)	0	16	16
	Homalodisca						
		Homalodisca liturata	COL	1 (E:0 I:1)	1	1	1
		Homalodisca vitripennis	COL	7 (E:6 I:1)	1	3	3
	Idiocerus						
		Idiocerus stigmaticalis	COL	1 (E:1 I:0)	0	0	0
	Japananus						
		Japananus hyalinus	COL	1 (E:1 I:0)	0	0	0
	Limotettix						
		Limotettix vaccinii	COL	1 (E:1 I:0)	0	0	0
	Macrosteles						
		Macrosteles septemnotatus	GBIF	1 (E:1 I:0)	0	0	0
	Scaphytopius						
		Scaphytopius magdalensis	COL	1 (E:1 I:0)	0	0	0
	Tapajosa						
		Tapajosa rubromarginata	COL	1 (E:0 I:1)	0	0	0
Coccidae							
	Ceroplastes						
		Ceroplastes ceriferus	COL	1 (E:1 I:0)	2	25	25
		Ceroplastes japanicus	-	1 (E:1 I:0)	0	0	0
		Ceroplastes rubens	COL	1 (E:1 I:0)	2	36	36
		Ceroplastes sinensis	COL	1 (E:1 I:0)	0	0	0
	Coccus						
		Coccus hesperidum	GBIF	4 (E:4 I:0)	1	12	12
		Coccus viridis	COL	3 (E:3 I:0)	1	3	3
	Protopulvinaria						
		Protopulvinaria pyriformis	COL	1 (E:1 I:0)	1	1	1
	Saissetia						
		Saissetia miranda	GBIF	2 (E:2 I:0)	0	0	0
		--appeared as Saissetia oleae					

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
Coreidae							
	Leptoglossus						
		Leptoglossus occidentalis	COL	1 (E:1 I:0)	1	6	0
Delphacidae		Delphacidae	COL	1 (E:1 I:0)	-	-	-
	Conomelus						
		Conomelus anceps	COL	1 (E:1 I:0)	0	0	0
	Nilaparvata						
		Nilaparvata lugens	COL	1 (E:0 I:1)	1	167	165
Diaspididae							
	Abgrallaspis						
		Abgrallaspis cyanophylli	COL	1 (E:1 I:0)	0	0	0
	Aonidiella						
		Aonidiella aurantii	COL	3 (E:3 I:0)	0	0	0
		Aonidiella inornata	COL	1 (E:1 I:0)	0	0	0
	Aspidiotus						
		Aspidiotus destructor	COL	2 (E:2 I:0)	0	0	0
		Aspidiotus nerii	COL	2 (E:2 I:0)	0	0	0
	Aulacaspis						
		Aulacaspis yasumatsui	COL	1 (E:1 I:0)	0	0	0
	Chrysomphalus						
		Chrysomphalus aonidum	COL	1 (E:1 I:0)	0	0	0
	Diaspis						
		Diaspis boisduvalii	COL	1 (E:1 I:0)	0	0	0
	Fiorinia						
		Fiorinia fioriniae	COL	1 (E:1 I:0)	0	0	0
	Lepidosphes						
		Lepidosphes beckii	-	1 (E:1 I:0)	0	0	0
	Parlatoria						
		Parlatoria cinerea	COL	1 (E:1 I:0)	0	0	0
	Pseudaonidia						
		Pseudaonidia trilobitiformis	COL	1 (E:1 I:0)	0	0	0
	Pseudaulacaspis						
		Pseudaulacaspis cockerelli	COL	1 (E:1 I:0)	0	0	0
		Pseudaulacaspis pentagona	COL	1 (E:1 I:0)	0	0	0
	Unaspis						
		Unaspis citri	COL	1 (E:1 I:0)	0	0	0
Flatidae							
	Metcalfa						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Metcalfa pruinosa	COL	1 (E:1 I:0)	1	73	0
Geocoridae							
	Geocoris						
		Geocoris punctipes	COL	2 (E:1 I:1)	0	0	0
Halimococcidae							
	Colobopyga						
		Colobopyga pritchardiae	COL	1 (E:1 I:0)	0	0	0
Issidae							
	Issus						
		Issus coleoptratus	COL	1 (E:1 I:0)	0	0	0
Lophopidae							
	Zophiuma						
		Zophiuma butawengi	COL	1 (E:1 I:0)	0	0	0
Lygaeidae		Lygaeidae	COL	1 (E:1 I:0)	-	-	-
Margarodidae							
	Icerya						
		Icerya purchasi	COL	2 (E:2 I:0)	0	0	0
Membracidae							
	Aconophora						
		Aconophora compressa	COL	1 (E:1 I:0)	1	1	1
Miridae		Miridae	COL	1 (E:1 I:0)	-	-	-
	Amblytylus	Amblytylus nasutus	COL	1 (E:1 I:0)	1	3	0
	Closterotomus	Closterotomus norvegicus	-	1 (E:1 I:0)	0	0	0
	Creontiades	Creontiades pallidus	GBIF	1 (E:1 I:0)	0	0	0
	Deraeocoris	Deraeocoris ruber	COL	1 (E:1 I:0)	0	0	0
		Deraeocoris brevis	COL	1 (E:1 I:0)	0	0	0
	Dicyphus						
		Dicyphus hesperus	COL	1 (E:1 I:0)	1	10	0
	Eccritotarsus						
		Eccritotarsus catarinensis	-	2 (E:2 I:0)	0	0	0
	Falconia						
		Falconia intermedia	-	1 (E:1 I:0)	0	0	0
	Heterotoma						
		Heterotoma meriopterum	-	1 (E:1 I:0)	0	0	0

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	Leptopterna						
		Leptopterna dolabrata	COL	1 (E:1 I:0)	1	1	0
	Lygus	Lygus	COL	1 (E:1 I:0)	-	-	-
		Lygus borealis	COL	1 (E:1 I:0)	1	7	0
		Lygus elisus	COL	1 (E:1 I:0)	1	12	0
		Lygus hesperus	COL	1 (E:1 I:0)	1	35	31
		Lygus keltoni	COL	1 (E:1 I:0)	0	17	0
		Lygus lineolaris	COL	2 (E:1 I:1)	1	497	249
		Lygus shulli	COL	2 (E:2 I:0)	0	1	0
		Lygus vanduzeei	COL	1 (E:1 I:0)	1	7	0
	Macrolophus						
		Macrolophus caliginosus	-	4 (E:0 I:4)	1	4	4
		Macrolophus pygmaeus	COL	4 (E:1 I:3)	4	18	18
	Melanotrichus						
		Melanotrichus coagulatus	COL	1 (E:1 I:0)	1	3	0
	Nesidiocoris						
		Nesidiocoris tenuis	COL	5 (E:0 I:5)	1	4	4
	Plagiognathus						
		Plagiognathus politus	COL	1 (E:1 I:0)	0	1	0
	Prepops						
		Prepops rubellicollis	COL	1 (E:1 I:0)	1	4	0
	Slaterocoris						
		Slaterocoris breviatus	COL	1 (E:1 I:0)	1	2	0
	Stenotus						
		Stenotus rubrovittatus	-	1 (E:1 I:0)	1	40	40
	Trigonotylus						
		Trigonotylus caelestialium	COL	1 (E:1 I:0)	1	3	2
Nabidae		Nabidae	COL	3 (E:3 I:0)	-	-	-
	Nabis	Nabis	COL	1 (E:1 I:0)	-	-	-
		Nabis alternatus	COL	1 (E:1 I:0)	1	4	0
		Nabis provencalis	GBIF	1 (E:1 I:0)	0	0	0
Pentatomidae		Pentatomidae	COL	1 (E:1 I:0)	-	-	-
	Nezara						
		Nezara viridula	COL	2 (E:1 I:1)	1	11	11
	Perillus						
		Perillus bioculatus	COL	1 (E:0 I:1)	0	0	0
	Plautia						
		Plautia stali	COL	1 (E:1 I:0)	1	1	1

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Podisus						
		Podisus maculiventris	COL	2 (E:2 I:0)	1	3	1
		Podisus nigrispinus	-	1 (E:1 I:0)	0	0	0
Pseudococcidae							
	Dysmicoccus						
		Dysmicoccus brevipes	COL	1 (E:1 I:0)	1	6	6
	Ferrisia						
		Ferrisia virgata	COL	1 (E:1 I:0)	1	3	3
	Nipaecoccus						
		Nipaecoccus nipae	COL	1 (E:1 I:0)	0	0	0
	Paracoccus						
		Paracoccus marginatus	COL	1 (E:1 I:0)	0	0	0
	Planococcus						
		Planococcus citri	COL	1 (E:1 I:0)	1	15	15
		Planococcus ficus	COL	2 (E:1 I:1)	2	6	6
	Pseudococcus						
		Pseudococcus longispinus	COL	1 (E:1 I:0)	1	4	4
		Pseudococcus viburni	COL	2 (E:2 I:0)	1	5	5
	Puto						
		Puto barberi	COL	1 (E:1 I:0)	0	0	0
Psyllidae		Psyllidae	COL	1 (E:1 I:0)	-	-	-
	Aphalara						
		Aphalara itadori	COL	1 (E:1 I:0)	0	0	0
	Cacopsylla						
		Cacopsylla mali	COL	1 (E:1 I:0)	0	0	0
		Cacopsylla pyri	COL	1 (E:1 I:0)	0	1	1
	Diaphorina						
		Diaphorina citri	COL	2 (E:2 I:0)	0	3	3
Saldidae		Saldidae	COL	1 (E:1 I:0)	-	-	-
Scutelleridae							
	Agonosoma						
		Agonosoma trilineatum	GBIF	1 (E:1 I:0)	0	0	0
Tingidae							
	Carvalhotingis						
		Carvalhotingis visenda	-	1 (E:1 I:0)	0	0	0
	Gargaphia						
		Gargaphia decoris	COL	1 (E:1 I:0)	0	0	0
Triozidae							

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	Bactericera						
		Bactericera cockerelli	COL	1 (E:1 I:0)	0	0	0
	Triozoida						
		Triozoida limbata	COL	1 (E:1 I:0)	0	0	0
Hymenoptera		Hymenoptera	COL	2 (E:2 I:0)	-	-	-
Aphelinidae		Aphelinidae	COL	1 (E:1 I:0)	-	-	-
	Aphelinus	Aphelinus	COL	1 (E:1 I:0)	-	-	-
		Aphelinus varipes	GBIF	1 (E:1 I:0)	1	35	2
	Aphytis						
		Aphytis africanus	COL	1 (E:1 I:0)	2	2	2
		Aphytis chrysomphali	COL	3 (E:3 I:0)	1	6	6
		Aphytis hispanicus	COL	1 (E:1 I:0)	1	1	1
		Aphytis lingnanensis	COL	1 (E:1 I:0)	1	6	6
		Aphytis melinus	COL	6 (E:4 I:2)	2	7	7
	Coccophagus						
		Coccophagus lycimnia	COL	2 (E:2 I:0)	1	1	1
	Encarsia	Encarsia	COL	1 (E:1 I:0)	-	-	-
		Encarsia acaudaleyrodis	COL	1 (E:1 I:0)	0	0	0
		Encarsia diaspidicola	COL	1 (E:1 I:0)	0	0	0
		Encarsia dispersa	COL	1 (E:1 I:0)	0	0	0
		Encarsia formosa	COL	2 (E:0 I:2)	1	3	3
		Encarsia guadeloupae	COL	2 (E:2 I:0)	0	0	0
		Encarsia perniciosi	COL	2 (E:2 I:0)	1	7	7
		Encarsia perplexa	COL	1 (E:1 I:0)	0	0	0
		Encarsia scapeata	COL	1 (E:1 I:0)	0	0	0
		Encarsia sofia	-	1 (E:1 I:0)	0	0	0
		Encarsia sophia	COL	1 (E:1 I:0)	0	0	0
		Encarsia tricolor	GBIF	1 (E:1 I:0)	0	0	0
	Encarsiella	Encarsiella	COL	1 (E:1 I:0)	-	-	-
	Eretmocerus						
		Eretmocerus hayati	COL	1 (E:1 I:0)	0	0	0
		Eretmocerus mundus	COL	2 (E:1 I:1)	0	5	5
		Eretmocerus sudanensis	-	1 (E:1 I:0)	0	0	0
	Marietta						
		Marietta picta	GBIF	1 (E:1 I:0)	0	0	0
Apidae		Apidae	COL	1 (E:1 I:0)	-	-	-
	Apis						
		Apis mellifera	COL	1 (E:1 I:0)	1	112	89

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
Argidae		Argidae	COL	1 (E:1 I:0)	-	-	-
Bethylidae		Bethylidae	COL	1 (E:1 I:0)	-	-	-
	Cephalonomia						
		Cephalonomia stephanoderis	-	2 (E:2 I:0)	0	0	0
	Goniozus	Goniozus	COL	4 (E:4 I:0)	-	-	-
		Goniozus playnotae	-	1 (E:1 I:0)	0	0	0
	Prorops						
		Prorops nasuta	-	2 (E:2 I:0)	0	0	0
	Sclerodermus						
		Sclerodermus harmandi	-	1 (E:0 I:1)	0	0	0
Braconidae		Braconidae	COL	3 (E:3 I:0)	-	-	-
Braconidae		Braconinae	GBIF	1 (E:1 I:0)	-	-	-
	Aleiodes						
		Aleiodes vaughanii	-	1 (E:1 I:0)	0	0	0
	Allobracon	Allobracon	COL	1 (E:1 I:0)	-	-	-
	Apanteles	Apanteles	COL	1 (E:1 I:0)	-	-	-
		Apanteles diatraeae	COL	1 (E:1 I:0)	0	0	0
		Apanteles taragamae	COL	2 (E:1 I:1)	0	0	0
		Aphaereta debilitata	GBIF	2 (E:2 I:0)	0	0	0
	Aphanta						
		Aphanta hospita	GBIF	1 (E:1 I:0)	0	0	0
	Aphidius	Aphidius	COL	2 (E:2 I:0)	-	-	-
		Aphidius avenae --appeared as Aphidius picipes	COL	1 (E:1 I:0)	0	0	0
		Aphidius colemani --also appears as Aphidius transcaspicus	COL	3 (E:1 I:2)	1	10	9
		Aphidius ervi	COL	7 (E:3 I:4)	5	114	96
		Aphidius gifuensis	COL	1 (E:1 I:0)	0	0	0
		Aphidius matricariae	COL	2 (E:2 I:0)	2	12	7
		Aphidius rhopalosiphi	COL	3 (E:3 I:0)	3	71	40
		Aphidius smithi	COL	1 (E:1 I:0)	0	0	0
		Aphidius urticae	COL	1 (E:1 I:0)	3	4	4
	Ascogaster	Ascogaster	COL	1 (E:1 I:0)	-	-	-
	Atanycolus	Atanycolus	COL	1 (E:1 I:0)	-	-	-
	Bassus	Bassus	COL	1 (E:1 I:0)	-	-	-
	Binodoxys						
		Binodoxys communis	COL	1 (E:0 I:1)	1	2	2

Class/Order/Family/Genus	Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
Bracon						
	Bracon cephi	COL	1 (E:1 I:0)	0	0	0
	Bracon lissogaster	COL	1 (E:1 I:0)	0	0	0
Centistes						
	Centistes diabroticae	COL	1 (E:1 I:0)	0	0	0
Chelonus						
	Chelonus cautus	COL	1 (E:1 I:0)	0	8	8
	Chelonus insularis	COL	1 (E:1 I:0)	0	8	8
Cotesia						
	Cotesia ayerza	COL	1 (E:1 I:0)	0	0	0
	Cotesia flavipes	COL	3 (E:2 I:1)	1	31	30
	Cotesia glomerata	COL	3 (E:1 I:2)	1	11	11
	Cotesia kazak	COL	1 (E:1 I:0)	0	0	0
	Cotesia marginiventris	COL	1 (E:1 I:0)	1	9	9
	Cotesia rubecula	COL	1 (E:1 I:0)	2	6	6
	Cotesia sesamiae	COL	3 (E:3 I:0)	2	24	24
	Cotesia vestalis	COL	1 (E:1 I:0)	2	12	12
Dacnusa						
	Dacnusa sibirica	COL	1 (E:1 I:0)	2	4	4
Diachasmimorpha						
	Diachasmimorpha fullawayi	COL	1 (E:1 I:0)	0	0	0
	Diachasmimorpha kraussii	COL	1 (E:0 I:1)	0	0	0
	Diachasmimorpha longicaudata	COL	3 (E:2 I:1)	3	14	14
	Diachasmimorpha tryoni	COL	2 (E:1 I:1)	0	0	0
Diaeretiella						
	Diaeretiella rapae	COL	2 (E:2 I:0)	2	23	9
Dinocampus						
	Dinocampus coccinellae	COL	2 (E:2 I:0)	1	4	1
Dolichogenidea						
	Dolichogenidea tasmanica	GBIF	1 (E:1 I:0)	0	0	0
Doryctobracon						
	Doryctobracon crawfordi	COL	1 (E:1 I:0)	0	0	0
Fopius						
	Fopius arisanus	COL	4 (E:0 I:4)	1	4	4
	Fopius ceratitivorus	COL	3 (E:2 I:1)	0	0	0
Habrobracon						
	Habrobracon hebetor	COL	3 (E:0 I:3)	0	0	0

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	Lipolexis						
		Lipolexis gracilis	COL	1 (E:1 I:0)	1	2	2
	Lysiphlebus						
		Lysiphlebus testaceipes	COL	2 (E:1 I:1)	1	29	21
	Macrocentrus						
		Macrocentrus linearis --appeared as Macrocentrus iridescens	COL	1 (E:1 I:0)	0	0	0
	Meteorus						
		Meteorus pulchricornis	COL	2 (E:1 I:1)	1	7	2
		Meteorus trachynotus	COL	1 (E:1 I:0)	1	40	0
	Microctonus	Microctonus	COL	1 (E:1 I:0)	-	-	-
		Microctonus aethiopoides	GBIF	3 (E:3 I:0)	5	63	63
	Microplitis						
		Microplitis croceipes	COL	3 (E:2 I:1)	0	0	0
		Microplitis mediator	COL	2 (E:0 I:2)	1	2	2
		Microplitis plutellae	COL	1 (E:1 I:0)	1	14	0
	Oncophanes						
		Oncophanes americanus	COL	1 (E:1 I:0)	0	0	0
	Opius	Opius	COL	1 (E:1 I:0)	-	-	-
		Opius hirtus	COL	1 (E:1 I:0)	0	0	0
	Perilitus						
		Perilitus brevicollis	COL	2 (E:2 I:0)	0	0	0
	Peristenus						
		Peristenus digoneutis	GBIF	1 (E:1 I:0)	0	0	0
		Peristenus howardi	-	1 (E:1 I:0)	0	0	0
		Peristenus relictus	GBIF	2 (E:2 I:0)	0	0	0
	Phanerotoma						
		Phanerotoma leucobasis	COL	1 (E:1 I:0)	0	0	0
	Praon						
		Praon volucre	COL	1 (E:1 I:0)	5	18	16
	Psyttalia						
		Psyttalia concolor	COL	2 (E:2 I:0)	1	6	6
		Psyttalia cosyrae	COL	1 (E:1 I:0)	2	4	4
		Psyttalia humilis	COL	3 (E:1 I:2)	1	6	6
		Psyttalia lounsburyi	COL	3 (E:1 I:2)	1	27	27
		Psyttalia perproxima	COL	1 (E:1 I:0)	1	4	4
	Spathius	Spathius	COL	1 (E:1 I:0)	-	-	-

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Spathius agrili	COL	3 (E:3 I:0)	1	4	4
	Stiropius						
		Stiropius letifer	COL	1 (E:1 I:0)	0	0	0
	Syngaster						
		Syngaster lepidus	-	2 (E:1 I:1)	1	1	1
	Trioxys						
		Trioxys pallidus	COL	1 (E:1 I:0)	1	2	2
	Utetes						
		Utetes anastrephae	-	1 (E:1 I:0)	0	0	0
Cephidae							
	Cephus						
		Cephus cinctus	COL	1 (E:1 I:0)	1	2	2
Ceraphronidae		Ceraphronidae	COL	1 (E:1 I:0)	-	-	-
	Aphanogmus	Aphanogmus	COL	1 (E:1 I:0)	-	-	-
Chalcididae		Chalcididae	COL	1 (E:1 I:0)	-	-	-
	Phasgonophora						
		Phasgonophora sulcata	COL	1 (E:1 I:0)	1	8	8
Chalcidoidea		Chalcidoidea	COL	1 (E:1 I:0)	-	-	-
Charipidae							
	Alloxysta	Alloxysta	COL	1 (E:1 I:0)	-	-	-
Cynipidae		Cynipidae	COL	1 (E:1 I:0)	-	-	-
Diapriidae		Diapriidae	COL	1 (E:1 I:0)	-	-	-
	Coptera						
		Coptera haywardi	-	1 (E:1 I:0)	0	0	0
		Coptera occidentalis	-	1 (E:0 I:1)	0	0	0
Dryinidae							
	Neodryinus						
		Neodryinus typhlocybae	GBIF	1 (E:0 I:1)	0	0	0
Encyrtidae		Encyrtidae	COL	1 (E:1 I:0)	-	-	-
	Anagyrus	Anagyrus	COL	2 (E:2 I:0)	-	-	-
	Avetianella						
		Avetianella longoi	COL	1 (E:1 I:0)	1	3	3
	Coccidoctonus						
		Coccidoctonus trinidadensis	COL	1 (E:1 I:0)	0	0	0
	Coccidoxenoides						
		Coccidoxenoides perminutus	COL	2 (E:2 I:0)	0	0	0
	Copidosoma						
		Copidosoma	COL	1 (E:1 I:0)	-	-	-

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Gahaniella						
		Gahaniella tertia	COL	1 (E:1 I:0)	0	0	0
	Leptomastix						
		Leptomastix dactylopii	COL	2 (E:2 I:0)	0	0	0
		Leptomastix epona	COL	1 (E:0 I:1)	0	0	0
	Metaphycus						
		Metaphycus angustifrons	COL	1 (E:1 I:0)	0	0	0
		Metaphycus flavus	COL	1 (E:1 I:0)	1	1	1
		Metaphycus galbus	COL	1 (E:1 I:0)	0	0	0
		Metaphycus helvolus	COL	1 (E:1 I:0)	0	0	0
		Metaphycus lounsburyi	COL	2 (E:2 I:0)	0	0	0
		Metaphycus luteolus	COL	1 (E:1 I:0)	0	0	0
		Metaphycus stanleyi	COL	1 (E:1 I:0)	0	0	0
	Microterys						
		Microterys nietneri	COL	1 (E:1 I:0)	0	0	0
	Oobius						
		Oobius agrili	COL	2 (E:1 I:1)	0	0	0
	Ooencyrtus						
		Ooencyrtus	COL	1 (E:1 I:0)	-	-	-
		Ooencyrtus telenomicida	COL	2 (E:2 I:0)	0	0	0
	Pseudaphycus						
		Pseudaphycus flavidulus	-	1 (E:0 I:1)	0	0	0
		Pseudaphycus maculipennis	COL	1 (E:1 I:0)	0	0	0
	Psyllaephagus						
		Psyllaephagus parvus	COL	1 (E:1 I:0)	0	0	0
		Psyllaephagus perplexans	-	1 (E:1 I:0)	0	0	0
	Syrphophagus						
		Syrphophagus aphidivorus	COL	1 (E:1 I:0)	2	2	2
Eucharitidae							
	Dilocantha						
		Dilocantha lachaudii	COL	1 (E:1 I:0)	0	0	0
	Isomerala						
		Isomerala coronata	COL	1 (E:1 I:0)	0	0	0
	Kapala						
		Kapala iridicolor	COL	1 (E:1 I:0)	0	0	0
		Kapala izapa	-	1 (E:1 I:0)	0	0	0
	Orasema						
		Orasema	COL	1 (E:1 I:0)	-	-	-

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Orasema aenea	COL	1 (E:1 I:0)	0	0	0
		Orasema salebrosa	COL	1 (E:1 I:0)	0	0	0
		Orasema simplex	COL	2 (E:2 I:0)	0	0	0
		Orasema xanthopus	COL	2 (E:2 I:0)	0	0	0
Eulophidae		Eulophidae	COL	3 (E:3 I:0)	-	-	-
	Aleuroctonus						
		Aleuroctonus vittatus	COL	1 (E:1 I:0)	0	0	0
	Cirrospilus	Cirrospilus	COL	1 (E:1 I:0)	-	-	-
		Cirrospilus brevis	COL	1 (E:1 I:0)	0	0	0
	Citrostichus						
		Citrostichus phyllocnistoides	COL	1 (E:1 I:0)	0	0	0
	Closterocerus	Closterocerus	COL	1 (E:1 I:0)	-	-	-
		Closterocerus chamaeleon	-	1 (E:1 I:0)	0	0	0
		Closterocerus cinctipennis	COL	1 (E:1 I:0)	0	0	0
		Closterocerus formosus --appeared as Neochrysocharis formosa	COL	1 (E:1 I:0)	0	0	0
	Colpoclypeus						
		Colpoclypeus florus	COL	1 (E:1 I:0)	0	0	0
	Diglyphus	Diglyphus	COL	1 (E:1 I:0)	-	-	-
		Diglyphus isaea	COL	1 (E:1 I:0)	6	42	42
	Elachertus	Elachertus	COL	1 (E:1 I:0)	-	-	-
		Elachertus fenestratus --appeared as Elachertus argissa	COL	1 (E:1 I:0)	0	0	0
	Euplectrus						
		Euplectrus comstockii	COL	1 (E:0 I:1)	0	0	0
	Horismenus	Horismenus	COL	1 (E:1 I:0)	-	-	-
	Miotropis	Miotropis	COL	1 (E:1 I:0)	-	-	-
	Neochrysocharis	Neochrysocharis	GBIF	1 (E:1 I:0)	-	-	-
		Neochrysocharis arvensis	GBIF	1 (E:1 I:0)	0	0	0
		Neochrysocharis chalybea	-	1 (E:1 I:0)	0	0	0
	Nesolynx						
		Nesolynx thymus	COL	1 (E:1 I:0)	0	0	0
	Ophelimus						
		Ophelimus maskelli	COL	1 (E:1 I:0)	1	1	1
	Pediobius						
		Pediobius pyrgo	COL	1 (E:1 I:0)	0	0	0
	Phymastichus						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Phymastichus coffea	COL	2 (E:2 I:0)	0	0	0
	Pnigalio	Pnigalio	COL	2 (E:2 I:0)	-	-	-
		Pnigalio agraules	COL	1 (E:1 I:0)	2	4	4
		Pnigalio sarasolai	COL	1 (E:1 I:0)	0	0	0
	Sympiesis						
		Sympiesis gregori	COL	1 (E:1 I:0)	0	0	0
	Tamarixia						
		Tamarixia radiata	COL	2 (E:1 I:1)	2	156	139
	Tetrastichus						
		Tetrastichus giffardianus	COL	1 (E:1 I:0)	0	0	0
		Tetrastichus planipennisi	-	2 (E:2 I:0)	0	0	0
	Zagrammosoma						
		Zagrammosoma lineaticeps	COL	1 (E:1 I:0)	0	0	0
		Zagrammosoma multilineatum	COL	1 (E:1 I:0)	0	0	0
Eupelmidae							
	Eupelmus						
		Eupelmus urozonus	COL	1 (E:1 I:0)	2	32	32
Eurytomidae							
	Eurytoma						
		Eurytoma curculionum	COL	1 (E:1 I:0)	0	0	0
		Eurytoma martellii	COL	1 (E:1 I:0)	0	0	0
	Tetramesa						
		Tetramesa romana	COL	2 (E:2 I:0)	0	0	0
Evaniidae							
	Evania						
		Evania appendigaster	COL	1 (E:1 I:0)	1	3	3
Figitidae							
	Anacharis						
		Anacharis zealandica	GBIF	2 (E:2 I:0)	0	0	0
	Anarchis	Anarchis	-	1 (E:1 I:0)	-	-	-
	Odontosema						
		Odontosema anastrephae	-	1 (E:1 I:0)	0	0	0
	Trybliographa						
		Trybliographa rapae	GBIF	1 (E:1 I:0)	0	0	0
Formicidae		Formicidae	COL	1 (E:1 I:0)	-	-	-
	Anoplolepis	Anoplolepis steingroeveri	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Azteca						
		Azteca instabilis	COL	1 (E:1 I:0)	0	0	0
		Azteca velox	COL	1 (E:1 I:0)	0	0	0
	Camponotus	Camponotus	COL	1 (E:1 I:0)	-	-	-
		Camponotus abscisus	COL	1 (E:1 I:0)	0	0	0
		Camponotus planatus	COL	1 (E:1 I:0)	0	0	0
		Camponotus striatus	COL	1 (E:1 I:0)	0	0	0
	Cataglyphis	Cataglyphis	COL	1 (E:1 I:0)	-	-	-
	Cephalotes	Cephalotes	COL	1 (E:1 I:0)	-	-	-
	Crematogaster	Crematogaster	COL	2 (E:2 I:0)	-	-	-
		Crematogaster peringueyi	COL	1 (E:1 I:0)	0	0	0
	Ectatomma	Ectatomma	COL	1 (E:1 I:0)	-	-	-
		Ectatomma ruidum	COL	1 (E:1 I:0)	0	0	0
		Ectatomma tuberculatum	COL	1 (E:1 I:0)	0	0	0
	Formica						
		Formica cunicularia	COL	1 (E:1 I:0)	2	4	4
		Formica fusca	COL	1 (E:1 I:0)	5	7	6
		Formica subrufa	COL	1 (E:1 I:0)	0	0	0
	Lasius						
		Lasius fuliginosus	COL	1 (E:1 I:0)	2	8	8
		Lasius grandis	COL	1 (E:1 I:0)	0	0	0
		Lasius niger	COL	1 (E:1 I:0)	3	7	7
	Linepithema						
		Linepithema humile	COL	1 (E:1 I:0)	1	34	28
	Messor	Messor	COL	1 (E:1 I:0)	-	-	-
	Monomorium						
		Monomorium floricola	COL	1 (E:1 I:0)	0	0	0
	Myrmica	Myrmica	COL	1 (E:1 I:0)	-	-	-
	Nesomyrmex						
		Nesomyrmex echinatinodis	-	1 (E:1 I:0)	0	0	0
	Pheidole	Pheidole	COL	1 (E:1 I:0)	-	-	-
		Pheidole pallidula	COL	1 (E:1 I:0)	0	0	0
	Plagiolepis	Plagiolepis	COL	1 (E:1 I:0)	-	-	-
		Plagiolepis pygmaea	COL	1 (E:1 I:0)	1	1	1
	Pseudomyrmex						
		Pseudomyrmex elongatus	COL	1 (E:1 I:0)	0	0	0
		Pseudomyrmex gracilis	COL	1 (E:1 I:0)	2	5	5
		Pseudomyrmex simplex	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Solenopsis						
		Solenopsis geminata	COL	2 (E:2 I:0)	1	30	2
		Solenopsis invicta	COL	7 (E:7 I:0)	2	5	4
		Solenopsis macdonaghi	COL	1 (E:1 I:0)	0	0	0
		Solenopsis picea	COL	1 (E:1 I:0)	0	0	0
		Solenopsis quinquecuspis	COL	1 (E:1 I:0)	0	0	0
		Solenopsis richteri	COL	3 (E:3 I:0)	0	2	2
		Solenopsis xyloni	COL	1 (E:1 I:0)	1	1	1
	Tapinoma	Tapinoma	COL	2 (E:2 I:0)	-	-	-
		Tapinoma sessile	COL	1 (E:1 I:0)	14	182	41
Ichneumonidae		Ichneumonidae	COL	2 (E:2 I:0)	-	-	-
Ichneumonidae		Ichneumoninae	-	1 (E:1 I:0)	-	-	-
Ichneumonidae		Cryptini	-	1 (E:1 I:0)	-	-	-
	Agrypon						
		Agrypon anxium	COL	1 (E:1 I:0)	0	0	0
	Australoglypta						
		Australoglypta latrobei	COL	1 (E:1 I:0)	0	0	0
	Campoletis						
		Campoletis chlorideae	COL	2 (E:2 I:0)	0	0	0
		Campoletis sonorensis	COL	1 (E:1 I:0)	1	11	10
	Campoplex						
		Campoplex capitator	COL	1 (E:1 I:0)	0	0	0
	Diadegma						
		Diadegma fenestralis	-	1 (E:1 I:0)	0	0	0
		Diadegma insulare	COL	2 (E:2 I:0)	1	87	3
		Diadegma majale	COL	1 (E:1 I:0)	0	0	0
		Diadegma semiclausum	COL	3 (E:3 I:0)	1	21	18
	Diadromus						
		Diadromus pulchellus	COL	1 (E:1 I:0)	1	7	7
	Dicaelotus						
		Dicaelotus inflexus	COL	1 (E:1 I:0)	0	0	0
	Eiphosoma						
		Eiphosoma vitticolle	COL	1 (E:1 I:0)	0	8	8
	Eriborus						
		Eriborus epiphyas	COL	1 (E:1 I:0)	0	0	0
	Exochus						
		Exochus albidrons	-	1 (E:1 I:0)	0	0	0
		Exochus lentipes	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		--appeared as Exochus notatus					
	Glypta	Glypta	COL	1 (E:1 I:0)	-	-	-
	Habronyx						
		Habronyx aclerivorus	GBIF	1 (E:1 I:0)	0	0	0
	Hyposoter						
		Hyposoter didymator	COL	1 (E:1 I:0)	1	5	0
		Hyposoter ebeninus	COL	1 (E:1 I:0)	0	0	0
	Itoplectis						
		Itoplectis maculator	COL	1 (E:1 I:0)	0	0	0
	Mastrus						
		Mastrus ridibundus	COL	1 (E:0 I:1)	0	0	0
	Ophion						
		Ophion flavidus	COL	1 (E:1 I:0)	0	0	0
	Phytodietus						
		Phytodietus celsissimus	COL	1 (E:1 I:0)	0	0	0
	Pristomerus						
		Pristomerus spinator	COL	1 (E:1 I:0)	1	8	8
	Scambus						
		Scambus pomorum	COL	1 (E:1 I:0)	0	0	0
	Triclistus						
		Triclistus meridiator	COL	1 (E:1 I:0)	0	0	0
	Venturia						
		Venturia canescens	COL	1 (E:1 I:0)	1	2	2
Megachilidae							
	Osmia						
		Osmia cornuta	COL	1 (E:1 I:0)	0	2	2
Megaspilidae							
	Dendrocerus						
		Dendrocerus	COL	1 (E:1 I:0)	-	-	-
Mymaridae		Mymaridae	COL	2 (E:2 I:0)	-	-	-
	Anagrus						
		Anagrus atomus	COL	1 (E:1 I:0)	2	3	3
		Anagrus epos	COL	1 (E: I:1)	0	0	0
		Anagrus nilaparvatae	COL	1 (E:1 I:0)	0	0	0
	Anaphes						
		Anaphes nitens	COL	1 (E: I:1)	0	0	0
	Cleruchoides						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Cleruchoides noackae	-	1 (E:1 I:0)	0	0	0
	Gonatocerus						
		Gonatocerus ashmeadi	COL	4 (E:4 I:0)	1	10	10
		Gonatocerus deleoni	-	1 (E:0 I:1)	0	0	0
		Gonatocerus morgani	COL	1 (E:1 I:0)	0	0	0
		Gonatocerus tuberculifemur	COL	1 (E:0 I:1)	0	0	0
	Parastethynium						
		Parastethynium maxwelli	-	1 (E:1 I:0)	0	0	0
Pergidae		Pergidae	COL	1 (E:1 I:0)	-	-	-
Platigastridae		Platigastridae	COL	1 (E:1 I:0)	-	-	-
	Amitus						
		Amitus hesperidum	COL	1 (E:1 I:0)	0	0	0
	Platygaster						
		Platygaster demades	COL	1 (E:1 I:0)	0	0	0
		Platygaster robiniae	-	1 (E:1 I:0)	0	0	0
	Synopeas						
		Synopeas myles	COL	1 (E:1 I:0)	0	0	0
Pteromalidae		Pteromalidae	COL	2 (E:2 I:0)	-	-	-
	Anisopteromalus						
		Anisopteromalus calandrae	COL	2 (E:2 I:0)	1	4	4
	Asaphes						
		Asaphes californicus	COL	1 (E:1 I:0)	0	0	0
		Asaphes suspensus	COL	2 (E:2 I:0)	2	2	2
	Enoggera						
		Enoggera nassaui	COL	2 (E:2 I:0)	1	1	0
	Halticoptera						
		Halticoptera circulus	COL	1 (E:1 I:0)	0	0	0
	Lariophagus						
		Lariophagus distinguendus	GBIF	3 (E:1 I:2)	0	0	0
	Mesopolobus						
		Mesopolobus morys	COL	1 (E:1 I:0)	0	0	0
	Nasonia						
		Nasonia vitripennis	COL	1 (E:0 I:1)	2	24	23
	Neopolycystus	Neopolycystus	COL	1 (E:1 I:0)	-	-	-
		Neopolycystus insectifurax	COL	2 (E:2 I:0)	0	0	0
	Pachycrepoideus						
		Pachycrepoideus near schedli	-	1 (E:1 I:0)	0	0	0
		Pachycrepoideus vindemmiae	COL	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Pachyneuron						
		Pachyneuron aphidis	GBIF	1 (E:1 I:0)	1	18	2
		Pachyneuron muscarum	COL	1 (E:1 I:0)	0	0	0
	Pteromalus	Pteromalus	COL	2 (E:2 I:0)	-	-	-
		Pteromalus cerealellae	COL	2 (E:0 I:2)	0	0	0
	Scutellista						
		Scutellista caerulea	COL	1 (E:1 I:0)	0	0	0
	Spalangia						
		Spalangia cameroni	COL	3 (E:1 I:2)	0	0	0
	Stenomalina						
		Stenomalina gracilis	COL	1 (E:1 I:0)	0	0	0
	Trichilogaster						
		Trichilogaster acaciaelongifoliae	COL	1 (E:1 I:0)	0	0	0
	Trichomalopsis						
		Trichomalopsis peregrina	COL	1 (E:1 I:0)	0	0	0
	Trichomalus						
		Trichomalus perfectus	COL	1 (E:1 I:0)	0	0	0
Scelionidae		Scelionidae	COL	1 (E:1 I:0)	-	-	-
	Gryon						
		Gryon pennsylvanicum	COL	1 (E:1 I:0)	0	0	0
	Telenomus						
		Telenomus busseolae	GBIF	3 (E:3 I:0)	0	1	1
		Telenomus isis	-	1 (E:0 I:1)	0	0	0
		Telenomus remus	GBIF	1 (E:1 I:0)	0	0	0
	Trissolcus						
		Trissolcus basalis	COL	1 (E:1 I:0)	1	1	1
Signiphoridae							
	Signiphora	Signiphora	COL	2 (E:2 I:0)	-	-	-
Sphecidae							
	Chlorions						
		Chlorions smaragdula	-	1 (E:1 I:0)	0	0	0
Tiphiidae		Tiphiidae	COL	1 (E:1 I:0)	-	-	-
Torymidae		Torymidae	COL	2 (E:2 I:0)	-	-	-
Torymidae							
	Torymus						
		Torymus beneficus	COL	1 (E:1 I:0)	0	19	19
		Torymus sinensis	COL	1 (E:1 I:0)	0	11	11

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
Trichogrammatidae		Trichogrammatidae	COL	2 (E:2 I:0)	-	-	-
	Brachyufens						
		Brachyufens osborni	COL	2 (E:2 I:0)	0	0	0
	Trichogramma	Trichogramma	COL	2 (E:2 I:0)	-	-	-
		Trichogramma acacioi	COL	1 (E:1 I:0)	0	0	0
		Trichogramma atopovirilia	COL	2 (E:1 I:1)	0	0	0
		Trichogramma bourarachae	COL	1 (E:1 I:0)	0	0	0
		Trichogramma bournieri	COL	1 (E:1 I:0)	0	0	0
		Trichogramma brassicae	COL	2 (E:2 I:0)	1	7	6
		Trichogramma cacaeciae --appeared as Trichogramma cacoeciae	COL	2 (E:2 I:0)	0	0	0
		Trichogramma chilonis	COL	1 (E:0 I:1)	1	2	1
		Trichogramma cordubensis	-	2 (E:2 I:0)	0	0	0
		Trichogramma embryophagum	COL	2 (E:2 I:0)	0	0	0
		Trichogramma euproctidis	COL	2 (E:2 I:0)	0	0	0
		Trichogramma evanescens	COL	3 (E:3 I:0)	2	3	3
		Trichogramma exiguum	COL	1 (E:0 I:1)	0	0	0
		Trichogramma lasallei	COL	1 (E:1 I:0)	0	0	0
		Trichogramma ostriniae	COL	1 (E:0 I:1)	1	1	1
		Trichogramma pintoi	GBIF	1 (E:1 I:0)	0	0	0
		Trichogramma pretiosum	COL	4 (E:3 I:1)	0	0	0
		Trichogramma rojasi	COL	1 (E:1 I:0)	0	0	0
		Trichogramma tshumakovae	COL	1 (E:1 I:0)	0	0	0
Vespidae		Vespidae	COL	1 (E:1 I:0)	-	-	-
Isoptera							
Rhinotermitidae							
	Coptotermes						
		Coptotermes formosanus	COL	1 (E:0 I:1)	2	49	49
Lepidoptera		Lepidoptera	COL	2 (E:2 I:0)	-	-	-
Arctiidae							
	Hyphantria						
		Hyphantria cunea	COL	1 (E:1 I:0)	2	77	12
Crambidae							
	Asciodes						
		Asciodes quietalis	GBIF	1 (E:1 I:0)	0	0	0
	Chilo						
		Chilo orichalcociliellus	-	1 (E:1 I:0)	0	0	0

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Chilo partellus	COL	2 (E:2 I:0)	1	2	0
	Maruca						
		Maruca vitrata	COL	2 (E:1 I:1)	2	7	1
	Ostrinia						
		Ostrinia nubilalis	COL	1 (E:0 I:1)	1	59	48
	Salbia						
		Salbia lotanalis	-	1 (E:1 I:0)	0	0	0
	Diatraea						
		Diatraea saccharalis	COL	1 (E:1 I:0)	3	39	39
Gelechiidae							
	Ardozyga						
		Ardozyga stratifera	-	1 (E:1 I:0)	0	0	0
	Pectinophora						
		Pectinophora gossypiella	COL	1 (E:1 I:0)	1	11	7
	Phthorimaea						
		Phthorimaea operculella	COL	1 (E:1 I:0)	1	7	7
	Sitotroga						
		Sitotroga cerealella	COL	5 (E:2 I:3)	1	1	0
	Tuta						
		Tuta absoluta	GBIF	3 (E:2 I:1)	1	18	18
Geometridae							
	Leuciris						
		Leuciris fimbriaria	COL	1 (E:1 I:0)	2	3	0
	Macaria						
		Macaria pallidata	-	1 (E:1 I:0)	0	0	0
	Pseudocoremia						
		Pseudocoremia suavis	COL	1 (E:1 I:0)	0	0	0
Glyphipterigidae							
	Acrolepiopsis						
		Acrolepiopsis assectella	GBIF	1 (E:1 I:0)	1	20	0
Gracillariidae							
	Phyllocnistis						
		Phyllocnistis citrella	COL	3 (E:3 I:0)	1	6	2
Lymantriidae							
	Euproctis						
		Euproctis chrysorrhoea	COL	1 (E:1 I:0)	1	5	0
	Lymantria						
		Lymantria dispar	COL	2 (E:1 I:1)	1	36	26

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Lymantria monacha	COL	1 (E:1 I:0)	1	40	29
Lyonetiidae							
	Leucoptera						
		Leucoptera coffeella	-	1 (E:1 I:0)	0	0	0
Noctuidae		Noctuidae	COL	1 (E:1 I:0)	-	-	-
	Abrostola						
		Abrostola asclepiadis	COL	1 (E:1 I:0)	1	11	0
	Agrotis						
		Agrotis ipsilon	COL	2 (E:1 I:1)	1	46	1
	Anticarsia						
		Anticarsia gemmatalis	COL	3 (E:3 I:0)	1	16	1
	Busseola						
		Busseola fusca	COL	4 (E:4 I:0)	2	12	10
		Busseola phaia	COL	1 (E:1 I:0)	2	7	7
	Copitarsia						
		Copitarsia decolora	GBIF	1 (E:0 I:1)	1	74	74
	Helicoverpa						
		Helicoverpa armigera	COL	8 (E:7 I:1)	1	83	68
		Helicoverpa zea	COL	2 (E:2 I:0)	1	18	8
	Heliothis						
		Heliothis virescens	-	2 (E:0 I:2)	2	127	115
	Hypena						
		Hypena opulenta	GBIF	1 (E:1 I:0)	0	0	0
	Mythimna						
		Mythimna separata	GBIF	3 (E:1 I:2)	1	39	3
		Mythimna unipuncta	GBIF	3 (E:2 I:1)	3	87	2
	Pseudoplusia						
		Pseudoplusia includens	GBIF	2 (E:2 I:0)	0	0	0
	Sesamia						
		Sesamia calamistis	COL	2 (E:2 I:0)	0	0	0
		Sesamia nonagrioides	COL	2 (E:2 I:0)	1	44	44
	Spodoptera						
		Spodoptera cosmioides	COL	1 (E:1 I:0)	1	37	29
		Spodoptera eridania	COL	1 (E:1 I:0)	1	39	32
		Spodoptera exigua	COL	3 (E:2 I:1)	2	36	26
		Spodoptera frugiperda	COL	8 (E:3 I:5)	2	33	11
		Spodoptera littoralis	COL	1 (E:1 I:0)	1	23	23
		Spodoptera litura	COL	3 (E:3 I:0)	1	42	33

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
	Trichoplusia						
		Trichoplusia ni	COL	4 (E:2 I:2)	2	4	0
	Xestia						
		Xestia c-nigrum	GBIF	1 (E:1 I:0)	1	53	0
Nymphalidae							
	Maniola						
		Maniola jurtina	COL	1 (E:1 I:0)	1	23	6
	Vanessa						
		Vanessa cardui	GBIF	1 (E:1 I:0)	1	39	14
Pieridae							
	Colias						
		Colias lesbia	COL	1 (E:1 I:0)	0	0	0
	Pieris						
		Pieris brassicae	COL	4 (E:2 I:2)	2	22	6
		Pieris napi	COL	1 (E:1 I:0)	1	23	3
		Pieris rapae	COL	7 (E:6 I:1)	2	134	108
Plutellidae							
	Plutella						
		Plutella xylostella	COL	7 (E:6 I:1)	1	546	395
Pyralidae		Pyralidae	COL	1 (E:1 I:0)	-	-	-
	Amyelois						
		Amyelois transitella	COL	1 (E:1 I:0)	0	0	0
	Anagasta						
		Anagasta Kuehniella	GBIF	3 (E:1 I:2)	0	1	1
	Corcyra						
		Corcyra cephalonica	COL	1 (E:1 I:0)	0	2	2
	Eldana						
		Eldana saccharina	COL	1 (E:1 I:0)	3	21	21
		Eldana sacharrina	-	1 (E:1 I:0)	0	0	0
	Ephestia						
		Ephestia kuehniella	COL	18 (E:6 I:12)	1	1	0
	Galleria						
		Galleria mellonella	COL	11 (E:7 I:4)	1	13	3
	Plodia						
		Plodia interpunctella	COL	3 (E:2 I:1)	1	18	10
Sesiidae							
	Synanthedon						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Synanthedon exitiosa	-	1 (E:1 I:0)	0	0	0
		Synanthedon pictipes	COL	1 (E:1 I:0)	0	0	0
	Vitacea						
		Vitacea polistiformis	-	1 (E:1 I:0)	1	3	0
Tortricidae		Tortricidae	COL	1 (E:1 I:0)	-	-	-
	Agapeta						
		Agapeta zoegana	COL	1 (E:1 I:0)	0	0	0
	Amorbia						
		Amorbia cuneana	-	1 (E:1 I:0)	0	0	0
	Choristoneura						
		Choristoneura rosaceana	COL	1 (E:1 I:0)	1	76	0
	Cydia						
		Cydia pomonella	COL	3 (E:0 I:3)	1	60	51
	Episimus						
		Episimus unguiculus	COL	1 (E:1 I:0)	0	0	0
	Lobesia						
		Lobesia botrana	COL	2 (E:2 I:0)	0	0	0
	Thaumatotibia						
		Thaumatotibia leucotreta	GBIF	2 (E:1 I:1)	1	1	1
Neuroptera		Neuroptera	COL	3 (E:3 I:0)	-	-	-
Chrysopidae		Chrysopidae	COL	3 (E:3 I:0)	-	-	-
	Ceraeochrysa	Ceraeochrysa	COL	1 (E:1 I:0)	-	-	-
	Chrysopa						
		Chrysopa formosa	COL	1 (E:1 I:0)	0	1	1
		Chrysopa nigricornis	COL	2 (E:2 I:0)	0	2	0
		Chrysopa oculata	COL	1 (E:1 I:0)	1	11	0
	Chrysoperla	Chrysoperla	COL	1 (E:1 I:0)	-	-	-
		Chrysoperla carnea	COL	11 (E:4 I:7)	1	22	21
		Chrysoperla genanigra	COL	1 (E:1 I:0)	0	0	0
		Chrysoperla nipponensis --appeared as Chrysoperla sinica	COL	1 (E:1 I:0)	0	0	0
		Chrysoperla plorabunda --appeared as Chrysopa plorabunda	COL	1 (E:1 I:0)	0	0	0
	Dichocrysa						
		Dichocrysa flavifrons	-	1 (E:1 I:0)	0	0	0
		Dichocrysa prasina	-	1 (E:1 I:0)	0	0	0
	Mallada						

Class/Order/Family/Genus		Taxa	Validation Source	Number of Occurrences in literature review	Number of BINs	Records on BOLD	Records in BOLD from GenBank
		Mallada basalis	COL	1 (E:1 I:0)	0	0	0
Coniopterygidae							
	Semidalis						
		Semidalis aleyrodiformis	COL	1 (E:1 I:0)	0	0	0
Hemerobiidae							
	Micromus						
		Micromus tasmaniae	COL	2 (E:2 I:0)	1	2	2
Orthoptera							
Acrididae							
	Angaracris						
		Angaracris barabensis	COL	1 (E:1 I:0)	1	1	1
	Bryodema						
		Bryodema luctuosum luctuosoma	-	1 (E:1 I:0)	0	0	0
	Cornops						
		Cornops aquaticum	COL	1 (E:0 I:1)	0	0	0
	Dasyhippus						
		Dasyhippus barbipes	COL	1 (E:1 I:0)	0	0	0
	Locusta						
		Locusta migratoria	COL	1 (E:0 I:1)	1	482	482
	Myrmeleotettix						
		Myrmeleotettix palpalis	COL	1 (E:1 I:0)	0	0	0
	Oedaleus						
		Oedaleus asiaticus	COL	1 (E:1 I:0)	0	0	0
Gryllidae							
	Acheta						
		Acheta domesticus	COL	1 (E:1 I:0)	1	2	2
Psocoptera		Psocoptera	GBIF	1 (E:1 I:0)	-	-	-
Thysanoptera							
Aeolothripidae							
	Aeolothrips						
		Aeolothrips fasciatus	COL	1 (E:1 I:0)	0	0	0
Phlaeothripidae							
	Gynaikothrips						
		Gynaikothrips ficorum	COL	1 (E:1 I:0)	3	11	11
		Gynaikothrips uzeli	COL	1 (E:1 I:0)	0	0	0
Thripidae		Thripidae	COL	1 (E:1 I:0)	-	-	-
	Frankliniella						

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		Frankliniella fusca	COL	1 (E:1 I:0)	0	3	3
		Frankliniella occidentalis	COL	8 (E:7 I:1)	4	352	273
		Frankliniella tritici	COL	1 (E:1 I:0)	2	193	6
	Neohydatothrips						
		Neohydatothrips variabilis	COL	1 (E:1 I:0)	0	0	0
	Scirtothrips						
		Scirtothrips aurantii	COL	1 (E:1 I:0)	2	2	2
		Scirtothrips dorsalis	COL	2 (E:2 I:0)	5	54	54
	Thrips						
		Thrips palmi	COL	1 (E:1 I:0)	6	172	172
		Thrips tabaci	COL	1 (E:1 I:0)	4	186	148