SYSTEMATICS OF THE GENUS *RHAGOLETIS* (DIPTERA: TEPHRITIDAE): NEW SPECIES, PHYLOGENY, AND JUSTIFICATIONS

By

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ABSTRACT

SYSTEMATICS OF THE GENUS *RHAGOLETIS* (DIPTERA: TEPHRITIDAE): NEW SPECIES, PHYLOGENY, AND JUSTIFICATIONS

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Flies of *Rhagoletis* (Diptera: Tephritidae) are economically important fruit pests (infesting specialty fruit crops including apples, blueberries and cherries), which also serve as models for studying modes of speciation and coevolutionary relationships with their hymenopteran parasitoids. There are new species within the genus which have not been previously formally described. One of these species is within the *tabellaria* species group. I describe the morphology of *Rhagoletis bushi* Hulbert & Smith, its geographic distribution, host association, phylogenetic relationships, and identify an associated species of parasitoid wasp. The new species infests the fruit of buffaloberry (*Shepherdia argentea*) in the Northern Great Plains of North America. There is a suite of morphological characters, and a unique host plant association, that are diagnostic of *R. bushi*. Further evidence for the validity of *R. bushi* and its placement within the *tabellaria* species group comes from DNA sequence data from multiple genetic loci. The phylogenetic relationships among *Rhagoletis* species groups remain unresolved despite analyses based on morphology, allozymes, and mitochondrial DNA. Most Nearctic *Rhagoletis* belong to one of five species groups (*pomonella*, *tabellaria*, *cingulata*, *suavis*, and *ribicola* groups), with two unplaced species (*R. fausta* and *R. juniperina*), all of which appear to be part of a larger monophyletic group that also includes some Palearctic taxa. Regarding the overall phylogeny of the genus, my goals were to 1) resolve phylogenetic relationships using mitochondrial (COI) and nuclear (28S, CAD, period, AATS) DNA sequences, and 2) to identify the monophyletic group containing these Nearctic species. Using Bayesian analysis of a

combined dataset with 4399 aligned nucleotides, I inferred a well-supported monophyletic group containing the five Nearctic Rhagoletis species groups, plus *R. fausta*, *R. juniperina*, and two Palearctic species: *R. batava* and *R. flavigenualis*. Within this larger monophyletic assemblage, the five Nearctic species groups together are monophyletic as are four of the five individual species groups (not *ribicola*). Palearctic and Neotropical *Rhagoletis* were resolved into well-supported clades of taxa often sharing closely related host plants. A well-resolved phylogeny of *Rhagoletis* is a valuable tool for future work addressing questions pertaining to how history, geography and ecology have shaped the phylogenetic patterns we observe in the genus.

It is often claimed that systematic biology is fundamental to all other areas of biology. I critically evaluate the acceptance of this claim by entomologists critically as it relates to the field of entomology. I also critically describe the justification and valuations for systematic biology using the framework of Boltanski and Thévenot's realms of worth and the philosophical framework for justification using virtues, desserts and outcomes. In order to accomplish these purposes, I critically analyze and review relevant entomological literature and interview practitioners of entomology and insect systematic biology. I find justification for systematic biology overwhelming takes the form of appeals to utilitarianism (both internally and externally focused) and are most relevant in the Industrial World. Additionally, some justifications given also pertain to the Civic World and to virtue. Evaluation of justification in systematic biology is important, especially as our globe becomes increasingly ecologically and politically unstable.

Dedicated to Jean Hulbert.

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PREFACE

My dissertation is likely somewhat unconventional when compared to others in entomology PhD programs and I would like to give an explanation before the main document. This dissertation has two chapters that would be considered typical entomology research where I detail my research on the systematics of the *Rhagoletis* (Diptera: Tephritidae). However, the third chapter addresses sociology of entomology and systematic biology. In that third chapter, I investigate and discuss the social dimensions of systematic biology. Why is that sociology chapter present in an otherwise conventional entomological dissertation of insect systematics? Early in my PhD program, I applied for and received the C. S. Mott Predoctoral Fellowship in Sustainable Agriculture. Part of the requirements of the fellowship included my participation in the Graduate Specialization in Ecological Food and Farming Systems (EFFS). The EFFS specialization "fosters understanding of interdisciplinary concepts critical to sustainable agriculture and food systems." Functionally, the EFFS specialization is designed to give bio/geo/chemical scientists a stronger and formal background in the social sciences and vice versa. In fulfillment of my participation in the EFFS specialization, I have worked on a research project investigating the social justifications for systematic biologists given by entomologists. It may initially seem that the three major chapters of my dissertation make strange neighbors however, as I elaborate in chapter three, I believe considerations of justification and social context are very important things for natural scientists to include in their work.

Additionally, the chapter of this dissertation titled "Description of a new species…" has already been published in the journal Insect Systematics and Diversity (Hulbert et al. 2018). As such, the chapter is not technically the nomenclatural act for the new species, although it does

contain a description of it. To address this, the “Nomenclature” section of the chapter has been altered relative to the published work to reflect the fact that the present dissertation does not contain any official nomenclatural acts. Also, I have removed all instances of the text “n. sp.” from the chapter. I believe these actions I have taken are sufficient to avoid any taxonomic confusion and blunders, but my purpose of including these details in the dissertation overview is to make my actions and intensions explicit.

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INTRODUCTION

Why study systematics?

Systematics defined

Systematic biology (systematics) may be defined as the study of the diversification of life and the relationships therein. There is sometimes confusion about the relationship between taxonomy and systematics. Taxonomy is best thought of as subfield within systematics. A concise definition of systematics accounting for taxonomy was written by Michener et al. (1970):

> Systematic biology (hereafter called simply systematics) is the field that (a) provides scientific names for organisms, (b) describes them, (c) preserves collections of them, (d) provides classifications for the organisms, keys for their identification, and data on their distributions, (e) investigates their evolutionary histories, and (f) considers their environmental adaptations. This is a field with a long history that in recent years has experienced a notable renaissance, principally with respect to theoretical content. Part of the theoretical material has to do with evolutionary areas (topics e and f above), the rest relates especially to the problem of classification. Taxonomy is that part of Systematics concerned with topics (a) to (d) above.

There may be room for reasonable disagreement on some points of the definition of systematic biology by Michener et al. (1970), such as which parts are more important than others, however I believe it to be a very useful working definition of the discipline. Explicitly considering a definition of systematic biology is important for my dissertation because of the

third chapter investigating the justifications used by the scientists within and adjacent to the discipline.

Justification

How is systematic biology, as a human activity, justified? We can use philosophical and sociological frameworks to analyze this question. In my dissertation the two frameworks I use are 1) the "Worlds of worth" by Boltanski and Thévenot (1991) and 2) the framework of virtue theory, rights theory and utilitarianism. These frameworks and how they organize justification for systematic biology is discussed in chapter three. Broad areas of justification are discussed briefly here as possible areas of justification for systematic biology.

Through analysis of texts which exemplify values certain domains of society, Boltanski and Thévenot (1991) infer and articulate what is considered "worthy" and valuable in these domains. The six domains they analyze are the Inspired world, the Domestic world, the world of Fame, the Market world, and the Industrial world. Justifications for systematics are most likely to draw from the Inspired, Industrial and Civic worlds. The Industrial world is defined by production, and value is conferred on the basis of functionality, reliability efficiency. Knowledge produced by systematic biology may contribute to the efficiency of agricultural production and the production of disease management measures. The Civic world is defined by the action and power of collectives for their benefit. Systematic biological knowledge may inform and provide evidence for certain legislation or other collective action around biodiversity (conservation). The Inspired world is defined by its value of enlightenment, worthy beings in this world are individuals able to reach inspired states. Knowledge production in systematics may be regarded

as a form of gaining enlightenment and scientists may use justifications relating to the Inspired World when talking about how they first became interested in their field.

Utilitarian justifications for systematics rely on the outcomes gained from the knowledge generated. Systematics may provide information leading to the control and prevention of diseases and pests. For example investigations into Zika virus phylogenetics revealed how the virus was entering the United States, helping efforts to stop its spread (Grubaugh et al. 2017). A similar investigation was undertaken on emerald ash borer in order to determine the region of origin for the invasive insects (Bray et al. 2011). The diagnosis and discrimination of organisms from all others is a direct utilization of systematic biological knowledge. One of systematic biology's primary goals is the definition and delineation of all organisms into a hierarchal organization. It is much easier to design diagnostic tools and tests for organisms that belong to well resolved areas of the phylogenetic tree of life. The ability to diagnose economically and epidemiologically significant organisms has obvious utilitarian benefit. Investigations into the production of reliable diagnostic methods are directly drawing upon existing systematic knowledge (and in some cases contributing to it) (Frey and Pfunder 2006, Dita et al. 2010, Frey et al. 2013). The conservation of biodiversity may also be invoked as a utilitarian justification for systematic biology.

Engaging in systematics research may be justified through "virtue". This justification states that the resolution of the tree of life is a good and virtuous scientific pursuit; it is an inherent good. This justification does seem open to criticisms (who decides what is a "good" thing to be working on), but that misses the point. The way scientists write and talk about systematic biology, they may invoke this justification whether meaning to or not. The Taxonomic Impediment refers to the problem of considerable gaps in the state of taxonomic

knowledge and insufficient resources (human, social, financial) to meaningfully address them. Addressing the taxonomic impediment may be presented as a justification for systematics, for providing more funding for the discipline. Fundamentally, this justification is a combination of a virtue justification (addressing the taxonomic impediment is an inherently good thing to do) and a utilitarian justification (increasing resource allocation to systematics will have the outcome of the taxonomic impediment being solved).

Rights Theory may also be used to justify investigations by systematic biologists. Rights theory asks, what are the rights and responsibilities actors have? By completing all the education and training necessary to become a systematic biologist, do those individuals have certain rights and responsibilities to then work on addressing the Taxonomic Impediment? A systematic biologist may refer to a certain "duty" to work on resolving the evolutionary tree of life when invoking Rights Theory justifications.

Introduction to *Rhagoletis*

Diversity of small organisms

Different patterns of biodiversity are observed in large animals compared to small animals. In larger animals (mostly vertebrates) sibling species are generally not in close physical proximity, while small animals (generally invertebrates) do occur in close geographic proximity to each other (Hutchinson 1959). The same environment looks very different to large and small animals. From the perspective of smaller animals there will be greater heterogeneity in the environment. This leads to many more niches to be exploited for smaller organisms (Hutchinson 1959). Additional niches to be exploited include plant resources: different plants and different parts of plants can be used to create niches by small insects in ways unavailable to larger ones.

These combination of factors (small size which leads to increased niche availability which is related to the reason for sibling species to be in close physical proximity) is why *Rhagoletis* has become such an important system for evolutionary biology research and for pest management (Bush 1993).

Overview of the genus

Rhagoletis is within the family Tephritidae (Ditpera), members of which are frugivorous and have highly specialized relationships with their host. There is (generally) a high level of fidelity for a species of *Rhagoletis* to its particular host plant's fruit. Certain species are direct pests of high value specialty crops including apples, cherries, blueberries. Additionally, because of their close host plant association, the genus provides an opportunity for studying speciation and evolution.

The life histories of *Rhagoletis* are closely associated with their host plant species. Courtship behavior and mating of adults takes place on the leaves and the fruit of their host plant (Bush 1966, Prokopy and Bush 1973, Boller and Prokopy 1976, Smith and Prokopy 1980). Larvae hatch from eggs (laid on the fruit) and burrow into the fruit where they feed on the flesh before dropping to the soil where they pupate and undergo diapause (in temperate climates). After diapause has been completed, the adults eclose and emerge from the soil. Timing (phenology) of the life history events in *Rhagoletis* are timed to correspond with their host.

Bush (1966) produced a major revision of the genus in a seminal monograph. Bush did extensive life history, cytological and taxonomic research into the genus with a focus on North American taxa. One of the major results of the work was the organization of *Rhagoletis* into a number of "species groups". Species groups, while not a formal taxonomic rank, were defined on

the basis of morphological similarity and common host plant use. The most well-known of these is the *pomonella* species group which has been a focus of research because of its economically important members and their patterns of speciation.

Species of *Rhagoletis* may commonly form host races (Boller and Prokopy 1976), which is a principle reason for research interest in the genus from both applied and fundamental science perspectives. A host race is an informal taxonomic grouping lower than species in which certain populations of the same species have specialized on different host plant fruits. In North America, a host shift occurred in *R. pomonella* from its native host plant *Crataegus* (hawthorn) host to introduced commercial apples (*Malus*) in the last ~170 years (Illingworth 1912). Host shifts in *Rhagoletis* have led to the formation of host races apples and hawthorns respectively. Importantly, the two host races are not reproductively isolated; their hosts are sympatric with each other and there is a consistent level of geneflow between them (Feder et al. 1994). The apple and hawthorn host races are hypothesized to be in an initial stage of speciation and provide an opportunity to study the fundamental nature of population divergence and the formation of new species (Feder et al. 1994). Through extensive research, the *Rhagoletis* system has become a non-model textbook example of speciation in the presence of gene flow (Schluter 2000, Coyne and Orr 2004, Nosil 2012). Studying evolutionary patterns in Rhagoletis may provide insights into when and why phytophagous insect outbreaks occur.

Rhagoletis differs from some other insect speciation systems in that the evolution of *Rhagoletis* does not follow patterns in plant chemistry. When *R. pomonella* shifted from hawthorn to apple, it did not shift to a plant phylogenetically or chemically close as might have been expected. This pattern is what is observed in other species groups across the genus like *R. cerasi* forming host races on *Lonicera* and *Prunus* (Boller et al. 1998). The opposing pattern is

also observed: closely related groups of species speciate in allopatry on very closely related host plants (the walnut flies for example). The results of my dissertation (chapter two) have implications for understanding how these patterns take place across *Rhagoletis.*

Previous phylogenetic analyses

There have been attempts to resolve evolutionary relationships between *Rhagoletis* species. Previous attempts to resolve the phylogeny have been based on patterns of host plant use, morphology, and limited DNA sequences. Past investigations have yielded important insights while also leaving some unanswered questions

Bush (1966) made the first hierarchical classification of *Rhagoletis* was done, organizing the North American taxa into species groups. He proposed five species groups which together make up the majority of the North American taxa (*pomonella*, *tabellaria*, *cingulata*, *suavis* and *ribicola*). While phylogenetic resolution was not a goal of Bush's monograph, he did include general hypotheses of evolutionary relationships. Bush focused on North American taxa and, with some adjustments, his classification scheme has been upheld by subsequent investigations including my own.

Phylogenetic analyses of *Rhagoletis* subsequent to Bush's (1966) monograph have used expanded taxonomic samples, morphological and molecular datasets to infer relationships. Berlocher and Bush (1982) used the signature of allozymes when electrophoresed on a gel to generate characters for use in phylogenetic inference. The allozyme-based phylogenies inferred generally resolved North American species groups, which were themselves part of a larger monophyletic group together.

Jenkins (1996) performed a massive taxonomic update of the genus by describing 247 morphological characters across 90 species (a subset was then used in a phylogenetic analysis). Morphological characters, especially unique wing banding patterns, are very useful for species-level identification. Jenkins (1996) identified and described 77 characters for use in a morphology-based phylogenetic analysis of *Rhagoletis* and found these characters to be generally uninformative. Smith and Bush (2000) reanalyzed the character-matrix generated by Jenkins using more liberal parameters (parsimony 50% majority rule consensus tree), but still did not infer species level relationships with confidence. In limited, narrow contexts morphological characters, especially male genitalia, may be phylogenetically informative (see the results and discussion in chapter one).

The development and expansion of DNA sequencing technology allowed the production of a very large number of potentially phylogenetically informative characters. Phylogenetic analyses based on mitochondrial DNA sequences of the cytochrome oxisase II gene (COII) and 16S ribosomal RNA (16S) showed strong support for the monophyly of individual species groups originally described by (Bush 1966), but not for phylogenetic resolution of relationships between the species groups (McPheron and Han 1997, Smith and Bush 1997, Smith et al. 2005). Specifically, the investigation of Smith et al. (2005) found an interesting result: all the members of the five North American species groups form a monophyletic group which also (and unexpectedly) includes two Palearctic taxa (*R. batava* and *R. flavigenualis*). A major goal of my dissertation (chapter two) was to test the hypothesis of the inclusion of the two palearctic taxa in the larger North American clade. Hamerlinck et al. (2016) did the most recent phylogenetic analysis of *Rhagoletis* prior to my dissertation, using sequences from mitochondrial cytochrome oxidase I (COI), 28S ribosomal RNA (28S), and carbamoyl-phosphate synthetase 2 aspartate

transcarbamylase and dihydroorotase (CAD) genes, however with a limited taxa sample. The phylogeny inferred from the three genes showed a sister relationship between the *pomonella* and *tabellaria* species groups which together were sister to a clade containing members of the *cingulata*, *suavis* and *ribicola* groups (Hamerlinck et al. 2016).

Dissertation overview

In chapter one I describe a new species of *Rhagoletis* in the *tabellaria* group and further analyze its parent species group. The previously undescribed species of *Rhagoletis,* infesting the fruits of buffaloberry (*Shepherdia argentea*), was first collected in 1982 by G. Bruce Neill and H. A. Worden near Indian Head Saskatchewan. Since it was first collected, the "buffaloberry fly" (*R. bushi*; described formally in a separate publication by myself: Hulbert et al. [2018]) has been part of several published analyses of *Rhagoletis* phylogeny and there is strong evidence that it belongs to the *tabellaria* species group (Jenkins 1996, Smith and Bush 1997, 2000, Smith et al. 2005). In chapter one of my dissertation, I present a description of *R. bushi* and provide three major lines of evidence supporting its status as a new species: 1) *R. bushi* uses a unique host plant among *Rhagoletis*. Host plant specialization is an important characteristic of the genus and there are no records of other *Rhagoletis* species infesting buffaloberry. 2) *R. bushi* has unique morphology. There is a set of easily observable diagnostic morphological characters to distinguish *R. bushi* from all other known species. No single morphological character is sufficient to diagnose *R. bushi*, but the suite of characters is a reliable means of diagnosis. 3) *R. bushi* has unique genetic sequences. There are unique genetic sequences for all individuals of *R. bushi* at all loci that I sequenced, and those individuals are resolved into a monophyletic group with high confidence when included in a phylogenetic analysis with its closest relatives.

In Chapter two I present a phylogenetic analysis of representatives from across the genus *Rhagoletis*. The analysis contains containing 86 individuals representing 37 species. I infer the phylogeny using alignments of genetic sequences from five different genes representing 4399 aligned states including: 1) mitochondrial cytochrome oxidase I (COI), 2) 28S ribosomal RNA (28S), 3) carbamoyl-phosphate synthetase 2 aspartate transcarbamylase and dihydroorotase (CAD), 4) period, and 5) Alanyl-tRNA synthetase (AATS). This dataset was sufficient to resolve the relationships between the major species groups of *Rhagoletis* including the North American species groups. One of the more exciting results of this chapter is the resolution of major monophyletic groups in *Rhagoletis* which are united by their use of closely related host plants, which suggests that diversification after colonization of new host plant groups was an important factor in the evolution of the genus.

In Chapter three, I present the results of an analysis into the role of systematics in entomology. It is often claimed that systematic biology is fundamental to all other areas of biology. I assess how entomologists think about the justifications, internal and external to science, for systematic biology. I also critically evaluate the societal justification for, and value of systematic biology using two major frameworks: 1) Boltanski and Thévenot's realm's of worth and 2) the framework of virtues, desserts and outcomes. In order to accomplish these purposes, I critically analyze and review relevant literature and interview practitioners of entomology and insect systematic biology. Overwhelmingly, I find utilitarianism and value to the Industrial World to be justifications given for systematic biology, but also some justifications given also pertain to the Civic World and to virtue.

CHAPTER 1: DESCRIPTION OF A NEW *RHAGOLETIS* (DIPTERA: TEPHRITIDAE) SPECIES IN THE *TABELLARIA* SPECIES GROUP

Abstract

Flies of *Rhagoletis* (Diptera: Tephritidae) are economically important fruit pests, which also serve as models for studying modes of speciation and coevolution with their hymenopteran parasitoids. We describe the morphology of *Rhagoletis bushi*, its geographic distribution, host association, phylogenetic relationships, and identify an associated species of parasitoid wasp. *Rhagoletis bushi* is in the *tabellaria* species group and infests the fruit of buffaloberry (*Shepherdia argentea*) in the Northern Great Plains of North America. There is a suite of morphological characters, and a unique host plant association, that are diagnostic of *R. bushi* Further evidence for the validity of *R. bushi*. and its placement within the *tabellaria* species group comes from a multilocus molecular phylogeny for representatives of species in the *tabellaria*, *pomonella* and *cingulata* groups inferred from five loci (COI, CAD, period, AATS and 28S) totaling. Additionally, we report a species of parasitoid, *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae), attacking *R. bushi*.

Introduction

Tephritid fruit flies of *Rhagoletis* Loew are economically injurious pests of apples, cherries, blueberries and walnuts (Boller and Prokopy 1976). In addition, *Rhagoletis* has provided an important system for fundamental evolutionary biology research (Mather and Roitberg 1987, Feder et al. 1988, 2003, 2005, Filchak et al. 2000, Jiggins and Bridle 2004, Schwarz et al. 2005, Michel et al. 2007, Xie et al. 2007, Arcella et al. 2015, Hood et al. 2015). Species within the genus are specialized frugivores with narrow host ranges (typically a single

host species), and host plant fidelity and specialization are important aspects of *Rhagoletis* biology (Bush 1966). Therefore, studying the patterns of host use and host shifts in *Rhagoletis* is valuable from an economic perspective and for fundamental evolutionary research.

Species of *Rhagoletis* have been documented to shift from their native host to economically important crops (Bush 1969). In North America, a host shift occurred in *R. pomonella* (Walsh) from its native host plant *Crataegus* Tourn *ex.* L. (hawthorn) to introduced commercial apples (*Malus pumila* Miller, 1768) in the last ~170 years (Walsh 1867). Host shifts in *Rhagoletis* have led to the formation of host races, the hypothesized initial stage of speciation-with-gene-flow, and have provided an opportunity to study the fundamental nature of population divergence and the formation of new species, especially in the *R. pomonella* species complex (Bush 1969, Feder et al. 1988, 2003, Drès and Mallet 2002, Xie et al. 2007, 2008, Schwarz et al. 2009, Hood et al. 2015, Doellman et al. 2018)which has become a textbook example of speciation in action (Schluter 2000, Coyne and Orr 2004, Nosil 2012). *Rhagoletis*, especially in the context of the pomonella species complex, is also a model system for studying coevolution and co-diversification with its numerous hymenopteran parasitoids. The ecological effects of host shifts in *Rhagoletis* extends through their associated parasitoid community and influence their diversification (Forbes et al. 2009, Hood et al. 2015, Hamerlinck et al. 2016). Thus, studying evolutionary patterns in *Rhagoletis* is important for understanding when and why *Rhagoletis* infestations occur.

In North America, *Rhagoletis* is composed of 25 described taxa, is arranged both phylogenetically and taxonomically into species groups i.e., presumably monophyletic groupings without official taxonomic status on the basis of morphology and distinct host plant associations (Bush 1966, Berlocher and Bush 1982, Foote et al. 1993, Smith and Bush 1997, Smith et al.

2005, Glover et al. 2018). The *tabellaria, pomonella, cingulata, suavis* and *ribicola* groups comprise 21 of the North American described species and are part of a larger monophyletic group that includes the Nearctic *R. juniperina* Marcovitch, *R. fausta* (Osten-Sacken), and the Palearctic *R. batava* Hering and *R. flavigenualis* Hering (Smith et al. 2005, Hamerlinck et al. 2016). Here we document a new species of *Rhagoletis* in the *tabellaria* group using a combination of morphological and molecular analysis, and surveys of host plant associations.

While morphological characters such as wing patterns and male genitalia have been useful for species-level identification in *Rhagoletis*, DNA sequences have proven to be more useful for phylogenetic studies (Bush 1966, Smith et al. 2005, Hood et al. 2012a). Jenkins (1996) identified and described 77 characters for use in a morphology-based phylogeny of *Rhagoletis*. However, these characters did not to resolve higher phylogenetic relationships in *Rhagoletis*. Analyses of mitochondrial COII and 16S ribosomal DNA showed strong support for the monophyly of species groups proposed by Bush (1966), but these data could not confidently resolve relationships among them (Han and McPheron 1997, Smith and Bush 1997, Smith et al. 2005). A Phylogeny of 15 North American *Rhagoletis* species inferred from the mitochondrial cytochrome oxidase I (COI), 28S ribosomal RNA, and carbamoyl-phosphate synthetase 2 aspartate transcarbamylase and dihydroorotase (CAD) genes gave better phylogenetic resolution of relationships between species groups (Hamerlinck et al. 2016) and provided evidence of a sister relationship between the *pomonella* and *tabellaria* species groups. The *tabellaria* species group is less-studied compared to the *pomonella* group likely due to lack of significant agricultural pests in the clade. The *tabellaria* species group provides a useful system for studying host plant use and diversification in a comparative context to the better-studied *pomonella* group

because the two groups have overlapping host ranges, similar biology and natural history, and may represent a comparison of modes of evolutionary processes.

There are four described species in the *tabellaria* group: *R. tabellaria* (Fitch), *R. electromorpha* Berlocher, *R. persimilis* Bush, and *R. ebbettsi* Bush (Foote et al. 1993). *Rhagoletis tabellaria* primarily infests the fruit of *Cornus stolonifera* Michx. (red osier dogwood). There are also records in the state of Washington of *R. tabellaria* commonly infesting *Vaccinium parvifolium* Sm. and *V. ovalifolium* Sm. 1787 (Plank 1923, Bush 1966), rarely *Prunus emarginata* (Dougl. ex Hook.) Eaton 1836 (bitter cherry) and *Maianthemum racemosum* (L.) Link (false Solomon's seal) (Yee and Goughnour 2008). Red osier dogwood has a transcontinental distribution in North America and *R. tabellaria* has been collected from across that range, although primarily from red osier dogwood (Figure 1.1, Table 1.1) (Little 1971, Foote et al. 1993).

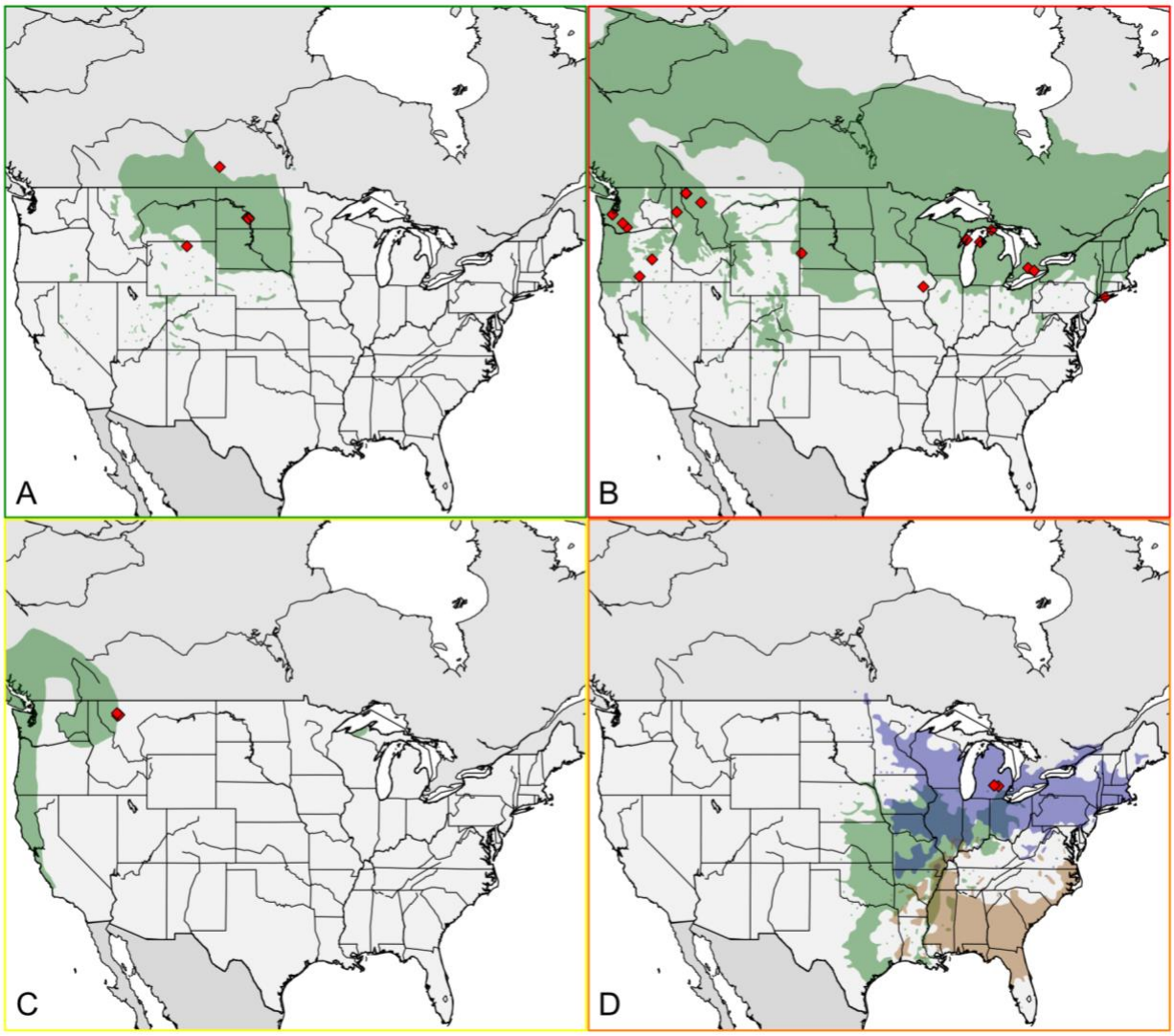

Figure 1.1. Map of collection locations (red diamonds) and approximate host plant ranges (shaded area) for the species included in this study (Little 1971, eFloras 2008): *R. bushi* infests *Shepherdia argentea* (A); *R. tabellaria* infests *Cornus stolonifera* (B); *R. persimilis* infests *Prosartes hookeri* (green) (C); *R. electromorpha* infests *C. drummondii* (green) *C. racemosa* (blue) and *C. foemina* (stricta) (light brown) (D). Individual map panels outlined in the same color as those associated with species in Figure 1.4.

Two species described by Bush (1966), *R. persimilis* and *R. ebbettsi*, are the least well-known members of the *tabellaria* species group. Adult *R. persimilis* were described and differentiated from *R. tabellaria* on the basis of male and female genitalia. The host plant of *R. persimilis* has now been established as *Prosartes hookeri* Torr. (Hooker's fairy bells) by rearing adult flies directly from the fruit. (D. Hulbert, pers. observ.), not *P. trachycarpum* as reported by

Smith and Bush (1997). *Prosartes hookeri* can be found in North-Central and Western regions of North America (eFloras 2008), with collection records of *R. persimilis* from Bear Lake and Robson, British Columbia, Canada (Bush 1966), and Flathead Lake, Montana (pers. observ.; Figure 1.1, Table 1.1). Little is known about *R. ebbettsi*, which was described from a single damaged specimen collected near Ebbets Pass, California on the basis of a unique wing pattern: the medial and subapical crossbands are joined by another band (Bush 1966). No individuals have been collected since that time and the host plant association of *R. ebbettsi* remains unknown. Due to a lack of material, we exclude *R. ebbettsi* from further analysis.

The most recent previously described species in the *tabellaria* group is *R. electromorpha*, whose morphology and life history parallel those of *R. tabellaria* (Berlocher 1984) described overlapping, comparative characters to distinguish between *R. electromorpha* and *R. tabellaria*, including differences in wing banding patten and body pigmentation, which are summarized by Foote et al. (1993). *Rhagoletis electromorpha* was first discovered and later described and named after its unique electrophoretic allozyme signature (Berlocher 1984). Three dogwood species are known to host *R. electromorpha: Cornus drummondii* (C. A. Mey.), *C. racemosa Lam.*, and *C. foemina* Mill. (*stricta* Lam.) (Berlocher 1984, Smith and Bush 1997), which are distributed across the eastern United States (Little 1971). Despite the broad range of its hosts, *R. electromorpha* has only been reported from three locations in Michigan (Figure 1.1, Table 1.1) and four in Illinois (Berlocher 1984).

Table 1.1. Collection information for flies in the Rhagoletis tabellaria species group including the buffaloberry fly, *R. bushi*.

State	Locality (County/ Municipality)	Latitude	Longitude	Host plant	Collection date	Collector	No. fruits	No. pupae	No. adults	No. wasps
				R. bushi						
ND	Mandan (Morton)	*46.7636*	*-100.9013*	*S. argentea*	10-Sep-95	GB/DB	ND	6*	5	0
		46.7631	-100.8441	*S. argentea*	12-Aug-14	DH	428	21	1	1
		46.7627	-100.8441	*S. argentea*	14-Aug-16	DH	1900	49	21	5
	Bismarck (Burleigh)	46.7677	-100.7664	*S. argentea*	13-Aug-14	DH	399	23	17	1
		46.6696	-100.7329	*S. argentea*	13-Aug-14 (9)	DH	676	61	33	0
		46.6695	-100.733	*S. argentea*	14-Aug-16 (7,8,10,11)	DH	2100	123	48	6
WY	W of Bighorn (Bighorn)	44.6538	-107.0033	*S. argentea*	5-Sep-97	GB/DB	881	2	2	0
SK	Indian Head (No. 156)	*50.5091*	-103.6856	*S. argentea*	May-82	GN/HW	ND	ND	60	0
				R. tabellaria						
ON	Zorra Twp (Oxford)	*43.0447*	*-80.9244*	*C. stolonifera*	30-Jul-97 (20)	DS	ND	91	36	9
	Simcoe (Simcoe)	*42.8393*	*-80.3041*	*C. stolonifera*	31-Jul-97	DS	ND	30	22	0
		42.8431	*-80.3067*	*C. stolonifera*	31-Jul-97	DS	ND	93	28	3
NY	Stony Brook (Suffolk)	*40.8925*	*-73.1158*	*C. stolonifera*	27-Jun-06	JLF	ND#	ND#	ND#	0
MI	Lake Leelanau (Leelanau)	*44.9803*	*-85.7173*	*C. stolonifera*	26-Jul-14	JS	1440	313	80	23
		44.9802	*-85.7185*	*C. stolonifera*	6-Aug-00	JS	1200	348	15	0
		44.9815	*-85.7114*	*C. stolonifera*	2-Aug-98	JS	1138	61	22	8

Table 1.1 (cont'd)

State	Locality (County/ Municipality)	Latitude	Longitude	Host plant	Collection date	Collector	No. fruits	No. pupae	No. adults	No. wasps
	Good Harbor Bay (Leelanau)	*44.9551*	*-85.8002*	*C. stolonifera*	4-Aug-01	JS	667	51	24	1
	Windemere Point (Mackinac)	*45.8466*	*-84.6191*	*C. stolonifera*	18-Jul-98	JS	106	4	2	2
IA	Iowa City (Johnson)	*41.6611*	*-91.5302*	*C. stolonifera*	11-Jul-11 (17)	AN	ND	ND	8	0
WI	Fish Creek (Door)	*45.1123*	*-87.2255*	*C. stolonifera*	5-Aug-95 (21)	GB	ND	106	27	32
	Mud Lake (Door)	*45.1347*	*-87.112*	*C. stolonifera*	5-Aug-95	GB	ND	174	44	46
SD	Crooks Tower (Lawrence)	44.1346	-103.8603	*C. stolonifera*	11-Aug-14	DH	307	2	1	0
MT	Yellow Bay (Lake)	47.8764	-114.0313	*C. stolonifera*	22-Aug-95	GB/DB	ND	53	16	10
	Libby (Lincoln)	48.5445	-115.5308	*C. stolonifera*	10-Aug-16	DH	350	1	1	0
		48.5639	-115.563	*C. stolonifera*	10-Aug-16 (22)	DH	2100	20	6	5
		48.5666	-115.5677	*C. stolonifera*	10-Aug-16	DH	400	1	0	0
		48.5664	*-115.5677*	*C. stolonifera*	24-Aug-95	GB/DB	ND	5	2	3
ID	Emida (Benewah)	*47.1765*	*-116.4966*	*C. stolonifera*	25-Aug-95	GB/DB	ND	3	2	0
OR	hwy 395 (Lake)	*42.4143*	*-120.2672*	*C. stolonifera*	27-Aug-95	GB/DB	ND	11	2	2

Table 1.1 (cont'd)

State	Locality (County/ Municipality)	Latitude	Longitude	Host plant	Collection date	Collector	No. fruits	No. pupae	No. adults	No. wasps
WA	Burns (Harney)	43.6764	-118.999	*C. stolonifera*	4-Aug-14	DH	1200	2	0	0
	Trout Lake (Klickitat)	*46.044*	*-121.5552*	*C. stolonifera*	30-Aug-95 (18,19)	GB/DB	ND	71*	40	15
	McLane (Thurston)	*47.0021*	*-123.0084*	*V. parvifolium*	25-Jul-96	RS	ND	ND	62	5
		47.0025	*-123.0081*	*V. parvifolium*	14-Aug-95	RS	ND	18*	14	0
WA	Strawberry Mt (Skamania)	46.3509	-121.9741	*V. parvifolium*	31-Aug-95	GB/DB	ND	301	186	45
				R. electromorpha						
	Hawk Meadow (Ingham)	*42.6658*	*-83.9315*	*C. foemina*	25-Aug-14 (15,16)	MD/JS	371	51	6	0
	Okemos (Ingham)	*42.7231*	*-84.3636*	*C. foemina*	13-Aug-12 (14)	DH	850	18	2	0
		42.7231	*-84.3636*	*C. foemina*	24-Sep-08	PS/JS	2139	16	0	0
				R. persimilis						
	Yellow Bay (Lake)	*47.8763*	*-114.0318*	*P. hookeri*@	22-Aug-95 (12)	GB/DB	ND	10*	2	4
	Flathead Lake (Lake)	47.8761	-114.0299	*P. hookeri*	9-Aug-16	DH	400	2	1	0
	Swan Lake (Lake)	47.9363	-113.8568	*P. hookeri*	10-Aug-16 (13)	DH	151	18	4	1
	Wayfarers St Pk (Flathead)	48.0539	-114.0814	*P. hookeri*	11-Aug-16	DH	112	1	0	1

Table 1.1 (cont'd)

State	Locality (County/ Municipality)	Latitude	Longitude	Host plant	Collection date	Collector	No. fruits	No. pupae	No. adults	No. wasps
				***pomonella* group (*R. pomonella, R. mendax* and *R. zephyria* respectively)**						
MI	East Lansing (Ingham)	42.7262	-84.4648	*Crataegus mollis*	23-Jun-09 (4)	JS	ND	ND	ND	ND
	Fennville (Allegan)	*42.595*	*-86.1557*	*Vaccinium sp.*	14-Aug-08 (5)	JS	ND	ND	ND	ND
MN	Bloomington (Hennepin)	*44.86*	*-93.2903*	*Symphoricarpos* sp.	3-Sept-13 (6)	JS	ND	ND	ND	ND
				R. cingulata						
MI	Rose Lake (Clinton)	44.0598	-85.3856	*Prunus serotina*	13-Jul-91 (1,2)	JS	ND	ND	ND	ND

Latitude and Longitude – Values in italics are approximations.
Collection Date – Date of fruit collection; numbers in parentheses following some dates specify individuals used in the phylogenetic analysis (Figure 1.4**)**.
Fruits – number of fruits collected from host plants.
Pupae – number of pupae recovered from host plant fruits.
Adults – number of adults emerged.
Collector – Initials: AN, A. E. Nelson; DB, Dorie Bush; DH, Dan Hulbert; DS, Dave Smitley; GB, Guy L. Bush; GN, G. Bruce Neill; HW, H. A. Worden; JLF, Jeff Feder; JS, Jim Smith; MD, Meredith Doellman; PS, Parita Shah; RS, Robert Sluss
* Two pupae taken for DNA analysis prior to rearing.
# Large collection made by Feder et al. with many adults reared in the greenhouse at the University of Notre Dame.
@ Misidentified as *Disporum trachycarpum* by Smith & Bush 1997.

A fifth putative species believed to belong to the *tabellaria* species group and which infests *Shepherdia argentea* (Pursh) Nutt. (buffaloberry), a deciduous shrub native to the Northern Great Plains of North America (Little 1971) (Figure 1.1A, Table 1.1), has been discussed repeatedly in the literature, despite remaining undescribed. This has, in turn, created a confusing and inconsistent history throughout the literature, as the buffaloberry-infesting species of *Rhagoletis* has been referred to by several provisional identifiers in published works and collection history. This buffaloberry-infesting *Rhagoletis* has been collected at least two times prior to the present study and has been part of studies on the genus under different provisional names. Jenkins (1996) first included this fly (referred to as *R.* "nr. *tabellaria*") in his morphological and phylogenetic analysis of the genus, and in doing so described diagnostic characters. Smith and Bush (1997) refer to it as *R.* "n. sp. B" in their molecular phylogenetic analysis and Smith and Bush (2000) included a specimen of the putative species as *R.* "nr. *electromorpha*" in their reanalysis of Jenkins' (1996) characters. Finally, Smith et al. (2005) published a molecular phylogeny expanding the taxon set of Smith and Bush (1997) in which they refer to the buffaloberry-infesting specimen as *R.* "nr. *tabellaria*"; in both of these analyses the individual is shown to be genetically distinct from described *Rhagoletis* based on mtDNA. Formal description of this unique fly is appropriate given the evidence that it represents a full species lineage and not a host race or minor variant. Here, we provide new molecular and morphological features to support the status of *Rhagoletis bushi*, as a new species and describe its life history, parasitoids, phylogenetic relationships, and provide diagnostic characters.

Methods

Insect collections

We collected *Rhagoletis bushi* individuals for analysis in this study from three locations in and around Bismarck and Mandan, North Dakota and a single location near Bighorn, Wyoming (Table 1.1). We sampled at similar times and locations to those made by Bush in 1995 and at locations known to have large stands of buffaloberry (W. Duckwitz pers. com.) All insects were collected as larvae within infested fruit sampled from the plant and the ground beneath the plant. North Dakota collections of *R. bushi* were made from the "Sakakawea" variety of *Shepherdia argentea*. We collected 285 pupae, of which 127 eclosed as adults following a simulated overwinter (see Insect Rearing). Of the adults, 29 individuals (15 female, 14 male), were point-mounted for morphological analysis, and five individuals were used for DNA sequencing. All individuals used for morphological and molecular analysis were collected near Bismarck, ND in August 2014 and 2016, near the location of Bush's 1995 collections. In addition, a series of specimens collected by G. Bruce Neill and H. A. Worden near Indian Head, Saskatchewan in 1982 and deposited in the Canadian National Collection of Insects, Arachnids, and Nematodes (CNCI) were included in the morphological examination. Specimens were deposited in the Albert J. Cook Arthropod Research Collection (MSUC) and the University of Guelph Insect Collection (DEBU). Over the course of the adult fly emergence, nine parasitoid wasps emerged.

For comparative molecular phylogenetic analyses, we also collected individuals representing three of the four remaining *tabellaria* group species. We made 25 collections from 18 different locations of *R. tabellaria* individuals from both *Cornus stolonifera* (red osier dogwood) and *Vaccinium parvifolium* Sm. (red huckleberry) and used a subset of six individuals

for phylogenetic analysis (Table 1.1). We also included *R. electromorpha* from two locations in Ingham County, MI (collected from *C. foemina*), and *R. persimilis* collected from *P. hookeri* at four locations in Montana. We included three *R. electromorpha* and two *R.* persimilis individuals in the phylogenetic analyses (Table 1.1). We were not able to collect *R. persimilis* and *R. electromorpha* across their respective ranges because of difficulties finding infested plants at the proper time. Members of the *pomonella* and *cingulata* species groups were collected and used as outgroups to the *tabellaria* group (Table 1.1).

Insect rearing

We used standard *Rhagoletis* rearing conditions as described by Frayer et al. (2015). Individuals from all species were reared as larvae to adulthood in the laboratory from field collected infested fruit. Field-collected fruit from each site and host plant were transported back to the laboratory, placed on moist fine vermiculite and held at ambient temperature (23-28°C) in separate, site- and host-specific 22.86 cm × 22.86 cm (9" × 9") plastic trays ("growers flats") for three to four weeks. During this time, larvae emerged from rotting fruit and pupated in the vermiculite. We sifted vermiculite and quantified pupae and fruit (to determine % infestation) and placed the pupae in Petri dishes (100 mm × 15mm) with a small amount of moist vermiculite to prevent desiccation. We then placed Petri dishes in a refrigerator (~4°C) for five months to simulate overwinter. Following overwinter, we placed closed Petri dishes at ambient temperature (23-28°C) to monitored adult eclosion. Plates were checked for eclosed adult flies and wasps every 2-4 days until emergence ceased. Flies and wasps that emerged were placed in plastic cages (16 oz. "deli cups") with water wicking and a paper tab impregnated with a solution of

sugar and yeast for approximately 48 hours (to allow exoskeletons to sclerotize) before being frozen at -20°C (with a subset pinned) for further analysis.

Characterization of morphological features

Morphological terminology follows Foote et al. (1993), while wing venation terminology follows the standard of (Cumming and Wood 2017).

DNA isolation, PCR amplification and DNA sequence alignment

We isolated DNA from adult whole-fly homogenates from individuals in the taxon set (Table 1.1) using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). For phylogenetic analyses, we used five loci totaling 4,270 nucleotides in length that have been useful in systematic analyses of insects, especially tephritids (Table B1) (Han et al. 2002, Hebert et al. 2004, Moulton and Wiegmann 2004, Barr et al. 2005, Smith and Brown 2008, Hamerlinck et al. 2016). We PCR-amplified regions of the mitochondrial protein coding gene cytochrome oxidase I gene (COI) (684 bp), the nuclear protein coding carbamoyl-phosphate synthase (CPS) domain of carbamoyl-phosphate synthetase 2, aspartate transcarbamylase and dihydroorotase gene (CAD) (990 bp), the large subunit of the nuclear ribosomal gene (28S) (1359 bp), the nuclear protein coding *period* gene (614 bp) and the nuclear protein coding alanyl t-RNA synthetase gene (AATS) (623 bp). Sequences from COI, 28S and CAD have been used previously in *Rhagoletis* phylogenetic research (Hamerlinck et al. 2016). We PCR amplified each gene separately in 25 μL reactions using GotaqFlexi (Promega, Madison, WI, USA) with the following reagents (and concentrations): reaction buffer (1X), $MgCl_2$ (8 mM), dNTP (0.5 mM each), forward and reverse primers (0.5 mM each), DNA polymerase (2.5 u), DNA template

(~18 – 92 ng). Primers and thermocycler conditions used for each of the five genes amplified are listed in (Table B1). Amplifications of COI, *period*, and AATS employed a single primer pair. For 28S we used two primer pairs to amplify two non-overlapping fragments separated by 58 bp (1359 bp total). For CAD, we used two primer pairs to amplify two overlapping fragments (990 bp total).

We developed new primers and a protocol for amplifying a region of the AATS gene in *Rhagoletis*. The primers were developed by using the predicted *R. zephyria* Snow alanine tRNA ligase cytoplasmic mRNA sequence (GenBank accession number: XM017616943) and Primer-BLAST as implemented by the NCBI website (Ye et al. 2012). We first selected primers that would amplify a DNA fragment of *R. zephyria* AATS similar to the one used by (Morita et al. 2016). We then compared potential primer sequences based on *R. zephyria* to those of *Ceratitis capitata* (Wiedemann) (accession number: XM020860749) and *Bactrocera latifrons* (Hendel) (accession number XM018939529) and found the sequences were conserved across three species of dipterans. *Ceratitis capitata* and *Bactrocera latifrons* sequences were chosen because they are the only other Tephritidae species for which AATS is entirely sequenced. These primers (AATSZ1F/AATSZ1R) were then used to amplify (and ultimately sequence) AATS using the conditions given in Table B1.

Verification of successful amplification for all PCR products was confirmed electrophoretically using agarose gel (1% w/v) prior to purification of PCR products using a QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's specifications. Sanger sequencing was performed at the Michigan State University Research Technology Support Facility via BigDye Terminator Sequencing on an Applied Biosystems 3730xl DNA Analyzer (Foster City, CA, USA) using the PCR primers as sequencing primers (Table B1).

We edited all sequences manually by visual comparison of the automatic base calls to the original electropherogram traces using MEGA (version 7.0.14) (Tamura et al. 2011). All sequences were deposited in GenBank (accession numbers MG825190- MG825320) (Table B2). Alignments of DNA sequences were constructed and edited in MEGA. We used the default parameters in MUSCLE (Edgar 2004) as implemented in MEGA to align DNA sequences. Alignments for all loci were unambiguous.

Molecular phylogenetic analysis

We first calculated descriptive statistics for the alignments at each locus individually, and then concatenated the 4270 bp combined five locus alignment. Using MEGA, we calculated the average uncorrected pairwise p-distance for each gene (including distances for each codon positions of protein-coding genes), the concatenated alignment, and for all transition and transversion mutations. We also used MEGA to determine the nucleotide composition for each alignment. Using PAUP* (version 4.0a152) (Swofford 2003) we counted the number of variable sites in each alignment (including and excluding parsimony informative sites). We also calculated the number of most parsimonious trees for each alignment in PAUP* using the heuristic search option with 100 random sequence additions and TBR branch swapping. The same parsimony settings were used for pairwise incongruence length difference (ILD) tests (Farris et al. 1995) between each gene alignment.

We used a maximum likelihood and Bayesian framework for our phylogenetic analyses of the five-locus alignment. We predefined the following biologically relevant partitions in the alignments per the recommendation of the PARTITIONFINDER software user-guide (Lanfear et al. 2017): 28S (fragments were concatenated and considered a single partition for phylogenetic

analysis), and separate partitions for each nucleotide position (1^{st}, 2^{nd}, 3^{rd} codon position) for COI, CAD, period and AATS. Next, we used PARTITIONFINDER v2.1.1 (Lanfear et al. 2017) to determine combinability of partitions and nucleotide substitution models. We then ran PARTITIONFINDER implementing PhyML (Guindon et al. 2010) with the "greedy" algorithm (Lanfear et al. 2012) using the corrected Akaike Information Criterion (AICc) to assess model and partition quality. We conducted separate runs of PARTITIONFINDER, one restricted to MRBAYES models and the other for RAxML models. The resulting partitioning and model scheme for MRBAYES is shown in Table B3. Because RAxML only allows the specification of one model rate of heterogeneity for all partitions in a concatenated analysis, we ran PARTITIONFINDER three times while restricting each run to one model of rate heterogeneity (GTR, GTR+G, or GTR+G+I) and comparing the AICc of each run. The GTR+G model had the lowest AICc and was thus used for subsequent RAxML analyses. Partitioning schemes and substitution models for RAxML are found in Table B3.

We inferred phylogenetic trees using RAxML (version 7.4.2) (Stamatakis 2014) as implemented by RAxMLGUI (version 1.31) (Silvestro and Michalak 2011) and MRBAYES (version 3.2.5) (Ronquist and Huelsenbeck 2003) using the model schemes described above (Table B3). For the maximum likelihood analysis, we ran RAxML for 1000 pseudoreplicates using the above partitioning scheme.

The Bayesian analysis used four independent runs each with four Metropolis-coupled chains with default heating parameters (one cold and three heated) in MRBAYES. The chains were sampled once every thousand generations for 10 million generations and the first 25% of samples were discarded as burn-in. All analyses converged to an average standard deviation of split frequencies below 0.01 and all branch lengths and substitution model parameters had

potential scale reduction factors less than 1.01 (Ronquist et al. 2012). We used FigTree (version 1.4.2) to visualize the phylogenetic trees (Rambaut 2014).

Characterization of parasitoids

We used the taxonomic key of Wharton and Yoder (2016) to identify the two individuals of a hymenopteran parasitoid species attacking the buffaloberry fly. In addition, we complemented morphological taxonomy with molecular barcodes for two individuals by PCR amplifying and sequencing the ~600 bp region of the cytochrome oxidase subunit I (COI) barcoding gene commonly used in insect barcoding studies (Hood et al. 2015, Hamerlinck et al. 2016) using the universal primers developed by Simon et al. (1994) utilized for other *Rhagoletis*-attacking parasitoid species (Forbes et al. 2009, Hood et al. 2012b). See mtDNA amplification protocols by (Hood et al. 2015) for details of PCR amplification.

Genomic DNA was extracted from whole adult body tissue for both individual parasitoids using Puregene extractions kits (Gentra Systems). Purified PCR products were DNA sequenced on an ABI 3700 sequencer with the ABI Prism BIGDYE Terminator v3.0 system (Applied Biosystems, Inc.) at the University of Notre Dame's genomic core facility. The generated barcodes were queried using the "identification request" tool on the Barcode of Life Database (Ratnasingham and Hebert 2007) to identify similar sequences from previously identified taxa. Two sequences were deposited on GenBank (accession numbers MG831937and MG831938).

Nomenclature

This chapter and the nomenclatural act(s) it contains were originally published in the journal Insect Systematics and Diversity (Hulbert et al. 2018) and was registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of that publication is urn:lsid:zoobank.org:pub:5C5EAC90-1213-45B3-A985-D35C525EC210.

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(Figure 1.2, Figure 1.3)

Rhagoletis "nr. *tabellaria*": Jenkins (1996): 40.

Rhagoletis "n. sp. B": Smith and Bush (1997): 34.

Rhagoletis "nr. *electromorpha*": Smith and Bush (2000): 195.

Rhagoletis "nr. *tabellaria*": Smith et al. (2005): 322.

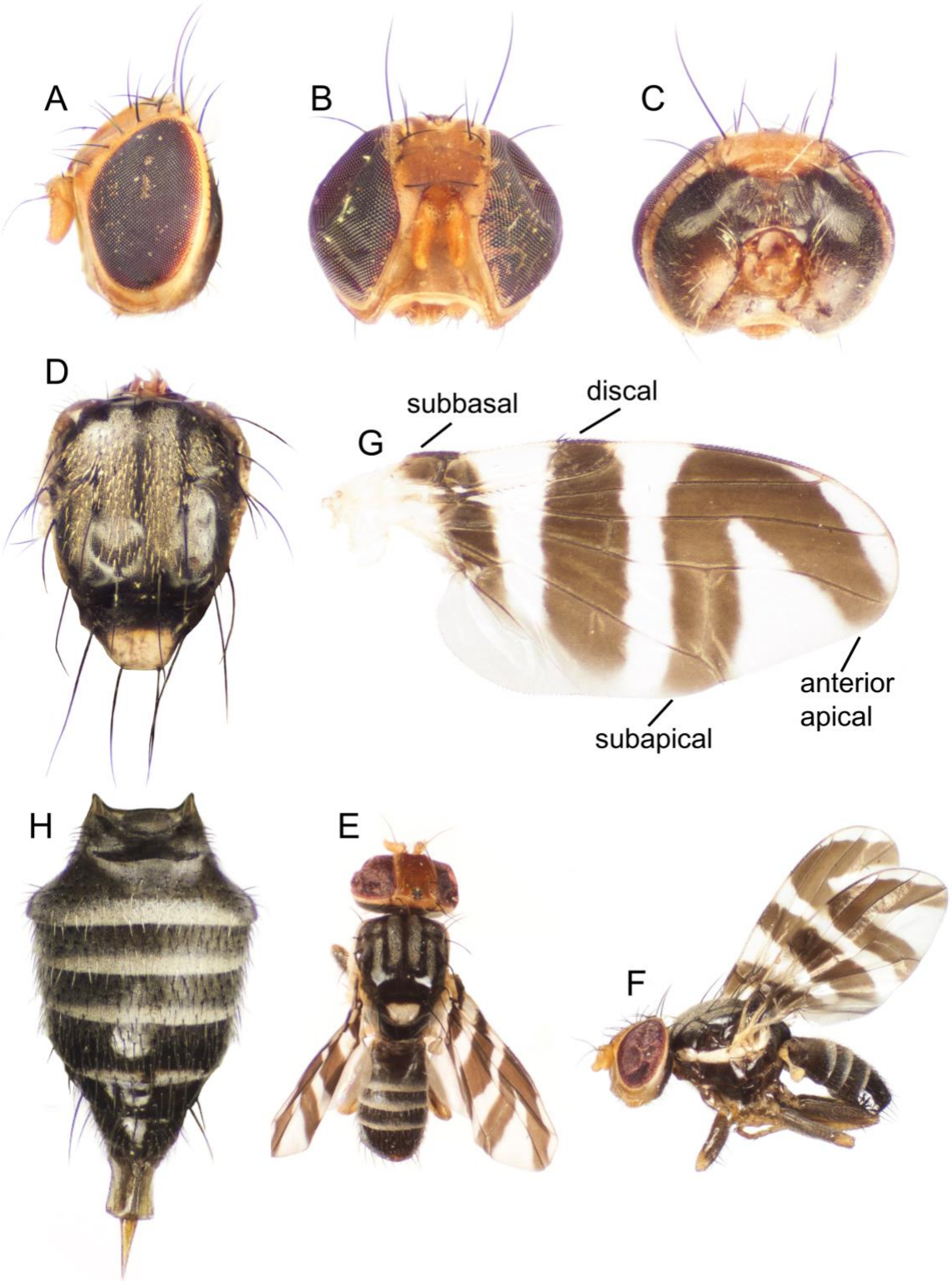

Figure 1.2. External morphology of *Rhagoletis bushi* including the lateral (A), anterior (B) and posterior (C) views of the head; dorsal view of thorax (D); the dorsal (E) and lateral (F) habitus of a male; the dorsal view of the female abdomen (H); dorsal view of the wing including band names (G). Photographs by M. D. Jackson, montage by D. Hulbert.

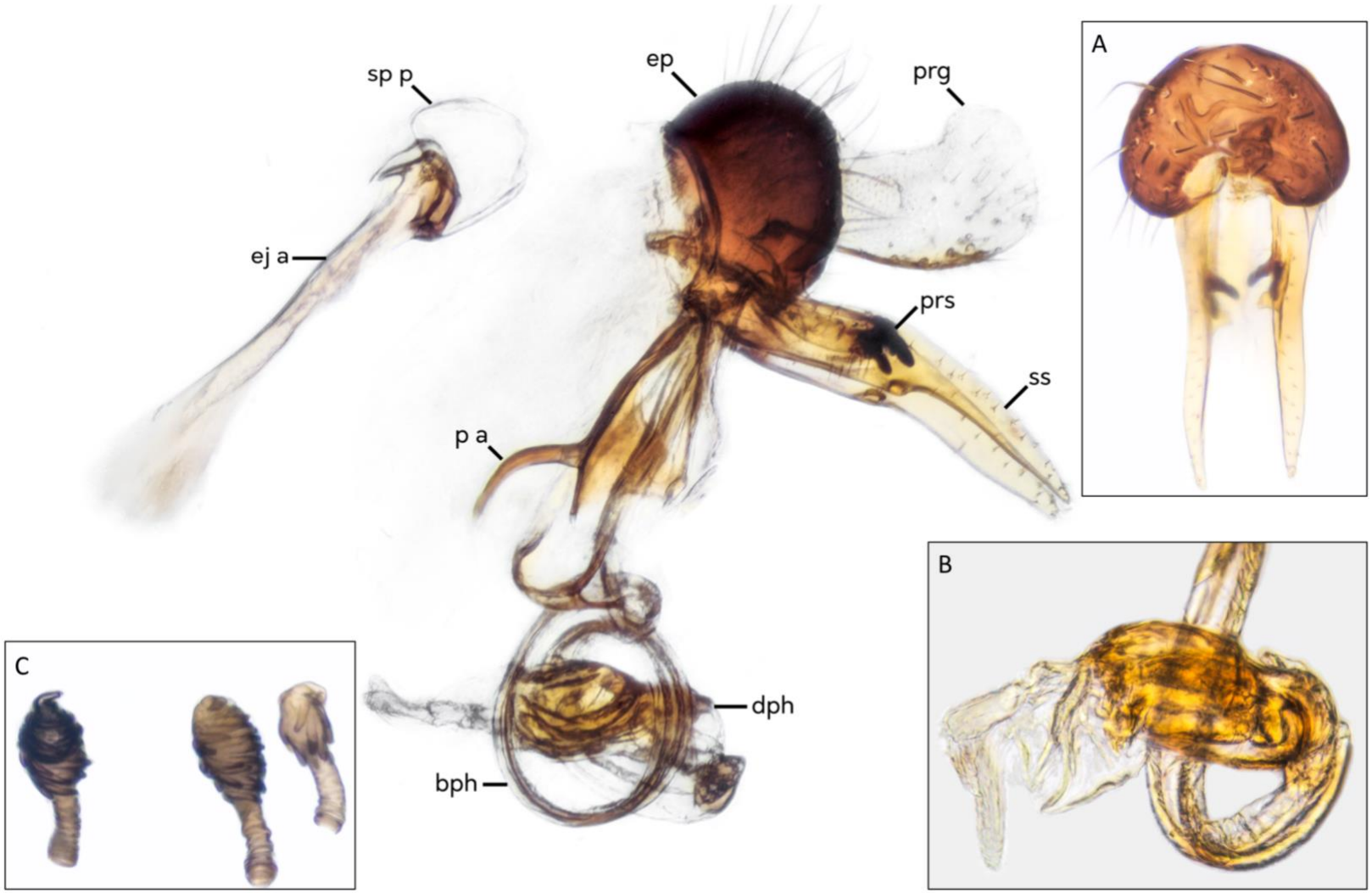

Figure 1.3. Genitalic morphology of *Rhagoletis bushi*. Lateral view of male genitalia including basiphallus (bph), distiphallus (dph), ejaculatory apodeme (ej a), epandrium (ep), phallic apodeme (p a), proctiger (prg), prensisetae (prs), sperm pump (sp p), and surstyli (ss). Insets showing the posterior view of the male genitalia (A), lateral view of the aedaegus (B) and spermathecae (C). Photographs by M. D. Jackson, montage by D. Hulbert.

Diagnosis

Rhagoletis bushi is distinguished from other *tabellaria* group species by a suite of morphological characters, unique DNA sequences, and distinct host fruit association. Morphological characters that diagnose *R. bushi* are shown in Table 1.2; wing pattern with subbasal and discal bands separated, anterior apical band reaching wing margin in cell r_{4+5}, the lateral scapular seta is not concolorous with the principle thoracic setae, tarsomere four and five are the same color as the rest of the tarsus, the midtibia does not have a distinct posterodorsal row of setae, in males, the basiphallic vesica is absent, the phallic apodeme is finger-like with a 90° bend anteriorly at the midpoint, the distiphallus has a unique triradiate appendage arising from the tip, while in females the oviscape has two pairs of subapical dorsal setae, and three spermathecae are present, one of which is definitively smaller than the other two. Additionally, DNA sequences from each of the genes used in the present analysis (COI, CAD, period, AATS, or 28S) will distinguish *R. bushi* from all other *Rhagoletis*. Within these genes there are autapomorphic nucleotides which diagnose *R. bushi* (Table B4).

Table 1.2. Morphological characters that define taxonomic classification of Rhagoletis tabellaria group flies (from Jenkins, 1996; terminology modified to conform with McAlpine et al. [1981] and White et al. [1999]). Morphologically, Jenkins (1996) character #29 serves as a synapomorphy for the tabellaria group within Rhagoletis.

Jenkins (1996) character #	Body part	Character description and states	States within			
			R. persimilis	*R. bushi*	*R. electromorpha*	*R. tabellaria*
18	Thorax	Lateral scapular seta concolorous (0) or not concolorous (1) to principle thoracic setae (exclu presutural acrostichal, and proepisternal setae)	0	1	0	1
28	Legs	Tarsomere 4 or 5 or both same color as rest of tarsus (usually yellowish) (0); or darker than basal segments (1)	1	0	0	0
29	Legs	Midtibia with distinct posterodorsal row of setae (0); or midtibia without distinct posterodorsal row of setae (1)	1	1	1	1
37	Genetalia	Basiphallic vesica present (1); or basiphallic vesica absent (0)	0	0	1	1
46	Abdomen	Syntergosternum 7+8 with one or more setae (0); or syntergosternum 7+8 with only setulae or bare (1)	0	0	1	1
63	Genetalia	Total number of spermathecae three (0); total number of spermathecae two (1); or total number of spermathecae four (2)	0	0	1	1
74	Genetalia	One spermatheca definitely smaller than other(s) (1); or spermathecae nearly same size (0)	1	1	0	0

Head: Orange-ish yellow color; face slightly lighter and more yellow. Genal seta similar color as face, sometimes slightly darker; all other major setae black. Ocellar triangle darker (light brown) than vertex. Black horseshoe-shaped pattern covering occiput; 6-12 (mean: 8.61; SE: 0.29; n: 28) post-ocular setae (Figure 1.2 A-C).

Thorax: Black dorsum; tomentum patterning: four well defined longitudinal stripes, outer pair broken by a tranverse groove (sulcus) that runs from the dorsocentral and presutual seta, inner stripes start at line drawn between the humeral seta, outer stripes start just posterior to where inner stripes begin, posterior end of outer stripes at line drawn between the acrostichal and intra-alar seta, posterior end of inner stripes at just anterior of outer stripes. Dorsocentral seta slightly anterior of anterior supraalar seta. Two pairs of scapular setae, both pairs slightly lighter and more yellow than other major setae (black); one or two anepisternal bristles. White notopleural stripe. White scutellar spot in shape of trapezoid; anterior limit of trapezoid limit even with pair of anterior scutellar setae, but lateral margin well separated from seta; posterior limit of trapezoid even with posterior scutellar setae, lateral margin encapsulating seta. Yellow to white halteres with the dorsal surface of the base of the bulb darkened (Figure 1.2 D-F).

Wing: General pattern of bands is two vertical lines and a "V" pattern. The basal and medial bands do not coalesce although slight infuscation in the area where they would (end of CuA+CuP and cell m_4) may be present. Apical and subapical bands joined on costa. Subapical band lightened on vein dm-m. Apical band reaching wing margin in cell r_{4+5}. Intercalary crossband absent (Figure 1.2 G).

Legs: All coxae and femora mostly black with some yellow at margins. Trochanters, tibiae, and tarsi concolorous, mostly dull yellow. Tibiae have no shading at connection with femur. Row of setae present on posterodorsal of tibia II and III (Figure 1.2 F).

Abdomen: Same as *R. tabellaria* description by Bush (1966): all segments brownish black with posterior margin of tergites II-V in female, and II-IV in males with pale gray to pale yellow band (Figure 1.2 E, F, H).

Genitalia: Male: Epandrium black. Proctiger longer than epandrium, with significant dorsal squarish swelling at apex (Figure 1.3). Surstyli dark yellowish orange, widest at base with distinct narrowing distal to prensisetae, abruptly tapering to sharp point at apex; prensisetae proximal to midpoint of surstyli, longer than internal projections of surstyli, right prensisetae proximal to left prensisetae; internal surface with 2-3 small setae proximal to prensisetae, posterior surface with small setae over much of surface, becoming more concentrated at apex of surstyli (Figure 1.3 A). Phallic apodeme finger-like and arising dorsally, bent nearly 90° anteriorly at mid-point (Figure 1.3). Ejaculatory apodeme long and narrow, apical flange barely wider than sperm pump (Figure 1.3). No gland-like tubular sac present at junction of basiphallus and distiphallus. Distiphallus convoluted; tip bearing triradiate appendage longer than distiphallic vesica with lateral extensions forming sharply-pointed flaps and dorsal extension finger-like with rugose tip (Figure 1.3 B).

Female: Ovipositor sheath black with two pairs of subapical dorsal setae. Three globular spermathecae present, two paired; paired spermathecae different in size, larger paired spermathecae equal in size to third, individual spermatheca; all spermathecae with long scale-like papillae on surface (Figure 1.3 C).

Material examined

Holotype: 1♂ pinned, USA: N. DAKOTA: Morton CO. 3 mi. E. Ft. Abraham Lincoln St. Pk., st. rte. 6; 10-IX-1995, Guy L. Bush collector // REARED FROM FRUIT: *Shepherdia argentea* emer: 13-16-VI-1996 killed: 20-VI-1996 // *Rhagoletis* near *tabellaria* det. J. Jenkins 1996. Deposited in Albert J. Cook Arthropod Research Collection (MSUC) cat.# 177673.

Paratypes: Same data as holotype (1♀, pinned, MSUC cat.# 177674); Reared from *Shepherdia argentea* USA: Bismarck, ND, Burleigh Co. USDA Plant Materials Lab N46°46.061' W100°45.982' 13 Aug., 2014 (Coll: D. Hulbert) (2♂ pinned, MSUC cat.# 177675, 177676; 3♂/5♀ pinned, DEBU); same data as paratypes from Bismarck except Morton Co. Ft. Lincoln State Park N46.7627 W100.8441' 14 Aug., 2016 (2♂/1♀, pinned, MSUC cat.# 177677-177679); same data as paratypes from Bismarck, ND except Morton Co. Ft. Lincoln State Park N46.7627 W100.8441' 14 Aug., 2016 (3♂/2♀, pinned, MSUC cat.# 177680-177684); same data as paratypes from Bismarck, ND except Burleigh Co. Kimball Bottoms Recreation Area N46°40.174' W100°43.976' 14 Aug., 2016 (2♂/4♀, pinned, MSUC cat.# 177685-177687, 177716-177718); Host: Buffaloberry seed BB-8 Emerged 23-VI-82 PFRA Tree Nursery Indian Head, Sask. H. A. Worden *Rhagoletis* nr. *tabellaria* det. J. Jenkins 1996 (2♂ pinned, CNCI); same data as paratypes from Indian Head, Sask. except BB-5 Emerged 18-VI-82 (1♀, pinned,

CNCI), BB-7 Emerged 18-VI-82 (1♂, pinned, CNCI); same data as paratypes from Indian Head, Sask. except BB-7 Emerged 19-21-VI-82 (1♀, pinned, CNCI); same data as paratypes from Indian Head, Sask. except BB-8 Emerged 18-21-VI-82 (1♀, pinned, CNCI); same data as paratypes from Indian Head, Sask. except BB-8 Emerged 21-VI-82 (1♂, pinned, CNCI); same data as paratypes from Indian Head, Sask. except BB-8 Emerged 16-VI-82 (1♂, pinned, CNCI); same data as paratypes from Indian Head, Sask. except BB-7 Emerged 24-VI-82 *Rhagoletis* n. sp. 82-1071 Det. J. F. McAlpine 1982 (1♀, pinned, CNCI); same data as paratypes from Indian Head, Sask. except BB-8 Emerged 25-VI-82 *Rhagoletis* n. sp. 82-1071 Det. J. F. McAlpine 1982 (1♂, pinned, CNCI); Indian Head Sask. PFRA tree nursery G. B. Neill Reared ex. Buffaloberry BB6 Coll. May 1982 em. 8-15 VI. 1982 *Rhagoletis* nr. *tabellaria* det. J. Jenkins 1996 (2♀, pinned, CNCI); Buffaloberry fly BB-5 emerged 10-VI-82 collected cocoons: May/ 82 PFRA Tree Nursery Indian Head, Sask G. Bruce Neill *Rhagoletis* n. sp. nr. *juniperina* 82-603 Det. J. F. McAlpine (1♀, pinned, CNCI).

Distribution

Rhagoletis bushi is currently only known from collections in the Bismarck area of North Dakota, a single collection site near Big Horn, Wyoming, and Indian Head, Saskatchewan (Table 1.1). The range of the host plant, *S. argentea*, is mainly in the Dakotas, Montana, Alberta, Saskatchewan, and Manitoba with isolated locations throughout western states (Figure 1.1 A).

Etymology

Rhagoletis bushi is named after Guy L. Bush who, beginning with his 1966 monograph, laid the foundation for the development of *Rhagoletis* into an evolutionary biology model

system, particularly for the study of sympatric speciation. Bush was also a proponent of describing and studying insect biodiversity (Bush 1966, 1992, 1993). We propose "buffaloberry fly" as the common name for this species.

Biology

Host Plant: *Rhagoletis bushi* is the only species in the genus known to infest fruit from *Sheperdia argentea*. There are no records of *Rhagoletis* infestation of the fruit of other members of the genus *Shepherdia*.

Parasitoids: We reared nine adult parasitoids from *R. bushi*. Morphological taxonomic identification suggested the wasps were *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae) a cosmopolitan idiobiont ectoparasitoid attacking the late pupal stage of its host, and developing on the pupa inside the host puparium (Van Alphen and Thunnissen 1982). The species typically parasitizes drosophilid hosts but has been reared from several *Rhagoletis* species including the eastern cherry fruit fly, *R. cingulata* (Loew) and *R. fausta*, and the western cherry fruit fly, *R. indifferens* Curran (Wang and Messing 2004, Wharton and Yoder 2016). The species has been used to help control non-*Rhagoletis* tephritid pests (Purcell 1998, Ovruski et al. 2000). We suggest that because *P. vindemmiae* were reared from *R. bushi* sampled from fruit as larvae or puparia these wasps likely oviposited directly on fly larvae.

The two mtDNA sequences obtained from the wasps were 98.9% identical to each other. Cytochrome oxidase I DNA barcode sequences have not previously been reported or databased for this species. BLAST search against the NCBI nucleotide database, revealed the sequences to be 92.4% and 92.3% identical to an unidentified parasitoid in the family Eulophidae

(Hymenoptera), and ~92% identical to two existing Pteromalids barcoded from Ontario and Alberta, Canada respectively reared from unknown hosts.

Results

Nucleotide alignments

We generated a five-locus dataset comprising 4270 aligned sites for 22 individuals across six species of *Rhagoletis* (Table 1.1). There were few insertions or deletions (indels) overall, with no indels observed in the COI, CAD, or AATS alignments. There was a single three-nucleotide insertion (TTA) in each *R. bushi* individual, starting at position 525 of the first 28S alignment (part "A") (Table B4). There is a single nucleotide insertion shared by *R. pomonella* and *R. mendax* Curran and a single nucleotide insertion in the *R. zephyria* sequence (Table B4). Within the *period* gene, there is an in-frame deletion of nine nucleotides (Thr-Ala-Ala) for *pomonella* group individuals starting at position 443 of the alignment. In addition, starting at position 460 in the *period* alignment, there is an in-frame insertion of three nucleotides (Thr) in the *R. tabellaria* individuals. Descriptive statistics for each gene alignment and the concatenated alignment are reported in Table B5.

Phylogeny of the tabellaria *species group*

Using both the individual genes and the concatenated alignment, we inferred phylogenetic relationships using a Bayesian and maximum-likelihood (ML) framework for the *tabellaria* species group. Both the Bayesian and maximum likelihood phylogenies inferred from the concatenated alignment strongly supported the monophyly of *R. bushi* and the other species within the *tabellaria* group (ML bootstrap = 100%, Bayesian posterior probability [BPP] = 1;

Figure 1.4). However, neither the Bayesian or maximum likelihood analyses resolved species level relationships with high support; a monophyletic group containing *R. tabellaria*, *R. electromorpha*, and *R. persimilis* was recovered (ML bootstrap = 53, BPP = 0.83) (Figure 1.4). Likewise, the individual gene trees did not generally resolve species-level relationships within the *tabellaria* group with high support (Figure B1-Figure ***B5***). The gene tree for the AATS alignment did, however, resolve species relationships with high support (≥ 98 ML bootstrap, ≥ 0.98 BPP) (Figure B4). The results of the incongruence length difference (ILD) tests did not give evidence of incongruence between gene alignments (Table B6)**.**

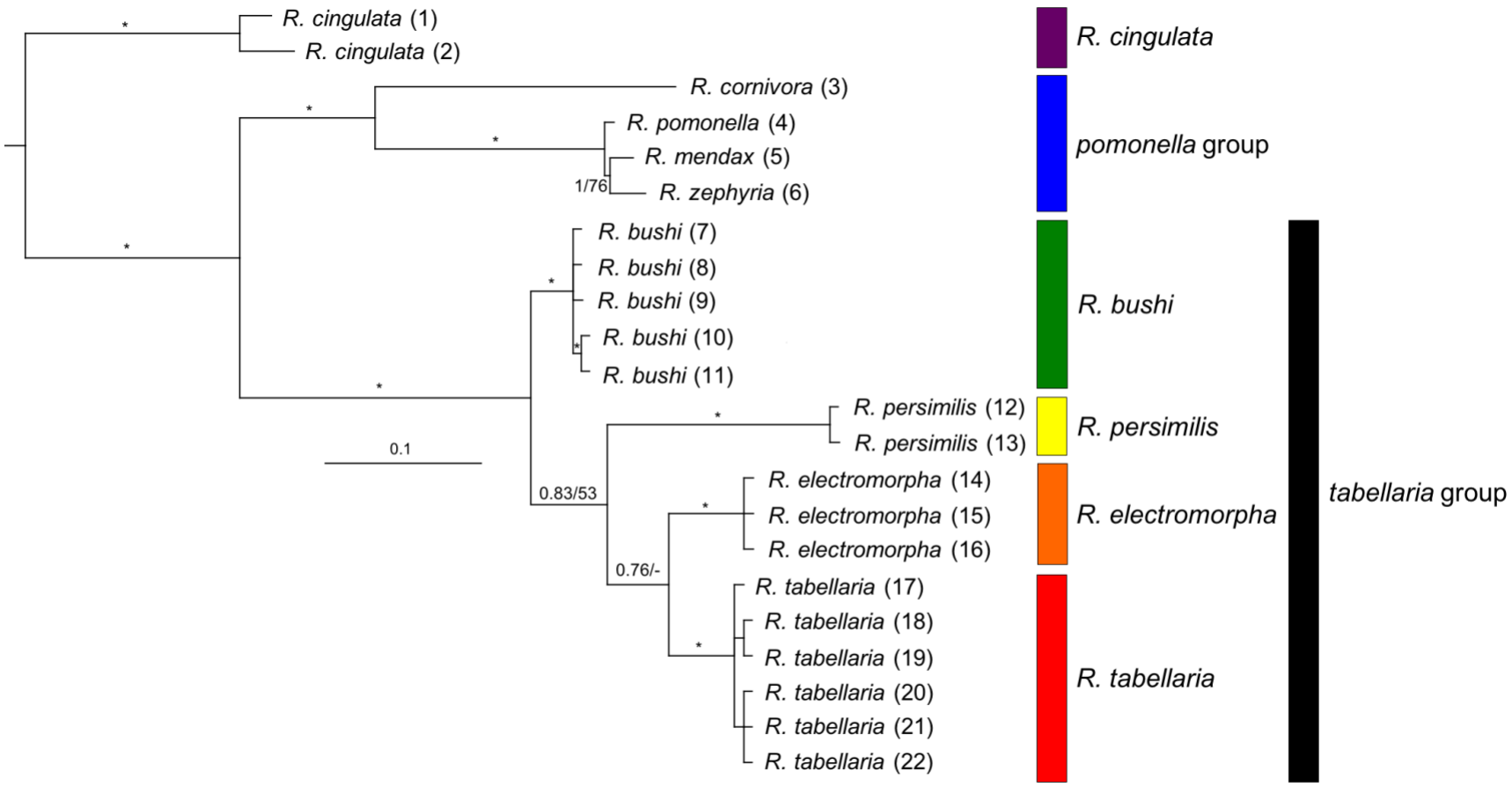

Figure 1.4. Bayesian phylogeny of the *tabellaria* species group, *pomonella* species group and outgroup *R. cingulata* inferred from a concatenated alignment of 4270 bp of DNA sequences from five genes, COI (684 bp), CAD (990 bp), period (614 bp), AATS (623 bp), and 28S (1359 bp). Numbers next to branches represent Bayesian posterior probabilities and maximum likelihood bootstrap support respectively. Asterisks (*) over branches indicate a Bayesian posterior probability of 1.0 and 100% maximum likelihood bootstrap support for the clade. The numbers in parentheses following the taxon designations in the *tabellaria* group correspond to specific collections in Table 1.1. Vertical colored blocks mark species group and species with the same colors as in Figure 1.1.

Morphology

Morphological characters in the *tabellaria* group, especially genitalia, support the results of the molecular phylogenetic analysis. The morphological characters of male and female genitalia support a close relationship between *R. tabellaria*:*R. electromorpha* and *R. bushi*:*R. persimilis* respectively. Both *R. tabellaria* and *R. electromorpha* have two spermathecae while *R. bushi and R. persimilis* have three (Table 1.2). Also, *R. tabellaria* and *R. electromorpha* both have basiphallic vesica, but this character is absent in *R. bushi* and *R. persimilis* (Table 1.2). The spermatheca of *R. tabellaria* are nearly the same size as each other, and this relative arrangement is the same in *R. electromorpha*. In contrast, the spermatheca are different sizes in *R. bushi* with one being larger than the other; this is also the case for *R. persimilis* (Table 1.2).

Revised key to the species group

Foote et al. (1993) published the most recent key to *Rhagoletis*. The key, while not phylogenetically informed, is based mostly on wing banding patterns and external morphology. *Rhagoletis bushi* keys out in the final couplet of Foote et al. (couplet #24) with *R. ribicola* Doane and *R. juniperina*, unlike the rest of the *tabellaria* species group which key to couplet #20. In order to distinguish *R. bushi* from *R. juniperina* and *R. ribicola* in Foote et al.'s key, we have rewritten the final couplet and added an additional couplet (figures referenced below are to Foote et al. [1993]):

24. Posterior surface of head with black horseshoe-shaped pattern (fig. 392); usually with one or two strong anepisternal bristles; cell cua not sharply pointed not (fig. 390, a); not infesting juniper cones (*Juniperus* spp.) 25

Posterior surface of head black only across upper 1/3; usually two or three strong anepisternal bristles; cell cua sharply pointed (fig. 391, a); wing pattern as in fig. 383; infesting juniper cones (*Juniperus* spp.) ..... ***juniperina* Marcovitch**

25. Apical band reaching costa at or beyond vein M_1, leaving extreme apex of cell r_{4+5} almost entirely hyaline, pattern as in fig. 382; usually with single strong anepisternal bristle (fig. 389, a); infesting currants and gooseberries (*Ribes* spp.) .... ***ribicola* Doane**

Infesting buffaloberry (*Shepherdia argentea*) (ancillary character); apical band reaching costa within cell r_{4+5}, anterior to vein M_1, wing pattern as in (Figure 1.2 D in present chapter); usually one or two strong anepisternal bristles..... ..... ***bushi* Hulbert & Smith**

Discussion

Rhagoletis bushi: *a new species*

The description of *R. bushi* represents a new North American *Rhagoletis* species. We present three lines of evidence to support the new species. First, *R. bushi* has unique and diagnostic morphological characters, second *R. bushi* infests the fruits of a unique host plant for *Rhagoletis*, and third *R. bushi* is genetically distinct from other species in the genus.

Morphological characters can be used to diagnose *R. bushi*. A combination of wing patterning, head patterning, wing cell shape, and pleural setae characters not described by

Jenkins (1996) are useful for readily identifying *R. bushi* without resorting to genitalic characters (but see Diagnosis). Although, *R. bushi* may be mistaken for *R. ribicola* (Doane), *R. berberis* (Curran) or *R. juniperia* in the absence of genitalic characters or host plant data.

The only known *Rhagoletis* species to infest *Shepherdia argentea* (buffaloberry) is *R. bushi*. The use of a particular host plant can be a proxy for species identification in *Rhagoletis*, although there are important but rare exceptions where host specific flies have been reared from "non-natal" hosts that likely do not represent established populations (Bush 1966, Yee and Goughnour 2008, Hood et al. 2012a, Yee et al. 2015). There are two other species in the genus *Shepherdia*: *S. canadensis* (L.) Nutt. (Canadian buffaloberry) and *S. rotundifolia* Parry (roundleaf buffaloberry), but it is unknown whether *R. bushi* or other *Rhagoletis* infest these species. *Rhagoletis bushi* is unlikely to infest *S. rotundifolia* as its range (Arizona and Utah) does not overlap with that of *S. argentea* and grows in a warmer, drier climate. The range of *S. canadensis* does overlap with *S. argentea* and the two species fruit at similar times (mid to late summer) (Soper and Heimburger 1982), thus *S. canadensis* may represent a viable host. However, Bush attempted to rear *Rhagoletis* from *S. canadensis* and did not find them infested (unpublished data). There are few published records of attempts to collect *Rhagoletis* from *Shepherdia* spp., and those that exist have either found *S. argentea* infested with *R. bushi* (Smith and Bush 1997) or with nothing (Glasgow 1933). There are few records of insects infesting the fruit of *S. argentea*, but buffaloberry is also a reported host plant of *Drosophila suzukii* (Matsumura) and, possibly, other *Drosophila* (Agbaba 2017).

Rhagoletis bushi is genetically distinct from other *Rhagoletis*. All of the gene sequences we used were able to discriminate *R. bushi* from other members of the genus. In phylogenetic analysis we found all loci to be reciprocally monophyletic with respect to *R. bushi* individuals.

Perhaps most useful for future genetic identification, the barcode region of COI is able to easily identify *R. bushi*. Similarly, the insertion in the 28S alignment for *R. bushi* may also serve as a useful diagnostic genetic character. Otherwise, we found a total of 17 autapomorphic positions for *R. bushi* across all gene alignments (three in COI, four in CAD, five in period, one in AATS, and four in 28S) (Table B4). The average sequence divergence (%) across all genes between *R. bushi* and *R. electromorpha*, *R. persimilis*, *R. tabellaria*, and *R. pomonella* was 1.46, 1.48, 1.43 and 2.89 respectively.

Phylogenetics and evolution of the tabellaria *species group*

The *tabellaria* species group is itself monophyletic and appears to be sister to the *pomonella* species group. The existence of *Rhagoletis* species groups has been reported by Bush (1966) and subsequent investigations have supported their existence (McPheron and Han 1997, Smith and Bush 1997, Smith et al. 2005). Hamerlinck et al. (2016) first reported evidence of a sister relationship between the *tabellaria* and *pomonella* groups based on DNA sequences of alleles at three loci (28S, CAD and COI), and this relationship has been further supported by the additional sequences presented here. Phylogenetic relationships within the genus are relatively unresolved beyond the *tabellaria* and *pomonella* groups. Within the North American species groups, there appears to be a close relationship between the *cingulata*, *suavis*, and *ribicola* groups (Hamerlinck et al. 2016).

We hypothesize a sister relationship between *R. tabellaria* and *R. electromorpha*, and present evidence to support this based on nucleotide sequences and morphological characteristics. Based on the Bayesian phylogenetic analysis performed on concatenated gene sequences, we inferred a sister relationship between *R. electromorpha* and *R. tabellaria*,

however, with only moderate confidence (BPP = 0.76; Figure 1.4). The maximum likelihood analysis did not resolve *tabellaria* group species level relationships. Nucleotide sequences are relatively phylogenetically inconclusive in the absence of other data. Future research plans for the *tabellaria* group include the use of next-generation sequencing techniques to generate draft genomes to resolve phylogenetic relationships and address other systematic questions.

Morphological data further support a sister relationship for *R. tabellaria* and *R. electromorpha*. Of the characters analyzed by Jenkins (1996) that are relevant to the *tabellaria* group, all the (male) genitalic characters support a *R. tabellaria – R. electromorpha* grouping. Specifically, both species have an aedeagus with a gland-like tubular sac (basiphallic vesica) which appears to be a derived character, shared by no other known *Rhagoletis* species. Another derived character shared by *R. tabellaria* and *R. electromorpha* is the presence of only two spermathecae (*R. persimilis* and *R. bushi* both have three) while possessing three spermathecal ducts. The spermathecal arrangement in *R. tabellaria* and *R. electromorpha* is consistent with the hypothesis that the common ancestor of the two species lost a spermatheca, but not the associated duct. It is worth noting that *R. ebbettsi*, which we did not include in our analysis because of lack of material, has the same pattern of spermathecae and spermathecal ducts as *R. tabellaria* and *R. electromorpha* (Bush 1966, Berlocher 1984). Collection and analysis of new *R. ebbettsi* individuals is necessary to have confidence in the species' phylogenetic placement. Looking deeper, one of the defining characteristics of the *pomonella* group is the presence of three spermathecae (organized in a pair of unevenly sized spermathecae and a separated single spermatheca). The *pomonella* group arrangement of spermathecae is the same as in *R. bushi* and *R. persimilis* giving further evidence in support of the hypothesis that the loss of a single spermatheca in *R. tabellaria* and *R. electromorpha* is a derived character of sister species.

There is one (non-genitalic) morphological character relevant to the *tabellaria* group that does not support a *R. tabellaria*: *R. electromorpha* sister relationship: *R. electromorpha* has lateral scapular seta the same color as principle thoracic setae while they are different in *R. tabellaria* (Table 1.2) (Jenkins 1996). However, we hypothesize this character is homoplasious. Characters associated with male insect genitalia are under sexual selection (Eberhard 1985, 2001, Huber and Eberhard 1997, Arnqvist 1998, Córdoba-Aguilar 2005, House and Simmons 2005, Arnqvist and Danielsson 2017) and evolve quickly relative to non-genitalic characters (Arnqvist 1997, Hosken and Stockley 2004, Méndez and Córdoba-Aguilar 2004) meaning these characters may have a better phylogenetic signal than non-genitalic characters for recently diverged taxa (Song and Bucheli 2010)such as those in the *tabellaria* group.

While the loci we sequenced allowed us to easily diagnose members of the *tabellaria* group they were insufficient to resolve species-level relationships within the group. We found a panel of molecular autapomorphies for each species in the *tabellaria* group that may be useful for diagnostic applications (Table B4). Using DNA sequences for diagnosis is a useful alternative for members of the *tabellaria* group which can be potentially difficult to distinguish from each other on the basis of morphological data alone. However, more extensive sampling is needed to assess intraspecific variation and hidden diversity. Of the taxa we sampled, only *R. tabellaria* was sampled across its geographic range. The ranges of the other *tabellaria* species group members remain relatively unknown, although the ranges of their respective host plants are well characterized. For example, *R. electromorpha* is known to infest three species of dogwoods: *C. drummondii*, *C. racemose* and *C. foemina* (*stricta*) in the Eastern United States (Berlocher 1984, Smith and Bush 1997), and diversity between populations infesting different species has not been characterized.

Hybridization between species in the *pomonella* group is known to occur and may also happen in the *tabellaria* group. The true phylogenetic relationship between species in the *tabellaria* group may be obscured in the presence of hybridization. In the *pomonella* group, *R. pomonella*, *R. mendax* and *R. zephyria* are all known to hybridize with each other (Bush 1966, Feder et al. 1999, Schwarz et al. 2005). Hybridization may have detrimental effects on subsequent generations, but it may also allow beneficial alleles to enter a population as evidenced by the introgression of alleles from *R. zephyria* to *R. pomonella* in western United States populations possibly conferring increased desiccation resistance on the latter (Arcella et al. 2015). Every species in the *tabellaria* group has naturally occurring zones of sympatry with at least on other member (Figure 1.1), and *Cornus* is the host plant genus for both *R. tabellaria* and *R. electromorpha* potentially creating favorable conditions for hybridization.

Dogwoods (*Cornus*) may represent the ancestral host plant genus of the *tabellaria* species group and the *pomonella* group. There are two species in the *tabellaria* group (*R. tabellaria* and *R. electromorpha*) and one in the *pomonella* group (*R. cornivora*) that infest dogwoods. The host dogwoods of the *tabellaria* and *pomonella* species are all in the subgenus *Kraniopsis*, and the hosts of *R. tabellaria* (*C. stolonifera*) and *R. cornivora* (*C. amomum*) are likely more closely related to each other than to the hosts of *R. electromorpha* (*C. foemina*, *C. drummondii* and *C. racemosa*) (Xiang et al. 1996, 2006). The flowering dogwood fly is an undescribed *pomonella* group species which attacks *Cornus florida* L., but we hypothesize that this represents an independent shift to a dogwood host because of the distant phylogenetic relationship between *C. florida* and the other dogwoods infested by *Rhagoletis* (Xiang et al. 1996, 2006). Dogwoods have a broad distribution throughout the northern hemisphere which overlaps with all members

of the *tabellaria* and *pomonella* species groups and would likely have been accessible to ancestral *Rhagoletis* populations.

Conclusion

We describe a new species of *Rhagoletis*, a classic non-model system for studying speciation and evolution. We also provide evidence for a hypothesis of phylogenetic relationships between *R. bushi* and its closest relatives. Members of *Rhagoletis* are closely associated with their host plants and often live in close physical proximity to their sister species; *R. bushi* is no exception. These qualities are very common in insects and are why *Rhagoletis* continues to be a rich area of research (Hutchinson 1959, Bush 1993). Well-studied systems like *Rhagoletis* still have new species yet to be described. Some of these new species may be members of the *pomonella* species complex (Payne and Berlocher 1995, Berlocher 1999) and, like the buffaloberry fly, have been informally recognized and studied for years, while others have yet to be discovered.

CHAPTER 2: MOLECULAR PHYLOGENY AND EVOLUTION OF *RHAGOLETIS*: RESOLUTION AND RELATIONSHIPS OF SPECIES GROUPS

Abstract

Flies of *Rhagoletis* (Diptera: Tephritidae) are economically important fruit pests (infesting specialty fruit crops including apples, blueberries and cherries), which also serve as models for studying modes of speciation and coevolutionary relationships with their hymenopteran parasitoids. The phylogenetic relationships among *Rhagoletis* species groups remain unresolved despite analyses based on morphology, allozymes, and mitochondrial DNA. Most Nearctic *Rhagoletis* belong to one of five species groups (*pomonella*, *tabellaria*, *cingulata*, *suavis*, and *ribicola* groups), with two unplaced species (*R. fausta* and *R. juniperina*). The main objectives of this study were 1) to circumscribe the monophyletic group containing these Nearctic species and 2) to resolve their phylogenetic relationships using a multilocus phylogeny based on mitochondrial (COI) and nuclear (28S, CAD, period, AATS) DNA sequences. Using Bayesian analysis of a combined dataset with 4399 aligned nucleotides, we inferred a well-supported monophyletic group containing the five Nearctic Rhagoletis species groups, plus *R. fausta*, *R. juniperina*, and two Palearctic species: *R. batava* and *R. flavigenualis*. Within this larger monophyletic assemblage, the five Nearctic species groups together are monophyletic as are four of the five individual species groups (not *ribicola*). Palearctic and Neotropical *Rhagoletis* were resolved into well-supported clades of taxa often sharing closely related host plants. A well-resolved phylogeny of *Rhagoletis* is a valuable tool for future work addressing questions pertaining to how history, geography and ecology have shaped the phylogenetic patterns we observe in the genus.

Introduction

Rhagoletis (Diptera: Tephritidae) is notable for its economic significance (Boller and Prokopy 1976, Foote 1981, Foote et al. 1993) and its use as an evolutionary model system (Bush 1966, Feder et al. 1988, Forbes et al. 2009, Hood et al. 2015). The genus is distributed through the Nearctic and Palearctic, and in the Neotropical regions. There are several species that are direct pests of high-value specialty crops such as *R. pomonella* (Walsh), which infests apples, *R. mendax* (Curran) which infest blueberries, and *R. cingulata* (Lowe) which infests cherries (Boller and Prokopy 1976). Pest species within *Rhagoletis* cause considerable damage in their respective host plants. For example, if not managed, apple orchards may lose 30-70% of their crop due to *R. pomonella* infestation, in part due to premature fruit drop caused by infestation (Howitt 1993). Additionally, the recent spread of *R. pomonella* in the Pacific Northwest threatens valuable apple production (Ali Niazee et al. 1981, McPheron 1990, Zhao et al. 2007, Green et al. 2013). As an injurious pest, the life history of *Rhagoletis* is closely intertwined with that of their host plant – a key reason why the genus has become such an important system to the field of evolutionary biology.

The host shift that *R. pomonella* underwent from hawthorn to apples has provided a classic case study of ecological speciation and a model for studying speciation in the presence of gene flow. Bush's (1966) seminal work modernizing the systematics of *Rhagoletis* also contained discussion of the phenomena of "host races" in *R. pomonella*, inspiring much research on the topic. Apples are an introduced crop to North America arriving in the 17th century and by the early 20th apple growers were reporting infestation of fruit by *R. pomonella* (Walsh 1864, 1867). A population of *R. pomonella* had shifted host from hawthorn to apples sometime during that time and formed what is referred to as a "host race" (Mayr 1963, Bush 1969). The two host

races (apple and hawthorn) of *R. pomonella* do have subtle differences in ovipositor length, phenologies (e.g. eclosion timing), and olfactory responses which more closely match their respective hosts (Bush 1969, Feder et al. 1993, Linn et al. 2003). Importantly, hawthorn and apple plants exist in sympatry making the possibility of gene flow between these populations likely and has been measured at about 6% per generation (Feder et al. 1994).

The study of genetic differences between the hawthorn and apple host races of *R. pomonella* shows how speciation happens in early stages in the presence of gene flow. There are differences in allele frequencies in the different host race populations and widespread polymorphisms across the genomes of individuals from the respective host races (Bush 1969, Feder et al. 1988, 2003, Drès and Mallet 2002, Michel et al. 2007, Xie et al. 2008, Schwarz et al. 2009, Hood et al. 2015, Doellman et al. 2018). Study of the *R. pomonella* species complex has led to the hypothesis that the host races are in the initial stage of speciation with gene flow and has provided an opportunity to study the fundamental nature of population divergence and the formation of new species. Studying these evolutionary patterns provides important clues for understanding how, when and why outbreaks of phytophagous insects occur.

Rhagoletis, especially in the context of the *pomonella* species complex, is also a model system for studying coevolution and co-diversification with its numerous hymenopteran parasitoids (Feder and Forbes 2010). Host shifts that occur in *Rhagoletis* have effects across trophic level in their wasp parasitoids (Forbes et al. 2009). While wasps spend part of their life cycle as obligate parasitoids they also have a free-living period where they must search for hosts, which likely has implications for their evolution. This is in contrast to other studied systems in which the parasite does not have a free-living period (Hafner and Nadler 1988, Hughes et al. 2007, Urban and Cryan 2012). Wasps (Hymenoptera) of *Diachasma* (late-instar larval

parasitoid) infest members of the *R. pomonella* species group including both host races of *R. pomonella*. There are genetic differences between the population of *Diachasma* infesting the haw-*R. pomonella* and the apple-*R. pomonella* based on evidence from allozymes, mitochondrial DNA sequences, microsatellites and eclosion timing (Forbes et al. 2009, 2010). These differences appear to make the populations of *Diachasma* better adapted to their respective host race in a similar manner to the apple and haw *Rhagoletis* host races to their host plant. The trophic cascading effect of host-shifting has also been observed in *Utetes* (egg and early-instar parasitoid) and *Diachasmimorpha* (late-instar larval parasitoid), and these examples are illustrative of how biodiversity begets biodiversity (Hood et al. 2015). At higher taxonomic levels, cophylogenetic analysis of *Rhagoletis* and three genera of wasp parasitoids (*Coptera, Utetes,* and *Diachasma*) showed that the current patterns of diversification in the groups were most explained by ancient cospeciation events rather than parasitoid shifts to unrelated host lineages (Hamerlinck et al. 2016).

The over 70 described species of *Rhagoletis* have been organized into species groups, informal groupings of taxa based on morphology and host plant associations (Bush 1966, Berlocher and Bush 1982, Foote et al. 1993, Smith and Bush 1997, Smith et al. 2005). The majority of North American taxa belong to one of species groups: *tabellaria, pomonella, cingulata, suavis* and *ribicola* first described by Bush (1966). Investigations by Berlocher and Bush (1982), Han and McPheron (1997), and Smith and Bush 1997 all support the existence of the original species groups proposed by Bush (1966) with some minor modifications. Specifically, *R. juniperina* Marcovitch was not found to be a member of the *tabellaria* group, and the *ribicola* group has either low support (Smith and Bush 1997) or is not recovered (Berlocher and Bush 1982). There are several other species that make up the North American

taxa: *R. fausta* (Osten Sacken) which has never been placed in a species group, *R. basiola* (Osten Saken) which is a member of the *alternata* group (all other members of which have a Palearctic distribution), *R. striatella* Wulp which is the eponymous member of its species group (all other members of which have a Neotropical distribution), and *R. acuticornis* (Steyskal) which infests *Lycium berlanderi* (Solanaceae) in the southwest USA and is not yet placed in a species group (Norrbom, A. L. 1989, Hernandez-Ortiz and Frías 1999, Smith and Bush 2000). There are also a large number of species in the Palearctic and Neotropical regions all within their own species groups.

There are at least 46 described species in nine different species groups outside of North America. In Central and South America are the *ferruginea*, *nova*, *psalida* and *striatella* groups, all the members of which infest plants in Solanaceae (Foote 1981, Hernandez-Ortiz and Frías 1999, Frías 2002). In the Palearctic region there are the *alternata*, *cerasi*, *flavicincta*, *meigenii*, and *zeryni* groups (Smith and Bush 2000, Korneyev et al. 2017). The most economically impactful of the Palearctic species is *R. cerasi* (L.), which is a pest of cherries in Europe (Daniel et al. 2012).

Previous investigations into the phylogeny of *Rhagoletis* have converged on some common conclusions while leaving some questions unanswered. Bush (1966) gave the first classification of *Rhagoletis* by the sorting of (mostly North American) taxa into species groups but did not include a hypothesis of phylogenetic relationship between these groups (Figure 2.1A). Electrophoresing allozymes (homologous proteins with different amino acid sequences) proved to be an effective way to distinguish between species (Berlocher 1980) and later this method was used to generate phylogenetically informative characters (Berlocher and Bush 1982). Phylogenetic analyses based on allozymes supported the existence of a monophyletic

clade of North American taxa, however relationships within this clade varied depending on the tree inference method used (Berlocher and Bush 1982) (Figure 2.1B). Similarly, a modern cladistic treatment described many useful morphological diagnostic characters for most *Rhagoletis* species (Jenkins 1996). Unfortunately, many morphological characters were not phylogenetically informative (Jenkins 1996, Smith and Bush 2000, Smith et al. 2005).

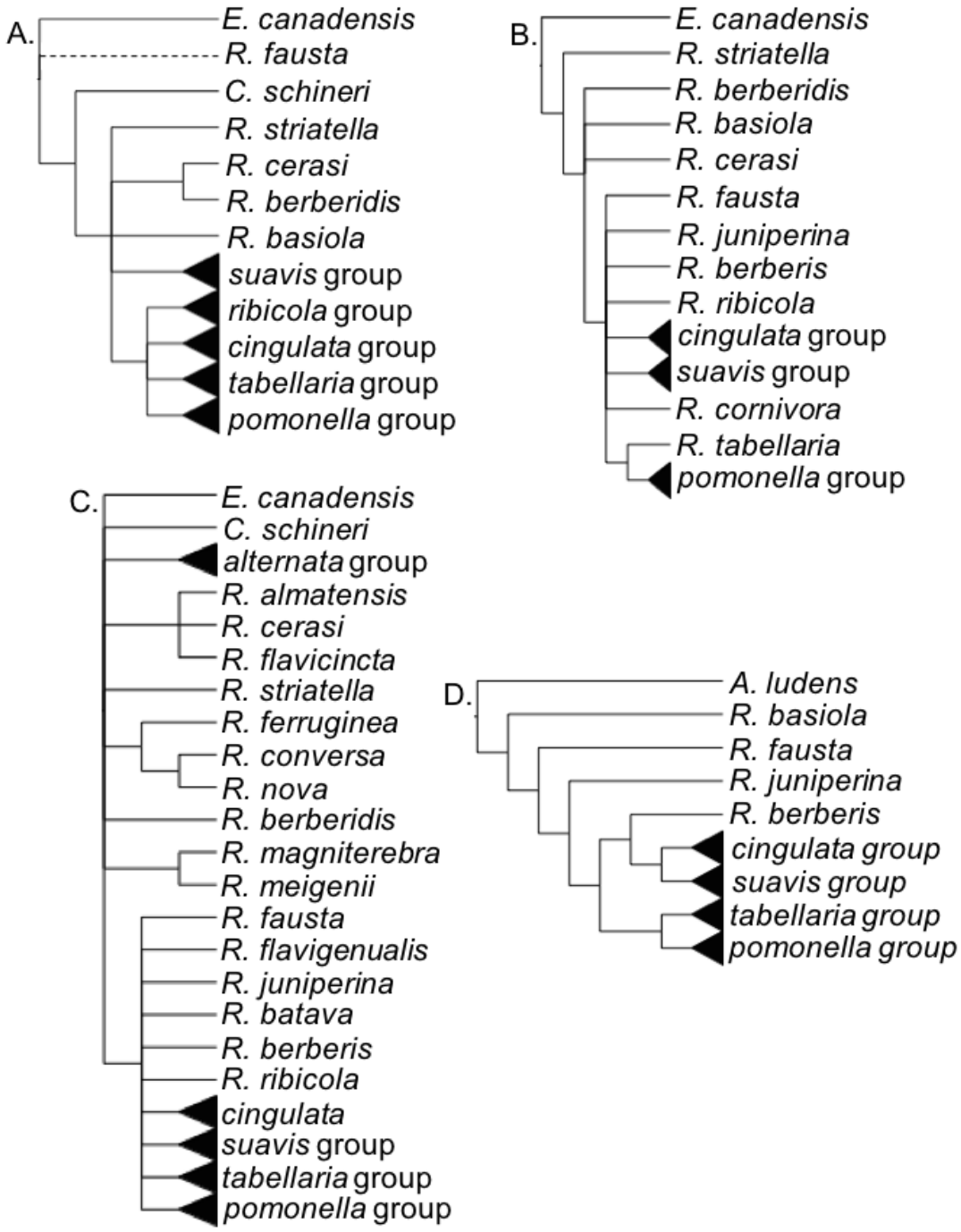

Figure 2.1. Summary of previous hypotheses of *Rhagoletis* phylogeny inferred from investigations by Bush (1966) based on morphology and cytology (A), by Berlocher and Bush (1982) based on allozymes (B), by Smith et al. (2005) based on mitochondrial (COII) DNA sequences (C), and by Hamerlinck et al. (2016) based on sequences from the genes COI, CAD and 28S (D). Only taxa that are also included on the present study are included in this figure.

As genetic tools for investigating systematics became more accessible, they were used to address phylogenetic questions in *Rhagoletis*. For example, mitochondrial DNA sequences of the genes 16S and COII were used (separately) to infer phylogenies of *Rhaogletis* which improved resolution: species groups were well supported, giving independent evidence of their existence, additionally these analyses supported the existence of a monophyletic clade containing the North American species groups (Han and McPheron 1997, Smith and Bush 1997). Unexpectedly, the clade containing the North American species groups also contained two Palearctic species, *R. flavigenualis* Hering and *R. batava* Hering when they were included in an analysis of COII sequences (Smith et al. 2005) (Figure 2.1C). The inclusion of these palearctic taxa in the aforementioned North American clade leads to some questions about global *Rhagoletis* phylogeography: are there other Palearctic taxa in this clade? What is the geographic origin of this clade? When did the members of this clade move to or from the Narctic and Palearctic?

Later, phylogenetic relationships of a subset of *Rhagoletis* species were inferred as part of an investigation into coevolution with hymenopteran parasitoids (see above) and was the first to use sequences from nuclear loci (CAD and 28S) in addition to mitochondrial COI (Hamerlinck et al. 2016). The subset of taxa included members of the five North American species groups (*pomonella*, *tabellaria*, *cingulata*, *suavis* and *ribicola*), *R. juniperina*, *R. fausta* and *R. basiola*. The phylogeny inferred from sequences of the three loci supported a monophyletic group containing representatives of the five North American species groups defined by Bush (1966) (Figure 2.1D). Also, the three-locus analysis generally supports two major sibling-clades within the five species groups: one including the *pomonella* and *tabellaria* groups and the other containing the *cingulata*, *suavis* and *ribicola* species groups (Figure 2.1D). The results of previous attempts to resolve *Rhagoletis* phylogeny are summarized in Figure 2.1.

Previous investigations into *Rhagoletis* systematics have converged on some common conclusions while still leaving important collective gaps in knowledge. There is strong support for the existence of each of the five North American species groups originally defined by Bush (1966) (with the exception of *R. juniperina*, see above) (Han and McPheron 1997, Smith and Bush 1997, Smith et al. 2005). These groups appear to be part of a larger monophyletic group containing at least *R. fausta, R. juniperina, R. batava* and *R. flavigenualis* (Figure 2.1C) (Smith et al. 2005). There is moderate support for a close relationship between the *tabellaria* and *pomonella* groups, and between the *cingulata, suavis* and *ribicola* groups, however with moderate support (Hamerlinck et al. 2016) (Figure 2.1D). It is unknown which, if any, other species are part of the clade containing the five North American species groups, *R. juniperina, R. fausta, R. batava,* and *R. flavigenualis*. The rose-infesting *alternata* group has been recovered by previous analyses (Smith and Bush 1997, Smith et al. 2005). Similarly, the Solanaceae-infesting species have shown an affinity for each-other in previous analyses, though with low confidence (Smith and Bush 1997, Smith et al. 2005). There have been few analyses that have investigated *Rhagoletis* phylogenetics of taxa outside North America (Ramírez et al. 2008). Finally, it has not been possible to analyze robustly the evolution of host plant use and coevolution with parasitoids across *Rhagoletis* without a resolved phylogeny of *Rhagoletis*.

Rhagoletis is consequential because of its importance to pest management and evolutionary biology. In spite of previous investigations into *Rhagoletis* phylogeny, there are still unanswered questions about relationships within the genus. In this paper, using a multilocus approach, we resolve the phylogenetic relationships of major species groups within *Rhagoletis*. The sequences used in our analysis have an established history of being successfully used in phylogenetic investigations of Diptera and especially Tephritidae (Wiegmann et al. 2000,

Moulton and Wiegmann 2004, Barr et al. 2005, Hamerlinck et al. 2016, Morita et al. 2016). Our analysis is the largest to date in terms of number of characters (aligned DNA states) of *Rhagoletis*. We analyze 86 individuals representing 36 species by sequencing five loci representing a mix of a mitochondrial gene (COI), nuclear ribosomal gene (28S), and nuclear protein coding genes (period, CAD, AATS) to infer phylogenetic relationships.

Methods

Taxon sampling and rearing

Collections were made by either sweep-netting or by collecting fruit infested with *Rhagoletis* larva by either the authors or our collaborators. The collected flies in our analysis comprise 86 individuals representing 37 species from four continents including three outgroup species on four continents (Table 2.1, Figure 2.2).There is at least one known host plant per species of *Rhagoletis* and these host relations are well established (Smith and Bush 2000). Sweeping for adult flies was done in the vicinity of, and on known *Rhagoletis* hosts. The collected flies were visually examined, and their species determined prior to further analysis. All fruit collections were made by collecting fruit from the known host plant species and directly beneath the plant. Infested fruit was brought back to the laboratory so that adults could be reared and identified. We used standard *Rhagoletis* rearing conditions similar to Frayer et al. (2015) and Hulbert et al. (2018).

Table 2.1. Specimen collection records.

Species group	Num.	Species/ taxon designation	Locality (County), State, Country	HP Family: *Genus species*	Date	Collector
Outgroup	1.	Anastrepha_ludens_GJ_006	El Jarro, Nuevo Leon, Mexico	Rutaceae: *Casimoroa greggii*	7-Mar-05	MA
	2.	Euphranta_canadensis_1	Ebbetts Pass, CA, USA	Grossulariaceae: *Ribes* sp.	19-Aug-97	GB /DB
	3.	Carpomya_schineri_X083199_3	Boom, Kyrgyzstan	Rosaceae: *Rosa* sp.	22-Jun-98	VK
	4.	Carpomya_schineri_X083199_5	Kashka-Suu, Bishkek, Kyrgyzstan	Rosaceae: *Rosa* sp.	7-Aug-98	VK
alterata	5.	R_alternata_Ger_A2	Kiel, Germany	Rosaceae: *Rosa rugosa*	unknown	TH
	6.	R_alternata_Kyrg_A1	Kashka-Suu, Bishkek, Kyrgyzstan	Rosaceae: *Rosa* sp.	8-Jul-98	VK
	7.	R_alternata_Kyrg_A2	Kashka-Suu, Bishkek, Kyrgyzstan	Rosaceae: *Rosa* sp.	8-Jul-98	VK
	8.	R_basiola_WA_A5	Clinton county, MI, USA	Rosaceae: *Rosa* sp.	Oct-93	JS
	9.	R_turanica_X050699_7	Kashka-Suu, Bishkek, Kyrgyzstan	Rosaceae: *Rosa* sp.	7-Aug-98	VK
	10.	R_turanica_X050699_8	Kashka-Suu, Bishkek, Kyrgyzstan	Rosaceae: *Rosa* sp.	7-Aug-98	VK
	11.	R_turanica_X051799_12	Kashka-Suu, Bishkek, Kyrgyzstan	Rosaceae: *Rosa* sp.	7-Aug-98	VK
flavicincta	12.	R_flavicincta_X040599_1	Kazakhstan	Caprifoliaceae: *Lonicera* sp.	23-Feb-98	VK
	13.	R_flavicincta_X040599_2	Kazakhstan	Caprifoliaceae: *Lonicera* sp.	23-Feb-98	VK
	14.	R_almatensis_X051799_13	Chatkal, Kyrgyzstan	Caprifoliaceae: *Lonicera stenatha*	2-Jul-98	VK
	15.	R_almatensis_X051799_14	Chatkal, Kyrgyzstan	Caprifoliaceae: *Lonicera stenatha*	2-Jul-98	VK

Table 2.1 (cont'd)

Species group	Num.	Species/ taxon designation	Locality (County), State, Country	HP Family: *Genus species*	Date	Collector
	16.	R_almatensis_X052899_7	Chatkal, Kyrgyzstan	Caprifoliaceae: *Lonicera stenatha*	2-Jul-98	VK
cerasi	17.	R_cerasi_Por_1	Castello Branco, Portugal	Rosaceae: *Prunus avium*	unknown	JL/ RP
	18.	R_cerasi_Por_2	Castello Branco, Portugal	Rosaceae: *Prunus avium*	unknown	JL/ RP
	19.	R_cerasi_012194_1	Hungary	Rosaceae: *Prunus avium*	unknown	SB/ GB
nova	20.	R_nova_1	Central Chile	Solanaceae: *Solanum* sp.	unknown	DF
	21.	R_nova_2	Central Chile	Solanaceae: *Solanum* sp.	unknown	DF
	22.	R_conversa_5	Las Cruces, Chile	Solanaceae: *Solanum nigrum*	11-Nov-96	GB/ DF
	23.	R_conversa_6	Las Cruces, Chile	Solanaceae: *Solanum nigrum*	11-Nov-96	GB/ DF
ferruginea	24.	R_ferruginea_1	Brasil	Solanaceae: *Solanum* sp.	unknown	DF
	25.	R_ferruginea_2	Brasil	Solanaceae: *Solanum* sp.	unknown	DF
striatella	26.	R_striatella_A5	Fish Creek (Fish Creek), WI, USA	Solanaceae: *Physalis heterophylla*	23-Aug-90	GB/ DB
	27.	R_striatella_A2	Fish Creek (Fish Creek), WI, USA	Solanaceae: *Physalis heterophylla*	23-Aug-90	GB/ DB
	28.	R_striatella_A4	Fish Creek (Fish Creek), WI, USA	Solanaceae: *Physalis heterophylla*	23-Aug-90	GB/ DB
*cerasi**	29.	R_berberidis_101194_4	Betten-Talstation, Switzerland	Berberidaceae: *Berberis vulgaris*	19-Jul-91	BM
	30.	R_berberidis_A1	Betten Talstation, Switzerland	Berberidaceae: *Berberis vulgaris*	19-Jul-91	BM

Table 2.1 (cont'd)

Species group	Num.	Species/ taxon designation	Locality (County), State, Country	HP Family: *Genus species*	Date	Collector
meigenii	31.	R_meigenii_101194_6	Rüdlingen, Switzerland	Berberidaceae: *Berberis* sp.	2-Jul-91	BM
	32.	R_meigenii_A4	Visperterminen, Switzerland	Berberidaceae: *Berberis* sp.	9-Jul-91	BM
	33.	R_chumsanica_X050699_12	Chatkal, Kyrgyzstan	Berberidaceae: *Berberis* sp.	2-Jul-98	VK
	34.	R_chumsanica_X050699_13	Surmatash, Kyrgyzstan	Berberidaceae: *Berberis* sp.	5-Jul-98	VK
unplaced	35.	R_magniterebra_X050499_11	Boom, Kyrgyzstan	Berberidaceae: *Berberis heteropoda*	22-Jun-98	VK
	36.	R_magniterebra_X051799_4	Naryn, Kyrgyzstan	Berberidaceae: *Berberis heteropoda*	13-Jul-98	VK
	37.	R_magniterebra_X051799_5	Naryn, Kyrgyzstan	Berberidaceae: *Berberis heteropoda*	13-Jul-98	VK
	38.	R_fausta_CA_A1	Cottage Springs (Calaveras), CA, USA	Rosaceae: *Prunus emarginata*	19-Aug-97	GB /DB
	39.	R_fausta_CA_A2	Cottage Springs (Calaveras), CA, USA	Rosaceae: *Prunus emarginata*	19-Aug-97	GB /DB
	40.	R_fausta_CA_B2	Cottage Springs (Calaveras), CA, USA	Rosaceae: *Prunus emarginata*	19-Aug-97	GB /DB
	41.	R_juniperina_CA_A1	Donner Pass county, CA, USA	Cupressaceae: *Juniperus grandis*	14-Aug	AF
	42.	R_juniperina_MI_A4	East Lansing (Ingham), MI, USA	Cupressaceae: *Juniperus virginiana*	9-Sep-10	MF
	43.	R_juniperina_NC_A1	Durham county, NC, USA	Cupressaceae: *Juniperus virginiana*	7-Aug-12	JS

Table 2.1 (cont'd)

Species group	Num.	Species/ taxon designation	Locality (County), State, Country	HP Family: *Genus species*	Date	Collector
zernyi	44.	R_flavigenualis_A1	Kashka-Suu, Bishkek, Kyrgyzstan	Cupressaceae: *Juniperus* sp.	7-Aug-98	VK
	45.	R_flavigenualis_X040599_7	Kashka-Suu, Bishkek, Kyrgyzstan	Cupressaceae: *Juniperus* sp.	7-Aug-98	VK
	46.	R_flavigenualis_2	Kashka-Suu, Bishkek, Kyrgyzstan	Cupressaceae: *Juniperus* sp.	7-Aug-98	VK
unplaced	47.	R_batava_X040599_5	Kara-Kol, Kyrgyzstan	Elaeagnaceae: *Hippophae rhamnoides*	13-Aug-98	VK
	48.	R_batava_X040599_6	Kara-Kol, Kyrgyzstan	Elaeagnaceae: *Hippophae rhamnoides*	13-Aug-98	VK
	49.	R_batava_X052899_5	Kara-Kol, Kyrgyzstan	Elaeagnaceae: *Hippophae rhamnoides*	13-Aug-98	VK
ribicola	50.	R_ribicola_OR_A2	Burns (Harney), OR, USA	Grossulariaceae: *Ribes* sp.	14-Aug-14	DH
	51.	R_ribicola_WA_A1	Cle Elum county, WA, USA	Grossulariaceae: *Ribes* sp.	Aug-13	WY
	52.	R_ribicola_WA_A2	Cle Elum county, WA, USA	Grossulariaceae: *Ribes* sp.	Aug-13	WY
	53.	R_berberis_WA_A2	Cle Elum county, WA, USA	Berberidaceae: *Mahonia* sp.	6-Sep-95	GB /DB
	54.	R_berberis_OR_A1	Oregon, USA	Berberidaceae: *Mahonia* sp.	Sep-14	WY
	55.	R_berberis_OR_A2	Oregon, USA	Berberidaceae: *Mahonia* sp.	Sep-14	WY
cingulata	56.	R_indifferens_WA_A1	Woodland (Cowlitz), WA, USA	Rosaceae: *Prunus cerasus*	Jun-11	WY

Table 2.1 (cont'd)

Species group	Num.	Species/ taxon designation	Locality (County), State, Country	HP Family: *Genus species*	Date	Collector
	57.	R_indifferens_WA_B1	Woodland (Cowlitz), WA, USA	Rosaceae: *Prunus avium*	Jun-11	WY
	58.	R_cingulata_MI_E1	Rose Lake (Clinton), MI, USA	Rosaceae: *Prunus serotina*	13-Jul-91	YM
	59.	R_cingulata_MI_E2	Rose Lake (Clinton), MI, USA	Rosaceae: *Prunus serotina*	13-Jul-91	YM
	60.	R_completa_OR_A1	Grand Junction county, CO, USA	Juglandaceae: *Juglans regina*	Sep-88	JJ
suavis	61.	R_completa_OR_A4	Grand Junction county, CO, USA	Juglandaceae: *Juglans regina*	Sep-88	JJ
	62.	R_suavis_IA_B3	Iowa City (Johnson), IA, USA	Juglandaceae: *Juglans nigra*	11-Sep	AF
	63.	R_suavis_MI_A4	East Lansing (Ingham), MI, USA	Juglandaceae: *Juglans nigra*	11-Sep	JS
tabellaria	64.	Buffaloberry_fly_101716_1	Mandan (Morton), ND, USA	Eleagnaceae: *Shepherdia argentea*	14-Aug-16	DH
	65.	Buffaloberry_fly_101716_2	Mandan (Morton), ND, USA	Eleagnaceae: *Shepherdia argentea*	14-Aug-16	DH
	66.	Buffaloberry_fly_101716_3	Bismarck (Burleigh), ND, USA	Eleagnaceae: *Shepherdia argentea*	13-Aug-14	DH
	67.	R_tabellaria_IA_A4	Iowa City (Johnson), IA, USA	Cornaceae: *Cornus stolonifera*	11-Jul-11	AN
	68.	R_tabellaria_100995_5	Trout Lake (Klickitat), WA, USA	Rosaceae: *Cornus stolonifera*	30-Aug-95	GB/ DB
	69.	R_tabellaria_v_051316_20	Stawberry Mt (Lewis), WA, USA	Rosaceae: *Vaccinium ovalifolium*	31-Aug-95	GB/ DB
	70.	R_persimilis_100995_3	Yellow Bay (Lake), MT, USA	Lilacaceae: *Prosartes hookeri*	Aug-95	GB

Table 2.1 (cont'd)

Species group	Num.	Species/ taxon designation	Locality (County), State, Country	HP Family: *Genus species*	Date	Collector
	71.	R_persimilis_102416_2	Swan Lake (Lake), MT, USA	Lilacaceae: *Prosartes hookeri*	10-Aug-16	DH
	72.	R_electromorpha_MI_A2	Hawk Meadow (Ingham), USA	Cornaceae: *Cornus foemina*	25-Aug-14	MD/ JS
	73.	R_electromorpha_MI_C1	Okemos (Ingham), USA	Cornaceae: *Cornus foemina*	13-Aug-12	DH
	74.	R_electromorpha_MI_C2	Okemos (Ingham), USA	Cornaceae: *Cornus foemina*	24-Sep-08	PS/ JS
pomonella	75.	R_cornivora_14_Shollow_051816_1	Sleepy Hollow prk (Clinton), MI, USA	Cornaceae: *Cornus obliqua*	Aug-14	JS
	76.	R_cornivora_14_Shollow_051816_2	Sleepy Hollow prk (Clinton), MI, USA	Cornaceae: *Cornus obliqua*	Aug-14	JS
	77.	R_pomonella_mex_3	Coajomulco, Morelos, Mexico	Rosaceae: *Crategus mexicana*	Oct-95	JG
	78.	R_pomonella_mex_7	Coajomulco, Morelos, Mexico	Rosaceae: *Crategus mexicana*	Oct-95	JG
	79.	R_pomonella_MI_A3	East Lansing (Ingham), MI, USA	Rosaceae: *Crataegus mollis*	23-Jun-09	JS
	80.	R_pomonella_MN_19	Staples (Todd), MN, USA	Rosaceae: *Crataegus mollis*	5-Sep-94	GB/ DB
	81.	R_mendax_090517_1	Fennville (Allegan), MI, USA	Rosaceae: *Vaccinium corymbosum*	Aug-14	RI
	82.	R_mendax_090517_2	Fennville (Allegan), MI, USA	Rosaceae: *Vaccinium corymbosum*	Aug-14	RI
	83.	R_mendax_090517_3	Fennville (Allegan), MI, USA	Rosaceae: *Vaccinium corymbosum*	Aug-14	RI

Table 2.1 (cont'd)

Species group	Num.	Species/ taxon designation	Locality (County), State, Country	HP Family: *Genus species*	Date	Collector
	84.	R_zephyria_ID_9	Elmira (Bonner), ID, USA	Caprifoliaceae: *Symphoricarpos* sp.	24-Aug-97	GB/ DB
	85.	R_zephyria_MN_A4	Bloomington (Hennepin), MN, USA	Caprifoliaceae: *Symphoricarpos* sp.	3-Sep-13	JS
	86.	R_zephyria_MN_7	Bloomington (Hennepin), MN, USA	Caprifoliaceae: *Symphoricarpos* sp.	3-Sep-13	JS

Species group: Follows designations by Smith and Bush (2000).

Collector – Initials: AN = A. E. Nelson, BM = Bernhard Merz, DB = Dorie Bush, DF = Daniel Frias, DH = Daniel Hulbert, GB = Guy Bush, JG = Jorge Graziano, JJ = John Jenkins, JL = J. P. Luz, JS = James Smith, MA = Martín Aluja, MD = Meredith Doellman, MF = Megan Frayer, PS = Parita Shah, RI = Rufus Isaacs, RP = R. Pavia, SB = Stewart Berlocher, TH = Thomas Hoffmeister, VK = Valery Korneyev, WY = Wee Yee, YM = Yue Ming

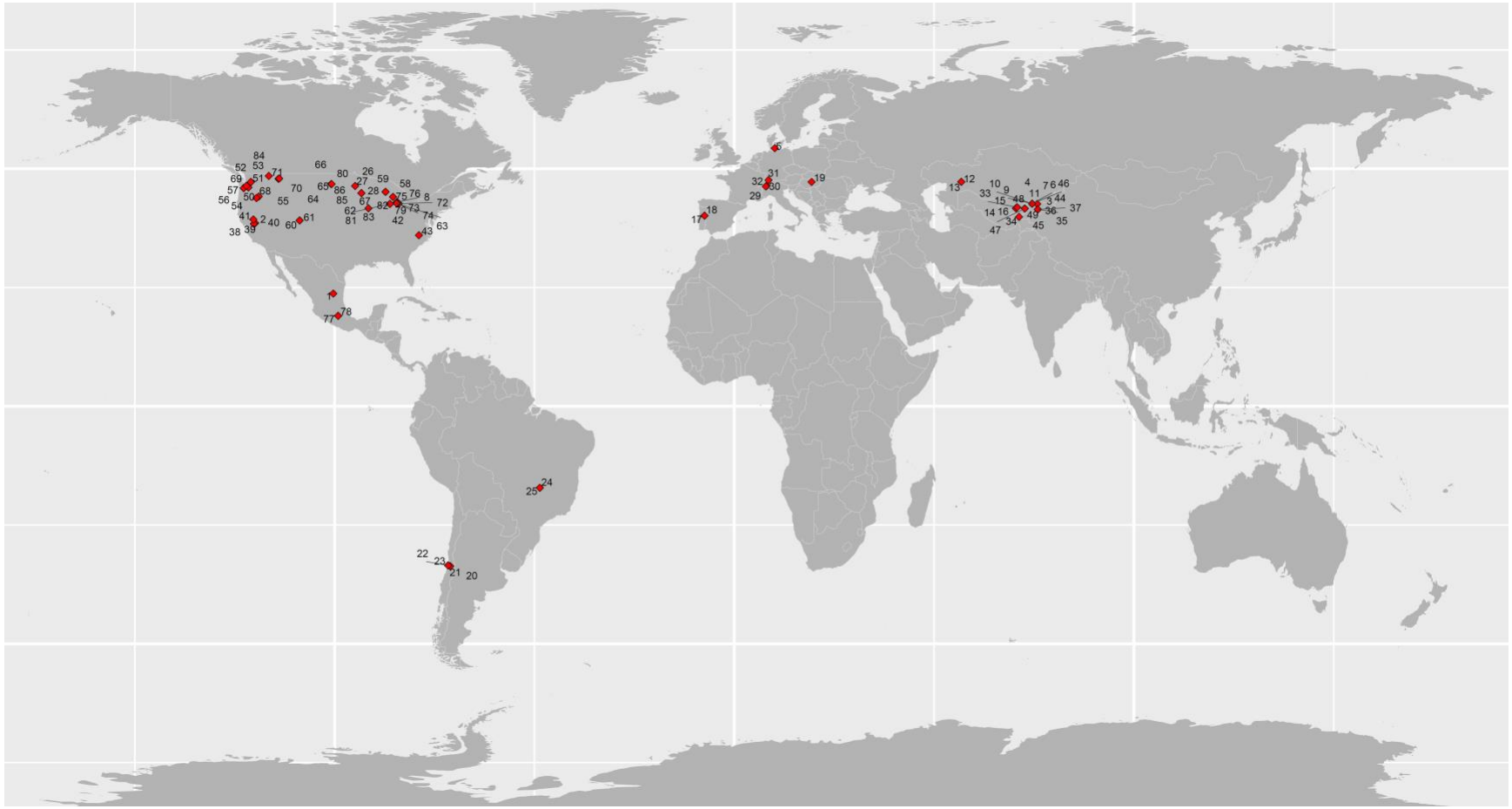

Figure 2.2. Map of collection locations. Numbers correspond to specimen numbers found in **Table 2.1**.

DNA isolation, PCR amplification and DNA sequence alignment

We isolated DNA from adult whole-fly homogenates from individuals in the taxon set (Table 2.1) using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). For phylogenetic analyses, we chose five loci that have been useful in systematic analyses of insects, especially tephritids: COI, CAD, period, AATS, and 28S (Han et al. 2002, Hebert et al. 2004, Moulton and Wiegmann 2004, Barr et al. 2005, Smith and Brown 2008, Hamerlinck et al. 2016). We PCR-amplified regions of the mitochondrial protein coding gene cytochrome oxidase I gene (COI) (685 bp), three nuclear protein coding genes: period (623 bp), CAD (1003 bp), and AATS (643 bp), and the gene encoding the large nuclear ribosomal subunit (28S) (1445 bp). We PCR-amplified each gene separately in 25 μL reactions using GotaqFlexi (Promega, Madison, WI, USA) with the following reagents (and final concentrations): reaction buffer (1X), $MgCl_2$ (8 mM), dNTP (0.5 mM each), forward and reverse primers (0.5 mM each), DNA polymerase (2.5 u), DNA template (~18 – 92 ng). Primers and thermocycler conditions used for each of the five genes amplified are listed in Table 2.2. Amplifications of COI, *period*, and AATS employed a single primer pair. For 28S we used two primer pairs to amplify two non-overlapping fragments separated by 58 bp (1445 bp total). For CAD, we used two primer pairs to amplify two overlapping fragments (1003 bp total).

Table 2.2. Primers and thermocycler conditions used to PCR amplify Rhagoletis DNA. All programs used a 30 second initial denaturation period at 95°C; followed by 35 cycles of 95°C for 30 seconds, the annealing temperature (below) for 30 seconds and 72°C for the extension time (below); followed by a final extension period of 10 minutes at 72°C (except when noted by *).

Locus	Primer pair	Primer sequence**	Reference	Annealing temp. (°C)	Extension time
COI	LepF1	5'-ATTCAACCAATCATAAAGATAT-3'	Hebert et al. 2004	46	2:00
	LepR1	5'-TAAACTTCTGGATGTCCAAAAA-3'	Hebert et al. 2004		
Period	Per2476F	5'-CAACGACGAAATGGAGAAATTC-3'	Barr and McPheron 2006	57	1:00
	Per3105R	5'-AABGACATGGGTTGGTACATC-3'	Barr et al. 2005		
AATS	AATSZ1F	5'-GGCACGGCTGATCCBAATAG-3'	Hulbert et al. 2018	62	1:00
	AATSZ1R	5'-TCWGRTGCACCTGTACCCTC-3'	Hulbert et al. 2018		
28S (A)	28SrDNA match F	5'-GTAAACAAGTACCGTGAGGG-3'	modified from Smith and Brown 2008	54	1:00
	28SrDNA match R	5'-TAGTTCACCATCTTTCGGGTCAC-3'	modified from Smith and Brown 2008		
28S (B)	S28C	5'-GTGCAAATCGATTGTCAGAA-3'	Han et al. 2002	65	1:30
	A28F	5'-TGGAACCGTATTCCCTTTCG-3'	Han et al. 2002		
CAD	54F	5'-GTNGTNTTYCARACNGGNATGGT-3'	Moulton and Wiegmann 2004	58-45 (touchdown)**	1:00
	414R	5'-AAACCACAATCGATCGCACAAAT-3'	Hamerlinck et al. 2016		
	405R	5'-GCNGTRTGYTCNGGRTGRAAYTG-3'	Moulton and Wiegmann 2004	58-45 (touchdown)**	1:00
	392F	5'-ATTTGTGCGATCGATTGTGGTTT-3'	Hamerlinck et al. 2016		

*The touchdown program, originally used in (Condon et al. 2008), employs an initial denaturation at 92°C for 2 minutes; followed by 12 cycles of 92°C for 10 seconds, 58-46°C (decreasing 1°C/ cycle) for 10 seconds, and 72°C for one minute; followed by 27 cycles of 92°C for 10 seconds, 45°C for 10 seconds, and 72°C for 1:30 minutes; followed by 72°C for 10 minutes.

**Nucleotides (including degenerate bases) follow the IUPAC naming conventions.

Verification of successful amplification for all PCR products was confirmed electrophoretically using agarose gels (1% w/v) prior to purification of PCR products using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's specifications. Sanger sequencing was performed at the Michigan State University Research Technology Support Facility via BigDye Terminator Sequencing on an Applied Biosystems 3730xl DNA Analyzer (Foster City, CA, USA) using the PCR primers as sequencing primers (Table 2.2).

We edited and aligned all sense and antisense strands manually by visual comparison of the automatic base calls to the original electropherogram traces using MEGA (version 7.0.14) (Tamura et al. 2011). All sequences were deposited in GenBank with accession numbers MH998667-MH999138 (Table 2.3). Alignments of DNA sequences were constructed and edited in MEGA. We used the default parameters in MUSCLE (Edgar 2004) as implemented in MEGA to align DNA sequences for all loci except 28S. For the 28S alignment, we used MAFFT with default parameters (Katoh et al. 2002). The alignment is available as a supplementary file.

Table 2.3. Accession numbers of the DNA sequences used in and generated by the present chapter.

Taxon designation	COI	CAD	period	AATS	28S part A	28S part B
Anastrepha_ludens_GJ_006	MH998894	MH998667	MH998825		MH998979	MH999061
Euphranta_canadensis_1	MH998895	MH998668		MH998752	MH998980	MH999062
Carpomya_schineri_X083199_3	MH998896	MH998669	MH998826	MH998753	MH998981	MH999063
Carpomya_schineri_X083199_5	MH998897	MH998670	MH998827	MH998754	MH998982	MH999064
R_alternata_Ger_A2	MH998902	MH998675	MH998832	MH998759	MH998987	MH999068
R_alternata_Kyrg_A1	MH998903	MH998676	MH998833	MH998760	MH998988	MH999069
R_alternata_Kyrg_A2	MH998904	MH998677	MH998834	MH998761	MH998989	MH999070
R_basiola_WA_A5	MH998901	MH998674	MH998831	MH998758	MH998986	MH999067
R_turanica_X050699_7		MH998671	MH998828	MH998755	MH998983	MH999065
R_turanica_X050699_8		MH998672	MH998829	MH998756	MH998984	MH999066
R_turanica_X051799_12		MH998673	MH998830	MH998757	MH998985	
R_flavicincta_X040599_1	MH998908	MH998681		MH998765	MH998993	MH999072
R_flavicincta_X040599_2	MH998909	MH998682		MH998766	MH998994	MH999073
R_almatensis_X051799_13	MH998905	MH998678		MH998762	MH998990	
R_almatensis_X051799_14	MH998906	MH998679		MH998763	MH998991	
R_almatensis_X052899_7	MH998907	MH998680		MH998764	MH998992	MH999071
R_cerasi_Por_1	MH998910	MH998683		MH998767	MH998995	MH999074
R_cerasi_Por_2	MH998911	MH998684			MH998996	MH999075
R_cerasi_012194_1		MH998685		MH998768	MH998997	MH999076
R_nova_1	MH998917	MH998691	MH998839	MH998771	MH999003	MH999082
R_nova_2	MH998918	MH998692	MH998840	MH998772	MH999004	MH999083
R_conversa_5	MH998919	MH998693	MH998841	MH998773	MH999005	MH999084
R_conversa_6	MH998920	MH998694	MH998842	MH998774	MH999006	MH999085
R_ferruginea_1	MH998915	MH998689	MH998838	MH998770	MH999001	MH999080
R_ferruginea_2	MH998916	MH998690			MH999002	MH999081
R_striatella_A5	MH998914	MH998686	MH998835		MH999000	MH999079
R_striatella_A2	MH998912	MH998687	MH998836		MH998998	MH999077

Table 2.3 (cont'd)

Taxon designation	COI	CAD	period	AATS	28S part A	28S part B
R_striatella_A4	MH998913	MH998688	MH998837	MH998769	MH998999	MH999078
R_berberidis_101194_4	MH998921	MH998695		MH998775	MH999007	MH999086
R_berberidis_A1	MH998922	MH998696	MH998843	MH998776	MH999008	MH999087
R_meigenii_101194_6	MH998923	MH998697	MH998844	MH998777		MH999088
R_meigenii_A4	MH998924	MH998698	MH998845	MH998778	MH999009	MH999089
R_chumsanica_X050699_12	MH998925	MH998699	MH998846	MH998779	MH999010	MH999090
R_chumsanica_X050699_13	MH998926	MH998700	MH998847	MH998780	MH999011	MH999091
R_magniterebra_X050499_11	MH998927	MH998701	MH998848	MH998781	MH999012	MH999092
R_magniterebra_X051799_4	MH998928	MH998702	MH998849	MH998782	MH999013	MH999093
R_magniterebra_X051799_5	MH998929	MH998703	MH998850	MH998783	MH999014	MH999094
R_fausta_CA_A1	MH998933	MH998707	MH998854	MH998787	MH999018	MH999098
R_fausta_CA_A2	MH998934	MH998708	MH998855		MH999019	MH999099
R_fausta_CA_B2	MH998935	MH998709	MH998856		MH999020	MH999100
R_juniperina_CA_A1	MH998936	MH998710			MH999021	MH999101
R_juniperina_MI_A4	MH998937	MH998711		MH998788	MH999022	MH999102
R_juniperina_NC_A1	MH998938	MH998712	MH998857		MH999023	MH999103
R_flavigenualis_A1	MH998939	MH998713		MH998789	MH999024	MH999104
R_flavigenualis_X040599_7	MH998940	MH998714	MH998858	MH998790	MH999025	MH999105
R_flavigenualis_2	MH998941	MH998715	MH998859	MH998791	MH999026	MH999106
R_batava_X040599_5	MH998930	MH998704	MH998851	MH998784	MH999015	MH999095
R_batava_X040599_6	MH998931	MH998705	MH998852	MH998785	MH999016	MH999096
R_batava_X052899_5	MH998932	MH998706	MH998853	MH998786	MH999017	MH999097
R_ribicola_OR_A2	MH998945	MH998719		MH998795	MH999030	MH999109
R_ribicola_WA_A1	MH998946	MH998720	MH998863		MH999031	MH999110
R_ribicola_WA_A2	MH998947	MH998721	MH998864		MH999032	MH999111
R_berberis_WA_A2	MH998944	MH998716	MH998862	MH998794	MH999029	
R_berberis_OR_A1	MH998942	MH998717	MH998860	MH998792	MH999027	MH999107

Table 2.3 (cont'd)

Taxon designation	COI	CAD	period	AATS	28S part A	28S part B
R_berberis_OR_A2	MH998943	MH998718	MH998861	MH998793	MH999028	MH999108
R_indifferens_WA_A1	MH998950	MH998724				MH999114
R_indifferens_WA_B1	MH998951	MH998725			MH999035	
R_cingulata_MI_E1	MH998948	MH998722	MH998865	MH998796	MH999033	MH999112
R_cingulata_MI_E2	MH998949	MH998723	MH998866	MH998797	MH999034	MH999113
R_completa_OR_A1	MH998952	MH998726	MH998867	MH998798	MH999036	
R_completa_OR_A4	MH998953	MH998727	MH998868	MH998799	MH999037	
R_suavis_IA_B3	MH998954	MH998728	MH998869	MH998800	MH999038	MH999115
R_suavis_MI_A4	MH998955	MH998729	MH998870	MH998801	MH999039	
Buffaloberry_fly_101716_1	MH998968	MH998742	MH998883	MH998814	MH999050	MH999128
Buffaloberry_fly_101716_2	MH998969	MH998743	MH998884	MH998815	MH999051	MH999129
Buffaloberry_fly_101716_3	MH998970	MH998744	MH998885	MH998816	MH999052	MH999130
R_tabellaria_IA_A4	MH998976	MH998750	MH998891	MH998822	MH999058	MH999136
R_tabellaria_100995_5	MH998977	MH998751	MH998892	MH998823	MH999059	MH999137
R_tabellaria_v_051316_20	MH998978		MH998893	MH998824	MH999060	MH999138
R_persimilis_100995_3	MH998974	MH998748	MH998889	MH998820	MH999056	MH999134
R_persimilis_102416_2	MH998975	MH998749	MH998890	MH998821	MH999057	MH999135
R_electromorpha_MI_A2	MH998971	MH998745	MH998886	MH998817	MH999053	MH999131
R_electromorpha_MI_C1	MH998972	MH998746	MH998887	MH998818	MH999054	MH999132
R_electromorpha_MI_C2	MH998973	MH998747	MH998888	MH998819	MH999055	MH999133
R_cornivora_14_Shollow_051816_1	MH998956	MH998730	MH998871	MH998802	MH999040	MH999116
R_cornivora_14_Shollow_051816_2	MH998957	MH998731	MH998872	MH998803		MH999117
R_pomonella_mex_3	MH998961	MH998735	MH998876	MH998807	MH999044	MH999121
R_pomonella_mex_7	MH998962	MH998736	MH998877	MH998808	MH999045	MH999122
R_pomonella_MI_A3	MH998963	MH998737	MH998878	MH998809	MH999046	MH999123
R_pomonella_MN_19	MH998964	MH998738	MH998879	MH998810	MH999047	MH999124

Table 2.3 (cont'd)

Taxon designation	COI	CAD	period	AATS	28S part A	28S part B
R_mendax_090517_1	MH998958	MH998732	MH998873	MH998804	MH999041	MH999118
R_mendax_090517_2	MH998959	MH998733	MH998874	MH998805	MH999042	MH999119
R_mendax_090517_3	MH998960	MH998734	MH998875	MH998806	MH999043	MH999120
R_zephyria_ID_9	MH998966	MH998739	MH998881	MH998812		MH999126
R_zephyria_MN_A4	MH998965	MH998740	MH998880	MH998811	MH999048	MH999125
R_zephyria_MN_7	MH998967	MH998741	MH998882	MH998813	MH999049	MH999127

Phylogenetic analyses

We calculated descriptive statistics for the alignments at each locus individually, and also for the concatenated five locus alignment. Using MEGA, we calculated the average uncorrected pairwise p-distance for each gene (including distances for each codon position of protein-coding genes), the concatenated alignment, and for all transition and transversion mutations. We also used MEGA to determine the nucleotide composition for each alignment. Using PAUP* (version 4.0a163) (Swofford 2003) we counted the number of variable sites in each alignment (including and excluding parsimony informative sites).

We used a maximum likelihood and Bayesian framework for our phylogenetic analyses of the five-locus alignment. When running PARTITIONFINDER, we predefined and used 13 biologically relevant partitions in the alignments following recommendations of the software user-guide (Lanfear et al. 2017): 28S (the two fragments were concatenated and considered a single partition for phylogenetic analysis), and separate partitions for each nucleotide position (1^{st}, 2^{nd}, 3^{rd} codon position) for COI, CAD, period and AATS. We used PARTITIONFINDER v2.1.1 (Lanfear et al. 2017) to determine combinability of partitions and nucleotide substitution models. We then ran PARTITIONFINDER implementing PhyML (Guindon et al. 2010) with the "greedy" algorithm (Lanfear et al. 2012) using the corrected Akaike Information Criterion (AICc) to assess model and partition quality. We conducted separate runs of PARTITIONFINDER, one restricted to MRBAYES (Ronquist et al. 2012) models and the other for RAxML (Stamatakis 2014) models. The resulting partitioning and model scheme for MRBAYES is shown in Table 2.4. Because RAxML only allows the specification of one model rate of heterogeneity for all partitions in a concatenated analysis, we ran PARTITIONFINDER three times while restricting each run to one model of rate heterogeneity (GTR, GTR+G, or

GTR+G+I) and compared the AICc of each run. The GTR+G model had the lowest AICc and was thus used for subsequent RAxML analyses. Partitioning schemes and substitution models for RAxML are shown in Table 2.4.

Table 2.4. The partitioning scheme results of the MrBayes and RAxML PARTITIONFINDER analyses. The predefined partitions within the same subset were combined in the phylogenetic analysis of the concatenated alignment.

Subset number	Model	Predefined Partition
1	GTR+I+G	AATS position 3
		period position 3
		CAD position 3
		COI position 1
2	GTR+I+G	AATS position 1
		period position 2
		28S
		AATS position 2
		period position 1
		CAD position 1
		CAD position 2
		COI position 2
3	GTR+I+G	COI position 3
		CAD non-coding

We inferred phylogenetic trees using RAxML (version 7.4.2) (Stamatakis 2014) as implemented by RAxMLGUI (version 1.31) (Silvestro and Michalak 2011) and MRBAYES version 3.2.5 (Ronquist et al. 2012) using the model schemes described above (Table 2.4). For the maximum likelihood analysis, we ran RAxML for 1000 pseudoreplicates using the partitioning scheme described above.

The Bayesian analysis used two independent runs each with four Metropolis-coupled chains with default heating parameters (one cold and three heated) in MRBAYES. The chains

were sampled once every thousand generations for 50 million generations and the first 25% of samples was discarded as burn-in. All analyses converged to an average standard deviation of split frequencies below 0.001 and all branch lengths and substitution model parameters had potential scale reduction factors less than 1.003 (Ronquist et al. 2012). We used FigTree (version 1.4.2) (Rambaut 2014) and Mesquite (version 3.5 build 888) (Maddison and Maddison 2011) to visualize the phylogenetic trees.

Results

Nucleotide alignments

We generated a five-locus dataset comprising 4399 aligned sites for 86 individuals representing 33 species of *Rhagoletis* and three outgroup species (Table 2.5). The complete alignment containing all five loci is found in the supplemental nexus file. The COI alignment had no insertions or deletions and few differences in amino acid sequence. The AATS alignment also had no insertions or deletions and few differences in amino acid sequence The CAD alignment had an inserted codon starting at position 352 in most taxa except for some *Rhagoletis* and the outgroups. Also, in the CAD alignment there was a stop codon or possibly an alternate reading frame starting at position 892 (Moulton and Wiegmann 2004). In the *period* alignment from position 445-471 there was a variable number of codons (coding for alanine and occasionally serine or threonine) ranging from zero to nine. The 28S alignment had several length variable regions in various taxa.

Table 2.5. Descriptive statistics for the nucleotide alignments used in this chapter.

Genetic marker	COI	CAD	period	AATS	28S	Concatenated
Total sites	685	1003	623	643	1445	4399
No. variable sites (PU)	27	79	33	42	55	236
No. PI sites	231	357	228	151	270	1237
% PI sites	33.72	35.59	36.60	23.48	18.69	28.12
% missing data	5.50	17.30	26.40	21.10	12.00	15.60
p-distance ± SE (%)						
1 pos.	4.47 ± (0.03)	4.58 ± (0.05)	4.18 ± (0.04)	2.32 ± (0.03)	NA	NA
2 pos.	0.40 ± (0.01)	3.23 ± (0.04)	2.77 ± (0.03)	0.77 ± (0.01)	NA	NA
3 pos.	22.77 ± (0.12)	18.54 ± (0.17)	14.87 ± (0.18)	11.98 ± (0.14)	NA	NA
total	9.20 ± (0.05)	8.77 ± (0.08)	7.29 ± (0.08)	2.09 ± (0.02)	2.25 ± (0.02)	6.28 ± (0.05)
Ts	5.71 ± (0.03)	5.27 ± (0.05)	4.57 ± (0.05)	3.25 ± (0.03)	0.94 ± (0.01)	3.68 ± (0.02)
Tv	3.49 ± (0.03)	3.50 ± (0.04)	2.73 ± (0.03)	1.75 ± (0.03)	1.31 ± (0.12)	2.60 ± (0.02)
R(s/v)	2.13 ± (0.04)	1.72 ± (0.02)	1.81 ± (0.02)	2.09 ± (0.02)	1.38 ± (0.03)	1.61 ± (0.01)
G+C%	32.9	47.5	53.0	42.0	34.2	40.4

PU = Parsimony uninformative
PI = Parsimony informative
Ts = Transition rate
Tv = Transversion rate
R(s/v) = rate of transitions to transversions

Descriptive statistics of each gene alignment and the concatenated alignment is reported in Table 2.5. The 28S gene had the lowest variation among the taxa we sequenced followed by AATS, *period*, CAD and COI respectively (Figure 2.3, Table 2.5). The gene *period* had the highest percentage of parsimony informative sites (36.6%), while CAD had the absolute largest number of parsimony informative sites (357).

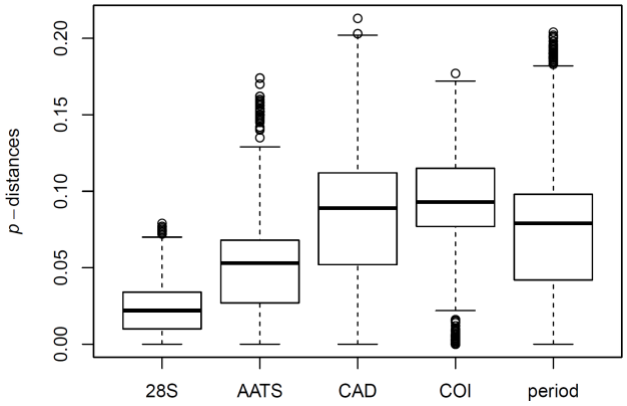

Figure 2.3. Genetic variation of gene fragments sequenced (uncorrected-*p*). Both adjacent (but not overlapping) fragments of 28S were combined for *p*-distance calculation. Among the fragments of genes sequenced, 28S varied the least variable while COI was the most variable.

Phylogeny of the genus

We inferred phylogenetic relationships among *Rhagoletis* species using the nucleotide alignments in a Bayesian and maximum likelihood framework and both methods gave the same topology (Figure 2.4). In order to facilitate the reporting and discussion of results, we have assigned numbers to certain clades of note in the inferred phylogeny (Figure 2.4). Some of the numbered clades represent species groups, while others represent higher level monophyletic groups of interest (themselves containing numbered species group clades). Some of the clades we define correspond to species groups previously identified by others (these include the *alternata* [I], *cingulata* [VIII], *suavis* [IX], *tabellaria* [X], and *pomonella* [XI] groups), while others are newly hypothesized relationships (reported and discussed below). We define clades I-XI which together contain all the *Rhagoletis* taxa included in our analysis.

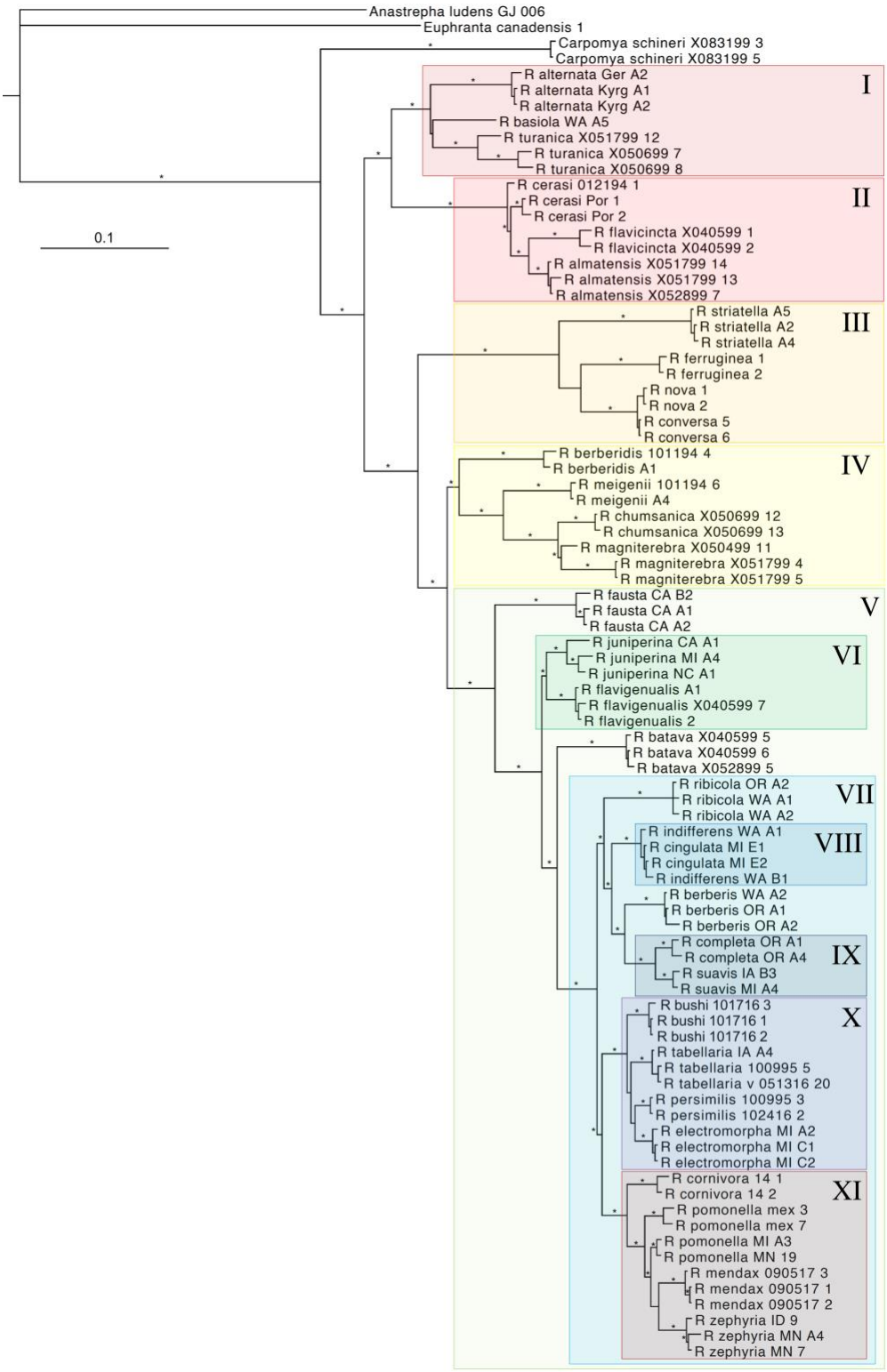

Figure 2.4. Phylogeny of the specimens included in our study inferred from DNA sequences from fragments of COI, CAD, period, AATS and 28S. The tree shown is the Bayesian consensus tree inferred using MrBayes with the models and partitioning scheme given by Partitionfinder. Asterisks (*) above branches indicate a Bayesian posterior probability of ≥0.99. Boxes and Roman numerals indicate groups of taxa discussed in the text and in **Figure 2.5**.

All members of *Rhagoletis* form a monophyletic group to the exclusion of the outgroups, *Anastrepha ludens* (Loew), *Euphranta canadensis* (Loew), and *Carpomya schineri* (Loew). The oldest division within *Rhagoletis* places clade I (members of the *alternata* group) and II (members of the *cerasi* group plus *R. flavicincta* (Loew)) together to the exclusion of all other *Rhagoletis* (Figure 2.4). Next, clade III (which includes members of *nova*, *striatella*, and *ferruginea* groups) is sister to clades IV-XI. Clade IV, which includes *R. berberidis* Jermy (previously thought to be a members of the *cerasi* group [Smith and Bush 2000]), *meigenii* group members, and *R. magniterebra* Rohdendorf (a previously unplaced species) is sister to clade V (mostly North American taxa and species groups, including clades VI-XI). Within clade V, *R. fausta* is sister to the remaining taxa, followed by clade VI (containing *R. juniperina* and *R. flavigenualis*) and *R. batava* respectively which are sibling to clade VII (containing clades VIII-XI, North American species groups).

Clade VII contains members of the five North American species groups first defined by Bush (1966). Within this clade, there are two main divisions, one containing members of the *cingulata* (clade VIII), *suavis* (IX) and *ribicola* (no clade) groups, and the other containing the *tabellaria* (clade X) and *pomonella* (clade XI) groups. Within the first division (clade) *R. ribicola* is sister to the remainder, and clade VIII is sister to a clade containing *R. berberis* Curran and clade IX. It is important to note that the *ribicola* group, as previously defined (Smith and Bush 2000), is not resolved as a monophyletic group in our analysis.

Host plant use

Several of the clades mentioned above contain taxa that all infest closely related host plants (Figure 2.5). Clade I contains the *alternata* group, all of which infest fruit of *Rosa* (rose

hips) in either the Nearctic (*R. basiola*) or Palearctic regions (*R. alternata* Fallén and *R. turanica* Rohdendorf). Species in clade II all infest *Lonicera* (honeysuckle) fruit in the Palearctic, although notable is *R. cerasi*, which also infests cherries and is therefore a considerable pest. Clade III contains species from three different species groups (*striatella*, *ferruinea*, and *nova*), but they all infest the fruit of Solanaceae in the Nearctic and Neotropical regions. Members of clade IV all infest Palearctic *Berberis* (barberry) fruit. Both species in clade VI infest cones of *Juniperus* (juniper) in either the Nearctic (*R. juniperina*) or Palearctic (*R. flavigenualis*). Clade IX contains species that have been previously sorted into the *suavis* species group (Bush, 1966) and all infest the drupes of *Juglans* (walnuts) in the Nearctic region. Clade VIII (members of the *cingulata* group) cannot be described as all infesting cherries, because other members of the cingulata group not included in our sampling (*R. osmanthi* Bush, *R. chionanthi* Bush, and *R. turpiniae* Hernández-Ortiz) infest non-*Prunus* hosts (*Osmanthus americana*, *Chionanthus virginicus*, and *Turpinia* spp. respectively).

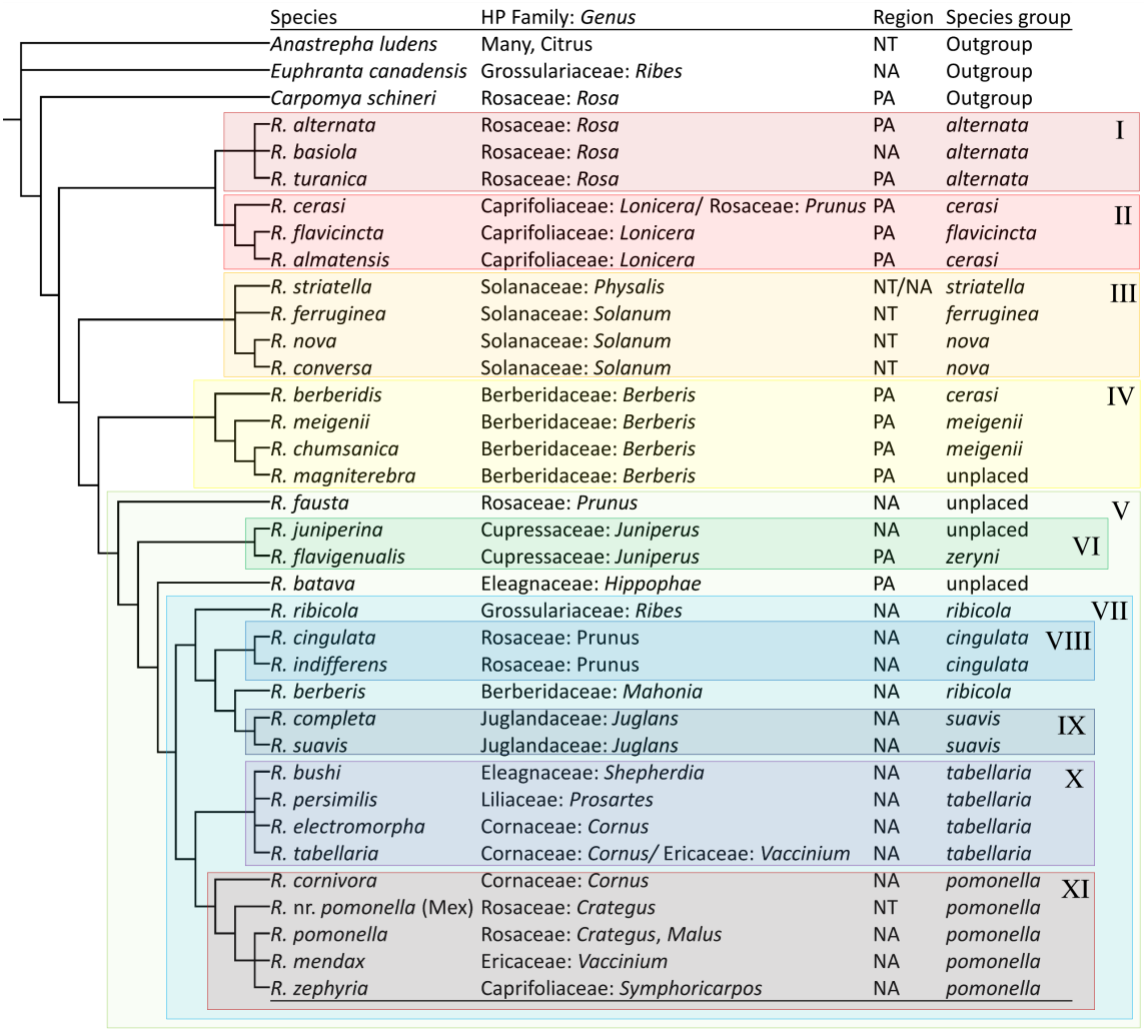

Figure 2.5. Cladogram inferred of the species included in this study in addition to their biogeographic information. All bipartitions with Bayesian posterior probability of ≤0.99 have been collapsed. Boxes and Roman numerals indicate groups of taxa discussed in the text and in Figure 2.4. Regions referred to are Neotropical (NT), Paleatctic (PA), and Nearctic (NA). Species groups are given as they are presented by Smith and Bush (2000).

Discussion

Resolved relationships genus-wide

Our analysis represents the first phylogenetic investigation into *Rhagoletis* phylogenetics that confidently resolves relationships between the major clades of the genus. A longstanding goal in *Rhagoletis* systematics research has been to resolve the relationships between the five North American species groups first defined by Bush (1966) (*pomonella, tabellaria, cingulata, suavis, ribicola*). We provide a hypothesis of species-group relationships that is highly supported by DNA sequence data. We infer resolved relationships within the clade containing the North American species groups (*pomonella, tabellaria, cingulata, suavis* and *ribicola* group members) plus *R. juniperina, R. flavigenualis,* and *R. batava* (Clade V; Figure 2.4, Figure ***2.5***). Outside Clade V, we resolve relationships of the Neotropical taxa (Clade III) and the other (mostly) palearctic taxa (clades I, II, and IV). Our analysis provides improved resolution and is largely consistent with previous investigations into *Rhagoletis* phylogeny.

Previous work generally supported the existence of *Rhagoletis* species groups and few other major clades (e. g. the *pomonella, suavis, cingulata* groups etc.), but did not resolve relationships between them. Morphological data, while very useful for species level diagnosis, is generally not a good source for broadly phylogenetically informative characters (Jenkins 1996, Smith and Bush 2000). However, consideration and analysis of morphological genitalic characters in the *tabellaria* group provided evidence for a hypothesis of species-level relationships that are unresolved based on molecular data collected by Hulbert et al. (2018) and also included in the present paper.

Previous investigations based on mitochondrial sequences supported the existence of major species groups and some higher level clades (Han and McPheron 1997, Smith and Bush

1997, Smith et al. 2005). One of the more interesting results to come out of these mtDNA analyses was the inclusion of two Palearctic *Rhagoletis* species (*R. batava*, and *R. flavigenualis*) in a larger monophyletic group containing most North American taxa (including the five North American species groups, *R. juniperina* and *R. fausta*). Our analysis provides further support for inclusion of *R. batava* and *R. flavigenualis* within the otherwise North American clade (clade V, Figure 2.4, Figure 2.5). Outside clade V, previous analyses (Smith et al. 2005) have supported monophyly of several species groups (*cerasi*, *alternata*, *meigenii* and one comprising (all of the?) Neotropical species, but not their relationship to other taxa. Our analysis here provides additional support for all the groups mentioned above in addition to a well-supported hypothesis of their relationships to each other (Figure 2.4, Figure 2.5).

There are, however, some important inconsistencies between our analysis and previous investigations. First, we inferred that *R. berberidis* was part of a clade that includes members of the *meigenii* species group (and *R. magniterebra*) (Figure 2.5). Previously, *R. berberidis* was thought to be part of the *cerasi* group and *R. magniterebra* was not officially thought to be part of any species group (Smith and Bush 2000), although the analysis by Smith et al. (2005) places *R. magniterebra* as sister to *R. meigenii*. Second, we place *R. flavicincta* in a clade containing *R. cerasi* and *R. almatensis*. The former was previously thought to be part of an eponymous species group and the latter two are part of the *cerasi* group. Our analysis supports placement of *R. flavicincta* with the other two *cerasi* group species that was originally reported by Smith et al. (2005). Finally, we do not recover the *ribicola* species group as monophyletic. The *ribicola* species group was proposed (as "tentative") by Bush (1996) on the basis of genitalic morphology. Later, *R. berberis* and *R. ribicola* were inferred to be sister species based on mitochondrial COII sequences, however this relationship had very low support (Smith and Bush

1997a) and subsequent re-analyses of those data do not support this relationship (Smith and Bush 2000, Smith et al. 2005). Therefore, given previous analyses and the data we collected, we are confident in our hypothesis that the *ribicola* species group, as previously conceived, does not exist.

The Nearctic taxa

A major goal of the present analysis was to determine the relationships of the members of the clade including the North American species groups (*pomonella*, *tabellaria*, *cingulata*, *suavis* and "*ribicola*"), *R. batava*, *R. flavigenualis*, *R. fausta*, and *R. juniperina*. The aforementioned taxa form a monophyletic group, an unexpected result, in previous analysis (Smith et al. 2005). Our analysis strongly supports the existence of this clade and further resolves relationships within it. We infer a monophyletic group composed of members of the North American species groups (clade VII; Figure 2.4, Figure 2.5) to the exclusion of *R. fausta*, *R. juniperina*, *R. flavigenualis*, and *R. batava*. Within clade VII, we infer strongly supported clades which make up each of the North American species groups, with the exception of the *ribicola* group (discussed above), in addition to relationships between the groups: the *pomonella* and *tabellaria* groups are sister to each other and the two together are sister to a clade containing the *suavis*, *cingulata*, and (former) *ribicola* group members. In previous analyses, there was support for the close relationship of the *tabellaria* and *pomonella* groups, and low support for a clade containing members of the *suavis*, *cingulata*, and (former) *ribicola* group (Hamerlinck et al. 2016, Hulbert et al. 2018). We do not include all members of each species groups mentioned above in our analysis, however, the unincluded taxa have been included in previous analyses which strongly

support their inclusion in their respective species groups (McPheron and Han 1997, Smith and Bush 1997, 2000, Rull et al. 2013, Glover et al. 2018).

From our analysis, we include *R. fausta*, *R. juniperina*, *R. flavigenualis*, and *R. batava* in a larger monophyletic group (clade V) that also includes clade VII, and this is consistent with previous analysis (Smith et al. 2005). Our analysis improves resolution: we find strong support for: *R. batava* as sister to all of clade VII; a sister-group relationship between *R. juniperina* and *R. flavigenualis*; tose two as sister to *R. batava* plus clade VII; and *R. fausta* as sister to the remainder of clade V. It is unknown whether any other species of *Rhagoletis* occupy this phylogenetic space (outside clade VII, but inside clade V), but there are several candidates. Specifically, there is a Siberian fly that infests sea-buckthorn (*Hippophae rhamnoides*) and may be a sub species (or possibly sister species) to *R. batava* (Stalažs and Balalaikins 2017). Also, *R. bagheera* Richter & Kandybina appears to be closely related to either *R. batava* or *R. flavigenualis* (Korneyev et al. 2017). Finally, there are at least two juniper-infesting *Rhagoletis* species not included in the present analysis that may be closely related to *R. juniperina* and *R. flavigenualis*: *R. mongolica* Kandybina and *R. zeryni* Hendel (Smith and Bush 2000) (discussed below).

Clades united by host plant associations

Our phylogenetic analysis of *Rhagoletis* revealed several well supported clades consisting solely of species that are united by their use of closely related host plant taxa. In each of these clades, the *Rhagoletis* species all infest the fruit of host plants in the same genus (or family, in the case of clade III). Host plant taxa infested by the above groups are also generally restricted to their respective clade. Additionally, these clades sometimes include both Nearctic and Palearctic

taxa. The existence of such clades united by close host plant relationships (in contrast to other clades containing *Rhagoletis* species infesting broader host species range) may be explained by the toxicity of the hosts, where genera (or families) of toxic hosts support clades of closely related *Rhagoletis* (while non-toxic hosts may host a more diverse, non-monophyletic group of species).

We inferred six clades united by closely related host plant species: I II, III, IV, VI and IX (Figure 2.4, Figure 2.5). Clade II contains palearctic *Lonicera*-infesting taxa and has been resolved in previous analysis (Smith et al. 2005) Included in clade II is *R. cerasi* which, while infesting *Lonicera,* is better known for also being a pest of cherries in Europe. Clade III contains Solanaceae-infesting species generally found in the Neotropical and Nearctic regions, these species may be pests of various nightshade family crops, including tomatillos. Clade III contains representatives from three species groups (*striatella*, *ferruginea*, and *nova*) and ours is the first analysis to confidently place them together in a monophyletic group. Given the close relationship of the Solanaceae-infesting species included in our analysis, we believe it likely that *R. acuticornis* (a previously unplaced species found in the Southwest United States and infesting Solanaceae: *Lycium berlandieri*) is a member of this clade. Clade IV contains palearctic *Berberis*-infesting (barberry-infesting) taxa and these species are not generally considered pests. Ours is the first analysis to confidently place all the *berberis*-infesting species in a clade, although it has been previously speculated that they formed a monophyletic group (Smith et al. 2005). Clade VIII contains the North American *suavis* species group. The existence of the *suavis* group has been well supported by previous investigations (Smith et al. 2005, Rull et al. 2013, Glover et al. 2018). All members of the *suavis* species group infest walnuts (*Juglans*). The rose-infesting species, *R. basiola*, *R. alternata*, and *R. turanica* are in clade I, with only *R. basiola*

found in North America. Recently, *R. emiliae* Richter (a previously unplaced species) was collected from roses in Tajikistan (unpublished data) and bears loose physical resemblance to *alternata*-group flies (Korneyev and Merz 1997), leading us to hypothesize that it is part of the *alternata* species group.

The juniper-infesting species in our analysis, *R. flavigenualis* and *R. juniperina*, are found in clade VI and are native to the Palearctic and Nearctic respectively. Ours is the first analysis to show a sister relationship between *R. juniperina* and *R. flavigenualis* and, we believe, suggests that they may be part of a larger clade that includes the other juniper-infesting *Rhagoletis* (which were not included in the present analysis because specimens were not able to be obtained). The other known juniper-infesting species include *R. zeryni* (found in Western Europe) and *R. mongolica* (found in central Asia). *Rhagoletis zeryni* and *R. flavigenualis* have both previously been placed in the *zeryni* species group and we believe it likely that all the juniper-infesting species belong in that group. Additionally, there is preliminary evidence that what is currently defined as *R. juniperina* may include at least three cryptic species. We found genetic differences between the *R. juniperina* individuals collected from *Juniperus grandis* and *J. virginiana* (Table 2.1, Figure 2.4). Similarly, *R. juniperina* individuals have been collected from *J. horozontalis* in the Bruce Peninsula of Ontario and analysis has revealed them to also be genetically distinct from individuals collected from *J. virginiana* (Frayer et al. 2015). Preliminary examination has also revealed morphological differences between individuals infesting *J. horozontalis* and *J. virginiana* (unpublished data). The diversity of the North American juniper-infesting *Rhagoletis* will be the subject of forthcoming manuscripts.

There are two main patterns observed in the major clades of *Rhagoletis* (Clades I, II, III, IV, VI, VIII, IX, X and XI) in relation to host plant use: clades that are united by use of closely

related host plants (clades I II, III, IV, VI and IX), and clades which are not (VIII, X and XI). One hypothesis is that host plants with more toxic fruit were colonized by *Rhagoletis* only once in evolutionary history and the specialization needed to survive on the toxic substrate is connected to an inability to shift on to other hosts (Bush 1966, 1969, Berlocher and Bush 1982). The most prominent examples of this phenomena are seen in the Solanaceae-infesting (clade VI), and walnut-infesting (clade IX) species. In both of these clades, the host plants have relatively toxic fruit and all known members of these respective groups feed only on nightshade fruits or walnut drupes respectively. At the other extreme, are the *pomonella* (XI), *cingulata* (VIII) and *tabellaria* (X) groups. Each of these groups have member species which infest less (or completely non) toxic fruit from across a wider taxonomic range (Smith and Bush 2000). The toxicity of host plants may, with further investigation, be able to explain diversification of *Rhagoletis* within its major clades, including when and why *Rhagoletis* pest outbreaks due to host shifts occur.

Conclusions

A resolved phylogeny for a genus as consequential as *Rhagoletis* will be an important tool. Resolving even a small portion of the genus previously facilitated research on coevolution between *Rhagoletis* and hymenopteran parasitoids (Hamerlinck et al. 2016). Future genus-wide investigations of *Rhagoletis* should explicitly address the origins of modern host plant and geographic distributions. There are relatively few *Rhagoletis* species that have truly unknown placement in any species group or clade discussed above, but they should be collected and analyzed; these species include *R. acuticornis*, *R. bezziana* Hendel, and *R. mongolica*. In North America, there is a need for *Rhagoletis* specimens from different species of juniper to test cryptic

species hypotheses. *Rhagoletis* continues to be an important and fruitful area of research in entomology and evolutionary biology, and our study on resolving phylogenetic relationships in the genus will be beneficial to future investigations.

CHAPTER 3: JUSTIFICATIONS FOR SYSTEMATIC BIOLOGY AND ITS RELATIONSHIP TO ENTOMOLOGY

Abstract

Systematics is a subfield of biology that is broadly concerned with organisms and their naming, description, collection, classification, identification, distribution, evolutionary histories, environmental adaptations. The purpose of systematic biology is to create an orderly structure of the evolutionary relationships of organisms to each other and to their environment. It is often claimed that systematic biology is fundamental to all other areas of biology. The first purpose of this chapter is to evaluate the acceptance of this claim by entomologists critically as it relates to the field of entomology. The second purpose of this chapter is to critically describe the justification and valuations for systematic biology using the framework of Boltanski and Thévenot's realms of worth and the philosophical framework for justification using virtues, desserts and outcomes. In order to accomplish these purposes, we critically analyze and review relevant entomological literature and interview practitioners of entomology and insect systematic biology. We find justification for systematic biology overwhelming takes the form of appeals to utilitarianism (both internally and externally focused) and are most relevant in the Industrial World. Additionally, some justifications given also pertain to the Civic World and to virtue. Evaluation of justification in systematic biology is important, especially as our globe becomes increasingly ecologically and politically unstable.

Introduction

Systematic biology and Entomology

Systematics is the study of biodiversity and diversification of life. Taxonomy, the field concerned with the naming and describing of organisms, is a subfield of systematics.. Michener et al. (1970) articulate a useful working definition of systematics:

> Systematic biology (hereafter called simply systematics) is the field that (a) provides scientific names for organisms, (b) describes them, (c) preserves collections of them, (d) provides classifications for the organisms, keys for their identification, and data on their distributions, (e) investigates their evolutionary histories, and (f) considers their environmental adaptations. This is a field with a long history that in recent years has experienced a notable renaissance, principally with respect to theoretical content. Part of the theoretical material has to do with evolutionary areas (topics e and f above), the rest relates especially to the problem of classification. Taxonomy is that part of Systematics concerned with topics (a) to (d) above. (Michener et al. 1970)

It is sometimes claimed by practitioners of systematic biology is that the field is fundamental to all other areas of biology (Cotterill 1995, Simpson and Cracraft 1995). Said another way, systematic biology is the foundation on which other areas biology are constructed. We will be assessing how entomologists think about the justifications, internal and external to science, for systematic biology.

Entomology is a broad discipline which is concerned with the study of insects. Insects are a class of arthropods which are the most diverse and among the most wide-spread group of

animals on earth. Entomologists study a range of basic and applied topics related to insects. Humans have been studying insects since prehistory especially in the context of apiculture and pest control (Dams and Dams 1977, Levinson and Levinson 2009, Roffet-Salque et al. 2016). The discipline developed in the 19th and 20th centuries and insects became important systems to study evolution, ecology and genetics.

A series of statutes in the United States established land grant universities (Morrill Land Grant Acts 1862, 1890; Land Grant Colleges 1994), agricultural experiment stations (Hatch Act, 1887) and university extension services (Smith-Lever Act 1914). Entomological research (in the context of pest management) was an important component to the mission of the land grant universities. The modern academic discipline of entomology in the United States has its origins in the land grant institution. However, the discipline has earlier origins in Europe during periods of colonial expansion which caused an influx of exotic insect specimens that were collected and curated by members of especially the British upper class. The "canonical" history of the modern discipline of Entomology is Eurocentric and especially Anglocentric.

The Entomological Society of America (EntSoc) is the largest scientific society for entomologists and the society defines the following four broad subject areas within entomology: 1) Medical, urban and veterinary entomology (MUVE); 2) Insect physiology, biochemistry and toxicology (PBT); 3) Plant-insect ecosystems (PIE); 4) Systematics, evolution and biodiversity of insects (SYSEB) ("ESA Sections | Entomological Society of America" 2018).

Insects play a foundational role in the development of modern systematic biology. Willi Hennig is considered to be the father of modern systematics because of his development of a conceptual and methodological framework for inferring phylogenetic relationships between organisms based on their scorable traits (characters) (Hennig 1966). Hennig's specific system

would be refined and later be called “cladistics”. Hennig designed cladistics while studying the evolutionary relationships between groups of flies (Diptera), a very diverse order of insects with a complex and an evolutionary history that is difficult to decipher (Wiegmann et al. 2011). Insects as a whole present a unique challenge to scientists concerned with their classification and evolution because of their extreme diversity, many cryptic taxa, and ambiguous or conflicting characters.

Overview

The primary questions this chapter addresses are: 1) How is systematic biology justified? 2) What is the value of systematic biology to other parts of biology? Due to the broadness of biology as a discipline we narrow our focus to the justification and value of systematic biology within an entomological context. Additionally, answering these questions in any absolute way is far beyond the scope of this chapter, therefore we evaluate the justification and value of systematic biology in entomology using interviews with systematic and non-systematic entomologists, statements made by systematic and nonsystematic-entomological scientific publications. To analyze the printed and spoken claims about justification and value, we use sociological paradigms on realms of value and a philosophical paradigm on justice.

Justification and value

How are human activities justified? Scholars have addressed the question using two different frameworks: 1) Scholars discuss what is important and represents the values of different groups and used the information to develop a framework for determining worth (an inductive approach) (Boltanski and Thévenot 1991) and 2) philosophers have discussed ethics and

identified main areas of justification (a deductive approach). As noted above, assessing absolute justification for systematic biology is well beyond the scope of this chapter so we use philosophical frameworks and the claims and perceptions of those involved with systematics to evaluate its worth.

Worlds of Worth

Boltanski and Thévenot (1991) analyze canonical texts from political philosophy to determine how different domains of society value and justify activities. Based on their review of literature, they identify six "Worlds" and what is considered of value or "worthy" within these Worlds. They identify what are worthy values and worthy "beings" in each of the worlds. The Six Worlds identified and analyzed are the Inspired World, The Domestic World, The World of Fame, The Civic World, The Market World, and The Industrial World. Human activities can be justified, according to Boltanski and Thévenot, by their contributions to one or more of these Worlds. In each of the worlds, discussed below, there are activities, qualities and entities that define what is valued. It is important to note that the entities within these worlds can take many forms, they may be individual people or collectives (individual people, corporations, NGOs) acting in certain ways, exemplifying the values of a particular world. In all worlds of worth, all types of social actors have various worth and the framework of Boltanski and Thévenot is asking, how (and in what social "realms" or "worlds") do these social actors get to be recognized as outstanding? What follows is a brief description of each of the Six Worlds described by Boltanski and Thévenot.

The Inspired world (St. Augustine): Concerned with gaining inspiration and enlightenment. Worthy beings are those that bring themselves to experience true inspiration. An

inspired state may be achieved by freeing oneself of internal and external mental and physical restraints (e.g., scientific revolutions). Those in an inspired state are able to do and see things that would appear beyond themselves. The path to inspiration may involve the rejection of worthy attributes from other Worlds; for example, rejection of the rational, wealth, structure, or efficiency among other things is valued if it helps achieve inspiration. The path towards inspiration is characterized as a voyage with ill-defined landmarks towards the creation of a masterpiece.

The Domestic World (Bossuet): This world is primarily concerned with interpersonal relationships and their dynamics. An especially important aspect of these relationships in the Domestic World is the hierarchical nature of them. This constrains the nature of engagement a person has with their relations and establishes what is "worthy" in a particular context. As the name suggests, these relationships may exist within a family unit, but the Domestic World is concerned with hierarchical aspects of interpersonal relationships in all areas of society. Deference and loyalty is valued when interacting with superiors, whereas authority and consideration is valued with interacting with subordinates. One's rank is crucially important in the Domestic world as it defines what is worthy for a particular context.

The World of Fame (Hobbes): This world exists in the realm of celebrity. Principally, Fame establishes worth here. Worth of people in this world is based on how well-known they are, and the worth of activities is based upon how they help achieve fame. The world of Fame exists on a short timeline, moments are fleeting (short memory, "15 minutes of fame"). As in the other worlds, worthy "beings" in the World of Fame may not be actual human individuals, they may be recognizable brands, companies, entities etc.

The Civic World (Rosseau): The most important unit in the Civic World is the collective. As such, organization is sacrosanct. Generally individual people are of little worth, but they may have increased value insofar as they help public collectives. However, these people become part of something very worthy if they are able to organize into a collective with power to enact a common will. A worthy collective is able to organize, break down the isolation of the individual members and wield collective power. Through collectives, worth is gained by sacrificing the particular short-term self-interests in favor of transcendent collective interests.

Industrial World (Saint-Simon): Primarily concerned with production. Objects, beings, scientific methods are all valued insofar as they are able to contribute to production. Functionality, efficiency, standardization and performance all confer the most worth in the Industrial World. Worthy beings are judged operationally, how well are they characterized by the above descriptors. Objects in the Industrial World are instruments, means mobilized for production. Human individuals may be worthy in the Industrial Realm if they can efficiently and accurately, they perform production tasks and work for long periods of time, their ability to produce quality product quickly, efficiently, accurately. An individual person may be part of a larger production unit (assembly line) or not, but whichever mode of production is more efficient is more worthy in the Industrial World.

The Market World (Adam Smith): The Market world has a close symbiotic relationship with the Industrial World and World of Fame. The Market World is concerned with the coordination of the marketplace. The nature of relationships in the Market world are transactional. A worthy object is one that is desirable, salable and marketable (in contrast to worth objects in the Industrial World which are efficient and functional). Worthy beings (individuals and organizations) are those that are rich; they own what others want. Entities in the

Market World are detached from one another and use the marketplace to facilitate transactions. The Marketplace strives to formalize transactions, moving from non-explicit exchanges to explicit exchanges as the market develops (the market is dis-embedded from social relations).

Systematic biology seems to be mostly justified in the Inspired World, Industrial World and Civic World. The fundamental nature of some systematic biology that motivates research may be seen as a desire for enlightenment. Systematic biology researchers may use the language and justifications of the Inspired World to describe why they chose their professions. In the Industrial World, knowledge produced by systematics may be used to make various forms of production more efficient. Especially in the areas of agriculture and medicine does systematic biology hold the most promise to increase production. In the Civic World, knowledge produced by Systematic Biologists may be useful for those that want to organize around causes such as the conservation of biodiversity. Recently, there has been a precipitous decline in insect populations (Shortall et al. 2009, Hallmann et al. 2017) and this has been publicized in the popular press (Guarino 2018, Jarvis 2018, McKie 2018). It may be that as the public's knowledge of anthropogenic biodiversity decline increases, justifications for systematic biology that explicitly invoke the Civic World will increase. Some of the other Worlds of Worth may have some relevance to characterizing all of systematic biology, however are not relevant for evaluating the justifications for the discipline. For example, interpersonal relationships in an academic laboratory setting are accurately characterized and explained by the Domestic World: understanding the close hierarchical interpersonal relationships found in the lab is an important aspect of the academic "ecosystem" in the natural sciences, but do not explain the overall justifications. Similarly, laboratory units engage with and consider the Market Worlds in their operation, but it is probably not important for justification either. Finally, while it is possible that

certain individual systematic biologists may have some of their personal motivations come from the World of Fame, but, again, it is not a justification for the discipline as a whole.

Ethics

An alternative to the inductive framework for assessing justification by Boltanski and Thévenot are deductive philosophical frameworks for ethics. Philosophical ethics may be thought of as a discipline for "asking better questions" and developing framework for thinking and perceiving ethical reasoning (Thompson 2015). An "agent" is an important piece of an ethical framework. An "agent" is an individual or entity that is capable of taking actions. These actions are constrained to varying degrees by technology, laws, and customs. The actions by an agent (conduct) will have some sort of consequence. Taken in aggregate, the effects of all consequences on all affected is the outcome. Three main theories we will be considering are 1) Utilitarianism, 2) Rights Theory, and 3) Virtue Theory, each of which are briefly described below with thoughts on how they relate to systematic biology.

Utilitarianism: Consequences and outcomes are most important in the Utilitarian school of thought. Other considerations are reducible to harm and benefit. Actions can be considered ethically "right" when for example they lead to the greatest good for the greatest number of people. In the context of systematic biology (especially entomological), a utilitarian justification is likely to fall into one of three categories: 1) Medical and pest, for example ZIKV phylogenetics revealed how the virus was entering the United States, helping efforts to stop its spread. (Grubaugh et al. 2017); emerald ash borer phylogenetic investigation determined what parts of Asia were the origin of the invasive species (Bray et al. 2011). 2) Diagnosis and discrimination of organisms. One of systematic biology's primary goals is the definition and

delineation of all organisms into a hierarchal organization. Organisms for which this organization is known may be diagnosed relatively easily. Being able to diagnose economically, epidemiologically, and forensically significant organisms has obvious utilitarian benefit (quickly diagnosing disease agents, vectors, pest organisms etc.). 3) Conservation of biodiversity: A motivating factor for the preservation of biodiversity is that a more biodiverse biome is more beneficial to humanity than a less diverse one. Part of the benefit of increased biodiversity comes from organisms with directly useful properties to human activity. Systematic biology would be fundamental to any research effort to exploring and discovering the beneficial organisms. The argument is different than one advocating for the preservation of natural areas because they provide ecosystem services.

Rights Theory: Rights theory asks how actions are based upon the actor's rights and constrained by the rights of others. Social interactions are a set of (sometimes) implicit promises, duties and rights: what are they? What is the social contract? When someone is in a particular position, what special rights are afforded to them and what are their duties? Rights theory examines these relationships and provides a framework for justifying activities. Justifications for systematic biology may be based on the framework of Rights Theory. These justifications would invoke the "dues" paid by systematic biologists for them to be where they are now, so they now have the right to be doing their research. These dues are likely in the form of formal education in undergraduate and graduate school, mentors they have learned from, and research projects they have been involved with. The case that resources are justly allotted to them for their research is made in presenting this history and these accomplishments. Because the systematic biologist has the right to do their studies, what duties and rights do they have to other parts of society? According to Rights Theory, they have a responsibility do all the parts of systematic biology.

Virtue Theory: Virtue Theory examines what is virtuous and vicious. This framework focuses on the individual, asking what a particular person's disposition or character is like when acting. The framework may also focus on the broader society that shapes norms and determines what is virtuous. The framework of virtue theory does not necessarily tell what is "good" to do in a prescriptive sense, rather it allows the exploration of what and how actions are always seen as "good to do". It is often stated, implicitly and explicitly, that the resolution of the evolutionary tree of all life, past and present, is a goal of systematic biology. Resolving the evolutionary tree of life may be seen as an inherently "virtuous" activity.

Methods

Literature analysis

Since it is impossible to determine in this chapter an absolute justification for systematic biology, we instead use tools to determine what are justifications given by those familiar with the science. One method to be used is interviewing those knowledgeable in the subject (discussed below), and another is a review of relevant literature. By reviewing scientific literature by systematic biologists and non-systematic biologists (who mention systematic biology in their work) we can examine how the authors write about justification. For practicality and to concentrate the scope of this project, we limited our analysis to the most recent literature published by the Entomological Society of America (Entsoc).

We reviewed literature published by the EntSoc to examine how authors discussed justification for systematic biology. We had two major categories for literature: 1) Systematic biology articles, and 2) articles published in the non-systematic biology journals (discussed further below). There are nine journals published by the EntSoc: American Entomologist, Annals

of the Entomological Society of America (AESA), Arthropod Management Tests, Environmental Entomology, Insect Systematics and Diversity (ISD), Journal of Economic Entomology (JEE), Journal of Insect Science, Journal of Integrated Pest Management, and Journal of Medical Entomology.

In order to find the most recent articles that dealt with explicitly systematic biology, we first chose the most recent articles published in Insect Systematics and Diversity (ISD). Because it is a very new journal, as of the time of writing the present chapter, there were 23 articles published in ISD. Prior to the establishment of ISD, systematic biology articles were published by EntSoc in the Annals of the Entomological Society of America (AESA), specifically in its "Systematics" section. Therefore, in addition to the most recent ISD articles, we also selected the most recent 24 articles from AESA in its Systematics section. These 47 articles were read and scored for the justifications they expressed. The justifications were scored in two broad categories: 1) the framework of worlds of worth given by Boltanski and Thévenot (1991) and , 2) the framework of utilitarianism, rights theory, and virtue theory.

A parallel sample of articles from the EntSoc journals with a more "industrial" focus was selected, these journals included the Journal of Economic Entomology (JEE), Journal of Integrated Pest Management (JIPM), and Journal of Medical Entomology (JME). In order to find articles that discussed systematic biology, we searched the journals with the keyword "systematics" and sorted the resulting articles by most recently published. Because versions of the word "systematics" may have meaning in certain contexts other than "systematic biology", we did not evaluate articles that used the word "systematics" in some other way. The sample of articles from the "industrial" journals was smaller than the sample from the "systematic biology" journals (ISD and AESA) because of the smaller number of articles (5 from JEE, 3 from JIPM, 5

from JME) found meeting the above requirements and the lack of diversity in the assessments. We felt that it was better to keep the same time frame across the two groups of journals than to obtain more industrial journal articles over a much greater time frame.

Interviews

As stated above, it is impossible to determine an absolute justification for systematic biology, we must use tools to determine what are justifications given by those familiar with the science. Other than reviewing the literature, conducted in-depth semi-structured interviews with science practitioners. Through interviews, we can probe scientists' opinions and feelings about the justifications for systematic biology. Like in the literature review and evaluation section above, we focus our sample on all entomologists, some of whom study systematic biology (in insects) and others who study other areas of entomology (such as MUVE, PBT, and PIE). We compared these two groups on their responses. The interviews discussed here are the first of a larger sample that will be conducted.

Conducted in-depth semi-structured interviews with PhD level scientists of entomology departments around the United States. All interviewees fall into one of two categories: 1) entomologists who primarily research systematic biology in insects, and 2) entomologists who primarily study something other than systematic biology. Those entomologists who do not study systematics have study areas that include agricultural production, toxicology, aquatic entomology, medical entomology, and ecology. All interviewees are PhD-level scientists in the United States. For the full sample of interviewees we will make an effort to compose our sample such that it is representative of gender, stage of career (early, middle or late), and (in the case of non-systematic entomologists) subject area.

We conducted in-depth semi-structured interviews with the interviewees on the value and justification of systematic biology. We gave the same questions in the same order to both the systematic entomologists and non-systematic entomologists with only minor alterations (explained below). The interviews consisted of five main sections with questions around a particular theme. The first section consisted of asking about their background in entomology, how they first got interested in the subject, and their current area of study in the discipline. The second part dealt with establishing what the interviewee understands systematic biology to be asking them to define the subject. If the interviewees left out important pieces in their definition, we asked them whether they feel those unincluded parts are within the scope of systematic biology. In general, the definition of systematic biology we used is the one given by Michener et al. (1970) (described above). In the third part we asked about how the interviewees relate to systematic biology in their own work. Do the interviewees consider any part of their research to be systematic biology (this specific question may be omitted for the systematic entomologists)? Does the interviewee feel that they use knowledge generated by systematic biology to inform their own research? In the fourth section, we asked the interviewees most directly about what they feel are the justifications for systematic biology. We asked what the interviewees feel is the justification for systematic biology in academic science, outside of academe, within industry, within broader society. The fifth and final section we asked about the interviewees previous experience and education in systematic biology. Has the interviewee had any formal education in systematic biology? Do the interviewees collaborate with any (other) systematic biologists, how? From the interviews that we will score the responses into the realms of worth and justice frameworks for justification described above.

Results and Discussion

Literature analysis

A total of 47 articles were evaluated from the EntSoc "systematic" journals (Table 3.1). Within Boltanski and Thévenot's "worlds of worth" framework, the most common appeals were made to either the Industrial world (15) or the Civic world (15). Within the deductive framework for justification, the 47 systematic articles all made appeals to utilitarianism, especially in the form of their usefulness to science or other scientists in the field. Appeals to utilitarianism other than usefulness to science were also made by some authors (20). Additionally, the authors of the systematic biology articles made appeals to virtue (22). We evaluated a total of 13 articles within the inductive and deductive frameworks described above. The authors of all these articles appealed only to the industrial world and to utilitarianism.

Table 3.1. Justifications given by the literature published by the EntSoc. Cells shaded in black have explicit justifications in the category while cells shaded in grey imply the respective justification. All articles contained internal utilitarian justifications, therefore only external utilitarian justifications are included in the table.

		World of Worth		Ethics		
Discipline	Citation	Civic	Industrial	Utilitarianism	Rights	Virtues
Systematic biology	(Leubner et al. 2017)					
	(Ortiz-Acevedo et al. 2017)					
	(Dietrich et al. 2017)					
	(Barden and Ware 2017)					
	(Caterino et al. 2017)					
	(Moreau and Wray 2017)					
	(Johnson et al. 2017)					
	(Jenkins et al. 2018)					
	(Chien and Heraty 2018)					
	(Dew et al. 2018)					
	(Schachat and Goldstein 2018)					
	(Huang 2018)					
	(Skvarla et al. 2018)					
	(Glover et al. 2018)					
	(Cognato et al. 2018)					
	(Théry et al. 2018)					
	(Brown et al. 2018)					
	(Song et al. 2018)					
	(Mugleston et al. 2018)					
	(Moulton et al. 2018)					
	(Burks et al. 2018)					
	(Anderson 2018)					
	(Blaimer et al. 2018)					
	(Catanach and Dietrich 2018)					

Table 3.1 (cont'd).

		Worlds of Worth		Ethics		
Discipline	Citation	Civic	Industrial	Utilitarianism	Rights	Virtues
	(Ide et al. 2018)					
	(Liang et al. 2017)					
	(Powell et al. 2017)					
	(Song et al. 2017)					
	(Dolan et al. 2017)					
	(Shirai et al. 2017)					
	(Przybyłowicz and Ochse 2017)					
	(Wilson et al. 2016)					
	(Liao et al. 2016)					
	(Ide and Abe 2016)					
	(Liang and Li 2016)					
	(Sohn 2016)					
	(Hosoishi and Ogata 2016)					
	(Gagliardi and Wagner 2016)					
	(Lagos-Kutz et al. 2016)					
	(Morgulis et al. 2016)					
	(Yao et al. 2016)					
	(González et al. 2016)					
	(Kadej and Háva 2016)					
	(Cole and Chiang 2016)					
	(Tan et al. 2016)					
	(Randrianandrasana et al. 2016)					
	(Wang et al. 2016)					
Other Entomology	(Shadmany et al. 2018)					
	(Wang et al. 2018)					
	(San Jose et al. 2018)					

Table 3.1 (cont'd).

		Worlds of Worth		Ethics		
Discipline	Citation	Civic	Industrial	Utilitarianism	Rights	Virtues
	(Adachi-Hagimori et al. 2018)		■	■		
	(Liu 2018)		▒	■		■
	(Schowalter and Ring 2017)		■	■		
	(Tofangsazi et al. 2014)		■	■		
	(Kho et al. 2018)		■	■		
	(Ciminera et al. 2018)		■	■		
	(Shang et al. 2018)		■	■		
	(Lam-Phua et al. 2018)		■	■		
	(Giordani et al. 2018)		■	■		

By far the most common justification for systematic biology given in the published articles was a utilitarian one. A utilitarian justification was given by authors of every article in the "systematic" journals and the "industrial" journals. Most utilitarian justifications took the form of describing how the author's work was useful to science and to other scientists, especially systematic biologists (internal utilitarianism). It is perhaps unsurprising that all the articles evaluated had this as a justification, it is a ubiquitous practice in scientific articles to describe how the work fits into a broader scientific context and how the results are expanding the boundary of knowledge. The other utilitarian justifications for systematic biology found in the articles mainly sought to describe how the work would be beneficial for pest management and medicine or, less commonly, how the work would be beneficial to conservation efforts (external utilitarianism).

A few examples will illustrate how the systematic biology published literature uses different justifications. Take, for instance, the opening sentence of the article by Moreau and Wray:

> Inferring the evolutionary relationships among the diversity of organisms on the planet has implications outside of systematics alone and includes fields as diverse as evolutionary biology, ecology, conservation science, food and crop security, and human health. (Moreau and Wray 2017)

In the above quote, there is an explicit acknowledgement that systematic biology has internal utilitarian benefit in addition to external utilitarian benefit (to ecology, conservation, crop production and medicine). Also, Moreau and Wray (2017) are stating that systematic biology has

value to the Industrial World by mentioning its benefit to crop production and medicine. Next, consider this quote from the end of an article by Shirai et al.:

> Knowing more about brassoline natural history also provides relevant knowledge for agricultural applications and for educational purposes, since their large size and beautiful wings are charismatic examples of ecology and evolution in action. Finally, in this study, we presented a framework that could be applied to the study of other insect groups by integrating traditional and modern methods of field and lab work, along with morphology and molecular biology. (Shirai et al. 2017)

For context, brassolines are colorful group of neotropical butterflies. In the quote above by Shirai et al. (2017), utilitarian justifications (internal and external) are given, but also an implicit appeal to virtue is made; studying charismatic insects is a good thing to do. It is worth noting that the appeal to virtue is weak and possibly debatable as it is embedded within a more obvious utilitarian justification.

Interviews

The interviews discussed here are the first of a larger sample that will be conducted. We interviewed three entomologists at research institutions in the United States. All of the interviewees (entomologists studying systematic biology, and those who did not) stated that they thought systematic biology was fundamental to other areas of biology. However, the entomologists who did not do systematic biology would not go so far to say whether they

thought systematic biology was fundamental to *all* other areas of biology, only that it was fundamental to *their* area.

All interviewees gave internal utilitarian justifications for systematic biology. For example, one ecological entomologist stated that systematic biology was necessary for their work because "the "operational units" that ecology studies are meaningless without systematic biology to study what makes up those units and gives names to the units". The systematic biologists interviewed gave similar examples relating to ecology.

When explaining how the systematic biologists first got interested in entomology, all gave responses that were consistent with the Inspired World. Given were examples of how they had first enjoyed spending time in outside and the sense of fascination with the natural world. Interviewees recount how these experiences began them on their current career trajectory and is one of the reasons they like their current jobs.

The systematic biologists both discussed insect collections and museums as important institutions of entomology and systematic biology. They gave internal utilitarian justifications for collection's existences, but also described them in terms of their civic and external utilitarian value. One example that was given multiple times was that of the emerald ash borer (EAB) invasion of North America. For context, in 2002, EAB was first discovered near Detroit, Michigan (it is native to northeast Asia). According to the systematic biologist interviewees, collected specimens of EAB were initially a mystery to local entomologists, but were able to be identified by working with local insect collections; this information was critical to the tracking and management of the invasion.

Another example given for the civic and external utilitarian benefit of systematic biology was the study of climate change. One way to assess the effects of climate change is by studying

how organisms are affected by changing temperatures and weather in different places on the planet. Climate change is causing the range of many organisms to shift, and in order to study this phenomenon, there must be good systematic knowledge of exactly what organisms are native to a particular area at a particular time.

Conclusions

The majority of the justifications given for systematic biology from both literature and interviews took the form of internal utilitarian justification. This is probably unsurprising given the academic context of the interviews and literature and the explicit need to justify one's scientific work in that environment, however other, external justifications were also given. It is important for various biological disciplines to be reflexive about their relationship to other parts of science and society.

In the interest of being reflexive, I will examine my own relationship to the present chapter. As a student practitioner of systematic biology, I am hardly an unbiased actor when it comes to investigating its social and scientific justifications. Prior to becoming a systematic biologist, I was an undergraduate student at a private liberal arts college and majored in biology generally. I began graduate school working on a master's degree in the Entomology Department at Michigan State University (MSU) with a research project investigating how rainfall effects the efficacy of insecticides. After graduating from the master's program, I began my PhD research studying the evolution and systematics of *Rhagoletis* (Diptera: Tephritidae). Shortly after beginning my PhD studies, I applied for and received the C.S. Mott predoctoral fellowship in Sustainable Agriculture. The fellowship funds a portion of my program and requires me to pursue the Ecological Food and Farming Systems (EFFS) specialization as part of my PhD

program. The EFFS specialization is designed for students who are interested in sustainable agriculture broadly. Specifically, the EFFS program gives natural science students (like me) courses and research opportunities in the social sciences and vice versa. I have completed the departmental requirements for a PhD and for the EFFS specialization at MSU thus far. The academic experiences and my own research and advocacy interests do not make me an unbiased individual when it comes to the subjects I am considering in the present chapter. However, I believe this does not impede my ability to investigate the topic and produce insights.

What are my own justifications given for the systematic biology research that I present in my other two data chapters? In the chapter describing a new species of *Rhagoletis*, I invoke internal utilitarianism (the lack of any formal previous description of *R. bushi* has caused "a confusing and inconsistent history throughout the literature"), and imply rights theory, that I have a responsibility to describe the new species. Similarly, I feel that the description of new species is inherently a good thing to do, a "virtuous" behavior, but whether or not this comes through in a strict reading of the chapter's text is debatable. In the first and second data chapters where I discuss why phylogenic investigations of *Rhagoletis* are justified, I make explicit appeals to external utilitarianism and the Industrial World ("…studying evolutionary patterns in *Rhagoletis* is important for understanding when and why *Rhagoletis* infestations occur" / "[patterns of host plant use may] explain diversification of *Rhagoletis* within its major clades, including when and why *Rhagoletis* pest outbreaks due to host shifts occur").

Examination of a researcher's relationship to a discipline and that discipline's relationship to other parts of society is important to consider and investigate. Without this kind of consideration and investigations, the academic science risks becoming too internally focused and perceived, rightly, as being at best irrelevant to the lives of laypeople. Lack of external focus can

lead to scientism as well. Briefly, scientism is the belief that modern western (nominally) academic science is the only valid way of producing knowledge and that its process should be applied to address all problems, social and political, in society. The ideas of scientism are articulated favorably by Sir Francis Bacon, "Workers, and managers, housewives and students, farmers and government officials would be subordinated to the scientifically organized industrial system" (Busch 2000). Contemporary popular scientists (and those in the recent past), who may be regarded as role models for young scientistsm publicly express opinions that are consistent with scientism (Burnett 2018). Reflexivity in systematic biology helps the discipline keep from being too internally focused, prevent it from being characterized as scientism, and improve its relationships with other parts of society.

APPENDICES

Appendix A: Record of deposition of voucher specimens

The specimens listed below have been deposited in the named museum (or their DNA sequences are deposited on NCBI GenBank) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _2018-6_____

Author and Title of thesis:
Daniel Hulbert: Molecular systematics of the genus *Rhagoletis* (Diptera: Tephritidae).

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)
Specimens:

Family	Genus-species	Life stage	Quantity	Preservation
Tephritidae	*Anastrepha ludens*	adult	1	pinned
Tephritidae	*Euphranta canadensis*	adult	1	pinned
Tephritidae	*Carpomya schineri*	adult	1	pinned
Tephritidae	*Rhagoletis alternata*	adult	1	pinned
Tephritidae	*R. basiola*	adult	1	pinned
Tephritidae	*R. turanica*	adult	1	pinned
Tephritidae	*R. cerasi*	adult	1	pinned
Tephritidae	*R. flavicincta*	adult	1	pinned
Tephritidae	*R. almatensis*	adult	1	pinned
Tephritidae	*R. striatella*	adult	1	pinned
Tephritidae	*R. ferruginea*	adult	1	pinned
Tephritidae	*R. chumsanica*	adult	1	pinned
Tephritidae	*R. magniterebra*	adult	1	pinned
Tephritidae	*R. fausta*	adult	1	pinned
Tephritidae	*R. juniperina*	adult	1	pinned
Tephritidae	*R. batava*	adult	1	pinned
Tephritidae	*R. ribicola*	adult	1	pinned
Tephritidae	*R. berberis*	adult	1	pinned
Tephritidae	*R. indifferens*	adult	1	pinned
Tephritidae	*R. cingulata*	adult	1	pinned
Tephritidae	*R. suavis*	adult	1	pinned
Tephritidae	*R. completa*	adult	1	pinned
Tephritidae	*R. bushi*	adult	1	pinned
Tephritidae	*R. tabellaria*	adult	1	pinned
Tephritidae	*R. persimilis*	adult	1	pinned
Tephritidae	*R. mendax*	adult	1	pinned
Tephritidae	*R. zephyria*	adult	1	pinned
Tephritidae	*R. cornivora*	adult	1	pinned

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Family	Genus-species	Life stage	Quantity	Preservation
Tephritidae	*R. pomonella*	adult	1	pinned

DNA-only specimens deposited on NCBI Genbank:

Family	Genus-species	Accession numbers
Tephritidae	*R. nova*	MH998917, MH998691, MH998839, MH998771, MH999003, MH999082
Tephritidae	*R. conversa*	MH998919, MH998693, MH998841, MH998773, MH999005, MH999084
Tephritidae	*R. berberidis*	MH998922, MH998696, MH998843, MH998776, MH999008, MH999087
Tephritidae	*R. meigenii*	MH998924, MH998698, MH998845, MH998778, MH999009, MH999089
Tephritidae	*R. flavigenualis*	MH998940, MH998714, MH998858, MH998790, MH999025, MH999105
Tephritidae	*R. electromorpha*	MH998971, MH998745, MH998886, MH998817, MH999053, MH999131

Appendix B: Supplementary material for chapter 1

Table B1. Primers and thermocycler conditions used to PCR amplify Rhagoletis DNA. All programs used a 30 second initial denaturation period at 95°C; followed by 35 cycles of 95°C for 30 seconds, the annealing temperature (below) for 30 seconds and 72°C for the extension time (below); followed by a final extension period of 10 minutes at 72°C (except when noted by *).

Locus	Primer pair	Primer sequence**	Reference	Annealing (°C)	Ext. (min:sec)
COI	LepF1	5'-ATTCAACCAATCATAAAGATAT-3'	Hebert et al. 2004	46	2:00
	LepR1	5'-TAAACTTCTGGATGTCCAAAAA-3'	Hebert et al. 2004		
Period	Per2476F	5'-CAACGACGAAATGGAGAAATTC-3'	Barr and McPheron 2006	57	1:00
	Per3105R	5'-AABGACATGGGTTGGTACATC-3'	Barr et al. 2005		
AATS	AATSZ1F	5'-GGCACGGCTGATCCBAATAG-3'	This study	62	1:00
	AATSZ1R	5'-TCWGRTGCACCTGTACCCTC-3'	This study		
28S (A)	28SrDNA match F	5'-GTAAACAAGTACCGTGAGGG-3'	modified from Smith and Brown 2008	54	1:00
	28SrDNA match R	5'-TAGTTCACCATCTTTCGGGTCAC-3'	modified from Smith and Brown 2008		
28S (B)	S28C	5'-GTGCAAATCGATTGTCAGAA-3'	Han et al. 2002	65	1:30
	A28F	5'-TGGAACCGTATTCCCTTTCG-3'	Han et al. 2002		
CAD	54F	5'-GTNGTNTTYCARACNGGNATGGT-3'	Moulton and Wiegmann 2004	58-45 (touchdown)*	1:00
	414R	5'-AAACCACAATCGATCGCACAAAT-3'	Hamerlinck et al. 2016		
	405R	5'-GCNGTRTGYTCNGGRTGRAAYTG-3'	Moulton and Wiegmann 2004	58-45 (touchdown)*	1:00
	392F	5'-ATTTGTGCGATCGATTGTGGTTT-3'	Hamerlinck et al. 2016		

*The touchdown program, originally used in (Condon et al. 2008), employs an initial denaturation at 92°C for 2 minutes; followed by 12 cycles of 92°C for 10 seconds, 58-46°C (decreasing 1°C/ cycle) for 10 seconds, and 72°C for one minute; followed by 27 cycles of 92°C for 10 seconds, 45°C for 10 seconds, and 72°C for 1:30 minutes; followed by 72°C for 10 minutes.
**Nucleotides (including degenerate bases) follow the IUPAC naming conventions.

Table B2. Accession numbers of the DNA sequences used in and generated by the present study. Numbers in parentheses following taxon designations correspond to individual numbers used in Table 1 and Figure 4.

Taxon designation	COI	CAD	period	AATS	28S part A	28S part B
R_bushi_101716_1 (7)	MG825300	MG825278	MG825190	MG825212	MG825234	MG825256
R_bushi_101716_2 (8)	MG825301	MG825279	MG825191	MG825213	MG825235	MG825257
R_bushi_101716_3 (10)	MG825302	MG825280	MG825192	MG825214	MG825236	MG825258
R_bushi_101716_4 (11)	MG825303	MG825281	MG825193	MG825215	MG825237	MG825259
R_bushi_C2 (9)	MG825304	MG825282	MG825194	MG825216	MG825238	MG825260
R_mendax_MI_A4 (5)	MG825305	MG825283	MG825195	MG825217	MG825239	MG825261
R_pomonella_MI_A3 (4)	MG825306	MG825284	MG825196	MG825218	MG825240	MG825262
R_cingulata_MI_E1 (1)	MG825307	MG825285	MG825197	MG825219	MG825241	MG825263
R_cingulata_MI_E2 (2)	MG825308	MG825286	MG825198	MG825220	MG825242	MG825264
R_electromorpha_MI_A2 (14)	MG825309	MG825287	MG825199	MG825221	MG825243	MG825265
R_electromorpha_MI_C1 (15)	KU511166	MG825288	MG825200	MG825222	MG825244	MG825266
R_electromorpha_MI_C2 (16)	MG825310	MG825289	MG825201	MG825223	MG825245	MG825267
R_persimilis_100995_3 (12)	MG825311	MG825290	MG825202	MG825224	MG825246	MG825268
R_persimilis_102416_2 (13)	MG825312	MG825291	MG825203	MG825225	MG825247	MG825269
R_tabellaria_10_ON_051316_1 (20)	MG825313	MG825292	MG825204	MG825226	MG825248	MG825270
R_tabellaria_95_2_051316_5 (21)	MG825314	MG825293	MG825205	MG825227	MG825249	MG825271
R_tabellaria_100995_5 (18)	MG825315	MG825294	MG825206	MG825228	MG825250	MG825272
R_tabellaria_100995_6 (19)	MG825316	MG825295	MG825207	MG825229	MG825251	MG825273
R_tabellaria_102416_3 (22)	MG825317	MG825296	MG825208	MG825230	MG825252	MG825274
R_tabellaria_IA_A4 (17)	MG825318	MG825297	MG825209	MG825231	MG825253	MG825275
R_zephyria_MN_A4 (6)	MG825319	MG825298	MG825210	MG825232	MG825254	MG825276
R_cornivora_14_Shollow_051816_1 (3)	MG825320	MG825299	MG825211	MG825233	MG825255	MG825277

Table B3. The partitioning scheme results of the MrBayes and RAxML PARTITIONFINDER analyses. The predefined partitions within the same subset were combined in the phylogenetic analysis of the concatenated alignment.

Program	Subset number	Model	Predefined Partition
MrBayes	1	GTR+I+G	COI position 1
			CAD position 1
			AATS position 1
			period position 1
	2	HKY+G	AATS position 2
			AATS position 3
			period position 2
			CAD position 2
			28S
			COI position 2
	3	GTR	COI position 3
	4	K80+G	CAD position 3
			period position 3
RAxML*	1	GTR+G	COI position 1
			CAD position 1
			AATS position 1
			period position 1
	2	GTR+G	AATS position 2
			AATS position 3
			period position 2
			CAD position 2
			28S
			COI position 2
	3	GTR+G	COI position 3
	4	GTR+G	CAD position 3
			period position 3

*RAxML only allows the specification of one model rate of heterogeneity for all partitions. The GTR+G model had the lowest AICc (16820.04); AICc for GTR = 16870.73 ; AICc for GTR+I+G = 16852.22.

Table B4. Autapomorphies for *tabellaria* group species based on the alignments we generated organized by locus. The position number represents the position within our alignment. Autapomorphies within the *tabellaria* group are highlighted in yellow.

	COI												
Position	27	69	96	105	171	214	312	420	441	471	495	538	621
R. bushi	T	T	C	G	T	T	T	A	T	T	A	T	G
R. tabellaria	T	T	T	A	T	T	T	A	T	C	G	T	A
R. electromorpha	T	T	T	A	T	T	C	A	T	T	A	C	A
R. persimilis	C	G	A	A	C	C	T	G	A	T	A	T	A
R. zephyria	T	T	A	A	T	T	T	A	T	A	A	T	T
R. pomonella	T	T	A	A	T	T	T	A	T	A	A	T	T
R. mendax	N	N	A	A	T	T	T	A	T	A	A	T	T
R. cornivora	N	T	G	A	T	T	T	A	T	A	A	T	T
R. cingulata	N	T	A	T	T	T	A	A	T	A	A	T	A

	CAD													
Position	156	417	489	645	650	735	741	823	844	856	857	876	896	945
R. bushi	C	G/A	G	G	T	C	C	G	G	A	C	A	A	A
R. tabellaria	A	T	A	A	G	T	C	T	G	C	A	C	A	A
R. electromorpha	C	G	G	A	G	T	T	G	G	A	C	C	A	T
R. persimilis	C	G	G	A	G	T	C	G	C	A	C	C	C	A
R. zephyria	N	G	G	A	G	T	C	G	G	A	C	C	A	A
R. pomonella	C	A	G	A	G	T	C	G	G	A	C	C	A	A
R. mendax	C	A	N	A	G	T	C	G	G	A	C	C	A	N
R. cornivora	C	G	G	A	G	T	C	G	G	A	C	C	A	N
R. cingulata	C	G	G	A	G	T	C	G	G	A	C	C	A	A

Table B4 (cont'd).

	period																		
Position	35	36	49	141	153	177	251	288	306	342	444	456	460	461	462	489	507	520	528
R. bushi	C	A	G	C	G	A	A	G	A	C	A	T	-	-	-	T	G	G	A
R. tabellaria	C	A	G	T	G	T	T	C	T	C	T	A	A	C	A	G	G	C	A
R. electromorpha	C	T	G	C	A	T	T	C	T	C	A	T	-	-	-	G	C	G	A
R. persimilis	T	A	A	C	G	T	T	C	T	T	A	T	-	-	-	G	G	G	C
R. zephyria	C	A	G	C	G	T	T	C	T	C	-	T	-	-	-	G	G	G	A
R. pomonella	C	A	G	C	N	T	T	C	T	C	-	T	-	-	-	G	G	G	A
R. mendax	C	A	G	C	G	T	T	C	T	C	-	T	-	-	-	G	G	G	A
R. cornivora	C	A	G	C	G	T	T	C	T	C	-	T	-	-	-	G	G	G	A
R. cingulata	C	A	G	C	G	T	T	C	T	C	-	T	-	-	-	G	G	G	A

	AATS				
Position	67	247	248	454	472
R. bushi	C	G	G	G	A
R. tabellaria	C	A	A	A	A
R. electromorpha	C	G	G	A	A
R. persimilis	T	G	G	A	G
R. zephyria	C	G	G	A	A
R. pomonella	C	G	G	A	A
R. mendax	C	G	G	A	A
R. cornivora	C	G	G	A	A
R. cingulata	C	G	G	A	A

Table B4 (cont'd).

	28SA								28SB					
Position	98	115	143	305	316	546	547	548	50	56	115	434	435	464
R. bushi	A	C	G	T	A	T	T	A	A	T	C	A	C	T
R. tabellaria	G	C	A	T	A	-	-	-	A	G	T	A	G	T
R. electromorpha	A	T	A	G	G	-	-	-	A	T	C	A	C	T
R. persimilis	A	C	A	T	A	-	-	-	C	T	C	G	A	A
R. zephyria	A	C	A	T	A	-	-	-	T	T	C	A	C	T
R. pomonella	A	C	A	T	A	-	-	-	T	T	C	A	C	T
R. mendax	A	C	N	T	A	-	-	-	T	T	C	A	C	T
R. cornivora	A	C	A	T	A	-	-	-	T	T	C	A	C	T
R. cingulata	A	C	A	T	A	-	-	-	T	T	C	A	C	T

Table B5. Statistics for nucleotide alignments. PI = parsimony informative, Ts = transition rate, Tv = transversion rate, R(s/v) = ratio of transitions to transversions, MP = most parsimonious, CI = consistency index, RI = retention index.

Genetic marker	COI	CAD	period	AATS	28S	Combined
Total sites	684	990	614	623	1359	4276
No. variable sites (excluding PI)	13	42	21	12	10	98
No. PI sites	86	58	44	17	27	235
% PI sites	12.57	5.86	7.17	2.73	1.99	5.5
Average *p*-distance ± SE (%)						
1 pos.	2.03 ± (0.10)	2.01 ± (0.06)	1.43 ± (0.08)	1.07 ± (0.04)	NA	NA
2 pos.	0.19 ± (0.02)	1.16 ± (0.04)	0.95 ± (0.05)	0.59 ± (0.03)	NA	NA
3 pos.	11.35 ± (0.48)	4.13 ± (0.14)	4.46 ± (0.17)	1.84 ± (0.14)	NA	NA
total	4.52 ± (0.20)	2.42 ± (0.07)	2.28 ± (0.09)	1.16 ± (0.06)	0.75 ± (0.03)	2.03 ± (0.07)
Ts	3.36 ± (0.14)	1.26 ± (0.04)	1.36 ± (0.07)	0.92 ± (0.04)	0.53 ± (0.02)	1.34 ± (0.05)
Tv	1.16 ± (0.07)	1.16 ± (0.03)	0.92 ± (0.03)	0.24 ± (0.02)	0.22 ± (0.01)	0.69 ± (0.02)
R (s/v)	5.07 ± (0.27)	1.17± (0.04)	1.79 ± (0.12)	2.89 ± (0.11)	2.62 ± (0.11)	1.92 ± (0.05)
G+C%	32.1	48.4	52.9	42.5	34.7	41
No. MP trees	4	191	11	8	2475	495
MP tree length	142	120	71	30	44	421
CI	0.81	0.87	0.93	0.97	0.91	0.85
CI (PI sites only)	0.79	0.79	0.9	0.94	0.88	0.8
RI	0.91	0.91	0.95	0.99	0.95	0.91

Table B6. Results of pairwise Incongruent Length Difference (ILD) tests performed on partitions.

Alignment	COI	CAD	period	AATS	28S
COI	-				
CAD	0.36	-			
period	0.13	0.22	-		
AATS	0.76	0.57	0.66	-	
28S	0.75	0.53	0.65	1.00	-

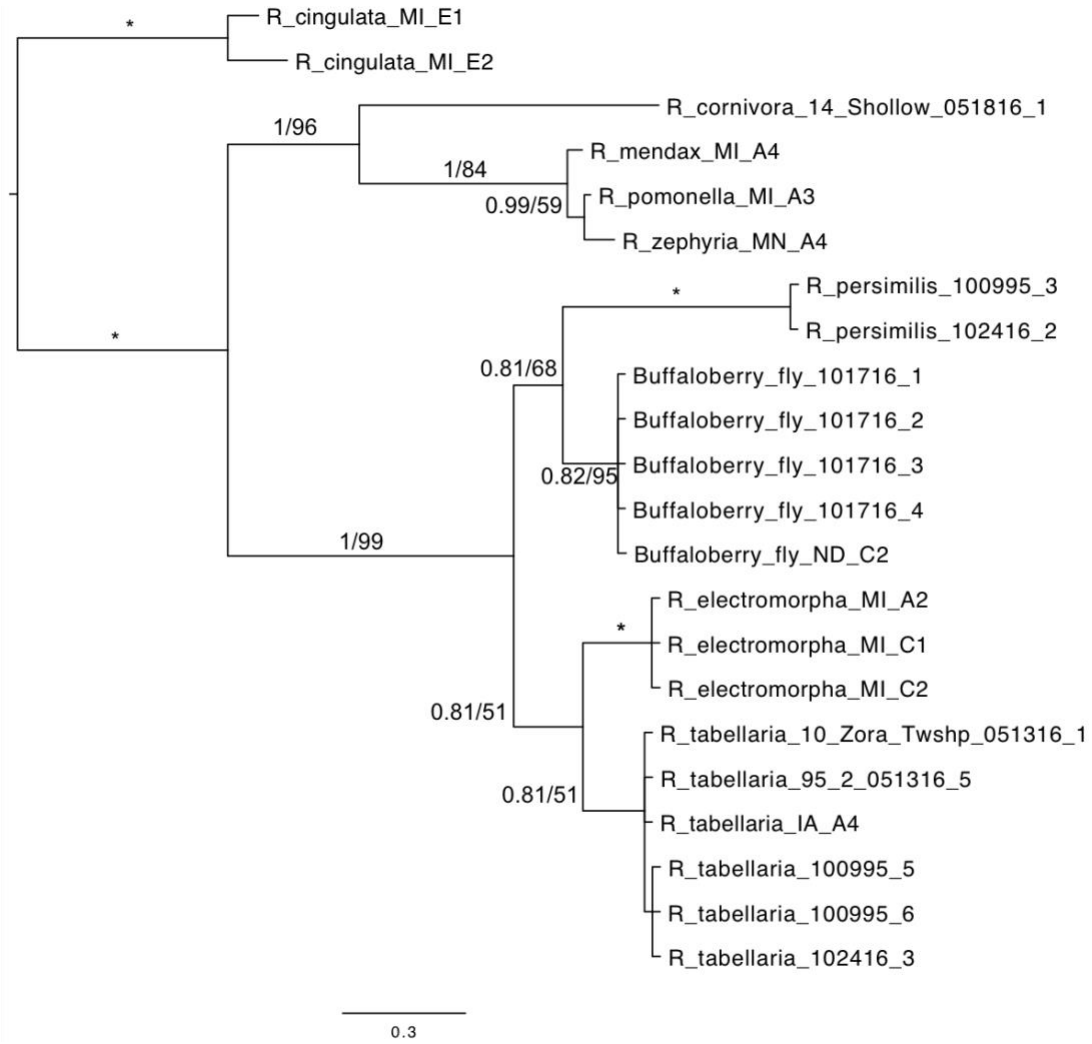

Figure B1. Bayesian phylogeny of the *tabellaria* species group, *pomonella* species group and outgroup *R. cingulata* inferred from an alignment of 684 bp of COI using MrBayes. Numbers next to branches represent Bayesian posterior probabilities and maximum likelihood bootstrap support respectively. Asterisks (*) over branches indicate a Bayesian posterior probability of 1.0 and 100% bootstrap support for the clade. Bayesian and maximum likelihood topologies were identical.

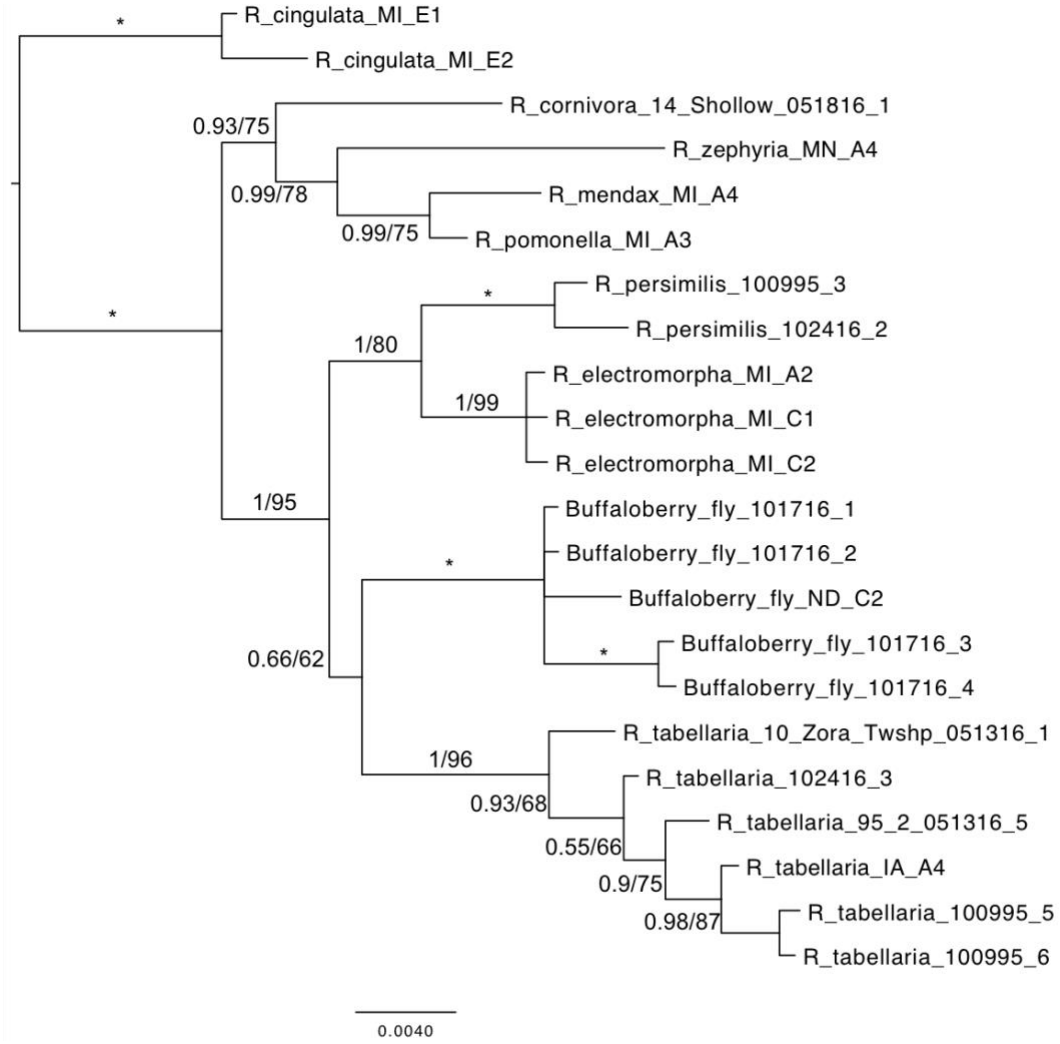

Figure B2. Bayesian phylogeny of the *tabellaria* species group, *pomonella* species group and outgroup *R. cingulata* inferred from an alignment of 990 bp of CAD using MrBayes. Numbers next to branches represent Bayesian posterior probabilities and maximum likelihood bootstrap support respectively. Asterisks (*) over branches indicate a Bayesian posterior probability of 1.0 and 100% bootstrap support for the clade. Bayesian and maximum likelihood topologies were identical.

Figure B3. Bayesian phylogeny of the *tabellaria* species group, *pomonella* species group and outgroup *R. cingulata* inferred from an alignment of 614 bp of period using MrBayes. Numbers next to branches represent Bayesian posterior probabilities and maximum likelihood bootstrap support respectively. Asterisks (*) over branches indicate a Bayesian posterior probability of 1.0 and 100% bootstrap support for the clade. Bayesian and maximum likelihood topologies were identical.

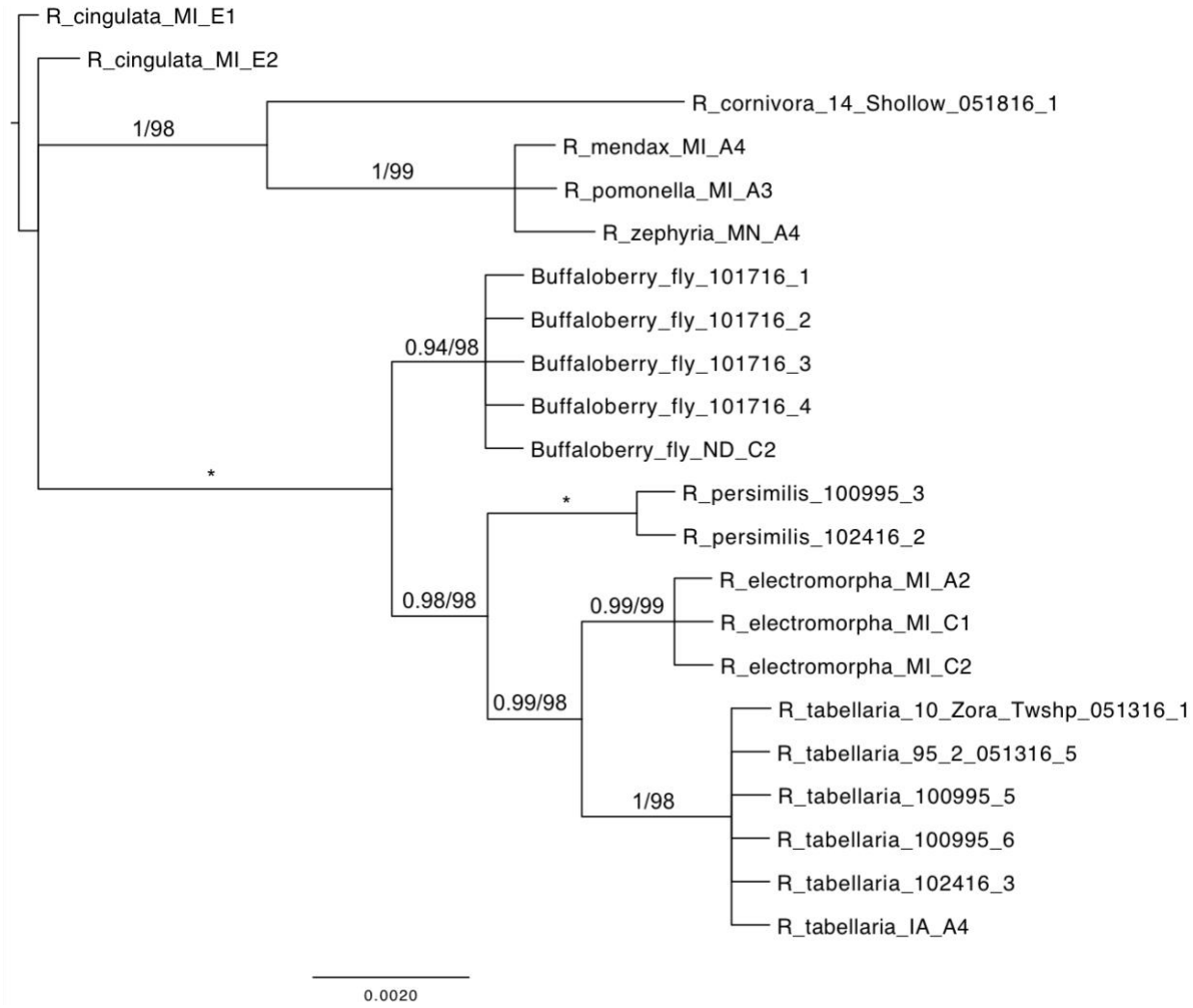

Figure B4. Bayesian phylogeny of the *tabellaria* species group, *pomonella* species group and outgroup *R. cingulata* inferred from an alignment of 623 bp of AATS using MrBayes. Numbers next to branches represent Bayesian posterior probabilities and maximum likelihood bootstrap support respectively. Asterisks (*) over branches indicate a Bayesian posterior probability of 1.0 and 100% bootstrap support for the clade. Bayesian and maximum likelihood topologies were identical.

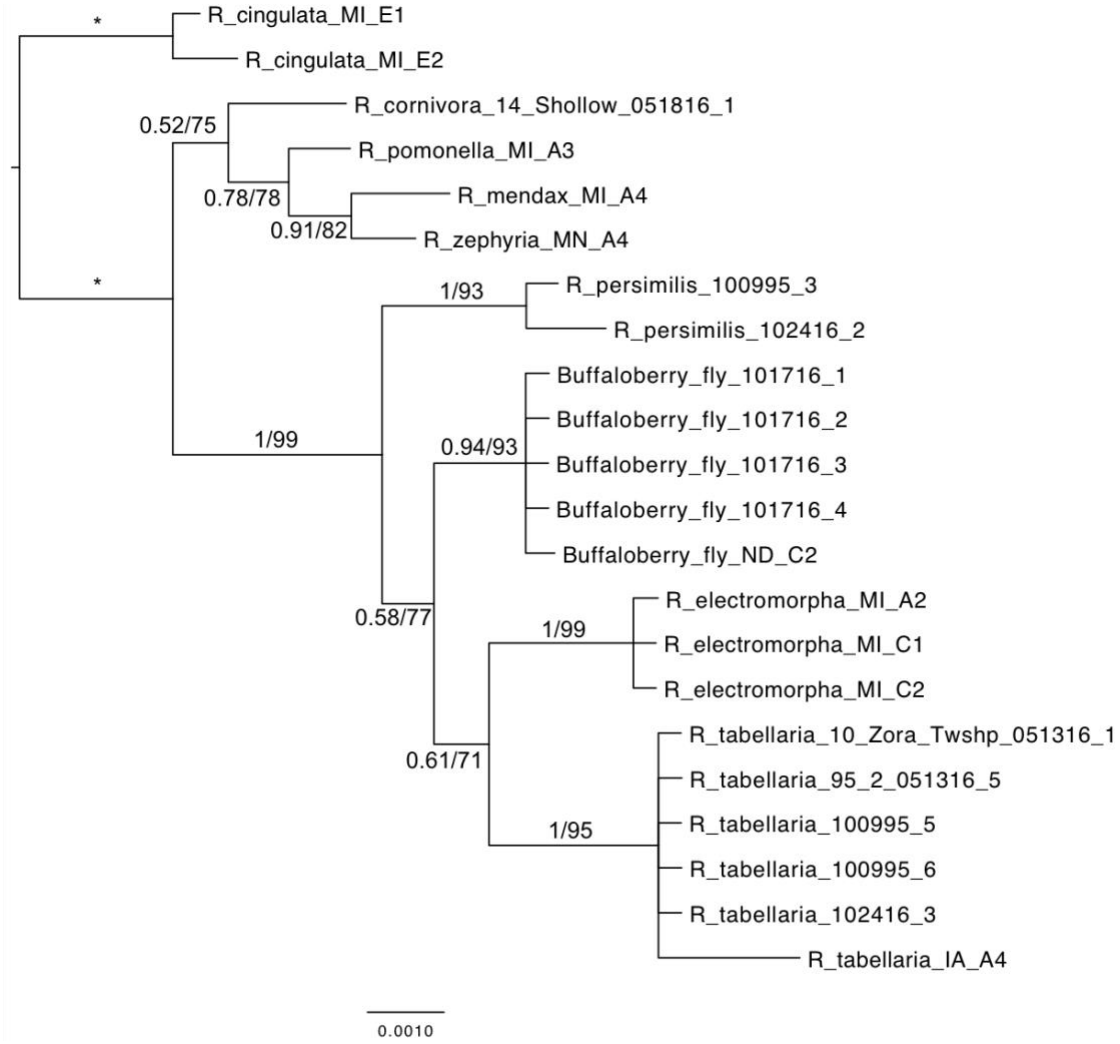

Figure B5. Bayesian phylogeny of the *tabellaria* species group, *pomonella* species group and outgroup *R. cingulata* inferred from an alignment of 1359 bp of 28S using MrBayes. Numbers next to branches represent Bayesian posterior probabilities and maximum likelihood bootstrap support respectively. Asterisks (*) over branches indicate a Bayesian posterior probability of 1.0 and 100% bootstrap support for the clade. Bayesian and maximum likelihood topologies were identical.

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