UNDERSTANDING A PEST—PHYLOGEOGRAPHY AND SYSTEMATICS OF THE PLUM CURCULIO (*CONOTRACHELUS NENUPHAR*)

by

SAMUEL N. CRANE

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2013

© 2013

SAMUEL NATHAN CRANE

All Rights Reserved

This manuscript has been read and accepted for the
Graduate Faculty in Biology in satisfaction of the
dissertation requirement for the degree of Doctor of Philosophy.

Date	**Chair of Examining Committee** Dr. Robert DeSalle, American Museum of Natural History
Date	**Executive Officer** Dr. Laurel A. Eckhardt
	Dr. Amy Berkov, The City College of New York
	Dr. Mike Hickerson, The City College of New York
	Dr. Andrea Sequeira, Wellesley College, MA
	Dr. Paul Goldstein, National Museum of Natural History **Supervisory Committee**
	Dr. Tracy Leskey, U.S. Department of Agriculture **Reader**

THE CITY UNIVERSITY OF NEW YORK

Abstract

UNDERSTANDING A PEST—PHYLOGEOGRAPHY AND SYSTEMATICS OF THE PLUM CURCULIO (*CONOTRACHELUS NENUPHAR*)

by

Samuel N. Crane

Advisor: Dr. Rob DeSalle

The plum curculio (*Conotrachelus nenuphar* Herbst) (Coleoptera: Curculionidae) is an economically and ecologically important pest in North America but is understudied despite having a long history in the scientific literature. Its chemical ecology, life history habits, and distribution are all well characterized. However, there is a dearth of knowledge concerning the population structure and evolutionary history of the species. This study aims to use methods from evolutionary biology to better understand an agricultural pest species and provide tools to aid in its management.

Existing taxonomic classifications of North American *Conotrachelus* species have been tested for the first time. Using a combined multigene approach, we have inferred a species phylogeny. Established species groups are well supported as monophyletic. However, the species groups identified in taxonomic keys are generally not recovered as monophyletic. Broadly sampling across the geographic distribution, we have sample over 1,000 individuals for mitochondrial DNA variation. We characterized population substructure of plum curculio populations from the full breadth of its range and reveal significant geographic and genetic structure. There is a significant north-south split that does not align with the current understanding of the plum curculio phenological strains. There are also highly structured populations corresponding to the Mississippi River and Apalachicola River basin, a pattern

thought to be associated with southern refugia along the Gulf Coast. There are likely multiple refugia used over the Last Glacial Maximum. Regions of the world that are most at threat of plum curculio beetle invasion, given the organism's habitat preferences, are identified across all continents and in every region where it is listed as a quarantine species. Molecular tools for diagnosing and managing the plum curculio, locally and internationally, are developed and provided for all life stages.

Acknowledgments

A project such as this bears one name but is the result of many contributions. To everyone who has supported me along the way, thank you.

I thank the following people for their efforts (successful or not) to collect specimens for this project: Tracy Leskey (Appalachian Fruit Research Station, USDA), Ted Cottrell (SE Fruit and Tree Nut Research Laboratory, USDA), Clement Akotsen-Mensah (Auburn University), Rick Weinzierl (University of Illinois), Donn Johnson (University of Arkansas), Russell Mizell (University of Florida), Karen Powers (Michigan State University), Willye Bryan & Mark Whalon (Michigan State University), John C. Wise (Michigan State University), Arthur Agnello & Harvey Reissig (NYS Agricultural Experiment Station), Celeste Welty (Ohio State University), Zachary Rinkes (Ohio State University), Ron Becker (Ohio State University), Phil Mulder & Andrew Puckett (Oklahoma State University), Daniel Mahr & Matt Stasiak (University of Wisconsin), Joanne Whalen (University of Delaware), Glen Koehler (University of Maine Cooperative Extension), Kathleen Leahy (Polaris Orchard Management), Bruce Barrett (University of Missouri), Alan Eaton (University of New Hampshire), Greg Krawczyk (Penn State University), Frank Hale (University of Tennessee), Thaddeus McCamant (Northland Community and Technical College), Kris Tollerup (Rutgers University), Gérald Chouinard (IRDA (Canada), Charles Vincent (Agriculture and Agri-Food Canada), Amanda Bakken (North Carolina State University).

Special thanks goes to Brian Hennings (Hoefler & Frere-Jones) for being an excellent driver and enthusiastic field assistant. This document is evidence that it turned out all right, so you're forgiven for the beetles you set free in the car.

Several museum collection visits were made in the course of this project and I would like to acknowledge my debt to the following individuals for hosting me and especially thank those who allowed me to sample for DNA from the museum collections: Charles O'Brien (private collection), Jens Prena (Smithsonian National Museum of Natural History), Bob Anderson (Canadian Museum of Nature), and Paul P. Tinerella (Illinois Natural History Survey).

My experience as a graduate student was much enriched by the teaching and mentoring that I was able to perform. I thank the following people for their guidance and support in helping me be the best instructor I could be: Victor S. Strozak (Center for Advanced Study in Education, CUNY), may he rest in peace; John H. Wahlert (Baruch College, CUNY); Jeanette Kim (College Now, CUNY); and the Education Department and REU Program at the American Museum of Natural History. I'd also like to thank my many students and mentees for being teachers in their own way and teaching me how to be a better teacher. Special thanks goes to Ella Massie-Schuh, Megrathea Cowan-Groden, and Berenice Villegas.

The research community at the American Museum of Natural History, in particular, and in New York City, in general, is a diverse and at times riotous family. I thank everyone who has ever talked with me in the halls or shared a thought over a beer with me during these many years. I collectively acknowledge and thank the many students, post-docs, and staff members at the AMNH, and especially members of the Section 20 genomics laboratory. Ellen Trimarco, in particular, was always so helpful in assisting me in the laboratory and listening patiently to my constant questions and comments. Some people have played an outsized role in my experience and have helped me not only professionally, but also personally: Lauren Esposito, for being more than just a colleague but also a friend; Sergios-Orestis Kolokotronis, with whom I had the good

fortune to share an office when first starting graduate school, for always being a reliable fount of information and ideas; and to Bryan Falk, we're almost there, it can't be long now.

Several people have helped me formulate my thoughts, given me critical introductions, or provided me with other forms of assistance that have been fundamental to the successful completion of this project and I would like to acknowledge my debt to them:

This project would not have happened without the help of Charlie O'Brien. He opened his home to me and I had the good fortune to spend my first spring break of graduate school working with Charlie in his collection. It was over these days of identifications and discussions of the taxonomic literature that we narrowed down the scope of my research project. The idea to study the plum curculio was all Charlie's, but I accept blame for any faults in the outcome. I still have all my notes from that week but I no longer have to refer to them to tell the difference between a mucron and an uncus;

Robert Anderson and Rocky Rockwell were both instrumental in getting into the CUNY program and helping me find my feet as a new doctoral student;

I'd like to thank Mark Siddall for always having an open door and a willingness to share his opinion;

I am grateful to John Flynn for giving me the opportunity to represent the collaborative program students in the new Richard Gilder Graduate School and for always being an enthusiastic supporter of the students;

It is difficult to overstate the contribution that George Amato makes in maintaining a collegial and productive laboratory environment at the Sackler Institute for Comparative Genomics. I have found it to be a wonderful and inspiring place to work as a graduate student and I am grateful for that.

Of course, I am deeply indebted to my advisory committee: Amy Berkov, Mike Hickerson, Andrea Sequeira, and Paul Goldstein. I'd also like to thank Richard Pearson, who sat on my proposal committee, and Tracy Leskey (USDA), who was a reader for the final dissertation.

Rob DeSalle, ultimately, is the reason all of this has happened. He not only supported me materially and professionally, but he also provided me with genuine encouragement and pointed advice when they were called for. I am forever indebted to his service for me and for creating the space for me to make my own mistakes along the way.

Finally, thanks go to my parents, Harold and Lavina Crane. Their love and truly unconditional support is the foundation from which I have been able to build everything else around me.

Funding was provided by National Science Foundation (IOS# 0922738) to Rob DeSalle, an NSF GK-12 Teaching Fellowship, an NSF-CUNY Alliance for Graduate Education and the Professoriate (AGEP) Summer Research Award, a Theodore Roosevelt Memorial Fund grant, and CUNY Dean K. Harrison Fellowship. Baruch College, CUNY Center for Advanced Study in Education, and CUNY's College Now Program also provided teaching opportunities. This research was supported, in part, under National Science Foundation Grants CNS-0958379 and CNS-0855217 and the City University of New York High Performance Computing Center.

Table of Contents

List of Tables

List of Figures

CHAPTER 1

Introduction: Understanding a Pest—Phylogeography and Systematics of the Plum Curculio (*Conotrachelus nenuphar*)

Samuel N Crane

Introduction

Increased productivity and yield from farm ecosystems has led to historic levels of human population growth. Sustained levels of economic growth and maintenance of low food prices on a global scale require enhanced gains in global food production, most of which is forecast to come from increased agricultural yields (FAO 2002; Sheppard 2004). A major cause of yield reduction is insect damage. These losses are mitigated largely by the application of insecticides, and pesticides will continue to be a necessary component of our collective efforts to achieve global crop production goals (Oerke *et al.* 1994; Pretty 2008). However, the deliberate release of neurotoxic poisons into the environment comes with a cost. These costs come in a variety of forms and are absorbed by the abiotic environment, non-target species, and human workers and consumers (New 2005). Over the long term, pesticide use is not a reliable option even for its intended purpose—pests become resistant and resurge after a time or secondary pests surge and become bigger problems than anticipated (Lewis *et al.* 1997). Ideally, farmers would be able to increase yields while simultaneously decreasing chemical inputs into agricultural ecosystems.

Integrated pest management (IPM) programs attempt to and often succeed in doing just that—reducing pesticide use while increasing yield (New 2005; Pretty 2008). IPM programs aim for effective management (vis-à-vis eradication) of pests by integrating ecological information (e.g. plant phenology) with control techniques (e.g. timing of pesticide sprays). Put most simply, IPM programs aim to control local populations at levels where the crop damage caused is below an economically damaging threshold (Thacker 2002). This target changes depending on the pest species, the crop, the relative local densities of crops and pests, and any number of other factors. Despite the need for ecological information in developing an IPM program, management plans are typically designed without knowledge of evolutionary history or pest population

demographics, including current population subdivisions. Phylogenetics and population genetics are two fields of biological research that are traditionally underutilized in agricultural systems, especially IPM programs. The goal of the research presented here is to apply the tools and methods of evolutionary biology to a problem in the agricultural sciences. The hope is that this demographic and evolutionary information can then be used to inform and improve management plans; ultimately contributing to increased yields from, and reduced chemical inputs into, agricultural ecosystems.

The focal system for this study is the plum curculio, *Conotrachelus nenuphar* (Herbst 1797) (Coleoptera: Curculionidae), a major agricultural beetle pest of commercial fruit. First, the plum curculio is briefly introduced and then a review of its biology given with an emphasis on current knowledge of the organism's status as a pest, its life history, distribution, and taxonomy. Next, the genus *Conotrachelus* will be discussed to provide taxonomic and historical context. Finally, the questions posed and hypotheses tested in this research program are briefly discussed and the overall aims stated in light of the goal of reducing pest pressure on commercial fruit operations.

The Plum Curculio, *Conotrachelus nenuphar*

> "That Plum Curculios are a most unmitigated nuisance, and, though most beautiful objects under the microscope, the fruit-growers of the United States, if they had their own way about the matter, would wish them swept from off the face of the Earth, at the risk even of interfering with the 'Harmony of Nature.'"
> Charles V. Riley, *4th Ann. Rept. St. Bd. Agr.*, 1868, pp 50-62.

The plum curculio, *Conotrachelus nenuphar* (Herbst 1797), is unique among *Conotrachelus* because of its prominence as a fruit pest in the United States and Canada. The species was likely known to pre-Columbian native American societies (Chapman 1938) and has been written about since colonial times, at least since the 1730's—predating its formal taxonomic description

by six decades. Records of European explorers between 1534 and 1542 note the use of plums by Native Americans, likely the Canada plum (*Prunus nigra*) and the American plum (*Prunus americana*). These records indicate that Native American groups dried plums to make prunes, and there's some evidence they had established methods to deal with a "worm" that would infest these fruits, of which plum curculio larvae are the only currently known such larvae (Chapman 1938). One of the earliest written records of the plum curculio was from a letter in 1736 between American botanist John Bartram and English merchant and botanist Peter Collinson (Darlington 1849). The insect is not mentioned by any name, only as being a pest of plums, apricots and nectarines in America. Peter Collinson advises John Bartram to control them by means of tobacco soaked water.

Very little was known about the beetle at this point but over the ensuing centuries, our knowledge of it has grown immensely. A German author formally described the species in 1797 (Herbst 1797). The first thorough report on the natural history and possible management practices for the beetle appears in the 3rd volume of *The Domestic Encyclopedia* published in the United States in 1804 (Tilton 1804). Dr. James Tilton, a Delaware physician and Surgeon General of the U.S. Army, wrote about the plum curculio, "the immense damage done, by an insect of this tribe, to the fruits of this country, of which there is no similar account in Europe, has given rise to the conjecture with some naturalists, that we have a peculiar and very destructive species in America." The species has remained a topic of interest for agriculturalists to the present day and is still acknowledged as peculiar and very destructive. The scientific literature for this species then spans over two centuries—from several decades before its taxonomic description in 1797 to the present day, some of which is reviewed in more depth in the following sections. Most studies of plum curculio have focused on understanding its life history patterns and

developing plans for controlling and mitigating the damage it causes. Reviews of this literature are given in (Quaintance & Jenne 1912; Chapman 1938; Racette *et al.* 1992; Vincent *et al.* 1999).

The species has received so much attention by agricultural researchers because of its broad host range, destructive capacity, and difficulty in monitoring. Without control, entire fruit crops will succumb to the depravations of the plum curculio. Today, everywhere it is found the beetle is a major agricultural pest on plums, apples, peaches, cherries, and blueberries. The adults feed on and the larvae develop within the fruit of these crops. The plum curculio is the greatest threat to peach and nectarine production in the southern U.S. and is a primary pest on apples in some areas, such as New England (Tracy Leskey, pers. comm.) and Minnesota (Thaddeus McCamant, pers. comm.).

To develop alternative, non-spray control techniques and improve monitoring efforts, recent studies have emphasized the phenology, host use patterns, and chemical ecology of the pest. Live laboratory colonies have been variously maintained since 1949 (Smith 1957a), with colonies currently maintained by Dr. Henry Fadamiro at Auburn University, Alabama; Dr. Mark Whalon at Michigan State University, Michigan; and Dr. Tracy Leskey at the USDA Appalachian Fruit Research Station, West Virgina.

Pest Status and Management

If left uncontrolled, plum curculio will decimate fruit crops. Pesticides are still the most commonly used management technology and the beetle is a major obstacle to organic and low-spray commercial fruit operations in the eastern United States and Canada. Crop damage comes from oviposition sites and from adult and larval feeding on fruits. The adult feeding punctures often deform the fruit and open up the skin to further damage by other insect pests or fungal

attacks. The developing larvae consume the flesh of the fruit and cause the fruit to drop from the tree before ripening. Both forms of damage are significant problems for fresh market fruits, and premature drop prevents the fruit even from being used as a processed food item. Some crops, such as cherries, are especially vulnerable because there is a zero-tolerance for pest infestation for processed food items (Hoffmann *et al.* 2010). The entire harvest from a cherry orchard will be denied if any sign of insect damage is found.

Plum curculio control strategies rely heavily upon pesticides. Historically, trees were physically jarred to remove adult beetles from infested trees and early chemical controls such as lead arsenate and DDT were employed (Quaintance & Jenne 1912; Smith 1957b). Traps are not sufficient to maintain populations below economic thresholds, but they are effectively deployed in monitoring programs (Pinero & Prokopy 2003; Leskey & Wright 2004). There are no standard biological control agents used against the plum curculio, though several nematode species are undergoing field trials (Shapiro-Ilan *et al.* 2002; 2008). Broad-spectrum insecticides are the only control measures that provide commercially acceptable levels of damage control and pest abatement. The organophosphate azinphos-methyl (Guthion®, Bayer CropScience), an acetylcholinesterase inhibitor, has been the most significant insecticide employed on fruit trees since its introduction in 1957. However, the Food and Quality Protection Act of 1996 (P.L. 104-170) has restricted registrations of azinphos-methyl for a number of years and the final registrations for the compound expired in September 2012. This chemical, the dominant chemistry deployed against plum curculio and other insect pests for more than 50 years, is no longer available to orchardists. The registration of another organophosphate, phosmet (Imidan®, Gowan Co.), remains in place but with further use restrictions. With the phase out of organophosphates, other less effective and more targeted chemicals will need to be optimized

and employed. Additional compounds under investigation for use against plum curculio are pyrethroids, insect growth regulators, neonicotinoids, and oxadiazines (Hoffmann *et al.* 2008). New methods of control and monitoring are also being actively researched to either replace or enhance the effectiveness of new limited-spectrum insecticides: biocontrol measures (Shapiro-Ilan *et al.* 2002; Alston *et al.* 2005; Jenkins, Mizell, *et al.* 2006a; Kim & Alston 2008; Shapiro-Ilan *et al.* 2008), pheromone attractants (Leskey & Prokopy 2000; 2001; Prokopy *et al.* 2001; Pinero *et al.* 2001; Prokopy *et al.* 2003; Leskey & Prokopy 2003; Pinero & Prokopy 2003; Leskey *et al.* 2005; Leskey & Zhang 2007; Leskey *et al.* 2008), growth regulators (Hoffmann *et al.* 2007; 2008), and novel trap design (Prokopy *et al.* 2000; Leskey & Prokopy 2002; Johnson *et al.* 2002; Leskey & Wright 2004; Leskey 2006; Lafleur *et al.* 2007; Lamothe *et al.* 2008; Leskey *et al.* 2008; Pinero *et al.* 2011). Without continued control of the pest, populations numbers will likely increase and fruit yields decrease.

Loss estimates for this species are rarely calculated, but there are a few reports available. The cost of control and loss to damage from fruit feeders (plum curculio being a dominate pest in this category) for peaches in the State of Georgia for the year 2004 was estimated at $2,336,650.00 USD (UG 2006). The same study reported the annual value of the Georgia peach crop for that year as $36,307,471.00, meaning the costs associated with fruit feeding pests for just peaches in Georgia represent 6.4% of the crop value. The 2010 production value of fruits for the entire U.S. are $2,220 million for apples, $761 million for sweet and tart cherries, $744 million for peaches and nectarines, and $6 million for plums (excluding California) (USDA-NASS 2012). So the value of the entire stone and pome fruit production in the U.S. is estimated at $3.7 billion annually. If the national economic damages from fruit crop loss and costs associated with pest management are estimated at a level consistent with peaches in Georgia,

then costs associated with fruit feeders in the US would be approximately $238 million annually. This is a very rough approximation but considering that the plum curculio is a pest in all States and Canadian Provinces east of the Rockies, a conservative estimate of the losses and management expenses due to plum curculio would be in the tens-of millions to low hundreds-of-millions of US dollars per year.

Conotrachelus nenuphar is listed as a quarantine pest by Regional Plant Protection Organizations covering Europe, Central America, and South America (EPPO, OIRSA, and COSAVE, respectively). Russia, South Africa, New Zealand, and several countries in Asia also categorize plum curculio as a quarantine pest. International trade of many stone and pome fruits is thus banned where plum curculio is a known problem. Plum curculio is a pest of global concern and proper species identification is of paramount importance for domestic control and foreign inspection and quarantine efforts.

Taxonomy of Plum Curculio

Excellent accounts of the classification and synonymy of *Conotrachelus nenuphar* Herbst are given in Quaintance and Jenne (1912), Chapman (1938), and Schoof (1942). The species was described or catalogued 7 times between 1797 and 1843. The original description is given by Johann Friedrich Wilhelm Herbst in 1797, predating the generic description of *Conotrachelus* (Herbst 1797). The description itself is a mere two short paragraphs, with only the dorsal view given as an illustration. The type locality was given as "North America" and the type specimen is likely at the Zoological Museum of Berlin, but this is uncertain. There are three generic treatments of North American *Conotrachelus* and each provided a specific description and key for *Conotrachelus nenuphar* (LeConte & Horn 1876; Blatchley & Leng 1916; Schoof 1942). Schoof's

description was the best documented and most thorough of them, but he did not examine the holotype. Nor did Leconte and Horn or Blatchley and Leng. In similar fashion, the holotype has not been examined as part of this phylogenetic and population genetics research. If the holotype is indeed lost then a neotype will need to be designated.

Much of the effort that went into these early descriptions and later classifications is poorly documented beyond the manuscripts themselves. LeConte and Horn (1876) states that, "I have many specimens before me, which show no variation worthy of note". A list of specimens examined is not given and the provenance of their specimens is not known. It is uncertain if they observed any, or the number if any, specimens from northern locations such as New England, upper Michigan, Wisconsin, or Minnesota. Blatchley and Leng (1916) and Schoof (1942) also did not provide a list of materials examined. The geographic distribution and provenance of their material is therefore unknown. The current location of Schoof's collection is also unknown. This is problematic because any effort to corroborate their findings with geographic populations will be unsatisfactory. Even during Schoof's time in the early 20th century, little was known about the life history differences between northern and southern populations (See Voltinism below). Certainly, JFW Herbst would not have had any knowledge about the distribution and variation of the plum curculio in the late 18th century when he described the species. Later authors gave no indication in their works that they did a better job of considering geographic variation across North America.

The evolutionary relationships of *Conotrachelus nenuphar* remain unresolved. The plum curculio belongs to one of Schoof's informal species groups (see Systematics and Taxonomy below): *Conotrachelus nenuphar*, *C. iowensis*, *C. juglandis*, *C. buchanani*, and *C. albicinctus*. Two more recently described species should be added to this grouping: *C. corni* and *C. downiei* because

of their close affinity to *C. iowensis* and *C. buchanani*, respectively (see Systematics and Taxonomy below). The phylogenetic relationships between these seven species are unknown. However, the species are morphologically highly similar, with a long, complex, and ongoing history of being confused for one another. Many of the anatomical characters used to discriminate the species vary only subtly, often overlapping, and identification is not trivial. These putatively closely related species use many different host families: Rosaceae (*C. nenuphar*), possibly Fagaceae (*C. iowensis*), Juglandaceae (*C. juglandis*), Cannabaceae (*C. buchanani* and *C. downiei*), and Cornaceae (*C. albicinctus* and *C. corni*). Different anatomical features group them together in different fashions (Schoof 1942). They all occur sympatrically in the eastern half of North America and have similar life history patterns.

The species is currently diagnosed from these closely related congeners by: the lateral shape of the mesoscutellum, vestiture of the elytra, the shape of a barb-like process on the tibia (the metauncus), and features of the male genitalia (Schoof 1942). These last two characters are particularly troublesome because they are restricted to adult males, and are therefore of limited utility in species recognition. Larval and pupal characters have not been adduced for discrimination of the plum curculio from its congeners. The larvae are the life stage most likely to be transported in late season fruit, especially from the southern extent of its range. Pupae may be transported in soil along with tree seedlings or transplants. To address adequately the taxonomic concerns about *Conotrachelus nenuphar* and improve diagnostics, an examination of molecular characters is warranted.

Life History and Distribution

The entire life cycle of one generation typically spans one year. In the spring, around the time young fruits are first developing and are approaching a centimeter in width, adult female plum curculios oviposit under the skin of the immature fruit, leaving a telltale crescent-shaped scar. A single female will only deposit one egg per cavity but may oviposit multiple times in the same fruit. Multiple individuals may oviposit on the same fruit. Individual females have been observed to lay as many as 9 eggs per day and on average may lay from 76 to over 300 eggs in a season (Quaintance & Jenne 1912; Smith & Salkeld 1964). The larvae burrow into the seed cavity where they spend several weeks maturing. Infected fruit drops from the tree prematurely and the grubs emerge and excavate a small cavity in the shallow soil, typically within the first few centimeters. There they pupate and the adult weevils emerge from the ground a month or more later. These summer generation adults will feed on mature fruits into the fall, when weather conditions prompt them to hibernate until the following spring.

The spring generation adults appear in numbers in April and May. Larval and pupal development occurs during May, June and early July. Summer generation adults emerge in June, July, and August. Adults may feed into October. The exact dates vary by year and latitude, often being subject to temperature more than host plant phenology. In the southern extent, individuals may go through a second round of breeding and oviposition in the summer, giving rise to a second annual brood—a late summer generation. The number of annual broods—voltinism—of the species is controlled by the necessity of reproductive diapause. Populations in the northern extent of the range have an obligate diapause whereas populations in the southern range have a facultative diapause (see Voltinism below). For this reason, summer and fall harvested fruit may have viable larvae in them in the southeastern United States, though this is rare.

The plum curculio is endemic and native to North America. Native hosts include hawthorn trees (*Crataegus spp.*), crabapple trees (*Malus spp.*), and wild plum (*Prunus spp.*), all Rosaceae. The adult beetles will feed on the fruits of a great many kinds of rosaceous and ericaceous plants: plums, apples, peaches, nectarines, cherries, apricots, pears, strawberries, quince, blueberries, haws, huckleberry, as well as grape (Vitaceae), gooseberry and currant (both Grossulariaceae), persimmon (Ebenaceae), and if given the opportunity will even feed on tropical fruits not available within its current range (Quaintance & Jenne 1912; Chapman 1938; Hallman & Gould 2004). The beetle discriminates among these potential food sources and prefers stone and pome fruits–especially plums, peaches, cherries, apricots, apples, and pears (Jenkins, Cottrell, *et al.* 2006b; Leskey & Wright 2007). Females will oviposit in these fruits, and larvae can successfully develop in any of them. Larvae have even been known to develop in fungal black knot (*Plowrightia morbosa*) on cherry trees (Quaintance & Jenne 1912; Jenkins, Cottrell, *et al.* 2006b).

The geographic range of the plum curculio is limited to the United States and Canada east of the Rocky Mountains. The limits of the weevil's distribution are illustrated in Figure 1, based on the account given in Chapman (1938). The western limit of occurrence was established in 1910 during an exhaustive survey beginning in Sherman, Texas and proceeding northward, crossing east and west along the route to determine where the species' abundance declined. A detailed account of these efforts is given in Quaintance and Jenne (1912). The northern limit is well established by recorded observations extending back to the late 1800's and does not extend much beyond the 49° latitude. Southern and eastern borders are fixed by the Gulf of Mexico and Atlantic Ocean. There are no established populations of plum curculio in the western United States, except for an infestation in Box Elder County, Utah dating to the 1980's, primarily of

fruit trees in home yards and wild plums (Alston *et al.* 2005). There are no known established populations of the plum curculio outside of North America.

The dashed line given in Figure 1 delimits the boundary below which the species is bivoltine, capable of having summer broods. This is the line proposed by Chapman (1938) and was determined empirically from reports of where the species was single brooded and where it was observed to be multiple brooded. Chapman's methods are given in full below:

> "The line in Fig. 6 [here, Figure 1] which indicates the boundary between the areas where the species is single and multiple brooded is, of course, only approximate. Its exact position varies from season to season. Furthermore, it has been necessary to guess where the boundary lies in several areas where information on this question is not available. In the northern portion of the multiple brooded zone there is normally only a small percentage of a second brood produced; in some seasons none. [A list of published sources for records of broods is given.]"

Little effort has been made to corroborate this observation, but among fruit growers and agricultural scientists currently working with the pest, it is regarded as valid. Still, there is little evidence to either support or refute the line proposed by Chapman. Multiple brooded individuals were observed in West Virginia (Leskey 2008), which would seem to contradict Chapman. However, Chapman has pointed out that his boundary is approximate and in reviewing his cited sources, he does not cite any workers from West Virginia. So there is no reason *a priori* to suppose that mid-Atlantic populations are univoltine. The transition zone from univoltine to multivoltine populations is therefore not well known and the possibility that some populations well north of Chapman's boundary have both strains remains plausible. Likewise, bivoltine populations may occur north of Chapman's boundary but have gone unrecognized all these years because in the wild they only ever have one summer generation and the local plum curculio fauna have not been adequately characterized from laboratory rearing and dissection.

Voltinism

Voltinism is defined as the number of generations an insect is capable of having per year. There are two phenological strains of the plum curculio. In the northern range of the species, the populations are univoltine and individuals experience an obligate reproductive diapause—oocytes in females do not develop until after hibernation (Smith & Salkeld 1964). In the southern portion of the range, populations are multivoltine and can continuously reproduce so long as conditions are favorable. These multivoltine populations have a facultative diapause—eggs can develop fully before hibernation. Not all populations always produce a late summer generation and even when they do, not all individuals within a brood will develop mature eggs, breed, and oviposit. So even under favorable conditions, the second summer generation is only partial.

Knowledge about plum curculio voltinism can be split into roughly three phases: early when it was not known that there was a multivoltine strain (prior to 1915), mid when it was known that there was a summer brood in the south but before the establishment of colonies to study the organism, and late (since 1964) when plum curculio colonies allowed more detailed study of the organisms anatomy and reproductive strategies.

The early literature on plum curculio is conflicted on accounts of the species' being univoltine or multivoltine, principally due to geographic variation in the locations of the workers studying the beetle. This was complicated by the fact that in the south there is only a partial second generation and in some places and some years, none at all. There were tendencies to ascribe to the whole of the species traits observed in only one region at one time.

An early and thorough account of the species as a pest of fruit trees was published in 1865 based on field observations in New Jersey by Isaac P. Trimble, entomologist of the State

Agricultural Society of New Jersey (Trimble 1865). At this time the plum curculio was already widely acknowledged as a common and particularly destructive pest. Trimble's book was written for the lay public and general orchardist—perhaps one of the earliest examples of agricultural extension service. Trimble writes that, "The idea of some writers, that there are two generations of this insect that prey upon the fruits the same year one generation early in the season, another later has been proved to be erroneous. Two generations there are undoubtedly living at the same time, but I have only been able to find the egg in the female of the older generation." Trimble acknowledges the account of some workers that there are two generations per year but here discounts those claims based on his observations that the summer generation adults are not developing mature eggs—the first clear record in the literature that establishes reproductive maturity as the diagnostic between plum curculio strains. From this he, and subsequent authors, concluded that the plum curculio as a whole has but one generation per year and the summer generation survive the winter as reproductively immature adults and it is these adults that appear and feed on fruit early in the season of the subsequent year. However, in the paragraph just before the preceding quote, Trimble notes in passing that, "[First summer generation plum curculio] that have been kept in a green-house until mid-winter, as they have been by Peter B. Mead, myself, and some others I have heard of, will be found pairing, but this must be considered as the effect of the artificial temperature." This is revealing in that contemporary authors have noted southern strain plum curculio with facultative diapause will often breed in the laboratory when given favorable conditions (temperature, photoperiod, food, and oviposition substrate) whereas northern strain with obligate diapause will not, regardless of favorable laboratory conditions. So the fact that Trimble has observed plum curculio populations around New Jersey mate in mid-winter under favorable artificial conditions implies that these mid-

Atlantic populations are in fact true southern strain. But Trimble could not have known this yet and so he concluded that plum curculio is single-brooded.

Other authors were equally conflicted and confused by their varied observations. This point is well illustrated by the vacillations of two colleagues, Benjamin D. Walsh of Illinois and Charles V. Riley of Missouri. Both men were born in England but became naturalized American citizens. Walsh was the first State Entomologist of Illinois from 1867 until his untimely death in 1869. Riley was the first State Entomologist for Missouri from 1868 to 1877, when he went on to work for the U.S. Department of Agriculture and as the first entomology curator at the Smithsonian, where many of his collections reside.

Walsh, a contemporary and correspondent of Charles Darwin (Sheppard 2004), stated in April 1867 that, "There is little doubt now, in my mind, that the 'Curculios' bred from the fruit of one year are the same individuals that puncture the fruit of the following year", coming to a similar conclusion as Trimble before him that the plum curculio is single-brooded and the beetles survive the winter as adults. Riley held opposing opinions around this time. In an anonymous article signed "V" to the *Prairie Farmer* in July 1867, Riley claimed that the plum curculio occasionally had two broods in a year. However, by September of that same year, Riley had been convinced that the plum curculio did in fact have only a single brood per year, as he stated during the 12th annual meeting of the Illinois State Horticultural Society (Riley 1868). He cited both Walsh (1867) and Trimble (1865) to support his claim. But Walsh and Riley would be in agreement on this issue only from September to December of 1867, when Walsh reversed his position and wrote in his first report as Illinois State Entomologist in 1867, "I find that there are two distinct broods of the Plum Curculio every year" (Walsh 1867). Riley, however, maintained that the plum curculio only had one brood. In his first report as Missouri State

Entomologist, he criticized Walsh's 1867 report and in reviewing Walsh's observations came to the opposite conclusion. So at this point, Walsh and Riley had reached reversed and opposing opinions as to the number of annual plum curculio generations.

Both men were esteemed State entomologists and together founded and edited the journal *American Entomologist*. Walsh and Riley had agreed to discuss the issue of plum curculio voltinism in front of the 14th annual meeting of the Illinois State Horticultural Society held from December 14th to 17th, 1869. However, a mere four weeks before that debate, on November 18th, B.D. Walsh died in a railroad accident. Riley addressed the Society by himself and in his published account of the address wrote, "It was on account of this difference of opinion between us, that we could never editorially touch upon the point in the columns of the *American Entomologist*; though we had each of us decided to come to an agreement, in accordance with the facts to be elicited in discussion at this meeting. Alas! How inscrutable are the ways of Providence!" One is left to wonder if they had not already come to some sort of an agreement on the issue prior to Walsh's sudden, unexpected death just weeks prior to the meeting. After a brief discussion of the significant impact that climate can have on insect physiology, Riley continued, "No one with knowledge of such facts, would for a moment doubt that in certain southerly latitudes, it is possible for the Curculio to be double-brooded, and, yet be single-brooded in more northerly regions." And so this, published in 1870, just after the death of B.D. Walsh, is the first instance in the literature that there was a proposed relationship between latitude and the number of plum curculio broods per year. Riley thereafter maintained that the plum curculio was primarily single-brooded everywhere but that in certain southern localities it would occasionally have a second summer brood: "as far south as St. Louis not more than one per cent of the beetles lay any eggs at all, until they have lived through one winter; or in other words, where one female

will pair and deposit a few eggs the same summer she was bred, ninety-nine will live on for nearly ten months and not deposit till the following spring. In more northern latitudes I doubt if any exception to the rule will be found" (Riley 1871).

In the early 20th century, a exhaustive report based on field experiments and literature reviews was published by Altus L. Quaintance and Eldred L. Jenne, both working for the U.S. Department of Agriculture (Quaintance & Jenne 1912). They also concluded, in support of Riley, that most populations of the plum curculio gave rise to a single generation per year. In their rearing experiments near Washington D.C. they observed very few late summer adults, but did successfully rear a second generation of adults on peaches.

So in the 50 years between Trimble (1865) and Quaintance and Jenne (1915), three clear facts had been established regarding plum curculio voltinism. First, that there was a single brood every year, with the spring adults being individuals from the previous summer generation that had over-wintered as adult beetles. Second, that in certain southern localities the climate was such that a second summer generation might occasionally develop, though the size of this second brood was never as large as that of the first brood. These early authors largely discounted the impact of the second brood to the point of disregarding it as an important feature of the organism's life history. Third and finally, that in localities as far north as Illinois and New Jersey, individuals could be brought into an insectary and observed to breed and oviposit under favorable artificial conditions. This behavior was often noted but never discussed at length, as the authors at the time did not know about the relationship between reproductive diapause physiology and the second brood.

Attitudes towards the second brood changed after a massive outbreak of plum curculio on peaches in Georgia in 1920. Most of the peach crop was destroyed and this prompted a

prolonged study of the life history and management techniques in use across the Georgia peach belt (Snapp *et al.* 1922; Snapp 1923; 1930). This work was conducted under the supervision of Oliver I. Snapp, Division of Deciduous Fruit, Bureau of Entomology. From these studies, he concluded that, "the most important truth revealed as a result of these studies was the establishment as a scientific fact that in the latitude of Central Georgia there occurs annually two generations of the plum curculio, and that a high precentage [sic] of the larvae that renders the best late varieties of peaches unmerchantable in Georgia are larvae of the second generation." And with this Snapp elevated the pest status of the summer brood to that of the overwintered, spring brood.

After Snapp's work on the beetle, a few reports came in from disparate places that acknowledged the existence of a second summer brood. Besides Georgia, reports of a second summer brood were filed from Illinois, Indiana, North Carolina, Maryland, Virginia, and Delaware. Perhaps the first instance of the northern strain and southern strain designations used in print were from a report out of Delaware in 1931 that found the state's plum curculio populations split, with southern Delaware populations capable of a summer brood and northern Delaware populations capable of only the single annual brood even when reared under identical conditions (Stearns 1931). The various reports on plum curculio voltinism were summarized and used as a basis for the plum curculio strain distribution map drawn in Chapman (1938).

Through the 1940s and early 1950s research continued on the plum curculio across the U.S. and Canada, with many regional management recommendations and new chemical control experiments being published. Significantly, in 1942 Schoof published his taxonomic manuscript of *Conotrachelus*. He cited very few articles from the agricultural literature: Quaintance and Jenne (1912), Snapp (1930), and Chapman (1938). Despite the rush of literature ten years prior

documenting variation in plum curculio voltinism, and his reference to Chapman's distribution map, he neglected to make any mention of the two strains.

In 1957, a method for rearing plum curculio continuously in a laboratory setting was published (Smith 1957a). This was followed a few years later by the first examination of ovary development in the two strains (Smith & Salkeld 1964) and further improvements in the rearing techniques (Hays & Cochran 1964; Broersma & Hays 1966). These studies allowed researchers to better understand the interaction between latitude, diapause, ovary development, and breeding cycles and marked the transition into the current stage of our understanding of plum curculio voltinism.

Smith and Salkeld (1964) dissected the maturing ovaries of northern and southern strain plum curculio adult females and laid out how the breeding behavior of the two strains was related to their diapause behavior. They established that northern strain females do not develop mature oocytes prior to diapause, and that southern strain females do. This was the first demonstration that the diapause behavior between the strains was different, with the northern strain required to diapause to develop oocytes. To this day, this is the only reliable method to distinguish the strains and their diapause behavior.

During the period between 1915 and the study of plum curculio oocyte development in 1964, the distribution and importance of plum curculio strains was worked out in some detail. It was recognized that the second summer generation could be as destructive as the spring and first summer generation. The transition between the two strains at a north-south gradient roughly along the 38th parallel north was established by the synthetic work of Chapman and encapsulated in his distribution map of 1938, which is still in use today. The first plum curculio colonies were

established and the relationship between ovary development and voltinism for this species was demonstrated.

Since this time, work has continued unabated towards understanding the behavior, host plant interactions, chemical control, regional impacts, monitoring, and trapping of the plum curculio. Over 120 original research articles on the plum curculio have been published in the scientific literature over the last 55 years. Research into the pheromone attractants, trap design, monitoring, and integrated pest management (IPM) programs of the plum curculio have been especially common since the prolific work of Ronald J. Prokopy in the 1990s at University of Massachusetts in Amherst. All through this time, the actual plum curculio individuals studied were often ascribed to the northern strain or southern strain group based solely on their geographic location. For example, the study that first isolated a male aggregation pheromone, grandisoic acid, from northern and southern strain adult plum curculios obtained their southern individuals from Florida and their northern individuals from central Illinois (Eller & Bartelt 1996). The authors noted that, "The single-brooded northern strain and the double-brooded southern strain are reported to be reproductively incompatible, but both strains were found to produce grandisoic acid. Therefore, it is unlikely that pheromone differences contribute to reproductive isolation of the strains" (Eller & Bartelt 1996). Note however, that the reproductive incompatibly mentioned was between laboratory-maintained multivoltine individuals in New York and a wild-collected univoltine individuals from New York (Padula & Smith 1971). Padula and Smith (1971) did not state the original provenance of the laboratory maintained individuals. The grandisoic acid study involved individuals from Illinois and Florida. There was no discussion of geographic variation across Illinois, Florida, and New York. The southern strain individuals are treated as a defined group and the northern strain individuals are treated as a second defined

group, seemingly based off geography. This was a reasonable approach to take because of our historic understanding of plum curculio voltinism as outlined above. There are two strains of plum curculio and their distribution was known, or was thought to be known. This was only problematic near Chapman's boundary. In these situations, the strain status of a locality could be determined by dissection of the summer generation adults, or as shown by Smith and Salkeld (1964), by continuous rearing in laboratory. More recent work in West Virginia has shown that, despite the fact that the state is entirely north of Chapman's boundary, there are multivoltine individuals there (Leskey & Wright 2004; Leskey 2008). Studies of plum curculio would benefit from a more accessible form of strain diagnosis and further characterization of the phenological strain distributions.

These strain designations have no taxonomic weight and merely indicate the assumed voltinism of any given population. To what degree the strains differ in other aspects—e.g., reproduction, host preference, phenology, morphology—is unknown or poorly characterized. The species status of the plum curculio has been unquestioned by agricultural researchers. Conversely, taxonomic workers have largely ignored the strain designation used by agricultural workers, even though such a distinction may point towards interesting (significant) systematic and evolutionary questions. This schism between taxonomists and ecologists perversely leaves open the question of taxonomic distinction between the ecotype strains.

Previous Molecular Research

Molecular work on the plum curculio started in 2004 with a strain discrimination study using RAPD-PCR (McClanan *et al.* 2004). The authors amplified polymorphic DNA regions from a single Massachusetts (MA) population and a single Georgia (GA) population. They

found 4 markers that resolved unique banding patterns for the MA northern strain and the GA southern strain. The authors cautioned that their findings were limited because of the small number of locations sampled and that there might exist hybrids between the strains. To date, there is still no reliable, non-destructive method for determining strain identity (without dissecting female ovaries).

A population genetic analysis was also undertaken in an attempt to diagnose the strains (Zhang *et al.* 2008). This study sampled 50 adult beetles from 11 locations along the mid-Atlantic, between Virginia and Massachusetts. The authors sequenced all individuals for a portion of the mitochondrial cytochrome oxidase *c* subunit I (*COI*) gene. The locus resolved two unique (disjunct) haplotype networks, one contained in the New York and Massachusetts populations and the other spread between New Jersey and Virginia. This roughly corresponds to the northern strain and southern strain distributions and supports the RAPD-PCR results. This study was the first of its kind for the plum curculio and was restricted in scope to the Mid-Atlantic States. The study area represents only one tenth of the total geographic range of the species and all localities sampled had fewer than 10 individuals, with 3 of the 11 localities being represented by only 2 individuals. The authors noted the promise of this marker for distinguishing the strains, but cautioned that, "In future studies, increasing sample size, testing more northern strain weevils; and inclusion of the *COII* gene or other molecular markers to enlarge the informative sites to more fully understand the evolution of plum curculio strains should be considered."

Systematics and Taxonomy of eastern North American *Conotrachelus*

Described by French Colonel and entomologist Pierre François Marie Auguste Dejean in 1835, *Conotrachelus* is a New World beetle genus with approximately 1,200 named species (Dejean 1837; O'Brien & Wibmer 1982). The generic name is based on the cone-shaped thorax typical of the group. The majority of *Conotrachelus* diversity is concentrated in South America and Central America. Despite containing numerous agricultural pests, the genus has not been subject to revisionary work in decades. Karl Fiedler published (1940) the most comprehensive revision and key to date, but treated members only from South America. For North American taxa, Leconte and Horn (1876), Blatchley and Leng (1916) and Schoof (1942) are the major works. In North America (NA) north of Mexico there are 63 currently described species. However, many authors have ignored the west, which has a slightly different *Conotrachelus* fauna than the east, and have focused their efforts on those species observed in eastern North America (ENA), here defined as north of Mexico and east of the Rocky Mountains. There are approximately 46 nominal species in eastern North America (O'Brien & Wibmer 1982). Leconte and Horn (1876), Blatchley and Leng (1916) and Schoof (1942) all dealt with a subset of these eastern NA species.

Leconte and Horn (1876) divided eastern North American species into 5 groups in their key. Division 1-A contains 9 species including *C. nenuphar*. Division I-B contains 9 species separated from I-A primarily by their uninterrupted elytral costae. Division II contains only 3 species, Division III only one, and Division IV has two species. Diagnostic characters for these divisions are discussed later (see Chapter 2). A total of 23 species from eastern North America were included in the key and their classification is summarized in Table 1.

Blatchley and Leng (1916) divided their focal taxa into 6 groups for convenience in navigating the key. Group I is referred to as the "*nenuphar* group" and contains 9 species. Group II ("*crataegi* group,") has 5 species; Group III ("*posticatus* group") 7 species; Group IV ("*anaglypticus* group") 4 species; Group V has only one species, *C. fissunguis*; finally, Group VI ("*erinaceus* group") has two species. A total of 27 species were treated, largely overlapping with Leconte and Horn (1876).

Schoof (1942) treated 28 species from the north-central US and placed them into 4 groups in his key. Schoof's intent was to "exhibit the phylogenetic relations of the species more clearly" but this was still a phenetic classification and could potentially reflect convergent evolution on the morphological traits studied. In Group I, Schoof rejected the "*nenuphar* group" designation because "only part of the group… is closely related to *nenuphar*", and included 15 species within it. In his conception, Group II has 8 species, Group III has 3 species, and Group IV is composed of only 2 species. Within Group I there are further divisions (species groups) that Schoof discussed but did not formally recognize in his key. Schoof acknowledges an affinity between species [*juglandis*, *nenuphar*, *buchanani*, *albicinctus*, + *iowensis*]. He also recognized within Group I two putatively closely related groups of species: [*retentus* + *affinis*] and [*elegans*, *hayesi*, + *aratus*]. The morphological characters that underlie his argument are given in each of his individual species descriptions.

There is considerable agreement in the groupings between the three classifications, which are compared in Table 1. The major difference is in Blatchley and Leng's Group II and Group III. They recognized an additional grouping in their classification that LeConte and Horn and Schoof did not, splitting Division I-B of LeConte and Horn and Group II of Schoof into two distinct groups. The other differences are mostly due to taxonomic sampling of the genus, with

some authors treating species that were ignored by the others (see Group I of Schoof). Despite these differences, group composition is overall very consistent between the classifications.

Two species have been added to the North American classifications since the 1940s. These are *C. corni* by (Brown 1966) and *C. downiei* O'Brien and Salsbury (in review). *Conotrachelus corni* falls out with *C. iowensis* in Schoof's key but lacks the white patch between the elytral costa, rather resembling the coloration pattern of *C. nenuphar* but being much smaller in size. *Conotrachelus downiei* is aligned with *C. buchanani* in Schoof's key and is separated from it primarily by features of the setae on the elytral declivities and the size differences between the two groups (*C. downiei* being smaller). Both new species are placed into Schoof's Group I.

Conotrachelus is species-rich, with a great diversity of morphological variation, host use patterns, feeding and foraging behaviors, and other life history traits. Our understanding of this diversity is poor. The genus contains several minor and major agricultural pests, including additional pests on pear and quince (Rosaceae; *Conotrachelus crategi*), walnuts (Juglandaceae; *Conotrachelus juglandis* and *Conotrachelus retentus*), pecans (Juglandaceae; *Conotrachelus hicoriae* and *Conotrachelus aratus*), guava (Myrtaceae; *Conotrachelus psidii*), avocados (Lauraceeae; *Conotrachelus perseae* and *Conotrachelus aguacatae*), and cocoa (Malvaceae; *Conotrachelus humeropictus*) among others. Developing a robust phylogenetic framework for comparative studies of *Conotrachelus* is an urgent need because of recent interest in developing non-spray approaches to pest abatement that capitalize on behavioral and physiological features of the pest species, reassessment of species status for agricultural pests, and ongoing research into the evolution of host-plant colonization and plant-animal interactions among these diverse insects (Pinzón-Navarro *et al.* 2010). The accurate inference of species boundaries is especially important in agricultural pests, because they have significant consequences for agriculture,

commerce, international trade, and law enforcement (e.g., quarantine and inspection efforts). However, the phylogenetic relationships among and evolutionary origins of these pest species are unknown. If Schoof's groupings are considered phylogenetic hypotheses, they have not been tested using modern analytical methods or genetic data.

Research Objectives

The preceding sections provided an overview of the state of knowledge concerning the plum curculio and its co-occurring congeners. The plum curculio is an economically and ecologically important pest but is understudied despite having a long history in the scientific literature. Its chemical ecology, life history habits, and distribution are all well characterized. However, there is a dearth of knowledge concerning the population structure and evolutionary history of the species. There is also cause to question the veracity of previous findings about the plum curculio because of the uncertainty surrounding differences between geographic populations and their voltinism. The genus has not been subject to revisionary taxonomic work in over 70 years and the established classifications have never been tested using phylogenetic systematic methods. *Conotrachelus nenuphar* pest populations have been genetically profiled from mid-Atlantic localities but this area represents less than a tenth of the total range of the species and hence these diagnostics are not reliable enough for implementation under a regulatory framework. The species' biogeographic history is utterly unknown. Despite its voracity and status as a quarantine pest, the invasion potential of plum curculio across significant fruit producing regions where it is not currently established has not been evaluated.

This research program aims to fill these gaps in our knowledge about the plum curculio, which is perhaps one of the most destructive insects in North America, but little studied

compared to other fruit pests because of the management successes of organophosphate insecticides. Now that the main tool against plum curculio (and other insect pests), azinphos-methyl, has been phased out phased out in the United States, the plum curculio may resurge locally and place more pressure on quarantine zones as native plum curculio populations are released from chemical management. With an eye toward future progress in pest management of plum curculio, the goals of this research program are to:

1.1 Use molecular data to test the established taxonomy and species groupings of *Conotrachelus* in eastern North America.
1.2 Explore the phylogenetic placement of *Conotrachelus nenuphar*.

2.1 Characterize population substructure of plum curculio from the full breadth of its range.
2.2 Elucidate the historical biogeographic patterns of the plum curculio—especially in relation to the observed genetic diversity and possible refugia during glacial periods.
2.3 Test the hypothesis that *Conotrachelus nenuphar* is a single species.

3.1 Identify regions of the world that are most at threat of plum curculio invasion given the organism's habitat preferences.
3.2 Provide molecular tools for diagnosing and managing the plum curculio, locally and internationally.

Table 1

Summary of eastern North American *Conotrachelus* classifications. The three classifications from Leconte and Horn (1876), Blatchley and Leng (1916) and Schoof (1942) are listed, with the species treated under each classification indicated by filled cells. Schoof has also been updated with the addition of *C. corni* and *C. downiei*. The divisions considered in this study are Group I (red), Group 2 (yellow), Group 3 (light blue), and Group 4 (dark blue). The Leconte and Horn (1876) classification is stippled. Blatchley and Leng (1916) is stripped and for this classification, the Group 2 is orange and the Group 3 is yellow as they were specified in the key. An unfilled cell indicates that that species was not treated in the respective classification. These groups correspond exactly to the classification proposed by Schoof. *Conotrachelus tibialis* has been synonymized with *C. schoofi*.

Table 1 Summary of eastern North American *Conotrachelus* Classifications

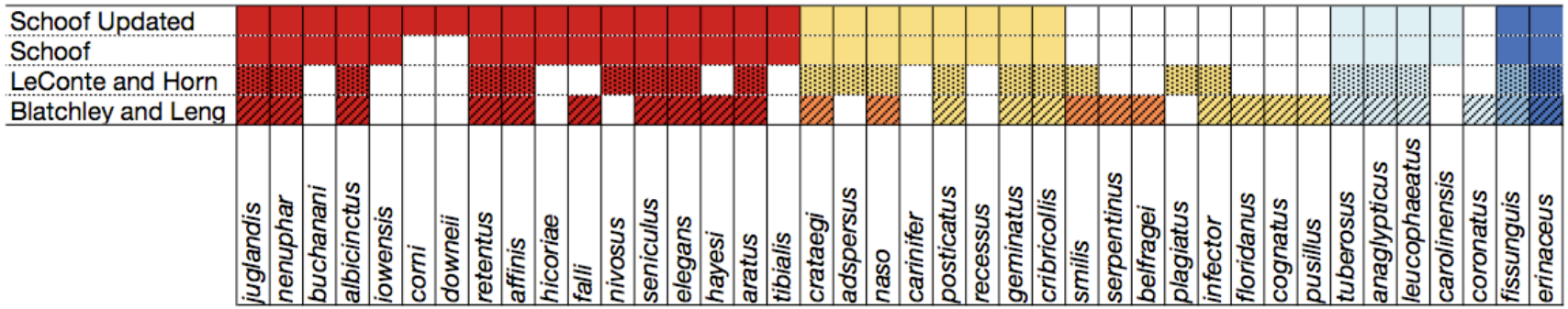
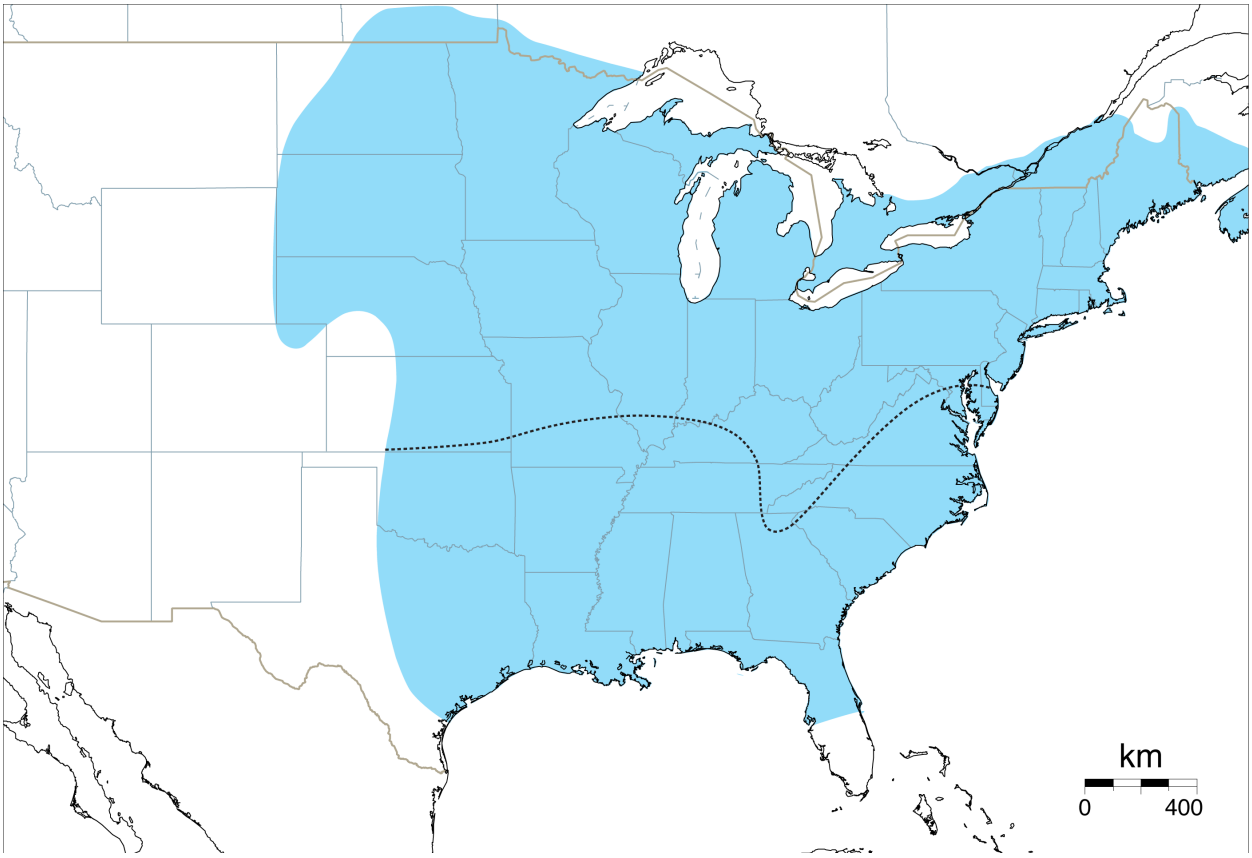


Figure 1

Distribution map for the plum curculio, *Conotrachelus nenuphar* Herbst, following Chapman 1938. The dashed line indicates the proposed and currently accepted transition zone from the univoltine northern strain to the multivoltine southern strain plum curculio.

Figure 1 Plum Curculio Distribution



CHAPTER 2

Molecular Phylogenetics of Eastern North American *Conotrachelus* (Coleoptera: Curculionidae)

Samuel N Crane

Introduction

The analysis of mitochondrial DNA (mtDNA) variation has a long history in intraspecific studies (Simon *et al.* 1994; Avise 2004; Hickerson *et al.* 2010) primarily because the shorter coalescence time (in the absence of selection) relative to nuclear DNA (nDNA) yields sufficient information for phylogeographic inferences. For this reason, mtDNA has been called a "leading indicator" of population divergence (Zink & Barrowclough 2008). To investigate population history, researchers will typically collect many individuals of one species and sample a few or even a single genetic marker, with mtDNA nearly always sampled (Caterino *et al.* 2000). Advances from such single species phylogeographic studies have aided our understanding of how microevolutionary processes lead to macroevolutionary patterns (Avise 2000; Knowles 2009). However, reliance on a single locus, mtDNA, for historical inferences can be problematic (Ballard & Rand 2005; Galtier *et al.* 2009). When considering the evolution of species rather than populations, gene histories do not necessarily reflect species history (the gene tree/species tree problem) and agreement between multiple markers increases confidence in the inferred species relationships. Well-supported disagreement between markers, while possibly reflecting faulty taxonomy, may actually be indicative of real historical, demographic events (Funk & Omland 2003). In the case of discordant relationships based on mtDNA and nDNA, the disagreement may be a result of mitochondrial introgression between populations or, especially in the case of rapidly radiating taxa, to incomplete lineage sorting. As part of a larger assessment of morphology, behavior, geography, and ecology—accurate delimitation of species necessitates the recognition of such disagreements between markers and an exploration of their origins.

This study uses the issue of concordance between multiple molecular markers among co-distributed close relatives to test long-standing taxonomic hypotheses in the beetle genus

Conotrachelus. This genus is found in the New World and contains numerous major and minor agricultural pest species, the most significant of which is the plum curculio, *Conotrachelus nenuphar*. This beetle is native to North America and, if left uncontrolled, is capable of complete destruction of tree fruit crops—especially apple, peaches, plums, and cherries. The accurate inference of species boundaries is especially important when considering agricultural pests, as the taxonomic inferences have consequences for agriculture, commerce, international trade, and law enforcement (e.g., quarantine and inspection efforts).

Systematics of Conotrachelus

Conotrachelus Dejean (Coleoptera: Curculionidae) contains approximately 1,200 named species (O'Brien & Wibmer 1982). For North American taxa, Leconte and Horn (1876), Blatchley and Leng (1916) and Schoof (1942) are the major works. In North America (NA) north of Mexico there are 63 currently described species and in just eastern NA (ENA), here defined as north of Mexico and east of the Rocky Mountains, there are approximately 46 nominal species (O'Brien & Wibmer 1982).

Conotrachelus species from eastern North America are divided into taxonomic infrageneric groups. The monophyly of these groups is uncertain. The most recent scheme comes from Schoof (1942) and places ENA species into 4 infrageneric groups. The 15 species treated by Schoof in Group 1 are united by: the number of processes, or teeth, on the femur (two); length of the first and second antennal segments; prothorax width relative to length; characteristics of the elytral costae; vestiture of the elytra; erectness of elytral setae; grooved metasternal plates (at least in males); and features of the dorsal plate of the aedeagus. There are a number of exceptions and qualifications to each of the characteristics used to define the group, so there is no set of pure morphological diagnostics. Writing in the early 1940s, Schoof intended his

classification to reflect species history and affinity, but by the nature of the work this is still a phenetic classification of these species.

Group 2 is identified by the same suite of characters used to unite Group 1, but with different states for the characters (e.g., one femoral tooth). Previous authors (Leconte and Horn, 1876; Blatchley and Leng, 1916) had divided Group 2 into two separate groups but, as Schoof argues, the characters used to split Group 2 by these authors were exclusive to female specimens and the distinctions disappear when male specimens are considered. Group 3 is composed of only 5 species and is identified by a dorsal median furrow on the prothrax, which other species in *Conotrachelus* lack. Group 4 contains species that have cleft tarsal claws that are close together, in contrast to the widely divergent tarsal claws of the other groups. Table 2 contains a summary list of all the species treated by Leconte and Horn (1876), Blatchley and Leng (1916), and Schoof (1942). Species groupings follow the Schoof classification.

Two species have been added to the North American classifications since the 1940s. These are *C. corni* by (Brown 1966) and *C. downiei* O'Brien and Salsbury (in review). *Conotrachelus corni* falls out with *C. iowensis* in Schoof's key but lacks the white patch between the elytral costa, instead resembling the coloration pattern of *C. nenuphar* but being much smaller in size. *Conotrachelus downiei* is aligned with *C. buchanani* and is separated from it primarily by features of the setae on the elytral declivities and the size difference between the two groups (*C. downiei* being smaller). Both new species are placed in Group I.

Higher-level weevil systematics are such that the generic relationships within the Curculionoidea are not well resolved. The working assumption among taxonomists is that the genus *Pheloconus* is sister to *Conotrachelus* (O'Brien, pers. comm.). Several species have been moved from *Conotrachelus* to *Pheloconus*, including *Pheloconus cameronensis, Pheloconus duplex,*

Pheloconus echinatus, Pheloconus erinaceus, Pheloconus nigromaculatus, Pheloconus texanus, Pheloconus parvulus (Quaintance & Jenne 1912; Smith & Salkeld 1964; O'Brien & Wibmer 1982). Superficially, they are highly similar to *Conotrachelus*.

In addition to studying species monophyly and the infrageneric groups used by all taxonomic workers of *Conotrachelus* for over a hundred years, several possible species groups can also be considered. Species were further divided into unnamed species groups discussed by Schoof but not formally recognized in his key. He acknowledges an affinity among [*juglandis*, *nenuphar*, *buchanani*, *albicinctus*, + *iowensis*]. The recently described species *C. corni* and *C. downiei* would fall within this subgroup, as discussed above. Presumably any one or any combination of these could represent the sister clade to the plum curculio, *C. nenuphar.* For the purposes of this study, species group I-a will be tested as [*juglandis*, *nenuphar*, *corni*, *buchanani*, *downiei*, *albicinctus*, + *iowensis*]. Schoof also recognizes within Group I two putatively closely related groups of species: [*retentus, hicoriae,* + *affinis*] and [*elegans*, *hayesi, schoofi* (synonymized with *tibialis*), + *aratus*]. These will be called subgroup I-b and I-c, respectively. Within Group 2, Schoof notes the affinity between [*naso*, *carinifer*, + *posticatus*]. This will here be refered to as subgroup II-a. These putative species groups are also indicated in Table 2.

The specific research aims of this study are to determine the phylogenetic relationships of *Conotrachelus nenuphar* and sympatric congeners in eastern North America, and test the monophyly of species in this group. To do this, we have (a) constructed a multilocus dataset for co-distributed *Conotrachelus* species in eastern North America, and (b) assessed gene tree concordance among the nuclear datasets and between nuclear and mitochondrial datasets.

Materials and Methods

Specimens

Species selected for this study were those treated by Schoof 1942, recently described species, species ascribed to the infrageneric species groups treated by Leconte and Horn (1876) and Blatchley and Leng (1916), and several members of *Pheloconus*. This sampling scheme represents the sampling within the classification proposed by the most recent taxonomic revision while incorporating additional species to test the boundaries of the genus and proposed infrageneric species groups.

The majority of specimens were loaned for destructive sampling of DNA from biological collections at the Smithsonian National Natural History Museum (NMNH), the Canadian Museum of Nature (CMN), and the personal collection of Dr. Charles O'Brien (CWOB). Specimens collected within the last 10 years were selected for this study, and all specimens used had been dried and either point mounted or pinned. Species identity was determined using Schoof's key and checked against how they were labeled in collection. Specimens from unsorted drawers were also processed from the NMNH collection. Additional specimens were field collected, identified against the reference collection provided by CWOB.

DNA Extraction, Sequencing, and Alignment

To preserve external and internal morphological characters of museum specimens, the head and prothorax of each individual was separated from the body and subjected to a modified version of the Qiagen® DNeasy® 96 Blood & Tissue Kit extraction protocol (Pinzón-Navarro *et al.* 2010). Whole tissue was soaked in lysis buffer without homogenization. This semi-destructive protocol allows for digestion of the soft internal tissue of the head and prothorax for

isolation of nucleic acids while preserving the hard sclerotized external anatomy. After lysis of internal soft tissue, the head and prothorax was reattached to each specimen.

Primers and PCR programs used to amplify target loci are listed in Table 3. Markers used in this study are the cytochrome oxidase *c* subunit I (*COI*) mitochondrial gene, the nuclear elongation factor 1 alpha gene (*EF1α*), and the non-coding nuclear internal transcribed spacer 2 (*ITS2*) marker. Target loci were sequenced in short overlapping fragments to compensate for the possibility of degraded DNA in older specimens. Successful PCR amplifications were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Inc.) on an ABI Prism™ 3730 DNA Analyzer. Sequences were assembled and edited in Geneious Pro™ 5.4 (Biomatters Ltd.).

Molecular sequences were aligned using MAFFT v6.814b (Katoh *et al.* 2002) using default settings, including automatic selection of algorithm based on data size, and a 200PAM / k=2 scoring matrix. Alignments were inspected visually and edited by hand in Geneious Pro™ 5.4 (Biomatters Ltd.). Alignments of coding regions (*COI* and *EF1α*) were translated using the invertebrate mitochondrial and standard genetic codes respectively to ensure there were no anomalous stop codons, as this would imply either that the alignments were poor or the potential sequencing of, in the case of mitochondrial DNA, a nuclear pseudogene.

Gene Trees

Phylogenies for each marker were reconstructed using maximum likelihood (ML) and Bayesian inference methods. ML trees were inferred using RAxML 7.2.8 (Stamatakis 2006; Stamatakis *et al.* 2008). Standard bootstrap analyses of the ML analysis were conducted with 1,000 pseudoreplicates. Bayesian trees were inferred with MrBayes 3.2.1 (Huelsenbeck &

Ronquist 2001; Ronquist & Huelsenbeck 2003; Altekar *et al.* 2004) by Markov chain Monte Carlo (MCMC) sampling for 10×10^6 generations. Analyses of *COI* and *EF1α* were initially run under three different partition schemes: unpartitioned (1P), first and second codon position plus third codon position (2P), and by codon positions (3P). The *ITS2* intron sequences were always unpartioned by codon. A Bayes factor analysis of the harmonic means of the marginal likelihoods from all runs of each of the three partition schemes was conducted in Tracer version 1.5 (Drummond *et al.* 2012) to determine the best scheme. Gamma shape, state frequencies, substitution rates, and transition/transversion rates were unlinked between partitions. Each analysis consisted of four independent runs of four Markov chains. Each partition was analyzed under the general time reversible (GTR) model of nucleotide substitution and rates were allowed to vary (Γ) across four rate categories. Since the Γ parameter can vary to zero, a proportion of invariable sites (I) was not specified. Use of both Γ and I can be problematic because estimates of the proportion of invariable sites and the gamma shape parameter may be correlated, which can effect branch length and tree length (Zhang *et al.* 2012). GTR is the most variable model of nucleotide substitution possible, with all other models being a subset of it. Since the MCMC process optimizes these parameters and computational resources were sufficient, a model selection analysis was not necessary. Convergence on a stationary phase by all four Markov chains across all four independent runs was checked by inspection of the –ln likelihood scores in Tracer v1.5 and by plotting posterior probability splits for each run and comparing the splits between runs using the SLIDE and COMPARE functions in Are We There Yet (AWTY) (Nylander *et al.* 2008). RaxML and MrBayes runs were conducted on the City University of New York High Performance Computing Center cluster.

Concatenation

The alignments used to generate the gene trees were concatenated and analyzed under various partitioning schemes in RAxML and MrBayes and with new search technologies under maximum parsimony with TNT (Goloboff *et al*. 2008). For maximum likelihood and Bayseian inference methods, three partition schemes were analyzed: 3-part (by marker), 5-part (COI codon position, EF1α, ITS2), and 7-part (COI codon position, EF1α codon position, ITS2). Again, all partitions were assigned the GTR+Γ substitution model. In RAxML, 1,000 bootstrap pseudoreplicates were generated and used to determine a majority-rule consensus tree. In MrBayes, four independent runs of four Markov chains were run for each partition scheme for 20×10^6 generations. Convergence was assessed in Tracer and AWTY, as above. TNT runs were performed by driven search and a strict consensus tree was used to generate 1,000 boostrap replicates to assess node support. RaxML and MrBayes runs were conducted on the City University of New York High Performance Computing Center cluster.

Results

DNA Extraction and Sequencing from Archival Museum Specimens

A total of 232 specimens from natural history collections were processed for whole genomic DNA. The average age of specimens was 12 years old and ranged from 1966 (DNA extraction and amplification was not successful) to 2010. Of these, 122 were successfully sequenced for a partial fragment of *COI* (53% success rate). Seven additional specimens were field collected. Identical sequences were removed from the datasets before alignment. There were 104 ingroup terminals and 5 outgroup terminals selected for the *COI* dataset. Fifty-eight specimens, including five outgroups, were sequenced for ITS2. Fifty-two specimens, including five outgroups, were successfully sequenced for *EF1α*. A total of 26 species was included in the

final concatenated data set, representing all major and minor infrageneric groups. The final dataset included two species from *Pheloconus* and five outgroup species: three from within subfamily Ceutorhynchinae, and two more distantly related species from the subfamily Entiminae. All sequences used in the final datasets came from the same set of individual beetles and no chimeric sequences were used to represent the species groups in the analyses.

Gene Trees

Bayes factor analysis of the *COI* dataset showed the 3P codon partition scheme to be the best scheme. Bayesian and ML inferences were largely concordant, and resolved many well-supported species groups as indicated in Figure 2 with bootstrap support values for the ML trees and the posterior probabilities from the Bayesian trees. However, deeper relationships between these clades were not well supported. The *COI* tree was the most taxonomically complete, with the most individuals and species groups sampled (Figure 2a). Group I was resolved as monophyletic with the exception of *C. seniculus*, or as monophyletic with *C. seniculus* with only weak support. Group II is paraphyletic with respect to Group I; however, several species groups were well supported by the analyses. Subgroup II-a with the addition of *C. pusillus* was recovered as monophyletic. Both *C. naso* and *C. posticatus* showed significant infraspecific structuring. Other monophyletic groups supported by ML and Bayesian inference were: all *C. analygpticus* individuals; a highly supported Group 3 clade made up of *C. carolinenesis*, *C. leucophaetus*, and *C. tuberosus*; and all *Pheloconus* individuals. The informal species groups I-a, I-b, and I-c were not monophyletic.

Both nuclear markers resolved fewer clades than *COI* but support for deeper nodes was recovered in each case. The nuclear intron ITS2 (Figure 2c) resolved a well-supported monophyletic Group I including *C. seniculus*, in agreement with the *COI* inference. A clade

composed of *C. iowensis* and *C. elegans* was weakly supported. Other species with high support included *C. buchanani* and *C. iownesis*; *C. retentus*, *C. aratus*, and *C. schoofi*; and a *C. nenuphar* group that did not include all *C. nenuphar* samples. *Conotrachelus leucophaetus* and *C. recessus* each were also well-supported as monophyletic in all analyses. An expanded Group II-a composed of *C. naso*, *C. posticatus*, *C. carinifer*, and *C.pusillus* was well supported, in agreement with *COI* results. Other groups were only weakly supported or unresolved.

In contrast to the other markers, *EF1α* did not recover Group I as monophyletic (Figure 2b). Of the three markers, this nuclear coding region had the least information and resolving power. The Bayesian analysis supported a monophyletic [*Conotrachelus* + *Pheloconus*], a large basal clade excluding *C. posticatus* and *C. naso*, and a clade composed of all Group 3 species plus *Pheloconus* and *C. recessus*. The maximum likelihood results indicated a few terminal species groups but overall gave little support to deeper level divergences, with the exception of a monophyletic [*Conotrachelus* + *Pheloconus*].

In none of the above gene trees do *Pheloconus* species resolve as sister to *Conotrachelus*. Pheloconus forms a clade with *C. fissunguis* in the nuclear markers and a distinct well-supported clade in the mitochondrial dataset.

Concatenated Tree

The concatenated analysis yielded a well-resolved tree that supported most nominal species as monophyletic. Overall, there were 5 primary clades, each composed of multiple well-supported species groups (Figure 3). Group I with *C. seniculus* had high support. Sister to this is subgroup II-a. Group 3 was monophyletic but a Group 2 species, *C. adspersus*, resolves as sister. Group IV was monophyletic with addition of the *Pheloconus* species or with *Pheloconus* as sister

group. Of the informal species groups, only group II-a was recovered. Subgroup I-a was polyphyletic with *C. juglandis* taking a basal position within Group 1. Subgroup I-b was also polyphyletic. *Conotrachelus affinis* and *C. hicoriae* form a well-supported monophyletic clade with no substantial difference within the group. Subgroup I-c showed a similar pattern in that *C. schoofi* and *C. aratus* form a monophyletic clade, and *C. elegans* resolves as sister to *C. iowensis*. Finally, *C. recessus* and *C. crataegi* form a clade with moderate support.

Discussion

Systematics of Eastern North American Conotrachelus

A preliminary 5-group system is proposed that follows the tradition in *Conotrachelus* of splitting the genus into diagnosable divisions. Three infrageneric groups from previous classifications are supported by the molecular data: Group 1, a limited Group 2, and Group 3. All markers and analyses recover Group 1 as monophyletic. Group 2 is paraphyletic. However, a more limited Group 2 in the sense of subgroup II-a as defined in Table 2 and conforming with Schoof's informal *naso*-group was commonly recovered. This restricted subgroup II-a is sister to Group 1. The *anaglypticus*-group, or Group 3, was also recovered as monophyletic although the support is weak. A monophyletic *C. recessus* plus *C. crataegi* is the fourth group, although the support is ambiguous and corresponds to ambiguous morphological support. The final clade recovered is Schoof's [Group 4 + *Pheloconus*]. Infrageneric Groups 1 and 2 form a crown clade that is sister to Group 4. The relationship between these three clades and Group 3 and Group 5 is unresolved, forming a polytomy from lack of resolution.

Interestingly, *C. fissunguis* appears as sister to a *Pheloconus* clade and there is no evidence here that *Pheloconus* is in fact sister to *Conotrachelus*. This implies that *Conotrachelus* is paraphyletic. There is considerable support in the data for a monophyletic clade composed of *C.*

fissunguis and the two *Pheloconus* representatives. The relative position of this basal group is unresolved but this result raises the possibility that *Pheloconus* falls within *Conotrachelus*, or that *Conotrachelus* needs revision. Indeed, one of the species treated here, *Pheloconus echinatus*, was originally described as *Conotrachelus echinatus* but is now considered a member of *Pheloconus* (O'Brien & Wibmer 1982).

Many of the species groups discussed in the introduction that were expected based on morphology were not supported by this 3-marker dataset. Perhaps the most surprising result was the sister species relationship recovered between *C. iowensis* and *C. elegans*. These species are not near each other in the taxonomic keys and it was suggested by Schoof that *C. iowensis* "is a close relative of *albicinctus* Lec., *buchanani n. sp*., and *nenuphar* (Hbst.)"(Schoof 1942). However, the mitochondrial and the nuclear intron data both support the sister relationship of *C. iowensis* and *C. elegans*. *Conotrachelus juglandis* was thought to be closely allied with C. *nenuphar*, but here it is recovered in a basal position within Group 1.

In this analysis, *C. nenuphar* was not in close topological proximity to *C. iowensis*, *C. juglandis*, or *C. albicnctus*. Rather, the *C. nenuphar* samples in this study resolve as two distinct clades, with an unresolved relationship to *C. buchanani*, *C. downiei*, and *C. corni*. *Conotrachelus downiei* was recovered as sister to *C. buchanani*. There is a three-terminal polytomy of [(*C. buchanani* + *C. downiei*), *C. nenuphar*, *C. corni*]. This lack of resolution may be due in large part to the missing data for *C. corni*. Despite DNA extractions from 6 specimens, only one successfully amplified and sequenced for *COI*, and none did for any of the nuclear primer sets (as indicated by the gene trees).

The data presented here suggest that several species may be split into more than one species. *C. affinis* and *C. hicoriae* specimens formed one clade, and indeed are nearly

indistinguishable morphologically. Schoof described *C. hicoriae* as a species distinct from *C. affinis* based on the metaunci shape and aedeagi of the males, but there are no distinguishing features for the females. The lack of resolution from the molecular data between these taxa would seem to suggest that *C. hicoriae* is synonymous with *C. affinis*.

Conclusion

Towards a Robust Taxonomy of Conotrachelus

This study is the first molecular phylogenetic study of eastern North American *Conotrachelus*, a group that was the subject of a number of taxonomic revisions in the late 19th and early 20th centuries. The systematics of the group have not been considered since Willi Henning and the advent of phylogenetic systematics. As the genus is an extraordinarily species rich genus with uncertain taxonomic boundaries, the existing classifications are a good starting point in elucidating the systematics of *Conotrachelus*. . This study has sampled species from the three taxonomic frameworks in an attempt to test competing classifications. None of them have held up to close scrutiny with molecular data. The one result consistent across all datasets and analyses is the monophyly of Group I species. We did not recover the other infrageneric groups proposed by earlier authors, but this study does point the way towards further taxonomic solutions for *Conotrachelus*.

Future research could include a more in depth analysis of the species included in the monophyletic Group I. The basal relationships in which are only weakly supported. The position of *C. seniculus* is also of interest. Only the combined dataset provided support for this species as an ingroup taxon for Group I. The *COI* and *ITS2* gene trees were conflicted. Further taxonomic and molecular sampling may help resolve the position of this species.

Another concern is the phylogenetic validity of the infrageneric groups when the full diversity of the genus is considered. There are many *Conotrachelus* species that extend their geographic ranges from Central America to South America, and only one study to date has looked at these species (Pinzón-Navarro *et al.* 2010). Karl Fiedler also used an infrageneric group system in his key of South American species that treated some 600 species(Fiedler 1940). There are also a great many economic pests in the South American *Conotrachelus* fauna and so this group would also benefit from a thorough molecular phylogenetic analysis of representative species across Fiedler's classification. Ultimately, the genus is simply in need of monographic revision.

On the Utility of Museum Specimens in Molecular Phylogenetics

Due to the ready availability of specimens in biological collections, phylogenetics and phylogeography would benefit immensely from access to this material locked behind cabinet doors. Other than concerns about permanent destructive sampling of specimens (let alone issues with sampling from type material), the main obstacle is the poor state of nucleic acid preservation in archived museum tissue. In this study, beetles were most often dried and point-mounted in fumigated cabinets. Many factors can affect the quality and integrity of tissue and the DNA preserved within it, and there is no way to determine in advance how well any particular specimen's DNA will amplify. Three steps were taken in this study to minimize the chance of DNA isolation and amplification failure. Since it's thought that DNA fragment size degrades with time, the most recently collected specimens of every target species were used. Of those specimens selected for analysis, PCR primers were designed to amplify multiple overlapping short fragments (typically 100-300bp in length), using sequences from recently

collected congeneric species. Finally, many more individuals were processed than were expected to be necessary to address the study's purposes. Here, we assumed that only a quarter of the amplifications would be successful, while in fact 53% were.

Processing fresh specimens results not only in a higher success rate per specimen, but also in longer fragments, ultimately reducing material costs and time requirements. An ideal approach would find a balance between reducing costs by using recent material (when available), and expanding the dataset by selectively leveraging the material stored in natural history collections (which already represent effort invested in collecting, curating, and preserving specimens). The issue of taxonomic sampling is not insignificant when dealing with very diverse groups such as weevils. Even in a geographically restricted study such as this, there about as many North American species in the genus *Conotrachelus* (~280) as there are, for example, mammals in the Order Carnivora (~280) across the whole planet. If access could be streamlined and the lab work refined, addressing the taxonomic impediment among Coleopterists would be aided by access to these great biodiversity storehouses. Infrequently collected species are already out there, waiting for their visitors.

Table 2

Taxonomic classifications of eastern North American *Conotrachelus* species. Group membership is specified according to the naming convention of each respective classification. A summary classification that incorporates all species treated by at least one author and includes the two recently described species, *C. corni* and *C. downiei* is provided as the Modified Schoof classification. The species groups of Schoof (1942) are indicated by the names outlined in the text. NA (not applicable) means that species was not treated in the given classification, or in the case of the species groups, that the species does not belong to that group.

Table 2 Taxonomic Classifications of eastern North American Conotrachelus

Species	Species group	Modified Schoof	Schoof	Blatchley and Leng	Leconte and Horn
juglandis	I-a	Group 1	Group 1	Group 1	Division 1-A
nenuphar	I-a	Group 1	Group 1	Group 1	Division 1-A
buchanani	I-a	Group 1	Group 1	NA	NA
albicinctus	I-a	Group 1	Group 1	Group 1	Division 1-A
iowensis	I-a	Group 1	Group 1	NA	NA
corni	I-a	Group 1	NA	NA	NA
downeii	I-a	Group 1	NA	NA	NA
retentus	I-b	Group 1	Group 1	Group 1	Division 1-A
affinis	I-b	Group 1	Group 1	Group 1	Division 1-A
hicoriae	I-b	Group 1	Group 1	NA	NA
falli	NA	Group 1	Group 1	Group 1	NA
nivosus	NA	Group 1	Group 1	NA	Division 1-A
seniculus	NA	Group 1	Group 1	Group 1	Division 1-A
elegans	I-c	Group 1	Group 1	Group 1	Division 1-A
hayesi	I-c	Group 1	Group 1	NA	NA
aratus	I-c	Group 1	Group 1	Group 1	Division 1-A
tibialis	I-c	Group 1	Group 1	NA	NA
crataegi	NA	Group 2	Group 2	Group 2	Division 1-B
adspersus	NA	Group 2	Group 2	NA	Division 1-B
naso	II-a	Group 2	Group 2	Group 2	Division 1-B
carinifer	II-a	Group 2	Group 2	NA	NA
posticatus	II-a	Group 2	Group 2	Group 3	Division 1-B
recessus	NA	Group 2	Group 2	NA	NA
geminatus	NA	Group 2	Group 2	Group 3	Division 1-B
cribricollis	NA	Group 2	Group 2	Group 3	Division 1-B
smilis	NA	Group 2	NA	Group 2	Division 1-B
serpentinus	NA	Group 2	NA	Group 2	NA
belfragei	NA	Group 2	NA	Group 2	NA
plagiatus	NA	Group 2	NA	NA	Division 1-B
infector	NA	Group 2	NA	Group 3	Division 1-B
floridanus	NA	Group 2	NA	Group 3	NA
cognatus	NA	Group 2	NA	Group 3	NA
pusillus	NA	Group 2	NA	Group 3	NA
tuberosus	NA	Group 3	Group 3	Group 4	Division 2
anaglypticus	NA	Group 3	Group 3	Group 4	Division 2
leucophaeatus	NA	Group 3	Group 3	Group 4	Division 2
carolinensis	NA	Group 3	Group 3	NA	NA
coronatus	NA	Group 3	NA	Group 4	NA
fissunguis	NA	Group 4	Group 4	Group 5	Division 3
erinaceus	NA	Group 4	Group 4	Group 5	Division 4

Table 3

PCR Primers used to amplify short overlapping DNA fragments from museum archival beetle specimens.

Table 3 PCR Primers and Protocols

	Primer Name	Marker	Sequence (5' – 3')	Direction	Pairs With	Citation
1	C1-J-2183 Jerry	COI	CAACATTTATTTTGATTTTTTGG	F	2	Simon et al 1994
2	TL2-N3014 Pat	COI	TCCAATGCACTAATCTGCCATATTA	R	1	Simon et al 1994
3	COI560F	COI	TTCCCRCAACACTTTTTAGGT	F	2	This study
4	COI235R	COI	TCCGGTAGGAACAGCRATAA	R	1	This study
5	COI459R	COI	CCTGCGATGATTGCAAATAC	R	1	This study
6	Forward416	COI	CATTATGTTTTATCAATAGGAGCAG	F	7	Pinzón-Navarro et al 2010
7	Reverse679	COI	CATAGAATAAAAATAAAGTAAAAA	R	6	Pinzón-Navarro et al 2010
8	EF1a1F	EF1a	TGATCACAGGAACCTCTCAA	F	10,11,12	This study
9	EF1a4F	EF1a	AACTTATCGTCGGTGTCAAC	F	10,11,12	This study
10	EF1a5R	EF1a	CTGGGTTGTAACCGATCTTC	R	8,9	This study
11	EF1a6R	EF1a	TCCTTTGAACCATGGCATTT	R	8,9	This study
12	EF1a7R	EF1a	ATTACCTGGAGAGGAAGACG	R	8,9	This study
13	ITS3	ITS2	GCTGCGTTCTTCATCGACCC	F	14	Sequeira et al 2008
14	ITS4	ITS2	GCATATTACTAAGCGGAGGA	R	13	Sequeira et al 2008
15	ITS184F	ITS2	CGTGTCGGAGCGAGTTGGACG	F	17,18	This study
16	ITS26F	ITS2	CAGGACACATGAACATCGAC	F	17,18	This study
17	ITS278R	ITS2	AATBGCTTTCGCATTTTAAGAC	R	15,16	This study
18	ITS427R	ITS2	CCGTGTTTCATTCGAAAATATCGG	R	15,16	This study

Figure 2

Majority rule consensus phylogenetic trees from the Bayesian analysis of individual molecular marker analyses. Node support values are indicated on the tree as posterior probabilities for Bayesian trees. Gene phylogenies: (a) *COI*, (b) *EF1α*, (c) *ITS2*.

Figure 2 *COI*, *EF1α* , and *ITS2* Single-Gene Phylogenies

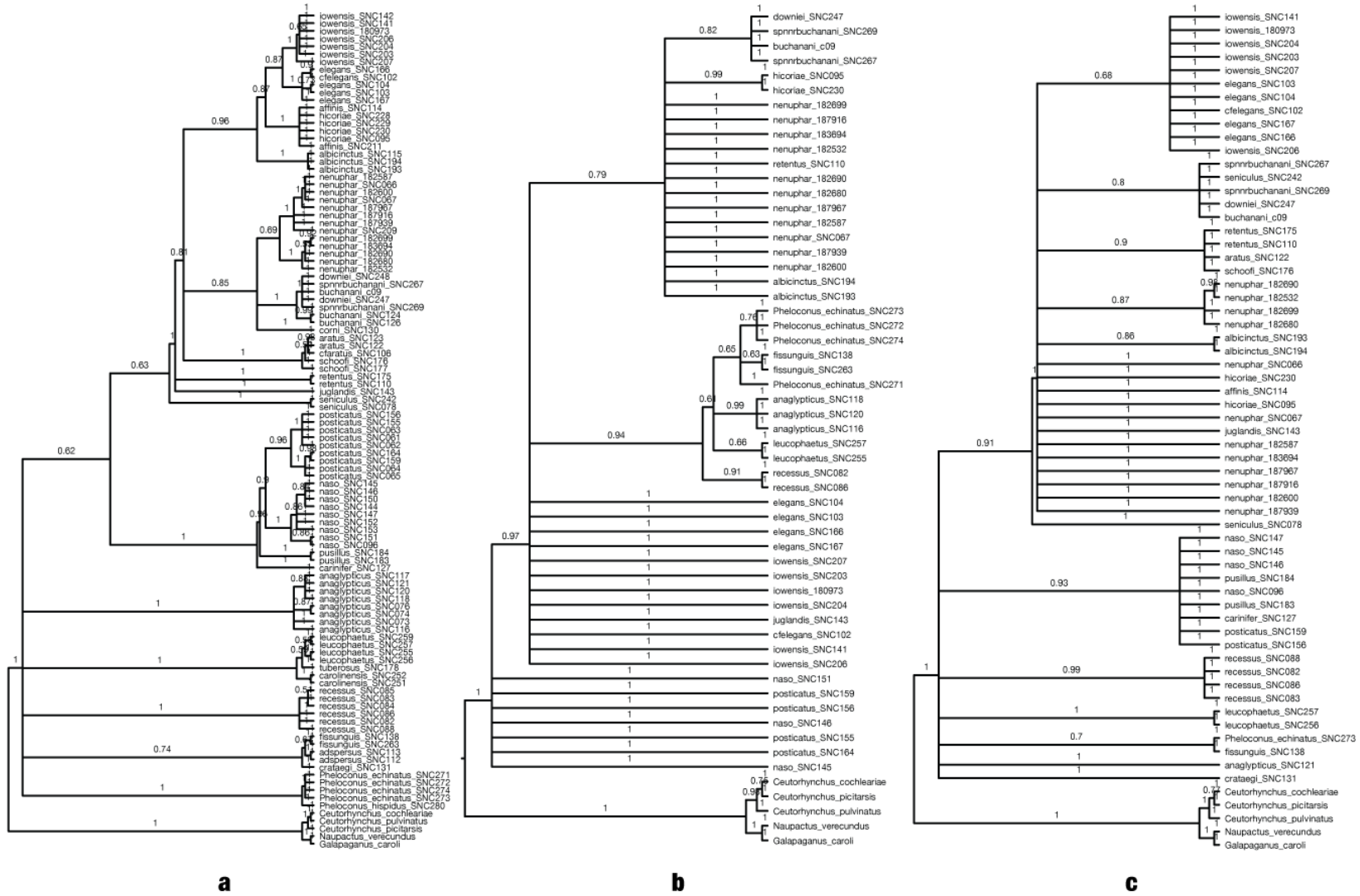
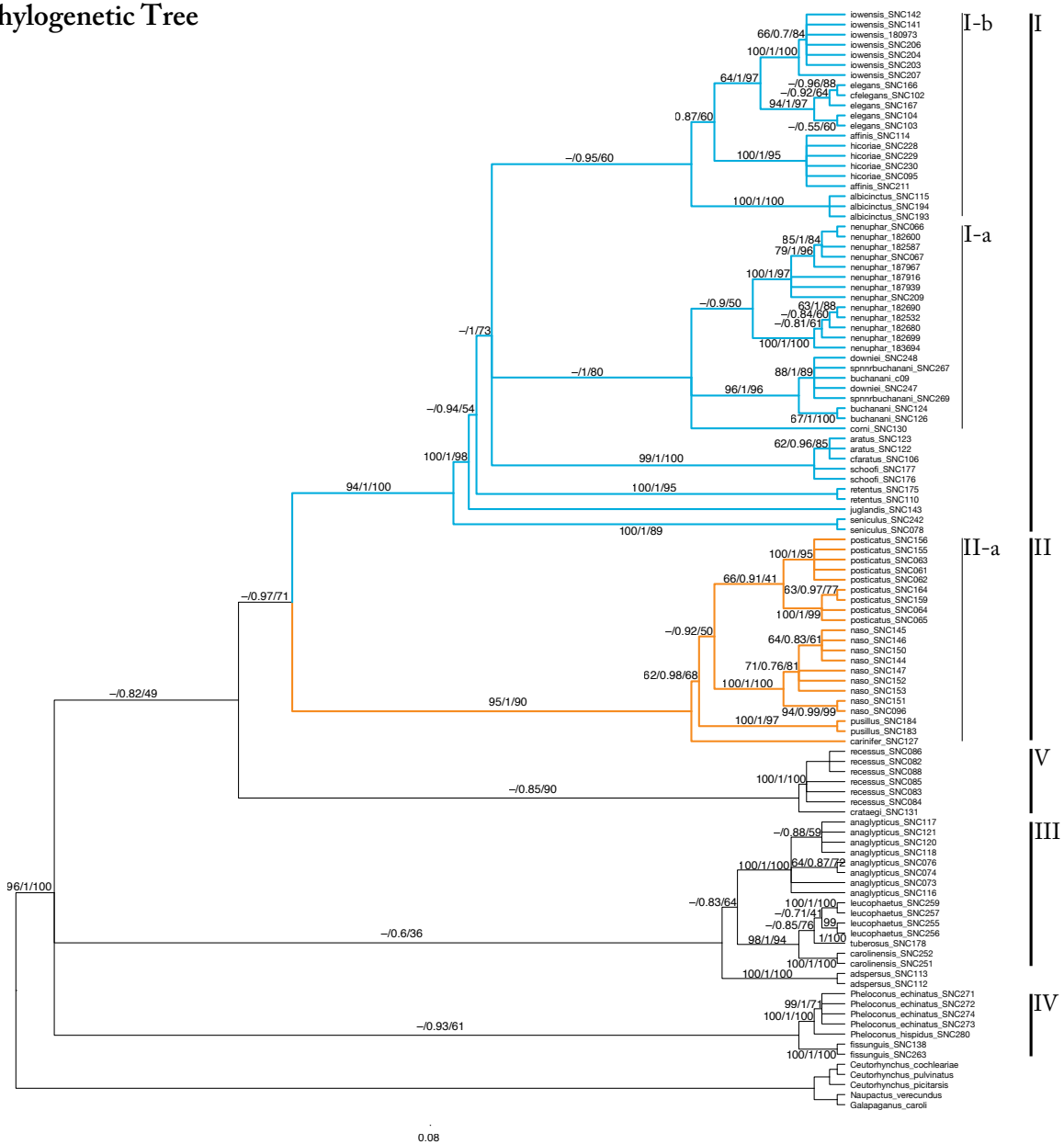


Figure 3

Summary tree and systematic conclusions. Mapped onto the Bayesian phylogeny of the concatenated dataset are node support values of maximum parsimony bootstraps, posterior probabilities for Bayesian trees, and bootstrap proportions for maximum likelihood trees (MP/PP/BS). The five major groups tested are indicated (I-V) along with the subgroups (I-a, I-b, II-a). The well-supported clade Group I is chaded blue and the monophyletic subgroup II-a is orange.

Figure 3 Summary Phylogenetic Tree



CHAPTER 3

Biogeography and Mitochondrial Divergence of the Plum Curculio Across Eastern North America.

Samuel N. Crane

Introduction

The plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae), is a native and pernicious fruit pest in the United States and Canada. Its geographic range extends from the eastern Rockies to the Atlantic and from southern Canada to the Gulf Coast. Across this range it uses as host and food source native American and Canada plums, Chickasaw plum, crabapple, and hawthorn. Following the introduction of domestic fruit trees by European settlers, the plum curculio became a major agricultural pest of orchard fruit—especially plums, peaches, nectarines, apples, cherries, and blueberries (Quaintance & Jenne 1912; Chapman 1938). Damage to crops is controlled to sub-economic levels by application of organophosphate pesticides, which are currently being phased out by the EPA (Food Quality Protection Act of 1996, PL 104-170). Novel approaches are needed to control this pest species and recent efforts have focused on alternative pesticides, trap design for monitoring, pheromone attractants for attract and kill efforts, and biological control (Racette *et al.* 1992; Hoffmann *et al.* 2008; Leskey *et al.* 2008; Shapiro-Ilan *et al.* 2008). The feature that all of these approaches share is that they depend on the particular biology of the plum curculio and leverage its particular chemical toxicology, foraging behavior, sexual or feeding behaviors, and immune system function, respectively. Effective experimental design to test the efficacy of these new approaches should account for and control biological differences within the species.

There are two strains of plum curculio, a northern strain and a southern strain. The number of generations per year is a defining characteristic of the strains. The northern strain plum curculio must diapause to become reproductively mature and has a single brood per year, with adults entering diapause in the late summer and early fall before female reproductive features have developed. The southern strain plum curculio often has only one brood per year but

has the ability to develop reproductively and have a second or even in rare cases a third generation in a single season (Smith & Salkeld 1964). In the case of the southern strain's facultative diapause, it is unclear what genetic, behavioral, or environmental factors trigger the development of the reproductive anatomy. The geographic boundary between the northern strain and southern strain plum curculio is thought to lie along an east-west axis roughly between the 37th and 39th north parallels (Chapman 1938). While both strains are the focus of alternative control efforts and IPM programs, the southern strain is the main focus of quarantine efforts since the summer brood has the potential to persist as viable larvae in fruit shipped to market, thought this is likely rare. Trade restrictions are placed on fruit originated from localities with multivoltine populations of plum curculio (EPPO 2013).

There have been two attempts to genetically characterize the northern and southern strains of plum curculio (McClanan *et al.* 2004; Zhang *et al.* 2008). Each of these efforts has revealed some consistent differences between the strains. McClanan *et al* (2004) used random amplified polymorphic DNA (RAPD) fragments to characterize two populations of the plum curculio. They found 4 loci that reliably discriminated between a population from Massachusetts and a population from Georgia. No other populations were screened. Zhang *et al* (2008) used mitochondrial cytochrome *c* oxidase subunit I (*COI*) partial gene fragment sequences from 11 populations along the Atlantic coast in an attempt to characterize the two strains. They recovered two well-supported clades in their sample. A locality in Massachusetts and a locality in New York clustered together and were distinct from all other localities from the mid-Atlantic and Southern States (NJ, WV, VA, GA, and FL). These efforts have suggested that molecular markers could be used to distinguish one strain from another, but remain inconclusive due to the limited number populations and individuals sampled and the limited geographic area covered.

Both studies sampled fewer than 10 individuals per population, which can skew population diversity measurements. The majority of the species distribution has never been sampled, including the northern limits in Canada and the entire western extent, from the Appalachians to the Rocky Mountains. A full geographic sample and extensive population level sampling is required to adequately characterize the species.

The aims of this study are to (1) genetically characterize populations of plum curculio from across the full breadth of its range. The mitochondrial *COI* gene was chosen to do this because it often serves as a leading indicator of population divergence and speciation, and has been used successfully and extensively across animals to characterize cryptic diversity and elucidate biogeographic patterns (Avise 2000; Funk & Omland 2003; Simon *et al.* 2006; Zink & Barrowclough 2008). This study also aims to (2) elucidate the historical biogeographic patterns of the plum curculio—especially in relation to the observed genetic diversity and possible refugia during glacial periods. This is achieved by analyzing the observed genetic variation in relation to its geographic structure. We test the hypothesis that the plum curculio existed in a single southern refugium during the last glacial maximum and make predictions about other possible refugia. Finally, (3) from a network and phylogenetic analysis we raise the issue of ongoing speciation in *Conotrachelus* and the possibility that the plum curculio represents two cryptic, functionally distinct and diagnosably different species.

Materials and Methods

Specimens

Adult specimens of *Conotrachelus nenuphar* were acquired during two field collection trips (2007 and 2009) and from agricultural specialists performing routine pest surveys in the United

States and Canada (2008 and 2009, see Collaborators and Acknowledgments). Specimens were captured and stored in 100% ethanol until deposition into the Ambrose Monell Cryo Collection at the American Museum of Natural History (New York), where they are permanently stored in liquid nitrogen cryogenic vats (Corthals & DeSalle 2005).

DNA Extraction and Sequencing

A single hind leg was removed from each specimen, homogenized and processed for genomic DNA using the Qiagen® DNeasy® 96 Blood & Tissue Kit standard protocol for insect tissue. All samples were processed for an 826bp region of the cytochrome *c* oxidase subunit I (*COI*) mitochondrial gene using the primers C1-J-2183 (Jerry) [5'-CAACATTTATTTTGATTTTTTGG-3'] and TL2-N-3014 (Pat) [5'-TCCAATGCACTAATCTGCCATATTA-3'] (Simon *et al.* 1994). Successful PCR amplifications were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Inc.) on an ABI Prism™ 3730 DNA Analyzer. Sequences were assembled and edited in Geneious Pro™ 5.4 (Biomatters Ltd.).

Haplotype Name Assignment

Before phylogenetic analysis, individual DNA sequences were collapsed to unique haplotypes using FaBox v.1.35 (Villesen 2007). Unique sequences for the 3' end of the *COI* marker were assigned alphanumeric haplotype names under the format (group-subgroup-identifier) using the following conventions. Two major haplotype groups were designated with the letters N and S corresponding to the two major plum curculio clades (see Results). Within these groups, a lower case letter distinguishes several subgroups (clades in the phylogeny). Finally, every haplotype is given a numeric identifier assigned by group and subgroup frequency, or

randomly in the case of singletons. For example, the most common haplotype is designated S1 and a related common haplotype from a subgroup is designated Sa1. This naming scheme is robust and transparent; the unique numeric identifier can be extended for haplotypes not found in this study and since the group and subgroup names are connected to the phylogeny, a reanalysis of the data with novel sequences will allow accurate placement and naming.

Phylogenetic and Network Inference

Phylogenetic trees were rooted with an outgroup of closely related *Conotrachelus* species, all of which belong to Group 1 as determined in Chapter 2: *C. iownesis, C. juglandis*, *C. albicinctus*, and *C. buchanani*. Parsimony analysis of the haplotypes was performed using PAUP* v. 4.10b (Swofford 2003)with heuristic searches. All characters were unweighted. Node support was measured by 1,000 bootstrap pseudoreplicates with heuristic searching and tree-bisection-reconnection branch swapping. Maximum likelihood analyses were performed with RAxML version 7.2.8 (Stamatakis 2006; Stamatakis *et al.* 2008) using the fast bootstrap method with 1,000 pseudoreplicates to assess node support. The GTR+Γ model of evolution was used and the sequences were partitioned by codon position. Bayesian inference was performed with MrBayes v.3.1.2. (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; Altekar *et al.* 2004) under the rate variable GTR model of nucleotide evolution. Four independent runs of four MCMC chains each were run for 10 million generations. Convergence across all runs and chains was assessed by plotting the likelihood scores against MCMC generation in Tracer and by plotting posterior probability splits for each run and comparing the splits between runs using the SLIDE and COMPARE functions in Are We There Yet (AWTY) (Nylander *et al.* 2008). Node support was assessed with posterior probabilities. A haplotype network was estimated

using the statistical parsimony method implemented in TCS v.1.21 (Clement *et al.* 2000), with mutational connections cut off at 95% probability of parsimony for pairwise differences. Haplotypes were visualized as a network graph and as frequency pie charts in a map of localities. The haplotype map was built using ArcGIS 9 (Esri, Inc.).

Population Genetics

Population specific estimates of molecular diversity were calculated from the full dataset, including nucleotide diversity (θ_π), haplotype diversity (h), and pairwise genetic differences (π). These estimates were made using DNAsp 5 (Librado & Rozas 2009) and Arlequin 3.5 (Excoffier & Lischer 2010). These summary statistics describe the overall genetic divergence of the sample. To assess levels of population differentiation as an approximation of gene flow between localities and regions, we conducted an analysis of molecular variance (AMOVA) within and between the major north and south haplotype clades in Arlequin, with 5,000 replicates to measure significance.

Ecological Niche Modeling

Ecological niche models (ENM) correlate data about the environment (e.g. temperature, precipitation, soil type) at locations where a species is found, creating a profile of environmental conditions suitable for the species. These models are used to generate maps of suitable habitat. Ecological niche models were developed for plum curculio using Maxent 3.2 (Phillips *et al.* 2004; 2006; Phillips & Dudik 2008). Three ecological niche models were generated: PC for all occurrence data, treating the sample as a single species; N for only those locations with haplotypes in the N clade; S for only those locations with haplotypes in the S clade. Predicted niche overlap for the N and S ENMs was measured using ENMTools (Warren *et al.* 2010).

Environmental data layers and Maxent output were visualized in ArcGIS 9. Climate layers used to infer ENMs were obtained from the WorldClim dataset (Hijmans *et al.* 2005) and selected based on assumed relevance to host tree survival, immature survival and development underground, and adult overwintering survival. In addition to temperature and precipitation data, soil characteristics were incorporated into the model because these features likely impact larval and pupal survival greatly. Soil data was obtained from the Oak Ridge National Laboratory's Global Gridded Surfaces of Selected Soil Characteristics (IGBP-DIS) dataset. Global climate layers were clipped to the training region, between 51°N, 24°N, 106°W, and 58°W (eastern North America, and roughly bounding the known native range of the species). All GIS layers were projected at a 5x5 arc-minute resolution and are listed in Table 6.

Results

DNA Sequencing and Alignment

A total of 1,040 individual plum curculio specimens, from 46 unique locations across the plum curculio's range, were successfully sequenced for the 3' region of the mitochondrial *COI* gene. The majority of samples came from areas not analyzed in previous molecular work. Alignment was unambiguous and there were no gaps; all sequences were 826 bp long. Of these, 133bp were variable and 32 unique haplotypes were recovered. Metadata on sampled locations and observed haplotypes for those locations are given in Table 7.

Phylogenetic and Network Inference

All three methods of phylogenetic inference agreed on overall topology, with 4 clades being recovered in all cases. Two deep clades labeled N and S were well supported. Within the N clade a further clade, labeled Na, was also recovered in all analyses although with weak support.

Within the S clade there is a paraphyletic group, labeled Sa, and a monophyletic crown group, labeled Sb. There were 76 parsimony informative sites across the 3' *COI* locus and a total of 225 most parsimonious trees were recovered. The log-likelihood of the ML tree is -2030.05588 and the log-likelihood average of two BI runs was -2098.271. The ML tree with maximum parsimony bootstrap, maximum likelihood bootstrap, and Bayesian posterior probabilities support values is given in Figure 4.

Statistical parsimony network inference yielded two disjoined haplotype networks at the 95% connection limit (see Figure 5). The most common haplotype by far (n=430) was S1. Most haplotypes were one step away from their nearest neighbor. There was very little structure and variation observed in the network, but what is there is highly correlated or structures geographically (Figure 6). The two networks each correspond to the N and S clades recovered by phylogenetic inference and also happen to be highly structured between the far northern limits of plum curculio distribution and the vast majority of the range in the south. Haplotype distributions are mapped in Figure 6.

The N haplotypes are only found in the far northern extremes of the plum curculio range, including New York, New England, Canada, upper Michigan, upper Wisconsin, and Minnesota. The S haplotypes are found from Iowa, lower Wisconsin, and upper Michigan southward from the mid-Atlantic to the Gulf. There is also structure apparent in the S clade in a west-to-east pattern, with Sa haplotypes only being found west of the Mississippi river, Sb haplotypes being found on the mid-Atlantic slope of the Appalachians, and the S haplotypes being found between the Mississippi and the Eastern Continental divide.

Population Genetics

The plum curculio populations were highly structured. Figure 8 shows a grid of pairwise genetic divergence within and between populations. The inter-population differences are given as pairwise nucleotide differences (π) and the net number of nucleotide differences (DA). The greatest differences are between northern and southern populations. There is very little variation observed between populations within the north or between populations within the south. The one location that has high intra-population differences is Traverse City in upper Michigan. This locality has both N and S haplotypes—the only such case observed. Directly to the south in Michigan there is a locality with only S haplotypes (Bear Lake) and directly to the north in Michigan is a locality with only N haplotypes (Northport). The straight-line distance between these two localities is 92km, with no natural barriers to plum curculio migration between them. Traverse City was the only locality recovered that seems to be a contact zone between the two clades. Specimens collected from Traverse City were collected from both cherry and apple and it is unclear if there is a consistent difference between those collected from cherries versus those collected from apples because the host data was not supplied at the specimen level.

An AMOVA analysis of the N and S group reveals a clear lack of gene flow between the two groups. At a highly significant level, the percent variance that can be attributed to differences between the N and S groups is 90.6% (Table 5). Theta statistics also support a lack of gene flow between the groups. The θ_{ST} for the N-S comparison is significant at $\theta_{ST} = 0.96013$. This is much higher than is typically observed between panmictic populations. Tajima's D is insignificant for both groups, indicating neutrality at this locus within the groups.

Ecological Niche Modeling

Ecological niche models reliability recover the species known range. A model built with all localities, including those from museum georeferenced specimens and from previous molecular work, predict eastern NA (east of the Rockies) as suitable, with a minimum training threshold indicating a sharp drop off in suitable habitat just west of the Mississippi and directly along the Gulf coast. This corresponds well with our knowledge of pest abundance (see Chapter 1); the plum curculio is not regarded as a major pest in these areas.

To test the ecological exchangeability of the two haplotype clades, ENMs were also constructed for each of these groups and their overlap assessed for significance. If their niches are highly similar, then the observed measured overlap should be within the limits of model estimates under a random MCMC based on the original data. The observed overlap between the N and S models extends from eastern Iowa, across the Great Lakes region, and into certain areas as far east as the New England Atalntic coast. The one area of contact between the clades falls within this zone, in upper Michigan.

Overlap is measured in two ways with ENMTools. Both the similarity and identity simulations showed that the samples were drawn from very similar backgrounds (D=0.95805 ±0.01006; I=0.99811 ±0.00091). The D and I statistics for the observed overlap were outside of the 95% confidence limit of the resampled models (D_{obvs}=0.45598; I_{obvs}=0.73249), indicating that the observed ENMs were statistically different. The simulations showed that the environmental space occupied by each haplogroup is not identical or even highly similar. The groups appear to as paraphyletic, and from the sampling done for this study, while the ENMs do predict some overlap in geographic space of the ENMs, overall the strains do not appear to be ecologically interchangeable.

Discussion

Genetic Diversity of the Plum Curculio

Knowledge of two distinct phenological strains of plum curculio dates back to the work of Oliver I. Snapp in Georgia. After a major outbreak in 1920 that nearly destroyed the entire Georgia peach crop, Snapp began a prolonged study of the species and was the first author to definitively establish that there is a second summer brood in the south and that this summer brood can be a major source of crop damage (Snapp *et al.* 1922; Snapp 1923; 1930). In 1938, Paul J. Chapman summarized all reports up to that point on plum curculio voltinism, and determined the boundary between northern and southern strains that is still used to this day and is indicated in Figure 6 (Chapman 1938). In 1957, a method for rearing plum curculio continuously in a laboratory setting was published (Smith 1957a). This was followed a few years later by the first examination of ovary development in the two strains, which showed that prior to diapause northern strain females do not develop mature oocytes, and that southern strain females do (Smith & Salkeld 1964). These strain designations have no taxonomic weight and merely indicate the assumed voltinism of any given population. To what degree the strains differ in other aspects—e.g., reproduction, host preference, phenology, morphology—is unknown.

This study has sampled the mitochondrial genetic diversity across the entire geographic range of the species. We found that the mitochondrial genetic diversity of the plum curculio does not correspond to voltinism strains. We detected 4 distinct mitochondrial haplotype groups, and the population differentiation tests and the analysis of molecular variance demonstrated that there is little evidence for gene flow between two of these groups. However, the geographic distribution of these haplogroups does not correspond with Chapman's boundary. For example West Virginia, despite being entirely north of Chapman's boundary, harbors both obligate and

facultative diapause plum curculio (Leskey & Wright 2004; Leskey 2008). This study shows that all individuals sampled from West Virginia and even points north to New Jersey are members of the south haplogroup. Although plum curculio from Delaware and New Jersey are north of or straddle Chapman's boundary, they were previously considered southern strain due to the expression of late summer breeding behavior under ideal artificial laboratory environments (Trimble 1865; Stearns 1931).

All locations in southern Michigan were previously thought to harbor only northern strain plum curculio, but in this study all individuals belong to the southern haplogroup. Indeed, the most common haplotype recovered in Michigan is the same as that found in Georgia and Florida. Thus, while there are diagnosable mitochondrial groups within plum curculio, there is no evidence here that the haplogroups correspond to voltinism strains. Alternatively, the southern strain may extend much further north than indicated by Chapman's boundary. This may not have been previously detected because at northern latitudes (e.g. downstate Illinois) their reproductive behavior is similar or identical to that of the northern strain plum curculio. To gain more insight into the genetic diversity of each strain, future sampling should be restricted to dissected and positively identified strains, and their *COI* haplotypes should be matched to the groups presented in this study.

The northern and southern haplogroups should be considered unique management units for pest management plans and experiments targeting biological traits. At the very least, the mitochondrial genome is in near complete isolation between the two groups, indicating a lack of female introgression and therefore gene flow between groups. Since there is no evidence that the males and females express different migration behaviors, this could reasonably be interpreted as leading evidence of genetic isolation.

Biogeographic History of the Plum Curculio

Insect mitochondrial sequences have been estimated to evolve at a rate of approximately 2.3% per million years (Brower 1994). Assuming neutrality (Tajima's test for all samples indicated neutrality for the *COI* marker used in this study), and considering that the average nucleotide difference between northern and southern populations was 4.3%, the split between these haplogroups appears to predate the LGM and extend back in time almost 2 million years (past several previous glacial maxima of the Quaternary period). Yet today the N-haplogroup occupies only the northern extremes of the range. Plausible explanations are that members of the northern group survived in the Driftless Zone refugium—a 42,000 km^2 area around southwestern Wisconsin and adjacent areas in Minnesota, Iowa, and Illinois that has largely remained unglaciated—and expanded out from there (this is supported by the high haplotype diversity of locality 32, which is just northwest of the Driftless Zone), or that members have moved northward after being displaced by the expanding S-haplogroup populations. This corresponds with patterns observed in other insect groups that exhibit univoltine and multivoltine strains or ecotypes, such as the European corn borer, *Ostrinia nubilalis*. European corn borer univoltine ecotypes are typically restricted to the far northern latitudinal limits of the group's distribution (Coates *et al.* 2004), possibly because there is a survival advantage to overwintering without developed reproductive structures. The energy that would go into developing reproductive structures can instead be diverted to fat reserves. The west-east structuring of the S-haplogroup corresponds with patterns previously associated with refugia across the Mississippi and Apalachicola river basins (Soltis *et al.* 2006). Southern refugia for these groups may have been demarcated by the major river basins.

Ecological Speciation and Taxonomy

Taxonomic revision requires the integration of multiple lines of evidence in order break out of the "taxonomic circle", or reject the null hypothesis of a single species (DeSalle *et al.* 2005). This study has presented evidence that the N-haplogroup and S-haplogroup of the plum curculio are genetically distinct. From this, a geographical hypothesis shows the haplogroups are diagnosable since they are parapatric. Additional evidence comes from the ecological niche model; identity tests reveals that the environmental niches are significantly different. Finally, two previous studies have revealed limited reproductive compatibility between northern and southern strain individuals that geographically correspond to the haplogroups recovered here. Taken collectively, the available information enables the diagnosability of the two strains and their legitimate potential for naming as two species.

Additional studies could further these lines of evidence by characterizing the diapause behavior of sampled populations and then sequencing the DNA from those individuals to correlate strain identity with the haplotypes defined here. Additionally, a detailed morphological study could be designed by first separating out the specimens according to their haplogroup membership and then looking for cryptic morphological differences between the groups; for example, a study could be performed to look at sclerotized male and female reproduction structures between these groups, as these anatomical features are commonly used in species descriptions. The research presented here does not go so far as a taxonomic revision of *Conotrachelus nenuphar*, but it is becoming clear that there are two diverging metapopulation lineages within the overall description of the plum curculio. Until further evidence is gathered and a taxonomic revision published, the northern and southern populations as diagnosed by the *COI* haplotypes should serve as management units.

Table 4

Locations sampled for plum curculio, *Conotrachelus nenuphar* Herbst. The locality name, U.S. State or Canadian Province, and GPS coordinates are given for each locality. The number of individuals sampled (N), number of *COI* haplotypes recovered (N_h), haplotype diversity (h), nucleotide diversity (Θ_π), and list of haplotypes are provided for each locality.

Table 4 Plum Curculio Localities

Locality	State	Latitude	Longitude	N	N_h	*h*	*h* s.d.	Θ_π	Θ_π s.d.	Haplotype (No.)
Clanton	AL	32.9192	-86.6735	48	2	0.0417	0.0395	0.0417	0.1220	S1 (47), S7 (1)
Fayetteville	AR	36.1061	-94.1056	12	1	0.0000	0.0000	0.0000	0.0000	Sa1 (12)
Rogers	AR	36.3157	-94.0960	1	1	1.0000	0.0000	0.0000	0.0000	Sa1 (1)
Byron	GA	32.6689	-83.7227	21	1	0.0000	0.0000	0.0000	0.0000	S1 (21)
Homestead	IA	41.7587	-91.8663	33	1	0.0000	0.0000	0.0000	0.0000	Sa1 (33)
Kalona	IA	41.4599	-91.7027	17	1	0.0000	0.0000	0.0000	0.0000	Sa1 (17)
Dixon Springs	IL	37.4375	-88.6722	6	1	0.0000	0.0000	0.0000	0.0000	S1 (6)
Pell	IL	40.0804	-88.1908	31	2	0.0645	0.5940	0.0645	0.1547	S1 (30), S10, (1)
Ringhausen Ridge	IL	39.0731	-90.6580	49	3	0.5272	0.0264	2.0612	1.3040	S1 (22), Sa1 (26), Sa3 (1)
Urbana	IL	40.0823	-88.2131	31	2	0.1247	0.0771	0.1247	0.2192	S1 (29), S4 (2)
Buckley Homestead	IN	41.2824	-87.3780	44	1	0.0000	0.0000	0.0000	0.0000	S1 (44)
Crown Point	IN	41.3840	-87.2541	12	2	0.1667	0.1343	0.1667	0.2704	S1 (11), S9 (1)
LaPorte	IN	41.7132	-86.6715	26	1	0.0000	0.0000	0.0000	0.0000	S1 (26)
Throckmorton	IN	40.2913	-86.8802	3	1	0.0000	0.0000	0.0000	0.0000	S1 (3)
West Lafayette	IN	40.4303	-86.9518	22	2	0.0909	0.0809	0.0909	0.1877	S1 (21), S4 (1)
Versailles	KY	37.9994	-84.6940	23	1	0.0000	0.0000	0.0000	0.0000	S1 (23)
Amherst	MA	42.3186	-72.5340	1	1	1.0000	0.0000	0.0000	0.0000	N1 (1)
Colrain	MA	42.7211	-72.7504	4	2	0.5000	0.2652	0.5000	0.6199	N1 (1), Na1 (3)
Bear Lake	MI	44.4030	-86.1824	20	2	0.1000	0.0880	0.1000	0.1983	S1 (19), S3 (1)
Fennville	MI	42.5969	-86.1568	76	3	0.0523	0.0353	0.0526	0.1365	S1 (74), S3 (1), S8 (1)
Lansing	MI	42.6892	-84.4987	20	2	0.1000	0.0880	0.1000	0.1983	S1 (19), S3 (1)
Northport	MI	45.1351	-85.6527	3	1	0.0000	0.0000	0.0000	0.0000	N2 (3)
Traverse City	MI	44.8670	-85.6750	68	4	0.5988	0.0447	15.1765	7.6128	S1 (20), N2 (38), N5 (4), Na1 (6)
Frazee	MN	46.7346	-95.7598	10	3	0.6000	0.1305	0.6667	0.6290	N2 (6), N6 (3), N8 (1)
Upsala	MN	45.8192	-94.6280	14	7	0.7582	0.1158	1.1209	0.8706	N2 (7), N3 (1), N6 (1), N8 (1), N9 (2), N11 (1), N12 (1)
New Franklin	MO	39.0211	-92.7586	7	1	0.0000	0.0000	0.0000	0.0000	Sa1 (7)
Madbury	NH	43.1686	-70.9344	23	3	0.5968	0.0737	0.7510	0.6407	N1 (3), Na1 (7), Na2 (13)
Bridgeton	NJ	39.5218	-75.2009	25	3	0.1567	0.0957	0.1600	0.2529	Sb1 (23), Sb2 (1), Sb3 (1)
Cream Ridge	NJ	40.1169	-74.5214	6	1	0.0000	0.0000	0.0000	0.0000	Sb1(6)
Geneva	NY	42.8739	-77.0081	49	2	0.0799	0.0520	0.0799	0.1710	N1 (47), N7 (2)
Columbus	OH	40.0092	-83.0376	3	2	0.6667	0.3143	0.6667	0.8315	S1 (1), S5 (2)
Perkins	OK	35.9986	-97.0480	23	1	0.0000	0.0000	0.0000	0.0000	Sa1 (23)
Brigham	QC	45.2542	-72.7306	30	3	0.4529	0.0805	0.4713	0.4700	N1 (8), Na1 (21), Na3 (1)
Frelighsburg	QC	45.0473	-72.8580	56	2	0.4929	0.0265	0.4929	0.4757	N1 (23), Na1 (33)
Granby	QC	45.4358	-72.6825	10	2	0.4667	0.1318	0.4667	0.5009	N1 (3), Na1 (7)
Henryville	QC	45.1346	-73.2200	6	2	0.5333	0.1721	0.5333	0.5863	N1 (2), Na1 (4)
Mont Saint Gregoire	QC	45.3490	-73.1199	4	3	0.8333	0.2224	1.1667	1.1081	N1 (2), Na1 (1), Na4 (1)
Mont Saint Hilaire	QC	45.5326	-73.1598	5	2	0.6000	0.1753	0.6000	0.6573	N1 (3), Na1 (2)
St. Bruno de Montarville	QC	45.5433	-73.3413	6	2	0.6000	0.1291	0.6000	0.6325	N1 (3), Na1 (3)
St. Hyacinthe	QC	45.6191	-72.9701	20	3	0.4684	0.1045	0.4947	0.4928	N1 (14), Na1 (5), N10 (1)
Shefford	QC	45.3689	-72.6468	41	2	0.2902	0.0780	0.2902	0.3483	N1 (34), Na1 (7)
Nashville	TN	36.0655	-86.7464	22	3	0.5671	0.0780	0.8009	0.6704	S1 (13), S2 (7), S6 (2)
Sturgeon Bay	WI	44.8813	-87.3237	76	3	0.6502	0.0221	0.8530	0.6768	N2 (34), N3 (25), N4 (17)
Sun Prairie	WI	43.1144	-89.2140	1	1	1.0000	0.0000	0.0000	0.0000	Sa1 (1)
Verona	WI	43.0542	-89.5359	17	2	0.3088	0.1222	0.3088	0.3740	Sa1 (14), Sa2 (3)
Kearneysville	WV	39.3551	-77.8750	16	2	0.1250	0.1064	0.7500	0.6531	S1 (1), Sb1 (15)

Figure 4

ML tree of *COI* plum curculio haplotypes. Overall topology was in agreement across MP, ML, and BI methods. Two well-supported clades were recovered across all methods of phylogenetic inference, here indicated as the N and S clades. Some weakly supported structuring was observed within these two clades, denoted by the haplotype names Na, Sa, and Sb. Node support values are given for universally recovered, well-supported nodes as bootstrap and posterior probabilities for MP, ML, and Bayesian trees, respectively (MP / ML / PP).

Figure 4 Phylogeny of Plum Curculio *COI* Haplotypes

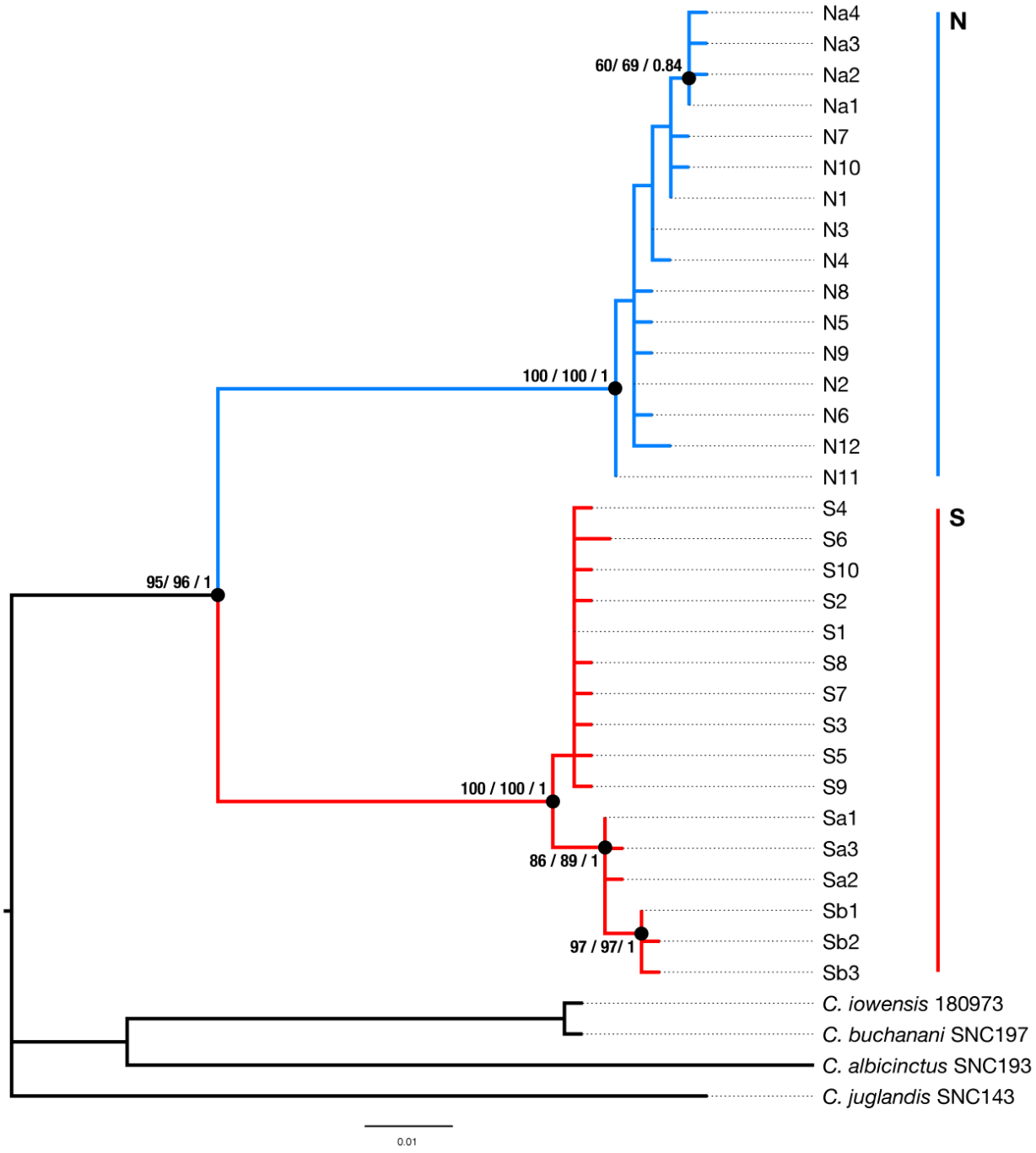
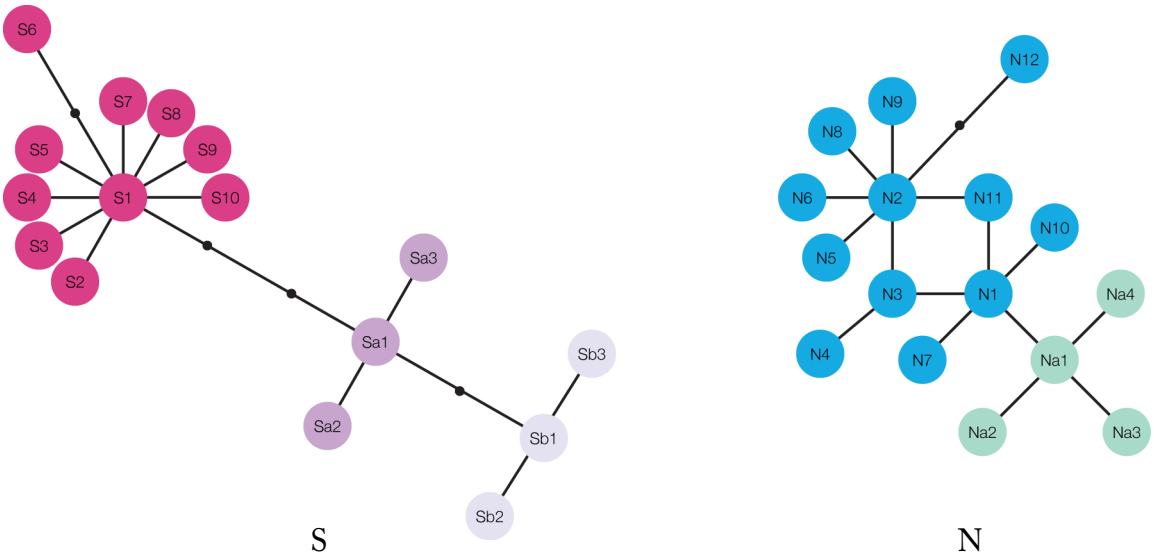


Figure 5

Statistical parsimony haplotype network for 1,040 plum curculio *COI* samples. Two disjunct networks are recovered at the 95% significance connection threshold used in a statistical parsimony network analysis. To force a connection between the two networks (N and S) requires the inference of 33 missing haplotypes (or steps). Haplotype names are the same as used in the phylogenetic tree.

Figure 5 Statistical Parsimony *COI* Haplotype Network



S N

Figure 6

Distribution map of plum curculio haplotypes. The map shows the locations and haplotype clade compositions for each sampled locality in eastern North America. See Table 4 for locality names and GPS coordinates. Haplotypes are colored according to their placement in the haplotype network (Figure 5) and named according to the phylogeny (Figure 4). Numbers are assigned location identifiers for reference only.

Figure 6 Haplotype Distribution Map.

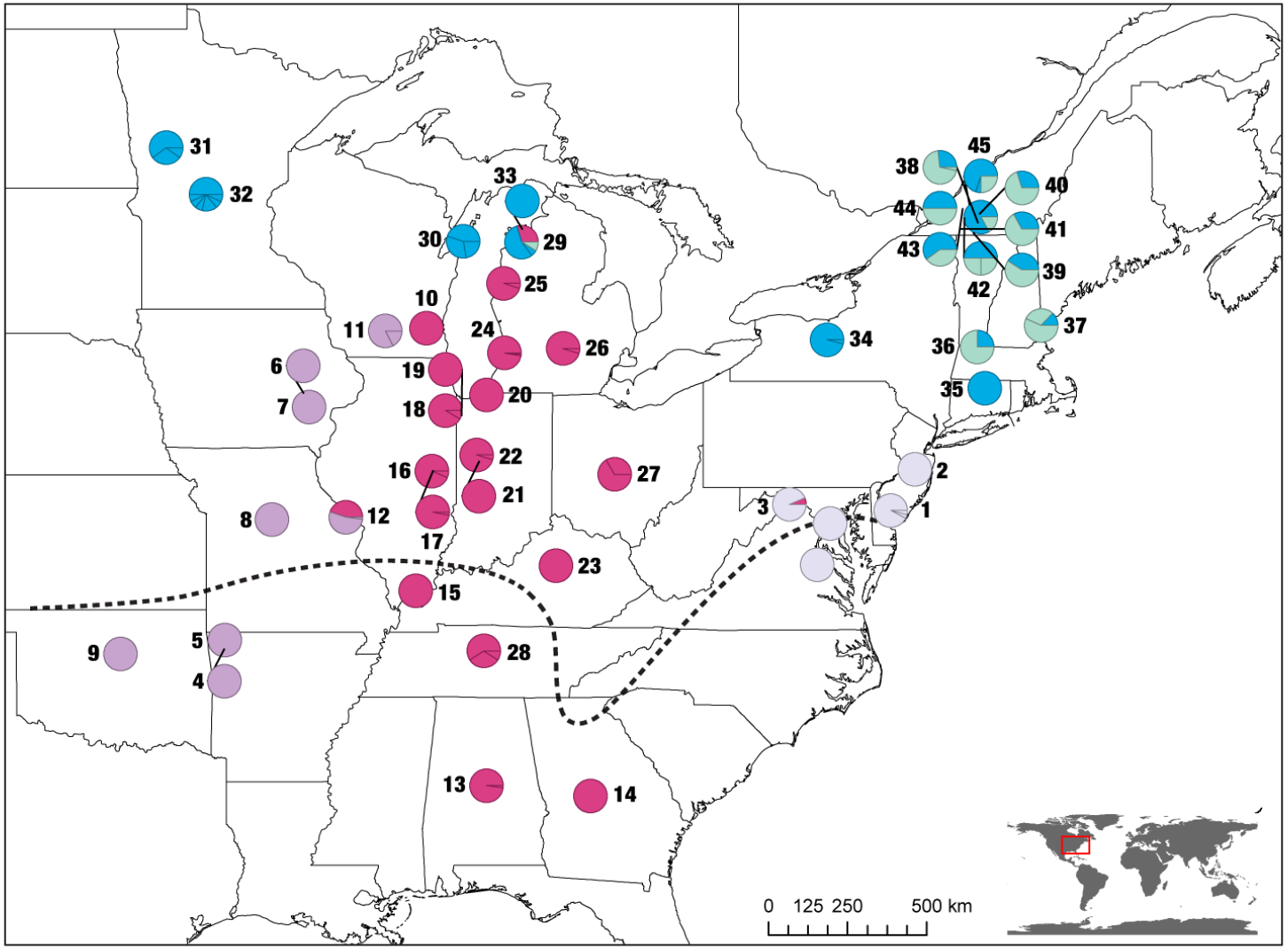


Table 5

Analysis of molecular variance (AMOVA) in the plum curculio across eastern North America. Two hypotheses of genetic variation were tested: all individuals as one group (H_1) and individuals forming two groups based on the majority haplotype clade membership, N or S (H_2). Genetic variation is reported in three hierarchical partitions: among groups (Θ_{CT}), among localities within groups (Θ_{CS}), and among individuals within localities (Θ_{ST}).

Table 5 Analysis of Molecular Variance (AMOVA)

Source of Variation	d.f.	Sum of Squares	Variance Components	% of Variation	Fixation Indices	P-value
H_1: One Group						
Among localities	45	8753.828	8.71109	92.74	Θ_{ST} = 0.92740	<0.00000
Within localities	995	678.512	0.68192	7.26		
Total	1040	9432.340	9.39301			
H_2: Two Groups (N vs. S Clade Members)						
Among groups	1	7841.660	15.50135	90.64	Θ_{CT} = 0.90637	<0.00000
Among localities/groups	44	912.168	0.91937	5.38	Θ_{SC} = 0.57414	<0.00000
Within localities	995	678.512	0.68192	3.99	Θ_{ST} = 0.96013	<0.00000
Total	1040	9432.340	17.10265			

Figure 7

COI haplotype compositions for 46 sampled localities. This chart shows the relative haplotype group composition for each individual locality. Haplotypes were binned into groups based on frequency, with only the most frequent haplotypes being shown individually and all other haplotypes binned into groups based on similarity (see figure legend). Southern haplogroup localities are characterized by low haplotype diversity, whereas northern haplogroup localities are more genetically diverse. Haplotype diversity measures (h) are listed in Table 4.

Figure 7 Locality Haplotype Composition Histogram.

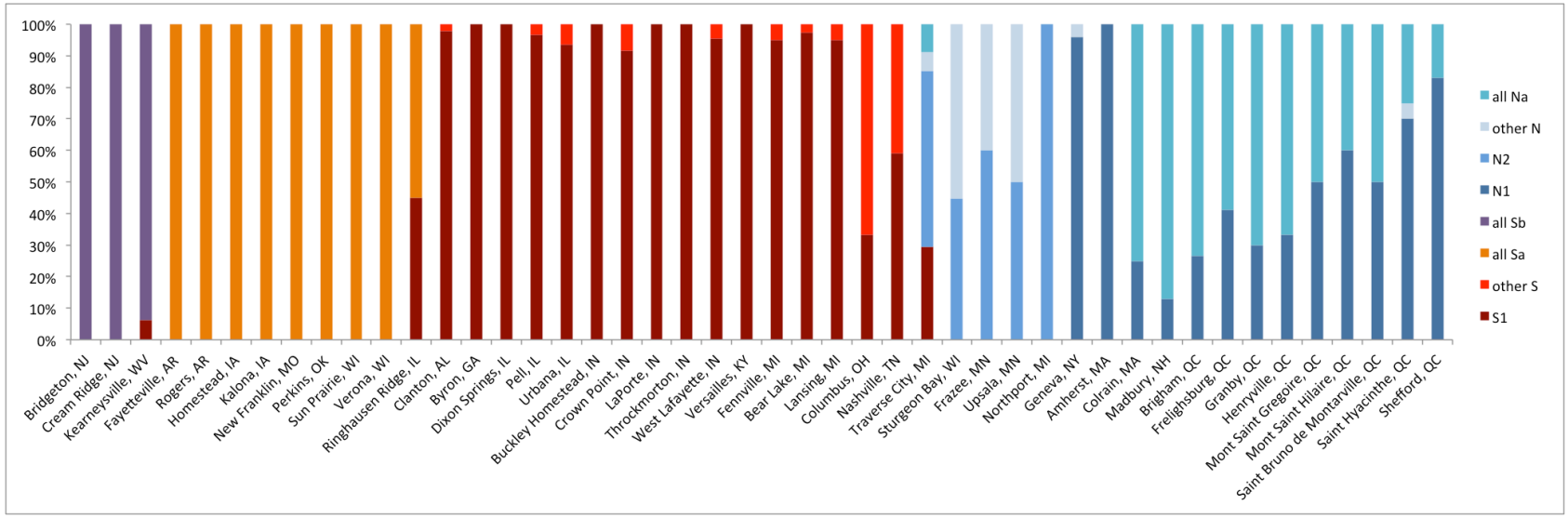


Figure 8

Average number of pairwise differences (π) between sampled localities. The diagonal shows π within localities, below the diagonal shows the net number of nucleotide differences between localities (D_A or [πxy-(πx+πy)/2]), and above the diagonal shows π_{xy}, pairwise differences between localities. Localities are ordered based on primary haplotype composition, as indicted along the y-axis.

Figure 8 Nucleotide Pairwise Differences.

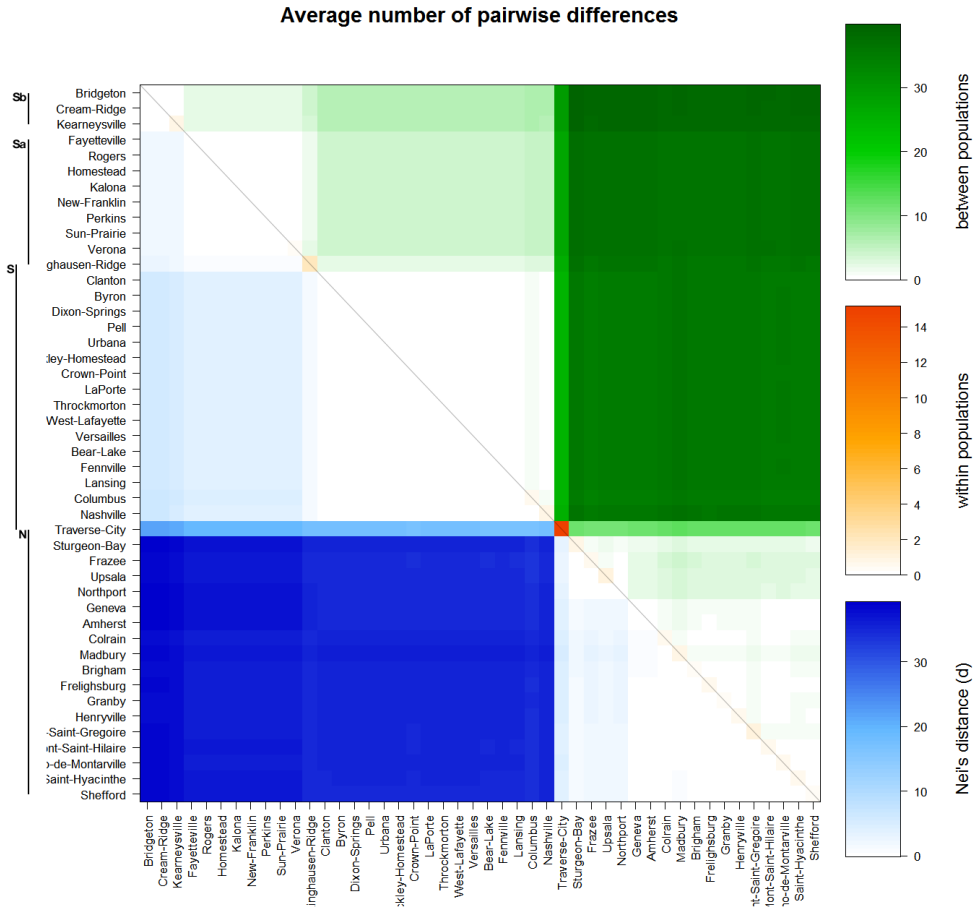


Table 6

Environmental layers and data sources used to construct the ecological niche models.

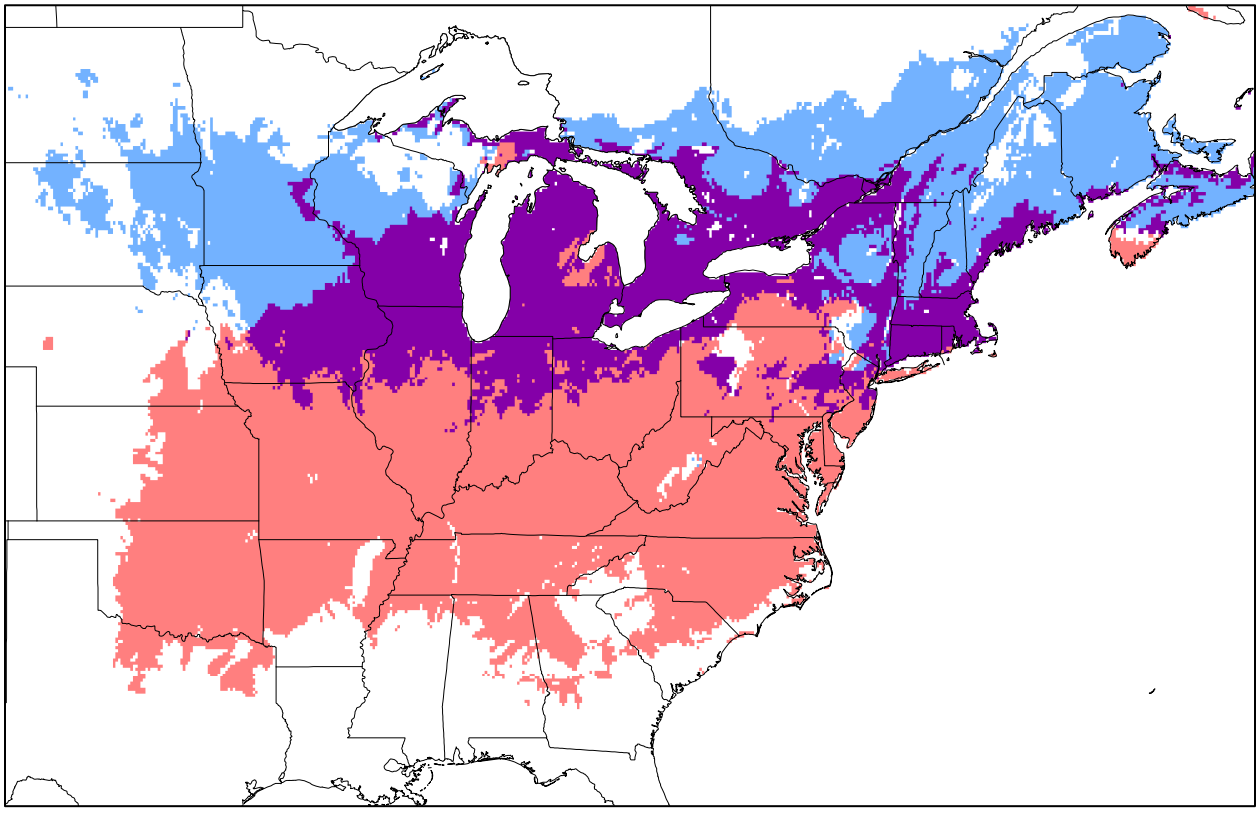
Table 6 GIS Layers: Climate and Soil

Environmental Layer	Data Source
Annual Mean Temperature [BIO1]	WorldClim
Isothermality (P2/P7) (* 100) [BIO3]	WorldClim
Max Temperature of Warmest Month [BIO5]	WorldClim
Mean Temperature of Coldest Quarter [BIO11]	WorldClim
Annual Precipitation [BIO12]	WorldClim
Precipitation of Driest Month [BIO14]	WorldClim
Precipitation of Driest Quarter [BIO17]	WorldClim
Precipitation of Warmest Quarter [BIO18]	WorldClim
soil carbon density (kg/m2) [soilcarb]	Global Gridded Surfaces of Selected Soil Characteristics (IGBP-DIS)
total nitrogen density (g/m2) [totaln]	Global Gridded Surfaces of Selected Soil Characteristics (IGBP-DIS)
profile available water capacity (mm) [pawc]	Global Gridded Surfaces of Selected Soil Characteristics (IGBP-DIS)
bulk density (g/cm3) [bulkdens]	Global Gridded Surfaces of Selected Soil Characteristics (IGBP-DIS)

Figure 9

Ecological niche models of the N and S clades. Blue indicates the predicted suitable habitat for the members of the N clade. The pink indicates the suitable habitat for member of the S clade. Overlap of the predictions is shown as purple. These binary models were determined by the suitable habitat predicted at the minimum value among the localities used to train the model (minimum training presence).

Figure 9 Environmental Niche Models



CHAPTER 4

Quantification of Invasion Risk and Diagnostics for Identification of the Plum Curculio (*Conotrachelus nenuphar*)

Samuel N Crane

Introduction

Biotic exchange is expected to increase in a non-linear relationship with trade (Levine & D'Antonio 2003; Pimentel *et al.* 2005). As the global economy continues to expand, more biotic exchanges are expected to occur and the impacts of these rapid long-range migrations will be difficult to predict. The enormous economic costs and increasing threat of invasive pest species calls for quantitative predictions about the risks associated with potential pests. To manage these risks, international organizations such as the United Nations Food and Agriculture Organization (FAO) and national regulatory bodies such the United States Department of Agriculture have established standards for assessing risks and setting regulatory guidelines based on those risks. A formal pest risk assessment considers risk as the product of the magnitude of potential economic losses and the probability of those losses occurring (FAO 2003). The probability of those losses occurring is a product of three factors: the likely pathways that would allow the pest to enter into the area under study (arrival phase), the probability that the pest will be established once it has reached the area (establishment phase), and the likelihood that it will spread once established (spread phase). These are the three stages of invasion ecology (Sakai *et al.* 2001; Liebhold & Tobin 2008). Considering the difficulty of eradication, which can be considered the irreversibility of establishment, the best defense against invasive pests is prevention. Regulatory and financial resources would be best utilized if scaled appropriately to the estimated risk. Attempts to prevent future invasions need to quantify the likelihood of arrival, establishment and spread, and these estimates need to be kept up-to-date.

Ecology and evolutionary biology offer many tools to enable the quantitative assessment of risk associated with invasive species. Propagule pressure is a well known determinate of invasion success and can be estimated for commercially traded species and their associates, for

example, by analyzing trade routes (Thuiller *et al.* 2005). Invasion potential can also be assessed by using environmental niche models (ENM) to identify those areas currently unoccupied that have abiotic features similar to the areas currently occupied (Guisan & Zimmermann 2000). This method is based upon the assumption that, all other factors being equal, if two disjunct areas have similar environmental characteristics and the species can persist in one area it should also be able to persist in the other area (Wiens & Graham 2005; Peterson *et al.* 2011). Niche models correlate data about the environment (e.g. temperature, precipitation, soil type) at locations where a species is found, creating a profile of environmental conditions suitable for the species. These models are used to generate maps of suitable habitat and are applied to a variety of goals, including but not limited to: exploring impacts of climate change on species distributions, setting conservation priorities, guiding field surveys, delimiting species, and assessing invasion potential (Guisan & Thuiller 2005; Elith *et al.* 2006; Kozak *et al.* 2008). Such predictions may be wrong to the extent that areas have biologically meaningful variation not captured in the models. For example, availability of host resources would be expected to have a limiting effect on the potential habitat predicted by any ENM (Peterson *et al.* 2011).

The environmental niche model provides quantitative information on the likelihood that a disjunct area is susceptible to establishment, either as a continuous probability surface based on the native range projected into unoccupied territory or as a binary prediction of the existence of suitable habitat. For phytophagous insects, the potential for spread beyond the initial area of colonization depends not only on suitable habitat but also on factors such as the distribution and abundance of suitable host resources. If a novel area has suitable habitat (as predicted by the model) for the pest species but lacks suitable hosts, the likelihood of spread is reduced. For pest species of commercial crops, suitable hosts will include both potential wild hosts and all

cultivated host species. These efforts at risk assessment are especially important prior to the arrival phase and are used to justify quarantine regulations and phytosanitary import requirements.

In this study I have attempted to quantify the risk associated with establishment and spread of a quarantined fruit pest from the United States and Canada. The plum curculio, *Conotrachelus nenuphar* (Herbst 1797) (Coleoptera: Curculionidae), is a beetle native to eastern North America (Quaintance & Jenne 1912). Native hosts include wild plum trees (*Prunus spp.*), crabapple trees (*Malus spp.*), and hawthorn trees (*Crataegus spp.*). The beetle is a crop pest on commercial cultivars of apples, peaches, plums, cherries, and other fruits (Chapman 1938; Vincent *et al.* 1999) throughout its range in North America. Where the beetle is a problem domestically, broad-spectrum insecticides are the only control measures that provide commercially acceptable levels of damage control and pest abatement. If left uncontrolled, plum curculio can decimate fruit crops.

The species is endemic to eastern North America and is a quarantined pest. Trade of many stone and pome fruits is limited where plum curculio is a known problem. By definition, quarantine pests are organisms of economic importance that are not yet established in the area under cnosideration or at least not yet widely distributed and being controlled (FAO 1997). Globally, pests are listed by Regional Plant Protection Organizations (RGGO) under the authority of the Food and Agriculture Organization's International Plant Protection Convention . *Conotrachelus nenuphar* is listed as a quarantine pest by RGGOs covering Europe, Central America, and South America (EPPO, OIRSA, and COSAVE, respectively). Russia, Turkey, Azerbajian, Moldova, Ukraine, Kazakhstan, South Africa, New Zealand, and several other countries also categorize plum curculio as a quarantine pest (EPPO 2013). Thus, the plum

curculio is a pest of global invasion concern and has not yet established itself outside of eastern North America. Managing the pest on a global scale requires robust estimates of the species potential for establishment and spread into novel areas.

There are no established populations of plum curculio in the western United States, except for an infestation in Box Elder County, Utah dating to the 1980's (Alston *et al.* 2005). Despite the fact that the plum curculio has not widely established itself outside of the native range, there are good reasons for being concerned about the locations of potentially suitable habitat. Possible modes for transport by human activity include movement of pupae in soil and movement of adults in packing and shipping material of fruits or trees for planting (considered to be the most likely mode of arrival by the European Plant Protection Agency) (EPPO 1997). Populations in the southern United States have the ability to breed in the late summer and fall before going into diapause, and so fruit going to market form these areas may harbor viable larvae.

Though the plum curculio is listed as an A1 quarantine pest, to the best of our knowledge an exhaustive and quantitative assessment of its invasion potential has never been carried out. As for global habitat suitability predictions, the European and Mediterranean Plant Protection Organization (EPPO) simply states without citation of analysis or data that, "Judging from its distribution in North America, *C. nenuphar* would appear to be capable of surviving throughout a large part of the EPPO [European and Mediterranean] region. The intensive cultivation of *C. nenuphar* host plants throughout the region could provide a basis of rapid multiplication of the pest and could possibly lead to immense losses and additional costs of control measures" (EPPO 1997). In this study we have used environmental data to build a niche model of this potentially invasive species and combine this model with international commercial fruit production data to

estimate the likelihood of establishment and spread in spatial and economic contexts. The synthetic niche model is used to predict potentially suitable habitat across the globe. These predictions are combined with host data and categorize the risk associated with host presence and habitat suitability in a spatial risk surface that can then inform the likelihood of establishment and spread.

As part of the international phytosanitary process, insect-infested product must be identified for the pest to determine if the insect is listed as quarantined. If the insects in shipped products are immatures, species-level identification is non-trivial. A rapid and reliable method of species diagnosis for plum curculio that works for all life stages would aid monitoring efforts. Therefore, we have also conducted additional sequencing and analysis of plum curculio specimens to generate DNA barcodes for the species. Molecular diagnostics for plum curculio identification at any life stage are presented in reference to regional variation, enabling not only species identification but also likely geographic area of origin from within eastern North America.

Methods

Data Sources

Plum Curculio Observations

Global position systems (GPS) coordinates for exact locations occupied by *Conotrachelus nenuphar* individuals were gathered from field surveys, published literature, specimen labels in biological collections, citizen science projects, and personal communications with extension agents and pest management professionals about their field sites. Locations without exact GPS coordinates (i.e. some of the published literature, specimens labels, and personal communications) were georeferenced with Google Earth (Google, Inc.) and precision was

verified when possible by consultation with the collector. Latitude and longitude, data source, locality names, and other relevant metadata for all occurrence points are provided in Table 7.

Plum curculio has two parapatric mitochondrial demes (Chapter 3). These demes show some evidence of reproductive isolation (for the mitochondrial genome), an observation that supports other reports of reduced fertility and lack of reproductive compatibility between northern and southern populations (Padula & Smith 1971; Zhang & Pfeiffer 2008). Based on these observed genetic differences, the occurrence data has been split between the mitochondrial clades: a northern group (N) and a southern group (S). Where direct molecular evidence for clade membership was not available from an observation locality, membership was assigned based on geographical location. Only one location of all the locations sampled for mitochondrial variation has contained individuals from both clades: Traverse City, Michigan (see Chapter 3).

Climate and Soil

Current climate data was acquired from the WorldClim database, Version 1.4, release 3 (Hijmans *et al.* 2005). The precipitation (mm) and temperature (°Cx10) data layers cover all land areas of the globe except Antarctica and were calculated by interpolation of average monthly climate data from weather stations between the years 1950 and 2000. For this study, derived bioclimatic variables were used that represent annual trends, seasonality, and climate extremes. These variables are potentially more biologically meaningful than annual means (e.g. monthly mean temperature versus temperature of the coldest month) and are made available by WorldClim in 19 different variable combinations. Data grids were acquired at 5x5 arc-minute spatial resolution.

Pupal *Conotrachelus nenuphar* develop at shallow soil depths (approximately 20mm). Thus, soil characteristics are very likely to effect range limits. Soil data were acquired from the Oak

Ridge National Laboratory's Global Gridded Surfaces of Selected Soil Characteristics (IGBP-DIS) database, which contains 7 global soil surfaces (Global Soil Data Task Group 2000). Data layers were at a 5x5 arc-minute spatial resolution covering a soil depth from 0–100cm. The current climate and soil data layers considered in this study are listed in Table 8. GIS layers were processed in ArcGIS version 9.2 (ESRI, Inc.).

Fruit Harvest Area

The plum curculio has a broad host range, and after introduction of domesticated species of stone and pome fruits into North America it has become a major pest on these fruits (Quaintance & Jenne 1912; Chapman 1938). While the beetle will attempt to use many kinds of fruit as host (Hallman & Gould 2004), it prefers those it is a known pest on—such as plums, apples, cherries, and peaches (Leskey & Wright 2007). Spatial data on fruit harvest areas for these four crops (plums, apples, cherries, and peaches) were acquired from the Center for Sustainability and the Global Environment (SAGE) at the University of Wisconsin-Madison (Monfreda *et al.* 2008). These data layers (M3-Crops) were generated from agricultural census and survey information at the subnational and national level for 175 different crops. These data are provided as percent area of each grid cell harvested per crop at 5 arc-minute resolution.

Environmental Niche Modeling

Variable Selection

Climate and soil data were used to build the environmental niche model. There are 19 bioclimatic variables available in the WorldClim dataset and 7 soil characteristics available in the IGBP-DIS soil dataset. Exploratory ENMs were built in Maxent, version 3.3.2 (Phillips *et al.* 2004; 2006; Elith *et al.* 2010), using all 26 variables and a jackknife test performed to assess the impact of each variable on the final model. Under this test, each variable is excluded in turn from

the model training process, and a model created with the remaining variables. Then a model is created using each variable in isolation. Finally, a model is created using all variables. The contribution of each variable to the final model is assessed as its contribution to the regularized training gain, or the average log probability of the presence locations. Informative variables will have a final training gain close to that of using all variables together while negatively impacting the gain when not included. However, if two or more variables are highly correlated then they may each show significant contributions to the overall model training gain.

To account for possible collinearity, environmental variable independence was assessed by Pearson correlation coefficients. GIS layers of the environmental variables were cropped to the modeling extent (see Model Estimation below) and Pearson's r was calculated for all pairs of variables within the climate and soil datasets using ENMTools, version 1.3 (Warren *et al.* 2010). A single variable from pairs with $r > 0.80$ was screened from the final analysis by dropping the correlated variable with the least contribution to training gain in the jackknife test (data not shown). A total of 6 bioclimatic variables and 3 soil variables were used to build the final niche model (Table 8).

Model Estimation

I used 71 plum curculio occurrence data points and 9 environmental data layers at 5x5 arc-minute resolution to build an environmental niche model for the plum curculio. The training background points were drawn randomly from an area restricted to the known native range of the plum curculio, here bounded between 24°N–51°N and 58°W–106°W (Quaintance & Jenne 1912; Chapman 1938).

Environmental niches were estimated using a machine-learning algorithm that estimates the probability distribution for a species' occurrence based on environmental constraints under a maximum entropy optimization procedure, implemented in the software package Maxent, version 3.3.2 (Phillips *et al.* 2004; 2006; Elith *et al.* 2010). As input, Maxent takes GPS coordinates of species occurrence records and GIS layers of environmental variables. The algorithm correlates the environmental conditions found at the places of known occurrence for the species and maps the environmental conditions back onto the geographic space. The output is a continuous probability surface of the likelihood of environmental suitability (not probability of occurrence). This presence-only modeling method performs well even with small sample sizes for the occurrence data (Pearson *et al.* 2007; Anderson & Gonzalez 2011) and has performed well in comparison to other modeling methods (Elith *et al.* 2006; Phillips & Dudik 2008). The logistic output of Maxent (a transformation of the raw output) is an estimate of the probability, from 0 to 1, that the species is present, given the environmental conditions, in a pixel (grid cell) of a coordinate system (Phillips & Dudik 2008). This continuous probability surface can be converted to a binary prediction of suitable and unsuitable habitat by setting a threshold (Liu 2008; Peterson *et al.* 2011). Binary models were specified by application of the minimum training presence value threshold, the lowest logistic score in the training dataset.

Three different ENMs were constructed. First, all occurrence data (n=71) were pooled for model training using all 9 variables (PC-model). Then, a model was built for the northern group (N-model, n=20) and for the southern group (S-model, n=52) using the same 9 variables. One location—Traverse City, Michgan—is shared by both the N-model and S-model since both haplotype groups were observed in this location. Otherwise the location sets are exclusive between the models (see Chapter 3 for further discussion of haplotype distributions). Each

model was replicated 10 times and the average output taken across these replicates. Models were smoothed by adjusting the regularization parameter for each data set: PC, N, and S models were each tuned by 0.1 intervals between 0.5 and 3.0 of the β multipier. All three models were projected globally to recover uninhabited areas that match the predicted habitat suitability in the native models (the potential distribution given the environmental variables).

Model Evaluation

I evaluated the models using two approaches. First, model performance was evaluated using the area under the curve (AUC) value from the receiver operating characteristic (ROC) curve as calculated by Maxent (Fielding & Bell 1997; Peterson *et al.* 2011). The AUC from the ROC curve varies between 0 and 1, with greater values indicating superior performance. The AUC was used to make comparisons across tuning parameter groups (β multiplier values) within each modeling set (PC, N, and S), with higher AUC values indicating better performance under a given regularization setting.

Once the regularization value was determined for each model, a threshold was applied to generate a binary model prediction—suitable and unsuitable. The minimum probability of presence observed in the training dataset was chosen to mark the threshold between suitable and unsuitable habitat. The performance of this binary model was evaluated with one-tailed binomial probabilities to determine whether evaluation localities in the model building process fell into suitable regions more often than expected by chance (p-values < 0.05 were considered significant).

Pest Invasion Risk Surface

Risk was categorized into 5 levels based on the presence of minimally suitable habitat (as predicted by the binary ENM) and abundance of cultivated stone and pome fruit trees. Fruit

harvest data were imported into ArcGIS and the proportion of each grid cell harvested for each of plum, apple, peach, and cherry was summed for each grid cell. The total harvested area for each grid cell was then binned into quartiles, representing increasing and equally proportioned intensities of commercial fruit production. The risk category is a function of the harvest area quartile and presence of suitable habitat, as indicted in Table 9. Grid cells with a suitable habitat and any level of commercial fruit production are at greater risk than areas with only suitable habitat, considering that the likelihood of spread is greater with more host species and trees present. Therefore, places with a greater proportion of their area under harvest (a higher density of fruit trees) are considered at greater risk of invasion and economic harm. The ENMs should be regarded as predictions of the species potential habitat (rather than the realized habitat). The realized habitat can be reduced from the potential habitat for a variety of reason, and the ENMs do not consider biotic interactions or anthropogeneic effects. Agriculture increases the total area and local densities of host plants, and so can be considered as both a biotic force and an anthropogenic effect on the distribution of pest species. For this reason, areas of high intensity commercial fruit production (as represented by the upper quartiles in the harvest area dataset especially) are expected to play a significant role in determining realized habitat—ultimately affecting the likelihood of both establishment and spread.

Plum Curculio Molecular Diagnostics

DNA Extraction and Sequencing

Extractions generated for a previous studies (Chapter 3) were used in this study to generate new 5' COI sequences for a subset of specimens (Table 10). Archived DNA extracts were amplified for the standardized DNA barcoding region using the primers LCO1490 [5'-GGTCAACAAATCATAAAGATATTGG-3'] and HCO2198 [5'-

TAAACTTCAGGGTGACCAAAAAATCA-3'] (Folmer *et al.* 1994). Successful PCR amplifications were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Inc.) on an ABI Prism™ 3730 DNA Analyzer. Sequences were assembled, edited, and aligned in Geneious Pro™ 5.4 (Biomatters Ltd.).

DNA Barcoding

DNA barcodes were generated in two ways. The standardized barcoding region for animals, a ~650bp locus on the 5' end of the *COI* gene, was targeted for use in the Barcode of Life Database (BOLD) (Ratnasingham & Hebert 2007). The Identification Engine on this platform uses a Hidden Markov model of the *COI* protein to make species-level identifications against a database of approximately 1 million animal barcodes. Only the 5' end of the *COI* gene is accepted by BOLD for identification of animal species. The plum curculio sequences were accessioned into the BOLD database.

DNA barcodes of *Conotrachelus nenuphar* were also generated using a diagnostic character based system. Character-based molecular diagnostics were determined using the characteristic attribute organization system (CAOS) (Sarkar *et al.* 2008; Bergmann *et al.* 2009). The CAOS algorithm identifies conserved character states from a guide tree and is therefore well situated for scaffolding on top of systematic work that has identified well-supported groups (Goldstein & DeSalle 2011). For this analysis, a barcode dataset was built of all Group 1 *Conotrachelus* species analyzed in Chapter 2. This approach was based on the highly informative 3' end of the *COI* gene and while not targeting the standardized barcode region, allowed for the creation of a molecular diagnostic in relation to the other congeneric species in eastern North America. A total of 39 *COI* sequences from the *Conotrachelus* species comprising the monophyletic Group 1 plus the 32 haplotypes for *Conotrachelus nenuphar* recovered in Chapter 3 were used to generate

species-level barcodes. These 71 sequences were aligned using the default settings in MAFFT (Katoh *et al.* 2002; Katoh & Standley 2013) and a neighbor-joining tree was constructed in PAUP* version 4.0 (Swofford 2003) using the GTR model of nucleotide evolution. Following the CAOS guidelines, this NJ tree was processed in Mesquite 2.72 (Maddison & Maddison) to resolve polytomies and collapse nodes around the defined species boundaries. This data was then analyzed with the P-Gnome algorithm on the CAOS workbench to identify diagnostic positions for each taxonomic grouping. The CAOS-generated barcodes were tested against an independent data set of *Conotrachelus nenuphar COI* sequences from GenBank (PopSet: 125657233).

Results

Ecological Niche Models

Three models of plum curculio habitat suitability were built from a total of 71 occurrence data points, 6 climate variables and 3 soil variables. The tuned regularization values, AUC average across 10 replicates, minimum training presence (MTP) threshold value of the averaged model, and the omission rate and associated p-value of the binary predictions from the MTP value are provided for each model in Table 11.

PC-model

The pooled observation model recovered the known distribution of the plum curculio (Figure 10b). The entire eastern United States was predicted as suitable under the binary model, which meets expectations. Areas in the western half of the Dakotas, Nebraska, Kansas, Oklahoma, and Texas were not predicted as suitable. Good habitat drops off quickly in southern Canada and at the southern extreme of Florida. The geographic limits of this binary prediction match the expected distribution, indicating good model performance. Threshold independent

and threshold dependent measures of model performance indicate a model that performs significantly better than random (test AUC=0.833 and omission rate p-value=0.0439).

The global projection predicts large swaths of suitable habitat across South America, Europe, Africa, Asia, and Australia (Figure 11). Very little area is predicted to be suitable in western North America. The PC-model does predict suitable habitat around the Great Salt Lake and Box Elder County in Utah, where the species is currently invasive, and an area that was not included in the model training dataset. Any good model should predict this area. The extent of the area predicted as suitable is very restricted and does not extent beyond where it is currently known to reside in Utah.

N-model

The northern deme model has very restricted range compared to the PC-model (Figure 10a). The southern extent was predicted as far south as a line roughly extending from Delaware to the border of Arkansas and Iowa. Large portions of the Midwest, from western Iowa westward were not predicted as suitable for these northern populations. Areas where the species is known to occur but is thought to be rare such as northern Maine (Glenn Kohler, pers. comm.) and North Dakota (Guy Hanely, pers. comm.) have very patchy predictions in the binary models and so meet expectations from prior knowledge. Evaluation statistics for this model indicate predictive performance better than random (test AUC=0.9261 and omission rate p-value=0.0039).

In contrast to the PC-model, the N-model projection (Figure 11a) does not predict areas across most continents as suitable. Regions in the Southern hemisphere are predicted as unsuitable. Large areas across eastern Europe and Asia (especially China) are predicted to have suitable habitat. Box Elder County is not predicted as suitable habitat for northern populations.

S-model

The model of the southern deme localities indicated that suitable habitat was more widespread then the N-model, but still restricted compared to the PC-model (Figure 10a). Large tracts along the Gulf Coast and southern Florida were not predicted as good habitat. The model did extend north into Wisconsin, Michigan and even southern Ontario. The entire mid-Atlantic and even areas extending into southern New England were predicted to contain suitable habitat by this model. Similar to the N-model, Midwestern States were not predicted to be habitable, especially areas north of Oklahoma. The S-model performed better than random (test AUC=0.8520 and omission rate p-value=0.0405).

Global predictions from the S-model (Figure 11a) cover a similar range as the PC-model, including large areas in South America, Europe, Africa, Asia, and Australia. The extent of predicted suitable areas in Europe and Asia is much smaller than under the N-model. Box Elder County, Utah is predicted as suitable as are a few other isolated patches in Idaho, Montana, and British Columbia. No areas in Washington or Oregon are predicted to be suitable. The PC-model overlaps in all areas with either the N-model (e.g. southwestern Russia), the S-model (e.g. South America and central Africa) or both (e.g. China and Europe).

Considering the extents of the overlapped N-model and S-model compared to the PC-model, there are a few large regions that the PC-model predicts as suitable but the individual deme models do not. For example, within the training region, the PC-model predicts large areas in the eastern portion of South Dakota, Nebraska, and Kansas. The species has been reported from these areas but the sightings and records are rare. The overlapped deme models do not predict these areas or predict relatively small suitable patches. Other areas that drop out of the ENM when the demes are modeled separately are mostly found in the tropics, including:

northern Amazon, southeastern Brazil, central and eastern Africa, and Southeast Asia. All models are statistically significant given their conditions, and the northern populations do occupy a slightly different environmental niche space than the southern populations as demonstrated by the niche identity and similarity tests in Chapter 3. The results taken together imply that the PC-model presented here is less conservative than a composite N/S-model.

Global Invasion Risk Surface

Harvest areas and levels for peaches (and nectarines), plums, cherries, and apples are illustrated in Figure 12. These fruit tree crop area layers were combined and categorized in quartile bins. These quartile groups were then overlaid on the PC-model ENM and an invasion risk surface generated (Figure 13). Areas of low risk are still at risk for invasion because the ENM predicts that the local environment is habitable, but the crop data shows that there is very little (bottom quartile and zero) area occupied by potential fruit tree hosts. Most of the plum curculio's native range falls into the low risk category. Such areas are at risk for invasion (if they do not currently harbor the species) but the risk is low since there is no commercial fruit production. Such areas could be, for example, suburbs or agricultural areas converted over to corn and soy production with very few and distant potential hosts.

Areas within the plum curculio's native range that also are significant fruit production regions, however, are in higher risk categories. New York (apples), Michigan (apples, peaches, cherries), and Georgia (peaches) all contain areas that are in the upper quartile of fruit production but the geographic extent of these areas are much less than those found in Europe and China (Figure 12). Thus, as a function of the environmental niche model and the availability of host resources, Europe and China are at very high risk of plum curculio establishment and

spread (not accounting for probability of arrival). Western North America, which has upper quartile levels of fruit harvest in Oregon (apples) and California (apples, cherries, plums, and peaches) are at low risk for invasion by plum curculio because of a lack of suitable habitat.

Molecular Identification Diagnostics

Standardized Barcoding Region and BOLD

New sequences covering the 5' end of the *COI* gene were sequenced for 161 specimens of *Conotrachelus nenuphar* from across its geographic range. The fragment size was 658bp and there was no length variation across samples. Average GC content was 34.85% (±0.022) and there were no ambiguous sites or anomalous stop codons. These sequences have been uploaded to the Barcode of Life Data Systems workbench (Project code: NAPCB). This data expands the species-level records for *Conotrachelus* in BOLD and captures additional sequence variation.

Character-based Molecular Diagnostics and CAOS

Table 12 lists 36 selected character attributes (CA) for the 3' region of the *COI* gene summarized for 13 *Conotrachelus* groups. The complete barcode, character attribute table, and associated FASTA for use in the CAOS workbench are available by request and will be made available via FigShare.com (Figshare, Inc.). All of the groups analyzed showed unique character combinations, allowing for discrimination. These 36 CAs were highlighted here because as a minimum set they allow for most species to be diagnosed by three simple pure CAs: *C. seniculus* (A-1, C-124, T-169), *C. retentus* (G-22, C-328, C-581), *C. juglandis* (T-232, G-304, G-628), *C. aratus*/*C. schoofi* (T-94, T-670, C-760), *C. corni* (C-627, G-724, G-733), *C. nenuphar* northern deme (A-59, C-127, A-718), *C. nenuphar* southern deme (C-172, G-607, A-271), *C. albicinctus* (C-639, C-659, A-741), *C. iowensis* (C-220, G-253, G-292). The two species *C. buchanani* and *C. downiei* can be grouped together with simple pure CAs (G-7, G-724, G-733) and can then be

distinguished from each other by complex pure or complex private CAs (not shown). *Conotrachelus elegans* can be diagnosed with simple and complex pure CAs (T-292, T-772, CT-667,668).

The DNA barcode is also able to diagnose regional variation within *Conotrachelus nenuphar*. Two geographically distinct mitochondrial groups are resolvable with simple pure CAs and enable the diagnosis to identify regional source populations in the case of mid-Atlantic (Sb haplotypes) and Midwestern (Sa haplotypes) The complete 3' COI barcode for *Conotrachelus nenuphar* and regional variation is shown in Table 13.

Discussion

Areas At Risk of Invasion

Invasion risk is quantified as the product of the probabilities of the three phases of invasion: arrival, establishment, and spread. Here, I have used environmental niche modeling to quantify the likelihood of establishment based on a binary prediction of minimally suitable habitat and the likelihood of spread as a function of host resource prevalence. The environmental niche models based on a combination of climatic and soil characteristics predict large regions across all continents (except Antarctica which was excluded from the analysis) as containing suitable habitat for the plum curculio fruit pest. However, not all areas predicted as suitable have host tree densities comparable to those found in the endemic range of the beetle—especially areas in the tropics. Given the finding that plum curculio larvae do not successfully develop in the flesh of tropical fruits (Hallman & Gould 2004), these areas are likely at a low risk of invasion. However, there are areas in Europe and Asia—the native ranges to the commercial fruit cultivars now grown in eastern North America—that have broad areas of high intensity fruit

cultivation that are also predicted by the ENMs to have suitable habitat. Should the plum curculio survive the arrival phase and avoid quarantine in these regions, the species is likely to establish. The invasion risk surface also shows that there is a high probability of spread in these regions given the relative densities of commercial fruit trees that are common hosts.

Molecular Diagnostics

This study has demonstrated that the *COI* gene is a useful marker for diagnosing *Conotrachelus nenuphar* from its congeners as well as identifying certain regional variants within the species. The northern and southern mitochondrial demes (Chapter 3) are readily diagnosable from each other, and within the southern populations, genetic variants found west of the Mississippi and in the mid-Atlantic are also diagnosable from the broader southern distribution. The standardized DNA barcode region for animals is able to identify *Conotrachelus nenuphar* samples within the BOLD platform using their online Identification Engine. The non-standard 3' end of the *COI* gene is also a good barcode for this species and using a character-based diagnostic approach in the CAOS workbench, regional variants and species-level identifications within *Conotrachelus* are possible.

Management Implications

The current quarantine status of *Conotrachelus nenuphar* is justified given the environmental niche model predictions of globally suitable habitats presented here. Countries with both suitable habitat and high levels of commercial fruit production should be especially vigilant in their quarantine efforts because the likelihood of establishment and spread is especially high in these areas. Eastern Europe and China are uniquely vulnerable since the size of the high-risk areas is so much greater than anywhere else. The potential for economic harm given the probability of invasion is greatest in these areas and preventative measures are sure to be more

cost effective that later eradication efforts (Sakai *et al.* 2001)—especially given the current costs of control efforts in the United States and Canada.

Given the identification success of the *COI* barcodes for plum curculio at the species and regional level, these molecular diagnostics should aid in the identification of insect fruit contaminants. Larval and pupal life stages are especially a risk for misidentification because of the lack of reliable identification diagnostics or keys for them. Additional sampling of regional variation and any novel genetic variations recovered in future analyses will still resolve to these regional and species-level barcodes given the flexibility of the HMM identification model of BOLD and the tree-based diagnostic character approach of CAOS.

Table 7 Occurrence Dataset

Locality	State / Province	Latitude	Longitude	GPS Source	3'-COI Clade	Source
Clanton	AL	32.91920	-86.67350	Field survey	S	AMCC
Fayetteville	AR	36.10610	-94.10560	Field survey	S	AMCC
Forrest City	AR	34.94698	-90.77051	Field survey	S	Pers. Comm. w/ Donn Johnson
Rogers	AR	36.31570	-94.09600	Field survey	S	AMCC
Wynne	AR	35.21667	-90.75000	Field survey	S	Pers. Comm. w/ Donn Johnson
Monticello	FL	30.53300	-83.83000	Georeference from label	S	CWOB
Quincy	FL	30.58300	-84.56700	Published GPS	S	Zhang_etal_2008
Athens	GA	33.88600	-83.41940	Georeference from publication	S	Lan and Scherm 2003
Byron	GA	32.66890	-83.72270	Field survey	S	AMCC & Zhang_etal_2008
Crawford	GA	32.67760	-84.00478	Published GPS	S	Jenkins_etal_2006
Houston	GA	32.49590	-83.55580	Published GPS	S	Jenkins_etal_2006
Macon	GA	32.47293	-83.04613	Published GPS	S	Jenkins_etal_2006
Taylor	GA	32.51657	-84.50152	Published GPS	S	Jenkins_etal_2006
Homestead	IA	41.75870	-91.86630	Field survey	S	AMCC
Kalona	IA	41.45990	-91.70270	Field survey	S	AMCC
Dixon Springs	IL	37.43750	-88.67220	Field survey	S	AMCC
Pell	IL	40.08040	-88.19080	Field survey	S	AMCC
Ringhausen Ridge	IL	39.07310	-90.65800	Field survey	S	AMCC
Urbana	IL	40.08230	-88.21310	Field survey	S	AMCC
Buckley Homestead	IN	41.28240	-87.37800	Field survey	S	AMCC
Crown Point	IN	41.38400	-87.25410	Field survey	S	AMCC
LaPorte	IN	41.71320	-86.67150	Field survey	S	AMCC
Throckmorton	IN	40.29130	-86.88020	Field survey	S	AMCC
West Lafayette	IN	40.43030	-86.95180	Field survey	S	AMCC
Versailles	KY	37.99940	-84.69400	Field survey	S	AMCC
Amherst	MA	42.31860	-72.53400	Field survey	N	AMCC & Zhang_etal_2008
Colrain	MA	42.72110	-72.75040	Field survey	N	AMCC
Hyattsville	MD	38.93333	-76.91667	Georeference from label	S	USNM
Monmouth	ME	44.23079	-70.06844	Field Survey	N	Pers. Comm. w/ Glenn Koehler
Bear Lake	MI	44.40300	-86.18240	Field survey	S	AMCC
Fennville	MI	42.59690	-86.15680	Field survey	S	AMCC
Lansing	MI	42.68920	-84.49870	Field survey	S	AMCC
Northport	MI	45.13510	-85.65270	Field survey	N	AMCC
Traverse City	MI	44.86700	-85.67500	Field survey	S, N	AMCC
Frazee	MN	46.73460	-95.75980	Field survey	N	AMCC
Upsala	MN	45.81920	-94.62800	Field survey	N	AMCC
Mountain Grove	MO	37.15330	-92.27000	Georeference from publication	S	Sarai 1969
New Franklin	MO	39.02110	-92.75860	Field survey	S	AMCC
Mills River	NC	35.42948	-82.56136	Field survey	S	AMCC
Spruce Pine	NC	35.87271	-82.02527	Field survey	S	AMCC
Minot	ND	48.23700	-101.31133	Field survey	N	Pers. Comm w/ Guy Hanley
Madbury	NH	43.16860	-70.93440	Field survey	N	AMCC

Bridgeton	NJ	39.52180	-75.20090	Field survey	S	AMCC & Zhang_etal_2008
Chatsworth	NJ	39.81700	-74.53300	Published GPS	S	Zhang_etal_2008
Cream Ridge	NJ	40.11690	-74.52140	Field survey	S	AMCC
Geneva	NY	42.87390	-77.00810	Field survey	N	AMCC & Zhang_etal_2008
Columbus	OH	40.00920	-83.03760	Field survey	S	AMCC
Perkins	OK	35.99860	-97.04800	Field survey	S	AMCC
Tenkiller	OK	35.59327	-95.02575	Georeference from database	S	http://bugguide.net/node/view/451838
Sewickley	PA	40.58334	-80.12764	Georeference from photograph EXIF	S	http://flic.kr/p/cSNFWo
Brigham	QC	45.25420	-72.73060	Field survey	N	AMCC
Frelighsburg	QC	45.04730	-72.85800	Field survey	N	AMCC
Granby	QC	45.43580	-72.68250	Field survey	N	AMCC
Henryville	QC	45.13460	-73.22000	Field survey	N	AMCC
Mont Saint Gregoire	QC	45.34900	-73.11990	Field survey	N	AMCC
Mont Saint Hilaire	QC	45.53260	-73.15980	Field survey	N	AMCC
Saint Bruno de Montarville	QC	45.54330	-73.34130	Field survey	N	AMCC
Saint Hyacinthe	QC	45.61910	-72.97010	Field survey	N	AMCC
Shefford	QC	45.36890	-72.64680	Field survey	N	AMCC
Nashville	TN	36.06550	-86.74640	Field survey	S	AMCC
Austin	TX	30.28432	-97.77826	Georeference from database	S	http://bugguide.net/node/view/724198
Blacksburg	VA	37.21700	-80.40000	Published GPS	S	Zhang_etal_2008
Fort AP Hill	VA	38.12428	-77.29490	Georeference from label	S	USNM
Troutville	VA	37.40000	-79.86700	Published GPS	S	Zhang_etal_2008
Washington	VA	38.68300	-78.13300	Published GPS	S	Zhang_etal_2008
Whitethorne	VA	37.19900	-80.21600	Published GPS	S	Zhang_etal_2008
Sturgeon Bay	WI	44.88130	-87.32370	Field survey	N	AMCC
Sun Prairie	WI	43.11440	-89.21400	Field survey	S	AMCC
Verona	WI	43.05420	-89.53590	Field survey	S	AMCC
Kearneysville	WV	39.35510	-77.87500	Field survey	S	AMCC & Zhang_etal_2008
Lost River	WV	38.91500	-78.84400	Georeference from label	S	USNM

Abbreviations: Ambrose Monell Cryo Collection (AMCC); Charles O'Brien Collection (CWOB); National Museum of Natural History, Smithsonian (USNM)

Table 8 GIS Layers: Climate, Soil, Agriculture

	Environmental and Agricultural Variables and Data Sources
1.	Annual Mean Temperature[a]
2.	Mean Diurnal Range (Mean of monthly (max temp - min temp))[a,†]
3.	Isothermality[a,†]
4.	Temperature Seasonality (standard deviation *100) [a]
5.	Max Temperature of Warmest Month[a,†]
6.	Min Temperature of Coldest Month[a]
7.	Temperature Annual Range[a]
8.	Mean Temperature of Wettest Quarter[a,†]
9.	Mean Temperature of Driest Quarter[a,†]
10.	Mean Temperature of Warmest Quarter[a]
11.	Mean Temperature of Coldest Quarter[a]
12.	Annual Precipitation[a]
13.	Precipitation of Wettest Month[a]
14.	Precipitation of Driest Month[a]
15.	Precipitation Seasonality (Coefficient of Variation) [a]
16.	Precipitation of Wettest Quarter[a]
17.	Precipitation of Driest Quarter[a,†]
18.	Precipitation of Warmest Quarter[a,†]
19.	Precipitation of Coldest Quarter[a]
20.	Bulk density[b,†]
21.	Field capacity[b]
22.	Profile available water capacity[b,†]
23.	Soil carbon density[b]
24.	Thermal capacity[b]
25.	Total nitrogen density[b,†]
26.	Wilting point[b]
27.	Apple harvest area[c]
28.	Plum harvest area[c]
29.	Peach harvest area[c]
30.	Cherry harvest area[c]

[†]Variable used in niche model. Data Sources: [a]WorldClim (Hijmans *et al.* 2005); [b]Oak Ridge National Laboratory (Global Soil Data Task Group 2000); [c]Center for Sustainability and the Global Environment, University of Wisconsin-Madison (Monfreda *et al.* 2008).

Table 9 Invasion Risk Categorization

Risk Category	Fruit Harvest Area	Habitat
Very Low	Zero harvest area	Not suitable
Low	Bottom quartile	Suitable
Moderate	Lower middle quartile	Suitable
High	Upper middle quartile	Suitable
Very High	Top quartile	Suitable

Table 10 DNA Barcode Specimen Metadata

AMCC ID	State	Locality	Latitude	Longitude	Date	Host
180409	AL	Clanton	32.9192	-86.6735	18-Jun-09	Peach
180422	AL	Clanton	32.9192	-86.6735	25-Jun-09	Peach
180618	IL	Dixon Springs	37.4375	-88.6722	28-Apr-09	Apple
180628	IL	Urbana	40.0823	-88.2131	22-May-09	Apple
180721	MA	Colrain	42.7211	-72.7504	31-May-09	Apple
180801	MI	Fennville	42.5969	-86.1568	27-May-09	Apple
180960	MI	Northport	45.1351	-85.6527	18-May-09	Cherry
180961	MI	Northport	45.1351	-85.6527	18-May-09	Cherry
181044	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181050	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181051	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181056	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181060	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181061	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181062	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181065	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181066	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181067	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181069	NH	Madbury	43.1686	-70.9344	31-May-09	Apple
181128	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181130	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181133	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181135	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181136	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181139	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181140	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181141	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181142	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181144	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181145	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181147	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181149	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181150	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181151	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181152	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181153	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181157	NY	Geneva	42.8739	-77.0081	28-May-09	Apple
181782	WI	Sturgeon Bay	44.8813	-87.3237	8-May-09	Apple
181783	WI	Sturgeon Bay	44.8813	-87.3237	8-May-09	Apple
181785	WI	Sturgeon Bay	44.8813	-87.3237	22-May-09	Apple
181790	WI	Sturgeon Bay	44.8813	-87.3237	22-May-09	Apple
181792	WI	Sturgeon Bay	44.8813	-87.3237	22-May-09	Apple
181802	WI	Sturgeon Bay	44.8813	-87.3237	22-May-09	Apple
181803	WI	Sturgeon Bay	44.8813	-87.3237	22-May-09	Apple
181809	WI	Sturgeon Bay	44.8813	-87.3237	29-May-09	Apple
181810	WI	Sturgeon Bay	44.8813	-87.3237	29-May-09	Apple
181811	WI	Sturgeon Bay	44.8813	-87.3237	29-May-09	Apple
182016	MI	Traverse City	44.8670	-85.6750	Jun-09	Cherry or Apple
182019	MI	Traverse City	44.8670	-85.6750	Jun-09	Cherry or Apple
182020	MI	Traverse City	44.8670	-85.6750	Jun-09	Cherry or Apple
182021	MI	Traverse City	44.8670	-85.6750	Jun-09	Cherry or Apple
182022	MI	Traverse City	44.8670	-85.6750	Jun-09	Cherry or Apple
182024	MI	Traverse City	44.8670	-85.6750	Jun-09	Cherry or Apple
182035	MI	Traverse City	44.8670	-85.6750	Jun-09	Cherry or Apple
182266	QC	Brigham	45.2542	-72.7306	5-Jun-09	Blueberry
182267	QC	Brigham	45.2542	-72.7306	5-Jun-09	Blueberry
182269	QC	Brigham	45.2542	-72.7306	5-Jun-09	Blueberry
182271	QC	Brigham	45.2542	-72.7306	5-Jun-09	Blueberry
182272	QC	Brigham	45.2542	-72.7306	9-Jun-09	Blueberry
182274	QC	Brigham	45.2542	-72.7306	9-Jun-09	Blueberry
182275	QC	Brigham	45.2542	-72.7306	9-Jun-09	Blueberry

182276	QC	Brigham	45.2542	-72.7306	9-Jun-09	Blueberry
182277	QC	Brigham	45.2542	-72.7306	9-Jun-09	Blueberry
182278	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182279	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182280	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182281	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182283	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182284	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182285	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182287	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182289	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182291	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182293	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182296	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182298	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182301	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182302	QC	Brigham	45.2542	-72.7306	12-Jun-09	Blueberry
182305	QC	Shefford	45.3689	-72.6468	5-Jun-09	Blueberry
182306	QC	Shefford	45.3689	-72.6468	5-Jun-09	Blueberry
182307	QC	Shefford	45.3689	-72.6468	5-Jun-09	Blueberry
182308	QC	Shefford	45.3689	-72.6468	5-Jun-09	Blueberry
182309	QC	Shefford	45.3689	-72.6468	5-Jun-09	Blueberry
182310	QC	Shefford	45.3689	-72.6468	5-Jun-09	Blueberry
182311	QC	Shefford	45.3689	-72.6468	5-Jun-09	Blueberry
182312	QC	Shefford	45.3689	-72.6468	5-Jun-09	Blueberry
182314	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182315	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182316	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182317	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182318	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182319	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182320	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182321	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182322	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182324	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182325	QC	Shefford	45.3689	-72.6468	9-Jun-09	Blueberry
182326	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182331	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182333	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182336	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182504	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182506	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182507	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182508	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182509	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182510	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182511	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182512	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182514	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182515	MN	Upsala	45.8192	-94.6280	24-Jun-09	Apple
182519	MN	Frazee	46.7346	-95.7598	14-Jun-09	Plum
182520	MN	Frazee	46.7346	-95.7598	14-Jun-09	Plum
182525	MN	Frazee	46.7346	-95.7598	14-Jun-09	Plum
182528	MN	Frazee	46.7346	-95.7598	14-Jun-09	Plum
182530	MN	Frazee	46.7346	-95.7598	9-Jun-09	Plum
182532	MN	Frazee	46.7346	-95.7598	9-Jun-09	Plum
182533	MN	Frazee	46.7346	-95.7598	14-Jun-09	Plum
182534	MN	Frazee	46.7346	-95.7598	9-Jun-09	Plum
182588	NJ	Bridgeton	39.5218	-75.2009	10-May-09	Peach
182595	NJ	Bridgeton	39.5218	-75.2009	22-May-09	Peach
182603	NJ	Bridgeton	39.5218	-75.2009	29-May-09	Peach
182609	NJ	Cream Ridge	40.1169	-74.5214	29-May-09	Apple
182679	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182680	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry

182681	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182684	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182685	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182686	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182687	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182688	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182689	QC	Shefford	45.3689	-72.6468	12-Jun-09	Blueberry
182690	QC	Mont-Saint-Grégoire	45.3490	-73.1199	16-May-09	Apple
182691	QC	Mont-Saint-Grégoire	45.3490	-73.1199	11-Jun-09	Apple
182693	QC	Mont-Saint-Grégoire	45.3490	-73.1199	11-Jun-09	Apple
182695	QC	Granby	45.4358	-72.6825	5-Jun-09	Blueberry
182696	QC	Granby	45.4358	-72.6825	5-Jun-09	Blueberry
182697	QC	Granby	45.4358	-72.6825	5-Jun-09	Blueberry
182698	QC	Granby	45.4358	-72.6825	5-Jun-09	Blueberry
182699	QC	Granby	45.4358	-72.6825	9-Jun-09	Blueberry
182700	QC	Granby	45.4358	-72.6825	9-Jun-09	Blueberry
182701	QC	Granby	45.4358	-72.6825	9-Jun-09	Blueberry
182703	QC	Granby	45.4358	-72.6825	12-Jun-09	Blueberry
182704	QC	Granby	45.4358	-72.6825	12-Jun-09	Blueberry
182705	QC	Granby	45.4358	-72.6825	12-Jun-09	Blueberry
183679	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183680	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183681	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183682	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183684	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183686	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183689	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183690	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183691	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183692	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183694	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183695	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183696	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183697	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183698	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple
183699	QC	Frelighsburg	45.0461	-72.3729	3-Jul-09	Apple

Table 11 Model Evaluation Statistics from ENM

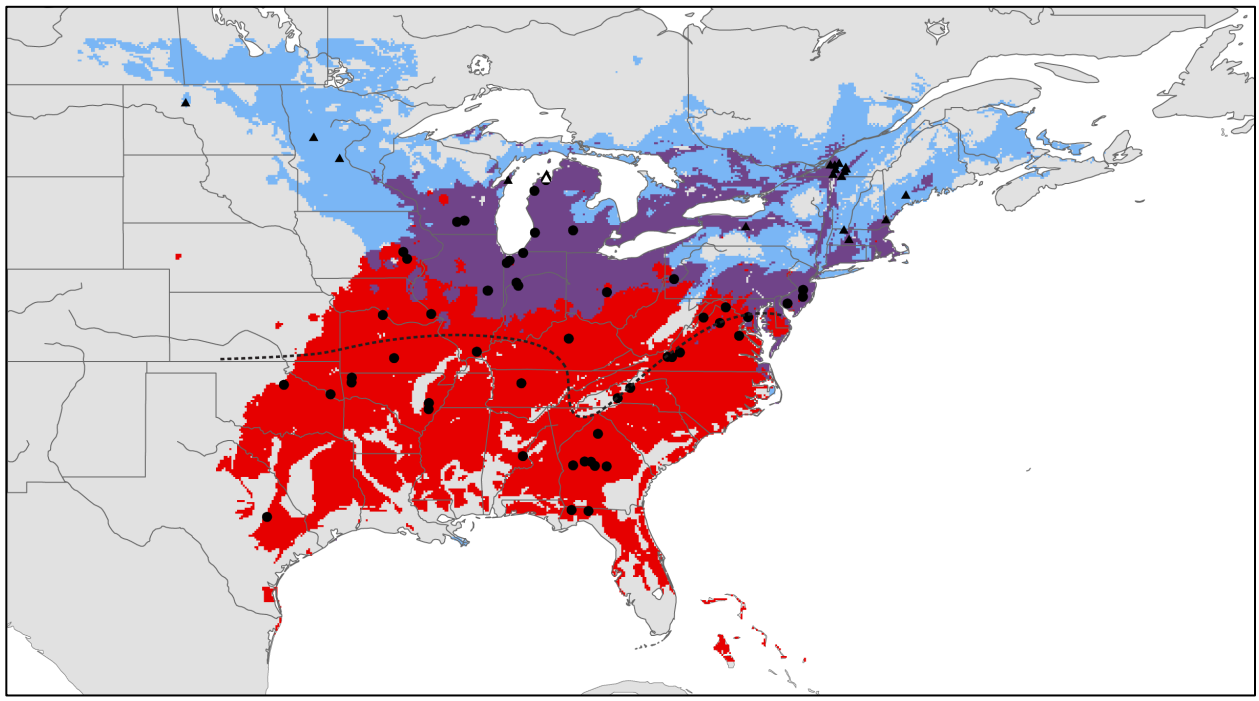
Model	Beta multiplier	AUC Average	MTP Threshold	Omission Rate	p-value
PC	1.6	0.8336	0.0891	0.0286	0.0439
N	1.7	0.9261	0.3185	0.0600	0.0039
S	1.5	0.8520	0.2621	0.1167	0.0405

Figure 10 ENM model.

The environmental niche model built using all occurrence points (10b) and split datasets of the northern group (blue, 10a) and southern group (red, 10a). The overlap between the N-model and S-model is indicated by purple shading in 10a. Chapman's boundary between the northern strain and southern strain plum curculio is indicated in 10a with a dashed line. Occurrence points used to build the ENMs are indicated by circles (S-model) and triangles (N-model) on the maps.

Figure 10 Environmental Niche Models.

a.



b.

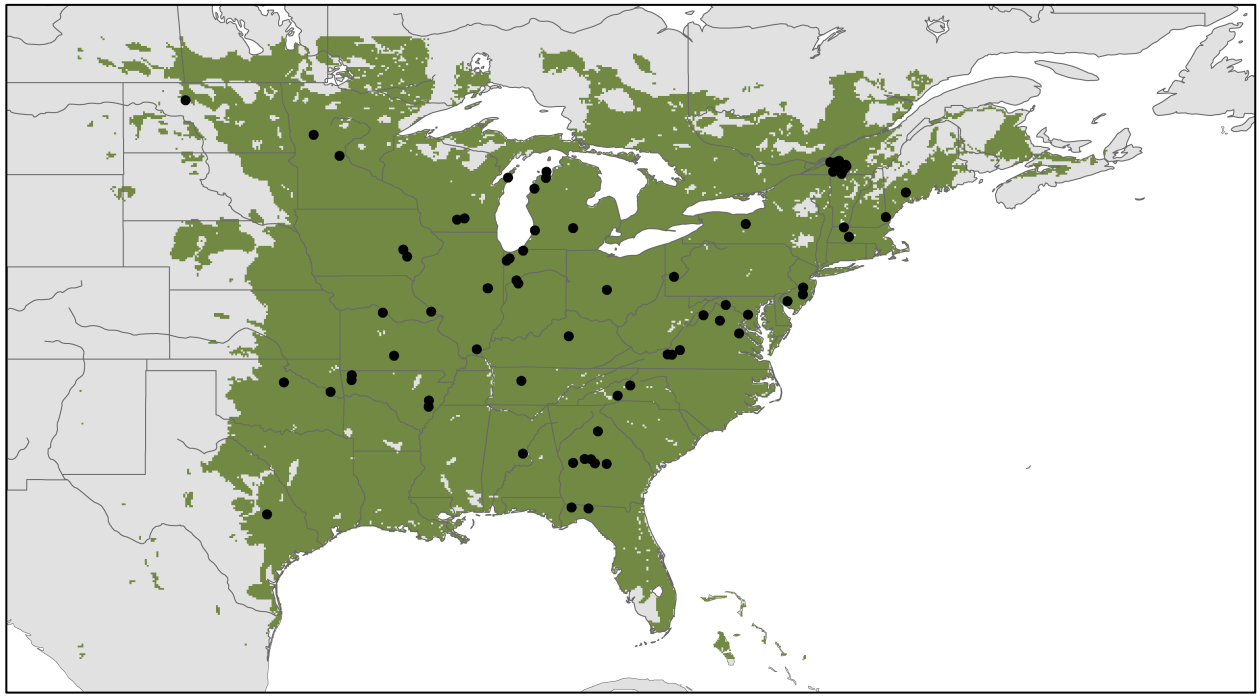
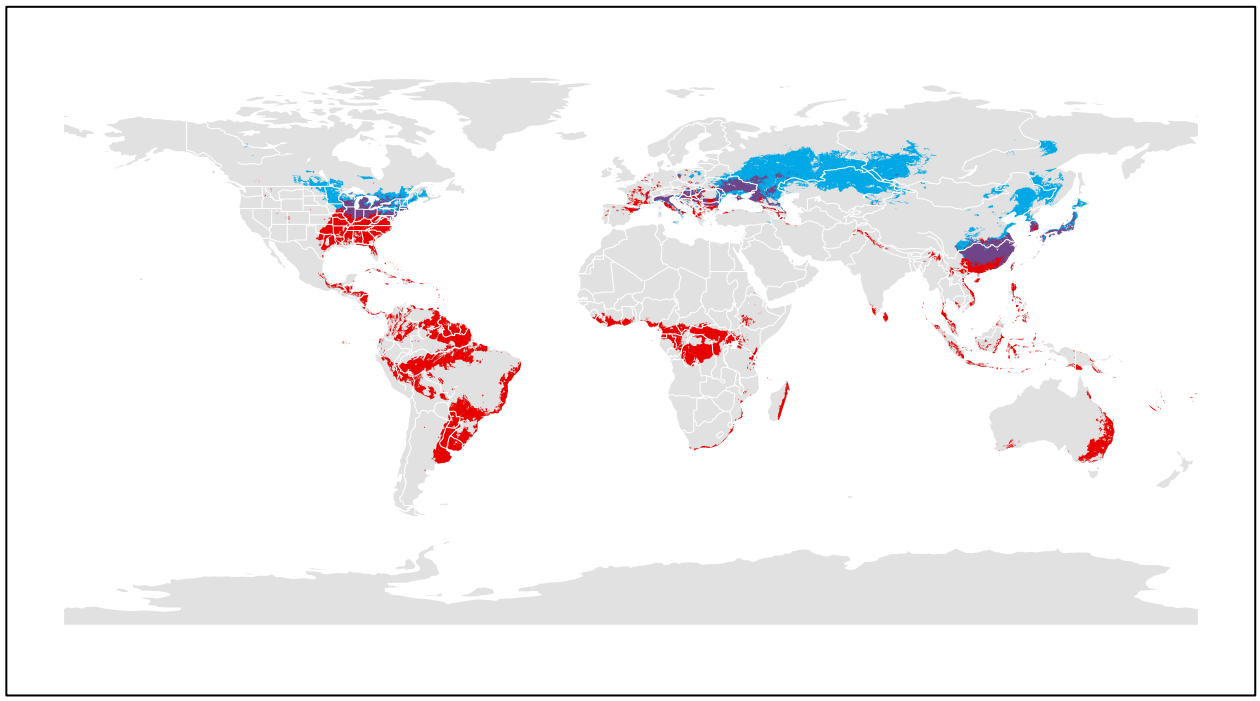


Figure 11 ENM Projections.

Global projections of the native ENMs for the PC-model (b) and the N-model and S-model (a). The overlap between the N-model and S-model is shown by the purple shading.

Figure 11 ENM Projections.

a.



b.

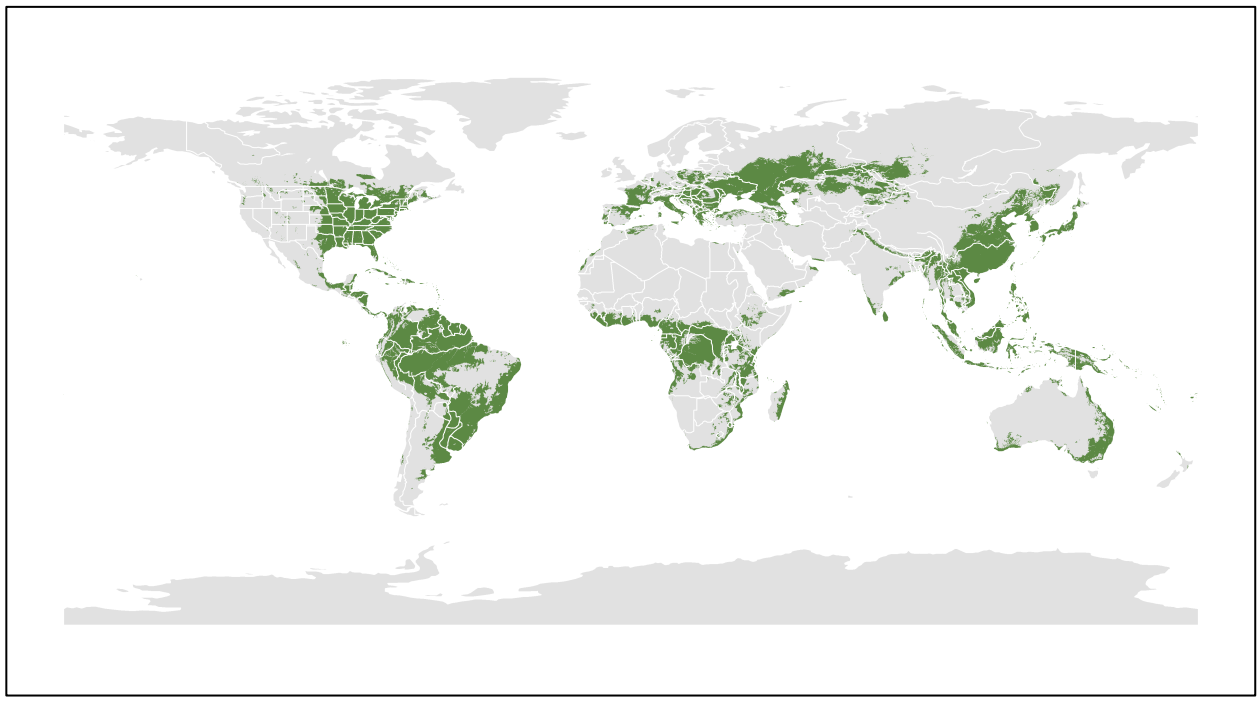


Figure 12 Fruit Tree Harvest Areas

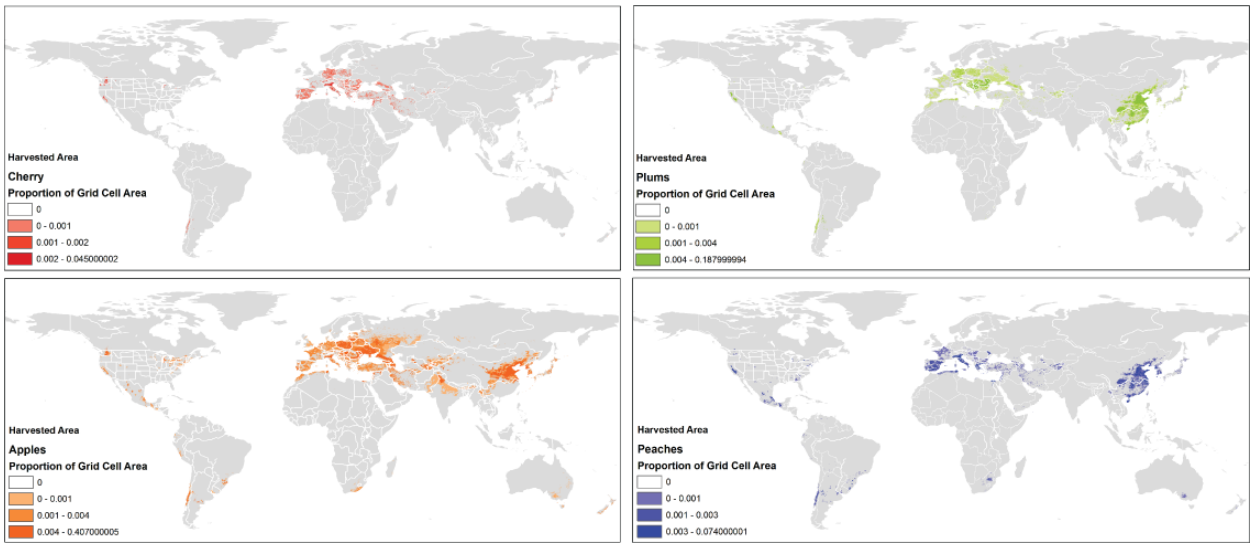


Figure 13 Plum Curculio Invasion Risk Surface

Prediction heat map of risk associated with establishment and spread of the plum curculio as a function of commercial host tree abundance and habitat suitability. The risk categories follow the scheme in Table 9. Large areas are at high or very high risk of plum curculio invasion in Europe and Asia.

Figure 13 Plum Curculio Invasion Risk Surface

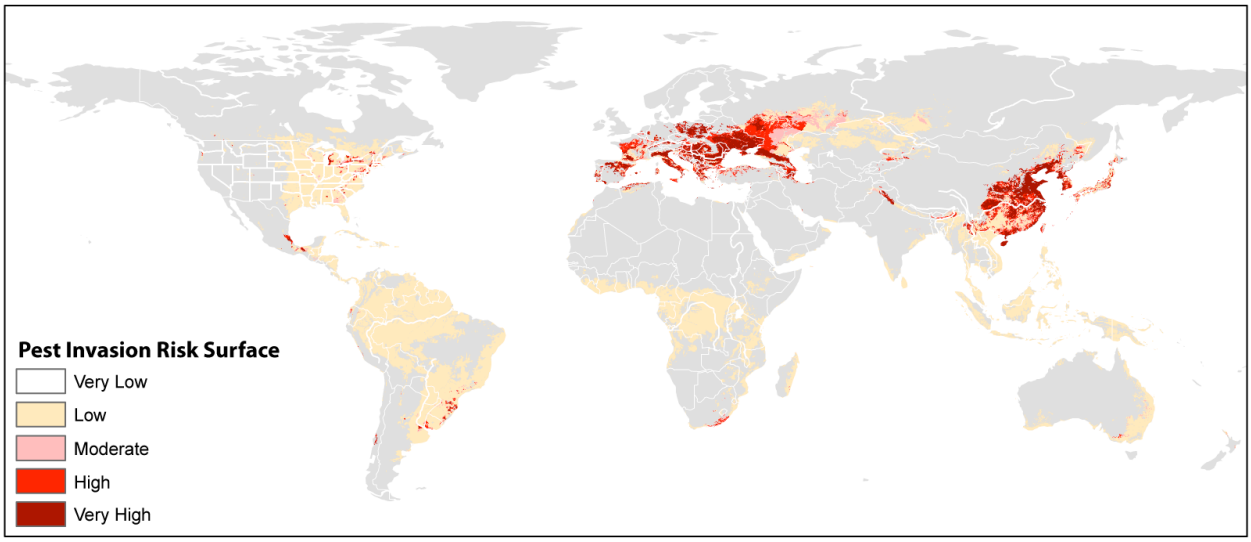


Table 13

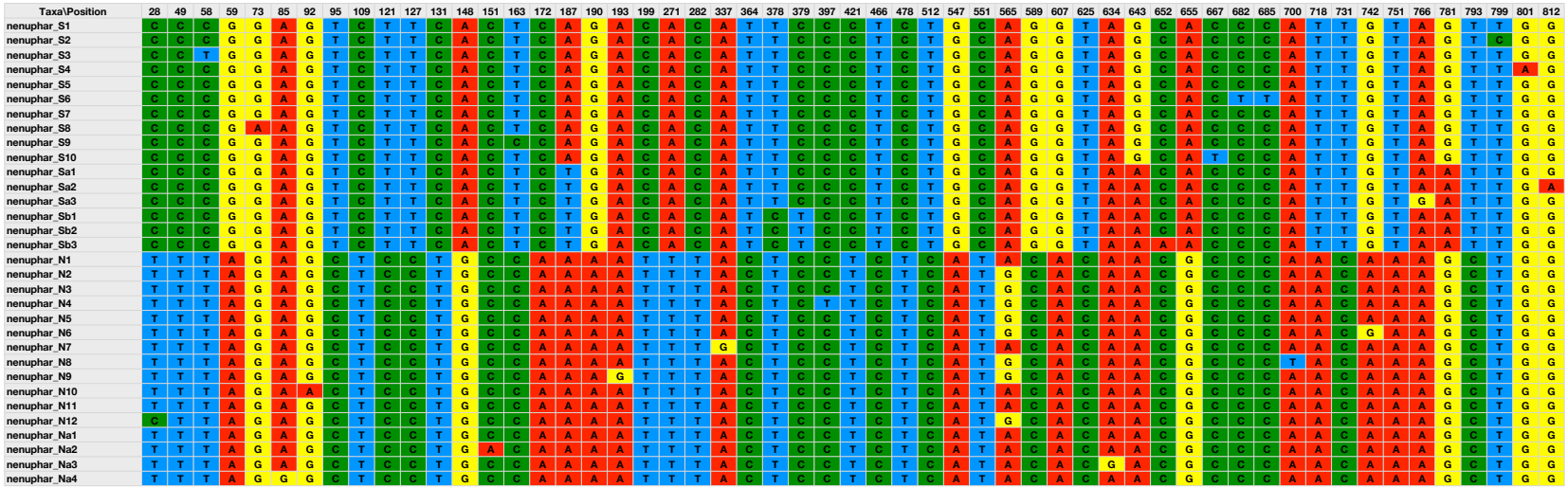
Selected (n=36) diagnostic nucleotides for *Conotrachelus* species and groups from the CAOS workbench (a). These characters were selected because they are able to resolve the listed species and groups using a combination of simple pure and complex pure character attributes. More detail is shown in (b) for the characters that diagnose the regional variation within *Conotrachelus nenuphar*.

Table 12 DNA Barcode Diagnostic Sites

a. 3' *COI Conotrachelus* species-level diagnostic characters

Taxa/Position	1	7	22	55	59	94	124	127	169	172	220	232	253	271	286	292	301	304	328	581	607	627	628	639	659	667	668	670	671	718	724	733	741	760	772
affinis/hicoriae	C	A	A	C	G	A	T	T	A	A	T	C	A	T	C	A	T	A	T	T	A	T	A	T	T	C	C	A	G	T	A	A	T	T	A
albicinstus	T	A	A	C	G	A	T	T	A	A	T	C	A	T	T	A	T	A	T	T	A	T	A	C	C	C	C	A	A	T	A	A	A	T	A
aratus/schoofi	T	A	A	C	G	T	T	T	A	A	T	C	A	T	T	A	A	A	T	T	A	T	A	T	T	T	C	T	A	T	A	A	T	C	A
buchanani	T	G	A	T	G	A	T	T	A	T	T	A	A	Y	T	A	T	A	T	T	A	T	A	T	T	C	C	A	A	T	A	A	T	T	C
corni	T	A	A	C	G	A	T	T	A	T	-	-	-	-	-	-	-	-	-	T	A	C	A	T	T	C	C	A	A	T	G	G	T	T	A
downiei	T	G	A	T	G	A	T	T	A	T	T	A	A	C	T	A	T	A	T	T	A	T	A	T	T	C	C	A	A	T	A	A	T	T	C
elegans	T	A	A	C	G	A	T	T	A	A	T	C	A	T	T	T	A	A	T	T	A	T	A	T	T	C	T	A	A	T	A	A	T	T	T
iowensis	T	A	A	C	G	A	T	T	A	A	C	C	G	T	T	G	C	A	T	T	A	T	A	T	T	C	C	A	A	T	A	A	T	T	A
juglandis	T	A	A	T	G	A	T	T	A	A	T	T	A	T	T	A	T	G	T	T	A	T	G	T	T	C	C	A	A	T	A	A	T	T	A
nenuphar_N	T	A	A	C	A	A	T	C	A	A	T	C	A	T	T	A	T	A	T	T	A	T	A	T	T	C	C	A	A	A	A	A	T	T	A
nenuphar_S	T	A	A	C	G	A	T	T	A	C	T	C	A	A	T	A	T	A	T	T	G	T	A	T	T	Y	C	A	A	T	A	A	T	T	A
retentus	T	A	G	T	G	A	T	T	A	A	T	C	A	T	T	A	A	A	C	C	A	T	A	T	T	C	C	A	A	T	A	A	T	T	A
seniculus	A	A	A	T	G	A	C	A	T	T	T	A	A	C	T	A	A	A	T	T	T	T	A	T	T	T	T	A	T	T	A	A	T	A	A

b. 3’ *COI Conotrachelus nenuphar* species-level and regional diagnostic characters



Taxa\Position	28	49	58	59	73	85	92	95	109	121	127	131	148	151	163	172	187	190	193	199	271	282	337	364	378	379	397	421	466	478	512	547	551	565	589	607	625	634	643	652	655	667	682	685	700	718	731	742	751	766	781	793	799	801	812
nenuphar_S1	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	C	C	C	A	T	T	G	T	A	G	T	T	G	G
nenuphar_S2	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	C	C	C	A	T	T	G	T	A	G	T	C	G	G
nenuphar_S3	C	C	T	G	G	A	G	T	C	T	T	C	A	C	T	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	C	C	C	A	T	T	G	T	A	G	T	T	G	G
nenuphar_S4	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	C	C	C	A	T	T	G	T	A	G	T	T	A	G
nenuphar_S5	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	C	C	C	A	T	T	G	T	A	G	T	T	G	G
nenuphar_S6	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	C	T	T	A	T	T	G	T	A	G	T	T	G	G
nenuphar_S7	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	C	C	C	A	T	T	G	T	A	G	T	T	G	G
nenuphar_S8	C	C	C	G	A	A	G	T	C	T	T	C	A	C	T	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	C	C	C	A	T	T	G	T	A	G	T	T	G	G
nenuphar_S9	C	C	C	G	G	A	G	T	C	T	T	C	A	C	C	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	C	C	C	A	T	T	G	T	A	G	T	T	G	G
nenuphar_S10	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	A	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	G	C	A	T	C	C	A	T	T	G	T	A	G	T	T	G	G
nenuphar_Sa1	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	T	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	A	C	A	C	C	C	A	T	T	G	T	A	A	T	T	G	G
nenuphar_Sa2	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	T	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	A	C	A	C	C	C	A	T	T	G	T	A	A	T	T	G	A
nenuphar_Sa3	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	T	G	A	C	A	C	A	T	T	C	C	C	T	C	T	G	C	A	G	G	T	A	A	C	A	C	C	C	A	T	T	G	T	G	A	T	T	G	G
nenuphar_Sb1	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	T	G	A	C	A	C	A	T	C	T	C	C	T	C	T	G	C	A	G	G	T	A	A	C	A	C	C	C	A	T	T	G	T	A	A	T	T	G	G
nenuphar_Sb2	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	T	G	A	C	A	C	A	T	C	T	C	C	T	C	T	G	C	A	G	G	T	A	A	C	A	C	C	C	A	T	T	G	T	A	A	T	T	G	G
nenuphar_Sb3	C	C	C	G	G	A	G	T	C	T	T	C	A	C	T	C	T	G	A	C	A	C	A	T	C	T	C	C	T	C	T	G	C	A	G	G	T	A	A	A	A	C	C	C	A	T	T	G	T	A	A	T	T	G	G
nenuphar_N1	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	A	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_N2	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	G	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_N3	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	G	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_N4	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	T	T	C	T	C	A	T	G	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_N5	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	G	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_N6	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	G	C	A	C	A	A	C	G	C	C	C	A	A	C	G	A	A	G	C	T	G	G
nenuphar_N7	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	G	C	T	C	C	T	C	T	C	A	T	A	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_N8	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	G	C	A	C	A	A	C	G	C	C	C	T	A	C	A	A	A	G	C	T	G	G
nenuphar_N9	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	G	T	T	T	A	C	T	C	C	T	C	T	C	A	T	G	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_N10	T	T	T	A	G	A	A	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	A	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_N11	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	A	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_N12	C	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	G	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_Na1	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	A	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_Na2	T	T	T	A	G	A	G	C	T	C	C	T	G	A	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	A	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_Na3	T	T	T	A	G	A	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	A	C	A	C	A	C	G	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G
nenuphar_Na4	T	T	T	A	G	G	G	C	T	C	C	T	G	C	C	A	A	A	A	T	T	T	A	C	T	C	C	T	C	T	C	A	T	A	C	A	C	A	A	C	G	C	C	C	A	A	C	A	A	A	G	C	T	G	G

References

Alston DG, Rangel DEN, Lacey LA *et al.* (2005) Evaluation of novel fungal and nematode isolates for control of *Conotrachelus nenuphar* (Coleoptera: Curculionidae) larvae. *Biological Control*, **35**, 163–171.

Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F (2004) Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. *Bioinformatics*, **20**, 407–415.

Anderson RP, Gonzalez I Jr (2011) Species-specific tuning increases robustness to sampling bias in models of species distributions: An implementation with Maxent. *Ecological Modelling*, **222**, 2796–2811.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press.

Avise JC (2004) *Molecular Markers, Natural History, and Evolution*. Sinauer Associates.

Ballard JWO, Rand DM (2005) The Population Biology of Mitochondrial DNA and Its Phylogenetic Implications. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 621–642.

Bergmann T, Hadrys H, Breves G, Schierwater B (2009) Character-based DNA barcoding: a superior tool for species classification. *Berliner und Münchener tierärztliche Wochenschrift*, **122**, 446–450.

Blatchley WS, Leng CW (1916) *Rhynchophora or weevils of north eastern America*. The Nature Publishing Company, Indianapolis, Indiana.

Broersma DB, Hays SB (1966) Improved methods for mass rearing plum curculio, *Conotrachelus nenuphar*. *Journal of Economic Entomology*, **59**, 235–236.

Brower AV (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 6491–6495.

Brown WJ (1966) Chrysomelinae and Curculionidae (Coleoptera): Descriptions and Notes. *The Canadian Entomologist*, **98**, 855–859.

Caterino MS, Cho S, Sperling FA (2000) The current state of insect molecular systematics: a thriving Tower of Babel. *Annual Review of Entomology*, **45**, 1–54.

Chapman P (1938) *The Plum Curculio as an Apple Pest*. New York State Agricultural Experiment Station, Geneva, NY.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Coates BS, Sumerford DV, Hellmich RL (2004) Geographic and voltinism differentiation among North American *Ostrinia nubilalis* (European corn borer) mitochondrial cytochrome c oxidase haplotypes. *Journal of insect science (Online)*, **4**, 35.

Corthals A, DeSalle R (2005) An Application of Tissue and DNA Banking for Genomics and

Conservation: The Ambrose Monell Cryo-Collection (AMCC). *Systematic Biology*, **54**, 819–823.

Darlington W (Ed.) (1849) *Memorials of John Bartram and Humphry Marshall*. Lindsay & Blakiston, Philadelphia, Pennsylvania.

Dejean PFMA (1837) *Catalogue des Coléoptères de la Collection de M. le Comte DeJean*. Chez Méquignon-Marvis Père et Fils, Paris.

DeSalle R, Egan MG, Siddall M (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*, **360**, 1905–1916.

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Elith J, Graham C, Anderson R *et al.* (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129.

Elith J, Phillips SJ, Hastie T *et al.* (2010) A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, **17**, 43–57.

Eller FJ, Bartelt RJ (1996) Grandisoic acid, a male-produced aggregation pheromone from the plum curculio, Conotrachelus nenuphar. *Journal of Natural Products*, **59**, 451–453.

EPPO (1997) *Quarantine pests for Europe. Data sheets on quarantine pests for the European Union and for the European and Mediterranean Plant Protection Organization*. European and Mediterranean

Plant Protection Organization.

EPPO (2013) PQR - EPPO database on quarantine pests.

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

FAO (1997) *International Plant Protection Convention*. Food and Agriculture Organization, Rome.

FAO (2002) *World agriculture: towards 2015/2030*. Food and Agriculture Organization, Rome, Italy.

FAO (2003) *International Standards for Phytosanitary Measures: Pest Risk Analysis for Quarantine Pests Including Analysis of Environmental Risks*. Food and Agriculture Organization, Rome, Italy.

Fiedler F (1940) *Monograph of the South American weevils of the genus Conotrachelus*. Brit. Mus. (Nat. Hist.), London.

Fielding AH, Bell JF (1997) A review of methods for the assessment of prediction errors in conservation presence/absence models. *Environmental conservation*, **24**, 38–49.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of

mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology*, **3**, 294–299.

Funk D, Omland K (2003) Species-Level Paraphyly and Polyphyly: Frequency, Causes, and Consequences, with Insights from Animal Mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 397–423.

Galtier N, Nabholz B, Glémin S, Hurst GDD (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology*, **18**, 4541–4550.

Global Soil Data Task Group (2000) Global Gridded Surfaces of Selected Soil Characteristics (IGBP-DIS).

Goldstein PZ, DeSalle R (2011) Integrating DNA barcode data and taxonomic practice: Determination, discovery, and description. *BioEssays*, **33**, 135–147.

Goloboff, P. A., Farris, J. S. and Nixon, K. C. (2008), TNT, a free program for phylogenetic analysis. *Cladistics*, 24: 774–786. doi: 10.1111/j.1096-0031.2008.00217.x

Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, **8**, 993–1009.

Guisan A, Zimmermann N (2000) Predictive habitat distribution models in ecology. *Ecological Modelling*, **135**, 147–186.

Hallman GJ, Gould WP (2004) Evaluation of subtropical and tropical fruits as potential hosts for the southern strain of plum curculio (Coleoptera: Curculionidae). *Florida Entomologist*, **87**, 241–243.

Hays SB, Cochran JH (1964) Modification of a Laboratory Rearing Method for the Plum Curculio, *Conotrachelus nenuphar*. *Journal of Economic Entomology*, **57**, 408–409.

Herbst J (1797) *Kafer, Natursystem aller Insekten*. Berlin.

Hickerson MJ, Carstens BC, Cavender-Bares J *et al.* (2010) Phylogeography's past, present, and future: 10 years after Avise, 2000. *MOLECULAR PHYLOGENETICS AND EVOLUTION*, **54**, 291–301.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hoffmann EJ, VanderJagt J, Whalon ME (2007) Pyriproxyfen activates reproduction in prediapause northern strain plum curculio (*Conotrachelus nenuphar* Herbst). *Pest Management Science*, **63**, 835–840.

Hoffmann EJ, Vandervoort C, Wise JC (2010) Plum Curculio (Coleoptera: Curculionidae) Adult Mortality and Associated Fruit Injury After Exposure to Field-Aged Insecticides on Tart Cherry Branches. *Journal of Economic Entomology*, **103**, 1196–1205.

Hoffmann E, Middleton S, Wise J (2008) Ovicidal activity of organophosphate, oxadiazine, neonicotinoid and insect growth regulator chemistries on the northern strain plum curculio, *Conotrachelus nenuphar*. *Journal of Insect Science*, **8**, Online: http://www.insectscience.org/8.29/.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Jenkins DA, Mizell RFI, Shapiro-Ilan D, Cottrell T, Horton D (2006a) Invertebrate predators and parasitoids of plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae) in Georgia and Florida. *Florida Entomologist*, **89**, 435–440.

Jenkins D, Cottrell T, Horton D, Hodges A, Hodges G (2006b) Hosts of plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), in central Georgia. *Environmental Entomology*, **35**, 48–55.

Johnson DT, Mulder PGJ, McCraw BD *et al.* (2002) Trapping plum curculio *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae) in the southern United States. *Environmental Entomology*, **31**, 1259–1267.

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, **30**, 772–780.

Katoh K, Misawa K, Kuma K-I, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059–3066.

Kim HG, Alston DG (2008) Potential of Two Entomopathogenic Nematodes for Suppression of Plum Curculio (*Conotrachelus nenuphar*, Coleoptera: Curculionidae) Life Stages in Northern Climates. *Environmental Entomology*, **37**, 1272–1279.

Knowles L (2009) Statistical Phylogeography. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 593–612.

Kozak KH, Graham CH, Wiens JJ (2008) Integrating GIS-based environmental data into evolutionary biology. *Trends in Ecology & Evolution*, **23**, 141–148.

Lafleur G, Chouinard G, Vincent C, Cormier D (2007) Impact of trap architecture, adjacent habitats, abiotic factors, and host plant phenology on captures of plum curculio (Coleoptera: Curculionidae) adults. *Journal of Economic Entomology*, **100**, 737–744.

Lamothe S, Chouinard G, Vincent C (2008) Abiotic Factors and Trap Design Modulate the Performance of Traps Used to Monitor the Plum Curculio. *Journal of Economic Entomology*, **101**, 1838–1846.

LeConte JL, Horn GH (1876) The Rhynchophora of America, north of Mexico. *Proceedings of the American Philosophical Society*, **15**, vii–442.

Leskey TC (2006) Visual cues and capture mechanisms associated with traps for plum curculio (Coleoptera: Curculiondae). *Journal of Entomological Science*, **41**, 97–106.

Leskey TC (2008) Reproductive development of female plum curculio (Coleoptera: Curculionidae) in the mid-Atlantic: presence of multivoltine populations. *Journal of Entomological Science*, **43**, 208–216.

Leskey TC, Prokopy RJ (2000) Sources of apple odor attractive to adult plum curculios. *Journal of Chemical Ecology*, **26**, 639–653.

Leskey TC, Prokopy RJ (2001) Adult plum curculio (Coleoptera: Curculionidae) attraction to

fruit and conspecific odors. *Annals of the Entomological Society of America*, **94**, 275–288.

Leskey TC, Prokopy RJ (2002) Developing a branch-mimicking trap for adult plum curculios. *Entomologia Experimentalis et Applicata*, **102**, 253–259.

Leskey TC, Prokopy RJ (2003) Influence of barometric pressure on odor discrimination and oviposition by adult plum curculios (Coleoptera: Curculionidae). *European Journal of Entomology*, **100**, 517–520.

Leskey TC, Wright SE (2004) Monitoring plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), populations in apple and peach orchards in the mid-Atlantic. *Journal of Economic Entomology*, **97**, 79–88.

Leskey TC, Wright SE (2007) Host preference of the plum curculio. *Entomologia Experimentalis et Applicata*, **123**, 217–227.

Leskey TC, Zhang A (2007) Impact of temperature on plum curculio (Coleoptera: Curculionidae) responses to odor-baited traps. *Journal of Economic Entomology*, **100**, 343–349.

Leskey TC, Pinero JC, Prokopy RJ (2008) Odor-Baited Trap Trees: A Novel Management Tool for Plum Curculio (Coleoptera: Curculionidae). *Journal of Economic Entomology*, **101**, 1302–1309.

Leskey TC, Zhang A, Herzog M (2005) Nonfruiting host tree volatile blends: novel attractants for the plum curculio (Coleoptera: Curculionidae). *Environmental Entomology*, **34**, 785–793.

Levine JM, D'Antonio CM (2003) Forecasting biological invasions with increasing international trade. *Conservation Biology*, **17**, 322–326.

Lewis WJ, van Lenteren JC, Phatak SC, Tumlinson JH (1997) A total system approach to sustainable pest management. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 12243–12248.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Liebhold AM, Tobin PC (2008) Population Ecology of Insect Invasions and Their Management. *Annual Review of Entomology*, **53**, 387–408.

Liu L (2008) BEST: Bayesian estimation of species trees under the coalescent model. *Bioinformatics*, **24**, 2542–2543.

Maddison PW, Maddison DR Mesquite: a modular system for evolutionary analysis.

McClanan ME, Luckhart S, Pfeiffer DG (2004) Use of random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to differentiate populations of plum curculio, *Conotrachelus nenuphar* (Herbst). *Journal of Entomological Science*, **39**, 117–121.

Monfreda C, Ramankutty N, Foley JA (2008) Farming the planet: 2. Geographic distribution of crop areas, yields, physiological types, and net primary production in the year 2000. *Global Biogeochemical Cycles*, **22**.

New TR (2005) *Invertebrate Conservation and Agricultural Ecosystems*. Cambridge University

Press, Cambridge, UK.

Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics*, **24**, 581–583.

O'Brien CW, Wibmer GJ (1982) *Annotated checklist of the weevils (Curculionidae sensu lato) of North America, Central America, and the West Indies (Coleoptera: Curculionoidea)*. The American Entomological Institute, Ann Arbor, Michigan.

Oerke EC, Dehne HW, Schönbeck F, Weber A (1994) *Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops*. Elsevier Science.

Padula AL, Smith EH (1971) Reproductive incompatibility between univoltine males and multivoltine females of the plum curculio. *Annals of the Entomological Society of America*, **64**, 665–668.

Pearson R, Raxworthy C, Nakamura M, Peterson A (2007) Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, **34**, 102–117.

Peterson AT, Soberon J, Pearson RG *et al.* (2011) *Ecological Niches and Geographic Distributions*. Princeton University Press, Princeton, New Jersey.

Phillips S, Dudik M (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, **31**, 161.

Phillips S, Anderson R, Schapire R (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

Phillips S, Dudík M, Schapire R (2004) A maximum entropy approach to species distribution modeling. *Proceedings of the twenty-first international conference on Machine learning*.

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, **52**, 273–288.

Pinero JC, Prokopy RJ (2003) Field evaluation of plant odor and pheromonal combinations for attracting plum curculios. *Journal of Chemical Ecology*, **29**, 2735–2748.

Pinero JC, Agnello AM, Tuttle A *et al.* (2011) Effectiveness of Odor-Baited Trap Trees for Plum Curculio (Coleoptera: Curculionidae) Monitoring in Commercial Apple Orchards in the Northeast. *Journal of Economic Entomology*, **104**, 1613–1621.

Pinero JC, Wright SE, Prokopy RJ (2001) Response of plum curculio (Coleoptera: Curculionidae) to odor-baited traps near woods. *Journal of Economic Entomology*, **94**, 1386–1397.

Pinzón-Navarro S, Barrios H, Múrria C, Lyal CHC, Vogler AP (2010) DNA-based taxonomy of larval stages reveals huge unknown species diversity in neotropical seed weevils (genus *Conotrachelus*): relevance to evolutionary ecology. *MOLECULAR PHYLOGENETICS AND EVOLUTION*, **56**, 281–293.

Pretty J (2008) Agricultural sustainability: concepts, principles and evidence. *Philosophical*

transactions of the Royal Society of London Series B, Biological sciences, **363**, 447–465.

Prokopy RJ, Chandler BW, Dynok SA, Pinero JC (2003) Odor-Baited Trap Trees: A New Approach to Monitoring Plum Curculio (Coleoptera: Curculionidae). *Journal of Economic Entomology*, **96**, 826–834.

Prokopy RJ, Chandler BW, Leskey TC, Wright SE (2000) Comparison of traps for monitoring plum curculio adults (Coleoptera: Curculionidae) in apple orchards. *Journal of Entomological Science*, **35**, 411–420.

Prokopy RJ, Phelan PL, Wright SE *et al.* (2001) Compounds from host fruit odor attractive to adult plum curculios (Coleoptera: Curculionidae). *Journal of Entomological Science*, **36**, 122–134.

Quaintance AL, Jenne EL (1912) The plum curculio. *Washington D.C. U. S. Dept. Agric. Bur. Ent. Bull.*, **No. 103**, (1–250).

Racette G, Chouinard G, Vincent C, Hill SB (1992) Ecology and management of plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae), in apple orchards. *Phytoprotection*, **73**, 85–100.

Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, **7**, 355–364.

Riley CV (1868) *Transactions of the Illinois State Horticultural Society for 1867*. Praire Farmer Company Steam Print, Chicago, Illinois.

Riley CV (1871) *Third annual report on the noxious, beneficial and other insects, of the state of Missouri*. State of Missouri, Jefferson City, Mo.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Sakai AK, Allendorf FW, Holt JS *et al.* (2001) The Population Biology of Invasive Species. *Annual Review of Ecology and Systematics*, 305–332.

Sarkar IN, Planet PJ, DeSalle R (2008) CAOS software for use in character-based DNA barcoding. *Molecular Ecology Resources*, **8**, 1256–1259.

Schoof HF (1942) *The genus Conotrachelus Dejean (Coleoptera, Curculionidae) in the north central United States*. University of Illinois Press, Urbana, Illinois.

Shapiro-Ilan DI, Mizell Iii RF, Cottrell TE, Horton DL (2008) Control of plum curculio, *Conotrachelus nenuphar*, with entomopathogenic nematodes: Effects of application timing, alternate host plant, and nematode strain. *Biological Control*, **44**, 207–215.

Shapiro-Ilan DI, Mizell RFI, Campbell JF (2002) Susceptibility of the plum curculio, *Conotrachelus nenuphar*, to entomopathogenic nematodes. *Journal of Nematology*, **34**, 246–249.

Sheppard CA (2004) Benjamin Dann Walsh: pioneer entomologist and proponent of Darwinian theory. *Annual Review of Entomology*, **49**, 1–25.

Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach AT (2006) Incorporating Molecular

Evolution into Phylogenetic Analysis, and a New Compilation of Conserved Polymerase Chain Reaction Primers for Animal Mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 545–579.

Simon C, Frati F, Beckenbach A *et al.* (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

Smith E, Salkeld E (1964) Ovary development and oviposition rates in the plum curculio, *Conotrachelus nenuphar* (Coleoptera: Curculionidae). *Annals of the Entomological Society of America*, **57**, 781–787.

Smith EH (1957a) A method for rearing the plum curculio under laboratory conditions including some biological observations. *J. econ. Ent., Washington*, **50**, 187–190.

Smith EH (1957b) Field and Laboratory Evaluations of Lead Arsenate, Wettable Sulfur and Hydrated Lime against the Plum Curculio. *Journal of Economic Entomology*, **50**, 177–183.

Snapp OI (1923) Recent Developments in Plum Curculio Investigations in Georgia. *Journal of Economic Entomology*, **16**, 275–283.

Snapp OI (1930) Life history and habits of the plum curculio in the Georgia peach belt. *Tech. Bull. U.S. Dept. Agric., Washington, D.C.*, **188**, 90 pp.

Snapp OI, Turner WF, Roberts JW (1922) *Controlling The Curculio, Brown-Rot, And Scab In The Peach Belt Of Georgia*. Kessinger Publishing, LLC.

Soltis DE, Morris AB, Mclachlan JS, Manos PS, Soltis PS (2006) Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, **15**, 4261–4293.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Stamatakis A, Hoover P, Rougemont J (2008) A Rapid Bootstrap Algorithm for the RAxML Web Servers. *Systematic Biology*, **57**, 758–771.

Stearns L (1931) The Broods of the Plum Curculio, *Conotrachelus nenuphar* Herbst, in Delaware. *Journal of Economic Entomology*, **24**, 62–66.

Swofford DL (2003) PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods).

Thacker JRM (2002) *An Introduction to Arthropod Pest Control*. Cambridge University Press, Cambridge, UK.

Thuiller W, Richardson DM, Pyšek P *et al.* (2005) Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Global Change Biology*, **11**, 2234–2250.

Tilton J (1804) *The Domestic Encyclopedia* (AFM Willich, Ed,). WY Birch & A Small, Philadelphia.

Trimble IP (1865) *A Treatise on the Insect Enemies of Fruit and Fruit Trees*. William Wood & Company, New York, New York.

UG (2006) *Summary of Losses from Insect Damage and Costs of Control in Georgia, 2004* (P Guillebeau, N Hinkle, P Roberts, Eds,). University of Georgia, Department of Entomology.

USDA-NASS (2012) *Crop Values 2011 Summary (February 2012)*. USDA National Agricultural Statistics Service.

Villesen P (2007) FaBox: an online toolbox for fasta sequences. *Molecular Ecology Notes*, **7**, 965–968.

Vincent C, Chouinard G, Hill SB (1999) Progress in plum curculio management: a review. *Agriculture, Ecosystems & Environment*, **73**, 167–175.

Walsh BD (1867) *First Annual Report on the Noxious Insects of the State of Illinois*. Illinois State Horticulture Society.

Warren DL, Glor RE, Turelli M (2010) ENMTools: a toolbox for comparative studies of environmental niche models. *Ecography*, **33**, 607–611.

Wiens JJ, Graham CH (2005) Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics*, 519–539.

Zhang C, Rannala B, Yang Z (2012) Robustness of Compound Dirichlet Priors for Bayesian Inference of Branch Lengths. *Systematic Biology*, **61**, 779–784.

Zhang X, Pfeiffer DG (2008) Evaluation of Reproductive Compatibility of Interstrain Matings Among Plum Curculio Populations in the Eastern United States. *Environmental Entomology*, **37**, 1208–1213.

Zhang X, Tu Z, Luckhart S, Pfeiffer D (2008) Genetic Diversity of Plum Curculio (Coleoptera: Curculionidae) Among Geographical Populations in the Eastern United States. *Annals of the Entomological Society of America*, **101**, 824–832.

Zink RM, Barrowclough GF (2008) Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*, **17**, 2107–2121.