Species Diversity of Hickory-feeding Phylloxerans (Hemiptera: Phylloxeridae) in the U.S.

by

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Abstract

During the spring months, hickories throughout the Eastern U.S. become adorned with abnormal growths or galls on the leaf or stem tissue. Many of these galls are caused by phylloxerans (Hemiptera: Phylloxeridae: *Phylloxera* spp. Boyer de Fonscolombe) which are small, cryptic, phytophagous insects that are closely related to aphids. Their galls come in an array of shapes, sizes, and colors and are considered extended phenotypes of their inducer (fundatrix). Hickory-feeding phylloxerans mainly cause aesthetic damage to the trees they infect, but some species, such as *Phylloxera russellae* Stoetzel, that infect pecans can cause economic damage. Despite their ubiquity and possible economic importance, hickory-feeding phylloxerans have been the focus of few taxonomic studies and have not been studied extensively for over 100 years. There are only 32 described hickory-feeding species in the U.S., and most of the taxonomy is based on collections from a small area around Washington, D.C. In addition to a lack of knowledge about their diversity in the U.S., even less is known about their evolutionary relationships. The three objectives of this dissertation are to: 1) Perform the first estimate of the phylogenetic relationships among phylloxeran species and test the monophyly of accepted genera and hosts; 2) Revise the species diversity; and 3) Characterize their gall morphologies and hosts. To accomplish these objectives, *Phylloxera* galls and/or phylloxerans were collected from across the U.S. on a variety of hosts including one species of *Castanea* Mill., 11 species of *Carya* Nutt., three species of *Quercus* L., and two species of *Juglans* L. DNA sequence information of mitochondrial COI and nuclear Ef-1α was used to estimate the phylogeny.

Phylloxera was determined to be monophyletic and *Phylloxera* spp. cluster by gall morphology rather than host. Fourteen putative new hickory and walnut-feeding *Phylloxera* spp. were described, and several new host records (black hickory, nutmeg hickory, sand hickory, and Northern California walnut) were discovered. With the additions made here and three species being designated as *nomina dubia* (*Phylloxera foveata* (Shimer), *Phylloxera globosa* (Shimer), and *Phylloxera minima* (Shimer)), the total number of *Phylloxera* spp. feeding on hickories and walnuts in the U.S. is now 44. A key is provided to gall forming phylloxerans in the U.S.

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Dedication

To my parents, Mary Ann and Paul Hamilton, for being my support system and for always encouraging me to work hard to reach my goals in life.

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List of Abbreviations

AMNH	American Museum of Natural History
AUMNH	Auburn University Museum of Natural History
BMNH	British Museum of Natural History
NMNH	National Museum of Natural History

Chapter 1

Literature Review

Overview on galls

Galls are abnormal growths of plant tissue that can occur on any plant organ and are caused by insects, mites, nematodes, bacteria, and fungi (Redfern 2011, Capinera 2012), with insects and mites being the most common (Capinera 2012). Galls are considered one of the most emblematic examples of plant manipulation by insects (Giron et al. 2016) and are distinguished from other insect made shelters (e.g. rolled leaves) by the active differentiation and growth of plant tissues that resemble a novel plant organ (Stone and Schonrogge 2003, Shorthouse et al. 2005, Giron et al. 2016). These abnormal growths are nutrient sinks that provide an abundant supply of carbohydrates, free amino acids, and minerals to their inducers (Bagatto and Shorthouse 1991, Larson and Whitham 1991, Koyama et al. 2004, Harris et al. 2006, Saltzmann et al. 2008). Their development is mainly controlled by insect genes and galls are considered extended phenotypes of their inducer (Dawkins 1982, Stone and Schonrogge 2003). Galls represent distinct microhabitats that provide their inducers with food, shelter, and some protection from natural enemies (Stone et al. 2002, Stone and Schonrogge 2003, Nabity et al. 2013).

Functional morphology of galls

Insect induced galls exhibit an array of colors, shapes, and sizes and this variation in gall morphology is not well understood. Thus, there has been a continuing debate of the adaptive significance of gall induction and gall morphology (Cornell 1983, Price et al. 1987). Three hypotheses have been used to explain the diversity of internal and external gall morphologies across insect taxa: Nutrition, Microenvironment, and Enemy Hypotheses (Stone and Schonrogge 2003).

The Nutrition Hypothesis posits that galls provide access to enhanced nutrition over other feeding modes (e.g. leaf chewing) by the presence of inner nutritive tissues (Stone and Schonrogge 2003). Nutritive tissue lines the inner surface of many closed galls (Shorthouse and Rohfritsch 1992) and is the site where immature insects actively feed to complete their development (Giron et al. 2016). Therefore, enhancement of the internal surface area in galls is key in providing increased nutrition to its developing inhabitants and is accomplished by the development of internal folds (e.g. aphids and thrips) or the division of galls into connected hollow radiating spines or passageways (e.g. aphids) (Stone and Schonrogge 2003).

The Microenvironment Hypothesis posits that gall tissues function to protect galling insects from harsh environmental conditions, which lead to desiccation (Stone and Schonrogge 2003). Modifications such as fully closed galls or galling insects being surrounded by waxy or corky tissue could help to protect against water loss (Stone and Schonrogge 2003). Additionally, modifications of gall tissue traits such as deposition of waxes and resins could also be important in response to microclimatic conditions (Stone and Schonrogge 2003).

Compared to the Nutrition and Microenvironment Hypotheses, the Enemy Hypothesis is the only one that can explain external gall morphology and states that galls function to protect their inducers from attack by natural enemies (Stone and Schonrogge 2003). Although galls do

provide some level of protection against attack by nonspecialist predators and pathogens (Hawkins et al. 1997), they do not represent enemy free space (Jeffries and Lawton 1984, Stone and Schonrogge 2003). Morphological features of the gall change throughout development (e.g. gall size, wall thickness and toughness, spine length, and density of hair cover) as well as the assemblages of insects that can exploit them (Stone and Schonrogge 2003). Smaller species have been found to attack earlier in gall development whereas larger species with long ovipositors attack later (Craig 1994, Briggs and Latto 1996, Abrahamson and Weis 1997, Stone et al. 2002).

Four structural traits have been shown to reduce the susceptibility of galling insects to enemy attack including: increased gall hardness (Weis 1982, Stone et al. 2002); increased thickness of gall tissue surrounding the insect (Rossi et al. 1992, Zwolfer and Arnold-Rinehart 1994, Abrahamson and Weis 1997, Stone et al. 2002); pubescence (Dixon et al. 1998); and recruitment of ant guards through nectar secretion (Seibert 1993, Abrahamson and Weis 1997). The Enemy Hypothesis would be supported if a defensive trait such as spines that are found across insect species in a particular taxon are correlated with lower mortality caused by a natural enemy (Stone and Schonrogge 2003).

Bailey et al. (2009) tested the Enemy Hypothesis by examining the parasitoid community associated with gall wasps (Hymenoptera: Cynipidae) on oak trees (*Quercus* spp. L.) in Hungary. Significant correlations were found between parasitoid community structure of oak gall wasps and external gall structural traits which included: toughness, hairiness, and stickiness (Bailey et al. 2009). Although many gall morphologies were sampled in this study, none were free of parasitoids, so the galls did not represent enemy free space (Bailey et al. 2009). Their results

indicated that the optimal way for a gall wasp to escape its community of parasitoids is to shift to a new organ on the oak host or to a new oak taxon (Bailey et al. 2009).

In addition to natural enemy pressure, competition may also be another factor that has led to the differentiation of gall traits among galling insects (Inbar et al. 2004). Gall inducers compete for optimal galling sites and nutrients in the same host plant (Whitham 1979, Inbar et al. 1995). Shifts to a new plant part may release galling insects from competition (Denno et al. 1995, Craig et al. 2000, and Inbar et al. 2004) and Cook et al. (2002) suggested that intraspecific competition among *Andricus* (Hartig) gall wasps for oviposition sites has promoted shifts to a novel part of the host plant. In the case of galling aphids such as *Pemphigus betae* Doane, habitat selection is considered key to their fitness and it is important for the fundatrix to find an area on the leaf which will have a high supply of nutrients and a low level of phenols (Zucker 1982). When multiple aphid fundatrices have been found on the same leaf, the aphids that induce galls near the base have higher reproductive success than aphids inducing galls near the apex (Whitham 1978, 1980).

More recently, several other hypotheses have been proposed to explain the variation in gall color and morphology. Many galls are brightly colored with hues of red or yellow and Inbar et al. (2010) proposed the Aposematic Gall Hypothesis, which posits that galls that are conspicuous in coloration and contain high levels of defensive chemicals (e.g. phenols and tannins) are aposematic. On the other hand, White (1993) proposed the Senescence Hypothesis in which galls that possess a bright coloration is a direct result of their inducers feeding on the outflow of nutrients from senescing tissues. Senescence feeders are primarily aphids, psyllids, and scale insects (White 1993). Lastly, the Caterpillar Mimicry Hypothesis was proposed by Yamazaki (2016) and states that galls that visually resemble caterpillars could deter herbivores

from feeding on them, which would lead to increased survival. Galls that resemble caterpillars have been found in Japan, Middle East, and the Palearctic region; these caterpillar mimics are formed by aphids (Aphididae), thrips (Phlaeothripidae), gall midges (Cecidomyiidae), and jumping plant lice (Triozidae) on a variety of host plants (Yamazaki 2016).

Mechanisms of gall induction

Although galling insects are known widely for their manipulation of plant tissue, the mechanisms involved in gall induction and formation still remain largely unresolved with the exception of some bacterial species (Stone and Schonrogge 2003). The crown gall caused by *Agrobacterium tumefasciens* is induced by the bacteria exporting plasmid DNA and the resulting expression of bacterial genes in the host (Davey et al. 1994). Similarly, root nodules caused by *Rhizobium* and *Frankia* spp. are induced by the export of lipochitooligosaccharide signal molecules (Denarie et al. 1996).

On the other hand, insect gall formation does not likely involve host genetic transformation since gall development ceases if the insect is removed (Richardson et al. 2017). Gall induction by insects occurs through a couple of different modes of action including larval feeding in plant tissue or from oviposition into plant tissue (Gagne 1989, Stone et al. 2002). Effectors (small molecules that change host cell structure and function) are injected into the host from the saliva of the immature insect or from maternal secretions to redirect plant development (Chen et al. 2010, Hogenhout and Bos 2011, Giron et al. 2016). Galling insects can introduce the effectors to the tissues from their salivary glands by their mouthparts or from their venom glands by their ovipositor (Vardal 2006, Stuart et al. 2012). Host tissue will dedifferentiate and hyperplasia (cell division) and hypertrophy (growth) will then follow (Oliveira and Isaias 2010; Dias et al. 2013; Carneiro et al. 2014, 2015; Suzuki et al. 2015). The chemical identity and mode

of action of the effectors causing these responses remain unknown (Giron et al. 2016), but phytohormones (auxins and/or cytokinins) (Cornell 1983, Shorthouse and Rohfritsch 1992, Suzuki et al. 2014, Tooker and Helms 2014), amino acids (Stone and Schonrogge 2003), proteins (Highton and Mabberly 1994), mutualistic viruses (Cornell 1983), and bacterial symbionts (Yamaguchi et al. 2012) have been proposed to be involved in gall induction.

Gall forming insects

There are approximately 15,000 species of insects that induce galls within which the insect feeds and develops (Richardson et al. 2017). The galling habit of insects has evolved repeatedly among and within insect orders (Stone and Schonrogge 2003). There are six known orders of insects that induce galls, including: Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, and Thysanoptera (Felt 1940, Gagne 1989, Shorthouse and Rohfritsch 1992, Williams 1994, Stone et al. 2002, Blanche 2012). Gall midges (Diptera: Cecidomyiidae) (Dreger-Jauffret and Shorthouse 1992) and gall wasps (Hymenoptera: Cynipidae) are considered the most speciose gall inducers (Ronquist and Liljebblad 2001).

Insect induced galls can be single chambered (unilocular) or multichambered (multilocular) and each chamber may have one insect or many (Gagne 1989, Stone et al. 2002, Blanche 2012); and they can range from simple structures such as leaf pits to more complex woody stem galls (Blanche 2012). Insect galls may have an opening for individuals to emerge from (eg. aphids and phylloxerans) or the gall may have no opening requiring the inhabitants to eat through the tissue to emerge (e.g. gall wasps) (Felt 1940). Variation in gall characters of the same species of insect can occur due to a gall being induced by a male or female (Blanche 2012), the time of the season the gall was induced (Pergande 1904), or the host plant species (Pergande 1904).

Gall structure can also vary widely among insect taxa and their morphology is indicative of the species of insect that induces it (Stone et al. 2002, Blanche 2012). The galls of scale insects (Coccoidea) can appear as enclosed swellings, tubes, blisters, pits, pouches, rosettes, and buds with one external opening on the stems, leaves, and flower buds of their host plants (Blanche 2012). Psyllid galls can have the appearance of pits, leaf rolls, or raised globular sacs with many of them being on leaves (Blanche 2012). Thrip galls can resemble leaf rolls, tubes, or pouches on leaves (Blanche 2012) and aphids also form pouch-like galls (Felt 1940). Cynipid galls come in a diversity of shapes and are thought to be the most complex structures of all insect induced galls (Schonrogge et al. 2000) with their surfaces often covered in spines of varying shapes and sticky substances excreted on the surface by glands (Stone and Cook 1998). Lastly, galls of *Phylloxera* spp. Boyer de Fonscolombe also occur in a vast array of shapes including disk, button, conical, elongate, and globular (Pergande 1904, Felt 1940).

Evolution of gall morphology and host plant relationships

The evolution of gall morphology and host plant relationships has been studied in numerous insect taxa including: aphids (Stern 1995, Inbar et al. 2004, Sano and Akimoto 2011), wasps (Stone and Cook 1998, Ronquist and Liljeblad 2001), scale insects (Cook and Gullan 2004), gall midges (Dorchin 2004, Joy and Crespi 2007), thrips (Crespi and Worobey 1998), and sawflies (Nyman et al. 2000). The general trend in many of these taxa is that related species were found to induce similar gall types (Dorchin et al. 2004). Nyman et al. (2000) found that gall morphology mirrored the phylogeny of nematine gallers and not their host plants; similar results were found with pemphigine aphids (Stern 1995), cynipid wasps (Stone and Cook 1998), and Australian gall thrips (Crespi et al. 1997, Crespi and Worobey 1998). Additionally, similar gall types were found to be close to each other on the phylogeny which indicates that gall types

evolve gradually (Nyman et al. 2000) and has also been observed with gall wasps (Stone and Cook 1998) and thrips (Crespi and Worobey 1998).

For example, with nematine sawflies the evolutionary sequence of gall morphology is as follows: 1) galling species evolved via leaf folders or rollers; 2) leaf blade gall; 3) apical leaf gall; 4) basal leaf gall; 5) petiole gall; 6) stem gall; and 7) bud gall (Nyman et al. 2000). Similarly, leaf folding was found to precede galling in thrips on *Acacia* Martius (Crespi and Worobey 1998). On the other hand, in the gall midge subtribe Baldratiina (Diptera: Cecidomyiidae), multichambered stem galls were determined to be the ancestral state followed by the evolution of mid-rib leaf galls and then other leaf gall types (single or multi-chambered) with some reversals to stem galls (Dorchin et al. 2004). Sano and Akimoto (2011) found that in galling aphids of the tribe Eriosomatini, the ancestral gall state was open leaf rolls followed by open and closed pouch galls.

Taxon of focus – *Phylloxera* spp.

Phylloxerans (Hemiptera: Phylloxeroidea: Phylloxeridae) are cryptic phytophagous insects that feed on the leaves, roots, and stems of their host plants. Phylloxerans are closely related to aphids (Aphididae) and adelgids (Adelgidae) (Favret et al. 2016). Across the world, Phylloxeridae is comprised of seven genera and about 70 described species that are found on a variety of hosts including: *Carya* spp. Nutt. (hickories), *Castanea* spp. Mill. (chestnuts), *Nyssa sylvatica* Marshall (black tupelo), *Populus* spp. L. (cottonwoods), *Pyrus* spp. L. (pears), *Quercus* spp. (oaks), *Salix* spp. L. (willows), *Ulmus* spp. L. (elms), and *Vitis* spp. L. (grapes) (Blackman and Eastop 2013). In the U.S., Phylloxeridae is composed of three genera (*Daktulosphaira* Shimer, *Phylloxera*, and *Phylloxerina* Borner) and 47 species that occur on *Carya* spp., *Castanea* spp., *Nyssa sylvatica*, *Populus* spp., *Quercus* spp., *Salix* spp., and *Vitis* spp. (Blackman and

Eastop 2013). Much of what we know about phylloxerans has resulted from studies of the grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), which is an economically important pest of grapevines (Medina et al. 2011, Favret et al. 2016).

Phylloxera is comprised of species that feed on chestnuts, hickories, and oaks (Pergande 1904). However, their feeding affects the hosts in different ways with *Phylloxera* spp. forming galls on hickories and causing yellow spotting and/or curling of the leaves on chestnuts and oaks (Blackman and Eastop 2013). Currently, there are 32 described species of hickory-feeding *Phylloxera* in the U.S. most of which were described from areas around Washington, D.C. on a few species of hickory – *Carya cordiformis* (Wangenh.) K. Koch (bitternut hickory), *C. glabra* (Mill.) Sweet (pignut hickory), *C. illinoinensis* (Wangenh.) K. Koch (pecan), *C. tomentosa* (Lam.) Nutt. (mockernut hickory), and *C. ovata* (Mill) K. Koch (shagbark hickory) (Pergande 1904, Stoetzel 1981, Blackman and Eastop 2013).

The current alpha taxonomy of hickory phylloxerans is based largely on host associations and gall morphologies (Pergande 1904). The appearance of hickory phylloxeran galls has considerable variation and Pergande (1904) classified the galls into four distinct groups: 1) galls that are thin, paper-like, and somewhat transparent; 2) galls that are fleshy and opaque; conical, globular, or pedunculate in shape; 3) galls formed from elongate folds between leaf veins; and 4) galls located on twigs or petioles. Felt (1940) colorfully described the interior of mature phylloxera galls as resembling the crystals of a geode due to the galls being densely lined with phylloxerans.

At first glance, phylloxerans look somewhat similar to aphids. However, they have several distinct characters that easily separate them from aphids. Phylloxerans lack cornicles (abdominal tubes projecting from the caudal end of the dorsum), most species have three-

segmented antennae, and alates (winged migrants) hold their wings flat over their back (Pergande 1904). On the other hand, most species of aphids have cornicles, five to six segmented antennae, and alates hold their wings roof-like over their body (Favret and Miller 2012).

The taxonomic placement of a couple of Fagaceae-feeding species was uncertain and for a time were placed in the genus *Moritziella* (Borner). These species are currently considered to be members of *Phylloxera*, but without phylogenetic evidence (Favret et al. 2016). The lack of abdominal spiracles is considered a key diagnostic feature of hickory-feeding species, and specimens in *Moritizella* also share this trait (Favret et al. 2016).

In addition to little being known about the diversity of *Phylloxera* spp. in the U.S., not much is known about their life cycle either. The life cycle of *Phylloxera caryaecaulis* (Fitch) was studied in detail by Pergande (1904), Morgan (1906), Whitehead (1934), and Caldwell and Schuder (1979). In this species, a fundatrix (a.k.a, stem-mother) hatches in the early spring from an overwintering egg and migrates to newly expanded leaf or stem tissue and begins to feed (Caldwell and Schuder 1979). The feeding initiates gall formation and the gall will grow around the fundatrix and enclose it (Caldwell and Schuder 1979). The fundatrix will reproduce parthenogenetically (asexually) and alates (winged migrants) will develop from the eggs (Caldwell and Schuder 1979). Once the gall reaches maturity and opens, the alates will emerge and migrate to nearby leaves and will also reproduce parthenogenetically and deposit eggs on the abaxial surface (underside) of leaves (Caldwell and Schuder 1979). Female and male sexuales will hatch from the eggs and will mate and each female will deposit one overwintering egg, which contains the fundatrix for the following year (Caldwell and Schuder 1979). From egg hatch of the fundatrix to deposition of an overwintering egg is approximately 50-60 days

(Stoetzel 1985). Overwintering eggs have been found in protected areas such as old galls and bark crevices (Kotinsky 1921).

Stoetzel (1985) conducted detailed studies on the biology of six *Phylloxera* spp. (*P. caryaecaulis*, *P. caryaevenae* Fitch, *P. deplanata* Pergande, *P. devastatrix* Pergande, *P. notabilis* Pergande, *and P. russellae* Stoetzel) and found that Pergande (1904), Whitehead and Eastep (1937), and Caldwell and Schuder (1979) incorrectly stated that sexually mature females and males hatched directly from eggs. Instead sexual males and females go through holometabolous development with progression through five instars while fundatrices, apterous females, and alate females go through hemimetabolous development with five instars (Stoetzel 1985). Pupiform larvae do not feed between molts because they lack mouthparts and the adults lack a rostrum and wing pads (Stoetzel 1985).

As in many species of aphids, at least some *Phylloxera* spp. have obligate seasonal alternations in host use. Stoetzel (1985) demonstrated host alternation of *Phylloxera texana* Stoetzel and *P. castaneae* (Haldeman). *Phylloxera texana* alates were isolated from galls on a *Carya* sp. and put on pecan leaves, but they did not lay eggs and died (Stoetzel 1985). On the other hand, when *P. texana* alates were placed on leaves of *Quercus falcata* Michx. and *Q. virginia* Mill. they laid eggs that hatched into nymphs (Stoetzel 1985). Similarly, *P. texana* alates were found laying eggs on *Quercus* spp. in the field (Stoezel 1985). In West Virginia, alates emerging out of a gall on *Carya tomentosa* were morphologically identical to the alates on chestnut trees (*Castanea* sp.) in a nearby nursery (Stoetzel 1985). A greenhouse experiment was conducted with galls from mockernut and large populations of *P. castaneae* developed on the chestnut leaves (Stoetzel 1985).

Hickories

Hickories (*Carya* spp.) belong to the family Juglandaceae, which also contains walnuts (*Juglans* spp. L.) (Kirkman et al. 2007). Seventeen species of hickories occur in Eastern North America and Eastern Asia (Zhang et al. 2013). There are 11 species of hickories native to the U.S. (bitternut, black, mockernut, nutmeg, pecan, pignut, sand, scrub, shagbark, shellbark, and water) and many of them have a Southeastern distribution, but some species are found as far north as Maine and as far west as Texas (Kirkman et al. 2007, Little 2013). Hickories can be distinguished from other trees by several key traits which include: compound leaves with five to 17 leaflets growing from a rachis (stalk); leaflets growing in oppositional pairs perpendicular to the stalk with a terminal leaflet at the end of the rachis; leaflets being generally larger toward the end of the rachis and having serrated edges; bark forming ridges in a vertical pattern; and nuts having a woody outer shell and being four-valved (Kirkman et al. 2007).

Hickories are considered an important component of temperate deciduous forests in the Eastern U.S. (McCarthy and Wistendahl 1988) because they provide food and shelter to many animal species (Fralish 2004, Nelson et al. 2014). In the Central Hardwood Forest, hickories have dominated the forest for thousands of years and many animal and plant species depend upon their presence, so they are considered keystone species (Fralish 2004). In addition to their importance in forest ecosystems, they are also economically important because their nuts are edible (pecan) and their wood is often used for tool handles, furniture, and preparation of food as fuel for cooking or smoking (Kirkman et al. 2007).

Previous taxonomic studies

Research examining the diversity of hickory phylloxerans in the U.S. has been limited. Shimer (1868) documented the diversity of hickory phylloxerans found near Mt. Carroll, Illinois and Pergande (1904) published 28 species descriptions of hickory phylloxerans, mainly from

material from around Washington, D.C. Pergande (1904) believed that his work treated only a fraction of the total diversity in the U.S., but since Pergande's research only two new species of *Phylloxera* on a *Carya* sp. have been described (Stoetzel 1981). Stoetzel (1981) described *Phylloxera russellae* and *Phylloxera texana* on *Carya illinoiensis* (pecan).

Objectives

Although hickory-feeding phylloxerans are ubiquitous in deciduous forests throughout Eastern North America, they have been the focus of very few studies. Their taxonomy is considered antiquated and incomplete because it has been neglected for more than 100 years, was only ever studied in detail in a few eastern states, and the true species diversity is likely to be much higher (Pergande 1904). In addition, the monophyly of hickory phylloxeran species has been questioned but not tested and little is known about their host-use patterns. This project uses a combination of morphological and molecular approaches to improve our understanding of the species diversity and phylogenetic history of hickory phylloxerans, as per recent research on Adelgidae (Havill et al. 2007, Foottit et al. 2009) and Aphididae (Yang et al. 2010, Wang et al. 2011). The objectives of my research are to: 1) Perform the first estimate of the phylogenetic relationships among phylloxeran species and test the monophyly of accepted genera and hosts; 2) Revise the species diversity; and 3) Characterize their gall morphologies and hosts.

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Chapter 2

Phylogeny of *Phylloxera* spp. on Juglandaceae and Fagaceae in the U.S.

Abstract

In the United States, Phylloxeridae (Hemiptera) is composed of three genera (*Daktulosphaira* Shimer; *Phylloxera* Boyer de Fonscolombe; *Phylloxerina* Borner) that include species that occur on an array of host plants. *Phylloxera* is comprised of species that are free-living on chestnuts (*Castanea* spp. Mill.) and oaks (*Quercus* spp. L.) as well as gall forming species that live on hickories (*Carya* spp. Nutt.). Currently, there are 32 described *Phylloxera* spp. on hickories in the U.S. Although they are ubiquitous throughout deciduous forests during the spring, few taxonomic studies have been done on this group and less is known about their evolutionary relationships. The objective of this study was to perform the first estimate of the phylogenetic relationships among phylloxeran species and to test the monophyly of accepted genera and hosts. Phylloxerans occurring on *Carya* spp., a *Castanea* sp., *Juglans* spp. L. (walnuts), *Populus* sp. L. (cottonwood), *Quercus* spp., and a *Vitis* sp. L. (grapevines) were collected from across the U.S. from 2015–2018. Relationships within Phylloxeridae were estimated with a Maximum likelihood analysis of DNA sequence data from the mitochondrial COI gene, and the nuclear Ef-1α gene. The phylogeny from the combined data set included exemplars of 17 putative new *Phylloxera* spp., 25 described *Phylloxera* spp., and three

undetermined *Phylloxera* spp. *Phylloxera* was determined to be monophyletic and *Phylloxera* spp. clustered by gall morphology rather than host plant use.

Introduction

Phylloxerans (Hemiptera: Phylloxeroidea: Phylloxeridae) are cryptic phytophagous insects that feed on the leaves, roots, and stems of their host plants. Phylloxerans are aphid-like in appearance and are closely related to aphids but differ from them by being oviparous in all generations and by lacking cornicles (Heie 1987). In the U.S., Phylloxeridae is represented by three genera (*Daktulosphaira* Shimer; *Phylloxera* Boyer de Fonscolombe; *Phylloxerina* Borner) and 47 species that occur on *Carya* spp. Nutt. (hickories), *Castanea* spp. Mill. (chestnuts), *Nyssa sylvatica* Marshall (black tupelo), *Populus* spp. L. (cottonwoods), *Quercus* spp. L. (oaks), *Salix* spp. L. (willows), and *Vitis* spp. L. (grapes) (Blackman and Eastop 2013).

Phylloxera is comprised of species that are free-living on chestnuts and oaks as well as gall forming species on hickories (Pergande 1904, Blackman and Eastop 2013). Recently, *Phylloxera castaneivora* (Miyazaki) and *Phylloxera corticalis* Kaltenbach were reassigned from *Moritziella* Borner because there were no distinct morphological differences between the two genera (Favret et al. 2016). *Moritziella* is now considered a junior subjective synonym of *Phylloxera* (Favret et al. 2016). However, nomenclatural issues still exist within *Phylloxera* with a distinction needing to be made between Palearctic and Nearctic species that differ in number of spiracles (Favret et al. 2016).

Currently, there are 32 described species of *Phylloxera* that occur on hickories in the U.S. (Favret et al. 2016). Similar to other gall inducing insects, phylloxeran galls are considered extended phenotypes of the inducer and are important in species level identifications (Stone and Schonrogge 2003). The taxonomy of hickory phylloxerans has been based largely on host

associations and gall morphologies (Pergande 1904, Caldwell and Schuder 1979). Pergande (1904) classified the galls into four distinct groups: 1) galls that are thin and somewhat transparent; 2) galls that are fleshy, opaque, conical to globular in shape, and have an opening located below leaf; 3) galls formed from elongate folds between leaf veins; 4) galls located on twigs or petioles.

Galls of *Phylloxera* spp. are commonly encountered on hickories during the spring months. Hickories (Juglandaceae) are considered an important component of deciduous forests (McCarthy and Wistendahl 1988) and are considered keystone species in some areas since animals and plants depend upon their presence (Fralish 2004). There are 11 species of hickories native to the U.S. (Kirkman et al. 2007, Fryer 2018), and the formation of geologic barriers such as the uplift of the Appalachian Mountains during the late Oligocene to Miocene and erosion that occurred until the end of the Miocene contributed to their divergence and speciation (Zhang et al. 2013).

Hickory-feeding phylloxerans have been worked on taxonomically several times (Shimer 1868, Pergande 1904, Stoetzel 1981). More recently, Favret et al. (2016) composed a catalogue of phylloxerids of the world and Zhao and Nabity (2017) constructed the first multi-gene tree of members of the Phylloxeridae including seven species found on hickories. Therefore, not much is known about their species diversity in the U.S. and very little is known about their evolutionary relationships. The objective of this study was to perform the first estimate of the phylogenetic relationships among phylloxeran species and test the monophyly of accepted genera and hosts.

Materials and Methods

Taxon sampling and data collection

Three hundred and sixty-nine samples were collected from 19 states in the U.S. from 2015–2018 (Figure 1) on 11 *Carya* spp. (*Phylloxera* spp.), one *Castanea* sp. (*Phylloxera castaneae* (Haldeman)), two *Juglans* spp. L. (*Phylloxera* spp.), one *Populus* sp. (*Phylloxerina popularia* (*Pergande*)), three *Quercus* spp. (*Phylloxera* spp.), and one *Vitis* sp. (*Daktulosphaira vitifoliae* (Fitch)). Approximately 150 samples were selected for DNA extractions and sequencing based on gall morphology, host plant, and geographic location.

Three aphid species (*Aphis sambuci* L., *Cinara pergandei* (Wilson), and *Hamemlistes spinosus* Shimer) were chosen as outgroups as well as species from two other genera in Phylloxeridae (*Phylloxerina salicis* (Lichtenstein), *Phylloxerina popularia*, and *Daktulosphaira vitifoliae*). These outgroup species do not occur on *Carya* spp. Sequences for the aphid outgroups and *Phylloxerina salicis* were taken from GenBank as were two sequences for the ingroups (*Phylloxera castaneivora* and *Phylloxera coccinea* (von Heyden)).

Galls were excised and/or phylloxerans were transferred from plant specimens to 95% ethanol and stored at 8°C until DNA extraction. Specimens from each collection were stored in 70% ethanol and slide-mounted in Canada balsam. Specimens were deposited at NMNH (National Museum of Natural History, Beltsville, MD). Phylloxerans were identified to species by using Pergande (1904) and Stoetzel (1981); identifications were based on morphological characters of the galls, specimens of *Phylloxera* spp., host identity, and phylogenetic inference. *Phylloxera* spp. identifications were confirmed by comparing them to the *Phylloxera* syntypes and dried gall specimens (if available) in the U.S. National Collection in Beltsville, MD. Any nomenclatural act in the dissertation should not be considered valid under ICZN. Hickories and walnuts were identified to species by using two field guides (Kirkman et al. 2007, Nelson et al.

2014) and identifications were confirmed by Dr. Nancy Loewenstein (Auburn University School of Forestry and Wildlife Sciences). Collection data are listed in Table 1.

DNA extractions, Polymerase Chain Reactions, and Gel Electrophoresis

DNA was extracted from 2–15 specimens derived from the same gall type or specimens identified as the same species in a sample if they were free-living. Phylloxerans were ground with a pestle directly in a 1.7 ml microcentrifuge tube containing 200 µl of TL Buffer including 25 µl OB Protease Solution and incubated in a water bath at 55°C for three hours. An E.Z.N.A.® Tissue DNA kit (OMEGA Bio-tek, Inc., Norcross, GA) was used for extractions and the enclosed protocol was followed.

Polymerase chain reactions were performed in 25 µl reactions consisting of 9 µl of ultra pure water, 12.5 µl of Q5® High-Fidelity 2X Master Mix (New England Biolabs Inc., Massachusetts, USA), 1.25 µl of forward primer, 1.25 µl of reverse primer, and 1 µl of DNA template. An approximately 700-bp DNA fragment of mitochondrial COI was amplified using the primers LepF (5'-ATTCAACCAATCATAAAGATATTGG-3') and LepR (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (Foottit et al. 2008). The primers Efs175 (5′-GGAAATGGGAAAAGGCTCCTTCAAGTAYGCYTGGG-3′) (Moran et al. 1999) and Efr1 (5′-GTGTGGCAATSCAANACNGGAGT-3′) (Hidalgo et al. 2012) were used to amplify an approximately 1000-bp DNA fragment of the nuclear gene Ef-1α. Polymerase chain reactions were performed in a T100 programmable thermal cycler (Bio-Rad, CA, USA). For COI amplification, after an initial denaturing step of 4 mins at 94°C, 35 cycles were performed with a denaturing for 45 secs at 94°C, an annealing step of 90 secs at 48°C or 50°C, primer extension for 120 secs at 72°C, and a final elongation step of 10 min at 72°C. For Ef-1α amplification, after an initial denaturing step of 3 mins at 94°C, 34 cycles were performed with a denaturing for 30

secs at 94°C, an annealing step of 52°C, 53°C, or 54°C for 1 min, primer extension for 90 secs at 68°C, and a final elongation step of 7 mins at 68°C.

PCR products were separated by gel electrophoresis. One percent agarose gels were prepared by adding one gram of agarose to 100 ml of 1x TAE buffer with 10 µl of Gel Red (Biotium Inc., Fremont, CA). Ten µl of 100 bp ladder (Biotium Inc., Fremont, CA) was added to the first lane of each gel and 10 µl of each 25 µL reaction was mixed with 1.67 µl of 6x loading buffer (Biotium Inc., Fremont, CA) and loaded onto the gel. The gels were run for 50 mins at a current of 100 V in an electrophoretic tank. DNA bands were viewed by ultraviolet transillumination using a G:BOX and the program GeneSnap 6.08 (Syngene, Cambridge, UK). Amplified products which produced a single band for each sample were purified by using an E.Z.N.A.® Cycle Pure kit (OMEGA Bio-tek Inc., Norcross, GA) following the enclosed protocol.

Sequencing

Cleaned amplicons were sent to Eurofins Genomics, LLC. and were Sanger sequenced in both directions. Geneious 11.1.4 (Biomatters Limited, Auckland, New Zealand) was used to assemble, analyze, and trim chromatograms of forward and reverse reactions. Each sample was then checked for contamination by inputting the sequence data into BLAST.

Analysis

A multiple sequence alignment was performed for COI, Ef-1α, and the combined data set using a Geneious alignment. Two Ef-1α introns were identified and removed prior to analysis using the program AliView (Larsson 2014). Three phylogenetic trees (COI, Ef-1α, and the combined data set) were constructed by RAxML 8.2.11 (Stamatakis 2014) with rapid bootstrapping and search for best-scoring ML tree. For each tree (COI, Ef-1α, and the combined

data set), a GTR GAMMA nucleotide model was used and run for 1,000 iterations. All sequences obtained in this study will be deposited in GenBank.

Results

COI was successfully sequenced for 131 samples and Ef-1α was sequenced for 113 samples. With sequences from GenBank, the phylogeny estimated from the combined data set contains sequences representing 146 specimens. Trees constructed from Maximum likelihood analyses of the combined data set, COI, and Ef-1α are shown in Figures 2, 3, and 4.

The phylogeny from the combined data set was comprised of 17 putative new *Phylloxera* spp., 25 described *Phylloxera* spp., and three innominate *Phylloxera* spp. *Phylloxera* and *Phylloxerina* were each recovered as monophyletic (Figure 2). *Phylloxera* spp. occurring on other host plant genera were nested with the hickory-feeding phylloxerans including: *Phylloxera castaneae* (*Castanea pumila* (L.) Mill. or dwarf chestnut), *Phylloxera glabra* (von Heyden) (*Quercus garryana* Douglas ex. Hook or Oregon white oak and *Quercus lobata* Nee or valley oak), *Phylloxera reticulata* Duncan (*Quercus kelloggii* Newberry or California black oak), "*Phylloxera stoetzelae*" (*Juglans hindsii* (Jeps.) Jeps. ex R.E. Sm. or Northern California walnut), *Phylloxera foveola* Pergande (*Juglans nigra* L. or black walnut), and an undetermined *Phylloxera* sp. (black walnut). Host-use groups in phylloxerans are not monophyletic.

Three major clades with conservative gall morphology are found in the phylogeny constructed from the combined data set (Figure 2). The first major clade is comprised of species that form an elongate fold along the leaf vein as their opening on the abaxial leaf surface. The second major clade is comprised of species that mainly have a round opening on the abaxial leaf surface. The third major clade is comprised of species that have a slit-like opening on the abaxial leaf surface or a conical or thorn-like opening.

Lastly, *Phylloxera caryaemagna* (Shimer) was originally considered a variety of *P. caryaecaulis* (Fitch). However, since this species occurs in a separate clade from *P. caryaecaulis* it is now considered a separate species.

Discussion

Regardless of host plant genus, *Phylloxera* spp. clustered together on the phylogeny. Favret et al. (2016) recently transferred phylloxerans from the genus *Mortiziella* (e.g. *P. castaneivora*) to the genus *Phylloxera* because they had the same number and placement of spiracles on the thorax. The placement of *P. castaneivora* on the phylogeny supports the recent transfer of *Moritziella* spp. to the genus *Phylloxera.*

However, the number and placement of spiracles do not seem to be important characters for identifying genera in Phylloxeridae. For example, an oak-feeding species, *Phylloxera glabra*, has additional spiracles present on the abdominal segments but clusters with the hickory-feeding species in the phylogeny. Zhao and Nabity (2017) found a similar result with another oak-feeding species *Phylloxera quercus* Boyer de Fonscolombe. *Phylloxera quercus* also has additional spiracles present on the abdominal segments, but clustered with the hickory-feeding phylloxerans.

Phylloxera spp. clustering into clades by gall morphology rather than host plant use is a common pattern seen in other gall forming insects such as aphids (Stern 1995, Inbar et al. 2004), Australian gall thrips (Crespi and Worobey 1998, McLeish et al. 2011), cynipid wasps (Stone and Cook 1998), and sawflies (Nyman et al. 2000). Since there can be variation in gall morphology of *Phylloxera* spp., the phylogeny also aided us in species determinations. For example, *Phylloxera caryasepta* (Shimer), *Phylloxera caryaeavellana* Riley, *Phylloxera conica* (Shimer), *Phylloxera spinosa* (Shimer), and *Phylloxera symmetrica* Pergande have variation in

gall forms, but the different forms of each species cluster together in the phylogeny. Therefore, it was not only important to study the gall morphology of specimens, but to also study the morphology of the *Phylloxera* specimens closely. For example, a gall that was very similar in form to *Phylloxera caryaesepta* was identified as being a new species since the *Phylloxera* specimens had different antennal characters in both the fundatrix and alate stages.

Pergande (1904) considered *P. caryaemagna* and *Phylloxera spinosa* as being varieties of *Phylloxera caryaeacaulis.* They each have very different gall morphologies (*Phylloxera caryaemagna* being globular and spongy, *P. spinosa* being globular and spiny, and *P. caryaecaulis* being globular and hard), but were recognized as being the same species because their inhabitants closely resembled one another (Pergande 1904). However, *Phylloxera caryaemagna and P. spinosa* occur in different clades from *P. caryaeacaulis*. In addition *to P. caryaemagna* being recognized as a separate species, *P. spinosa* has also recently been recognized as being a distinct species (Favret et al. 2016)

A novel gall-forming *Phylloxera* sp., "*Phylloxera stoetzelae*," was collected from *Juglans hindsii* (Northern California walnut) which represents a new host genus. Walnuts (*Juglans* spp.) are in the same family as hickories (Juglandaceae) and are sister to them phylogenetically (Manos and Stone 2001). Walnuts and hickories are morphologically similar to one another with their pinnately compound leaves, small flowers, and large nuts (Nelson et al. 2014) as well as being chemically similar with both of them containing the same allelochemicals (juglone and juglone's hydroquinone form) (Rietveld 1983). Allelochemicals are toxic chemicals produced by plants that protect them from herbivorous insects; insects must be able to neutralize this defense in order to successfully survive and reproduce on a host plant (Walling 2000, Nishida 2014) .

Perhaps since walnuts are both morphologically and chemically similar to hickories (Rietveld 1983, Nelson et al. 2014), *Phylloxera* spp. are able to colonize and inhabit this genus as well. Alates of two different species (*Phylloxera foveola* and an unidentified *Phylloxera* sp.) were collected from *Juglans nigra* (black hickory) in Arkansas and Oklahoma. *Juglans nigra* could possibly be used as a secondary host for the alates to lay their eggs on of the sexual generation which would represent another example of host switching. Stoetzel (1985) successfully demonstrated host switching with *Phylloxera texana* Stoetzel and *Phylloxera castaneae* and observed that alatae from both species gave rise to populations on *Quercus* spp. and *Castanea* spp. Similarly, Pergande (1904) noted that he found *Phylloxera perniciosa* Pergande alates that had emerged from galls on a mockernut hickory depositing eggs on other genera of trees, shrubs, and weeds.

Insect gallers are considered highly specialized since they reprogram plant metabolism to their benefit (Ali and Agrawal 2012) and the phylogeny helps to elucidate which *Phylloxera* spp. are monophagous (feeding on only one host plant species) versus oligophagous (feeding on several host plant species). The majority of *Phylloxera* spp. seem to be oligophagous since they were collected on more than one host plant species including such species as *P. caryaeavellana*, *P. caryaemagna*, *P. caryaescissa* Riley, *P. caryaesepta*, *P. caryaevenae* Fitch, *P. conica*, *P. foveola*, *P. spinosa*, and *P. symmetrica*. In contrast, species occurring on *Carya illinoiensis* (Wangenh.) K. Koch (pecan) such as *Phylloxera devastatrix* Pergande and *Phylloxera russellae* Stoetzel seem to be monophagous since they were not collected on any other hickory species. Similarly, many of the new species collected were only collected on one hickory species including: "*Phylloxera auburnensis",* "*Phylloxera bispinae*", "*Phylloxera chippokesiensis*",

"*Phylloxera crypta*", "*Phylloxera falsostium*", "*Phylloxera floridana*", "*Phylloxera myristica*", "*Phylloxera paludis",* "*Phylloxera wiedenmanni*", and "*Phylloxera williamsi*".

Two factors may contribute to the diversification of phylloxerans including geographic isolation and host range expansion. Allopatric speciation or speciation by geographic isolation is important in the divergence of insect species (Nyman et al. 2010). For example, *P. caryaesemen* Walsh is thought to be a western species and has been collected in Arkansas, Missouri, and Illinois whereas its sister species "*P. chippokesiensis"* was collected in Virginia. Host range expansions allow insects to become more widely distributed and shift to novel host plant species (Janz et al. 2006). For example, *P. russellae* is found only on *C. illinoinensis* and its sister species "*P. myristica*" was found on *C. myrisiticiformis*. Similarly, *P. castaneae* is a free-living species on *Castanea* spp. and its sister species "*P. crypta*" is a gall forming species found on *C. aquatica*.

This is the first molecular phylogeny of *Phylloxera* spp. collected from across the U.S. (with a couple taxa collected from outside the U.S.). The phylogeny not only illustrates the host-use patterns of 25 described species and 17 putative new species, but also demonstrates that species that are closer together on the phylogeny have more similar gall morphologies. This study provides the framework for future studies that may look into the correlation between gall morphology and natural enemy associations (predators or parasitoids) of *Phylloxera* spp. or further examination of the diversity of *Phylloxera* spp. present in other areas of the U.S. such as the Northeast or Midwest.

Table 1. Accessions of *Phylloxera* spp. and outgroup species with life history and collection information. Species followed by a superscript denote sequences that were taken from GenBank.

Acc. no.	Species	Life history/Shape	Collection Date	Collection Locality	Host
211	*"Phylloxera floridana"*	Leaf gall: button	10/03/15	Archbold Biological Station, FL	*Carya floridana*
191	*Phylloxera caryaecaulis*	Leaf gall: globular	04/13/16	Davis Arboretum, AL	*Carya glabra*
207	*"Phylloxera auburnensis"*	Leaf gall: globular	04/13/16	Davis Arboretum, AL	*Carya pallida*
009	*"Phylloxera bispinae"*	Leaf gall: thorny	04/19/16	Bankhead National Forest, AL	*Carya glabra*
010	*Phylloxera foveola*	Leaf gall: button	04/19/16	Bankhead National Forest, AL	*Carya glabra*
011	*Phylloxera spinosa*	Leaf gall: globular	04/19/16	Bankhead National Forest, AL	*Carya glabra*
022	*Phylloxera caryaescissa*	Leaf gall: button	04/19/16	Bankhead National Forest, AL	*Carya tomentosa* hybrid
025	*Phylloxera spinosa*	Petiole gall: globular	04/19/16	Bankhead National Forest, AL	*Carya glabra*
032	*Phylloxera caryaescissa*	Leaf gall: button	04/19/16	Bankhead National Forest, AL	*Carya tomentosa*
012	*Phylloxera symmetrica*	Leaf gall: pear	04/20/16	Talladega National Forest, AL	*Carya tomentosa x C. pallida*
018	*Phylloxera caryaescissa*	Leaf gall: button	04/20/16	Talladega National Forest, AL	*Carya cordiformis*
041	*Phylloxera caryaescissa*	Leaf gall: button	04/22/16	Florida Caverns State Park, FL	*Carya glabra*
042	*Phylloxera caryaesepta*	Leaf gall: thorny	04/22/16	Florida Caverns State Park, FL	*Carya glabra*
044	*Phylloxera caryaemagna*	Leaf gall: globular	04/22/16	Florida Caverns State Park, FL	*Carya cordiformis*
070	*Phylloxera caryaefallax*	Leaf gall: round	04/23/16	A.J. Henry Park, FL	*Carya glabra*
072	*Phylloxera* sp. 3	Leaf gall: elongate	04/23/16	A.J. Henry Park, FL	*Carya glabra*
083	*Phylloxera* sp. 2	Leaf gall: elongate	04/24/16	Dauset Trails, GA	*Carya glabra*
084	*Phylloxera spinosa*	Leaf gall: globular	04/24/16	Dauset Trails, GA	*Carya glabra*
085	*Phylloxera conica*	Leaf gall: globular	04/24/16	Dauset Trails, GA	*Carya glabra*
087	*"Phylloxera flavoconica"*	Leaf gall: conical	04/24/16	Dauset Trails, GA	*Carya tomentosa*
089	*Phylloxera notabilis*	Leaf gall: oval	04/25/16	J.F. Gregory City Park, GA	*Carya aquatica*
090	*"Phylloxera stoetzelae"*	Leaf gall: round	04/25/16	J.F. Gregory City Park, GA	*Juglans hindsii*
091	*Phylloxera pilosula*	Leaf gall: globular	04/25/16	Tom Triplett Park, GA	*Carya tomentosa*
092	*Phylloxera caryaevenae*	Leaf gall: elongate	04/25/16	Tom Triplett Park, GA	*Carya tomentosa*

Table 1, Cont.

Acc. no.	Species	Life history/Shape	Collection Date	Collection Locality	Host
095	*Phylloxera conica*	Leaf gall: globular	04/25/16	Tom Triplett Park, GA	*Carya tomentosa*
097	*Phylloxera conica*	Leaf gall: globular	04/25/16	Tom Triplett Park, GA	*Carya tomentosa*
101	*Phylloxera caryaescissa*	Leaf gall: button	04/27/16	Edisto Nature Trail, SC	*Carya cordiformis*
102	*Phylloxera caryaemagna*	Leaf gall: globular	04/27/16	Edisto Nature Trail, SC	*Carya tomentosa*
103	*Phylloxera notabilis*	Leaf gall: oval	04/27/16	Edisto Nature Trail, SC	*Carya cordiformis*
104	*Phylloxera caryaevenae*	Leaf gall: elongate	04/27/16	Francis Marion National Forest, SC	*Carya tomentosa x C. cordiformis*
109	*Phylloxera conica*	Leaf gall: globular	04/27/16	Francis Marion National Forest, SC	*Carya glabra*
110	*"Phylloxera crypta"*	Leaf gall: round	04/27/16	Francis Marion National Forest, SC	*Carya aquatica*
113	*Phylloxera caryaevenae*	Leaf gall: elongate	04/27/16	Francis Marion National Forest, SC	*Carya aquatica*
114	*"Phylloxera myristica"*	Leaf gall: round	04/27/16	Francis Marion National Forest, SC	*Carya myristiciformis*
118	*Phylloxera caryaefoliae*	Leaf gall: conical	04/28/16	Sumter National Forest, SC	*Carya glabra*
127	*Phylloxera foveola*	Leaf gall: button	04/28/16	Sumter National Forest, SC	*Carya glabra*
131	*Phylloxera picta*	Leaf gall: round	04/28/16	Sumter National Forest, SC	*Carya glabra*
133	*Phylloxera picta*	Leaf gall: round	04/28/16	Sumter National Forest, SC	*Carya tomentosa*
135	*Phylloxera caryaeavellana*	Leaf gall: hazelnut	04/28/16	Sumter National Forest, SC	*Carya pallida*
136	*Phylloxera conica*	Leaf gall: globular	04/28/16	Sumter National Forest, SC	*Carya myristiciformis*
142	*Phylloxera pilosula*	Leaf gall: globular	04/29/16	Nantahala National Forest, NC	*Carya tomentosa*
147	*Phylloxera caryaemagna*	Leaf gall: globular	04/30/16	Warriors' Path State Park, TN	*Carya pallida*
150	*Phylloxera caryaemagna*	Leaf gall: globular	04/30/16	Warriors' Path State Park, TN	*Carya cordiformis*
154	*"Phylloxera flavoconica"*	Leaf gall: conical	04/30/16	Warriors' Path State Park, TN	*Carya ovata*
155	*"Phylloxera flavoconica"*	Leaf gall: conical	04/30/16	Warriors' Path State Park, TN	*Carya ovata*
157	*Phylloxera caryaemagna*	Leaf gall: globular	04/30/16	Warriors' Path State Park, TN	*Carya pallida*
172	*Phylloxera russellae*	Leaf gall: round	05/13/16	ALFA building AU, AL	*Carya illinoinensis*
174	*Phylloxera devastatrix*	Stem gall: globular	05/13/16	ALFA building AU, AL	*Carya illinoinensis*
175	*Phylloxera devastatrix*	Leaf gall: globular	05/13/16	ALFA building AU, AL	*Carya illinoinensis*

Table 1, Cont.

Acc. no.	Species	Life history/Shape	Collection Date	Collection Locality	Host
176	*Phylloxera notabilis*	Leaf gall: oval	05/13/16	Davis Arboretum, AL	*Carya laciniosa*
208	*Phylloxera* sp. 8	Leaf gall: round	06/06/16	Talladega National Forest, AL	*Carya tomentosa*
210	*Phylloxera castaneae*	Free-living	09/01/16	Mobile, AL	*Castanea pumila*
12	*Phylloxera glabra*	Free-living	09/04/16	Ankeny National Wildlife Refuge, OR	*Quercus garryana*
1p5	*Phylloxera spinosa*	Leaf gall: globular	04/25/17	Mehan, OK	*Carya texana*
rb9	*Phylloxera picta*	Leaf gall: round	05/03/17	Bienville National Forest, MS	*Carya tomentosa*
rb4	*Phylloxera caryaeavellana*	Leaf gall: hazelnut	05/03/17	Bienville National Forest, MS	*Carya tomentosa*
rb0	*Phylloxera* sp. 6	Leaf gall: conical	05/03/17	Bienville National Forest, MS	*Carya tomentosa*
rbr	*Phylloxera picta*	Leaf gall: round	05/03/17	Bienville National Forest, MS	*Carya tomentosa*
rb7	*Phylloxera symmetrica*	Leaf gall: pear	05/03/17	Bienville National Forest, MS	*Carya tomentosa*
eup	*"Phylloxera paludis"*	Leaf gall: round	05/04/17	Delta National Forest, MS	*Carya aquatica*
rbo	*"Phylloxera stoetzelae"*	Leaf gall: round	05/04/17	Delta National Forest, MS	*Carya aquatica*
rbm	*Phylloxera caryaevenae*	Leaf gall: elongate	05/05/17	Kisatchie National Forest, LA	*Carya tomentosa*
rbc	*Phylloxera caryaeavellana*	Leaf gall: hazelnut	05/05/17	Kisatchie National Forest, LA	*Carya pallida*
of1	*Phylloxera* sp. 5	Leaf gall: elongate	05/05/17	Kisatchie National Forest, LA	*Carya cordiformis*
ofo	*Phylloxera* sp. 1	Leaf gall: elongate	05/06/17	Louisiana State Arboretum, LA	*Carya tomentosa* hybrid
ofy	*Phylloxera russellae*	Leaf gall: round	05/06/17	Mercy Regional Medical Center, LA	*Carya illinoinensis*
wed	*Phylloxera caryaefallax*	Leaf gall: round	05/07/17	Martin Dies, Jr. State Park, TX	*Carya texana*
wev	*Phylloxera caryaevenae*	Leaf gall: elongate	05/08/17	Davy Crockett National Forest, TX	*Carya texana*
we4	*Phylloxera* sp. 5	Leaf gall: elongate	05/08/17	Fort Boggy State Park, TX	*Carya texana*
we2	*Phylloxera* sp. 5	Leaf gall: elongate	05/08/17	Fort Boggy State Park, TX	*Carya myristiciformis*
we1	*Phylloxera caryaesepta*	Leaf gall: thorny	05/08/17	Fort Boggy State Park, TX	*Carya myristiciformis*
wey	*Phylloxera* sp. 5	Leaf gall: elongate	05/08/17	Fort Boggy State Park, TX	*Carya texana*
we0	*Phylloxera conica*	Leaf gall: globular	05/08/17	Fort Boggy State Park, TX	*Carya myristiciformis*
2qx	*Phylloxera conica*	Leaf gall: globular	05/09/17	Lake Bob Sandlin State Park, TX	*Carya texana*
cms	*Phylloxera caryaescissa*	Leaf gall: button	05/09/17	Lake Bob Sandlin State Park, TX	*Carya tomentosa*

Table 1, Cont.

Acc. no.	Species	Life history/Shape	Collection Date	Collection Locality	Host
2qd	*"Phylloxera wiedenmanni"*	Leaf gall: button	05/09/17	Lake Bob Sandlin State Park, TX	*Carya cordiformis*
cmq	*Phylloxera caryaefallax*	Leaf gall: round	05/09/17	Lake Bob Sandlin State Park, TX	*Carya tomentosa*
5o0	*Phylloxera caryaescissa*	Leaf gall: button	05/11/17	Ouachita National Forest, OK	*Carya tomentosa*
2q5	*Phylloxera caryaesepta*	Leaf gall: thorny	05/11/17	Ouachita National Forest, OK	*Carya texana*
2qk	*Phylloxera caryaeavellana*	Leaf gall: hazelnut	05/11/17	Ouachita National Forest, OK	*Carya cordiformis*
2q1	*Phylloxera caryaefallax*	Leaf gall: round	05/11/17	Ouachita National Forest, OK	*Carya texana*
5or	*Phylloxera caryaefoliae*	Leaf gall: conical	05/11/17	Lake Wister State Park, OK	*Carya texana*
ts4	*Phylloxera* sp. 5	Leaf gall: elongate	05/11/17	Lake Wister State Park, OK	*Carya texana*
jq0	*Phylloxera foveola*	Leaf gall: button	05/12/17	Ouachita National Forest, AR	*Carya tomentosa*
5oc	*Phylloxera caryaeavellana*	Leaf gall: hazelnut	05/12/17	Ouachita National Forest, AR	*Carya texana*
5og	*Phylloxera caryaeavellana*	Leaf gall: hazelnut	05/12/17	Ouachita National Forest, AR	*Carya cordiformis*
5ok	*Phylloxera caryaescissa*	Leaf gall: button	05/12/17	Ouachita National Forest, AR	*Carya tomentosa*
v3b	*Phylloxera caryaescissa*	Leaf gall: button	05/12/17	Ouachita National Forest, AR	*Carya texana*
ts3	*Phylloxera foveola*	Leaf gall: button	05/12/17	Ouachita National Forest, AR	*Carya texana*
m0b	*Phylloxera caryaecaulis*	Petiole gall: globular	05/12/17	Lake Wilson City Park, AR	*Carya ovata*
m0g	*Phylloxera symmetrica*	Leaf gall: pear	05/12/17	Lake Wilson City Park, AR	*Carya texana*
m0u	*Phylloxera foveola*	Alate only	05/13/17	Natural Falls State Park, OK	*Juglans nigra*
m0s	*"Phylloxera wiedenmanni"*	Leaf gall: button	05/13/17	Natural Falls State Park, OK	*Carya cordiformis*
m0o	*"Phylloxera falsostium"*	Leaf gall: round	05/13/17	Natural Falls State Park, OK	*Carya cordiformis*
jzg	*Phylloxera picta*	Leaf gall: round	05/13/17	Lake Wedington, AR	*Carya tomentosa*
yrt	*Phylloxera caryaecaulis*	Petiole gall: globular	05/14/17	Devil's Den State Park, AR	*Carya ovata*
m07	*Phylloxera caryaesepta*	Leaf gall: thorny	05/14/17	Ozark National Forest, AR	*Carya cordiformis*
m02	*Phylloxera symmetrica*	Leaf gall: pear	05/14/17	Ozark National Forest, AR	*Carya texana*
m04	*Phylloxera caryaeavellana*	Leaf gall: hazelnut	05/14/17	Ozark National Forest, AR	*Carya cordiformis*
jz6	*Phylloxera caryaesemen*	Leaf gall: round	05/15/17	St. Francis National Forest, AR	*Carya cordiformis*

Table 1, Cont.

Acc. no.	Species	Life history/Shape	Collection Date	Collection Locality	Host
yr3	*Phylloxera caryaesemen*	Leaf gall: conical	05/15/17	St. Francis National Forest, AR	*Carya cordiformis*
jz9	*Phylloxera* sp. 7	Alate only	05/15/17	St. Francis National Forest, AR	*Juglans nigra*
g4v	*Phylloxera* sp. 2	Leaf gall: elongate	05/15/17	St. Francis National Forest, AR	*Carya texana*
n8l	*Phylloxera* sp. 5	Leaf gall: elongate	05/15/17	St. Francis National Forest, AR	*Carya tomentosa*
n8k	*Phylloxera* sp. 4	Leaf gall: elongate	05/16/17	Holly Springs National Forest, MS	*Carya ovata*
cme	*Phylloxera picta*	Leaf gall: round	05/17/17	Tombigbee National Forest, MS	*Carya tomentosa*
cmc	*Phylloxera caryaescissa*	Leaf gall: button	05/17/17	Tombigbee National Forest, MS	*Carya tomentosa*
16	*Phylloxera caryaecaulis*	Leaf gall: globular	05/23/17	Arnold Arboretum, MA	*Carya ovata*
17	*Phylloxera subelliptica*	Petiole gall: oval	05/23/17	Arnold Arboretum, MA	*Carya ovata*
19	*Phylloxera caryaeavellana*	Leaf gall: hazelnut	05/24/17	Arnold Arboretum, MA	*Carya glabra*
20	*Phylloxera foveola*	Leaf gall: button	05/24/17	Arnold, Arboretum, MA	*Carya glabra*
21	*Phylloxera caryaecaulis*	Stem gall: globular	05/24/17	Arnold Arboretum, MA	*Carya glabra*
bwu	*Phylloxera russellae*	Leaf gall: round	05/29/17	Stillwater, OK	*Carya illinoinensis*
13	*Phylloxera reticulata*	Free-living	11/17/17	Jasper Ridge Biological Preserve, CA	*Quercus kelloggii*
11	*Phylloxera glabra*	Free-living	04/05/18	UC Riverside, CA	*Quercus lobata*
qzb	*Phylloxera foveola*	Leaf gall: button	05/16/18	George Washington National Forest, VA	*Carya cordiformis*
qzv	*Phylloxera caryaeglobuli*	Leaf gall: globular	05/16/18	George Washington National Forest, VA	*Carya ovata*
tzq	*"Phylloxera falsostium"*	Leaf gall: round	05/17/18	Chippokes Plantation State Park, VA	*Carya cordiformis*
tnh	*"Phylloxera chippokesiensis"*	Leaf gall: thorny	05/17/18	Chippokes Plantation State Park, VA	*Carya cordiformis*
qze	*Phylloxera foveola*	Leaf gall: button	05/17/18	Eastern Shore of VA National Wildlife Refuge, VA	*Carya glabra*
hl0	*Phylloxera picta*	Leaf gall: round	05/17/18	Eastern Shore of VA National Wildlife Refuge, VA	*Carya tomentosa*
bwf	*Phylloxera caryaesepta*	Leaf gall: thorny	05/17/18	Eastern Shore of VA National Wildlife Refuge, VA	*Carya tomentosa x C. pallida*
brm	*Phylloxera foveola*	Leaf gall: button	05/18/18	Trap Pond State Park, DE	*Carya pallida*
qzn	*Phylloxera symmetrica*	Leaf gall: pear	05/18/18	Trap Pond State Park, DE	*Carya pallida*
brx	*Phylloxera* sp. 1	Leaf gall: elongate	05/18/18	Trap Pond State Park, DE	*Carya pallida*

Table 1, Cont.

Acc. no.	Species	Life history/Shape	Collection Date	Collection Locality	Host
tnw	*Phylloxera* sp. 1	Leaf gall: elongate	05/18/18	Trap Pond State Park, DE	*Carya pallida*
s3x	*Phylloxera caryaeavellana*	Leaf gall: hazelnut	05/18/18	Trap Pond State Park, DE	*Carya pallida*
tn8	*Phylloxera symmetrica*	Leaf gall: pear	05/18/18	Trap Pond State Park, DE	*Carya pallida*
1pv	*Phylloxera caryaemagna*	Leaf and stem gall: globular	05/18/18	White Clay Creek State Park, DE	*Carya cordiformis*
mph	*Phylloxera* sp. 4	Leaf gall: elongate	05/19/18	Cunningham Falls State Park, MD	*Carya ovata*
pga	*Phylloxera foveola*	Leaf gall: button	05/19/18	Cunningham Falls State Park, MD	*Carya ovata*
tnc	*Phylloxera caryaeglobuli*	Leaf gall: globular	05/19/18	Cunningham Falls State Park, MD	*Carya ovata*
1pe	*Phylloxera* sp. 2	Leaf gall: elongate	05/20/18	Yankauer Nature Preserve, WV	*Carya glabra*
tnq	*Phylloxera* sp. 2	Leaf gall: elongate	05/20/18	Yankauer Nature Preserve, WV	*Carya ovata*
qzl	*"Phylloxera williamsi"*	Leaf gall: globular	05/20/18	Yankauer Nature Preserve, WV	*Carya cordiformis*
bru	*Phylloxera caryaeglobuli*	Leaf gall: globular	05/21/18	Monongahela National Forest, WV	*Carya ovata*
tnm	*Phylloxera caryaeglobuli*	Leaf gall: globular	05/21/18	Hawks Nest State Park, WV	*Carya ovata*
tnp	*Phylloxera symmetrica*	Leaf gall: pear	05/21/18	Hawks Nest State Park, WV	*Carya tomentosa*
2ic	*Daktulosphaira vitifoliae*	Leaf galls	07/10/17	Mehan, OK	*Vitis vulpina*
10	*Phylloxerina popularia*	Free-living	07/18/17	Tilman Bishop State Wildlife Area, CO	*Populus deltoides*
JQ920928.1	*Phylloxerina salicis*[3]	Free-living	08/24/03	China	*Salix paraplesia*
CNC#HEM054286	*Aphis sambuci*[2]	Free-living	07/12/05	British Colombia, Canada	Unknown
CNC#HEM049208.2	*Cinara pergandei*[2]	Free-living	05/27/02	Danville, VA	Unknown
CNCHEM054843	*Hamamelistes spinosus*[4]	Stem gall and free-living	06/28/99	Madison, WI	*Betula nigra*
EF418795.1	*Phylloxera castaneivora*[5]	Free-living	08/29/05	Shandong, China	*Castanea crenata*
BIOUG28363-C04	*Phylloxera coccinea*[1]	Free-living	08/27/14	British Colombia, Canada	Unknown

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Figure 1. Localities across U.S. where *Phylloxera* spp. were collected from 2015–2018 indicated by circles. Squares indicate where additional samples of Phylloxeridae (*Phylloxera* spp., *Phylloxerina* spp., and *Daktulosphaira vitifoliae*) were collected.

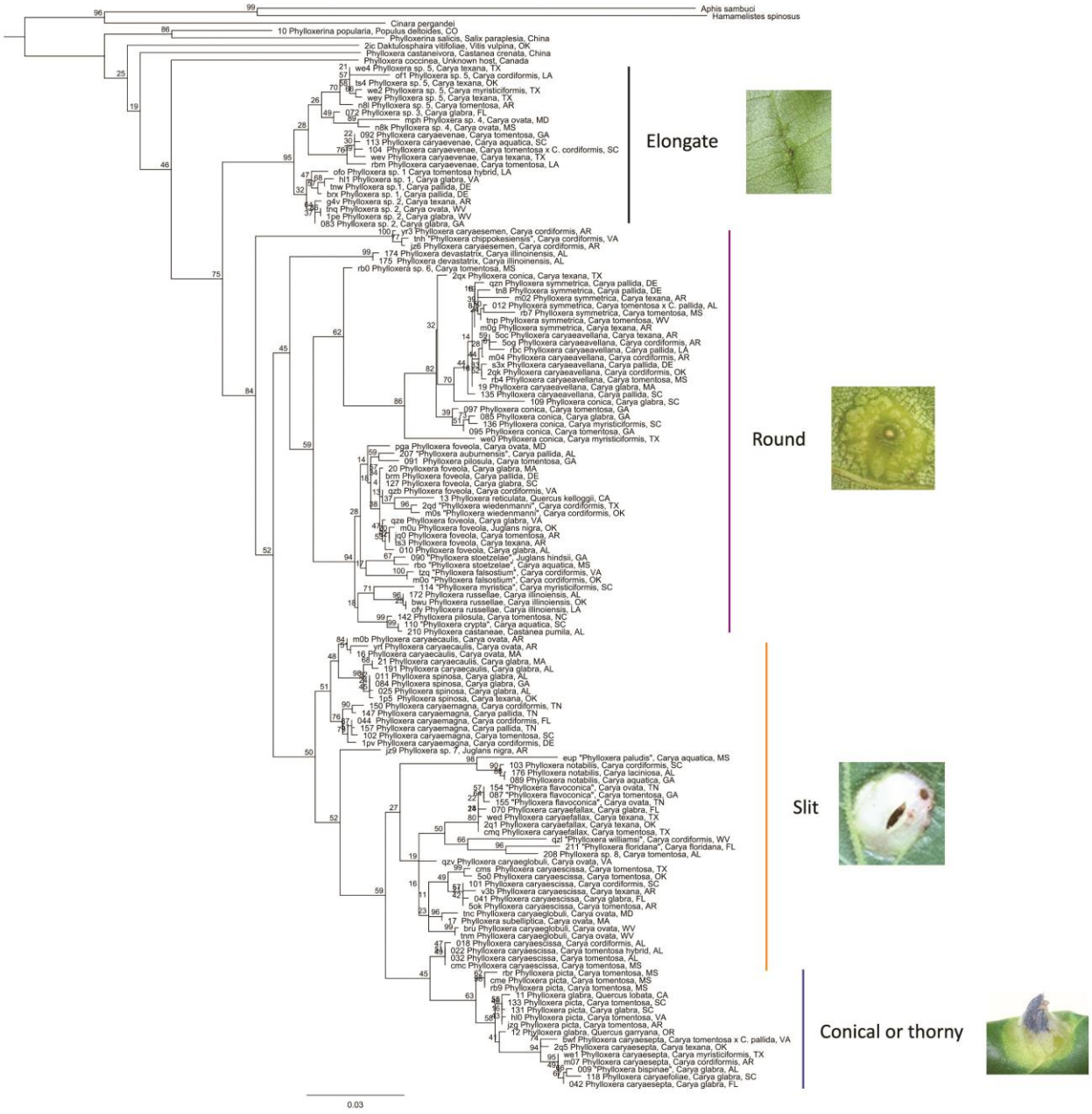

Figure 2. Maximum likelihood tree of the combined dataset. Bootstrap values are located at nodes. Each sample name contains the accession number, *Phylloxera* or outgroup species, host plant species, and state abbreviation or country where sample was collected. Pictures to the right indicate opening type for each major clade.

Figure 3. COI gene tree constructed from a Maximum likelihood analysis. Bootstrap values are located at nodes. Sample names include accession number and *Phylloxera* or outgroup species.

Figure 4. Ef-1α gene tree constructed from a Maximum likelihood analysis. Bootstrap values are located at nodes. Sample names include accession number and *Phylloxera* or outgroup species.

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Chapter 3

Fourteen new species of *Phylloxera* (Hemiptera: Phylloxeridae) described on Juglandaceae in the U.S.

Abstract

Temperate deciduous forests are found across the Eastern U.S. and are dominated by broad-leafed trees that shed their leaves once a year such as hickories (*Carya* spp. Nutt.) and oaks (*Quercus* spp. L.). Phylloxerans (Hemiptera: Phylloxeridae: *Phylloxera* spp. Boyer de Fonscolombe) are small aphid-like insects that are common in these forests on hickories and form galls (abnormal growths of plant tissue) during the spring in which the insects grow and develop. Despite their ubiquity, this group has not been worked on taxonomically for over 100 years and much of what is known about their species diversity is from a small area around Washington, D.C. *Phylloxera* galls and specimens were collected from 53 sites distributed in 15 states across the Eastern U.S. from 2015–2018 to revise their species diversity, characterize their gall morphologies, and describe their host-use. The collection was comprised of 358 samples of phylloxerans which included 19 putative new species, 21 described species, and six undetermined *Phylloxera* spp. Herein, we describe 14 new species of *Phylloxera* along with their respective galls and provide a key to all accepted hickory and walnut-feeding species in the U.S.

Introduction

Galls are considered one of the most emblematic examples of plant manipulation by insects (Giron et al. 2016) and are distinguished from other insect made shelters (e.g. rolled leaves) by the active differentiation and growth of plant tissues that resemble a novel plant organ (Stone and Schonrogge 2003, Shorthouse et al. 2005, Giron et al. 2016). There are approximately 15,000 described species of insects that induce galls within which the insect feeds and develops (Richardson et al. 2017). Galls are thought to confer their inducers with access to enhanced nutrition as well as protection from harsh abiotic conditions and biotic interactions (Stone et al. 2002, Stone and Schonrogge 2003).

On deciduous trees such as hickories (*Carya* spp. Nutt.) and oaks (*Quercus* spp. L.), galling insects cause aesthetic damage and when abundant may cause premature leaf drop and branch die back (Caldwell and Schuder 1979, Buffington et al. 2013). In some cases, galling insects which inhabit deciduous trees may cause economic damage such as the invasive gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) that infests all species of chestnut (*Castanea* spp. Mill.) and threatens the chestnut industry (Cooper and Rieske 2007). On pecan trees (*Carya illinoinensis* (Wangenh.) K. Koch), *Phylloxera russellae* Stoetzel (Hemiptera: Phylloxeridae) can destroy the current year's nut crop as well as the next year's nut crop when infestations are high (Stoetzel and Tedders 1981).

Hickories are considered an important component of temperate deciduous forests in the Eastern U.S. (McCarthy and Wistendahl 1988). There are 11 species of hickories native to the U.S. and many of them have a Southeastern distribution, but some species are found as far north as Maine and as far west as Texas (Kirkman et al. 2007, Fryer 2018). Hickories provide food and shelter to many animal species (Fralish 2004, Nelson et al. 2014) and are also economically

important since their nuts are edible (e.g. pecans) and their wood is often used for tool handles, furniture, and preparation of food as fuel for smoking (Kirkman et al. 2007).

Phylloxerans (Hemiptera: Phylloxeridae: *Phylloxera* spp. Boyer de Fonscolombe) are small aphid-like insects that feed on the phloem of chestnuts, hickories, and oaks (Pergande 1904). Their feeding affects their host plants in different ways with *Phylloxera* spp. forming galls on hickories and causing yellow spotting and/or curling of the leaves on chestnuts and oaks (Blackman and Eastop 2013). Currently, there are 32 described species of hickory-feeding *Phylloxera* in the U.S. with many of them being collected on *Carya cordiformis* (Wangenh.) K. Koch (bitternut hickory), *C. glabra* (Mill.) Sweet (pignut hickory), *C. illinoinensis* (pecan), *C. tomentosa* (Lam.) Nutt. (mockernut hickory), and *C. ovata* (Mill.) K. Koch (shagbark hickory) (Pergande 1904, Stoetzel 1981, Blackman and Eastop 2013).

Hickory-feeding phylloxerans induce galls on their hosts in the early spring when fundatrices (stem-mothers) emerge from their overwintering eggs and begin to feed on the leaves or stems shortly after bud-break and leaf expansion (Pergande 1904, Caldwell and Schuder 1979). Their feeding induces a gall to form around them and the fundatrix will mature and reproduce parthenogenetically within the gall (Caldwell and Schuder 1979). Once the gall matures, the orifice or orifices will open and the alates (winged migrants) will emerge to reproduce parthenogenetically and lay the eggs of the sexual generation (Caldwell and Schuder 1979). The sexual males and females will then mate and lay the egg of the fundatrix for the next spring in an old gall or bark crevice (Caldwell and Schuder 1979).

Although phylloxerans are ubiquitous thoughout deciduous forests during the spring, they have not been the focus of many taxonomic studies. Shimer (1868) documented the diversity of hickory phylloxerans found near Mt. Carroll, Illinois and Pergande (1904) published 28 species

descriptions of hickory phylloxerans, mainly from material around Washington, D.C. Pergande (1904) believed that his work treated only a fraction of the total diversity in the U.S., but since Pergande's research only two new species of *Phylloxera* on pecan have been described (Stoetzel 1981).

Despite the possibility of many new species of *Phylloxera* awaiting to be discovered on hickories across the eastern U.S., this group has been overlooked and not worked on taxonomically for over 100 years until now. During this study, we collected hickory-feeding phylloxerans from 15 states in three geographic regions of the U.S. (Mid-Atlantic, Southeast, and South Central) to revise their species diversity, characterize their gall morphologies, and describe their host-use. A key to all hickory and walnut-feeding *Phylloxera* spp. in the U.S. is provided.

Materials and Methods

Phylloxera galls and/or specimens were collected on hickories, walnuts, and a chestnut from 53 sites in 15 states across the Eastern U.S. during fall 2015 and spring 2016–2018. Field sites were selected based on geographic location and hickory species distribution. KoBoCollect, a mobile device application, was used to record field data for galls and their hosts at each site. Data types included date, GPS coordinates, gall pictures, and host pictures (rachis, bark, nuts, and bud). Each gall type collected from an individual tree at a site was assigned an accession number and galls collected from each tree were placed in a gallon-size ziplock plastic bag with their corresponding accession numbers for later processing. A rachis, terminal bud, and nut (if present) were collected from each tree for herbarium vouchers for later host identification. A rachis was preserved on herbarium paper using a plant press and a nut (if present), terminal bud, and additional rachis were placed in a paper lunch bag with a small amount of silica gel. The corresponding accession numbers were put on the herbarium paper and bag. Hickories were

identified to species by using two field guides (Kirkman et al. 2007, Nelson et al. 2014) and identifications were confirmed by Dr. Nancy Loewenstein of the Auburn University School of Forestry and Wildlife Sciences. Herbarium samples are deposited at AUMNH.

Photographs and measurements of galls were taken prior to dissection and during dissection under a dissecting microscope and were recorded by using KoBoCollect. A ruler and a 5 mm Micro Ruler were used to take measurements. Measurements included: 1) number of galls on leaf/stem; 2) number of gall types on leaf/stem; 3) gall description; 4) location of gall; 5) distance to closest gall; 6) gall surface (pubescent, glabrous, smooth, or rugose); 7) location of opening/openings (above, below, or both); 8) opening type (nipple, round, slit, or thorn); 9) state of opening/openings (open, slightly open, or closed); 10) opening length; 11) opening surrounded by (bracts, pubescence, tubercles, or nothing); 12) opening pubescence on outside (dense, fair, or none); 13) opening color (green/yellow, white, crimson, or brown); 14) opening a different color than rest of gall (yes or no); 15) horizontal diameter of gall on adaxial leaf surface, 16) vertical diameter of gall on adaxial leaf surface; 17) gall height on adaxial leaf surface; 18) horizontal diameter of gall on abaxial leaf surface; 19) vertical diameter of gall on abaxial leaf surface; 20) gall height on abaxial leaf surface; 21) wall thickness; 22) opening pubescence on inside (dense, fair, or none); and 23) life stages present (Pergande 1904). Measurements were recorded and phylloxerans were removed from each gall type contained in each bag and were put in separate vials containing 70% ethanol for slide-mounting and 95% ethanol for molecular work.

Phylloxerans were prepared for slide-mounting by putting them in vials containing 10% KOH and were left to sit in the solution overnight. Specimens were then transferred to a petri dish where the body contents were pumped out; no incision on the abdomen was necessary as the

specimens were very small and delicate. They were then transferred to a plate containing three rows of wells with the first row of wells filled with specimen clearing fluid (BioQuip Products, Inc., Rancho Dominguez, CA) and a couple drops of double stain (BioQuip Products, Inc., Rancho Dominguez, CA), the second row of wells filled with 95% ethanol, and the third row of wells were filled with clove oil (Spectrum Chemical MFG Corp, Gardena, CA). Specimens were put in the well containing the specimen clearing fluid and double stain for at least 10 minutes. The specimens were then transferred to a well containing 95% ethanol for at least 10 minutes. Next, the specimens were put in the well containing clove oil for at least 10 minutes. A small drop of Canada balsam (Alfa Aesar™, Heysham, England) thinned with xylenes was put on a slide and an individual phylloxeran was put in the balsam with the head facing down and covered with a coverslip (15 mm). The slide-mounted specimens were then transferred to a slide warmer for at least one month to allow the Canada balsam to thoroughly dry.

Specimens were viewed through a phase contrast light microscope. Phylloxerans were identified to species by using the key of Pergande (1904), the published descriptions of Stoetzel (1981), and DNA sequence data generated from molecular work completed from 2016–2018. *Phylloxera* spp. identifications were confirmed by comparing them to the *Phylloxera* syntypes in the U.S. National Collection in Beltsville, MD. Any nomenclatural act in the dissertation should not be considered valid under ICZN. There were no *Phylloxera* specimens or dried gall specimens in the U.S. National Collection for *Phylloxera foveata* (Shimer), *Phylloxera globosa* (Shimer) , or *Phylloxera minima* (Shimer) so they have been designated as *nomina dubia*. NIS-Elements software was used to take photographs and measurements. All of the given measurements are maximum dimensions and are expressed as a range across specimens. Digital images of phylloxeran specimens were traced and inked. Illustrations were refined with Adobe

Photoshop. In each of the illustrations, the dorsal body surface is displayed on the left side and the ventral body surface on the right side. Measurements are given in millimeters (mm) and micrometers (µm). The morphological terminology used in the descriptions follows that of Pergande (1904), Stoetzel (1981), and Metz et al. (2017). *Phylloxera* specimens are deposited at AUMNH (Auburn University Museum of Natural History, Auburn, AL), BMNH (British Museum of Natural History, London, England), and NMNH (National Museum of Natural History, Beltsville, MD).

Taxonomy

Phylloxera Boyer de Fonscolombe, 1834; Pergande 1904; Stoetzel 1981; Favret et al. 2016; Metz et al. 2017

Type species. *Phylloxera quercus* Boyer de Fonscolombe, 1834, by original monotypy.

Generic diagnosis. Fundatrix. Body shape of adult female fundatrix variable: elliptical, elongate, globular, obovate, or oval. Apterous. Cuticle smooth or with minute granulation, tiny points, conical tubercles, or round tubercles. Cuticle with or without dusky markings. Setae on body acuminate or capitate. Eye a triommatidion. Antennae with three segments; annulated, scaly, or smooth. Third antennal segment longest and with a thumb-like sensory organ, 2–4 sensory pits, and two or more setae. Digitules present on tarsi.

Alate. Body shape of alate variable: slender or stout. Cuticle smooth or with minute granulation, tiny points, or conical tubercles. Setae on body acuminate. Compound eyes with a triommatidion and medial and lateral ocelli present. Antennae with 3–4 segments; annulated, scaly, or smooth. Third antennal segment longest and with or without a basal sensorium, an upper sensorium always present, 3–5 sensory pits, and two or more setae. Digitules present on

tarsi. Forewings and hindwings flat against dorsum when not spread. Forewings with a pterostigma and the following veins: subcosta, radius, media, cubitus, and anal.

***Phylloxera auburnensis* Hamilton, sp. n.**

Figures 1–3

Material examined. Holotype: adult female fundatrix, slide-mounted. Five galls collected on *Carya pallida* leaves at Donald E. Davis Arboretum (32.59529042, -85.48221744), Auburn University, Lee County, AL, USA, F.B. Hamilton, 13.IV.16, Acc. 207. Holotype deposited at NMNH.

Paratypes: 5 adult female alates. Same data as holotype. Deposited at AUMNH, BMNH, and NMNH.

Description. Fundatrix. Slide-mounted adult female 0.39 mm long and 0.22 mm wide (Figure 1). Body outline oval. Cuticle with minute sharp points apparent around margins and head. *Antenna*: Length ~ 89 µm; segment I wider than long, with one long seta on anterior margin and one short submedial seta; segment II slightly longer than wide, with one long seta on anterior margin and one short medial seta; segment III longest with coarsely annulated surface, one thumb-like sensory organ, three sensory pits, one seta at apex and another seta proximal to it. *Mouthparts*: Two setae on anterior end of clypeus; rostrum extends to metacoxa, labial segments I and II without setae, segment II longest, segment III with one pair of marginal setae, segment IV with one pair of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter of ~ 20 µm. *Legs*: Coxa with 1–5 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, 0–1 seta on anterior margin, and 0–1 seta on apical margin; tibia with 1–2 setae on

posterior margin, 0–1 seta on anterior margin, and 1–4 setae on apical margin; tarsal segments I and II fused with 4–9 digitules (setae that appear broadly dilated or expanded at tips) and claws reduced. Vulva not apparent due to distortion of cuticle.

Setae of similar size distributed across dorsum and venter with average length ~ 13 µm. *Dorsal setae*: Head with four marginal setae; prothorax with one pleural seta and two marginal setae; mesothorax with one medial seta, one pleural seta, and one marginal seta; metathorax with one pleural seta and two marginal setae; abdominal segment I with one pleural seta and two marginal setae. Distribution of setae undetermined for rest of abdominal segments due to distortion of cuticle of preserved specimens. *Ventral setae*: Head with one marginal seta, one seta anterior to clypeus, and one seta near eye; one seta at level of coxa on prothorax, mesothorax, and metathorax. Distribution of setae undetermined for abdominal segments due to distortion of cuticle of preserved specimens.

Description. Alate. Slide-mounted adult female 0.43–0.53 mm long and 0.20–0.29 mm wide (Figure 2). *Wings*: Forewing ~ 0.8 mm long and ~ 0.3 mm wide; hindwing ~ 0.5 mm long and ~ 0.2 mm wide. *Antenna*: Length of ~ 119 µm; segment I wider than long with one short seta and one long seta along posterior margin; segment II about as long as wide, scaly, and with two long setae near posterior margin; segment III longest, annulate on basal one-third, rest of segment scaly with large sensorium along posterior margin ~ one-half to three-fourths length of segment, four setae near apex. *Mouthparts:* Setae absent on clypeus; rostrum extends to above mesocoxa, labial segment I without setae, segment II with two pairs of marginal setae, segment III with one pair of marginal setae, segment IV longest with two pairs of marginal setae, and segment V shortest with one pair of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 20 µm. *Legs*: Coxa with 0–2 setae; trochanter and femur fused, one pair of

campaniform sensilla and one long seta on posterior margin, 0–1 seta on anterior margin, and one seta on apical margin; tibia with 0–2 setae on posterior margin, 1–4 medial setae, 0–1 seta on anterior margin, and 2–4 setae on apical margin; tarsal segments I and II fused with 6–7 digitules (toe-like setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and well-developed claws. Vulva indicated by striations in derm.

Setae of similar size distributed across dorsum and venter with average length ~ 6 µm. *Dorsal setae*: Head with one medial seta; prothorax with one medial seta and one pleural seta; mesothorax with one medial seta and five pleural setae; metathorax with one medial seta; abdominal segments with varying number of setae ranging from 1–5 per segment. *Ventral setae*: Head with two marginal setae; prothorax with one pleural seta; one seta at level of coxa on mesothorax and metathorax; abdominal segments I–III fused, abdomen with varying number of setae ranging from 1–3 per segment, and terminal abdominal segment with three long setae with average length ~ 27 µm.

Galls. Green, glabrous, and raised to a point on adaxial leaf surface; on abaxial leaf surface more convex, light green, pubescent, and globular; distributed along midvein of leaf (Figure 3). On adaxial leaf surface: horizontal diameter ~ 3.6 mm (2.5–4.5 mm), vertical diameter ~ 3.4 mm (2.5–4.5 mm), and height ~ 0.6 mm; on abaxial leaf surface: horizontal diameter ~ 4.3 mm (3–5.5 mm), vertical diameter ~ 3.9 mm (3–5 mm), and height ~ 2.6 mm. Opening round and surrounded by 5–6 short pubescent bracts that extend out from gall surface when open.

Remarks. *Carya pallida* (Ashe) Engl. & Graebn. (sand hickory) represents a novel host for *Phylloxera* spp. The gall of this species most resembles that of *Phylloxera caryaeavellana* Riley, 1880, a species known to occur on *Carya tomentosa* near Washington, D.C (Pergande

1904). Galls of the two species are green and round on adaxial leaf surface; on abaxial leaf surface both species are light green, globular, and pubescent. The gall of this species differs from that of *P. caryaeavellana* by the following character (with the corresponding *P. caryaeavellana* state in parentheses): gall on adaxial leaf surface raised to a point (vs. not raised to a point). The fundatrix of this species differs from *P. caryaeavellana* by the following characters (with corresponding *P. caryaevellana* states in parentheses): two dark, median puncture marks near eyes absent (vs. present), dark depression on each side of head absent (vs. present), and antennal segment III with two setae near apex (vs. three setae near apex) (Pergande 1904). This species differs from *P. caryaeavellana* by the following characters of the alate (with corresponding *P. caryaeavellana* states in parentheses): antennal segment III annulate at basal one-third with remainder of segment scaly (vs. annulate for entire length) and antennal segment III with four setae near apex (vs. three setae near apex) (Pergande 1904). The alate has similar antennae to *Phylloxera pilosula* Pergande with antennal segment III lacking a lower sensorium, upper sensorium about two-thirds the length of the joint, and being a similar diameter thoughout. Alates of *P. auburnensis* differ from *P. pilosula* by being much shorter. The fundatrix of *P. auburnensis* differs from *P. pilosula* by the presence of minute granulation on the cuticle, absence of two puncture marks between the eyes, and absence of six rows of dusky tubercles on dorsum.

Etymology. Specific epithet (*auburnensis*) refers to the location (Auburn University, Auburn, AL) where the galls were collected.

Phylloxera bispinae Hamilton, sp. n.

Figures 4–6

Material examined. Holotype: adult female fundatrix, slide-mounted. One gall collected on *Carya glabra* leaves at Bankhead National Forest (34.17686637, -87.27817928), AL, USA, F.B. Hamilton, 19.IV.16, Acc. 028. Holotype deposited at NMNH.

Paratypes: 4 adult female fundatrices, 1 adult alate, and 6 nymphs. Fifteen galls collected on *Carya glabra* leaves at Bankhead National Forest, AL, USA, F.B. Hamilton, 19.IV.16, Accs. 009, 023, and 037. Paratypes deposited at AUMNH, BMNH, and NMNH.

Description. Fundatrix. Slide-mounted adult female 0.38–0.57 mm long and 0.29–0.39 mm wide (Figure 4). Body outline globular to oval. Cuticle with some conical granulations and more prominent on head. *Antenna*: Length ~ 87 µm; segment I wider than long with one long seta on anterior margin and one short seta near base; segment II longer than wide with one long seta on anterior margin, one short seta near middle, and one long seta on posterior margin; segment III longest with coarsely annulated surface, one sensory organ apparent in some specimens near middle of posterior margin, one thumb-like sensory organ, three sensory pits, and one long seta at apex with four setae proximal to it. *Mouthparts*: One seta on anterior end of clypeus; rostrum extends to metacoxa, labial segment I without setae, segment II with three pairs of marginal setae, segment III with one pair of medial setae, segment IV with one pair of medial setae and one pair of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter of ~ 31 µm. *Legs*: Coxa with 4–7 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, 1–3 setae on anterior margin, and 1–3 setae on apical margin; tibia with 1–2 setae on posterior margin, 0–1 seta on anterior margin, and 4–5 setae on apical margin; tarsal segments I and II fused with 6–8 digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and well-developed claws. Vulva indicated by striations in derm.

Setae distributed across dorsum with average length ~ 11 µm. *Dorsal setae*: Head with two medial setae, two pleural setae, and two marginal setae; prothorax with one medial seta, two pleural setae, and one marginal seta; mesothorax with one medial seta, four pleural setae, and two marginal setae; metathorax with one medial seta, four pleural setae, and one marginal seta; abdominal segments with varying number of setae ranging from 2–5 per segment. Two long setae present on terminal abdominal segment ~ 16 µm. *Ventral setae*: Setae with average length of ~ 8 µm; head with one marginal seta and one seta anterior to clypeus; one seta at level of coxa on prothorax, mesothorax, and metathorax along with two pleural setae on prothorax; abdominal segments I–III fused, varying number of setae on abdominal segments ranging from 1–5 per segment. Terminal abdominal segment with two long setae ~ 19 µm and one short seta at opening.

Description. Alate. Slide-mounted adult female 0.52 mm long and 0.24 mm wide (Figure 5). *Wings*: Forewings and hindwings broken off. *Antenna*: Length of ~ 123 µm; segment I wider than long, scaly, and with one long seta along anterior margin; segment II longer than wide, scaly, and with two long setae along anterior margin and one sensory pit near posterior margin; segment III long and stout, about equal diameter throughout, lower sensorial membrane absent or indistinct, annulate from base to upper sensorial membrane, scaly from upper sensorial membrane to apex, upper sensorial membrane ~ three-fourths length of segment, distinct nipple at apex with one long seta and four shorter setae. *Mouthparts*: Rostrum extends to mesocoxa, labial segment I with one pair of medial setae, segment II with one pair of marginal setae, segment III with one pair of marginal setae, segment IV longest with two pairs of marginal setae, and segment V shortest with one pair of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 24 µm. *Legs*: Coxa with 1–3 setae; trochanter and femur fused, one pair of

campaniform sensilla and one long seta on posterior margin, 0–1 seta on anterior margin, and 1–2 setae on apical margin; tibia with one seta on posterior margin, 0–1 seta near middle, setae absent on anterior margin, and 5–6 setae on apical margin; tarsal segments I and II fused with 5–7 digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and well-developed claws. Vulva indicated by striations in derm.

Setae of similar size distributed across dorsum and venter with average length ~ 5 µm. *Dorsal setae*: Head with one medial seta, one pleural seta, and two marginal setae; prothorax with one medial seta, one pleural seta, and one marginal seta; mesothorax with one medial seta and two pleural setae; metathorax with one medial seta and two pleural setae; abdominal segments with varying number of setae ranging from 3–5, abdominal segment VIII with two long setae (~23 µm) and one short seta. *Ventral setae*: Head with one seta anterior to clypeus; one seta at level of coxa on prothorax and one medial seta; one seta above coxa on mesothorax and one pleural seta; one seta at level of coxa on metathorax. Abdominal segments I–III fused; segments with varying number of setae ranging from 1–6.

Galls. Elongate, slender, conical projections on both sides of leaf; green, crimson, or green and crimson; distributed along midvein of leaf (Figure 6). On adaxial leaf surface: horizontal diameter ~ 3.5 mm (1–7 mm), vertical diameter ~ 3.4 mm (1–6 mm), and height ~ 4 mm; on abaxial leaf surface: horizontal diameter ~ 3.5 mm (1–7 mm), vertical diameter ~ 3.8 mm (1–8 mm), and height ~ 3.2 mm. Opening surrounded by slender bracts on both surfaces.

Remarks. The gall of this species most resembles that of *Phylloxera caryaesepta subspecies perforans* Pergande, 1904, a species known to occur on *Carya glabra* in New York and Virginia (Pergande 1904). Galls of the two species are elongate and conical on both sides of the leaf with each having a thorn-like opening (Pergande 1904). The fundatrices of both species

have an irregular distribution of minute conical tubercles with more being found on the head and two dusky puncture marks between the eyes (Pergande 1904). Additionally, the alates of both species have stout bodies and long, slender legs (Pergande 1904). This species differs from *P. caryaesepta subspecies perforans* by the following gall characters (with corresponding *P. caryaesepta subspecies perforans* states in parentheses): vertical diameter of gall from 6.5–11 mm (vs. 4–6 mm) and opening on adaxial leaf surface surrounded by 11–12 slender pubescent bracts (vs. ~ eight pubescent bracts) (Pergande 1904). This species differs from *P. caryaesepta subspecies perforans* by the following characters of the fundatrix (with corresponding *P. caryaesepta subspecies perforans* states in parentheses): diameter across thorax ~ 0.4 mm (vs. ~ 0.7 mm), outline of body oval (vs. pyriform or globular), antennal segment I wider than long (vs. longer than wide), antennal segment II lacking 3–4 scaly annulations (vs. with 3–4 scaly annulations), and an intermediate sensorium present (vs. absent) near middle of posterior margin on antennal segment III (Pergande 1904). This species differs from *P. caryaesepta subspecies perforans* by the following characters of the alate (with corresponding *P. caryaesepta subspecies perforans* states in parentheses): antennal segment III stout (vs. long and slender), antennal segment III of almost uniform diameter throughout (vs. varying in diameter throughout), a basal sensorial membrane absent (vs. present) on antennal segment III, upper sensorial membrane about three-fourths the length of antennal segment III (vs. two-fifths the length of antennal segment III), antennal segment III scaly (vs. annulated) by upper sensorial membrane, and apex of antennal segment III with five setae (vs. with two setae) (Pergande 1904).

Etymology. Specific epithet (*bispinae*) from the Latin *bi* meaning two and *spinae* meaning spines; in relation to the gall resembling two thorn-like structures on a leaf.

Phylloxera chippokesiensis Hamilton, sp. n.

Figures 7–9

Material examined. Holotype: adult female fundatrix, slide-mounted. Five galls collected on *Carya cordiformis* leaves at Chippokes Plantation State Park (37.14611399, -76.73876058), VA, USA, F.B. Hamilton, 17.V.18, Acc. tnh. Holotype deposited at NMNH.
Paratypes: 8 adult female sexuparae. Same data as holotype and four additional galls collected, Acc. qz7. Paratypes deposited at AUMNH, BMNH, and NMNH.

Description. Fundatrix. Slide-mounted adult female 0.21 mm long and 0.11 mm wide (Figure 7). Body outline oval. Cuticle almost smooth with minute granulation. Dorsum with cluster of dusky markings present on head and two clusters of dusky markings present on prothorax. *Antenna*: Length ~ 61 µm; segment I wider than long with one long seta on anterior margin, one short seta near base, and rounded at apex; segment II wider than long with one long seta on anterior margin, one long seta near middle, and rounded at apex; segment III longest with annulated surface, widest near middle of segment and tapering sharply towards apex, one thumb-like sensory organ and three circular sensory pits near apex with one long seta proximal to them, and one very long seta (~ 17 µm) at apex with two shorter setae. *Mouthparts*: One seta present on clypeus; rostrum extends to metacoxa, labial segment II with two pairs of marginal setae, segment III with one pair of marginal setae, segment IV with two pairs of marginal setae, segment V shortest with two pairs of setae at apex and inner pair longer than usual ~ 5 µm. *Spiracles*: Anterior spiracles with diameter of ~ 14 µm and posterior spiracles with diameter of ~ 10 µm. *Legs*: Coxa with 2–4 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, one seta on anterior margin, and 1–3 setae on apical margin; tibia with 0–1 seta on posterior margin, 0–1 seta on anterior margin, and 5–6 setae on

apical margin with some being very long ~ 17 µm; tarsal segments I and II fused with 7–9 digitules (setae that appear broadly dilated or expanded at tips), 0–1 short seta on anterior margin, and well-developed claws.

Setae of similar size distributed across dorsum and venter with average length ~ 4 µm. *Dorsal setae*: Capitate setae (slightly thickened and expanded at tips) extend from head to abdominal segment IV; head with one pleural seta and three marginal setae; prothorax with two medial setae, one pleural seta, and two marginal setae; mesothorax with one medial seta, one pleural seta, and two marginal setae; metathorax with one medial seta, one pleural seta, and two marginal setae; abdominal segments I–VII with two setae per segment, setae absent on abdominal segment VIII and terminal abdominal segment. *Ventral setae*: One marginal seta and one medial seta present on head; seta absent at level of coxa on prothorax, mesothorax, and metathorax; abdominal segments I–III fused; abdomen with 1–2 setae per segment.

Description. Sexupara. Slide-mounted adult female 0.43–0.72 mm long and 0.31–0.48 mm wide (Figure 8). Apterous and body outline obovate. Cuticle densely covered in minute granulation. Dorsum with two clusters of dusky markings on head and one cluster of dusky markings on prothorax. Eye a triommatidion. *Antenna*: Length ~ 70 µm; segment I slightly wider than long with one long seta on anterior margin and one short seta near middle, and several striations present on surface; segment II about as long as wide with one long seta on anterior margin, one long seta near middle, and almost straight at apex; segment III longest with some scalloped annulations on surface, almost of equal length or slightly shorter than first two antennal segments combined, widest near middle of segment and narrowest at apex, one thumb-like sensory organ with one short seta proximal to it and two long setae at apex. *Mouthparts*: Seta absent on clypeus; rostrum extends to mesocoxa, labial segment II with one pair of marginal

setae, segment III with one pair of medial setae, segment IV with one pair of medial setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter of ~ 31 µm. *Legs*: Coxa with 3–5 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, 1–2 setae on anterior margin, and 1–3 setae on or near apical margin; tibia with 0–1 seta on posterior margin, 1–2 setae on anterior margin, and 4–6 setae on apical margin; tarsal segments I and II fused with 2–7 digitules (setae that appear broadly dilated or expanded at tips), 0–1 short seta on anterior margin, and well-developed claws. Vulva indicated by striations in derm.

Setae of similar size distributed across dorsum and venter with average length ~ 7 µm. *Dorsal setae*: Head with one medial seta, two pleural setae, and one marginal seta; prothorax with one medial seta, two pleural setae, and one marginal seta; mesothorax with one medial seta, one pleural seta, and one marginal seta; metathorax with one medial seta, one pleural seta, and two marginal setae; abdominal segments with varying number of setae ranging from 1–4 setae per segment. *Ventral setae*: One marginal seta on head; one seta at level of coxa on prothorax, mesothorax, and metathorax; abdominal segments I–III fused; abdominal segments with varying number of setae ranging from 1–3 setae per segment.

Galls. Round and convex with depression or central elevation near middle, shiny and slightly pubescent, and green or yellowish-green on adaxial leaf surface. On abaxial leaf surface gall thorny and more convex, light green, transparent especially near base, and slightly pubescent (Figure 9). On adaxial leaf surface: horizontal diameter ~ 2.0 mm (1.4–2.4 mm), vertical diameter ~ 2.0 mm (1.5–2.6 mm), and height ~ 0.5 mm (0.2–0.6 mm). On abaxial leaf surface: horizontal diameter ~ 2.4 mm (1.9–3 mm), vertical diameter ~ 2.4 mm (1.8–3 mm), and height ~

2 mm (0.5–4 mm). Opening thorn-like on abaxial leaf surface and splits into 4–5 slender long bracts.

Remarks. The gall of this species most resembles that of *Phylloxera caryaesemen*, (Shimer 1869) a species known to occur on *Carya glabra* in Illinois and Missouri as well as *C. cordiformis* in Illinois. Galls of the two species are small and have a depression in center on adaxial leaf surface (Pergande 1904). The fundatrices of both species are small and oval. This species differs from *P. caryaesemen* by the following gall characters (with corresponding *P. caryaesemen* states in parentheses): adaxial leaf surface yellowish-green (vs. red or yellow), slightly pubescent on both leaf surfaces (vs. pubescent only on abaxial leaf surface), more convex on abaxial leaf surface (vs. on adaxial leaf surface), thorn-like (vs. almost flat) on abaxial leaf surface, long and slender bracts (vs. short and stout bracts) surrounding opening, and opening green (vs. white) (Pergande 1904). This species differs from *P. caryaesemen* by the following characters of the fundatrix (with corresponding *P. caryaesemen* states in parentheses): setae capitate (vs. acuminate) on dorsum, antennal segment III with sharp annulations (vs. several coarse annulations), antennal segment III with an extremely long seta ~ 16.8 µm (vs. with a long seta ~ 6.3 µm) at apex, claws long and slender ~ 12 µm (vs. short ~ 5.8 µm), and digitules extremely long ~ 21 µm (vs. short ~ 9.2 µm).

Etymology. Specific epithet (*chippokesiensis*) named after the site where the specimens were collected: Chippokes Plantation State Park in Surry County, VA.

***Phylloxera crypta* Hamilton, sp. n.**

Figures 10-11

Material examined. Holotype: adult female fundatrix, slide-mounted. Two galls collected on *Carya aquatica* leaves at Francis Marion National Forest (33.1125611, -79.79275649), Berkeley County, SC, USA, F.B. Hamilton, 27.IV.16, Acc. 110. Holotype deposited at NMNH. Paratype: 1 adult female fundatrix. Same data as holotype. Deposited at NMNH.

Description. Fundatrix. Slide-mounted adult female 0.47–0.57 mm long and 0.38–0.43 mm wide (Figure 10). Body outline elliptical. Cuticle covered in minute granulations. Dusky circular marks present on dorsum near midline of body on prothorax, mesothorax, metathorax, and abdominal segment I. *Antenna*: Length ~ 123 µm; segment I about as long as wide with one long seta on anterior margin and one short seta on posterior margin; segment II longer than wide with one long seta on anterior margin, one short seta on posterior margin, and one circular sensory pit on apical margin; segment III longest with distinct petiole at base, two shallow annulations present on petiole with more distinct annulations on remainder of segment, one thumb-like sensory organ on posterior margin with three small, circular sensory pits anterior to it, and one long seta at apex with three long setae proximal to it. *Mouthparts*: One seta present on anterior end of clypeus; rostrum extends to mesocoxa, labial segment I without setae, segment II with one medial seta and two pairs of marginal setae, segment III with one pair of marginal setae, segment IV longest with two pairs of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 28 µm. *Legs*: Coxa with 3–5 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, 1–2 setae on anterior margin, and 1–3 setae on apical margin; tibia with 1–2 setae on posterior margin, one seta on anterior margin, and five setae on apical margin; tarsal segments I and II fused with 7–8 digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and claws reduced. Vulva indicated by striations in derm.

Setae of varying lengths distributed across dorsum and venter with average length ~ 9 µm. *Dorsal setae*: Head with two medial setae, two pleural setae, and one marginal seta; prothorax with two medial setae, two pleural setae, and one marginal seta; mesothorax with one medial seta and two pleural setae; metathorax with one medial seta, six pleural setae, and one marginal seta; abdominal segments with a varying number of setae ranging from 2–6 per segment; terminal abdominal segment with two long setae each ~ 19 µm, one medium seta ~ 7.5 µm, and one short seta ~ 6 µm. *Ventral setae*: Head with one marginal seta; one seta at level of coxa on prothorax with two pleural setae; one seta at level of coxa on mesothorax with one medial seta, five pleural setae, and four marginal setae; one seta at level of coxa on metathorax with one medial seta and one marginal seta; abdominal segments I–III fused, abdomen with varying number of setae per segment ranging from 1–6, terminal abdominal segment with two long setae ~15 µm and two short setae ~ 6 µm.

Galls. Round, slightly convex, pubescent, and concolorous to surrounding leaf tissue on both surfaces with slight depression in center on adaxial leaf surface (Figure 11). On adaxial leaf surface: horizontal diameter ~ 4.1 mm (3.5–4.6 mm), vertical diameter ~ 4 mm (3.3–4.6 mm), and height ~ 0.3 mm. On abaxial leaf surface: horizontal diameter ~ 4 mm (3–5 mm), vertical diameter ~ 3.7 mm (3.2–4.2 mm), and height ~ 0.6 mm. Opening a green, stem-like projection on abaxial leaf surface.

Remarks. The gall of this species most resembles that of *Phylloxera deplanata* Pergande, 1904, a species known to occur on *Carya tomentosa* in Washington, D.C (Pergande 1904). Galls of the two species are slightly convex on adaxial leaf surface with a shallow depression; on abaxial leaf surface gall is green, convex, and with an opening (Pergande 1904). This species differs from *P. deplanata* by the following gall characters (with corresponding *P. deplanata*

states in parentheses): greenish-yellow (vs. red with a yellow depression or greenish-yellow) on adaxial leaf surface, depression in center (vs. depression towards one side) on adaxial leaf surface, and opening is long and stem-like with short pubescence (vs. a short nipple with short pubescence) (Pergande 1904). This species differs from *P. deplanata* by the following characters of the fundatrix (with corresponding *P. deplanata* states in parentheses): cuticle with minute granulation (vs. with small conical tubercles); conical protuberance absent (vs. present) in front of eyes; dusky circular marks present (vs. absent) on dorsum near midline of prothorax, mesothorax, metathorax, and abdominal segment I; antennal segment III with a distinct petiole (vs. absent); and four setae (vs. two setae) at apex of antennae (Pergande 1904).

Phylloxera crypta is sister to *Phylloxera castaneae* (Haldeman) on the phylogeny and differs from it by the following characters (with corresponding *P. castaneae* states in parentheses): gall forming species (vs. free-living species), stout tubercles absent (vs. four stout tubercles present) on dorsum near anterior end of body, dusky markings present (vs. absent) on dorsum, and four setae present (vs. two setae present) near apex of antennae (Pergande 1904).

Etymology. Specific epithet (*crypta*) refers to the inconspicuous appearance of the galls.

***Phylloxera echinus* Hamilton, sp. n.**

Figures 12–13

Material examined. Holotype: adult female fundatrix, slide-mounted. Four galls collected on *Carya aquatica* leaves at Francis Marion National Forest (33.11278907, -79.79273561), Berkeley County, SC, USA, F.B. Hamilton, 27.IV.16, Acc. 112. Holotype deposited at NMNH.

Description. Fundatrix. Slide-mounted adult female 0.41 mm long and 0.23 mm wide (Figure 12). Body outline elliptical. Cuticle with minute granulations across body. *Antenna*:

Length ~ 68 µm; segment I wider than long with one short seta near base; segment II longer than wide with one short seta near anterior margin and one long seta near posterior margin; segment III longest with annulated surface, one prominent thumb-like sensory organ on posterior margin with three circular sensory pits anterior to it, and one long seta and one short seta at apex with one seta proximal to them. *Mouthparts*: Two setae on posterior end of clypeus; rostrum extends to mesocoxa, labial segment II with two pairs of marginal setae, segment III with one pair of marginal setae, segment IV longest with two pairs of marginal setae, and segment V shortest with one pair of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 23 µm. *Legs*: Coxa with 2–5 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, one seta on anterior margin, and 0–2 setae on apical margin; tibia with 0–1 seta on anterior margin and six setae on apical margin; tarsal segments I and II fused with 7–9 digitules (setae that appear broadly dilated or expanded at tips) and one short seta on anterior margin. Vulva indicated by striations in derm.

Setae stout and of varying lengths distributed across dorsum and venter with average length of ~ 16 µm. *Dorsal setae*: Many setae distributed across dorsum and decreasing in number towards apex; last four abdominal segments with 2–4 setae per segment. *Ventral setae*: One seta at level of coxa on prothorax with three pleural setae; one seta at level of coxa on mesothorax and metathorax; abdominal segments I–III fused; a varying number of setae distributed across abdomen ranging from 2–5 per segment.

Galls. Globular, pubescent, pink, and more convex on adaxial leaf surface and slightly convex, pubescent, and green on abaxial leaf surface (Figure 13). On adaxial leaf surface: horizontal diameter ~ 1.5 mm (1.3–1.8 mm), vertical diameter ~ 1.5 mm (1.3–1.8 mm), and height ~ 0.5 mm. On abaxial leaf surface: horizontal diameter ~ 1.5 mm (1.2–1.7 mm), vertical

diameter ~ 1.5 mm (1.3–1.6 mm), and height ~ 0.1 mm. Opening round on abaxial leaf surface and surrounded by short white pubescence.

Remarks. The gall of this species most resembles that of *Phylloxera pilosula* Pergande, 1904, a species known to occur on *Carya glabra* near Washington, D.C (Pergande 1904). Galls of the two species are densely covered with long, yellowish-white hairs on both adaxial and abaxial leaf surfaces, and the opening is surrounded by whitish pubescence on the abaxial leaf surface (Pergande 1904). This species differs from *P. pilosula* by the following gall characters (with corresponding *P. pilosula* states in parentheses): globular and raised (vs. flat or slightly raised with a central depression) on adaxial leaf surface, pink (vs. pale green) on adaxial leaf surface, and flat with opening surrounded by only pubescence (vs. convex with a distinct nipple opening) on abaxial leaf surface (Pergande 1904). This species differs from *P. pilosula* by the following characters of the fundatrix (with corresponding *P. pilosula* states in parentheses): cuticle with minute granulation (vs. lacking granulation), two blackish median puncture marks absent (vs. present) on head, six rows of dusky tubercles absent (vs. present) on dorsum, and antennal segment III annulate (vs. scaly) (Pergande 1904).

Etymology. Specific epithet from the Latin *echinus* for the resemblance of its gall to a sea urchin; treated as a noun in apposition.

***Phylloxera falsostium* Hamilton, sp. n.**

Figures 14–15

Material examined. Holotype: adult female fundatrix, slide-mounted. Five galls collected on *Carya cordiformis* leaves at Natural Falls State Park (36.17396029, -94.6680864), Delaware County, OK, USA, F.B. Hamilton, 13.V.17, Acc. m0n. Holotype deposited at NMNH.

Paratypes: 6 adult female fundatrices and 1 alate nymph. Same data as holotype and Acc. m0o. In addition, 4 fundatrices from six galls collected on *Carya cordiformis* leaves, Chippokes Plantation State Park (37.14601448, -76.73859791), Surry, VA, USA, F.B. Hamilton, 17.V.18, Acc. tzq. Deposited at AUMNH, BMNH, and NMNH.

Description. Fundatrix. Slide-mounted adult female 0.26–0.51 mm long and 0.13–0.25 mm wide (Figure 14). Body outline elongate to pyriform. Cuticle covered in minute granulations. One dusky puncture mark present on each side of dorsum of head; elongate dusky markings present on prothorax; two to three groups of dusky markings present on mesothorax through abdominal segment I. *Antenna*: Length ~ 62 µm; segment I wider than long with one long seta along anterior margin and one short seta near middle; segment II longer than wide, narrowest at base and edges rounded at apex, one long seta on anterior margin and one long seta on posterior margin with one small sensory pit near base on posterior margin; segment III longest with annulated surface, one distinct thumb-like sensory organ and three circular sensory pits near apex, one long seta at apex along with two shorter setae, and one short seta near middle of segment. *Mouthparts*: Rostrum extends past mesocoxa and to level of metacoxa in some specimens, labial segment II with three pairs of marginal setae, segment III with one pair of marginal setae, segment IV with two pairs of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 32 µm. *Legs*: Coxa with 2–5 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, one seta on anterior margin, and 1–3 setae on apical margin; tibia with 0–1 seta on posterior margin, 0–1 seta on anterior margin, and 5–6 setae near apical margin; tarsal segments I and II fused with 7–10 digitules (setae that appear broadly dilated or expanded at tips) and one short seta on anterior margin. Vulva indicated by striations in derm.

Setae of varying lengths distributed across dorsum and venter. *Dorsal setae*: Setae with average length ~ 14 µm and marginal setae slightly longer with average length ~ 18 µm; head with one medial seta, two pleural setae, and four marginal setae; prothorax with two medial setae and three pleural setae with pleural setae overlapping one another; mesothorax with two medial setae and four pleural setae, and couple of pleural setae overlapping one another; metathorax with two medial setae, one pleural seta, and one marginal seta; abdominal segments with varying number of setae ranging from 0–5 per segment. *Ventral setae*: Setae with average length of 6 µm; head with one marginal seta; two seta at level of coxa on prothorax; one seta at level of coxa on mesothorax and metathorax; abdominal segments I–III fused; varying number of setae distributed across abdomen ranging from 1–5 per segment; terminal abdominal segment with one short seta at opening ~ 4 µm.

Galls. Round and convex, yellowish-green and somewhat transparent, with or without slit-like opening in middle, and rim formed around circumference on adaxial leaf surface; more raised above than below; round, pubescent, light green and much lighter compared to adaxial leaf surface, and sunken below surface with rim formed around circumference on abaxial leaf surface (Figure 15). On adaxial leaf surface: horizontal diameter ~ 3.2 mm (2.3–4.5 mm), vertical diameter ~ 3.1 mm (1.6–4 mm), and height ~ 1.2 mm; on abaxial leaf surface: horizontal diameter ~ 3.1 mm (2.1–4.1 mm), vertical diameter ~ 3.2 mm (1.8–5.1 mm), and height ~ 0.62 mm. False opening present on adaxial surface of some galls that appears slit-like and opens into a false chamber. Opening on abaxial leaf surface round and surrounded by 5–6 small bracts which open to expose white pubescence.

Remarks. The gall of this species most resembles that of *Phylloxera rimosalis* Pergande, 1904, a species known to occur on *Carya tomentosa* in Washington, D.C. (Pergande 1904). Galls

of the two species are slightly raised and round on both surfaces of the leaf, gall on adaxial leaf surface is green and somewhat transparent with a distinct rim around it, and gall on abaxial leaf surface with a round opening covered in white pubescence (Pergande 1904). Additionally, the fundatrices of both species have two dusky puncture marks on head, dusky markings on prothorax, antennal segment I globular, antennal segment II longer than wide, and antennal segment III annulate (Pergande 1904). This species differs from *P. rimosalis* by the following gall characters (with corresponding *P. rimosalis* states in parentheses): somewhat transparent near center (vs. somewhat transparent around margins) on adaxial leaf surface, a slit-like opening present (vs. absent) on adaxial leaf surface, a false chamber present (vs. absent) inside gall, more raised on adaxial leaf surface (vs. on abaxial leaf surface), and opening level with surface (vs. slightly protruding) (Pergande 1904). This species differs from *P. rimosalis* by the following characters of the fundatrix (with corresponding *P. rimosalis* states in parentheses): dusky markings present on mesothorax, metathorax, and abdominal segment I (vs. absent on mesothorax and metathorax, but present on first three abdominal sutures), setae stout (vs. slender) on dorsum, pleural setae overlap one another (vs. do not overlap one another) on prothorax and mesothorax, and antennae ~ 62 µm long (vs. ~ 179 µm long) (Pergande 1904).

Etymology. Specific epithet (*falsostium*) is from the Latin *falsus* meaning false and *ostium* meaning door in reference to the false opening on the adaxial leaf surface.

***Phylloxera flavoconica* Hamilton, sp. n.**

Figures 16–18

Material examined. Holotype: adult female fundatrix, slide-mounted. Eleven galls collected on *Carya ovata* at Warriors' Path State Park (36.48793807, -82.48459229), Sullivan County, TN, USA, F.B. Hamilton, 30.IV.16, Acc. 154. Holotype deposited at NMNH.
Paratypes: 8 female fundatrices, slide-mounted. Same data as holotype including Acc. 155. In addition, 9 female fundatrices and 1 adult female sexupara, slide-mounted and collected from five galls on *Carya tomentosa* leaves at Dauset Trails Nature Center (33.23612682, -83.95234807), Butts County, GA, USA, F.B. Hamilton, 24.IV.16, Acc. 087. Paratypes deposited at AUMNH, BMNH, and NMNH.

Description. Fundatrix. Slide-mounted adult female 0.22–0.53 mm long and 0.12–0.35 mm wide (Figure 16). Body outline obovate or elongate. Cuticle with conical granules across surface. One prominent linear depression on each side of dorsum extending from anterior margin of head to prothorax. Clusters of dusky circular markings distributed across dorsum from head to about abdominal segment III. *Antenna*: Length ~ 87 µm; segment I about as long as wide with two long setae on anterior margin, one short seta near base, and one circular sensory pit on posterior margin; segment II longer than wide with one long seta on anterior margin, one small sensory pit near apex, and one long seta on posterior margin; segment III longest with annulated surface, one thumb-like sensory organ on posterior margin with three small, circular sensory pits anterior to it, one stout seta and four shorter setae at apex, and one short seta proximal to them. *Mouthparts*: One long seta present on anterior end of clypeus; rostrum extends to metacoxa, labial segment II longest with three pairs of medial setae, segment III with one pair of medial setae, segment IV with two pairs of medial setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 20 µm. *Legs*: Coxa with 4–5 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on

posterior margin, two setae on anterior margin, and one seta on apical margin; tibia with 0–1 seta on posterior margin, one seta on anterior margin, and 5–7 setae on apical margin; tarsal segments I and II fused with seven digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and well-developed claws. Vulva indicated by striations in derm.

Setae of varying lengths distributed across dorsum and venter with average length ~ 8 µm. Setae along dorsal margins longer with average length ~ 11 µm. *Dorsal setae*: Head with one medial seta, one pleural seta, and two marginal setae; prothorax with one marginal seta; mesothorax with one medial seta and two marginal setae; metathorax with one medial seta, one pleural seta, and two marginal setae; abdominal segments with varying number of setae ranging from 1–3, abdominal segment VIII and terminal abdominal segment each with one long seta ~ 25 µm. *Ventral setae*: Head with one marginal seta; one seta at level of coxa on prothorax and one pleural seta; one seta at level of coxa on mesothorax; one seta at level of coxa on metathorax; abdominal segments I–III fused; varying number of setae per abdominal segment ranging from 1–4.

Description. Sexupara. Slide-mounted adult female 0.63 mm long and 0.27 mm wide (Figure 17). Apterous and body outline obovate. Cuticle mostly smooth except for conical granulations distributed on head. Eye a triommatidion with small ocular facets surrounding it. *Antenna*: Length ~ 111 µm; segment I wider than long with one short seta and one long seta near posterior margin; segment II about as long as wide with one short seta and one small sensory pit near anterior margin and one long seta on posterior margin; segment III longest with annulated surface, one slender thumb-like sensory organ on posterior margin with two small circular sensory pits anterior to it, one short seta proximal to thumb-like sensory organ, and one stout seta at apex along with four shorter setae. *Mouthparts*: Seta absent on clypeus; rostrum extends to

mesocoxa, labial segment II longest with one pair of marginal setae, segment III with one pair of marginal setae, segment IV with two pairs of marginal setae, and segment V with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 20 µm. *Legs*: Coxa with 2–5 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, one seta on anterior margin, and 1–3 setae on apical margin; tibia with 1–2 setae on posterior margin, 0–1 seta on anterior margin, and 4–6 setae on apical margin; tarsal segments I and II fused with 4–7 digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and well-developed claws. Vulva indicated by striations in derm.

Setae of varying lengths distributed across dorsum and venter with average length ~ 8 µm. *Dorsal setae*: Head with one pleural seta and two marginal setae; prothorax with two medial setae and two marginal setae; mesothorax with two medial setae and two marginal setae; metathorax with two medial setae, one pleural seta, and one marginal seta; abdominal segments with varying number of setae ranging from 1–4 per segment. *Ventral setae*: Head with one medial seta, one pleural seta, and one marginal seta; one seta at level of coxa on prothorax, mesothorax, and metathorax; abdominal segments I–III fused; varying number of setae across abdomen ranging from 1–4 per segment; terminal abdominal segment with one long seta ~ 22 µm and one short seta.

Galls. Conical and yellow, glabrous, and more prominent on adaxial leaf surface; on abaxial leaf surface slightly convex and light yellowish-green (Figure 18). On adaxial surface of leaf: horizontal diameter ~ 3 mm (1.1–5 mm), vertical diameter ~ 3 mm (1.2–5.5 mm), and height ~ 1.7 mm. On abaxial surface of leaf: horizontal diameter ~ 2.7 mm (0.9–5.5 mm),

vertical diameter ~ 2.8 mm (1.1–5.5 mm), and height ~ 0.5 mm. Opening slit-like on abaxial leaf surface with protruding lips covered in white pubescence.

Remarks. The gall of this species most resembles that of *Phylloxera caryaefallax* Riley, 1874, a species known to occur on *Carya ovata* in Illinois and Missouri as well as states along the Mississippi River (Pergande 1904). Galls of the two species are yellow and somewhat conical on adaxial leaf surface (Pergande 1904). The openings for both species are located on the abaxial leaf surface. Additionally, the cuticle of the fundatrices of both species have conical granules distributed across the surface (Pergande 1904). This species differs from *P. caryaefallax* by the following gall characters (with corresponding *P. caryaefallax* states in parentheses): conical (vs. flattened and round or conical) on adaxial leaf surface and opening a transverse slit (vs. a nipple with a round or oval orifice or slit-like) (Pergande 1904). This species differs from *P. caryaefallax* by the following characters of the fundatrix (with corresponding *P. caryaefallax* states in parentheses): fundatrix obovate (vs. almost globular), dusky circular markings present (vs. absent) on dorsum, capitate setae absent (vs. present) on terminal abdominal segment, antennae ~ 87 µm (vs. ~ 181 µm), antennal segment III with coarse annulations (vs. with scaly annulations), and antennal segment III with ~ 10 annulations (vs. with ~ 20 annulations) (Pergande 1904).

Etymology. Specific epithet (*flavoconica*) refers to their appearance as yellow, conical galls.

***Phylloxera floridana* Hamilton, sp. n.**

Figures 19–20

Material examined. Holotype: adult female fundatrix, slide-mounted. Unrecorded number of galls collected on *Carya floridana* leaves at Archbold Biological Station (27.17098998, -81.34805597), Highlands County, FL, USA, F.B. Hamilton, 3.X.I5, Acc. 211. Holotype deposited at NMNH.

Paratype: 1 immature female fundatrix, slide-mounted. Same data as holotype. Deposited at NMNH.

Description. Fundatrix. Slide-mounted female 0.41–0.67 mm long and 0.28–0.33 mm wide (Figure 19). Body outline globular to oval. Cuticle with conical granulations. *Antenna*: Length ~ 133 µm; segment I about as long as wide with one long seta on anterior margin and one short seta near middle; segment II longer than wide with two long setae near anterior margin and one sensory pit near posterior margin; segment III longest with sharply annulated surface, one thumb-like sensory organ on posterior margin without any apparent sensory pits anterior to it, and one stout, long seta at apex with three shorter setae proximal to it. *Mouthparts*: Seta absent on clypeus; rostrum extends to mesocoxa, labial segment II with three pairs of medial setae, segment III with one pair of medial setae, segment IV longest with two pairs of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 28 µm. *Legs*: Coxa with 4–5 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, one seta on anterior margin, and 1–2 setae on apical margin; tibia with 1–3 setae on posterior margin, one seta on anterior margin, and 5–6 setae near apical margin; tarsal segments I and II fused with 5–7 digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and well-developed claws.

Setae of varying lengths distributed across dorsum and venter with average length ~ 9 µm. *Dorsal setae*: Head with one medial seta, one pleural seta, and one marginal seta; prothorax with two medial setae and one pleural seta; mesothorax with one medial seta and two marginal setae; metathorax with two medial setae and three marginal setae; abdominal segments with varying number of setae ranging from 1–4 per segment, abdominal segment VIII with one long seta ~ 25 µm, and terminal abdominal segment with one short seta ~ 9 µm. *Ventral setae*: Head with one marginal seta anterior to eye; one seta at level of coxa on prothorax and one pleural seta; one seta at level of coxa on mesothorax and metathorax; abdominal segments I–III fused; varying number of setae distributed across abdomen ranging from 2–9 per segment; terminal abdominal segment with one long marginal seta ~ 39 µm long, one medium seta ~ 12 µm long, and two grain-shaped scleroses ~ 16 µm long.

Galls. Round and slightly convex, yellow, and with or without central depression on adaxial leaf surface; somewhat conical and convex on abaxial leaf surface (Figure 20). Measurements taken on dried gall specimens. On adaxial leaf surface: horizontal diameter ~ 3.3 mm (1.1–5.2 mm), vertical diameter ~ 2.8 mm (1.4–4 mm), and height ~ 0.4 mm. On abaxial leaf surface: horizontal diameter ~ 3.2 mm (1.1–5.2 mm), vertical diameter ~ 2.7 mm (1.2–4 mm), and height ~ 0.7 mm (Figure 14). Slit-like opening on abaxial leaf surface fringed with whitish pubescence.

Remarks. This is the first *Phylloxera* sp. described from *C. floridana* Sarg. (scrub hickory). Scrub hickory occurs throughout the central Florida peninsula and is found in sand scrub (Nelson et al. 2014). The gall of this species most resembles that of *Phylloxera caryaefallax* Riley, 1874, a species known to occur on *Carya ovata* in Illinois and Missouri as well as states along the Mississippi River (Pergande 1904). Galls of the two species are yellow

with a slit-like opening on abaxial leaf surface. Additionally, the cuticle of the fundatrices of both species have conical granulations distributed across surface (Pergande 1904). This species differs from *P. caryaefallax* by the following characters of the fundatrix (with corresponding *P. caryaefallax* states in parentheses): fundatrix oval (vs. almost globular), cuticle with some small conical granulations (vs. densely covered in conical granulations), two grain-like scleroses present (vs. absent) at caudal end of venter, capitate setae absent (vs. present) on terminal abdominal segment, antenna with sharp annulations (vs. with scaly annulations), antennae ~ 133 µm (vs. ~181 µm), and antennae with four setae near apex (vs. with two setae near apex) (Pergande 1904).

Etymology. Specific epithet (*floridana*) refers to the host plant species, *Carya floridana*.

***Phylloxera killianae* Hamilton, sp. n.**

Figures 21–22

Material examined. Holotype: female fundatrix, slide-mounted. Four galls collected on *Carya glabra* leaves at Nantahala National Forest (35.16564806, -83.54076799), Macon County, NC, USA, F.B. Hamilton, 29.IV.16, Acc. 141. Holotype deposited at NMNH.

Description. Fundatrix. Slide-mounted female 0.15 mm long and 0.09 mm wide (Figure 21). Body outline obovate. Cuticle smooth. *Antenna*: Length ~ 45 µm; segment I wider than long with one long seta on anterior margin; segment II wider than long with one long seta on anterior margin, one sensory pit at base, and one short seta near middle; segment III longest and slightly longer than first two antennal segments put together, surface scaly, one thumb-like sensory organ near posterior margin with two circular sensory pits anterior to it and one seta proximal to it, and one stout seta at apex with two shorter setae. *Mouthparts*: Rostrum apparently broken off.

Spiracles: Anterior spiracles with diameter of ~ 22 µm and posterior spiracles with diameter of ~ 11 µm. *Legs*: Stout; coxae with 2–3 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, setae absent on anterior margin, one seta near middle, and three setae on apical margin; tibia with 4–6 setae near apical margin; tarsal segments I and II fused with 5–7 digitules (setae that appear broadly dilated or expanded at tips), and 0–1 short seta on anterior margin.

Setae of varying lengths distributed across dorsum and venter with average length ~ 5 µm. *Dorsal setae*: Head with one marginal seta; prothorax with one medial seta and two marginal setae; mesothorax with one medial seta, three pleural setae, and one marginal seta; metathorax with one medial seta and one marginal seta; abdominal segments with varying number of setae ranging from 0–6 per segment. *Ventral setae*: Head with one marginal seta; one seta at level of coxa on prothorax, mesothorax, and metathorax; abdominal segments I–III fused; abdomen with 0–2 setae per segment.

Galls. Round and slightly convex, covered in crimson resin-like droplets which make it appear to sparkle with several long white hairs present on adaxial surface and on abaxial surface of leaf around opening (Figure 22). On adaxial leaf surface: horizontal diameter ~ 1.2 mm (0.9–1.5 mm), vertical diameter ~ 1 mm (0.8–1.1 mm), and height ~ 0.3 mm. On abaxial leaf surface: horizontal diameter ~ 1 mm (0.9–1.1 mm), vertical diameter ~ 0.9 mm, and height ~ 0.2 mm. Opening on abaxial leaf surface circular and surrounded by long white pubescence.

Remarks. The gall of this species most resembles that of *Phylloxera pilosula* Pergande, 1904, a species known to occur on *Carya glabra* near Washington, D.C (Pergande 1904). Galls of the two species have long whitish pubescence on both surfaces with the opening being found on the abaxial leaf surface (Pergande 1904). The fundatrices of both species have a smooth

cuticle, slender last several abdominal segments, and a scaly third antennal segment (Pergande 1904). This species differs from *P. pilosula* by the following gall characters (with corresponding *P. pilosula* states in parentheses): crimson (vs. light green), crimson resin-like droplets present (vs. absent) on both surfaces of leaf, depression absent (vs. present) in middle of gall on adaxial leaf surface, and opening surrounded by only long white pubescence (vs. surrounded by 5–6 slender filaments and long white pubescence) (Pergande 1904). This species differs from *P. pilosula* by the following characters of the fundatrix (with corresponding *P. pilosula* states in parentheses): two median puncture marks absent (vs. present) on head, six rows of pale dusky tubercles absent (vs. present) on dorsum, and legs stout (vs. long and slender) (Pergande 1904).

Etymology. Specific epithet (*killianae*) named in honor of Dr. Joella Killian, Professor Emeritus of Biology, at the University of Mary Washington in Fredericksburg, VA. Dr. Killian inspired me to become an entomologist through her passion for entomology and enthusiasm towards teaching it.

***Phylloxera myristica* Hamilton, sp. n.**

Figures 23–25

Material examined. Holotype: adult female fundatrix, slide-mounted. Five galls collected on *Carya myristiciformis* leaves at Francis Marion National Forest (33.12628563, -79.77577303), Berkeley County, SC, USA, F.B. Hamilton, 27.IV.16, Acc. 114. Holotype deposited at NMNH. Paratypes: 1 adult female alate and three nymphs. Same data as holotype. Deposited at NMNH.

Description. Fundatrix. Slide-mounted adult female 0.31 mm long and 0.20 mm wide (Figure 23). Body outline oval. Cuticle with minute granulations across body. One dusky puncture mark present on dorsum of head each side of midline and one cluster of dusky cells on

prothorax of dorsum. *Antenna*: Length ~ 76 µm; segment I wider than long with one short seta on anterior margin and one short seta near base; segment II about as long as wide with one short seta on anterior margin, one short seta near posterior margin, and one small sensory pit on posterior margin; segment III longest with scalloped annulations, minute thumb-like sensory organ on posterior margin with two small circular sensory pits anterior to it, one stout seta at apex with two shorter setae, and one short seta near middle of segment. *Mouthparts*: Setae absent on clypeus; rostrum extends to metacoxa, labial segment I with one medial seta, segment II with three pairs of marginal setae, segment III with one pair of medial setae, segment IV longest with two pairs of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 21 µm. *Legs*: Coxa with 4–7 setae; trochanter and femur fused, one pair of campaniform sensilla and one seta on posterior margin, one seta on anterior margin, and 1–2 setae on apical margin; tibia with 0–1 seta on posterior margin, one seta on anterior margin, and 4–5 setae near apical margin; tarsal segments I and II fused with 7–8 digitules (setae that appear broadly dilated or expanded at tips) and one short seta on anterior margin. Vulva indicated by striations in derm.

Setae of varying lengths distributed across dorsum and venter with average length ~ 6 µm. *Dorsal setae*: Head with two medial setae, one pleural seta, and four marginal setae; prothorax with two medial setae, eight pleural setae, and three marginal setae; mesothorax with two medial setae, 12 pleural setae, and one marginal seta; metathorax with two medial setae and seven pleural setae; abdominal segments with varying number of setae ranging from 1–6 per segment. *Ventral setae*: Head with one marginal seta and one seta anterior to clypeus; one seta at level of coxa on prothorax and two pleural setae; one seta at level of coxa on mesothorax; two

setae at level of coxa on metathorax; abdominal segments I–III fused; 1–2 setae found on each abdominal segment.

Description. Alate. Slide-mounted adult female 0.72 mm long and 0.34 mm wide (Figure 24). *Wings*: Undetermined size due to forewings and hindwings being broken off. *Antenna*: Length of ~ 179 µm; segment I about as long as wide with one short seta near middle; segment II about as long as wide with one short seta on anterior margin and one small, circular sensory pit near posterior margin; segment III longest, annulate on ~ basal one-half and rest of segment scaly, large circular sensorium near base and indented above it, short slender sensorium on posterior margin with three small, circular sensory pits anterior to it, one stout seta at apex with additional two short setae and two long setae proximal to them on anterior margin. *Mouthparts:* Rostrum extends past procoxa to well above mesocoxa, labial segment II with one pair of medial setae, segment III with one pair of medial setae, segment IV longest with two pairs of marginal setae, and segment V with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 29 µm. *Legs*: Coxa with 1–8 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, 1–2 setae on anterior margin, 0–1 seta near middle, and 0–2 setae on apical margin; tibia with 2–3 setae on posterior margin, 1–3 setae on anterior margin, and 1–4 setae on apical margin; tarsal segments I and II fused with 7–8 digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and well-developed claws. Vulva indicated by striations in derm.

Setae of varying lengths distributed across dorsum and venter with average length ~ 8 µm. *Dorsal setae*: Head with two medial setae and three pleural setae; prothorax with one medial seta; mesothorax with two medial setae and two pleural setae; metathorax with one medial seta and three pleural setae; 2–4 setae on last few abdominal segments, and distribution of setae on

other segments undetermined due to distortion of cuticle on preserved specimen. *Ventral setae*: Setae absent on head; three pleural setae and one marginal seta on prothorax; one seta at level of mesocoxa and two pleural setae; one seta at level of metacoxa; abdominal segments I–III fused; abdominal segments with 1–6 setae per segment; long setae present on last two abdominal segments with length ~ 23 µm.

Galls. Round, flattened, and yellowish-green with central depression in middle on adaxial leaf surface; round, more convex, and green with reticulated pattern on abaxial leaf surface (Figure 25). On adaxial leaf surface: horizontal diameter ~ 2.4 mm (2–2.9 mm), vertical diameter ~ 2.4 mm (2–2.9 mm), and height ~ 0.1 mm. On abaxial leaf surface: horizontal diameter ~ 2.3 mm (1.7–2.7 mm), vertical diameter ~ 2.2 mm (1.8–2.8 mm), and height ~ 0.9 mm. Opening on abaxial leaf surface a brown closed nipple that likely breaks open into several bracts when gall matures.

Remarks. *Carya myristiciformis* (Michx. f.) Nutt. (nutmeg hickory) represents a novel host for *Phylloxera* spp. Nutmeg hickory is the rarest species of hickory in the U.S. and is found in river bottoms, stream margins, and hillsides in disjunct populations from the coast of South Carolina to central Texas (Nelson et al. 2014). The gall of this species most resembles that of *Phylloxera russellae* Stoetzel, 1981, a species known to occur on *Carya illinoinensis* in AR, GA, LA, MS, OK, and TX (Stoetzel 1981). Galls of the two species are round, somewhat flattened, and greenish with a round opening on the abaxial leaf surface (Stoetzel 1981). This species differs from *P. russellae* by the following gall characters (with corresponding *P. russellae* states in parentheses): a reticulated pattern present only on abaxial leaf surface (vs. present on both leaf surfaces), slightly raised (vs. raised) on adaxial leaf surface, a distinct central depression present (vs. absent) on adaxial leaf surface, white pubescence absent (vs. present) around opening on

abaxial leaf surface (Stoetzel 1981). This species differs from *P. russellae* by the following characters of the fundatrix (with corresponding *P. russellae* states in parentheses): cuticle with some granulations (vs. densely granulated), concavity absent (vs. present) in center of head, rostrum extends to metacoxa (vs. extends past mesocoxa), claws well-developed (vs. reduced), antennal segment III with scalloped annulations (vs. with sharp annulations), an indistinct (vs. distinct) thumb-like sensory organ on antennal segment III, two sensory pores present (vs. three sensory pores present) along posterior margin of antennal segment III, three setae (vs. four setae) present at apex of antennal segment III (Stoetzel 1981). In addition, Stoetzel (1981) stated that there were no alatae known from *P. russellae* and there was no host alternation.

Etymology. Specific epithet from the Latin *myristica* named after its host plant species (nutmeg hickory).

***Phylloxera paludis* Hamilton, sp. n.**

Figures 26–27

Material examined. Holotype: adult female fundatrix, slide-mounted. Nine galls collected on *Carya aquatica* leaves at Delta National Forest (32.81699665, -90.80930258), Sharkey County, MS, USA, F.B. Hamilton, 4.V.17, Acc. eup. Holotype deposited at NMNH.
Paratypes: 3 adult female fundatrices. Same data as holotype as well as one gall collected under Acc. rbn on same host species and location. Deposited at BMNH and NMNH.

Description. Fundatrix. Slide-mounted adult female 0.50–0.56 mm long and 0.27–0.40 mm wide (Figure 26). Body outline globular, obovate, or oval. Cuticle covered in conical granulations that extend from head to ~ abdominal segment IV with minute scale-like points extending to terminal abdominal segment. Two dusky puncture marks present on head with one

on each side of midline of dorsum. In addition, dusky group of cells present on prothorax of dorsum. *Antenna*: Length ~ 111 µm; segment I about as long as wide with one long seta along anterior margin; segment II longer than wide with one short seta on anterior margin and one long seta on posterior margin; segment III longest and slightly clavate with sharply annulated surface, one slender thumb-like sensory organ along posterior margin and three circular and one oval sensory pit anterior to it, and one long seta at apex with 3–4 short setae. *Mouthparts*: One seta present near midline of clypeus; rostrum extends well past mesocoxa and to level of metacoxa in some specimens, labial segment I with one marginal seta, segment II longest with two pairs of marginal setae, segment III with one pair of marginal setae, segment IV with two pairs of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 32 µm. *Legs*: Coxa with 3–4 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, one seta on anterior margin, and 0–2 setae on apical margin; tibia with 1–2 setae on posterior margin, one seta on anterior margin, and six setae near apical margin; tarsal segments I and II fused with eight digitules (setae that appear broadly dilated or expanded at tips) and one short seta on anterior margin. Vulva indicated by striations in derm; tooth-like lobe with length ~ 18 µm and width ~ 5 µm present on each side of opening.

Setae of varying lengths distributed across dorsum and venter with average length ~ 10 µm. Marginal setae on dorsum slightly longer ~ 12 µm. *Dorsal setae*: Head with one medial seta, one pleural seta, and three marginal setae; prothorax with one medial seta, three pleural setae, and two marginal setae; mesothorax with two medial setae, four pleural setae, and two marginal setae; metathorax with one medial seta, three pleural setae, and two marginal setae; abdominal segments with varying number of setae ranging from 2–4 per segment; terminal abdominal

segment with one medium seta ~ 12 µm and one long seta ~ 26 µm. *Ventral setae*: Head with three marginal setae and one seta anterior to clypeus; one seta at level of coxa on prothorax and two pleural setae; one seta at level of coxa on mesothorax and metathorax; abdominal segments I–III fused; varying number of setae distributed across abdomen ranging from 1–3 per segment; terminal abdominal segment with two long marginal setae ~ 20 µm and 28 µm.

Galls. Round and shiny, convex, light green to brownish-green, slightly pubescent, and with shallow or deep depression in center on adaxial leaf surface; round and shiny, flattened and slightly convex, light green to brownish-green, and slightly pubescent on abaxial leaf surface; more convex above than below (Figure 27). Occurs along midvein of leaf. On adaxial leaf surface: horizontal diameter ~ 3.8 mm (3–4.5 mm), vertical diameter ~ 3.3 mm (2.5–4 mm), and height ~ 1 mm; on abaxial leaf surface: horizontal diameter ~ 3.1 mm (2.1–4.5 mm), vertical diameter ~ 3.0 mm (2.1–4.9 mm), and height ~ 0.2 mm. Opening on abaxial leaf surface round and surrounded by 5–6 short bracts fringed with pubescence at tips.

Remarks. The gall of this species most resembles that of *Phylloxera notabilis* Pergande, 1904, a species known to occur on *Carya illinoiensis* throughout eastern and southern U.S. (Pergande 1904, Blackman and Eastop 2013). Galls of the two species are more convex on adaxial leaf surface, pubescent, and greenish. Additionally, the fundatrices share common characters with them being of similar size and shape, cuticle being covered in conical tubercles, and antennal segment III being annulate and slightly clavate (Pergande 1904). This species differs from *P. notabilis* by the following gall characters (with corresponding *P. notabilis* states in parentheses): flattened (vs. globular to conical) on abaxial leaf surface, slightly pubescent (vs. densely pubescent) on abaxial leaf surface, and red coloration absent (vs. present) (Pergande 1904). This species differs from *P. notabilis* by the following characters of the fundatrix (with

corresponding *P. notabilis* states in parentheses): notch absent (vs. present) in middle of head, conical tubercles are wider and more rounded at tips (vs. longer and more acute at tips) on dorsum, two dusky puncture marks present (vs. absent) on dorsum of head, antennal segment III ~ 65 µm long (vs. ~ 128 µm long), antennal segment III with one oval and three circular sensory pits (vs. with three circular sensory pits), and tooth-like lobes present (vs. absent) around vulva (Pergande 1904).

Etymology. Specific epithet (*paludis*) is the Latin genitive of swamp and refers to the bayou near where the specimens were collected; treated as a noun in apposition.

***Phylloxera stoetzelae* Hamilton, sp. n.**

Figures 28–29

Material examined. Holotype: adult female fundatrix, slide-mounted. Seven galls collected on *Carya aquatica* leaves at Delta National Forest (32.81543961, -90.80947761), Sharkey County, MS, USA, F.B. Hamilton, 4.V.17, Acc. rbo. Holotype deposited at NMNH.
Paratypes: 4 fundatrices and 6 alate nymphs. Same data as holotype. In addition, five galls and 1 fundatrix collected on *Juglans hindsii* leaves at J.F. Gregory City Park (31.95036364, -81.30175912), Bryan County, GA, USA, F.B. Hamilton, 25.IV.16, Acc. 090. Deposited at AUMNH, BMNH, and NMNH.

Description. Fundatrix. Slide-mounted adult female 0.50–0.80 mm long and 0.24–0.55 mm wide (Figure 28). Body outline globular or elliptical. Cuticle covered in minute granulation. *Antenna*: Length ~121 µm; segment I about as long as wide with one long seta on anterior margin and one short seta near base; segment II longer than wide with one long seta on anterior margin, one long seta near posterior margin, and one short seta near base; segment III longest

with annulated surface, one thumb-like sensory organ along posterior margin and three circular sensory pits anterior to it, and one long seta at apex with two short setae. *Mouthparts*: Rostrum extends past mesocoxa, labial segment II longest with two pairs of marginal setae, segment III with one pair of marginal setae, segment IV with two pairs of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 36 µm. *Legs*: Coxa with 3–4 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, 1–2 setae on anterior margin, and 0–1 seta on or near apical margin; tibia with 0–1 seta on posterior margin, 0–1 seta on anterior margin, and 2–6 setae near apical margin; tarsal segments I and II fused with 7–8 digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and claws reduced. Vulva indicated by striations in derm.

Setae of varying lengths distributed across dorsum and venter with average length ~ 7 µm. *Dorsal setae*: Head with two marginal setae and one pleural seta; prothorax with two medial setae, two pleural setae, and two marginal setae; mesothorax with two medial setae and four pleural setae; metathorax with one medial seta, two pleural setae, and one marginal seta; abdominal segments with varying number of setae ranging from 0–5 per segment. *Ventral setae*: Head with two marginal setae and one seta anterior to clypeus; one seta at level of coxa on prothorax, mesothorax, and metathorax; abdominal segments I–III fused, varying number of setae distributed across abdomen ranging from 1–4 per segment, terminal abdominal segment with three short setae and one long marginal seta ~ 14 µm.

Galls. Round and yellowish-gold or crimson, slightly convex or sunken below leaf tissue, with central elevation or depression, shiny and glabrous on adaxial leaf surface; globular, much more convex, yellowish-gold or green with a hint of pink, shiny, and glabrous on abaxial leaf

surface (Figure 29). On adaxial leaf surface: horizontal diameter ~ 6.4 mm (4–9 mm), vertical diameter ~ 6.2 mm (3.9–9.5 mm), and height ~ 0.1 mm. On abaxial leaf surface: horizontal diameter ~ 6.8 mm (4–10 mm), vertical diameter ~ 6.7 mm (4–10 mm), and height ~ 2.9 mm. Opening on abaxial surface surrounded by white pubescence and at maturity, four white pubescent bracts exposed.

Remarks. *Juglans hindsii* (Jeps.) Jeps. ex R.E. Sm. (Northern California walnut) represents a novel host for *Phylloxera* spp. The gall of this species most resembles that of *Phylloxera russellae* Stoetzel, 1981, a species known to occur on *Carya illinoinensis* in AR, GA, LA, MS, and TX (Stoetzel 1981). Galls of the two species are round, glabrous, and flattened with a round opening below surrounded by white pubescence. Additionally, the fundatrices share a common feature with the rostrum extending past the mesocoxa (Stoetzel 1981). This species differs from *P. russellae* by the following gall characters (with corresponding *P. russellae* states in parentheses): yellowish-gold or crimson and green (vs. green), a reticulated pattern absent (vs. present), and rim of elevated tissue absent (vs. present) around opening (Stoetzel 1981). This species differs from *P. russellae* by the following characters of the fundatrix (with corresponding *P. russellae* states in parentheses): cuticle with dense uniform granulation (vs. with acute points and dense granulation), labial segment II with two pairs of marginal setae (vs. with one medial seta and two pairs of marginal setae), posterior end of body semicircular (vs. tapering), and antennal segment III with one long seta at apex and two shorter setae proximal to it (vs. with one long seta at apex and three shorter setae proximal to it) (Stoetzel 1981). In addition, this species differs from *P. russellae* life history (with corresponding *P. russellae* states in parentheses): alates present (vs. absent) in life cycle; and sexuparae absent (vs. present) in life cycle (Stoetzel 1981).

Etymology. Specific epithet (*stoetzelae*) named in honor of Dr. Manya Stoetzel, former Research Entomologist and Research Leader at the USDA Systematic Entomology Laboratory (Beltsville, MD), for her significant contributions to *Phylloxera* research in the U.S.

Phylloxera wiedenmanni Hamilton sp. n.

Figures 30–32

Material examined. Holotype: adult female fundatrix, slide-mounted. Five galls collected on *Carya cordiformis* leaves at Natural Falls State Park (36.17380934, -94.66989952), Delaware County, OK, USA, F.B. Hamilton, 13.V.17, Acc. m0s. Holotype deposited at NMNH. Paratypes: Five alates and two nymphs, slide-mounted. Same data as holotype. In addition, one fundatrix, slide-mounted. Three galls collected on *Carya cordiformis* leaves at Lake Bob Sandlin State Park (33.05545423, -95.0964349), Camp County, TX, USA, F.B. Hamilton, 09.V.17, Acc. 2qd. Deposited at AUMNH, BMNH, and NMNH.

Description. Fundatrix. Slide-mounted adult female 0.70–0.71 mm long and 0.44–0.48 mm wide (Figure 30). Body outline elliptical. Cuticle with minute granulation. *Antenna*: Length ~ 100 µm; segment I wider than long with one short seta along anterior margin and one small sensory pit near base along posterior margin; segment II about as long as wide with one long seta on anterior margin and one long seta on posterior margin; segment III longest with coarsely annulated surface, stout thumb-like sensory organ on posterior margin with three small and circular sensory pits anterior to it, one long seta at apex, and 1–3 shorter setae proximal to it. *Mouthparts*: Rostrum extends past mesocoxa and almost to level of metacoxa, labial segment I with one pair of medial setae, segment II longest and setae absent, segment III with one pair of medial setae, segment IV with two pairs of marginal setae, and segment V shortest with two

pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 41 µm. *Legs*: Coxa with 2–4 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, one seta on anterior margin, and 1–2 setae on apical margin; tibia with 1–2 setae on posterior margin, 0–1 seta on anterior margin, and six setae near apical margin; tarsal segments I and II fused with 7–8 digitules (setae that appear broadly dilated or expanded at tips), 0–1 short seta on anterior margin, and claws reduced.

Setae of varying lengths distributed across dorsum and venter with average length ~ 9 µm. *Dorsal setae*: Head with one medial seta, one pleural seta, three marginal setae, two dusky puncture marks near middle of head, and couple of dusky markings near margin; prothorax with two medial setae, two pleural setae, one marginal seta, elongate dusky marking in suture and couple of dusky markings near margin; mesothorax with one medial seta, two pleural setae, one marginal seta, dusky marking in suture and one dusky marking near margin; metathorax with one medial seta, four pleural setae, one marginal seta, two dusky markings with one near middle of segment and one near margin; abdominal segments with varying number of setae ranging from 2–5 per segment, and circular dusky marking near margin of abdominal segment III and IV. *Ventral setae*: Head with one seta along margin, one seta on inner edge of eye, and one seta anterior to clypeus; one seta at level of coxa on prothorax, mesothorax, and metathorax; three setae near margin on prothorax and three setae near margin on mesothorax; abdominal segments I–III fused, varying number of setae distributed across abdomen ranging from 1–4 per segment. Vulva indicated by striations in derm.

Description. Alate. Slide-mounted adult female 0.60–0.77 mm long and 0.30–0.35 mm wide (Figure 31). *Wings*: Forewing ~ 0.8 mm long and ~ 0.4 mm wide; hindwing ~ 0.5 mm long and ~ 0.2 mm wide. Cuticle with minute granulation. *Antenna*: Length of ~ 135 µm; segment I

wider than long with one long seta on anterior margin and scaly surface; segment II longer than wide, scaly, and with one short seta on anterior margin and one long seta on posterior margin; segment III longest, ~ 8 coarse annulations on basal one-fourth and rest of segment scaly, pedicel present at basal end with distinct projecting pointy sensorium on anterior margin, upper sensorium present on posterior margin that ~ three-fourths length of segment and 4–5 circular sensory pits anterior to it, and one long seta present at apex along with two shorter setae. *Mouthparts:* Rostrum extends to well above mesocoxa, labial segment I without setae, segment II with two pairs of marginal setae, segment III with one pair of marginal setae, segment IV longest with two pairs of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter ~ 23 µm. *Legs*: Coxa with 2–3 setae; trochanter and femur fused, one pair of campaniform sensilla and one long seta on posterior margin, one seta on anterior margin, and 0–2 setae on apical margin; tibia with 0–2 setae on posterior margin, 0–2 setae near middle, 0–1 seta on anterior margin, and 5–7 setae on apical margin; tarsal segments I and II fused with 6–8 digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and well-developed claws. Vulva indicated by striations in derm.

Setae of varying lengths distributed across dorsum and venter with average length ~ 8 µm. *Dorsal setae*: Head with one medial seta, two pleural setae, and one marginal seta; prothorax with one marginal seta; mesothorax with one medial seta and one pleural seta; metathorax with one medial seta and one pleural seta; abdominal segments with 1–2 setae except for terminal abdominal segment with four setae, and two long setae on terminal abdominal segment with length ~ 19 µm. *Ventral setae*: Head with one marginal seta; one seta at level of coxa on prothorax, mesothorax, and metathorax and one additional pleural seta on mesothorax;

abdominal segments I–III fused; varying number of setae distributed across abdomen ranging from 1–6 per segment.

Galls. Round or oval, convex, with pubescent yellowish-green circumference and transparent light yellow depression in center, occurs above plane of leaf or slightly sunken below plane forming a rim around it on adaxial leaf surface; round or oval, convex, constricted at base, light green, pubescent, and with round opening surrounded by 6–7 pubescent bracts on abaxial leaf surface (Figure 32). On adaxial leaf surface: horizontal diameter ~ 7.1 mm (5.0–9.1 mm), vertical diameter ~ 6.2 mm (4.1–8.0 mm), and height ~ 2.3 mm (0.1–3.5 mm); on abaxial leaf surface: horizontal diameter ~ 7.6 mm (6.0–9.0 mm), vertical diameter ~ 6.0 mm (4.5–8.0 mm), and height ~ 1.7 mm (1.0–2.1 mm).

Remarks. The gall of this species most resembles that of *Phylloxera foveola* Pergande, 1904, a species known to occur on *Carya glabra* in Virginia. The galls of both species are round and convex with a depression in middle on adaxial leaf surface and below convex with a round opening surrounded by bracts (Pergande 1904). The fundatrices of both species have minute granulation on the surface and are of a similar size (Pergande 1904). The alates of both species have both an annulated and scaly antennal segment III (Pergande 1904). This species differs from *P. foveola* by the following gall characters (with corresponding *P. foveola* states in parentheses): raised circumference pubescent (vs. glabrous) on adaxial leaf surface, pubescent (vs. glabrous) on abaxial leaf surface, depression light yellow to whitish (vs. light red with a darker dimple or yellowish-green with a pink dimple) on adaxial leaf surface, and constricted at base (vs. not constricted at base) on abaxial leaf surface (Pergande 1904). This species differs from *P. foveola* by the following characters of the fundatrix (with corresponding *P. foveola* states in parentheses): dusky markings present (vs. absent) on dorsum; and abdomen gradually tapering

(vs. pointed posteriorly with last several abdominal segments slender) (Pergande 1904). This species differs from *P. foveola* by the following characters of the alate (with corresponding *P. foveola* states in parentheses): antennal segment III with upper sensorial membrane ~ three-fourths the length of segment (vs. ~ one-half the length of segment), antennal segment III with ~ 8 annulations (vs. ~ 15 annulations), antennal segment III with a sharp sensorium present at base (vs. a sharp sensorium absent at base) (Pergande 1904). On the phylogeny, *P. wiedenmanni* is more closely related to *Phylloxera reticulata* Duncan which is a free-living species on *Quercus kelloggii* Newberry in California, USA.

Etymology. Specific epithet (*wiedenmanni*) named in honor of Dr. Robert N. Wiedenmann, Professor of Entomology, at University of Arkansas in Fayetteville, AR. I am forever grateful for Dr. Wiedenmann giving me the opportunity to pursue my M.S. in Entomology at the University of Arkansas and for serving as a mentor to me throughout graduate school.

Phylloxera williamsi Hamilton, sp. n.

Figures 33–34

Material examined. Holotype: adult female fundatrix, slide-mounted. One gall collected on *Carya cordiformis* leaves at Yankauer Nature Preserve (39.50127146, -77.85210293), Berkeley County, WV, USA, F.B. Hamilton, 20.V.18, Acc. qzl. Holotype deposited at NMNH.

Description. Fundatrix. Slide-mounted adult female 0.59 mm long and 0.42 mm wide (Figure 33). Body outline globular. Cuticle with irregular to conical granulations from head to about abdominal segment III and scale-like points arranged in rows on abdominal segments. *Antenna*: Length ~ 133 µm; segment I wider than long with one long seta on anterior margin, one

short seta near middle, and rounded at apex; segment II about as long as wide with one long seta on anterior margin, one short seta near middle, and nearly straight at apex; segment III longest with annulated surface, slightly wider at base and apex, one thumb-like sensory organ on posterior margin and three sensory pits anterior to it, one long seta and two short setae at apex. *Mouthparts*: Setae absent on clypeus; rostrum extends past metacoxa, labial segment I without setae, segment II with two pairs of marginal setae, segment III with two pairs of medial setae, segment IV with two pairs of marginal setae, and segment V shortest with two pairs of setae at apex. *Spiracles*: Anterior and posterior spiracles with diameter of ~ 47 µm. *Legs*: Coxa with 3–4 setae; trochanter and femur fused, one pair of campaniform sensilla and 1–2 setae on posterior margin, 1–2 setae on anterior margin, and 1–2 setae on apical margin; tibia with 1–2 setae on posterior margin, 0–1 seta on anterior margin, and 4–6 setae on apical margin; tarsal segments I and II fused with 5–8 digitules (setae that appear broadly dilated or expanded at tips), one short seta on anterior margin, and well-developed claws. Vulva indicated by striations in derm; long sclerosis ~ 37 µm present on each side of vulva.

Setae of similar size distributed across dorsum and venter with average length ~ 8 µm. *Dorsal setae*: Setae from head to about abdominal segment III slightly elevated on wart-like structures; head with three medial setae, two pleural setae, and two marginal setae; prothorax with one medial seta, one pleural seta, and one marginal seta; mesothorax with one medial seta, three pleural setae, and two marginal setae; metathorax with two medial setae and two marginal setae; abdominal segments with varying number of setae ranging from 0–3 per segment. *Ventral setae*: Setae absent on head; one seta at level of coxa on prothorax, mesothorax, and metathorax; abdominal segments I–III fused, varying number of setae on abdominal segments ranging from 1–4 per segment.

Galls. Oval and convex, yellowish-green, and with long white pubescence on both sides of leaf; slightly more convex above than below (Figure 34). On adaxial leaf surface: horizontal diameter ~ 2.2 mm, vertical diameter ~ 2.4 mm, and height ~ 1.5 mm; on abaxial leaf surface: horizontal diameter ~ 2.2 mm, vertical diameter ~ 2.2 mm, and height ~ 1.2 mm. Opening slit-like on abaxial leaf surface and densely covered in pubescence.

Remarks. The gall of this species most resembles that of *Phylloxera floridana* Hamilton sp. n., a species occurring on *Carya floridana* in central Florida. Galls of the two species are convex and yellowish with an opening below. Fundatrices of both species have conical tubercles on cuticle. This species differs from *P. floridana* by the following gall characters (with corresponding *P. floridana* states in parentheses): oval (vs. round) on both surfaces of leaf, pubescent with long white hairs (vs. glabrous) on adaxial leaf surface, depression absent (vs. present) in center on adaxial leaf surface, and opening densely covered in pubescence (vs. with some pubescence). This species differs from *P. floridana* by the following characters of the fundatrix (with corresponding *P. floridana* states in parentheses): cuticle densely covered in irregular to conical tubercles (vs. with some minute conical tubercles), rostrum extends past metacoxa (vs. extends to mesocoxa), rostrum ~ 181 µm long (vs. ~ 112 µm long), antennal segment III with three setae at apex (vs. with four setae at apex), a long sclerosis present (vs. absent) around each side of vulva, and grain-like scleroses absent (vs. present) on caudal end of venter.

Etymology. Specific epithet (*williamsi*) named in honor of Dr. Mike Williams, Professor Emeritus of Entomology, at Auburn University for his significant contributions to scale insect research and in gratitude for him helping me to reach my goals.

Discussion

Collections occurring across the U.S. proved to be advantageous since 14 new species of *Phylloxera* were collected and described. Collecting in different geographic regions was also advantageous since new species of *Phylloxera* were described from hickory species that were not previously known as hosts including: nutmeg hickory, sand hickory, and scrub hickory. Nutmeg hickory is the rarest species of hickory found in disjunct populations throughout the southern U.S., sand hickory is found throughout the Southeast and west to eastern Missouri and Louisiana and north to Delaware, and scrub hickory is found only in Central Florida (Nelson et al. 2014, Kirkman et al. 2007). In addition, a novel gall-forming *Phylloxera* sp. (*P. stoetzelae*) was described from a new host plant genus (*Juglans hindsii*). These results help to confirm Pergande's belief that only a fraction of the *Phylloxera* spp. diversity in the U.S. had been treated and there are likely many more new species awaiting to be discovered.

Key to galls of hickory and walnut-feeding *Phylloxera* spp. in the U.S.

1. Spines present on gall surface……..……...…..……..2

1'. Spines absent on gall surface…….…….…….……..3

2(1). Spines short and stout; gall globular; crimson, white, white with crimson, or yellowish-green; diameter 5.0–25.0 mm……..……*Phylloxera spinosa* (Shimer)

2'. Spines long and slender; gall globular; greenish-yellow or brownish-yellow; diameter 5.0–15.0 mm…….....……..*Phylloxera spinuloides* Pergande

3(1'). Elongate sclerotized ridge above leaf with a protruding pubescent fold of tissue below leaf on each side of elongate slit opening……..,,……...……..*Phylloxera caryaevenae* Fitch

3'. Button-like, conical, globular, nut, pear, or thorn-like without an elongate slit opening below leaf……....4

4(3’). Slit-like opening present…….…….…….....5

4’. Round, conical, or thorny opening present…..…..……14

5(4). Button-like and somewhat flattened……..…….……6

5’. Conical or globular……...…….……...……..7

6(5). Convex above leaf and with or without an elevated center; red, light green, yellowish-green, or white; diameter 8.0–14.0 mm; below leaf level with leaf tissue or convex, light green, and opening with whitish pubescence…….……..*Phylloxera caryaescissa* Riley

6’. Slightly convex above leaf and with or without a depression in center, yellow; diameter 1.1–5.2 mm; more convex below leaf with a protruding opening fringed with whitish pubescence ……..…….…….....*Phylloxera floridana* sp. n.

7(5’). Conical……..…..……....…….8

7’. Globular……...…….……...……...9

8(7). More convex above leaf than below, yellow, diameter 1.1–5.0 mm; below leaf light yellowish-green and opening with white pubescence…...……..*Phylloxera flavoconica* sp. n.

8’. Often more convex below leaf than above, conical or round and somewhat flattened above leaf, yellow or red, diameter 1.0–5.0 mm; below leaf light green with a protruding clam-like opening with white pubescence…….....……..……..*Phylloxera caryaefallax* Riley

9(7’). Long white pubescence present above leaf and a protruding opening below leaf...…… …….…..…....……...…….....*Phylloxera williamsi* sp. n.

9’. Long white pubescence absent above leaf and opening flush with surface below leaf….…...10

10(9’). Occurs between lateral veins and often with several small openings near apex above leaf ……..…….....*Phylloxera caryaeglobuli* Walsh

10’. Occurs along midvein of leaves, petioles, or stems and without several small openings near apex above leaf……11

11(10’). Occurs only on petioles, somewhat flattened, and mottled green……..*Phylloxera subelliptica* (Shimer)

11’. Occurs along midvein of leaves and petioles; not flattened; light green, yellowish-white, or yellowish-green and with or without a hint of crimson……..12

12(11’). Spongy and with short pubescence……*Phylloxera caryaemagna* (Shimer)

12’. Hard and with or without pubescence……13

13(12’). Glabrous, occurs singly or in clusters, diameter 5.0–25.0 mm, does not split at maturity into two equal halves…….*Phylloxera caryaecaulis* (Fitch)

13’. Densely pubescent, occurs in clusters, diameter 2.0–15.0 mm, gall splits at maturity into two equal halves…….*Phylloxera caryaeren* Riley

14(4’). Conical or thorn-like opening present……15

14’. Round opening present…..23

15(14). Occurs on petioles, stems, or along midvein of leaves; yellowish-white; confluent on petioles and stems…….*Phylloxera perniciosa* Pergande

15’. Occurs only on leaves; yellow, yellowish-green, crimson, crimson and green, pink and green, green and purple; can be confluent or separate when occurring in groups……16

16(15’). Opening present on each side of leaf……17

16’. Opening present only on one side of leaf……21

17(16). Opening above leaf jagged……*Phylloxera intermedia* Pergande

17’. Opening above leaf smooth……18

18(17’). Slightly convex on both sides of leaf with a short conical nipple opening on each side……...……..*Phylloxera picta* Pergande

18’. Convex with a thorn-like opening on one or both sides of leaf…….……19

19(18’). Conical above leaf and flat beneath, opens from above; below leaf a round opening surrounded by slender pubescent filaments that remains closed…...*Phylloxera caryaefoliae* Fitch

19’. Conical or thorn-like on both sides of leaf; usually opens from above leaf, but can also open from below leaf……...…….……..…...…...20

20(19’). Slender conical projections on both sides of leaf; green, crimson, or green and crimson; diameter 1.0–7.0 mm. Fundatrix has an intermediate sensorium on antennal segment III and alate with a stout antennal segment III, which lacks a basal sensorium and upper sensorium three-fourths the length of segment……..….…….*Phylloxera bispinae* sp. n.

20’. Elongate and slender or short and stout conical projections on both sides of leaf; light rose, crimson, yellowish-green, or light green and crimson towards apex; elongate galls with a diameter 2.0–4.0 mm; short and stout galls with a diameter of 5.0–12.0 mm. Fundatrix lacks an intermediate sensorium on antennal segment III and alate with a slender, long antennal segment III with a basal sensorium present and upper sensorium two-fifths the length of segment…….

…….....……...……...*Phylloxera caryaesepta* (Shimer)

21(16’). Convex above and below leaf; a light green thorn-like projection below leaf, which is transparent and opening splits into several long bracts…….....…*Phylloxera chippokesiensis* sp. n.

21’. Slightly convex above and below leaf; a light green thorn-like projection absent below leaf, opaque, and long bracts absent around opening…...…….……..…..……22

22(21’). Concolorous to surrounding leaf tissue on both surfaces of leaf with a slight depression in center above leaf; diameter 3.5–4.6 mm; below opening slender and stem-like……..

…….……*Phylloxera crypta* sp. n.

22’. Red with a yellow central depression or entirely greenish-yellow above leaf; diameter 1.0–5.0 mm; below leaf purplish or greenish-yellow, opening with a short nipple…….

…….*Phylloxera deplanata* Pergande

23(14’). Crimson resin-like droplets present on both gall surfaces…....*Phylloxera killianae* sp. n.

23’. Crimson resin-like droplets absent on both gall surfaces…….……..……24

24(23’). Long yellowish-white pubescence present above leaf……..…….....……...25

24’. Long yellowish-white pubescence absent above leaf…….……26

25(24). Globular and more convex above leaf, pink, diameter 1.3–1.8 mm; below leaf slightly convex, light green, and pubescent; opening below flat and surrounded by short yellowish-white pubescence…….....*Phylloxera echinus* sp. n.

25’. Round and flat or slightly convex above leaf with a shallow depression, light green, diameter 3.0–6.0 mm; below leaf more convex, green to almost white at apex, a nipple opening densely covered in long yellowish-white pubescence……..…….....*Phylloxera pilosula* Pergande

26(24’). Slit-like opening in center above leaf, which leads down into a false chamber…….....

…..……..…….....*Phylloxera falsostium* sp. n.

26’. Slit-like opening absent above leaf and a false chamber inside absent…….……27

27(26’). Opening above leaf with apex of gall splitting into several stout bracts; below leaf raised to a point…….……...…….……*Phylloxera texana* Stoetzel

27’. Opening below leaf and not raised to a point…….……...……28

28(27’). A reticulated pattern present above and below leaf.....*Phylloxera russellae* Stoetzel

28’. A reticulated pattern absent on both sides of leaf…….…..……...……29

29(28’). Rugose above leaf and globular….……..…..…….....*Phylloxera conica* (Shimer)

29’. Smooth above leaf and button-like, flattened, or globular…….……...…….…....30

30(29’). Raised to a rounded point in center above leaf…….....*Phylloxera auburnensis* sp. n.

30’. About evenly convex or with a depression in center above leaf…….……...31

31(30’). Spindle-shaped below leaf and suspended from leaf by a slender filament; surface sticky

……...…..*Phylloxera caryaegummosa* Riley

31’. Conical, flattened, globular, hazelnut, or pear shaped below leaf and not suspended from leaf by a slender filament; surface dry…….……..…32

32(31’). A transparent depression present in center above leaf……..…….…….…….33

32’. A transparent depression absent in center above leaf…..…….……...……34

33(32). Yellowish-green circumference with a pale red depression or completely yellowish-green with a pink dimple; circumference and depression glabrous; depression may be shallow or deep; diameter 3.0–5.0 mm…….…..…*Phylloxera foveola* Pergande

33’. Light green circumference with a whitish or yellow depression, a pink dimple absent; circumference pubescent and depression glabrous; depression shallow; diameter 5.0–9.1 mm…...

……..…….....*Phylloxera wiedenmanni* sp. n.

34(32’). Seed-like above leaf and often with a depression in center that extends to circumference; diameter 0.3–2.0 mm….....…….……...*Phylloxera caryaesemen* (Shimer)

34’. Not seed-like above leaf and lacking a depression in center that extends to circumference; diameter 2.0–15.0 mm…….…..……35

35(34’). Occurs along midvein of leaves……..……..36

35’. Does not occur along midvein of leaves…….……...…….38

36(35). Globular or irregular; about equally convex above and below leaf; occurs along midvein of leaves, petioles, stems, leaf buds, and flower buds; opening flat and round surrounded by pubescence or nipple-like and found below leaf when present on leaf…….

…...……...*Phylloxera devastatrix* Pergande

36’. Oval or round; more convex above leaf than below; occurs only along midvein of leaves; opening stem-like, nipple-like, or surrounded by short bracts……..…….……..……..37

37(36’). Yellowish-green or slightly red, with or without a central depression, pubescent, and diameter 2.0–10.0 mm; below leaf somewhat conical, green or yellow, and pubescent; opening below leaf stem-like which splits into several long bracts……*Phylloxera notabilis* Pergande

37’. Light green to brownish-green, with a shallow or deep depression in center, slightly pubescent, and diameter 3.0–4.5 mm; below leaf flattened and round, light green to brownish-green, and slightly pubescent; opening below leaf round and surrounded by 5–6 short bracts with pubescence at tips……..……..….*Phylloxera paludis* sp. n.

38(35’). Globular or irregular; occurs singly or in clusters on petioles or stems; opening with or without a short nipple….....*Phylloxera georgiana* Pergande

38’. Button-like or flattened above leaf; occurs singly or in clusters on leaves……..

…….....39

39(38’). About equally convex above and below leaf….**.....***Phylloxera depressa* (Shimer)

39’. Galls more convex below leaf…….……...40

40(39’). Button-like above leaf and often with a distinct rim around circumference……

……..….....*Phylloxera rimosalis* Pergande

40’. Flat above leaf and without a distinct rim around circumference…...……...……..….41

41(40’). Below leaf a brown nipple opening……..……*Phylloxera myristica* sp. n.

41’. Below leaf without a brown nipple opening…….....…….……..…..42

42(41’). Glabrous and shiny above and below leaf……...…..……..*Phylloxera stoetzelae* sp. n.

42’. Glabrous and shiny or dull above leaf; pubescent and dull below leaf……..…….……...43

43(42’). Globular below leaf and usually with a hazelnut shape; light green and apex splits into several bracts at maturity; diameter 5.0–10.0 mm.……...…....*Phylloxera caryaeavellana* Riley

43’. Conical, globular, or pear-shaped below leaf; crimson, light green, yellow, or white; opening below leaf surrounded by 4–8 short pubescent bracts; diameter 2.0–8.0 mm……...

……...*Phylloxera symmetrica* Pergande

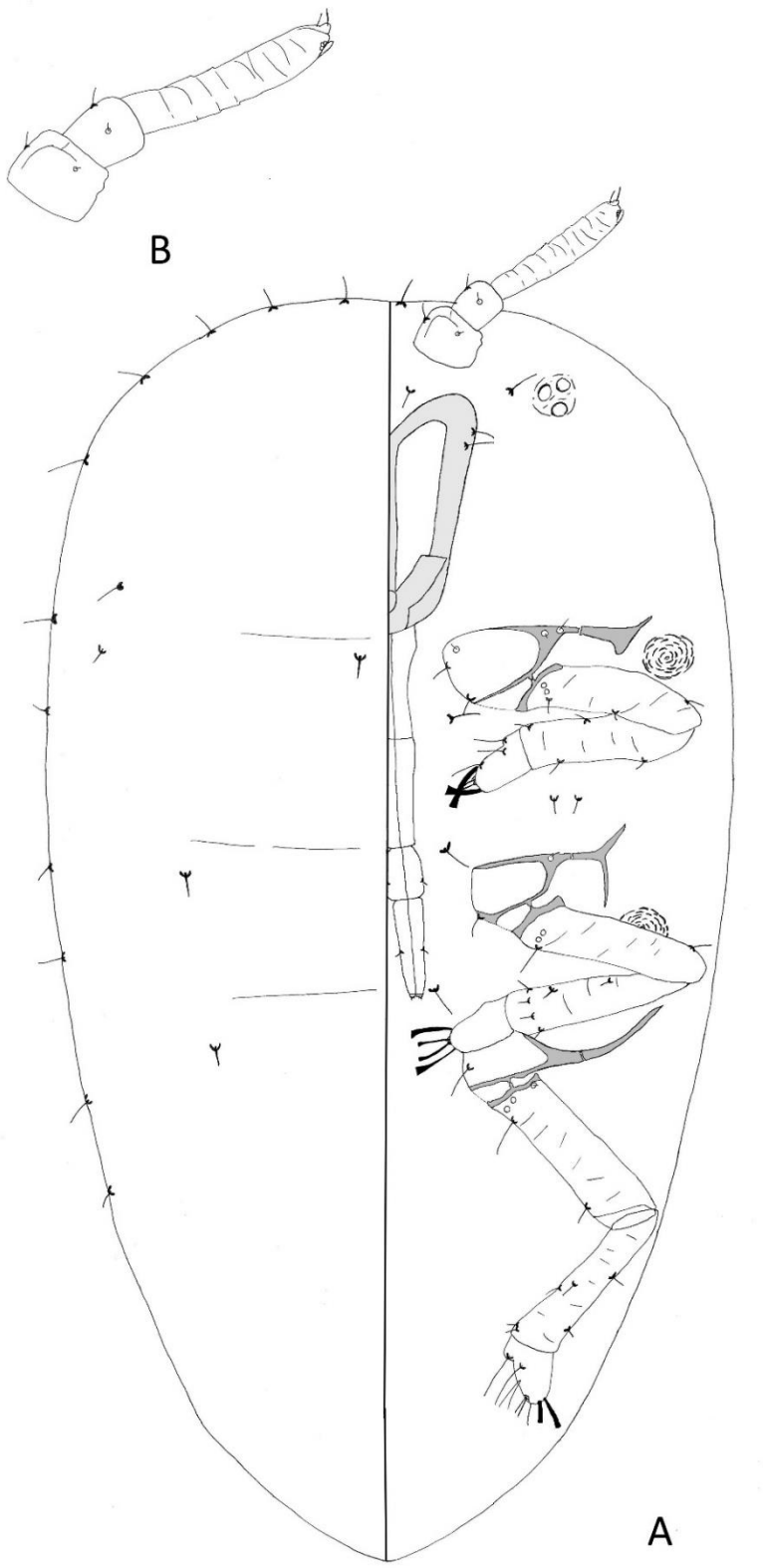

Figure 1. *Phylloxera auburnensis* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

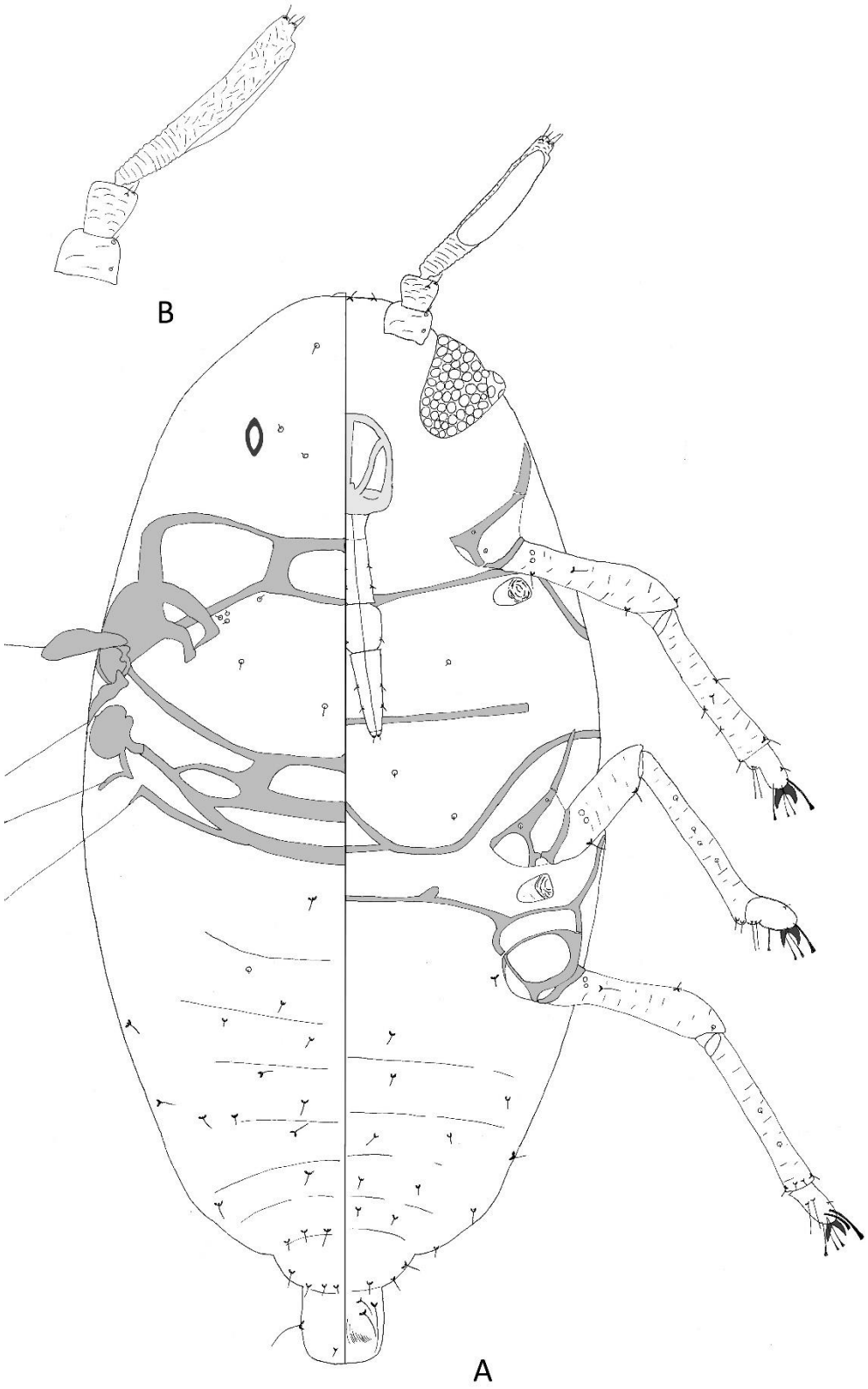

Figure 2. *Phylloxera auburnensis* Hamilton, sp. n., alate; **(A)** whole body (**B)** antenna.

Figure 3. Gall of *Phylloxera auburnensis* on adaxial surface of leaf **(A)**, abaxial surface of leaf **(B)**, and distributed in a group along midvein of leaf **(C)**.

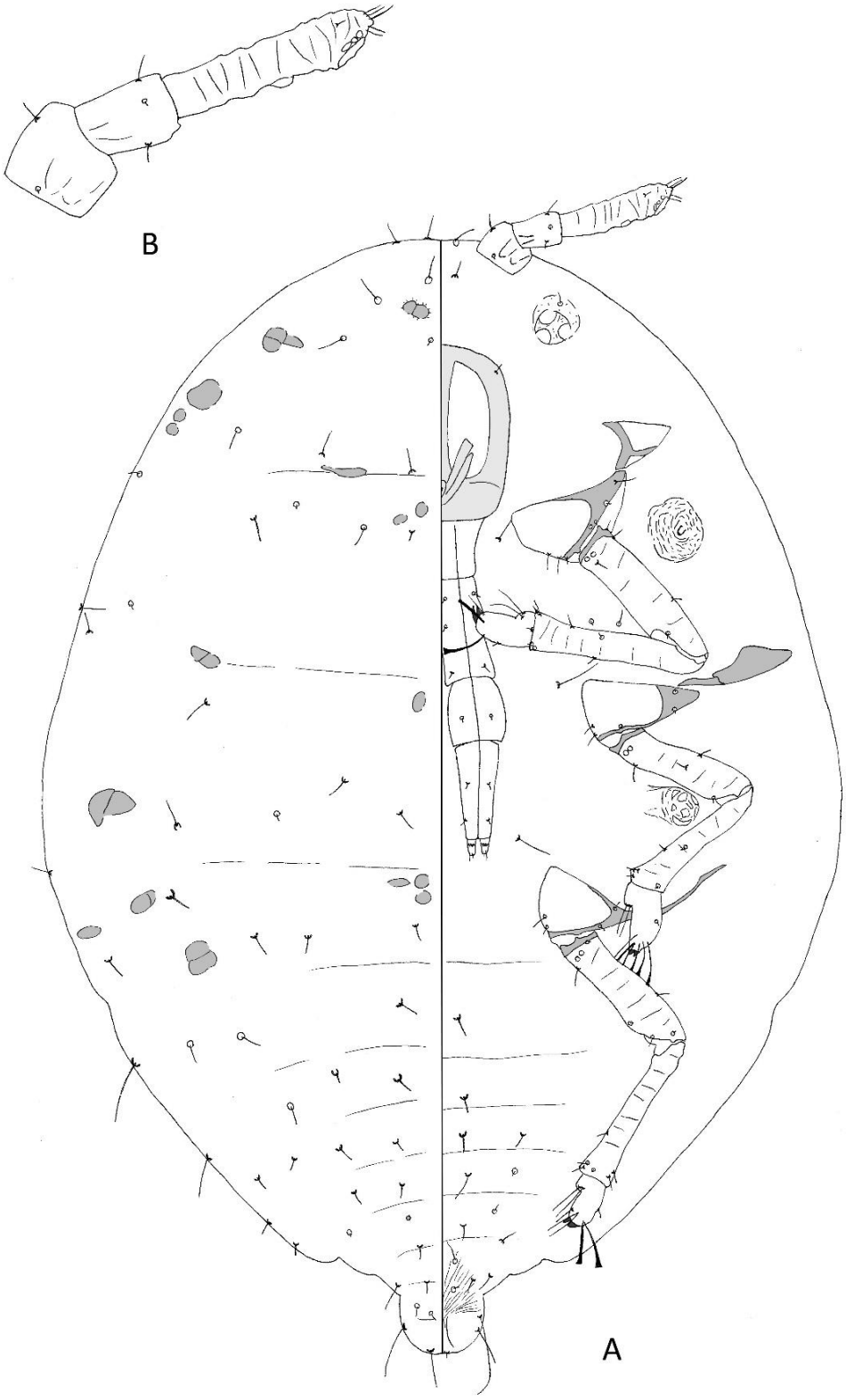

Figure 4. *Phylloxera bispinae* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

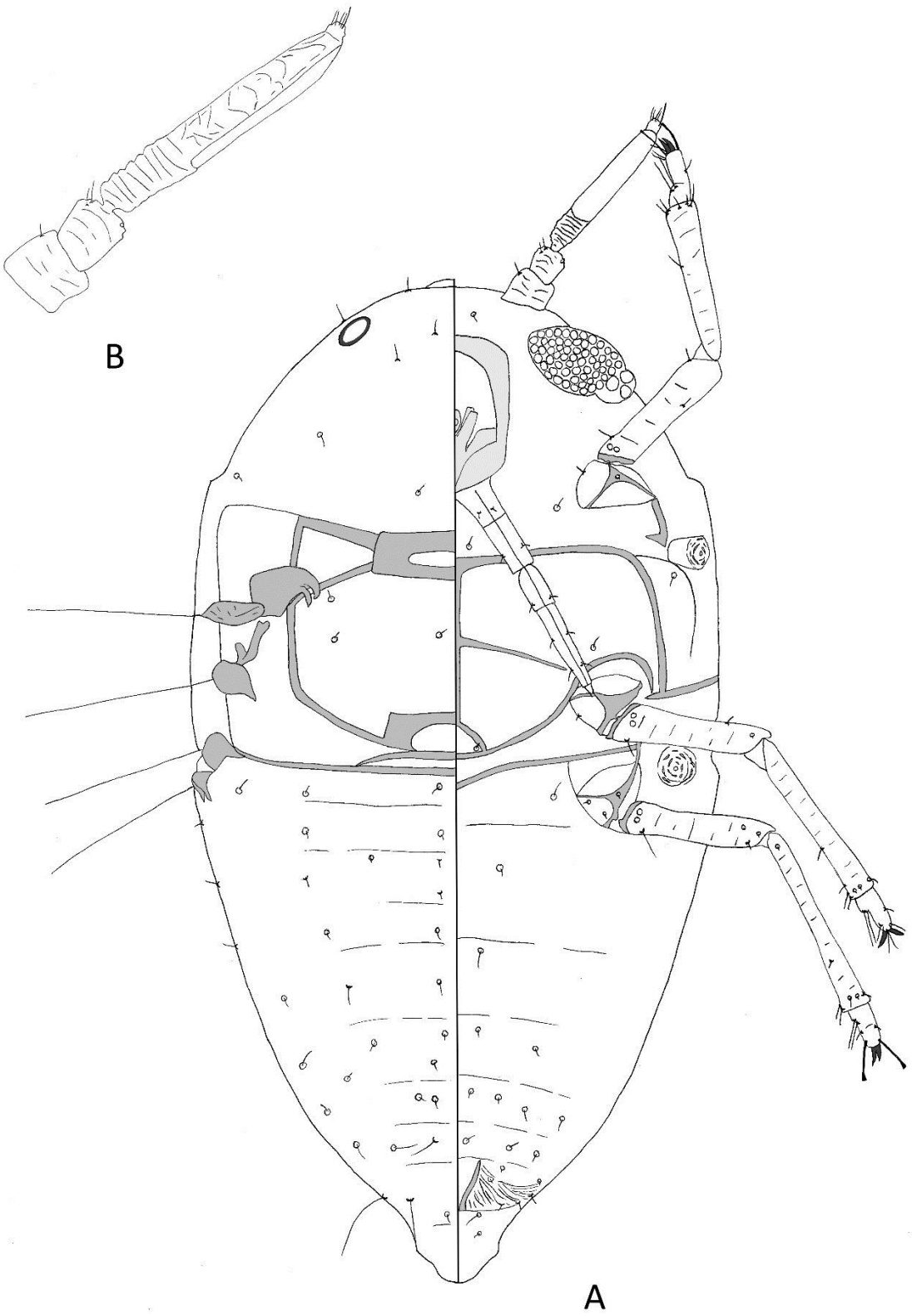

Figure 5. *Phylloxera bispinae* Hamilton, sp. n., alate; **(A)** whole body **(B)** antenna.

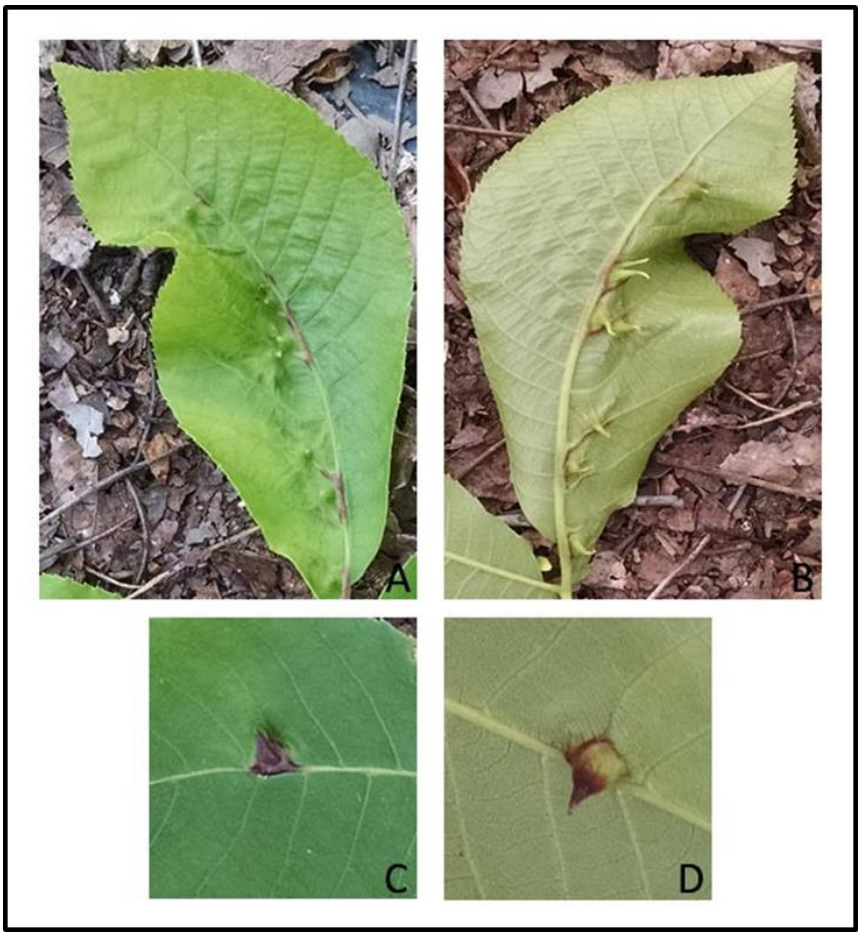

Figure 6. Young galls of *Phylloxera bispinae* on adaxial surface of leaf **(A)** abaxial surface of leaf **(B)** mature gall with crimson coloration on adaxial surface **(C)** and with crimson and green coloration on abaxial surface **(D)**.

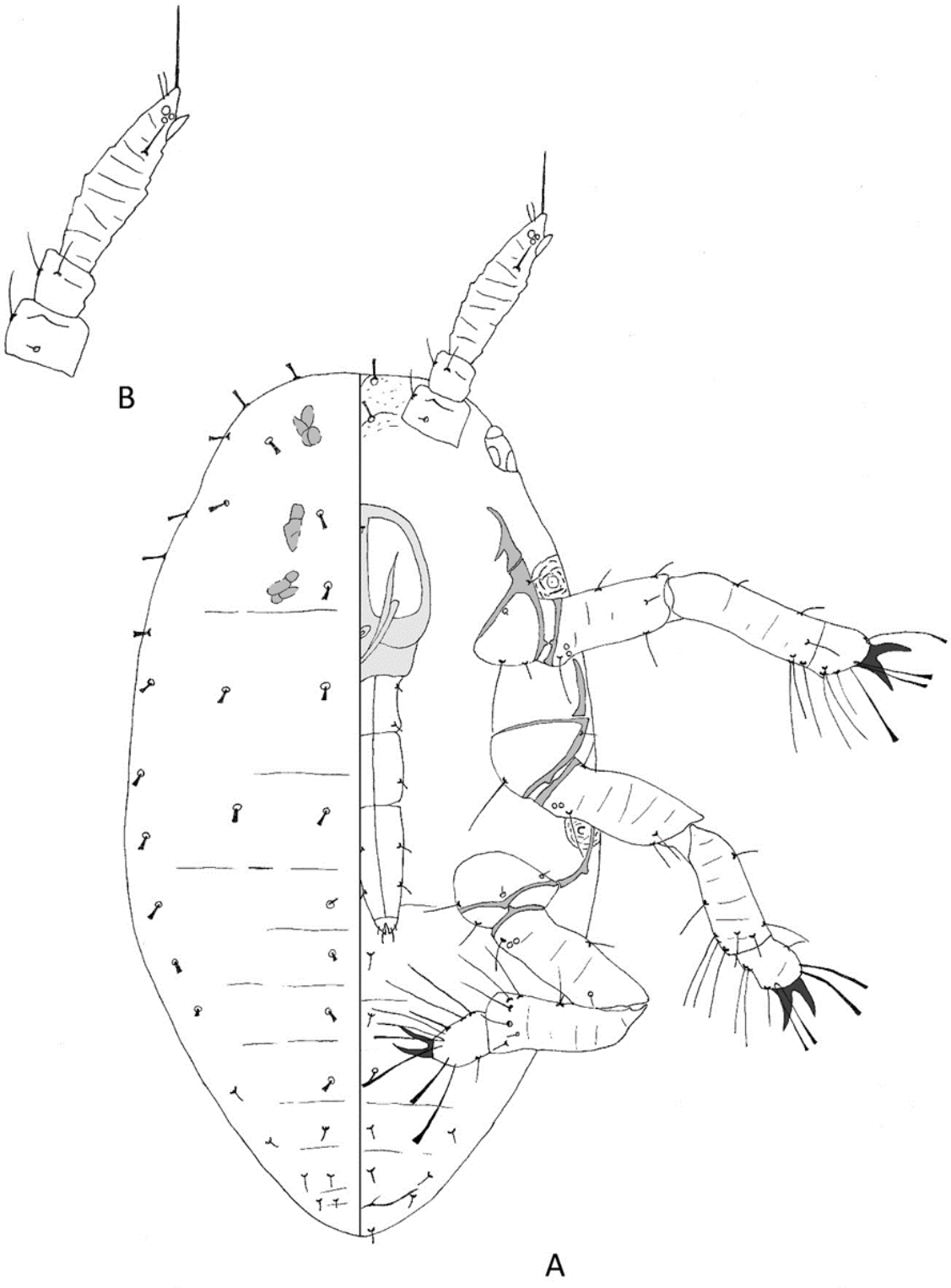

Figure 7. *Phylloxera chippokesiensis* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

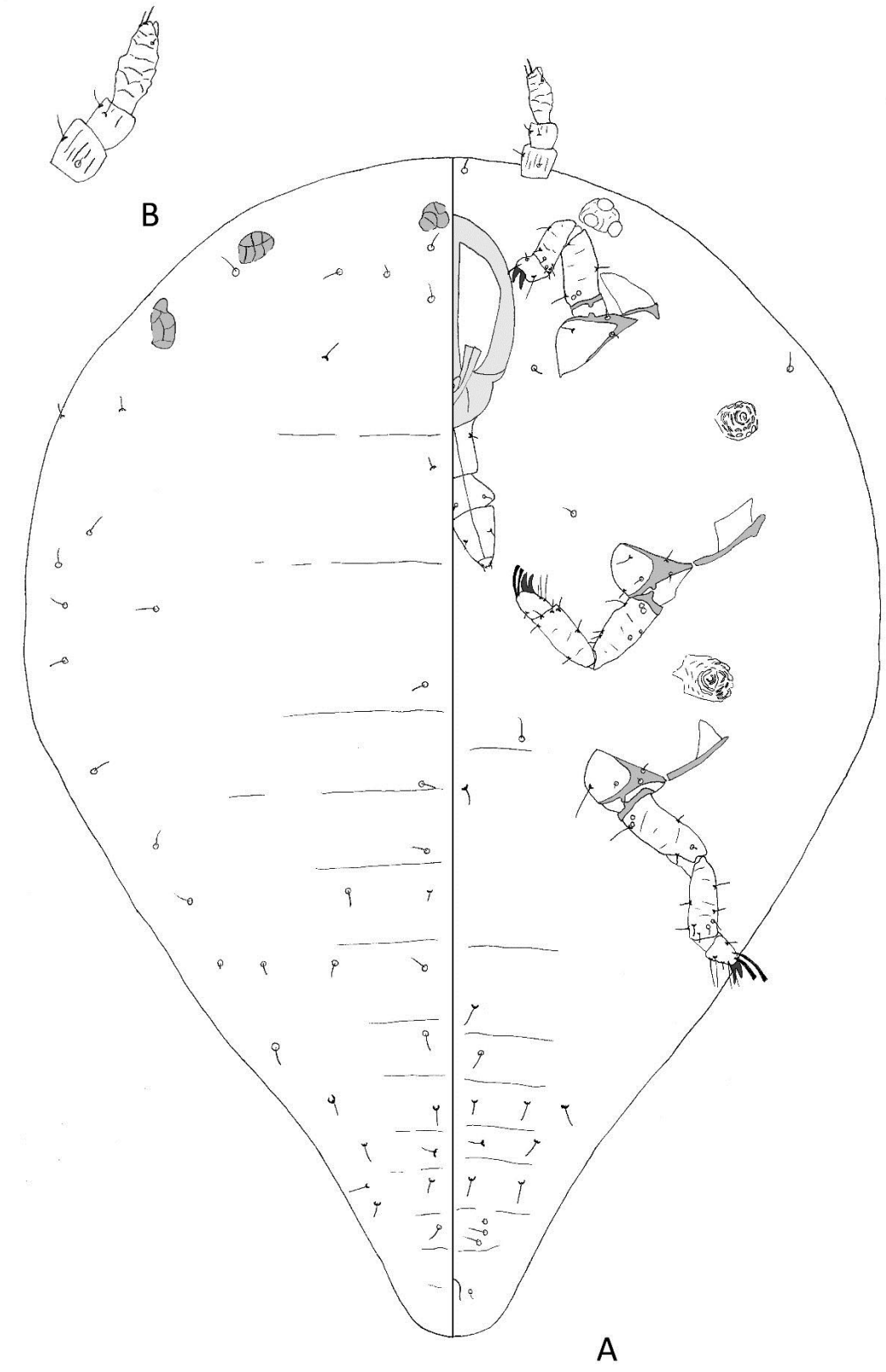

Figure 8. *Phylloxera chippokesiensis* Hamilton, sp. n., sexupara; **(A)** whole body **(B)** antenna.

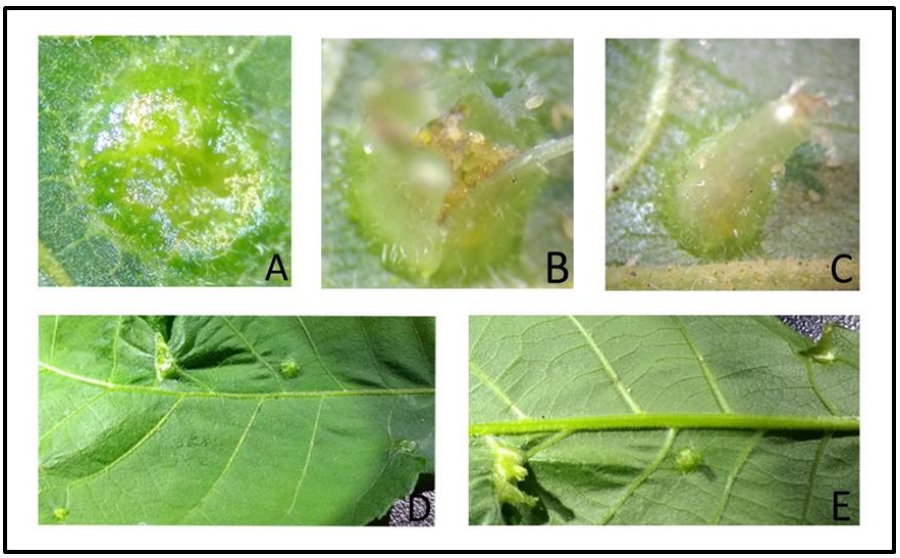

Figure 9. Gall of *Phylloxera chippokesiensis* on adaxial surface of leaf **(A)** bracts open on abaxial surface of leaf **(B)** bracts closed on abaxial surface of leaf **(C)** along midvein on adaxial surface of leaf **(D)** along midvein on abaxial surface of leaf **(E)**.

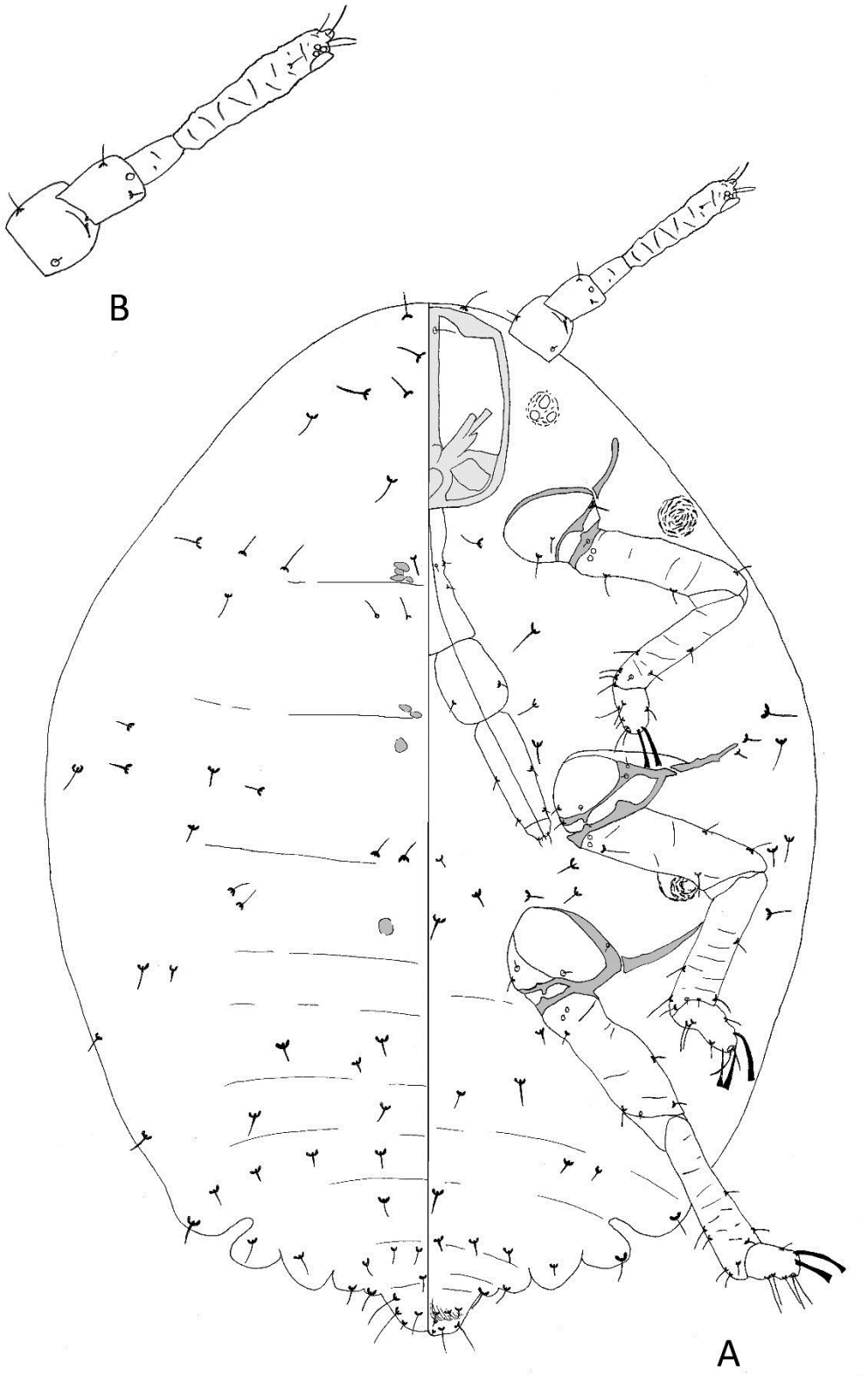

Figure 10. *Phylloxera crypta* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

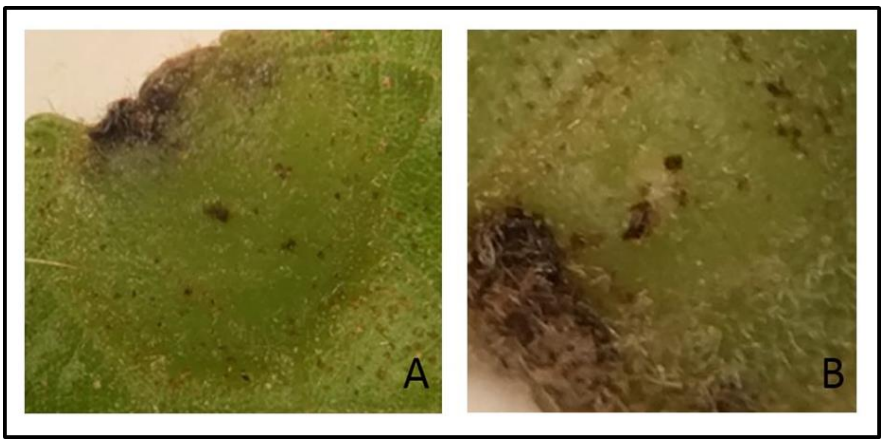

Figure 11. Gall of *Phylloxera crypta* on adaxial surface of leaf **(A)** and abaxial surface of leaf **(B)**.

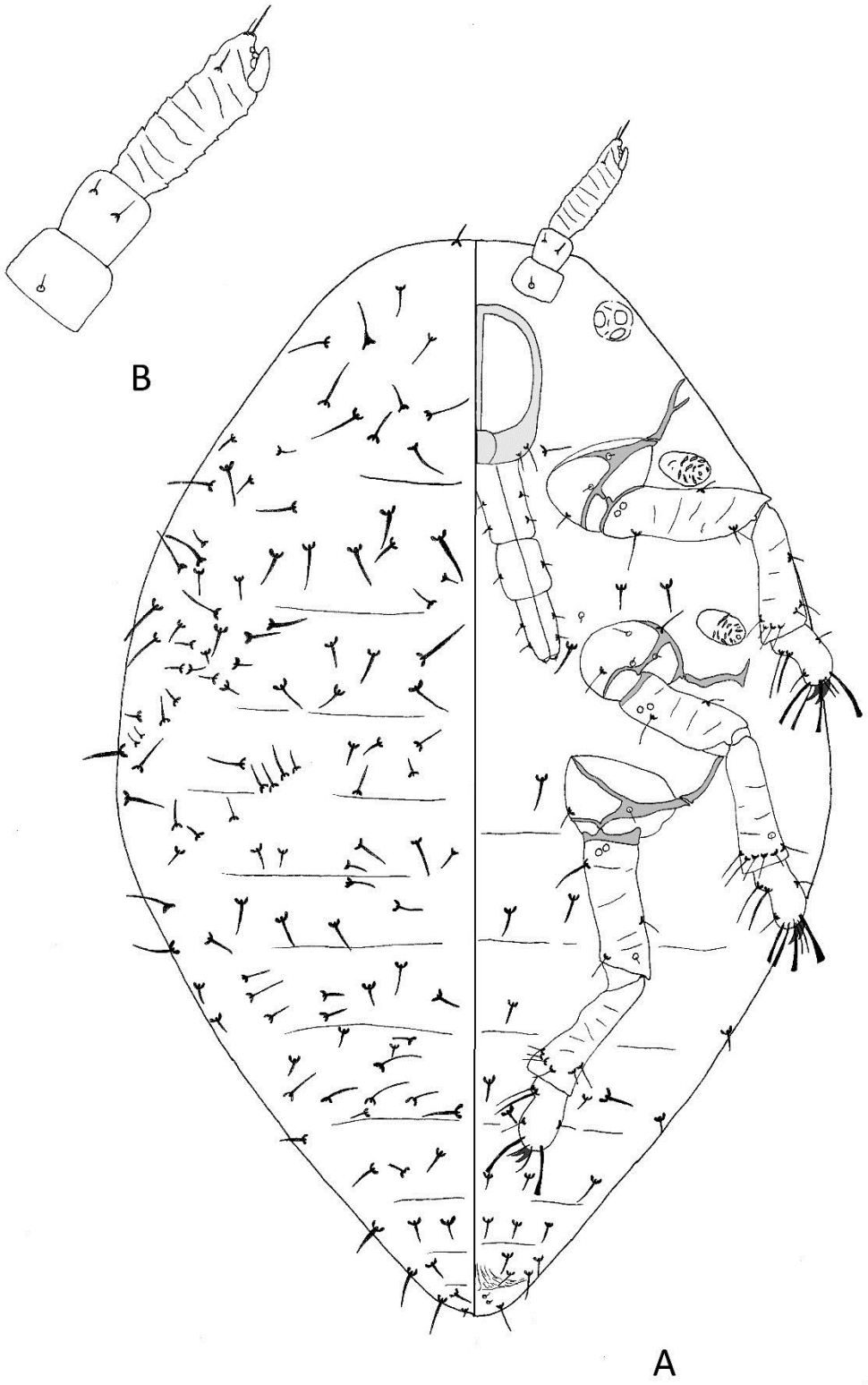

Figure 12. *Phylloxera echinus* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

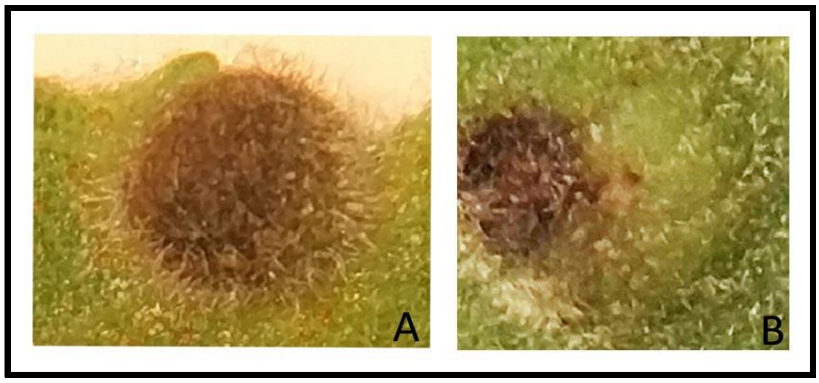

Figure 13. Gall of *Phylloxera echinus* on adaxial surface of leaf **(A)** and abaxial surface of leaf **(B).**

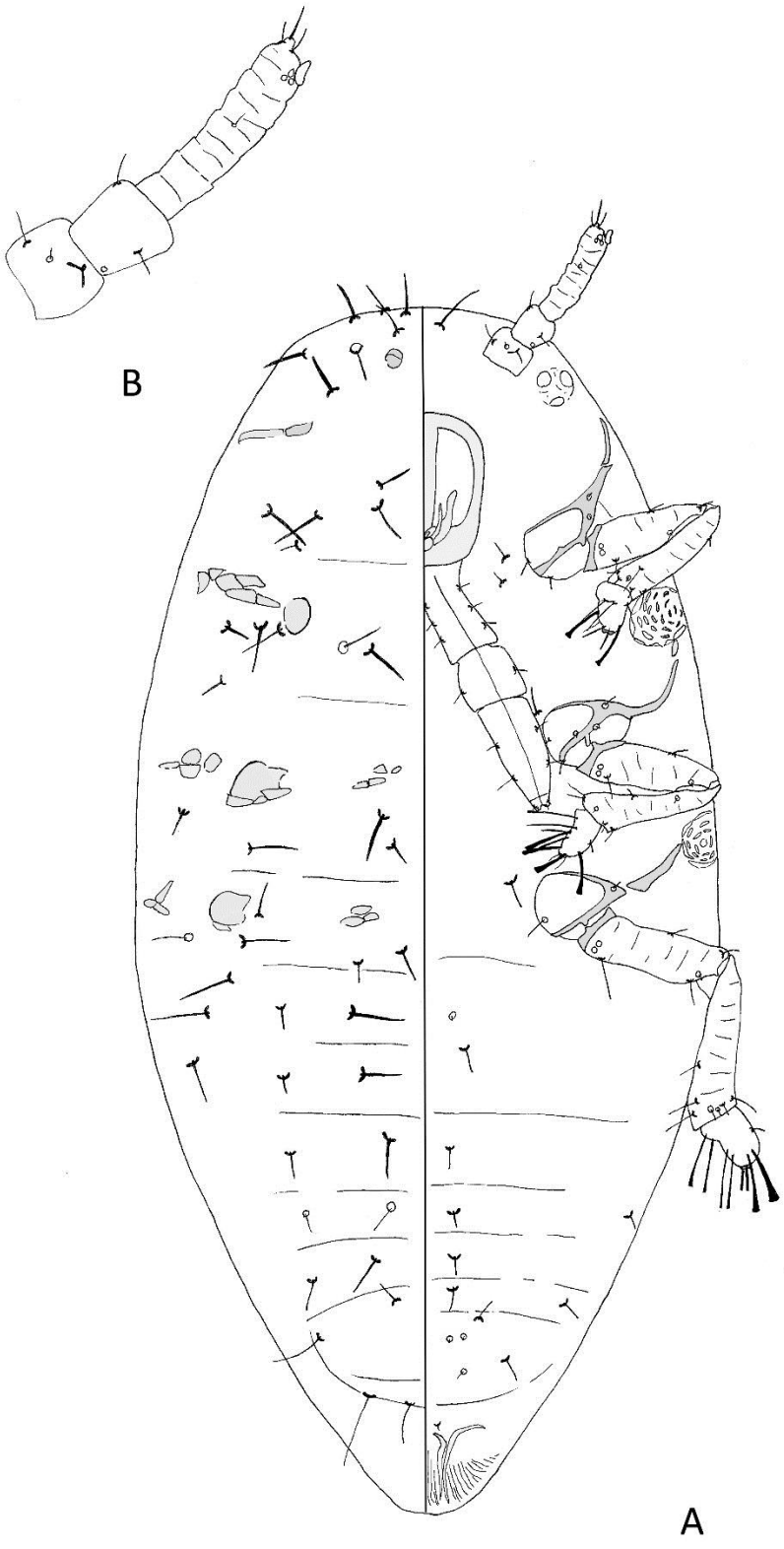

Figure 14. *Phylloxera falsostium* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

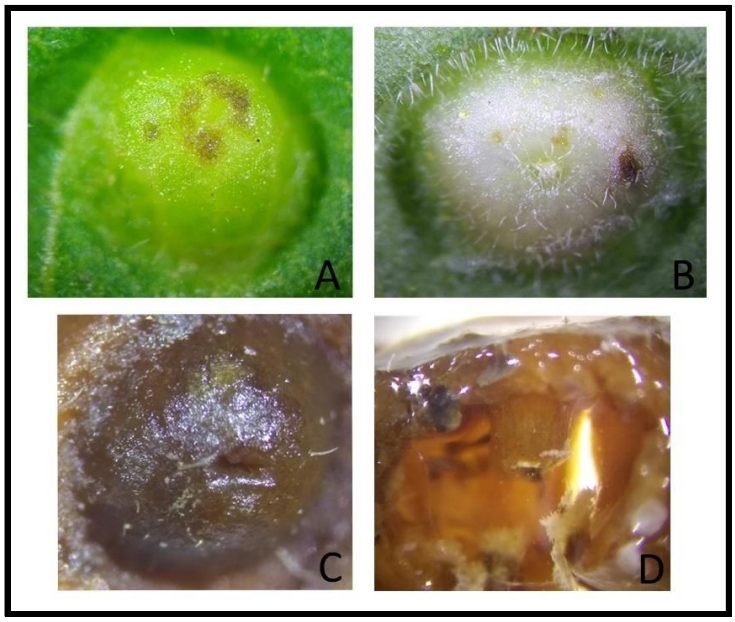

Figure 15. Gall of *Phylloxera falsostium* on adaxial surface of leaf **(A)** abaxial surface of leaf **(B)** false opening on adaxial surface **(C)** and false chamber inside gall **(D).**

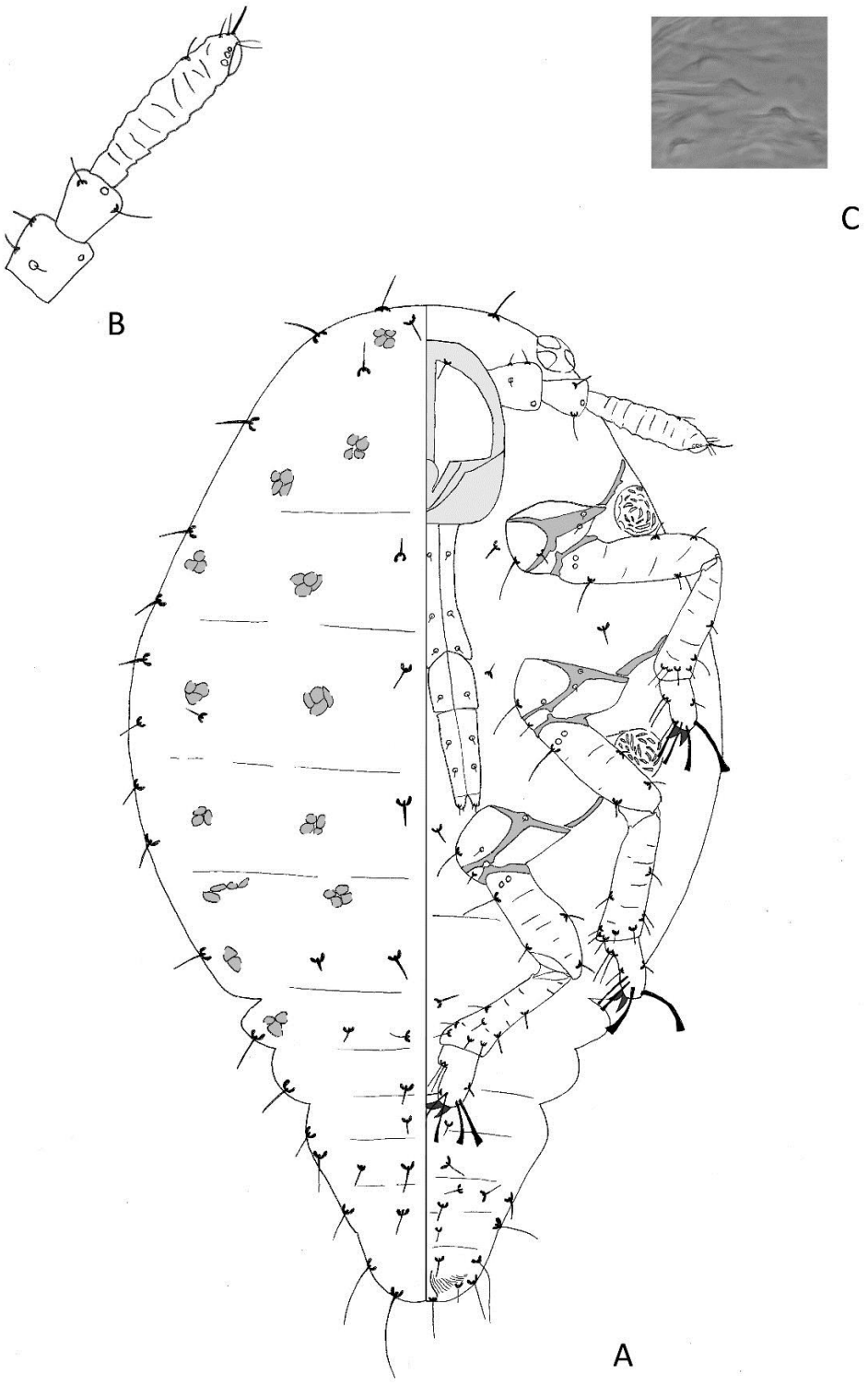

Figure 16. *Phylloxera flavoconica* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna **(C)** cuticle.

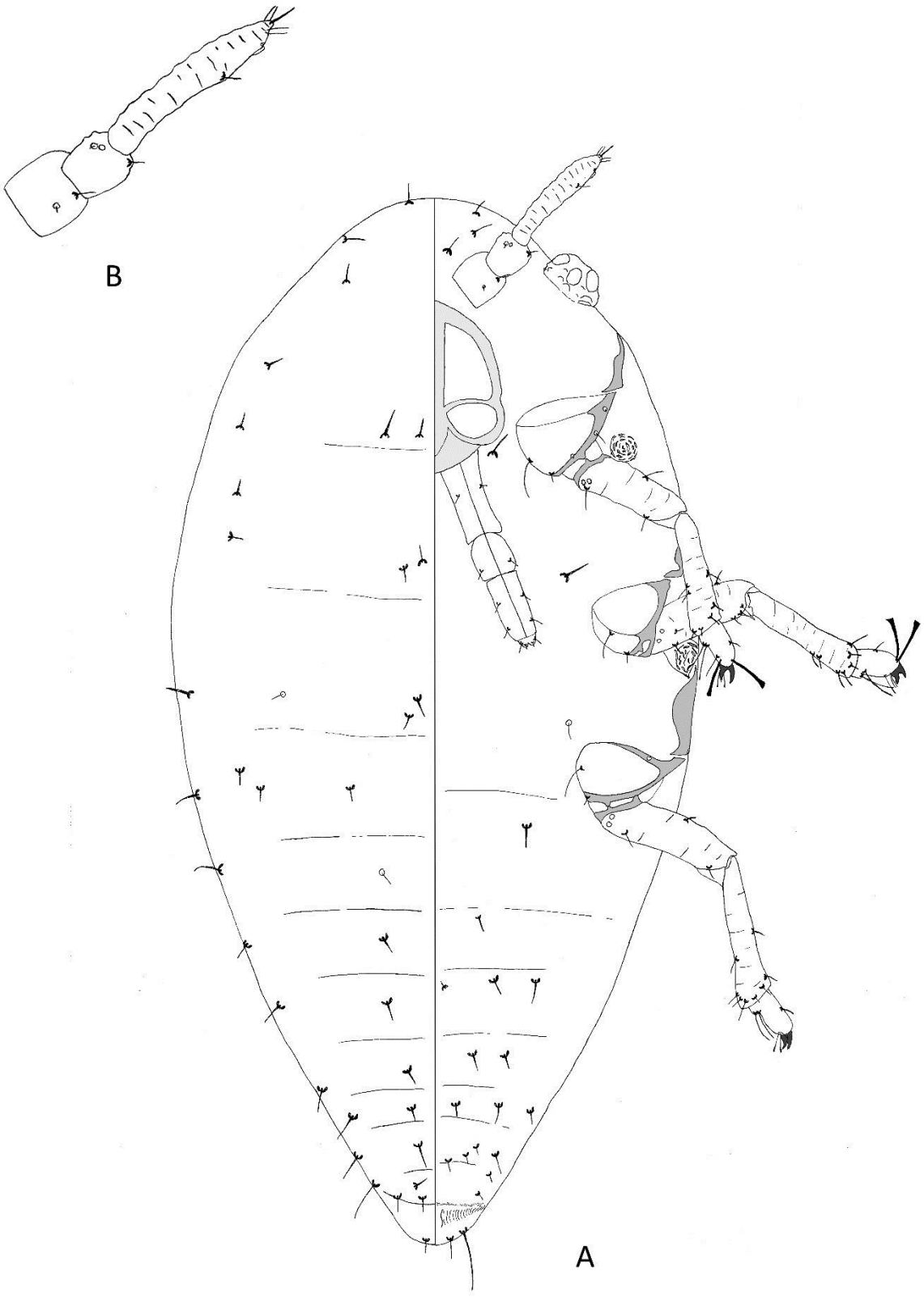

Figure 17. *Phylloxera flavoconica* Hamilton, sp. n., sexupara; **(A)** whole body **(B)** antenna.

Figure 18. Gall of *Phylloxera flavoconica* on adaxial surface of leaf **(A)** abaxial surface of leaf **(B)** and distributed in a group **(C)**.

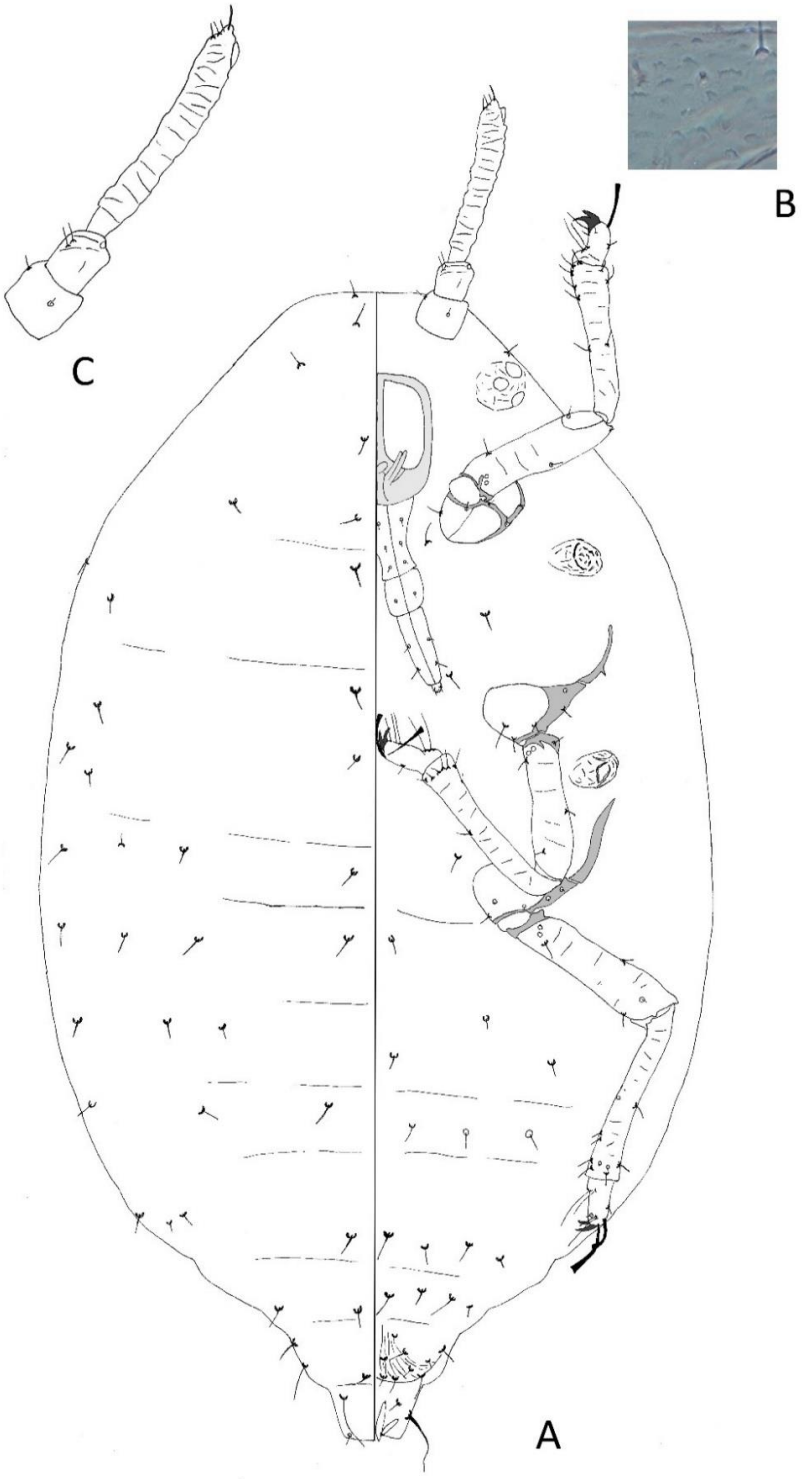

Figure 19. *Phylloxera floridana* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** cuticle **(C)** antenna.

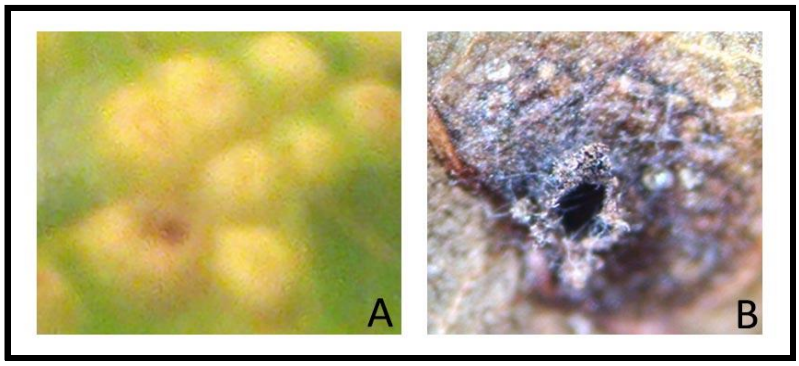

Figure 20. Gall of *Phylloxera floridana* on adaxial surface of leaf **(A)** and abaxial surface of leaf **(B).**

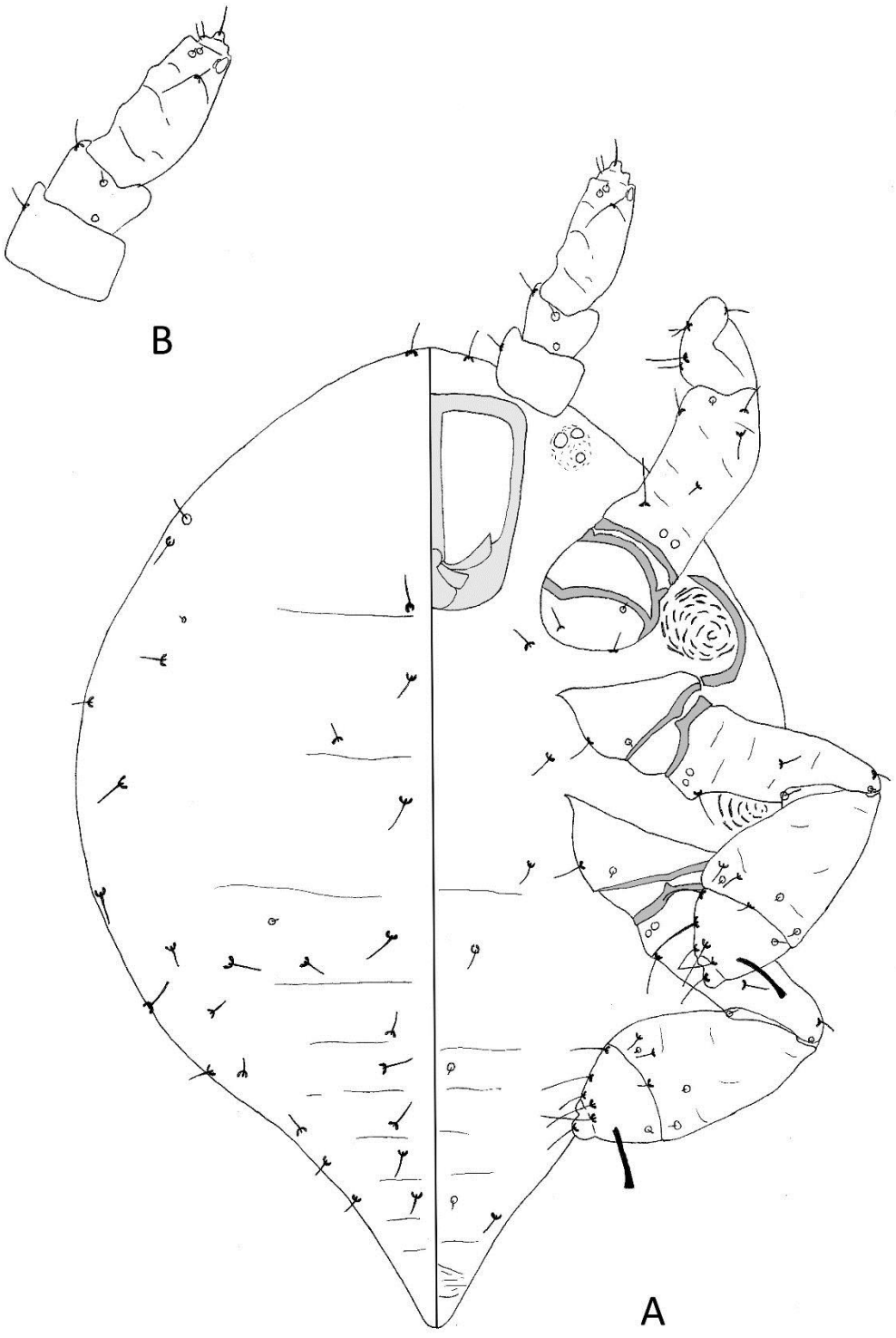

Figure 21. *Phylloxera killianae* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

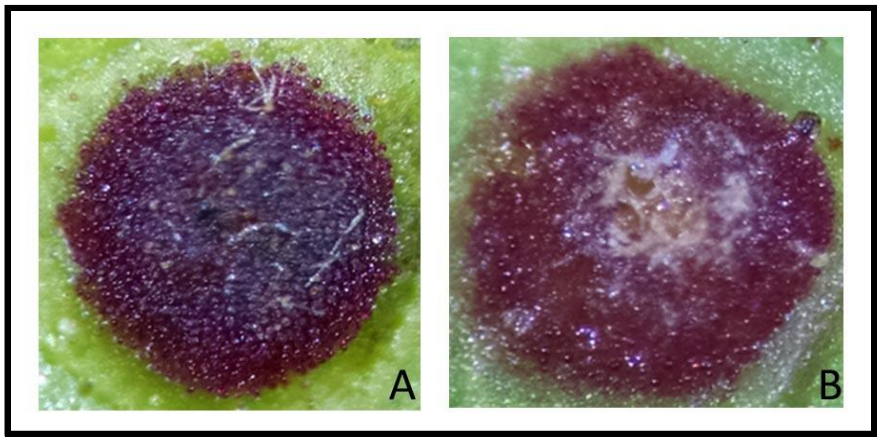

Figure 22. Gall of *Phylloxera killianae* on adaxial surface of leaf **(A)** and abaxial surface of leaf **(B).**

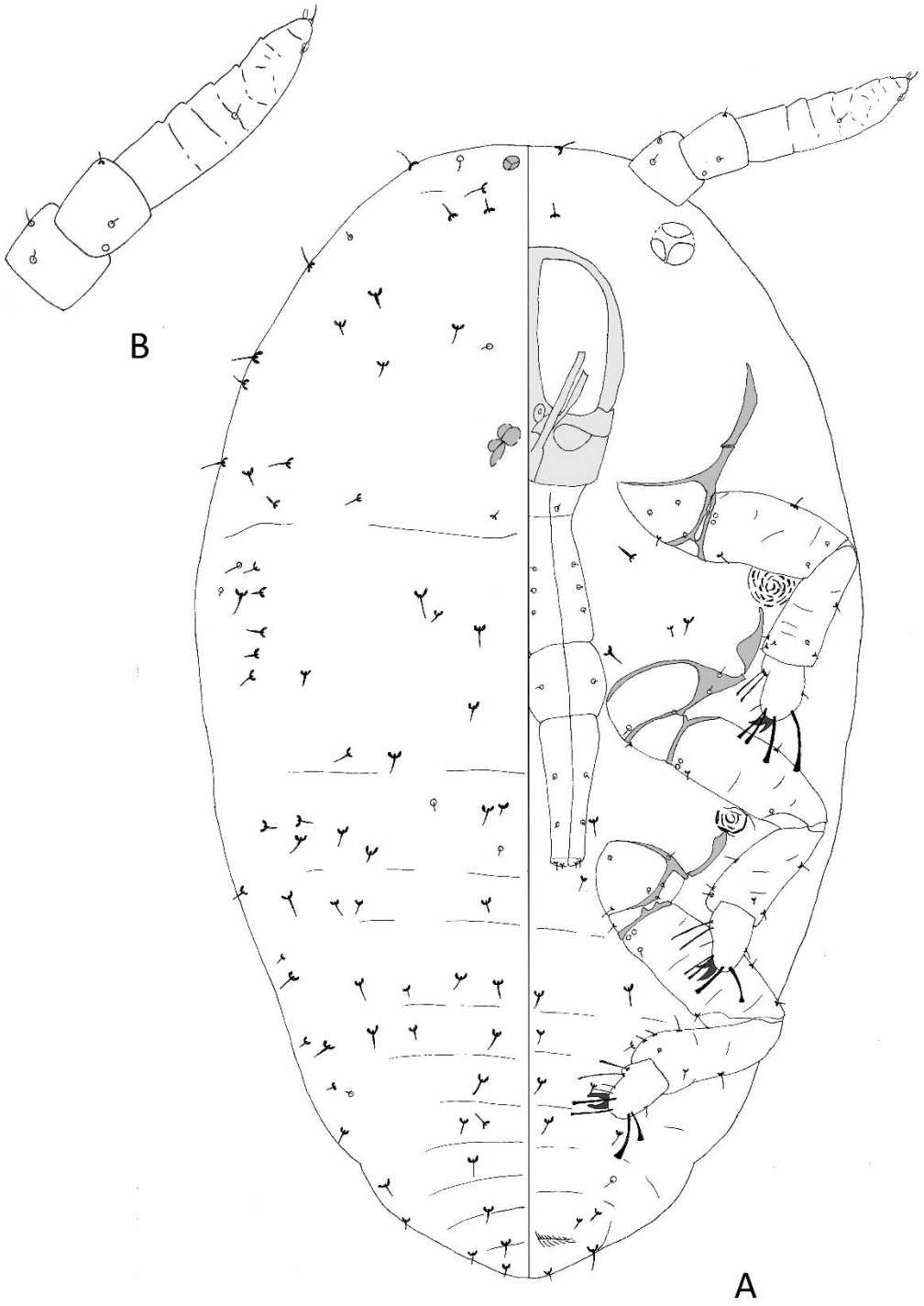

Figure 23. *Phylloxera myristica* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

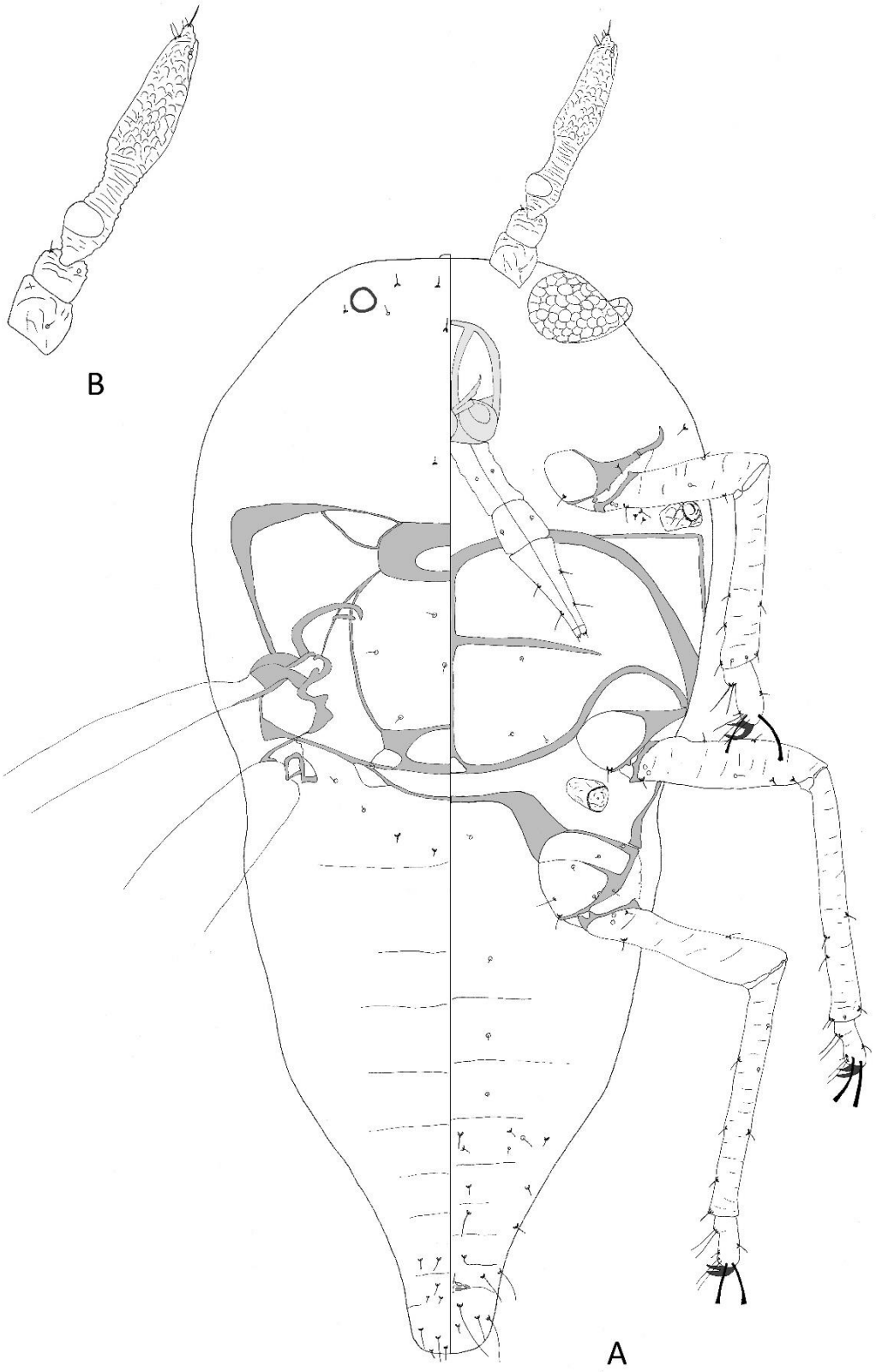

Figure 24. *Phylloxera myristica* Hamilton, sp. n., alate; **(A)** whole body **(B)** antenna.

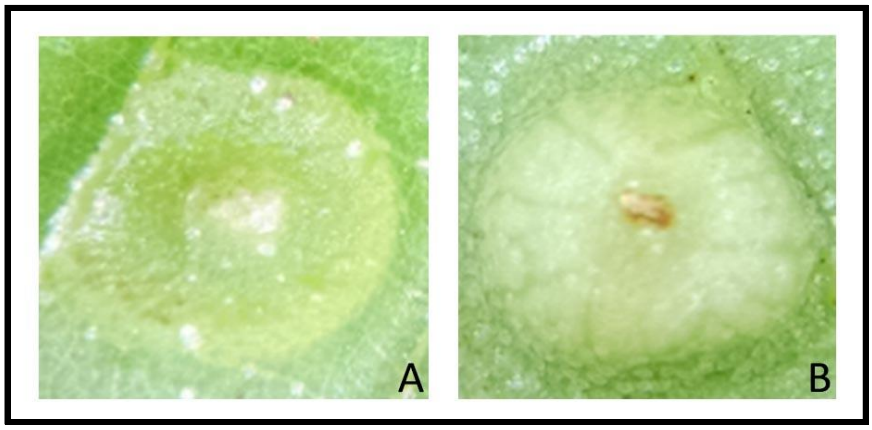

Figure 25. Gall of *Phylloxera myristica* on adaxial surface of leaf **(A)** and abaxial surface of leaf **(B).**

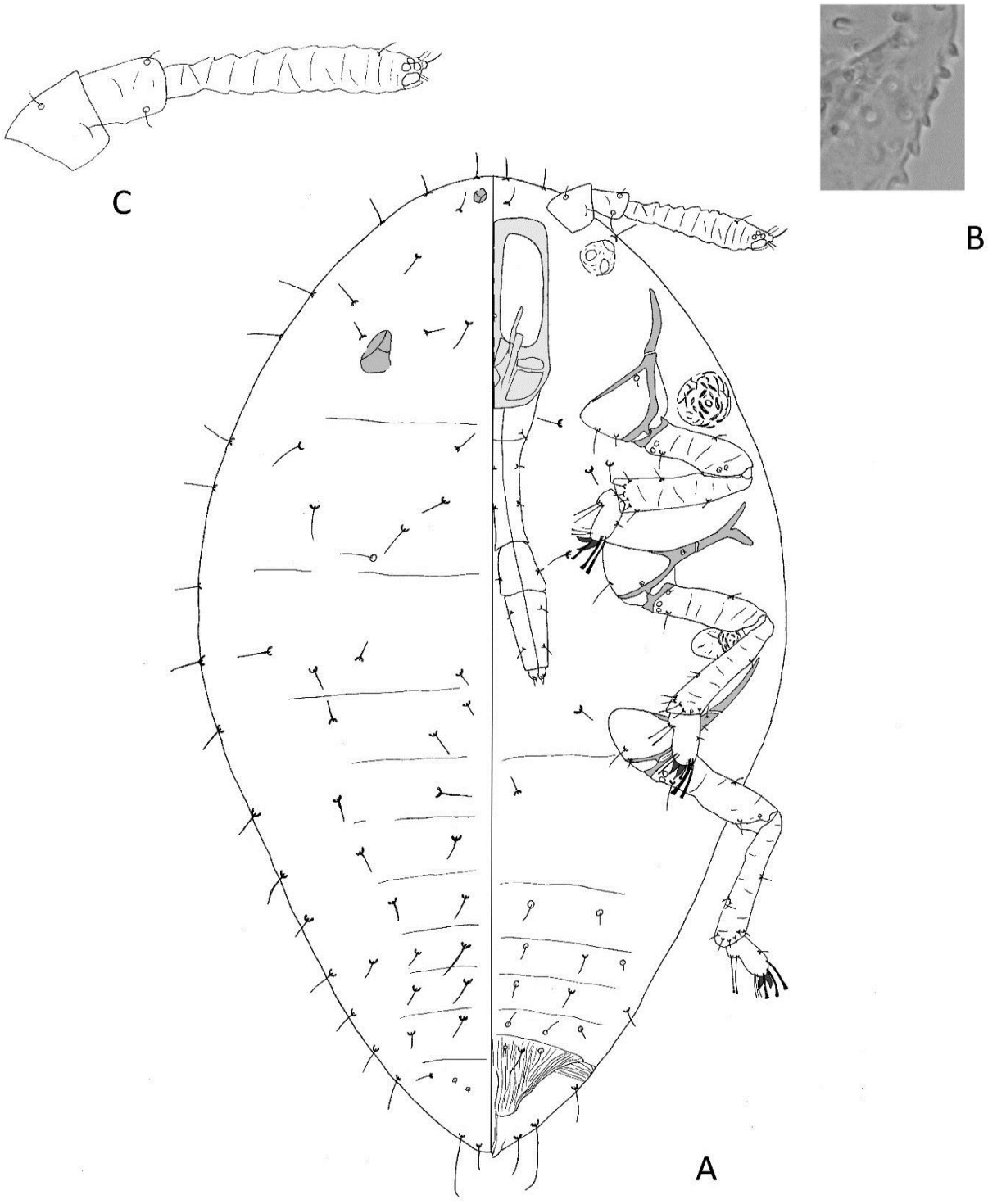

Figure 26. *Phylloxera paludis* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** cuticle **(C)** antenna.

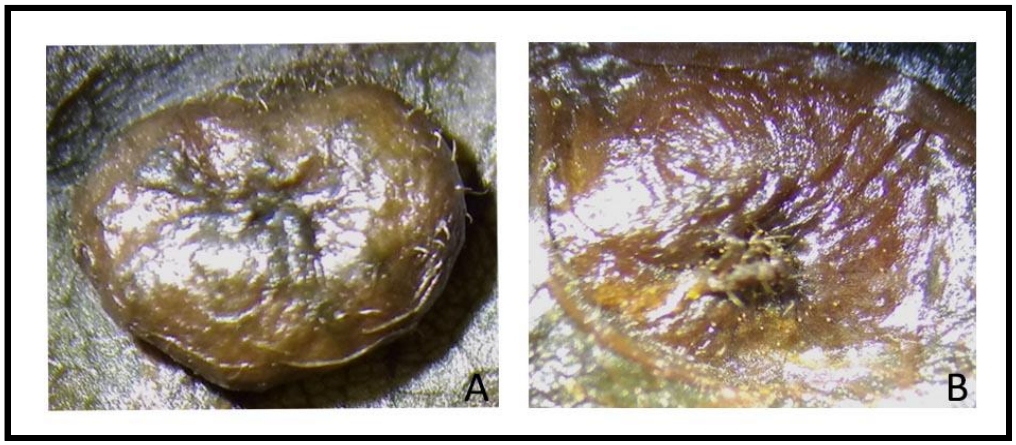

Figure 27. Gall of *Phylloxera paludis* on adaxial surface of leaf **(A)** and abaxial surface of leaf **(B).**

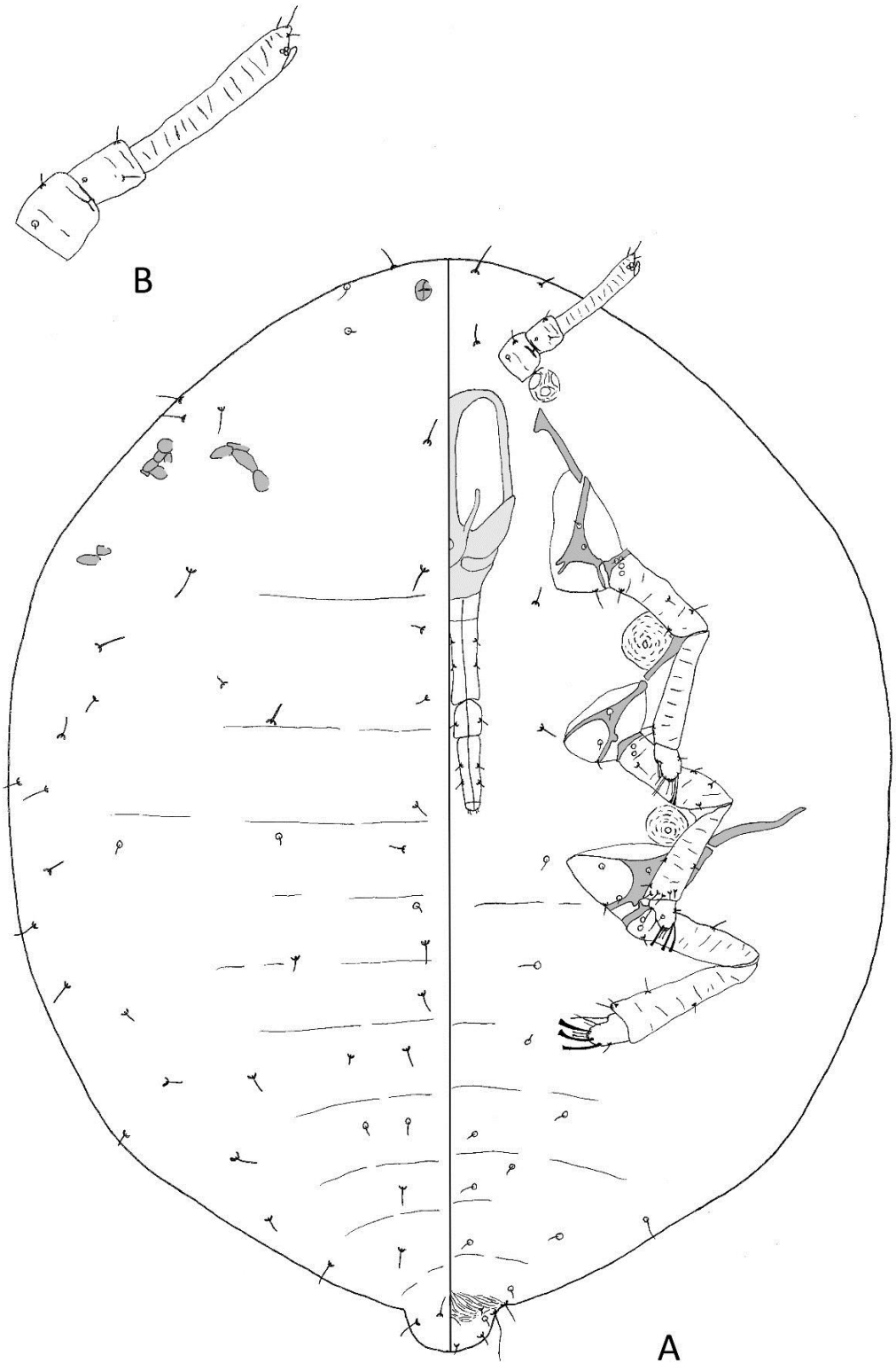

Figure 28. *Phylloxera stoetzelae* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

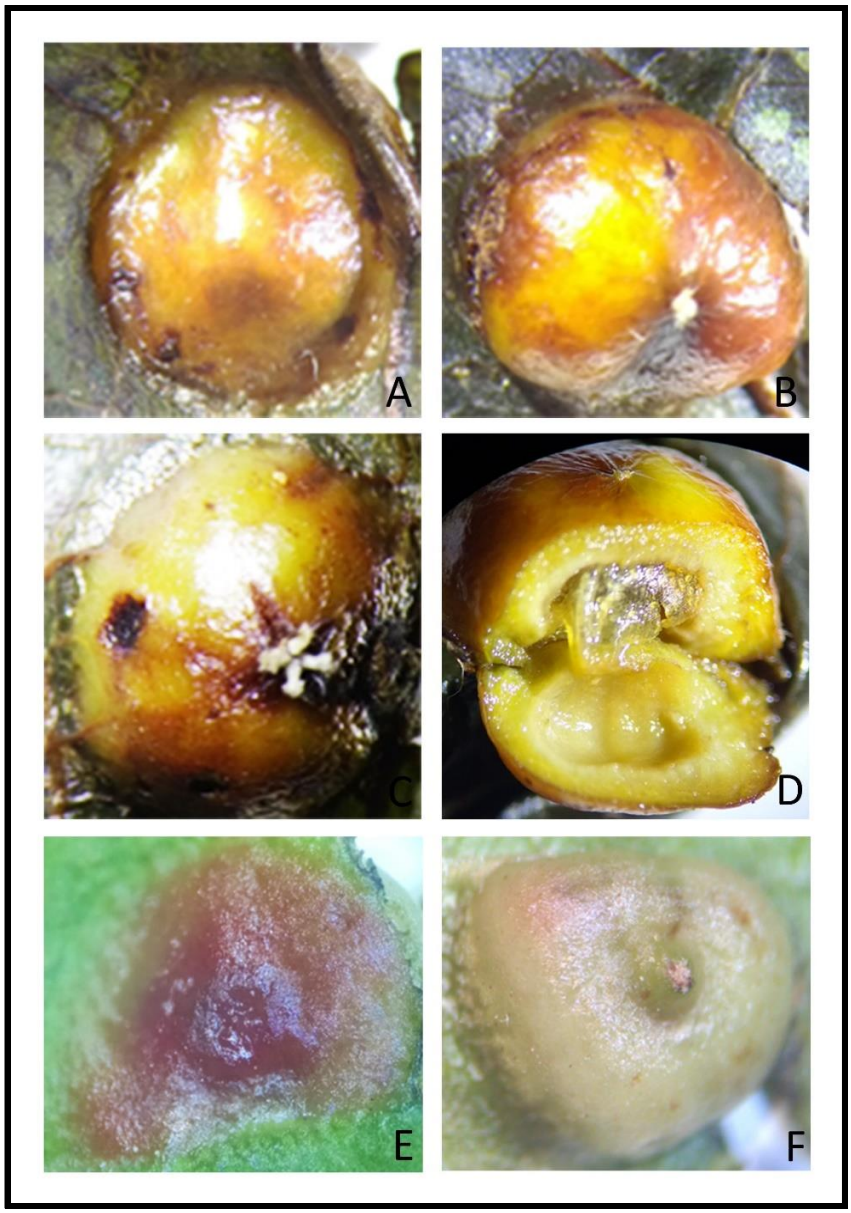

Figure 29. Gall of *Phylloxera stoetzelae* on adaxial surface of leaf on *Carya aquatica* **(A)** abaxial surface of leaf with opening tightly closed on *Carya aquatica* **(B)** abaxial surface of leaf with orifice slightly open exposing four white pubescent bracts **(C)** cross-section of gall exposing the yellowish-gold nutritive layer with phylloxerans attached to it **(D)** adaxial surface of leaf on *Juglans hindsii* **(E)** abaxial surface of leaf on *Juglans hindsii* **(F)**.

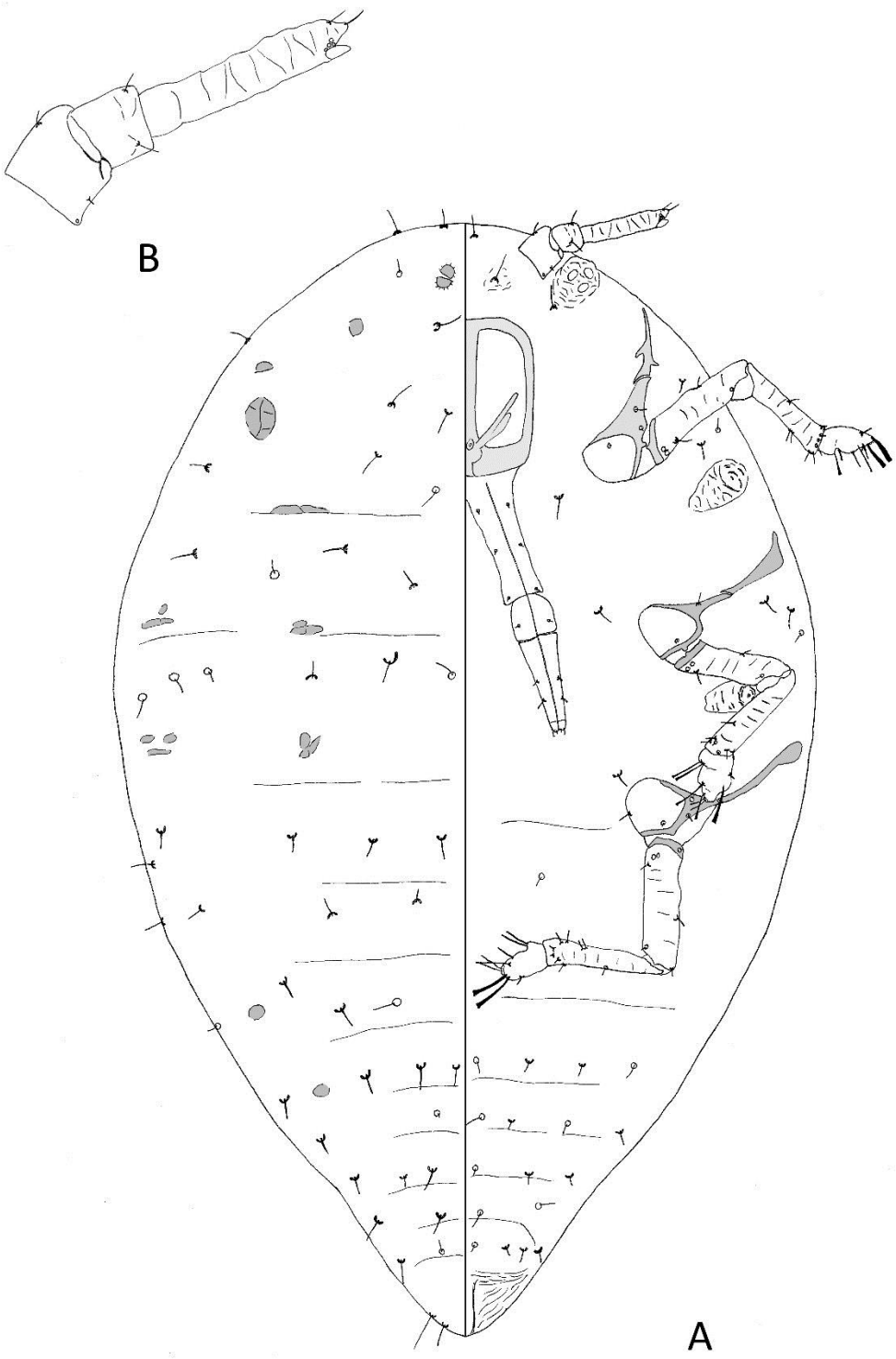

Figure 30. *Phylloxera wiedenmanni* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna.

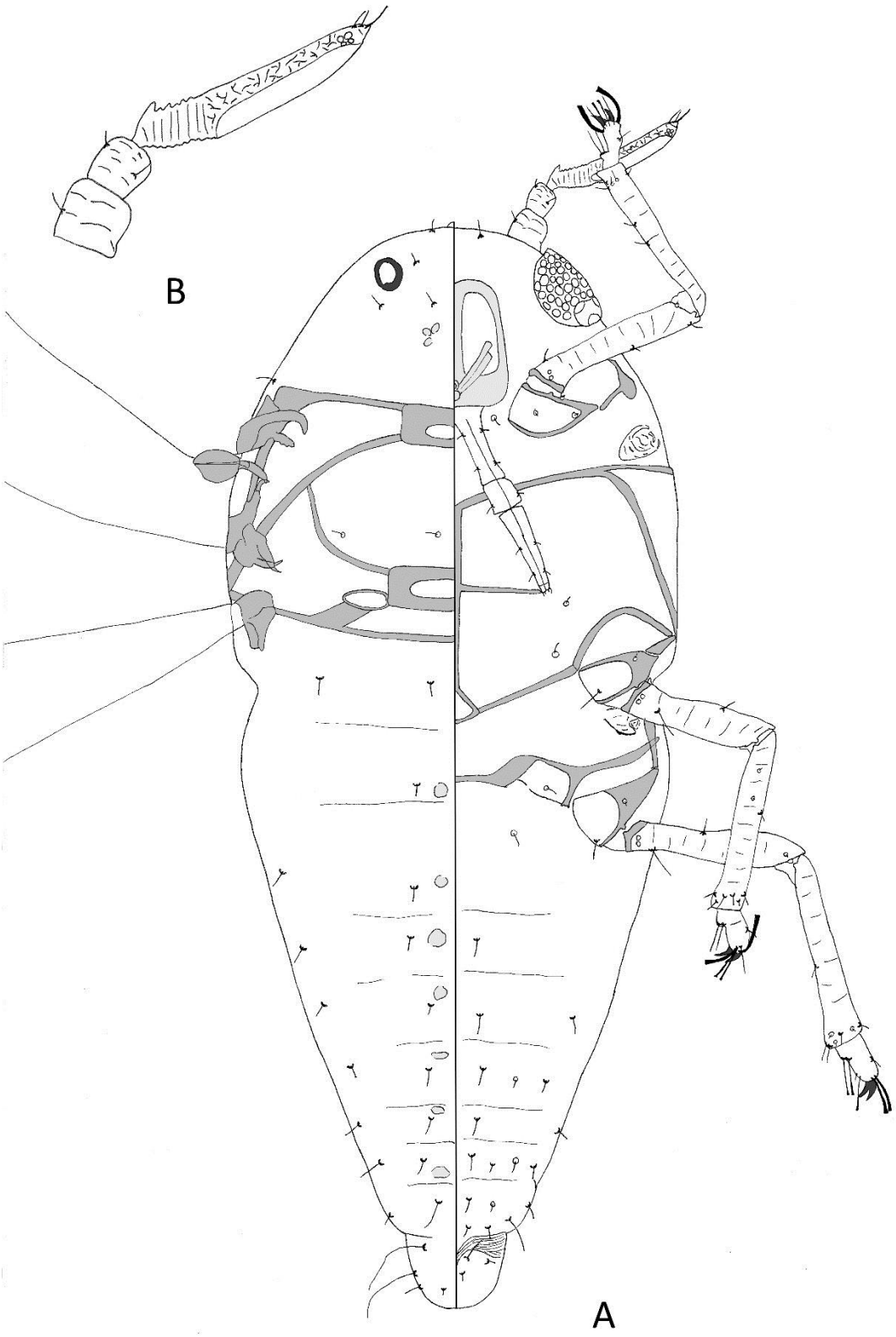

Figure 31. *Phylloxera wiedenmanni* Hamilton, sp. n., alate; **(A)** whole body **(B)** antenna.

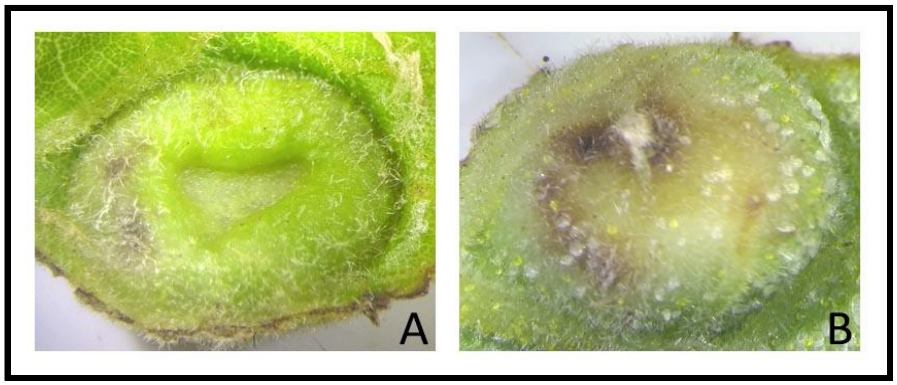

Figure 32. Gall of *Phylloxera wiedenmanni* on adaxial surface of leaf **(A)** and abaxial surface of leaf **(B)**.

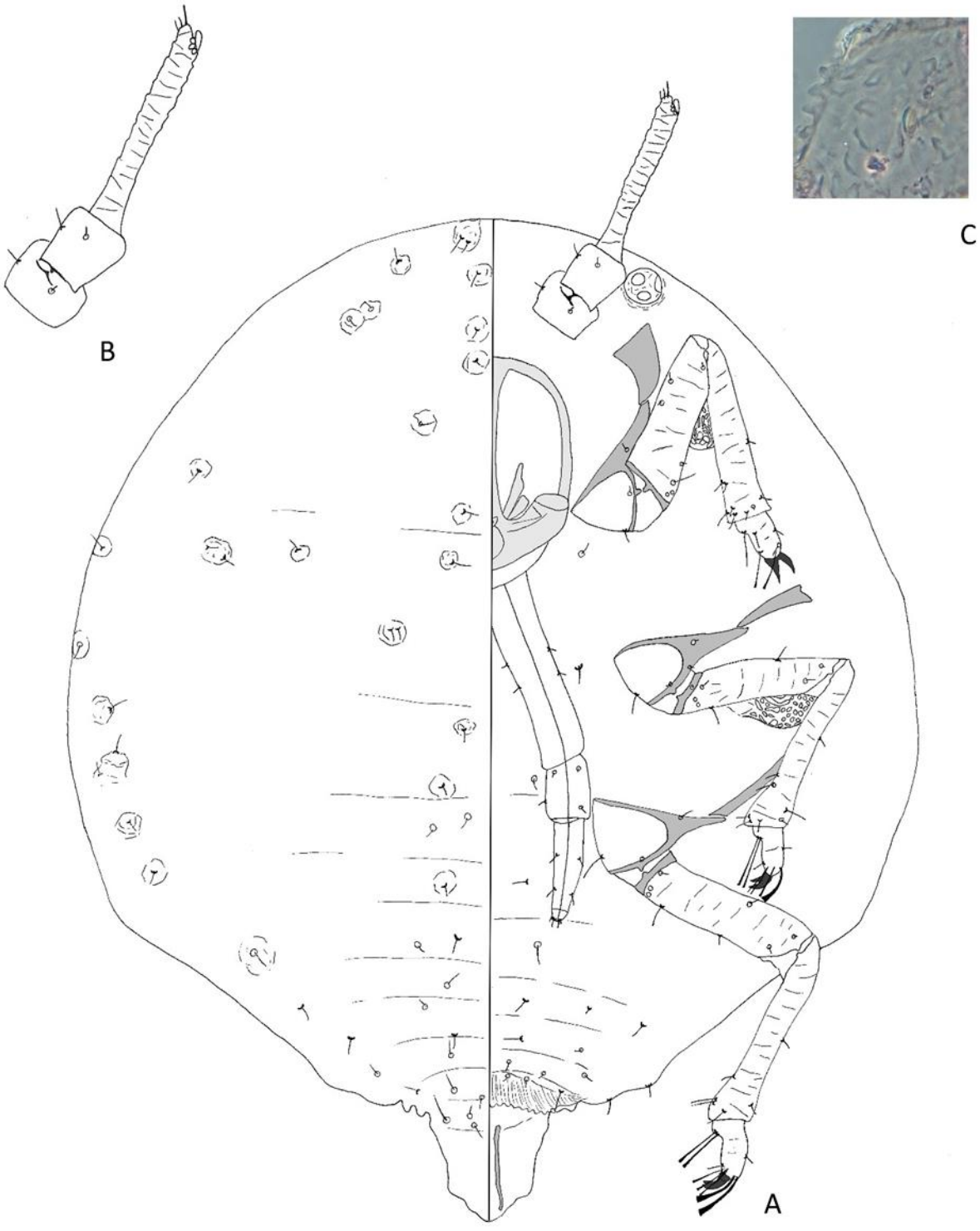

Figure 33. *Phylloxera williamsi* Hamilton, sp. n., fundatrix; **(A)** whole body **(B)** antenna **(C)** cuticle.

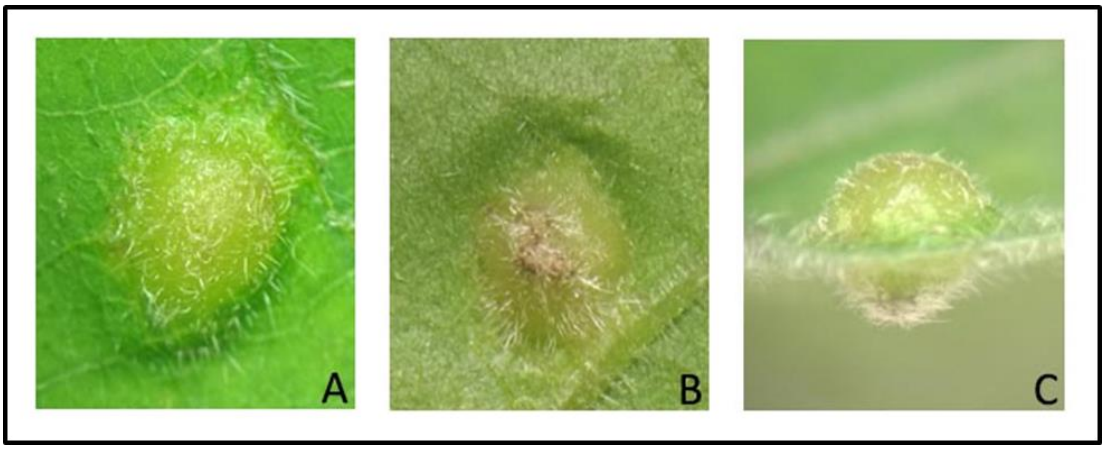

Figure 34. Gall of *Phylloxera williamsi* on adaxial surface of leaf **(A)** abaxial surface of leaf **(B)** and laterally **(C)**.

Acknowledgments

We thank the U.S. Forest Service, U.S. Fish and Wildlife Service, The Nature Conservancy, and state parks for the issuance of collecting permits and waivers to collect specimens from the following locations: Bankhead National Forest, Talladega National Forest, Francis Marion National Forest, Sumter National Forest, Nantahala National Forest, Bienville National Forest (Permit No. 0412171), Delta National Forest (Permit No. 0412171), Holly Springs National Forest (Permit No. 0412171), Tombigbee National Forest (Permit No. 0412171), Kisatchie National Forest, Davy Crockett National Forest, Ouachita National Forest, Ozark National Forest (Permit No. OZF-FW-FY17-05), St. Francis National Forest (Permit No. OZF-FW-FY17-05), George Washington National Forest, Monongahela National Forest (Permit No. FS-2400-8), Hawks Nest State Park, Eastern Shore of Virginia National Wildlife Refuge (Permit No. R18-001), Yankauer Nature Preserve, Florida Caverns State Park (Permit No. 16040411), Warriors Path State Park (Permit No. 2016-015), Martin Dies Jr. State Park, Fort Boggy State Park, Lake Bob Sandlin State Park, Lake Wister State Park, Natural Falls State Park, Devil's Den State Park (Permit No. 017-2017), Louisiana State Arboretum, Chippokes Plantation State Park (Permit No. CPPO-RCP-032118), Trap Pond State Park (Permit No. 2018-WSC-009), White Clay Creek State Park (Permit No. 2018-WSC-009), and Cunningham Falls State Park (Permit No. 2018DNR028). We thank the American Museum of Natural History for the Theodore Roosevelt Travel Grant, which supported our third year of field work. We also thank Chloe Kaczvinsky, Micaela Finney, and Mayrolin Garcia Morales for assistance with field collections and Cara Ratterman for assistance with slide-mounting. We greatly appreciate Dr. Gary Miller for granting us access to study the national collection of *Phylloxera* spp. at the USDA Systematic Entomology Laboratory in Beltsville, MD.

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Conclusion

This dissertation addresses three objectives: 1) Perform the first estimate of the phylogenetic relationships among phylloxeran species and test the monophyly of the accepted genera and hosts; 2) Revise the species diversity; and 3) Characterize their gall morphologies and hosts.

Chapter II presents the first phylogeny of *Phylloxera* spp. that were collected from across the U.S. on all 11 species of hickory, three species of oak, two species of walnut, and two species of chestnut (with one of the chestnut-feeding species collected from China and *Phylloxera coccinea* collected from Canada). The phylogeny from the combined dataset is composed of seventeen putative new *Phylloxera* spp., 25 described *Phylloxera* spp., and three undetermined *Phylloxera* spp. *Phylloxera caryaemagna* is now considered a separate species rather than a variety of *P. caryaecaulis* due to its placement in a separate clade. Phylloxerans clustered into three major clades, which correspond with their gall morphologies and include species that produce galls with elongate openings, galls with round openings, and galls with slit openings or galls with conical or thorny openings. Therefore, species that are more closely related have more similar gall morphologies. *Phylloxera* and *Phylloxerina* were determined to be monophyletic. Host-use groups in phylloxerans were determined to not be monophyletic.

Chapter III describes 14 new hickory and walnut-feeding *Phylloxera* spp. Three previously described species (*P. foveata, P. globosa,* and *P. minima*) lacked type specimens and

were designated as *nomina dubia*. The new total of described hickory and walnut-feeding *Phylloxera* spp. in the U.S. is raised to 44 which increases their diversity by 50%. Several new host records were recorded including *Carya texana* (black hickory), *Carya myriticiformis* (nutmeg hickory), and *Carya pallida* (sand hickory) as well as *Juglans hindsii* (Northern Califonia walnut) and *Juglans nigra* (black walnut), which represents a new host genus. Lastly, a dichotomous key to galls of hickory and walnut-feeding *Phylloxera* spp. was composed.

This research has laid the groundwork for future *Phylloxera* studies in the U.S. First, new *Phylloxera* spp. were described from only two of the three major clades, which form galls with openings that are round, slit, or conical or thorny. The third clade that forms galls with elongate openings along leaf veins requires further taxonomic work since five putative new species from this group have been identified and are awaiting description.

Second, *Phylloxera* galls exhibit many different colors, shapes, and sizes. This variation in gall morphology is not well understood and is thought to be driven by natural enemy interactions (Stone and Schonrogge 2003, Bailey et al. 2009). For each gall dissected, approximately 23 different measurements were taken and these could be used to study gall architecture. In addition, natural enemies were collected from dissected galls and could be used to examine the correlation between certain gall morphological traits such as opening type and natural enemies associated with them.

Third, walnuts need to be the focus of a *Phylloxera* study. A new gall forming species, "*Phylloxera stoetzelae*," was collected on *J. hindsii* near Savannah, GA. Two other species (*Phylloxera foveola* and an undetermined *Phylloxera* sp.) were collected on *J. nigra* in Oklahoma and Arkansas. Two walnut species have overlapping ranges with hickory species including: *Juglans cinerea* (butternut) occurs throughout the Mid-Atlantic, Midwest, Northeast,

and parts of the southern U.S.; *J. nigra* has a more widespread distribution occurring throughout the Mid-Atlantic, Midwest, Northeast, Southeast, and the South Central U.S (Kirkman et al. 2007, Fryer 2018). Since walnuts are chemically and morphologically similar to hickories (Rietveld 1983, Nelson et al. 2014), it seems likely that there are other undescribed *Phylloxera* spp. inhabiting them.

Lastly, future collections may be made in the Northeast and Midwest since not much is known about the diversity of phylloxerans in these areas. In addition, Mexico should also be sampled since several hickory species (*Carya illinoensis*, *Carya myristiciformis*, and *Carya ovata*) are distributed there (Maisenhelder and Francis 1990, Fryer 2018) and no phylloxerans have been recorded or described from there.

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Appendix 1. List of sites where *Phylloxera* spp. were collected from 2015–2018. Additional samples that were sent to me have collector's name in parentheses.

1. Archbold Biological Station, FL
2. Davis Arboretum, AL
3. Lake Wilmore Park, AL
4. Hickory Dickory Park, AL
5. Bankhead National Forest, AL
6. Talladega National Forest, AL (Collector – Dr. Charles Ray)
7. Florida Caverns State Park, FL
8. A.J. Henry Park, FL
9. Dauset Trails, GA
10. J.F. Gregory City Park, GA
11. Tom Triplett Park, GA
12. Edisto Nature Trail, SC
13. Francis Marion National Forest, SC
14. Sumter National Forest, SC
15. Nantahala National Forest, NC (site 1)
16. Warriors' Path State Park, TN
17. Mills Park, TN
18. Nantahala National Forest, NC (site 2)
19. Chattahoochee National Forest, GA
20. ALFA building AU, AL
21. Mobile, AL (Collector – John Olive)
22. Bienville National Forest, MS
23. Delta National Forest, MS
24. Kisatchie National Forest, LA
25. Louisiana State Arboretum, LA
26. Mercy Regional Medical Center, LA
27. Martin Dies, Jr. State Park, TX
28. Davy Crockett National Forest, TX

29. Fort Boggy State Park, TX
30. Lake Bob Sandlin State Park, TX
31. Ouachita National Forest, OK
32. Lake Wister State Park, OK
33. Ouachita National Forest, AR
34. Lake Wilson City Park, AR
35. Natural Falls State Park, OK
36. Stillwater, OK (Collector – Dr. Mike Palmer)
37. Mehan, OK (Collector – Dr. Mike Palmer)
38. Lake Wedington, AR
39. Devil's Den State Park, AR
40. Ozark National Forest, AR
41. St. Francis National Forest, AR
42. Holly Springs National Forest, MS
43. Tombigbee National Forest, MS
44. George Washington National Forest, VA (site 1)
45. George Washington National Forest, VA (site 2)
46. Chippokes Plantation State Park, VA
47. Eastern Shore of VA National Wildlife Refuge, VA
48. Trap Pond State Park, DE
49. White Clay Creek State Park, DE
50. Cunningham Falls State Park, MD
51. Yankauer Nature Preserve, WV
52. Monongahela National Forest, WV
53. Hawks Nest State Park, WV

Appendix 2. Alternative key to gall forming *Phylloxera* spp. in the U.S. In species descriptions, above means adaxial leaf surface and below means abaxial leaf surface. Pictures for described species (if available) are below each description with left picture being of the gall above and the right picture being of the gall below. If species epithet is followed by sp. n. please refer to Figures section for gall pictures.

Group I

Elongate gall occurring along leaf vein with an elongate slit opening beneath

1. Elongate sclerotized ridge above that is green, yellow, or pink with a protruding pubescent fold of tissue below on each side of elongate slit opening which is light green or pink; length 6.0–14.0 mm ……..……...…..……..*Phylloxera caryaevenae* Fitch

Group II

Galls that are button-like, conical, or globular with a slit-like opening beneath. Some species may also occur on petioles or stems.

Galls that are button-like

2. Round and convex above; red, light green, yellowish-green, or white in color; diameter 8.0–14.0 mm; below level with leaf tissue or convex, light green, and opening a pubescent transverse slit….. ……...……..*Phylloxera caryaescissa* Riley

3. Round and slightly convex above, yellow, and with or without a depression in center; diameter 1.1–5.2 mm; below more convex with a protruding slit-like opening fringed with whitish pubescence…..…….....*Phylloxera floridana* sp. n.

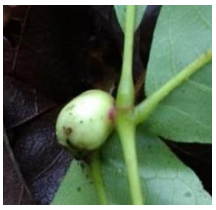
Galls that are conical

4. Conical and yellow above, more convex above than below, diameter 1.1–5.0 mm; light yellowish-green and with a pubescent slit-like opening below…… ……..…….. …….*Phylloxera flavoconica* sp. n.

5. Conical or round and somewhat flattened above, convex, yellow or red, diameter 1.0–5.0 mm; below light green, often more convex below than above, and a protruding clam-like opening with white pubescence……..……..*Phylloxera caryaefallax* Riley

Galls that are somewhat globular

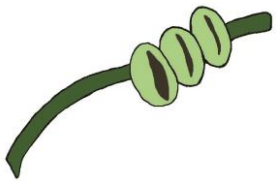
6. Globular and smooth, hard, pale yellowish-green with a hint of crimson, diameter 5.0–25.0 mm; occurs on petioles with an irregular opening or along mid-vein of leaves with a slit-like opening below……..……..……*Phylloxera caryaecaulis* (Fitch)



7. Globular above, much more convex above than below, light green or yellowish-green sometimes with a hint of red, often with several small openings near apex of gall, and occurs between leaf veins; below almost white and opening a pubescent transverse slit …….....*Phylloxera caryaeglobuli* Walsh

8. Globular and light green or yellowish-white, some with a hint of crimson, spongy with short pubescence, diameter 4.0–20.0 mm; occurs along midvein of leaf with gall tissue appearing to protrude through leaf tissue above and below much more convex with a slit-like opening when mature; also occurs on petioles……*Phylloxera caryaemagna* (Shimer)

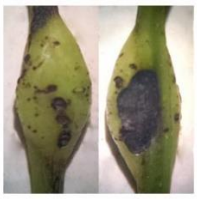
9. Kidney-shaped and hard, light green, and densely pubescent; diameter 2.0–15.0 mm; occurs on petioles, stems, and along underside of midvein of leaves; usually occurs in clusters; opening a transverse slit that spreads apart as the gall matures dividing the gall into two equal halves……..…….....…..…….*Phylloxera caryaeren* Riley



10. Globular and covered in short spines; crimson, white, white with crimson, or yellowish-green; occurring along midvein of leaf or petiole; opening slit-like or irregular…….. …..……..……..……*Phylloxera spinosa* (Shimer)

11. Globular and densely covered in long slender spines, greenish-yellow or brownish-yellow, and diameter 5.0–15.0 mm; occurs on petioles or along midvein of leaves; opening slit-like or irregular……..*Phylloxera spinuloides* Pergande

12. Oval and mottled green, glabrous, diameter about 10.0 mm and length about 20.0 mm; occurring on side of petiole or completely surrounding it with a slit-like opening apparent when mature..……..…...…….…..……..*Phylloxera subelliptica* (Shimer)



13. Globular and yellowish-green above with some long white pubescence, slightly more convex above than below, and diameter 2.2 mm; below pubescent and with a protruding slit-like opening fringed with white pubescence……...…*Phylloxera williamsi* sp. n.

Group III

Galls that are somewhat conical or thorny with nipple or elongate thorn-like openings

Galls with nipple or thorn-like openings on both sides of leaf

14. Slender conical projections on both sides of leaf; green, crimson, or green and crimson; diameter 1.0–7.0 mm; occurs along midvein and more convex above than below. Openings thorny and surrounded by slender bracts on both surfaces……
……..……*Phylloxera bispinae* sp. n.

15. Elongate and slender or short and stout conical projections on both sides of leaf; light rose, crimson, yellowish-green, or light green and crimson towards apex; more convex above than below; elongate and slender galls with a diameter 2.0–4.0 mm; short and stout galls with a diameter of 5.0–12.0 mm; resembling either a thorn or sea anemone. An opening at apex on each side fringed with filaments……*Phylloxera caryaesepta* (Shimer)

16. Slightly convex on both sides of leaf with a conical nipple opening on each side; diameter 3.0–6.0 mm; color variable: dark green and nipple and ring around base red, concolorous with leaf surrounded by a purple ring and buff around base, pink and margin yellow or green, green with the nipple buff and yellow ring around base, or yellowish-green with a purple ring surrounding nipple. Upper nipple splits into several recurved pubescent bracts at maturity and lower nipple remains closed ……*Phylloxera picta* Pergande

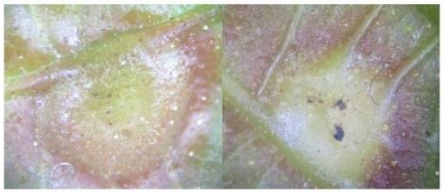
Galls with a nipple or thorn-like opening on one side of leaf or stem

17. Conical and pale yellowish-green above with a pale ring surrounding base, apex splits at maturity into several bracts, and diameter about 5.0 mm; flat beneath with a round opening surrounded by slender pubescent filaments……*Phylloxera caryaefoliae* Fitch

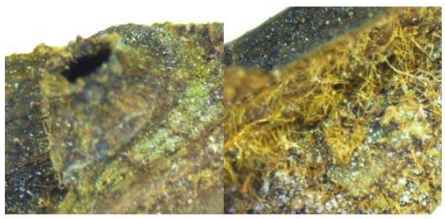
18. Round and convex above, a depression or a central elevation near middle, green or yellowish-green, and shiny and slightly pubescent; diameter 1.4–2.4 mm; below more convex with a light green thorny projection; transparent especially near base; thorn-like opening splits into several long bracts at maturity……..*Phylloxera chippokesiensis* sp. n.

19. Round, slightly convex, and concolorous to surrounding leaf tissue on both surfaces of leaf with a slight depression in center above; diameter 3.5–4.6 mm. Below opening a green stem or thorn-like projection……*Phylloxera crypta* sp. n.

20. Round and slightly convex above, red with a yellow central depression or entirely greenish-yellow, and diameter 1.0–5.0 mm; below more convex and often conical, purplish or greenish-yellow, and nipple with oval orifice fringed with pale pubescence…. …….*Phylloxera deplanata* Pergande



21. Convex or conical above, red or yellow surrounded by a pale ring, and diameter 3.0–6.0 mm; before maturity there is no apparent opening and at maturity bracts split at apex into a jagged opening; below slightly convex and paler than leaf with a small closed nipple which may or may not open …...…….……..*Phylloxera intermedia* Pergande



22. Thorny, yellowish-white, and smooth; diameter 2.0–8.0 mm; occurs on petioles, stems, or along midvein of leaves with the underside usually more convex. Opening is elongate and thorn-like……..……..*Phylloxera perniciosa* Pergande

Group IV

Galls that are button-like, globular, nut, pear, or spindle-shaped with a round opening

Galls that are more convex above

23. Round and convex above, red or yellowish-green with a depression in center, small and seed-like, diameter 0.3–2.0 mm; slightly convex below and light green. Nipple opening whitish surrounded by several short pubescent bracts…*Phylloxera caryaesemen* (Shimer)

24. Globular and more convex above; crimson, greenish-yellow, or purplish-brown; diameter 3.0–7.0 mm; slightly convex below or sunken below leaf tissue and light green. Nipple opening round, pubescent, and splits into 4–8 short bracts at maturity…….……..……...…….*Phylloxera conica* (Shimer)

25. Globular and more convex above, pinkish, and with long yellowish-white pubescence; diameter 1.3–1.8 mm; below slightly convex, light green, and pubescent. Opening round and surrounded by short yellowish-white pubescence …….....*Phylloxera echinus* sp. n.

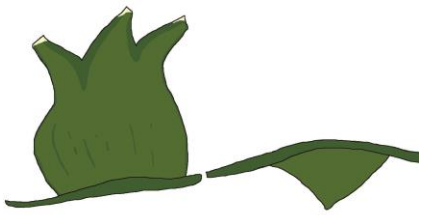
26. Round and convex or slightly sunken below leaf surface with a distinct rim above and yellowish-green; diameter 2.3–4.5 mm; with or without an apparent slit-like opening in middle which leads down into a false chamber; below round, pubescent, almost white, and sunken below leaf surface with a rim around circumference. Opening below round and surrounded by white pubescence which splits into 5–6 small bracts at maturity……...
…….……..……*Phylloxera falsostium* sp. n.

27. Button-like and more convex above than below, yellowish-green circumference with a pale red depression or completely yellowish-green with a pink dimple, depression may be shallow or deep, and diameter 3.0–5.0 mm; below slightly convex or depressed and yellowish-green with a short nipple which splits into 4–8 flat recurved bracts…….
…… ……..…..…*Phylloxera foveola* Pergande

28. Oval or round and more convex above, yellowish-green or slightly red, with or without a central depression, pubescent, and diameter 2.0–10.0 mm; below somewhat conical, green or yellow, and pubescent. Occurs along midvein of leaf and opening stem-like which splits into several long bracts……...……..*Phylloxera notabilis* Pergande

29. Round and more convex above, light green to brownish-green, shiny and slightly pubescent, with a shallow or deep depression in center, some with a seam along middle of circumference, and diameter 3.0–4.5 mm; below flattened and round, light green to brownish-green, shiny, and slightly pubescent. Opening below round and surrounded by 5–6 short bracts with pubescence at tips……...…….*Phylloxera paludis* sp. n.

30. Globular and much more convex above; diameter 4.0–6.0 mm; opening occurs above with apex of gall splitting into several stout bracts; below raised to a point……. ……*Phylloxera texana* Stoetzel



31. Button-like and with a depression in center; more convex above than below; occurs above plane of leaf or slightly sunken below plane forming a rim around it;

circumference light green and pubescent; depression almost white or yellow and translucent; diameter 5.0–9.1 mm; below round or oval, light green, and pubescent. Opening below round and surrounded by 6–7 pubescent bracts……...

……...……...*Phylloxera wiedenmanni* sp. n.

Galls that are more convex below

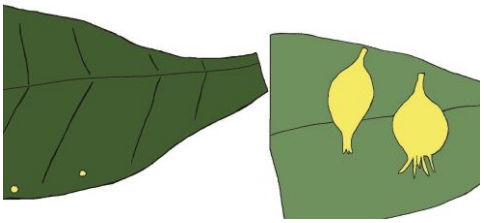
32. Slightly convex and raised to a point above, green and glabrous, and diameter 2.5–4.5 mm; below globular and more convex, light green, and densely pubescent; distributed along midvein of leaf. Opening round and surrounded by 5–6 short pubescent bracts which extend out from the gall surface when open……*Phylloxera auburnensis* sp. n.

33. Round and flat above; often slightly convex or sunken below leaf surface; crimson, green, or yellowish-green; diameter 5.0–10.0 mm; below globular and often with a hazelnut shape, light green, pubescent, and apex splits into several bracts at maturity

……..…….……..…….…….*Phylloxera caryaeavellana* Riley

34. Globular and suspended by a slender filament from below; yellow or white and pubescent and sticky; apex splits at maturity into several filaments……..

…….……...….*Phylloxera caryaegummosa* Riley



35. Round and flattened above, yellowish-green and with a round depression in middle, diameter 2.0–2.9 mm; below round, more convex, and light green with a reticulated pattern. Opening below a brown nipple...……...……...…….*Phylloxera myristica* sp. n.

36. Round and flat or slightly convex above with a shallow depression, light green, and with long yellowish-white pubescence; diameter 3.0–6.0 mm; below more convex with a nipple composed of slender bracts, green to almost white at apex, and densely covered in long yellowish-white pubescence……...*Phylloxera pilosula* Pergande

37. Button-like and often with a distinct rim around circumference above, light green, and shiny; diameter 3.0–11.0 mm; below more convex, light green with yellow pubescence, and opening a conical nipple with a round orifice……....*Phylloxera rimosalis* Pergande

38. Round and slightly convex or slightly sunken below surface above, yellowish-gold or crimson, glabrous and shiny, and with a central depression or elevation; diameter 4.0–9 mm; below more convex and globular, yellowish-gold or light green, and glabrous and shiny. Opening below surrounded by white pubescence and at maturity splits open into four white pubescent bracts……..……..……..……...*Phylloxera stoetzelae* sp. n.

39. Round and flat above; some can be slightly sunken below leaf surface or slightly convex; a depression in center; concolorous with leaf tissue, crimson, light green, or yellow; diameter 2.0–8.0 mm; below more convex and conical, globular, or pear shaped; base with or without constriction; pubescent; crimson, light green, yellow, or white. Opening below surrounded by 4–8 short pubescent bracts…….....
……...*Phylloxera symmetrica* Pergande

Galls that are about *equally convex above and below*

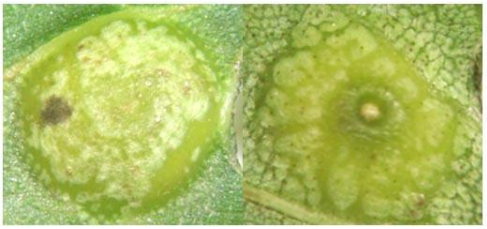
40. Round and smooth above, light yellowish-green, and diameter 3.8–6.2 mm; about equally convex above and below; below lighter with a nipple-like opening surrounded by many bracts…….....*Phylloxera depressa* (Shimer)

41. Globular or irregular; about equally convex above and below; smooth; green, yellowish-green, or red on one side; diameter 3.0–15.0 mm; occurs on midvein of leaves, petioles,

stems, leaf buds, and flower buds. Opening flat and round surrounded by pubescence or nipple-like and found below when present on leaf ……...*Phylloxera devastatrix* Pergande

42. Round and slightly convex above and below, covered in crimson resin-like droplets with several long white hairs on surface above, and diameter 0.9–1.5 mm; below opening round and surrounded by long white hairs……..…..……*Phylloxera killianae* sp. n.

43. Button-like and flattened, about equally convex above and below, and green or yellowish-green with a reticulated pattern; diameter 3.2–7.2 mm; opening below surrounded by white pubescence which splits into 2–5 bracts at maturity……
……..……...*Phylloxera russellae* Stoetzel



Galls that occur only on petioles

44. Globular or irregular; occurs singly or in clusters on petioles or stems; greenish-yellow or red on base or one side; diameter 5.0–10.0 mm; opening with or without a short nipple……...…….…….…….....*Phylloxera georgiana* Pergande

References Cited

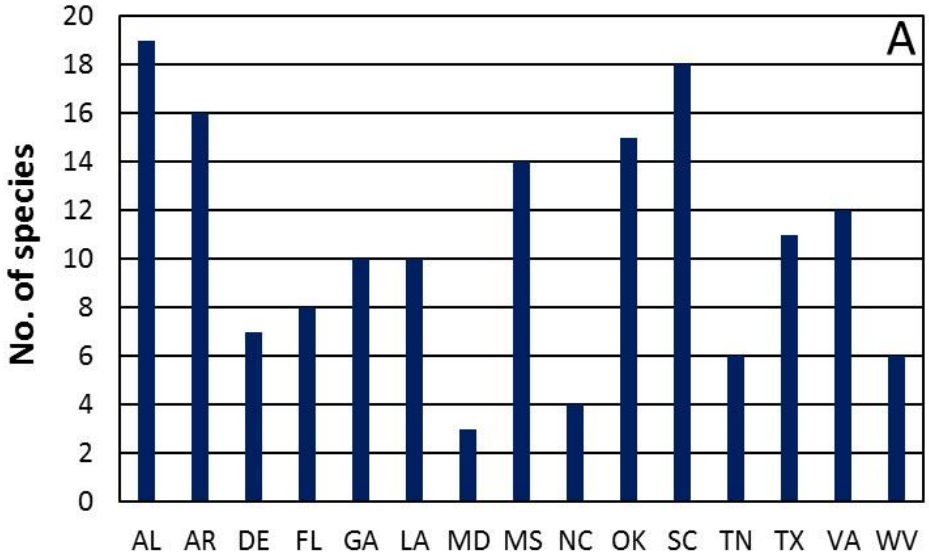
Pergande, T. 1904. North American Phylloxerinae affecting Hicoria (*Carya*) and other trees. Proceedings of the Davenport Academy of Sciences. 9: 185–271.

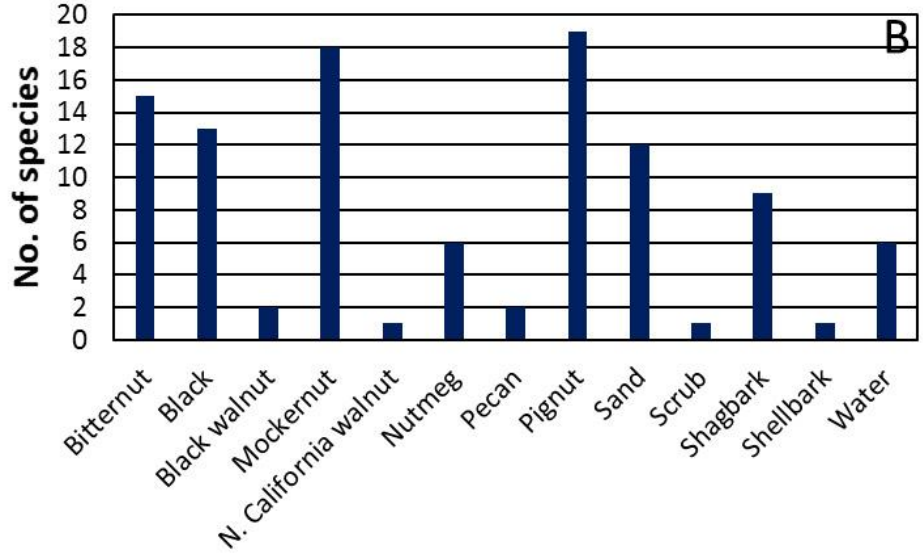
Stoetzel, M.B. 1981. Two new species of *Phylloxera* (Phylloxeridae: Homoptera) on pecan. J Georgia Entomol So. 16: 127–144.

Appendix 3. Number of samples of *Phylloxera* spp. collected in each state from 2015–2018.

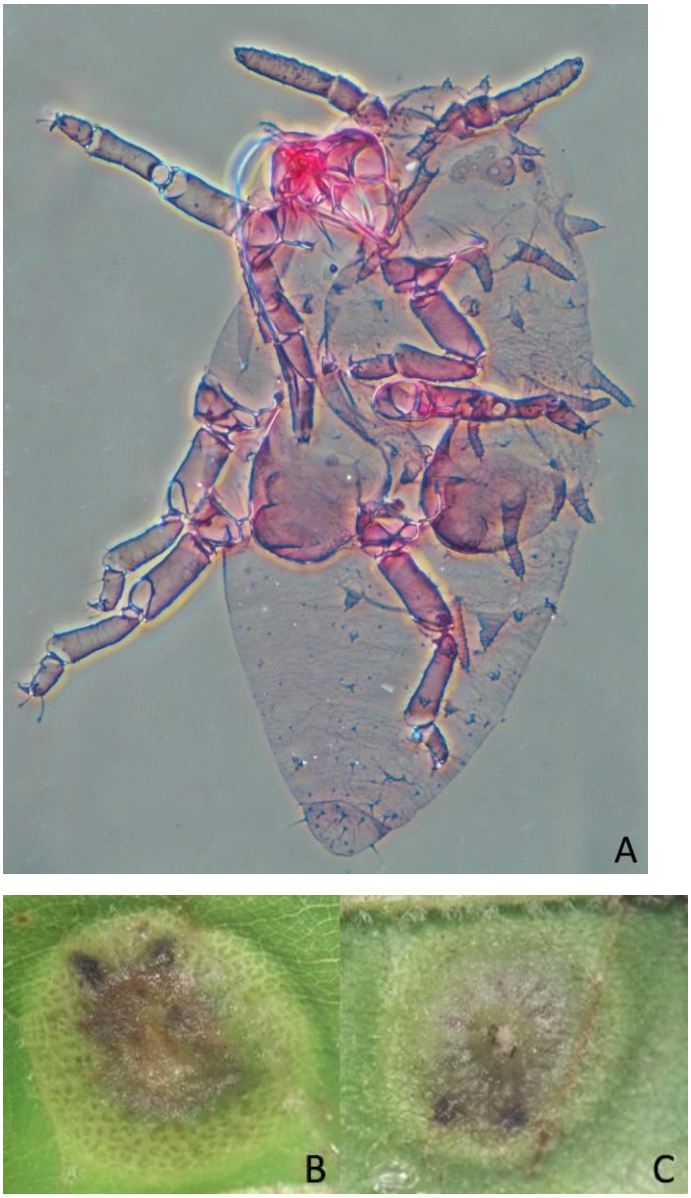
Species	AL	AR	DE	FL	GA	LA	MD	MS	NC	OK	SC	TN	TX	VA	WV	Total no. samples per species
Phylloxera auburnensis sp. n.	1															1
Phylloxera bispinae sp. n.	4															4
Phylloxera caryaeavellana	5	14	1			1		4		6	2		3			36
Phylloxera caryaecaulis		2									1					3
Phylloxera caryaefallax		3		1				3		1	1		4			13
Phylloxera caryaefoliae										1	2		1			4
Phylloxera caryaeglobuli		1					2	1					1	1	5	11
Phylloxera caryaemagna	2	2	3	2					3	1	1	4		2	2	22
Phylloxera caryaescissa	11	10		3	2	3		7		11	2		3	1		53
Phylloxera caryaesemen		2														2
Phylloxera caryaesepta	1	4		1	1	2		1		5	1		2	1		19
Phylloxera caryaevenae	3	4		2	1	1		4		2	2		3	3		25
Phylloxera castaneae	1															1
Phylloxera chippokesiensis sp. n.														2		2
Phylloxera conica	2			4	3	1		1		2	4	1	6			24
Phylloxera crypta sp. n.											1					1
Phylloxera deplanata	1															1
Phylloxera devastatrix	2					1										3
Phylloxera echinus sp. n.											1					1
Phylloxera falsostium sp. n.										2				1		3
Phylloxera flavoconica sp. n.					1							2				3
Phylloxera floridana sp. n.				1												1
Phylloxera foveola	2	6	4				1			3	2			3	1	22
Phylloxera killianae sp. n.									1							1
Phylloxera myristica sp. n.											1					1
Phylloxera notabilis	1				1						1					3
Phylloxera paludis sp. n.								2								2
Phylloxera picta	1	3	2			1		5			3		1	2		18
Phylloxera pilosula					2				2			2		3		9
Phylloxera rimosalis											2					2
Phylloxera russellae	1					1				1						3
Phylloxera spinosa	6	1			4				1	2	3					17
Phylloxera subelliptica	1															1
Phylloxera stoetzelae sp. n.					1			1								2
Phylloxera symmetrica	2	3	2					1		1		1			1	11
Phylloxera wiedenmanni sp. n.										1			1			2
Phylloxera williamsi sp. n.															1	1
Phylloxera sp. 1			4			1								2		7
Phylloxera sp. 2		1			1						2				2	6
Phylloxera sp. 3				1												1
Phylloxera sp. 4							1	1								2
Phylloxera sp. 5		1				1		1		2			3			8
Phylloxera sp. 6								1								1
Phylloxera sp. 7		1														1
Phylloxera sp. 8	1															1
Phylloxera sp. 9												1				1
Phylloxera sp. 10														1		1
Phylloxera sp. 11			1													1
Total no. samples per state	48	58	17	15	17	13	4	33	7	41	32	11	28	22	12	358

Appendix 4. Bar graphs displaying number of *Phylloxera* spp. collected per state (A) and number of *Phylloxera* spp. collected on each hickory or walnut species (B).





Appendix 5. One alate nymph of *Phylloxera* sp. 10 (A), Acc. tnu, collected on *Carya tomentosa* x *C. pallida* at Eastern Shore of Virginia National Wildlife Refuge (37.13588766, -75.9602065), Northhampton County, VA on 5/17/18. No described hickory-feeding *Phylloxera* alates have long tubercles projecting from cuticle, so this is likely a new species. The galls of this species closely resemble those of *P. foveola*. Gall of *Phylloxera* sp. 10 on adaxial leaf surface (B) and abaxial leaf surface (C).



Appendix 6. An alate nymph of *Phylloxera caryaevenae* (Acc. 104) collected on *Carya tomentosa* x *C. cordiformis* in Francis Marion National Forest (33.11242695, -79.79253625), SC on 04/27/16. This is the first record of an immature alate of this species being collected.