DIVERSITY OF SOUTH AMERICAN AMBROSIA BEETLES (CURCULIONIDAE: SCOLYTINAE: XYLEBORINI) AND THEIR FUNGAL PARTNERS

By

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ABSTRACT

DIVERSITY OF SOUTH AMERICAN AMBROSIA BEETLES (CURCULIONIDAE: SCOLYTINAE: XYLEBORINI) AND THEIR FUNGAL PARTNERS

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Ambrosia beetles from the tribe Xyleborini (Coleoptera: Curculionidae: Scolytinae) small, haplodiploid beetles that farm nutritional fungi on the walls of tunnels they excavate in the xylem of dead or nearly dead trees. These biological traits make them successful participants in worldwide wooded ecosystems and facilitate their human-mediated invasion beyond their native ranges. A minority of these introduced species are classified as pests because of the physical damage they cause to their plant hosts, or because they vector pathogenic fungi that infect ornamental, lumber, and forest trees. Most of the current knowledge on the diversity of xyleborine beetles and their fungi centers around species found in North America, Asia, and Europe. Little is known about the ambrosia partnerships in the Neotropics, which is concerning because South America is a strong trading partner with the US and the potential for new invasive Xyleborini to be imported from this area is significant. Continuing forest damage caused by invasive Xyleborini/fungi inspires robust research efforts to describe these symbionts and document their biological traits. Considerable efforts are required to enhance such endeavors in underrepresented regions such as South America and Africa. To increase understanding of the South American Xyleborini and their associated fungi, I compiled current knowledge of their historical and contemporary taxonomic records, biological records, and ecological studies. I also completed surveys throughout Ecuador to collect beetles and fungi. Molecular analysis of fungi isolated from Ecuadorian beetles reveals that several *Coptoborus* species associate with *Fusarium* fungi, including the ambrosia *Fusarium* Clade (AFC) that has previously been

recovered from *Euwallacea* spp. and *Xyleborus ferrugineus* in Central America, Florida, California, Israel, and Asia. Examination of the morphology of some South American xyleborine specimens previously classified as *Coptoborus* spp. suggests a high similarity to *Xyleborus* spp. from Africa. Phylogenetic analysis of these South American and African beetles as well as morphological assessment of additional specimens necessitates the designation of a new genus *Xenoxylebora* gen. nov. containing species endemic to both continents. This unusual distribution demonstrates the ability of these ambrosia beetles to survive long-distance trans-oceanic dispersal.

This dissertation is dedicated to my father, Dr. Michael Vernon Osborn, who would be so proud. And to my husband, Davin Taddeo whose love and support made this work possible.

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INTRODUCTION

As global ecological homogeny increases, the economic and ecological impact of invasive species continues to increase in the United States (Seebens et al. 2017). Bark and ambrosia beetles from the weevil subfamily Scolytinae are especially likely to be introduced to the United States from exotic locations. All life stages of these small beetles are spent inside woody plants, which makes them easily overlooked as they cross boarders via international trade networks inside ornamental plants of wood packing material. (Rabaglia et al. 2019, Lantschner et al. 2020).

Most bark and ambrosia beetles prefer recently deceased host trees because these plant hosts cannot defend themselves against the tunneling beetles (Hubbard 1897). Such trees are also possess optimal physical conditions for brood development, provide protection from predators, and are unlikely to house competing fungi or wood-boring animals. Only a small number of scolytine species attack living trees and are thus destructive to forests and food crop and ornamental trees (Smith and Hulcr 2015).

Ambrosia beetles differ from bark beetles in that they burrow into the xylem of their host trees and rely on the growth of symbiotic fungi that they farm inside these galleries for nutrition. Bark beetles tunnel under the bark and feed on the phloem of their plant hosts. Ambrosia beetles have the potential to vector devastating plant diseases if they are partnered with phytopathogenic fungi (Hulcr and Dunn 2011, Carrillo et al. 2014). For instance, *Coptoborus ochromactonus* Smith and Cognato has caused significant damage to trees in balsa plantations in Ecuador beginning in the 1990s and was recently discovered to associate with a putatively lethal strain of *Fusarium* fungus (Stilwell et al. 2014, Osborn et al. 2022).

The beetle-fungus mutualism also makes ambrosia beetles more efficient at expanding their geographical range with (Rassati et al. 2016, Lantschner et al. 2020) and without (Gohli et al. 2016) human mediation. Their reliance on fungi for nutrition affords them a greater variety of possible host taxa than bark beetles which have a more constrained host breadth (Francke-Grosmann 1967, Bright 1968). Ambrosia beetles require host trees that have adequate moisture and nutrition for their fungal symbionts, and which are free from contaminating fungi that might outcompete the ambrosia fungi (Freeman et al. 2016, Cavaletto et al. 2021, Nuotclà et al. 2021). Outside of these habitat requirements, ambrosia beetles can successfully colonize a variety of trees. The host range available to a given bark beetle is smaller because their phloem-feeding lifestyle places them in direct interaction with the chemistry of their hosts (Kirisits 2004). Thus, when bark beetles are introduced to novel environments, they are unlikely to survive unless they encounter host trees that meet their specific nutritional needs. Ambrosia beetles carry their fungal partners with them as they disperse to new trees. They readily establish in new locales because growth requirements of many ambrosia fungi can be fulfilled by a phylogenetically diverse group of trees.

This invasion potential is enhanced in the ambrosia beetle tribe Xyleborini because these genera have a haplodiploid mating system. Females lay fertilized eggs which develop into female offspring, and unfertilized eggs which become males. The male offspring stay inside the ambrosia gallery and mate with their sisters. Therefore, when a female xyleborine is introduced to a new ecosystem, she needs only to find a suitable place to plant a new ambrosia fungal garden since she was already fertilized in her natal gallery (Jordal et al. 2001, Peer and Taborsky 2005).

These biological traits allowed xyleborine ambrosia beetles to establish new populations on distant continents. Lowered Allee Effect thresholds likely contributed to the success of the first individuals as they arrived in the Neotropics from the Nearctic region 23 million years ago (Jordal et al. 2000, Liebhold and Kean 2018, Lantschner et al. 2020). These early xyleborines experienced a rapid rate of speciation, resulting in the hyperdiversity of xyleborines present in the South America today (Jordal et al. 2000, Gohli et al. 2017). This radiation, in turn, has no doubt fueled further dispersal and colonization of ambrosia beetles throughout the Americas, and has caused perpetual challenges to scientists as they catalogue species and untangle taxonomic relationships. This is particularly true of the South American members of the genus *Xyleborus* (Jordal et al. 2000, Cognato et al. 2011).

About 40 xyleborine species have established invasive populations beyond their native geographic ranges (Rabaglia et al. 2006, Kirkendall and Faccoli 2010, Haack and Rabaglia 2013, Gomez et al. 2018, Lin et al. 2021). This includes several *Xylosandrus* native to Asia that now infest a broad range of trees, damaging forests, lumber plantations, nurseries, and food crop trees all around the world (Atkinson et al. 2000, Kirkendall and Ødegaard 2007, Egonyu et al. 2015, Reding et al. 2015, Flechtmann and Atkinson 2016, Dzurenko et al. 2021, Carreras-Villaseñor et al. 2022, Urvois et al. 2022). *Xyleborus glabratus* Eichhoff, which is also native to Asia, was first discovered in the United States in 2002 and has since spread throughout the Southern states where it attacks live individuals of several tree species, especially those in the Lauraceae family (Haack 2006). The symbiotic relationship between *X. glabratus* and the pathogenic fungus *Harringtonia lauricola* (Harrington, Fraedrich and Aghayeva) (= *Raffaelea lauricola*) exemplifies the danger of virulent fungi paired with invasive ambrosia beetles. *Xyleborus glabratus* and *H. lauricola* continue to cause significant damage to avocado trees, ornamental

plants, and forest ecosystems from Florida to Texas (Inch et al. 2012, Ploetz et al. 2017, Wingfield et al. 2017).

Losses caused by Xyleborine invaders and their fungi from the Eurasia have inspired extensive research regarding their distributions, evolutionary histories, and biology. However, there is less information about Neotropical xyleborine beetles and their fungi (Wood 2007, Smith et al. 2017). The United States increasingly imports food, plants, and other raw materials from South America, and therefore risks importing invasive ambrosia beetles from our southern neighbors – and potentially harmful fungi (Marini et al. 2011, Meurisse et al. 2019, Lantschner et al. 2020).

This dissertation examines current knowledge on the taxonomy, evolutionary history, and ecology of the xyleborine-fungal symbiome and adds to this knowledge through a survey of endemic ambrosia beetles and their fungi from Ecuador. I use the knowledge and material from this research to address four questions: (i) what fungi were associated with xyleborine species in previous research? (ii) which fungi are carried by various *Coptoborus* species collected in Ecuador? (iii) what new taxonomic boundaries can be established to better-reflect the evolutionary past of Neotropical xyleborine genera? And (iv) do new generic boundaries need to be created?

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CHAPTER 1:

Diversity of xyleborine ambrosia beetles, their associated fungi, and the imperative of global collaboration

ABSTRACT

Ambrosia beetles from the tribe Xyleborini are part of forest ecosystems on every continent except Antarctica. Because of their small size, haplodiploid mating structure, and protected lives inside the sapwood of woody plants, they have a unique ability to expand into new regions via inadvertent human transport. A small number of invasive xyleborines cause significant damage to forests, lumber concerns, and agricultural systems. The most dangerous of these transmit pathogenic fungi capable of causing disease in or killing living trees. The relationships between these fungi and their beetle vectors range from mutualistic symbiosis to facultative association and many are not understood. Unstable taxonomies, convergent morphologies and the difficulty of obtaining and isolating ambrosia fungi accross their entire global ranges make comprehensive surveys of ambrosia fungi difficult to achieve. Ambrosia fungi from Asia, Europe and North America are fairly well documented, however we have yet to sufficiently document those from Africa, and South America. Worldwide cooperation to improve and standardize scientific study of the ambrosia symbiome is needed to better understand these economically impactful organisms.

INTRODUCTION

Forest biomes, with their abundance of trees and other flora, are prominent ecosystems on every continent except Antarctica. As a biological system, forests experience a constant cycle of death,

decomposition, and renewal which provides a steady supply of dead trees as a ubiquitous source of carbon and nutrients for terrestrial environments (Ulyshen 2016, Klemm et al. 2005). Although cellulose, hemicellulose, and lignin that form plant cell walls are indigestible to most organisms, many animals rely on these molecules to provide much of their energetic needs. The difficult task of converting plant polysaccharides into shorter sugars is dominated by bacteria, fungi, and some protists (Flint et al. 2008).

Many mammals rely on plant digesting microbes as endosymbionts (Martins et al. 2014), and several groups of insects independently evolved strategies involving microbes to gain access to the nutrients within plant cell walls. For example, most termites employ a highly structured community of bacteria, archaea, and co-evolved protozoa that allow them to degrade plant cell walls more efficiently than any other herbivorous animal (Brune and Okhuma 2011). Several groups of insects use plant-degrading fungi to convert plant material into a nutritional food source. The relationships between cellulolytic fungi and these insects take various forms ranging from obligate mutualism with attine ants and macrotermitine termites, to commensal associations with the bee species *Scaptotrigona depilis* (Moure), silphid and lymexylid beetles, and inchoate interactions with *Euops* attelabid beetles, and *Doubledaya* erotilid beetles (Kobayashi et al. 2008, Paludo et al. 2019, Biedermann and Vega 2020, Toki and Aoki 2021).

Bark and ambrosia beetles from the curculionid subfamily Scolytinae contain species with a variety of relationships with wood-degrading fungi. Many bark beetles transmit spores and maintain an opportunistic relationship with them as cohabitates of the same host plants (Six 2003, Harrington 2005). They supplement their diets with fungi and transport them in specialized organs called mycangia. However, they do not require these fungi to survive. Scolytine species that maintain a mutualistic symbiosis with fungi are called ambrosia beetles. Wood-degrading

fungi are the sole food source for adults and larvae of these beetles. Ambrosia fungi benefit from this association because they are protected from parasites and competing fungi (Batra 1967, Kirisits et al. 2004, Biedermann and Vega 2020) and given access to suitable growing substrates with appropriate nutrient and moisture levels (Rassati et al. 2016, Nuotclá et al. 2021). Independent phylogenetic analyses found that the ambrosia fungal feeding evolved at least 12 times in Scolytinae and once in the weevil subfamily Platypodinae (Jordal and Cognato 2012, Gohli et al. 2017, Johnson et al. 2018, Pistone et al. 2018). Some ambrosia beetle lineages are quite diverse, and research suggests that fungus farming is one factor that contributes to their species richness (Jordal and Cognato 2012, Gohli et al. 2017).

Ecology of Xyleborini

The tribe Xyleborini contains ~1260 species (Smith, unpublished), all of which are ambrosia beetles. They are found in forested habitats worldwide, experiencing rapid speciation events on more than one continent (Jordal et al. 2000, Jordal and Cognato 2012, Cognato et al. 2018, Eliassen and Jordal 2021). They also successfully invade new habitats at a higher rate than non-ambrosia scolytines (Rabaglia et al. 2019, Lantschner et al. 2020). Xyleborines possess a suite of biological characteristics that make them uniquely equipped for transport to, and proliferation in, novel environments. Life inside sapwood protects them from the elements and allows them to survive long distance dispersal via ocean currents (Cognato 2013, Jordal 2015, Cognato et al. 2018) and trade (Seebens et al. 2017, Grousset et al. 2020). Once in a new environment, xyleborine beetles are likely to reproduce and successfully establish a population because they carry their food source with them. When the females leave the fungal garden, they carry the nutritional fungi with them inside specialized exoskeletal structures called mycangia (Francke-

Grosmann 1956, Schedl 1962). Since ambrosia fungi can grow on many species of trees given the correct environmental and moisture conditions, dispersing females are likely to find suitable hosts in unfamiliar environments if the climate is relatively similar to that in their native range (Rassati et al. 2016). Furthermore, female xyleborines lay unfertilized eggs that mature into flightless haploid males which mate with their mother (if unfertilized) and sisters inside their natal gallery. Thus, dispersing females do not need to find a mate before founding a new ambrosia colony and reproducing.

These traits allow one foundress to establish a population, which makes them excellent colonizers and potential pests in non-native ranges. Their subcortical habitat and small size (1–3 mm long) conceal them from customs and border inspectors (Seebens et al. 2017). Approximately 40 xyleborine species have established populations beyond their native ranges due to human accidental introduction (Table 1.1) (Rabaglia et al. 2006, Kirkendall and Faccoli 2010, Haack and Rabaglia 2013, Gomez et al. 2018, Lin et al. 2021).

History of research regarding harmful Xyleborini

Ambrosia beetles have long caused ecological and financial harm to forests and the lumber industry in Europe and North America (Schmidberger 1836, Hubbard 1897). However, despite knowing that ambrosia beetles bore into the sapwood and often vector undesirable fungi, the earliest researchers did not appreciate the symbiotic nature of the beetle-fungal association. Many noted that ambrosia beetles ate a white powery substance that was never observed to be associated with bark beetles (Schmidberger 1836, Ratzeburg 1839). Schmidberger (1836) established the term “ambrosia” to describe the beetles’ diet because of its fruity odor, he suspected it was made by females out of tree sap and saliva to feed her offspring (Beling 1873).

When Hartig (1844) documented the fungal nature of ambrosia he classified the fungus associated with *Anisandrus dispar* (Fabricius) as *Monila candida* (Persoon). More than five decades later, Hubbard (1897) recognized that ambrosia fungi provide nutrition to the beetles and are actively cultivated in a symbiotic relationship.

Before the middle of the twentieth century, investigations into the ambrosia symbiome were generally flawed with the assumption that each ambrosia beetle species lives with one obligate fungal symbiont (e.g., Neger 1909, Doane and Gilliand 1929, Leach et al. 1940) and common misidentifications of ambrosia fungi (e.g., Neger 1911, Muller 1933, Verall 1943). As ambrosia research continued, scientists discovered complex assemblages of fungi inhabiting ambrosia beetle galleries (Norris 1965, Batra 1966, Norris 1979). Batra (1967) reclassified the known ambrosia fungi at the time into eight genera, of which three were associated with xyleborines: *Ascoidea, Ambrosiella,* and *Monacrosporium*.

Recent ambrosia research revealed the complex and varied range of relationships shared among xyleborine ambrosia beetles and fungi (Kostovcik et al, 2015, Skelton et al. 2018). This includes the identification of many fungal symbionts (von Arx and Hennebert, 1965, Harrington et al. 2008, Freeman et al. 2013, Harrington et al. 2014, Li et al. 2016, Lynch et al. 2016, Simmons et al. 2016a, 2016b, Aoki et al. 2018, 2019, Lynn et al. 2020, Aoki et al. 2021) and recognition that the same fungal species might grow in the galleries of both non-xyleborines xyleborines (Gebhart et al. 2004). The discovery and identification of the mycangium (Francke-Grosmann 1956, Schedl 1962, Batra 1963) revealed the complex role this organ plays in the development and maintenance of the ambrosia symbiome (Mayers et al. 2022). The evolution of mycangia correlates with the ambrosia lifestyle throughout Scolytinae (Mayers et al. 2015), and mycangia and their corresponding fungal partners from several ambrosia lineages are coadapted

(Johnson et al. 2018, Mayers et al. 2020b). The mycangium/fungi interaction also allows beetles to ensure vertical transmission of the most desirable fungal partners (Skelton et al. 2019), which extends their ability to survive variable conditions such as changing moisture levels inside the gallery and defensive compounds from new host plants (Hulcr and Dunn 2011, Nuotclá et al. 2021).

AMBROSIA FUNGI OF THE XYLEBORINI

Xyleborines have established mutual symbioses with a taxonomically diverse group of fungi within three orders of Ascomycota and one from Basidiomycota (Table 1.2; Figure 1.1) (Bateman 2018). Given the beetles' unique capacity for range expansion, their fungal partners share similarly large geographical ranges. Molecular dating estimates indicate that the first fungus farming xyleborines appeared ~21 million years ago followed by a rapid evolutionary radiation fueled by global warming during the early Miocene (Jordal and Cognato 2012). This timing is incongruent with the origins of the xyleborine fungal lineages. Phylogenetic analysis of *Raffaelea/Harringtonia* shows that the three clades of ambrosia fungi in the genus emerged 86 Ma, 67 Ma, and 33 Ma ago, before the current estimate for the origin of Xyleborini (Vanderpool 2017). Similarly, the Ambrosia *Fusarium* Clade (AFC), which includes both *Fusarium* and *Neocosmospora* species, is ~24 million years old (O'Donnell et al. 2015, de Beer et al. 2022). Mayers et al. (2020b) determined the first appearance of the *Ambrosiella* lineage associated with xyleborines to be ~12 million years ago, ~9 million years after the origin of Xyleborini. The ages of the ambrosia fungi *Dryadomyces* and *Irpex subulatus* (Ryvarden) have not been estimated, but further investigation of the timing of their entrance into the ambrosia symbiome could further illuminate the mechanisms of ambrosial evolution.

Current knowledge of the dissimilar patterns of emergence between the Xyleborini and their ambrosia fungi in *Raffaelea/Harringtonia, Fusarium/Neocosmospora* and *Ambrosiella* suggests that xyleborine beetles obtained their current ambrosia fungi through lateral transmission (partner switching) (O'Donnell et al. 2015, Peris et al. 2021). *Raffaelea/Harringtonia* is known to have experienced frequent partner switching (Miller et al. 2019) including at least three independent transitions into symbiosis with xyleborine beetles (Vanderpool 2017). Frequent symbiont switching, including a recent shift to Neotropical Xyleborini was found to be the most likely explanation for the confused phylogenetic patterns in *Fusarium* (AFC) (O'Donnell et al. 2015, Peris 2021, Osborn et al. 2022a). A constrained lineage of *Ambrosiella* was also found to have transferred from their historical platypodine beetle hosts to *Xylosandrus* spp. within Xyleborini (Mayers et al. 2020a).

After the first appearance of the invasive xyleborine *Xyleborus glabratus* Eichhoff and the destructive fungus *Harringtonia lauricola* (T. C. Harr., Fraedrich and Aghayeva) in the United States, surveys detected *H. lauricola* in the mycangia of *Xyleborus* spp., *Xylosandrus* spp. and two *Ambrosiodmus* spp. that commonly inhabit the same tree hosts as *X. glabratus* (Carrillo et al. 2014, Ploetz et al. 2017). *Xyleborus bispinatus* Eichhoff fed and reproduced when experimentally reared in a garden of *H. lauricola* (Saucedo et al. 2017). The fungus is also known to cohabitate with other *Raffaelea* species, both inside the mycangium and upon beetle gallery walls (Harrington et al. 2010, 2011, Simmons et al. 2016b). These studies show that the fungal symbiont of one beetle can enter the mycangium of a different species when the galleries exist in the same tree. They also support the hypothesis posited by Hulcr and Dunn (2011) that invasive ambrosia beetles are more likely to switch to a pathogenic fungus when they expand

into new geographic regions or exploit novel host species, because new fungi may enhance their ability to survive tree defenses.

CURRENT AND EMERGING PESTIFEROUS XYLEBORINE BEETLE/FUNGAL PARTNERSHIPS

Xyleborus glabratus and *Harringtonia lauricola*

The invasive *X. glabratus* was first detected in North America in 2002 in Chatham County, Georgia (Table 1.3) (Haack 2006, Rabaglia et al. 2006). Although its association with host trees throughout its large native range in Southeast Asia is not well understood (Cognato et al. 2019), it attacks living Lauraceae trees throughout the southeastern United States (Haack 2006, Harrington et al. 2011, Ploetz et al. 2017). *Xyleborus glabratus* carries several ophiostomatoid fungi originally assigned to the genus *Raffaelea*. De Beer et al. (2022) recently described the new genus *Harringtonia* to accommodate three former *Raffaelea* species: *R. aguacate* Simmons Dreaden and Ploetz, *R. brunnea* Batra (associated with Corthylini ambrosia beetles), and *Raffaelea lauricola* Harrington Fraedrich and Aghayeva, the causal agent of laurel wilt (Fraedrich et al. 2008, Harrington et al. 2008). *Harringtonia lauricola* quickly spreads throughout its tree host after inoculation; it prevents water transport by blocking the xylem, causing wilting symptoms within 14 days (Inch et al. 2012). This beetle-phytopathogen partnership likely originated in Asia and was co-imported to the southeastern United States (Harrington et al. 2011) where laurel wilt disease causes widespread damage to forest ecosystems (Ploetz et al. 2017, Wingfield et al. 2017) and commercial avocado (*Persea americana* Mill.) plantations (Inch et al. 2012). Phylogenetic analysis of the cytochrome c

oxidase I gene analysis of *X. glabratus* across its US population revealed only one haplotype, suggesting that the beetle-fungal pair was introduced once to southern North America (Hughes et al. 2017) followed by rapid proliferation to Georgia and throughout 10 southern states (Olatinwa et al. 2021). Within 20 years, the beetle and fungus advanced west to Texas and north to Kentucky, killing over 300 million trees, especially redbay and sassafras (Hughes et al. 2017, Olatinwa et al. 2021). The beetle and fungus may eventually extend their range as far north as Michigan, given the trend of global warming (Formby et al. 2017).

As discussed above, *H. lauricola* is commonly found in the mycangia of cohabitating xyleborine species and supports survival and reproduction of a closely related *Xyleborus*. Thus, the fungus may be likely to expand its range by jumping to other beetle partners, especially those with preoral mycangia (Ploetz et al. 2017). Investigators have developed rapid field detection techniques (Abdulridha et al. 2018, Hamilton et al. 2021) and have begun to increase the understanding of the pathogenicity of *H. lauricola* with molecular screening (Zhou et al. 2020) and metabolic examination (Joseph et al. 2021). However, effective methods for controlling the movement of *X. glabratus* and other vectors are very limited (Rivera et al. 2020), and there are few effective treatments for trees infected with *H. lauricola* (Olatinwa et al. 2021). Thus, laurel wilt continues to cause significant ecological and economic damage in the southeastern United States (Hughes et al. 2017).

***Xylosandrus* spp. and *Ambrosiella* spp**.

The genus *Ambrosiella* is one of three lineages in the Microascales family Ceratocystidaceae that independently evolved ambrosial associations with beetles (Mayers et al. 2015). Each of the 11 *Ambrosiella* species maintain tight associations with one ambrosia beetle species (Miller et al.

2019, Mayers et al. 2020b). Due to their long evolutionary history with ambrosia beetles, the genus is highly phenotypically converged which makes morphology-only based identification and taxonomic assignments unreliable (Cassar and Blackwell 1996, Alamouti et al. 2009, Mayers et al. 2020b). *Ambrosiella* also appears to be well adapted to beetles possessing large, complex mycangia that are associated with internal glands (Harrington et al. 2014, Johnson et al. 2018, Mayers et al. 2020b). The most recent phylogenetic treatment of the genus (Mayers et al. 2020b) identified two clades, one of which has been associated with Scolyplatypodini (Curculionidae: Platypodinae) ambrosia beetles since the origin of *Ambrosiella* ~18 million years ago. The more derived clade contains the majority of *Ambrosiella* species and has been carried by several xyleborine genera since its origin ~12 million years ago.

The granulate ambrosia beetle (*Xylosandrus crassiusculus* (Motschilsky)) has a history of human-aided range expansion that stretches back more than a century to Africa (Hagedorn 1908), North America (Anderson 1974), and most recently to Central/South America (Kirkendall and Ødegaard 2007, Flechtmann and Atkinson 2016) and Europe (Table 1.3) (Pennacchio et al. 2003, Nageleisen et al. 2015, Gallego et al. 2017, Kavčič 2018). The ability of *X. crassiusculus* to establish and spread beyond its native Asia is fueled by two factors that are difficult to disentangle because of the complex genetic diversity this species possesses in both its native and introduced ranges (Ito and Kajimura 2009, Flechtmann and Atkinson 2016, Storer et al. 2017). First, the beetle quickly proliferates after establishing a new population. This is evidenced by the fact that it was one of the most prevalent ambrosia beetle species in the Costa Rica just ten years after its first detection in the country (Kirkendall and Ødegaard 2007). Second, repeated introductions of this species are common, and this increases the rate of success for nascent populations to expands their geographic range (Rabaglia et al. 2019). Both factors are

responsible for the rapid proliferation of the granulated ambrosia beetle throughout the North America after its first appearance in Dorchester County, South Carolina, United States in 1974 (Anderson 1974, LaBonte et al. 2005, Flechtmann and Atkinson 2016, Rabaglia et al. 2019), and its range expansion throughout South America and Africa (Flechtmann and Atkinson 2016, Landi et al. 2017, Nel et al. 2020).

Xylosandrus crassiusculus maintains an obligate relationship with its fungal symbiont *Ambrosiella roeperi* T.C. Harr & McNew throughout its global geographic range (Harrington et al. 2014, Mayers et al. 2020b, Nel et al. 2020, Saragih et al. 2021). This fungus is not known to cause disease in host plants; however infestation with *X. crassiusculus* can be associated with unidentified fungal infections that may opportunistically exploit the holes created by dispersing foundresses (Atkinson et al. 1988). The granulated ambrosia beetle impacts ornamental plant nurseries and lumber productivity by killing young saplings (Kirkendall Ødegaard 2007) and damaging stored logs (Atkinson et al. 2000). It also attacks and reproduces in a vastly large range of angiosperms including food crop trees like coffee (*Coffea canephora* Pierre ex A. Froehner), cacao (*Theobroma cacao* L.), mango (*Mangifera indica* L.), papaya (*Carica papaya* L.), rubber (*Hevea brasiliensis* Willd. Ex A. Juss), camphor (*Cinnamomum camphora L.*), mahogany (*Swietenia* Jacq. sp.), teak (*Tectona grandis* L.), and stone fruits (*Prunus* L. spp.) (Schedl 1963, Atkinson et al. 1988, Wood and Bright 1992). Cavaletto et al. (2021) discovered that host preference of *X. crassiusculus* was more influenced by ethanol levels in the wood than plant taxon. Harrington et al. (2014) anticipated that *X. crassiusculus* was unlikely to efficiently vector plant pathogens from *Raffaelea,* but studies on the lateral transfer of pathogenic fungi between sympatric ambrosia beetles have call this prediction into question. Carrillo et al. (2014) and Ploetz et al. (2017) discussed the potential for the granulated ambrosia beetle to spread of laurel

wilt disease after finding its causal agent, *H. lauricola*, inside the mycangia of *X. crassiusculus* specimens.

Like *X. crassiusculus,* the black twig borer (*Xylosandrus compactus* (Eichhoff)) uses a wide variety of dicotyledonous angiosperm hosts (Ngoan et al. 1976, Hara and Beardsley 1979). This ambrosia beetle attacks small-diameter twigs and healthy trees of diameter 3 mm or larger (Wood 1982), causing wilting and death in young individuals (Oliviera et al. 2008). It is a significant pest of coffee, cacao, avocado, mango, and many forest and nursery trees (Brader 1964, Ngoan et al. 1976, Oliveira et al. 2008).

The native range of *X. compactus* probably extends through Asia, although it has been widely introduced to many regions and lives in a pantropical distribution today (Table 1.3) (Brader 1964, Rabaglia et al. 2006, Haack and Rabaglia 2013, Urvois et al. 2022). Brader (1964) noted that *X. compactus* was limited to the paleotropics but predicted it would eventually spread to the Neotropics. A phylogeographical analysis of global patterns of genetic diversity of *X. compactus* confirmed that two Asian linages independently invaded Africa and the Americas, Pacific Islands and Europe through international trade (Urvois et al. 2022). The introduction to Africa probably occurred several hundred years ago, early in the history of the continent's participation in international trade (Egonyu et al. 2015). The occurrence of *X. compactus* in the Americas, Pacific Islands and Europe is more recent. In North America, it was first recorded in Ft. Lauderdale, Florida in 1941 before quickly establishing pestiferous populations in avocado plantations in Florida and Georgia (Wood 1982). Estimates place the introduction of the black twig borer to South America in the 1970s. It was first collected in Peru in 1973 (Oliviera et al. 2008) and in Brazil during the 1980s (Delgado and Couturier 2017). *Xylosandrus compactus* was introduced to Oahu, Hawaii in 1961; it has since spread to all the Hawaiian Islands where it

threatens coffee crops and several native trees (Hara and Beardsley 1979, Greco and Wright 2015). Most recently, this ambrosia species was discovered in Italy in 2011 (Garonna et al. 2012) then France, Spain, and Greece by 2019 (Faccoli 2021). The population in central Europe causes commercial and ecological damage by attacking bay (*Laurus nobilis* L.) and carob *Ceratonia siliqua* L.) trees, producing five generations per year (Gugliuzzo et al. 2020).

Xylosandrus compactus is associated with two fungi which lead to several years of confusion as investigators uncovered inconsistent results when culturing samples from the beetles' gallery walls (Hara and Beardslet 1979). Species from two genera have the most consistent association with *X. compactus*: *Ambrosiella xylebori* Brader ex von Arx & Hennebert (Mayers et al. 2015, Bateman et al. 2016, Gugliuzzo et al. 2020) and *Fusarium* spp. from the *solani* species complex (FSSC) (Muller 1933, Brown 1954, Ngoan et al. 1976, Gugliuzzo et al. 2020). Bateman et al. (2016) suggested that *A. xylebori* is likely the nutritional symbiont because they repeatedly recovered it from beetle mycangia and galleries.

However, the role (if any) of *Fusarium* in the life cycle of the black twig borer, is less clear. A pathogenicity study in Uganda found that *Fusarium* spp. vectored by *X. compactus* are the causal agent of wilting in infested cacao plants (Kagezi et al. 2017). Bateman et al. (2016) found FSSC was consistently recovered from the external surface of the beetles, but not found in the mycangium. They suggested that FSSC has conidiophores that are adapted for insect dissemination and the pathogenicity of FSSC allows *X. compactus* to overcome plant defenses when it attacks healthy hosts. These fungi may be prone to developing associations with arthropods because the FSSC contains several insect-vectored plant pathogens (Aoki et al. 2003, Summerell and Leslie 2011) and is vectored by several insect and mite orders (O'Donnell et al. 2012). However, these possible explanations for the association between *X. compactus* and FSSC

require further investigation (Bateman et al. 2016). The beetle is responsible for vectoring a fungal disease that is capable of causing significant economic and ecological damage (Egonyu et al. 2015).

Xylosandrus morigerus (Blandford) was described in England from specimens taken from infested orchids originating in Papua New Guinea (Blandford 1894a). Thus, its potential for introduction to many non-native regions was clearly notable from the beginning (Table 1.3) (Browne 1961, Kalshoven 1961). This ambrosia beetle is probably native to tropical Asia, with introduced populations worldwide (Wood and Bright 1992, Kirkendall and Ødegaard 2007, Cognato and Rubinoff 2008, Kirkendall and Faccoli 2010). A study of the mitochondrial genetic diversity of the invasive population in Costa Rica suggested that despite widely divergent mitochondrial lineages, *X. morigerus* maintains the same host generalism throughout the contry (Andersen et al. 2012). This ecological consistancy may contribute to the extreme mobility of the species outside of its native range. Like *X. compactus, X. morigerus* attacks twigs and small diameter samplings from a wide variety of angiosperms (Browne 1961, Kalshoven 1961). This can cause significant damage to the coffee, cacao, avocado, mahogany, teak, legumes, and many forest trees (Kalshoven 1961, Carreras-Villaseñor et al. 2022).

The mycangial contents of *X. morigerus* have not yet been studied, but the nutritional symbiont of this beetle is presumably an unstudied *Ambrosiella* species (Batemena et al. 2018). Two isolates from the FSSC were recovered from *X. morigerus* collected in Veracruz, Mexico and found to cause wilting and necrosis in coffee, and several forest tree species (Carreras-Villaseñor et al. 2022). The full nature of the relationship between *X. morigerus* and this pathogenic FSSC is still uncertain.

Xylosandrus germanus was described by Blandford (1894b) from 16 specimens collected from the Japanese island of Honshu. It was not collected outside of Asia until Felt (1932) reared several hundred from infested grapevines in Nassau County, New York. Initial biological descriptions of this species noted that it occasionally infested healthy trees but had better success attacking freshly fallen or sick hosts (table 1.3) (Hoffmann 1941). Hoffmann (1941) also observed that *X. germanus* relied on a fungus for food and concluded that this fungus was unlikely to be a plant pathogen. As the beetle spread through the eastern United States (Weber and McPherson 1982, LaBonte et al. 2005), it was linked to a *Fusarium* fungal disease responsible for killing young walnut trees in plantations in southern Illinois, Indiana, Missouri, and Iowa (Kessler et al. 1974). It was first reported in Europe in Germany in 1951 (Groschke 1953) and established populations thorough central Europe by the 1990s (Wood and Bright 1992). In 2018, the beetle spread to the Mediterranean scrubland (Contarini et al. 2020). In 2007 *X. germanus* was discovered in Hawaii (Cognato and Rubinoff 2008). Phylogeographic analysis across its native and introduced ranges indicates that *X. germanus* populations in Europe and North America each descended from independent invasions by Japanese founders, with Europe having been invaded once, and North America several times (Dzurenko et al. 2021). Non-native populations of *X. germanus* are so abundant they are a common model for studies about xyleborine sex ratios and inbreeding rates (Peer and Taborsky 2005, Keller et al. 2011).

Although *X. germanus* generally attacks trees that are already physiologically compromised (Ranger et al. 2018) it is a significant pest causing death and dieback in nurseries (Reding et al. 2015) and orchards (Agnello et al. 2017) in the United States. In Europe, it causes significant losses in forests (Grégoire et al. 2003), vineyards (Ruzzier et al. 2021), and the lumber industry (Galko et al. 2018). The mycangial symbiont of *X. germanus* is *Ambrosiella*

grosmanniae McNew, C. Mayers, and T.C. Harr which is closely related to *A. roeperi* and *A. xylobori* and has repeatedly been isolated from specimens in the United States, Germany, Netherlands, and Switzerland (Mayers et al. 2015). Molecular data from the fungi from *X. germanus* Japan (within the native range) indicated that there may be more genetic diversity than proposed by Mayers et al. (2015) (Ito and Kajimura 2017).

Additional fungi, including *Fusarium* spp., have been recovered from *X. germanus* galleries (Yang et al. 2008) but their relationship to *X. germanus* is currently unknown. Disease in New York apple orchards associated with *Fusarium* sp. is thought to be vectored by *X. germanus,* but this link remans unconfirmed because specific identification and pathogenicity studies have not been performed (Agnello et al. 2017).

Similar to other species of *Xylosandrus, X. germanus* can live inside an exceptionally wide species of plants including conifers (Weber and McPherson 1983). Multi-year monitoring of non-native *X germanus* in forests in Slovakia showed that populations did not decline significantly after an exceptionally cold winter, suggesting that they may be capable of continuing to expand into colder areas (Dzurenko et al. 2022). In addition, as global climate change continues to bring milder winters and trigger stress in host trees, this beetle's range will likely continue to expand throughout its non-native range (Henin and Versteirt 2004).

Ambrosia *Fusarium* Clade and *Euwallacea* spp. + *Coptoborus* spp.

As discussed above, three *Xylosandrus* spp. vector FSSC pathogens that cause a significant global impact but are not nutritional symbionts for the beetles (Carreras-Villaseñor et al. 2022). The genera *Euwallacea* and *Coptoborus* maintain nutritional relationships with an unusual group of *Fusarium* species collectively referred to as the Ambrosia *Fusarium* Clade (AFC) (Li et al.

2016, Lynch et al. 2016, Osborn et al. 2022a). These fungi form a monophyletic group that diverged from the FSSC ~21–24 million years ago (Kasson et al. 2013, O'Donnell et al. 2015). The AFC consists of 19 species, 11 of which have been formally described (Gadd and Loos 1947, Nirenberg 1990, Freeman et al. 2013, Na et al. 2018, Aoki et al. 2018, 2019, Lynn et al. 2020, Aoki et al. 2021).

Euwallacea fornicatus (Eichhoff) has been a well-documented pest of tea since the late 19th century (Table 1.3) (Walgama 2012). However, there was little examination of its gallery fungus until Gadd and Loos (1947) described *Monacrosporium ambrosium* Gadd and Loos from beetles infesting tea plantations in Sri Lanka. In 1987, Brayford also noticed the fungus vectored by *E. fornicatus* in tea in the Indian state of Maharashtra and wrote a duplicate description providing the name *Fusarium bugnicourtii* (Gadd and Loos). These two names were synonymized three years later (Nirenburg 1990) and there was no further discussion of this ambrosia fungus until 2012 when *E. fornicatus* was discovered vectoring an undescribed *Fusarium* causing Fusarium Dieback Disease in avocado in California, United States, and Israel (Eskalen et al. 2012, Mendel et al. 2012). An inclusive survey examining the fungi from worldwide *Euwallacea* showed that several species including *Euwallacea interjectus* (Blanford), *Euwallacea validus* (Eichhoff), *E. fornicatus,* and *Euwallacea sp.* cultivate AFC (Kasson et al. 2013). Interestingly, this study also recovered the AFC species AF-9 from *Xyleborus ferrugineus* (Fabricius) from Costa Rica (Kasson et al. 2031). Testing for co-cladogenesis in *Euwallacea* and AFC revealed that the AFC are vertically transmitted by the beetles and share a deep and dynamic relationship with *Euwallacea* that includes frequent partner switching (O'Donnell et al. 2015). The beetles and fungi likely became associated close to the origin of Euwallacea (19–24

million years ago). However, the nutritional relationship between the beetles and fungi has not been examined and needs investigation (O'Donnell et al. 2015).

Despite the long history of research and monitoring of *E. fornicatus*, identification of this species based on morphology is quite difficult. The taxonomy of the species has historically been unstable with scientists describing several species with nearly identical diagnoses, placing them into synonymy, and resurrecting some (see Stouthammer et al. 2017 and Gomez et al. 2018). The rediscovery of a syntype from the initial type series of *Xyleborus fornicatus* Eichhoff provided clarity about the identity of the original description (Smith et al. 2019). However, considerable uncertainty around the diagnosis of *E. fornicatus* and the correct use of its common name, polyphagous shot hole borer remains (Carrillo et al. 2020a, 2020b).

Members of the *E. fornicatus* species complex spread beyond their native Asia and Oceania into Central America in the 1980s (Wood 1982). Between 2006 and 2018, they spread further to North America (California and Florida, United States) (Rabaglia et al. 2006), Israel (Mendel et al. 2012), and South Africa (Paap et al. 2018). Some AFC fungi associated with *Euwallacea* spp. cause plant disease (Eskalen et al. 2012, Mendel et al. 2012, Paap et al. 2018), yet pathogenicity among all members of the AFC has not been evaluated.

Beetles from the Neotropical genus *Coptoborus* were linked to a dieback disease affecting balsa (*Ochroma pyramidale* Cavanilles ex Lamark) plantations in Ecuador in the early 1990s (Stilwell et al. 2014). The beetle was described as *Coptoborus ochromactonus* Smith and Cognato after its association with dying balsa trees (Table 1.3) (Stilwell et al. 2014). Morphological and molecular analysis both identified the fungus associated with this beetle-vectored disease to be a *Fusarium* sp. (Stilwell et al. 2014, Castro et al. 2019) and a study of infested balsa plantations in western Ecuador showed that tree age and stress levels had the most

influence on infection and mortality rates (Martínez et al. 2020). Osborn et al. (2022a) completed a survey of the fungi in the mycangia of several *Coptoborus* from Ecuador including *C. ochromactonus* and concluded that they carry the unnamed species AF-9 from the AFC. Close investigation of the AFC associated with *C. ochromactonus* is still needed to fulfil Koch's postulates for disease causality (Smith 1905).

Ambrosiodmus minor and *Irpex subulatus*

The sister ambrosia beetle genera *Ambrosiodmus* and *Ambrosiophilus* are the only xyleborines with documented symbioses with the basidiomycete fungus *I. sublatus* (= *Flavodon subulatus*) (You et al. 2015, Simmons et al. 2016a, Li et al. 2017). The presence of this fungus, which was first assigned to *Flavodon* and recently was moved into *Irpex* by Tian et al. (2022), is very uncommon among the Xyleborini. *Ambrosius minor* (Stebbing) is native to Eastern Asia (Wood and Bright 1992, Lin et al. 2019), but it has been introduced to the southeastern United states, spreading from Nassau County, Florida to Georgia, Alabama, and Mississippi by 2017 (Table 1.3) (Schiefer 2018). The ambrosia partnership between *A. minor* and *I. subulatus* could result in unique ecological consequences and challenges as the beetle continues to spread. *Irpex sublatus,* like other white-rot fungi, can efficiently degrade lignin, one of the most recalcitrant structural molecules that is responsible for the rigidity of plant walls (Eriksson et al. 1990). Because this fungus colonizes dead wood efficiently and may outcompete similar native fungi, it may alter the carbon cycle in forests and increase falling limbs and damage to trees in human environments (Gomez and Hulcr 2020, Jusino et al. 2020).

AMBROSIA BEETLE/FUNGAL RESEARCH IN REGIONAL CENTERS OF DIVERSITY

There is a greater understanding of introduced xyleborine beetles and their fungi compared to the species residing in their native range. Approximately 1300 xyleborine species are distributed in the tropics but adequate knowledge of taxonomy, ecology, and fungal symbioses is lacking (Wood 2007, Smith et al. 2017, Eliassen and Jordal 2021). Similarly, most studies exploring ambrosia fungus diversity and their relationships to ambrosia beetles focus on the minority of fungi that cause economic or ecological harm (Batra 1967, Alamouti et al. 2009, Dreaden et al. 2014, Hughs et al. 2017, Short et al, 2017). In many ways, this bias is necessary because of the urgency required to mitigate the damage caused by a newly discovered pathogen (Leibold and Kean 2018). However, future-oriented studies aimed at gaining a holistic understanding of the diversity of ambrosia symbioses and the biological mechanisms maintaining them may be the best way to fully understand the ambrosia symbiome and prevent the ecological consequences of waiting until after a new pest presents itself to begin research (e.g., Hulcr and Dunn 2011, Skelton et al. 2018, Miller et al. 2019, Mayers et al. 2020b).

Xyleborine diversity is thoroughly documented in North America (Bright 1968, Wood 1982), Europe (Pfeffer 1995), and Asia (Smith et al. 2020). Detailed catalogues also exist for the Xyleborini of Africa (Schedl 1963) and South America (Wood 2007). However, these regions have not been fully explored and probably contain undescribed species and genera (Wood 2007, Smith et al. 2017, Eliassen and Jordal 2021, Osborn et al. 2022b). Despite considerable historical discourse regarding the classification of ambrosia fungi and their connection to the Xyleborini (see Leach et al. 1940, Francke-Grosmann 1967), the identities and ecological impacts of most xyleborine ambrosia fungi are poorly understood or unknown. There are several comprehensive

discussions of beetle-fungal interactions, but most focus broadly on Scolytinae (Harrington 2005, Six 2012, Hulcr and Stelinski 2017), or contrast ambrosia and bark beetles with other fungus-farming insects (Farrell et al. 2001, Biedermann and Vega 2020). Norris (1979) reviewed the fungi associated with xyleborines more than four decades ago, but there have been taxonomic changes and new discoveries since then (Figure 1.1).

During the last two and a half decades, several phylogenetic studies of the ambrosia fungi from Hypocreales, Ophiostomatales, and Microascales created robust classifications of these groups based on isolates obtained from fungal repositories and molecular sequences from GenBank (Cassar and Blackwell 1996, Jones and Blackwell 1998, Alamouti et al. 2009, Dreaden et al. 2014, Vanderpool 2017 Mayers et al. 2020b, de Beer et al. 2022). The global scope of these is useful for understanding broad evolutionary patterns, but finer understanding of the evolutionary interactions between ambrosia fungi and beetles requires the collection of beetles and isolation of the fungi for comparison across geographic space. Studies accomplishing this have focused on beetles from Asia (Gadd and Loos 1947, Brayford 1987, Gebhardt 2005, Li et al. 2016, Lin et al. 2017, Carrillo et al. 2019, Lynn et al. 2020), North America (Six et al. 2009, Harrington et al. 2008, Eskalen et al. 2013, Harrington et al. 2014, Lynch et al. 2016, Simmons et al. 2016a, Mayers et al. 2017, Aoki et al. 2018), or both (Harrington et al. 2010, Freeman et al. 2013, Simmons et al. 2016b, Na et al. 2018, Aoki et al. 2021). A few included specimens from Australia (von Arx and Hennebert 1965, Kasson et al. 2013, Aoki et al. 2019), Europe (Nirenberg 1990, Mayers et al. 2015), and Africa (von Arx and Hennebert 1965, Scott and du Toit 1970), but the xyleborine ambrosia fungi from these areas are sparsely studied. The fungi living with xyleborines native to South America are virtually unknown despite a few studies

exploring one endemic genus from Ecuador (Stilwell et al. 2014, Castro et al. 2019, Osborn et al. 2022a).

ENHANCING GLOBAL RESEARCH OF AMBROSIA SYMBIOSIS.

The nature of the xyleborine ambrosia beetle-fungus relationship remains poorly understood because of varying levels of fidelity/promiscuity, evolutionary histories, and taxonomic diversity of the fungi. Yet xyleborines are found globally and are common invaders in every biogeographic region. They cause harm through mass-aggregation on weakened trees and structural damage to wood, but they are the most ecologically and economically impactful when they inoculate host trees with pathogenic fungi (Hulcr and Stelinski 2017).

The recent formation of the Bark Beetle Mycobiome collaborative group (Hulcr et al. 2020) and its framework for standardizing methods and communication is a crucial first step towards studying and combating harmful ambrosia beetles as a global community rather than as individual countries or regions. Invasive ambrosia beetles are monitored and studied by well-established research programs in several regions, but there are parts the world that currently lack such sustained efforts. Many of these are developing nations located around the equator where there is the richest diversity of xyleborine beetles. These countries may be the source of future invasive populations as well as suffer from the consequences of new introductions, yet they rarely have robust and enduring programs for studying the ambrosia symbiosis and monitoring for non-native species.

To better strengthen all regions against harmful new invasive xyleborines and their possibly destructive fungi, research needs to be supported equitably around the world to enhance understudied areas. The founding consortium of researchers that crafted the Bark Beetle

Mycobiome includes an impressive group from institutions in the United States and South Africa. To maximize its potential, the collaborative relationships codified in its foundation, as cited in this review, should be expanded to benefit research programs in Central and South America, Oceania, Asia, and Sub-Saharan Africa (apart from South Africa) should be developed and supported. Partnerships between traditionally privileged nations and these underrepresented regions would be mutually beneficial because local knowledge, infrastructure and ingenuity can be harnessed to gain knowledge that benefits the global community.

APPENDIX

Genus	species	Common Name, Notes	Origin Region(s)
Ambrosiodmus	*lewisi*		Asia
Ambrosiodmus	*minor*	punky wood ambrosia beetle	Asia
Ambrosiodmus	*rubricollis*		Asia/East Asia
Ambrosiophilus	*aratus*		Asia/East Asia
Ambrosiophilus	*nodulosus*		Asia
Anisandrus	*dispar*		Europe
Anisandrus	*maiche*		Asia
Cnestus	*mutilatus*	camphor shot borer	Asia
Coptoborus	*coartatus*		South America
Coptoborus	*crinitulus*		South America
Coptoborus	*ricini*		South America
Coptoborus	*villosulus*	(= *theobromae*)	South America
Cyclorhipidion	*bodoanum*	(= *californicus*)	North Asia
Cyclorhipidion	*fukiense*		Asia
Cyclorhipidion	*pelliculosum*		Asia
Dryocoetoides	*cristatus*		South America
Dryoxylon	*onoharaense*		Asia
Euwallacea	*fornicatus*	polyphagous shothole borer	Asia
Euwallacea	*interjectus*		Asia
Euwallacea	*similis*		Africa, Asia
Euwallacea	*validus*		Asia
Xyleborinus	*alni*		Asia, Europe
Xyleborinus	*andrewesi*		Asia
Xyleborinus	*attenuatus*		East Asia
Xyleborinus	*exiguus*		Asia
Xyleborinus	*octiesdentatus*		Asia
Xyleborinus	*saxesenii*		Asia, Europe

Table 1.1: Introduced Xyleborini, their native region, and region(s) of introduction. Data inferred from Wood and Bright 1992, Pennacchio et al. 2003, Rabaglia et al. 2006, Kirkendall and Ødegaard 2007, Wood 2007, Cognato and Rubinoff 2008, Kirkendall and Faccoli 2010, Haack and Rabaglia 2013, Gomez et al. 2018, Schiefer 2018 Lin et al. 2021, and Urvois et al. 2022.

Table 1.1 (cont'd)

Genus	species	Common Name, Notes	Origin Region(s)
Ambrosiodmus	*lewisi*		Asia
Ambrosiodmus	*minor*	punky wood ambrosia beetle	Asia
Ambrosiodmus	*rubricollis*		Asia/East Asia
Ambrosiophilus	*aratus*		Asia/East Asia
Ambrosiophilus	*nodulosus*		Asia
Anisandrus	*dispar*		Europe
Anisandrus	*maiche*		Asia
Cnestus	*mutilatus*	camphor shot borer	Asia
Coptoborus	*coartatus*		South America
Coptoborus	*crinitulus*		South America
Coptoborus	*ricini*		South America
Coptoborus	*villosulus*	(= *theobromae*)	South America
Cyclorhipidion	*bodoanum*	(= *californicus*)	North Asia
Cyclorhipidion	*fukiense*		Asia
Cyclorhipidion	*pelliculosum*		Asia
Dryocoetoides	*cristatus*		South America
Dryoxylon	*onoharaense*		Asia
Euwallacea	*fornicatus*	polyphagous shothole borer	Asia
Euwallacea	*interjectus*		Asia
Euwallacea	*similis*		Africa, Asia
Euwallacea	*validus*		Asia
Xyleborinus	*alni*		Asia, Europe
Xyleborinus	*andrewesi*		Asia
Xyleborinus	*attenuatus*		East Asia
Xyleborinus	*exiguus*		Asia
Xyleborinus	*octiesdentatus*		Asia
Xyleborinus	*saxesenii*		Asia, Europe

Table 1.1 (cont'd)

Genus	species	Introduced Region(s)
Ambrosiodmus	*lewisi*	North America (USA)
Ambrosiodmus	*minor*	North America (USA)
Ambrosiodmus	*rubricollis*	Australia, Europe (Italy), North America (Mexico, USA)
Ambrosiophilus	*aratus*	Europe (Italy), North America (USA)
Ambrosiophilus	*nodulosus*	North America (USA)
Anisandrus	*dispar*	North America (USA)
Anisandrus	*maiche*	North America (USA)
Cnestus	*mutilatus*	North America (USA)
Coptoborus	*coartatus*	Africa
Coptoborus	*crinitulus*	Africa
Coptoborus	*ricini*	Africa
Coptoborus	*villosulus*	Africa
Cyclorhipidion	*bodoanum*	Europe, North America (USA)
Cyclorhipidion	*fukiense*	North America (USA)
Cyclorhipidion	*pelliculosum*	North America (USA)
Dryocoetoides	*cristatus*	Africa
Dryoxylon	*onoharaense*	North America (USA)
Euwallacea	*fornicatus*	Australia, Central America, Hawaii, North America (USA), South America (Brazil)
Euwallacea	*interjectus*	North America (USA)
Euwallacea	*similis*	North America (USA), South America (Brazil)
Euwallacea	*validus*	North America
Xyleborinus	*alni*	North America
Xyleborinus	*andrewesi*	Hawaii, North America (USA)
Xyleborinus	*attenuatus*	Europe (Austria), North America
Xyleborinus	*exiguus*	Central America
Xyleborinus	*octiesdentatus*	North America (USA)
Xyleborinus	*saxesenii*	Africa, Australia, Hawaii, New Zealand, North America (USA), South America

Table 1.1 (cont'd)

Genus	species	Introduced Region(s)
Xyleborus	*abberrans*	South America (Brazil)
Xyleborus	*affinis*	Africa, Asia, Australia, Europe, Hawaii, North America (USA)
Xyleborus	*ferrugineus*	Africa, Asia Australia, Hawaii
Xyleborus	*glabratus*	North America (USA)
Xyleborus	*pfeilii*	Europe, New Zealand, North Amercia
Xyleborus	*seriatus*	North America (USA)
Xyleborus	*spinulosus*	North America (USA), Hawaii
Xyleborus	*xylographus*	Asia
Xylosandrus	*amputatus*	North America (USA)
Xylosandrus	*compactus*	Africa, Central America, Europe, Hawaii, New Zealand, North America (USA), South America
Xylosandrus	*crassiusculus*	Africa, Australia, Central America, Europe (Italy), Hawaii, North America, South America
Xylosandrus	*germanus*	Europe, North America
Xylosandrus	*morigerus*	Africa, Central America, Europe, Hawaii, Middle East (Jordon, Lebanon), North America, Pacific Islands, South America

Genus	species	Beetle(s)
Ambrosiella	*batrae*	*Anisandrus sayi*
Ambrosiella	*beaveri*	*Cnestus mutilatus*
Ambrosiella	*catenulata*	*Eccoptopterus* spp., *Hadrodemius* spp.
Ambrosiella	*cleistominuta*	*Anisandrus maiche*
Ambrosiella	*grosmanniae*	*Xylosandrus germanus*
Ambrosiella	*hartigii*	*Anisandrus dispar*
Ambrosiella	*nakashimae*	*Xylosandrus amputatus*
Ambrosiella	*roeperi*	*Xylosandrus crassiusculus*
Ambrosiella	*xylebori*	*Xylosandrus compactus*
Dryadomyces	*amasae*	*Amasa concitatus*, *Amasa* aff. *glaber*
Dryadomyces	*sulphureus*	*Xyleborinus saxexenii*
Fusarium	AF-6 AF-8	*Euwallacea* sp.
Fusarium	*duplospermum*	*Euwallacea* sp.
Fusarium	AF-9 AF-10	*Coptoborus* spp. *Xyleborus ferrugineus*
Fusarium	*drepaniforme*	*Euwallacea fornicatus*
Fusarium	AF-11 *papillatum*	*Euwallacea* sp. from Taiwan
Fusarium	AF-13	*Euwallacea* sp. from Taiwan
Fusarium	AF-14	*Euwallacea* sp. from Taiwan
Fusarium	AF-15	*Euwallacea* sp. from Taiwan
Fusarium	AF-16	*Euwallacea* sp. from Taiwan
Fusarium	AF-17	*Euwallacea* sp.
Fusarium	AF-18	*Euwallacea* sp.

Table 1.2: Ambrosia fungal species associated with xyleborine beetles and their beetle partners. Inferred from von Arx and Hennebert 1965, Batra 1967, Scott and du Toit 1970, Brayford 1987, Gebhardt 2005, Harrington et al. 2008, Six et al. 2009, Harrington et al. 2010, Kasson et al. 2013, Mayers et al. 2015, O'Donnell et al. 2015, Simmons et al. 2016a, 2016b, Lin et al. 2017, Mayers et al. 2017, Na et al. 2018, Carrillo et al. 2019, Lynn et al. 2020, Nel et al. 2021, and Osborn et al. 2022a.

Table 1.2 (cont'd)

Genus	species	Beetle(s)
Harringtonia	*lauricola*	*Xyleborus glabratus*
Irpex	*subulatus*	*Ambrosiodmus minor*
Neocosmospora	AF-1 *ambrosia* AF-2	*Euwallacea fornicatus*
Neocosmospora	*euwallaceae*	*Euwallacea* sp.
Neocosmospora	AF-3 *floridana*	*Euwallacea interjectus*
Neocosmospora	AF-4 *oligoseptata* AF-7	*Euwallacea validus*
Neocosmospora	*obliquiseptata*	*Euwallacea* sp.
Neocosmospora	AF-12 *kuroshio*	*Euwallacea* sp.
Neocosmospora	AF-19 *rekana*	*Euwallacea perbrevis*
Raffaelea	*arxii*	*Xyleborus vovulus* (= *X. torquatus*)
Raffaelea	*campbelliorum*	*Xyleborus glabratus*
Raffaelea	*cyclorhipidii*	*Cyclorhipidion ohnoi*
Raffaelea	*ellipticospora*	*Xyleborus glabratus*
Raffaelea	*fusca*	*Xyleborus glabratus*
Raffaelea	*promiscua*	*Xyleborinus saxesenii*
Raffaelea	*subalba*	*Xyleborus glabratus*
Raffaelea	*subfusca*	*Xyleborus glabratus*
Raffaelea	*xyleborini*	*Xyleborinus andrewsii*

Table 1.2 (cont'd)

Genus	species	Notes
Ambrosiella	*batrae*	
Ambrosiella	*beaveri*	
Ambrosiella	*catenulata*	
Ambrosiella	*cleistominuta*	
Ambrosiella	*grosmanniae*	
Ambrosiella	*hartigii*	
Ambrosiella	*nakashimae*	
Ambrosiella	*roeperi*	
Ambrosiella	*xylebori*	
Dryadomyces	*amasae*	
Dryadomyces	*sulphureus*	= *Raffaelea sulphurea*
Fusarium	AF-6	
Fusarium	AF-8 *duplospermum*	
Fusarium	AF-9	
Fusarium	AF-10 *drepaniforme*	= *Fusarium bugnicourtii*
Fusarium	AF-11 *papillatum*	
Fusarium	AF-13	
Fusarium	AF-14	
Fusarium	AF-15	
Fusarium	AF-16	
Fusarium	AF-17	
Fusarium	AF-18	

Table 1.2 (cont'd)

Genus	species	Notes
Harringtonia	*lauricola*	= *Raffaelea lauricola*
Irpex	*subulatus*	= *Flavodon ambrosius*
Neocosmospora	AF-1 *ambrosia* AF-2	= *Fusarium ambrosium*
Neocosmospora	*euwallaceae*	= *Fusarium euwallaceae*
Neocosmospora	AF-3 *floridana*	= *Fusarium floridanum*
Neocosmospora	AF-4 *oligoseptata*	= *Fusarium oligoseptatul*
Neocosmospora	AF-7 *obliquiseptata*	= *Fusarium obliquiseptatum*
Neocosmospora	AF-12 *kuroshio*	= *Fusarium kuroshium*
Neocosmospora	AF-19 *rekana*	= *Fusarium rekanum*
Raffaelea	*arxii*	
Raffaelea	*campbelliorum*	
Raffaelea	*cyclorhipidii*	
Raffaelea	*ellipticospora*	
Raffaelea	*fusca*	
Raffaelea	*promiscua*	
Raffaelea	*subalba*	
Raffaelea	*subfusca*	
Raffaelea	*xyleborini*	

Species	Nutrutional symbiont	Pathogenic fungus	Asia
Xyleborus glabratus	*Harringtonia lauricola*	*Harringtonia lauricola*	Native
Xylosandrus crassiusculus	*Ambrosiella roeperi*	…	Native
Xylosandrus compactus	*Ambrosiella xylebori*	*Fusarium solani* sp.	Native
Xylosandrus morigerus	*Ambrosiella* sp.	*Fusarium solani* sp.	Native
Xylosandrus germanus	*Ambrosiella grosmanniae*	*Fusarium solani* sp.	Native
Euwallacea fornicatus spp.	*Fusarium* spp. (AFC)	*Fusarium* spp. (AFC)	Native
Coptoborus ochromactonus	*Fusarium* sp. AF-9	*Fusarium* sp. AF-9	N/A
Ambrosiodmus minor	*Irpex Subulatus*	…	Native

Table 1.3: Harmful Xyleborini, their nutritional symbionts, associated pathogenic fungi, native region, year of first report in introduced regions, and significant plant hosts. Inferred from Blanford 1894a, Hagedorn 1908, Hoffmann 1941, Groschke 1953, Anderson 1974, Kessler et al. 1974, Hara and Beardsley 1979, Wood 1982, Weber and McPherson 1983, Nirenburg 1990, Pennacchio et al. 2003, Haack 2006, Rabaglia et al. 2006, Kirkendall and Ødegaard 2007, Cognato and Rubinoff 2008, Olivera et al. 2008, Eskalen et al. 2012, Garonna et al. 2012, Mendel et al. 2012, Stilwell et al. 2014, Egonyu et al. 2015, Mayers et al. 2015, Nageleisen et al. 2015, You et al. 2015, Flechtmann and Atkinson 2016, Simmons et al. 2016a, Gallego et al. 2017, Li et al. 2017, Kavčič 2018, Paap et al. 2018, Schiefer 2018, Carreras-Villaseñor et al. 2022, and Osborn et al. 2022a.

Table 1.3 (cont'd)

Species	North America	Africa	Europe
Xyleborus glabratus	2002	N/A	N/A
Xylosandrus crassiusculus	1700s?	1700s?	2003
Xylosandrus compactus	1941	1700s?	2011
Xylosandrus morigerus	unknown	unknown	unknown
Xylosandrus germanus	1941	N/A	1951
Euwallacea fornicatus spp.	2006	2018	Native
Coptoborus ochromactonus	N/A	N/A	N/A
Ambrosiodmus minor	2017	N/A	N/A

Table 1.3 (cont'd)

Species	**Central/South America**	**Oceania**	**Australia**
Xyleborus glabratus	N/A	N/A	N/A
Xylosandrus crassiusculus	1996	N/A	N/A
Xylosandrus compactus	1970s	1961 (Hawaii)	N/A
Xylosandrus morigerus	unknown	Native	N/A
Xylosandrus germanus		2007 (Hawaii)	N/A
Euwallacea fornicatus spp.	1980s	1980s	Native
Coptoborus ochromactonus	Native	N/A	N/A
Ambrosiodmus minor	N/A	N/A	N/A

Table 1.3 (cont'd)

Species	Significant hosts
Xyleborus glabratus	Lauraceae*, Persea*
Xylosandrus crassiusculus	*Carica, Cinnamomum, Coffea, Hevea, Magnifera, Prunus, Swietenia, Tectona, Theobroma*
Xylosandrus compactus	*Ceratonia, Coffea, Laurus, Magnifera, Persea, Theobroma,*
Xylosandrus morigerus	*Coffea, Cacao, Fabaceae, Persea, Swietenia, Tectona*
Xylosandrus germanus	*Malus, Pinus, Vitis*
Euwallacea fornicatus spp.	*Persea*
Coptoborus ochromactonus	*Ochroma*
Ambrosiodmus minor	*…*

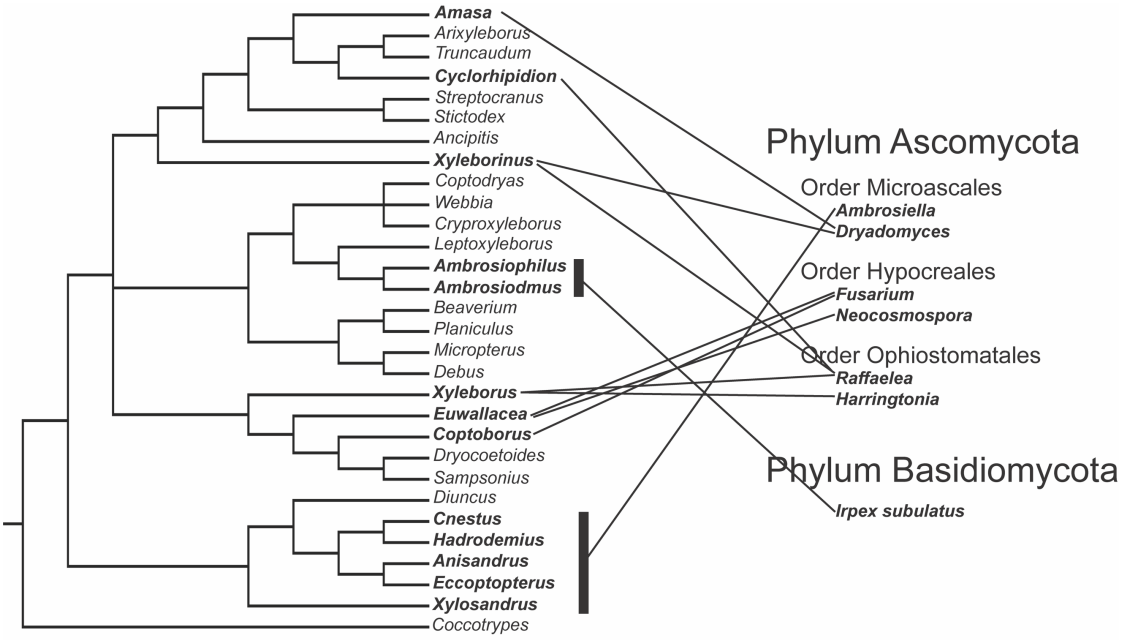

Figure 1.1: Schematic representation of ambrosia associations between xyleborine and fungal genera. Cladogram of the Xyleborini summarized from Cognato et al. 2011, Cognato et al. 2018, and Johnson et al. 2018. Relationships between the beetles and fungi from von Arx and Hennebert 1965, Batra 1967, Scott and du Toit 1970, Brayford 1987, Gebhardt 2005, Harrington et al. 2008, Six et al. 2009, Harrington et al. 2010, Kasson et al. 2013, Mayers et al. 2015, O'Donnell et al. 2015, Simmons et al. 2016a, 2016b, Lin et al. 2017, Mayers et al. 2017, Na et al. 2018, Carrillo et al. 2019, Lynn et al. 2020, Nel et al. 2021, Osborn et al. 2022a.

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CHAPTER 2:

Ecuadorian *Coptoborus* beetles harbor *Fusarium* and *Graphium* fungi previously associated with *Euwallacea* ambrosia beetles

ABSTRACT

Ambrosia beetles from the scolytine tribe Xyleborini (Curculionidae) are important to the decomposition of woody plant material on every continent except Antarctica. These insects farm fungi on the walls of tunnels they build inside recently dead trees and rely on the fungi for nutrition during all stages of their lives. Such ambrosia fungi rely on the beetles to provide appropriate substrates and environmental conditions for growth. A small minority of xyleborine ambrosia beetle/fungi partnerships cause significant damage to healthy trees. The xyleborine beetle *Coptoborus ochromactonus* vectors a *Fusarium* (Hypocreales) fungus that is lethal to balsa (*Ochroma pyramidale* (Malvaceae)) trees in Ecuador. Although this pathogenic fungus and its associated beetle are not known to be established in the United States, several other non-native ambrosia beetle species are vectors of destructive plant diseases in this country. This fact and the acceleration of trade between South America and the United States demonstrate the importance of understanding fungal plant pathogens before they escape their native ranges. Here we identify the fungi accompanying *Coptoborus* ambrosia beetles collected in Ecuador. Classification based ribosomal internal transcribed spacer 1 (ITS) sequences revealed the most prevalent fungi associated with *Coptoborus* are *Fusarium* spp. and *Graphium* spp. (Microascales: Microascaceae), which have been confirmed as ambrosia fungi for xyleborine ambrosia beetles, and *Clonostsachys* sp. (Hypocreales), which is a diverse genus found abundantly in soils and associated with plants. Phylogenetic analyses of the *Fusarium* strains

based on ITS, translation elongation factor (*EF1*-α), and two subunits of the DNA-directed RNA polymerase II (*RPB1* and *RPB2*) identified them as *Fusarium.* sp. AF-9 in the Ambrosia *Fusarium* Clade (AFC). This *Fusarium* species was previously associated with a few xyleborine ambrosia beetles, most notably the species complex *Euwallacea fornicatus* (Eichhoff 1868) (Curculionidae: Scolytinae: Xyleborini). Examination of ITS and *EF1*-α sequences showed a close affinity between the *Graphium* isolated from *Coptoborus* spp. and other xyleborine-associated *Graphium* as well as the soil fungus *Graphium basitruncatum*. This characterization of ambrosia fungi through DNA sequencing confirms the identity of a putative plant pathogen spread by *Coptoborus* beetles and expands the documented range of *Fusarium* and *Graphium* ambrosia fungi.

INTRODUCTION

The weevil subfamily Scolytinae includes bark beetles and ambrosia beetles that are ecologically important in decomposing dead and dying trees and shrubs worldwide (Hofstetter et al. 2015). Bark and ambrosia beetles share an evolutionary history, although they inhabit different ecological niches (Jordal and Cognato 2012, Johnson et al. 2018). Bark beetles excavate tunnels (galleries) underneath and parallel to the bark of their host, feed on phloem, and facultatively interact with fungi that already exist in their environment (Wood 2007, Hulcr et al. 2015). Ambrosia beetles carry symbiotic fungi in specialized cuticular organs known as mycangia. They bore into the xylem of their host, then inoculate the gallery walls with symbiotic fungi that provide nutrition throughout the entire life cycle of the beetles (Hubbard 1897, Hofstetter et al. 2015). The bark beetle lifestyle is ancestral within Scolytinae, but ambrosia feeding evolved 12–16 independent times (Johnson et al. 2018) with several different fungal lineages.

Ambrosia fungi provide three essential services to their beetle partners. They extract carbon and nutrients from the xylem substrate and pass them to the beetles (Bleiker and Six 2007, Six 2012, Hofstetter et al. 2015). As they spread and decompose the wood, the fungi probably protect the beetles from plant defensive responses (Klepzig et al. 2001, Six 2003, Hofstetter et al. 2015), and the invasion of less desirable fungi in the gallery (Klepzig 2006). The saprotrophic action of ambrosia fungi may also soften the wood, allowing the beetles to expand their galleries more easily (Barta 1967). In return, ambrosia beetles disperse the fungi and provide a protected habitat inside the xylem with optimal humidity (Batra 1967).

The nature and diversity of the ambrosia symbiosis has always been enigmatic, because ambrosia beetles are small and live most of their lives within their galleries where they cannot be observed easily. Ambrosia fungi are often highly adapted to life inside beetle tunnels (Francke-Grosmann 1967, Malloch and Blackwell 1993, Cassar and Blackwell 1996, Jones and Blackwell 1998), and convergence challenges their morphological identification and description (Francke-Grossman 1967, Alamouti et al. 2009). The relationships they share inside the beetle gallery are complex, often involving nematodes, protozoa, mites, other filamentous fungi, yeasts and bacteria (Hofstetter et al. 2015).

Ambrosia beetles and their fungi generally inhabit dead or dying plants and thus are considered harmless. However, a handful of ambrosia beetles vector destructive fungi capable of killing otherwise healthy trees. These partnerships cause serious ecological and economic damage to forest, urban and ornamental trees (Hubbard 1897, Smith and Hulcr 2015). Some of the most impactful vectors are exotic xyleborine ambrosia beetles.

The invasive beetle species complex *Euwallacea fornicatus* (Eichhoff 1868), which originated in Asia, was first documented in the southeastern United States in 2002 (Haack 2006).

This group, which contains seven morphologically cryptic beetle species (Smith et al. 2019) was introduced multiple times in North and Central America (Wood and Bright 1992, Haack 2006, Rabaglia et al. 2006, Kirkendall and Ødegaard 2007). Like fungi associated with other invasive xyleborines, the mycangial partners of the *Euwallacea fornicatus* species group are diverse and include fungi from the nectrioid genera *Paracremonium* and *Fusarium* (Hypocreales), and *Graphium* (Microascales: Microascaceae) (Freeman et al. 2013, 2016, Li et al. 2016, Lynch et al. 2016, O'Donnell et al. 2016, Carrillo et al. 2020a).

Many of the species in the genus *Graphium* are plant pathogens, including some that exploit physically damaged trees (Musvuugwa et al. 2020). Several *Graphium* species disperse with scolytine beetles, including many bark beetles (Wingfield and Gibbs 1991, Jacobs et al. 2003, Okada et al. 2003, Malacrinó et al. 2017). Baker and Norris (1968) recovered *Graphium* from the mycangium of the xyleborine beetle *Xyleborus ferrugineus* (F.), although they never confirmed that it acted as a nutritional symbiont (Kok 1979). Recent work established two *Graphium* species as ambrosia fungi associated with the *E. fornicatus* species complex: *Graphium euwallaceae* Twizeyimana, Lynch & Eskalen and *Graphium kuroshium* Na, Carrillo & Eskalen (Freeman et al. 2016, Lynch et al. 2016, Na et al. 2018, Carrillo et al. 2020a, 2020b). Interestingly, both *Graphium* ambrosia fungi (as well as the undescribed species associated with *X. ferrugineus*) coexist with *Fusarium* (Baker and Norris 1968, Freeman et al. 2016, Lynch et al. 2016).

The genus *Fusarium* is diverse in species and ecology. Although the taxonomic status of many *Fusarium* species is still poorly understood (Summerell et al. 2010, Crous et al. 2021), several species complexes cause disease including in numerous important grain and food crops (Aoki et al. 2003, Ploetz 2006, Saremi et al. 2008, Arie 2019), trees (Gardner 1980, Stilwell et al.

2014), humans and other animals (O'Donnell et al. 2012, Smyth et al. 2019). Some species in the *Fusarium solani* (Mart.) Sacc. species complex are vectored by *Euwallacea* ambrosia beetles (see Aoki et al. 2021 and references therein). These fungi form a monophyletic lineage and are collectively referred to as the Ambrosia *Fusarium* Clade (AFC) (Kasson et al. 2013, O'Donnell et al. 2015). As with other *Fusarium* species, some AFC isolates are phytopathogens, causing damage to avocado and forest trees in California and Israel (Eskalen et al. 2013, Kasson et al. 2013, Cognato et al. 2015, Lynch et al. 2016, Short et al. 2017). Researchers examining the AFC uncovered evidence of a longstanding cooperative relationship with *Euwallacea* beetles beginning 19–24 MA (O'Donnell et al. 2015). The origin of the AFC (~21–24 MA) roughly coincides with the origin of their xyleborine beetle associates (~19 MA) (Jordal et al. 2000, Kasson et al. 2013, O'Donnell et al. 2015). Several AFC species also produce large, club-shaped conidia, presumably an adaptation to their nutritional partnership with beetles (O'Donnell et al. 2015). However, the relationship between the fungi and their beetle hosts appears to be promiscuous, with at least five host-switching events over 19–24 MA (O'Donnell et al. 2015). Furthermore, there is evidence that species from other xyleborine genera such as *Xyleborus ferrugineus* (Baker and Norris 1968, Norris and Baker 1968) and *Xyleborinus saxesenii* (Ratzeburg 1837) (Malacrinó et al. 2017) associate with *Fusarium*.

The most destructive xyleborine-fungal partnerships originated in Asia and were imported to the Americas (Haack 2016). Nevertheless, potentially threatening beetle-ambrosia pairings from Central and South America deserve attention (Liebhold and Kean 2018, Stillwell et al. 2014). In the early 1990's the xyleborine *Coptoborus ochromactonus* Smith & Cognato (Stilwell et al. 2014) was discovered to vector an undescribed *Fusarium* pathogen that causes a wilting disease that can kill up to 90% of infected balsa trees (*Ochroma pyramidale* (Cav. Ex

Lam.) Urb.) in Ecuador (Stilwell et al. 2014, Castro et al. 2019). This newly described beetle is a member of a recently revised genus native to the Neotropics comprising 77 species (Smith and Cognato 2021). The identities of *Coptoborus*-associated fungi are mostly unknown (Wood 2007, Hulcr et al. 2015). The location of the mycangium of *Coptoborus* spp. has not been confirmed (Castro et al. 2019), although examination of the mycangial diversity within Xyleborini suggests that mandibular mycangia (hereafter referred to as preoral mycangia after Li et al. 2015) are the ancestral state for the tribe (Schedl, 1962, Francke-Grosmann 1967, Cognato et al. 2011). Additionally, phylogenetic analysis indicates that the genus shares a close evolutionary relationship with *Xyleborus*, which have preoral mycangia (Cognato et al. 2011).

Given the damage caused by *C. ochromactonus* to the Ecuadorian balsa industry and the increasing ecological homogenization (Fisher et al. 2012) from mounting international trade and global climate change (Liebhold and Kean 2018, Aukema et al. 2010, Marini et al. 2011, Rabaglia et al. 2019), further investigation of fungi associated with South American xyleborines has become increasingly relevant. To complement the increasing knowledge of Neotropical xyleborine diversity (Wood 2007, Smith et al. 2017, Bright 2019, Smith and Cognato 2021), this paper identifies and phylogenetically resolves the fungi associated with six Ecuadorian *Coptoborus* species.

METHODS

Material collection

Live ambrosia beetles were collected from dead and dying trees and woody plant material at the Yasuní Research Station (Orellana Province, Ecuador, –0.661111, –76.400389) between January

and March 2018. Specimens from each wood sample were sorted to morphospecies, based on characters of the antennae, protibiae, and declivital sculpturing. They were kept alive on damp Kimwipe tissues in 50 ml vials and transported to Pontifica Universidad Católica del Ecuador in Quito.

Depending on availability, up to five specimens per morphospecies were selected for fungal isolation, for a total of 24 beetle specimens. Each individual was surface-sanitized by vortexing for 15 s in sterile phosphate-buffered saline (PBS). To isolate the preoral mycangia, a sterile scalpel was used to separate the head of each beetle from the pronotum. Each head was pulverized with a sterile micropestle and combined with 500 µL of sterile PBS. Fifty µL of this solution was transferred into 500 µL of sterile PBS to create a 9x dilution. The remainder of the body of each specimen was preserved in 100% ethanol and vouchered in The A.J. Cook Arthropod Research Collection at Michigan State University.

Fifty µL each from the 1x solution and the 9x dilution were transferred to Petri dishes filled with potato dextrose agar (PDA) fortified with 1.6% agar, 0.2% yeast extract, and 1% Penicillin-Streptomycin antibiotics (Life Technologies; Waltham, Massachusetts). The plated mycangial samples were incubated at 23–25 ºC in the dark and checked for growth as over the course of ten days. Colonies on the plates were visually examined to characterize them into morphotypes and the growing edge of one representative from each morphotype was transferred to PDA in a Petri dish to obtain pure cultures. After incubating the pure cultures for up to five days, plugs with fungal growth were preserved in 100% ethanol for subsequent use.

Molecular data

DNA was extracted from the fungal samples with a Qiagen plant Mini Kit (Hilden, Germany) according to the manufacturer's instructions. The ribosomal internal transcribed spacer 1 (ITS) (Schoch et al. 2012) from each isolate was amplified and sequenced for preliminary identification to genus with the nucleotide BLAST search function of the National Center for Biotechnology Information (NCBI) GenBank database (Altschul et al. 1990). Twenty-six fungal samples were identified as the ambrosia lineages *Fusarium* or *Graphium* (Table 2.1). We amplified and sequenced two additional loci from these isolates based on their known phylogenetic utility: translation elongation factor (*EF1*-α), and the second largest subunit of DNA-directed RNA polymerase II (*RPB2*) (Mendel et al. 2012, O'Donnell et al. 2012, Eskalen et al. 2013, Freeman et al. 2013, Kasson et al. 2013, O'Donnell et al. 2015). PCR primers and protocols followed Jacobs et al. (2004), Lynch et al. (2016), O'Donnell et al. (1998, 2007, 2010), and White et al. (1990) (Tables 2.2 and 2.3).

Previous work has also demonstrated the phylogenetic utility of DNA-directed RNA polymerase I (*RPB1*) (O'Donnell et al. 2012, Freeman et al. 2013, Kasson et al. 2013, O'Donnell et al. 2015), thus we included this locus in our data set when data were available from GenBank (Table 2.4). Repeated attempts to amplify this locus from our Ecuadorian fungal isolates were not successful, however, inclusion of *RPB1* data from additional *Fusarium* strains from GenBank added resolution and branch support to our analyses. Thus, we incorporated *RPB1* data into the phylogenetic analysis for *Fusarium*, but not for *Graphium*. The total volume of each PCR cocktail for all loci was 25 μL per 50–5 ng DNA template and included 1x buffer, 1.75 mM $MgCl_2$, 200 μM dNTPs, 0.3 μM each forward and reverse primers, and 1.25 units HotStar Taq (Qiagen) (See Table 2.3 for PCR conditions). PCR products were readied for cycle sequencing

with Exo-SAP-IT (Applied Biosystems; Waltham, Massachusetts) and sequenced with BIGDYE TERMINATOR 1.1 (Applied Biosystems) at the Michigan State University Research Technology Support Facility. Opposing strands were compiled, trimmed, and edited to correct ambiguous or incorrect base judgements with SEQUENCHER 5.0 BUILD 7082 (Gene Codes Corporation; Ann Arbor, Michigan). Completed sequences were submitted to GenBank (see Table 2.1 for accession numbers). Sequence alignments for both *Fusarium* spp. and *Graphium* spp. were created using the default settings of MUSCLE (Madeira et al. 2019).

Phylogenetic analyses

To identify the putative ambrosia fungi to species, the data from *Fusarium* spp. and *Graphium* spp. were each subjected to independent phylogenetic analyses on each locus and on combined datasets. Loci used for *Fusarium* were ITS*, EF1-α, RPB1,* and *RPB2*. Those used for *Graphium* were ITS*,* and *EF1-α*. Both datasets were also supplemented with corresponding data from GenBank (Table 2.4).

Data from *Fusarium* isolates included 17 AFC fungal samples from Yasuní and molecular data from similar ambrosia fungi obtained from GenBank. The additional sequences are GenBank data from 95 known ambrosia strains isolated from xyleborines or wood infested with xyleborines (Mendel et al. 2012, Kasson et al. 2013, O'Donnell et al. 2015, Na et al. 2018, Carrillo et al. 2020b). We also included three representative strains from non-ambrosia *Fusarium* species (Zhang et al. 2006, O'Donnell et al. 2007, O'Donnell et al. 2010, Kasson et al. 2013). *Fusarium neocosmosporiellum* O'Donnell and Geiser was used to root the trees based on previous phylogenies of the AFC (see Kasson et al. 2013, Lynn et al. 2020).

We evaluated the combined *Fusarium* dataset using PARTITIONFINDER 2.1.1 to establish appropriate partitions within the data and to choose the best-fit model of nucleotide substitution for each (Guindon et al. 2010, Lanfear et al. 2016). An exhaustive search (using the 'all' search algorithm) of seven data blocks (a single data block for the non-coding ITS gene, and the 1st, 2nd and 3rd codon positions of *EF1-α*, *RPB1,* and *RPB2*) established seven partitions selected based on the corrected Akaike information criterion (AICc). The ITS*, EF1-α* genes, and a subset that combines the third codon positions from *RPB1* and *RPB2* were each assigned to a separate general time-reversible model with variable nucleotide frequencies, and gamma distributed rate of variation (GTR+I+G) (Lanave et al. 1984, Rodríguez et al. 1990). A general time-reversible model with variable nucleotide frequencies (GTR+I) (Lanave et al. 1984, Rodríguez et al. 1990) fit a combined subset of the first codon positions of *RPB1* and from *RPB2*. The second codon position of *RPB1* was fit to the general time-reversible model with a gamma distribution (GTR+G) (Lanave et al. 1984, Rodríguez et al. 1990). The second codon position of *RPB2* fit the Hasegawa-Kishino model with variable nucleotide frequencies and a gamma distribution (HKY+I+G) (Hasegawa et al. 1985).

Sequence data of ITS*,* and *EF1-α,* from the nine *Graphium* isolates were concatenated with data from the same two loci belonging to 65 *Graphium* ambrosia strains found in GenBank (Table 2.4). Thirty-nine of these were isolated from wood infested by scolytine beetles (Cruywagen et al. 2010, Lynch et al. 2016, Na et al. 2018), 16 were isolated from xyleborine beetles (Na et al. 2018, Carrillo et al. 2020b), seven were isolated from non-xyleborine Scolytinae (Jacobs et al. 2003, Hulcr et al. 2007, Cruywagen et al. 2010), and three were isolated from *Pissodes* weevils (Molytinae) (Cruywagen et al. 2010, Paciura et al. 2010). We also included a three-member outgroup consisting of the non-ambrosia species *Graphium*

basitruncatum (Matsush.) Seifert & G. Okada*, Ophiostoma ulmi* (Buisman) Nannf.*,* and *Geosmithia putterillii* (Thom) Pitt (formerly *G. pallida* (G. Sm.) M. Kolarík, Kubátová & Pazoutová) after previous work concerning ambrosia-beetle-associated *Graphium* (Kolarík and Hulcr 2008, Cruywagen et al. 2010, Lynch et al. 2016, Na et al. 2018, Carrillo et al. 2020b).

The combined *Graphium* dataset was divided into seven data blocks using PARTITIONFINDER 2.1.1 (Guindon et al. 2010, Lanfear et al. 2016). The non-coding gene ITS was assigned to a single block, whereas the coding genes *EF1-α* was divided into three blocks containing the first, second and third codon positions, respectively. A complete search of these data blocks identified five subsets for partitioning. ITS and the second codon position of *EF1-α* each fit a separate general time-reversible model with a gamma distribution (GTR+G) (Lanave et al. 1984, Rodríguez et al. 1990). The first and third codon positions from *EF1-α* best fit was Hasegawa-Kishino with gamma distributed rate of variation (HKY+G) (Hasegawa et al. 1985).

We imported the model schemes for *Fusarium* and *Graphium* into MRBAYES 3.2.5 (Ronquist and Huelsenbeck 2003) and used them to search the treespace of each combined dataset with Monte Carlo Markov chains. Both analyses used identical parameters including two independent runs, each consisting of three heated chains and one cold chain (metropolis coupling), searching across 40 million generations using Monte Carlo rules for tree acceptance or rejection at each generation. We sampled the tree at every 100th generation and discarded the first 25% (100,000 trees) as burn-in before using the remaining 300,000 trees to build a majority-rule consensus tree.

The data from both genera were also subjected to independent heuristic searches using PAUP* 4.0A BUILD 167 (Swofford 2002) to search for most parsimonious trees. Gaps were treated as missing. Characters were unordered and equally weighted during the heuristic search with

2000 repetitions of random stepwise addition of taxa using tree bisection/reconnection. Bootstrap support was calculated using the same character parameters and 2000 full heuristic searches with simple stepwise addition.

We created gene trees for *Fusarium* ITS, *EF1-α*, *RPB1,* and *RPB2,* and *Graphium* ITS and *EF1-α* with the same parameters used on the combined parsimony and Bayesian analyses. Alignments from each locus in the combined data sets (ITS, *EF1-α*, *RPB1*, and *RPB2* for *Fusarium*, and ITS, and *EF1-α*, for *Graphium*) were separated to create seven individual gene alignments. The PARTITIONFINDER 2.1.1 partition schemes from the combined data sets were applied to each of the genes for both genera and each was subjected to Bayesian analysis using to settings specified above for the combined data. We also used PAUP* 4.0A BUILD 167 to conduct combined parsimony searches on each gene alignment.

RESULTS

Fungal identification via ITS

We recovered 50 fungal cultures from 24 *Coptoborus* specimens. Each beetle yielded up to three morphologically distinct fungal cultures from their mycangial region. Searched with BLAST based on ITS sequences preliminarily identified these fungi as belonging to nine taxa that included one basidiomycete order (Exobasidiales) and seven genera in the ascomycete class Pezizomycotina (Table 2.5). Four isolates were only recovered from single beetles, significantly aligning to *Penicillium griseofulvum* Dierckx, *Hypocrea virens* P. Chaverri, Samuels & E.L. Stewart, *Chaetomium globosum* Kunze, and Exobasidiales. The remaining four fungal taxa were recovered with greater frequency. Among them, two strains tentatively identified as

Phialemoniopsis sp. and *Clonostachys* sp. (Hypocreales) were isolated from only *Coptoborus tolimanus* (Eggers).

One known and two putative ambrosia fungi were recovered from the examined specimens. Isolates with top BLAST matches to *Graphium euwallaceae* Twizeyimana, Lynch and Eskalen was cultured from the mycangia of one individual each of *Coptoborus coartatus* (Sampson 1921) and *Coptoborus osbornae* Smith & Cognato 2021. The BLAST search also identified two *Fusarium* species previously isolated from dead and diseased wood. *Fusarium* sp. 1 RJ2014 was isolated from two individuals of *C. coartatus,* four *Coptoborus cracens* Wood 2007*,* five *C. osbornae,* and one *Coptoborus pseudotenuis* (Schedl 1936). *Fusarium solani* was isolated from one individual each of *C. pseudotenuis* and *C. osbornae*. Given the close association both *Fusarium* share with ambrosia beetle habitat and plant disease (Mohali and Stewart 2017, Jankowiak et al. 2019), it is possible that further analysis will place them within the AFC. However, additional molecular data and phylogenetic analysis are required to confirm this.

Specific identification of ambrosia fungi via phylogenetic analyses

Comparison of the Yasuní fungal isolates with GenBank sequences preliminarily identified 26 isolates as possible ambrosia fungi. Of these, 17 were tentatively placed into *Fusarium* and nine were assigned to *Graphium euwallaceae.* We used combined data of four and two genes to provide specific identification of the *Fusarium* and *Graphium* sequences, respectively.

Length-variable regions were not found in the alignment of the four *Fusarium* loci. We subjected the concatenated alignment to Bayesian and parsimony analyses. Two Bayesian runs converged within 40 million generations with a split distribution between analyses that reached a

mean standard deviation of 0.015. Most of the clades of the Bayesian consensus tree were well supported with greater than 0.9 posterior probabilities (PP). The *Fusarium* isolates from Ecuadorian beetles were placed in a clade with *Fusarium.* sp. AF-9 with very strong support (PP = 1.00) (Figures 2.1 and 2.2). The parsimony analysis of the *Fusarium* data (293 informative characters) evaluated 1.0 x 10^{11} rearrangements and yielded 909 000 trees with a best score of 641 steps. A strict consensus of these shared general agreement with the consensus tree generated from the Bayesian analysis. Together, the parsimony and Bayesian analyses provided strong support placing the Ecuadorian *Fusarium* within the AFC AF-9 lineage (Kasson et al. 2013, O'Donnell et al. 2015).

Gene trees created from single-gene alignments and subjected to identical analyses as the combined *Fusarium* data were largely congruent with the combined tree. Most differences stemmed from lack of resolution in the gene trees. The gene trees for ITS, *EF1-α* and *RPB2* placed the *Coptoborus* fungal isolates inside the *Euwallacea* AFC lineage and support the identification of them as *F.* sp. AF-9 (Figures 2.3–2.6).

Several length-variable regions were found in the alignment of the ITS and *EF1-α* data from *Graphium.* These length-variable regions were most evident in the sequences other than those from the Ecuadorian fungal cultures. After 40 million generations, the two Bayesian runs had converged. The mean standard deviation of the split distribution between them was 0.002. The consensus tree resulting from Bayesian analysis was poorly resolved (Figures 2.7 and 2.8). Some lineages associated with non-xyleborine beetles (*Graphium pseudormiticum* M. Mouton & M.J. Wingf.*, Graphium fimbriasporum* (M. Morelet) K. Jacobs, Kristis & M.J. Wingf.*,* and *Graphium scolytodis* M. Kolařík & J. Hulcr) and several *Graphium* species associated with storm-injured trees (*Graphium adansoniae* Cruwy., Z.W. de Beer & Jol. Roux*, Graphium*

madagascariense Cruwy., Z.W. de Beer & Jol. Roux*, Graphium fabiforme* Cruwy., Z.W. de Beer & Jol. Roux, and *Graphium penicillioides* Corda) formed well supported clades (Figures 2.7 and 2.8).

However, the *Graphium* isolated from *Coptoborus* and those associated with wood-infesting insects, including *Euwallacea,* formed a weakly supported clade (PP = 0.64) that also included the soil fungus *G. basitruncatum.* Notably, the ambrosia species *Graphium euwallaceae* remained unresolved within this group. Parsimony analysis of the combined *Graphium* dataset included 280 informative characters and evaluated 6.7 x 10^{10} combinations. The best tree score, 1115, was shared by 1 989 000 trees. Gene trees from *EF1-*α were consistent with the combined tree while the ITS tree showed low branch support and poor resolution for the ambrosia species. (Figures 2.9–2.10).

DISCUSSION

This study is the first to survey Neotropical xyleborine beetles for symbiotic fungi within their native range. We obtained 50 fungal cultures representing at least eight taxa from the mycangia of 24 beetles collected in Yasuní, Orellana Province, Ecuador. Fungi putatively identified as representing the order Exobasidiales and the species *Penicillium griseofulvum*, *Hypocrea virens*, and *Chaetomium globosum* were each recovered from only a single beetle. However, several taxa were repeatedly isolated from more than one beetle: *Phialemoniopsis* sp., a poorly understood genus, and *Clonostachys* sp., commonly associated with both plants and soil where it functions as an endophyte, saprotroph, and parasite to nematodes and *Neonectria* fungi (Yu and Sutton 1997, Schroers et al. 1999, Zhang et al. 2008, Stauder et al. 2020). Notably, *Clonostachys rosea* (Link: Fries) Schroers, Samuels, Seifert, and Gams is a known biological control agent

against several plant parasites, including *Fusarium graminearum* Schwabe, which causes *Fusarium* head blight on wheat and barley (Xue et al. 2014, Nygren et al. 2018, Demissie et al. 2018). *Clonostachys* spp. have also been recovered from the wood of trees affected by the ambrosia beetle-vectored disease Fusarium Dieback (Carrillo et al. 2020b). Notably in this study, *Clonostachys* spp. was cultured from *C. tolimanus* from the same infested wood sample as *C. coartatus* and its cultured *Fusarium* and *Graphium* fungi. Given that contamination of ambrosia beetle galleries with competing saproxylic fungi is common (Franke-Grosmann 1967, Malacrinó et al. 2017), and that neither C*lonostachys* nor *Phialemoniopsis* have been reported in association with ambrosia beetles, it is unlikely that they participate in the ambrosia symbiome. However, if these fungi are found in the mycangia of other xyleborines, they may deserve further investigation to rule out a symbiotic association.

More broadly, fungi identified as *Fusarium* sp AF-9. and *Graphium euwallaceae* were associated with *Coptoborus* collected from Yasuní. These fungi were previously shown to function as nutritional partners with other ambrosia beetles (e.g., Norris and Baker 1968, Freeman et al. 2016, Carrillo et al. 2020a). We propose that they may be symbionts of xyleborines of the area.

Our recovery of *Fusarium* sp. AF-9 in Ecuador is a novel discovery from neotropical xyleborines. While the Ambrosia *Fusarium* Clade has been well documented from beetles in the related genera *Euwallacea* and *Xyleborus,* this is the first time the fungus has been isolated from mycangia of *Coptoborus* spp. with confirmation from DNA data. With the exception of a single Costa Rican specimen of *Xyleborus ferrugineus* whose identification is questionable and unconfirmed (Kasson et al. 2013, O'Donnell et al. 2015), genetic data have only documented *Fusarium* to associate with *Euwallacea* spp. in the Middle East (Israel), Australia (Queensland),

Asia (Taiwan, India, Sri Lanka, Malaysia, Singapore), and North America (Florida, Pennsylvania, and California, USA) (Mendel et al. 2012, Kasson et al. 2013, O'Donnell et al. 2015, Na et al. 2018). Most of these records are from invasive beetle species living outside their native ranges.

Phylogenetic analyses that included wide representation of the previously collected AFC placed our *Fusarium* isolates from *Coptoborus* with Ambrosia *Fusarium* sp. AF-9 supported by strong posterior probability and bootstrap values (Figure 2.1). We isolated this fungus from three *Coptoborus* species living in two different plant hosts. As previously reported, this group is sister to *Fusarium pseudensiforme* Samuels, Nalim & Geiser and closely related to *F. duplospermum* (sp. AF-8, Figure 2.1) (Kasson et al. 2013, Aoki et al. 2021).Together with previous work on the AFC, we show that *Fusarium* lives with their beetle partners in a variety of plant hosts and associates with several *Euwallacea* and *Coptoborus* species, including some living within their native ranges and some exotic invasive species (Mendel et al. 2012, Eskalen et al. 2013, Freeman et al. 2013, Stilwell et al. 2014, O'Donnell et al. 2015, Li et al. 2016, Lynch et al. 2016, Short et al. 2017, Na et al. 2018, Castro et al. 2019).

The fidelity between ambrosia beetles and their associated fungi spans a spectrum where some partnerships are constant, and others are promiscuous. Even within Xyleborini, some lineages share the same fungal genus as a symbiont. For instance, *Ambrosiodmus* and *Ambrosiophilus* maintain a symbiotic relationship with the basidiomycete fungus *Flavodon* (Li et al. 2015, Kasson et al. 2016). The clade including *Cnestus, Hadrodemius, Diuncus, Anisandrus, Eccoptopterus,* and *Xylosandrus* associates with various fungi from a single lineage in the genus *Ambrosiella* (Mayers et al. 2015, Skelton et al. 2019). The relationships among other ambrosia beetle genera and their fungi are looser and characterized by multiple partner-switching events.

Various *Xyleborus* species carry multiple fungi in their mycangia (Norris 1965, Baker and Norris 1968). Although many rely on *Raffaelea* spp. for nutrition (Gebhardt et al. 2004, Harrington et al. 2010, Saucedo et al. 2017), lateral transfer of the laurel wilt fungus *Harringtonia lauricola* (T.C. Harr., Fraedrich & Aghayeva) Z.W. de Beer & M. Procter (= *Raffaelea lauricola*) appears to be common among *Xyleborus* species (Carrillo et al. 2014, Ploetz et al. 2017).

Euwallacea spp. rely on two nutritional symbionts from the fungal genera *Fusarium* and *Graphium* (Freeman et al. 2013, 2016, Li et al. 2016, Lynch et al. 2016, Carrillo et al. 2020a) and likely underwent several host-switching events in their evolutionary histories (O'Donnell et al. 2015). Factors mediating the specificity of scolytine-fungal relationships are poorly understood. It is likely that some characteristics of the beetle, such as internal mycangium structure and the associated glands help regulate which fungi can successfully survive inside the mycangium (Schedl 1962, Skelton et al. 2019, Mayers et al. 2020a, 2020b). However, there is also evidence that fungal preference for specific environmental conditions, and other taxonomically constrained characteristics play a role in determining whether ambrosia fungi are associated with a limited number of ambrosia beetle species, or if they are likely to switch partners frequently (Francke-Grosmann 1967, Rassati et al. 2016 Miller et al. 2019).

Considering previous work on relationships of xyleborine beetles and fungal associates, the isolation of *Fusarium* sp. AF-9 from *Coptoborus* is not surprising. *Coptoborus* and the other Neotropical xyleborine genera are sister to *Xyleborus* (Cognato et al. 2011, Smith and Cognato 2021), which use *Raffaelea* spp. as symbionts but appear to switch fungal partners readily (Freeman et al. 2013, 2016, Li et al. 2016, Lynch et al. 2016, Carrillo et al. 2020a). *Fusarium*-associating *Euwallacea* beetles are not monophyletic with *Coptoborus* but more distantly related (Cognato et al. 2011, Gohli et al. 2017). This indicates a potential lateral transfer of *Fusarium* sp.

AF-9 among non-related beetle taxa given the close phylogenetic distance between the *Fusarium* sp. AF-9 we recovered, and those previously isolated from *Euwallacea* (Kasson et al. 2013, O'Donnell et al. 2015).

Graphium isolates from *Coptoborus* spp. were weakly associated with other *Graphium* from *Euwallacea* spp. and other wood-infesting beetles and *G. basitruncatum*. More informative sequence data are required to fully reveal the relationships within the genus and between various ambrosia *Graphium* spp.

Similarly, more work is needed to illuminate the nature of the relationship between *Coptoborus* and *Fusarium* sp. AF-9 and *Graphium*. While association with multiple specimens and species is compelling evidence, repeated discoveries of the association from multiple locations and a more in-depth nutritional study is needed before their association can be categorized with confidence as symbiotic or mutualistic. More inclusive sampling, including a wider sampling of infested tree hosts, more representative species, and from throughout their neotropical range, is necessary to fully document fungal associations of *Coptoborus* and other Neotropical xyleborine genera. This should include morphological study of the fungi to characterize the reproductive structures.

Our confirmation that *Coptoborus* spp. in Ecuador associate with AFC fungi could be important for the management of balsa and other crop trees in South America. The new discovery of *Graphium* spp. living with these beetles may also be important for silviculture and ecological conservation. Additionally, knowledge about these potentially pathogenic fungi vectored by *Coptoborus* beetles will enhance protective measures if they establish themselves beyond their native ranges.

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APPENDIX

Species	Strain	Xyleborine Associate
Fusarium sp. AF-9*	ROF4	*Coptoborus coartatus*
Fusarium sp. AF-9*	ROF7	*Coptoborus coartatus*
Fusarium sp. AF-9*	ROF8	*Coptoborus coartatus*
Fusarium sp. AF-9*	ROF40	*Coptoborus cracens*
Fusarium sp. AF-9*	ROF49	*Coptoborus pseudotenuis*
Fusarium sp. AF-9*	ROF50	*Coptoborus pseudotenuis*
Fusarium sp. AF-9*	ROF52	*Coptoborus cracens*
Fusarium sp. AF-9*	ROF53	*Coptoborus cracens*
Fusarium sp. AF-9*	ROF55	*Coptoborus cracens*
Fusarium sp. AF-9*	ROF56	*Coptoborus cracens*
Fusarium sp. AF-9*	ROF57	*Coptoborus osbornae*
Fusarium sp. AF-9*	ROF59	*Coptoborus osbornae*
Fusarium sp. AF-9*	ROF60	*Coptoborus osbornae*
Fusarium sp. AF-9*	ROF62	*Coptoborus osbornae*
Fusarium sp. AF-9*	ROF64	*Coptoborus osbornae*
Fusarium sp. AF-9*	ROF67	*Coptoborus osbornae*
Fusarium sp. AF-9*	ROF70	*Coptoborus osbornae*
Graphium sp.	ROF5	*Coptoborus coartatus*
Graphium sp.	ROF6	*Coptoborus coartatus*
Graphium sp.	ROF58	*Coptoborus osbornae*
Graphium sp.	ROF61	*Coptoborus osbornae*
Graphium sp.	ROF63	*Coptoborus osbornae*
Graphium sp.	ROF65	*Coptoborus osbornae*
Graphium sp.	ROF66	*Coptoborus osbornae*
Graphium sp.	ROF69	*Coptoborus osbornae*
Graphium sp.	ROF71	*Coptoborus osbornae*

Table 2.1: *Fusarium* and *Graphium* fungal isolates cultured from Ecuadorian *Coptoborus* beetles collected in Yasuní, Orellana, Ecuador and used in phylogenetic analyses.

Table 2.1 (cont'd)

	GenBank accession no.		
Strain	**ITS**	***EF1a***	***RPB2***
ROF4	OL711912	OM416955	OM304845
ROF7	OL711913	OM416956	…
ROF8	OL711914	OM416957	…
ROF40	OL711915	OM416958	OM304846
ROF49	OL711916	OM416959	OM315195
ROF50	OL711917	OM416960	OM315196
ROF52	OL711918	OM416961	OM315197
ROF53	OL711919	OM416962	OM304847
ROF55	OL711920	OM416963	OM315198
ROF56	OL711921	OM416964	OM304848
ROF57	OL711922	OM416965	OM315199
ROF59	OL711923	OM416966	OM315200
ROF60	OL711924	OM416967	OM315201
ROF62	OL711925	OM416968	OM297005
ROF64	OL711926	OM416969	OM304849
ROF67	OL711927	OM416970	…
ROF70	OL711928	OM416971	…
ROF5	OL963597	…	…
ROF6	OL963598	…	…
ROF58	OL963599	…	…
ROF61	OL963600	…	…
ROF63	OL963601	…	…
ROF65	OL963602	…	…
ROF66	OL963603	…	…
ROF69	OL963604	…	…
ROF71	OL963605	…	…

Locus	Primer	Sequence 5'-3'	Source
	ITS4	TCCTCCGCTTATTGATATGC	
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al., 1990
	EF1F	TGCGGTGGTATCGACAAGCGT	
EF1-α	EF1R	AGCATGTTGTCGCCGTTGAAG	Lynch et al., 2016
	Fa	CAYAARGARTCYATGATGGGWC	O'Donnell et al.,
RPB1	G2R	GTCATYTGDGTDGCDGGYTCDCC	2010
	52f	GGGGWGAYCAGAAGAAGGC	
RPB2	7cr	CCCATRGCTTGYTTRCCCAT	Lynch et al., 2016

Table 2.2: Amplification primers used for gene sequencing.

Locus	Description	Temperature	Time	# Cycles
ITS, *RPB1*	Hot Start	95.0°C	15 min	1
	Denature	94.0°C	30 sec	40
	Annealing	55.0°C	30 sec	
	Extension	72.0°C	1 min	
	Final Extension	72.0°C	10 min	1
EF1-α	Hot Start	95.0°C	15 min	1
	Denature	94.0°C	30 sec	40
	Annealing	57.0°C	30 sec	
	Extension	72.0°C	1 min	
	Final Extension	72.0°C	10 min	1
RPB2	Hot Start	95.0°C	15 min	1
	Denature	94.0°C	30 sec	40
	Annealing	59.0°C	30 sec	
	Extension	72.0°C	1 min	
	Final Extension	72.0°C	10 min	1

Table 2.3: PCR conditions used for gene sequencing.

Species	Strain	Insect Associate
Fusarium ambrosium (AF-1)	NRRL 62942	*Euwallacea* sp.
Fusarium ambrosium (AF-1)	NRRL 22345**	*Euwallacea fornicatus*
Fusarium ambrosium (AF-1)	NRRL 36510**	*Euwallacea fornicatus*
Fusarium ambrosium (AF-1)	NRRL 46583**	*Euwallacea fornicatus*
Fusarium ambrosium (AF-1)	NRRL 62605**	*Euwallacea fornicatus*
Fusarium euwallaceae (AF-2)	NRRL 62626	*Euwallacea* sp.
Fusarium floridanum (AF-3)	NRRL 62606**	*Euwallacea* sp.
Fusarium floridanum (AF-3)	NRRL 62608	NA
Fusarium floridanum (AF-3)	NRRL 62628	*Euwallacea interjectus*
Fusarium floridanum (AF-3)	NRRL 62629	*Euwallacea interjectus*
Fusarium oligoseptatum (AF-4)	NRRL 62578	*Euwallacea validus*
Fusarium oligoseptatum (AF-4)	NRRL 62579	*Euwallacea validus*
Fusarium oligoseptatum (AF-4)	NRRL 62580	*Euwallacea validus*
Fusarium oligoseptatum (AF-4)	NRRL 62581	*Euwallacea validus*
Fusarium oligoseptatum (AF-4)	NRRL 62582	*Euwallacea validus*
Fusarium tuaranense (AF-5)	NRRL 22231	NA
Fusarium tuaranense (AF-5)	NRRL 46518	NA
Fusarium tuaranense (AF-5)	NRRL 46519	NA

Table 2.4: *Fusarium* and *Graphium* fungal sequences obtained from GenBank and used in phylogenetic analysis. Sources: [a]O'Donnell et al. 2015, [b]Kasson et al. 2013 [c]Carrillo et al. 2019, [d]Mendel et al. 2012, [e]Na et al. 2018, [f]Zhang et al. 2006, [g]O'Donnell direct submission, [h]O'Donnll et al. 2007, [i]O'Donnell et al. 2010, [j]Lynch et al. 2016, [k]Cruywagen et al. 2010, [l]Paciura et al. 2008, [m]Jacobs et al. 2003, [n]Hulcr et al. 2007, [o]Okada et al. 2000, [p]Twizeyimana et at Direct Submission, [q]Kolarik et al. unknown date, and [r]Hameline et al. Direct Submission. [*]Outgroup. [**]Fungus was isolated from xyleborine gallery. [T]Ex-type strain. [?]Identification of this beetle partner is questionable (O'Donnell et al., 2015).

Table 2.4 (cont'd)

Species	Strain	Insect Associate
Fusarium sp. AF-6	NRRL 62590**	*Euwallacea* sp.
Fusarium sp. AF-6	NRRL 62591**	*Euwallacea* sp.
Fusarium obliquiseptatum (AF-7)	NRRL 62610	*Euwallacea* sp.
Fusarium obliquiseptatum (AF-7)	NRRL 62611**	*Euwallacea* sp.
Fusarium duplospermum (AF-8)	NRRL 62583	*Euwallacea* sp.
Fusarium duplospermum (AF-8)	NRRL 62584	*Euwallacea* sp.
Fusarium duplospermum (AF-8)	NRRL 62585	*Euwallacea* sp.
Fusarium duplospermum (AF-8)	NRRL 62586	*Euwallacea* sp.
Fusarium duplospermum (AF-8)	NRRL 62587	*Euwallacea* sp.
Fusarium duplospermum (AF-8)	NRRL 62589	*Euwallacea* sp.
Fusarium sp. AF-9	NRRL 22643	Xyleborus ferrigineus?
Fusarium sp. AF-9	NRRL 66088	unknown
Fusarium pseudensiforme	NRRL 46517	NA
Fusarium drepaniforme (AF-10)	NRRL 62941	unknown
Fusarium papillatum (AF-11)	NRRL 62943	*Euwallacea* sp.
Fusarium papillatum (AF-11)	NRRL 62944	*Euwallacea* sp.
Fusarium kuroshium (AF-12)	NRRL 62945	*Euwallacea* sp.
Fusarium kuroshium (AF-12)	NRRL 62946	*Euwallacea* sp.
Fusarium sp. AF-13	UCR 5584	*Euwallacea* sp.
Fusarium sp. AF-13	UCR 6394	*Euwallacea* sp.
Fusarium sp. AF-13	UCR 6403	*Euwallacea* sp.
Fusarium sp. AF-13	UCR 6409	*Euwallacea* sp.
Fusarium sp. AF-13	UCR 6432	*Euwallacea* sp.
Fusarium sp. AF-14	TW 2	NA
Fusarium sp. AF-14	TW 56	NA
Fusarium sp. AF-14	UCR 5499	*Euwallacea* sp.
Fusarium sp. AF-14	UCR 5509	*Euwallacea* sp.
Fusarium sp. AF-14	UCR 5546	*Euwallacea* sp.
Fusarium sp. AF-14	UCR 6436	*Euwallacea* sp.
Fusarium sp. AF-15	UCR 6395	*Euwallacea* sp.

Table 2.4 (cont'd)

Species	Strain	Insect Associate
Fusarium sp. AF-15	TW 15	NA
Fusarium sp. AF-15	TW 45	NA
Fusarium sp. AF-16	TW 4	NA
Fusarium sp. AF-16	TW 25	NA
Fusarium sp. AF-16	TW 34	NA
Fusarium sp. AF-16	TW 37	NA
Fusarium sp. AF-16	UCR 5508	*Euwallacea* sp.
Fusarium sp. AF-16	UCR 5513	*Euwallacea* sp.
Fusarium sp. AF-16	UCR 6405	*Euwallacea* sp.
Fusarium sp. AF-17	TW 40	NA
Fusarium sp. AF-17	UCR 5545	*Euwallacea* sp.
Fusarium sp. AF-17	UCR 6414	*Euwallacea* sp.
Fusarium sp. AF-18	TW 1	NA
Fusarium sp. AF-18	TW 44	NA
Fusarium sp. AF-18	TW 55	NA
Fusarium sp. AF-18	UCR 5557	*Euwallacea* sp.
Fusarium sp. AF-18	UCR 6411	*Euwallacea* sp.
Fusarium sp. AF-18	UCR 6417	*Euwallacea* sp.
Fusarium euwallaceae (AF-2)	NRRL 54722	*Euwallacea* sp.
Fusarium euwallaceae (AF-2)	NRRL 54723	*Euwallacea* sp.
Fusarium euwallaceae (AF-2)	NRRL 54724	*Euwallacea* sp.
Fusarium euwallaceae (AF-2)	NRRL 54725	*Euwallacea* sp.
Fusarium euwallaceae (AF-2)	NRRL 54726	*Euwallacea* sp.
Fusarium sp.	NRRL 54727	*Euwallacea* sp.
Fusarium sp.	NRRL 54728	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 3641	NA
Fusarium sp. JM-2017a	UCR 3644	NA
Fusarium sp. JM-2017a	UCR 3651	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 3652	NA
Fusarium sp. JM-2017a	UCR 3653	NA

Table 2.4 (cont'd)

Species	Strain	Insect Associate
Fusarium sp. JM-2017a	UCR 3654	NA
Fusarium sp. JM-2017a	UCR 3657	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 3659	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 3660	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 3661	NA
Fusarium sp. JM-2017a	UCR 4672	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 4673	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 4674	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 4675	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 4676	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 4677	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 4678	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 4679	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 4680	*Euwallacea* sp.
Fusarium sp. JM-2017a	UCR 4681	*Euwallacea* sp.
Fusarium kuroshium	TW 43	NA
Fusarium kuroshium	UCR 6408	*Euwallacea* sp.
Fusarium neocosmosporiellum	NRRL 22468*	NA
Fusarium neocosmosporiellum	NRRL 43467*	NA
Fusarium lichenicola	NRRL 32434*	NA
Graphium kuroshium	UCR 4593	NA
Graphium kuroshium	UCR 4594^{T}	NA
Graphium kuroshium	UCR 4606	NA
Graphium kuroshium	UCR 4607	NA
Graphium kuroshium	UCR 4608	*Euwallacea* sp.
Graphium kuroshium	UCR 4609	*Euwallacea* sp.
Graphium kuroshium	UCR 4616	NA
Graphium kuroshium	UCR 4617	NA
Graphium kuroshium	UCR 4618	*Euwallacea* sp.
Graphium kuroshium	UCR 4622	*Euwallacea* sp.

Table 2.4 (cont'd)

Species	Strain	Insect Associate
Graphium sp.	UCR 5497	*Euwallacea* sp.
Graphium sp.	UCR 5501	*Euwallacea* sp.
Graphium sp.	UCR 5506	*Euwallacea* sp.
Graphium sp.	UCR 5512	*Euwallacea* sp.
Graphium sp.	UCR 5517	*Euwallacea* sp.
Graphium kuroshium	UCR 5519	*Euwallacea* sp.
Graphium sp.	UCR 5528	*Euwallacea* sp.
Graphium sp.	UCR 5531	*Euwallacea* sp.
Graphium sp.	UCR 5548	*Euwallacea* sp.
Graphium kuroshium	UCR 5549	*Euwallacea* sp.
Graphium sp.	UCR 6662	*Euwallacea* sp.
Graphium sp.	UCR 6667	*Euwallacea* sp.
Graphium sp. II	UCR 2132	NA
Graphium sp. II	UCR 2137	NA
Graphium sp. II	UCR 2140	NA
Graphium sp. I	UCR 2159	NA
Graphium sp. I	UCR 2160	NA
Graphium sp. I	UCR 2162	NA
Graphium sp. I	UCR 2163	NA
Graphium sp. I	UCR 2164	NA
Graphium sp. I	UCR 2165	NA
Graphium sp. I	UCR 2166	NA
Graphium sp. III	UCR 2289	NA
Graphium sp. III	UCR 2291	NA
Graphium carbonarium	UCR 2300	NA
Graphium euwallaceae	UCR 2308	NA
Graphium carbonarium	UCR 2325	NA
Graphium carbonarium	UCR 2329	NA
Graphium euwallaceae	UCR 2974	NA
Graphium euwallaceae	UCR 2975	NA
Graphium euwallaceae	UCR 2976	NA

Table 2.4 (cont'd)

Species	Strain	Insect Associate
Graphium euwallaceae	UCR 2977	NA
Graphium euwallaceae	UCR 2978	NA
Graphium euwallaceae	UCR 2979	NA
Graphium euwallaceae	UCR 2980^{T}	NA
Graphium euwallaceae	UCR 2981	NA
Graphium pseudormiticum	CMW 12285	*Pissodes* sp. (Molytinae)
Graphium adansoniae	CMW 30617	NA
Graphium adansoniae	CMW 30618^{T}	NA
Graphium adansoniae	CMW 30620	NA
Graphium fabiforme	CMW 30626^{T}	NA
Graphium fabiforme	CMW 30627	NA
Graphium madagascariense	CMW 30628^{T}	NA
Graphium madagascariense	CMW 30629	NA
Graphium penicillioides	CMW 5292	NA
Graphium penicillioides	CMW 5295	NA
Graphium carbonarium	CMW 12418	*Pissodes* sp. (Molytinae)
Graphium carbonarium	CMW 12420^{T}	*Pissodes* sp. (Molytinae)
Graphium pseudormiticum	CMW 503^{T}	*Orthotomicus erosus*
Graphium laricis	CMW 5601^{T}	*Ips cembrae*
Graphium laricis	CMW 5603	*Ips cembrae*
Graphium fimbriasporum	CMW 5605^{T}	*Ips typographus*
Graphium fimbriasporum	CMW 5606	*Ips typographus*
Graphium scolytodis	CCF 3566	*Scolytodes unipunctatus*
Graphium scolytodis	CCF 3570	*Scolytodes unipunctatus*
Graphium basitruncatum	JCM 9300*	NA
Geosmithia putterillii	U 160*	NA
Ophiostoma ulmi	Q 412T-0*	NA

Table 2.4 (cont'd)

Strain	Host Plant/Source	Origin
NRRL 62942	*Camellia sinensis* (tea)	Sri Lanka
NRRL 22345**	*Camellia sinensis* (tea)	India
NRRL 36510**	*Camellia sinensis* (tea)	India
NRRL 46583**	*Camellia sinensis* (tea)	India
NRRL 62605**	*Camellia sinensis* (tea)	India
NRRL 62626	*Persea americana* (avocado)	California, USA
NRRL 62606**	*Acer negundo* (box elder)	Florida, USA
NRRL 62608	*Acer negundo* (box elder)	Florida, USA
NRRL 62628	*Acer negundo* (box elder)	Florida, USA
NRRL 62629	*Acer negundo* (box elder)	Florida, USA
NRRL 62578	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
NRRL 62579	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
NRRL 62580	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
NRRL 62581	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
NRRL 62582	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
NRRL 22231	*Hevea brasiliensis* (Pará rubber)	Malasia
NRRL 46518	*Hevea brasiliensis* (Pará rubber)	Malasia
NRRL 46519	*Hevea brasiliensis* (Pará rubber)	Malasia
NRRL 62590**	*Persea americana* (avocado)	Florida, USA
NRRL 62591**	*Persea americana* (avocado)	Florida, USA
NRRL 62610	Unknown	Queensland, Australia
NRRL 62611**	*Persea americana* (avocado)	Queensland, Australia
NRRL 62583	*Persea americana* (avocado)	Florida, USA
NRRL 62584	*Persea americana* (avocado)	Florida, USA
NRRL 62585	*Persea americana* (avocado)	Florida, USA
NRRL 62586	*Persea americana* (avocado)	Florida, USA
NRRL 62587	*Persea americana* (avocado)	Florida, USA

Table 2.4 (cont'd)

Strain	Host Plant/Source	Origin
NRRL 62589	*Persea americana* (avocado)	Florida, USA
NRRL 22643	Unknown	Costa Rica
NRRL 66088	*Delonix regia* (royal poinciana)	Florida, USA
NRRL 46517	Unknow recently dead tree	Sri Lanka
NRRL 62941	Unknown	Singapore, Malaysia
NRRL 62943	*Camellia sinensis* (tea)	Sri Lanka
NRRL 62944	*Camellia sinensis* (tea)	Sri Lanka
NRRL 62945	*Platanus racemosa* (sycamore)	California, USA
NRRL 62946	*Platanus racemosa* (sycamore)	California, USA
UCR 5584	Unknown	Taiwan
UCR 6394	Unknown	Taiwan
UCR 6403	Unknown	Taiwan
UCR 6409	Unknown	Taiwan
UCR 6432	Unknown	Taiwan
TW 2	*Persea americana* (avocado)	Taiwan
TW 56	*Persea americana* (avocado)	Taiwan
UCR 5499	Unknown	Taiwan
UCR 5509	Unknown	Taiwan
UCR 5546	Unknown	Taiwan
UCR 6436	Unknown	Taiwan
UCR 6395	Unknown	Taiwan
TW 15	*Persea americana* (avocado)	Taiwan
TW 45	*Persea americana* (avocado)	Taiwan
TW 4	*Persea americana* (avocado)	Taiwan
TW 25	*Persea americana* (avocado)	Taiwan
TW 34	*Persea americana* (avocado)	Taiwan
TW 37	*Persea americana* (avocado)	Taiwan

Table 2.4 (cont'd)

Strain	Host Plant/Source	Origin
UCR 5508	Unknown	Taiwan
UCR 5513	Unknown	Taiwan
UCR 6405	Unknown	Taiwan
TW 40	*Persea americana* (avocado)	Taiwan
UCR 5545	Unknown	Taiwan
UCR 6414	Unknown	Taiwan
TW 1	*Persea americana* (avocado)	Taiwan
TW 44	*Persea americana* (avocado)	Taiwan
TW 55	*Persea americana* (avocado)	Taiwan
UCR 5557	Unknown	Taiwan
UCR 6411	Unknown	Taiwan
UCR 6417	Unknown	Taiwan
NRRL 54722	*Persea americana* (avocado)	Israel
NRRL 54723	*Persea americana* (avocado)	Israel
NRRL 54724	*Persea americana* (avocado)	Israel
NRRL 54725	*Persea americana* (avocado)	Israel
NRRL 54726	*Persea americana* (avocado)	Israel
NRRL 54727	*Persea americana* (avocado)	Israel
NRRL 54728	*Persea americana* (avocado)	Israel
UCR 3641	*Platanus racemosa* (sycamore)	California, USA
UCR 3644	*Platanus racemosa* (sycamore)	California, USA
UCR 3651	Unknown	California, USA
UCR 3652	*Persea americana* (avocado)	California, USA
UCR 3653	*Persea americana* (avocado)	California, USA
UCR 3654	*Persea americana* (avocado)	California, USA
UCR 3657	Unknown	California, USA
UCR 3659	Unknown	California, USA

Table 2.4 (cont'd)

Strain	Host Plant/Source	Origin
UCR 3660	Unknown	California, USA
UCR 3661	*Persea americana* (avocado)	California, USA
UCR 4672	Unknown	Taiwan
UCR 4673	Unknown	Taiwan
UCR 4674	Unknown	Taiwan
UCR 4675	Unknown	Taiwan
UCR 4676	Unknown	Taiwan
UCR 4677	Unknown	Taiwan
UCR 4678	Unknown	Taiwan
UCR 4679	Unknown	Taiwan
UCR 4680	Unknown	Taiwan
UCR 4681	Unknown	Taiwan
TW 43	*Persea americana* (avocado)	Taiwan
UCR 6408	Unknown	Taiwan
NRRL 22468*	Stored peanuts	Guinea
NRRL 43467*	Human eye	Louisiana, USA
NRRL 32434*	Human	Germany
UCR 4593	*Persea americana* (avocado)	California, USA
UCR 4594T	*Persea americana* (avocado)	California, USA
UCR 4606	*Persea americana* (avocado)	California, USA
UCR 4607	*Persea americana* (avocado)	California, USA
UCR 4608	unknown	California, USA
UCR 4609	unknown	California, USA
UCR 4616	*Persea americana* (avocado)	California, USA
UCR 4617	*Persea americana* (avocado)	California, USA
UCR 4618	unknown	California, USA
UCR 4622	unknown	California, USA

Table 2.4 (cont'd)

Strain	Host Plant/Source	Origin
UCR 5497	unknown	Taiwan
UCR 5501	unknown	Taiwan
UCR 5506	unknown	Taiwan
UCR 5512	unknown	Taiwan
UCR 5517	unknown	Taiwan
UCR 5519	unknown	Taiwan
UCR 5528	unknown	Taiwan
UCR 5531	unknown	Taiwan
UCR 5548	unknown	Taiwan
UCR 5549	unknown	Taiwan
UCR 6662	unknown	Florida, USA
UCR 6667	unknown	Florida, USA
UCR 2132	*Durio* sp. (Durian)	Thailand
UCR 2137	*Durio* sp. (Durian)	Thailand
UCR 2140	*Durio* sp. (Durian)	Thailand
UCR 2159	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
UCR 2160	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
UCR 2162	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
UCR 2163	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
UCR 2164	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
UCR 2165	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
UCR 2166	*Ailanthus altissima* (tree of heaven)	Pennsylvania, USA
UCR 2289	*Acacia auriculiformis* (earleaf acacia)	Veitnam
UCR 2291	*Acacia auriculiformis* (earleaf acacia)	Veitnam
UCR 2300	*Acacia auriculiformis* (earleaf acacia)	Veitnam
UCR 2308	*Acacia auriculiformis* (earleaf acacia)	Veitnam
UCR 2325	*Ricinus communis* (castor bean)	Veitnam

Table 2.4 (cont'd)

Strain	Host Plant/Source	Origin
UCR 2329	*Ricinus communis* (castor bean)	Veitnam
UCR 2974	*Ricinus communis* (castor bean)	California, USA
UCR 2975	*Acer negundo* (box elder)	California, USA
UCR 2976	*Ricinus communis* (castor bean)	California, USA
UCR 2977	*Acacia floribunda* (weeping acacia)	California, USA
UCR 2978	*Erythrina atitlanensis*	California, USA
UCR 2979	*Quercus agrifolia* (coast live oak)	California, USA
UCR 2980^{T}	*Persea americana* (avocado)	California, USA
UCR 2981	*Persea americana* (avocado)	California, USA
CMW 12285	*Tsuga dumosa* Himalayan hemlock	China
CMW 30617	*Adansonia digitata* (African baobab)	South Africa
CMW 30618^{T}	*Adansonia digitata* (African baobab)	South Africa
CMW 30620	*Adansonia digitata* (African baobab)	South Africa
CMW 30626^{T}	*Adansonia rubrostipa* (fony boabab)	Madagascar
CMW 30627	*Adansonia rubrostipa* (fony boabab)	Madagascar
CMW 30628^{T}	*Adansonia rubrostipa* (fony boabab)	Madagascar
CMW 30629	*Adansonia rubrostipa* (fony boabab)	Madagascar
CMW 5292	*Populus nigra* (black poplar)	Czech Republic
CMW 5295	*Populus nigra* (black poplar)	Czech Republic
CMW 12418	*Salix babylonica* (weeping willow)	China
CMW 12420^{T}	*Salix babylonica* (weeping willow)	China
CMW 503^{T}	*Pinus* sp. (pine)	South Africa
CMW 5601^{T}	*Larix decidua* (European larch)	Austria
CMW 5603	*Larix decidua* (European larch)	Austria
CMW 5605^{T}	*Picea abies* (Norway spruce)	France
CMW 5606	*Picea abies* (Norway spruce)	Austria

Table 2.4 (cont'd)

Strain	Host Plant/Source	Origin
CCF 3566	*Cercropia* sp.	Costa Rica
CCF 3570	*Cercropia* sp.	Costa Rica
JCM 9300*	Soil	Solomon Islands
U 160*	*Ulmus pumila* (Siberian elm)	Colorado, USA
Q 412T-0*	*Ulmus americana* (American elm)	…

Table 2.4 (cont'd)

	GenBank accession no.			
Strain	**ITS**	***EF1a***	***RPB1***	***RPB2***
NRRL 62942	KM406631[a]	KM406624[a]	KM406638[a]	KM406645[a]
NRRL 22345**	KC691557[b]	KC691529[b]	KC691586[b]	KC691618[b]
NRRL 36510**	KC691558[b]	KC691530[b]	KC691588[b]	KC691619[b]
NRRL 46583**	KC691556[b]	KC691528[b]	KC691585[b]	KC691617[b]
NRRL 62605**	KC691559[b]	KC691531[b]	KC691589[b]	KC691620[b]
NRRL 62626	KC691560[b]	KC691532[b]	KC691590[b]	KC691621[b]
NRRL 62606**	KC691561[b]	KC691533[b]	KC691591[b]	KC691622[b]
NRRL 62608	KC691562[b]	KC691534[b]	KC691592[b]	KC691623[b]
NRRL 62628	KC691563[b]	KC691535[b]	KC691593[b]	KC691624[b]
NRRL 62629	KC691564[b]	KC691536[b]	KC691594[b]	KC691625[b]
NRRL 62578	KC691565[b]	KC691537[b]	KC691595[b]	KC691626[b]
NRRL 62579	KC691566[b]	KC691538[b]	KC691596[b]	KC691627[b]
NRRL 62580	KC691567[b]	KC691539[b]	KC691597[b]	KC691628[b]
NRRL 62581	KC691568[b]	KC691540[b]	KC691598[b]	KC691629[b]
NRRL 62582	KC691569[b]	KC691541[b]	KC691599[b]	KC691630[b]
NRRL 22231	KC691570[b]	KC691542[b]	KC691600[b]	KC691631[b]
NRRL 46518	KC691571[b]	KC691543[b]	KC691601[b]	KC691632[b]
NRRL 46519	KC691572[b]	KC691544[b]	KC691602[b]	KC691633[b]
NRRL 62590**	KC691574[b]	KC691546[b]	KC691604[b]	KC691635[b]
NRRL 62591**	KC691573[b]	KC691545[b]	KC691603[b]	KC691634[b]
NRRL 62610	KC691575[b]	KC691547[b]	KC691605[b]	KC691636[b]
NRRL 62611**	KC691576[b]	KC691548[b]	KC691606[b]	KC691637[b]
NRRL 62583	KC691581[b]	KC691553[b]	KC691611[b]	KC691642[b]
NRRL 62584	KC691582[b]	KC691554[b]	KC691612[b]	KC691643[b]
NRRL 62585	KC691577[b]	KC691549[b]	KC691607[b]	KC691638[b]
NRRL 62586	KC691578[b]	KC691550[b]	KC691608[b]	KC691639[b]
NRRL 62587	KC691579[b]	KC691551[b]	KC691609[b]	KC691640[b]

Table 2.4 (cont'd)

	GenBank accession no.			
Strain	**ITS**	***EF1a***	***RPB1***	***RPB2***
NRRL 62589	KC691580[b]	KC691552[b]	KC691610[b]	KC691641[b]
NRRL 22643	KC691583[b]	…	…	KC691644[b]
NRRL 66088	KM406632[a]	KM406625[a]	KM406639[a]	KM406646[a]
NRRL 46517	KC691584[b]	KC691555[b]	KC691615[b]	KC691645[b]
NRRL 62941	KM406633[a]	KM406626[a]	KM406640[a]	KM406647[a]
NRRL 62943	KM406635[a]	KM406628[a]	KM406642[a]	…
NRRL 62944	KM406634[a]	KM406627[a]	KM406641[a]	KM406648[a]
NRRL 62945	KM406636[a]	KM406629[a]	KM406643[a]	KM406649[a]
NRRL 62946	KM406637[a]	KM406630[a]	KM406644[a]	KM406650[a]
UCR 5584	MK432880[c]	MK435457[c]	MK435509[c]	MK435541[c]
UCR 6394	MK432881[c]	MK435458[c]	MK435510[c]	MK435542[c]
UCR 6403	MK432883[c]	MK435460[c]	MK435512[c]	MK435544[c]
UCR 6409	MK432886[c]	MK435463[c]	MK435515[c]	MK435547[c]
UCR 6432	MK432890[c]	MK435467[c]	MK435519[c]	MK435551[c]
TW 2	MK432862[c]	MK435439[c]	MK435491[c]	MK435523[c]
TW 56	MK432872[c]	MK435449[c]	MK435501[c]	MK435533[c]
UCR 5499	MK432873[c]	MK435450[c]	MK435502[c]	MK435534[c]
UCR 5509	MK432875[c]	MK435452[c]	MK435504[c]	MK435536[c]
UCR 5546	MK432878[c]	MK435455[c]	MK435507[c]	MK435539[c]
UCR 6436	MK432891[c]	MK435468[c]	MK435520[c]	MK435552[c]
UCR 6395	MK432882[c]	MK435459[c]	MK435511[c]	MK435543[c]
TW 15	MK432861[c]	MK435438[c]	MK435490[c]	MK435522[c]
TW 45	MK432870[c]	MK435447[c]	MK435499[c]	MK435531[c]
TW 4	MK432866[c]	MK435443[c]	MK435495[c]	MK435527[c]
TW 25	MK432863[c]	MK435440[c]	MK435492[c]	MK435524[c]
TW 34	MK432864[c]	MK435441[c]	MK435493[c]	MK435525[c]
TW 37	MK432865[c]	MK435442[c]	MK435494[c]	MK435526[c]

Table 2.4 (cont'd)

	GenBank accession no.			
Strain	**ITS**	***EF1a***	***RPB1***	***RPB2***
UCR 5508	MK432874[c]	MK435451[c]	MK435503[c]	MK435535[c]
UCR 5513	MK432876[c]	MK435453[c]	MK435505[c]	MK435537[c]
UCR 6405	MK432884[c]	MK435461[c]	MK435513[c]	MK435545[c]
TW 40	MK432867[c]	MK435444[c]	MK435496[c]	MK435528[c]
UCR 5545	MK432877[c]	MK435454[c]	MK435506[c]	MK435538[c]
UCR 6414	MK432888[c]	MK435465[c]	MK435517[c]	MK435549[c]
TW 1	MK432860[c]	MK435437[c]	MK435489[c]	MK435521[c]
TW 44	MK432869[c]	MK435446[c]	MK435498[c]	MK435530[c]
TW 55	MK432871[c]	MK435448[c]	MK435500[c]	MK435532[c]
UCR 5557	MK432879[c]	MK435456[c]	MK435508[c]	MK435540[c]
UCR 6411	MK432887[c]	MK435464[c]	MK435516[c]	MK435548[c]
UCR 6417	MK432889[c]	MK435466[c]	MK435518[c]	MK435550[c]
NRRL 54722	JQ038014[d]	JQ038007[d]	JQ038021[d]	JQ038028[d]
NRRL 54723	JQ038015[d]	JQ038008[d]	JQ038022[d]	JQ038029[d]
NRRL 54724	JQ038016[d]	JQ038009[d]	JQ038023[d]	JQ038030[d]
NRRL 54725	JQ038017[d]	JQ038010[d]	JQ038024[d]	JQ038031[d]
NRRL 54726	JQ038018[d]	JQ038011[d]	JQ038025[d]	JQ038032[d]
NRRL 54727	JQ038019[d]	JQ038012[d]	JQ038026[d]	JQ038033[d]
NRRL 54728	JQ038020[d]	JQ038013[d]	JQ038027[d]	JQ038034[d]
UCR 3641	KX262196[e]	KX262216[e]	KX262236[e]	KX262256[e]
UCR 3644	KX262197[e]	KX262217[e]	KX262237[e]	KX262257[e]
UCR 3651	KX262198[e]	KX262218[e]	KX262238[e]	KX262258[e]
UCR 3652	KX262199[e]	KX262219[e]	KX262239[e]	KX262259[e]
UCR 3653	KX262200[e]	KX262220[e]	KX262240[e]	KX262260[e]
UCR 3654	KX262201[e]	KX262221[e]	KX262241[e]	KX262261[e]
UCR 3657	KX262202[e]	KX262222[e]	KX262242[e]	KX262262[e]
UCR 3659	KX262203[e]	KX262223[e]	KX262243[e]	KX262263[e]

Table 2.4 (cont'd)

	GenBank accession no.			
Strain	**ITS**	***EF1a***	***RPB1***	***RPB2***
UCR 3660	KX262204[e]	KX262224[e]	KX262244[e]	KX262264[e]
UCR 3661	KX262205[e]	KX262225[e]	KX262245[e]	KX262265[e]
UCR 4672	KX262206[e]	KX262226[e]	KX262246[e]	KX262266[e]
UCR 4673	KX262207[e]	KX262227[e]	KX262247[e]	KX262267[e]
UCR 4674	KX262208[e]	KX262228[e]	KX262248[e]	KX262268[e]
UCR 4675	KX262209[e]	KX262229[e]	KX262249[e]	KX262269[e]
UCR 4676	KX262210[e]	KX262230[e]	KX262250[e]	KX262270[e]
UCR 4677	KX262211[e]	KX262231[e]	KX262251[e]	KX262271[e]
UCR 4678	KX262212[e]	KX262232[e]	KX262252[e]	KX262272[e]
UCR 4679	KX262213[e]	KX262233[e]	KX262253[e]	KX262273[e]
UCR 4680	KX262214[e]	KX262234[e]	KX262254[e]	KX262274[e]
UCR 4681	KX262215[e]	KX262235[e]	KX262255[e]	KX262275[e]
TW 43	MK432868[c]	MK435445[c]	MK435497[c]	MK435529[c]
UCR 6408	MK432885[c]	MK435462[c]	MK435514[c]	MK435546[c]
NRRL 22468[*]	DQ094318[f]	AF178349[g]	KC691616[e]	…
NRRL 43467[*]	EF453092[h]	EF452940[h]	HM347178[i]	…
NRRL 32434[*]	DQ094444[f]	DQ246977[f]	HM347156[i]	…
UCR 4593	KX262276[e]	KX262286[e]	…	…
UCR 4594[T]	KX262277[e]	KX262287[e]	…	…
UCR 4606	KX262278[e]	KX262288[e]	…	…
UCR 4607	KX262279[e]	KX262289[e]	…	…
UCR 4608	KX262280[e]	KX262290[e]	…	…
UCR 4609	KX262281[e]	KX262291[e]	…	…
UCR 4616	KX262282[e]	KX262292[e]	…	…
UCR 4617	KX262283[e]	KX262293[e]	…	…
UCR 4618	KX262284[e]	KX262294[e]	…	…
UCR 4622	KX262285[e]	KX262295[e]	…	…

Table 2.4 (cont'd)

	GenBank accession no.			
Strain	**ITS**	***EF1a***	***RPB1***	***RPB2***
UCR 5497	MK432903[c]	MK435469[c]	…	…
UCR 5501	MK432902[c]	MK435470[c]	…	…
UCR 5506	MK432901[c]	MK435471[c]	…	…
UCR 5512	MK432900[c]	MK435472[c]	…	…
UCR 5517	MK432899[c]	MK435473[c]	…	…
UCR 5519	MK432898[c]	MK435474[c]	…	…
UCR 5528	MK432897[c]	MK435475[c]	…	…
UCR 5531	MK432896[c]	MK435476[c]	…	…
UCR 5548	MK432895[c]	MK435477[c]	…	…
UCR 5549	MK432894[c]	MK435478[c]	…	…
UCR 6662	MK432893[c]	MK435479[c]	…	…
UCR 6667	MK432892[c]	MK435480[c]	…	…
UCR 2132	KM592367[j]	KM363259[j]	…	…
UCR 2137	KJ131236[j]	KJ131246[j]	…	…
UCR 2140	KJ131237[j]	KJ131247[j]	…	…
UCR 2159	KJ131228[j]	KJ131238[j]	…	…
UCR 2160	KJ131229[j]	KJ131239[j]	…	…
UCR 2162	KJ131231[j]	KJ131241[j]	…	…
UCR 2163	KJ131232[j]	KJ131242[j]	…	…
UCR 2164	KJ131233[j]	KJ131243[j]	…	…
UCR 2165	KJ131234[j]	KJ131244[j]	…	…
UCR 2166	KJ131235[j]	KJ131245[j]	…	…
UCR 2289	KM592368[j]	KM592360[j]	…	…
UCR 2291	KM592369[j]	KM592361[j]	…	…
UCR 2300	KM592370[j]	KM592362[j]	…	…
UCR 2308	KM592371[j]	KM592363[j]	…	…
UCR 2325	KM592372[j]	KM592364[j]	…	…

Table 2.4 (cont'd)

	GenBank accession no.			
Strain	**ITS**	***EF1a***	***RPB1***	***RPB2***
UCR 2329	KM592373[j]	KM592365[j]	…	…
UCR 2974	KF540218[j]	KF534799[j]	…	…
UCR 2975	KF540219[j]	KF534800[j]	…	…
UCR 2976	KF540220[j]	KF534801[j]	…	…
UCR 2977	KF540221[j]	KF534802[j]	…	…
UCR 2978	KF540222[j]	KF534803[j]	…	…
UCR 2979	KF540223[j]	KF534804[j]	…	…
UCR 2980[T]	KF540224[j]	KF534805[j]	…	…
UCR 2981	KF540225[j]	KF534806[j]	…	…
CMW 12285	HM630608[k]	HM630587[k]	…	…
CMW 30617	GQ200610[k]	HM630596[k]	…	…
CMW 30618[T]	GQ200611[k]	HM630598[k]	…	…
CMW 30620	GQ200613[k]	HM630597[k]	…	…
CMW 30626[T]	GQ200616[k]	HM630592[k]	…	…
CMW 30627	GQ200617[k]	HM630593[k]	…	…
CMW 30628[T]	GQ200619[k]	HM630595[k]	…	…
CMW 30629	GQ200620[k]	HM630594[k]	…	…
CMW 5292	HQ335310[k]	HM630600[k]	…	…
CMW 5295	HQ335311[k]	HM630601[k]	…	…
CMW 12418	FJ434980[l]	HM630602[k]	…	…
CMW 12420[T]	FJ434979[l]	HM630603[k]	…	…
CMW 503[T]	AY148186[m]	HM630586[k]	…	…
CMW 5601[T]	AY148183[m]	HM630588[k]	…	…
CMW 5603	AY148182[m]	HM630589[k]	…	…
CMW 5605[T]	AY148177[m]	HM630590[k]	…	…
CMW 5606	AY148180[m]	HM630591[k]	…	…

Table 2.4 (cont'd)

	GenBank accession no.			
Strain	**ITS**	***EF1a***	***RPB1***	***RPB2***
CCF 3566	AM267264[n]	…	…	…
CCF 3570	AM267265[n]	…	…	…
JCM 9300[*]	AB038427[o]	KJ131248[p]	…	…
U 160[*]	HF546278[q]	HG799859[q]	…	HG799911[q]
Q 412T-0[*]	KF854010[r]	KF899888[r]	…	…

Beetle Identity	Beetle voucher	Fungal Strain	Fungal Identification
Coptoborus catulus	B88	ROF3	Exobasidiales
Coptoborus tolimanus	B13	ROF9	*Phialemoniopsis* sp.
		ROF10	*Clonostachys* sp.
	B14	ROF11	*Phialemoniopsis* sp.
		ROF12	*Clonostachys* sp.
	B15	ROF13	*Clonostachys* sp.
		ROF72	*Phialemoniopsis* sp.
	B28	ROF14	*Phialemoniopsis* sp.
		ROF15	*Penicillium griseofulvum*
	B29	ROF16	*Clonostachys* sp.
		ROF17	*Phialemoniopsis* sp.
	B30	ROF18	*Clonostachys* sp.
		ROF19	*Clonostachys* sp.
	B31	ROF20	*Clonostachys* sp.
		ROF21	*Clonostachys* sp.
		ROF22	*Clonostachys* sp.
	B32	ROF23	*Clonostachys* sp.
		ROF24	*Clonostachys* sp.
		ROF25	*Phialemoniopsis* sp.
	B33	ROF26	*Clonostachys* sp.
		ROF27	*Clonostachys* sp.
		ROF28	*Clonostachys* sp.

Table 2.5: BLAST identities of fungi isolated from *Coptoborus* spp.

Table 2.5 (cont'd)

Beetle Identity	Beetle voucher	Fungal Strain	Fungal Identification
Coptoborus pseudotenuis	B88	ROF49	*Fusarium solani*
		ROF50	*Fusarium* sp. 1 RJ2014
Coptoborus cracens	B83	ROF40	*Fusarium* sp. 1 RJ2014
	B89	ROF52	*Fusarium* sp. 1 RJ2014
	B90	ROF53	*Fusarium* sp. 1 RJ2014
		ROF54	*Chaetomium globosum*
	B91	ROF55	*Fusarium* sp. 1 RJ2014
		ROF56	*Fusarium* sp. 1 RJ2014
Coptoborus coartatus	B9	ROF4	*Fusarium* sp. 1 RJ2014
	B10	ROF5	*Graphium euwallaceae*
		ROF6	*Graphium euwallaceae*
	B11	ROF7	*Fusarium* sp. 1 RJ2014
		ROF8	*Fusarium* sp. 1 RJ2014
Coptoborus osbornae	B92	ROF58	*Graphium euwallaceae*
		ROF59	*Fusarium* sp. 1 RJ2014
		ROF57	*Fusarium* sp. 1 RJ2014
	B93	ROF60	*Fusarium solani*
		ROF61	*Graphium euwallaceae*
	B94	ROF62	*Fusarium* sp. 1 RJ2014
		ROF63	*Graphium euwallaceae*
	B95	ROF64	*Fusarium* sp. 1 RJ2014
		ROF65	*Graphium euwallaceae*
		ROF66	*Graphium euwallaceae*
	B96	ROF67	*Fusarium* sp. 1 RJ2014
		ROF68	*Hypocrea virens*
		ROF69	*Graphium euwallaceae*
	B97	ROF70	*Fusarium* sp. 1 RJ2014
		ROF71	*Graphium euwallaceae*

Table 2.5 (cont'd)

Fungal Strain	Top GenBank Match	% Seq Similarity	% Coverage
ROF3	KP229361	98.67	85
ROF9	MT887369	99.57	93
ROF10	KP006352	98.53	98
ROF11	MT887369	99.17	93
ROF12	KP006352	98.53	98
ROF13	KP006352	99.17	99
ROF72	MT887369	99.17	93
ROF14	MT887369	99.17	92
ROF15	KT898767	99.61	99
ROF16	KP006352	98.9	98
ROF17	MT887369	99.59	93
ROF18	KP006352	98.53	98
ROF19	KP006352	99.27	98
ROF20	KP006352	99.63	98
ROF21	KP006352	98.9	98
ROF22	KP006352	98.9	98
ROF23	KP006352	99.63	98
ROF24	KP006352	98.9	98
ROF25	MT887369	99.17	94
ROF26	KP006352	99.27	98
ROF27	KP006352	98.9	98
ROF28	KP006352	98.17	98

Table 2.5 (cont'd)

Fungal Strain	Top GenBank Match	% Seq Similarity	% Coverage
ROF49	KR350652	97.94	99
ROF50	MF782769	97.75	100
ROF40	MF782769	97.75	99
ROF52	MF782769	98.15	99
ROF53	MF782769	97.78	99
ROF54	MF476064	99.66	99
ROF55	MF782769	97.78	99
ROF56	MF782769	97.78	99
ROF4	MF782769	97.41	98
ROF5	EF165016	98.01	98
ROF6	EF165016	98.41	80
ROF7	MF782769	97.78	99
ROF8	MF782769	97.78	99
ROF58	EF165016	98.01	99
ROF59	MF782769	97.76	99
ROF57	MF782769	97.41	99
ROF60	KR350652	98.08	98
ROF61	EF165016	98.41	80
ROF62	MF782769	98.14	99
ROF63	EF165016	98.41	99
ROF64	MF782769	97.05	99
ROF65	EF165016	98.41	99
ROF66	EF165016	98.41	99
ROF67	MF782769	97.05	99
ROF68	GU046491	99.37	99
ROF69	EF165016	98.41	99
ROF70	MF782769	98.47	99
ROF71	EF165016	97.61	99

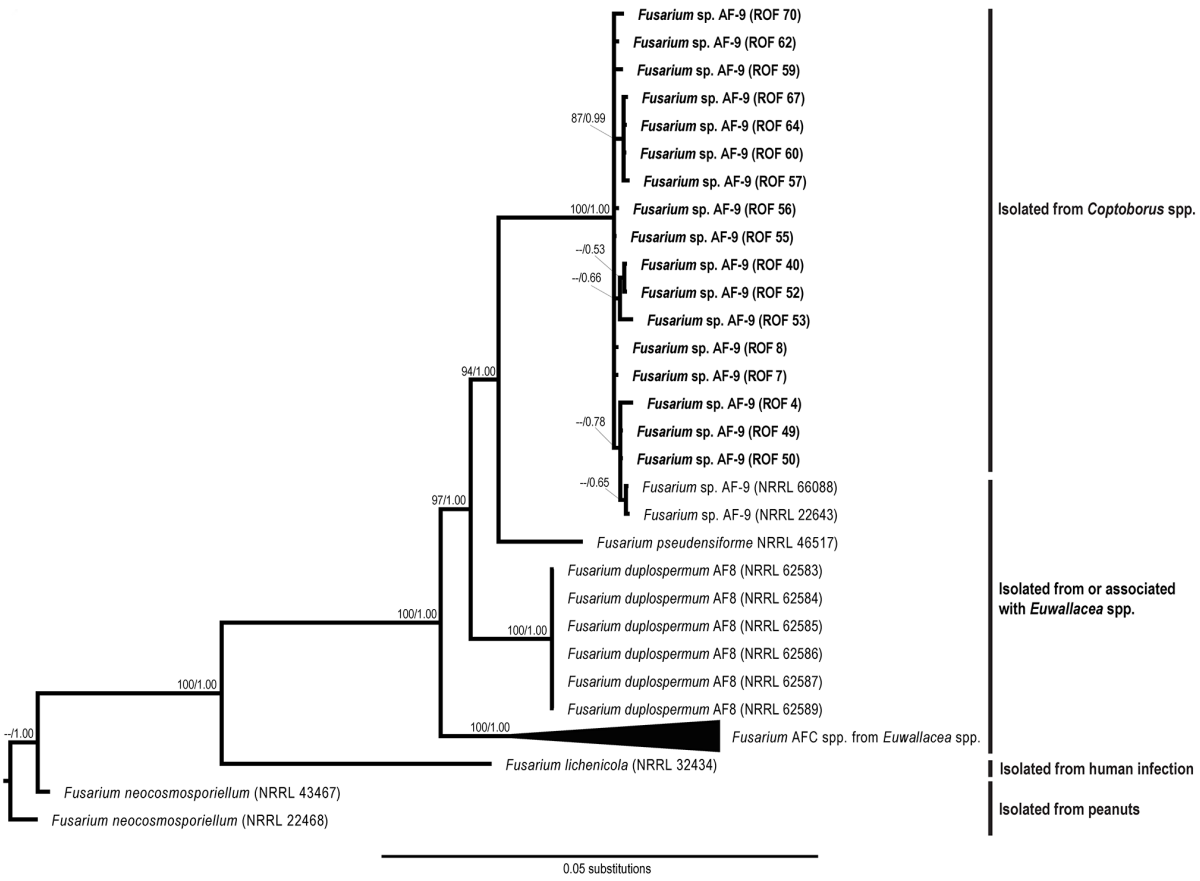

Figure 2.1: Condensed Bayesian consensus tree of *Fusarium* strains reconstructed from ITS, *EF1*-α, *RPB1*, and *RPB2* sequence data. Nodes are labeled with bootstrap values/posterior probabilities. Clades containing AFC strains associated with *Euwallaceae* spp. beetles have been collapsed. *Fusarium* sp. isolated from *Coptoborus* sp. for this study are written in **bold**. See Figure 2.2 for the complete tree.

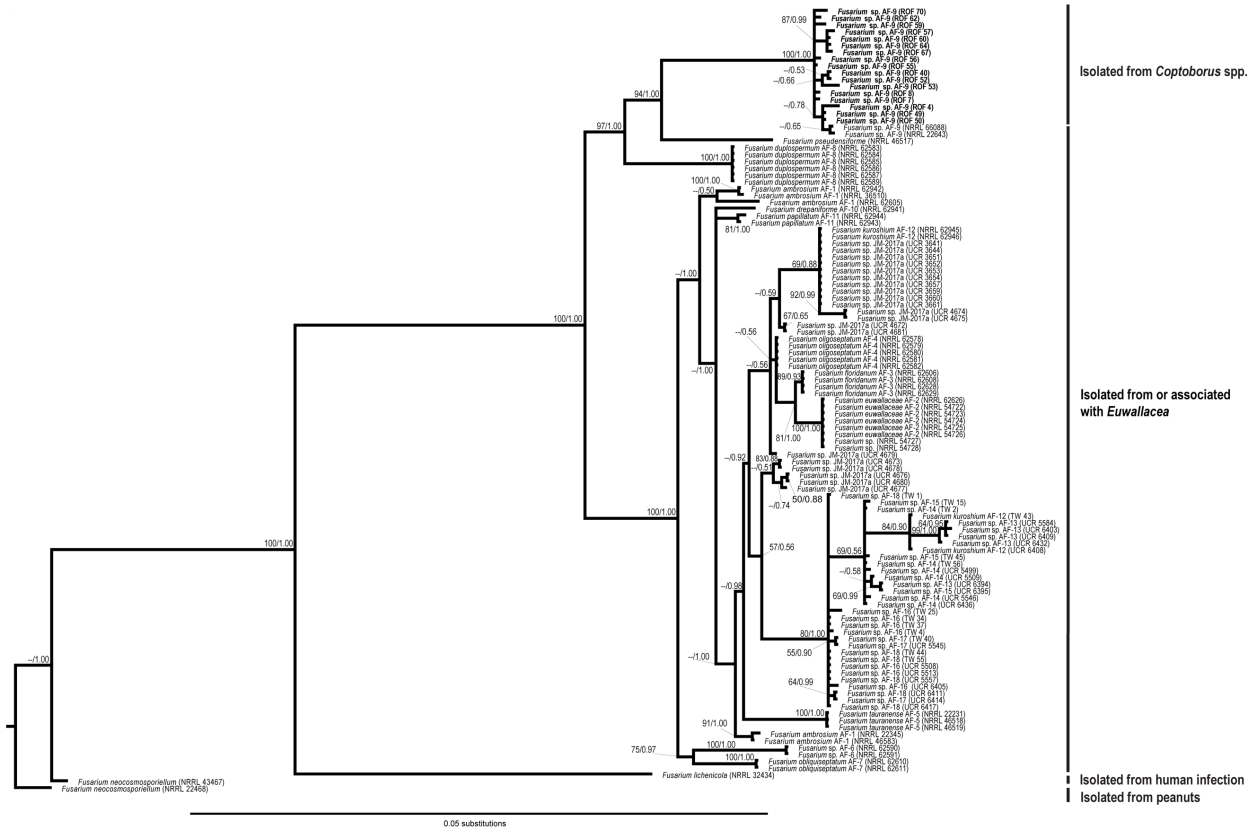

Figure 2.2: Complete Bayesian consensus tree of *Fusarium* strains reconstructed from ITS, *EF1-α*, *RPB1*, and *RPB2* sequence data. Nodes are labeled with bootstrap values/posterior probabilities. *Fusarium* sp. isolated from *Coptoborus* sp. for this study are written in **bold**.

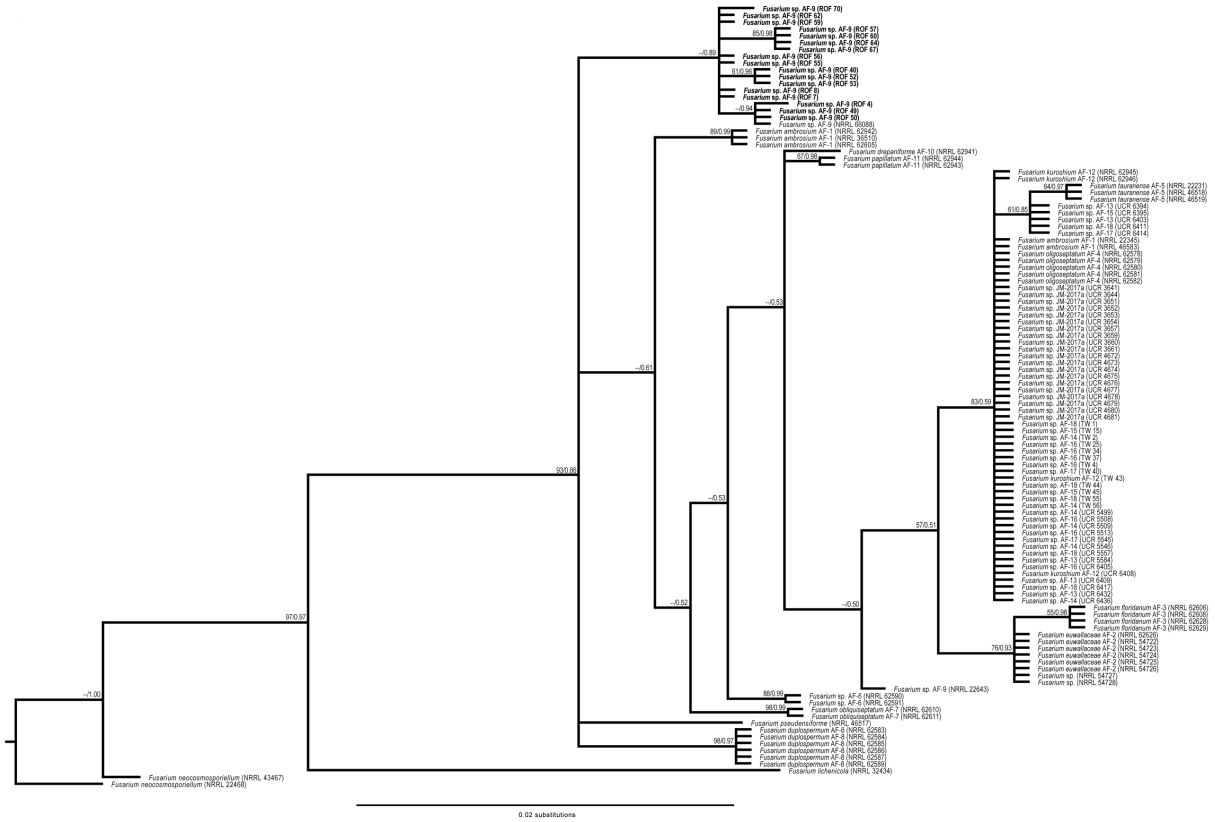

Figure 2.3: Bayesian consensus tree of *Fusarium* strains built from gene sequence data of *EF1-α*. Nodes are labeled with bootstrap values/posterior probabilities. Strains isolated from *Coptoborus* sp. for this study are written in **bold**.

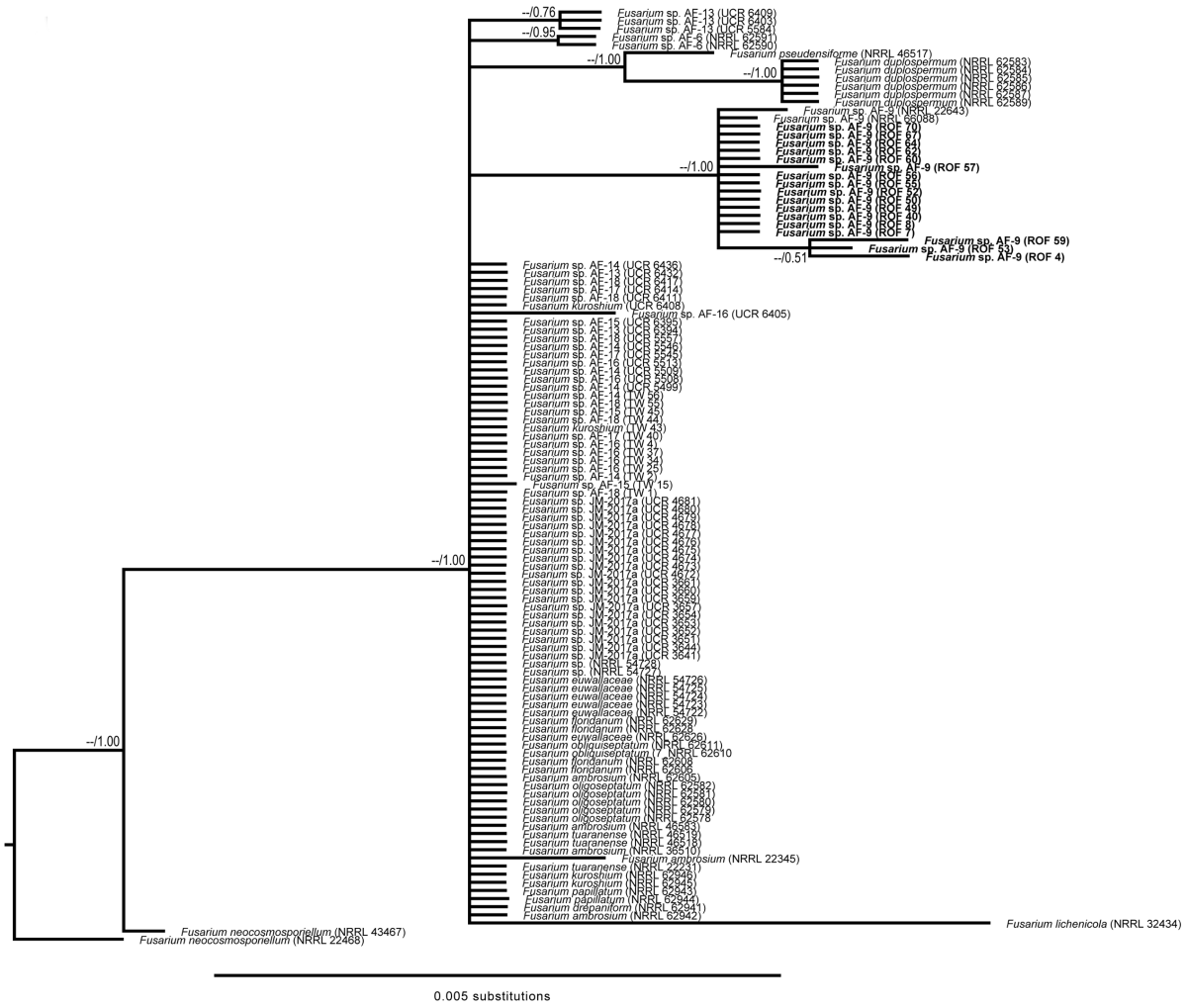

Figure 2.4: Bayesian consensus tree of *Fusarium* strains built from gene sequence data of ITS. Nodes are labeled with bootstrap values/posterior probabilities. Strains isolated from *Coptoborus* sp. for this study are written in **bold**.

Figure 2.5: Bayesian consensus tree of *Fusarium* strains built from gene sequence data of *RPB1*. Nodes are labeled with bootstrap values/posterior probabilities. Strains isolated from *Coptoborus* sp. for this study are written in **bold**.

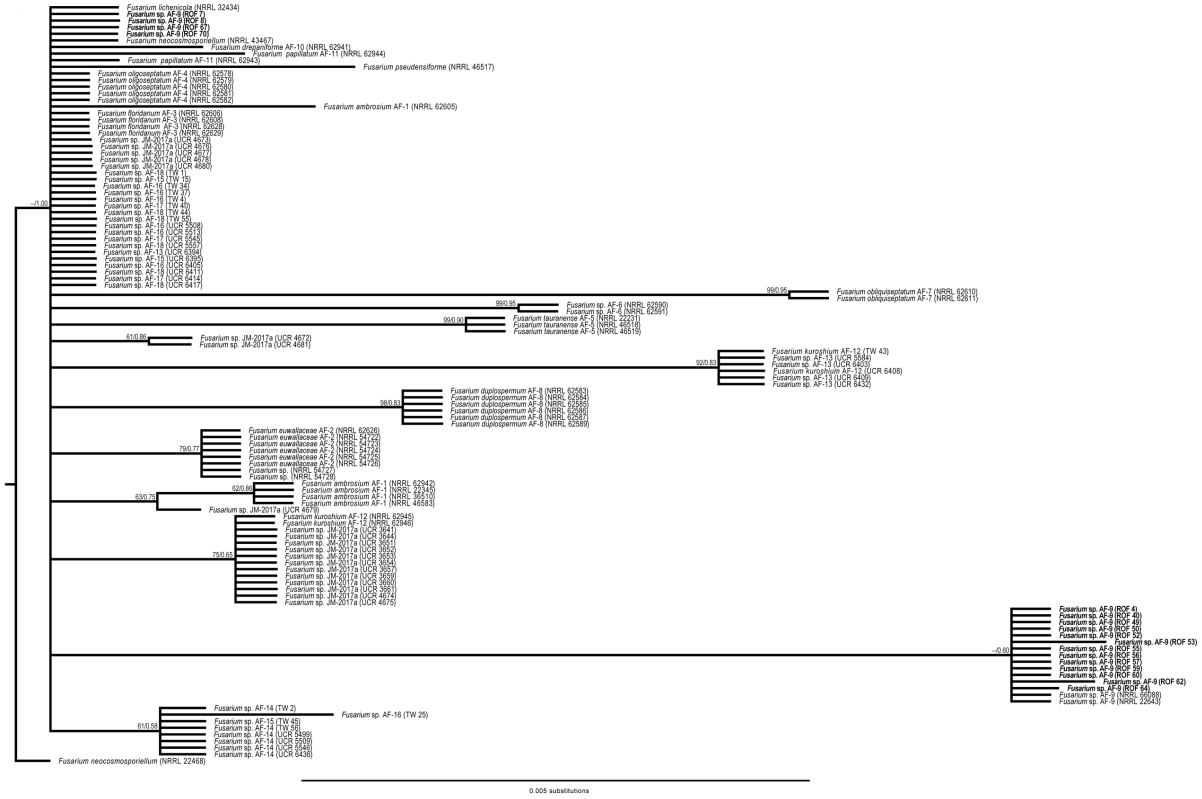

Figure 2.6: Bayesian consensus tree of *Fusarium* strains built from gene sequence data of *RPB2*. Nodes are labeled with bootstrap values/posterior probabilities. Strains isolated from *Coptoborus* sp. for this study are written in **bold**.

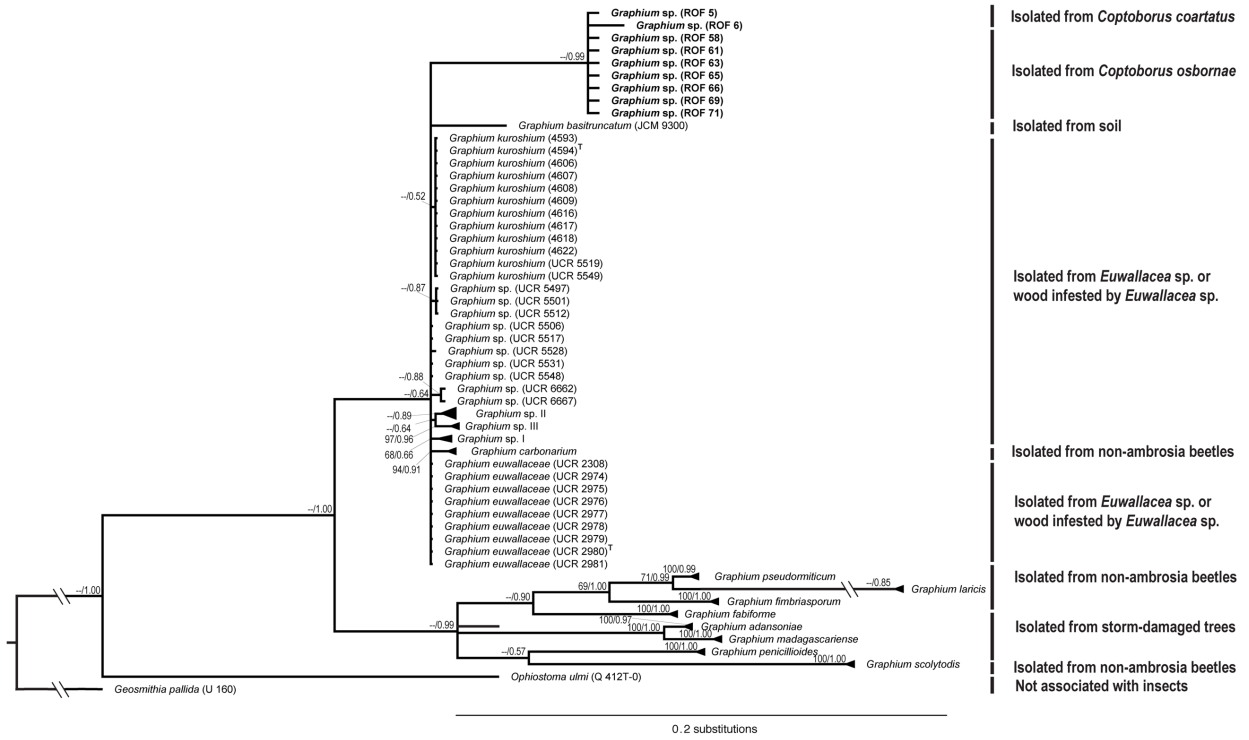

Figure 2.7: Condensed Bayesian consensus tree of *Graphium* strains reconstructed from ITS, and *EF1*-α sequence data. Nodes are labeled with bootstrap values/posterior probabilities. Clades containing strains from *G.* sp. I, *G.* sp. II, *G.* sp. III, *G. carbonarium, G. pseudormiticum, G. laricis, G. fimbriasporum, G. fabiforme, G. adansoniae, G. madagascariense, G. penicillioides, and G. scolytodis* have been collapsed. *Graphium* sp. isolated from *Coptoborus* spp. for this study are written in **bold**. ᵀDenotes ex-type strains. See Figure 2.8 for the complete tree.

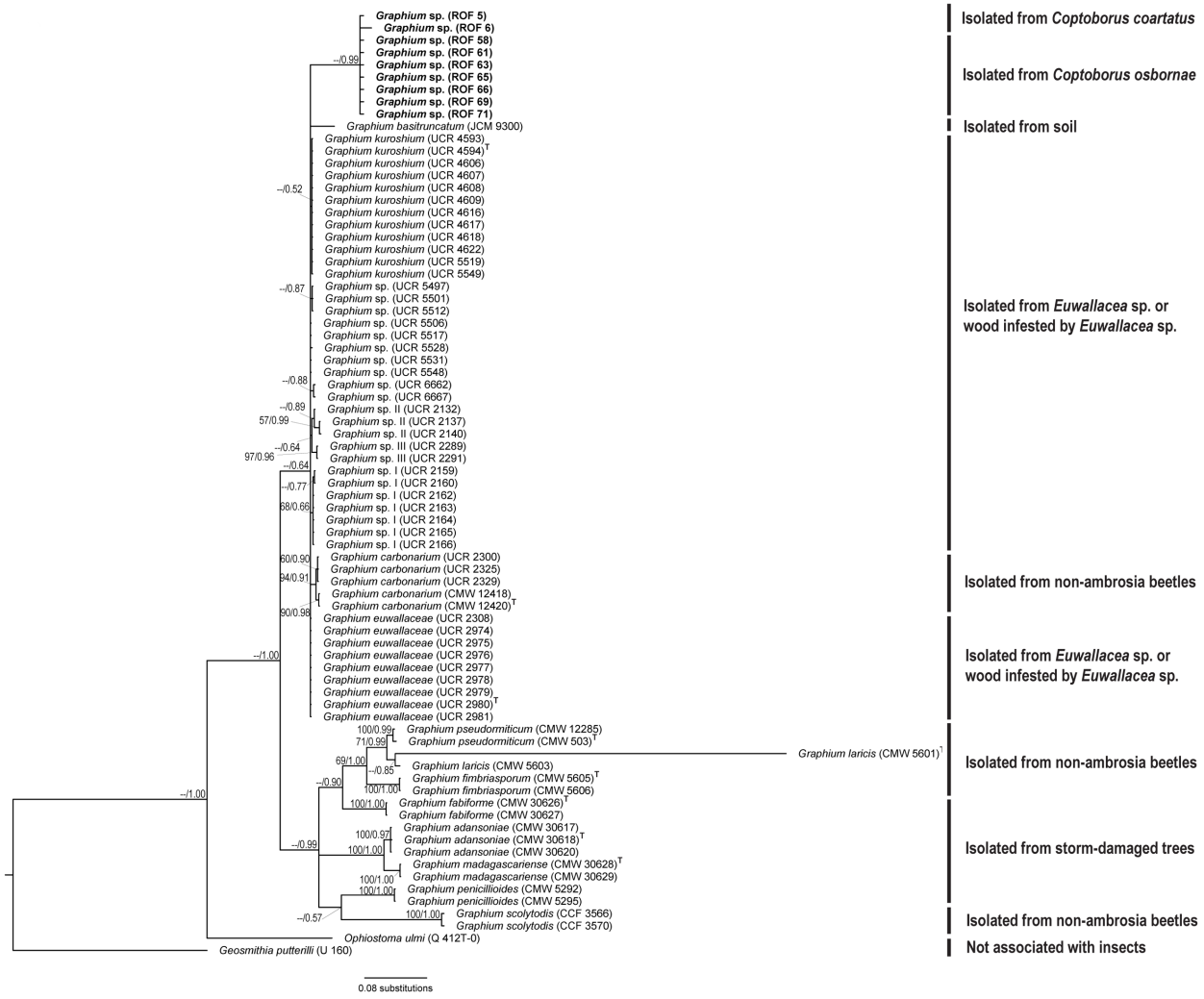

Figure 2.8: Complete Bayesian consensus tree of *Graphium* strains reconstructed from ITS, *EF1*-α, and *RPB2* sequence data. Nodes are labeled with bootstrap values/posterior probabilities. *Graphium* sp. isolated from *Coptoborus* sp. for this study are written in **bold**. ᵀDenotes ex-type strains.

Figure 2.9: Bayesian consensus tree of *Graphium* strains built from gene sequence data of *EF1-α*. Nodes are labeled with bootstrap values/posterior probabilities. Strains isolated from *Coptoborus* sp. for this study are written in **bold**. ᵀDenotes ex-type strains.

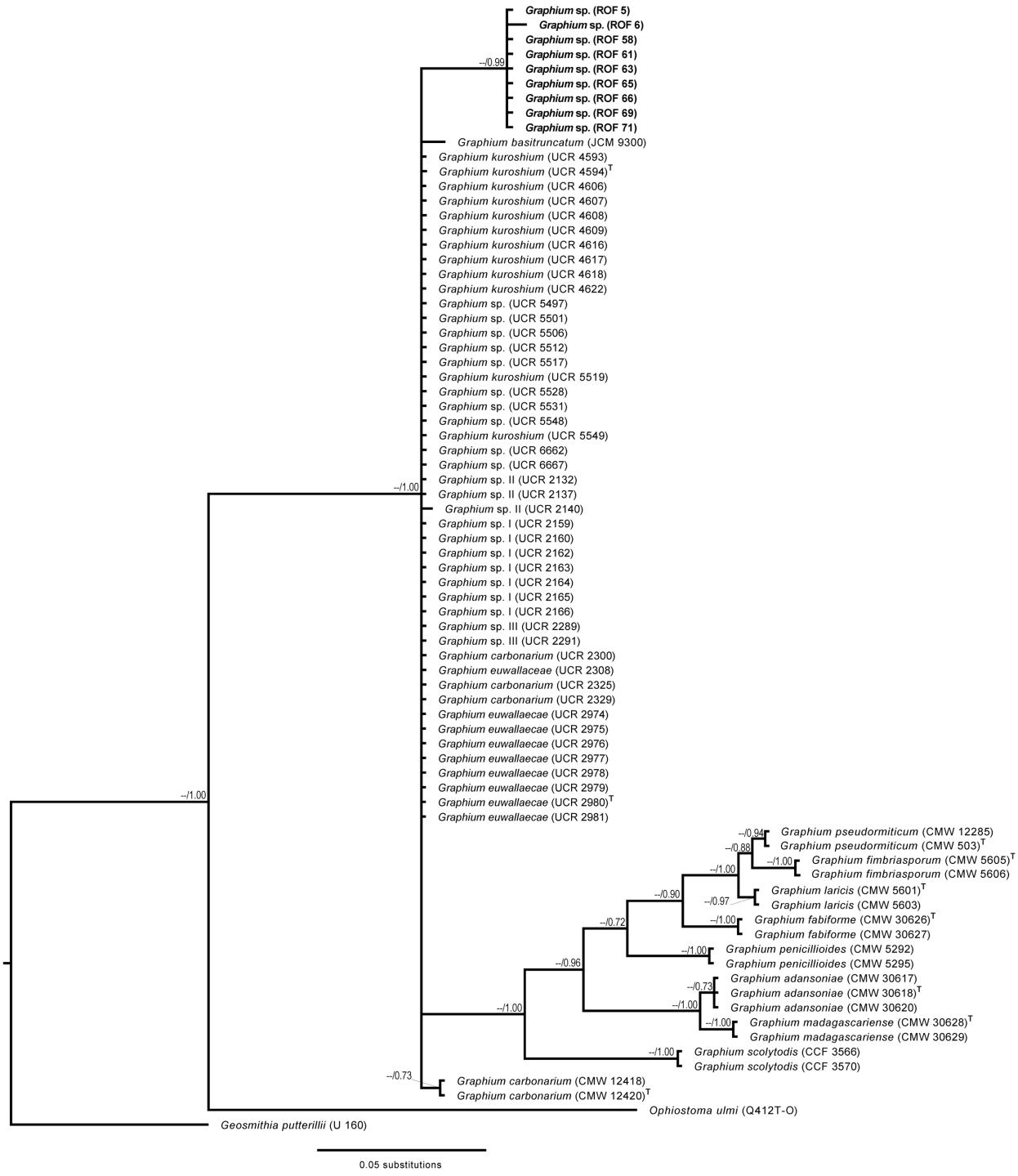

Figure 2.10: Bayesian consensus tree of *Graphium* strains built from gene sequence data of ITS. Nodes are labeled with bootstrap values/posterior probabilities. Strains isolated from *Coptoborus* sp. for this study are written in **bold**. ᵀDenotes ex-type strains.

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CHAPTER 3:

New xyleborine (Coleoptera: Curculionidae: Scolytinae) genus with an Afrotropical-Neotropical distribution

ABSTRACT

Plant-associated arthropods have been shown to cross large oceanic distances on floating plant material and to establish themselves on distant landmasses. Xyleborini (Coleoptera: Curculionidae: Scolytinae) ambrosia beetles occur in forests worldwide and are likely capable of long range dispersal. In less than 20 million years, this group dispersed from Asia to tropical regions of Africa and South America. The phylogeny, taxonomy, and biogeography of one *Xyleborus* species group which occurs on both continents are reviewed for this study. Based on a well-resolved molecular phylogeny resulting from parsimony, likelihood, and Bayesian analyses of four gene loci, we describe a new monophyletic genus, *Xenoxylebora* Osborn, Smith & Cognato, gen. nov., for this bicontinental *Xyleborus* species group with seven Afrotropical and six Neotropical species. Six new species are described: *Xenoxylebora pilosa* Osborn, Smith & Cognato, sp. nov. from Africa, and *Xenoxylebora addenda* Osborn, Smith & Cognato, sp. nov., *Xenoxylebora calculosa* Osborn, Smith & Cognato, sp. nov., *Xenoxylebora hystricosa* Osborn, Smith & Cognato, sp. nov., *Xenoxylebora serrata* Osborn, Smith & Cognato, sp. nov., and *Xenoxylebora sulcata* Osborn, Smith & Cognato, sp. nov., from South America. Seven new combinations from *Xyleborus* are proposed: *Xenoxylebora caudata* (Schedl, 1957) comb. nov., *Xenoxylebora collarti* (Eggers, 1932) comb. nov., *Xenoxylebora perdiligens* (Schedl, 1937) comb. nov., *Xenoxylebora sphenos* (Sampson, 1912) comb. nov., *Xenoxylebora subcrenulata* (Eggers, 1932) comb. nov., and *Xenoxylebora syzygii* (Nunberg, 1959) comb. nov. from Africa,

and *Xenoxylebora neosphenos* (Schedl, 1976) comb. nov. from South America. One new synonym is proposed: *Xenoxylebora sphenos* (Sampson, 1912) =*Xyleborus tenellus* Schedl, 1957 syn. nov.. Descriptions, diagnoses, images, and a key to the identification of all 13 species are provided. The sequence of colonization between Africa and South America is uncertain for *Xenoxylebora*. Prevailing ocean currents and predominant locality patterns observed for other organisms suggests an African *Xenoxylebora* origin. However, the phylogeny and biogeographical analyses suggest a possible South American origin for African *Xenoxylebora,* which is supported by the occurrence of ocean counter currents between the continents and evidence of dispersal from South America to Africa among some plant and arthropod taxa.

INTRODUCTION

The occurrence of related organisms across multiple continents has received much scientific attention because distributional patterns of species contain important clues about the geological history of landmass movements and the mechanics of evolution. Patterns of biodiversity are complex mosaics often resulting from several historical forces (Upchurch 2007). These include vicariance and long-distance dispersal via wind and water as well as geodispersal whereby previously confined species expand their ranges when geographical barriers erode or disappear (Renner 2004a, Couvreur et al. 2011, Condamine et al. 2012, Praz and Packer 2014, Luo et al. 2020). Extinction and taxon sampling biases also affect the documentation of biodiversity and understanding of the factors responsible for observed geographic patterns of species (Upchurch 2007).

Despite the challenge of untangling all elements contributing to the arrangement of biodiversity, evidence suggests that some taxa can travel over oceans between continents. This

includes many plants (Langenheim and Lee 1974, Dick et al. 2003, Givnish et al. 2004, Renner 2004b, Cook and Crisp 2005, Won and Renner 2006, Couvreur et al. 2011), vertebrates (Raxworthy et al. 2002, Bond et al. 2015), and arthropods (Erwin 1979, Bonte et al. 2003, Jordal 2015, Gohli et al. 2016, Murray and Heraty 2016, Luo et al. 2020, Eliassen and Jordal 2021). Plants travel across oceans relatively easily as seeds, or rafts of tangled plant material (floating islands) (Renner 2004a, Peck and Perez-Gelabert 2012). For instance, the plant genus *Hymenaea* (Fabaceae) produces tough, buoyant seedpods that are probably capable of delivering a viable seed after floating in the ocean for several weeks (Langenheim and Lee 1974, de Queiroz 2005). Some animals – especially insects – are also likely to disperse by rafting across oceans because of their close relationships with plants. Peck and Perez-Gelabert (2012) discovered evidence of widespread trans-oceanic dispersal among the beetle genera native to the West Indies. Most of these genera belong to three families (Cerambycidae, Chrysomelidae, and Curculionidae) whose larvae or adults and adults live inside wood, under bark, or are associated other plant structures such as leaves, fruits, and roots. For example, beetles from the weevil tribe Tesserocerini (Curculionidae: Platypodinae) probably arrived in the Neotropics after drifting there from Africa inside *Hymenaea* seeds (Peris et al. 2015).

Scolytine bark and ambrosia beetles (Curculionidae) are preadapted for trans-oceanic travel because most of their life cycles occur inside their woody plant hosts thus presumably protecting them from saltwater and UV radiation. Bark beetles eat tree phloem and live inside or just underneath tree bark. Several species are known to have traveled relatively short distances among Caribbean islands and South and Central America (Kirkendall and Jordal 2006). Other scolytines including the crypturgine genus *Aphanarthrum* and several species from the tribe Hypoborini have dispersed in this manner among the Macaronesian Islands (Jordal and Hewitt

2004) and between Madagascar and the African continent (Jordal 2021a). Trans-ocean rafting carried *Araptus* (Corthylini) from Central America to Cocos Island (Kirkendall and Jordal 2006), and two clades of Scolytoplatypodini from Africa to Madagascar (Jordal 2013). The fossil species †*Electroborus brighti* Cognato, 2013 (Hylesinini) from Dominican amber is sister to the African genus *Strombophorus* and thus presumably evolved from a common ancestor that traveled from Africa to the Americas (Cognato 2013). Several more bark beetle taxa have experienced extreme long-distance dispersal between Africa and the Americas (Figure 1) (Jordal 2012, 2017, 2021b).

Ambrosia beetles farm symbiotic fungi as food for larvae and adults in burrows made in the sapwood of plant hosts. This lifestyle has independently evolved in at least 12 scolytine lineages and once in the Platypodinae (Jordal and Cognato 2012, Gohli et al. 2017, Johnson et al. 2018, Pistone et al. 2018). Nutritional fungus farming is associated with high species diversity – especially in the tribe Xyleborini (Jordal et al. 2000, Farrell et al. 2001, Cognato et al. 2011, Gohli et al. 2017) and is likely an asset to long-range habitat expansion. Given that ambrosia beetles only require host plant conditions that can support fungal growth, they are less constrained in their choice for plant hosts compared to bark beetles, which may survive on only certain tree species (Farrell et al. 2001, Malacrinó et al. 2017, Johnson et al. 2018).

The hyperdiverse monophyletic ambrosia beetle tribe Xyleborini originated ~20 million years ago in Asia (Cognato et al. 2011, Jordal and Cognato 2012, Johnson et al. 2018) and currently contains ~1260 species (Smith, unpublished). Xyleborini species exhibit a suite of traits that make them especially well-prepared to successfully colonize new land masses. Their association with woody plants means that they have the potential to be included in floating islands that may move between continents. Dispersing females inoculate insipient galleries with

fungus they carry with them when they leave their natal colonies. This ensures that food is readily available once a subcortical farm is established. The entire tribe also exhibits haplodiploid inbreeding in which haploid flightless males mate with their diploid sisters inside the burrow before the females disperse. Thus, they do not need to find a mate after arriving to a new environment (Jordal et al. 2000, 2001, Gohli et al. 2016, 2017). These traits allow xyleborine ambrosia beetles to experience low Allee effect thresholds because small colonizing populations are able to produce nutritious food in novel habitats and are less susceptible to inbreeding depression and extinction by genetic drift (Jordal et al. 2001, Peer and Taborsky 2005, Kirkendall and Jordal 2006, Lantschner et al. 2020). Several Xyleborini have been discovered to have completed ancient long-distance dispersal (Kirkendall and Jordal 2006, Gohli et al. 2016, Cognato et al. 2018, Eliassen and Jordal 2021).

Xyleborine ambrosia beetles are known from Africa (206 species), and South America (236 species) (Figure 3.1) (Gohli et al. 2016, Eliassen and Jordal 2021, Smith unpublished) however, these species estimates are low and much of the fauna awaits discovery (e.g., Smith et al. 2017, Eliassen and Jordal 2021, Smith and Cognato 2021). Their generalist host needs, inbreeding reproductive system, and the similar tropical climate shared between the Afrotropics and Neotropics allow this group to successfully invade novel habitats with greater ease than other insects (Jordal et al. 2001, Rassati et al. 2016, Lantschner et al. 2020). *Xyleborus* – the largest Xyleborini genus lives in every biogeographic realm except the Antarctic (Hulcr et al. 2015). Several species within the genus have a history of ancient trans-oceanic dispersal (Kirkendall and Jordal 2006, Gohli et al. 2016, Cognato et al. 2018) and many species have efficiently colonized new habitats via anthropogenic transfer (Haack 2006, Smith et al. 2015, Morgan et al. 2017, Rabaglia et al. 2019).

Early researchers identified *Xyleborus* as a large and confusing genus because of the morphological similarity of many of its members (Hubbard 1897). Indeed, molecular phylogenies have confirmed that *Xyleborus* is not monophyletic (Jordal et al. 2000, Hulcr et al. 2007, Dole et al. 2010, Cognato et al. 2011) and several new genera have recently been described from species previously designated as *Xyleborus* (*e.g.* Hulcr and Cognato 2009, Cognato et al. 2020). In this paper we reconstruct a dated DNA-based phylogeny of African and South American xyleborine species, infer dispersal scenarios for the African and South American species via parametric historical biogeography analyses (eg. Sanmartín 2012), and as a result describe a new genus from *Xyleborus* with an unusual Afro/Neotropical distribution that presumably experienced a historical bifurcation between Africa and the Americas.

METHODS

Taxon Sampling

Molecular data were obtained from beetle specimens collected in Africa (Cameroon, Kenya, and Uganda), South and Central America (Brazil, Costa Rica, Ecuador, Guyana, Panama, Peru, and Suriname), and North America (United States) (Table 3.1). Given that one species of the suspected new genus was previously considered to be *Coptoborus* (Wood and Bright 1992), we included a dense sampling of species from this genus along with other Neotropical Xyleborini genera, and *Xyleborus* species. Several other Xyleborini genera including *Ambrosiodmus Anisandrus*, *Cnestus*, *Euwallacea*, *Xyleborinus* were used as the outgroup based on prior phylogenetic analyses of the tribe (Cognato et al. 2011, Cognato et al. 2019). In total, we sampled 59 xyleborine specimens (belonging to 52 species in 11 genera). The ingroup included

six specimens belonging to the newly described genus (five of the 13 known species) and 37 specimens from Neotropical xyleborine genera (31 species in five genera), and the outgroup consisted of 16 specimens (representing 16 species) belonging to the five genera listed above. Most specimens were excised from their galleries inside infested wood; the remaining individuals were trapped with ethanol-baited bottle traps (Reding et al. 2011) or modified flight intercept traps (Nikulina et al. 2015). All specimens were preserved in in 95−100% ethanol before transportation to the Holistic Insect Systematics Laboratory at Michigan State University where they were stored at -80 ºC.

Molecular dataset

DNA extraction from the head and pronotum was accomplished using Qiagen DNeasy Blood and Tissue Kits (Qiagen; Hilden, Germany) following manufacturer protocols. Each beetle was dissected to separate the meso-, metathorax and abdomen from the head and pronotum to expose the thoracic muscle tissue for more efficient DNA extraction, and to remove potentially contaminating organisms in the digestive tract. After the DNA was extracted, each head/pronotum was reunited with its corresponding meso-, metathorax and abdomen for future examination. These voucher specimens were deposited in the Albert J. Cook Arthropod Research Collection (MSUC).

The resulting DNA templates were used for amplification of four loci: *COI, CAD, EF1-α* and 28S. Each PCR reaction contained 1.25 units hot star Taq (Qiagen), 0.3µM each forward and reverse primers (Table 3.2), 200µM dNTPs, 1x buffer, 1.75 mM magnesium chloride and 50–5 ng of DNA template. Amplification protocols consisted of 15 minutes initial denaturation (95 ºC) and 38 cycles of 30 seconds denaturation (95 ºC), 30 seconds annealing (50 ºC for *COI,* 55 ºC for

CAD, EF1-α and 28S), and 5 minutes extension (72 ºC). To confirm amplification success and rule out contamination, each reaction was subjected to gel electrophoresis run through a 1.5% agarose gel stained with ethidium bromide. PCR products that were consistent with the fragment size predicted for each locus were cleaned using the ExoSAP-IT enzymatic reagent (Thermo Fisher Scientific; Waltham, Massachusetts, USA). Sanger sequencing was performed at the Michigan State University Research Technology Support Facility using Bigdye terminator 1.1 chemistry (Applied Biosystems; Waltham, Massachusetts, USA).

Complementary strands from each Sanger reaction were assembled and trimmed with Sequencher 5.0 build 7082 (Gene Codes Corporation; Ann Arbor, Michigan, USA). Adjustments to the sequences were also made at this time to fix mistaken or unclear base assignments. Sequences generated for this study were submitted to the NCBI GenBank database (Table 3.1). Each locus was aligned with MUSCLE using the default settings (Madeira et al. 2019) and merged into a single, interleaved nexus file containing all four loci.

Phylogenetic analyses

Models of evolution were determined with PartitionFinder 2.1.1 (Lanfear et al. 2016) following the corrected Akaike information criterion (AICc). A single data block was established for the ribosomal LSU gene 28S, whereas the first, second, and third codon positions of the mitochondrial (*COI*) and protein-coding genes (*CAD* and *EF1-α*) were evaluated separately with linked branch lengths via the 'all' search algorithm. PartitionFinder 2.1.1 returned nine substitution models for each of the suggested partitions (Table 3.3).

This partition assembly was used for Bayesian phylogenetic inference via MrBayes 3.2.5 (Ronquist and Huelsenbeck 2003). We ran two independent Monte Carlo runs with 40 million

generations each using metropolis coupling (one cold chain and three heated chains per run). Trees were sampled every 100th generation, and the first 100,000 trees were discarded (25% burn-in). The remainder were used to build a majority-consensus tree and to determine posterior probabilities. The nine-partition structure was also evaluated with ModelFinder with the MF+MERGE option to possibly merge partitions during maximum likelihood search with IQ-TREE 2.1.3 using nearest-neighbor interchange and 1000 bootstrap pseudoreplications (Table 3.3) (Nguyen et al. 2015, Chernomor et al. 2016, Kalyaanamoorthy et al. 2017).

We also used PAUP* 4.0a build 167 (Swofford 2002) to search the combined interleaved data for most parsimonious trees. Gaps were treated as missing and each character as unordered and equally weighted. The heuristic search consisted of 2000 repetitions with random stepwise addition via bisection/reconnection. A bootstrap analysis of 500 pseudoreplications was also conducted using identical heuristic conditions and simple stepwise addition. For all phylogenetic analyses, an outgroup species *Anisandrus sayi* (Hopkins, 1915) was used to root the resulting trees because it represents the most distantly related xyleborine genus to the ingroup (Cognato et al. 2011).

Biogeographical analyses

To estimate the geographic origin of ancestral nodes within the new genus, we evaluated 50,000 trees resulting from the Bayesian inference with Statistical Dispersal Extinction Cladogenesis (SDEC) and BioGeoBEARS using the default settings implemented in RASP version 4.2 (Beaulieu et al. 2013, Matzke 2013a, 2013b, 2014, Massana et al. 2015, Yu et al. 2015, R Core Team 2017). The geographic areas of the taxa were based on Wood and Bright (1992) (Table 3.4). Both analyses used the default settings and included all possible paired combinations of the

five relevant geographical regions for comparison (Table 3.4). To create a binary consensus tree, we resolved the six polytomies in the Bayesian consensus tree by grouping unresolved clades based on morphological similarity. This did not affect the analysis for the new genus because its corresponding clade is fully resolved (Figure 3.2).

Taxonomy

Type material, when available, and additional non-type specimens were examined from the following institutions:

CNCI—Canadian National Collection of Insects, Ottawa

ICB—Instituto de Ciencias Biologicas, Escuela Politécnica Nacional, Quito

MSUC—Albert J. Cook Arthropod Research Collection, Michigan State University, East Lansing

NHMUK—Natural History Museum, London

NHMW—Naturhistorisches Museum Wien, Vienna

NMNH—National Museum of Natural History, Smithsonian Institution, Washington, D.C.

NZCS—National Zoological Collection of Suriname, Paramaribo

RMCA—Musée Royal de l'Afrique Centrale, Tervuren

SEMC— Biodiversity Institute & Natural History Museum, The University of Kansas, Lawrence

UNMSM—Universidad Nacional Mayor de San Marcos, Lima

Each species was photographed using a Visionary Digital Passport II system (Dun Inc; Palmyra, Virginia, USA) using a Canon EOS 5D Mark II (Tokyo, Japan), 65.0 mm Canon Macro photo lens, two Dynalite (Union, New Jersey, USA) MH2015 road flash heads, Dynalite RoadMax MP8 power pack and a Stack Shot (Cognisys, Inc; Traverse City, Michigan, USA).

Montage images were assembled using Helicon Focus Mac Pro 6.7.1 (Helicon Soft; Kharkov, Ukraine) and improved using Adobe Photoshop 2020 (Adobe Systems; Mountain View, California, USA).

Specimens were examined using Leica (Wetzlar, Germany) MZ125 and MZ16 stereomicroscopes and illuminated with a Shott (Southbridge, Massachusetts, USA) 150W halogen light source (model ACE®1). Length and width of the body, pronotum, and elytra was measured on up to seven specimens per putative species using a Leica MZ6 stereomicroscope. Length was measured at the longest point of the body or structure and width was measured at the widest. Protruding tubercles were included in length measurements. We followed the pronotal and antennal club types proposed by Hulcr et al. (2007) and excluded the pedicel from the number funicle segments. Distribution and host records were obtained from the following publications: Schedl 1963, Browne 1965, Medler 1980, Wood 1982, Beaver and Löyttyniemi 1985, Wood and Bright 1992, Wood 2007, Smith et al. 2017.

Previous work has established that *COI* pairwise differences of >10% are useful for xyleborine species demarcation (Dole et al. 2010, Cognato et al. 2020). We used this intraspecific threshold as well as congruence between the molecular phylogeny and morphological divergence to delineate species.

Data availability

DNA sequences are available at NCBI GenBank (Table 3.1). The following files were deposited in DRYAD (https://doi.org/10.5061/dryad.zs7h44jc9): NEXUS files with aligned DNA sequences, most parsimonious trees, a consensus tree resulting from the Bayesian analysis, a resolved tree used in the RASP analysis and a Phylip file with aligned DNA sequences.

RESULTS

Phylogenetic analysis

The combined data included six *Xyleborus* specimens from Africa and South America, 30 specimens from the Neotropical genera (*Coptoborus, Dryocoetoides, Sampsonius* and *Taurodemus*), and 23 specimens representing several widespread genera (*Ambrosiodmus, Anisandrus, Cnestus, Euwallacea, Xyleborinus,* and *Xyleborus*) (Table 3.1). The four-gene multisequence alignment contained 2463 bp (606 *COI*, 477 *CAD*, 373 *EF1-α*, and 1007 28S), 666 bp of which were parsimony-informative. The heuristic search evaluated over 3.4 billion rearrangements and located 36 most parsimonious trees (score = 4176). Bayesian inference of the treespace reached a final average split frequency standard deviation of 0.003 and produced a consensus tree that has moderate posterior probability (PP) support (0.60–0.89 PP) at the basal nodes and strong PP (>0.95) (Erixon et al. 2003) for most derived nodes (Figure 3.2). Likelihood analysis using nearest-neighbor-interchange located a most likely tree (log-likelihood = -20108.9524) which was identical to the trees found in the parsimony and Bayesian analyses except for two *Euwallacea*. However, some bootstrap values were lower compared to parsimony bootstrap values and posterior probabilities (Figure 3.3). Potentially this is an artefact of applying poor models of nucleotide substitution to bootstrapped data sets.

In agreement with previous phylogenetic work on Xyleborini, the Neotropical genera *Coptoborus, Dryocoetoides,* and *Sampsonius* are monophyletic; together forming a well-supported lineage sister to the widely distributed genus *Xyleborus*. Six specimens from tropical Africa and the Amazon formed a robust clade: *Xenoxylebora neosphenos* (previously *Xyleborus neosphenos* Schedl, 1976) (Ecuador), *Xenoxylebora addenda* sp. nov. (previously *Coptoborus*

sp.) (Suriname), *Xenoxylebora calculosa* sp. nov. (previously *Xyleborus* sp.) (Peru), *Xenoxylebora collarti* (Eggers, 1932) (previously *Xyleborus*) (Kenya), and two *Xenoxylebora sphenos* (Sampson, 1912) (previously *Xyleborus*) (Uganda and Cameroon). This group has strong posterior probability, maximum likelihood, and parsimony bootstrap support (1.00, 100 and 96, respectively), however its placement within Xyleborini as sister to *Taurodemus* and *Xyleborinus* exhibits mixed support (0.84 PP, 51 maximum likelihood bootstrap and no parsimony bootstrap support). Thus, its position is uncertain. Nevertheless, this group clearly represents a separate origin from the other Neotropical genera. This well-supported grouping of one putative *Coptoborus* specimen with five *Xyleborus* species necessitates taxonomic revision of this clade.

Biogeographical analyses

The SDEC analysis indicated that the most likely place of origin of the new genus is the Neotropics (0.66 Neotropics/0.33 Afrotropics and Neotropics). The clade including the African species could have originated in either the Neotropics or Africa (0.81 Neotropics/0.19 Afrotropics and Neotropics (Figure 3.2). The BioGeoBears analysis was largely congruent with these results and differ by eight or fewer reported probabilities (0.69 Neotropics/0.28 Afrotropics and Neotropics, and 0.27 Neotropics/0.72 Afrotropics and Neotropics, respectively). The biogeographical results for the other xyleborines included in this study are not reported because Xyleborini is greatly under-sampled (59/1260 species; 11/42 genera) and details analysis and discussion of these taxa are beyond the scope of this study.

Taxonomy

Xenoxylebora Osborn, Smith & Cognato **gen. nov.**

Type species. Xenoxylebora neosphenos (Schedl, 1976).

Diagnosis. 1.70–2.70 mm long; 2.38–4.60 × as long as wide. *Xenoxylebora* is distinguished from all other xyleborine genera by the following combination of characters: antennal club obliquely truncate, typically type 1, with segment 1 encircling the anterior face (Figure 3.4.3) or type 2, with segment 1 nearly covering the posterior face; antennal club wider than long (Figure 3.4.3); protibia slender, posterior face unarmed (Figure 3.4.4); mycangial tufts absent; and scutellum small, flush with elytra and elytral apex typically armed by large subquadrate or quadrate tubercles (Figure 3.4.5).

Xenoxylebora is superficially similar in appearance to *Xyleborinus* with which it shares an elongate form, declivital sculpturing and obliquely truncate antennal club (types 1 and 2). It can be distinguished by the scutellum small, flush with the elytra and flat, the lack of mycangial tufts. In *Xyleborinus* the scutellum is minute, conical, disconnected from elytra and surrounded by a dense tuft of mycangial setae. *Xenoxylebora* is also similar to some elongate *Coptoborus* and can be distinguished by the antennal club type 1, without sutures on posterior face, club wider than long, and protibia slender. *Coptoborus* species have antennal flat club types 3, or 4 (rarely type 2), with two or three sutures on posterior face, club round or longer than wide (Smith and Cognato 2021).

Female. 1.70–2.70 mm long; 2.38–4.60 × as long as wide. Body light to dark brown, pronotum anterior slope and elytral declivity often darker. *Head:* epistoma entire, transverse, weakly sinuate or sinuate, bearing hair-like setae. Frons shagreened, rarely shining, glabrous or sparsely

setose. Eyes narrowly or broadly and moderately emarginate. Submentum large, triangular, deeply impressed. Antennal scape short and thick, shorter than club, funicle 4-segmented. Pedicel shorter than funicle. Club circular, obliquely truncate, slightly wider than long, type 1 or 2; segment 1 corneous, weakly concave, occupying basal 1/3 – 1/2, nearly covering or covering posterior face (Figure 3.4.3). *Pronotum:* 1.00–1.80 × as long as wide. In dorsal view rounded anteriorly with sides parallel in basal 2/3 (type 7); base subtransverse, posterior angles narrowly rounded. In lateral view rounded anteriorly, slope occupying anterior ~1/4; disc much longer than basal slope (type 8). Anterior slope densely covered in broad or narrow asperities and erect hair-like setae; disc shiny to subshiny, finely punctate, glabrous or with erect hair-like setae; lateral margins obliquely costate. *Elytra:* 1.33 – 2.80 × as long as wide, 1.22–1.53 × as long as pronotum. Scutellum small, flat, flush with elytra. Base transverse, humeral angles rounded. Sides parallel for basal ~ 1/3 – 3/4 then weakly to acutely rounded to apex; apex entire, rounded to acuminate, rarely truncate and bearing two round to quadrate tubercles at sutural apex, one on each elytron, tubercles small and inconspicuous to large and prominent or elongate (Figure 3.4.5). Disc subshiny to strongly shining, rarely dull; strial punctures deep, each bearing a seta the height of the diameter of a puncture, rarely longer; interstriae flat, impunctate or finely punctate, glabrous or with hair-like setae. Declivity gradually or steeply rounded, occupying apical ~1/4–3/5 of elytra, shagreened, subshiny, rarely strongly shagreened or dull; declivital face weakly convex to convex, rarely concave. Striae flat to impressed, glabrous or setose; impunctate, or punctures smaller than those on disc. Interstriae flat to impressed, glabrous or with erect or semi-recumbent setae, bearing various tubercles from inconspicuous round granules to large prominent triangular denticles. Interstriae 1–3 often bearing largest tubercles near summit. Interstriae 4–6 often ending short of posterolateral margin. Interstriae 7 usually with

prominent series of tubercles along posterolateral margin. Posterolateral margin usually costate along interstriae 7. *Legs:* protibiae very slender, outer edge weakly rounded bearing 6–8 socketed denticles, posterior face flat and unarmed (Figure 3.4.4). Meso- and metatibiae flat, evenly rounded, bearing 6–11 socketed denticles.

Male. Unknown.

Distribution. AFROTROPICAL (Cameroon, Democratic Republic of the Congo, Ghana, Kenya, United Republic of Tanzania) and NEOTROPICAL (Brazil, Ecuador, Guyana, Peru, Suriname).

Biology. Schedl (1963) described and illustrated the gallery systems of *X. collarti*, *X. sphenos* and *X. syzygii* (Nunberg, 1959) (as *Xyleborus submontanus* Schedl, 1960). The cave-tunnel gallery system reported for all these species is comprised of a short unbranched entrance tunnel that leads to one or two irregularly shaped brood chambers in the longitudinal plane. Specimens of the genus have been recorded from a wide variety of hardwood hosts ranging in size from 2–12 cm (Schedl, 1963). Numerous African specimens have been collected from leaf litter (Schedl 1963). Fungal associates are unknown.

Etymology. G. *Xeno* = strange (referring to the unusual Afrotropical-Neotropical distribution), *xyle* (G) = wood, and *bora* (G) = gluttonous (traditionally interpreted as "borer" by scolytine taxonomists).

Xenoxylebora addenda Osborn, Smith & Cognato **sp. nov.**

(Figures 3.5.6–3.5.8)

Type material. Holotype, female: SURINAME, Sipaliwini [District], 2.977312°N, 55.38500°W, 200 m., Camp 4 (low), Kasikasima, 20–25.III.2012, T. Larson, SR12-0320-TN1, 2012 CI-RAP survey, DNA voucher Cop. neo 1 (NZCS).

Diagnosis. 2.00 mm long (n = 1); 2.86 × as long as wide. This species is distinguished by declivital interstriae 1 with denticles larger than those of interstriae 2 or 3, declivital interstriae 2 moderately impressed and appearing weakly bisulcate. *Xenoxylebora addenda* is similar to *X. sulcata* sp. nov..

Female. 2.00 mm long (n = 1), 2.86 × as long as wide. Body brown, pronotum anterior slope and elytra darker. *Head:* epistoma entire, weakly sinuate, bearing hair-like setae. Frons shagreened, sparsely setose. Eyes narrowly and moderately emarginate. Club type 2, segment 1 corneous, weakly concave, occupying basal 1/3, nearly covering posterior face. *Pronotum:* 1.29 × as long as wide. Anterior slope with dense, broad, coarse asperities; disc finely punctate, with abundant erect hair-like setae. *Elytra:* 1.57 × as long as wide, 1.22 × as long as pronotum. Sides parallel for basal ~1/2 then rounded to apex, apex entire, bearing two small, round sutural tubercles as large as denticles on interstriae 7. Disc strongly shining, strial punctures deep, each with a recumbent seta the height of the diameter of a puncture; interstriae finely punctate, with two confused rows of hair-like setae slightly longer than width of an interstria. Declivity gradually rounded, occupying ~1/2 of elytra, shagreened, subshiny; declivital face weakly convex; striae bearing recumbent hair-like setae as long as those on disc, punctures smaller than those on disc, striae 1 and interstriae 2 moderately impressed; interstriae bearing two confused rows of semi-recumbent to erect hair-like setae slightly longer than width of an interstria; interstriae 1–3 with small uniformly spaced granules, those on interstriae 1 larger than those on 2 and 3; interstriae 7 with larger denticles. Posterolateral margin costate and bearing tubercles along interstriae 7. *Legs:* protibiae with six socketed denticles. Metatibiae with eight socketed denticles.

Male. Unknown.

Distribution. NEOTROPICAL: Suriname (Sipaliwini).

Biology. The holotype was collected in a flight intercept trap.

Etymology. L. *addenda* = to be added, referring to the late identification of this species, necessitating its addition to the manuscript. An adjective.

Xenoxylebora calculosa Osborn, Smith & Cognato **sp. nov.**

(Figures 3.5.9–3.5.11)

Type material. Holotype, female: PERU, Madre de Dios, Los Amigos Biological Station, CM2, GPS: S12.4492' W70.2517', 17–18.V.2008, [S. M.] Smith, [J.] Hulcr, sample Peru 81, ex 9 cm diameter trunk (UNMSM). Six paratypes, female: PERU, as holotype, except Peru 74, ex 2 cm diameter branch (3, MSUC; 1, NHML; 1, NMNH); as previous except: SMS 111, *Coptoborus neosphenos*, 16.IX.2011 [DNA voucher] (1, MSUC).

Diagnosis. 1.90–2.10 mm long (mean = 1.96 mm, n = 7); 2.71–3.00 × as long as wide. This species is distinguished by the tubercles of interstriae 1–3 subequal, declivital striae 2 and interstriae 2 weakly impressed, elytral disc subequal to length of elytral declivity and declivital face shining. *Xenoxylebora calculosa* is similar to *X. serrata* sp. nov. and *X. neosphenos.*

Female. 1.90–2.10 mm long (mean = 1.96 mm, n = 7); 2.71–3.00 × as long as wide (*holotype* 1.90 mm long; 2.17 × as long as wide). Body light brown, pronotum anterior slope and elytra darker. *Head:* epistoma entire, sinuate, bearing hair-like setae. Frons shagreened, glabrous. Eyes narrowly and moderately emarginate. Club type 2, segment 1 corneous, weakly concave, occupying basal 1/3, nearly covering posterior face. *Pronotum:* 1.14–1.29 × as long as wide (*holotype* 1.14 × as long as wide). Anterior slope with dense, broad, coarse asperities; disc sparely covered in minute setae, almost glabrous. *Elytra:* 1.43–1.71 × as long as wide (*holotype* 1.57 × as long as wide), 1.37 × as long as pronotum. Sides parallel for basal ~1/2–2/3 then

rounded to apex, apex entire, bearing two small, round sutural tubercles, as large as denticles on interstriae 7. Disc strongly shining; strial punctures deep each with a minute seta the length of a diameter of a puncture; interstriae glabrous, punctures biseriate. Declivity gradually rounded, occupying ~1/2 of elytra, shiny; declivital face weakly convex; striae 2 weakly impressed with recumbent hair-like setae longer than those on disc, punctures smaller than those on the disc; interstriae bearing semi-recumbent bristle-like setae slightly longer than the width of an interstria; interstriae 2 weakly impressed; interstriae 1–3 with small, uniformly spaced granules, each ~1/2 the width of an interstria, and bearing a long, erect seta; interstriae 7 with large denticles near the declivital base, gradually transitioning to large, round tubercles at apex. Posterolateral margin costate and bearing moderate subquadrate tubercles along interstriae 7.

Legs: protibiae with six socketed denticles. Meso- and metatibiae with six and eight socketed denticles, respectively.

Male. Unknown.

Distribution. NEOTROPICAL: Peru (Madre de Dios).

Biology. Specimens collected from canopy fogging and extracted from infested wood 2–9 cm in diameter.

Etymology. L. *calculosa* = pebbly, referring to the texture and appearance of small denticles on the declivity. An adjective.

Xenoxylebora caudata (Schedl, 1957) **comb. nov.**

(Figures 3.5.12–3.5.14)

Xyleborus caudatus Schedl, 1957: 110, orig. spelling.

Material Examined. Holotype, female: Congo Belge [= DEMOCRATIC REPUBLIC OF THE CONGO], [Tshopo Prov.], Yangambi, 10.X.1952, Dr. [K.E.] Schedl. Paratype, female: as holotype, except: 14.X.1952 (1). (Holotype and paratype in RMCA).

Diagnosis. 2.00 mm long (mean = 2.00 mm, n = 2); 2.86 × as long as wide. This species is distinguished by the tubercles of declivital interstriae 3 larger and much more prominent than those of interstriae 1 or 2, sutural tubercles on elytral apex distinctly elongated, laterally angled, and closely grouped to three large denticles on interstriae 1. *Xenoxylebora caudata* is similar to *X. hystricosa* sp. nov., *X. perdiligens* (Schedl, 1937) and *X. sphenos*.

Distribution. AFROTROPICAL: Democratic Republic of the Congo (South Kivu, Tschopo), Zambia.

Biology. Breeds in small twigs and branches (Beaver and Löyttyniemi 1985). Host plants: Anacardiaceae (*Antrocaryon*), Fabaceae (*Pterocarpus*), Irvingiaceae (*Klainedoxa*), Urticaceae (*Musanga*).

Xenoxylebora collarti (Eggers, 1932) **comb. nov.**

(Figures 3.5.15–3.5.17)

Xyleborus collarti Eggers, 1932: 300.

Xyleborinus collarti (Eggers): Wood and Bright 1992: 806.

Xyleborus collarti Eggers: Hulcr et al. 2007: 577.

Xyleborus semipilosus Eggers, 1932: 300. Synonymy: Schedl 1963: 490.

Material Examined. Holoytpe, female: *X. collarti*: [DEMOCRATIC REPUBLIC OF THE CONGO], Forêt de Kawa, 22.IV.[19]24, A. Collart, USNMENT 01547104 (NMNH); images examined. Holotype, female: *X. semipilosus*, [DEMOCRATIC REPUBLIC OF THE CONGO],

Katanga [= Tanganyika + Haut-Lomami + Lualabba + Haut-Katanga Prov.], Lufudizi, 22.IX.1924, C. Seydel, Musee du Congo (RMCA). Seven females designated *X. collarti*: KENYA, Kakamega District [= County], Isecheno, Isecheno Forest Reserve, 13.II.2002, R.R. Snelling, ex sifted litter #02-47, SM0677729 (1); as previous except: Yala River Forest Reserve, 0.204ºN 34.873ºE, 1450–1470 m, 15.II.2002, ex sifted litter between buttresses #02-058, SM0668771, SMS 373 [DNA voucher] (1), SM0698653 (1), SM0698704, SMS 372 [DNA voucher] (1); as previous except: 1450 m, 28.II.2002, #02-096, SM0678399 (1); as previous except: 8.III.2002, ex sifted *Ficus* litter between buttresses #02-0111, SM0698291 (1), SM0698280 (1) (all in SEMC).

Diagnosis. 2.20–2.30 mm long (mean = 2.26 mm; n = 5); 2.81–3.29 × as long as wide. This species is distinguished by the tubercles of interstriae 1–3 subequal and declivity convex with a gradual slope. *Xenoxylebora collarti* is similar to *X. pilosa* sp. nov., and *X. subcrenulata* (Eggers, 1932).

Distribution. AFROTROPICAL: Angola, Cameroon, Côte d'Ivoire, Democratic Republic of the Congo (Haut-Katanga, Haut-Lomami, Lualabba), Ghana, Kenya (Kakamega), Nigeria, United Republic of Tanzania.

Biology. Xenoxylebora collarti is reported to colonize branches ranging from 2–5 cm in diameter. Females construct a cave-tunnel gallery system comprised of a short unbranched entrance tunnel that leads to one irregularly shaped brood chamber in the longitudinal plane. (Schedl, 1963). Host plants: Apocynaceae (*Conopharyngia*), Fabaceae (*Acacia*, *Millettia*), Hypericaceae (*Haronga*), Meliaceae (*Trichilia*, *Turraeanthus*), Myrtacae (*Syzygium*), Rubiaceae (*Cinchona*, *Galiniera*), Solanaceae (*Solanum*). Specimens designated *X. collarti* were sifted from *Ficus* leaf litter.

Comments. Schedl (1963) placed *X. semipilosus* in synonymy without comment. Strangely both species were described in same publication and page (Eggers, 1932: 300) however, Eggers failed to recognize the species were conspecific and compared the form of *X. semipilosus* to that of *Xyleborinus saxesenii* (Ratzeburg, 1837) and that of *Xenoxylebora collarti* to *Fraudatrix melas* (Eggers, 1927). Both species are clearly conspecific and we agree with the synonymy of *X. semipilosus*.

Xenoxylebora hystricosa Osborn, Smith & Cognato **sp. nov.**

(Figures 3.5.18–3.5.20)

Type material. Holotype, female: BRAZIL, Amazonas, 60 K[ilo]m[eters] N[orth] Manaus, Fazenda Esteio, ZF-3 Km-23, 10.V.1985, B.C. Klein, ex arm Malaise (NMNH).
Paratypes, 3 females: BRAZIL, as holotype, except: 8.III.1985 (1); 14.V.1986 (1, NMNH); SURINAME, Mariwijine, Palumeu, 160 m, 7–8.VIII.1999, Z.H. Falin, ex FIT (1, CNCI).

Diagnosis. 2.15–2.30 mm long (mean = 2.24 mm, n = 4); 2.44–2.88 × as long as wide.
This species is distinguished by declivital interstriae 1 and 2 unarmed, spines on interstriae 3 very prominent, significantly larger than those on interstriae 4–7. *Xenoxylebora hystricosa* is similar to *X. caudata*, *X. perdiligens* and *X. sphenos*.

Female. 2.15–2.30 mm long (mean = 2.24 mm, n = 4); 2.44–2.88 × as long as wide (*holotype* 2.20 mm long; 2.44 × as long as wide). Body light brown, pronotum anterior slope and elytra slightly darker. *Head:* epistoma entire, weakly sinuate, with hair-like setae. Frons shining, sparsely setose, finely punctate. Eyes broadly and moderately emarginate. Club type 2, segment 1 corneous, weakly concave, occupying basal 1/3 of segment 1, nearly covering posterior face. *Pronotum:* 1.13–1.43 × as long as wide (*holotype* 1.33 × as long as wide). Anterior slope with

dense, broad, coarse asperities; disc shining, finely punctate with erect hair-like setae. *Elytra:* 1.33–1.75 × as long as wide (*holotype* 1.33 × as long as wide), 1.29 × as long as pronotum. Sides parallel for basal ~ 1/3–1/2 then attenuate to apex, apex entire, bearing two subquadrate sutural tubercles similar in size to denticles on interstriae 3. Disc strongly shining, strial punctures deep each with a minute seta the length of a diameter of a puncture; interstriae finely punctate with single row of minute erect hair-like setae ~1 ½ × as long as the width of an interstria. Declivity gradually rounded, occupying ~1/2 of elytra, shagreened, subshiny; declivital face weakly convex; striae flat, with recumbent hair-like setae, thicker and as long as those on disc; punctures smaller than those on disc; interstriae flat with erect hair-like setae ~2 × as long as the width of an interstria; interstriae 1 and 2 unarmed on declivital face, interstriae 1 with two or three denticles at the summit; interstriae 3 with large, distinct, uniformly spaced denticles ~2 × as tall as the width of an interstria extending from declivital base to apex and bearing two similarly sized subquadrate sutural tubercles at apex; interstriae 4–7 with smaller denticles ~2/3 the width of an interstria. Posterolateral margin costate and bearing denticles along interstriae 7. *Legs:* protibiae with six socketed denticles. Meso- and metatibiae with nine socketed denticles.

Male. Unknown.

Distribution. NEOTROPICAL: Brazil (Amazonas), Suriname (Marowijine).

Biology. Specimens were passively collected in Malaise and FIT traps.

Etymology. L. *hystricosa* = thorny, referring to the appearance of the distinctive row of large denticles on declivital interstriae 3. An adjective.

Comments. Specimens collected in malaise and flight intercept traps.

Xenoxylebora neosphenos (Schedl, 1976) **comb. nov.**

(Figures 3.6.21–3.6.23)

Xyleborus neosphenos Schedl, 1976: 76.

Coptoborus neosphenos (Schedl): Wood and Bright 1992: 663.

Xyleborus neosphenos Schedl: Smith and Cognato 2021: 620.

Material Examined. Holotype, female: BRAZIL, Rondônia, Vilhena, XI.1973, M. Alvarenga (NHMW). ECUADOR, Napo Prov. [= Orellana Prov.], Res[erva]. Ethnica Waorani, 1 km S. Okone Gare Camp, Trans[ect]. Ent[omology]., 00°39'10"S, 076°26'W, January 1994, T. L. Erwin et al., 220 m, insecticidal fogging, terra firme forest, trans[ect] 1, sta[tion] 5, Erwin-lot # 594 (1, ICB); Orellana Prov., Tiputini Biodiversity Station, S00°38.189' W76°08.965', 223 m, 3–9.VI.2011, S.M. Smith, SMS 102 *Coptoborus neosphenos*, 16.IX.2011 [DNA voucher] (1, MSUC). PERU, Madre de Dios, Los Amigos Biological Station, CM2, GPS: S12.4492' W70.2517', 17–18.V.2008, [S.M.] Smith, [J.] Hulcr, Peru 74, ex 2 cm diameter (MSUC); as previous except Peru 88c, ex 3 cm diameter twig (MSUC).

Diagnosis. 1.90–2.10 mm long (mean = 2.00 mm, n = 2); 2.71–3.00 × as long as wide. This species is distinguished by the tubercles of interstriae 1–3 subequal, acuminate elytral apex, and tubercles on elytral apex subquadrate and very prominent. *Xenoxylebora neosphenos* is similar to *X. calculosa* and *X. serrata.*

Distribution. NEOTROPICAL: Brazil (Rondônia, Mato Grosso), Ecuador (Orellana), Peru (Cusco, Madre de Dios).

Biology. One specimen (NMNH) from Mato Grosso (E78) was collected from small 3.3 cm diameter uprooted tree of *Pouteria* sp. (Sapotaceae) in gallery forest (Roger A. Beaver, personal

communication). Specimens have also been collected from canopy fogging and extracted from additional infested wood 2–3 cm in diameter.

Xenoxylebora perdiligens (Schedl, 1937) **comb. nov.**

(Figures 3.6.24–3.6.26)

Xyleborus perdiligens Schedl, 1937: 399.

Material Examined. Lectotype, female: [UNITED REPUBLIC OF TANZANIA], Urw.[ald] hint.[er] d[em] Randbg. [= Randbgebirge] N.W. Tanganjikasees, 18[00]–2200 m, S[ammler] Grauer (NHMW). Nine females: Congo [= DEMOCRATOC REPUBLIC OF THE CONGO], [North Kivu Prov.], Dorsale de Lubero, Mt. Muleke, June/July 1963, M. J. Célis, Coll. Mus. Tervuren (3, RMCA); as previous except: P.N.A, Massif Ruwenzori, Kalonge, 2.IX.1952, P. Vanschuytbroeck & J. Kekenbosch, alt. 2210 m, 922–26 (3, RMCA); as previous except 9–11.VIII.1952, 741–42 (1, RMCA); Lubero, route Kimbulu, June 1954, R. P. M. J. Célis, alt. 18300 m, Tamisage Tamisage de terreau sous fougéres arbor; (1, RMCA); as previous except ruiss prés de Kimbulu, alt. 1750 m, (1, RMCA).

Diagnosis. 2.5–2.7 mm long (mean = 2.56 mm, n = 8); 2.78–3.38 × as long a wide. This species is distinguished by the tubercles of declivital interstriae 3 larger and much more prominent than those of interstriae 1 or 2, declivital interstriae 1 and 2 sparsely granulate, distance between granules equal to width of at least three granules, and declivital slope very steep, occupying apical quarter of elytra. *Xenoxylebora perdiligens* is similar to *X. caudata*, *X. hystricosa* and *X. sphenos*.

Distribution. AFROTROPICAL: Democratic Republic of the Congo (North Kivu), United Republic of Tanzania (Taganyika).

Biology. Specimens were collected by sieving soil and sifting leaf litter under tree ferns.

Comments. A series large of *X. perdiligens* (RMCA; listed above) was incorrectly identified as *X. tenellus* by M. Nunberg and F. G. Browne. Browne however referred to the specimens and "*tenellus* Schedl large form". It is probable other specimens of *X. perdiligens* are misidentified as *X. tenellus* in other collections.

Xenoxylebora pilosa Osborn, Smith & Cognato **sp. nov.**

(Figures 3.6.27–3.6.29)

Type material. Holotype, female: Tanganyika Terr[itory]. [= United Republic of Tanzania]s, [Morogoro Region], Bunduki, Uluguru M[oun]t[ain]s, 1500 m, gorge Mungula, 1–6.V.1957, Mission Zoolog. I.R.S.A.C. en Afrique orientale, P. Basilewsky et N. LeLeup, ex forêt transition dans l'humus (RMCA).

Diagnosis. 2.20 mm long (n = 1); 3.14 × as long as wide. This species is distinguished by declivital interstriae 1 and 3 bearing small, round granules, interstriae 2 with very minute granules, almost indistinct, and declivity convex with a gradual slope. *Xenoxylebora pilosa* is similar to *X. collarti,* and *X. subcrenulata*.

Female. 2.20 mm long (n = 1); 3.14 × as long as wide. Body medium brown, pronotum anterior slope and elytra darker. *Head:* epistoma entire, sinuate, with hair-like setae. Frons shagreened, sparsely setose. Eyes narrowly and moderately emarginate. Club type 2, segment 1 corneous, weakly concave, nearly covering posterior face. *Pronotum:* 1.29 × as long as wide. Anterior slope with dense broad, coarse asperities; disc subshiny, finely punctate, with erect hair-like setae. *Elytra:* 1.86 × as long as wide, 1.44 × as long as pronotum. Sides parallel for basal ~2/3 then broadly rounded to apex, apex entire, bearing two round sutural tubercles larger than other

declivital tubercles. Disc strongly shining; strial punctures deep, each with a seta the height of the distance between punctures; interstriae finely punctate, with two confused rows of hair-like setae slightly longer than width of interstriae. Declivity gradually rounded, occupying ~1/3 of elytra, shagreened, subshiny; declivital face convex, highly obscured by dense setae; striae flat with semi-erect hair-like setae ~3/4 as long as interstrial setae; punctures slightly smaller than those on disc; interstriae flat with two confused rows of erect bristle-like setae as long as the width of an interstria; interstriae 1 with two rows of hair-like setae, small granules and three denticles as tall as the width of an interstria close to apex; interstriae 2 bearing very minute granules; interstriae 3–6 with small, rounded granules; interstriae 7 with slightly larger rounded granules ~ ½ as tall as the width of an interstria. Posterolateral margin costate and bearing tubercles along interstriae 7. *Legs:* protibiae with seven socketed denticles. Meso- and metatibiae with eight and seven socketed denticles, respectively.

Male. Unknown.

Distribution. AFROTROPICAL: Democratic Republic of the Congo (Tanganyika).

Biology. Specimens were collected from humus in a forest transition zone.

Etymology. L. *pilosa* = hairy, referring to the abundant hair-like setae covering the elytra. An adjective.

Comments. The holotype was previously identified as *Xyleborus semipilosus* (= *Xenoxylebora collarti*) by M. Nunberg. It is probable that other specimens of this species have been misidentified as *Xyleborus semipilosus* in collections.

Xenoxylebora serrata Osborn, Smith & Cognato **sp. nov.**

(Figures 3.6.30–3.6.32)

Type material. Holotype, female: GUYANA, [Potaro-Siparuni Region = Region 8], Iwokrama Forest, Turtle M[oun]t[ain]n, GPS: N04.44.081' W058.42.830', Guy 33, 4–9.III.2007, McCall, [A. I.] Cognato, [J.] Hulcr, [S. M.] Smith, [S.] Dole (MSUC).

Diagnosis. 1.70 mm long (n = 1); 2.83 × as long as wide. This species is distinguished by the tubercles of interstriae 1–3 subequal, declivital striae 2 and interstriae 2 weakly impressed, elytral disc shorter than length of elytral declivity and declivital face shagreened. *Xenoxylebora serrata* is similar to *X. calculosa* and X. *neosphenos.*

Female. 1.70 mm long (n = 1); 2.83 × as long as wide. Body light brown, pronotum anterior slope and elytra distinctly darker. *Head:* epistoma entire, weakly sinuate, with hair-like setae. Frons shagreened, sparsely setose. Eyes broadly and moderately emarginate. Club type 1, segment 1 corneous, weakly concave, occupying basal 1/3, encircling anterior face. *Pronotum:* 1.17 × as long as wide. Anterior slope with dense, broad, coarse asperities; disc shining, finely punctate and glabrous. *Elytra:* 2.00 × as long as wide, 1.43 × as long as pronotum. Sides parallel for basal ~2/3 then broadly rounded to apex, apex entire, bearing two round sutural tubercles as large as denticles on interstriae 7. Disc strongly shining; strial punctures deep, each with a minute seta the length of a diameter of a puncture; interstriae finely punctate, glabrous. Declivity gradually rounded, occupying ~3/5 of elytra, shagreened, subshiny; declivital face weakly convex; striae bearing with recumbent hair-like setae; punctures smaller than those on disc; striae 2 weakly impressed; interstriae bearing semi-recumbent bristle-like setae slightly longer than the width of an interstria; interstriae 2 weakly impressed; interstriae 1–4 with evenly spaced minute granules; interstriae 5 with four denticles close to summit, ~1/2 as tall as the width of an

interstria diminishing to small granules toward apex; interstriae 6 with rounded tubercles smaller than those on interstriae 7, diminishing in size toward apex; interstriae 7 with large, round tubercles about as tall as the width of an interstria. Posterolateral margin costate and bearing denticles along interstriae 7. *Legs:* protibiae with six socketed denticles. Meso- and metatibiae with nine and eight socketed denticles, respectively.

Male. Unknown.

Distribution. NEOTROPICAL: Guyana (Potaro-Siparuni).

Biology. Unknown.

Etymology. L. *serrata* = toothed like a saw, named in reference to the appearance of the denticles on declivital interstriae 7. An adjective.

Xenoxylebora sphenos (Sampson, 1912) **comb. nov.**

(Figures 3.6.33–3.6.35)

Xyleborus sphenos Sampson, 1912: 247.

Xyleborus perdiligens diligens Schedl, 1954: 79. Synonymy: Wood and Bright 1992: 775.

Xyleborus montanus tenellus Schedl, 1957: 107. **syn. nov.**

Material Examined. Lectotype, *X. perdiligens diligens*, female: Gold Coast [= GHANA], [Eastern Region], Mpraeso, 23.I.1947, G.H. Thompson (NHMW); paralectotype, female, as lectotype (NHMUK). Holotype *X. montanus tenellus*, female: Congo Belge [= DEMOCRATIC REPUBLIC OF THE CONGO], [Tshopo Prov.], Yangambi, 12.IX.1952, Dr. [K.E.] Schedl, Nr. 852 (RMCA). Six females designated *X. sphenos*: CAMEROON, Southwest Region, Limbe, 22.IX.2007, B. Jordal, ex EtOH trap, (3, MSUC); as previous except: SMS 368 [DNA voucher]. Congo Belge [= DEMOCRATIC REPUBLIC OF THE CONGO], [South Kivu Prov.], Hembe-

Bitale, 8.VIII.1952, Dr. [K.E.] Schedl, ex s. 593 (1, NHMW). KENYA, Kakamega District, Isecheno, Isecheno For.[est] Res.[ersve], 0.24°N, 34.85°E, 1600 m, 13.II.2002, R.R. Snelling, sifted litter #02–044, SM0677385 (1 SEMC). 6 females designated *X. tenellus* (RMCA): Congo Belge [= DEMOCRATIC REPUBLIC OF THE CONGO], [North Kivu Prov.], P.N.A, Massif Ruwenzori, Kalonge, 2210 m, 2.IX.1952, P. Vanschuytbroeck & J. Kekenbosch, 922–26 (2); as previous except: 31.VII-3–5.VIII.1952, 688–69 (1). Face N. Ruwenzori, Kikura, Vallée de la Kafuko, 2000 m, P. N. Virunga, VII/VIII.[19]74, R.P., M. Lejeune, ex dans bois morts (1); as previous except: sol suspend (1). Dorsale de Lubero, Mt. Muleke, JVI/VII. 1963, M.J. Célis (1).

Diagnosis. 1.70–2.40 mm long (mean = 2.09 mm, n = 14); 2.38–4.60 × as long as wide. This species is distinguished by the tubercles of declivital interstriae 3 larger and much more prominent than those of interstriae 1 or 2, declivital interstriae 1 and 2 densely granulate, granules subcontiguous, declivital slope gradual, and occupying apical third of elytra. *Xenoxylebora sphenos* is similar to *X. caudata*, *X. perdiligens*, and *X. hystricosa*.

Distribution. AFROTROPICAL: Cameroon (Southwest), Democratic Republic of the Congo (North Kivu, South Kivu, Tshopo), Ghana (Eastern), Kenya (Kakamega), Uganda.

Biology. Xenoxylebora sphenos is reported to colonize branches ranging from 2–7 cm in diameter. Females construct a cave-tunnel gallery system comprised of a short unbranched entrance tunnel that leads to one or two irregularly shaped brood chamber in the longitudinal plane. Females deposit eggs in a single heap and brood size ranges from 27–38 individuals (Schedl, 1963). Specimens were collected from liter sifting and alcohol trapping. Host plants: Clusiaceae (*Pentadesma*), Fabaceae (*Albizzia*), Malpighiaceae (*Acridocarpus*), Meliaceae (*Lovoa, Turraeanthus*).

Comments. The holotype of *X. sphenos* (NHMUK) is not on its point and is likely lost. Our concept of *X. sphenos* is based on Sampson's description and specimens independently identified by R. A. Beaver and B. H. Jordal. *Xyleborus montanus tenellus* Schedl, 1957 is here synonymized with *X. sphenos* because the specimens examined are morphologically indistinguishable. An additional investigation utilizing additional specimens as part of a molecular analysis will be required to further assess species limits.

Xenoxylebora subcrenulata (Eggers, 1932) **comb. nov.**

(Figures 3.7.36–3.7.38)

Xyleborus subcrenulatus Eggers, 1932: 301, orig. spelling.

Type material. Holotype, female: Congostaat [= DEMOCRATIC REPUBLIC OF THE CONGO], [Kongo Central Prov.], Madimba, 15.iv.[19]30, A. Collart, USNMENT 01547123 (NMNH); images examined.

Diagnosis. 1.8 mm long (n = 1); 2.89 × as long as wide. This species is distinguished by the tubercles of interstriae 1–3 subequal and declivity flat with a steep slope. *Xenoxylebora subcrenulata* is similar to *X. collarti* and *X. pilosa*.

Distribution. AFROTROPICAL: Democratic Republic of the Congo (Kongo Central).

Biology. Unknown.

Xenoxylebora sulcata Osborn, Smith & Cognato **sp. nov.**

(Figures 3.7.39–3.7.41)

Type material. Holotype, female: ECUADOR, Napo Prov. [= Orellana Prov.], Res[erva]. Ethnica Waorani, 1 km S. Okone Gare Camp, Trans[ect]. Ent[omology]., 00°39'10"S, 076°26'W, 220 m,

January 1994, T.L. Erwin et al., insecticidal fogging, terra firme forest, trans[ect] 1, sta[tion] 6, Erwin-lot # 595 (ICB).

Diagnosis. 1.90 mm long (n = 1); 2.71 × as long as wide. This species is distinguished by the tubercles of interstriae 1–3 subequal, declivity sulcate between striae 1 and interstriae 4, and declivity appearing bisulcate. *Xenoxylebora sulcata* is similar to *X. addenda*.

Female. 1.90 mm long (n = 1); 2.71 × as long as wide. Body light brown, elytra slightly darker. *Head:* epistoma entire, transverse, with hair-like setae. Frons shagreened, sparsely setose. Eyes broadly and moderately emarginate. Club type 1, segment 1 corneous, weakly concave, occupying basal 1/3, encircling anterior face. *Pronotum:* 1.14 × as long as wide. Anterior slope with dense, broad, coarse asperities; disc shining, finely punctate with erect hair-like setae. *Elytra:* 1.57 × as long as wide, 2.88 × as long as pronotum. Sides parallel for basal ~2/3 then rounded to apex, apex entire, bearing two small, round sutural tubercles as large as denticles on interstriae 7. Disc subshiny, strial punctures small and shallow, each with one minute recumbent seta the height of a diameter of a puncture; interstriae with two confused rows of hair-like setae ~1 ½ × as tall as the width of an interstria. Declivity gradually rounded, occupying ~1/2 of elytra, shagreened, subshiny; declivital face bisulcate between striae 1 and interstriae 4; striae with recumbent hair-like setae longer than those on disc; punctures smaller than those on disc; interstriae with semi-recumbent hair-like setae slightly longer than the width of an interstria; interstriae 2 and 3 impressed; interstriae 1–3 with small, uniformly spaced granules, each ~1/2 the width of an interstria, and bearing a long, erect seta; interstriae 7 with large denticles near the declivital base, gradually transitioning to large, round tubercles at apex. Posterolateral margin costate and bearing denticles and tubercles along interstriae 7. *Legs:* pro-, meso-, and metatibiae with seven socketed denticles each.

Male. Unknown.

Distribution. NEOTROPICAL: Ecuador (Orellana).

Biology. The holotype was collected by canopy fogging.

Etymology. L. *sulcata* = furrowed, referring to the sulcate declivity. An adjective.

Xenoxylebora syzygii (Nunberg, 1959) **comb. nov.**

(Figures 3.7.42–3.7.44)

Xyleborus montanus Schedl, 1957: 106. Preoccupied by Niisima 1910.

Xyleborus syzygii Nunberg, 1959: 167.

Xyleborus submontanus Schedl, 1960: 106. Unnecessary replacement name.

Material Examined. Holotype, female: Congo Belge [= DEMOCRATIC REPUBLIC OF THE CONGO], [South Kivu Prov.], Hembe-Bitale, 15.VIII.1952, Dr. [K.E.] Schedl. Paratypes, 3 females: as holotype. (Holotype and paratypes in RMCA).

Diagnosis. 2.00–2.05 mm long (mean = 2.01, n = 4); 2.86–2.93 × as long as wide. This species is distinguished by the declivity obliquely truncate, declivital face concave between suture and interstriae 4, and declivital interstriae 1–3 bearing two or three confused rows of round equally sized sub-contiguous granules.

Distribution. AFROTROPICAL: Democratic Republic of the Congo (South Kivu).

Biology. Xenoxylebora syzygii is reported to colonize branches ranging from 3–12 cm in diameter. Females construct a cave-tunnel gallery system comprised of a short unbranched entrance tunnel that leads to one irregularly shaped brood chamber in the longitudinal plane. Females deposit eggs in a single heap and brood size ranges from 27–38 individuals (Schedl, 1963). Host plants: Euphorbiaceae (*Alchornea*), Moraceae (*Ficus*), Myrtaceae (*Syzygium*).

Key to *Xenoxylebora* species (females only)

1 Tubercles on declivital interstriae 3 much more prominent than those of interstriae 1 or 2 (if present) (Figures 3.5.18, 3.6.24) … **2**

– Tubercles on declivital interstriae 3 of similar size as those of interstriae 1 and 2 or tubercles of interstriae 1 larger (Figures 3.5.9, 3.7.39) … **5**

2 Sutural tubercles on elytral apex distinctly elongated, laterally angled, and closely grouped to three large denticles on interstriae 1 (Figures 3.5.12–3.5.14) … ***caudata***

– Sutural tubercles on elytral apex large but not elongated or laterally angled, and widely separated from subapical tubercles (Figures 3.5.18, 3.6.24) …**3**

3 Declivital interstriae 1 and 2 unarmed, spines on interstriae 3 very prominent, significantly larger than those on interstriae 4–7 (Figures 3.5.18–3.5.20); Neotropical…***hystricosa* sp. nov.**

– Declivital interstriae 1 and/or 2 granulate, denticles on interstriae 3 moderate or similar size to those on interstriae 4–7 (Figures 3.6.24, 3.6.33); Afrotropical…**4**

4 Declivital interstriae 1 and 2 sparsely granulate, distance between granules equal to width of at least three granules; declivital slope very steep, occupying apical quarter of elytra (Figures 3.6.24–3.6.26) … ***perdiligens***

– Declivital interstriae 1 and 2 densely granulate, granules subcontiguous; declivital slope gradual, occupying apical third of elytra (Figures 3.6.33–3.6.35) …***sphenos***

5 Declivity obliquely truncate; declivital face concave between suture and interstriae 4; declivital interstriae 1–3 bearing two or three confused rows of round, sub-contiguous granules (Figs 3.7.42–3.7.44) … ***syzygii***

– Declivity rounded (Figure 3.5.9) or acuminate (Figure 3.6.21); declivital face weakly convex (Figure 3.6.22) to convex (Figure 3.5.7), interstriae flat or impressed; declivital interstriae with uniseriate granules or denticles …**6**

6 Declivity with one or more striae or interstriae clearly impressed (Figures 3.5.6, 3.7.39); Neotropical …**7**

– Declivity flat (Figure 3.7.37) to convex (Figure 3.5.15); Afrotropical…**11**

7 Elytral apex acutely acuminate in dorsal profile; tubercles on elytral apex subquadrate and very prominent (Figures 3.6.21–3.6.23) …***neosphenos***

– Elytral apex rounded in dorsal profile; sutural tubercles on elytral apex round and not significantly enlarged (Figures 3.5.9, 3.6.30) …**8**

8 Declivital striae 1 and interstriae 2 moderately (Figure 3.5.6) or deeply impressed, appearing bisulcate (Figure 3.7.39) …**9**

– Declivital striae 2 and interstriae 2 weakly impressed (Figures 3.5.9, 3.6.30) …**10**

9 Declivity sulcate between striae 1 and interstriae 4, appearing bisulcate; denticles of interstriae 1–3 subequal (Figures 3.7.39–3.7.41) …***sulcata* sp. nov.**

- Declivital interstriae 2 weakly impressed; denticles of interstriae 1 larger than those of interstriae 2 and 3 (Figures 3.5.6–3.5.8) …***addenda* sp. nov.**

10 Elytral disc shorter than length of elytral declivity; declivital face shagreened (Figures 3.6.30–3.6.32) …***serrata* sp. nov.**
- Elytral disc subequal to length of elytral declivity; declivital face shining (Figures 3.5.9–3.5.11) …***calculosa* sp. nov.**

11 Declivity flat, declivital slope steep (Figures 3.7.36–3.7.38) …***subcrenulata***
- Declivity convex, declivital slope gradual (Figure 3.5.16) …**12**

12 Declivital interstriae 1–3 bearing denticles of consistent size (Figures 3.5.15–3.5.17) …***collarti***
- Declivital interstriae 1 and 3 bearing small round granules, interstriae 2 with very minute granules, nearly indistinct (Figures 3.6.27–3.6.29) … ***pilosa***

DISCUSSION

With 315 known species (Smith, unpublished), *Xyleborus* is the most speciose xyleborine genus and researchers have long recognized the need for its taxonomic revision (Hubbard 1897, Wood 1986, Jordal et al. 2000, Cognato et al. 2011). Modern techniques using molecular data have provided better understanding of taxon limits within this polyphyletic genus (Hulcr and Cognato 2009, Cognato et al. 2020) and this study provides further revision through the designation of the new genus *Xenoxylebora* from African and South American *Xyleborus* species.

Xenoxylebora was recovered in all phylogenetic analyses and well-supported by the Bayesian, likelihood, and parsimony analyses (Figures 3.2 and 3.3). It is morphologically distinguished from other xyleborine genera by characters of the protibiae, antennal club, and scutellum. Its phylogenetic position within Xyleborini clearly places the genus outside *Xyleborus, senso stricto*, and the other Neotropical genera, i.e., *Coptoborus*, *Dryocoetoides* and *Taurodemus* (Figure 3.2). These results demonstrate that *Xenoxylebora* colonized the Neotropics independent of the main radiation of endemic Neotropical Xyleborini including *Coptoborus*, *Dryocoetoides* and *Sampsonius* (Cognato et al. 2011).

There are many examples of bark and ambrosia beetles dispersing among and between islands as well as the closest continent (Jordal and Hewitt 2004, Kirkendall and Jordal 2006, Jordal 2013, Cognato et al. 2018, Jordal 2021b). Dispersal across large oceanic distances is less frequent and bicontinental scolytine lineages spread between Africa and South America (Jordal 2012, 2015, Gohli et al. 2016, Jordal 2017, Eliassen and Jordal 2021) are especially notable because this distribution is uncommon in the subfamily (Figure 3.1) (Jordal 2012, Hulcr et al. 2015).

The most likely place of origin for *Xenoxylebora* is the Neotropics followed by a dispersal to Africa. This is contrary to the more common westward dispersal from Africa to the Americas in which the strong South Equatorial Current can transport terrestrial arthropods inside floating plant material (Fratantoni et al. 2000, Renner 2004a, Cognato 2013, Jordal 2015). The African *Xenoxylebora* clade renders the Neotropical *Xenoxylebora* species paraphyletic. This, and the biogeography analyses suggest that the ancestor of the African clade originated in either Africa or South America (Figure 3.2).

Interaction between storm winds and the North Equatorial Countercurrent is probably responsible for the eastward migration of several plant taxa, arthropods and scolytine clades from the Americas to Africa (Richardson et al. 1992, Fratantoni et al. 2000, Givnish et al. 2004, Renner 2004a, Won and Renner 2006, Murray and Heraty 2016, Jordal 2017). *Xenoxylebora* could have dispersed to Africa through this migration pathway. However, future phylogenetic analyses should include *Xenoxylebora* specimens from additional localities to better test the relationship between Neotropical and African *Xenoxylebora.* Also, a denser sampling of xyleborines would allow for further testing of the current biogeographic observations. Only then will the evolutionary and biogeographical histories of *Xenoxylebora* be more clearly understood.

Species and generic diversity of African and South American ambrosia beetles is under-described and would benefit from increased sampling and taxonomic study (Wood 2007, Smith et al. 2017, Cognato et al. 2020, Dole et al. 2021, Jordal 2021a, 2021b). Collection of more *Xenoxylebora* throughout its known geographic range for the extraction of additional molecular data would likely reveal additional species and help to better define proposed species limits within the genus. *Xenoxylebora sphenos* was observed to vary morphologically between collection locations and the phylogenetic analysis provides only moderate support of the monophyly of the species (Figure 3.2). However, this study had insufficient data to justify revision of the species.

The preponderance of xyleborine ambrosia beetles distributed between Africa and South America makes it clear that there has been an historically important exchange of scolytine fauna between the two continents that likely profoundly impacted the biodiversity of both regions (Figure 3.1) (Jordal 2012, Cognato 2013, Gohli et al. 2016, Jordal 2017, 2021a). Continued

investigation of these shared faunas is likely to provide a better understanding of the evolution and biogeographical history of Xyleborini and in general Scolytinae.

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APPENDIX

Voucher Name	Genus	species
Cop.neo1	*Xenoxylebora*	*addenda*
Xyleb_sp_111	*Xenoxylebora*	*calculosa*
SMS373	*Xenoxylebora*	*collarti*
Copto neo 102	*Xenoxylebora*	*neosphenos*
SMS368	*Xenoxylebora*	*sphenos*
AF187142.1	*Xenoxylebora*	*sphenos*
Ambobl	*Ambrosiodmus*	*obliquus*
Theo sp 89	*Ambrosiodmus*	sp. nov.
Ambtac	*Ambrosiodmus*	*tachygraphus*
Xylsay	*Anisandrus*	*sayi*
Xyomul	*Cnestus*	*mutilatus*
Theo.sp1	*Coptoborus*	*capillisoror*
Cop.sp2	*Coptoborus*	*chica*
The.coa1	*Coptoborus*	*coartatus*
Copto pseu 108	*Coptoborus*	*exilis*
Cop.tol1	*Coptoborus*	*furiosa*
SMS371	*Coptoborus*	*leeloo*
Cop.pse1	*Coptoborus*	*martinezae*
Cop.och1	*Coptoborus*	*ochromactonus*

Table 3.1: Specimens used for molecular phylogenetic reconstruction including voucher name, specific identification, collection location, publication source, and GenBank numbers.

Table 3.1 (cont'd)

Voucher Name	Genus	species
Cop.sp1	*Coptoborus*	*papillicauda*
Cop.sp3	*Coptoborus*	*pristis*
Cop.neo2	*Coptoborus*	*pristis*
Copto sp 105	*Coptoborus*	*pseudotenuis*
Copto bellus 94	*Coptoborus*	*sagitticauda*
SMS369	*Coptoborus*	*scully*
Cop.ves1	*Coptoborus*	*vespatorius* 1
Copto vesp 98	*Coptoborus*	*vespatorius* 98
Theo sp 110	*Coptoborus*	*villosulus*
Theo theo 101	*Coptoborus*	*villosulus* 101
Theo.sp2	*Coptoborus*	*villosulus* 2
Theo sp 87	*Coptoborus*	*villosulus* 87
Theo sp 88	*Coptoborus*	*villosulus* 88
Dryoc gran 109	*Dryocoetoides*	*granulicauda*
Dry.cap1	*Dryocoetoides*	nr. *capucinus* 1
Dryoc cap 107	*Dryocoetoides*	nr. *capucinus* 107
Dry.sp1	*Dryocoetoides*	*sp. 1*
Dryoc sp 97	*Dryocoetoides*	*sp. 97*
Xylpos	*Euwallacea*	*posticus*

Table 3.1 (cont'd)

Voucher Name	Genus	species
Xylsim	*Euwallacea*	*similis*
Euwsp02_273	*Euwallacea*	*wallacei*
Euwxan_283	*Euwallacea*	*semirudis*
Samdam 357	*Sampsonius*	*dampfi*
Sam ens 128	*Sampsonius*	*ensifer*
Tau god 1	*Taurodemus*	*godmani*
Tau_var_1	*Taurodemus*	*varians*
Xyipex	*Xyleborinus*	*exiguus*
Xyleb_grac_106	*Xyleborinus*	*gracilis*
Xyiint	*Xyleborinus*	*intersetosus*
Xyiqua	*Xyleborinus*	*quadrispinosus*
Xyleb_rec_96	*Xyleborinus*	*reconditus*
Xyisax	*Xyleborinus*	*saxsesenii*
Xyisig	*Xyleborinus*	*signatipennis*
Xylaff	*Xyleborus*	sp. F
Xylfer_352	*Xyleborus*	*bispinatus*
Xylgla	*Xyleborus*	*glabratus*
Xylperf	*Xyleborus*	*perforans*
Xylall	*Xyleborus*	*principalis*
Xylvol_356	*Xyleborus*	*volvulus*
Xylxyl_355	*Xyleborus*	*xylographus*

Table 3.1 (cont'd)

Voucher Name	Coll Location	Source
Cop.neo1	Suriname: Sipaliwini	This study
Xyleb_sp_111	Peru: Madre de Dios	This study
SMS373	Kenya: Kakamega District	This study
Copto neo 102	Ecuador: Napo Province, Parque Nacional Yasuní	This study
SMS368	Cameroon: Southwest Region, Limbe	This study
AF187142.1	Uganda: Kibale National Park	Normark et al. 1999
Ambobl	USA: SC	Cognato et al. 2011
Theo sp 89	Ecuador: Manabí Province	This study
Ambtac	USA: MD	Cognato et al. 2011
Xylsay	USA: MD	Cognato et al. 2011
Xyomul	USA: MS	Cognato et al. 2011
Theo.sp1	Brazil: Bahia, Serra Bonita Reserve	This study
Cop.sp2	Suriname: Sipaliwini	This study
The.coa1	Ecuador: Los Ríos Province, Samama Nature Reserve	This study
Copto pseu 108	Panama: Panamá, Parque Nacional Soberanía	This study
Cop.tol1	Ecuador: Los Ríos Province, Samama Nature Reserve	This study
SMS371	Ecuador: Napo Province, Parque Nacional Yasuní	This study
Cop.pse1	Ecuador: Los Ríos Province, Samama Nature Reserve	This study
Cop.och1	Ecuador: Cotopaxi Province, Yacusinchi Reserve	This study

Table 3.1 (cont'd)

Voucher Name	Coll Location	Source
Cop.sp1	Suriname: Sipaliwini	This study
Cop.sp3	Brazil: Bahia, Serra Bonita Reserve	This study
Cop.neo2	Ecuador: Cotopaxi Province, Yacusinchi Reserve	This study
Copto sp 105	Ecuador: Napo Province, Parque Nacional Yasuní	This study
Copto bellus 94	Guyana: Region 8, Iwokrama Forest	This study
SMS369	Ecuador: Napo Province, Parque Nacional Yasuní	This study
Cop.ves1	Ecuador: Los Ríos Province, Samama Nature Reserve	This study
Copto vesp 98	Guyana: Region 8, Iwokrama Forest	This study
Theo sp 110	Peru: Madre de Dios Dept., Los Amigos Biological Station	This study
Theo theo 101	Guyana: Region 8, Iwokrama Forest	This study
Theo.sp2	Brazil: Bahia, Serra Bonita Reserve	This study
Theo sp 87	Ecuador: Manabí	This study
Theo sp 88	Ecuador: Napo Province, Parque Nacional Yasuní	This study
Dryoc gran 109	Peru: Madre de Dios Dept., Los Amigos Biological Station	This study
Dry.cap1	Brazil: Bahia, Serra Bonita Reserve	This study
Dryoc cap 107	Panama: Panamá, Cerro Azul	This study
Dry.sp1	Ecuador: Cotopaxi Province, Yacusinchi Reserve	This study
Dryoc sp 97	Guyana: Region 8, Iwokrama Forest	This study
Xylpos	Costa Rica	Cognato et al. 2011

Table 3.1 (cont'd)

Voucher Name	Coll Location	Source
Xylsim	Papua New Guinea	Cognato et al. 2011
Euwsp02_273	Papua New Guinea	Cognato et al. 2011
Euwxan_283	Papua New Guinea	Cognato et al. 2011
Samdam 357	Ecuador	Cognato et al. 2011
Sam ens 128	Ecuador: Napo Province, Parque Nacional Yasuní, Tiputini	This study
Tau god 1	Panama: Chiriquí Province	This study
Tau_var_1	Peru: Madre de Dios	This study
Xyipex	Papua New Guinea	Cognato et al. 2011
Xyleb_grac_106	Panama: PAN 32	This study
Xyiint	Costa Rica	Cognato et al. 2011
Xyiqua	Madagascar	Cognato et al. 2011
Xyleb_rec_96	Guyana: Region 8, Iwokrama Forest	This study
Xyisax	USA: OH	Cognato et al. 2011
Xyisig	Madagascar	Cognato et al. 2011
Xylaff	Costa Rica	Cognato et al. 2011
Xylfer_352	Papua New Guinea	Cognato et al. 2011
Xylgla	USA: GA	Cognato et al. 2011
Xylperf	Papua New Guinea	Cognato et al. 2011
Xylall	Ghana	Cognato et al. 2011
Xylvol_356	Papua New Guinea	Cognato et al. 2011
Xylxyl_355	USA:MI	Cognato et al. 2011

Table 3.1 (cont'd)

Voucher Name	GenBank Numbers			
	COI	***CAD***	***EF1-α***	**28s**
Cop.neo1	TBD	TBD	TBD	TBD
Xyleb_sp_111	TBD	TBD	TBD	TBD
SMS373	TBD	TBD	TBD	TBD
Copto neo 102	TBD	TBD	TBD	TBD
SMS368	TBD	TBD	TBD	TBD
AF187142.1	AF187142	N/A	AH011661	N/A
Ambobl	HM064048	HM064227	HM064154	HM099667
Theo sp 89	TBD	TBD	TBD	TBD
Ambtac	HM064053	HM064232	HM064159	HM099671
Xylsay	GU808704	GU808626	GU808741	GU808589
Xyomul	GU808719	GU808641	GU808755	GU808603
Theo.sp1	TBD	TBD	TBD	TBD
Cop.sp2	TBD	TBD	TBD	TBD
The.coa1	TBD	TBD	TBD	TBD
Copto pseu 108	TBD	TBD	TBD	TBD
Cop.tol1	TBD	TBD	TBD	TBD
SMS371	TBD	TBD	TBD	TBD
Cop.pse1	TBD	TBD	TBD	TBD
Cop.och1	TBD	TBD	TBD	TBD

Table 3.1 (cont'd)

Voucher Name	GenBank Numbers			
	COI	***CAD***	***EF1-α***	**28s**
Cop.sp1	TBD	TBD	TBD	TBD
Cop.sp3	TBD	TBD	TBD	TBD
Cop.neo2	TBD	TBD	TBD	TBD
Copto sp 105	TBD	TBD	TBD	TBD
Copto bellus 94	TBD	TBD	TBD	TBD
SMS369	TBD	TBD	TBD	TBD
Cop.ves1	TBD	TBD	TBD	TBD
Copto vesp 98	TBD	TBD	TBD	TBD
Theo sp 110	TBD	TBD	TBD	TBD
Theo theo 101	TBD	TBD	TBD	TBD
Theo.sp2	TBD	TBD	TBD	TBD
Theo sp 87	TBD	TBD	TBD	TBD
Theo sp 88	TBD	TBD	TBD	TBD
Dryoc gran 109	TBD	TBD	TBD	TBD
Dry.cap1	TBD	TBD	TBD	TBD
Dryoc cap 107	TBD	TBD	TBD	TBD
Dry.sp1	TBD	TBD	TBD	TBD
Dryoc sp 97	TBD	TBD	TBD	TBD
Xylpos	HM064133	HM064312	N/A	HM099748

Table 3.1 (cont'd)

Voucher Name	GenBank Numbers			
	COI	***CAD***	***EF1-α***	**28s**
Xylsim	HM064139	HM064317	HM064217	HM099754
Euwsp02_273	N/A	HM064265	HM064183	HM099704
Euwxan_283	HM064086	HM064267	HM064185	HM099706
Samdam 357	HM064095	HM064276	HM064190	HM099713
Sam ens 128	TBD	TBD	TBD	TBD
Tau god 1	TBD	TBD	TBD	TBD
Tau_var_1	TBD	TBD	TBD	TBD
Xyipex	HM064109	N/A	N/A	HM099725
Xyleb_grac_106	TBD	TBD	TBD	TBD
Xyiint	HM064108	N/A	HM064200	N/A
Xyiqua	HM064111	HM064289	HM064201	HM099726
Xyleb_rec_96	TBD	TBD	TBD	TBD
Xyisax	HM064112	HM064290	HM064202	HM099727
Xyisig	HM064113	HM064291	N/A	HM099728
Xylaff	GU808696	GU808621	GU808735	GU808581
Xylfer_352	HM064126	HM064305	HM064211	HM099741
Xylgla	HM064127	HM064306	N/A	HM099742
Xylperf	HM064132	HM064311	N/A	HM099747
Xylall	HM064118	HM064296	N/A	HM099733
Xylvol_356	HM064149	HM064327	HM064222	HM099763
Xylxyl_355	HM064150	HM064328	N/A	HM099764

Gene	Primer	Primer sequence (5'-3')
COI	1495b	AACAAATCATAAAGATATTGGRAC
	rev750	GAAATTATNCCAATTCCTGG
CAD	ApCADfor4	TGGAARGARGTBGARTACGARGTGGTYCG
	ApCADrev1mod	GCCATYRCYTCBCCYACRCTYTTCAT
EF1-α	eflafor1	TACGTAACCATCATTGATGCTYCC
	eflarev1	CCTTCTTTACGTTCAATGGACCATCC
28s	D2F1	ACTGTTGGCGACGATGTTCT
	D3R2	TCTTCGCCCCTATACCC
	3665	AGACAGAGTTCAAGAGTACGTG
	4048	TTGCTCCGTGTTTCAAGACGGG

Table 3.2: Primers used for PCR reactions.

	Partition (Gene-Codon Position)	Phylogeny Model
PartitionFinder	*COI*-1st	F81+I+G
	COI-2nd	GTR+I+G
	COI-3rd	GTR+I+G
	CAD-1st	K80+G
	CAD-2nd	HKY+I
	CAD-3rd	K80+I+G

Table 3.3: Models of nucleotide substitution assigned by PartitionFinder 2.1.1 and ModelFinder, respectively, and used for phylogeny reconstruction by likelihood and Bayesian analyses. JC = Jukes Cantor model of evolution with equal base frequencies and equal substitution rates (Jukes and Cantor 1969); F81 = Felsenstein model with unequal base frequencies and equal rates of substitution (Felsenstein 1981); K80 = Kimura model with equal base frequencies and unequal rates of transition and transversion substitutions (Kimura 1980); HKY = Hasegawa model with unequal base frequencies and unequal rates of transition and transversion substitutions (Hasegawa et al. 1985); TN = Tamura Nei model with unequal rates of purine and pyrimidine rates and unequal rates of transition and transversion substitutions (Tamura and Nei 1993); TIM2e =Transition model with equal base frequencies and AC and CG substitution rates equal to AT and GT, respectively; TIM3e = Transition model with equal base frequencies and AC and AT substitution rates equal to CG and GT, respectively; GTR = general time reversible model with unequal base frequencies and unequal rates of substitution (Lanave et al. 1984, Rodríguez et al. 1990); I = variable nucleotide frequencies; F = nucleotide frequencies determined from the data; FQ = nucleotides with equal frequencies; G = gamma distributed rates of variation; R = FreeRate model of distributed rates of variation (Yang 1995, Soubrier et al. 2012).

Table 3.3 (cont'd)

	Partition (Gene-Codon Position)	**Phylogeny Model**
PartitionFinder	*EF1-α*-1st	JC+I
	EF1-α-2nd	HKY+I+G
	EF1-α-3rd	GTR+I+G
	28S	GTR+G
ModelFinder	*COI*-1st, *CAD*-2nd, EF1-*α*-1st	HKY+F+I+G
	COI-2nd	TN+F+R3
	*COI*3rd	TIM3e +FQ+G
	CAD-1st, *EF1-α*-3rd	TIM3e+FQ_I+G
	CAD -3rd, EF1-*α*-2nd	TIM2e+FQ+I+G
	28s	GTR+F+I+G

Taxon Label	Genus	species
Coptoborus_n_sp_Surinam	*Xenoxylebora*	*addenda*
Xyleb_sp_111	*Xenoxylebora*	*calculosa*
Xyleborus_collarti_Kenya	*Xenoxylebora*	*collarti*
Coptoborus_neospenos_Tiputini	*Xenoxylebora*	*neosphenos*
Xyleborus_sphenos	*Xenoxylebora*	*sphenos*
Xyleborus_sphenos_Camaroon	*Xenoxylebora*	*sphenos*
Ambrosiodmus_obliquus	*Ambrosiodmus*	*obiquus*
Ambrosiodmus_sp_Ecuador_Lalo_Loor	*Ambrosiodmus*	*sp. nov.*
Ambrosiodmus_tachygraphus	*Ambrosiodmus*	*tachygraphus*
Anisandrus_sayi	*Anisandrus*	*sayi*
Cnestus_mutilatus	*Cnestus*	*mutilatus*
Theoborus_capillisoror_Brazil_Bahia	*Coptoborus*	*capillisoror*
Coptoborus_chica_Surinam	*Coptoborus*	*chica*
Theoborus_coartatus_Ecuador_Samama	*Coptoborus*	*coartatus*
Coptoborus_exilis_Panama	*Coptoborus*	*exilis*
Coptoborus_furiosa_Ecuador_Samama	*Coptoborus*	*furiosa*
Coptoborus_leeloo	*Coptoborus*	*leeloo*
Coptoborus_martinezae_Ecuador_Samama	*Coptoborus*	*martinezae*
Coptoborus_ochromactonus_Ecuador_Yacusinchi	*Coptoborus*	*ochromactonus*
Coptoborus_papillicauda_Surinam	*Coptoborus*	*papillicauda*

Table 3.4: Biogeographical states used for biogeography analyses.

Table 3.4 (cont'd)

Taxon Label	Genus	species
Coptoborus_pristis_Ecuador_Yacusinchi	*Coptoborus*	*pristis*
Coptoborus_pristis_Brazil_Bahia	*Coptoborus*	*pristis*
Coptoborus_pseudotenuis_Ecuador	*Coptoborus*	*pseudotenuis*
Coptoborus_sagitticauda_Guyana	*Coptoborus*	*sagitticauda*
Coptoborus_scully	*Coptoborus*	*scully*
Coptoborus_vespatorius_Ecuador_Samama	*Coptoborus*	*vespatorius* 1
Coptoborus_vespatorius_Guyana	*Coptoborus*	*vespatorius* 98
Theoborus_villosulus_Peru	*Coptoborus*	*villosulus*
Theoborus_villosulus_Guyana	*Coptoborus*	*villosulus* 101
Theoborus_villosulus_Brazil_Bahia	*Coptoborus*	*villosulus* 2
Theoborus_villosulus_Ecuador	*Coptoborus*	*villosulus* 87
Theoborus_villosulus_Tiputini	*Coptoborus*	*villosulus* 88
Dryocoetoides_grandulicauda_Peru	*Dryocoetoides*	*granulicauda*
Dryocoetoides_nr_capicinus_2_Brazil_Bahia	*Dryocoetoides*	nr. *capusinus* 1
Dryocoetoides_nr_capicinus_1_Panama	*Dryocoetoides*	nr. *capusinus* 97
Dryocoetoides_sp_1Ecuador_Yacusinchi	*Dryocoetoides*	sp. 1
Dryocoetoides_sp_2_Guyana	*Dryocoetoides*	sp. 97
Xylpos	*Euwallacea*	*posticus*
Xylsim	*Euwallacea*	*similis*
Euwsp02_273	*Euwallacea*	*wallacei*

Table 3.4 (cont'd)

Taxon Label	**Genus**	**species**
Euwxan_283	*Euwallacea*	*semirudis*
Samdam_357	*Sampsonius*	*dampfi*
Sam_ens_128	*Sampsonius*	*ensifer*
Taurodemus_godmani	*Taurodemus*	*godmani*
Tau_var_1	*Taurodemus*	*varians*
Xyipex	*Xyleborinus*	*exiguus*
Xyleb_grac_106	*Xyleborinus*	*gracilis*
Xyiint	*Xyleborinus*	*intersetosus*
Xyiqua	*Xyleborinus*	*quadrispinosus*
Xyleb_rec_96	*Xyleborinus*	*reconditus*
Xyisax	*Xyleborinus*	*saxesenii*
Xyisig	*Xyleborinus*	*signatipennis*
Xylfer_352	*Xyleborus*	*bispinatus*
Xyleborus_glabratus	*Xyleborus*	*glabratus*
Xylperf	*Xyleborus*	*perforans*
Xylall	*Xyleborus*	*principalis*
Xyleborus_affinis	*Xyleborus*	sp. F
Xylvol_356	*Xyleborus*	*vovulus*
Xylxyl_355	*Xyleborus*	*xylographus*

Table 3.4 (cont'd)

Taxon Label	Region of Origin
Coptoborus_n_sp_Surinam	Neotropical
Xyleb_sp_111	Neotropical
Xyleborus_collarti_Kenya	Afrotropical
Coptoborus_neospenos_Tiputini	Neotropical
Xyleborus_sphenos	Afrotropical
Xyleborus_sphenos_Camaroon	Afrotropical
Ambrosiodmus_obliquus	Nearctic
Ambrosiodmus_sp_Ecuador_Lalo_Loor	Neotropical
Ambrosiodmus_tachygraphus	Nearctic
Anisandrus_sayi	Australasian & Oceanian
Cnestus_mutilatus	Australasian & Oceanian
Theoborus_capillisoror_Brazil_Bahia	Neotropical
Coptoborus_chica_Surinam	Neotropical
Theoborus_coartatus_Ecuador_Samama	Neotropical
Coptoborus_exilis_Panama	Neotropical
Coptoborus_furiosa_Ecuador_Samama	Neotropical
Coptoborus_leeloo	Neotropical
Coptoborus_martinezae_Ecuador_Samama	Neotropical
Coptoborus_ochromactonus_Ecuador_Yacusinchi	Neotropical
Coptoborus_papillicauda_Surinam	Neotropical

Table 3.4 (cont'd)

Taxon Label	Region of Origin
Coptoborus_pristis_Ecuador_Yacusinchi	Neotropical
Coptoborus_pristis_Brazil_Bahia	Neotropical
Coptoborus_pseudotenuis_Ecuador	Neotropical
Coptoborus_sagitticauda_Guyana	Neotropical
Coptoborus_scully	Neotropical
Coptoborus_vespatorius_Ecuador_Samama	Neotropical
Coptoborus_vespatorius_Guyana	Neotropical
Theoborus_villosulus_Peru	Neotropical
Theoborus_villosulus_Guyana	Neotropical
Theoborus_villosulus_Brazil_Bahia	Neotropical
Theoborus_villosulus_Ecuador	Neotropical
Theoborus_villosulus_Tiputini	Neotropical
Dryocoetoides_grandulicauda_Peru	Neotropical
Dryocoetoides_nr_capicinus_2_Brazil_Bahia	Neotropical
Dryocoetoides_nr_capicinus_1_Panama	Neotropical
Dryocoetoides_sp_1Ecuador_Yacusinchi	Neotropical
Dryocoetoides_sp_2_Guyana	Neotropical
Xylpos	Nearctic
Xylsim	Australasian & Oceanian
Euwsp02_273	Australasian & Oceanian

Table 3.4 (cont'd)

Taxon Label	Region of Origin
Euwxan_283	Australasian & Oceanian
Samdam_357	Neotropical
Sam_ens_128	Neotropical
Taurodemus_godmani	Neotropical
Tau_var_1	Neotropical
Xyipex	Australasian & Oceanian
Xyleb_grac_106	Neotropical
Xyiint	Neotropical
Xyiqua	Afrotropical
Xyleb_rec_96	Neotropical
Xyisax	Worldwide
Xyisig	Afrotropical
Xylfer_352	Worldwide
Xyleborus_glabratus	Neotropical
Xylperf	Worldwide
Xylall	Afrotropical
Xyleborus_affinis	Worldwide
Xylvol_356	Worldwide
Xylxyl_355	Nearctic

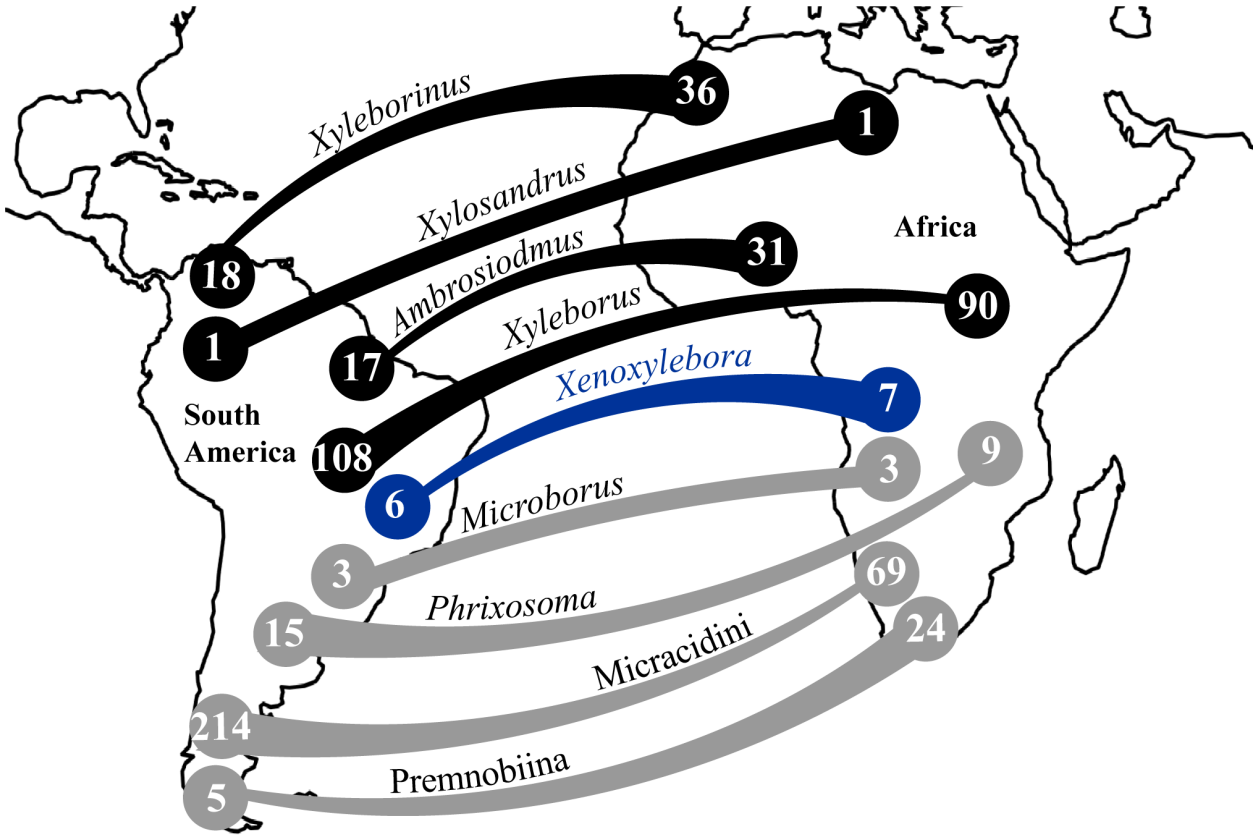

Figure 3.1: Map of Scolytinae with native distributions across Africa and South America (Wood and Bright 1992, Jordal 2012, Hulcr et al. 2015, Gohli et al. 2016, Bright 2010, 2019, Atkinson 2021, Eliassen and Jordal 2021, Jordal 2021b, 2021c, 2021d, 2021e, 2021f). Each circled number represents the number of species from the corresponding group endemic or established in the indicated continent. The expanded side of each line indicates the continent containing a plurality of species. Two *Premnobius* species have cosmopolitan distributions and are therefore each counted once on each continent: *P. cavipennis* and *P. ambitiosus*. *Xenoxylebora* is depicted in blue; black indicates xyleborine genera, and grey indicated non-xyleborine groups.

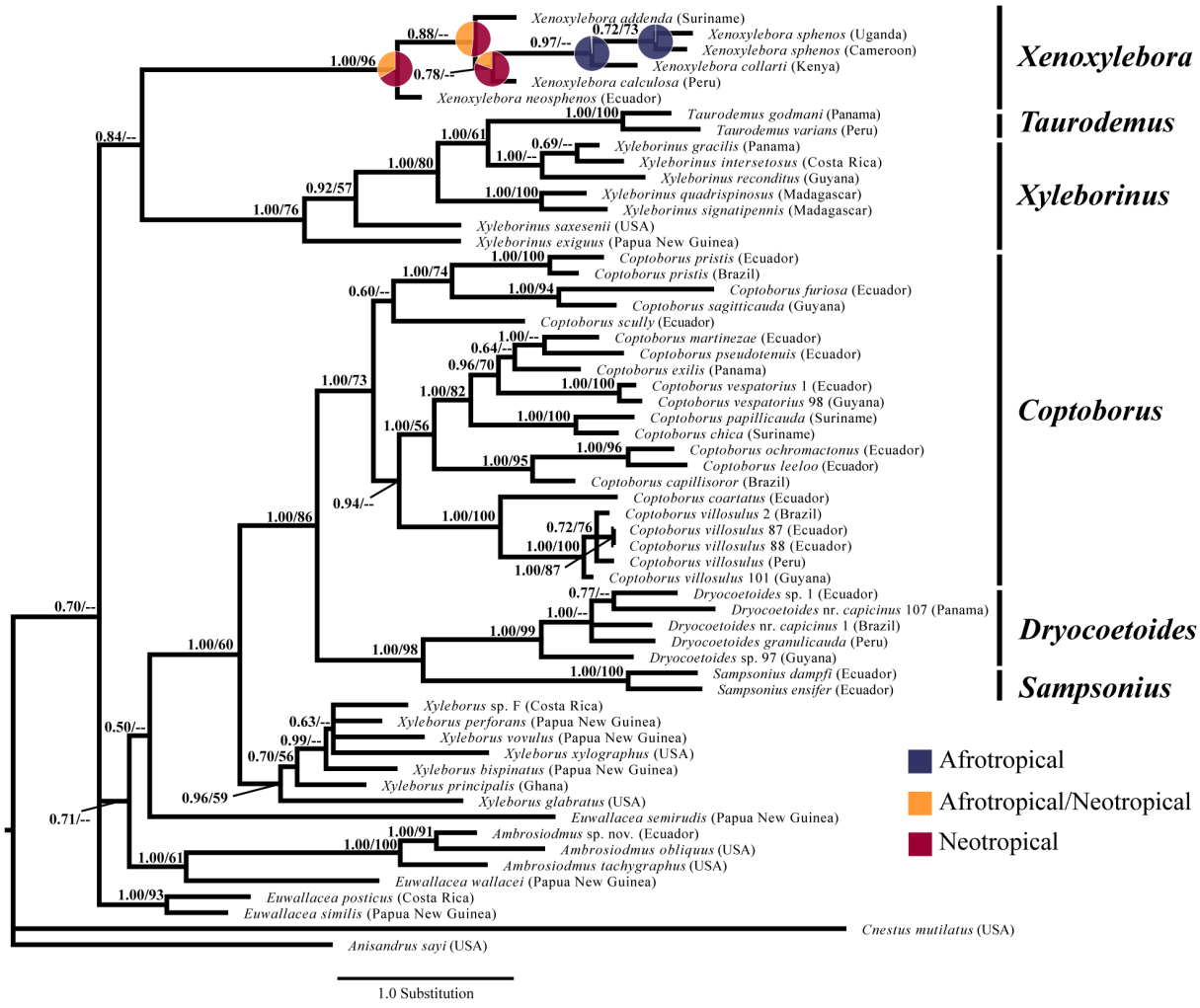

Figure 3.2: Phylogenetic tree resulting from a Bayesian analysis of *CO1*, *CAD*, *EF1-α* and 28S. Nodes are labeled with posterior probability/bootstrap support. Posterior probabilities > 0.95 are considered strong clade support. Nodes within *Xenoxylebora* are additionally labeled with pie diagrams indicating the relative probabilities of origin in the Neotropics, Afrotropics, and Neotropics/Afrotropics. An outgroup *Anisandrus sayi* was used to root the tree.

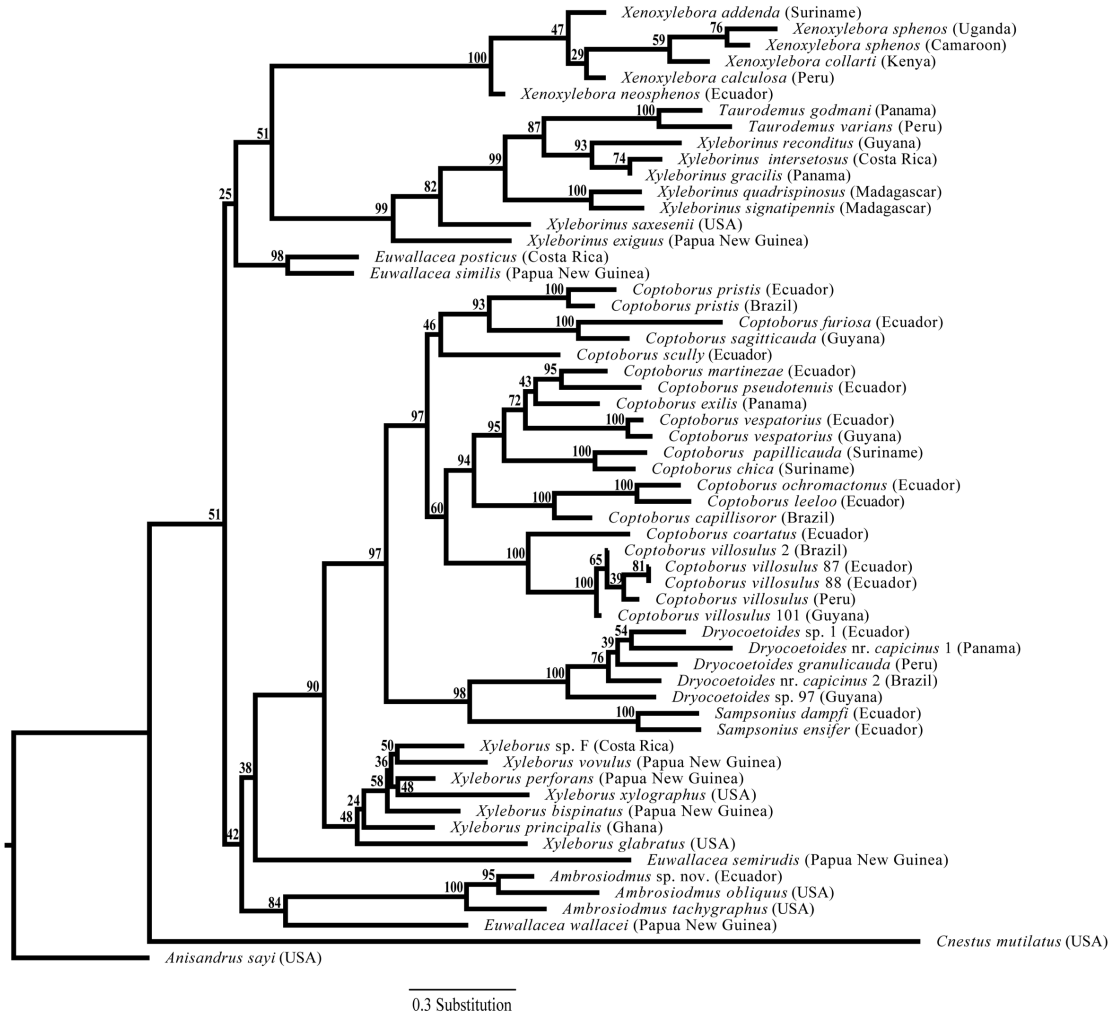

Figure 3.3: Maximum likelihood tree resulting from Nearest Neighbor Interchange search of *CO1*, *CAD*, *EF1-α* and 28S sequences using IQ-TREE version 2.1.3. Nodes are labeled with bootstrap support from 1000 pseudoreplications. *Anisandrus sayi* was used to root the tree.

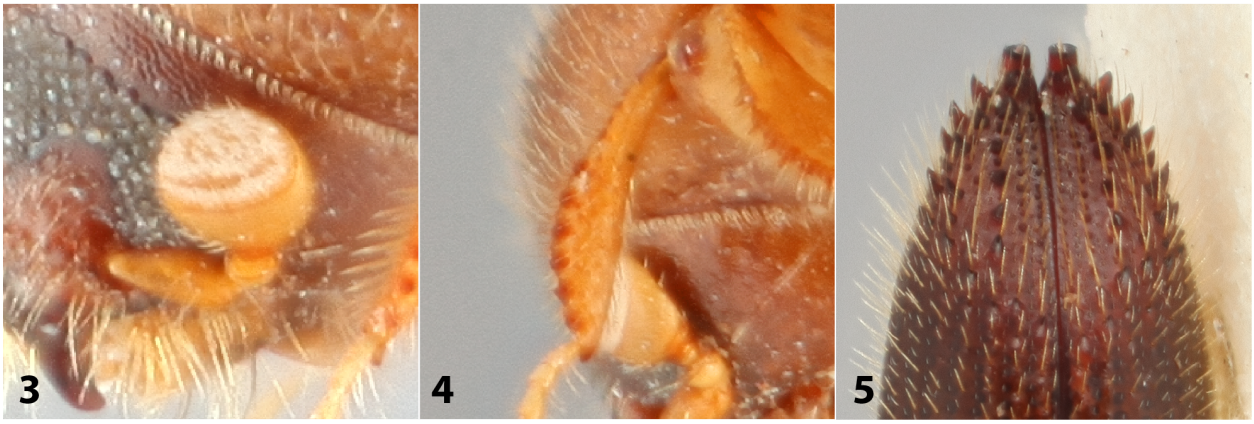

Figure 3.4: Diagnostic characters for *Xenoxylebora.* (3.4.3) type 1 antennal club. (3.4.4) slender protibia, outer edge weakly rounded, posterior face flat and unarmed. (3.4.5) sutural tubercles on the declivital apex.

Figure 3.5: Dorsal, lateral, and declivital aspects of *Xenoxylebora addenda* holotype (3.5.6–3.5.8); *Xenoxylebora calculosa* holotype (3.5.9–3.5.11); *Xenoxylebora caudata* paratype (3.5.12–3.5.14); *Xenoxylebora collarti* (3.5.15–3.5.17); *Xenoxylebora hystricosa* holotype (3.5.18–3.5.20).

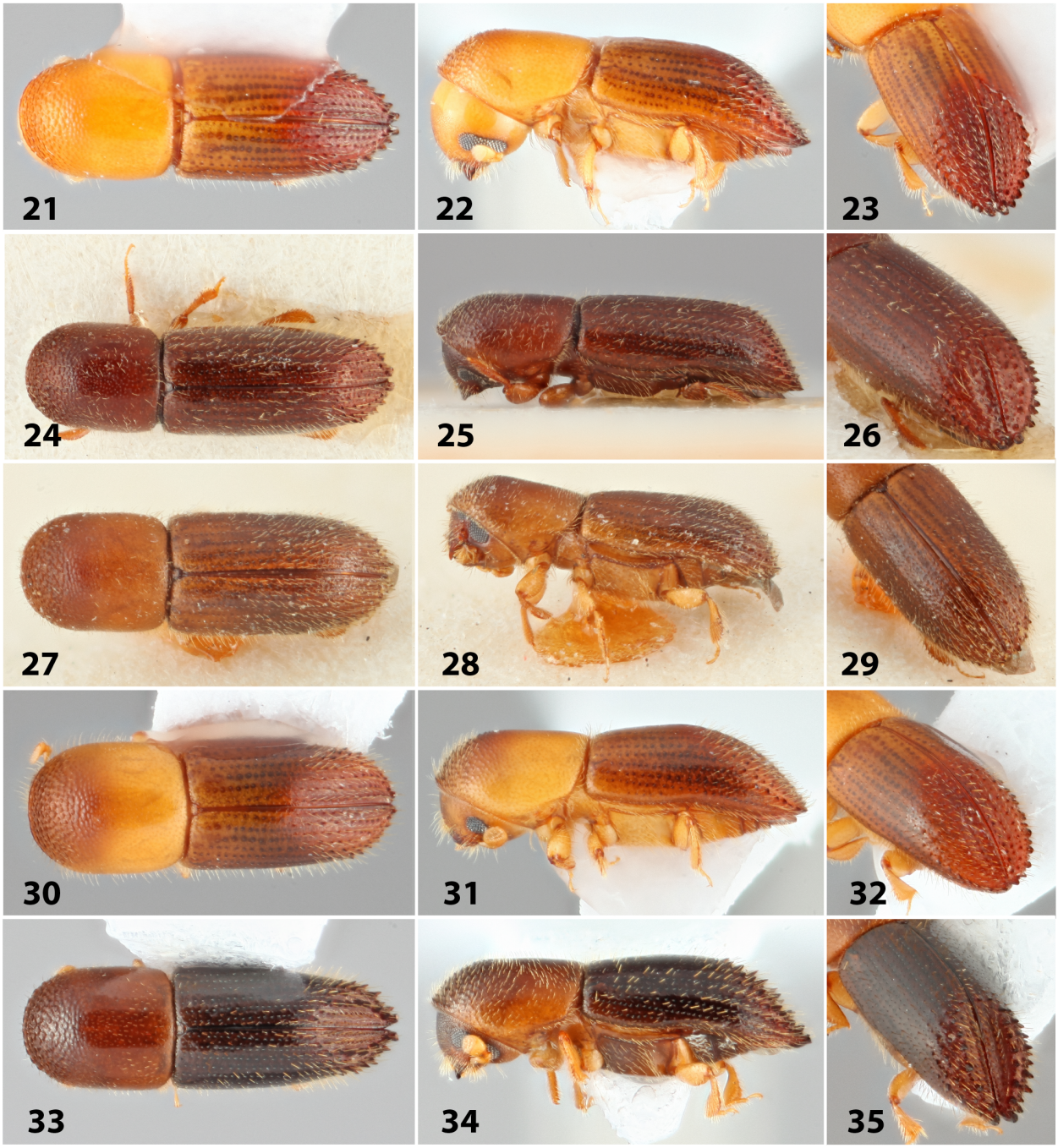

Figure 3.6: Dorsal, lateral, and declivital aspects of *Xenoxylebora neosphenos* (3.6.21–3.6.23); *Xenoxylebora perdiligens* (3.6.24–3.6.26); *Xenoxylebora pilosa* holotype (3.6.27–3.6.29); *Xenoxylebora serrata* holotype (3.6.30–3.6.32); *Xenoxylebora sphenos* (3.6.33–3.6.35).

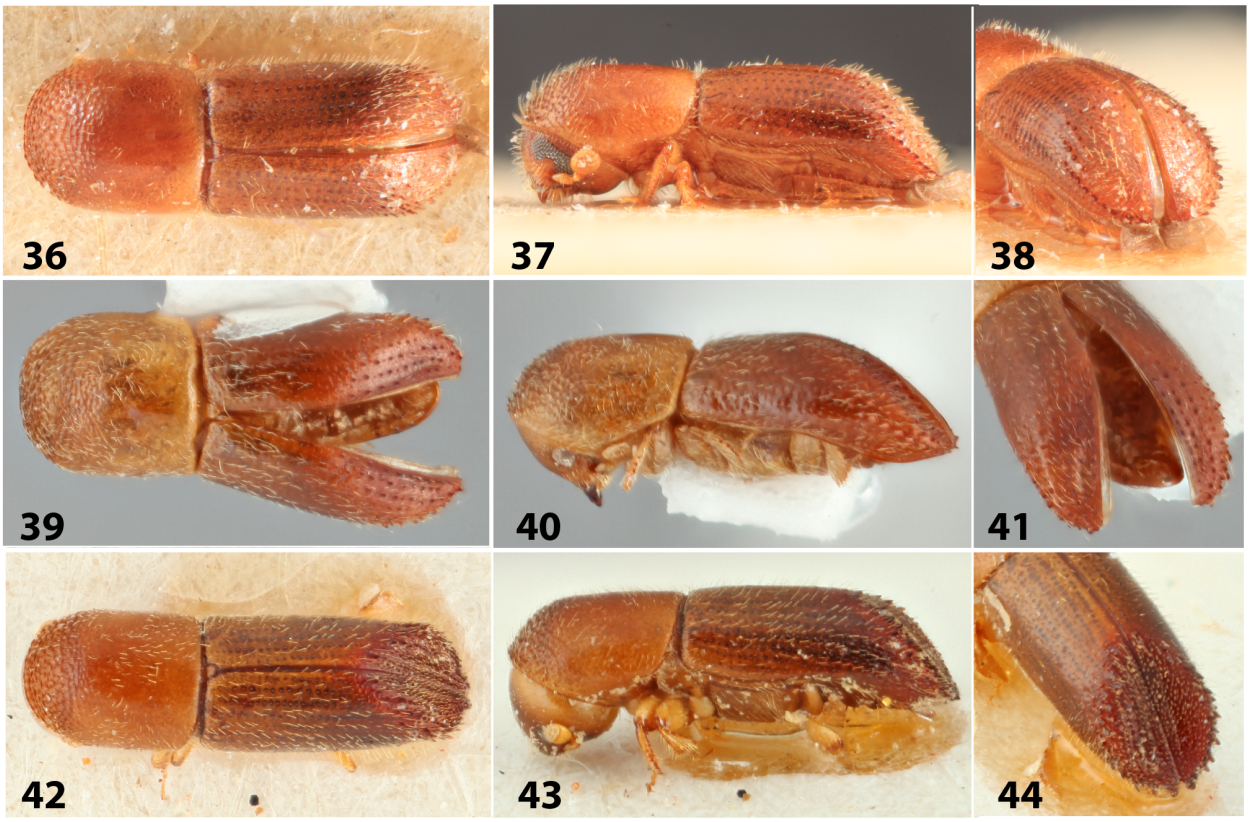

Figure 3.7: Dorsal, lateral, and declivital aspects of *Xenoxylebora subcrenulata* holotype (3.7.36–3.7.38); *Xenoxylebora sulcata* holotype (3.7.39–3.7.41); *Xenoxylebora syzygii* paratype (3.7.42–3.7.44).

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