BIOGEOGRAPHY AND PHYLOGENETICS OF GRASSLAND AUCHENORRHYNCHA

BY

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DISSERTATION

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ABSTRACT

Grasslands cover a significant portion of the Earth's terrestrial surface, yet we know little about the historical biogeography of grassland-restricted lineages. Previous work on the biogeography of grassland taxa has largely focused on large mammals. While these studies have generated some general patterns for the origin and dispersal of grassland animals, these patterns may not be applicable to less studied groups of organisms due to differences in natural history traits, such as dispersal mechanisms. To address this limitation, I examined the historical biogeography of three tribes of Deltocephalinae leafhoppers and a subfamily of planthoppers (Caliscelinae) and compared these data to biogeographic patterns observed in other grassland restricted lineages. In order to correctly infer biogeographic patterns, accurate phylogenies of each lineage are required. Using molecular sequence data from multiple genes, I inferred phylogenies for each Auchenorrhyncha lineage with a thorough sampling of each lineage including representatives from all major grasslands of the world. Along with individual phylogenies of each lineage, a combined dataset of Hecalini, Paralimnini, Deltocephalini, and the taxa included by Zahniser and Dietrich (2013) was also constructed.

My phylogenetic analyses were used to both infer biogeographic patterns and to estimate divergence times. Grass feeding in Deltocephalini was inferred to be Palearctic in origin, and Old World origins were inferred for each target lineage. Hecalini and Paralimnini were inferred to be Palearctic in origin, while Deltocephalini + Tetartostylini was more widespread and Caliscelinae was inferred to be Ethiopian. All three leafhopper lineages were estimated to have diverged from their sister tribes around 50 MYA while Caliscelinae was inferred to be significantly older at 71MYA. These lineages therefore predate the formation of large contiguous grasslands, but as grasses were present for tens of millions of years prior to climatic conditions favoring grassland formation, these dates are reasonable. Each group was inferred to have invaded the New World, and in many lineages this invasion occurred approximately 40 MYA (with New World Caliscelinae again predating the Deltocephalinae). All lineages underwent periods of radiation corresponding to the rise of grasslands and the diversification of C_4 grasses. In general these insect lineages show different biogeographic patterns from other lineages for which grassland biogeography has been reconstructed. Both horses and camels were inferred to have originated in the New World and then spread to the Old World, while bovids showed a similar Old World to New World spread. These patterns are similar to the Chiasmini, a related tribe of grassland Deltocephalinae, which based on an intuitive interpretation of the phylogeny, was thought to have originated in the Old World and then spread to the New World. Future work should examine additional grassland insect lineages to determine if these patterns are limited to Auchenorrhyncha or are found in a wider variety of grassland insects, such as some beetles and moths. Additional taxon sampling could also allow biogeographic reconstruction to be performed on more regional scales where different factors play a role in determining species ranges.

To my family, friends, and jellyfish

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CHAPTER ONE: INTRODUCTION

Large contiguous grasslands cover significant portions of many continents while smaller patches occur where local characteristics of soil, topography, or disturbance fail to support forest communities. Grasslands are one of the largest biomes with land cover estimates ranging from 24-40% of Earth's terrestrial surface (up to 52.5 million square kilometers worldwide) (World Resources Institute 2000; Shantz 1954). Estimates of grassland cover vary greatly because there are many ways to delineate grassland ecosystems. Sharp definitions are impossible because small grasslands often exist in forests and deserts and in many areas changes in disturbance regimens quickly lead to woody plant encroachment. The World Resources Institute (2000) defines grassland as any landscape which is dominated by herbaceous and shrubby vegetation and maintained by fire, grazing, or climatic conditions like periodic drought or freezing. This definition is broad enough that it includes some areas of woodland and tundra. Others, such as Scholes and Hall (1996), define grasslands as areas with less than 10% tree cover. Shantz (1954) identifies two types of grasslands, those formed by climatic conditions on pedocal soils (soils high in calcium carbonate and low in organic matter commonly associated with arid or semiarid areas), unleached, and with dry subsoils, or those which replaced forest after cutting or fire, then maintained as grassland by fire. Lastly, there are use-based definitions like those of the NRC (1994), McNaughton (1993), and Graetz (1994), which identify regions supporting grazing systems as grasslands.

True grasslands typically have highly fertile soils and moderate climates. While these characters lead to a highly productive and diverse ecosystem, they also make grasslands attractive for human use. Grasslands played a pivotal role in both human evolution and the rise of civilization, as soil fertility in these areas was high enough to support a sedentary culture, allowing a transition from hunter-gather nomads to farming. Grasses themselves were domesticated, leading to greater and more predictable harvests, and thus greatly increased human populations (Olsson 2001). Globally, grasslands are under threat due to agricultural practices and urbanization although the scale of this threat varies regionally. For example, in North America, only 9% of tallgrass prairie remains, while grasslands in Africa and Australia are more (at least 50%) intact (World Resource Institute 2000). Interestingly, grassland extent is on the rise in some areas, such as Australia, due to forest clearing, although conversion of historic grassland to woodlands is also occurring (State of the Environment Advisory Council, 1996). Not only has the overall size of grasslands been reduced, but those that remain tend to be on marginal lands. Buringh and Dudal (1987) estimated that only one-sixth of remaining grasslands are located on high to medium quality land.

The overall decline in size and quality of grasslands has adversely affected grassland obligate species throughout the world. North American grassland birds have been in decline for decades although the full scale of this decline has only recently become apparent (Brennan and Kuvlesky 2005). The authors point towards a number of causes ranging from habitat fragmentation and conversion to agricultural land, to degradation in rangeland through invasive species and changes in fire regimens. Many grassland inhabiting species are on the IUCN red list as endangered or critically endangered. These include 8 arthropods, 32 mollusks, 145 amphibians, 50 birds, 108 mammals, and 28 reptiles. In addition, climate change will result in changes to climatological patterns including the seasonality of rainfall which could negatively impact existing grasslands, lead to shifts in C_3 vs. C_4 plant dominance, and help invasive grass species establish in existing grasslands (Kang et al. 2007; Fay et al. 2008; Pau et al. 2013). Because many organisms found in grasslands are closely tied to specific grassland microclimates and host plants, the survival of these species is threatened by grassland degradation and loss.

My dissertation infers global biogeographic patterns for three lineages: the planthopper subfamily Caliscelinae and two leafhopper groups: Hecalini and Deltocephalini + Paralimnini to better understand how grass specialist lineages diversified in grasslands. In order to explicitly infer biogeographic patterns, accurate phylogenies of each lineage are required. Chapter 2 provides a historical overview of the grasslands of the world and evidence supporting historical grassland delineation. Chapters 3, 4, and 5 are phylogenetic analyses of three grassland lineages: Caliscelinae (Hemiptera: Caliscelidae), Hecalini (Hemiptera: Cicadellidae: Deltocephalinae), and a clade of Deltocephalinae with linear connectives (Hemiptera: Cicadellidae: Deltocephalinae: Deltocephalini, Paralimnini, and Tetartostylini). Chapter 6 is a biogeographic analysis of each lineage and includes divergence time estimation to test biogeographic reconstructions.

CHAPTER TWO: A HISTORY OF GLOBAL GRASSLANDS

Inferring ancient patterns in the origin and spread of native grasslands

The timing of grassland formation and grass diversification varied regionally. Because grasses are rarely fossilized (but see below), the exact timing and patterns of diversification in grasses, along with the rise and spread of grasslands, remain uncertain. Most studies have placed the origin of grasses (Poaceae) in northern Gondwana during the Late Cretaceous between 70 and 55 MYA based on pollen samples (Jacobs et al. 1999). Recently however, the inclusion of new fossil evidence and more refined molecular clocks has suggested grasses originated much earlier, with estimates as old as 129 MYA being inferred, although a Gondwanan origin is still supported (Prasad et al. 2011; Bouchenak-Khelladi et al. 2010). These later dates result from the inclusion of a number of rice (Oryzeae) fossils that are used as calibration points. While these fossils are placed within the Oryzeae clade based on morphological features, their exact placement is unknown. Dating analyses using these fossils under different placement scenarios infer the origin of Poaceae between 107 and 129 MYA (Prasad et al. 2011). Regardless of the timing of grass origination, grasslands themselves did not begin to establish as a dominant biome until the forests began opening between the Paleocene and the Eocene (Kellogg 2001; Strömberg 2011). By the middle Eocene, grasses were found on all continents except Antarctica (Jacobs et al. 1999), and grass diversity increased steadily through the middle Tertiary. These primitive grasses were all C_3 grasses, which thrive under high atmospheric carbon dioxide levels and groundwater along with moderate temperatures and sunlight, compared to C_4 plants, which can cope with lower levels of carbon dioxide, higher temperatures, and survive in areas that undergo periodic droughts. Although the earliest probable origin of C_4 grasses had been placed between 30 and 32 MYA, recent fossils suggest this origin was much earlier, possibly the middle Eocene, which was a time marked by warm climates and high CO_2 levels (Vicentini et al. 2008). Grasses utilizing the latter photosynthetic pathway did not become dominant components of grasslands until significantly later, suggesting that the drivers for their expansion are more complex than previously suspected. Edwards and Smith (2010) constructed a phylogeny to examine origins of C_4 grasses and found that C_4 grasses originated as grass moved out of closed-canopy forests and into open-canopy areas, as opposed to originating when grasses spread into temperate regions. This is similar to other plant lineages, which developed C_4 photosynthesis to overcome reduced water availability. This shift occurred in areas where precipitation decreased to below 1500mm a year, which is considered the threshold between closed and open canopy systems, and allowed grass dominated ecosystems to spread into previously uninhabitable regions and also resulted in changes in grass communities in areas previously dominated by C_3 vegetation.

While C_4 photosynthetic pathways evolved independently in at least 62 lineages across flowering plants, grasses represent about 60% of plant species (representing at least 22 C_4 lineages plus 2 C_3-C_4 intermediate lineages) utilizing this pathway (Edwards and Smith 2010; Grass Phylogeny Working Group 2012; Sage et al. 2011). Because C_4 pathways have evolved many times, lumping all C4 plants into a single pathway is probably an oversimplification and could hide traits which are important to understand the rise of these grasses and their incorporation into existing C_3 grasslands. One potential confounding effect stems from the fact that studies comparing C_3 and C_4 grasses typically focused on distantly related taxa (in many cases species which last shared a common ancestor over 50 MYA). Common garden experiments involving *Alloteropsis semialata* (which includes both C_3 and C_4 subspecies) suggest that C_3 and C_4 plants behave as expected under most conditions (i.e. higher summer temperatures favoring C_4 plants), however,C_4 plants were better equipped to survive simulated droughts but when subjected to natural droughts the C_3 varieties performed better (Ibrahim et al. 2008). Pau et al. (2013) examined traits associated with C_4 grasses in a phylogenetic framework and found that while the widely accepted views that C_4 grasses are more dominant in areas with higher temperatures, lower precipitation, and lower tree cover were supported, the effects of each of these factors was different compared to taking a nonphylogenetic approach, in which all C_4 grasses were lumped together and compared to all C_3 grasses. Conversely, Edwards and Smith (2010) found C_4 and C_3 grasses had similar temperature profiles except 2 lineages of C_3 grasses, which grew in much cooler environments than expected. Lastly, the various C_4 pathways have centers of origin in different biogeographic regions, which could help explain major differences in the timing and patterns of spread of C_4 dominated grasslands (Sage et al. 2011).

Inference of historical grasslands from faunal components

Although grasslands are a relatively recent land cover type, their predominance has led to colonization and specialization by animals. Because animals are better represented in the fossil record than grasses, the occurrence of various groups, particularly Perissodactyls (odd toed ungulates such as horses and zebras) and Artiodactyls (even toed ungulates such as deer and antelope), have been used to infer a region as historically grass dominated. Equids are among the best-studied groups as they are well represented in the fossil record and exhibit a number of morphological features indicative of being exclusively grazers. In areas without ungulates, other mammal groups (for example marsupials in the case of Australia) filled the grazing niche, and showed similar adaptations. Some rodent clades have also been used to identify grasslands, as have notoungulates - a now extinct group of ungulates that was a dominant group in South America. Grazing ungulates and grassland specialized lineages of other mammalian groups have adapted to grassland life through changes in dental, skull, and skeletal morphology. These characteristics include possession of hypsodont (high crowned) dentition; square straight premaxilla, broad muzzles, large masserteric chewing musculature, and elongated legs for running in open areas. Although possession of this suite characters strongly suggests reliance on grasslands there are some non-grassland mammals with one or more of these characteristics (Janis et al. 2002). Hypsodont teeth are one of the most common characters to identify animals as grass feeders because the extra enamel and tooth height allows the teeth to resist wear caused be consuming fibrous grasses and associated grit. Additionally, hypsodont teeth are found in Glires (rodents and lagomorphs which also possess hypselodont teeth, which grow continuously through life) and other mammal groups feeding on vegetation. These tooth types were first recorded in the late Eocene in North America and then gradually became more common in the fossil record. However, recent evidence from the North American fossil record shows high-crowned teeth became common before the formation of large grasslands in North America, so other factors, such as an increase in grit intake, contributed to the rise this character (Jardine et al. 2012). In addition to these morphological characters, the teeth themselves can be examined for microwear. Feeding on different types of plants results in different microwear patterns allowing what an animal was feeding on prior to death to be inferred. However, the last few meals consumed tend to override older meals; studies on extant animals suggest microwear patterns only reflect meals consumed in the last few hours or at most days before death (Solounias et al. 1994). Diseased or impaired animals may feed on nonstandard foods, leading to incorrect assumptions about an animal's diet.

Inference of historical grasslands from floral components

More recently, direct methods have become available to identify actual grasses and grasslands rather than relying on indirect evidence from fossilized animals. These direct methods rely on plant macrofossils and pollen grains to document changes in vegetation over time. Pollen is well represented but only useful at the family level, as grass pollen in general is too morphologically similar to be identified at lower taxonomic levels. Grass phytoliths (opaline silica remains of silica cells deposited in the leaf epidermis) can be used for subfamily level ID (Strömberg et al. 2007). Although macrofossils are very rare, some grass reproductive parts have been fossilized, especially in North America. Other forms of direct and indirect sampling, such as stable carbon isotopes both of teeth and fossilized soils can be used to identify historical grasslands. Stable carbon isotopes from fossils can be used to estimate the photosynthetic pathways of ancient vegetation, and also to assess whether assumptions about morphology (i.e. high crowned teeth being indicative of grass feeders) are justified (Clementz 2012). Stable isotope analysis of enamel from mammal teeth can be used to establish what types of vegetation animals were feeding on because plant chemicals are incorporated into the animal's teeth (Ambrose and Deniro 1986; Stevens and Hedges 2004).   By determining the isotopic makeup of the teeth, inferences may be made about the diet, specifically, whether the animal fed on C_3, C_4, or a mixture of plant types. This provides evidence of regional vegetation composition, although there are potential limitations because animals may preferentially feed on certain plants over others. In addition, fossilized soils themselves can be used to infer the climate of an area at a given time, although this is of limited use in areas subjected to high erosion. In particular, the presence of carbonates is a good indicator that the soils once supported grasslands. Carbonates generally precipitate in dry soils where net evaporation exceeds precipitation (common in grass dominated or mixed ecosystems). Stable isotope composition of soil has also been used to identify proportions of C_3 and C_4 grasses. However, results can be obscured by atmospheric carbon-13 incorporation in areas of low productivity when using the more commonly preserved pedogenic carbonates, which leads to an overestimate of C_4 vegetation abundance (Cotton et al. 2012). Recently, organic material preserved in Miocene paleosols have been used to reconstruct the C_3/C_4 grass transition between 10.2 and 8.9 MYA for a location in southwestern Montana, and checked against a reconstruction built using phytoliths collected from the same paleosols. The authors inferred this ancient ecosystem had similar ratios of C_3/C_4 plants as modern ecosystems in this same region (10 and 20% C_4), one of the higher percentages during the late Miocene. Similar studies inferred a complete lack of C_4 plants in Pakistan until 8 MYA and C_4 plants comprising only 5 to 10% in Argentina during this same period. Coupled with reconstructions from the Great Plains showing C_4 percentages comparable or greater than in the Montana site this suggests that the C_4 photosynthetic pathway in grasses first evolved in North America, although biogeographic patterns are obscured by the sheer number of convergent lineages of C_4 grasses (Sage et al. 2011).

Historical grasslands by continent

North America - Based on macrofossil assemblages (plants, spikelets, and inflorescence) grasslands occurred as early as the early Eocene, but little pollen is available from this period, and some available fossil evidence is equivocal (Crepet and Feldman 1991). The fact that grass pollen is rarely found in samples from Eocene through Oligocene suggests grasslands were not a dominant vegetation type during this period. Mountain building events in the western United States during the Oligocene created a rain shadow in the western central United States, which led to short grass grasslands becoming more widespread in this area. Tall grass prairie developed more recently in areas farther east as temperatures warmed and rainfall decreased (Bredenkamp et al. 2002). Based on plant fossils and the radiations of grass associated mammals like camels and horses, it would appear grass-dominated ecosystems became more common in North America during the middle Miocene and the number of distinct grass species increased dramatically until all modern subfamilies were represented by the late Miocene (7 MYA) (Thomasson 1990).

Hypsodont teeth were first recorded in North America in the late Eocene, as were other morphological features suggestive of living in open areas. For example, the genus *Protoptychus* was a North American rodent living during the middle Eocene that possessed a number of characteristics such as elongate hind limbs with shortened forelimbs suggesting it lived in open, arid regions (Wahlert 1973). Adaptations for open habitats have also been observed in equids and camelids, both of which underwent a rapid radiation in the early Miocene followed by a rapid decline in diversity approximately 5 MYA. At that point, rodents with high-crowned teeth again became common. While these adaptations are suggestive of animals inhabiting grasslands, and the presence of hypsodont dentition suggests these animals fed on grasses, it is not until approximately 10 MYA that unequivocal evidence is available. At the Poison Ivy Quarry in Nebraska, a rhinoceros with grass remains in its oral cavity and rib cage were uncovered, demonstrating animals which ate grass were present at this time (Voorhies and Thomasson 1979).

While incorporating C4 grasses into a grazing diet appears to be widespread during the Miocene in North America, the C_4/C_3 composition varied with latitude. The earliest evidence for C_4 plants in diet is 15.6 MYA and C_4 plants were common in diets by 6.8 Ma. As inclusion of C_4 plants in the diet became common in other lower latitude areas (Pakistan for example) at about this same it is thought that a rapid expansion in the dominance of C_4 plants occurred globally (Cerling et al. 1993, Quade et al. 1989). As this occurred the number of equid genera declined greatly (from 9 to 3 in North America). This is possibly due to a decrease in woody plants or because C_4 grasses have lower nutritional values than C_3 grasses. The exact timing is related to latitude, with lower latitudes consistently showing evidence of earlier C_4 incorporation compared to higher latitudes, along with making up a greater percentage of diet.

While diets containing purely C_4 plants were first recorded near the Miocene-Pliocene boundary (5.7 Ma) at lower latitudes, grazers never developed a pure C_4 diet at higher latitudes (Cerling et al. 1997a). However, a study of ungulate communities in Nebraska and Texas by Fraser and Theodor (2013) raises questions about this latitudinal progression. The authors reconstructed ungulate and proboscidean communities in these two regions and scored various tooth characteristics along with identifying species as hind or foregut fermenters. They found that while both areas had animals that incorporated C_4 plants in their diets, it appeared that those in Texas utilized C_4 more commonly, suggesting that such plants made up a larger part of the flora in this area. As the two communities did not differ in the prevalence of morphological traits associated with grasslands (such as tooth type and having hind-gut fermentation) they hypothesized that the expansion of C_4 plants into higher latitudes did not come at the expense of woodlands or other non-grass vegetation. C_4 grassland expansion at the expense of C_3 grasslands could also be ruled out because paleosol carbon isotopes and phytolith assemblages from the same areas do not show latitudinal differences. This led the authors to conclude that this time period was not a time of major range expansion of C_4 grasses and that previous findings of strong latitudinal gradients could be due to sampling biases or preferential feeding on C_3 grasses in Nebraska. This highlights the need for multiple lines of evidence when attempting to reconstruct ancient ecosystems.

Eurasia - While most of Europe is forested and has been since the Tertiary, parts of Asia and eastern Europe have evidence of grasslands in the Oligocene (although significant cover did not exist until the early Miocene) and late Miocene respectively (Bernor et al. 1996). In northwest India pollen samples from the early Miocene include 8% grass pollen, which increased throughout the Miocene (Mathur 1984). Additionally, vertebrate fossils from the Oligocene suggest that central Asia was mainly an open ecosystem. Horses entered Asia from North America between 10.9 and 10.7 MYA and quickly radiated to include grazing species. While C_4

grasses have been documented in Eurasia as early as 32MYA (Urban et al. 2010), they did not form a major component of the ecosystem. Isotopic study of fossils from Pakistan suggested that C_4 grasses entered diets around 9.4 MYA and increased steadily until 5 MYA when some groups fed exclusively on C_4 grasses (Cerling et al. 1997b). This contrasts with the abrupt shift found in other parts of Asia between 8.1 and 6.5 MYA (Quade and Cerling 1995).

Europe and North America were connected by a land bridge either directly or indirectly (through Asia) throughout the Tertiary (Lindsay et al. 1989). However, most ungulates in Europe were thought to be browsers based on teeth wear. While it had been thought that the dispersal of hipparionine horses from North America into Eurasia occurred as grasslands spread, microwear analysis of teeth suggests that only some species, in Eurasia were grazers while most species had a mixed diet (Hayek et al. 1992). In Europe, the earliest hipparionines appear to be forest dwelling species which is supported by stable isotope analysis. According to Asian soil deposits, which are rich in carbonates, C_4 vegetation appeared in the late Miocene and quickly became dominant (although how quickly is debated) (Quade and Cerling 1995; Morgan et al 1994). This contrasts with Europe, where there is no evidence supporting a shift to C_4 grass dominance.

South America - South America has supported many grassland dominated ecosystems ranging from tropical savannah to temperate grasslands. Grass pollen from as early as the early Paleocene (57MYA) has been found in northern South America. Additionally, grass pollen is a common feature of samples taken in the Pantropical zone (northern South America and western Africa) in the early Eocene (49-45 MYA), suggesting an ancient grassland was found in this region (Germeraad et al. 1968).

Further support for early grasslands comes from the dung beetle genus *Coprinisphaera,* which was found in deposits at Gran Barranca (a deposit in southern Argentina) from the late middle Eocene, approximately 39.8 MYA (Sanchez et al. 2010). Since dung beetles are associated with large mammals and open habitats, their presence suggests grass dominated areas occurred in this region at that time, and more closed habitats before this period. Although equating lack of fossil evidence with absence of a taxon is risky, the condition of the deposit (well exposed older material, a gradual increase in the number of ichnospecies (species based on the fossilized work of the species rather than an actual fossil) and similar recent deposits which show abundant *Coprinisphaera* evidence) suggests that if the beetles had been present fossil evidence would have been recovered (Sanchez et al. 2010). However, conditions in this region appear to be highly variable as there are extended periods where there is no evidence of beetles in the deposits.

Because South America was isolated for long periods from other land masses, a number of unique groups evolved. One of these, the Notoungulates is a now extinct group of mammals thought to have been similar to ungulates. Tooth crown height began increasing in the late Paleocene and by 31.5 MYA high-crowned teeth were the dominant form while similar aged notoungulates had muzzle shapes similar to those of other grazers. Both of these features are indicative of grazers occurring in South America much earlier than in North America (Wyss et al. 1993; Shockey 1997).

Stable isotope analysis supports a prolonged utilization of primarily C_3 plants. In fact, the earliest evidence of C_4 plant consumption does not occur until approximately 10 MYA (in a Bolivian fossil which appears to have a mixed C_3/C_4 diet) and the earliest exclusive C_4 diet is not documented until 5.5 MYA (in Argentina) (MacFadden et al. 1994, MacFadden et al. 1996). There is some evidence of C_4 grasses occurring as early as 16MYA based on stable isotope analysis of paleosols, but the authors could not rule out diagenetic factors causing these results Kleinert and Strecker (2001). This suggests that while C_4 plants were present they were not widespread or found in great numbers.

Africa - The earliest record of grass in Africa is a Paleocene pollen sample from Nigeria (Adegoke et al. 1978). Since that time grasslands have been present in Africa (although many areas repeatedly switched between grasslands and forests) as supported by pollen samples and fossils. Particularly in southern Africa, one of the main drivers of grassland origination was climate- during the Oligocene; this region was significantly cooler than at present, leading to replacement of tropical or subtropical forests with grasses which could better tolerate the cooler climate (Bredenkamp et al. 2002). Grass pollen is well represented in pollen cores but typically in low abundances, and examination of vertebrate communities supports a landscape of mixed grass/woodland habitats. Beginning 8.5 MYA high-crowned teeth became common in many animals including rabbits, rodents, and porcupines suggesting smaller grass patches were transitioning into larger contiguous regions.

While C_4 grasses first appeared in Africa and entered animal diets by the mid Miocene, they do not make up a large portion of diet until the late Miocene (Leakey et al 1996; Morgan et al. 1994). Stable isotope analysis of mid Miocene soils and ungulate teeth from Kenya did not yield evidence of C_4 plant utilization, although plant macrofossils identified as C_4 grasses have been found (Cerling et al. 1991; Cerling et al. 1997a). As stable isotopes cannot differentiate between types of C_3 vegetation, the teeth were examined and microwear patterns were found to be congruent with a mixed diet of browse and grazing. Additionally, it is thought that while C_4 biomass increased over the last 10 million years, at no time during the Neogene did this grass type dominate in the Rift Valley. Between 8.5 and 6.5 MYA C_4 plants became an important or exclusive dietary component in parts of Africa

Australia - Based on the fossil record and inferences about natural history, much of Australia was forest and dominated by arboreal species until the Miocene when the forests opened up and grazers became more common although the earliest record of grasses are mid Eocene pollen samples (Martin 1994). Evidence points to a forest dominated landscape with small grassy patches. Starting around 13 MYA grass pollen becomes slightly more abundant, then becomes significantly more abundant after 4.5 MYA. Because of Australia's long isolation, browsing and grazing roles were played by kangaroos and wombats (Archer et al 1994). Additionally, the fossil record is poor in Australia compared to other regions. Although marsupial tooth structure is substantially different from that of eutherians, high-crowned teeth are still present in grazers.

To summarize, grasses were documented in the pollen record on all continents (except Antarctica) by the Eocene, however they do not become widespread until the Miocene with the exception of a northern South America/western Africa grassland in the Paleocene (Jacobs et al. 1999) (See Table 2.1 and Figure 2.1 for a summary of important dates by continent). C_3 grasses were replaced over time by C_4 grasses in many areas although the timing varied by region. With the rise of the grasslands, grazing mammals evolved and became widespread. Teeth, jaw, and limb morphology all changed to better support life in open areas and to accommodate the shift from browsing to grazing.

Modern Grasslands

Grasslands are home to a variety of plants and animals many of which are reliant on grasslands for all or part of their life history. According to World Resource Institute (2000) approximately 110 of the 234 Centers of Biodiversity identified by the IUCN-World Conservation Union and World Wildlife Fund-US include portions of grasslands. Some of these areas contain upward of 2400 species of vascular plants alone. While temperate grasslands are dominated by perennial bunchgrasses, those in tropical areas are dominated by annual grasses. Shaw (2000) found African grasslands and savannahs were dominated by Panicoideae and Andropogoneae; mesic grasslands are favorable to Paniceae; and arid environments favored the Chloridoideae and Eragrosteae. South American grasslands and savannahs were dominated by Panicoideae and Arundinoideae. Groves (2000) found that Aveneae and Agrosteae were underrepresented and the Paniceae overrepresented in South America; Agrosteae and Paniceae were underrepresented while Aveneae was overrepresented in South Africa; and three of six tribes (Agrosteae, Aveneae, and Festuceae) were overrepresented while the remaining 3 were underrepresented or absent in Oceania. Plant compositions in temperate grasslands are more similar to other temperate grasslands than to more closely located tropical grasslands. Groves (2000) also noted that there are four floral groups in temperate grasslands-perennial C_3 grasses (growing mainly in spring and fall), warm season C_4 grasses which grow in the summer, forbs which are interspersed with the grasses, and nonnative plant species.

Invasive species have become established in many grasslands, either through intentional introduction as livestock forage or erosion control or accidently through human and animal movement. In some areas invasive species now dominate grasslands. This, coupled with the fact that grasslands have been widely converted into agricultural systems (both for crop/biofuel production on the rich, fertile soils and as rangeland for livestock) has led to great declines in grassland size worldwide. The native vegetation remaining tends to be in areas undesirable for agriculture, for example, rocky soils and hilltops. These remaining patches are often small and highly fragmented which has resulted in very few functioning grasslands remaining, especially in developed countries. Grasslands in less developed regions of the world have fared better as pastoral systems cause less degradation but even grasslands in these regions have suffered much degradation (World Resource Institute 2000).

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Tables and Figures

	North America	South America	Africa	Eurasia	Australia
First Grass	55MYA (macrofossil; Crepet and Feldman 1991)	60MYA (Pollen, Jacobs et al. 1999) *Strömberg (2011) places it at 70 MYA	Paleocene (Pollen, Adegoke et al. 1978)	52MYA (macrofossils and pollen, Jacobs et al. 1999)	52MYA (pollen, Segalen et al. 2007)
First Grassland	24MYA (pollen and ungulate morphology; Janis et al. 2000)	40MYA (Pollen, Germeraad et al. 1968; dung beetles, Sanchez et al. 2010)	16MYA	20MYA (pollen, Jimenez-Moreno et al. 20007)	6MYA, although pollen evidence suggests wet grasslands are much older (Martin 1994)
First C_4 grass	19MYA (phytolithys, with fossils occurring later; Strömberg 2005)	10MYA (stable isotopes MacFadden et al. 1996; Kleinert and Strecker (2001) suggest 16 MYA, but couldn't rule out diagenetics)	16MYA (carbon isotopes)	32MYA (stable isotopes, Urban et al. 2010)	15MYA
First C_4 dominated grassland	7-5.5MYA(phytoliths, paleosols, stable isotopes of teeth)	8MYA (enamel, MacFadden et al. 1996)	9MYA (stable isotopes, Cerling et al. 1997)	Never	Never

Table 2.1: Key events in grassland formation by continent. Dates from Strömberg (2011) if no citation given

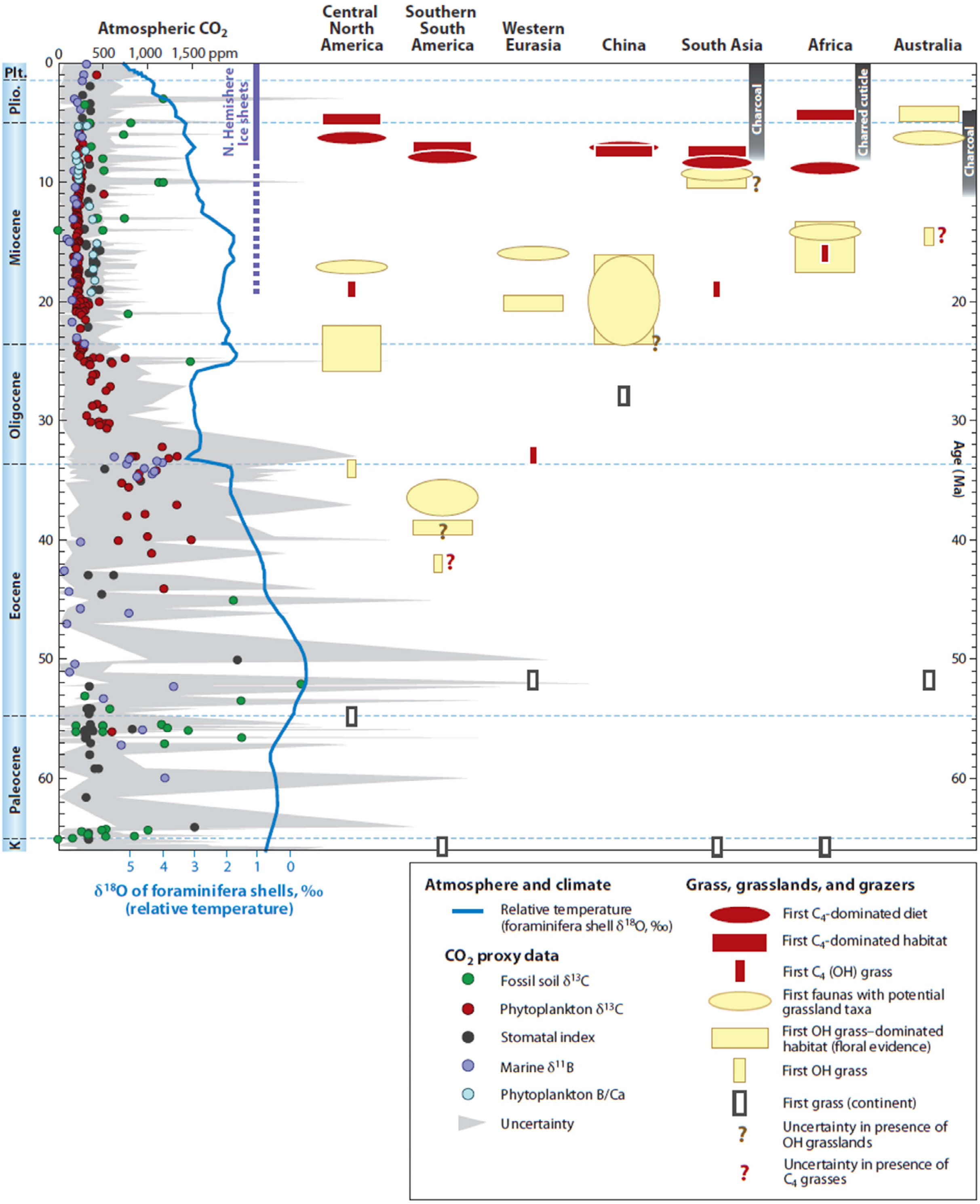

Figure 2.1: Summary of grassland formation by region (Taken from Strömberg et al. 2011)

CHAPTER THREE: A MOLECULAR PHYLOGENY AND DIVERGENCE TIME ESTIMATION FOR THE PLANTHOPPER SUBFAMILY CALISCELINAE (FULGOROIDEA: CALISCELIDAE)

Introduction

Caliscelidae is a widespread family of mostly grass feeding planthoppers that are most diverse and abundant in native grasslands. Although Caliscelidae is relatively low in species diversity (approximately 70 genera and 200 species) compared to some other lineages of grassland Auchenorrhyncha, they are geographically widespread. Caliscelids are found in all biogeographic regions although in Australia they are represented by just a single genus that is also found in southeast Asia and various Pacific Islands. This makes Caliscelidae a lineage for which historical biogeographic reconstruction can be utilized to elucidate patterns of diversification and spread of grassland insects. Caliscelids have not been the subject of a comprehensive taxonomic revision, and the status of most genera and species has not been formally tested. Additionally, recent regional treatments have resulted in a large number of new taxa being described including 6 new genera and 8 species of Afrotropical taxa by Gnezdilov and Bourgoin (2009). However, recent work by Gnezdilov and others has resulted in better resolution of relationships between species (using only morphological evidence) and synonomy of taxa (Gnezdilov 2008, Gnezdilov and Wilson 2006, Gnezdilov and Bourgoin 2009).

While caliscelids have been included in a number of recent molecular phylogenies focusing on Fulgoromorpha as a whole (Yeh et al. 2005, Urban and Cryan 2007, Song and Liang 2013), the monophyly of this family has not been adequately tested. Existing phylogenies only include a few exemplar specimens from this family, include taxa only from the Northern

Hemisphere, and do not include representatives from multiple subfamilies (Gnezdilov and Bourgoin 2009). Additionally, the placement of various genera to subfamily is questionable. For example, the placement of *Papagona* the only New World representative of Ommatidiotinae (excluding *Asarcopus palmarum* which has been introduced from Africa onto dates in California), has been called into question (Gnezdilov 2011). Excluding *Papagona*, Ommatidiotinae comprises 20 Old World genera while Caliscelinae contains approximately 50 genera distributed worldwide. While Ommatidiotinae is known to feed on a variety of hosts including dates and buckwheat along with grasses, Caliscelinae is thought to be grass specialists (Che et al. 2009; Wilson 2005; Gnezdilov and Wilson 2011).

Gnezdilov and Bourgoin (2009) suggested a Gondwanan origin for Caliscelidae based on their presence in all biogeographic regions and affinities between Oriental and Madagascan taxa. However, their hypothesis appears to be based at least in part on a misinterpretation of Shcherbakov (2007) who, contrary to Gnezdilov and Bourgoin (2009), did not suggest that caliscelids are an ancestor of the higher planthoppers and that Perforissidae is an early offshoot of Caliscelidae rather than a distinct, distantly related family.

This study tests the monophyly of Caliscelidae using DNA sequence data from 4 genes (2 nuclear and 2 mitochondrial) for approximately 30 species of caliscelids from all biogeographic regions except Australia, representing the largest molecular phylogeny of the family to date. Additionally, the monophyly of some widespread genera are tested, the tribal placement of *Papagona* is examined, and divergence times are estimated for lineages within the family.

Materials and Methods

Taxonomic history - This group was traditionally treated as a subfamily of Issidae, and only recently elevated to family status based on a variety of characteristics including patterns of acoustic signals, morphological characters and molecular phylogenetics (Tishechkin 1998, Yeh et al. 1998, Emeljanov 1999). However, no single morphological synapomorphy uniting the family has been identified. Instead a suite of characters including strongly reduced wings; the anterior connective lamina of the 8th gonapophysis narrow, with 1-9 large teeth, and lacking a comb; flat gonoplacs lacking teeth; and characters of the coryphe (Emeljanov 1999, Gnezdilov 2003) is used to recognize the group. Gnezdilov and Wilson (2006) provided a key to subfamilies and tribes (although only applicable to immatures), moved additional genera from Issidae into Caliscelidae, and attempted to place genera already in Caliscelidae into natural groups. Additional authors have worked to transfer various taxa previously placed in Issidae into Caliscelidae (Gnezdilov and Bourgoin 2009). Currently two subfamilies and 4 or 5 tribes are recognized; however, because the faunas of many regions are poorly known, additional higher taxa may eventually need to be established.

One of the first molecular studies of Fulgoroidea included the then issid subfamily Caliscelinae in order to test for monophyly of Issidae (Yeh et al. 1999). This study was based on portions of mitochondrial 16S and cytochrome b sequenced for 10 taxa. The results showed polyphyly of Issidae (although the subfamilies placed outside of the issid clade varied), supporting the proposal to elevate the Caliscelidae to family status. In a follow-up study using only 16S, and with broader taxon sampling (53 species from 15 families), the monophyly of the family Caliscelidae was confirmed, although its relationship to other planthopper families was equivocal (Yeh et al. 2005). Using a dataset comprising sequence data from four genes, Urban and Cryan (2007) identified Ricaniidae as the sister family of Caliscelidae, although support for this relationship was low and the analysis included only 3 Nearctic caliscelid taxa. Using different sets of genes and exemplar taxa (although the two caliscelid taxa included were represented by sequences generated and used by Urban and Cryan (2007)), Song and Liang (2013) recovered Tettigometridae as the sister family to Caliscelidae, but again this was poorly supported. Additionally, both Caliscelidae and Tettigometridae were lacking 2 of the 4 genes sampled (and many Ricaniidae taxa were only represented by 18S). Such missing data could result in incorrectly inferring relationships (or conversely low support values). Song and Liang also attempted to date the divergence times of various radiations using an uncorrelated, lognormal relaxed molecular clock and root age of 260 million years (plus or minus 10 million years) for Fulgoroidea. This resulted in an estimate of Caliscelidae radiating 24 million years ago (with a 95% credibility interval ranging from 60 to 10 million years). Interestingly, the two included caliscelid taxa are both Nearctic in origin, and this date coincides with the first grass-dominated habitat in North America, which is thought to have arisen between 26 and 22 million years ago (Strömberg 2011).

While the planthopper fossil record is extensive there are no known fossils which can be placed in Caliscelidae, possibly due to differences between caliscelids and other planthoppers in regards to habitat preferences and time of diversification (Shcherbakov 2006). Shcherbakov (2007) described Perforissidae, a new family of planthopper from amber inclusions that has many morphological features including sensory pits on various segments and an angular (in profile) anteclypeus, in common with Caliscelidae. However, the author treats these as homoplastic characters because characters of the wing venation, mesonotum, tarsi, and ovipositor place the Perforissidae more basal in Fulgoroidea. A single Canadian Amber (70-90 MYA) fossil in the Canadian National Collection, #852, identified as Caliscelidae by K.G.A. Hamilton (Skidmore 1999) has been re-examined and belongs to Perforissidae. Thus, true Caliscelidae still have not been documented in the fossil record.

Shcherbakov (2007) presented an intuitive assessment of higher fulgoroid evolution based on his interpretation of the fossil record, hypothesizing that the so-called "higher" fulgoroid families (i.e., those with the spines of the second hind tarsomere reduced or absent, including Caliscelidae) were derived from subbrachypteous ancestors. According to Shcherbakov, this explains why fully winged taxa in this group do not have homologous wing venation to each other or to primitive fulgoroids such as Cixiidae; he suggests that such complex venational patterns arose independently multiple times. He places Issidae as one of the more basal members of the higher planthopper group, and suggests that caliscelids are intermediate members that have neotenous characteristics such as retention of sensory pits in adults and reduced wing venation. He further suggests that this was an evolutionary attempt to become more “cicadellid like” and that Issidae s.l. (in which he includes caliscelids) are as diverse and widespread as they are because of this body plan.

The oldest extant family of higher fulgoroids represented in the fossil record is Nogodinidae, which was found in material from the Early Paleocene, and common throughout the Paleocene. While Caliscelidae are not known from fossils, Ricaniidae, one of the potential sister families of Caliscelidae, are known from a few fossils, the earliest of which is from the

Miocene (older fossils previously placed in this family have been reassigned to other families or orders) (Shcherbakov 2006).

Taxon sampling - A total of 31 specimens representing 29 morphospecies were included as ingroup taxa. These taxa include representatives from all major grasslands of the world except for Australia. As no formal test of the monophyly of Caliscelidae has been attempted, a number of outgroups were included in this study to test the status of Caliscelidae. Based on Urban and Cryan's (2007) phylogeny 7 outgroups from 5 families (2 nogodinids, 2 ricaniids, and single representatives of Lophopidae, Flatidae, and Tropiduchidae) were included in this study. Additionally, given that Song and Liang (2013) indicated a possible sister group relationship between Caliscelidae and Tettigometridae, 3 exemplars of Tettigometridae, representing 2 genera, were included.

Specimen acquisition and DNA extraction - The majority of specimens were field collected, preserved in 95% ethanol, and stored at -20°C until DNA extraction occurred, however some taxa were only represented by specimens originally collected into ethanol but later dried, point mounted, and deposited in the Illinois Natural History Survey Insect Collection. DNA extraction, typically from the abdomen only, was equally successful from both types of specimens. However, sequences obtained from representatives of Tettigometridae were of much poorer quality that those of other taxa, even compared to other specimens with similar collecting and preservation history (i.e. collected during the same field expedition).

DNA was extracted from each specimen using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following a modified version of the manufacturer's protocol for "Total DNA from Animal Tissues." Changes to the protocol included lengthening the incubation period in step 2 to 36 hours and decreasing the amount of Buffer AE in step 7 to 50µl (which was repeated twice in different 1.5mL collection tubes rather than using the same collection tube as in the standard protocol). After extraction the cleared specimens were placed in microvials with glycerin as voucher specimens.

PCR and DNA sequencing - Following initial screening of 6 candidate genes, the 4 gene regions selected for further study were those that amplified readily across a range of taxa, had the best phylogenetic signal and number of parsimony informative characters, and evolved at various rates. Four genes (D2 region of 28S (860bp), Histone H3 (350bp), 12S (430bp), and 16S (670bp)) were amplified and sequenced for all taxa (see Appendix B and C for PCR primers and reaction conditions). All PCR was performed using 25µL reactions with *Taq* polymerase (Promega, Madison, WI).

Products were submitted for high-throughput sequencing at the Keck Biotechnology Center of the University of Illinois. Sequences were assembled automatically in Sequencher 4.8 (minimum match = 60; minimum overlap = 20), each contig for a given gene was assembled into a single alignment and exported as FASTA file. FASTA files were opened in seaview 4.3.0 (Gouy et al. 2010) and then aligned using the built in version of MUSCLE (Edgar 2004) with all parameters set at default, except in the case of 12S where a higher gap opening penalty was used to align selected sites after the initial alignment introduced extraneous single gaps in the majority of sequences.

Resulting alignments were then adjusted by eye and all sequenced regions were included. Sequences were trimmed to exclude primer regions, but no other regions were removed, and any gaps were treated as missing data. Sequences will be deposited in GenBank.

Phylogenetic analysis - Models were selected for each gene using ModelGenerator (nset=6) with evolutionary models yielding the highest Akaike's information criterion (AIC) score selected (Keane et al., 2006). Before individual genes were combined, individual gene trees were inferred using 20 million Bayesian MCMC generations (logged every 1000 generations; 25% burnin) in BEAST using default priors and models selected by ModelGenerator (Drummond and Rambaut 2007). These gene trees were compared and because there was no conflict between well-supported clades (using posterior probabilities greater than 0.90), data were combined, although treated as individual partitions for both Bayesian and likelihood analyses.

Complete phylogenies were inferred using parsimony (PAUP* 4.0b10: 10,000 random addition sequences, TBR branch swapping), maximum likelihood (Garli: 10 independent runs, default settings, and an automated stop criterion if *ln*L score remained constant for 50,000 generations), and Bayesian MCMC methods (MrBayes: 20 million generations; 4 runs and 4 chains; and BEAST: 40 million generations; tree prior = speciation: birth-death process; for both programs trees sampled every 1000 generations with a burnin = 25% and log files were viewed in Tracer to ensure convergence was reached) (Swofford 2003; Huelsenbeck and Ronquist 2001; Ronquist & Huelsenbeck 2003; Drummond and Rambaut 2007). Both posterior probabilities and bootstrap values (1000 replicates of 100 random addition sequences) were calculated to estimate branch support. Additionally, analyses with and without Tettigometridae and utilizing different suites of outgroups, were performed to examine the effects of outgroup choice on topology, branch support, and branch length.

Divergence time estimation - Although there are many fossil planthoppers, no fossil caliscelids are currently available. To estimate divergence times, geographically restricted clades can be used as calibration points. Biogeographic events such as the formation of land bridges, mountains, and islands are commonly used for divergence time estimation, based on the assumption that a given species or group could not have originated before the area they are currently restricted to originated (Weir and Schluter 2008; Heads 2006; Worobey et al 2010). This assumption is not without its drawbacks, including the possibility of species living outside the selected region that have gone extinct and uncertainty in the timing of biogeographic events (Wilke et al 2009). Also, node ages inferred using this method should be treated as maximum ages because colonization of a region by any given clade may have occurred more recently than the earliest date of origin of that region.

Based on the assumption that a grass feeding species could not have colonized and radiated in a region prior to the expansion of grasslands, I used dates of the first grassland ecosystem in a given biogeographic region based on Strömberg (2011). To do this, the distribution of each exemplar genus was coded by major biogeographic region and mapped on the resulting topology for the Bayesian analysis. Clades known from single biogeographic regions with a widely accepted date of first grassland formation were identified and these dates were used in a BEAST divergence time estimation analysis under a relaxed lognormal molecular clock prior. I used a normal distribution with a 24 MYA mean, and 3 MYA standard deviation for the 10 taxa clade containing the Mexican representative of *Aphelonema* a number of *Bruchomorpha* species, *Fitchiella*, and one *Papagona* species from Mexico. This date was used both because it is the earliest time with known grass dominated ecosystems in North America (based on floral evidence), and also was the date inferred by Song and Liang (2013) as the time of divergence between their two included caliscelid species (*Aphelonema* and *Bruchomorpha* both from North America). Ideally, many calibration points should be used and spread throughout the tree. However, because setting node ages to the age of oldest grassland in an area could bias dates to the favored hypothesis of a tight correlation between the origin and spread of grasslands and the diversification of caliscelids, I chose to use a single calibration point based on a relatively well sampled clade of Nearctic taxa. This allows all other dates calculated to be compared to the dates of major events in grassland history such as the rise of grasslands on other continents or the development of C_4 photosynthetic pathways.

Results

PCR amplification, sequencing and alignment - Most taxa were represented by complete sequences of all four genes (Table 3.1). The D2 region of 28S from *Ommatidiotus dissimilis* was omitted from analysis because the resulting sequences were of extremely poor quality and base calls were suspect. These reactions were repeated using different amplification protocols but continued to fail. Table 3.2 includes summary statistics for each gene and the total dataset.

Phylogenetic Analyses - Based on AIC scores the model GTR + I + G was selected for each gene except for 12s for which GTR + G was selected. Trees inferred from individual genes using both parsimony and Bayesian techniques did not include any well-supported conflicts, so data were concatenated into a final alignment of 4 partitions (one for each gene) for subsequent analyses.

Trees resulting from parsimony, likelihood, and Bayesian techniques were generally congruent, with a few exceptions discussed below. Therefore, only the tree (Figure 3.1) resulting from a Bayesian analysis of all ingroup and outgroup taxa, minus tettigometrids (the overall poor quality of these sequences led to areas of questionable alignment, lower support values, and a lessening of topological stability) is shown. All resulting phylogenies included a monophyletic ingroup, although support for this was weak in some analysis, particularly those including Tettigometridae. Caliscelid relationships were mostly unaffected by outgroup choice, with the exception of *Ommatidiotus dissimilis*. In most analyses this species is sister to the rest of Caliscelidae. However, in some analyses in which Tettigometridae are included, it is inferred to be within one of two major clades of Caliscelinae or within the outgroup although support is very low for these alternate topologies. Relationships between included ingroup taxa were well supported with the majority of nodes approaching 1.0 posterior probability. Excluding *Ommatidiotus,* the ingroup topology was unaffected by outgroup selection. Two major clades were recovered, although support for these clades was weak in some analyses, one consisting solely of Old World taxa and the other a mix of New and Old World species. While the different analyses resulted in trees that were generally congruent, the placement of Argentina 2 was not congruent between the Bayesian (where is was placed in a clade with the rest of the Argentina taxa, posterior probably of 1.0, but as the unsupported sister to a *Bruchomorpha* from Mexico) and parsimony analyses (where it was the weakly supported sister to the rest of the New + Old World mixed clade). Additionally, several genera, including *Aphelonema*, *Bruchomorpha*, *Fitchiella*, and *Papagona*, were found not to be monophyletic.

Divergence time estimation - A single calibration point was used for a 10 taxa clade containing the Nearctic representative of *Aphelonema,* four *Bruchomorpha* species, *Fitchiella*, and one *Papagona* (Figure 3.2). A normal prior was used based on the oldest known age of grasslands in North America. By using a single calibration point, the bias introduced by restricting clade ages to grassland ages (and therefore decreasing the chance for Type 1 error, in this case falsely concluding that Caliscelidae divergence times tightly correlate to periods of grass evolution) Caliscelidae was found to have originated approximately 80 MYA, a date which is within the suspected age for higher fulgoroids (based on divergence time estimation by Song and Liang 2013), and the two subfamilies diverged approximately 72 MYA. The two major Caliscelinae clades were estimated to have diverged 62 MYA. In the Old World-only clade the Asian taxa began radiating approximately 29 MYA, while the New World clade underwent radiations between 29 and 22 MYA and again between 14 and 8 MYA.

Discussion

Caliscelidae was found to be monophyletic, albeit with low branch support, and the monophyly of the grass-specialist lineage, Caliscelinae, also received only weak support. A more thorough test of the monophyly of the family will require addition of data for two tribes of Ommatidiotinae not included in the current dataset. The relationship between Caliscelidae and other planthopper families is still unclear and will also require further taxon sampling with specimens representing a wider diversity of taxa in potential sister families and sequences for additional genes, particularly those such as 18S or additional regions of 28S which have been used in other planthopper phylogenetic work. Inclusion of these genes would allow data generated by other authors such as Urban and Cryan (2007) and Song and Liang (2013) to be included which could result in a more robust assessment of the placement of Caliscelidae as a whole. Previous attempts to infer a phylogeny of planthoppers have had very limited taxon sampling, have produced conflicting results, and have also included only caliscelids that are closely related; therefore, they did not adequately test the monophyly of the family. Additionally, the single representative of Ommatidiotinae, *Ommatidiotus dissimilis*, is sister to the Caliscelinae although, to test the monophyly of Ommatidiotinae, additional representatives are required. My phylogeny also confirmed that *Papagona* should be placed within Caliscelidae as suggested by Gnezdilov (2011), leaving Ommatidiotinae without any native New World members while Caliscelinae includes two major, well supported clades one of which includes only Old World species while the other is globally distributed. Caliscelidae is currently divided into 4 or 5 tribes, with Caliscelinae including only a single tribe. More taxa of Ommatidiotinae will need to be included in the dataset before monophyly of the tribes can be tested.

None of the included caliscelid genera for which specimens of multiple species were available were recovered as monophyletic. *Bruchomorpha*, one of the most diverse genera of Caliscelidae comprising 26 species found in North and Central America, was represented by 5 species on my phylogeny. One of these fell outside the main *Bruchomorpha* clade, while the others formed a well-supported monophyletic clade with the inclusion of *Fitchiella* (represented by one species) and one of the included *Papagona* species. As presently defined, the globally distributed genus *Aphelonema* was represented by two species in my phylogeny. The New World representative was placed basally in a clade containing a number of other New World taxa, while the Old World species, *Aphelonema eoa,* formed a clade with an unidentified species from Taiwan, suggesting that *Aphelonema* comprises at least two independent lineages. Additionally, the recently described genus *Calampocus*, previously only known from Madagascar, and now known from central Africa was not monophyletic.

The origin and diversification of grasses is widely thought to have occurred between 75 and 90 MYA based on fossil evidence and molecular clocks, although inclusion of recent grass fossils could push this date back as far as 130 MYA (Bouchenak-Khelladi et al. 2010, Prasad et al. 2011). Therefore, the inferred 80 MYA origin of Caliscelidae fits within the potential dates for grass origination. As grassland ecosystems developed and diversified between 40 and 20 MYA, caliscelids radiated and spread throughout the world. A second caliscelid radiation was inferred to have occurred between 15 and 8 MYA, which corresponds with the evolution and diversification of C_4 grasses and grasslands. The present results are compelling because use of a single calibration point based on the time of origin of grasslands in North America resulted in age estimates for the origins of other geographically restricted calisceline clades that are consistent with independently derived estimates for the origins of grasslands in these areas and for the expansion of C4-dominated grasslands.

The present results potentially shed light on the origin of grasslands in Madagascar. Although Madagascar's grasslands were previously thought to be recent and anthropogenic in origin (Klein 2002), the recent description of endemic Madagascar genera and species by Gnezdilov and Bourgoin (2009) provides further support for the hypothesis presented by Bond et al. (2008), who found evidence supporting the presence of ancient grasslands on Madagascar, possibly invading the region during the late Miocene. This ancient grassland was potentially maintained by climatic changes, fire, and herbivores, although most large grazing herbivores on Madagascar have since gone extinct (Willis et al. 2008). One genus included in the present analysis, *Calampocus*, was previously thought to be endemic to Madagascar

(Gnezdilov and Bourgoin 2009) but is now known to occur in Africa. Inclusion of Malagasy caliscelids could shed further light on the evolution of grasslands in this region.

Unless caliscelid fossils are located, dating divergence times in this group will continue to be difficult. The dates inferred by constraining geographic nodes to the dates when grasslands first developed on a given continent result in estimates that roughly correlate to two major periods of grassland evolution- the first being the rise of grassland biomes and the second being the rise of C4 grasses (and their subsequent dominance both in existing grasslands and in new areas of grassland expansion). However, these dates are still based on the assumption that the clades restricted to single continents originated about the same time as the grasslands themselves formed. While using a normal prior allowed for some variation (in this case a standard deviation of 3 million years was used to capture uncertainty in grassland origination) this assumption is not without potential flaws. These flaws include the fact a normal distribution does not exactly capture the expected distribution of dates around this node because insects could colonize an area significantly later than the grasslands themselves formed. To better describe the data a new prior is required.

Other potential problems with the present analysis include the lack of information on host associations for many caliscelid taxa. While most extant caliscelid taxa with known host associations feed on grass, *Asarcopus,* a member of Ommatidiotini, feeds on date palm, and host associations of most other non-calisceline caliscelids remain unknown. Thus, it is not clear whether grass specialization is limited to the subfamily Caliscelinae or whether it occurs more widely within the family. Another potential source of error is the assumption that the clades used for dating did not diversify during the lag time between the first appearance of grass on the given continent, but instead happened after grassland communities developed.

Future studies should include Madagascar endemics and additional taxa from both southern Africa and southeast Asia (particularly India) to identify affinities between these faunas and estimate divergence times. While an explicit biogeographic analysis is outside the scope of this chapter (see chapter 5 for biogeographic analyses), recovered clades tend to be geographically structured with an Old World clade (containing two clades of African species and a clade of Oriental species) and a mixed clade (containing separate Nearctic and Neotropical clades along with some Palearctic taxa). This implies that isolation of continents has played a role in the diversification of Caliscelinae.

Taxonomic Implications - *Bruchomorpha*, *Calampocus*, *Aphelonema*, and *Papagona* were not recovered to be monophyletic in my study. This result is not surprising because this family has not been revised and many genera are poorly defined. Species placement has historically been complicated by the strong sexual dimorphism and variations in wing length exhibited by Caliscelidae, resulting in many examples where males and females of the same species have been given different species names. Additional work should focus on defining genera and examining the placement of species.

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Tables and Figures

Name on Tree	Collecting Location	Extraction Code	D2 region of 28S	Histone	12S	16S	Biogeographical Coding
Thailand_1	Thailand	C1	-	-	-	-	E
Thailand_2	Thailand	C2	-	-	-	-	E
Thailand_3	Thailand	C3	-	-	-	-	E
Thailand_4	Thailand	C4	-	-	-	-	E
Argentina_1	Argentina	C5	-	-	-	-	C
Argentina_2	Argentina	C6	-	-	-	-	C
Argentina_3	Argentina	C7	-	-	-	-	C
Argentina_4	Argentina	C8	-	-	-	-	C
Gwurra_aphrodite_1	Zambia	C9	-	-	-		D
Gwurra_1	Zambia	C10	-	-	-	-	D
Populonia_1	Zambia	C11	-	-	-	-	D
Calampocus_1	Zambia	C12	-	-	-	-	D
Gwurra_aphrodite_2	Swaziland	C13	-	-	-	-	D
Calampocus_2	Swaziland	C14	-	-	-	-	D
Papigona_1	Mexico	C15	-	-	-	-	B
Papigona_2	Mexico	C16	-	-	-	-	B
Bruchomorpha_sp_3	Mexico	C17	-	-	-	-	B
Aphelonema sp	Mexico	C18	-	-	-	-	B
Bruchomorpha_sp1	Mexico	C19	-	-	-	-	B
Bruchomorpha_sp2	Mexico	C20	-	-	-	-	B
Bruchomorpha_sp1	Mexico	C21	-	-	-	-	B
Bruchomorpha_sp1	Mexico	C22	-	-	-	-	B
Bruchomorpha_dorsata_1	USA: Illinois	C23	-	-	-	-	B
Bruchomorpha_dorsata_2	USA: Illinois	C24	-	-	-	-	B
Bruchomorpha oculata	USA: Illinois	C25	-	-	-	-	B
Aphelonema eoa	Kyrgyzstan	C26	-	-	-	-	A
Caliscelis_1	Kyrgyzstan	C27	-	-	-	-	A
Ommatidiotus dissimilis	Kyrgyzstan	C28		-	-	-	A
Fitchella_1	USA: Kentucky	C29	-	-	-	-	B
Taiwan_1	Taiwan	C30	-		-	-	A
Tanzania_1	Tanzania	C31	-	-	-	-	D
Nogodinidae_1	Panama	O1	-	-	-	-	ACDEF
Nogodinidae_2	Thailand	O2	-	-	-	-	ACDEF
Leophid	Tanzania	O3	-	-	-	-	ACDEF
Flatidae	Puerto Rico	O4	-	-	-	-	ABCDEF
Tropiduchidae	Puerto Rico	O5		-	-	-	ABCDEF
Riccinidae_1	Thailand	O6		-	-	-	ACDEF
Riccinidae_2	Taiwan	O7	-		-	-	ACDEF
Tetogometridae_1	Thailand	O8		-	-	-	not included
Tetogometridae_2	Thailand	O9		-	-	-	not included
Tetogometridae_3	Thailand	O10		-	-	-	not included

Table 3.1: List of included taxa, "-" denotes successful sequencing of gene; Biogeographic coding as follows: A- Palearctic; B- Nearctic; C- Neotropical; D- Ethiopian; E- Oriental; F- Australasian

	D2 region of 28S	Histone	12S	16S	Total
Total Characters	845	351	400	520	2116
Constan	375	222	135	230	962
Parsimony uninformative	143	13	60	58	274
Parsimony informative	327	116	205	232	880

Table 3.2: PAUP summary statistics for each gene and total dataset

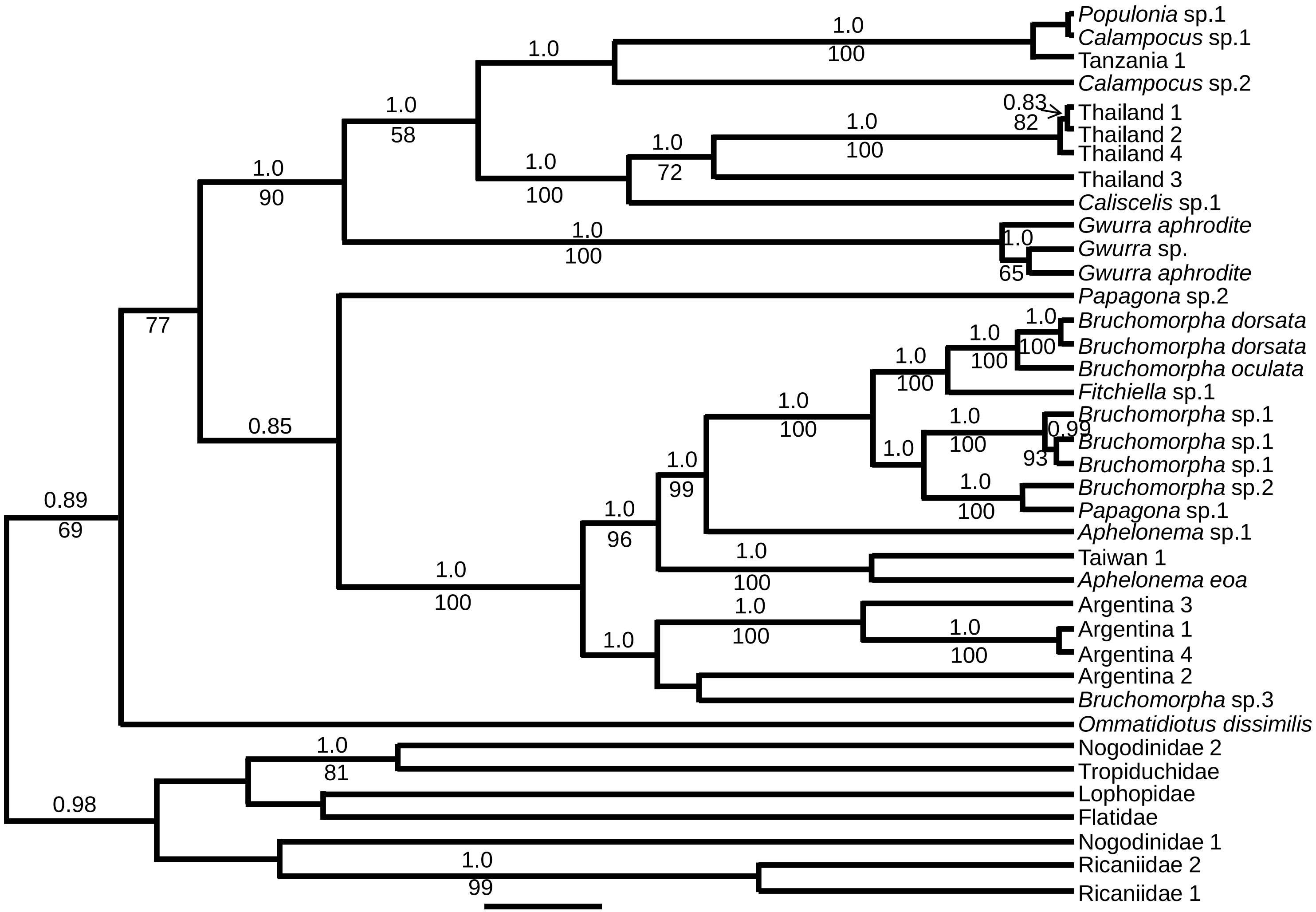

Figure 3.1: Molecular phylogeny of Caliscelidae and select outgroups using the combined dataset (12S, 16S, H3, and D2 region of 28S) analyzed using BEAST. Numbers above nodes represent posterior probability while numbers below the notes are parsimony bootstrap values. Missing values indicate less than 0.80 posterior probability or below 50% bootstrap support.

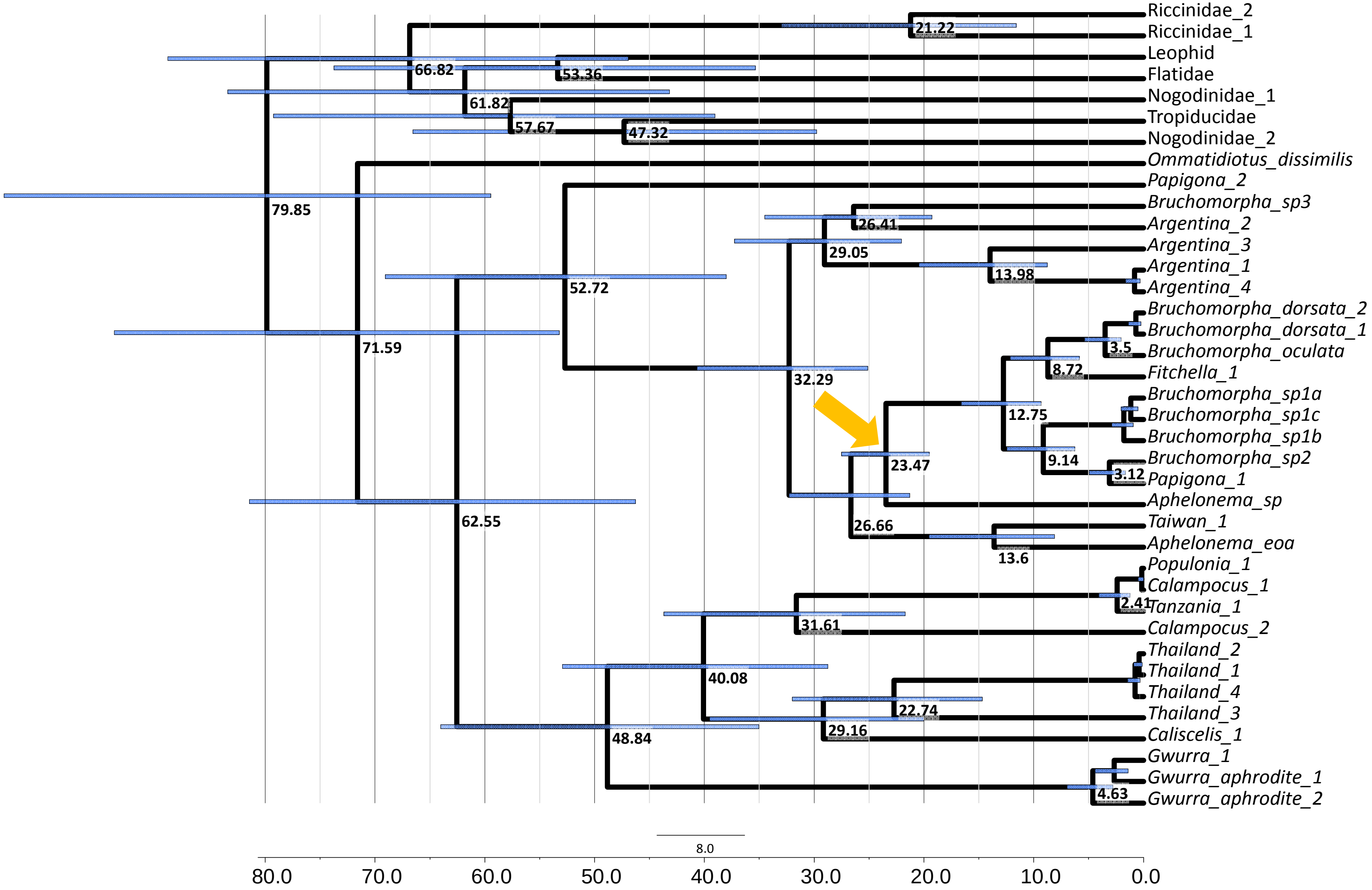

Figure 3.2: Results of BEAST divergence time estimation. Yellow arrow identifies a clade of Nearctic Caliscelidae which was used as a calibration point. Divergence time (in MYA, along X axis) are note at each node, node bar and numbers above each branch represent 95% confidence interval in this estimate

CHAPTER FOUR: A MOLECULAR PHYLOGENY OF HECALINI (HEMIPTERA: CICADELLIDAE: DELTOCEPHALINAE) WITH NOTES ON THE MONOPHYLY OF *HECALUS* AND THE PLACEMENT OF SOME GENERA

The leafhopper tribe Hecalini is a small group (23 genera and 180 species) of leafhoppers in the subfamily Deltocephalinae. Relationships within this group are poorly understood, and not all analyses support its monophyly (Zahniser and Dietrich 2013). While Hecalini are well represented in museum collections a comprehensive molecular phylogeny has not been attempted and the monophyly of some genera, particularly *Hecalus* is questionable. Although this tribe is distributed globally, most genera and species have narrow ranges, characters favorable for global biogeographic reconstruction.

Hecalines are medium to large leafhoppers, all with some degree of dorsoventral flattening. The crown is often produced and flattened, sometimes to the point of being concave in lateral view. Additionally, the ocelli are closer to the eyes than the laterofrontal sutures (Zahniser and Dietrich 2013). These leafhoppers are grassland specialists, and all are cryptically colored with green, yellow, or brown (occasionally with red or orange markings). This group contains monophagous and polyphagous taxa, although all known hosts are grasses. While most species appear to have relatively narrow host associations, a few widespread taxa are found on grasses that are distantly related (Hamilton 2000). Sexual dimorphism is common in Hecalini, particularly in terms of size (females being significantly larger). Also, some species have short and long winged forms (Hamilton 2000).

The status of Hecalini, the taxa placed within it, and its relationship to other tribes has not been well tested. There is considerable overlap of characters with the tribe Dorycephalini, species of which are also dorosventrally flattened with an elongated head, leading to genera being placed incorrectly (Hamilton 2000). Hamilton (2000) provided a thorough review of the classification and history of this and the morphologically similar tribe Dorycephalini. More recently, molecular phylogenetic analyses of Deltocephalinae by Zahniser and Dietrich (2010) based on 28S and histone H3 sequence data also pointed to a close relationship between Hecalini and Dorycephalini. However *Attenuipyga*, the exemplar Dorycephalini included in this analysis, has at times been placed within Hecalini so it was unclear if the entire tribe should be synonymized or if the genus itself is misplaced.

Recently, Zahniser and Dietrich (2013) inferred a phylogeny which included additional taxa (although no additional hecalines) which further supported the monophyly of Hecalini (although *Glossocratus* is occasionally not placed with the other Hecalini this has no support) while suggesting *Dorycephalus* was not closely related to Hecalini. As a result of this analysis, the concept of Dorycephalini was narrowed to include only the type genus, restricted to the Palearctic, and the two New World genera (*Attenuipyga* and *Neoslossonia*) previously placed in Dorycephalini were transferred to Hecalini (Zahniser and Dietrich 2013). Additionally, the placement of Hecalini itself was unresolved with different analytical methods resulting in it being closely related to Arrugadini, Macrostelini, or Athysanini and no position particularly well supported. However, in all instances Hecalini is represented by just a few exemplar taxa, which do not cover the range of diversity in this group.

Currently, the tribe includes 23 genera (180 species) divided between two subtribes: Glossocratina and Hecalina. Glossocratina is a monotypic subtribe containing the genus *Glossocratus*, which is recognized by the keeled laterofrontal sutures and the serrate second valvula (Zahniser and Dietrich 2013). This subtribe has an Old World distribution, although it is not found in Australia. The other subtribe, Hecalina, now contains the remaining members of the tribe and is distributed worldwide. Hecalina are recognized by the presence by a number of characters including an unkeeled laterofrontal suture, an ovipositor extending beyond the pygofer apex, and the second valvula without dorsal teeth. While most Hecalini genera are fairly restricted in distribution (often from only a single biogeographic region), two genera, *Hecalus* and *Memnonia* have representatives in both the New and Old World. In particular, *Hecalus* has over 70 valid species and occurs in all biogeographic regions, although it is sparsely represented in Australia and South America.

This paper represents the first extensive molecular phylogeny of Hecalini. Previous studies have included too few representatives of the tribe to provide an adequate test of its monophyly, and the placements of the majority of Hecalini genera have never been tested using molecular methods. Additionally, the placement of *Attenuipyga* and *Dorycephalus*, both of which have at times been placed either in Hecalini or within a separate subfamily is tested in this broader dataset. Finally, the monophyly of many genera, including *Hecalus* is tested.

Materials and methods

Specimen acquisition, taxon sampling, and DNA extraction - Ingroup sampling included 29 specimens representing 27 species in 13 different genera from all major grasslands regions of the world. In cases where genera are from multiple biogeographic regions or the monophyly of the genus is in question multiple species were included. This sampling includes two species of *Attenuipyga* (included by Zahniser and Dietrich (2010) in Hecalini), to further test the placement of this genus. As the monophyly of this group has not been tested with a dataset covering the range of Hecalini diversity nine outgroup taxa from six other grass-specialist Deltocephalinae tribes were included. These taxa were selected based on Zahniser and Dietrich (2010), which found Hecalini was in the a larger clade of Deltocephalinae comprising exclusively grass feeding leafhoppers but its position within this clade was not well resolved. Because Hecalini are relatively large bodied and can be abundant in grasslands, they are commonly collected by non-specialists, including many recent general biodiversity inventories, facilitating specimen availability. This has allowed for many genera to be represented by multiple species so their monophyly can be tested. In most cases DNA was extracted from recently collected specimens preserved in 95% ethanol and stored at -20°C prior to extraction. In a few instances fresh material was not available, so pinned specimens were used for DNA extraction. DNA was extracted from each specimen using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following a modified version of protocol for Total DNA from Animal Tissues. Modifications include lengthening the incubation period in step 2 to 36 hours and decreasing the amount of Buffer AE in step 7 to 50µ (which was repeated using a different 1.5mL collection tube, rather than a single tube). Because Hecalini are large leafhoppers, abdomens were removed for DNA extraction while the rest of the insect was mounted. Abdomens were punctured with 2-4 small holes to ensure buffers permeated the specimen. After extraction cleared specimens were placed in microvials with glycerin and stored with the point mounted thorax and head as voucher specimens. In general fresh specimens yielded better quality DNA, but sequences from pinned specimens were of high enough quality to be included in this study.

PCR and DNA sequencing - A pilot study of 8 genes (12S, 16S, histone 3, 28S, COI, COII, wingless, and arginine kinase) was first performed to select genes that amplified readily across the tribe, and to insure the genes were appropriate for phylogenetic study. Three genes (12S (401 bp), 28S (2716 bp), and Histone H3 (351bp)) were amplified and sequenced for all taxa. All PCR reactions were 25µL and used *Taq* polymerase (Promega, Madison, WI) ((see Appendix 2 and 3 for reaction conditions). Products were submitted for high-throughput sequencing at the Keck Biotechnology Center of the University of Illinois. Sequencher 4.8 was used to automatically assemble contigs (minimum match = 60; minimum overlap = 20), and each contig for a given gene was assembled into a single alignment and exported as FASTA file. FASTA files were aligned in seaview 4.3.0 (Gouy et al. 2010) using the built in version of MUSCLE (Edgar 2004) with all parameters set at default, except in the case of 12S which required a higher gap opening penalty in some regions where the original alignment resulted in high numbers of extraneous gaps. Resulting alignments were then adjusted by eye.

Phylogenetic analysis - ModelGenerator (nset=6) was used to select evolutionary models for each gene with the one yielding the highest Akaike's information criterion (AIC) score picked (Keane et al., 2006). Gene trees were inferred using 20 million generation BEAST runs and the model selected by ModelGenerator and default priors (Drummond and Rambaut 2007). Resulting gene trees were compared and as there was no conflict between well supported clades (using posterior probabilities greater than 0.90) data were combined as individual partitions. Sequences were trimmed to exclude primer regions, but no other regions were removed, and any gaps were treated as missing data. Complete phylogenies were inferred using a variety of techniques: parsimony (PAUP* 4.0b10: 10,000 random addition sequences, TBR branch swapping), maximum likelihood (Garli: 10 independent runs, default settings, and an automated stop criterion if *ln*L score remained constant for 50,000 generations), and Bayesian methods (MrBayes: 20 million generations, runs = 4 chains = 4; and BEAST: 40 million generations, tree prior = speciation: birth-death process; for both methods burnin = 25% and log files viewed in Tracer to ensure convergence was reached) (Swofford 2003; Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Both posterior probabilities and parsimony bootstrap values (1000 replicates of 100 random addition sequences) were calculated to estimate branch support.

Results

PCR amplification and sequencing alignment - Most taxa were represented by complete sequences of all three genes (See Appendix A). Failed reactions were repeated using different amplification protocols but continued to be unsuccessful. The final alignment included a total of 3482 characters, of which 2823 were constant, 220 were variable but parsimony-uninformative, and 439 were parsimony-informative (Table 4.1 contains a complete summary by gene).

Phylogenetic Analysis - Using AIC in ModelGenerator GTR + I + G was picked for both histone and 28S. AIC slightly favored K81uf + G over HKY + G (a difference in AIC scores of less than 0.4, while the third most favored model was about 2.0 points worse). Additionally HKY + G was selected over K81uf + G by the other criteria ModelGenerator uses to rank models. However, this model cannot be implemented in MrBayes or BEAST so HKY + G was used. Trees inferred from individual genes did not include any well supported topological conflicts and data was concatenated. Each gene was treated as a separate partition to allow for different models to be used for each gene.

Tree topologies were consistent regardless of analytical technique although minor differences, particularly towards the tips of the trees were found. All places where the parsimony or likelihood tree differed from the Bayesian tree bootstrap values showed the node in question to be unsupported (values less than 50), while the nodes in question were marginally supported (or better) in the Bayesian analyses (posterior probability greater than 0.80) so a single tree, based on the Bayesian analysis is shown here (Figure 4.1). The monophyly of Hecalini (including *Attenuipyga* but excluding *Hecalusina*) was consistently supported, although support values themselves were low. *Glossocratus* (and therefore the Glossocratina), was sister to the Hecalina and both subtribes were well supported. Within Hecalina there are two main clades, one containing only New World genera, and the other which is globally distributed. While most genera were found to be monophyletic, *Hecalus*, the most speciose and widely distributed genus was not monophyletic but rather formed two distinct geographically based clades. *Attenuipyga* was embedded within the Hecalina, and sister to a clade containing four strictly New World genera. Finally, Dorycephalini, represented here by one the two species included in the type genus, was firmly placed within the outgroup.

Discussion

The monophyly of Hecalini (with the inclusion of *Attenuipyga*) was upheld in all analyses. Additionally, Dorycephalini was not found to be closely related to Hecalini, further supporting the results of Zahniser and Dietrich (2013). Additionally, the monophyly of both subtribes, Glossocratina and Hecalina were both recovered and well supported (posterior probability of one for both, and bootstrap values of 100 and 96 respectively). Glossocratina only includes a single genus that is fairly diverse (29 valid species) and widely distributed in the Old World. This clade is quite divergent from the rest of Hecalini. Conversely, Hecalina includes approximately 150 species distributed worldwide. This subtribe contains two major well-supported clades.

One clade is found only in the New World, and contains *Spangbergiella*, *Neohecalus*, *Dicyphonia*, *Jiutepeca*, and *Attenuipyga*, with *Attenuipyga* as sister to the remaining members of this clade. Of these, *Spangbergiella*, *Dicyphonia*, and *Attenuipyga* were represented by multiple species, and all were found to be monophyletic. Additionally, this clade contains an unidentified species from Argentina which is sister to *Spangbergiella*. The second clade contains taxa from around the world, and includes *Hecalus*, *Parabolocratalis*, *Memnonia*, *Thomsoniella*, *Hecalusina*, *Linnavuoriella*, and a new genus of Oriental Hecalini. Of these all but *Parabolocratalis* and *Hecalusina* are represented by multiple specimens. Hamilton (2000) concluded that only the Holarctic species should be placed in *Hecalus* (although he then goes on to say there are approximately 40 true *Hecalus* species in the Old World, mostly found the Ethiopian (where the type species occurs) or Oriental regions; along with 9 Nearctic species). Additionally, he suggests that *Hecalus chilensis* McKamey and Hicks, 2007 (as *H. australis* Linnavuori and DeLong, 1977, *nec* Evans 1941) could potentially also be placed as a true *Hecalus*. However, based on the specimens included in my analysis *Hecalus* is instead divided into New and Old World clades.

The Old World clade (here composed of two species from Australia (including *H. australis*), two African species, and a currently unidentified southeast Asian specimen) is sister to the single representative of *Parabolocratalis* while the New World clade (containing 2 Nearctic species (*H. major* and *H. viridis* which were explicitly identified as true *Hecalus* by Hamilton) and an unidentified specimens also from the Nearctic) is sister to *Memnonia*. While *Memnonia* is currently known from both North America and Asia, only North American representatives were included in my phylogeny. Future work should include the Asian representatives of *Memnonia* to confirm the correct placement of these species. Interestingly, an undescribed Asian genus, is sister to *Memnonia* + New World *Hecalus*, so *Memnonia* having both New and Old World taxa could be valid. These two *"Hecalus*" containing clades form a clade although support for it was generally low. The clade containing *Thomsoniella*, *Linnavuoriella*, and an unidentified southeast Asian specimen was well supported. *Thomsoniella* was monophyletic and well supported in all analyses. Two specimens of *Linnavuoriella* (both identified as *arcuata*) were included in this analysis; and the unidentified specimen was placed in this group. Lastly, *Hecalusina*, a recently described genus was placed outside of Hecalini with quite high support. While it had been placed in Hecalini due to the presence of an anterior carina on the margin of the head and positioning of the laterofrontal sutures, a number of atypical characters of the male and female genitalia, wings, and general body form were noted. Analyses including a wider range of tribes will be required to place this genus to tribe.

Taxonomic Implications - The monophyly of Hecalini (with the exclusion of *Hecalusina*) and placement of *Attenuipyga* was confirmed. However, the globally distributed genus *Hecalus* was not monophyletic but rather partitioned into New and Old World clades. The type species of *Hecalus*, *Hecalus paykulli*, was not included in this analysis, but is a widespread Old World species. Synapomorphies are required to define these two clades of “*Hecalus*”. Argentina 1 is closely allied with *Spangbergiella*, representing either a new species or a sister genus. Further morphological study will be required to determine the correct placement of this species.

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Tables and Figures

	Total	Histone	28S	12S
Total	3482	353	2725	404
Constant	2823	252	2406	165
Parsimony Uninformative	220	15	154	51
Parsimony Informative	439	86	165	188

Table 4.1: PAUP Summary statistics for each gene

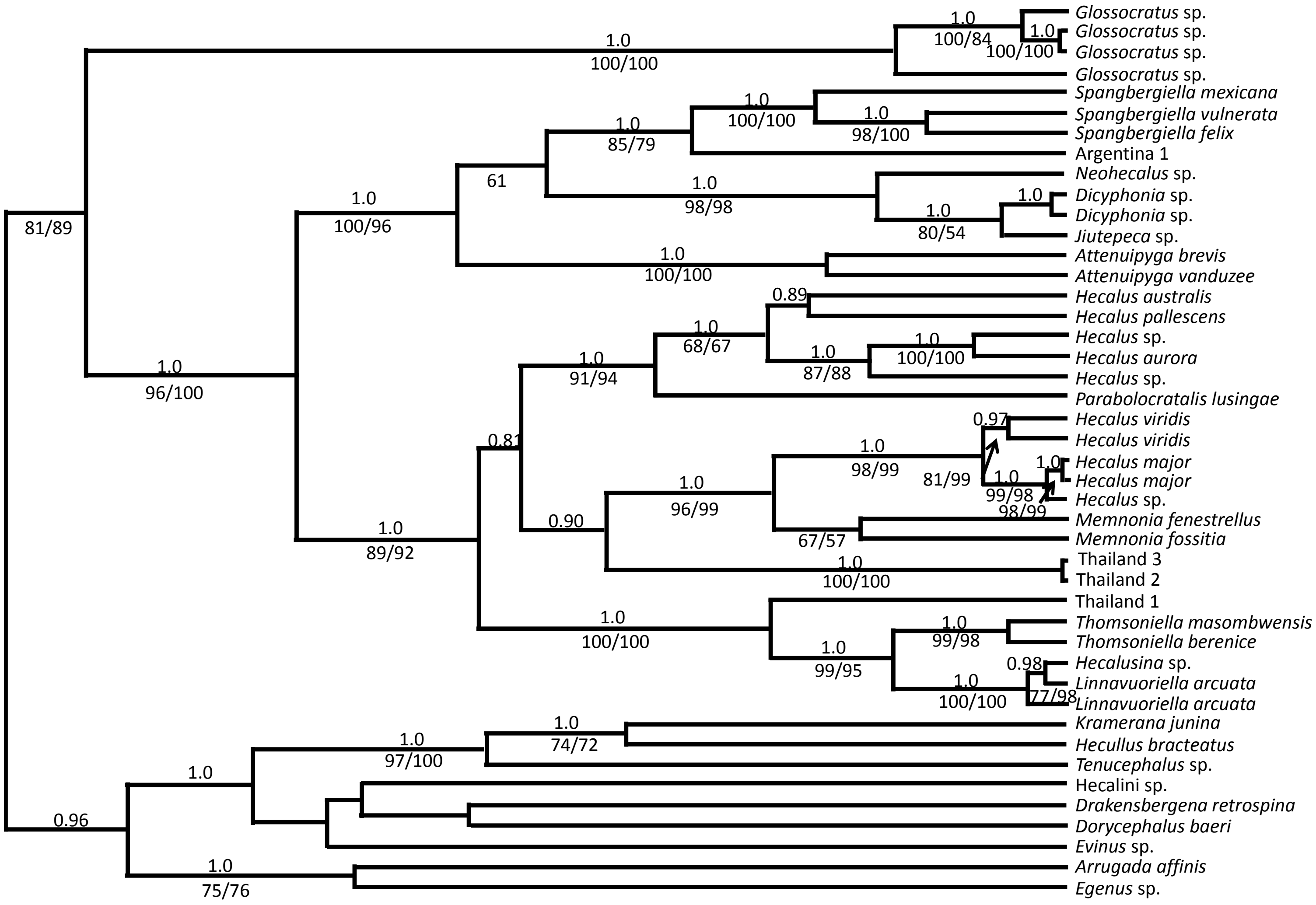

Figure 4.1: Molecular phylogeny of Hecalini and select outgroups using the combined dataset (12S, H3, and 28S) analyzed using BEAST. Numbers above branches represent posterior probability while numbers below the branches are bootstrap values (parsimony/likelihood). Missing values indicate less than 0.80 posterior probability or below 50% bootstrap support.

CHAPTER FIVE: A MOLECULAR PHYLOGENY OF DELTOCEPHALINE LEAFHOPPERS WITH LINEAR CONNECTIVES (HEMIPTERA: CICADELLIDAE: DELTOCEPHALINAE: DELTOCEPHALINI, PARALIMNINI, AND TETARTOSTYLINI)

The largest leafhopper subfamily, Deltocephalinae, contains over 900 valid genera. Of these, 139 are in the tribe Paralimnini, 68 are in Deltocephalini, and a single genus is placed within Tetartostylini (Zahniser and Dietrich 2013). Both Paralimnini and Deltocephalini are distributed globally while Tetartostylini is found only in the Palearctic and Ethiopian regions. All three groups are members of a large grass specialist clade and many have very narrow host associations, feeding on a single species of grass or at most a group of closely related grass species. Additionally, many important domestic crops are grasses, and insects that fed on their wild ancestors also feed on the domestic varieties. This results in some members of these tribes being pests and has led to accidental introduction of these pest species in novel areas (Nielson 1968). Although these lineages are incredibly diverse and well represented in collections, no large-scale molecular phylogeny had elucidated relationships within and between these tribes.

There is considerable overlap between these three tribes morphologically, and there are no defining synapomorphies. However, certain characters particularly in the head and male genitalia tend to be associated with a given tribe. These three tribes possess a linear connective (except in a few species, for example *Micrelloides polemon* which has a "Y" shaped connective) and previous analyses by Zahniser and Dietrich (2010, 2013) have suggested they form a single clade, although there is no firm support that the two larger tribes are reciprocally monophyletic or that Tetartostylini warrants tribal status. Deltocephalini is generally recognized by having a linear connective that is fused to the aedeagus, while both Paralimnini and Tetartostylini have a linear connective articulated to the aedeagus. However, in both Deltocephalini and Paralimnini there are examples of species having the nontraditional aedeagal attachment, for example a species of *Wyshinamia* (a member of Deltocephalini) that has an articulated aedeagus. All tribes also have the clypellus tapering or parallel-sided, the lorum narrower than the clypellus at the base, the anterior arms of the connective closely appressed, and the first valvula of the female with dorsal sculpture imbricate (Zahniser and Dietrich 2013). Both Paralimnini and Deltocephalini include brachypterous species while all species in Tetartostylini are macropterous.

This study will infer a molecular phylogeny of the clade of Deltocephalinae containing tribes with linear connectives (Deltocephalini, Paralimnini, and Tetartostylini) to test the monophyly of each group and the placement of individual genera. Additionally, the monophyly of select widespread genera will also be tested.

Materials and methods

Taxonomic history - Oman (1949) had a much broader concept of Deltocephalini than used today, including both Deltocephalini, Paralimnini, and parts of several other tribes. He split Deltocephalini (sensu Oman) into 4 groups based on the shape of the connective (linear or Y-shaped) and the attachment of the aedeagus to the connective (fused or articulated). Group 1 (following the group numbers of Fang et al 1993) contained those members of the tribe with a linear connective and fused aedeagus (currently the concept of Deltocephalini); group 2 contained members with a linear connective and articulated aedeagus (currently the concept of

Paralimnini); while groups 3 and 4 included members with Y-shaped connectives (Athysanini and others). Emeljanov (1962) erected the tribe Jassargini to receive members of the Deltocephalini (primarily members of group 2) that lacked carinae on the sides of the pronotum. However, the family-group name Paralimnini was already available, so Jassargini was in fact a junior synonym of Paralimnini.

Fang et al. (1993) undertook one of the earliest molecular phylogenies of Deltocephalinae. Using 21 taxa in 19 genera (all found in the New World) from Oman's group 1, 5 genera from group 2, two genera from group 3, and a distantly related species of *Macrosteles*, they sequenced 562 bases of the 3' end of 16S mitochondrial ribosomal gene [16S]. The resulting phylogeny found group 1 to be monophyletic, excluding *Cabrulus*, which was placed in the group 2 clade. The authors stated the placement of this genus had been problematic so this result was not surprising. The authors also found 16S was substitutionally saturated because transversions were more frequent than transitions in all but representatives of different populations of *Sanctanus balli*. Based on *Drosophila* studies by DeSalle et al (1987) showing a linear relationship between trasnsitions and percent divergence Fang et al. (1993) estimated that these taxa are relatively old (over 200 MYA) which would make the clade much older than even the earliest grasses.

Fang et al. (1995) used 76 morphological characters in adults to elucidate relationships among genera in the Deltocephalinae both to compare with a previously published molecular phylogeny (Fang et al. 1993) and as part of a combined DNA and morphology analysis. They included 25 Nearctic *Deltocephalus*-like genera (Oman 1949 group 1, all currently placed in Deltocephalini), along with 7 genera currently placed in Paralimnini (Oman 1949 group 2), and 3 more distantly related tribes. They concluded that group 1 was a monophyletic lineage, united by the presence of a fused linear connective, although in some analyses one Deltocephalini genus, *Cabrulus*, was placed inside the group 2 clade. Group 2 often formed a monophyletic lineage, although in some analyses one genus (not consistently the same one) could not be unequivocally placed in the clade. The authors also concluded that group 1 and group 2 were sister clades united by the presence of a linear connective. Lastly, they concluded that grass feeding is a derived state in Deltocephalinae while more basal members fed on a variety of dicots and woody shrubs. However, as this study only included Nearctic taxa and many genera were not represented, this finding could be an artifact of taxon sampling.

Kamitani (1999) produced a phylogeny of the Japanese Deltocephalinae, including a number of Paralimnini and Deltocephalini, which resulted in redefining the boundaries of the two tribes. He coded 64 morphological characters for 41 genera and concluded that Deltocephalini was composed of 2 paraphyletic lineages, and the members of one lineage were moved to Paralimnini. He identified 5 synapomorphies supporting the monophyly of the Deltocephalini tribe, 3 synapomorphies supporting the Paralimnini clade, and 4 synapomorphies supporting the Deltocephalini + Paralimnini clade. While most of Kamitani's characters had been previously used by other authors, he identified some novel characters and states. However, Kamitani did not include non-Japanese species except for two members of Deltocephalini and one of Doraturini (now Chiasmini), which were included because they were type species of the tribes of interest or because they possessed several unique characters.

Deltocephalinae was the subject of a phylogenetic analysis of morphological and molecular characters by Zahniser and Dietrich (2010). Zahniser and Dietrich coded 119 characters, including a number which had previously been neglected or underutilized in Deltocephalinae, including female genitalia and extensive studies of leg chaetotaxy, along with molecular data from 353 bp of the nuclear protein coding gene Histone 3 [H3]and 2908 bp of the 28S large subunit ribosomal gene [28S]. The resulting phylogeny led to the current understanding of Deltocephalinae to encompass approximately 6200 species and 36 tribes, including a number of groups previously recognized as separate subfamilies. They identified a clade containing all grass/sedge specializing tribes (and depending on analysis some non grass/sedge feeders). Relationships between these tribes were variable although the monophyly of many groups (including Paralimnini and Deltocephalini) was well supported, even though the relationships between tribes were often not well supported. Unfortunately, due to the breadth of this study taxon sampling within tribes was light and in many cases even hyper diverse tribes were represented by a few exemplars that were all from the same geographic region. For example both Paralimnini and Deltocephalini were represented by 5 taxa each, of which all but one in each tribe was collected in the United States. This geographic concentration on North America is not indicative of distribution patterns in these two tribes and could influence classification. Zahniser and Dietrich also found support for a single clade of grass and sedge specialists, which includes the majority of Deltocephalinae species. This clade contains only 1/3 of the tribes (including Deltocephalini and Paralimnini) although many of these tribes are quite diverse and include hundreds of species. This high species richness is possibly due to the high amount of host specificity exhibited by grass-feeding Deltocephalinae. While host plants are poorly known for the majority of species, in groups that have been intensely studied, such as the Paralimnini genus *Flexamia*, many individual leafhopper species feed on single species of grasses or at most a group of congeneric species (Whitcomb and Hicks 1988).

Most recently, Zahniser and Dietrich (2013) followed up on Zahniser and Dietrich (2010) with an analysis including additional taxa (although no additional Deltocephalini or Paralimnini). This analysis again found support for Deltocephalini and Paralimnini as closely related, monophyletic tribes. In all analyses the authors found Deltocephalini and Paralimnini to be sister tribes, but this was only well supported in the Bayesian analysis. This relationship could also be driven by morphological data, as partitioned Bremer support values show topologies with fewer steps were available for molecular only partitions while the morphological partition strongly supported a topology including Deltocephalini as sister to Paralimnini. This larger dataset again supported a single origin of grass/sedge specialization.

Specimen acquisition, taxon sampling, and DNA extraction - Ingroup sampling included approximately 40 Deltocephalini taxa, 90 Paralimnini taxa, and a single *Tetartostylus*. As species level keys are unavailable for much of the world's fauna many included specimens are identified only to genus, and 23 specimens are undetermined to genus (many of these are thought to represent new genera). While most species have narrow ranges, a number of genera are widely distributed, including a number with Holarctic ranges. In instances where taxa from different parts of the ranges were available multiple representatives of the genus were included to test the monophyly of these groups. This resulted in 20 different Deltocephalini genera and 40 Paralimnini genera (and an additional 20 unidentified specimens which are thought to be Paralimnini based on morphological characters) included in my phylogeny. Although the monophyly of each tribe has been well supported in various studies, the relationship between these tribes has not been tested using this wide of a range of taxa. For this reason, I also included 20 outgroup taxa, both taxa included in Zahniser and Dietrich (2013) (for which I also sequenced a 436bp region of the 12S mitochondrial ribosomal gene [12S], using genomic DNA provided by J. Zahniser, so that gene coverage of the two studies would match) and some newly included taxa. These outgroups were all found by Zahniser and Dietrich (2013) to be members of the large grass feeding clade (of which Deltocephalini and Paralimnini are members).

In most cases DNA was extracted from recently collected specimens preserved in 95% ethanol and stored at -20°C prior to extraction. In a few instances fresh material was not available, so pinned specimens were used for DNA extraction. DNA was extracted from each specimen using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following a modified version of protocol for Total DNA from Animal Tissues. Modifications include lengthening the incubation period in step 2 to 36 hours and decreasing the amount of Buffer AE in step 7 to 50µ (which was repeated twice in different 1.5mL collection tubes rather than using the same collection tube as the protocol calls for). As these tribes contain leafhoppers of variable size, two different extraction techniques were used. For most leafhoppers, abdomens were removed for DNA extraction while the rest of the insect was mounted. Abdomens were punctured with 2-4 small holes to ensure buffers permeated the specimen. After extraction cleared specimens were placed in microvials with glycerin and stored with the pin- or point-mounted thorax and head as voucher specimens in the insect collection of the Illinois Natural History Survey. In some instances however, DNA extraction was performed on the entire leafhopper, particularly in cases of exceptionally small leafhoppers. In these cases, 2-3 small holes were poked both in the abdomen and thorax, and a final hole was created between the thorax and head to allow buffers to enter the entire specimen. In these cases, the entire leafhopper was stored in glycerin in a microvial after extraction was completed. In general fresh, ethanol-preserved specimens yielded better quality DNA, although this was not always the case, as a number of pinned Kyrgyzstani samples yielded better sequences than those from the same series of collecting trips approximately 15 years ago but which had been stored in 95% ethanol at -20°C since collection. Completed extracts were stored at -20°C.

PCR and DNA sequencing - First, 9 genes (12S, 16S, H3, 28S, cytochrome c oxidase I and II, the nuclear gene wingless, NADH dehydrogenase I, and the nuclear gene coding for arginine kinase), which had been used in previous leafhopper studies, were tested in a pilot DNA sequencing study including a number of genera from each tribe representative of the diversity of the groups. PCR and sequencing for these taxa were undertaken to identify genes that amplified readily across the two tribes and to insure the genes were appropriate for phylogenetic study. Three genes (12S (417 bp), 28S (2748 bp), and Histone H3 (351bp)) were amplified and sequenced for all taxa. All PCR reactions were 25µL and used *Taq* polymerase (Promega, Madison, WI) (see Appendix B and C for reaction conditions). Products were submitted for high-throughput sequencing at the Keck Biotechnology Center of the University of Illinois. Raw forward and reverse strands of each sequence were aligned and assembled in Sequencher 4.8 (minimum match = 60, minimum overlap = 20) and manually adjusted using chromatograms. Each gene was then assembled into a single contig and exported to seaview 4.3.0 as a FASTA file. The built in MUSCLE aligner was used to produce multiple alignments with all alignment settings at default values followed, when necessary, by manual adjustments by eye (Edgar 2004, Gouy et al. 2010). In regions of the 28S and 12S alignment where the original computer alignment was problematic groups of sites were selected and realigned using a higher gap open cost and then rechecked by eye. Sequences were trimmed to exclude primer regions, but no other regions were removed, and any gaps were treated as missing data. All novel sequences will be deposited in GenBank. Outgroups were selected based on the phylogeny from Zahniser and Dietrich (2013) which identified a large clade of grass feeding leafhoppers which included Deltocephalini and Paralimnini. Outgroup sequences included all members of this clade (although only specimens with sequence data for each gene were used) with 28S and Histone sequence data provided by Zahniser and Dietrich with the addition of 12S which was sequenced from the same extracts.

Phylogenetic analysis - Each gene was first analyzed separately to insure that gene trees were not in conflict. This included selecting an evolutionary model for each gene using modelgenerator with the model having the best AIC score selected (Keane et al., 2006). Gene trees were inferred using 40 million generation BEAST runs under the model selected by modelgenerator. Resulting gene trees were compared and as there was no conflict between well supported clades (using posterior probabilities) data were combined as individual partitions. Complete phylogenies were inferred using a variety of techniques: parsimony (PAUP* 4.0b10: 10,000 random addition sequences, TBR branch swapping), maximum likelihood (Garli: 10 independent runs, default settings, and an automated stop criterion if *ln*L score remained constant for 50,000 generations), and Bayesian (MrBayes: 20 million generations, nrun=4, nchain=4 and BEAST: 40 million generations) methods (Swofford 2003; Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; Drummond & Rambaut 2007).

Both posterior probabilities and bootstrap values (MP: 1000 replicates of 100 random addition sequences; ML: 500 replicates of 10 runs, automated stop criterion if *ln*L score remained constant for 50,000) were calculated to estimate branch support. Because some taxa were missing large portions of sequence data (for example all of 28s) phylogenetic analysis was also performed on a dataset in which each included taxon was represented by at least 2 of the three genes. Lastly, to further explore the relationships among these taxa and Deltocephalinae as a whole my data set was added to the Zahniser and Dietrich (2013) data set of 152 taxa (with the addition of 12s) and analyzed as above.

Results

PCR amplification and sequencing alignment - Most taxa were represented by complete sequences of all three genes (see Appendix A). Each reaction that failed was repeated using various PCR conditions but continued to fail. Table 5.1 includes summary statistics for each gene and the total dataset.

Phylogenetic Analyses - Using AIC in modelgenerator GTR + I + G was picked for both histone and 28s while GTR + G was favored for 12s. Trees inferred from individual genes did not include any well supported topological conflicts so genes were concatenated. Each gene was treated as a separate partition to allow for different models to be used for each gene.

Tree topologies were largely congruent between analytical methods (with incongruences generally weakly supported) and broadly speaking both Paralimnini and Deltocephalini are monophyletic clades which were well supported in all analyses. All topologies included a well-supported (Posterior Probably = 0.99) clade containing all sampled members of Deltocephalini, Paralimnini, and Tetartostylini (sensu Zahniser and Dietrich 2013), excluding *Agudus*, a South American genus typically placed within Paralimnini (Figure 5.1). The placement of *Agudus* (here represented by 4 species) was equivocal, in this dataset it was placed with *Scaphotettix* (tribe Mukariini) while in a larger dataset not included here, it was placed as the sister to all other Paralimnini. The tribe Tetartostylini (represented here by a South African species) was placed within Deltocephalini, although often in a relatively basal position. In most analyses this finding was well supported, however under parsimony the placement of Tetartostylini was unresolved (but still placed within the Deltocephalini). Paralimnini (excluding *Agudus*) was constantly recovered as the sister to Deltocephalini + Tetartostylini, and this arrangement was well supported (Posterior Probability = 0.99). Within both the Deltocephalini and Paralimnini there are a number of clades, many of which are to some extent geographically clustered. Additionally, in many instances where multiple species from a genus were included they did not form monophyletic groups, which is not surprising since the ranges of many such taxa span multiple continents.

Some analyses or different combinations of included taxa lead to some taxa being placed in unexpected portions of the tree. The genus *Agudus*, a Neotropical genus currently placed within Paralimnini was never placed with support inside Paralimnini. In fact, in all but a single analysis (Bayesian with all taxa from Zahniser and Dietrich (2013) only including taxa with at least 2 genes) *Agudus* was placed in a clade which is sister to Deltocephalini + Tetartostylini + Paralimnini.

Discussion

Paralimnini and Deltocephalini are closely related Deltocephalinae tribes that are morphologically similar. Additionally Tetartostylini, a tribe of 11 species in a single genus that shares many characters with both Deltocephalini and Paralimnini, is thought to be closely related to these tribes based on molecular and morphological phylogenies (Zahniser and Dietrich 2013). My study found good support (posterior probability of .99) for a clade containing these three tribes and the placement of Tetartostylini within Deltocephalini. Previous studies had suggested a close relationship among these three tribes, but limits to taxon sampling and missing data in these studies may have resulted in artificially upholding the tribal status of Tetartostylini. This should be further tested with the inclusion of more representatives both of Tetartostylus and Palearctic representatives of Deltocephalini. Additional genes could also shed further light on the relationships among these tribes. Analyses of the entire dataset (our dataset plus that from Zahniser and Dietrich (2013)) supported Deltocephalini + Tetartostylini as sister to Paralimnini.

The placement of *Agudus* is equivocal, and my dataset did not provide support for its inclusion within Paralimnini or even the Paralimnini + Deltocephalini + Tetartostylini clade. Additional genes and the inclusion of more South American taxa are required to better elucidate the placement of *Agudus*.

Various genus groups were recovered in my analyses although often genera with New and Old World representatives were not monophyletic. I found evidence for a close relationship between a number of Old World genera with a well-supported (posterior probability = 1.0, MP bootstrap = 95) clade including *Maiestas* and the Old World representative of *Deltocephalus* as suggested by Webb and Viraktamath (2009). The second major clade (posterior probability = 1.0, MP bootstrap = 99) recovered in Deltocephalini includes 14 genera of Nearctic Deltocephalini (some of which include Neotropical species), including the New World representatives of *Deltocephalus*. Also embedded in this clade was *Toldoanus*, a South American monotypic genus and two unidentified South American specimens (one of which is morphologically similar to *Lonatura*, a Nearctic genus also included in this clade). The only other Neotropical Deltocephalini specimens were placed in a clade containing Oriental and Australian species although support for this clade was low (posterior probability = 0.88). Paralimnini also included a number of well supported clades which in general were geographically restricted by biogeographic region.

Sorhoanus, a Holarctic genus was not monophyletic, with the Neartic representative, *Sorhoanus orientalis* clustering with other Nearctic genera while the representative from Kyrgyzstan is placed with other Palearctic taxa. This finding was repeated in *Deltocephalus*, where included New and Old World species were not recovered as monophyletic. However there are examples, such as *Psammotettix*, where a true Holarctic distribution is supported in my phylogeny. The monophyly of these and the many other genera occurring across many biogeographic regions should be further tested with the addition of more taxa, particularly in the case of *Deltocephalus* and *Psammotettix*, which are highly diverse and occur worldwide. In general the inferred phylogeny supports the monophyly of Paralimnini and Deltocephalini and excluding a few exceptions characters of the male genitalia can be used to place genera to tribe. I also found Tetartostylini was placed within the Deltocephalini although it has male genitalia similar to Paralimnini.

Taxonomic Implications - My analysis places Tetartostylini within the Deltocephalini with high branch support suggesting Tetartostylini should be synonymized with Deltocephalini (which has priority). The monophyly of Deltocephalini (with the inclusion of Tetartostylini) and Paralimnini (with the exclusion of *Agudus*) was well supported. *Agudus*, a South American genus, requires further investigation to resolve its placement with any certainty. Although there are a few exceptions (i.e. one species of *Wyshinamia*) members of Paralimnini possess an aedeagus articulated with connective while Deltocephalini has it fused to the connective. However, *Tetartostylus* possesses an aedeagus which is articulated to the connective, suggesting that this character is more complex than previously realized.

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Tables and Figures

	Combined	28S	Histone	12S
Total	3485	2699	351	436
Constant	2442	2092	236	114
Parsimony Uniformative	419	336	26	58
Parsimony Informative	624	271	90	263

Table 5.1: summary statistics for each gene

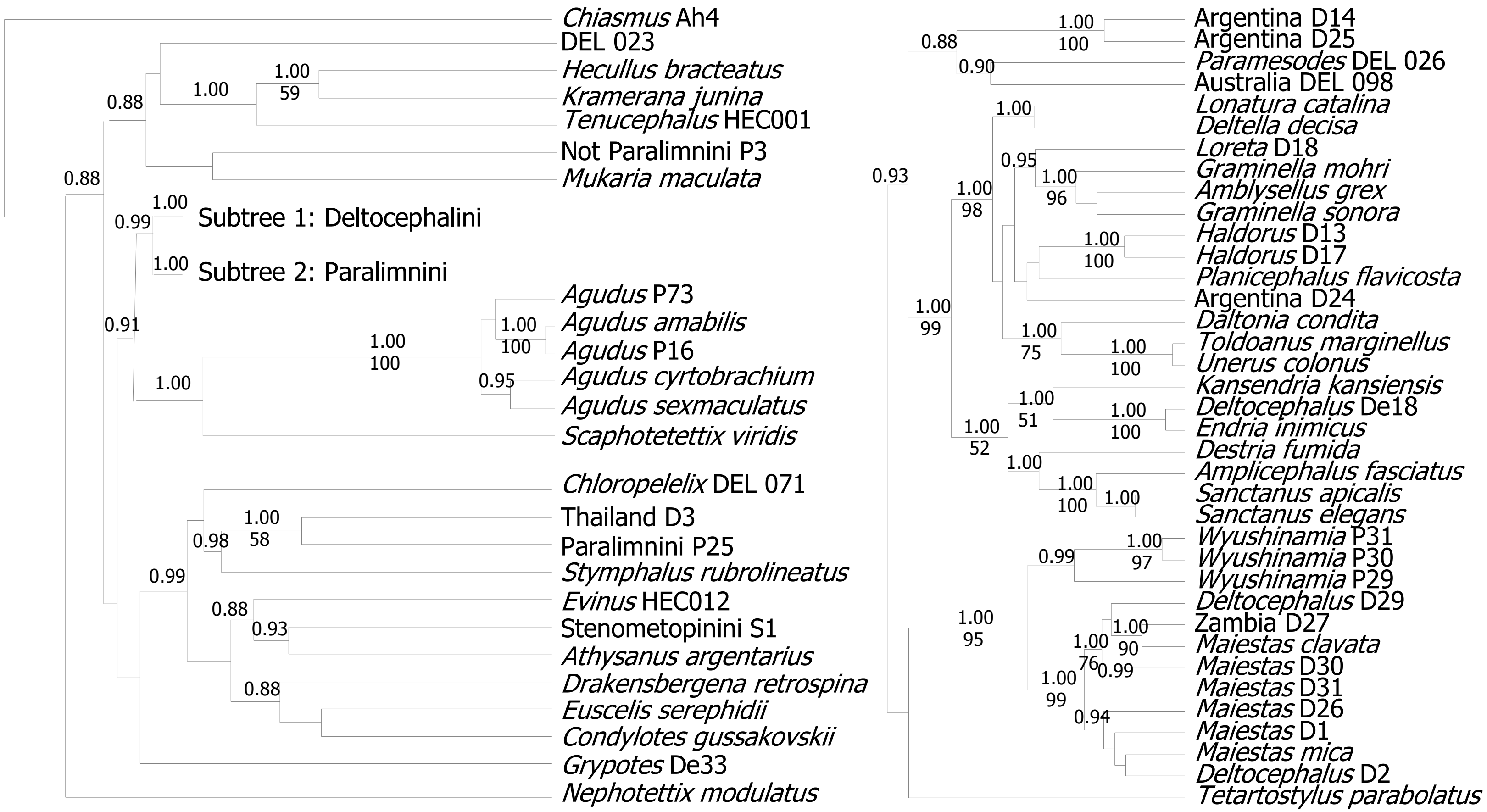

Figure 5.1: Molecular phylogeny of Deltocephalini, Paralimnini, Tetartostylini, and select outgroups using the combined dataset (12S, H3, and 28S) analyzed using BEAST. Numbers above nodes represent posterior probabilities while numbers below the notes are bootstrap values. (parsimony) Missing values indicate less than 0.80 posterior probability or below 50% bootstrap support. Subtree 1(Deltocephalini, on left) and Subtree 2 (Paralimnini) on following page.

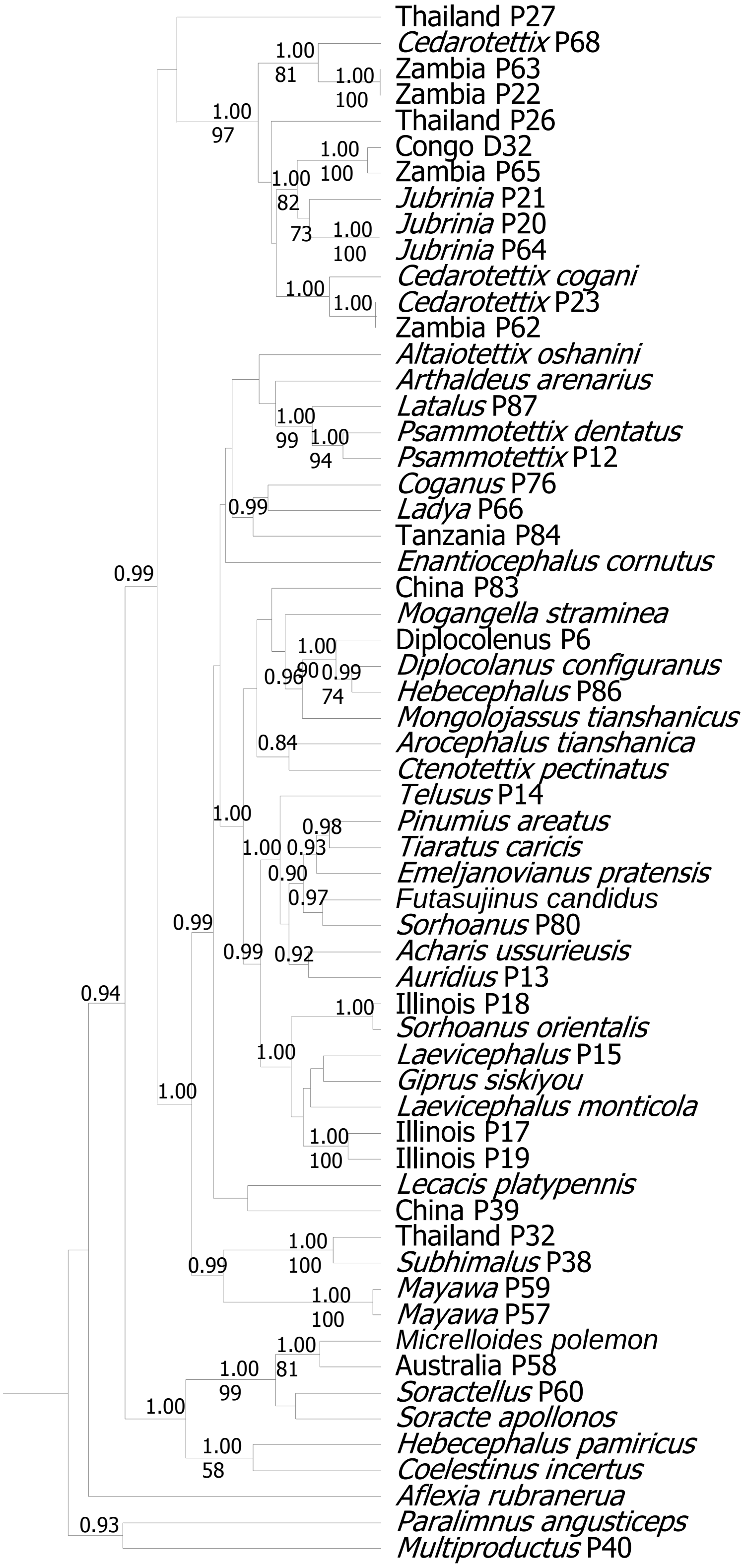

Figure 5.1: (continued)

CHAPTER SIX: BIOGEOGRAPHY OF GRASSLAND ORGANISMS

While the study of global-scale grassland biogeography is in its infancy, research on individual clades of grassland species has identified some interesting patterns. For example, while temperate grasslands are dominated by perennial bunchgrasses, annual grasses dominate in tropical areas. Shaw (2000) and Groves (2000) compared grass species composition of tropical and temperate grasslands and found plants in temperate grasslands are more phylogenetically related to those of other temperate grasslands than to those occurring in more closely located tropical grasslands. These studies, however, were descriptive in nature and did not employ rigorous biogeographic methods. Peterson et al. (2010) inferred a phylogeny of the grass subfamily Chloridoideae that suggests an African or Asian origin with subsequent invasions of Australasia and the Americas, although these findings also were based on an intuitive interpretation of the phylogenetic trees rather than formal biogeographic analysis. The most derived clade includes a number of tribes found mainly in the New World, including Muhlenbergiinae, which is thought to have originated in North America and later invaded South America.

In animal lineages that originated and diversified exclusively within grasslands, and have at times been globally distributed, a phylogeny-based biogeographic study to reveal global patterns is appropriate. Relationships among grassland animals at a global scale, and the processes that created the associated biogeographic patterns are essentially unknown outside ungulate mammals (Hassanin & Douzery 2003, Maguire & Stigall 2008). Various ungulate groups have been studied in a phylogenetic and biogeographic framework. The best known are the equids which are believed to have originated in North America and then dispersed into Eurasia via the Bering Land Bridge (MacFadden 1992). A similar path was seen in camelids, which also originated in the New World then dispersed to Eurasia and South America via the Bering land bridge and the Isthmus of Panama respectively. Interestingly, cervids follow the opposite pattern of dispersal, originating in central Asia and then crossing the Bering Land Bridge during the Miocene- Pliocene boundary (Gilbert et al. 2006). Bovids also appear to have an Old World (possibly eastern African or Middle Eastern) origin with dispersal into the New World via the Bering Land Bridge (Bibi 2011). These studies are not without their limitations mainly stemming from the natural history of the focal organisms which had limited dispersal opportunities (i.e., land bridges), and comprise clades consisting of a few, mostly widespread, species. This arrangement of few species and large ranges limits studies to the observation of large-scale biogeographic patterns. In contrast, many grassland insects contain widespread subfamilies or tribes often with hundreds of species which themselves have very narrow ranges allowing more fine scale patterns to be observed.

Although insects are one of the most diverse lineages on earth, relatively few groups diversified in close association with grasslands. However, the few clades that did diversify in grasslands tend to be highly speciose. Jameson et al. (2007) focused on the scarab beetle subtribe Anisopliina, which feeds on various parts of grass plants (seeds, pollen, roots) at all life stages. This subtribe is distributed in the Palearctic, Oriental, Ethiopian, Nearctic, and Neotropical biogeographic regions, and contains approximately 100 species. Based on phylogenetic analysis of 91 morphological characters Jameson et al. concluded that Anisopliina itself was not monophyletic and instead only composed a clade of Mediterranean species.

However, the authors were able to propose a potential biogeographic pattern based on the discovered sister group relationships between their geographically restricted Anisopliina clade and a New World species, *Callirhinus metallescens*. Based on the inferred phylogeny, the authors suggest a New World origin of the clade with subsequent spread and radiation in the Old World. More recently, Zahniser (2008) found that Chiasmini (a lineage of grassland leafhoppers which includes 21 genera distributed worldwide) includes 2 clades, both of which originated in the Old World and independently colonized and diversified in the New World, although more fine scale patterns were ambiguous. Additionally, the relationships between C_3 and C_4 feeding species have been examined in one planthopper tribe (Delphacidae: Delphacini) found in grasslands. Urban et al. (2010) found that the earliest diverging lineages are primarily C_3 feeders while the most derived clade primarily contains C_4 feeders. They also suggest that this switch between grass types is at least partially responsible for the diversification of the group.

The hemipteran suborder Auchenorrhyncha (leafhoppers, planthoppers, cicadas, and relatives) contains a number of previously identified monophyletic groups that are restricted to grasslands. These include clades within the planthopper families Caliscelidae (202 species) and Delphacidae (2086 species), the froghopper family Cercopidae (1500 species), and 12 leafhopper tribes (totaling ca. 2264 species). Some of these groups are particularly suited to biogeographic reconstruction as they are highly diverse and distributed throughout the grassland regions of the world. As far as is known, all are host-plant specialists on grasses and many utilize a narrow range of grass species. This leads to many species having highly localized distributions, although at the tribal level they are widespread across many biogeographic regions. This mix of narrow species ranges with widespread lineages allows for finer-grained resolution of grassland biogeographic patterns. Conversely, birds, mammals, and grasses themselves have characters that tend to obscure biogeographic patterns because they include many widespread species, are limited to a few zoogeographic regions, or have relatively few species.

Based on previous studies, grassland-specialist lineages have arisen in various parts of the world, and historical patterns of dispersal vary among these groups, even among related lineages. For example, while camels and horses both have New World origins with subsequent dispersals into other regions, bovids do not follow this trend. Instead bovids originated in the Old World, though the timing of the radiations of camels, horses, and bovids are different. It has even been suggested that the spread of horses into the Old World actually drove the radiation of bovids as horses ate the coarser grasses allowing access to more nutritious vegetation (Janis 1982). The two grassland insect clades that have been studied in a biogeographic framework (based on intuitive assessments of the phylogeny) also suggest these lineages arose in different regions. These previous analyses did not include attempts to estimate times of origin of grassland-associated clades.

My dissertation infers global biogeographic patterns for three lineages: the planthopper subfamily Caliscelinae and two leafhopper groups: Hecalini and Deltocephalini + Paralimnini to better understand how grass specialist lineages diversified in grasslands. Each of these groups is thought to represent an independent lineage, is quite speciose, and has representatives in each biogeographic region, although numbers of species in each region vary greatly (Zahniser & Dietrich 2010; Zahniser and Dietrich 2013). Additionally, these groups are well represented in recently collected material, facilitating specimen availability. To test biogeographic hypotheses I also performed divergence time estimation in order to not only test the plausibility of the scenarios proposed, but also to look for broad temporal patterns across lineages, which has not previously been attempted for grassland insects.

Methods

Molecular phylogenies were inferred for each lineage of interest (Caliscelinae, Hecalini, and Deltocephalini + Paralimnini) (see Chapters 3, 4, 5 for detailed methods). Additionally, because a single origin of grass feeding has been inferred in Deltocephalinae (which includes Hecalini, Deltocephalini, and Paralimnini) my data were combined with Zahniser and Dietrich's (2013) dataset representing all tribes and all but two subtribes (Opsiini: Achaeticina and Paralimnini: Aglenina) of Deltocephalinae. The mitochondrial ribosomal gene 12S was sequenced as described in previous chapters from extracts used in Zahniser and Dietrich (2013). After exclusion of taxa missing entire genes this dataset contained 279 taxa (4 outgroups, 140 Deltocephalini, Paralimnini, and Hecalini, with the remainder being representatives of other tribes in Deltocephalinae). This combined dataset allowed me to explore the effect of outgroup choice and taxon coverage on inferred biogeographic patterns and divergence time estimation.

Range coding - The Earth's terrestrial surface is often broken into six biogeographic regions (Australasian, Ethiopian, Nearctic, Neotropical, Oriental, and Palearctic, reflecting long term isolation making them home to distinct lineages and endemic taxa. These regions were treated as areas in the biogeographic analyses. The known range of each specimen was coded into a multistate character matrix in a variety of ways to explore the effects of coding methods

(Appendix A). Introduced species were always coded to reflect their native range and specimens that could not be identified were coded based solely on the exemplar. In instances where a genus or species was known from multiple biogeographic regions, this case was coded as a polymorphic character.

Character coding was as follows:

Method 1: The collecting locality for each specimen was coded, resulting in each tip having exactly one area of distribution. This method is an oversimplification because some species in my dataset are known to have ranges extending in multiple biogeographic regions. In the case of poorly known taxa, the range may be much broader than specimen data would indicate.

Method 2: The known native range was coded for each species, which resulted in each tip having ranges between 1 and 3 regions.

Method 3: The known range of each included genus was coded.

These broader coding methods were utilized because taxa (particularly the outgroups) were used as exemplars so coding based on individual species resulted in misrepresenting the known geographic diversity of a group, for example Drabescini, a widespread Old World tribe is represented here by three species, all from Taiwan although each genus occurs in multiple biogeographic regions. Coding methods 1 and 2 would result in each Drabescini tip occurring in a single region while method 3 would result in these tips having wider ranges. Exemplar based coding has drawbacks, particularly in poorly known lineages as it assumes monophyly of taxa which may not be tested. I incorporated two ways to guard against inclusion of ranges from non-monophyletic taxa. First, when possible, if a genus was recorded from multiple regions additional specimens were included to represent the different areas so monophyly could be tested. Secondly, each multiregional coding was evaluated on a case-by-case basis, and ranges constrained to the core range if outlying taxa were suspected to be misplaced or a result of dubious records (this occurred for 21 of 279 terminals, although many of these are at the tribal level so a single change affects multiple specimens). Many of these instances are recently described taxa which have resulted in the known range of the genus expanding; typically this has resulted in genera previously known only from the New World now containing a single Old World species. Other instances are related to the tribe Selenocephalini, which is known only from the Old World with the exception of *Citorus rugipennis*, a species described from Argentina based on a single specimen. By removing these records, limiting introduced taxa to their native ranges, and identifying instances of nonmonophyly at the genus level, an accurate biogeographic reconstruction is possible even when all members of the group are not available for inclusion.

Biogeographic study - Biogeographic patterns were identified using RASP (Reconstruct Ancestral State in Phylogenies) using Statistical Dispersal-Vicariance Analysis (S-DIVA), Dispersal-Extinction-Cladogenesis (DEC), and Bayesian Binary MCMC (BBMCMC) (Ronquist, 1996; Ree, 2008; Yu et al. 2011), and Mesquite (Madison and Madison 2011). While these programs all map geographic distributions of modern taxa on a phylogenetic tree and infer range patterns for historical nodes, the assumptions in each program are different as are the techniques used to infer ancestral ranges.

S-DIVA, which is based on Dispersal-Vicariance Analysis (DIVA) (Ronquist 1997), was the first major attempt to explicitly reconstruct ancestral ranges. This method assumes vicariance is the simplest explanation for historical changes in species distributions, and so a vicariance event is favored over dispersal or extinction (which are both penalized). However, this assumption is false in some instances including global grassland biogeography, because grassland formation did not begin until after the continents had already broken apart and large grasslands tend to have developed in isolation from each other (although vicariance is possible at a regional level as grasslands are subdivided by climate change or formation of barriers). Because of its preference of vicariance over all else, reconstructions tend to result in ancestral nodes with widespread ancestors, often one found globally. To account for this bias, each analysis was performed twice, one in which ancestral ranges were unlimited (typically results in widespread ancestors) and one where these ranges were limited to the maximum number of ranges observed in a single tip, which assumes range size is similar to those observed in taxa today.

Using RASP, I performed BBMCMC, which allows for phylogenetic uncertainty to be taken into account rather than assuming a given topology is fixed and correct. For the BBMCMC analysis I set cycles = 5,000,000; chains = 10; frequency of samples = 1000; discard = 1000. For all analyses in RASP, the BEAST tree file from the preferred analysis in previous chapters was loaded and condensed using a 25% burnin. This tree was then used in character reconstructions.

Because the distribution patterns of grassland taxa are more likely to be explained by dispersal than vicariance, I also used Mesquite to reconstruct ancestral states (which assumes all changes in biogeographic patterns are due to dispersal) using parsimony (Sanmartin et al. 2008). This was done by character mapping on the preferred Bayesian topologies inferred in each of the previous chapters. While several most parsimonious reconstructions were identified, the differences were located at the very tips of the trees and did not affect basal nodes.

Ree et al. (2005) developed the likelihood based approach DEC (implemented in the software package lagrange) which allowed a taxa to be found in multiple regions, and while dispersal and extinction are free parameters, cladogenesis models are not. For example, a widespread ancestor could diverge into two, one of which was limited to a single part of the range while the other inherited the remainder of the range. Because DEC does not treat vicariance as the null model over dispersal or extinction, widespread ancestors are not as commonly reconstructed (Ronquist and Sanmartin 2011; Webb and Ree 2012). DEC also allows users to create dispersal matrices (which can be different for various time periods) taking into account the changing connectivity of regions. DEC can be run either with user-defined matrices or with a default dispersal matrix where connectivity between regions is treated as equal. Defining a matrix can be useful in instances where taxa are limited to certain kinds of dispersal (such land bridges, the times of appearance/disappearance of which can be dated) but, because insects are less constrained in their movements (rather can be blown between regions over oceans or other boundaries), I chose to use the default matrix.

Divergence time estimation - Biogeographic events such as mountain building and island formation are commonly used as calibration points for divergence time estimation, based on the assumption that a given species or group could not have originated before the area they are currently restricted to originated (Weir and Schluter 2008; Heads 2006; Worobey et al 2010). This assumption is not without its drawbacks, including the possibility of extinct species living outside the selected region and the often large uncertainty in the timing of biogeographic events (Wilke et al 2009). Based on the assumption that a grass feeding species could not have colonized and radiated in a region prior to the expansion of grasslands, I used dates of the first grassland ecosystem in a given biogeographic region a reported by Strömberg (2011). To do this, coding method 3 from the range coding section above was mapped on the consensus trees for three different analyses- the Hecalini tree from Chapter 3, the Deltocephalini + Paralimini tree from Chapter 4, and the combined 279 taxon tree described above. Clades known from single biogeographic regions with a widely accepted date of first grassland formation were identified and these dates were then used in a BEAST divergence time estimation analysis under a relaxed lognormal molecular clock prior. For the Hecalini-only tree I used a single calibration point: 24 MYA mean, and 3 MYA standard deviation (normal distribution) for the largest clade containing *Hecalus viridis* and *Hecalus major* but excluding *Memnonia fossitia* (to create a Nearctic only clade). For the Paralimnini + Deltocephalini tree I used 3 calibration points: the largest clade containing Paralimnini 26 and Paralimnini 64 but excluding Paralimnini 59 (normal distribution with a 16 MYA mean, and 2 MYA standard deviation; Ethiopian clade); the largest clade containing *Laevicephalus monticola* and *Giprus siskiyou* but excluding *Sorhoanus orientalis* (normal distribution with a 24 MYA mean, and 3 MYA standard deviation; Nearctic clade); the clade containing all representatives of *Agudus* (normal distribution with a 39 MYA mean, and 2 MYA standard deviation; South American clade). For the 279 taxa tree I also included a clade containing *Aflexia rubranerura* and *Flexamia areolata* (normal distribution with a 6 MYA mean, and 1 MYA standard deviation; C_4 plant specialists). Ideally, many calibration points should be used and spread throughout the tree. However, because setting node ages to the age of oldest grassland in an area could bias dates to the favored hypothesis of a tight correlation between the origin and spread of grasslands and the diversification of these leafhoppers, I chose to use a limited number of calibration points and then compare dates calculated for other nodes to be compared to the dates of major events in grassland history such as the rise of grasslands on other continents or the development of C_4 photosynthetic pathways.

Results

Caliscelidae - An Old World origin of Caliscelidae was recovered, with the Palearctic region being the most preferred under BBMCMC. The ancestor for Caliscelinae was less resolved, although an Old World (most likely Ethiopian) distribution was preferred. This differed from SDIVA which reconstructed a widespread Old and New World ancestor for Caliscelidae and an Ethiopian + Palearctic origin for Caliscelinae when numbers of regions per node were limited to two regions per ancestral node or a widespread Old and New World ancestor at both nodes when limits were not enforced. DEC favored a Palearctic range for the origin of Caliscelidae although it also included New + Old World distributions as less favored options. The clade containing primarily New World taxa (with the inclusion two Old World taxa which diverged from the North American group about 26 MYA) is inferred to favor a Nearctic distribution (BBMCMC) or either a Nearctic or Nearctic + Palearctic distribution (DEC). The Neotropical group diverged from the Nearctic group around 32 MYA, and the +/- 8MYA confidence interval encompasses periods where these two regions are bridged by an archipelago (Gingerich 1981; Lissinna 2005; Wegner et al. 2011). The strictly Old World clade is inferred to have an Ethiopian ancestor (BBMCMC), which spread to the Palearctic then Oriental Regions while DEC inferred a more widespread (Palearctic + Ethiopian) distribution. As expected, SDIVA (and to a limited extent DEC) favored widespread ancestors for all nodes excluding the terminal tips, which tend to be geographically clustered, and so support a reconstruction of ancestors with the same distributions. Ancestral state reconstruction in Mesquite also favored more widespread distribution including the ancestor of Caliscelidae having a Palearctic + Ethiopian distribution and Caliscelinae having a Palearctic + Ethiopian + Nearctic distribution.

Hecalini - All biogeographic reconstructions favored an Old World origin for Hecalini, although the different methods preferred different origins, and in some cases also included the Nearctic region as part of a widespread ancestral range (see Figure 6.1 for the tree with divergence time estimates and Table 6.1 for a summary of key nodes; note that divergence time estimates based solely on the Hecalini tree are substantially older than grasses and do not fit with prior knowledge of leafhopper evolution, so dates referenced here are from the subfamily tree). Glossocratina was inferred to have a Palearctic + Oriental ancestral range while Hecalina was inferred to have either a Nearctic + Ethiopian or Nearctic + Oriental ancestral range. These areas were not in contact with each other during the time at which this divergence took place (47 MYA) making this hypothesis unlikely. At this same node, both BBMCMC and Mesquite using range coding method 3 inferred an Oriental ancestral range for the subtribe, which possibly predates the evolution of grasslands (although not grasses) in this region by tens of millions of years. All methods strongly favored a Nearctic origin for the strictly New World clade (although DEC and Mesquite under certain conditions favor a widespread New World ancestral range) while results were variable for the mixed clade with Oriental region only or Nearctic + Ethiopian being the most commonly reconstructed ancestral range. The Old World *Hecalus* + *Parabolocratalis* clade was most commonly inferred to have an Ethiopian ancestral range, while the New World *Hecalus* + *Memnonia* + New Oriental genus had an ancestral distribution which was Oriental, Nearctic, or both. Mesquite, SDIVA, and BBMCMC strongly supported an Oriental origin for the clade containing *Thomsoniella* and *Linnavuoriella* while DEC favored an Ethiopian + Oriental ancestor.

Deltocephalini + Paralimnini - Most analyses inferred a Palearctic origin for both Deltocephalini + Tetartostylini and Paralimnini (see Table 6.2 for reconstructions and divergence time estimates for select nodes and Figure 6.2 for divergence time estimations). However, SDIVA equally favored many widespread (but mostly Old World combinations) areas. This was a common result for SDIVA in this dataset, particularly at more basal nodes where it equally favored a number of widespread region combinations. The clade containing all members of Deltocephalinae with linear connectives (Deltocephalini + Tetartostylini + Paralimnini + *Agudus*) was also inferred to have a Palearctic origin. Within the Deltocephalini there were two main clades, an Old World clade, which also contained Tetartostylini and a New World clade which included a number of New World genera. Most analyses favored either an Ethiopian or Palearctic origin for the Old World group, while the New World clade was almost always inferred to have originated in the Nearctic. Within the Paralimnini, a clade containing a mix of Nearctic and Palearctic genera (including many found in both regions) was inferred to have originated in the Palearctic. In general, divergence time estimates inferred using this data set were in line with dates of known grassland events. While major clades diverged before the rise of true grasslands (but after grasses themselves evolved) they did not diversify until grasslands became widespread. A second round of diversification represented by a number of species pairs included in the phylogeny occurred between 5 and 15MYA, which corresponds to the evolution and diversification of C_4 grasses.

Combined Deltocephalinae tree - Patterns were quite different when the combined analysis was used compared to those done for lineages separately, particularly at the basal nodes (See Table 6.3 for reconstructions and divergence time estimates for select nodes and Figure 6.3 for divergence time estimations). Grass feeding in Deltocephalinae has previously been inferred to have a single origin (Zahniser and Dietrich 2010, Zahniser and Dietrich 2013), which I inferred to have a Palearctic origin and dated to have occurred 60MYA. Hecalini was inferred to have either a Palearctic or Ethiopian + Palearctic origin and diverged 52MYA. Glossocratina was always inferred to have an Old World origin, although the analyses did not agree on where this origin was. Hecalina was most often inferred to be African in origin. The clade containing members of Deltocephalinae with linear connectives (Deltocephalini + Tetartostylini + Paralimnini) was inferred to have either a Palearctic or a Palearctic + Neotropical origin (although a number of analyses did not favor any particular solution), due to the relatively basal position of *Agudus*, an endemic South America genus which has not been conclusively placed in a tribe. As the origin of this clade was inferred to be 52MYA a Palearctic + Neotropical origin is not supported, so I favor a Palearctic origin for this clade. Paralimnini + *Agudus*, and Paralimnini itself were both inferred to have Palearctic origins.

Discussion

Old World origins of Caliscelidae (Figure 4), Hecalini, Paralimnini, and Deltocephalini (Figure 6.5) were recovered under virtually all reconstruction techniques, except in a few instances where the entire world was reconstructed as the preferred ancestral range. The family Caliscelidae was inferred to have a Palearctic origin while the subfamily Caliscelinae (which is a grass feeding lineage) first diversified in the Ethiopian region. Caliscelinae was estimated to have diverged from *Ommatidiotus dissimilis* (our only representative of the Ommatidiotinae, a clade of mixed vegetation feeders) 71.5 MYA, which is within the Maastrichtian, a time in which pollen samples suggest grasses (although not grasslands) were present in parts of Africa (Strömberg 2011). Approximately 60 MYA Caliscelinae split into a primarily New World clade and a strictly Old World clade. As there was still limited connectivity between North America and Eurasia continental vicariance cannot be ruled out for this split (Beard and Dawson 1999). The New World Clade contains two Old World taxa which were dated to have diverged from the New World members of this clade during the Late Oligocene. The strictly Old World clade was inferred to have diversified in the Ethiopian region, then spread to the Palearctic about 40 MYA then the Oriental region about 29MYA. While these times predate the formation of large grasslands in these regions, grasses themselves were present (Strömberg 2011).

Grass feeding was inferred to be Palearctic in origin, and although Caliscelinae began to diversify at about 60.3 MYA, Caliscelinae and Ommatidiotinae diverged approximately 71.5 MYA. Both of these dates are after the origin of grasses themselves, but before the rise of grasslands. This time scale is in line with the most commonly cited estimate of the origin of grasses at 80 MYA, although inclusion of recently described 65MYA rice phytoliths and cuticles as calibration points for divergence time estimation suggests grasses might be more ancient than previously thought, up to 129 MYA (Strömberg 2011; Prasad 2011). Each tribe was also inferred to have originated in the Old World although individual tribes were inferred to have originated in different Old World continents. All three lineages were inferred to have diverged approximately 50 MYA, well before the origin of grasslands. However, generic level diversification happened much later, between 20 and 40 MYA while species divergence between 5 and 15 MYA; which are within the ranges for grassland diversification and the rise of C_4 grasses respectively. Further taxon sampling to include more species is needed, particularly in groups with well-known host association to further test if the spread of C_4 plants is correlated to the increase in leafhopper species diversity.

When biogeographic patterns of Hecalini were inferred based on a small number of outgroups the tribe was inferred to have arisen from a widespread ancestor both at the tribe and subtribe level which contrasts with the Deltocephalinae analysis which prefers a Palearctic origin for this tribe. Both Hecalina and Glossocratina were inferred to have originated in the Old World in the subfamily analysis while the smaller Hecalini-focused analyses often included the Nearctic region as part of a widespread distribution for the ancestor of Hecalina (which is not supported by dating analysis showing these groups diverged long after the continents broke apart). Within the Hecalina, a clade containing only New World members was inferred to have been Nearctic in origin and then spread into the Neotropics in both datasets and under virtually all methods. While this clade diverged from the mixed clade about 40 MYA, it only diversified in the last 30 MYA, well within the timeframe in which large grasslands became widespread on these continents (Strömberg 2011).

While grasses themselves were present for tens of millions of years before the formation of grasslands, large clades of grassland specialists would not have been expected to have diversified until grasses became more diverse and grasslands became widespread, because host specialization is uncommon in instances where the host is patchily distributed or rare. Interestingly, the Neotropical taxa represented here appear to have begun diversifying earlier than the Nearctic taxa (even though those lineages split off earlier), which agrees with grassland reconstructions, suggesting that Neotropical regions supported grasslands significantly earlier than the Nearctic (Strömberg 2011).

Reconstructions for clades within the mixed Old and New World Hecalina clade were virtually identical between the large and small datasets. The first lineage, which includes the Old World *Hecalus* (and *Parabolocratalis* in the small dataset) was generally inferred to have either an Oriental or Ethiopian origin, while the group containing the New World *Hecalus* plus *Memnonia* (a genus with members in both the New and Old World), and a strictly Old World undescribed genus was generally inferred to have either an Oriental (BBMCMC and Mesquite) or an Oriental + Nearctic (SDIVA) distribution. However, as these two regions were not connected during the time the lineages diverged from the others (30 MYA), an Oriental + Nearctic distribution seems implausible, so the strictly Oriental distribution is favored. The final major clade, a mix of genera from various parts of the Old World, was also inferred to be Oriental in origin. Divergence time estimation for Hecalini based only on Hecalini and select outgroups yielded dates which were significantly older than expected, with the tribe arising long before grasses themselves evolved. I suspect this can be traced to the use of a single calibration point and limited outgroup selection, both of which were addressed by divergence time estimation of the entire subfamily.

In contrast to the results from analysis of Hecalini, biogeographic patterns inferred using the limited Deltocephalini + Paralimnini dataset were similar to those inferred using the dataset encompassing the entire subfamily, although the success of different analytical techniques was variable. Many basal nodes reconstructed using SDIVA inferred many different combinations of ranges, with no single combination favored, particularly when using the limited dataset. However, DEC did not produce usable results in this large dataset, reconstructing ancestral ranges where no single range (or combination) was represented by greater than 5% probability. The placement of *Agudus* in the large dataset was problematic, its placement as sister (with low branch support and on a very long branch) to the rest of Paralimnini as opposed to in a different clade resulted in a favoring of an ancestral range of Palearctic + Neotropical in some reconstructions, while in the smaller dataset where *Agudus* was not placed in this position these basal nodes were typically inferred to have Palearctic only distributions. With the addition of more South American Paralimnini taxa and denser sampling in the rest of Deltocephalinae, it is possible that *Agudus* will be more firmly placed and biogeographic reconstructions will be able to better resolve the ancestral ranges of these nodes.

Importance of taxon sampling and divergence time estimation in biogeographic study- This study highlights the importance of taxon sampling, particularly in regards to outgroups when reconstructing ancestral ranges. This is especially important when nodes of interest would be located in basal portions of the tree used to infer ancestral ranges. In these cases, nodes were often inferred to have ancestral ranges that spanned the globe or a large number of equally probable range/range combinations were inferred. Nodes towards the tips of the trees were, in general, less susceptible to this problem. In my data set, reconstruction involving single lineages with select outgroups often resulted in less accurate reconstructions than when the lineage was placed in a larger context, i.e. the Deltocephalinae as a whole compared to the individual tribes. This was most apparent at basal nodes, while nodes less basally placed tended not to be as affected. This was particularly problematic for the Caliscelidae analysis. While the dataset included representatives of Caliscelinae from many biogeographic regions, Ommatidiotinae was represented only by a single species. Additionally, outgroup representatives were extremely limited, with one or two exemplar species representing diverse families. To address this, a more representative sampling of Ommatidiotinae, both at the tribal and generic levels along with denser outgroup sampling is required.

Divergence time estimation is of paramount importance for testing biogeographic hypothesis produced by various reconstruction methods because estimated divergence times can be used to evaluate the plausibility of each reconstruction. Unfortunately, accurately estimating divergence times in the absence of fossils of known age that may be used to constrain the ages of nodes, is difficult. For example, divergence time estimation of Hecalini relying solely on the Hecalini tree from Chapter Four and utilizing the only calibration point available for that group (the age of North American grasslands) significantly predates the origin of grasses. However, in using the preferred tree from the Deltocephalinae matrix additional calibration points from Paralimnini and Deltocephalini can be included, which result in divergence time estimations that fit within accepted ages for grass and grassland evolution.

These estimates can then be utilized to choose between the various hypothesis generated by ancestral range reconstruction, such that ranges which do not match inferred dates, regions which were not in contact during the inferred time, or ranges which were uninhabitable during certain geologic periods can be rejected. Rogue taxa can also result in erroneous reconstructions, especially when these taxa are placed in basal positions relative to the nodes of interest, as is the case with *Agudus*.

Conclusions

The Old World origins of Caliscelidae, Hecalini, Deltocephalini, and Paralimnini agree with the intuitive biogeographic assessment of the leafhopper tribe Chiasmini (Zahniser 2008), which also suggested an Old World origin with later spread to the New World. Although further sampling of Caliscelidae is required, the origin of Caliscelinae, the grass feeding subfamily, is inferred to be earlier than the origin in Deltocephalinae. While the grass feeding Deltocephalinae tribes all diverged before grasslands themselves became widespread, the diversification of these lineages did not begin until later, with geographically restricted genus groups radiating shortly after grasslands became more widespread in a given region, and included species pairs diverging between 5 and 15 MYA, a timescale corresponding to the rise of C_4 grasses. Previously, all systematic investigations of global biogeographic patterns have centered on ungulates, which diversified as grasslands became widespread during the Miocene. Origins of ungulate groups are variable, bovids and cervids both have Old World origins, equids and camelids are New World in origin. While all these groups eventually were found globally, the dates for these movements are significantly later than those inferred for insect lineages utilized in this study. My findings were consistent with the order of grassland formation outlined by Strömberg (2011). In general regions with older grasslands, for example South America, support older leafhopper lineages compared to regions with younger grasslands. Timing of clade diversification is also consistent with the proposed ages of major periods of grassland formations, for example the expansion of grasslands and the rise of C_4 photosynthetic pathways.

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Tables and Figures

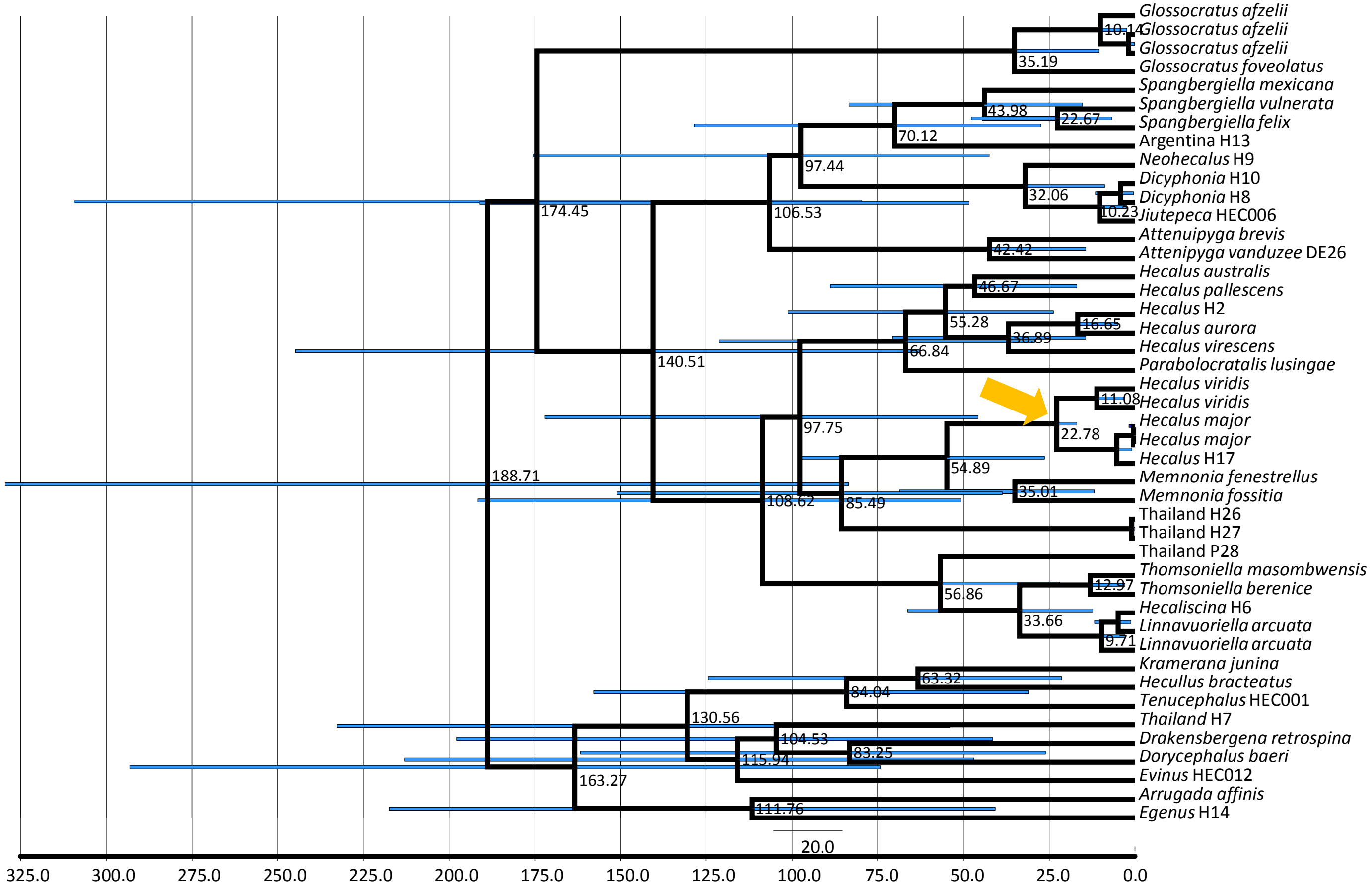

Figure 6.1: Results of BEAST divergence time estimation. Yellow arrow identifies a clade of Neartic Hecalini which was used as a calibration point. Divergence time (in MYA, along X access) are noted at each node, bar at each branch represents 95% confidence interval in this estimate.

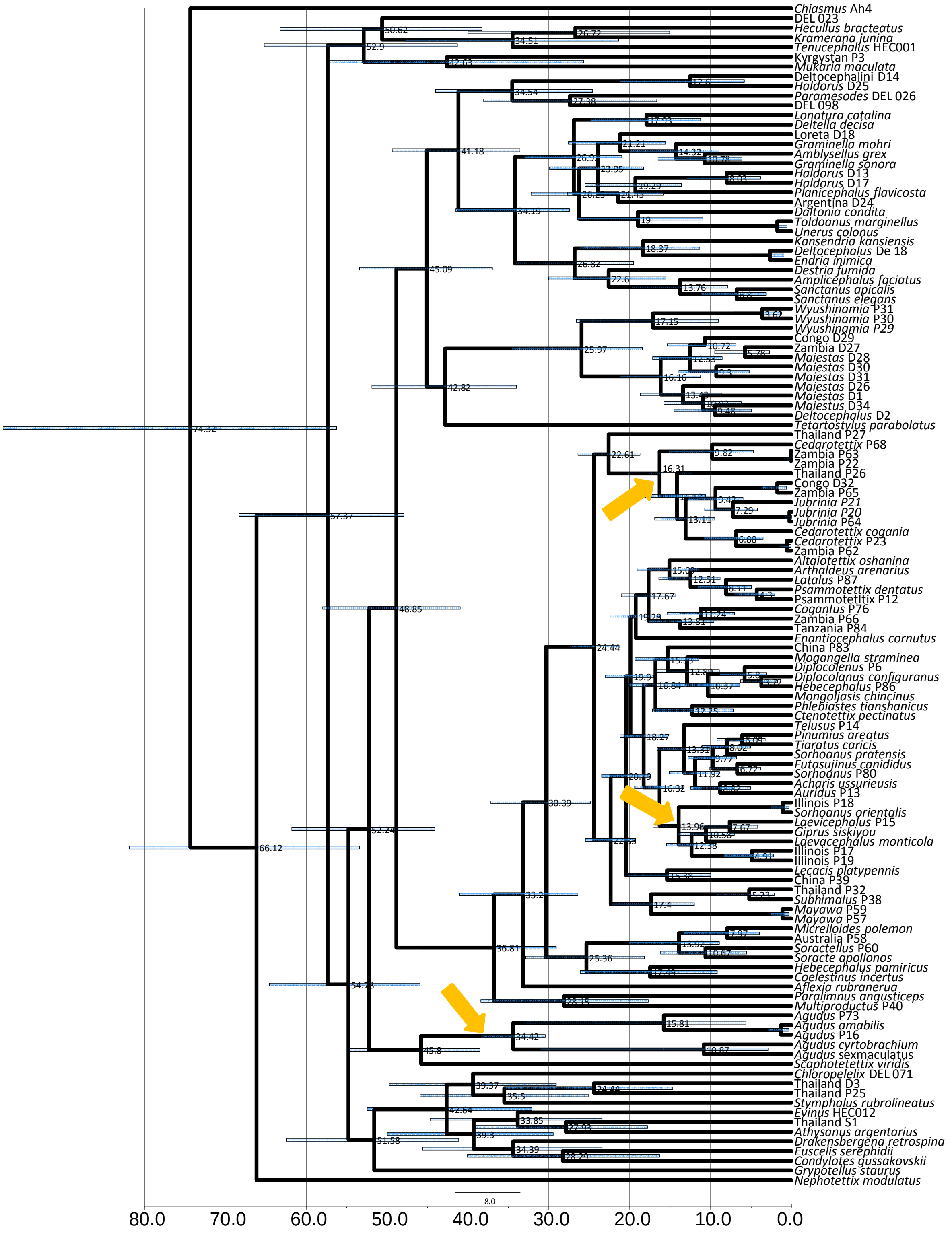

Figure 6.2: Results of BEAST divergence time estimation for Deltocephalini + Tetartostylini + Paralimnini and selected outgroups. Yellow arrows identify clades used for calibration points. Divergence time (in MYA, along X axis) are noted at each node, bars represent 95% confidence interval in this estimate

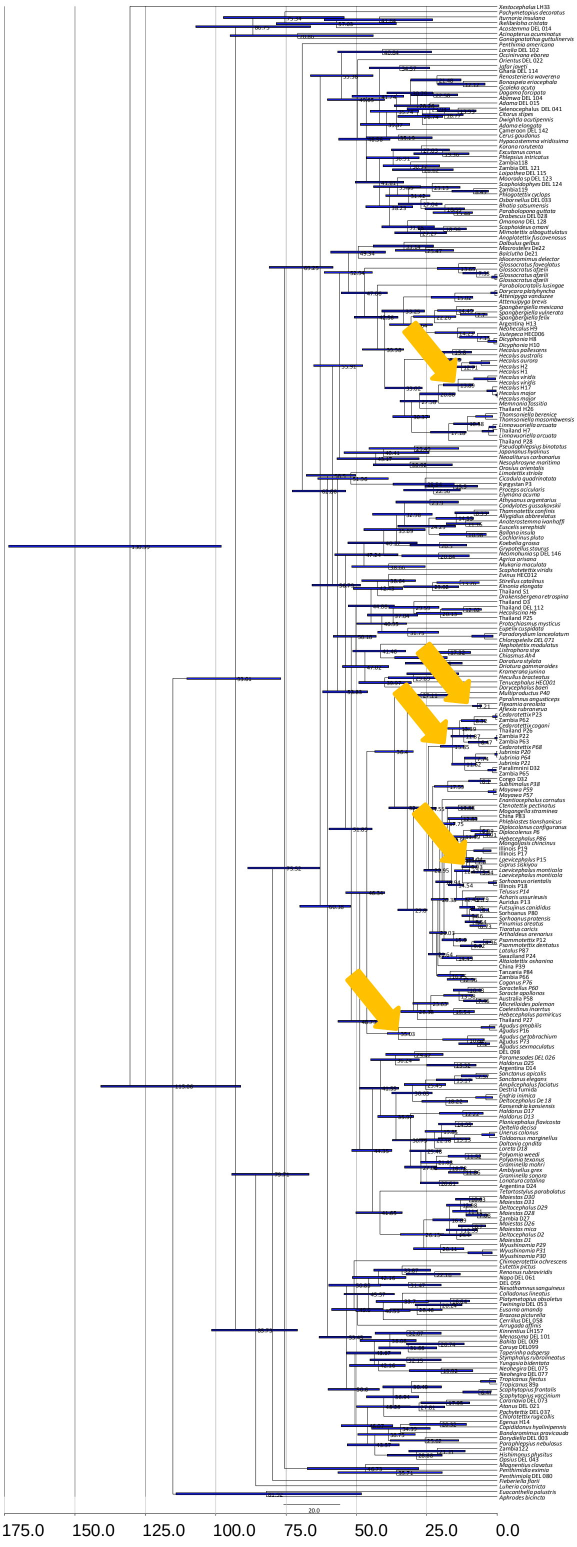

Figure 6.3: Results of BEAST divergence time estimation for Deltocephalinae. Yellow arrows identify clades used for calibration points. Divergence time (in MYA, along X axis) are noted at each node, bars represent 95% confidence interval in this estimate.

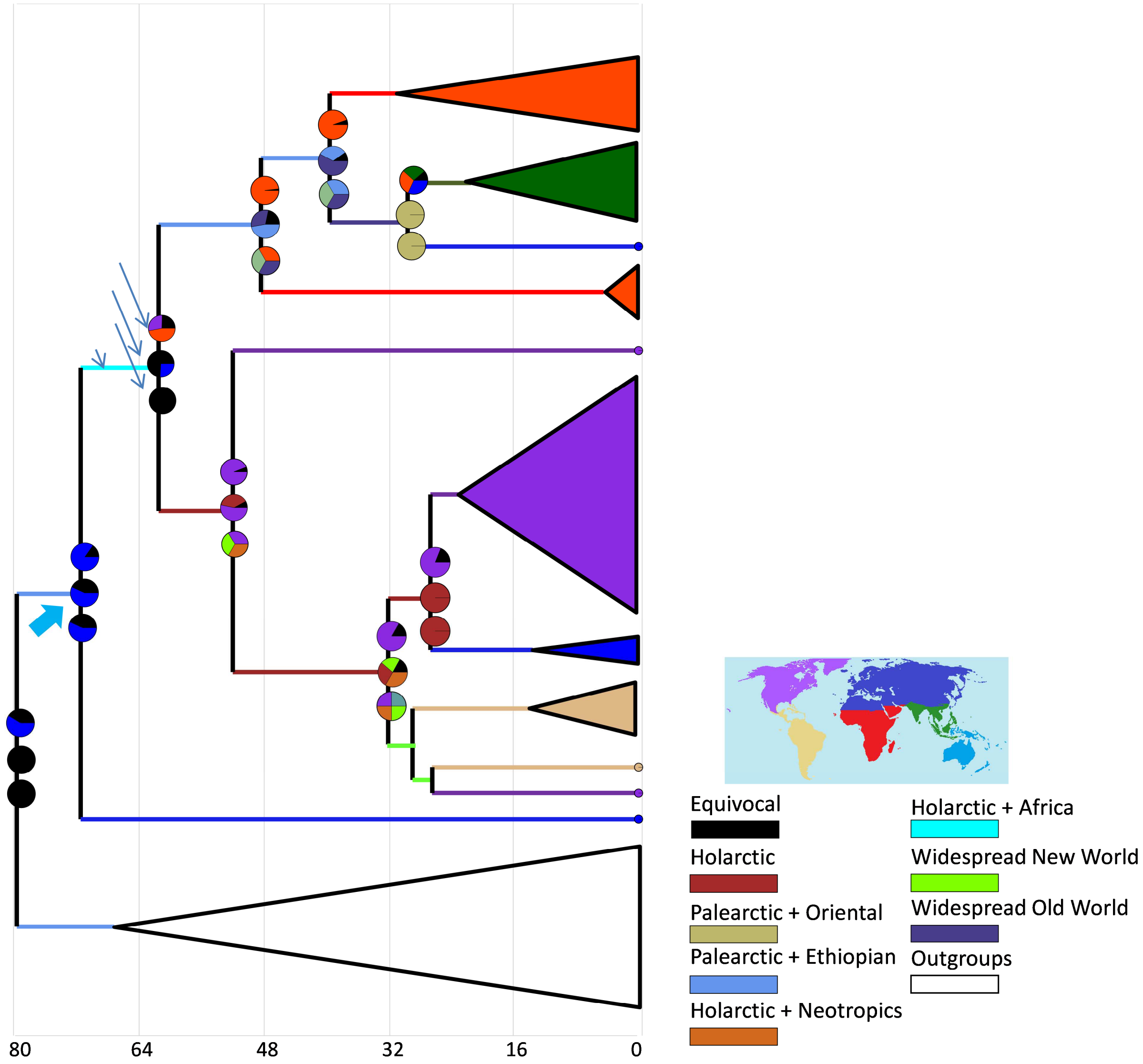

Figure 6.4: Combined results of biogeographic reconstruction of Caliscelidae. Light gray, vertical lines represent age in 16 MY increments (labeled in MYA along X axis).

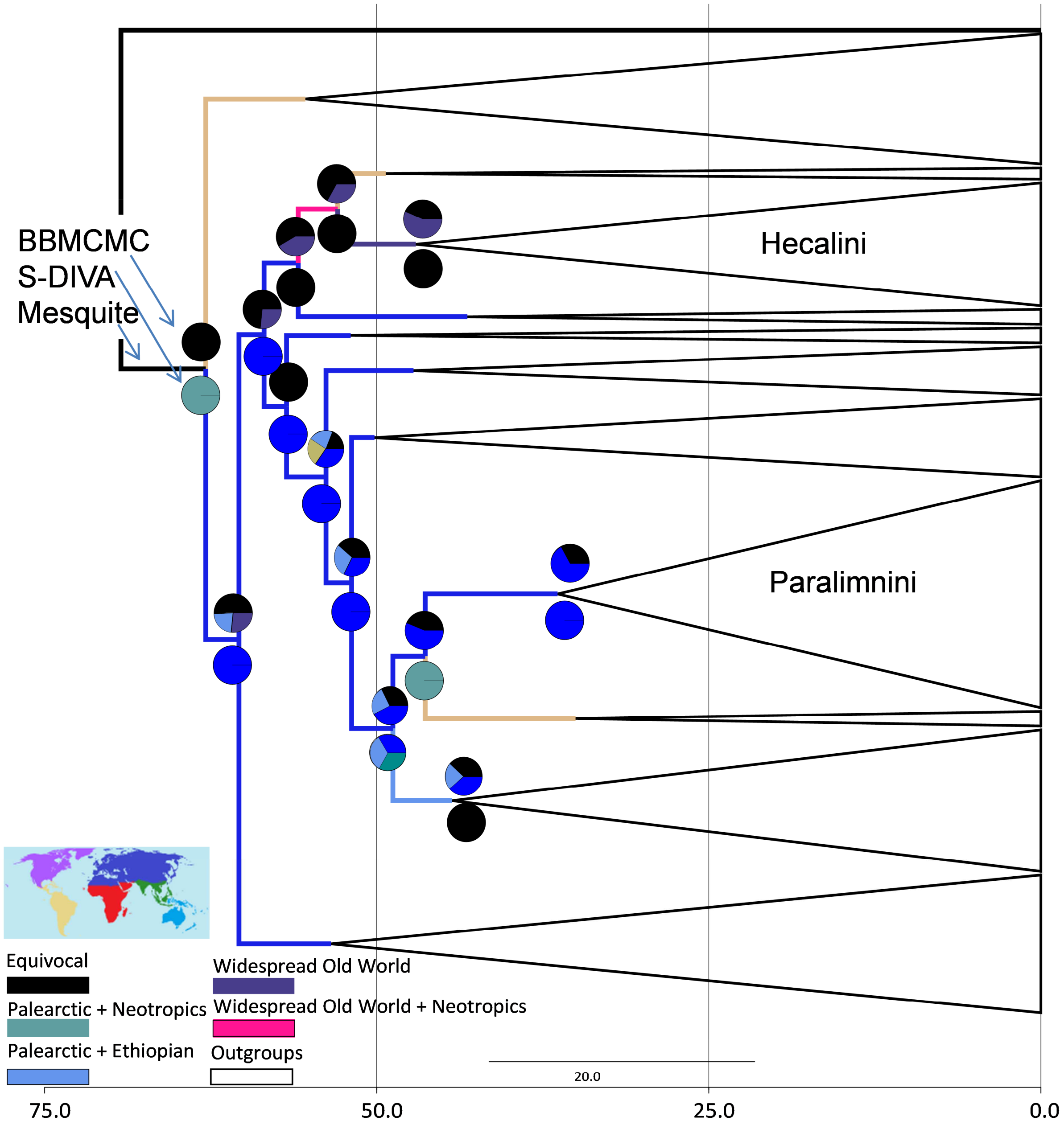

Figure 6.5: Biogeographic reconstruction of Hecalini, Paralimnini, and Deltocephalini + Tetartostylini. Light gray, vertical lines represent age in 25 MY increments (labeled in MYA along X axis).

	SDIVA (no limit)	SDIVA (with limits)	DEC	BBMCMC	Mesquite	Node Age	95% Confidence Interval
Hecalini	(ABCD)/*/*	*/*/*	D/(BD)/*	D/A/E	(ABCDE)/(ABCDE)/E	188.71	not applicable
Hecalina	(BE)/*/*	*/*/(BE)	(BD)/(BD)/*	B/B/(E)	(ABCDE)/(ABCDE)/E	174.75	79.63, 309.12
Glossocratina	(AD)/*/*	(AD)/(A)/*	(AD)/(AD)/(ADEF)	A/A/(ADE)	(ABCDE)/A/E	174.75	79.63, 309.12
New World Clade	B/B/B	B/B/B	B/B/(BC)	B/B/B	(BC)/(BC)/B	140.51	62.99, 244.77
Mixed Clade	E/*/*	*/*/E	(BD)/(BD)/(ADEF)	E/E/E	(BDE)/(BE)/E	140.51	62.99, 244.77
Clade "A" *(Parabolocratalis* + Old World *Hecalus)*	D/*/*	D/D/*	(BD)/D/(ADEF)	D/D/DE	D/D/(DE)	97.75	45.79, 172.19
Clade "B" "New Genus" + *Memnonia* + New World Hecalus)	(BE)/(BE)/E	(BE)/(BE)/*	B/*/*	E/E/E	(BDE)/(BE)/E	97.75	45.79, 172.19
Clade "C" (Thailand_H28 + *Thomsoniella + Linnavuoriella)*	E/E/*	*/E/E	(DE)/(DE)/(ADEF)	E/E/E	(DE)/E/E	108.62	50.67, 191.78

Table 6.1: Summary of ancestral states at select nodes by each reconstruction technique when the preferred Hecalini tree from Chapter 3 is used. Node age refers to a divergence time estimation preformed on this same tree. A: Palearctic, B: Nearctic, C: Neotropical, D: Ethiopian, E: Oriental, F: Australasian. * refers to reconstructions where multiple areas are equally plausible.

	SDIVA (no limit)	SDIVA (with limits)	DEC	BBMCMC	Mesquite	Node Age	95% Confidence Interval
"linear connective clade"	*/*/A	*/*/A	A/*/A	A/A/A	(ACE)/(ACE)/A	54.78	45.92, 64.55
Agudus + *Scaphotettix*	(CE)/(CE)/*	(CE)/(CE)/*	(CE)/(AC)	E/E/A	(ACE)/(ACE)/A	52.78	44.12,61.80
Deltocephalini + Tetartostylini	*/*/A	*/*/A	A/E/A	A/A/A	(ABCEF)/(ABCDEF)/A	48.85	40.96, 57.94
Old World Deltocephalini + *Tetartostylus*	D/*/*	*/*/*	D/D/(AE)	D/D/A	(DE)/(DE)/A	45.09	36.97, 53.41
New World Deltocephalini	B/*/*	B/*/*	B/B/B	B/B/B	E/(BC)/(BC)/AB)	41.18	33.56, 49.39
Paralimnini	*/*/A	*/*/A	A/A/A	A/A/A	(AE)/(AE)/A	48.85	40.96, 57.94
Ethiopian clade	*/*/*	*/*/*	(DE)/(DE)/(DE)	D/D/D	(DE)/(DE)/(DE)	22.61	18.76, 26.41
Holarctic clade	A/A/A	A/A/A	A/A/A	A/A/(AD)	A/A/A	20.49	17.48, 23.48

Table 6.2: Summary of ancestral states at select nodes by each reconstruction technique when the preferred Paralimnini + Deltocephalini tree from Chapter 4 is used. Node age refers to a divergence time estimation preformed on this same tree. A: Palearctic, B: Nearctic, C: Neotropical, D: Ethiopian, E: Oriental, F: Australasian.* refers to reconstructions where multiple areas are equally plausible.

	SDIVA (no limit)	SDIVA (with limits)	DEC	BBMCMC	Mesquite	Node Age	95% Confidence Interval
Grassfeeding clade	*/A/A	*/A/A		A/A/(ADE)	(ABCDE)/A/A	60.38	52.07, 70.04
Hecalini	*/(AD)/*	*/(AD)/*		B/A/(ADE)	(AD)/A/A	52.94	44.42, 61.14
Hecalina	D/D/*	D/D/*		B/D/D	D/D/(ABDE)	47.06	39.01, 55.45
Glossocratina	(AD)/A/*	(AD)/A/*		B/A/(ADE)	A/D/(ADE)	47.06	39.01, 55.45
New World Clade	B/B/B	B/B/B		B/B/B	B/B/B	39.98	33.23, 47.87
Mixed Clade	*/*/*	(DE)*/*		D/D/(ADE)	D/D/(ADE)	39.98	33.23, 47.87
Clade "A" (Old World *Hecalus)*	E/E/*	E/E/*		D/D/(ADEF)	D/D/(ADE)	33.02	26.69, 39.99
Clade "B" "New Genus" + *Memnonia* + New World *Hecalus)*	(BE)/(BE)/*	(BE)/(BE)/*		E/E/E	E/E/E	33.02	26.69, 39.99
Clade "C" (Thailand_H28 + *Thomsoniella* + *Linnavuoriella)*	E/E/E	E/E/E		E/E/E	E/E/E	33.02	26.69, 39.99
"linear connective clade"	*/*/*	*/*/*		A/A/A	(AC)/(AC)/A	51.89	44.49,59.72
Deltocephalini + Tetartostylini	*/*/*	*/*/*		A/D/A	(ABCDEF)/(ABCDEF)/(AD)	48.79	42.56, 56.53
Old World Deltocephalini	*/*/*	*/*/*		D/D/(AD)	(DE)/(DE)/(ADE)	44.35	37.41, 51.62
New World Deltocephalini	B/B/B	B/B/B		B/B/B	B/B/B	44.35	37.41, 51.62
Paralimnini + *Agudus*	(AC)/(AC)/(AC)	(AC)/(AC)/(AC)		A/A/A	(AC)/(AC)/A	48.79	42.56, 56.53
Paralimnini	A/A/A	A/A/A		A/A/A	A/A/A	46.34	40.00, 53.81
Ethiopian clade	D/D/D	D/D/D		D/D/D	D/D/D	24.55	21.61,27.41
Holarctic clade	A/A/A	A/A/A		A/A/A	A/A/A	21.64	18.62,24.56

Table 6.3: Summary of ancestral states at select nodes by each reconstruction technique when the preferred Deltocephalinae tree. Node age refers to a divergence time estimation preformed on this same tree. A: Palearctic, B: Nearctic, C: Neotropical, D: Ethiopian, E: Oriental, F: Australasian. * refers to reconstructions where multiple areas are equally plausible.

APPENDIX A: LIST OF TAXA

A list of taxa included in the study with voucher numbers and GenBank accession numbers. [1]Fragments I, II, IV, and V from *B. insula* and fragment III from *B. ortha*. [2]Fragments I-III and V from *P. compacta* and fragment IV from *P. weedi*. Species with no biogeographic data were excluded from biogeographic study, and where a region is italicized it was excluded. **28S data taken from previous study (Dietrich et al., 2001; Rakitov, unpublished); 28S and H3 data from "DEL", "CHI", "HEC", "LH" vochers provided by Zahniser and Dietrich (2013). Vouchers are deposited at INHS.

Tribe	Subtribe	Species	28S	Histone H3	12S	Voucher #	Locality	Specimen Coding	Species Coding	Genus Coding
Aphrodinae / Aphrodini		*Aphrodes bicincta*	AF304579	GU123794	-	71**	USA: Maryland	PAL	PAL	NEA PAL
Aphrodinae / Portanini		*Portanus* sp. (undescribed sp.)	AF304674	--		**	Ecuador: Napo			
Aphrodinae / Xestocephalini		*Xestocephalus desertorum*	AF304619	GU123892		LH33**	USA: Illinois	NEA	NEA	NEA
Euacanthellinae		*Euacanthella palustris*	GU123728	GU123827	-	DEL078	Australia: NSW	AUS	AUS	AUS
Neocoelidiinae		*Chinaia* sp. (undescribed sp.)	AF304676	--		lh104**	Ecudaro: Napo			
Acinopterini		*Acinopterus acuminatus*	JX845484	GU123790	-	DEL141	USA: Illinois	NEA	NEO NEA	NEA NEO
Acostemmini		*Acostemma stilleri*	GU123696	GU123791	-	DEL014	Madagascar: Toliara Prov.	ETH	ETH	ETH ORI
Acostemmini		*Eryapus* sp.	GU123727	--		DEL081	Madagascar: Toliara Prov.			
Acostemmini		*Ikelibeloha cristata*	JF835026	JN177306		LH177	Madagascar: Province d'Antsiranana	ETH	ETH	ETH
Acostemmini		*Iturnoria insulana*	JN177307	JN177308		DEL132	Madagascar: Province d'Antsiranana	ETH	ETH	ETH
Arrugadini		*Arrugada affinis*	GU123699	GU123795	-	HEC005	Peru: Huanuco Prov.	NEO	NEO	NEO
Athysanini		*Allygidius abbreviatus*	JX845485	JX845526		DEL126	France: Prov-Alpes-Côte d'Azur	PAL	PAL	PAL
Athysanini		*Anoterostemma ivanhoffi*	JX845487	JX845528		DEL130	Scotland: Kirkcudbrightshire	PAL	PAL	PAL
Athysanini		*Atanus* sp. (undescribed sp.)	GU123700	GU123796		DEL021	Peru: Huanuco Prov.	NEO	NEO	NEA NEO
Athysanini		*Athysanus argentarius*	GU123701	GU123797	-	DEL044	USA: Illinois	NEA	NEA NEO	AUS ETH NEA NEO PAL
Athysanini		*Brazosa picturella*	GU123709	GU123806	-	DEL006	Brazil: Goias	NEO	NEO	NEO
Athysanini		*Caranavia separata*	GU123710	GU123807		DEL073	Peru: Junin Prov.	NEO	NEO	NEO
Athysanini		*Cerrillus* sp.	GU123711	GU123808	-	DEL058	Peru: San Martín Prov.	NEO	NEO	NEO
Athysanini		*Chimaerotettix ochrescens*	JX845489	JX845530		DEL020	Ecuador: Orellana	NEO	NEO	NEO
Athysanini		*Colladonus lineatus*	GU123718	GU123815	-	DEL052	USA: California	NEA	NEA	NEA NEO PAL
Athysanini		*Dagama forcipata*	GU123720	GU123817	-	DEL055	South Africa: WCape Prov.	ETH	ETH	ETH
Athysanini		*Egenus*	-	-	-	H14	Argentina	NEO	NEO	NEO
Athysanini		*Eusama amanda*	AF304590	GU123829		77h**	Mexico: Durango	NEA	NEA	NEA
Athysanini		*Euscelis seriphidii*	GU123729	GU123830	-	DEL070	Kyrgyzstan: Dzhalal-abad	PAL	PAL	NEA PAL
Athysanini		*Eusceloidea nitida*	JX845494	--	-	DEL060	Peru: San Martín Prov.			
Athysanini		*Eutettix pictus*	GU123730	GU123831		DEL100	USA: Illinois	NEA	NEA	NEA NEO
Athysanini		*Idioceromimus delector*	GU123740	GU123844	-	DEL065	Brazil: Amazonas	NEO	NEO	NEO
Athysanini		*Loralia* sp.	GU123746	GU123851	-	DEL102	Australia: South Australia	AUS	AUS	AUS
Athysanini		*Napo* sp. (undescribed sp.)	GU123751	GU123856	-	DEL061	Peru: San Martin Prov.	NEO	NEO	NEO
Athysanini		*Neohegira breviceps*	GU123753	GU123858	-	DEL077	Peru: Huanuco Prov.	NEO	NEO	NEO
Athysanini		*Neohegira* sp. 075	GU123786	GU123891	-	DEL075	Peru: Huanuco Prov.	NEO	NEO	NEO
Athysanini		*Nesothamnus sanguineus*	GU123755	GU123860	-	DEL072	Ecuador: Orellana	NEO	NEO	NEO
Athysanini		*Orientus* sp.	GU123757	GU123862	-	DEL022	Taiwan: Taipei Co.	ORI	ORI	*NEA* ORI PAL
Athysanini		*Pachytettix* sp. (undescribed sp.)	GU123761	GU123865	-	DEL037	Peru: Huanuco Prov.	NEO	NEO	NEO
Athysanini		*Platymetopius obsoletus*	GU123771	GU123875	-	DEL013	Kyrgyzstan: Dzhalal-abad	PAL	PAL	ORI *NEO* PAL
Athysanini		*Renonus rubraviridis*	JX845524	JX845552		DEL127	Mexico: Jalisco	NEO	NEO	NEO
Athysanini		*Thamnotettix confinis*	GU123783	GU123888		De1**	USA: Colorado	NEA	NEA PAL	NEA NEO PAL ETH ORI
Athysanini		*Twiningia pellucida* *	GU123785	GU123890	-	DEL053	USA: California	NEA	NEA	NEA
Athysanini		*Yungasia bidentata*	GU123787	GU123893	-	DEL074	Peru: Huanuco Prov.	NEO	NEO	NEO

Tribe	**Subtribe**	**Species**	**28S**	**Histone H3**	**12S**	**Voucher #**	**Locality**	**Specimen Coding**	**Species Coding**	**Genus Coding**
Athysnini		*Condylotes gussakovskii*	-	-	-	P44	Kyrgystan	PAL	PAL	PAL
Bahitini		*Bahita* sp. (undescribed sp.)	GU123702	GU123798	-	DEL009	Peru: Huanuco Prov.	NEO	NEO	NEO
Bahitini		*Caruya* sp.	GU123703	GU123799	-	DEL099	Peru: Huanuco Prov.	NEO	NEO	NEO
Bahitini		*Kinrentius* sp.	JX845523	JX845549		LH157	Peru: Junín Prov.	NEO	NEO	NEO
Bahitini		*Menosoma* sp. ca. *elegans*	GU123749	GU123854	-	DEL101	Peru: Huanuco Prov.			
Bahitini		*Oxycephalotettix tiputini*	GU123759	--		DEL018	Ecuador: Orellana			
Bahitini		*Taperinha adspersa*	GU123780	GU123885	-	DEL032	Peru: Huanuco Prov.	NEO	NEO	NEO
Bonaspeiini		*Bonaspeia eriocephala*	JX845521	GU123804		DEL049	South Africa: WCape Prov.	ETH	ETH	ETH
Bonaspeiini		*Cerus goudanus*	GU123712	GU123809	-	DEL050	South Africa: WCape Prov.	ETH	ETH	ETH
Bonaspeiini		*Renosteria waverena*	GU123772	GU123878	-	DEL048	South Africa: WCape Prov.	ETH	ETH	ETH
Chiasmini		*Chiasmus varicolor*	GU123713	GU123810	-	Ah4**	Australia: NSW	AUS	AUS ETH	AUS ETH ORI PAL
Chiasmini		*Doratura stylata*	AF304589	GU123821		LH19**	USA: Maryland	NEA	ETH NEA PAL	ETH NEA PAL
Chiasmini		*Driotura gammaroides*	JX845492	JX845533		LH96	USA	NEA	NEA	NEA
Chiasmini		*Gurawa minorcephala*	JX845495	JX856131		CHI066	Thailand: Chiang Mai			
Chiasmini		*Listrophora styx*	JX845500	JX845539		DEL138	South Africa: Mpumalanga	ETH	ETH	ETH
Chiasmini		*Nephotettix modulatus*	GU123754	GU123859	-	CHI007	Madagascar: Toliara Prov.	ETH	PAL ETH	PAL ETH ORI AUS
Chiasmini		*Protochiasmus mysticus*	GU123708	GU123805		DEL035	Brazil: Goias	NEO	NEO	NEO
Cicadulini		*Cicadula quadrinotata*	GU123717	GU123813	-	DEL106	Kyrgyzstan	PAL	PAL	ETH NEA ORI PAL
Cicadulini		*Elymana acuma*	GU123726	GU123826	-	DEL092	USA: Illinois	NEA	NEA	ETH NEA PAL
Cicadulini		*Proceps acicularis*	JX845511	JX845550		DEL110	France: Prov-Alpes-Côte d'Azur	PAL	PAL	PAL
Cicadulini		*Stenometopiellus sigillatus*	JX845515	--		DEL064	Kyrgyzstan: Chuy			
Cochlorhinini		*Ballana insula* [1]	AF304582/ AF304580	--	-	LH52**	USA: California	NEA	NEA	NEA
Cochlorhinini		*Ballana ortha* [1]	AF304581	GU123801		LH17**	USA: Wyoming			
Cochlorhinini		*Cochlorhinus pluto*	AF304586	GU123814		LH08**	USA: California	NEA	NEA	NEA
Deltocephalini		"DeltocephAUS" (undesc. gen.sp.)	GU123722	GU123820	-	DEL098	Australia	AUS	AUS	AUS
Deltocephalini		*Amblysellus grex*	-	-	-	D4	Mexico	NEA	NEA	NEA NEO *ORI*
Deltocephalini		*Amplicephalus fasciatus*	-	-	-	D20	Mexico	NEA	NEA	NEA NEO
Deltocephalini		Argentina D14	-	-	-	D14	Argentina	NEO	NEO	NEO
Deltocephalini		Argentina D24	-	-	-	D24	Argentina	NEO	NEO	NEO
Deltocephalini		*Daltonia condita*	-	-	-	D19	Mexico	NEA	NEA	NEA
Deltocephalini		*Deltella decisa*	-	-	-	D22	USA: Illinois	NEA	NEA	NEA
Deltocephalini		*Deltocephalus* D2	-	-	-	D2	Thailand	ORI	ORI	AUS ETH ORI PAL
Deltocephalini		*Deltocephalus* D29	-	-	-	D29	Congo	ETH	ETH	AUS ETH ORI PAL
Deltocephalini		*Deltocephalus fuscinervosus*	-		-	D35	USA: California			
Deltocephalini		*Deltocephalus minutus*			-	D37	USA: California			
Deltocephalini		*Deltocephalus* sp.	GU123721	GU123819	-	De18**	USA	NEA	NEA	NEA NEO
Deltocephalini		*Destria fumida*	-	-	-	D15	USA	NEA	NEA	NEA
Deltocephalini		*Endria inimica*	-	-	-	D5	USA: Illinois	NEA	NEA	NEA PAL
Deltocephalini		*Graminella mohri*	-		-	D36	USA: Nebraska	NEA	NEA	NEA NEO
Deltocephalini		*Graminella sonora*	-	-	-	D6	Mexico	NEA	NEA	NEA NEO
Deltocephalini		*Haldorus* D13	-	-	-	D13	Argentina	NEO	NEO	NEA NEO

Tribe	Subtribe	Species	28S	Histone H3	12S	Voucher #	Locality	Specimen Coding	Species Coding	Genus Coding
Deltocephalini		*Haldorus* D17	-	-	-	D17	Mexico	NEA	NEA	NEA NEO
Deltocephalini		*Haldorus* D25	-	-	-	D25	Argentina	NEO	NEO	NEA NEO
Deltocephalini		*Kansendria kansiensis*	-	-	-	D16	USA	NEA	NEA	NEA
Deltocephalini		*Lonatura catalina*	-	-	-	D7	USA: Illinois	NEA	NEA	NEA *AUS*
Deltocephalini		*Loreta* D18	-	-	-	D18	Argentina	NEO	NEO	NEO
Deltocephalini		*Maiestas clavata*	-	-	-	D28	Zambia	ETH	ETH	AUS ETH ORI PAL
Deltocephalini		*Maiestas* D1	-	-	-	D1	United Arab Emirates	PAL	PAL	ORI ETH PAL AUS
Deltocephalini		*Maiestas* D26	-	-	-	D26	Australia	AUS	AUS	ORI ETH PAL AUS
Deltocephalini		*Maiestas* D30	-	-	-	D30	Congo	ETH	ETH	AUS ETH ORI PAL
Deltocephalini		*Maiestas* D31	-	-	-	D31	Congo	ETH	ETH	AUS ETH ORI PAL
Deltocephalini		*Maiestas mica*	-		-	D34	Tanzania	ETH	ETH	ORI ETH PAL AUS
Deltocephalini		*Maiestas schmidtgeni*	-	-	-	D10	Kyrgystan			
Deltocephalini		*Maiestas* sp. Near *mica* D33	-	-	-	D33	Swaziland			
Deltocephalini		*Paramesodes* sp. (undesc. sp.)	GU123764	GU123868	-	DEL026	Taiwan: Taichung Co.	ORI	ORI	ETH ORI PAL
Deltocephalini		*Planicephalus flavicosta*	-	-	-	D8	Mexico	NEA	NEO NEA	NEA NEO
Deltocephalini		*Polyamia compacta*[2]	AF304609/ AF304607	GU123876		113**	USA: Colorado			
Deltocephalini		*Polyamia texanus*	-	-	-	D9	Mexico	NEA	NEO NEA	NEA NEO *PAL*
Deltocephalini		*Polyamia weedi*[2]	AF304608	--	-	LH90**	USA: Illinois	NEA	NEA	NEA NEO *PAL*
Deltocephalini		*Sanctanus apicalis*	-	-	-	D11	Mexico	NEA	NEA	NEA NEO
Deltocephalini		*Sanctanus elegans*	-	-	-	D21	Mexico	NEA	NEA	NEA NEO
Deltocephalini		*Toldoanus marginellus*	-	-	-	D23	Argentina	NEO	NEO	NEO
Deltocephalini		*Unerus colonus*	-	-	-	D12	USA	NEA	NEO NEA	NEA NEO
Deltocephalini		*Wyushinamia* P29	-	-	-	P29	Thailand	ORI	ORI	ORI
Deltocephalini		*Wyushinamia* P30	-	-	-	P30	Thailand	ORI	ORI	ORI
Deltocephalini		*Wyushinamia* P31	-	-	-	P31	Thailand	ORI	ORI	ORI
Deltocephalini		Zambia D27	-	-	-	D27	Zambia	ETH	ETH	ETH
Dorycephalini		*Dorycephalus baeri*	JX845491	JX845532		HEC010	Kyrgyzstan: Naryn	PAL	PAL	PAL
Drabescini	Drabescina	*Drabescus* sp.	GU123724	GU123824	-	DEL028	Taiwan: Taipei Co.	ORI	ORI	AUS ETH ORI PAL
Drabescini	Paraboloponina	*Bhatia satsumensis*	GU123706	GU123803	-	DEL030	Taiwan: Taipei Co.	PAL	PAL	ORI PAL
Drabescini	Paraboloponina	*Parabolopona guttata*	GU123762	GU123866	-	DEL029	Taiwan: Nantou Co.	PAL	ORI PAL	PAL ORI
Drakensbergenini		*Drakensbergena retrospina*	GU123725	GU123825	-	DEL051	South Africa: KZN Prov.	ETH	ETH	ETH
Eupelicini	Eupelicina	*Eupelix cuspidata*	AF304644	GU123828		lh118**	Kyrgyzstan: Chuy	PAL	ETH PAL	ETH ORI PAL
Eupelicini	Paradorydiina	*Chloropelix canariensis*	GU123715	GU123811	-	DEL071	Portugal: Madeira Isl.	PAL	ETH PAL	ETH ORI PAL
Eupelicini	Paradorydiina	*Paradorydium paradoxum*	AF304637	GU123877		lh116**	Kyrgyzstan: Dzhalal-abad	PAL	PAL	PAL ETH ORI AUS
Faltalini		*Hecullus bracteatus*	GU123737	GU123841	-	HEC011	USA: New Mexico	NEA	NEA	NEA NEO
Faltalini		*Kramerana junina*	GU123743	GU123848	-	HEC004	Peru: Junin Prov.	NEO	NEO	NEO
Faltalini		*Tenucephalus* sp. (undesc. sp.)	GU123781	GU123886	-	HEC001	Brazil: Goias	NEO	NEO	NEA NEO
Fieberiellini		*Fieberiella florii*	AF304594	GU123834		LH23**	USA: Illinois	PAL	PAL	PAL
Goniagnathini		*Goniagnathus guttulinervis*	GU123736	GU123838	-	DEL002	Kyrgyzstan: Dzhalal-abad	PAL	PAL ORI ETH	AUS ETH ORI PAL
Hecalini	Glossocratina	*Glosocratus afzelii*	-	-	-	H3	Congo	ETH	PAL ETH	ETH ORI PAL

Tribe	**Subtribe**	**Species**	**28S**	**Histone H3**	**12S**	**Voucher #**	**Locality**	**Specimen Coding**	**Species Coding**	**Genus Coding**
Hecalini	Glossocratina	*Glosocratus afzelii*	-	-	-	H5	Cameroon	ETH	PAL ETH	ETH ORI PAL
Hecalini	Glossocratina	*Glosocratus foveolatus*	-	-	-	H28	Kyrgyzstan	PAL	PAL	ETH ORI PAL
Hecalini	Glossocratina	*Glossocratus afzelii*	GU123735	GU123837	-	HEC009	Madagascar: Toamasina Prov.	ETH	PAL ETH	ETH ORI PAL
Hecalini	Hecalina	Argentina H13	-	-	-	H13	Argentina	NEO	NEO	NEO
Hecalini	Hecalina	*Attenuipyga brevis*	-	-	-	HEC013		NEA	NEA	NEA
Hecalini	Hecalina	*Attenuipyga vanduzei*	AF304653	GU123822		De26	USA: Illinois	NEA	NEA	NEA
Hecalini	Hecalina	*Dicyphonia*	-	-	-	H10		NEA	NEA	NEA
Hecalini	Hecalina	*Dicyphonia*	-	-	-	H8	Mexico	NEA	NEA	NEA
Hecalini	Hecalina	*Hecalus aurora*	-	-	-	H4	Ghana	ETH	ETH	AUS ETH ORI PAL
Hecalini	Hecalina	*Hecalus australis*	-	-	-	H20	Australia	AUS	AUS	AUS ETH ORI PAL
Hecalini	Hecalina	*Hecalus* H17	-	-	-	H17	USA: Montana	NEA	NEA	NEA NEO
Hecalini	Hecalina	*Hecalus* H2	-	-	-	H2	Thailand	ORI	ORI	AUS ETH ORI PAL
Hecalini	Hecalina	*Hecalus major*	-	-	-	H23	USA: Illinois	NEA	NEA	NEA NEO
Hecalini	Hecalina	*Hecalus major*	-	-	-	H24	USA: Illinois	NEA	NEA	NEA NEO
Hecalini	Hecalina	*Hecalus pallescens*	-	-	-	H21	Australia	AUS	AUS	AUS ETH ORI PAL
Hecalini	Hecalina	*Hecalus virescens*	-	-	-	H1	Swaziland	ETH	ETH	AUS ETH ORI PAL
Hecalini	Hecalina	*Hecalus viridis*	-	-	-	H18	USA: Illinois	NEA	NEA	NEA NEO
Hecalini	Hecalina	*Hecalus viridis*	AF304596	GU123840		De27**	USA: Colorado	NEA	NEA	NEA NEO
Hecalini	Hecalina	*Jiutepeca*	-	-	-	HEC006		NEA	NEA	NEA
Hecalini	Hecalina	*Linnavuoriella arcuata*	-	-	-	H22	Australia	AUS	AUS PAL ORI	AUS PAL ORI
Hecalini	Hecalina	*Linnavuoriella arcuata*	-	-	-	H25	Taiwan	AUS	AUS PAL ORI	AUS PAL ORI
Hecalini	Hecalina	*Memnonia fenestrella*			-	HEC002		NEA	NEA	NEA ORI PAL
Hecalini	Hecalina	*Memnonia fossitia*	-	-	-	H19	Mexico	NEA	NEA	NEA ORI PAL
Hecalini	Hecalina	*Neohecalus*	-	-	-	H9		NEA	NEA	NEA
Hecalini	Hecalina	*Spangbergiella felix*	-	-	-	H12	Argentina	NEO	NEO	NEA NEO
Hecalini	Hecalina	*Spangbergiella mexicana*	-	-	-	H15	Mexico	NEO	NEO NEA	NEA NEO
Hecalini	Hecalina	*Spangbergiella vulnerata*	-	-	-	H11	Argentina	NEO	NEO NEA	NEA NEO
Hecalini	Hecalina	Thailand H26	-	-	-	H26	China	ORI	ORI	ORI
Hecalini	Hecalina	Thailand H27	-		-	H27	China	ORI	ORI	ORI
Hecalini	Hecalina	Thailand H7	-	-	-	H7	Thailand	ORI	ORI	ORI
Hecalini	Hecalina	Thailand P28	-	-	-	P28	Thailand	ORI	ORI	ORI
Hecalini	Hecalina	*Thomsoniella berenice*	-	-	-	H16	Zambia	ETH	ETH	ETH PAL AUS ORI
Hecalini	Hecalina	*Thomsoniella masombwensis*	-	-	-	HEC020		ETH	ETH	ETH PAL AUS ORI
Hypacostemmini		*Hypacostemma viridissima*	GU123739	GU123843	-	DEL094	South Africa: KZN Prov.	ETH	ETH	ETH
Koebeliini	Grypotina	*Grypotellus staurus*	AF304651/ AF30652	GU123839	-	De33**	Greece: Delphi	PAL	PAL	PAL
Koebeliini	Koebeliina	*Koebelia grossa*	AF304599	GU123846		LH56**	USA: California	NEA	NEA	NEA
Limotettigini		*Limotettix striola*	GU123745	GU123850	-	DEL004	Kyrgyzstan: Osh	PAL	PAL NEA	NEA PAL AUS
Luheriini		*Luheria constricta*	JX845502	GU123852	-	DEL105	Argentina: Salta	NEO	NEO	NEO
Macrostelini		*Balclutha neglecta*	GU123704	GU123800		De21**	USA	NEA	NEA NEO *ORI*	AUS ETH NEA NEO ORI PAL

Tribe	**Subtribe**	**Species**	**28S**	**Histone H3**	**12S**	**Voucher #**	**Locality**	**Specimen Coding**	**Species Coding**	**Genus Coding**
Macrostelini		*Dalbulus gelbus*	AF304587	GU123818		D7**	USA: OH (OSU lab colony)	NEA	NEO NEA	NEA NEO
Macrostelini		*Evinus peri*	GU123731	GU123832	-	HEC012	Kyrgyzstan	PAL	PAL	PAL
Macrostelini		*Macrosteles quadrilineatus*	GU123748	GU123853		De22	USA	NEA	NEA	NEA NEO ETH PAL ORI
Magnentiini		*Magnentius clavatus*	JX845503	JX845541		LH199	India: Karnataka	ORI	ORI	ORI ETH
Mukariini		"Mukariini 146" (undesc. gen.sp.)	JX845505	JX845543		DEL146	China: Shaanxi Prov.	PAL	PAL	PAL
Mukariini		*Agrica arisana*	GU123779	GU123884	-	DEL023	Taiwan: Nantou Co.	PAL	PAL	PAL
Mukariini		*Mukaria maculata*	GU123750	GU123855	-	DEL024	Taiwan: Changhua Co.	ORI	PAL ORI	ORI PAL AUS
Mukariini		*Scaphotettix viridis*	GU123774	GU123879	-	DEL068	Taiwan: Tainan Co.	ORI	ORI	ORI PAL
Occinirvanini		*Occinirvana eborea*	JX845507	JX845545		DEL143	Australia: W. Aust.	AUS	AUS	AUS
Opsiini	Circuliferina	*Neoaliturus carbonarius*	GU123752	GU123857	-	DEL012	Kyrgyzstan: Dzhalal-abad	PAL	PAL	ETH PAL ORI AUS NEO NEA
Opsiini	Eremophlepsiina	*Pseudophlepsius binotatus*	JX845512	JX845551	-	DEL125	Kyrgyzstan: Issyk-Kul	PAL	PAL	PAL
Opsiini	Opsiina	*Alishania formosana*	-		-	P79	Thailand			
Opsiini	Opsiina	*Hishimonus phycitis*	GU123738	GU123842	-	DEL031	Taiwan: Taipei Co.	ORI	AUS ORI	AUS ETH ORI PAL
Opsiini	Opsiina	*Japananus hyalinus*	JX845499	JX845538		DEL129	USA: Illinois	PAL	PAL	PAL ORI
Opsiini	Opsiina	*Nesophrosyne maritima*	JX845506	JX845544		DEL109	USA: Hawaii	NEA	NEA	NEA *NEO PAL*
Opsiini	Opsiina	*Opsius versicolor*	GU123756	GU123861	-	DEL043	Israel: Negvev	PAL	ORI PAL	ETH PAL ORI NEO NEA
Opsiini	Opsiina	*Orosius orientalis*	JX845509	JX845547		DEL139	Australia: New South Wales	AUS	PAL AUS	ORI ETH PAL AUS
Opsiini		"Zambia122Ops" undescribed genus near *Libengaia*	JX845520	JX845560		DEL122	Zambia: Northwest Prov.	ETH	ETH	ETH
Paralimnini	Paralimnina	*Acharis ussurieusis*	-	-	-	P82	China	PAL	PAL	PAL
Paralimnini	Paralimnina	*Aflexia rubranura*	GU123698	GU123793	-	De16**	USA	NEA	NEA	NEA
Paralimnini	Paralimnina	*Agudus amabilis*	-	-	-	P70	Argentina	NEO	NEO	NEO
Paralimnini	Paralimnina	*Agudus cyrtobrachium*	-	-	-	P71	Argentina	NEO	NEO	NEO
Paralimnini	Paralimnina	*Agudus* P16	-		-	P16	Argentina	NEO	NEO	NEO
Paralimnini	Paralimnina	*Agudus* P73	-	-	-	P73	Argentina	NEO	NEO	NEO
Paralimnini	Paralimnina	*Agudus sexmaculatus*	-	-	-	P72	Argentina	NEO	NEO	NEO
Paralimnini	Paralimnina	*Altaiotettix oshanini*	-	-	-	P1	Kyrgystan	PAL	PAL	PAL
Paralimnini	Paralimnina	*Arocephalus roborovskii*		-	-	P8	Kyrgystan			
Paralimnini	Paralimnina	*Arocephalus tianshanica*	-		-	P48	Kyrgystan	PAL	PAL	PAL
Paralimnini	Paralimnina	*Arthaldeus arenarius*	-	-	-	P42	Kyrgystan	PAL	PAL	PAL
Paralimnini	Paralimnina	*Auridius* P13	-	-	-	P13	USA	NEA	NEA	NEA
Paralimnini	Paralimnina	Australia P58	-	-	-	P58	Australia	AUS	AUS	AUS
Paralimnini	Paralimnina	*Cedarotettix cogani*	-	-	-	P67	Swaziland	ETH	ETH	ETH
Paralimnini	Paralimnina	*Cedarotettix* P23	-	-	-	P23	Zambia	ETH	ETH	ETH
Paralimnini	Paralimnina	*Cedarotettix* P61	-		-	P61	Zambia			
Paralimnini	Paralimnina	*Cedarotettix* P68	-	-	-	P68	Swaziland	ETH	ETH	ETH
Paralimnini	Paralimnina	China P39	-	-	-	P39	China	PAL	PAL	PAL
Paralimnini	Paralimnina	China P83	-	-	-	P83	China	PAL	PAL	PAL
Paralimnini	Paralimnina	*Cleptochiton pantherinus*		-	-	P51	Kyrgystan			
Paralimnini	Paralimnina	*Coelestinus incertus*	-	-	-	P43	Kyrgystan	PAL	PAL	PAL
Paralimnini	Paralimnina	*Coganus* P76	-	-	-	P76	Swaziland	ETH	ETH	ETH
Paralimnini	Paralimnina	Congo D32	-	-	-	D32	Congo	ETH	ETH	ETH
Paralimnini	Paralimnina	*Ctenotettix pectinatus*	-	-	-	P53	Kyrgystan	PAL	PAL	PAL
Paralimnini	Paralimnina	*Diplocolenus configuratus*	-		-	P34	USA: Montana	NEA	NEA	NEA PAL
Paralimnini	Paralimnina	*Diplocolenus* P6	-	-	-	P6	Kyrgystan	PAL	PAL	NEA PAL
Paralimnini	Paralimnina	*Emeljanovianus medius*	-	-	-	P9	Kyrgystan			

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Paralimnini	Paralimnina	*Emeljanovianus pratensis*	-	-	-	P7	Kyrgystan	PAL	PAL	PAL
Paralimnini	Paralimnina	*Enantiocephalus cornutus*	-	-	-	P41	Kyrgystan	PAL	PAL	PAL
Paralimnini	Paralimnina	*Flexamia areolatus*	GU123733	GU123835		De38**	USA	NEA	NEA	NEA
Paralimnini	Paralimnina	*Futasujinus candidus*	-	-	-	P78	Thailand	ORI	ORI	ORI
Paralimnini	Paralimnina	*Giprus siskiyou*	-		-	P89	USA: Nevada	NEA	NEA	NEA
Paralimnini	Paralimnina	*Hebecephalus* P86	-		-	P86	USA: Nebraska	NEA	NEA	NEA PAL
Paralimnini	Paralimnina	*Hebecephalus pamiricus*	-	-	-	P45	Kyrgystan	PAL	PAL	NEA PAL
Paralimnini	Paralimnina	*Henschia collina*	-		-	P37	USA: Montana			
Paralimnini	Paralimnina	Illinois P17	-	-	-	P17	USA	NEA	NEA	NEA
Paralimnini	Paralimnina	Illinois P18	-	-	-	P18	USA	NEA	NEA	NEA
Paralimnini	Paralimnina	Illinois P19	-	-	-	P19	USA	NEA	NEA	NEA
Paralimnini	Paralimnina	*Jubrinia* P20	-	-	-	P20	Zambia	ETH	ETH	ETH
Paralimnini	Paralimnina	*Jubrinia* P21	-	-	-	P21	Zambia	ETH	ETH	ETH
Paralimnini	Paralimnina	*Jubrinia* P64	-	-	-	P64	Zambia	ETH	ETH	ETH
Paralimnini	Paralimnina	Kyrgystan P10	-	-	-	P10	Kyrgystan			
Paralimnini	Paralimnina	*Ladya* P66	-	-	-	P66	Zambia	ETH	ETH	ETH
Paralimnini	Paralimnina	*Laevicephalus monticola*	-	-	-	P75	Mexico	NEA	NEA	NEA
Paralimnini	Paralimnina	*Laevicephalus monticola*	GU123744	GU123849		60b**	USA	NEA	NEA	NEA
Paralimnini	Paralimnina	*Laevicephalus monticola*	-		-	P54	Mexico	NEA	NEA	NEA
Paralimnini	Paralimnina	*Laevicephalus* P15	-	-	-	P15	USA	NEA	NEA	NEA
Paralimnini	Paralimnina	*Latalus curtus*	-		-	P33	USA: Montana			
Paralimnini	Paralimnina	*Latalus* P87	-		-	P87	USA: California	NEA	NEA	NEA PAL
Paralimnini	Paralimnina	*Latalus personatus*	-		-	P35	USA: Montana			
Paralimnini	Paralimnina	*Lecacis platypennis*	-	-	-	P24	Swaziland	ETH	ETH	ETH
Paralimnini	Paralimnina	*Mayawa* P57	-	-	-	P57	Australia	AUS	AUS	AUS
Paralimnini	Paralimnina	*Mayawa* P59	-	-	-	P59	Australia	AUS	AUS	AUS
Paralimnini	Paralimnina	*Micrelloides polemon*	-	-	-	P55	Australia	AUS	AUS	AUS
Paralimnini	Paralimnina	*Mogangella straminea*	-	-	-	P46	Kyrgystan	PAL	PAL	PAL
Paralimnini	Paralimnina	*Mogangina bromi*	-	-	-	P47	Kyrgystan			
Paralimnini	Paralimnina	*Mongolojassus tianshanicus*	-	-	-	P5	Kyrgystan	PAL	PAL	NEA PAL
Paralimnini	Paralimnina	*Multiproductus* P40	-	-	-	P40	Thailand	PAL	PAL	PAL
Paralimnini	Paralimnina	*Paralimnus angusticeps*	GU123763	GU123867	-	DEL001	Kyrgyzstan: Issyk-Kul	PAL	PAL	PAL ETH ORI
Paralimnini	Paralimnina	*Paramesus major*		-	-	P11	Kyrgystan			
Paralimnini	Paralimnina	*Paramesus major*	-	-	-	P69	Kyrgystan			
Paralimnini	Paralimnina	*Peconus* P90	-			P90				
Paralimnini	Paralimnina	*Peconus scriptanus*	-	-	-	P77	USA: New Mexico			
Paralimnini	Paralimnina	*Pinumius areatus*	-	-	-	P4	Kyrgystan	PAL	PAL NEA	PAL NEA
Paralimnini	Paralimnina	*Praganus hofferi*	-	-	-	P49	Kyrgystan			
Paralimnini	Paralimnina	*Psammotettix dentatus*	-		-	P88	USA: California	NEA	NEA	AUS ETH NEA ORI PAL
Paralimnini	Paralimnina	*Psammotettix* P12	-	-	-	P12	Mexico	NEA	NEA	AUS ETH NEA ORI PAL
Paralimnini	Paralimnina	*Psammotettix* P81	-	-	-	P81	China			
Paralimnini	Paralimnina	*Rhoananus hypochlorus*	-	-	-	P50	Kyrgystan			
Paralimnini	Paralimnina	*Soracte apollonos*	-	-	-	P56	Australia	AUS	AUS	AUS
Paralimnini	Paralimnina	*Soractellus*	-	-	-	P60	Australia	AUS	AUS	AUS ORI ETH PAL
Paralimnini	Paralimnina	*Sorhoanus orientalis*	-		-	P36	USA: Montana	NEA	NEA	NEA PAL
Paralimnini	Paralimnina	*Sorhoanus* P80	-	-	-	P80	China	PAL	PAL	NEA PAL
Paralimnini	Paralimnina	*Spartopyge mexicana*	-	-	-	P74	Mexico			
Paralimnini	Paralimnina	*Subhimalus* P38	-		-	P38	Thailand	ORI	ORI	ORI PAL
Paralimnini	Paralimnina	Tanzania P84	-		-	P84	Tanzania	PAL	PAL	PAL

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Paralimnini	Paralimnina	Tanzania P85	-		-	P85	Tanzania			
Paralimnini	Paralimnina	*Telusus* P14	-	-	-	P14	USA	NEA	NEA	NEA
Paralimnini	Paralimnina	Thailand P25	-	-	-	P25	Thailand	ORI	ORI	ORI
Paralimnini	Paralimnina	Thailand P26	-	-	-	P26	Thailand	ORI	ORI	ORI
Paralimnini	Paralimnina	Thailand P27	-	-	-	P27	Thailand	ORI	ORI	ORI
Paralimnini	Paralimnina	Thailand P32	-	-	-	P32	Thailand	ORI	ORI	ORI
Paralimnini	Paralimnina	*Tiaratus caricis*	-	-	-	P2	Kyrgystan	PAL	PAL	PAL
Paralimnini	Paralimnina	*Triasargus ancoratus*	-	-		P52	Kyrgystan			
Paralimnini	Paralimnina	Zambia P22	-	-	-	P22	Zambia	ETH	ETH	ETH
Paralimnini	Paralimnina	Zambia P62	-	-	-	P62	Zambia	ETH	ETH	ETH
Paralimnini	Paralimnina	Zambia P63	-	-	-	P63	Zambia	ETH	ETH	ETH
Paralimnini	Paralimnina	Zambia P65	-	-	-	P65	Zambia	ETH	ETH	ETH
Pendarini		*Bandaromimus parvicauda*	GU123705	GU123802	-	DEL076	Peru: Huanuco Prov.	NEO	NEO	NEO
Pendarini		*Chlorotettix rugicollis*	GU123716	GU123812	-	DEL042	USA: Illinois	NEA	NEA	NEA NEO *ORI*
Pendarini		*Chlorotettix unicolor*	GU123714	--		138**	USA			
Pendarini		*Copididonus hyalinipennis*	GU123719	GU123816	-	DEL007	Brazil: Goias	NEO	NEO	NEO
Pendarini		*Dorydiella kansana*	GU123723	GU123823	-	DEL003	USA: Nebraska	NEA	NEA	NEA
Pendarini		*Paraphlepsius nebulosus*	GU123765	GU123869	-	DEL045	USA: Illinois	NEA	NEA	NEA
Pendarini		*Tropicanus chiapasus*	GU123784	GU123889	-	89a**	Mexico	NEO	NEO	NEA NEO
Pendarini		*Tropicanus flectus*	JX845517	JX845557		DEL131	Mexico: Chiapas	NEO	NEO	NEA NEO
Penthimiini		*Jafar javeti*	JX845498	JX845537		DEL113	Togo: Sotouboua Region	ETH	ETH	ETH
Penthimiini		*Penthimia americana*	AF304606	GU123870		LH34	USA: Illinois	NEA	NEA	ETH NEA *NEO* ORI PAL
Penthimiini		*Penthimidia eximia*	JX845510	JX845548		DEL148	Rep. of Congo: Iboubikro	ETH	ETH	ETH
Penthimiini		*Penthimiola bella* *	GU123766	GU123871	-	DEL080	Madagascar: Toamasina Prov.	ETH	ETH *NEO*	ETH
Phlepsiini		*Excultanus conus*	GU123732	GU123833	-	DEL005	Mexico: Chiapas	NEO	NEO	NEA NEO
Phlepsiini		*Korana rorulenta*	GU123742	GU123847	-	DEL095	Burkina Faso: Yako	ETH	ETH	ETH
Phlepsiini		*Phlepsius intricatus*	GU123768	GU123873	-	DEL017	Kyrgyzstan: Dzhalal-abad	PAL	PAL ETH	PAL ETH *ORI*
Scaphoideini		*Anoplotettix fuscovenosus*	JX845486	JX845527		DEL147	France: Provence-Alpes-Côte d'Azur	PAL	PAL	PAL
Scaphoideini		*Loipothea* sp. (undescribed sp.)	JX845501	JX845540		DEL115	Zambia: Northwest Prov.	ETH	ETH	ETH
Scaphoideini		*Mimotettix alboguttulatus*	JX845504	JX845542		DEL117	Zambia: Northwest Prov.			
Scaphoideini		*Omanana* sp. (undescribed sp.)	JX845508	JX845546		DEL128	Mexico: Jalisco	NEA	NEA	NEA
Scaphoideini		*Osbornellus* sp. (undescribed sp. near *O. linnavuori* DeLong)	GU123758	GU123863	-	DEL033	Peru: Huanuco Prov.	NEO	NEO	NEA NEO PAL ETH ORI
Scaphoideini		*Phlogotettix cyclops*	GU123769	GU123874	-	DEL027	Taiwan: Ilan Prov.	PAL	PAL	PAL ORI
Scaphoideini		*Scaphoideus alboguttatus*	GU123773	--		DEL040	Taiwan: Nantou Co.			
Scaphoideini		*Scaphoideus omani*	JX845513	JX845553		DEL120	Zambia: Northwest Prov.	ETH	ETH	AUS ETH NEA ORI PAL
Scaphoideini		*Scaphoideus* sp. SA	GU123775	--		DEL063	South Africa: WCape Prov.			
Scaphoideini		*Scaphoidophyes* sp. near *pyrus* Barnett & Freytag	JX845525	JX845554		DEL124	Zambia: Copperbelt Prov.	ETH	ETH	ETH
Scaphytopiini		*Scaphytopius frontalis*	JX845514	JX845555		DEL116	USA: Maryland	NEA	NEO NEA	NEA NEO
Scaphytopiini		*Scaphytopius vaccinium*	GU123776	GU123880		lh09b**	USA	NEA	NEA	NEA NEO
Selenocephalini	Adamina	*Adama (Krisnella) elongata*	GU123694	GU123788		DEL083	Zambia: Copperbelt Prov.	ETH	ETH	ETH PAL
Selenocephalini	Adamina	*Adama (Paracostemma)* sp.	GU123697	GU123792	-	DEL015	Rwanda: Nyungwe Forest	ETH	ETH	ETH PAL

Tribe	**Subtribe**	**Species**	**28S**	**Histone H3**	**12S**	**Voucher #**	**Locality**	**Specimen Coding**	**Species Coding**	**Genus Coding**
Selenocephalini	Dwightlina	*Dwightla acutipennis*	JX845493	JX845534		DEL111	Cameroon: Southwest Region	ETH	ETH	ETH
Selenocephalini	Ianeirina	*Abimwa knighti*	GU123695	GU123789	-	DEL104	Zambia: Northwestern Prov.	ETH	ETH	ETH
Selenocephalini	Selenocephalina	*Citorus stipes*	JX845490	JX845531		DEL144	Zambia: Northwest Prov.	ETH	ETH	ETH *NEO*
Selenocephalini	Selenocephalina	*Selenocephalus deserticola*	GU123777	GU123881	-	DEL041	Israel: Negvev	PAL	PAL	ETH PAL
Stegelytrini		*Pachymetopius decoratus*	GU123760	GU123864	-	DEL025	Taiwan: Taichung Co.	ORI	ORI	ORI PAL
Stegelytrini		*Placidellus* sp.	GU123770	--		**	Thailand			
Stenometopiini		*Kinonia elongata*	GU123741	GU123845		75g**	USA	NEA	NEA	NEA
Stenometopiini		*Stirellus catalinus*	AF304614	GU123882		82k**	Mexico: Durango	NEA	NEA	AUS ETH NEA NEO ORI PAL
Stenometopiini		Thailand S1	-	-	-	S1	Thailand	ORI	ORI	ORI
Tetartostylini		*Tetartostylus parabolatus*	GU123782	GU123887	-	DEL047	South Africa: WCape Prov.	ETH	ETH	ETH PAL
Vartini		*Stymphalus rubrolineatus*	GU123778	GU123883	-	DEL062	South Africa: WCape Prov.	ETH	ETH	ETH
-		*Hecaliscina*	-	-	-	H6	Thailand	ORI	ORI	ORI
---		"Cameroon142" undescr. gen.sp.	JX845488	JX845529		DEL142	Cameroon: Southwest Region	ETH	ETH	ETH
---		"Ghana114" undescr. gen.sp.	JX845522	JX845535		DEL114	Ghana: Western Region	ETH	ETH	ETH
---		"Peru059" undescr. gen.sp.	GU123767	GU123872	-	DEL059	Peru: San Martin Prov.	NEO	NEO	NEO
---		"Thailand112" undescr. gen.sp.	JX845516	JX845556		DEL112	Thailand: Chaiyaphum	ORI	ORI	ORI
---		"Zambia118" undescr. gen.sp.	JX845518	JX845558		DEL118	Zambia: Northwest Prov.	ETH	ETH	ETH
---		"Zambia119" near *Phlogothamnus*	JX845519	JX845559		DEL119	Zambia: Northwest Prov.	ETH	ETH	ETH
---		"Zambia121" near *Houtbayana / Acacimenus*	JX845496	JX845536		DEL121	Zambia: Copperbelt Prov.	ETH	ETH	ETH
---		Kyrgystan P3	-	-	-	P3	Kyrgystan	PAL	PAL	PAL
---		Thailand D3	-	-	-	D3	Thailand	ORI	ORI	ORI

APPENDIX B: PRIMERS FOR AMPLIFICATION AND SEQUENCING

Locus	Primer	Sequence	Citation
16s	+1	5'-CCG GT(CT) TGA ACT CA(AG) ATC A(AT)G T -3'	Dietrich et al (1997)
16s	-1	5'-CTGTTTA(AT)CAAAAACATTTC -3'	Dietrich et al (1997)
Histone 3	AF	5'-ATG GCT CGT ACC AAG CAG ACG GC -3'	Ogden and Whiting (2003)
Histone 3	AR	5'-ATA TCC TTG GGC ATG ATG GTG AC -3'	Ogden and Whiting (2003)
28S	P1	5'-AGT CGK GTT GCT TGA KAG TGC AG -3'	Dietrich et al., 2001; Hillis & Dixon, 1991
28S	M_{2alt}	5'-TTC GGG TCC CAA CGT GTA CG -3'	Dietrich et al., 2001; Hillis & Dixon, 1991
28S	EE	5'-CCG CTA AGG AGT GTG TAA-3'	Dietrich et al., 2001; Hillis & Dixon, 1991
28S	MM	5'-GAA GTT ACG GAT CTA RTT TG -3'	Dietrich et al., 2001; Hillis & Dixon, 1991
28S	L	5'-CCT CGG ACC TTG AAA ATC C -3'	Dietrich et al., 2001; Hillis & Dixon, 1991
28S	*X*	5'-CAC AAT GAT AGG AAG AGC C -3'	Dietrich et al., 2001; Hillis & Dixon, 1991
12S	ai	5'-AAA CTA GGA TTA GAT ACC CTA TTA T - 3'	Simon et al., 1994
12S	b1	5'-AAG AGC GAC GGG CGA TGT GT	Simon et al., 1994

APPENDIX C: PCR CONDITIONS

Step	28S and Histone	16S	12S
1	3 minutes (94ƒC)	5 minutes (95ƒC)	2 minutes (94ƒC)
2	1 minute (94ƒC)	1 minute (92ƒC)	30 seconds (94ƒC)
3	1 minute (55ƒC)	1 minute (48ƒC)	30 seconds (50ƒC)
4	2 minutes (72ƒC)	1.5 minutes (72ƒC)	1 minute (65ƒC)
	Repeat steps 2-4 27 times	Repeat 2-4 11 times	Repeat 2-4 34 times
5	7 minutes (72ƒC)	1 minute (92ƒC)	7 minutes (65ƒC)
6		35 seconds (54ƒC)	
7		1.5 minutes (72ƒC)	
		Repeat 5-7 33 times	
8		7 minutes (72ƒC)	