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Background: *Leptoloma* Chase is a genus of grasses comprising five species, all of which are endemic to North America. An overview of these species can be seen in Table 1. *Leptoloma* is often treated as an infrageneric taxon within the genus *Digitaria* Haller and is distinguished morphologically from other closely related clades by having an open paniculate inflorescence, with spikelets usually being solitary on elongated pedicels. A morphological phylogeny from 2009 placed species from *Leptoloma* sister to species from *Digitaria* sect. *Pennatae* (Stapf) Henrard in the 'Tumbleweed' clade (Vega, 2009). The name of this clade is due to the nature of most species in this group having an inflorescence that dehisces and forms a tumbleweed at maturity. This trait, however, is also seen in other species of *Digitaria* outside this clade. To date, no molecular phylogeny performed on this group has included any *Leptoloma* species other than *L. cognatum*, leaving the taxonomic status of this clade unclear.

Table 1. List of species in *Leptoloma*, their geographic range, and diagnostic characters within the genus.

Species	Geographic Range	Diagnostic characters
Leptoloma arenicola Swallen	Gulf Coast, Texas into N. Mexico	Spikelets 3.5–4.6 mm, solitary; upper glume 5-7 veined; rhizomatous
L. clarkiae (Sánchez-Ken) Wipff & Shaw	Central Mexico	Spikelets 2.7-3.2 mm long, paired; upper glume 3-veined; lower lemma 7-veined; without rhizomes
L. cognatum (Schult.) Chase	Eastern U.S., as far west as Texas	Spikelets 2.2-3.3 mm, solitary; upper glume 3(-5)-veined; lower lemma 7-veined; without rhizomes
L. pubiflorum (Vasey) Wipff & Shaw	Southern Oklahoma, Texas, west to Arizona and south into N. Mexico	Spikelets 2.2-3.3 mm, solitary; upper glume 3(-5) veined; lower lemma 5-veined, densely pubescent; without rhizomes
L. syrticola Wipff & Shaw	Texas and S.E. New Mexico	Spikelets 2.2-3.3 mm, solitary; upper glume 3(-5) veined; lower lemma 5-veined, densely pubescent; rhizomatous

Objectives: The objective of this project is to begin to provide evidence for whether or not *Leptoloma* is monophyletic by constructing a phylogeny with molecular data. Additionally, multiple methods of phylogenetic inference will be compared to see how the results of each differ.

Methods: This project utilized available sequence data for a variety of species from *Digitaria* and *Leptoloma* that was downloaded from NIH Genbank (https://www.ncbi.nlm.nih.gov/genbank/). A large number of datasets currently exist for whole genomes and individual genes that have been sequenced from species in *Digitaria*. The only *Leptoloma* species for which open-source sequences are available is *Leptoloma cognatum*. This study utilized genetic data for the *rbcL* gene from seven species of *Digitaria* plus *Leptoloma congatum*. *Anthephora pubescens* and *Chaetopoa pilosa* have also been included because these two species often appear nested within *Digitaria* in molecular phylogenies, impying that *Digitaria* is not monophyletic or that these genera should be included within *Digitaria*. *Panicum repens* and *Dichanthelium acuminatum* subsp. *acuminatum* were used as outgroup taxa as they often are in phylogenies of this group. The *rbcL* gene is a chloroplast gene that is commonly used in phylogenetics for grasses and other plants. A list of the species used in this study can be found in Table 2.

**Table 2.** List of species in used in this study.

Species	GenBank Accession Number	*rbcL* Sequence Length (bp)
Leptoloma cognatum	KY627334.1	561
Anthephora pubescens	FN870763.1	637
Digitaria sanguinalis	HQ590067.1	607
Digitaria californica	MF963129.1	1237
Digitaria ciliaris	KX282695.1	580
Digitaria bicornis	KY627255.1	559
Digitaria eriantha	HE573375.1	1342
Digitaria villosa	KY626716.1	561
Digitaria ischaemum	MF596731.1	603

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Chaetopoa pilosa	HE573362.1	1342
Dichanthelium acuminatum subsp. *acuminatum*	KY627164.1	557
Panicum repens	GU135141.1	567

First, sequence alignment was performed using the program MAFFT and its default settings. Maximum likelihood analysis was perfromed using RAxML-NG version 1.1.0 using the GTR+G model. The resulting tree was visualized in FigTree and is shown below in Figure 1. A maximum parsimony tree was also generated using PAUP*. A bootstrap parsimony analysis was performed using the heuristic search option along with random stepwise addition sequence, TBR branch swapping, and 100 replicates. *D. acuminatum* subsp. *acuminatum* and *P. repens* were set as the outgroups. The resulting tree, visualized in FigTree, can be seen in Figure 2. Finally, a Bayesian analysis was performed using BEAST2. Model settings were left at defaults, with the exception of the MCM chain length being set to 1,000,000. The maximum clade credibility tree was found using TreeAnnotator and was visualized in FigTree with color coded.
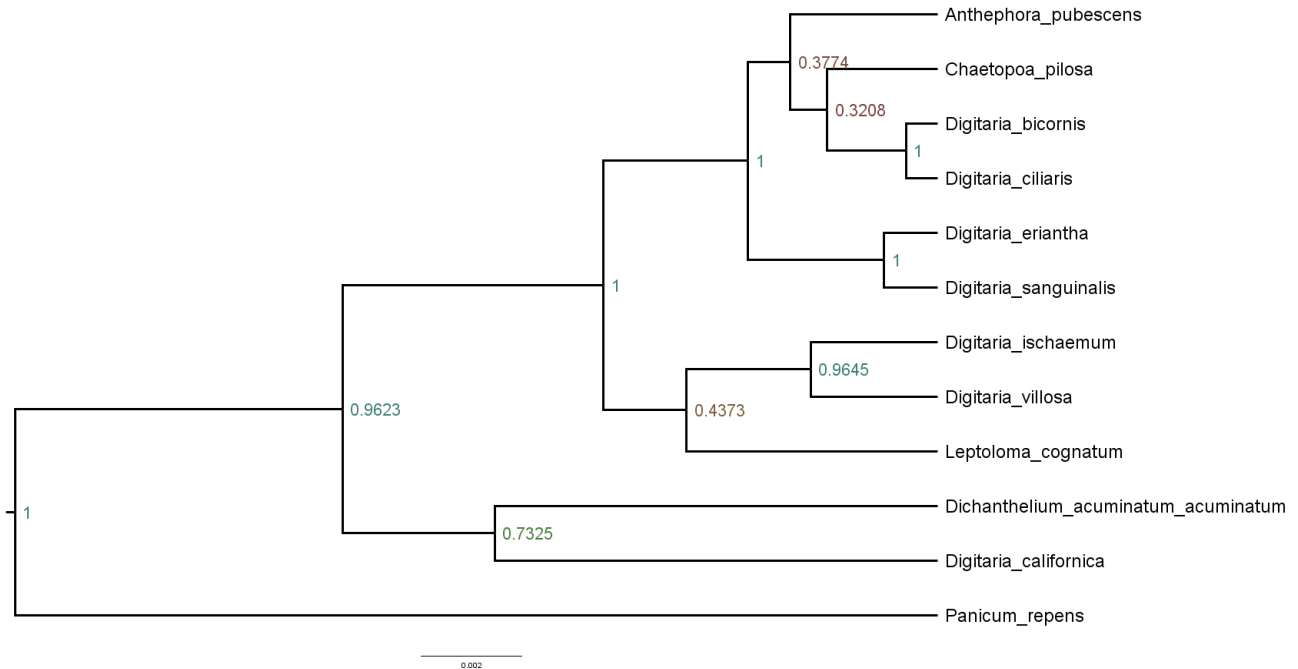
Results:

Three separate phylogenies were generated as described in the methods above. Each of these are displayed below and show noticeably different levels of accuracy and indicate different relationships among the taxa studied. Figure 1, the phylogeny generated via maximum likelihood in RAxML-NG, shows a relatively high level of uncertainty, with multiple polytomies. It also does not parse out *Panicum repens* and *Dichanthelium acuminatum* subsp. *acuminatum* as the outgroup taxa. Figure 2, a parsimony tree with bootstrap values, is essentially all polytomies and really provides no information as to the relationships between any of the species in this study. Figure 3 is by far the highest quality tree obtained and was done using Bayesian analysis in MrBayes. Although it does place *Digitaria californica* as sister to *Dichanthelium acuminatum* subsp. *acuminatum*, it contains no polytomies and seven of the eleven nodes have support values of 0.96 or higher.
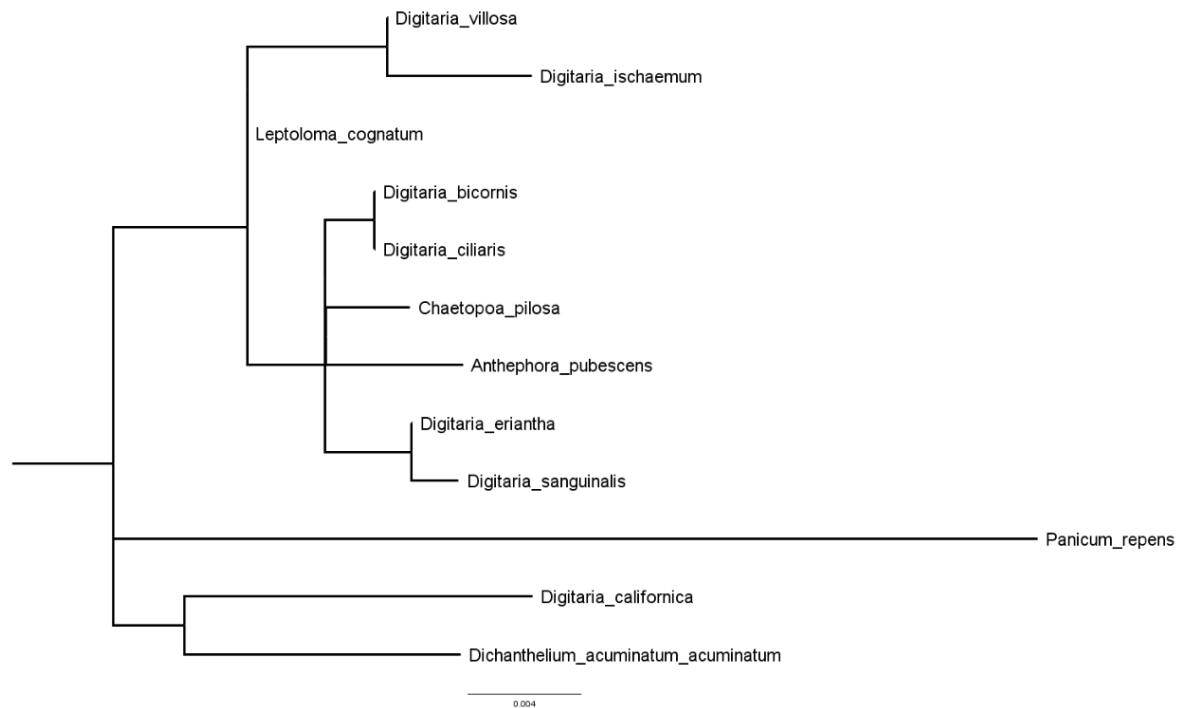
Figure 1. Maximum likelihood tree generated using RAxML-NG
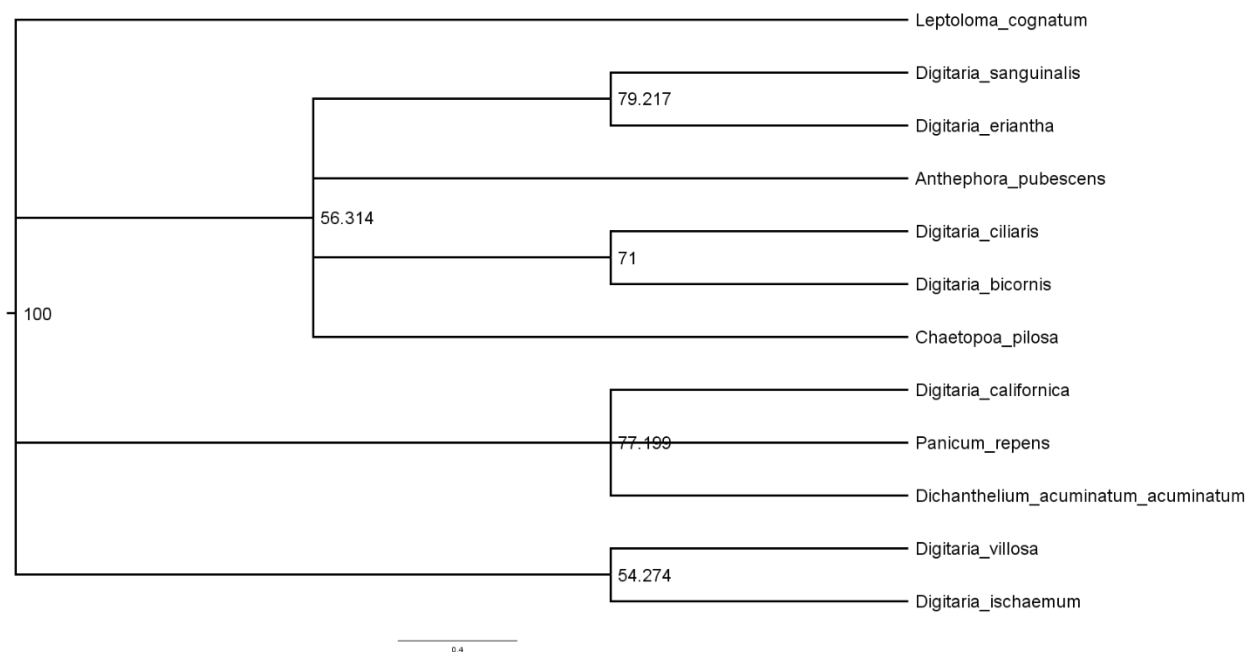
Figure 2. Parsimony bootstrap analysis tree generated using PAUP*.

Figure 3. Maximum clade credibility tree done using Bayesian analysis in MrBayes.

Discussion:

While the results of this project do not provide any striking novel information about relationships amongst the studied taxa, it was good practice in understanding how to generate different types of phylogenies using various types of computer programs. It also serves to illustrate the differences in effectiveness and usefulness of the different methods of phylogenetic inference. As we would expect, the more rudimentary method, parsimony analysis, generated a very poorly resolved tree. We also see a degree of improvement in resolution between the maximum likelihood tree and the tree generated using Bayesian inference, which is generally the most widely accepted form of phylogenetic inference currently. The tree in Figure 3 indicates that, according to sequence data from the *rbcL* gene, *Digitaria californica* may be relatively distantly related to the rest of *Digitaria*, if not in its own genus. This is also supported by morphological data, which often places *Digitaria californica* in *Digitaria* section *Trichachne* (Figure 4, from Vega, 2009). We also see that *Leptoloma cognatum* is likely an intrageneric taxon within *Digitaria*, along with *Chaetopoa pilosa* and *Anthephora pubescens*. While some of the relationships of the core *Digitaria* are not exactly what we would expect, this tree is overall not entirely unexpected and shows support from some other previous phylogenies of this group (Morrone, 2012). In general, this also indicates that a phylogeny of this group could either benefit from using more taxa, or from using a different set of moleuclar data or this data in conjuction with additional data.

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