PHYLOGEOGRAPHY OF A COASTAL GRASS IN EASTERN NORTH AMERICA: RECONSTRUCTING AN EVOLUTIONARY HISTORY OF SEA OATS (*UNIOLA PANICULATA* L., POACEAE)

A Thesis
by
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FOREWORD

The research detailed in this thesis will be submitted to the *Journal of Biogeography*, an international peer-reviewed journal. The thesis has been prepared according to the format required by this journal.

ABSTRACT

PHYLOGEOGRAPHY OF A COASTAL GRASS IN EASTERN NORTH AMERICA: RECONSTRUCTING AN EVOLUTIONARY HISTORY OF SEA OATS (*UNIOLA PANICULATA* L., POACEAE). (August 2011)

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Aim I tested the hypothesis that *Uniola paniculata* populations are divided into eastern and western lineages, with the southern tip of Florida possibly acting as the primary geographic break, as is the case in co-distributed animal taxa. Additionally, I asked: 1) Whether the geographic distribution of chloroplast DNA (cpDNA) variation in *U. paniculata* corresponds to genetic structure in nuclear variation as reported in previous studies, and 2) whether the geographic distribution of cpDNA variation in *U. paniculata* corresponds to the geographic distribution of morphological adaptive traits reported in previous studies.

Location Southeastern North America

Methods I sampled 47 populations of *U. paniculata* throughout its natural range in the United States. I used sequence variations in maternally inherited cpDNA to perform phylogeographical analyses. I used TCS software to reconstruct the intraspecific

phylogenetic network and Monmonier's algorithm to identify phylogeographic breaks in the species.

Results I found four cpDNA haplotypes and two major lineages: eastern (Atlantic Coast) and western (Gulf Coast). The eastern lineage is ancestral to the western lineage, and the phylogeographic break separating the two occurs at the southern tip of Florida.

Main conclusions The phylogeographic analysis suggests that *U. paniculata* populations survived the last glacial maximum (LGM) in refugia in southern Florida (including the Keys) and the Bahamas, and possibly in other locations, including Cuba, Texas and the Gulf Coast of Mexico. Following the LGM, a combination of vicariance and dispersal explains the current distribution of haplotypes into an eastern and western lineage. There are seven populations that contain a haplotype that is not in its native range; at least five of these populations are very likely explained by human-mediated transplantation. The phylogeographical pattern observed in *U. paniculata* is concordant with co-distributed animal taxa that experience a maritime discontinuity at the southern tip of Florida. The genetic structure of cpDNA sequence variations has a weak correlation with the genetic structure of nuclear DNA variation, and there is partial concordance between the geographic distribution of cpDNA and morphological variation reported by previous studies.

DEDICATION

For my parents, Margaret and Richard Hodel, who taught me the value of education and lifelong learning.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my thesis committee chairperson, Eva B. Gonzales, for teaching me everything I know about phylogeography and lab techniques, for tons of editorial help on this thesis, at all hours of the day, and finally, for taking a chance on a music major who decided he wanted to be a scientist when he grows up. I would also like to thank my committee members, Mike D. Madritch and Howard S. Neufeld, for editorial help, sampling advice and teaching me a lot about many different aspects of biology along the way. The sampling in this study would have not nearly been as complete without help from Dr. Mike Kane of the University of Florida, who provided samples from many locations along the Florida coast. This project was funded by NC SeaGrant, the Appalachian State University Office of Student Research, the Appalachian State University Cratis D. Williams Graduate School and the Appalachian State University Graduate Student Senate. Additionally, the following people provided assistance in numerous ways: Ray Williams, Zach Murrell, Gary Walker, Ciara Lockstadt, Patrick Sullins, Jackie Helwege, Shayla Murray, Kyle Flores, Margo Pray, Jim Sobieraj, Sarah Pate and Derick Poindexter.

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Chapter One: Introduction

Impact of glaciation on species distributions in eastern North America

Historical climate change has impacted species by causing shifts in their distributions (Hewitt, 2000). During the mid-Pliocene (3.3 – 3 Mya), the average global temperature was 2-3 ^{o}C warmer than current conditions and sea level was 25 m higher (Dwyer & Chandler, 2009). Subsequent global climate oscillations during the Pleistocene (2.6 M – 12,000 ya) led to repeated cycles of glacial advance and retreat in North America. As glaciers advanced, many species shifted their ranges southward, while as glaciers retreated, these species then re-radiated northward (Cronin, 1988; Delcourt & Delcourt, 1993; Morris *et al.*, 2010). Although southeastern North America was not covered with ice, the climate changes and sea level fluctuations associated with glaciation likely affected the historical distributions of many organisms, including coastal plant species (Bert, 1986; Felder & Staton, 1994). During periods of glacial advance, more water was confined to glaciers and consequently global sea levels fell. During the last glacial maximum (LGM), around 20,000 years BP, lower sea levels caused the coastline in the southeast to more closely resemble the current 200 m depth contour rather than the current coastline, and the coastline was significantly shifted on both the Atlantic Coast and throughout the Gulf of Mexico (Watts, 1980). The distribution of plant species with an exclusively coastal range changed during the Pleistocene climate oscillations, although in different ways than most other terrestrial species because of their

narrow, linear distribution. Coastal plants are uniquely suited to the ecology of the coastal ecosystem, and they are found over widespread longitudes and latitudes (Wagner, 1964; Delcourt & Delcourt, 1993, Christensen, 2000). Current rapid global climate changes highlight the importance of understanding species' historical distribution and migration patterns and rates. The ability of species to adjust their ranges or adapt to new climatic conditions affects whether they survive these climate changes (Thomas *et al.*, 2004). By studying the past distributions and migrations of species, we may infer the future geographical ranges of species as the climate changes. It is especially important to understand the processes that determine the geographical distribution of coastal species, as they are likely to be impacted by sea level fluctuations caused by global climate changes to a greater degree than inland species.

Several types of data are available to track how species' ranges shifted during glacial cycles. Fossil pollen and seeds can be used to infer changes in distribution of vegetation as the climate changes (Delcourt & Delcourt, 1981, 1991, 1993; Davis, 1983; Jackson *et al.*, 2000). However, fossil seed data are very scarce in the literature and the fossil pollen record can be misinterpreted. Because of the long distance dispersal ability of pollen, the presence of fossil pollen does not necessarily indicate that a species persisted in that location (McLachlan & Clark, 2004; Davis *et al.*, 2005; Gonzales *et al.*, 2008). Furthermore, the absence of fossil pollen data does not conclusively preclude the historical presence of a species in a certain region (McLachlan & Clark, 2004). Finally, because of high-energy waves and frequent disturbances, coastal ecosystems have a relative dearth of fossil data (Barbour & Christensen, 1993). It is important to utilize all other records (i.e., molecular markers) available when reconstructing evolutionary history, especially in coastal species.

Molecular markers can complement the fossil record to improve the understanding of the historical distribution of a species, without requiring researchers to solely rely on interpreting incomplete paleoecological data (Avise, 2000). Seed dispersal leaves a genetic footprint that can be tracked with appropriate molecular tools. Organelle DNA, from mitochondria or chloroplasts, is maternally inherited in most angiosperms and is only passed on to future generations in seeds. Thus, chloroplast DNA (cpDNA) can be used to map seed movement and track thousands of years of maternal lineages (e.g., Stehlik, 2002). The chloroplast genome is non-recombining like the mitochondrion genome, although the chloroplast genome has a much lower mutation rate. It can be assumed that most haplotypes identified from cpDNA from living plants will pre-date the LGM (Wolfe *et al.*, 1987).

The geography and topography of the southeastern United States has determined how the ranges of species were impacted as climate varied and several patterns have emerged from the evolutionary histories of various co-distributed taxa (Avise, 2000; Soltis *et al.*, 2006). One geographical feature that affects the distribution of species is the long, narrow peninsula of Florida, which currently divides marine and coastal species into two distinct units: Atlantic and Gulf of Mexico (Avise, 1992, 2000). In the mid-Pliocene, relatively warm temperatures caused sea level rises that led to the inundation of Florida. Then, during the Pleistocene, the expansion of the Florida peninsula (and subsequent contraction during glacial minima) contributed to the isolation of Gulf populations from Atlantic populations (Gold *et al.*, 1999; Avise, 2000). Additionally, carbonate sediments, ocean currents and mangrove-dominated ecosystems in southern Florida could also be responsible for preventing migration between Gulf and Atlantic populations of coastal and marine species (Wise *et al.*, 2004).

Phylogeography of coastal southeastern North America

In the southeastern United States, the majority of phylogeography studies use mitochondrial DNA (mtDNA) to examine the evolutionary histories of animal species (Avise, 2000). Despite rapid improvements in molecular techniques, there is still a dearth of phylogeography studies of many organisms in the southeastern United States (Soltis *et al.*, 2006). The lack of data is even more apparent when one considers phylogeography studies on southeastern plant species; the Soltis *et al.* (2006) review revealed that only 11% of phylogeography studies on organisms in the southeastern United States were on plants. Furthermore, to my knowledge, none of the previously published plant phylogeography studies focus on a species with an exclusively coastal distribution.

This study examines the cpDNA structure and diversity in populations of sea oats (*Uniola paniculata* L., Poaceae) throughout their native range to test the hypothesis that there is an east-west disjunction in this species, as seen in other co-distributed animal taxa. *Uniola paniculata* is a semi-tropical coastal grass that grows in sand dunes in the southeastern United States, from southern Virginia to eastern Mexico, and in Cuba and the Bahamas. It is typically the dominant plant species on the most exposed areas of sand dunes and it is very rare that *U. paniculata* is found more than 200 meters inland (Barbour & Christensen, 1993; Wagner, 1964). The species is adapted to a stressful habitat and is frequently exposed to high temperatures, unstable substrates, drought conditions, heavy winds and salt spray (Wagner, 1964). *Uniola paniculata* can reproduce both sexually and clonally. The seed heads may be dispersed large distances, primarily by ocean currents, but also by wind and animals. Additionally, the plants can propagate vegetatively through rhizomes (Wagner, 1964). Their fibrous roots and rhizomatous growth habit enable the grass to efficiently bind

sand that builds and sustains coastal sand dunes, thus preventing erosion (Snyder & Boss, 2002). As such, they provide valuable ecosystem services by building and sustaining coastal dunes, which act as a first line of defense to protect the land behind the dunes from storm surges, whether that land is coastal habitat for other plant and animal species or valuable coastal real estate (Degner *et al.*, 2007). As a result, *U. paniculata* is frequently used in coastal restoration programs following storm damage, since it can quickly stabilize dunes and reduce subsequent damage arising from erosion and wave action. However, these restoration efforts are often done using plants of either unknown geographic or genetic origin. Transplantation with no regard to origin can lead to the introduction of plants poorly adapted to local conditions, which may reduce survival. Furthermore, it can harm the genetic integrity of existing populations, leading to problems such as outbreeding depression (Broadhurst *et al.*, 2008)

Phylogeography studies using mtDNA have detected shared patterns among multiple animal species in the southeastern United States (Bowen & Avise, 1990; Gold & Richardson, 1998; Avise, 2000; Soltis *et al.*, 2006). An understanding of the evolutionary history of a species such as *U. paniculata* may elucidate whether we see common phylogeographical patterns in unrelated taxa, indicating whether species' distributions are governed by common environmental conditions or determined individualistically for each species. I expected that *U. paniculata*, as a coastal species whose seeds are primarily water-dispersed, may follow patterns observed in coastal animals. The main goal of this study was to investigate whether coastal plant species follow previously identified patterns in animal species by examining the evolutionary history of *U. paniculata* and how it affects the geographic distribution of genetic diversity within the species. I tested the hypothesis that *U. paniculata* populations

are divided into eastern and western lineages, with the southern tip of Florida possibly acting as the primary geographic break, as is the case in co-distributed animal taxa (Table 1). Additionally, I asked: 1) Whether the geographic distribution of cpDNA variation in *U. paniculata* corresponds to genetic structure in nuclear variation as reported in two previous studies (Franks *et al.*, 2004; Subudhi *et al.*, 2005) and 2) whether the geographic distribution of cpDNA variation in *U. paniculata* corresponds to the geographic distribution of morphological adaptive traits reported in two previous studies (Seneca, 1972; Harper & Seneca, 1974).

This study will be valuable in several ways: it will contribute to the theoretical conservation discussion of the Evolutionarily Significant Unit (ESU) concept, it will provide a scientific basis for future coastal dune restoration strategies, and it will give researchers access to another plant phylogeography study when looking for broad phylogeographic trends. The goal of an ESU is to determine conservation value at a level below species, at either the subspecies or population level (Ryder, 1986). Identifying intraspecific maternal lineages is a crucial step for delineating ESUs (Moritz, 1994). Currently, there are no restrictions in place controlling the origin of *U. paniculata* propagules used in dune restoration. This study will help determine whether guidelines should be imposed on how these plants are used in dune building projects. Because of a lack of plant phylogeography studies in southeastern North America, thus far it has been difficult to determine if plant species follow similar patterns as animal species. This study will contribute to our understanding of phylogeographic patterns in co-distributed taxa, and whether plants follow their own phylogeographic patterns or fit into established patterns observed in animal species.

Table 1. Studies of animal species in the southeastern North America showing a Gulf-Atlantic phylogeographic break between maternal lineages based on mitochondrial DNA variation. Adapted from Soltis *et al.* (2006) with permission.

Species name	Common name	References
Sciaenops ocellatus	red drum	Gold *et al.* (1999)
Pogonias cromis	black drum	Gold & Richardson (1998)
Cynoscion nebulosus	spotted seatrout	Gold & Richardson (1998)
Opsanus beta & *tau*	toadfish	Avise *et al.* (1987)
Carcharhinus limbatus	blacktip shark	Keeney *et al.* (2005)
Centropristis striata	black seabass	Bowen & Avise (1990)
Malaclemys terrapin	diamondback terrapin	Lamb & Avise (1992)
Ammodramus maritimus	seaside sparrow	Avise & Nelson (1989)
Loligo pealei	longfin squid	Herke & Foltz (2002)
Busycon perversum	sinestral whelk	Wise *et al.* (2004)
Brachidontes exustus	scorched mussel	Lee & Foighl (2004)
Crassotrea virginica	oyster	Reeb & Avise (1990)
Crepidula convexa	marine gastropod	Collin (2001)
Spisula solidissima	surfclam	Hare & Weinberg (2005)
Limulus polyphemus	horseshoe crab	Saunders *et al.* (1986)
Pagurus pollicaris & *longicarpus*	hermit crab	Young *et al.* (2002)
Emerita talpoida	mole crab	Tam *et al.* (1996)
Cicindella dorsalis	tiger beetle	Vogler & DeSalle (1993)

Chapter Two: Methods

Sampling methods

Forty-seven populations were sampled across the range of *U. paniculata* in the United States (Fig. 1 & Table 4). The sampled populations were spaced 2-200 km along the coast. At each sampling location, I collected 10 cm^2 leaf tissue samples from 10 individuals, which were spaced at least 10 m apart. All Florida samples except Jacksonville and Key West were obtained from tissue cultures grown by Dr. Mike Kane of the University of Florida. Additionally, I obtained leaf tissue samples from two commercial growers of *U. paniculata*, the Green Seasons Nursery (Parrish, Florida, USA) and the Oak Island Greenhouse (Oak Island, North Carolina, USA). Finally, in Louisiana, where some populations of *U. paniculata* have been extirpated due to a sand-deficient coastal environment (Hester & Mendelssohn, 1987), and in the Bahamas, I used herbarium specimens (one gram of seed head tissue) from the Smithsonian U.S. National Herbarium (Washington, D.C., USA) for DNA extraction. Specimen vouchers were catalogued and are stored in the Appalachian State University Herbarium (Boone, N.C., USA; accession numbers 21774-21785).

Laboratory Analyses

The collected leaf tissue (10 cm^2 per individual) was flash frozen in liquid nitrogen and stored at -80 ^oC in the lab until DNA extraction. Total genomic DNA was extracted

from disrupted leaf tissue using the DNeasy Plant Mini (Qiagen, Inc., Valencia, California, USA) following the protocol of the manufacturer. I amplified non-coding regions of cpDNA using PCR with universal primers (Taberlet *et al.*, 1991; Hamilton,1999; Ebert & Peakall, 2009). I ran PCR in the Biometra T-gradient (Whatman Biometra, Goettingen, Germany) thermoblock following the PCR protocol recommended by the designer of the primers. PCR reactions included 12.5 uL goTaq Master Mix (Promega Corporation, Madison, Wisconsin, USA), 8.5 µL nuclease free water, 2 µL DNA, 1 µL forward primer and 1 µL reverse primer. The PCR reaction was one cycle (94 ^{o}C for five minutes), 40 cycles (94 ^{o}C for 30 seconds, varying annealing temperature for 30 seconds, 72 ^{o}C for one minute), one cycle (72 ^{o}C for seven minutes). The annealing temperature depended on the primer pair used in the reaction and was 1-2 ^{o}C below the mean melting temperature of the two primers.

Initially, I tested 56 universal primer pairs on five geographically distant samples (Taberlet *et al.*, 1991; Hamilton,1999; Ebert & Peakall, 2009) to see if they would amplify cpDNA from our study species. I used 1% agarose gel stained with GelRed (Biotium Inc., Hayward, California, USA) to visualize the amplified cpDNA fragments. Twenty-five primer pairs yielded a single band and could be successfully sequenced for multiple samples. I sequenced 10-15 geographically distant individuals to identify which of the 25 primer pairs amplified variable regions. Five of the 25 cpDNA fragments show genetic variation resulting in six cpDNA variable regions (Table 2). I used cpDNA sequence variations to identify distinct haplotypes (Table 3, Table 4, Fig.1).

Amplified fragments were sequenced by Retrogen, Inc. (San Diego, California, USA), and were then aligned and compared using Sequencher for Mac version 4.10.1 (Gene Codes Corp., Ann Arbor, Michigan, USA). All cpDNA fragments were initially sequenced using

the forward primer. In cases where there were sequence ambiguities, the cpDNA fragments were also sequenced using the reverse primer for clarification. Additionally, I extracted DNA from a second individual in every population except two to investigate whether there is variation among individuals within a population. When herbarium specimens were used (NPBBa, TmILa), I was able to extract DNA from only one individual per population.

Table 2. Primers that amplify variable regions of chloroplast DNA, the genes where they anneal, primer sequence and primer developer.

Primer Name	Gene	Primer Sequence (5'-3')	Author
rpl 20	*rpl20*	TTT GTT CTA CGT CTC CGA GC	Hamilton (1999)
5' -rps 12	*rps12*	GTC GAG GAA CAT GTA CTA GG	Hamilton (1999)
ANU_cp007-L	*rps16* ex1	CTT CGA GAT CGA ACA TCA AT	Ebert & Peakall (2009)
ANU_cp008-R	*rps16* ex2	AAA ACG ATG TGG TAG AAA GC	Ebert & Peakall (2009)
ANU_cp016-L	*trnG* ex1	GCG GGT ATA GTT TAG TGG TAA AAG	Ebert & Peakall (2009)
ANU_cp017-R	*trnG* ex2	CGT TAG CTT GGA AGG CTA GG	Ebert & Peakall (2009)
ANU_cp035-L*	*rpoB*	TGT GGA CAT TCC CTC ATT TC	Ebert & Peakall (2009)
ANU_cp036-R*	*rpoC*	TGC AGT CCC CTG CCT TAC	Ebert & Peakall (2009)
ANU_cp047-L	*psbC*	GGC GTA GCT ACC GAG ATC AA	Ebert & Peakall (2009)
ANU_cp048-R	*psbZ*	TGC AAA AAC AGC TAA TTG GAA A	Ebert & Peakall (2009)

* = two variations found in the fragment amplified by this primer pair.

Analyses of cpDNA variation

I reconstructed the evolutionary relationships among cpDNA haplotypes (representing maternal lineages) using maximum parsimony (MP), maximum likelihood (ML) and unweighted pair group with arithmetic mean (UPGMA) techniques. Maximum parsimony analyses were performed with all nucleotide substitutions weighted equally. I followed the approaches of Clement *et al.* (2000) and Templeton *et al.* (1992) using TCS

software (http://darwin.uvigo.es/software/ tcs.html, 2/28/2011) to create a network of haplotypes justified by a 95% parsimony criterion. Maximum likelihood analyses were conducted using the PHYLIP version 3.69 (Felsenstein, 1989) program DNAML with the default settings (http://evolution.genetics.washington.edu/phylip.html, 3/5/2011). UPGMA analyses were completed using the PHYLIP program DNADIST to construct a genetic distance matrix (Table 3) and the program NEIGHBOR to create an UPGMA tree. Default settings were used for both programs and the Jukes-Cantor Model was used in the calculation of genetic distance among haplotypes.

Uniola pittieri, Eragrostis pilosa and *Sorghum bicolor* were used as outgroups to root the phylogenetic trees in all three approaches (MP, ML, UPGMA). The sequences for all three species were obtained from GenBank (accession numbers: *U. pittieri*, AY509526; *E. pilosa*, AY136859; *S. bicolor*, EF115542). However, GenBank only had sequence coverage for either *U. pittieri* or *E. pilosa* corresponding to the variable region in *U. paniculata* flanked by primers ANU_cp007-L and ANU_cp008-R (Ebert & Peakall, 2009), whereas *S. bicolor* had complete coverage of all six variable regions. *Uniola pittieri* is the only other widespread New World *Uniola* species and *E. pilosa* has been shown to be closely related to *Uniola* in published phylogenies of the grasses (Barker *et al.*, 2001; Peterson *et al*., 2010), making them suitable outgroups. *Sorghum bicolor* is the closest relative that had complete coverage of all variable regions identified in *U. paniculata* in this study, making it suitable for rooting the TCS cpDNA haplotype tree; it shares 83% sequence identity. The phylogenetic relationships among *U. paniculata* cpDNA lineages were combined with their geographical distribution to gain insights into their evolutionary history.

I used the most parsimonious haplotype network combined with the geographic data

associated with each haplotype to infer likely phylogeographical relationships. I then used BARRIERS 2.2 (http://www.mnhn.fr/mnhn/ecoanthropologie/software/barrier.html, 4/17/2011) to identify geographic barriers using Monmonier's algorithm (Manni *et al.*, 2004, Morris *et al.*, 2010). This algorithm computes genetic barriers by testing how geographic and genetic distances correspond among the populations. I generated 100 bootstrapped genetic distance matrices using our cpDNA sequence data and the PHYLIP 3.69 programs DNADIST and SEQBOOT. When these matrices are inputted into BARRIERS 2.2, they provide bootstrap support for proposed geographic barriers.

I calculated total genetic diversity (h_T) and genetic diversity within populations (h_S) using the software PERMUT (http://www.pierroton.inra.fr/genetics/labo/ Software/Permut/, 6/14/2011). For this analysis, I combined populations into groups identified by BARRIERS with greater than 95% bootstrap support. Additionally, I measured the level of genetic differentiation among groups of populations by calculating G_{ST} (Nei, 1987) and N_{ST} (Pons & Petit, 1995, 1996) in PERMUT. G_{ST} measures genetic differentiation among sample locations using haplotype frequencies. N_{ST} is another measure of genetic differentiation that takes similarities among haplotypes into account, unlike G_{ST} (Petit et al., 2005). PERMUT tests whether N_{ST} is significantly greater than G_{ST} by measuring how many permuted values of G_{ST} are higher than N_{ST}. If N_{ST} is significantly greater, we can infer that the relative distribution of phylogenetically related haplotypes contributes to the overall geographic structure of the species, which is one definition of phylogeographic structure (El Mousadik & Petit, 1996; Saeki *et al.*, 2011). Furthermore, I tested the barrier displaying the strongest support by using AMOVA in GenAlEx (http://www.anu.edu.au/BoZo/GenAlEx/, 4/21/2011). For the AMOVA analysis, I divided the populations into two groups, an eastern group and a

western group, based on which side of the strongest-supported barrier they were located. Then AMOVA calculates the portion of statistically significant genetic variation that exists across the barrier and within each unit on opposing sides of the barrier. Statistically-supported barriers can be used to explain patterns observed from the cpDNA variation-based phylogeny.

Comparison with previous studies

I compared the phylogeographical pattern identified in *U. paniculata* to patterns found in co-distributed taxa in previous studies (Avise, 2000; Soltis *et al.*, 2006) in order to determine if *U. paniculata* exhibits the same east-west break as many southeastern animal species. Additionally, I examined the similarities between patterns in cpDNA variation and patterns of variation observed using nuclear DNA markers in previous studies (Franks *et al.*, 2004; Subudhi *et al.*, 2005) to identify any phylogeographical similarities between different types of markers. Finally, I compared the variations in cpDNA collected in this study to variations in morphological characters detected using the same species in previous studies (Seneca, 1972; Harper & Seneca, 1974), which investigated how several morphologically variable characters in *U. paniculata* correlated with geography. By comparing the geographic distribution of evolutionary lineages with the distribution of morphological variations, I can infer whether independent evolutionary lineages contain unique adaptive variations or whether the evolution of morphological adaptive traits is governed by other processes.

Chapter Three: Results

Chloroplast DNA diversity

I sequenced and aligned a total of approximately 16,000 base pairs of cpDNA, which represents regions amplified by 25 primer pairs. Of these 25 primer pairs, 20 pairs amplified regions that showed no variation among the samples and five pairs amplified variable regions (Table 1). Four of the pairs amplified a region with one variable site, while one primer pair amplified a region with two variable nucleotides. In total, I identified six variable characters, all of which were single nucleotide substitutions and parsimony-informative. Combined, these six variations in cpDNA sequences identified four distinct haplotypes, labeled A-D (Tables 3 & 4). Forty-two of the 47 populations were fixed for a single haplotype; the populations BSPMs, AtBNC, KwISC, HtHSC and OcINC contained two haplotypes.

Table 3. Pairwise matrix indicating number of nucleotide substitutions among the four haplotypes (A-D) above the diagonal. Pairwise genetic distances (%) based on the PHYLIP program DNADIST, using the Jukes-Cantor Model, are below the diagonal.

Pairwise Distance Matrix				
Haplotype	**A**	**B**	**C**	**D**
A	0	3	5	6
B	0.000783	0	2	3
C	0.001306	0.000522	0	1
D	0.001568	0.000783	0.000261	0

Table 4. *Uniola paniculata* populations, their geographical locations and haplotype group. The samples are grouped by state or country, from southwest to northeast.

Site	State	Sample ID	Latitude (N)	Longitude (W)	Haplotype
South Padre Island	TX	SPITx	26.1456	97.1697	C
Padre Island NS	TX	PdITx	27.3986	97.3083	C
Matagorda	TX	MatTx	28.5975	95.9764	C
Timbalier Island	LA	TmILa	29.0600	90.4711	A
Buccaneer SP	MS	BSPMs	30.2661	89.3878	A , C
Dauphin Island Sea Lab	AL	DpIAl	30.2467	88.0772	C
Green Seasons Nursery	FL	GSNFl	30.2919	87.4619	C
Perdido Key SP	FL	PdKFl	30.3219	87.3150	C
Navarre Beach SP	FL	NvBFl	30.3839	86.8608	C
Henderson Beach SRA	FL	HnBFl	30.3836	86.4439	C
Saint George Island SP	FL	StGFl	29.6572	84.8747	C
Honeymoon Island SP	FL	HmIFl	28.0656	82.8336	C
Delnor Wiggins Pass SP	FL	DWPFl	26.2753	81.8283	D
Key West	FL	KyWFl	24.5455	81.0879	D
New Providence Beach	BA	NPBBa	25.0707	77.3910	A
Bill Baggs SP	FL	BBgFl	25.6706	80.1533	A
John U. Lloyd SP	FL	JULFl	26.0611	80.1114	A
John D. MacArthur SP	FL	JDMFl	26.8208	80.0378	A
Sebastian Inlet SP	FL	SbIFl	27.8547	80.4444	A
Gamble Rogers SRA	FL	GmRFl	29.4394	81.1078	A
Anastasia SRA	FL	AnaFl	29.8928	81.2761	A
Jacksonville	FL	JaxFl	30.3228	81.3900	D
Little Talbot Island	FL	LTIFl	30.4592	81.4111	A
Jekyll Island	GA	JkIGa	31.0447	81.4119	A
Saint Simon's Island	GA	StSGa	31.1456	81.3722	A
Sapelo Island	GA	SpIGa	31.3906	81.2642	A
Tybee Island	GA	TyIGa	32.0039	80.8378	A
Hilton Head	SC	HtHSC	32.1944	80.6981	A , C
South Hunting Island	SC	HISSC	32.3417	80.4517	A
Edisto Island	SC	EdISC	32.5422	80.2344	A
North Hunting Island	SC	HINSC	32.5600	80.1761	A
Kiawah Island	SC	KwISC	32.5803	80.1306	A , B
South Folly Beach	SC	FBSSC	32.6603	79.9294	A
North Folly Beach	SC	FBNSC	32.6658	79.9536	D
Fort Sumter	SC	FtSSC	32.6992	79.8883	B
Sullivan's Island	SC	SvISC	32.7586	79.8503	C
Breach Inlet	SC	BrISC	32.7750	79.8147	A
South Isle of Palms	SC	IPSSC	32.7864	79.7872	A
North Isle of Palms	SC	IPNSC	32.8028	79.6914	C
Huntington SP	SC	HSPSC	33.5008	79.0589	A
Oak Island Greenhouse	NC	OIGNC	33.9133	78.1478	A
Wrightsville Beach	NC	WrBNC	34.2150	77.7906	A
Emerald Isle	NC	EmINC	34.6769	76.9586	B
Atlantic Beach	NC	AtBNC	34.6750	76.7386	A , B
Ocracoke Island	NC	OcINC	35.1069	75.9533	A , B
Kill Devil Hills	NC	KDHNC	36.0072	75.6525	A
Assateague Island	VA	AsIVa	37.9428	75.3014	A

NS = National Seashore, SP = State Park, SRA = State Recreation Area.

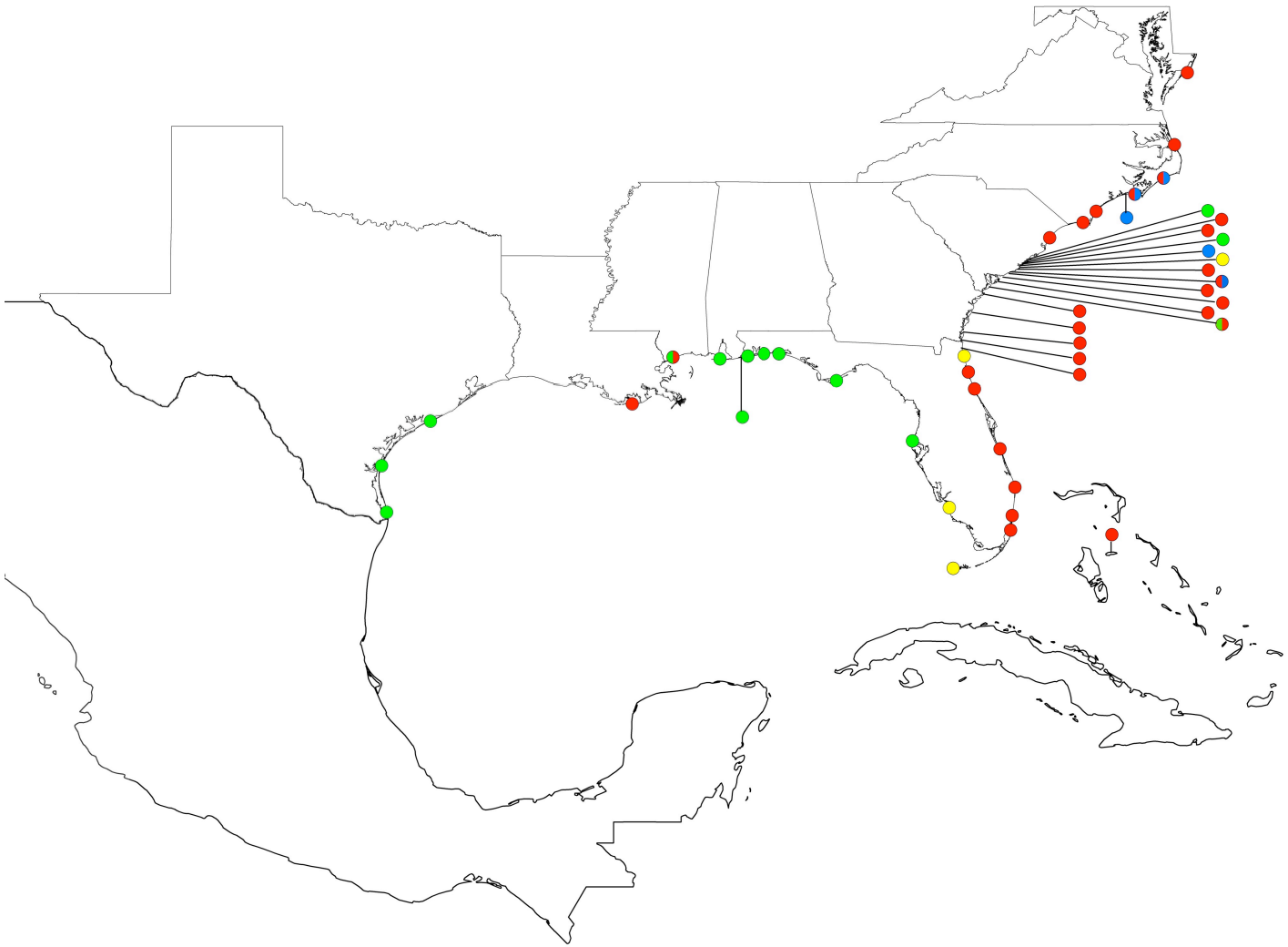

Figure 1. Map of geographical distribution of haplotypes. Red = haplotype A, blue = haplotype B, green = haplotype C, yellow = haplotype D.

Geographic distribution

Two haplotypes (A and C) are widespread. Haplotype A is abundant predominantly on the Atlantic Coast; 24 of the 32 populations sampled on the Atlantic Coast are exclusively composed of haplotype A (Fig. 1). Haplotype B is rare, occurring in only four populations, and is unique to North and South Carolina. Haplotype C is widespread on the Gulf Coast. Of the 14 populations sampled on the Gulf Coast, 10 of them contained only haplotype C individuals. Haplotype D is rare, occurring in only four disjunct populations on the Gulf and Atlantic Coasts of Florida, and in South Carolina (Fig. 1).

I used the cpDNA haplotype data and TCS software to form an unrooted phylogenetic haplotype network using the most parsimonious connections (>95%). TCS adds intermediate extinct or non-existent haplotypes to create the most parsimonious network connections. Outgroup sequences to root the tree were chosen based on maximum identity to the query sequence, in the cases of *U. pittieri* and *E. pilosa*, and maximum coverage for *S. bicolor*. Using all three outgroups resulted in a consistently rooted phylogeny indicating that haplotype A is ancestral to B, which is ancestral to C, which is ancestral to D (Fig. 2). The other analyses (ML and UPGMA) yielded identical haplotype trees for all three outgroups (not shown).

BARRIERS 2.2 (Manni *et al.*, 2004) identified six significant geographic boundaries with bootstrap support over 95% (Fig. 3). The software identified one barrier that had bootstrap support of 99%, which is located at the southern tip of Florida and separates the range of *U. paniculata* into two geographic regions. The AMOVA analysis comparing the two groups (eastern, western) on either side of the barrier at the southern tip of Florida revealed that 66% of the molecular variance existed between the two groups, while 34% was

within the two groups ($p<0.001$). Total genetic diversity (h_T) was 0.654, while average population genetic diversity (h_S) was 0.390, when a population was defined as a group of populations isolated by geographic breaks with greater than 95% bootstrap support identified using BARRIERS. Using 1000 permutations, N_{ST} was 0.509, making it significantly higher than G_{ST}, which was 0.404 ($p < 0.001$). Because N_{ST} was significantly greater than G_{ST}, the evolutionary history of the populations likely influenced the geographic distribution of cpDNA lineages within this species.

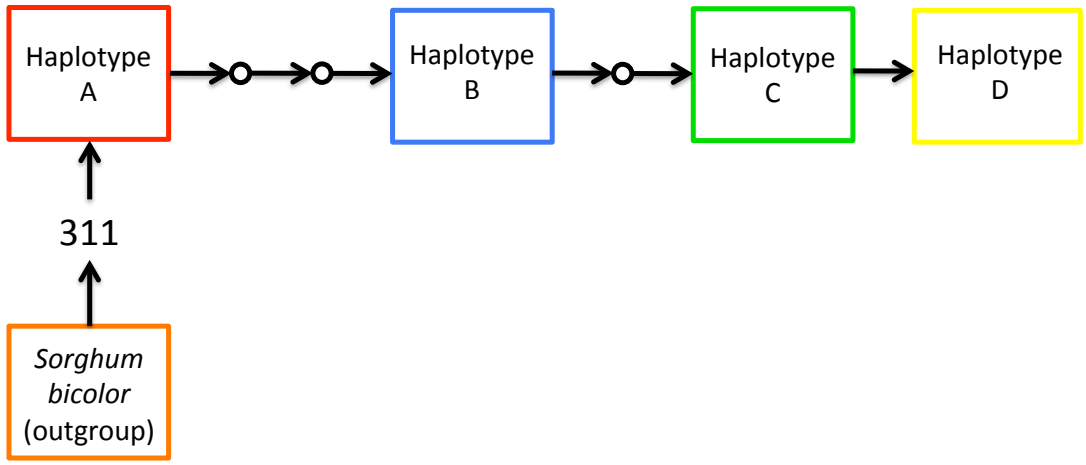

Figure 2. The cpDNA haplotype network resulting from the TCS analysis using maximum parsimony (>95%), composed of the four *U. paniculata* haplotypes (A-D) and the outgroup *S. bicolor*. Each circle represents an intermediate, non-sampled or non-existent haplotype. Each line within the *U. paniculata* network represents a mutation (single nucleotide substitution). There are 312 mutations separating the outgroup and Haplotype A; the 311 circles (intermediate haplotypes) are not individually represented.

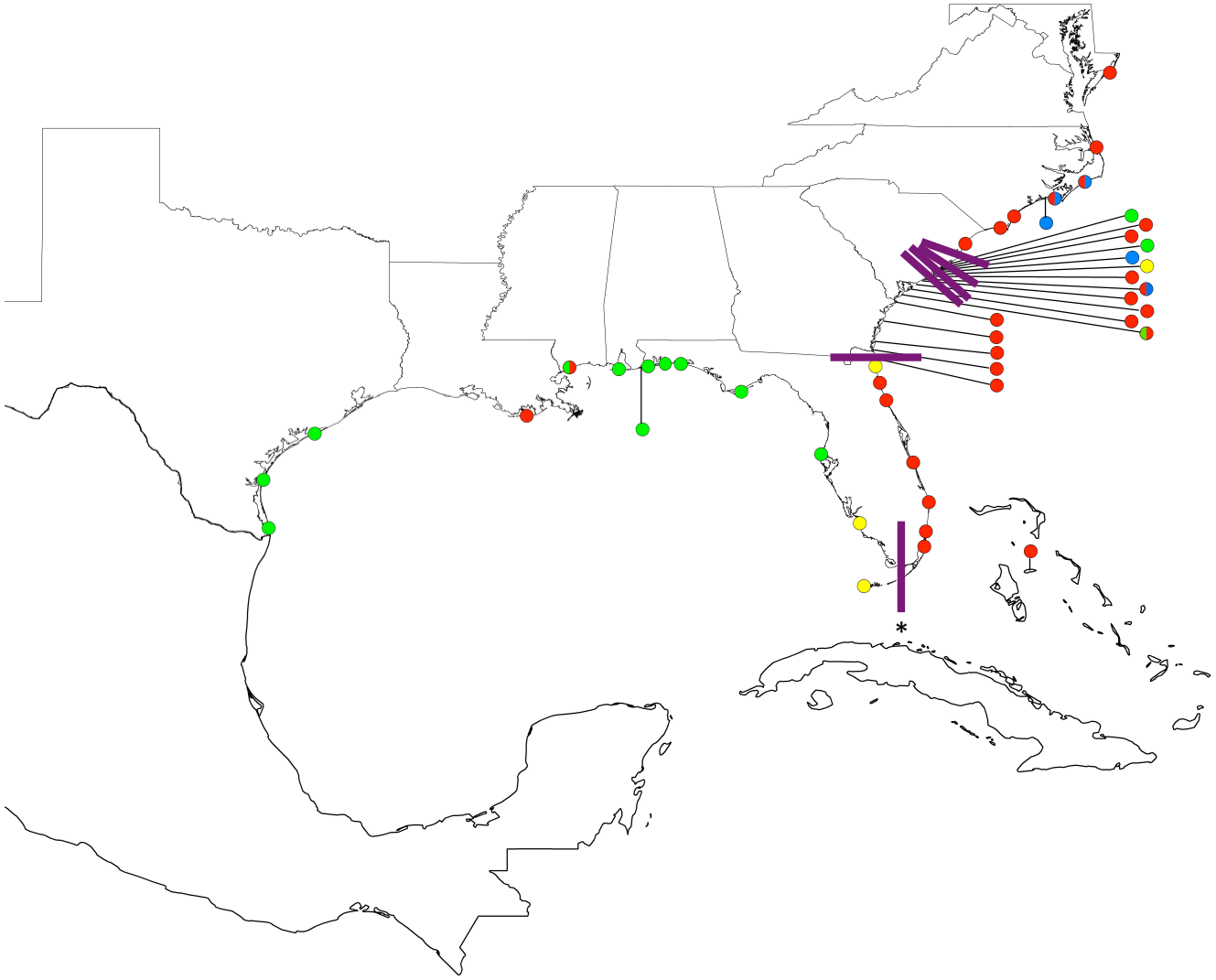

Figure 3. Geographic breaks in the range of *Uniola paniculata* based on Monmonier's algorithm using the program Barriers 2.2 (Manni *et al*., 2004). Breaks are indicated with purple lines; all lines displayed have bootsrap values of at least 95 %. The asterisk below the line in southern Florida indicates bootstrap support of 99 %.

Chapter Four: Discussion

Geographic distribution of cpDNA haplotypes

My study supported the hypothesis that there is an east-west phylogeographic break that divides the range of *U. paniculata* into two main genealogical lineages (Atlantic and Gulf of Mexico). This east-west disjunction, referred to as a maritime discontinuity, is concordant with previously reported patterns in co-distributed animal species (Table 1) (Avise *et al*., 1987; Gold & Richardson, 1998; Avise, 2000; Soltis *et al.*, 2006). Organisms that follow this pattern typically divide into eastern and western lineages, with the split between the lineages occurring on the southern portion of the Florida peninsula (Avise, 2000; Soltis *et al.*, 2006). Many distantly-related species, such as the oyster (C*rassotrea virginica),* dusky seaside sparrow (*Ammodramus maritimus*) and blacktip shark (*Carcharhinus limbatus*) show a phylogeographic break at a similar point on the Florida peninsula as *U. paniculata* (Fig. 3) (Avise & Nelson, 1989; Reeb & Avise, 1990; Keeney *et al.*, 2005).

The phylogeographical break observed among the eastern and western lineages of *U. paniculata* is likely caused by historical events in the southeastern United States, including sea level changes due to glaciation, the associated changes in the size and shape of the Florida peninsula, carbonate sediments and mangrove-dominated ecosystems in southern Florida (Wise *et al.*, 2004). The observed patterns of cpDNA variation suggest that *U. paniculata* survived in southern refugia during a Pleistocene glacial maximum, and a

combination of vicariance and dispersal events divided the species into two intraspecific units during the subsequent northward spread of the species following the LGM. The inland habitats of the Florida peninsula, unsuitable for *U. paniculata*, acted as a barrier to dispersal and subsequent gene flow among Atlantic and Gulf populations. Similarly, the mangroves of southern Florida created a barrier between the Atlantic and Gulf populations, because they prevent coastal sand dunes and beaches from forming in a portion of southern Florida.

The Atlantic (eastern) lineage is ancestral and composed of two cpDNA haplotypes (A and B), while the Gulf Coast lineage is primarily represented by more recently derived haplotypes (C and D). Each lineage has one dominant, widespread haplotype: A on the Atlantic Coast, and C on the Gulf Coast, and rare haplotypes: B on the Atlantic Coast and D on the Gulf Coast. Of the 32 populations sampled on the Atlantic Coast, 27 contained haplotype A, of which 23 were solely haplotype A. Of the fourteen populations sampled on the Gulf Coast, 11 contained haplotype C, while 10 were solely haplotype C. The majority of populations (42 out or 47) were fixed for a single haplotype. This low level of polymorphism is consistent with other plant phylogeography studies and the low mutation rate of cpDNA (Wolfe *et al.*, 1987; Avise, 2000; Soltis *et al.*, 2006; Morris *et al.*, 2008; Morris *et al.*, 2010). Surprisingly, of the 47 populations, seven contained a haplotype outside of its expected range (i.e., an Atlantic haplotype existing in a population located on the Gulf Coast, or vice versa).

The observed pattern is consistent with multiple glacial refugia for *U. paniculata.* The current distribution of cpDNA haplotypes provides genetic evidence for refugia in the Bahamas and southern Florida, including the Florida Keys. It is also possible that *U. paniculata* survived in Cuba and along the Gulf Coast in Florida, Texas or Mexico. Based on

the slow mutation rate of cpDNA, with an average synonymous nucleotide substitution rate of 0.1-0.3% per million years, it is very likely that the cpDNA haplotypes predate the LGM (Wolfe *et al.*, 1987; Dorken & Barrett, 2004). Furthermore, it is possible that some of these haplotypes diverged before multiple glaciation events in the Pleistocene (Zurawski *et al.*, 1984). The fact that the most recently derived haplotype (D) was observed in one of the southernmost locations, the Florida Keys, is further evidence that the haplotypes may have diverged prior to the LGM. With rising temperatures and receding glaciers, the species migrated northward, and the unsuitable inland Florida habitats acted as a barrier to gene flow between the Atlantic and Gulf coasts. Haplotypes A and B would have migrated north-south along the Atlantic Coast, while haplotypes C and D would have migrated east-west along the Gulf Coast.

Anthropogenic introductions may be responsible for the seven populations containing unexpected haplotypes. Interviews with commercial growers of *U. paniculata* revealed that the majority of greenhouses in Florida grow *U. paniculata* from seeds collected from a single location on the Gulf Coast of Florida, Perdido Key State Park. Genetic analyses indicated that plants naturally growing in this location and seedlings commercially grown with seed from Perdido Key were both haplotype C, a haplotype with unexpected occurrences on the Atlantic Coast. Seedlings can be purchased and planted hundreds of kilometers away from where the seeds were harvested, possibly leading to the introduction of non-native haplotypes (personal communication with owner of Green Seasons Nursery, Parrish, Florida) and could explain the occurrences of haplotypes C on the Atlantic Coast. Interestingly, all the Atlantic populations containing haplotype C occur near Charleston, SC. This region is heavily populated, has lots of coastal real estate development and is a busy shipping port. It is

possible that some of the unexpected haplotypes were introduced by humans, either intentionally to restore dunes or unintentionally via trade. The Atlantic populations comprised of the Gulf haplotype D (JaxFl and FBNSC) are also in heavily populated locations, where it is possible that dune restoration projects introduced this non-native haplotype, although there is no evidence thus far that haplotype D has been as extensively used in restoration as haplotype C has been.

Human transport can also explain the presence of Atlantic haplotypes on the Gulf Coast, where one of the populations, Buccaneer State Park (BSPMs), is comprised of a mix of haplotypes A and C. This sampling location underwent recent extensive beach restoration after its coastline was devastated by Hurricane Katrina, causing Buccaneer State Park to be closed from 2005 to 2010 (personal communication, Mississippi Wildlife, Fisheries and Parks). When I sampled this area, the beach was devoid of any *U. paniculata* plants more than a meter tall and the plants were growing in evenly spaced rows. It is very likely that these seedlings were introduced from multiple sources after Hurricane Katrina. Another Gulf Coast population (TmILa) containing haplotype A is now extirpated and as a result, I had to use a herbarium specimen for DNA extraction. This population was used in dune building experiments in the 1970s and 1980s, which may have introduced *U. paniculata* propagules with an Atlantic Coast origin (Mendelssohn *et al.*, 1991).

The only haplotype obtained from the Caribbean is haplotype A, sampled in the Bahamas (NPBBa), east of the southern tip of Florida, providing evidence that there may have been a refugium for the Atlantic haplotype A. While the Bahamas were not physically connected to the Florida peninsula during glacial maxima, they were closer to the Florida peninsula than they are today--less than 100 km at the closest point (Haq *et al.*, 1987, Maskas

& Cruzan, 2000). It is certainly possible that *U. paniculata*, with its water dispersed seeds, could have radiated northward along the Atlantic Coast from a refugium in the Bahamas because of the movement of the Gulf Stream. My sampling was limited to herbarium specimens in the Bahamas, which made it difficult to obtain additional individuals from this location. Furthermore, populations of Atlantic haplotypes may have survived in the southern portion of the Florida peninsula. The climate in southern Florida during the LGM was likely warm enough to allow the populations of *U. paniculata* to persist.

The population sampled from the more western Florida Keys (KyWFl) is made up of haplotype D, suggesting that the Keys may have served as one of the southernmost refugia and propagule sources for the Gulf haplotype D. The Florida Keys were part of a continuous land mass with the Florida peninsula during the last Pleistocene glacial maximum. At that time, inland Florida habitats would have been an even larger barrier separating the Atlantic Coast from the Gulf Coast than they are currently, suggesting that the Florida Keys are a likely southern refugium for modern day Gulf haplotypes. It is also possible that populations in Cuba, Texas and Mexico persisted along the Gulf Coast during the LGM. However, there were no Cuban or Mexican samples available to investigate this possibility, and I did not find any genetic footprint that would either confirm or exclude this scenario.

The TCS phylogenetic network indicates that haplotype B is ancestral to haplotype C (Fig. 2). This ancestry network does not suggest an obvious explanation of how haplotype B gave rise to haplotype C; haplotype B has an Atlantic distribution restricted to the Carolinas, while haplotype C has a widespread Gulf distribution. Several scenarios are possible: the lineages may have split in the Carolinas and a long-distance dispersal event moved haplotype C to the Gulf, or the lineages may have diverged in a southern refugium and the two

haplotypes migrated to separate coasts following the LGM, or homoplasy may have occurred in both haplotypes. However, neither homoplasy nor long distance dispersal appear to be plausible explanations. Homoplasy would have required three independent identical mutations in both haplotypes, while a dispersal event would have required seeds from the Carolinas to travel hundreds of kilometers against the Gulf Stream and around the tip of Florida and into the Gulf of Mexico. Therefore, all available evidence suggests that haplotype C arose from haplotype B in a glacial refugium.

Synthesis with nuclear genetic diversity studies

Chloroplast DNA is maternally inherited and can be used to track the genetic footprint left by seed movement, whereas nuclear DNA markers are biparentally inherited. Two studies of *U. paniculata* used nuclear markers to characterize genetic diversity and its distribution across the species' geographic range. Franks *et al.* (2004) measured genetic structure and diversity in populations of *U. paniculata* using allozymes. Their analysis revealed less population genetic structure than the cpDNA data, and they found that several geographically disjunct populations were more similar to one another than to neighboring populations. For example, Virginia Beach (VA), Sapelo Island (GA) and Cape Canaveral (FL), geographically distant Atlantic Coast populations, formed a clade with two populations on the far western edge of the Gulf Coast, Mustang Island (TX) and South Padre Island (TX).

Another study used amplified fragment length polymorphisms (AFLPs) to assess genetic diversity among populations of *U. paniculata* (Subudhi *et al.,* 2005). Their data revealed higher population genetic structure among *U. paniculata* populations than the Franks *et al.* (2004) allozyme study. The Carolinas clustered together, all the Florida

populations formed a cluster, Mississippi and Alabama populations clustered together and Texas, Louisiana and Virginia populations form a cluster. With the exception of Virginia populations forming a cluster with very distant populations from the western Gulf (Texas and Louisiana), the populations grouped together based on geography. However, Subudhi *et al.* (2005) did not sample on the Florida peninsula, making conclusions about congruence between patterns of cpDNA and AFLP markers difficult. The nuclear DNA data do not show a clear east-west phylogeographic break, most likely because of the combination of anthropogenic introduction of non-native populations with subsequent long distance pollen mediated gene dispersal among neighboring populations.

Synthesis with morphological variation studies

The genetic discontinuity identified by cpDNA variation shows partial congruence with the patterns of morphological variation found in previous studies of *U. paniculata* (Seneca, 1972; Harper & Seneca, 1974), which examined morphological variations among populations. Whereas the cpDNA lineages split into two major units, Seneca (1972), who sampled a significant portion of the species' range, found that morphological variation separated the species into three units. Seneca (1972) found significant divergence in adaptive traits of plants collected from different geographic locations: plants differed in germination response following a period of cold, and seedlings responded differently to salinity, temperature and photoperiod treatments. Harper & Seneca (1974) identified a significant latitudinal gradient in floral initiation timing, but this study was conducted only in North Carolina, which has a limited latitudinal range of just a few degrees. The surveyed populations clustered into *three* geographically structured groups (Fig. 4) based on

differences in morphological characters: populations from North Carolina and Virginia grouped together (1), as did populations from the Atlantic Coast of Florida (2), and the populations from the Gulf of Mexico formed the final group (3). These results partially correspond to the east-west discontinuity identified by the cpDNA lineages. The western cpDNA lineage correlates with one of the morphological groups (3), which indicates that historical separation may be accompanied by morphological divergence. Seneca's (1972) study also found that the eastern populations were further subdivided into distinct northern and southern groups. This subdivision of the eastern lineage can be best explained by the effect of local environmental conditions on the evolution of adaptive traits. Thus, there is only partial congruence between the genetic and morphological structure. However, Seneca's (1972) sampling was limited, with a large gap between the northern (1) and southern (2) Atlantic groups, and no populations from the Gulf Coast of Florida were included in the analysis. Future studies of morphological variation can help determine more precisely the relationship between cpDNA lineages and morphological variation.

Evolutionarily Significant Units and conservation implications

Ryder (1986) coined the term 'Evolutionarily Significant Unit' (ESU) to address the problem of conserving biodiversity at a level below species. He acknowledged that identifying ESUs would be challenging, and that multiple types of data should be used to define an ESU including morphological data, geographical distribution, and nuclear and mitochondrial DNA data. Moritz (1994) proposed using solely molecular techniques to define ESUs. By using nuclear and maternally inherited DNA markers, an ESU could be defined as a group of populations (i.e., an evolutionary independent lineage) that has been

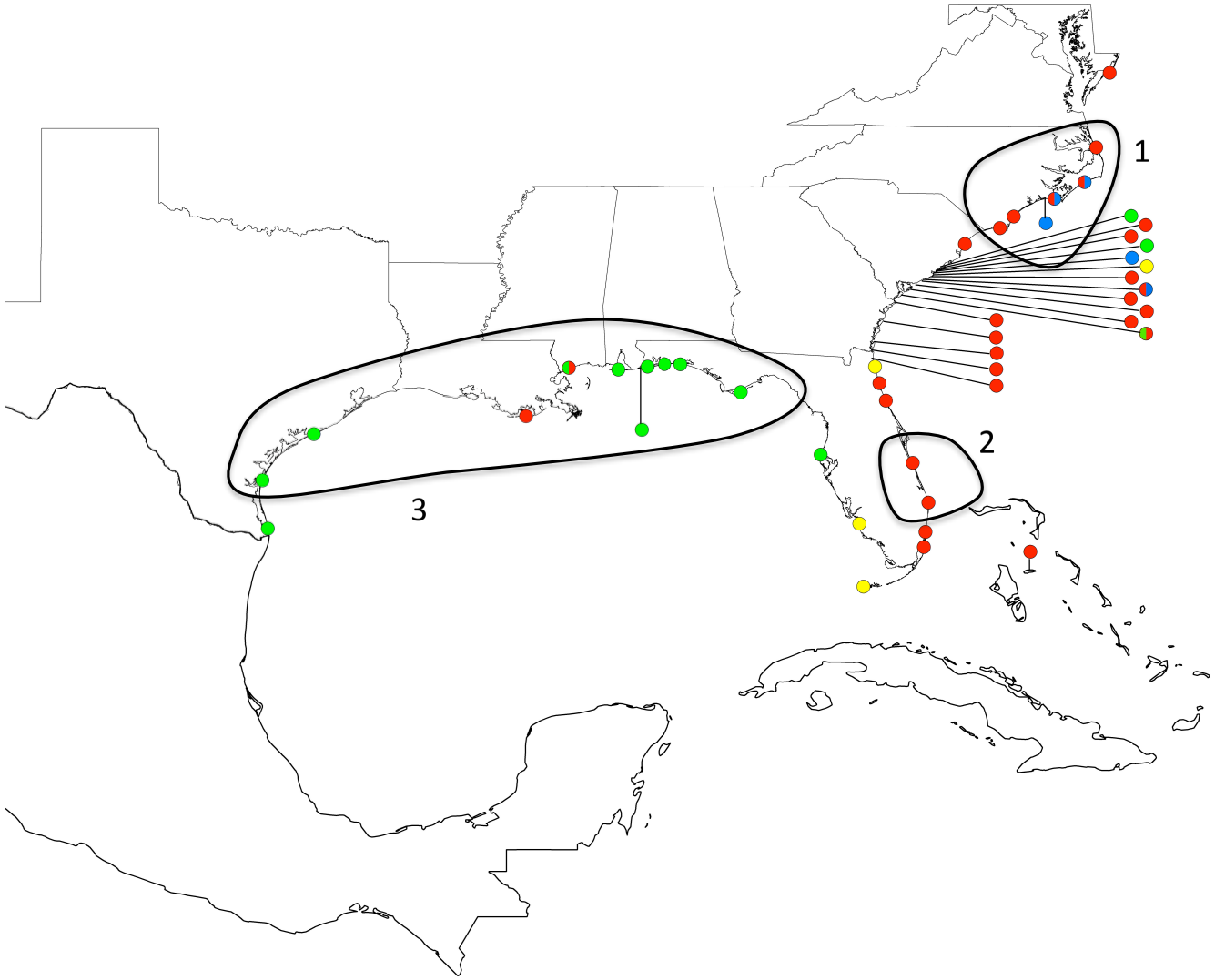

Figure 4. Map of haplotypes and geographic locations of the three units delineated by significant morphological variations in Seneca (1972).

historically isolated. Moritz (1994) suggested that an ESU should be defined as a set of populations that are reciprocally monophyletic for maternally inherited DNA alleles, and that show significant divergence of allele frequencies at nuclear loci. This definition of ESUs, based only on genetic data, has the advantage of being easy to implement, especially as molecular phylogeny tools become increasingly affordable and available. However, an assumption of this definition is that populations with independent evolutionary histories naturally contain the genetic material to adapt to changing environments. Crandall (2000) called for the inclusion of both ecological and genetic data and advocated incorporating the concepts of ecological and genetic exchangeability (*sensu* Templeton, 1994) into the definition of ESUs so that adaptive variation and intraspecific evolutionary history would be preserved.

The question that arises is whether ESUs can be delineated for *U. paniculata* based on the results of this study and previously published investigations of nuclear genetic diversity and morphological variation. The cpDNA variation collected in this study lays a foundation for defining ESUs within *U. paniculata* by identifying two independent evolutionary lineages. The allozyme study (Franks *et al.*, 2004) showed less geographic structure and congruence with cpDNA than the AFLP study (Subudhi *et al.*, 2005), making it difficult to combine organelle and nuclear DNA data to define ESUs *sensu* Moritz (1994). The morphologically variable characters group the species into three groups while the cpDNA sequence variations divide the species into only two units. One cpDNA unit corresponds geographically to one of the morphology-based clusters, while the other cpDNA unit correlates geographically to the other two morphology-based clusters. Thus, ecological data (i.e., morphological variations from Seneca [1972]) and genetic data (i.e., cpDNA

variations from this study) can be combined to delineate two ESUs (*sensu* Crandall, 2000) within the species, an eastern unit and a western unit. More complete AFLP data and more information about morphological variations across the entire range of the species are needed to fully grasp how ESUs would be best implemented. However, at this point, the data available suggest that the eastern and western maternal lineages should be treated as separate ESUs.

Initial indications from comparing cpDNA variation with morphological variation from the Seneca (1972) study show there are at least two evolutionary independent lineages that are morphologically distinct. Each lineage may have unique adaptive variation vital to surviving future environmental changes. *Uniola paniculata* occupies a highly specialized environment, yet there is considerable variation in the environmental conditions throughout its range: there is a large difference in factors such as temperature between the northern and southern range limits (Wagner, 1964; Seneca, 1972; Barbour & Christensen, 1993). Controlled experiments, such as reciprocal transplant and common garden experiments are needed to test the success of plants from different lineages in different environmental conditions. These experiments can determine whether the variation within the lineages has adaptive value in the environment (e.g., if lineages native to Texas can survive conditions in North Carolina).

Because *U. paniculata* is used so frequently in coastal habitat restoration, it is important to understand how to use individuals that are well adapted to the restoration location. Currently, there are few regulations controlling introductions of *U. paniculata*, and seedlings are often introduced to non-native regions (e.g. haplotype C, the Gulf Coast native, being introduced to Atlantic Coast beaches). Thus far, there is no evidence that haplotypes

of *U. paniculata* show reduced fitness or survival when planted in non-native regions, yet the general consensus is that using local seeds in restoration is optimal to preserve evolutionary potential (Broadhurst *et al.*, 2008). Based on the distribution of cpDNA lineages, it would be prudent to place a restriction on the importation of *U. paniculata* from a different coast (Atlantic or Gulf) for dune restoration to ensure that propagules have an evolutionary history of succeeding in the environment where they are introduced. Then, following the completion of experiments to test the survival of plants of different origins in different environmental conditions, the transplantation restrictions for the species can be refined based on the results of the experiments. This study has documented the evolutionary history of different populations of *U. paniculata*; the next step is to determine if the independent lineages contain the necessary adaptive variation and how to best conserve this variation.

Conclusions

This study investigated phylogeographical patterns in *U. paniculata*, the dominant coastal dune grass in the southeastern United States. It identified two widespread cpDNA lineages, appearing predominantly on opposite sides of a phylogeographical discontinuity at the southern tip of the Florida peninsula. Many marine animal species and a few coastal animal species have been observed to follow this same phylogeographical pattern, suggesting a common causal factor for those species and *U. paniculata*. Thus far, there have been few phylogeographical studies on plant species in the southeastern United States, and to my knowledge, no phylogeographical studies on coastal plant species in this region. Thus, there are no analogous coastal plant studies with which to compare my findings, making it difficult at this point to draw generalized conclusions about the phylogeography of coastal plant

species in the southeastern United States. As the global climate changes at an unprecedented rate, it is important to understand how different species might react (Petit *et al.*, 1999). The responses of organisms such as *U. paniculata* are particularly relevant, as this species provides one of the first lines of defense protecting coastal habitats from storm surges and rising sea levels that will accompany global climate change.

Uniola paniculata may be especially vulnerable to rapid climate change because the plants can only disperse in two directions due to the linear distribution of the species. *Uniola paniculata* is capable of lateral vegetative spread of up to 2 m per year, while its seeds can disperse much greater distances. In the 1900s, the coastline in the western Gulf of Mexico retreated at a rate of 1-50 m per year (Hester & Mendelssohn, 1987). Unable to vegetatively propagate or disperse quickly enough, *Uniola paniculata* populations were extirpated in many areas of the western Gulf Coast, especially in Louisiana, where there is presently no suitable sandy beach habitat remaining for the species to colonize. If the rate of sea level change and frequency of storms continue to increase, *U. paniculata* may be extirpated from other locations in the future.

The results of this study identify eastern and western independent evolutionary lineages, and combined with results from Seneca (1972), suggest that each of the two lineages be designated an ESU, with the Atlantic lineage further subdivided into northern and southern management units for conservation and restoration purposes. Future experiments will test the success of various lineages in different environmental conditions, providing improved ESU delineation. For now, it is wise to ensure that *U. paniculata* individuals have an evolutionary history of succeeding in the environment they occupy. The better we protect

these plants, the better they can maintain sand dunes, which will provide coastal habitat for themselves and many other species in the future.

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APPENDIX A

Molecular Sequences

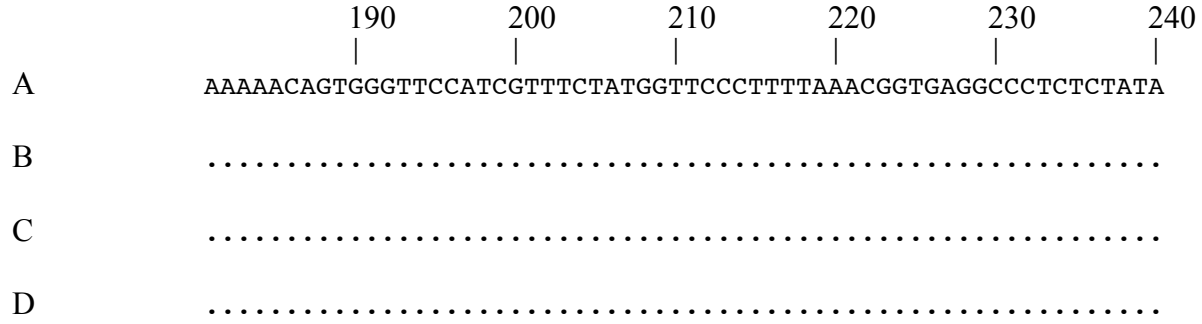
Chloroplast DNA Sequences Amplified by Primers rpl 20 and 5' -rps 12 (Hamilton, 1999)

Sequenced with rpl 20

```
Haplotype   Sequence  10         20         30         40         50         60
                      |          |          |          |          |          |
A                     ATAACTAATGATTTCTCTTCTTTTAACCGCTCTTTTTCCCNTTAATAACAAAACGGATTA
B                     ............................................................
C                     ............................................................
D                     ............................................................

                      70         80         90         100        110        120
                      |          |          |          |          |          |
A                     TTCCGATATATAAAATATTAATTCCAATGGCTTTTGCTACTATAACCTTCCCAACCACGA
B                     ....T.......................................................
C                     ....T.......................................................
D                     ....T.......................................................

                      130        140        150        160        170        180
                      |          |          |          |          |          |
A                     TTTTTTATTCTATCCCCTTCAGTTATTTCGCATAGAAATAACAAATTCTAACGATACTAA
B                     ............................................................
C                     ............................................................
D                     ............................................................
```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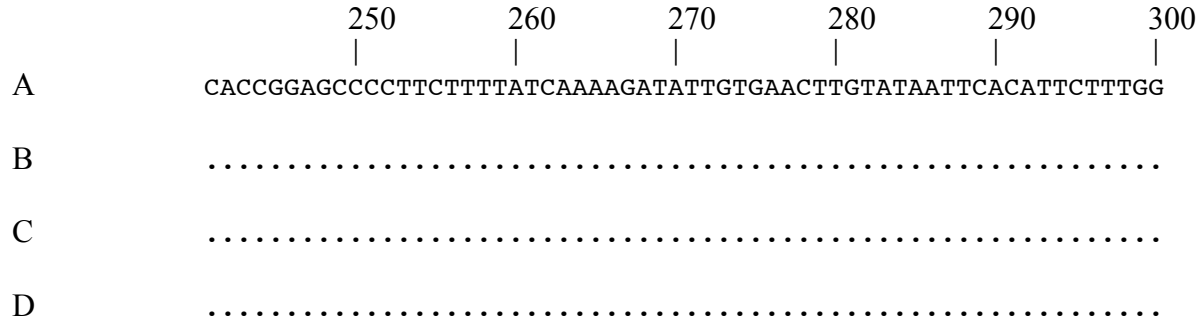
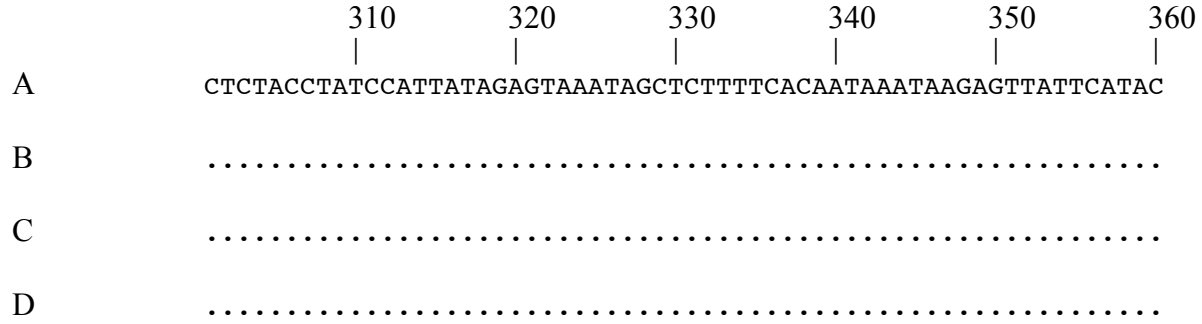
A AAAAACAGTGGGTTCCATCGTTTCTATGGTTCCCTTTTAAACGGTGAGGCCCTCTCTATA
B .....
C .....
D .....


250 260 270 280 290 300
A CACCGGAGCCCCTTCTTTTATCAAAAGATATTGTGAACTTGTATAATTCACATTCTTTGG
B .....
C .....
D .....


310 320 330 340 350 360
A CTCTACCTATCCATTATAGAGTAAATAGCTCTTTTCACAATAAATAAGAGTTATTCATAC
B .....
C .....
D .....

```
                370         380         390         400         410         420
                |           |           |           |           |           |
A               AGTGACGGTATTTAATTATGAAAGTTGGCTAAGTAGCTGACCCTTTAGTCCGTTCTTTTA

B               ..........................................................

C               ..........................................................

D               ..........................................................
```

```
                430         440         450         460         470         480
                |           |           |           |           |           |
A               AGATAAAGGAGCATAAGCCTTTTCTTTTTATTACTATTTCCTCCGCTTAATGGATAACCA

B               ..........................................................

C               ..........................................................

D               ..........................................................
```

```
                490         500         510         520         530         540
                |           |           |           |           |           |
A               TTTGCTACCAATGGGGGGGGATTGCTTCTTCCAATCTAGATGATTGGATTTGCACCAAAG

B               ..........................................................

C               ..........................................................

D               ..........................................................
```

```
                  550         560         570         580         590         600
                  |           |           |           |           |           |
A         GAAACCATAAATTCCATATACCGTAGAAATCTAAGATAGAGCTTCTCTATCCTATTCATT

B         ............................................................

C         ............................................................

D         ............................................................
```

```
                  610         620         630         640         650         660
                  |           |           |           |           |           |
A         GGTACCGATCATGGATACTTCAAAATTTGTCTTTATTTGTTTGAACTCATGATCTGAACG

B         ............................................................

C         ............................................................

D         ............................................................
```

```
                  670
                  |
A         AGTCGCACATACACCCTA

B         ..................

C         ..................

D         ..................
```

Chloroplast DNA Sequences Amplified by Primers ANU_cp007-L and ANU_cp008-R

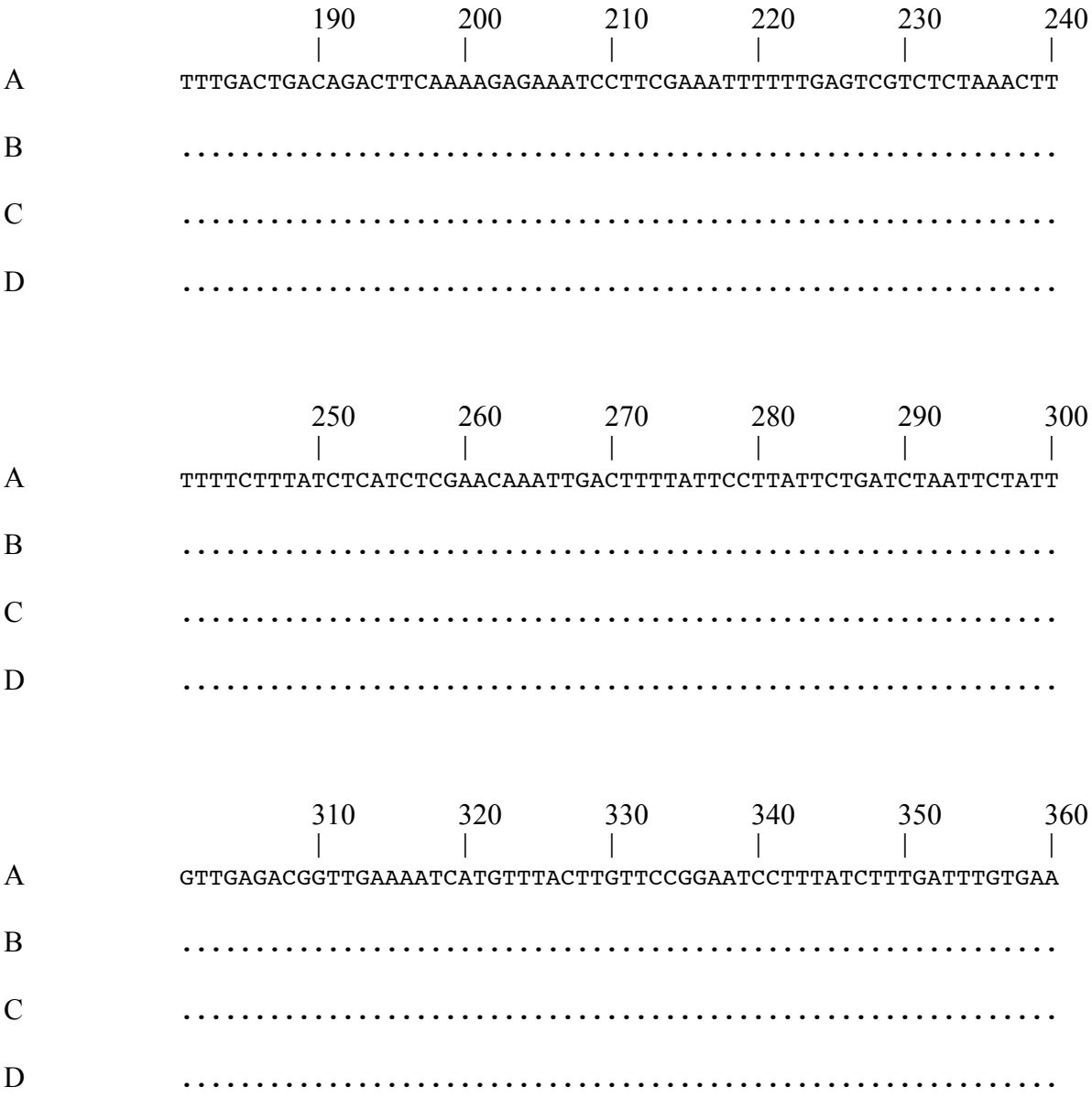
(Ebert & Peakall, 2009)

Sequenced with ANU_cp007

```
Haplotype  Sequence  10         20         30         40         50         60
                     |          |          |          |          |          |
A                    GTAGATAAACAATGCCCCCCCTAGAAACGTATAGGAGGTTTTCTCCTCATACGGCTCGAG
B                    ............................................................
C                    ............................................................
D                    ............................................................

                     70         80         90         100        110        120
                     |          |          |          |          |          |
A                    AAAATGATTCGAATTTCTATCTATAATACAAGTAGATAAAAATTAGACTATGACTTGCAT
B                    ............................................................
C                    ............................................................
D                    ............................................................

                     130        140        150        160        170        180
                     |          |          |          |          |          |
A                    TAATTTCCTTATAGAAGAAAAAACAAATTTCATTTATACTCATGACTCAAGTTGGCTAAT
B                    ............................................................
C                    ............................................................
D                    ............................................................
```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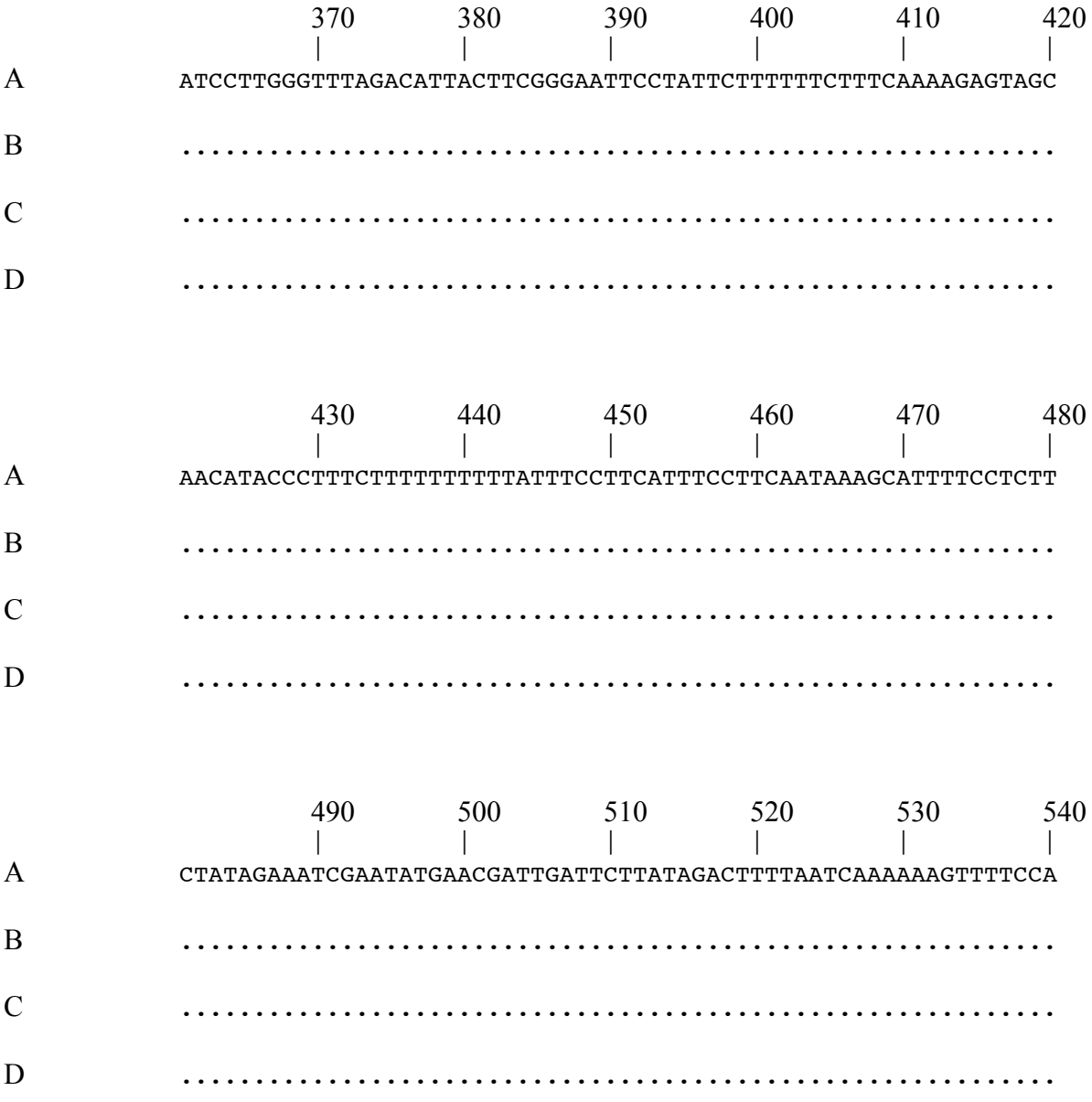
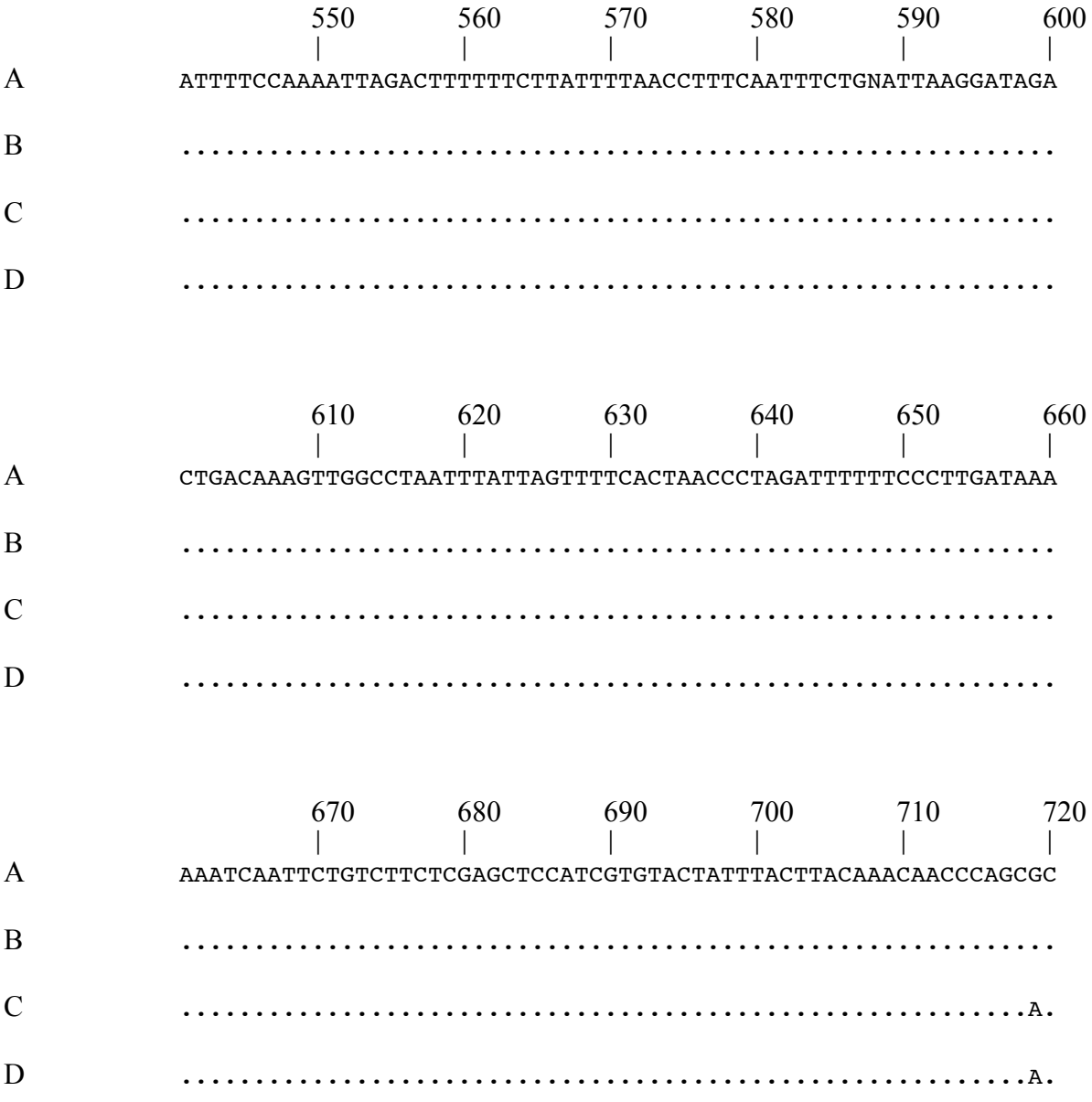
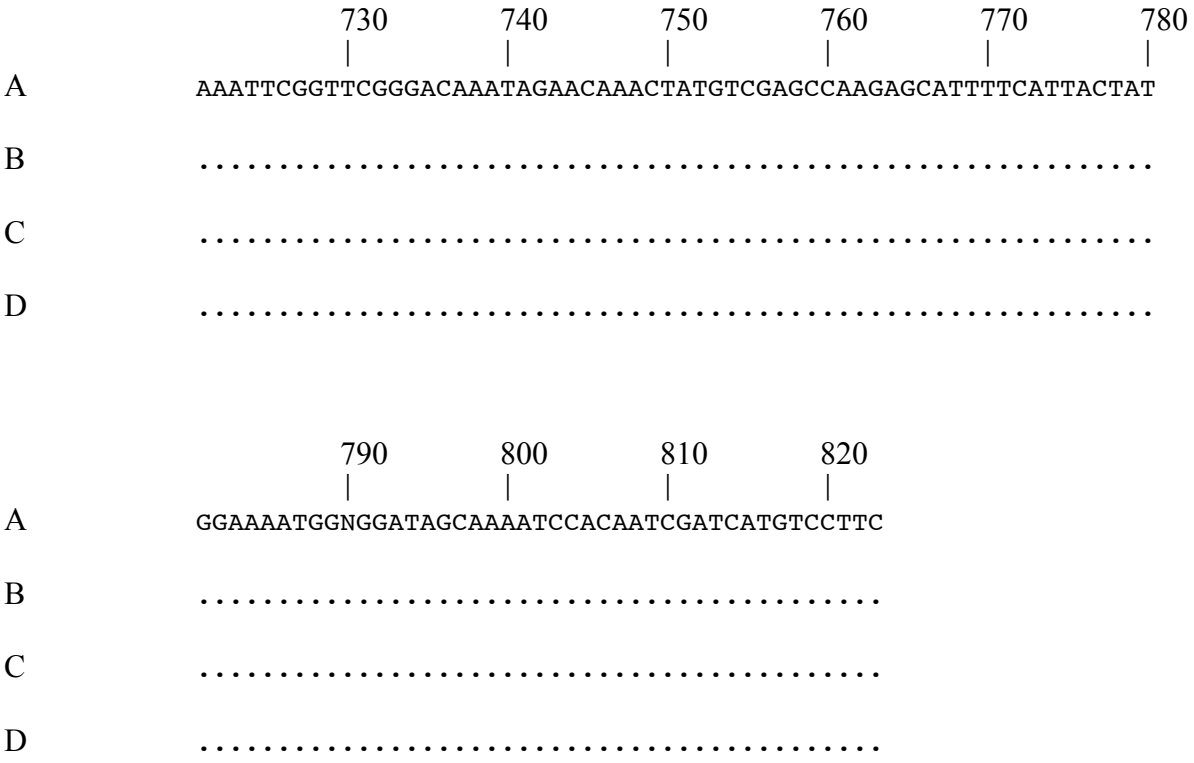
A TTTGACTGACAGACTTCAAAAGAGAAATCCTTCGAAATTTTTTGAGTCGTCTCTAAACTT
B .....
C .....
D .....
250 260 270 280 290 300
A TTTTCTTTATCTCATCTCGAACAAATTGACTTTTATTCCTTATTCTGATCTAATTCTATT
B .....
C .....
D .....
310 320 330 340 350 360
A GTTGAGACGGTTGAAAATCATGTTTACTTGTTCCGGAATCCTTTATCTTTGATTTGTGAA
B .....
C .....
D .....


370 380 390 400 410 420
A ATCCTTGGGTTTAGACATTACTTCGGGAATTCCTATTCTTTTTTCTTTCAAAAGAGTAGC
B .....
C .....
D .....
430 440 450 460 470 480
A AACATACCCTTTCTTTTTTTTTTATTTCCTTCATTTCCTTCAATAAAGCATTTTCCTCTT
B .....
C .....
D .....
490 500 510 520 530 540
A CTATAGAAATCGAATATGAACGATTGATTCTTATAGACTTTTAATCAAAAAAGTTTTCCA
B .....
C .....
D .....


550 560 570 580 590 600
A ATTTTCCAAAATTAGACTTTTTTCTTATTTTAACCTTTCAATTTCTGNATTAAGGATAGA
B .....
C .....
D .....
610 620 630 640 650 660
A CTGACAAAGTTGGCCTAATTTATTAGTTTTCACTAACCCTAGATTTTTTCCCTTGATAAA
B .....
C .....
D .....
670 680 690 700 710 720
A AAATCAATTCTGTCTTCTCGAGCTCCATCGTGTACTATTTACTTACAAACAACCCAGCGC
B .....
C .....A.
D .....A.

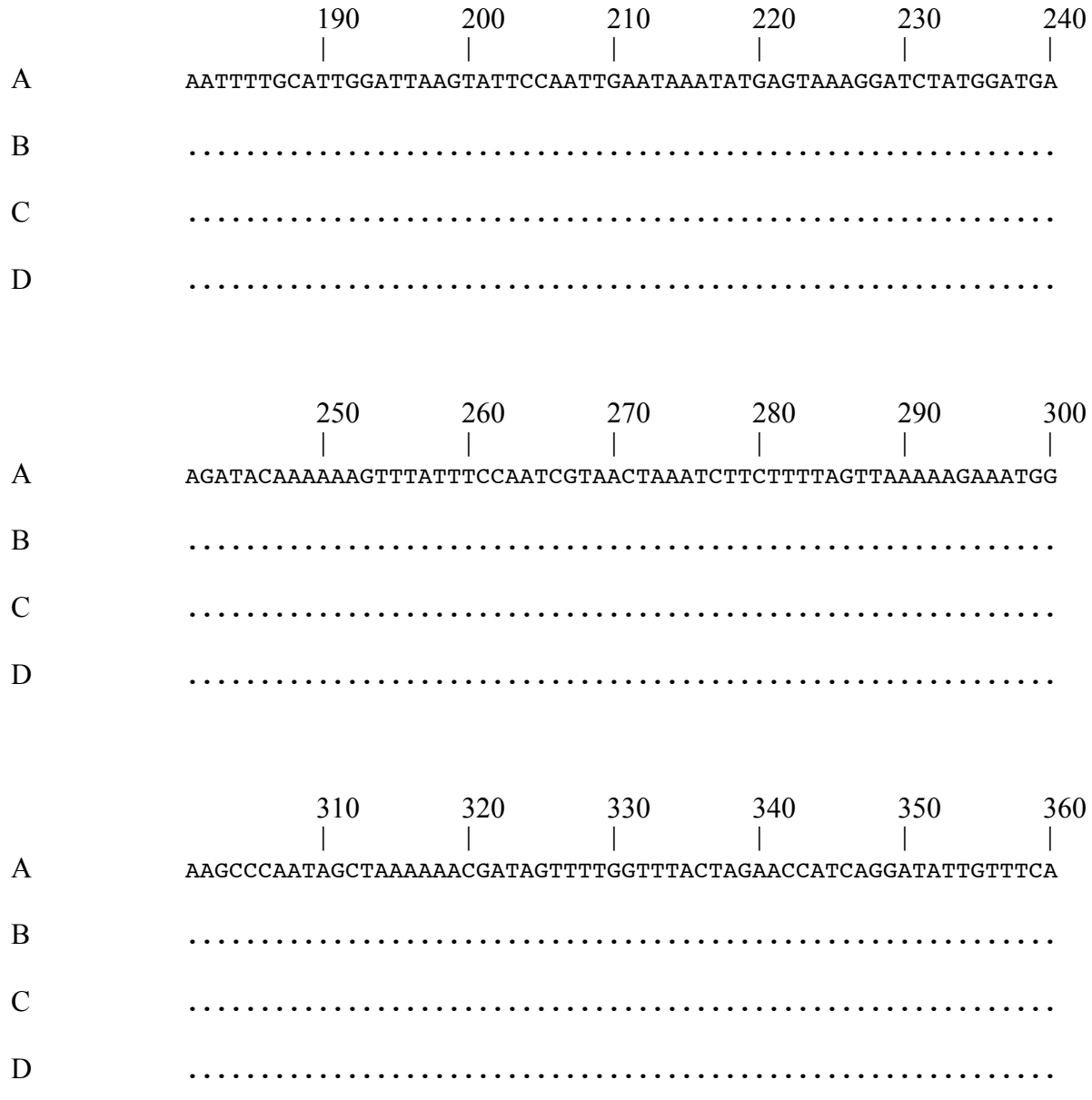

730
740
750
760
770
780
A
AAATTCGGTTCGGGACAAATAGAACAAACTATGTCGAGCCAAGAGCATTTTCATTACTAT
B
.....
C
.....
D
.....
790
800
810
820
A
GGAAAATGGNGGATAGCAAAATCCACAATCGATCATGTCCTTC
B
.....
C
.....
D
.....

Chloroplast DNA Sequences Amplified by Primers ANU_cp016-L and ANU_cp017-R

(Ebert & Peakall, 2009)

Sequenced with ANU_cp016-L

```
Haplotype   Sequence  10         20         30         40         50         60
                      |          |          |          |          |          |
A                     ATCCTTAAGTTTTTTATATTCATATTCCGATGAAAACTTTAGTTCTTATAAAGGGTTTAA

B                     ............................................................

C                     ............................................................

D                     ............................................................


                      70         80         90         100        110        120
                      |          |          |          |          |          |
A                     TCCTTTTCCTCTCAATAGCATATTGAGGAAGAATATACATTCTCGCGATTAGTATCCAAA

B                     ............................................................

C                     ............................................................

D                     ............................................................


                      130        140        150        160        170        180
                      |          |          |          |          |          |
A                     GACTAATTAAATTTGCATGCAAAATACAAATTTGATTATGAGTACAGAGTCGCGAAGCAT

B                     ............................................................

C                     ........................A...................................

D                     ........................A...................................
```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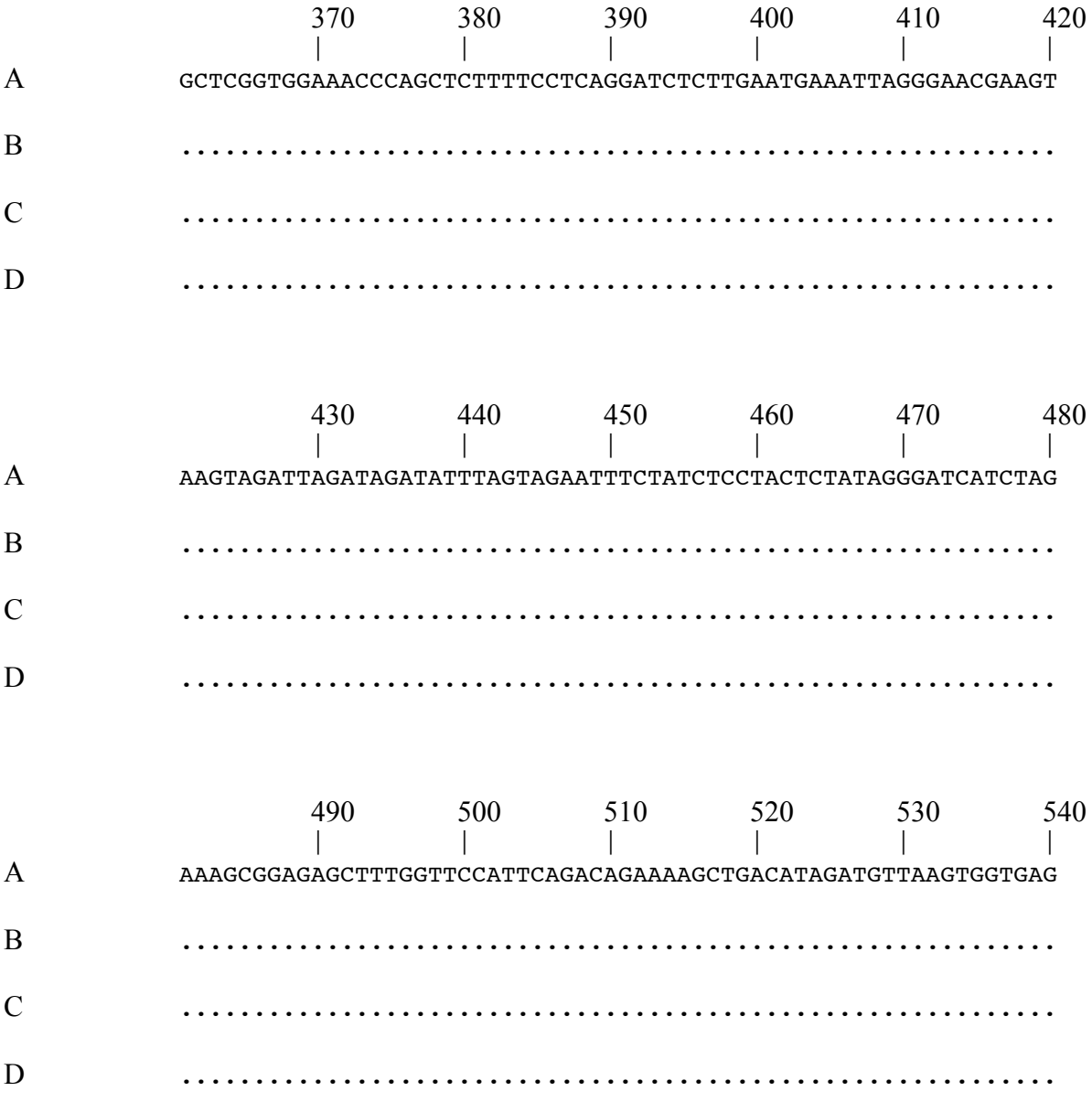
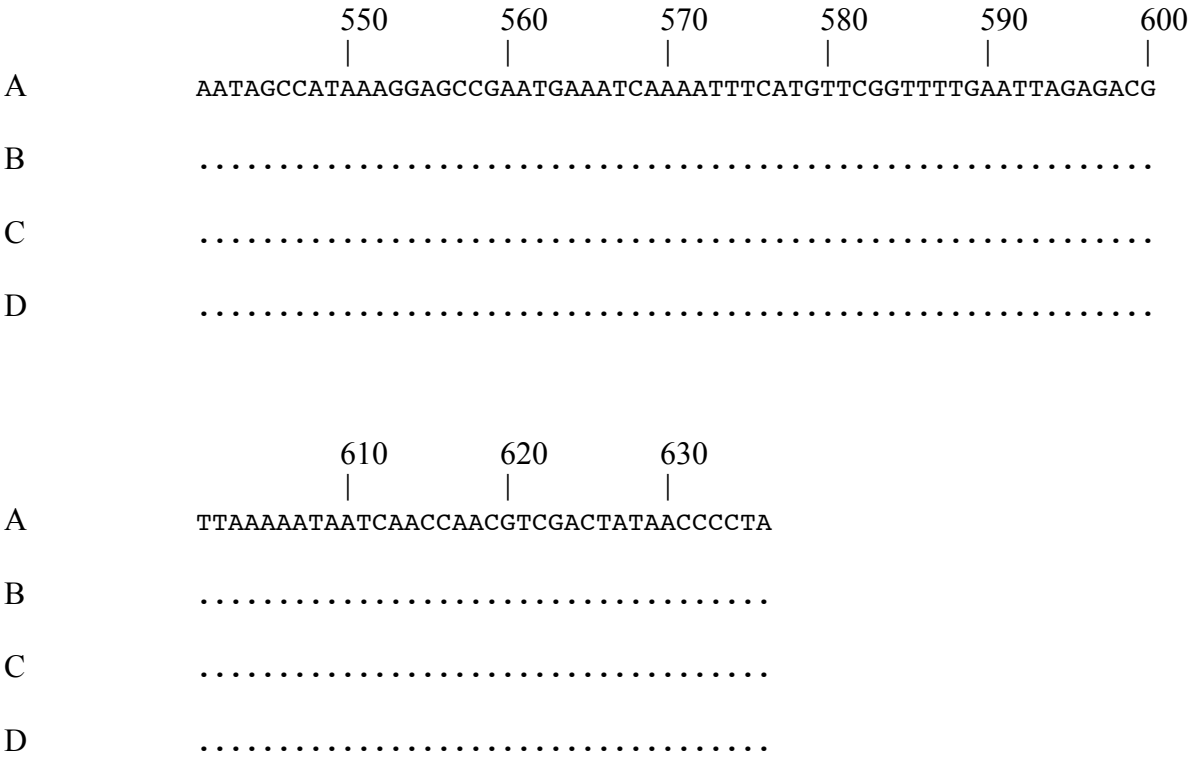
A AATTTTGCATTGGATTAAGTATTCCAATTGAATAAATATGAGTAAAGGATCTATGGATGA
B .....
C .....
D .....
250 260 270 280 290 300
A AGATACAAAAAAGTTTATTTCCAATCGTAACTAAATCTTCTTTTAGTTAAAAAGAAATGG
B .....
C .....
D .....
310 320 330 340 350 360
A AAGCCCAATAGCTAAAAAACGATAGTTTTGGTTTACTAGAACCATCAGGATATTGTTTCA
B .....
C .....
D .....


370 380 390 400 410 420
A GCTCGGTGGAAACCCAGCTCTTTTCCTCAGGATCTCTTGAATGAAATTAGGGAACGAAGT
B .....
C .....
D .....
430 440 450 460 470 480
A AAGTAGATTAGATAGATATTTAGTAGAATTTCTATCTCCTACTCTATAGGGATCATCTAG
B .....
C .....
D .....
490 500 510 520 530 540
A AAAGCGGAGAGCTTTGGTTCCATTCAGACAGAAAAGCTGACATAGATGTTAAGTGGTGAG
B .....
C .....
D .....


550
560
570
580
590
600
A
AATAGCCATAAAGGAGCCGAATGAAATCAAAATTTCATGTTCGGTTTTGAATTAGAGACG
B
.....
C
.....
D
.....
610
620
630
A
TTAAAAATAATCAACCAACGTCGACTATAACCCCTA
B
.....
C
.....
D
.....

Chloroplast DNA Sequences Amplified by Primers ANU_cp035-L and ANU_cp036-R

(Ebert & Peakall, 2009)

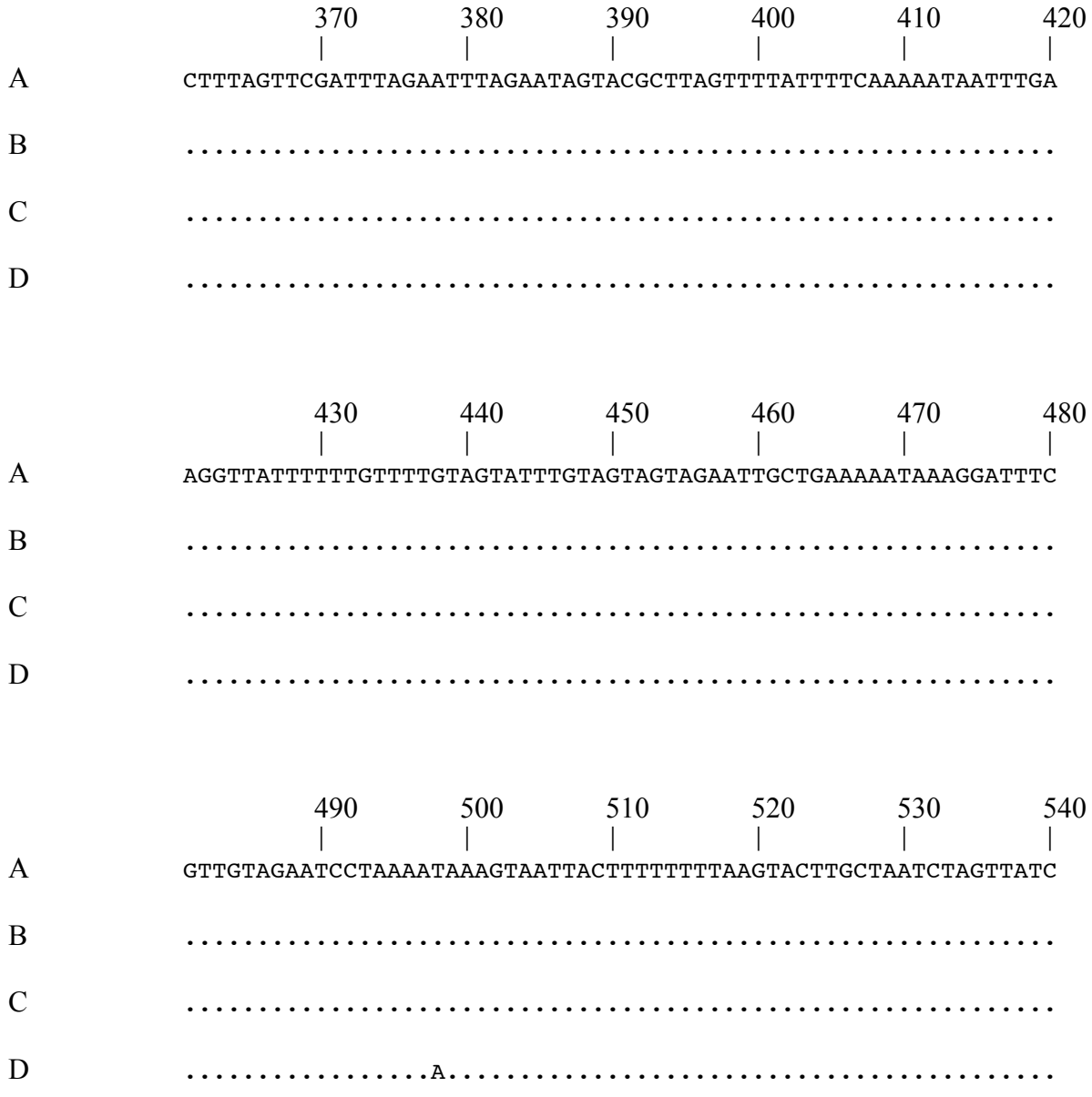
Sequenced with ANU_cp035-L

```
Haplotype   Sequence  10         20         30         40         50         60
                      |          |          |          |          |          |
A                     CTATTTATCGAAGANAATTCCATTATCAGCTCACTCTTCATCAATCCCTACGGATCAATC
B                     ............................................................
C                     ............................................................
D                     ............................................................

                      70         80         90         100        110        120
                      |          |          |          |          |          |
A                     TAGCAATCATGGAATCTATATTCTGTTTACTGAATCACATGAAATTCTAGGAAACTCCAC
B                     ............................................................
C                     ............................................................
D                     ............................................................

                      130        140        150        160        170        180
                      |          |          |          |          |          |
A                     ATACGTATTTCATATATGTATTTCATACATATGAATAGAGATAATTTTGACGAAAGTTCC
B                     ............................................................
C                     ............................................................
D                     ............................................................
```

```
                 190         200         210         220         230         240
                  |           |           |           |           |           |
A        AATTTTGCAGGGGTAGAAATGGAATTAAAATGAATTGATAAAAAACTCCTAGAAAAAATT

B        ....................................................................

C        ....................................................................

D        ....................................................................
```

```
                 250         260         270         280         290         300
                  |           |           |           |           |           |
A        CTGCCACTTAAACTTTTCATAATCATATTCTAATCAGATTGGATAGGAAAGATTTCTCGT

B        ....................................................................

C        ....................................................................

D        ....................................................................
```

```
                 310         320         330         340         350         360
                  |           |           |           |           |           |
A        TTTAGCTCCTTTCTATGAAAGAAATAAAGCCATAAAATTTATAAGCACTAGAAAGTTTTA

B        ....................................................................

C        ....................................................................

D        ....................................................................
```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A CTTTAGTTCGATTTAGAATTTAGAATAGTACGCTTAGTTTTATTTTCAAAAATAATTTGA
B .....
C .....
D .....
430 440 450 460 470 480
A AGGTTATTTTTTGTTTTGTAGTATTTGTAGTAGTAGAATTGCTGAAAAATAAAGGATTTC
B .....
C .....
D .....
490 500 510 520 530 540
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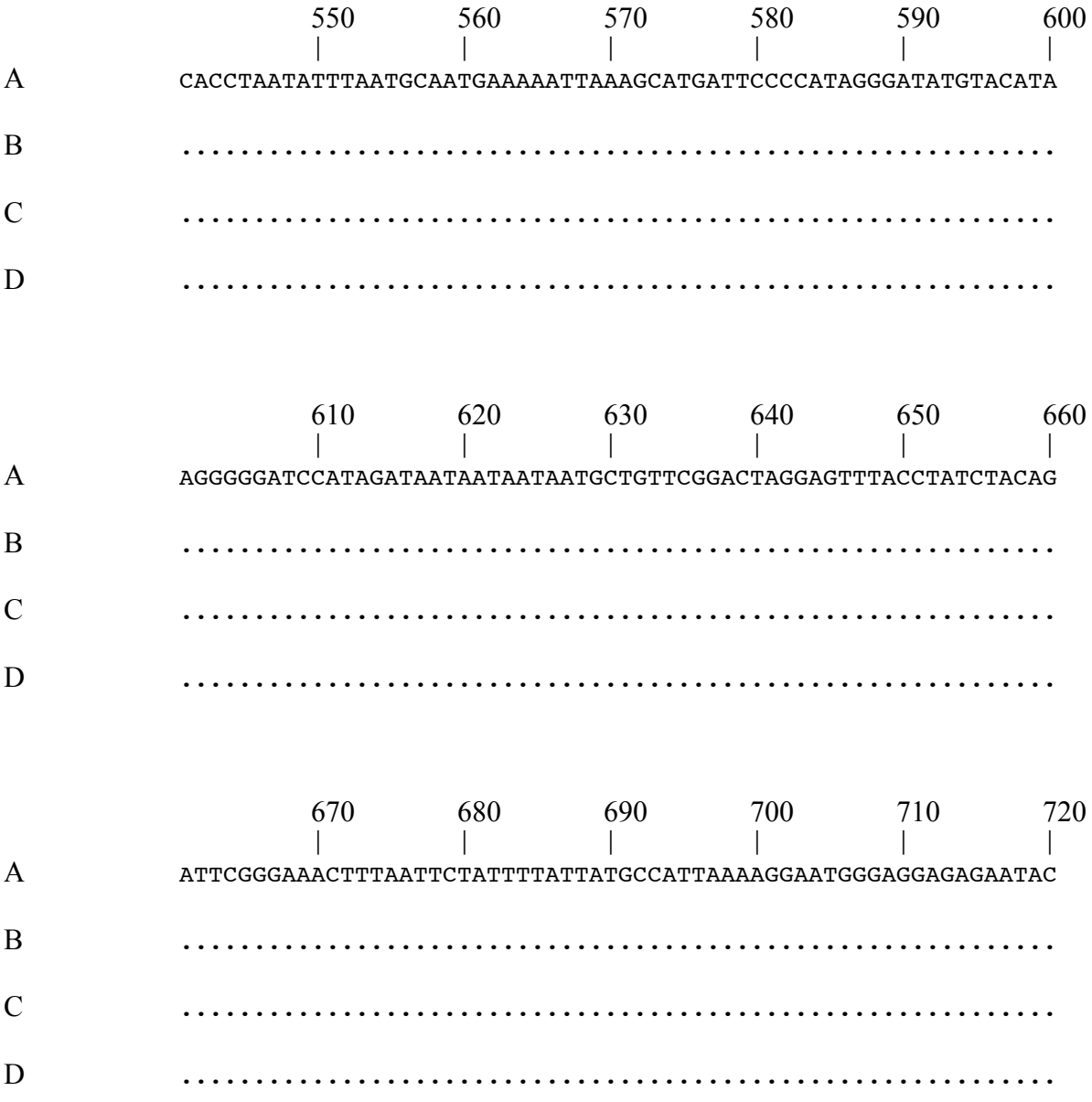
B .....
C .....
D .....A.....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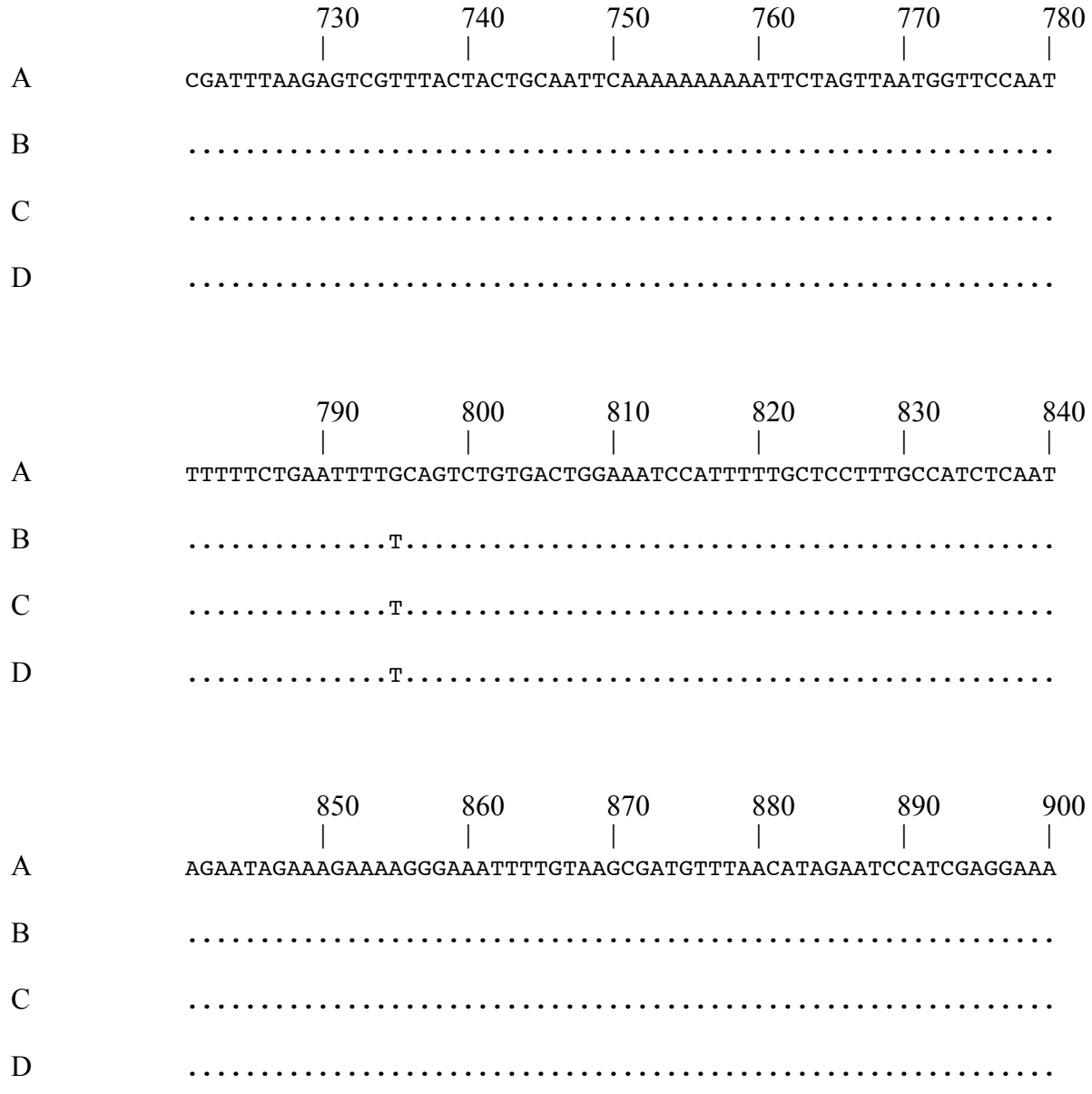
A CACCTAATATTTAATGCAATGAAAAATTAAAGCATGATTCCCCATAGGGATATGTACATA
B .....
C .....
D .....
610 620 630 640 650 660
A AGGGGGATCCATAGATAATAATAATAATGCTGTTCGGACTAGGAGTTTACCTATCTACAG
B .....
C .....
D .....
670 680 690 700 710 720
A ATTCGGGAAACTTTAATTCTATTTTATTATGCCATTAAAAGGAATGGGAGGAGAGAATAC
B .....
C .....
D .....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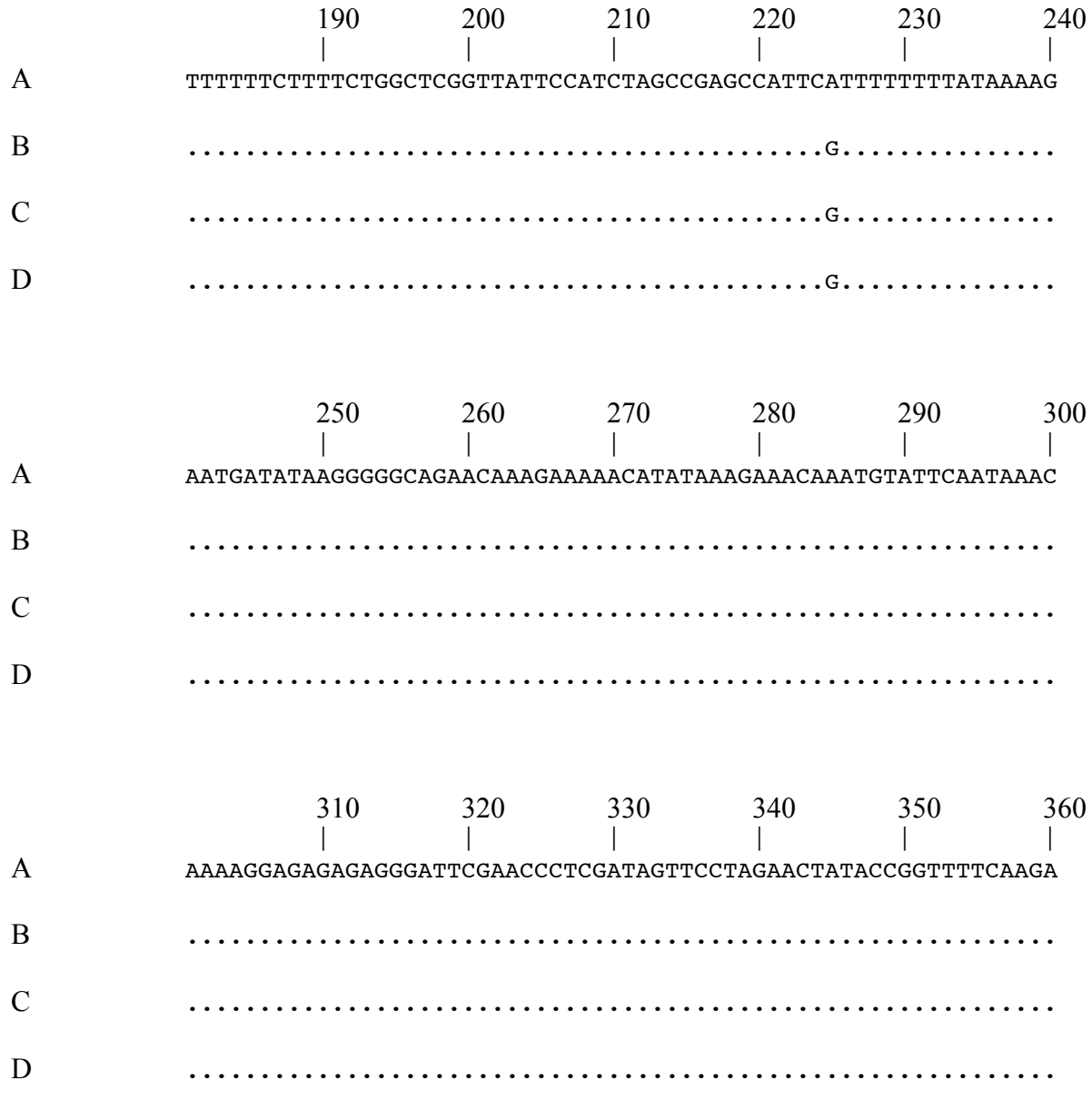
A CGATTTAAGAGTCGTTTACTACTGCAATTCAAAAAAAAAAATTCTAGTTAATGGTTCCAAT
B .....
C .....
D .....
790 800 810 820 830 840
A TTTTTCTGAATTTTGCAGTCTGTGACTGGAAATCCATTTTTGCTCCTTTGCCATCTCAAT
B .....T.....
C .....T.....
D .....T.....
850 860 870 880 890 900
A AGAATAGAAAGAAAAGGGAAATTTTGTAAGCGATGTTTAACATAGAATCCATCGAGGAAA
B .....
C .....
D .....

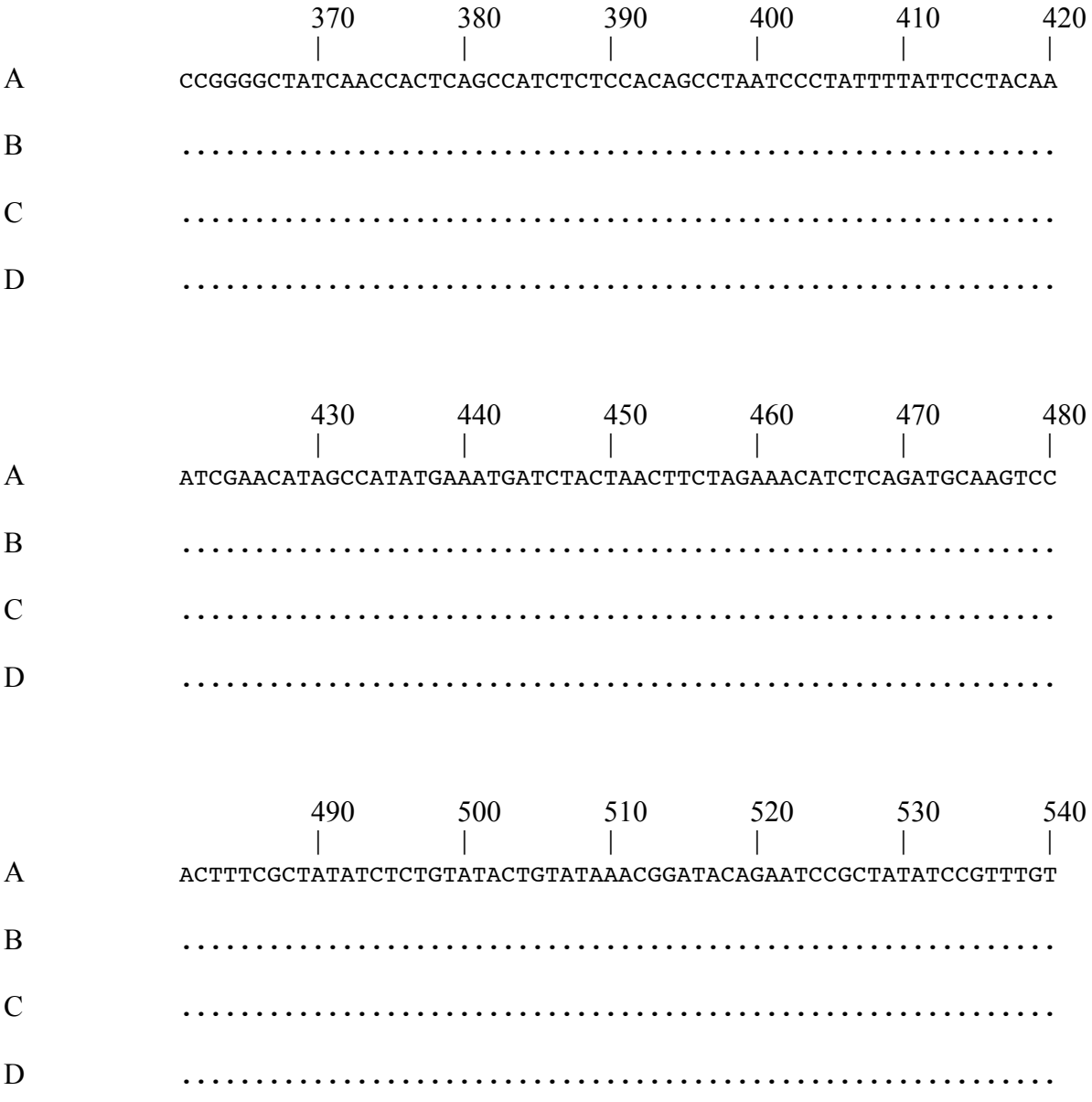
Chloroplast DNA Sequences Amplified by Primers ANU_cp047-L and ANU_cp048-R

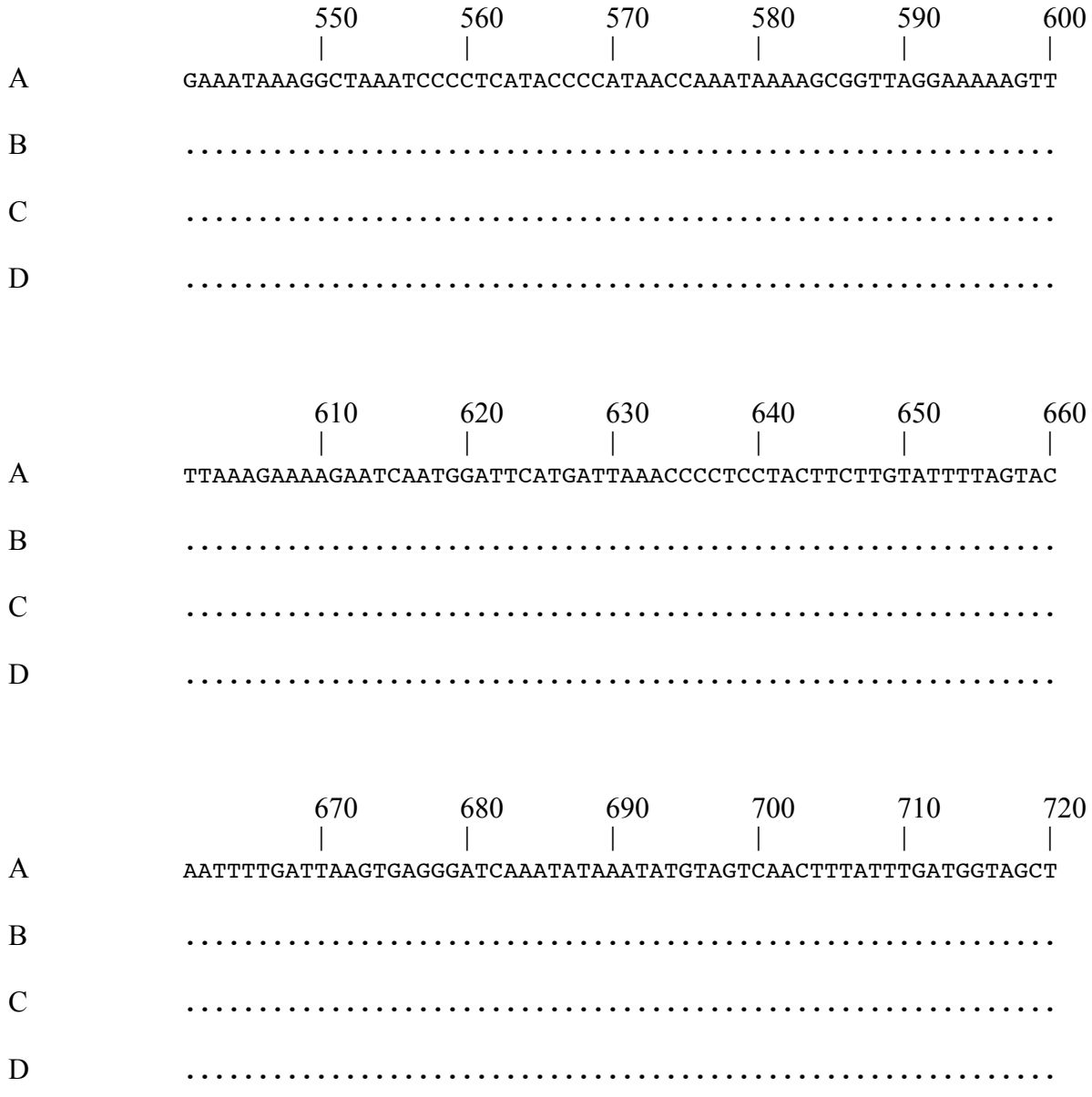
(Ebert & Peakall, 2009)

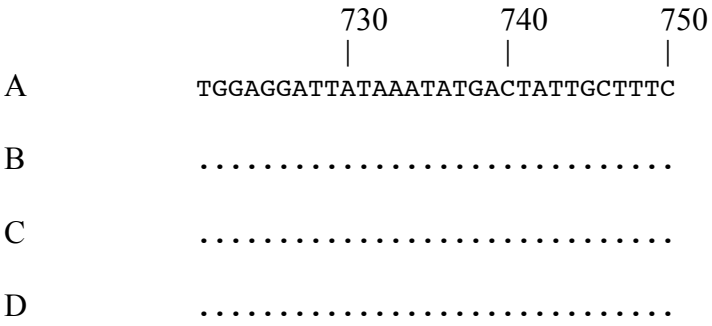
Sequenced with ANU_cp047-L

```
Haplotype   Sequence  10         20         30         40         50         60
                      |          |          |          |          |          |
A                     TTGGTTAGCGACTTCCCATTTTGTTCTAGGATTCTTCTTTTTTGTGGGCCATTTGTGGCA
B                     ............................................................
C                     ............................................................
D                     ............................................................

                      70         80         90         100        110        120
                      |          |          |          |          |          |
A                     TGCAGGAAGAGCCCGAGCTGCTGCAGCCGGCTTTGAAAAGGGAATTGATCGTGATTTGGA
B                     ............................................................
C                     ............................................................
D                     ............................................................

                      130        140        150        160        170        180
                      |          |          |          |          |          |
A                     ACCTGTTCTTTACATGACCCCTCTTAACTAAGATTTTCTTATTTATACCTGTTCTACTGT
B                     ............................................................
C                     ............................................................
D                     ............................................................
```


190 200 210 220 230 240
A TTTTTTCTTTTCTGGCTCGGTTATTCCATCTAGCCGAGCCATTCATTTTTTTTATAAAAG
B .....G.....
C .....G.....
D .....G.....
250 260 270 280 290 300
A AATGATATAAGGGGGCAGAACAAAGAAAAACATATAAAGAAACAAATGTATTCAATAAAC
B .....
C .....
D .....
310 320 330 340 350 360
A AAAAGGAGAGAGAGGGATTCGAACCCTCGATAGTTCCTAGAACTATACCGGTTTTCAAGA
B .....
C .....
D .....


370 380 390 400 410 420
A CCGGGGCTATCAACCACTCAGCCATCTCTCCACAGCCTAATCCCTATTTTATTCCTACAA
B .....
C .....
D .....
430 440 450 460 470 480
A ATCGAACATAGCCATATGAAATGATCTACTAACTTCTAGAAACATCTCAGATGCAAGTCC
B .....
C .....
D .....
490 500 510 520 530 540
A ACTTTCGCTATATCTCTGTATACTGTATAAACGGATACAGAATCCGCTATATCCGTTTGT
B .....
C .....
D .....


550 560 570 580 590 600
A GAAATAAAGGCTAAATCCCCTCATACCCCATAACCAAATAAAAGCGGTTAGGAAAAAGTT
B .....
C .....
D .....
610 620 630 640 650 660
A TTAAAGAAAAGAATCAATGGATTCATGATTAAACCCCTCCTACTTCTTGTATTTTAGTAC
B .....
C .....
D .....
670 680 690 700 710 720
A AATTTTGATTAAGTGAGGGATCAAATATAAATATGTAGTCAACTTTATTTGATGGTAGCT
B .....
C .....
D .....


730
740
750
A
TGGAGGATTATAAATATGACTATTGCTTTC
B
.....
C
.....
D
.....

APPENDIX B

Photographs of the Study Species

Photograph 1. *U. paniculata* population, Emerald Isle, NC.

Photograph 2. The reproductive structures of *U. paniculata*, Emerald Isle, NC.

Photograph 3. *U. paniculata* population, Padre Island National Seashore, TX.

Photograph 4. Rows of *U. paniculata* planted after destruction from Hurricane Katrina, near Buccaneer State Park, MS.

Photograph 5. Reproductive structures of *U. paniculata* with anthers visible, Honeymoon Island, FL.

VITA

Richard Groth Jones Hodel was born on June 11, 1980 in Durham, NC. He wanted to be a mathematician or scientist in high school, but ended up deciding to be a music major in college. He received a Bachelor of Arts in Music at Amherst College in 2002. Then his interest in the natural sciences was rekindled after taking a summer course in biology at the University of North Carolina in 2006. This one course led to many more and prompted him to begin work on a Master of Science degree in Biology at Appalachian State University in the fall of 2009. He finished this degree in the summer of 2011, and plans to pursue a Ph.D. in Botany at the University of Florida under the supervision of Drs. Douglas and Pamela Soltis beginning in the fall of 2011. He maintains an interest in music, but is thrilled about starting a career as a biologist.