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POPULATION DYNAMICS AND GENETIC DIFFERENTIATION OF THE THREATENED FRESHWATER MUSSEL SPECIES FUSCONAIA ASKEWI, FUSCONAIA LANANENSIS, AND PLEUROBEMA RIDDELLII

Edith D. Plants-Paris
University of Texas at Tyler

POPULATION DYNAMICS AND GENETIC DIFFERENTIATION OF THE THREATENED FRESHWATER MUSSEL SPECIES *FUSCONAIA ASKEWI*, *FUSCONAIA LANANENSIS*, AND *PLEURobema RIDDELLII*

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EDITH PLANTS-PARIS

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Neil B. Ford, PhD., Committee Chair

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EDITH PLANTS-PARIS

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for the Master of Science degree

Approvals:

Thesis Chair: Neil B. Ford, PhD.

Member: John S. Placyk Jr., Ph.D.

Member: Lance R. Williams, Ph.D.

Chair, Department of Biology

Dean, College of Arts and Sciences

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Table of Contents

Table of Contents (Continued)

List of Tables

List of Figures

Abstract

POPULATION DYNAMICS AND GENETIC DIFFERENTIATION OF THE THREATENED FRESHWATER MUSSEL SPECIES *FUSCONAIA ASKEWI*, *FUSCONAIA LANANENSIS*, AND *PLEUROBEMA RIDDELLII*

Edith Plants-Paris

Thesis Chair: Neil Ford, Ph.D.

The University of Texas at Tyler

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North America has the most diverse freshwater mussel fauna in the world with approximately 300 species; unfortunately, extinction rates for freshwater mussels rivals the rates of many other groups of organisms. Population-level natural life history data is essential in the management of species of conservation concern, yet basic information about freshwater mussel life-history and demographic traits are unknown for many species. To further complicate matters, taxonomic uncertainty exists among some members of the group. The work detailed herein had two goals: to gain further understanding of the taxonomic relationship between *Fusconaia lananensis* and *F. askewi* by sequencing genes that had not been previously examined for these species, genes *16S* and *ITS1,* and collect data on the population size, density, and structure for both *F. askewi* and *F. lananensis*, as well as for *Pleurobema riddellii*, all of which are classified as state threatened in Texas. The second goal was accomplished via qualitative analysis of data from 0.25 m^2 quadrats and through mark-recapture studies at field sites where the highest densities of these species have been recorded. Specifically, quadrat

surveys were conducted at seven mark-recapture sites in the Neches, Sabine, and Angelina Rivers during the summers of 2014 and 2015. In terms of my genetic analysis, data collected from the *16S* gene has provided additional support that *F. askewi* and *F. lananensis* are one single species, as recently proposed by other researchers. Data collected from the *ITS1* gene showed no genetic differentiation between *F. askewi*, *F. lananensis*, and *F. flava*, though recently published research indicates that there is low genetic variation within the *ITS1* gene for several different species found in genus *Fusconaia*. Sites on the Neches and Angelina Rivers had significantly higher recapture rates between 2014 and 2015 than sites on the Sabine River, likely because of a flooding event that occurred in the Sabine River during that time. The largest population estimate for a *F. askewi* population was 302±26.72 in 2015 within the 25 m area while the largest population estimate for a *P. riddellii* population was 101±4.99 in 2015 within the 25 m area. Fewer juvenile *P. riddellii* were detected than *F. askewi*, leading to left-skewed size class distributions for *P. riddellii.* As conservation efforts for freshwater mussels increase, continued analysis of established freshwater mussel populations will be crucial.

Chapter 1: Introduction to Freshwater Mussels

North America has the most diverse freshwater mussel fauna in the world with approximately 300 species (Williams et al. 1993). Often a large percentage of the benthic biomass in freshwater systems is comprised of freshwater mussels, and thus mussels likely play an integral role in these systems. Specifically, through their burrowing and filtration feeding behaviors freshwater mussels likely are critical in nutrient cycling (Vaughn and Hakenkamp 2001). At the same time, freshwater mussels are an important food source for other organisms at higher trophic levels such as mammals, birds, and fishes (Haag 2012). Freshwater mussels and their shells also play a role in substrate stability and in habitat heterogeneity (Gutiérrez et al. 2003).

Historically freshwater mussels have been recognized as efficient suspension feeders that consume primarily plankton (Dame et al. 1985, Dame et al. 1991). Freshwater mussels have been observed filtering large amounts of water within short periods of time (Kryger and Riisgard 1988, McIvor 2004, Strayer et al. 2004). Along with plankton, dissections of mussel digestive tracts have also contained zooplankton and detritus. Freshwater mussels have also been documented feeding off of sentiment through the use of their foot and through use of their siphon on the sentiment surface (Strayer et al. 2004, Nichols et al. 2005). Feeding methods employed by freshwater mussels have been shown to affect nutrient cycling in their ecosystem in several ways such as transferring nutrients from the water column to the riverbed and stimulating both primary and secondary production through nutrient excretion (Spooner and Vaughn 2006, Spooner 2007, Vaughn et al. 2008).

Unlike most marine mussels, freshwater mussels have specialized larvae, called glochidium, which parasitically feed from the gills of host fish (Strayer et al. 2004). Techniques and adaptations to transmit glochidia to hosts vary between freshwater mussel species. One specialized method described is the use of lures by the females of some species to mimic fish or invertebrates (Haag and Warren 2000, Haag and Warren 2003). In other species, female mussels release their glochidia in conglutinates that mimic fish food sources such as eggs or larvae and thus are attacked by host fish (Jones and Neves 2002, Haag and Warren 2003). These two methods typically attract a few specific fish species and are seen within mussel species that are considered host specialists. A third method of glochidia transmission is used by mussels considered host generalists in which a mucous web of glochidia that entangle fish indiscriminately is released (Haag and Warren 2003). These adaptations in glochidia transmission to fish-hosts indicate a close evolutionary link between mussel life-history traits and their use of host-fish (Strayer et al. 2004).

Unfortunately, extinction rates for freshwater mussels rival the rates of many other groups of organisms (Ricciardi and Rasmussen 1999). As of 2014, 28% of freshwater mussels were federally listed as imperiled but some researchers suggest that this number could be as high as 65% (Haag and Williams 2014). There are multiple factors that may impact freshwater mussel fauna, but the destruction of river systems by the creation and use of dams may cause the most impact with both positive and negative consequences on mussel populations (Singer and Gangloff 2011, Haag 2012, Gangloff 2013). In the last decade or so, mussel conservation efforts have greatly increased via federal and state agencies in the United States, as well as various other conservation

groups, though freshwater mussel populations still face threats of decline (Haag 2012). Knowledge of the various aspects of life-history traits is crucial for protecting freshwater mussels; unfortunately, much of this information is unknown for many species of freshwater mussels.

Freshwater Mussels of East Texas

52 species of freshwater mussels in the family unionidae are found in Texas and 15 are currently listed as state threatened by the Texas Parks and Wildlife Department. Of these 15 species, 6 are found in the East Texas area including: Southern Hickorynut (*Obovaria jacksoniana)*, Texas Heelsplitter (*Potamilus amphichaenus*), Sandbank Pocketbook (*Lampsilis satura*), Louisiana Pigtoe (*Pleurobema riddelli)*, Texas Pigtoe (*Fusconaia askewi)*, and the Triangle Pigtoe (*F. lananensis)*. Despite being of conservation concern, little is known about the various aspects of these species' population biology, genetics, and their life history traits (Howells, unpublished). Though general ranges are known for these species, there are few sites that are known to contain a high abundance of any of these threatened species.

The species targeted in the current study were the 3 state-threatened pigtoe species *P. riddellii, F. askewi,* and *F. lananensis.* Though there has been taxonomic uncertainty between *F. lananensis* and *F. askewi*, *P. riddellii* is morphologically and genetically distinct from *Fusconaia* species (Burlakova et al. 2012, Howells et al. 2012). While previous studies have gathered additional information about these three species, including the creation of habitat suitability models (Ford 2013) and the identification of a fish host for *F. askewi* (Marshall 2014, Bertram 2015), much remains to be learned about these three species. The goal of this project was to 1) to gain further understanding of the

taxonomic relatedness of *F. lananensis* and *F. askewi* by sequencing genes that have not been previously sequenced for these species (*16S* and *ITS1),* and 2) to study population size, density, and structure for *P. riddellii*, *F. askewi*, and *F. lananensis* through quantitative analysis of data from the use of 0.25 m^2 quadrats and through mark-recapture studies at high-density field sites.

Chapter 2: Genetic Differentiation of *Fusconaia askewi* and *F. lananensis*

Traditionally, species descriptions for freshwater mussels were based largely on shell morphology, which can vary greatly between individuals in a population and along environmental gradients (Haag 2012). This creates difficulties in defining species based solely on morphology and has led to researchers incorporating molecular genetic data to help define and identify species, with the mitochondrial genes *ND1* and *COX1*, and the nuclear genes *ITS1* and *ITS2* most commonly used for freshwater mussels (e.g. Burlakova et al. 2012, Kallersjo et al. 2005, Inoue et al. 2014). Although mitochondrial genes are commonly used when conducting molecular phylogenetic analyses with freshwater mussels, species found within bivalve families Unionidae, Veneridae, and Mytilidae are known to use a unique method of mitochondrial inheritance that makes them less useful. Specifically, female mussels transmit their mitochondria to all of their offspring (F-type), but male mussels can also transfer their mitochondria (M-Type) to their sons resulting in heteroplasmic males. Some studies have sequenced both mitochondrial types but the effect of heteroplasmy on conclusions from genetic analyses is not understood. Therefore nuclear genes such as *ITS1* and *ITS2* in addition to mitochondrial genes should be used in studies involving freshwater mussels (Krebs 2004, Mock et al. 2004, Kallersjo et al. 2005).

F. lananensis (Triangle Pigtoe) and *F. askewi* (Texas Pigtoe) are difficult to distinguish and currently both are listed as State threatened by the Texas Parks and Wildlife Department. Since, *F. lananensis* and *F. askewi* are often sympatric, proper identification of the two is critical. *Fusconaia askewi* occurs from the San Jacinto River north to the Red River system and *F. lananensis* is found in the Angelina River, the

Neches River, and the Attoyac River (Howells 2014). Along with range overlap, *F. lananensis* and the *F. askewi* also possess morphological similarities in external and internal characteristics. Both *F. lananensis* and the *F. askewi* may possess a sub-rectangular shape and similar external coloration ranging from chestnut brown to black (Howells et al. 2012, Howells 2014). Because of their similar external morphology and with additional variation existing within each species, these species typically cannot be positively identified in the field (Figure 2.1, Howells 2014). Additional variation can typically be seen inside the shell for both species, particularly with coloration of the nacre. *Fusconaia askewi* is often characterized as having white nacre with pink or red nacre outside the palatal line and *F. lananensis* is often identified as having solid pink nacre with occasional pearly bumps and yellow blotches (Figure 2.1, Howells 2014). *F. askewi* has also been observed having solid pink, orange or solid white nacre. Given that such variation exists in the nacre, using coloration may not be a reliable method for species identification if the distinguishing pearly bumps and yellow blotches are not seen for *F. lananensis* or the white and pink coloration of the nacre in *F. askewi.* Both species are described as having three pseudocardinal teeth (two left, one right) triangular and compressed and three lateral teeth (two left, one right) straight to slightly curved (Howell 2014).

Figure 2.1. Visual comparison of *Fusconaia lananensis (left)* and *F. askewi (right)* external and internal morphology. Both specimens were collected in the Angelina River off 343.

Similarities in morphology and geographic ranges lead Burlakova et al. (2012) to hypothesize that *F. lananensis* and *F. askewi* are not separate species. She found low genetic variation within the *ND1* and *COX1* genes between *F. askewi* and *F. lananensis* (1% for *ND1* and 0.7% for *COX1*) (Burlakova et al. 2012). However, other authors expressed concern over the methodology in this study, including: 1) the full range of morphological variation between *F. lananensis* and *F. askewi* were not acknowledged in this study and 2) collection sites for mussels were not identified (Howells et al. 2012). However, other recent studies using the *ND1* and *COX1* genes also support the idea that *F. askewi* and *F. lananensis* are not separate species (Marshall 2014, Bertram 2015). While both species are currently listed as threatened in the state of Texas because of the

rarity of *F. lananensis*, the difficulty distinguishing *F. askewi* from *F. lananensis* has inhibited determination of whether *F. askewi* might actually not be in need of protection. The goal of this study was to include genes *16S* and *ITS1* to the analysis of the genetic relationship between *F. askewi* and *F. lananensis* to add more genetic data to this analysis. Genes *ND1* and *COX1*, which have been used in earlier studies with these species, were also included in the analysis as additional localities for specimens were sampled. All study site locations were recorded for all sequenced individuals and a larger sample size of *F. lananensis* was included in this study. All specimens were collected and preserved to help confirm species identification with the addition of internal morphological characteristics.

Materials and Methods

I collected *F. lananensis* and *F. askewi* in the summers of 2014 and 2015 from the Neches and Angelina Rivers (Table 2.1). Whole specimens were frozen so that the shells could be referred to during later analyses and to confirm species identification through internal morphology. Foot and mantle tissue was removed and preserved in 95% ethanol and stored at -20°C for DNA analysis. DNA from preserved tissue samples were extracted using E.Z.N.A. Gel Extraction Kits (Omega bio-tek, Norcross, GA). The mitochondrial genes (mtDNA) *ND1, COX1, 16S* and nuclear gene *ITS1* were then amplified via polymerase chain reactions (PCRs) for each individual. Primers used were as follows:

ND1: 5’ -TG GCAGAAAAGTGCATCAGATTAAAGC-3’

5’ -TCGGAATTCTCCTTCTGCAAAGTC-3’

(Serb et al. 2003

COX1: 5’ -GTTCCACAAATCATAAGGATATTGG-3’

5’ -TACACCTCAGGGTGACCAAA AAACCA-3’

(Campbell et al. 2005)

16S: 5’ -CCGTTCTGAACTCAGCTCATGT-3’

5’ -CGACTGTTTAACAAAAACAT-3’

(Campbell et al. 2005)

ITS1: 5′-AAAAAGCTTCCGTAGGTGAACCTGCG-3′

5′-AGCTTGCTGCGTTCTTCATCG-3′

(King et al. 1999)

PCR parameters for *ND1, COX1,* and *ITS1* were as follows: 94˚ C for 5 m, 30 cycles of 94˚ C for 45 s, 54˚ C for 60 s, and 72˚ C for 60 s followed by a final extension of 72˚C for 5 m (King et al. 1999, Campbell et al. 2005, Serb et al. 2003). PCR parameters for *16S* were as follows: 92˚C for 5 m; 92˚C for 40 s, 50˚C for 60 s, 68˚C for 90 s, x 35; 72˚C for 10 m (Campbell et al. 2005). An Eppendorf Mastercycler gradient thermal cycler was used to amplify all PCR reactions. Gel electrophoresis was used to test the quality of amplification and successfully amplified PCR products were purified using E.Z.N.A. cycle pure kits (Omega bio-tek, Norcross, GA) following the standard protocol with an additional 30 µL of purified water for resuspension. Purified DNA was concentrated to 17-20 ng/ µL with a 260/280 ratio around 1.8 to 2.0 as recommended by Eurofins MWG Operon where reactions were shipped for sequencing using BigDye Terminator v 3.1 Cycle Sequencing kits (Applied Biosystems).

Table 2.1 Tissue samples from *Fusconaia lananensis* and *F. askewi* used in the final DNA analysis

Species	Sample #	Location Collected	Genes used in analysis
F. askewi	EP130	Neches, Downstream 294	*ND1, ITS1*
F. askewi	EP134	Neches, Downstream 294	*ND1, ITS1*
F. askewi	EP136	Neches, Downstream 294	*ND1, 16S, ITS1*
F. askewi	EP138	Neches, Downstream 294	*ND1, 16S*
F.askewi	EP139	Neches, Downstream 294	*ND1, 16S, ITS1*
F. lananensis	EP106	Angelina, Downstream 343	*COX1, ITS1*
F. lananensis	EP107	Neches, of 79	*ND1, COX1, ITS1*
F. lananensis	EP145	Angelina, Upstream 343	*ND1, COX1, 16S, ITS1*
F. lananensis	EP146	Angelina, Upstream 343	*ND1, COX1, ITS1*
F. lananensis	EP150	Angelina, Upstream 343	*ND1, COX1, 16S, ITS1*
F. lananensis	EP151	Angelina, Upstream 343	*ND1, COX1, 16S*
F. lananensis	EP152	Angelina, Upstream 343	*ND1, 16S, ITS1*
F. lananensis	EP153	Angelina, Upstream 343	*ND1, 16S, ITS1*
GenBank Sequences			
F. flava	AY613793	Campbell et al. 2005	*ND1*
F .askewi	JN180998	Burlakova et al. 2012	*COX1*
F. askewi	KT285626	Pfeiffer et al. 2015	*COX1*
F. flava	KT285636	Pfeiffer et al. 2015	*COX1*
F. flava	AY238481	Krebs et al. 2003	*16S*
F. flava	DQ383442	Campbell et al. 2008	*ITS*

After the DNA sequences were obtained, the sequences were edited and aligned using programs Sequencher, Clustal X, and Mesquite (Gene Codes 2000, Larkin et al. 2007, Maddison and Maddison 2004). Program AliView was used alongside Mesquite for visualizing the DNA sequences (Larson 2014). Sequences from GenBank (http://www.ncbi.nlm.nih.gov) were used to compare to out putative *F. lananensis* and *F. askewi* sequences to related species *Fusconaia flava*, the Wabash Pigtoe, and to provide additional *F. askewi* sequences for analysis of the *COX1* gene (additional COX1 sequences for *F. lananensis* were not available via GenBank). Percent divergence values

were calculated for all genes, comparing 1) individuals identified as *F. lananensis* and *F.askewi* based on morphology and collection locale and 2) *F. lananensis* and *F. askewi* to *F. flava* to compare divergence values with a species that is genetically distinct from the study species.

Results

The *ND1* gene was successfully sequenced for a total of 14 samples (Table 2.2). Sequencing was less successful with the *COX1* gene only sequenced for 7 individuals, the *16S* gene sequenced for 10 individuals, and the *ITS1* gene sequenced for 12 individuals (Table 2.2). The *COX1* gene could not be sequenced from the *F. askewi* samples. For this gene, two *F. askewi COX1* sequences were obtained via GenBank and compared to the *F. lananensis* sequences for analysis. *ND1* sequences were trimmed to a length of 764 bases long and were compared to a *F. flava* sequence from GenBank (Table 2.1). When comparing the *F. lananensis* and *F. askewi ND1* sequences to the *F. flava ND1* sequence, the sequences differed by 3.14% (Table 2.2). When comparing *F. lananensis* sequences to *F. askewi* sequences, the percent divergence dropped to 0.39% (Table 2.2) *F. askewi* and *F. lananensis* both had percent divergence values of 0.26% when looking at variation within each species (Table 2.3). *COX1* sequences were trimmed to a length of 604 bases long. When compared to the *F. flava* sequence a percent divergence of 4.14% was calculated (Table 2.2). When removing the *F. flava* sequence *F. lananensis* and *F. askewi* were 0.99% divergent (Table 2.2). Looking at the variation with the two species, *F. lananensis* had a slightly higher percent divergence value than *F. askewi* within the *COX1* gene (Table 2.3). The *16S* sequences were trimmed to a length of 444 bases long. When compared to *F. flava* a percent difference of 5.63% was calculated for all

sequences (Table 2.2). When excluding *F. flava* a percent difference value of 1.351% was calculated for *F. lananensis* and *F. askewi* (Table 2.2). Most of this variation came from a single sequence, EP151; when this sequence was removed from the analysis the percent difference dropped to 0.23%. For gene *16S* no individuals shared mutations; all observed mutations were unique. Looking at the variation within each species, there was no divergence within the *F. askewi* samples for the 16S gene (Table 2.3). Gene *ITS1* was trimmed to a length of 507 bases long. A percent divergence value of 0.80% was calculated for analysis with and without *F. flava* (Table 2.2). Comparing the variation within each species, *F. lananensis* had a higher percent divergence value than *F. askewi*, with *F. lananensis* having a value of 0.39% and *F. askewi* having a value of 0.79% (Table 2.3).

Table 2.2. Percent divergence values for *Fusconaia lananensis* and *F. askewi*, with the number of individuals used in the final analysis for each gene is included. All *F. flava* sequences used in the analyses were obtained through GenBank.

	ND1	***COX1***	***16S***	***ITS1***
Number of *Fusconaia askewi*	5	2*	4	5
Number of *Fusconaia lananensis*	7	6	5	7
		Percent Divergence		
Fusconaia askewi/Fusconaia lananensis	0.39	0.99	1.35	0.80
Fusconaia askewi/Fusconaia lananensis + Fusconaia flava	3.14	4.14	5.63	0.80

* Indicates samples obtained from GenBank for use in the analysis

Table 2.3 Percentage divergence values for *F. lananensis* and *F. askewi*, with divergence values within species.

	ND1	***COX1***	***16S***	***ITS1***
Fusconaia askewi	0.26	0.50	0	0.39
Fusconaia lananensis	0.26	0.66	1.35	0.79
Fusconaia askewi/ Fusconaia lananensis	0.39	0.99	1.35	0.80

Discussion

Percent divergence values obtained from this study for the *ND1* and *COX1* genes are similar to values found in other genetic studies involving *F. lananensis* and *F. askewi*; specifically, a divergence value of 0.39% was calculated for the *ND1* gene when comparing *F. lananensis* with *F. askewi* in the current study and this is congruent with the values arrived at by Burlakova et al. (2012) and Marshall (2014). When *F. flava* is included in the analysis I calculated a value of 3.14% while Burlakova et al. (2012) calculated a range from 2.59-3.43%. Divergence values calculated for *COX1* were slightly higher than values obtained in a previous study; specifically, a divergence value of 0.99% was calculated for *F. lananensis* and *F. askewi* individuals in my study while values ranging from 0.3-0.7% were seen in Burlakova et al. (2012). Divergence values between *F. lananensis* and *F. askewi* with the addition of *F. flava* for the *COX1* gene were similar to values obtained with Burlakova et al. (2012).; a value of 4.14% was calculated in the current study when including *F. flava* while Burlakova et al. (2012) had a range of 2.92-4.91%. Studies looking at percent divergence for the genus *Fusconaia* for the *16S* have not been conducted prior to the current study; however, the amount of divergence found between *F. flava* and *F. lananensis*/*F. askewi* for this study was 5.63%, similar to the amount of variation seen within different species of the same genus in other studies (Kallersjo et al. 2005). The amount of variation within just *F. lananensis*/*F. askewi* dropped to a much lower value of 1.35% without the presence of *F. flava*. There was no difference in divergence values between the *F. lananensis* and *F. askewi* samples and the *F. flava* sample (Table 2.2). This is similar to results for individuals of different species in *Fusconaia* that show little to no variation with the *ITS1* gene (Manendo et al.

2008, Schilling 2015). This could indicate that these species within *Fusconaia* are more recently diverged; yet there is also evidence that variation within the *ITS* gene regions could be species specific (Kallersjo et al. 2005, Manendo et al. 2008). There are three regions within the *ITS* gene that can be used for sequencing: the *ITS1*, *5.8s*, and the *ITS2* region. The *ITS1* and the *ITS2* regions can have a large amount of diversity based on species, with values of <1% reported within species and 4.7%-15.3% between species (Kallersjo et al. 2005). As the *ITS1* gene was used in this analysis it is possible that the *ITS2* region could present more variation for *Fusconaia*.

Overall, the percent divergence values based on the amount of variation within the *ND1*, *COX1*, and *16S* genes from the *F.lananensis* and *F. askewi* do not support the current classification of these individuals as separate species and validates the work of Burlakova et al. (2012). Divergence values obtained with the *ND1* and *COX1* genes were similar to those found with Burlakova et al. (2012) both within the *F. lananensis* and *F. askewi* and with the inclusion of *F. flava*. The addition of the results for the *16S* gene further support the hypothesis that *F. lananensis* and *F. askewi* are not separate species. Though the *ITS1* gene did not confirm nor deny this, further analysis using different regions of the *ITS* gene could bring further support for combining the two species.

Chapter 3: Population Dynamic of Freshwater Mussels

The population structure and rate of growth for individuals in freshwater mussel populations can be determined through life history traits such as individual growth rates, life spans, and host interactions (Haag 2012). For example, traits related to the local population sizes of mussels in the Red River basin, such as regional abundance and time spend brooding, were strong predictors of local extinction (Vaughn 2012). Determining life history traits for freshwater mussel species can also help to understand species distribution in rivers (Haag and Warren 1998, Daniel and Brown 2014). In addition, freshwater mussel species dependent on host fish density are often restricted to sites with stable host populations (Haag and Warren 1998). Unfortunately, population structures and individual growth rates are not known for most species of freshwater mussels and generalizations are made about life history only based on well-studied species such as *Margaritifera margaritifera*, the Freshwater Pearl Mussel, found throughout Europe and parts of eastern Canada (Hastie et al. 2000, Outeiro et al. 2007). For example, depictions of all freshwater mussel species as being slow growing and long lived are not accurate. Though many species of mussels do have long lifespans, lifespans between freshwater mussels species vary from 4 to 200 years (Haag and Rypel 2011). As other species of freshwater mussels have been studied they have shown great differences in life-history traits, the need to study these traits in individual species has become more apparent (Haag 2012).

Age and size structure of populations have been studied in some species of freshwater mussels (Bauer 1983, Hastie et al. 2000, Rogers et al. 2001, Haag 2012) and

left-skewed size-class distributions are most commonly found (Rogers et al. 2001, Haag 2012). This pattern has been observed with species known to have low recruitment rates and high survival, but this left-skewed distribution may be caused by two other factors: 1) human impacts may be suppressing recruitment rates of some species and 2) surveys used with freshwater mussels tend to be biased against smaller individuals (Bauer 1983, Hastie et al. 2000, Haag 2012). To obtain unbiased information about a population, intensive excavation methods have been used to increase detectability of smaller individuals (Miller and Payne 1988, Haag and Warren 2007, Haag 2012). Using these methods, three types of size distributions which differ from traditional left-skewed distributions can be seen in a healthy stream: 1) cohort-dominated, 2) uniform, and 3) right-skewed distributions (Haag 2012). Cohort-dominated freshwater mussel populations are dominated by one or few size classes representing size/age cohorts with other classes represent by few individuals (Payne and Miller 1989, Payne and Miller 2000, Haag 2012). Populations with uniform distributions have a relatively even frequency of individuals across size classes and may be dominated by mid-sized or large individuals (Haag 2012). This occurs due to of the accumulation of older individuals as growth slows and is often seen with longer-lived species (Miller and Payne 1993, Haag and Warren 2007, Haag and Warren 2010, Haag 2012). Finally, right-skewed populations consist of classes dominated by younger individuals with smaller numbers of older individuals and are often seen with short-lived species (Crabtree and Smith 2009, Haag and Warren 2010, Jones and Neves 2011, Haag 2012).

Several different methods have been employed to determine different population characteristics, such as density of a species. The two most common methods of mussel

surveying include 1) quadrat sampling and 2) timed surveys (Vaughn et al. 1997). Quadrat surveys are sometime also used in conjunction with timed surveys, as there are observed benefits and disadvantages to both methods. For example, timed surveys tend to underestimate small species and quadrat surveys tend to underestimated species richness in an area (Vaughn et al. 1997). Both 0.25 m^2 and 1 m^2 quadrats are commonly use to determining specific population characteristics such as species abundance, population density, distribution, and sizes (Vaughn et al. 1997, Kuenzler 2003, Strayer and Smith 2003).

Mark-recapture is another method used to study freshwater mussel populations although some concerns have been raised over potential bias towards larger individuals within a mussel population (Anthony et al. 2001, Haag 2009, Hua et al. 2015). Depending on the models used, mark-recapture studies can be used to calculate several different aspects in a population including survival, recruitment, and population size (Matter et al. 2013). In addition, measurements of individual mussels can be tracked over a period of months or years to determine both individual and class growth rates (Villella et al. 2004). Results obtained from mark-recapture can be further analyzed to examine relationships between aspects such as population density and population growth with external influences (Lauzon-Guay et al. 2005, Widarto 2007). Recently mark-recapture studies have even been used in the field of freshwater mussel propagation to assess both the growth of lab-reared mussels in the wild and impacts they have when released on established populations (Hua et al. 2015). Several different methods of tagging have been used in mussel mark-recapture studies including glued tags, carved numbers on shells, and passive integrated transponder (PIT) tags (Peterson et al. 2011, Kurth et al. 2007,

Hua et al. 2015). Though animals marked with PIT tags have a much higher recapture rate than the use of glued tags and carved tags, glued tags and carved tags are currently cheaper to use (Kurth et al. 2007, Hua et al. 2015).

The goal of this project was to estimate population size, density, and structure for the state-threatened species *Pleurobema riddellii, Fusconaia askewi*, and *F. lananensis*. This was done by using 0.25 m^2 quadrats and mark-recapture at field sites where the highest densities of these three species have been recorded. Multiple mark-recapture sites were set up for each species to monitor population size and growth over the course of a year. As information on the population ecology for these threatened species is limited, additional data about these populations are crucial for conservation efforts.

Materials and Methods

Field Sites

Locations for the field sites used were selected from sites with the top highest densities for the target species in previous surveys (Ford 2013). From those locations, I selected sites that had easy accessibility via boat ramps as it was necessary to visit several times during the course of this study. During the summer of 2014, I established a total of seven field sites with three in the Neches River, three in the Sabine River, and one in the Angelina River (Table 1). Populations of *P. riddellii* were examined at the sites in the Neches River (Table 1). Populations of *F. askewi* were studied at sites on the Sabine River (Table 1). Finally, I established one site on the Angelina River to study a population consisting of both *F. askewi* and *F. lananensis* (Table 1). For this study, all

individuals under study were classified as *Fusconaia* because of the difficulty in differentiation between *F. askewi* and *F. lananensis* in the field.

Random 0.25 m^2 surveys

During the summer of 2014 and 2015 I conducted random 0.25 m^2 surveys at each of the seven field sites. Specifically, a 150-m segment of the river was marked off at the field site and divided into three 50-m segments. Within each 50-m segment, 27 0.25 m^2 quadrats were sampled using a stratified randomize design with three starts: one near each bank and one in the middle of the river (Strayer and Smith 2003, Pooler and Smith 2005). All mussels within each quadrat were excavated by hand by student workers. I recorded all live and recently dead mussel species and measured length, height, and width of the 3 study species. I used the results from these surveys to help choose where the 5 m x 5 m mark-recapture site would be set up for the mark-recapture study. The 0.25 m^2 quadrat with the largest number of the desired species was the point where the 25 square meter mark recapture location was established. Therefore those sites had the highest density of mussels. Most of the 0.25 m^2 surveys were conducted during the summer of 2014, except for sites Sabine 2 and Sabine 3. The 0.25 m^2 survey data for these sites for the summer of 2015 were obtained from surveys conducted by another graduate student (Jared Dickson, unpublished).

Table 3.1. Site names, locations, coordinates, and species of study for each field site.

Site Name	***River***	***Location***	***Coordinates***	***Species***
Neches 1	Neches	Upstream Hwy 294	N 31.643610, W-95.285900	*Pleurobema riddellii*
Neches 2	Neches	Cherokee Hunting Club	N 31.715680, W-95.332570	*P. riddellii*
Neches 3	Neches	Downstream Hwy 79	N 31.841370, W-94.425150	*P. riddellii*
Sabine 1	Sabine	Downstream Hwy 14	N 32.553450, W-95.200690	*Fusconaia askewi*
Sabine 2	Sabine	Hwy 14 Bridge	N 32.557638, W-95.205906	*F. askewi*
Sabine 3	Sabine	Upstream Hwy 43	N 32.377156, W-94.465937	*F. askewi*
Angelina 1	Angelina	Upstream 343	N 31.753400, W-94.961610	*Fusconaia spp.*

Mark-Recapture

A total of seven sites were chosen for mark and recapture research: three on the Sabine River, three on the Neches River, and one on the Angelina River. As indicated above I chose these sites as they had the highest density locations for the targeted species. The sites on the Sabine were chosen for *F. askewi* mark/recapture, the sites on the Neches were chosen for *P. riddellii* mark/recapture, and the site on the Angelina was chosen for *F.lananensis* and *F. askewi* mark/recapture. Each 5 m x 5 m area was bounded by rebar rod positioned in the banks of the river, and by spray painting trees on the bank, and by taking a GPS point within the middle of the mark-recapture location. We placed the 1 m^2 quadrats within the area and excavated all of mussels by hand. After we completed excavating one the quadrat, we moved the quadrat and excavated the next 1 m^2 area. We excavated the entire 5 m x 5 m area for a total of 25 quadrats. In instances where the river was too deep for excavation without diving or unsuitable habitat for mussels would have fallen within the 5 m x 5 m square, a different shape equaling approximately 25 m^2 was

used instead. I marked all live *P. riddellii*, *F.askewi*, and *F. lananensis* mussels by gluing bee queen-marking tags on the mussel shell with super glue. The tag color, tag number, mussel species, and mussel length were recorded for each mussel marked. After marking, each mussel was carefully placed back within the quadrat.

All mark-recapture sites were visited an additional two times during the summer of 2015 for the 1-year mark-recapture period and for the 2-3 week mark-recapture period. During the first visit during the summer of 2015, we re-excavated the sites for mussels through use of the 1 m^2 quadrats, including the immediate area outside the 5 m x 5 m quadrat. I recorded the tag color, tag number, species, and length for all previously marked mussels found. I also recorded the number of marked and unmarked target species mussels and tagged and recorded all new mussels found during that time. For the field sites in the Neches River and the Angelina River, we tagged all mussels with the Biomark PIT tag. PIT tags were attached to the outer shell with superglue, covered with a marine epoxy, and then allowed to dry for approximately 15 minutes. I recorded the PIT tag identification number for each PIT-tagged mussel. After all the mussels were marked, we placed all mussels back within the quadrat. Approximately 2-3 weeks later, we resurveyed the 5 m x 5 m sites again using a Biomark HPR Plus Reader to locate pit tagged mussels in the Neches River sites (*Neches 1, Neches 2, and Neches 3*) and the Angelina site (*Angelina 1*). After scanning the site we also excavated the 5 m x 5 m quadrat by hand with the use of the 1m^2 quadrats to locate any unmarked mussels and I recorded the tag number, tag color, and the number of unmarked target mussels.

Mark-Recapture Analysis

Mark-recapture models were created from the data for each location using Program MARK version 8.0 (White and Burnham 1999). Program MARK is used to provide parameter estimates from organisms that are marked and reencountered, whether alive or dead, and can provide population size estimates within closed populations (White and Burnham 1999). The POPAN model was chosen for this study because in addition to estimating capture probability, survival probability, and overall population size this model has the capability to estimate the population size at each encounter (Arnason and Schwarz 1995, Schwarz and Arnason 1996). I created models for each of the seven field sites and both real values and derived values were extracted from the top model(s) for each site. I recorded the real and derived estimates for φ_1 (survival probability between the initial visit and the 2nd visit), p_2 (probability of capture at 2nd visit), N_1 (initial population estimate), N_2 (population at 2nd visit), and Gross N (overall population estimate during course of study) for the top model(s) at each field site. These values are reflective of the population with the 5 m x 5 m mark-recapture areas and not the entire field site. In addition, I also calculated the recapture frequencies for each site visit using data collected during the mark-recapture study to determine any significant difference between visits and rivers.

0.25 m^2 Quadrat Survey Analysis and Size Classes

The data we collected from the initial 0.25 m^2 quadrat surveys was used to calculate mean m^2 densities and the densities of the mark-recapture sites by using the total number of individuals divided by the total mark-recapture area (25 m^2). As two of the sites on the Sabine River had quadrat data collected in 2015 instead of 2014, I

compared the densities from the quadrat data to the densities of the mark-recapture site obtained during the first visit in 2015 (2nd mark-recapture visit). Initial quadrat survey data from all other sites were obtained in 2014 and were compared to mark-recapture site densities during the first visit in 2014. In addition to densities, I calculated the mean lengths of each species from both the 0.25 m^2 quadrat surveys and the mark-recapture sites and compared them to each other. T-tests were used to compare data collected by the different survey methods. The size classes were created by using the lengths of mussels sampled from both the mark-recapture sites and from the 0.25 m^2 quadrat surveys. Survey data from 2014 was pooled with mark-recapture lengths from 2014 and survey data from 2015 was pooled with mark-recapture lengths from 2015. Histograms were created with R Studio, with packages dplyr and ggplot2 (R Core Team 2013, Wickham and Francois 2013, Wickham 2009).

Results

POPAN Estimates

Model selection in MARK resulted in a total of 10 top models as three sites had two top models of equal values (Table 3.2). All ranked models created for each site are included in Appendix A. The largest population sizes were estimated for *F. askewi* populations (Table 3.2) Largest Gross N values were also estimated for *F. askewi* populations (Table 3.2) An increase in estimated population size was seen in most populations between 2014 and 2015 except at sites *Sabine 2* and *Neches 3* (Table 3.2). Though the largest population estimate from the models was for *F. askewi*, there were no significant difference in estimated population sizes between the Neches River and the

Sabine River within the 25 m^2 mark-recapture area. This can be seen both during 2014 and 2015 (Table 3.2). Though there was no significant difference in population sizes, the largest population size estimated for *F.askewi* was higher than the largest estimated population size for *P. riddellii*. The largest population estimate for a *F. askewi* population was at site *Sabine 1* with a population estimate of 302 ± 26.72 in 2015 while the largest population estimate for a *P. riddellii* population was at site *Neches 1* with a population estimate of 101±4.99 in 2015 within the 25 m^2 mark-recapture area (Table 3.2). Both sites *Neches 3* and *Sabine 2* saw a decrease in estimated population sizes between 2014 and 2015 while all other sites saw an increase in estimated population size during this time within the 25 m^2 mark-recapture area (Table 3.2). Though site *Neches 3* saw a small decrease in estimated population size in 2015, site *Neches 3* had a larger gross N value than site *Neches 2*. A value of 91 ± 2.33 was calculated for site *Neches 3* and 71 ± 2.89 for site *Neches 2* (Table 3.2). This larger gross N value indicates that model estimated that there were more individual mussels present in the 25 m^2 mark-recapture area over the course of the study, which can also be seen when including the number of individuals found during the final visit (Table 3.3).

Table 3.2. Real and derived estimates with standard error values from top POPAN models for all seven mark-recapture sites. Neches 3, Sabine 2, and Angelina 1 had two models top models with equal real and derived estimates. These values are reflective of the 25 m^2 mark-recapture site and not the entire field site.

Site	***Model****	***Survival (φ_1)***	***Capture (p_2)***	***N_1 at 2014***	***N_2 at 2015***	***Gross N***
Pleurobema riddellii						
Neches 1	φ(t)pent(t)p(t)	0.92 ± 0.03	0.85 ± 0.06	64 ± 5.78	101 ± 4.99	150 ± 3.75
Neches 2	φ(t)pent(t)p(t)	0.98 ± 0.01	1 ± 0	29 ± 3.96	60 ± 3.12	71 ± 2.89
Neches 3	φ(.)pent(t)p(t)	0.98 ± 0.01	1 ± 0	58 ± 4.12	54 ± 4.21	91 ± 2.33
	φ(.)pent(t)p(.)					
Fusconaia askewi						
Sabine 1	φ(.)pent(t)p(.)	0.92 ± 0.01	0.44 ± 0.04	280 ± 34.56	302 ± 26.72	909 ± 97.03
Sabine 2	φ(t)pent(t)p(t)	0.02 ± 0.07	1 ± 0	90 ± 6.12	64 ± 6.12	381 ± 63.95
	φ(t)pent(t)p(.)					
Sabine 3	φ(.)pent(t)p(.)	0.97 ± 0.01	0.44 ± 0.05	116 ± 20.19	169 ± 18.61	293 ± 32.75
Fusconaia						
Angelina 1	φ(t)pent(t)p(.)	0.77 ± 0.04	1 ± 0	42 ± 4.3	95 ± 4.71	181 ± 13.49
	φ(.)pent(t)p(t)					

** φ, survival probability; p, capture probability; pent, recapture probability; N, population estimates; (.), constancy; (t), temporal variation*

Mean density values for F. askewi and P. Riddellii

Fusconaia askewi was found in the Neches, Sabine, and Angelina Rivers during 0.25 m^2 surveys (Table 3.3). Although *F. askewi* was found in all three rivers, width and height measurements for sampled *F. askewi* were only gathered at sites on the Sabine River. With the 0.25 m^2 surveys we did not detect *F. askewi,* live or dead, at site *Sabine 2* though this site was still used as a mark-recapture site (Table 3.2, Table 3.3). Site *Sabine 1* had the highest mean live m^2 density for *F. askewi* at 5.63 ± 5.90 and also had the highest mean m^2 density for dead *F. askewi* at 9.04 ± 10.26. Site Sabine 3 during the summer of 2015 had a mean m^2 density for live *F. askewi* at 0.89 ± 2.56 (Table 3.3). No dead *F.askewi* were found at site Sabine 3 during quadrate surveys (Table 3.3). *P. riddellii* was found only in the Neches River during 0.25 m^2 surveys (Table 3.3). The

0.25 m^2 surveys did not detect *P.riddellii*, live or dead, at site *Neches 3* though this site was still used as a mark-recapture site (Table 3.2, Table 3.3). Site Neches 2 had the highest mean m^2 density for live *Pleurobema riddellii* at 2.82 ± 7.34 and site Neches 1 had the lowest mean m^2 density for live *P. riddellii* at 0.22 ± 0.51 (Table 3.3). Neither site had any dead individuals during the 0.25 m^2 surveys (Table 3.3).

Table 3.3. Mean m^2 densities for *Fusconaia askewi* and *Pleurobema riddellii* from surveys between 2014-2015.

	Fusconaia askewi		*Pleurobema riddellii*	
Site	***Mean m² density Live***	***Mean m² density Dead***	***Mean m² density Live***	***Mean m² density Dead***
Neches 1	0.54 ± 1.45	0.15 ± 0.70	0.222 ± 0.51	0 ± 0
Neches 2	4.37 ± 7.58	0.07 ± 0.54	2.815 ± 7.34	0 ± 0
Neches 3	0.22 ± 1.21	0.22 ± 0.93	--	--
Sabine 1	5.63 ± 5.90	9.04 ± 10.26	--	--
*Sabine 2**	--	--	--	--
*Sabine 3**	0.89 ± 2.56	0 ± 0	--	--
Angelina 1~	2.22 ± 9.35	0 ± 0	--	--

**Indicates sites surveyed during summer 2015. All other sites were surveyed during summer 2014.*
~All individuals surveyed were identified as Fusconaia askewi

Comparison of size distributions over a 1-year period

I used lengths collected from both mark-recapture sites and from 0.25 m^2 quadrat surveys were used to create size classes for the mussels from all seven study sites, separated by year (Appendix B). Lengths obtained from the 0.25 m^2 quadrat surveys were pooled within the year they were collected. Overall, sites on the Sabine River exhibited a larger number of size classes compared to the Neches River. Site *Neches 1* saw a shift in lengths, with more individuals in 2015 being measured between 30-50 mm as opposed to 2014, which saw a larger number of individuals in the 20-40 mm range (Figure 3.2). Site

Neches 2 both years had the 40-50 mm size class as the most common group (Figure 3.3). Between 2014 and 2015 at site *Neches 2* also saw a decrease in the smallest size class and an increase in the largest size class (Figure 3.3). Site *Neches 3* had a size class of 0-10 mm that did not reappear in 2015 (Figure 3.4). Size classes at site Sabine 1 skewed-left with more individuals being recorded in larger size classes in 2015 (Figure 3.5). *Sabine 2* saw a large shift in size distribution becoming left-skewed; smaller individuals were measured in 2014 and individuals measured in 2015 were larger (Figure 3.6). Though the larger sizes classes at *Sabine 3* did see an increase in the number of individuals measured in 2015, smaller individuals between 10-40 mm were found in 2015 and not seen in 2014 (Figure 3.7). All sizes at site *Angelina 1* saw in increase in number of individuals between 2014 and 2015, with a new 10-20 mm size class added in 2015 (Figure 3.8).

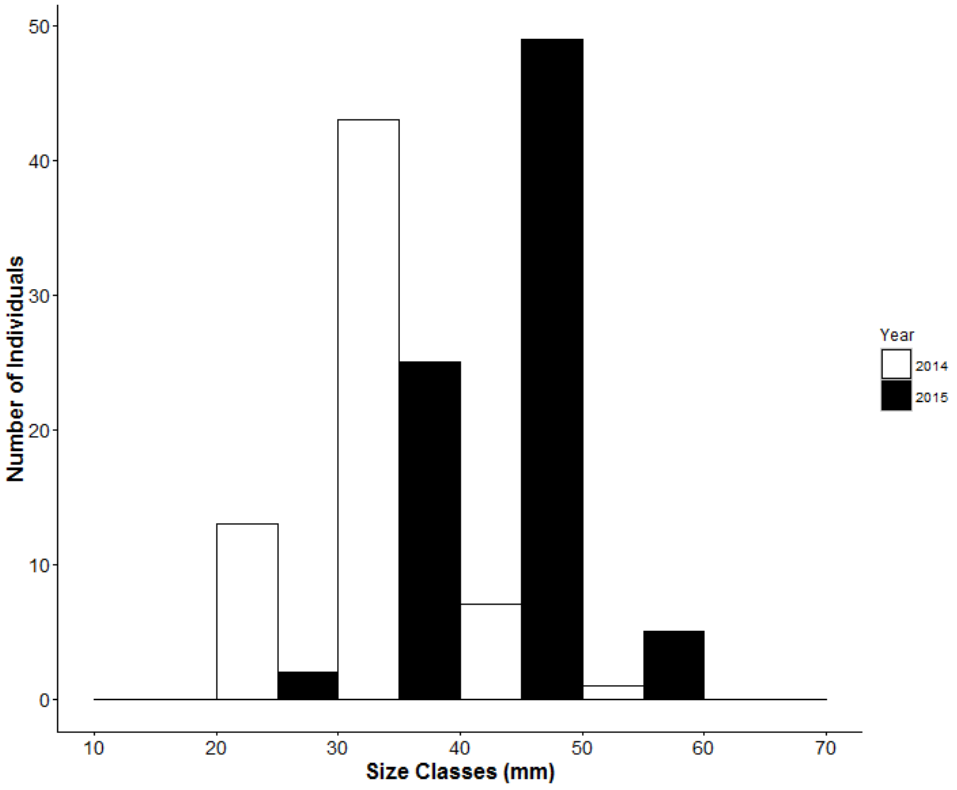

Figure 3.1. Sizes classes for *Pleurobema riddellii* at site *Neches 1*, by year.

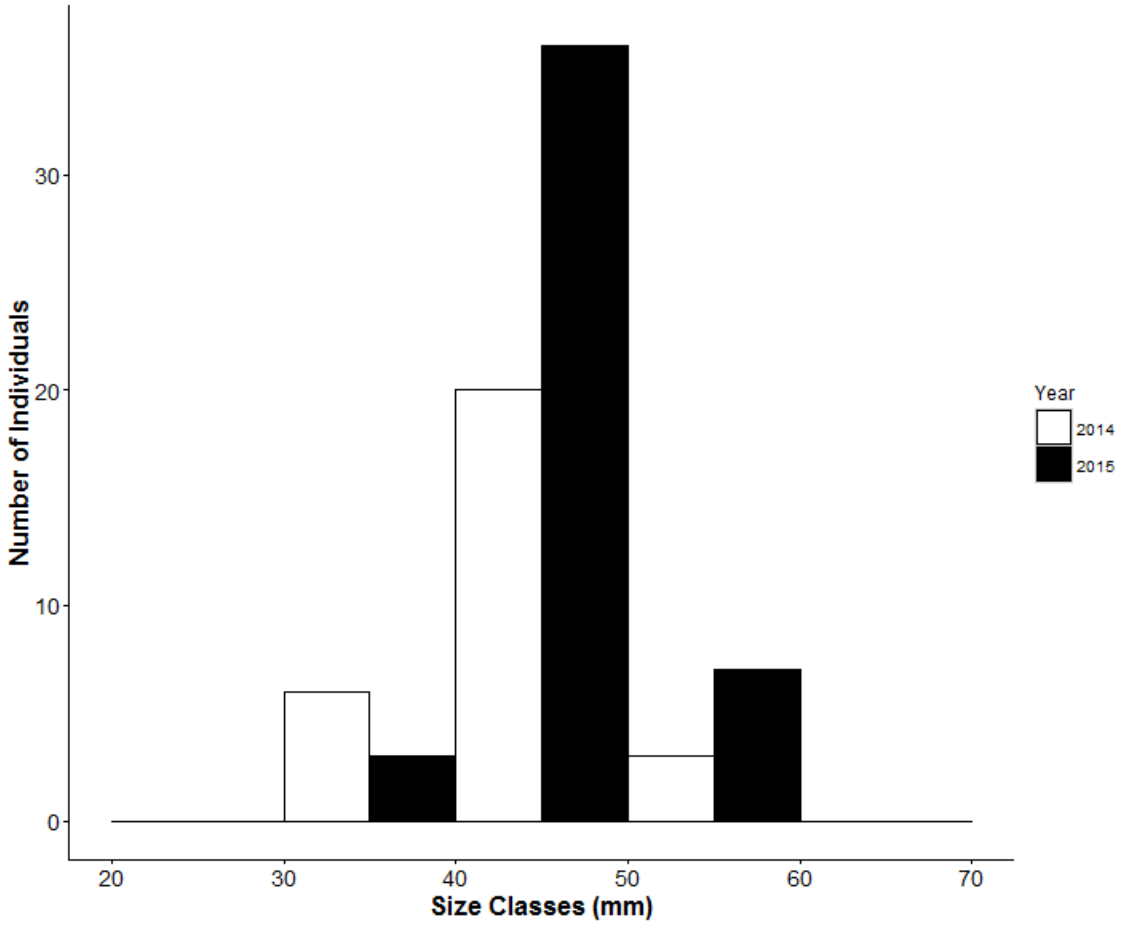

Figure 3.2. Sizes classes for *Pleurobema riddellii* at site *Neches 2*, by year.

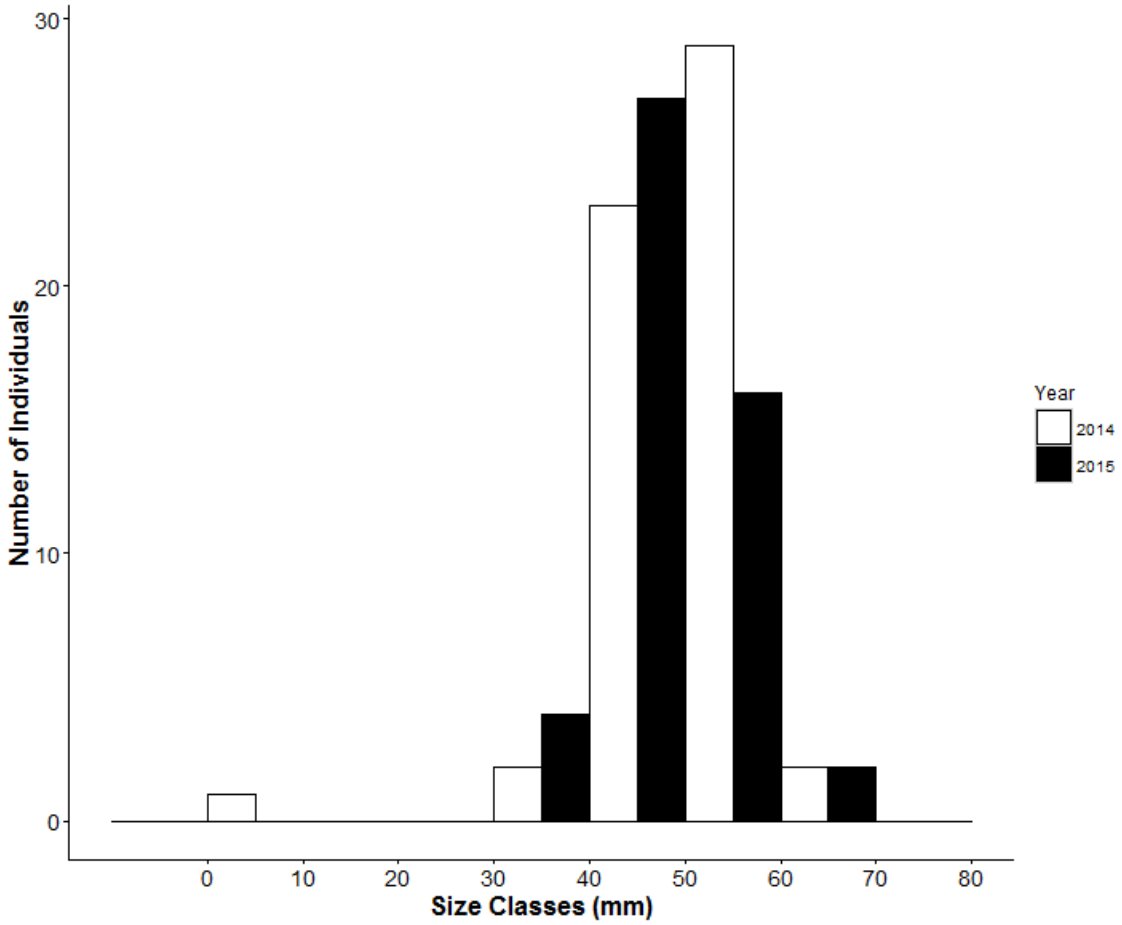

Figure 3.3. Sizes classes for *Pleurobema riddellii* at site *Neches 3*, by year.

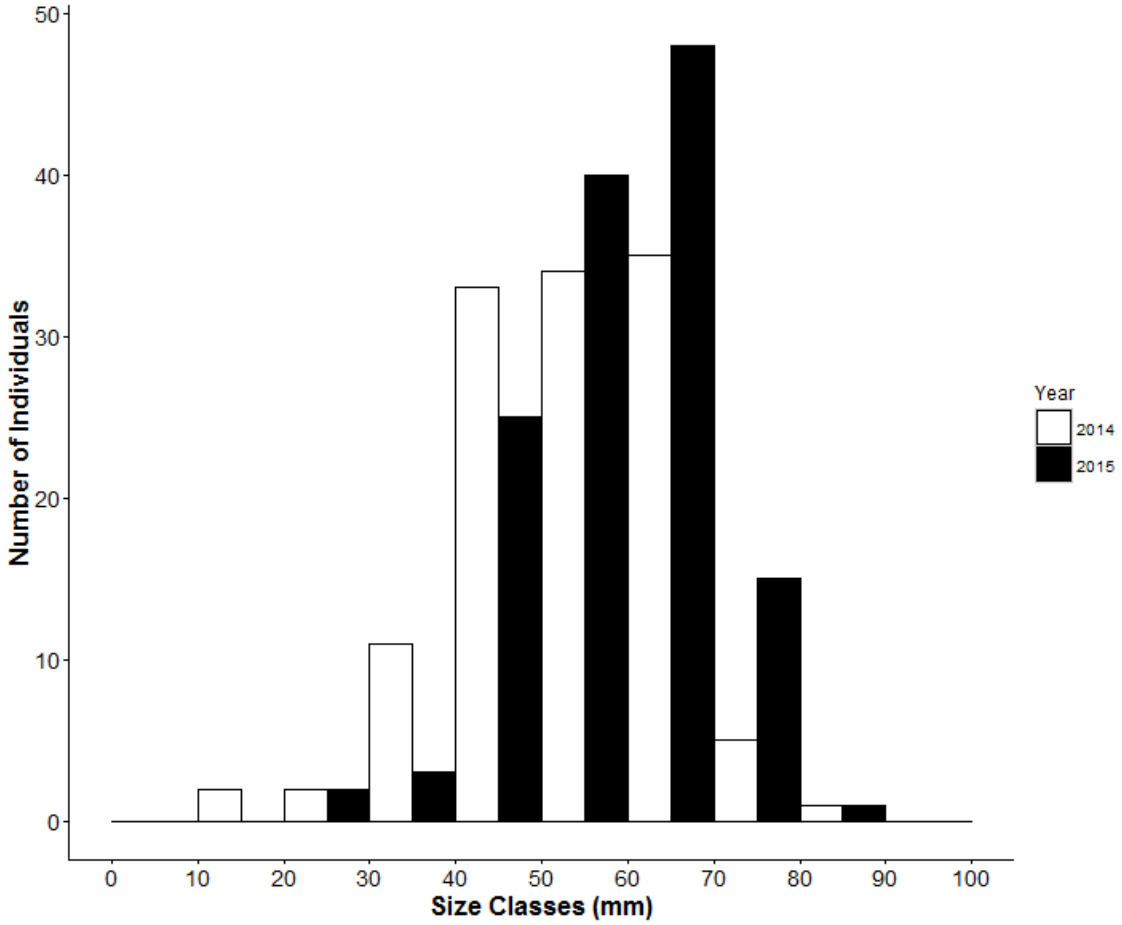

Figure 3.4. Sizes classes for *Fusconaia askewi* at site *Sabine 1*, by year.

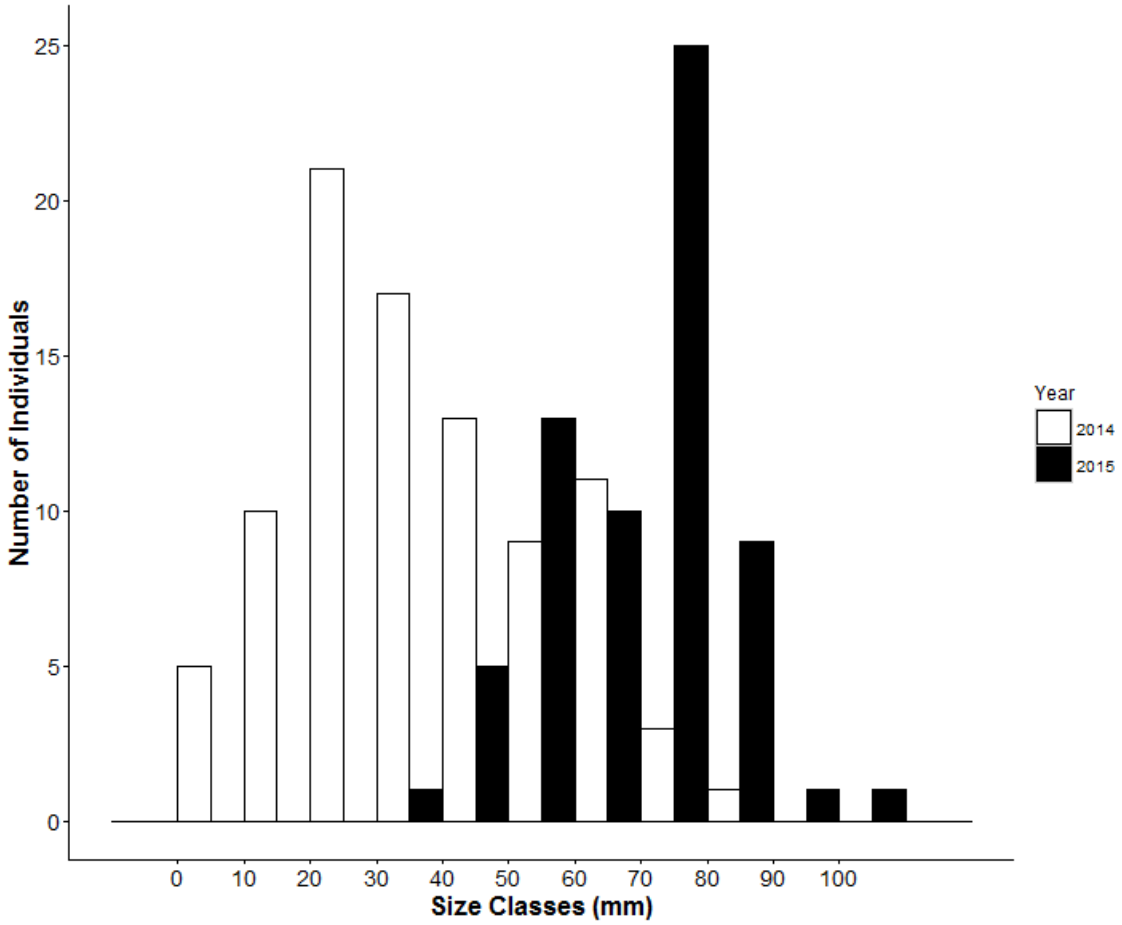

Figure 3.5. Sizes classes for *Fusconaia askewi* at site *Sabine 2*, by year.

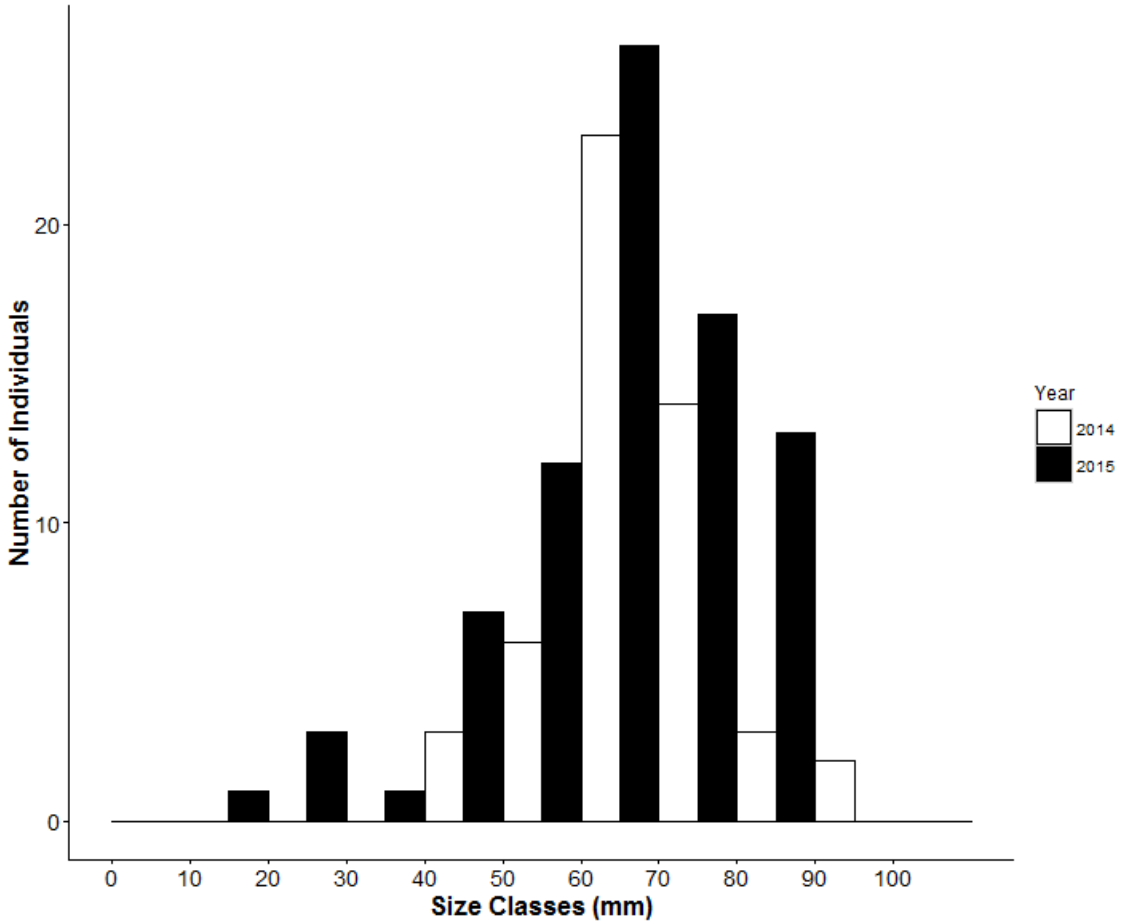

Figure 3.6. Sizes classes for *Fusconaia askewi* at site *Sabine 3*, by year.

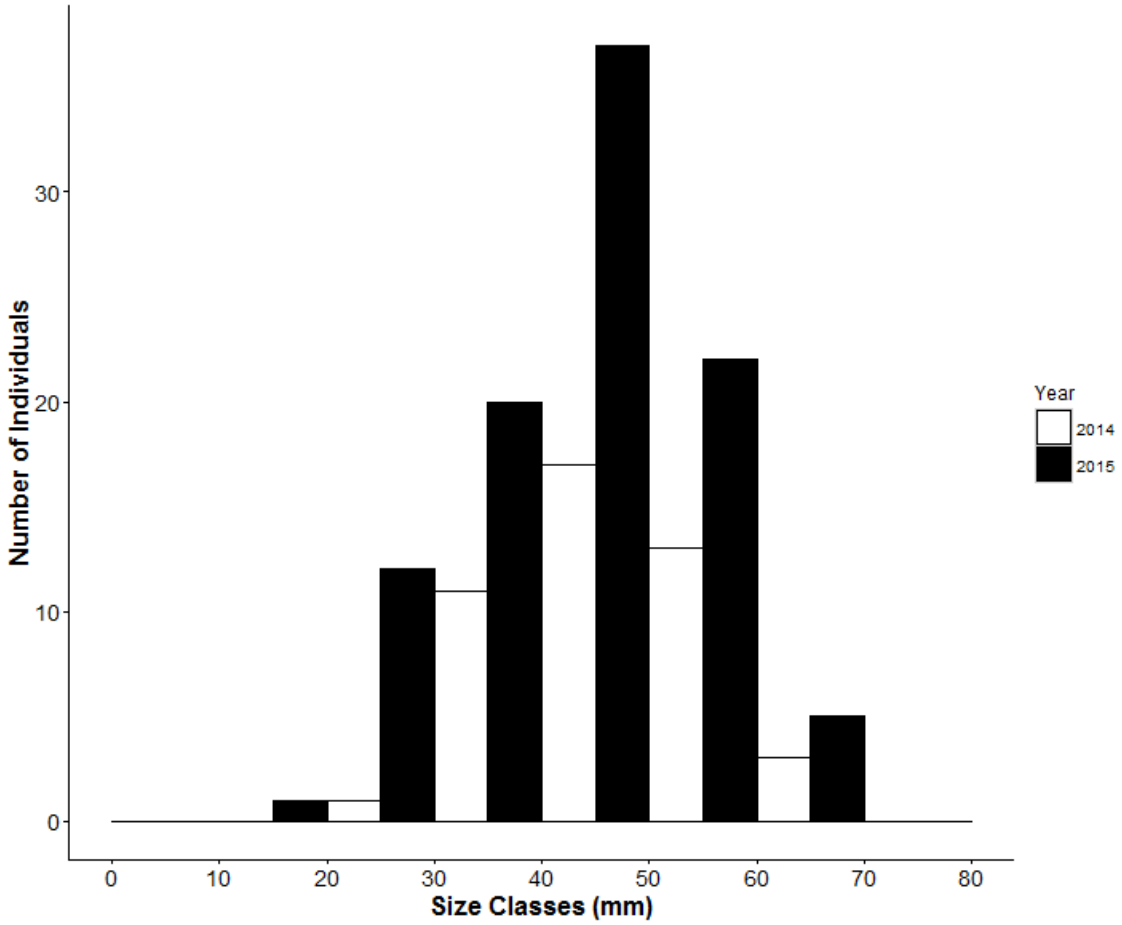

Figure 3.7. Sizes classes for *Fusconaia* at site *Angelina 1*, by year.

Comparison of recapture rates

Values obtained from the mark-recapture study were also used to calculate the percentages of individuals recaptured between the three site visits. All sites had a higher recapture rate between the 2-week periods in 2015 versus the one-year period between 2014-2015 (Table 3.4). All sites that had PIT tags added to the mussels during the 2nd site visit had significantly higher recapture values between the 2nd and 3rd site visits (Table 3.4; p-value < 0.05, t-value = 12.99, df = 4,). All sites on the Neches River had a significantly larger recapture rate between the one-year period (1st and 2nd site visit) than the sites on the Sabine River (Table 3.4; p-value < 0.05, t-value = 9.33, df= 4). Additionally, all sites on the Neches River had a marginally significant increase in recapture rates when the PIT tags were added compared to the sites on the Sabine River where PIT tags were not used (Table 3.4; p-value < 0.06, t-value = 3.99, df = 4). Approximately 9% of mussels at site *Angelina 1* were identified as *F. lananensis* during 2014 based on external morphology while all other specimens captured were classified as *Fusconaia* (Table 3.4).

Table 3.4. Recapture Rates and Total Captured for all seven mark-recapture sites within the 25 m^2 sites.

Site	***1st Visit, 2014***	***Recapture Rate between Visit 1 and 2***	***2nd Visit, 2015 Total***	***Recapture Rate between Visit 2 and 3***	***3rd Visit, 2015 Total***
*Neches 1**	63	0.49	77	1	100
*Neches 2**	28	0.61	45	1	50
*Neches 3**	58	0.55	56	1	61
Sabine 1	122	0.07	125	0.49	126
Sabine 2	89	0.01	64	0.02	2
Sabine 3	50	0.14	72	0.53	65
*Angelina 1**	40	0.33	81	0.94	76

**Indicates sites where all individuals in 2015 were marked with PIT Tags in addition to bee tags*

Quadrat densities versus mark recapture densities

Mean m^2 densities were also calculated for all mark-recapture sites for comparison to the values calculated from the 0.25 m^2 surveys (Table 3.6). Estimates of density for the mark-recapture portion of the study were calculated by dividing the total number of individuals found during the visit divided by 25 m (Table 3.5). There was no significant difference in m^2 density between the use of 0.25 m^2 quadrats or mark-recapture sites (Table 3.5). Higher densities were found using 0.25 m^2 quadrats for sites Neches 2, Sabine 1, and Angelina 1 while other sites had higher values calculated from mark-recapture data (Table 3.5). In addition to mean densities, mean lengths of individuals sampled during both 0.25 m^2 quadrat surveys and mark-recapture sites were compared (Table 3.6). There was no significant difference in mean lengths between mussels in the 0.25 m^2 quadrat surveys or mark-recapture sites (Table 3.6). Greater mean lengths were found at sites Neches 1 and Sabine 1 through the use of 0.25 m^2 quadrats while other sites had higher values calculated through the mark-recapture study (Table 3.6).

Table 3.5. Comparison of mean m^2 densities between quadrat surveys and mark-recapture values. Mean densities for mark-recapture sites were obtained by dividing the total captured by 25 m.

	Mean m^2 density	
Site	***m^2 Quadrats***	***Mark-Recapture***
Pleurobema riddellii		
Neches 1	0.22±0.51	2.52
Neches 2	2.82±7.34	1.12
Neches 3	0	2.32
	Mean for *Pleurobema riddellii*	
	1.01	1.99
Fusconaia askewi		
Sabine 1	5.63±5.90	4.88
*Sabine 2**	0±0	2.52*
*Sabine 3**	0.89±2.56	3.16*
	Mean for *Fusconaia askewi*	
	2.17	3.52
Fusconaia		
Angelina 1	2.22±9.35	1.76

**Indicates sites where 0.25^2 surveys were conducted during summer 2015. Mark-recapture density values for these sites reflect values obtained during 2015.All other values reflect the number captured during initial visits in 2014.*

Table 3.6. Comparison of lengths (mm) of individuals measured during quadrat surveys and mark-recapture. Includes both mean and range for each method.

	Lengths (mm)			
Site	**m^2 Quadrats**		**Mark-Recapture**	
	Mean	Range	Mean	Range
Pleurobema riddellii				
Neches 1	42.30±3.68	36.55-47.00	34.3±5.86	21.6-53.3
Neches 2	38.63±6.18	28.2-51.1	44.7±4.12	36.2-53.3
Neches 3			48.5±8.05	38.7-63.3
Fusconaia askewi				
Sabine 1	54.23±15.88	12-85	53±11.69	16.1-85.8
Sabine 2	46.85±13.91	14.8-79.4	68.7±12.75	42.5-104.3
Sabine 3			64.1±15.29	16.5-88.1
Fusconaia				
Angelina	43.57±10.51	31.7-64.4	45.7±8.67	29.9-69.9

**Indicates sites where 0.25^2 surveys were conducted during summer 2015. Mark-recapture length values for these sites reflect values obtained during 2015.All other values reflect the lengths recorded during initial visits in 2014.*

~Indicates sites where no individuals were captured during 0.25 m^2 quadrat surveys.

Discussion

Population dynamics at high density sites

Size classes suggest that *P. riddellii* may reach an adult size between 30-40mm, as this size class had the largest number of individuals (Figure 3.1, Figure 3.2, Figure 3.3). Therefore I suggest *P. riddellii* below a length of 30 mm may be considered juveniles although this should be analyzed with assessment of gametes in the gonads. The population of *P. riddellii* at site *Neches 3* had a greater range of size classes than other sites on the Neches River (Figure 3.3). The *P. riddellii* populations at site Neches 1 and Neches 3 had individuals less than 30 mm in length, indicating some recruitment at these sites (Figure 3.1, Figure 3.3). The lack of size classes smaller than 30 mm for *P. riddellii* populations could also be the result of juveniles burrowing, which would decrease our detection of juveniles (Figure 3.1, Figure 3.2, Figure 3.3, Vaughn and Hakenkamp 2001).

For *F. askewi*, individuals appear to reach an adult size after reaching approximately 40 mm (Figure 3.4, Figure 3.5, Figure 3.5, Figure 3.7). Therefore I suggest *F. askewi* below 40 mm may be considered juveniles although this should be analyzed with assessment of gametes in the gonads. Size distributions for the *F. askewi* in the Sabine River showed a wide diversity in size classes even though mark-recapture studies have been known to be biased against smaller individuals, *Fusconaia askewi* populations at three field sites had shifts in the second year to larger size classes which could be due, in part, to the growth of individuals over the one year period and the immigration of new adults into the site (Figure 3.4, Figure 3.5). Overall, my *F. askewi* populations had lower recapture rates between 2014-2015 than my *P. riddellii* populations (Table 3.2). The *F.*

askewi population at site *Sabine 1* had small size classes that were detected again in 2015; it is possible these individuals grew into the next size classes, were dislocated or killed during the flooding, or burrowed further into the sediment (Figure 3.4). The *F. askewi* population at site *Sabine 3* gained additional sizes classes in 2015; this addition of smaller size classes indicates recruitment may have occurred in the population at *Sabine 3* (Figure 3.6). It is also possible that the new juveniles were moved into the study site during flooding. Overall sites *Sabine 1* and *Sabine 2* had almost normal distributions that were skewed slightly left, which would be expected of a population with a uniform distribution of a longer lived species (Miller and Payne 1993, Haag and Warren 2007, Haag and Warren 2010, Haag 2012).

The largest population sizes within the mark-recapture areas were found in the Sabine and Angelina Rivers where *F. askewi* populations were tracked (Table 3.2). In addition, the *F. askewi* population at *Sabine 1* also had a large gross N value of 909 ± 97.03 (Table 3.2). This difference in population estimates for these species agreed with previous research where *F. askewi* was found in much higher densities than *P. riddellii* (Burlakova et al. 2010, Ford et al. 2012, Ford et al. 2014). Unlike other surveys, no difference was found between the mean density of *F. askewi* in the Neches River or the Sabine River. *F. askewi* was typically found in higher densities in the Sabine River (Ford et al. 2012). More apparent juveniles for species *F. askewi* were detected than for *P. riddellii* during the study. Indeed the lack of small size classes suggest recruitment may not be high with these *P. riddellii* populations but is occurring with the *F.askewi* populations, particularly site *Sabine 2* (Figure 3.5, Nalepa and Gauvin 1988, Haag 2012).

Average m^2 at high density sites: comparisons to other studies

Mean m^2 densities calculated for *P. riddellii* were 1.99 per m^2 and mean densities for *F. askewi* were 3.52/m^2 (Table 3.5). Surveys for Texas threatened species *Popenaias popeii,* the Texas Hornshell, calculated densities were between 0-0.186 individuals per m^2 (Karatayev et al. 2015). Species density for a locally rare species *Quadrula pustulosa*, the Pimpleback, in Wisconsin had estimates of approximately 0.25 individuals per m^2 (Sethi et al. 2004). In comparison, common mussel species had values of up to 1.25 individuals per m^2 in the same area (Sethi et al. 2004). When surveys were conducted in locations in French Creek, Ohio, *Epioblasma torulosa rangiana,* the Northern Rifle Shell, had m^2 densities ranging from 0.01 – 6.67 m^2 (Crabtree and Smith 2009). When comparing to those found in other studies, both *P. riddellii* and *F. askewi* had higher densities than expected for a rare mussel species. The density values obtained for *P. riddellii* and *F. askewi* are more comparable to densities one might expect for common mussel species, not rare mussel species. These higher values for *P. riddellii* and *F. askewi* should be expected as my study were conducted at sites with apparently high suitable habitat that historically had higher densities of these species. Quadrat surveys for these species at random site locations would likely produce densities similar to those found in other studies involving rare species.

Effect of flooding on recapture rates in the Sabine River

Between the 1st and 2nd mark-recapture visits, 2014 and 2015, the mussels at Neches River sites had higher recapture rates than sites on the Sabine River (Table 3). One significant event that occurred in the Sabine River between 2014 and 2015 was heavy flooding. Both the Sabine River and the Neches River experienced higher water

levels during the winter of 2014-2015 as opposed to the winter of 2013-2014 (Figure 3.9, Figure 3.10, Figure 3.11, Figure 3.12). Data from gauges on both rivers near the mark-recapture sites show that the sites on the Sabine River measured more flooding during the 1st and 2nd visits than sites on the Neches River (Figure 3.9, Figure 3.11). Flooding has been found to have negative effects on mussel population, including killing a significant portion of the population (Strayer 1999, Hastie et al. 2001). Flooding may have impacted mussels in the Sabine River; however, the number of *F. askewi* found at these sites in 2015 were similar to number found before the flooding event. Potentially, the flooding dislocated the marked *F. askewi* out of the mark-recapture areas, resulting in the smaller recapture rates in the Sabine River sites.

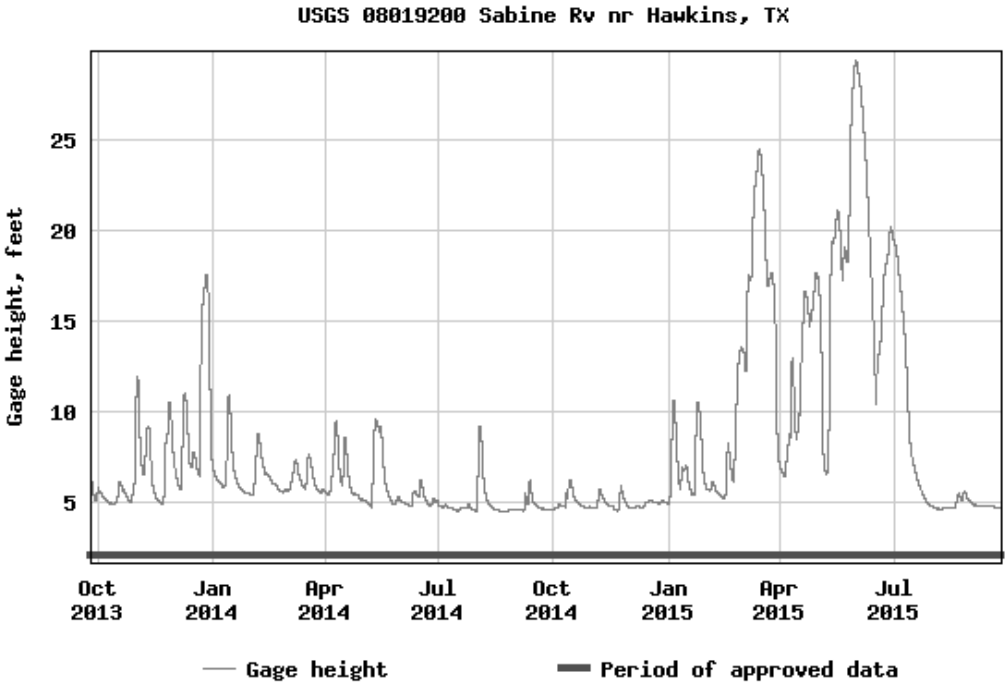

Figure 3.8. Gauge for the Sabine River in Hawkins, TX upstream from sites Sabine 1 and Sabine 2. Date ranges are from September 2013 through September 2015. Graph courtesy of the USGS.

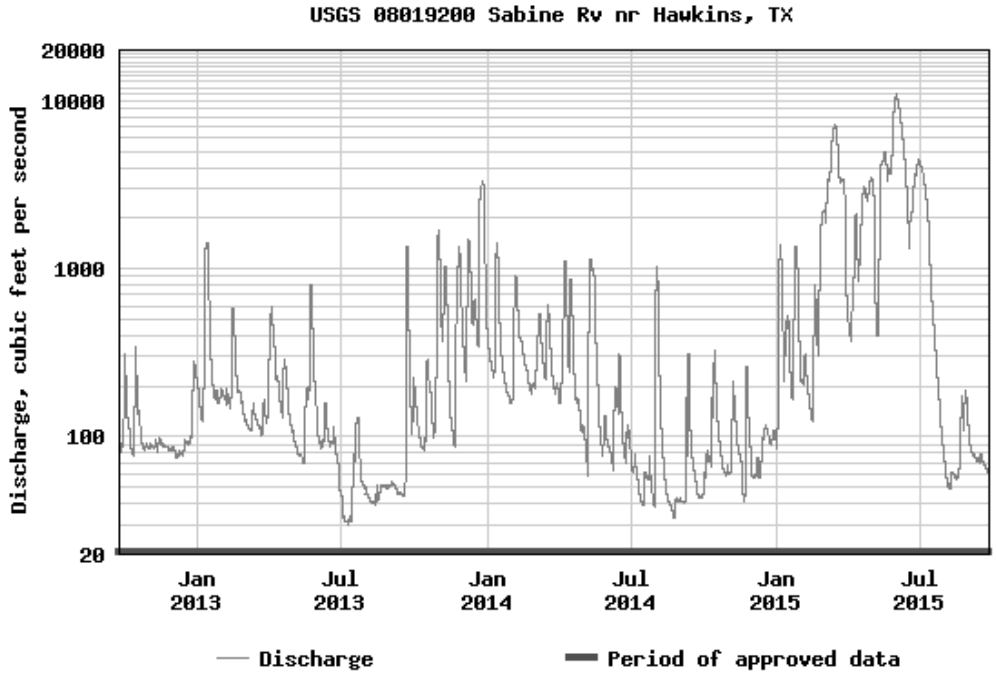

Figure 3.9. Discharge for the Sabine River in Hawkins, TX upstream from sites Sabine 1 and Sabine 2. Date ranges are from September 2013 through September 2015. Graph courtesy of the USGS.

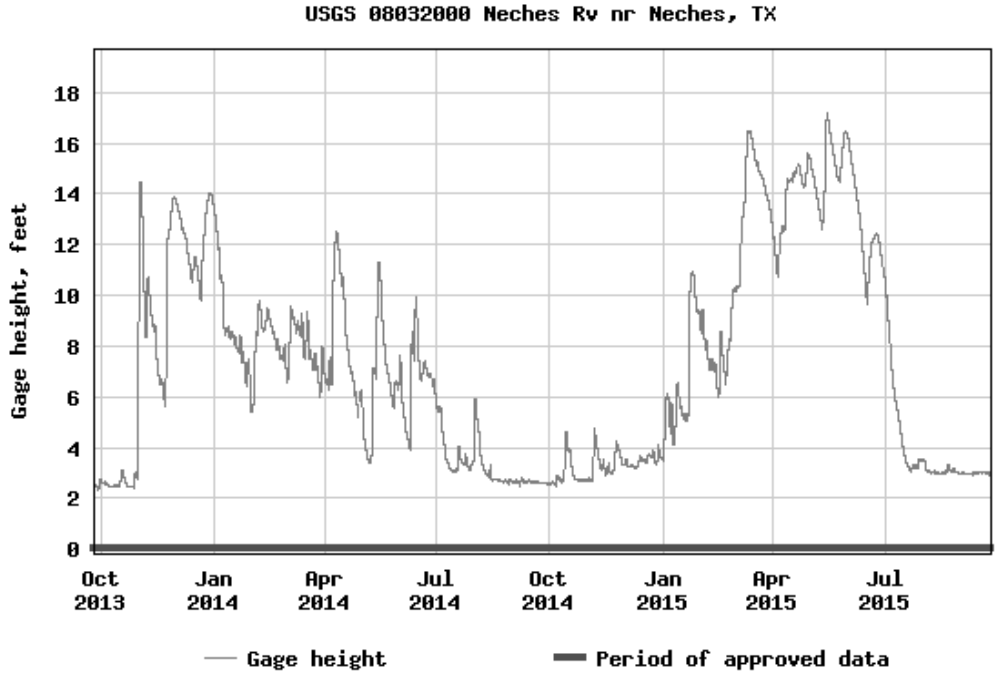

Figure 3.10. Gage heights for Neches River outside Neches, TX near site Neches 3. Date ranges are from September 2013 through September 2015. Graph courtesy of the USGS.

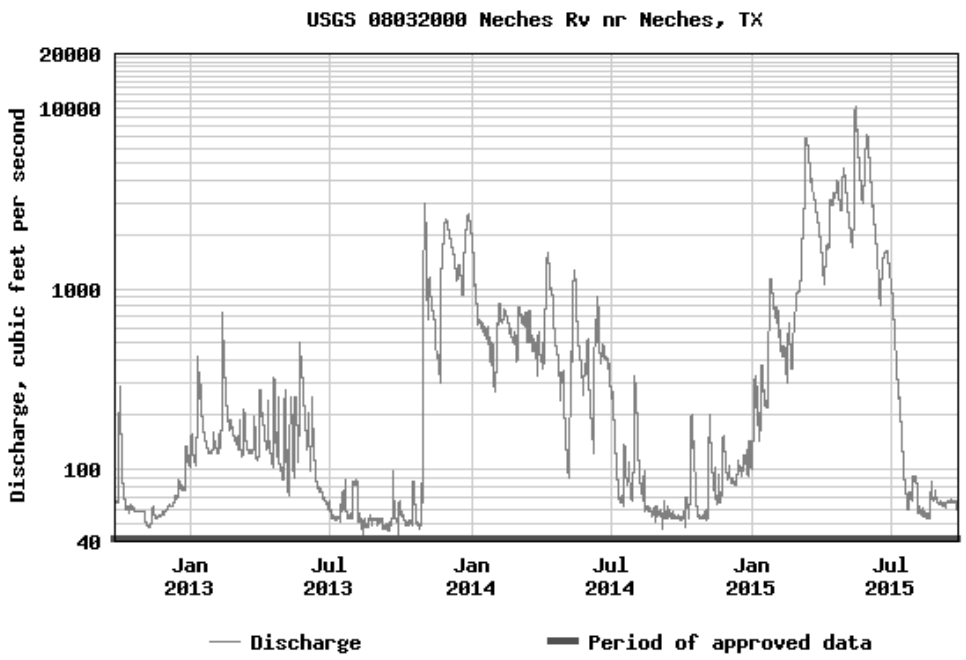

Figure 3.11 Discharge for Neches River outside Neches, TX near site Neches 3. Date ranges are from September 2013 through September 2015. Graph courtesy of the USGS.

Site Sabine 2

One site on the Sabine River had survival rates much lower than initially anticipated for *F. askewi.* Site *Sabine 2* has been previously used in other studies and has also been used in class field trips because of easy accessibility and the abundance of freshwater mussels, particularly *F. askewi* (Bakken 2013). However a low number of *F. askewi* were recaptured during each return visit and the top POPAN model estimated a low survival rate of 0.02 ± 0.02, much lower than all other field sites (Table 3.2, Table 3.3). The top models for this *F. askewi* population also showed temporal variation with the survival probability (Table 3.2). In addition, size class distribution changed dramatically between 2014 and 2015, changing from a right-skewed distribution to a left skewed distribution, i.e. changing from a juvenile dominated distribution to an adult dominated distribution (Figure 3.5). This *F. askewi* population also had a right-skewed

during the year 2014; this type of distribution is typically not seen in other studies freshwater mussels (Bauer 1983, Hastie et al. 2000, Rogers et al. 2001, Haag 2012). This type of distribution is often seen with short-lived species; however, as *F. askewi* is believed to be a longer-lived species this type of distribution could indicate high recruitment in this population (Crabtree and Smith 2009, Haag and Warren 2010, Jones and Neves 2011, Haag 2012). Distribution changed dramatically in 2015 with the smallest size classes disappearing, likely related to the large amount of flooding between 2014 and 2015 (Figure 3.8, Figure 3.9).

Like the other sites on the Sabine River, *Sabine 2* experienced a large amount of flooding between the one-year mark-recapture time period between 2014-2015 (Table 3.2, Figure 3.8, Figure 3.9). The *F. askewi* populations at sites *Sabine 1* and *Sabine 3* did not have a dramatic drop in survival probability (Table 3.2, Figure 3.8, Figure 3.9). Additionally *F. askewi* at site *Sabine 2* also had a very low recapture rate between the 2nd and 3rd site visit, much lower than other recapture rates during this time period for other *F. askewi* populations (Table 3.4). It is possible that the effects of the flooding were stronger at this particular site than the other *F. askewi* populations. Yet Site *Sabine 2* is about 0.7 km upstream from site *Sabine 3* and *Sabine 3* had a higher recapture rate than site *Sabine 2* between the 2nd and 3rd visit (Table 3.1, Table 3.4). Site *Sabine 2* is located a short distance downstream from a bridge, about 200 m, which may have had an effect on the *F. askewi* population during the study. Mussel abundance and bank stability have both been observed declining immediately downstream of bridges (Levine et al. 2003).

Effect of PIT Tags on recapture rates

The *P. riddellii* populations and the *F. askewi* population on the Angelina River all had a top model where p, or the capture probability, was time dependent while all sites on the Sabine River had a model where capture probability was constant (Table 3.2). This was likely because of the addition of the PIT tags at the Neches River and the Angelina River sites as these sites showed a marginally significant increase in recapture rates between the 2nd and 3rd visit (Table 3). All *F. askewi* populations on the Sabine River, where PIT tags were not used, did not show this amount of increase between the 2nd and 3rd visit (Table 3.4). This indicates that the addition of the PIT tags likely influenced the recapture probability on the Neches River sites and the Angelina Site. It should also be noted that during the period where only bee tags were used at all sites, between the 1st and 2nd visits, the Neches River already had significantly higher recapture rates than the Sabine River (Table 3.4). Again, the Sabine River had more flooding during this time period, which may have decreased recapture rates (Figure 3.8, Figure 3.9).

Comparison of quadrat densities and mark recapture densities

Mean m^2 densities and mean lengths were calculated for both the 0.25 m^2 quadrat surveys and the mark-recapture surveys (Table 3.5, Table 3.6). No difference was found between the m^2 densities or mean length of individuals found through the 0.25 m^2 quadrat surveys and the mark-recapture project, for both *P. riddellii* populations and the *F. askewi* (Table 3.5). Quadrat sampling has been known to underestimate the abundance of rare freshwater mussel species (Vaughn et al. 1997). In this study 0.25 m^2 quadrat surveys estimated similar densities to the mark-recapture sites. No difference between mean lengths were found between 0.25 m^2 quadrat surveys and the mark-recapture

surveys (Table 3.5). This may relate to the fact that these sites were high density sites for the species relative to overall distributions in the rivers. Though the mark-recapture sites in this study did track smaller individuals, mark-recapture studies involving freshwater mussels can be biased towards larger individuals in the population (Miller and Payne 1988, Haag and Warren 2007, Haag 2009). The handling of mussels during these studies have also negatively impact growth rates of handled mussels, leading to overestimates in age and size in mark-recapture studies (Haag and Commens-Carson 2008, Haag 2009). In contrast, quadrat sampling has been known to be an effective way to sample for smaller individuals such as juveniles, especially when compared to timed surveys (Amyot and Downing 1991, Vaughn et al. 1997). In this study the number of individuals collected during the 0.25 m^2 quadrat surveys was smaller than the number of individuals collected from the mark-recapture sites. It is possible that employing a larger number of 0.25 m^2 quadrats would have increased the probability of finding smaller individuals within these locations. In addition timing of the surveys may have biased the size of individuals measured, as juveniles of some species have been known to descend into deeper substrate during mid- to late summer (Amyot and Downing 1991, Vaughn et al. 1997).

Population dynamics: Conclusions

Overall, my *P. riddellii* populations and the *F. askewi* populations showed differences in population responses over the course of this study. The *F. askewi* populations had strong evidence of recruitment and a wide diversity of size classes; in contrast, the *P. riddellii* populations had little diversity in size class and little evidence of recruitment. Although the *P. riddellii* populations did not have evidence of recruitment occurring, the *P. riddellii* populations were much more stable than the *F. askewi*

populations in the Sabine River over the course of this study. The flooding that occurred during this study had a stronger impact on the population structure of the *F. askewi* populations than the *P. riddellii* populations. Flooding dislocated the original *F. askewi* populations on the Sabine River; however, though the new individuals present in the population had strong signs of recruitment and high abundance.

Chapter 4: Conclusions and Future Research

Additional molecular genetic analysis supports the conclusion that *Fusconaia lananensis* and *F. askewi* should not be designated as separate species. Along with the strong morphological similarities I suggest that *F. lananensis* and *F. askewi* should be combined into one species. However, as the specimens in the study were only from two locations is would be useful to analyze other populations.

The models I created provided estimates of population size at three sites for each species in addition to survival, capture, and entry probabilities for species *P. riddellii* and *F.askewi*. Though the population estimates are from 25 m^2 areas with high densities, with additional data recapture probabilities from other locations the models could be used to estimate population sizes over larger stretches of river (Inoue et al. 2014) which is the critical information needed for protection of threatened mussels. The use of PIT tags greatly increased the recapture rates, though the cost for this tagging method may remain an issue for some time. Bee tags may be better suited for short-term studies as they are easily applied and last for at least a year.

As expected, population sizes within the 25 m^2 mark-recapture areas for *P. riddellii* were lower than population estimates for *F. askewi.* Though earlier studies have found locations with large numbers of *F. askewi*, *F. askewi* is endemic to the Sabine, Neches, and Angelina Rivers (Ford et al. 2012). In comparison to the mean m^2 densities of rare mussels sampled during studies, the mean densities for *P. riddellii* and *F. askewi*, had values comparable to more common species than rare species (Table 3.5, Sethi et al. 2004, Karatayev et al. 2015). An explanation of these higher densities for *P. riddellii* and *F. askewi* is that these studies were conducted at sites that historically had the highest densities of these species. Quadrat surveys for these species at random site locations would more likely produce densities similar to those found in other studies involving rare species. Though *P. riddellii* have been located in several sites in the Neches River, *P. riddellii* is extremely rare in other river systems (Ford et al. 2012, Ford et al. 2014).. In addition, the results of the size distributions suggest little evidence of recruitment for *P. riddellii*; however further steps should be taken to increase detection of juveniles for species *P. riddellii*.

As conservation efforts for freshwater mussels increase, continued analysis of established freshwater mussel populations will be crucial to determine population status. *Fusconaia askewi* appears to be stable in the Sabine River because of the abundance of both adults and juveniles; however, *F. askewi* is endemic to this region (Ford et al. 2012) and so may merit continued protection considering it is less abundant in other river systems. The *P. riddellii* at all three Neches sites were marked with PIT tags during the summer of 2015 so these populations can continue to be monitored to gather more information for this species. It should be note that because these sites were chosen based

on historical sites with high density, the measures obtained from this study cannot be extrapolated to the entire population of river system for each species. Additional surveys in random river locations and more detailed research on mesohabitats would be needed before population level information could be extrapolated for the entire species. However, the sites set up in this study can continue to be monitored to gather information at established sites at areas with historically high densities of these species. If the sites for *P. riddellii* are monitored, the population size and the size class distributions can be measured from year to year to watch for changes in population size or distribution. Detection of juvenile *P. riddellii* will be essential to provide evidence for recruitment at these sites; if *P. riddellii* is a species with burrowing juveniles, additional intensive sampling methods may be needed. As these sites are representative of high density sites for *P. riddellii*, revisiting these sites in the future may be useful for additional studies involving these species, including topics such as reproductive seasonality and other life history traits.

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Appendix A: POPAN Model Rankings

Table A.1 Ranking of POPAN models for site *Neches 1*.

Model*	***AICc***	***Delta AICc***	***AICc Weight***	***Model Likelihood***	***Parameters***
φ(t)pent(t)p(t)	188.58	0	0.57	1	5
φ(.)pent(t)p(t)	190.26	1.68	0.25	0.43	5
φ(t)pent(t)p(.)	192.31	3.72	0.09	0.15	5
φ(.)pent(t)p(.)	193.07	4.49	0.06	0.1	5
φ(t)pent(.)p(t)	194.16	5.58	0.03	0.06	6
φ(.)pent(.)p(.)	90595.57	90406.99	0	0	3
φ(t)pent(.)p(.)	90599.64	90411.06	0	0	5
φ(.)pent(.)p(t)	-90599.64	0	0	0	0

** φ, survival probability; p, capture probability; pent, recapture probability; (.), constancy; (t), temporal variation*

Table A.2 Ranking of POPAN models for site *Neches 2*.

Model*	***AICc***	***Delta AICc***	***AICc Weight***	***Model Likelihood***	***Parameters***
φ(t)pent(t)p(t)	53.45	0	0.71	1	3
φ(.)pent(t)p(t)	55.27	1.82	0.29	0.403	3
φ(t)pent(t)p(.)	41020.94	40967.49	0	0	1
φ(.)pent(t)p(.)	41022.76	40969.31	0	0	1
φ(t)pent(.)p(.)	41023.01	40969.56	0	0	2
φ(.)pent(.)p(t)	41024.83	40971.38	0	0	2
φ(.)pent(.)p(.)	41024.83	40971.37	0	0	2
φ(t)pent(.)p(t)	41029.41	40971.96	0	0	5

** φ, survival probability; p, capture probability; pent, recapture probability; (.), constancy; (t), temporal variation*

Table A.3 Ranking of POPAN models for site *Neches 3*.

Model*	***AICc***	***Delta AICc***	***AICc Weight***	***Model Likelihood***	***Parameters***
φ(.)pent(t)p(t)	99.15	0	0.41933	1	2
φ(.)pent(t)p(.)	99.15	0	0.41933	1	2
φ(t)pent(t)p(.)	101.12	1.98	0.1561	0.3723	3
φ(t)pent(t)p(t)	107.91	8.77	0.00524	0.0125	4
φ(.)pent(.)p(t)	132.42	33.27	0	0	2
φ(t)pent(.)p(t)	82175.96	82076.82	0	0	3
φ(.)pent(.)p(.)	82176.06	82076.92	0	0	3
φ(t)pent(.)p(.)	82178.06	82078.92	0	0	4

** φ, survival probability; p, capture probability; pent, recapture probability; (.), constancy; (t), temporal variation*

Table A.4 Ranking of POPAN models for site *Sabine 1*.

Model*	***AICc***	***Delta AICc***	***AICc Weight***	***Model Likelihood***	***Parameters***
φ(.)pent(t)p(.)	287.29	0	0.363	1	4
φ(t)pent(t)p(t)	288.17	0.89	0.233	0.6409	5
φ(.)pent(t)p(t)	288.17	0.89	0.233	0.6409	5
φ(t)pent(t)p(.)	288.79	1.51	0.171	0.4698	5
φ(.)pent(.)p(t)	172454.4	172167.13	0	0	3
φ(t)pent(.)p(.)	172515.1	172227.79	0	0	5
φ(.)pent(.)p(.)	172607.3	172320.06	0	0	3
φ(t)pent(.)p(t)	NONE	NONE	NONE	NONE	NONE

** φ, survival probability; p, capture probability; pent, recapture probability; (.), constancy; (t), temporal variation*

Table A.5 Ranking of POPAN models for site *Sabine 2*.

Model*	***AICc***	***Delta AICc***	***AICc Weight***	***Model Likelihood***	***Parameters***
φ(t)pent(t)p(t)	37	0	0.49357	1	4
φ(t)pent(t)p(.)	37	0	0.49357	1	4
φ(.)pent(t)p(t)	44.3	7.29	0.01286	0.0261	5
φ(.)pent(t)p(.)	113.1	76.1	0	0	4
φ(t)pent(.)p(t)	113.31	76.31	0	0	3
φ(.)pent(.)p(t)	127288.69	127251.69	0	0	2
φ(t)pent(.)p(.)	127326.87	127289.86	0	0	3
φ(.)pent(.)p(.)	127415.54	127378.54	0	0	3

** φ, survival probability; p, capture probability; pent, recapture probability; (.), constancy; (t), temporal variation*

Table A.6 Ranking of POPAN models for site *Sabine 3*.

Model*	***AICc***	***Delta AICc***	***AICc Weight***	***Model Likelihood***	***Parameters***
φ(.)pent(t)p(.)	219.27	0	0.32062	1	4
φ(t)pent(t)p(t)	219.85	0.58	0.24014	0.749	5
φ(.)pent(t)p(t)	219.85	0.58	0.24014	0.749	5
φ(t)pent(t)p(.)	220.22	0.95	0.19911	0.621	5
φ(.)pent(.)p(t)	70628.44	70409.17	0	0	5
φ(t)pent(.)p(.)	72191.57	71972.3	0	0	4
φ(.)pent(.)p(.)	72212.54	71993.27	0	0	3
φ(t)pent(.)p(t)	NONE	NONE	NONE	NONE	NONE

** φ, survival probability; p, capture probability; pent, recapture probability; (.), constancy; (t), temporal variation*

Table A.7 Ranking of POPAN models for site *Angelina 1*.

Model*	***AICc***	***Delta AICc***	***AICc Weight***	***Model Likelihood***	***Parameters***
φ(t)pent(t)p(.)	166.08	0	0.42486	1	3
φ(.)pent(t)p(t)	166.08	0	0.42486	1	3
φ(t)pent(t)p(t)	168.16	2.08	0.15027	0.3537	4
φ(.)pent(.)p(t)	59493.78	59327.7	0	0	2
φ(t)pent(.)p(.)	59510.56	59344.48	0	0	2
φ(t)pent(.)p(t)	59512.61	59346.54	0	0	3
φ(.)pent(t)p(.)	59535.05	59368.97	0	0	2
φ(.)pent(.)p(.)	59538.06	59371.98	0	0	3

** φ, survival probability; p, capture probability; pent, recapture probability; (.), constancy; (t), temporal variation*

Appendix B. Size Classes, by Year (Alternative)

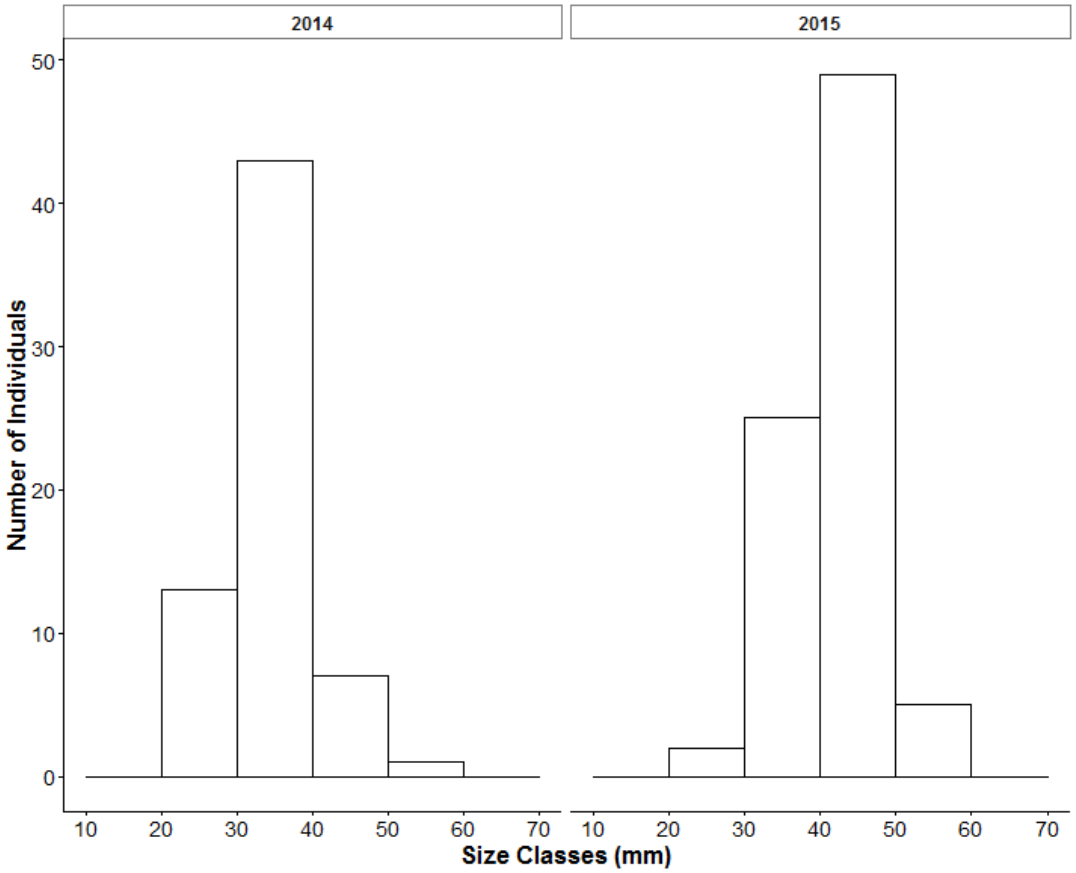

Figure B.1 Sizes classes for *Pleurobema riddellii* at *Neches 1* site.

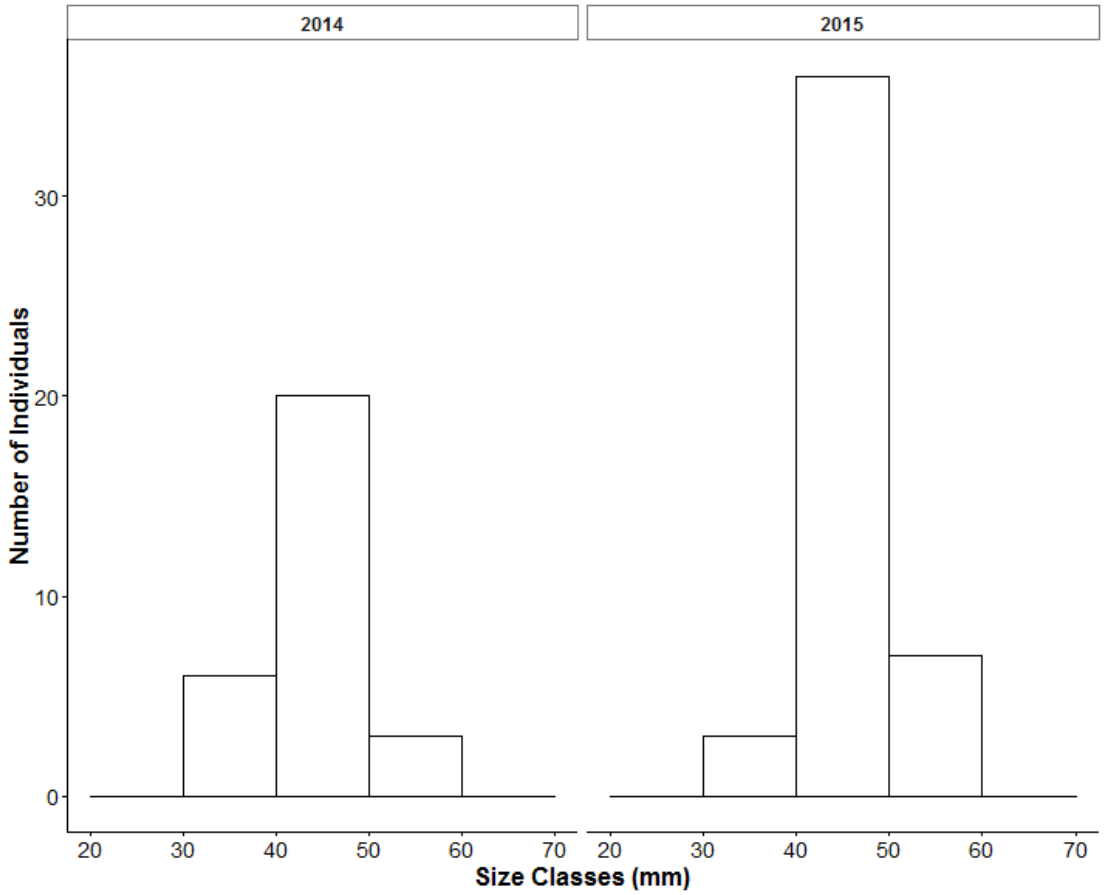

Figure B.2 Sizes classes for *Pleurobema riddellii* at *Neches 2* site.

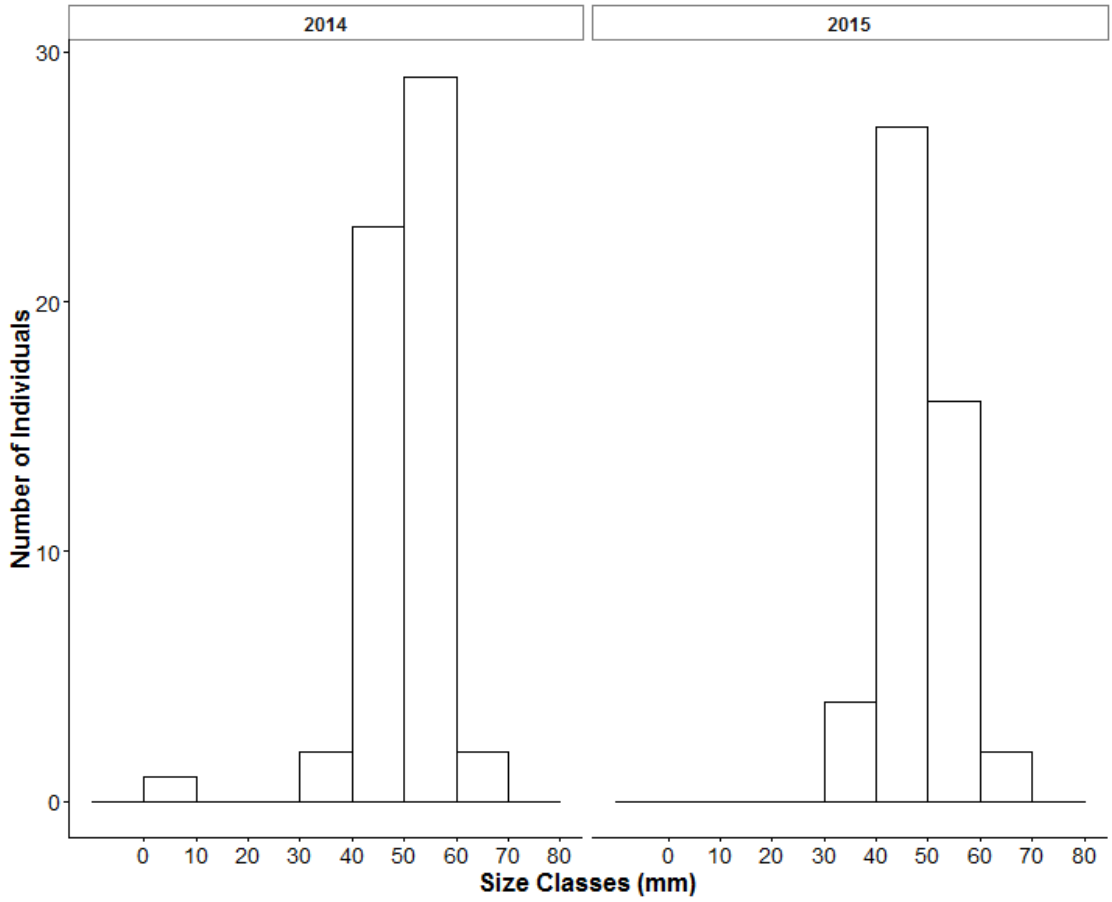

Figure B.3 Sizes classes for *Pleurobema riddellii* at *Neches 3* site.

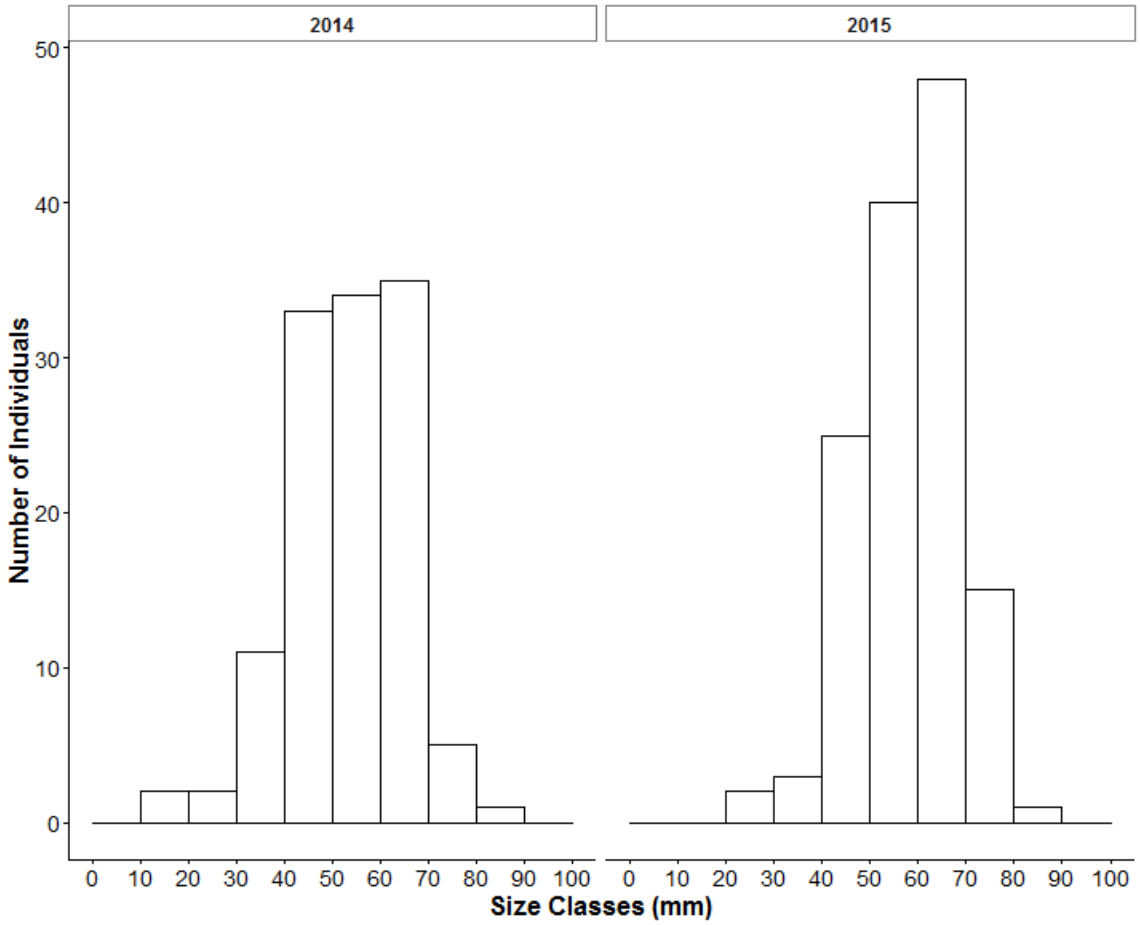

Figure B.4 Sizes classes for *Fusconaia askewi* at *Sabine 1* site.

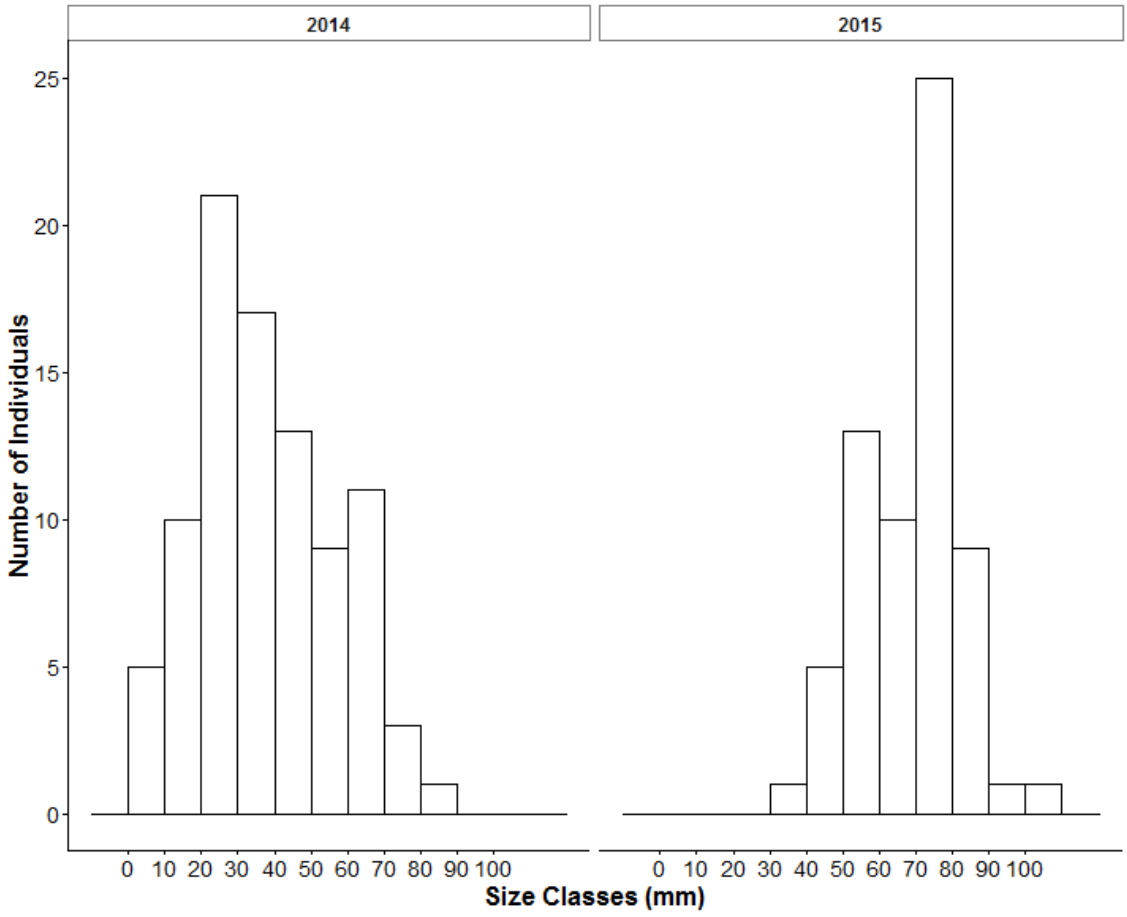

Figure B.5 Sizes classes for *Fusconaia askewi* at *Sabine 2* site.

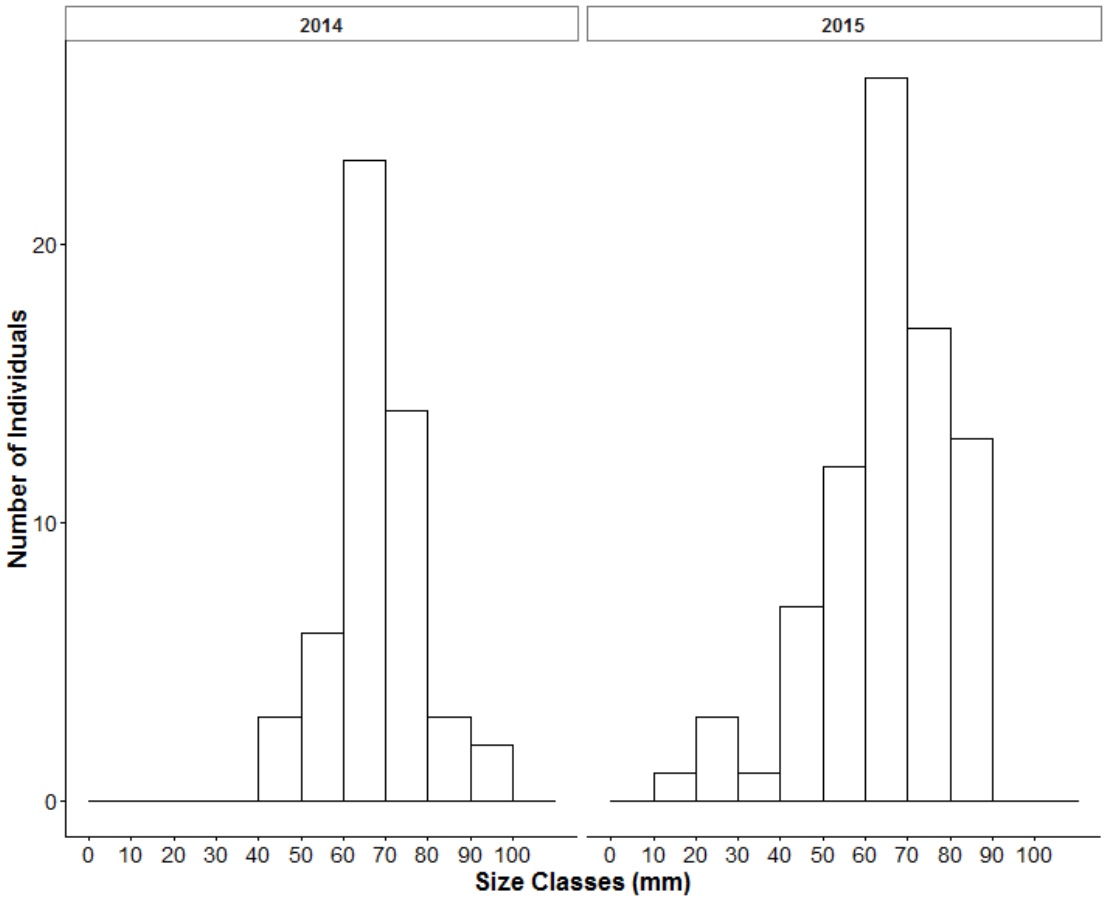

Figure B.6 Sizes classes for *Fusconaia askewi* at *Sabine 3* site.

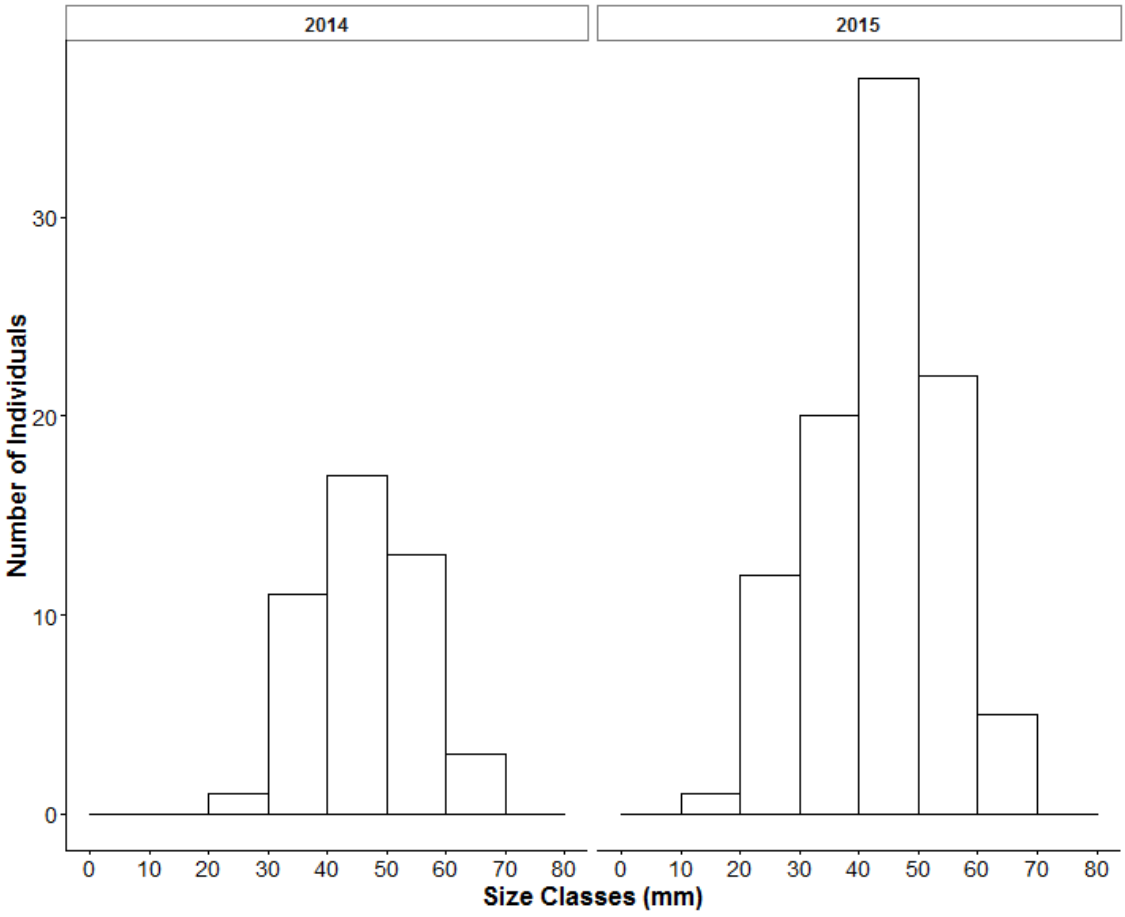

Figure B.7 Sizes classes for *Fusconaia askewi* at *Angelina 1* site.

Appendix C: Nucleotide alignment of the *ND1* gene

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Faskewi EP138           ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Faskewi EP134           ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Faskewi EP139           ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Faskewi EP136           ACATAACCTCCACACTTATTACATATCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Faskewi EP130           ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Flananensis EP146       ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Flananensis EP151       ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Flananensis EP107       ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Flananensis EP154       ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Flananensis EP152       ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Flananensis EP153       ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Flananensis EP147       ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Flananensis EP145       ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
Flananensis EP150       ACATAACCTCCACACTTATTACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
GENBANK Fflava          ACATAACCTCCACACTTATCACATACCTTCTAATCTTACTAGGCGTAGCATTCTTTACCC
AY613793

Faskewi EP138           TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Faskewi EP134           TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Faskewi EP139           TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Faskewi EP136           TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Faskewi EP130           TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Flananensis EP146       TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Flananensis EP151       TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Flananensis EP107       TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Flananensis EP154       TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Flananensis EP152       TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Flananensis EP153       TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Flananensis EP147       TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Flananensis EP145       TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
Flananensis EP150       TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
GENBANK Fflava          TTCTTGAACGCAAAGCTTTAGGGTACTTTCAAATCCGAAAAGGCCCAAACAAAGTTGGAA
AY613793
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Appendix C (Continued)

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Faskewi EP138           TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Faskewi EP134           TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Faskewi EP139           TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Faskewi EP136           TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Faskewi EP130           TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Flananensis EP146       TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Flananensis EP151       TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Flananensis EP107       TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Flananensis EP154       TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Flananensis EP152       TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Flananensis EP153       TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Flananensis EP147       TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Flananensis EP145       TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
Flananensis EP150       TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
GENBANK Fflava          TTATAGGAATCCCACAACCACTAGCAGACGCCCTAAAACTTTTTGTGAAAGAATGAGTAA
AY613793

Faskewi EP138           TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Faskewi EP134           TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Faskewi EP139           TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Faskewi EP136           TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Faskewi EP130           TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Flananensis EP146       TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Flananensis EP151       TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Flananensis EP107       TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Flananensis EP154       TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
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Flananensis EP153       TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Flananensis EP147       TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Flananensis EP145       TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
Flananensis EP150       TACCCACATCCTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
GENBANK Fflava          TACCCACATCTTCAAACTACTTACCATTTATTTTAACCCCAACAATCATATTAATTTTAG
AY613793
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Appendix C (Continued)

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Faskewi EP134           CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Faskewi EP139           CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Faskewi EP136           CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Faskewi EP130           CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Flananensis EP146       CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Flananensis EP151       CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Flananensis EP107       CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Flananensis EP154       CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Flananensis EP152       CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Flananensis EP153       CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Flananensis EP147       CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Flananensis EP145       CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
Flananensis EP150       CACTTAGGCTATGACAGCTATTCCCATCCTTTATACTCTCATTTCAAATAACCCTAGGAA
GENBANK Fflava          CACTTAGACTATGACAACTATTTCCATCCTTTATACTCTCATTTCAAATAGCCCTAGGAA
AY613793
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Faskewi EP138           TACTCCTATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Faskewi EP134           TACTCATATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Faskewi EP139           TACTCATATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Faskewi EP136           TACTCCTATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Faskewi EP130           TACTCCTATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Flananensis EP146       TACTCCTATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Flananensis EP151       TACTCCTATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Flananensis EP107       TACTCCTATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Flananensis EP154       TACTCCTATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Flananensis EP152       TACTCATATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Flananensis EP153       TACTCATATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Flananensis EP147       TACTCCTATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Flananensis EP145       TACTCATATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
Flananensis EP150       TACTCCTATTCTTATGTATTTCTTCTTTAACCGTCTACACAACCTTAATAGCAGGTTGGG
GENBANK Fflava          TACTCTTATTCTTATGTATCTCTTCCTTAACTGTCTATACAACCTTAATAGCAGGTTGGG
AY613793
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Appendix C (Continued)

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Faskewi EP138           CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Faskewi EP134           CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Faskewi EP139           CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Faskewi EP136           CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Faskewi EP130           CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Flananensis EP146       CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Flananensis EP151       CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Flananensis EP107       CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Flananensis EP154       CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Flananensis EP152       CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Flananensis EP153       CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Flananensis EP147       CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Flananensis EP145       CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
Flananensis EP150       CCTCAAACTCGAAGTATGCTCTACTAGGAGCCATTCGAGCCATGGCCCAAACCATCTCAT
GENBANK Fflava          CCTCAAACTCGAAATATGCTCTACTAGGGGCCATTCGAGCCATGGCCCAAACCATCTCAT
AY613793
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Faskewi EP138           ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Faskewi EP134           ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Faskewi EP139           ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Faskewi EP136           ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Faskewi EP130           ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Flananensis EP146       ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Flananensis EP151       ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Flananensis EP107       ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Flananensis EP154       ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Flananensis EP152       ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Flananensis EP153       ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Flananensis EP147       ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Flananensis EP145       ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
Flananensis EP150       ATGAAGTAACAATAACACTAATTATCATTTTCTACCTATTCTTGATTATACAAATAGACA
GENBANK Fflava          ATGAGGTAACAATAACACTAATTATCATCTTCTACCTATTCTTAATTATACAAATAGACA
AY613793
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Appendix C (Continued)

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Faskewi EP138           TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Faskewi EP134           TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Faskewi EP139           TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Faskewi EP136           TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Faskewi EP130           TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Flananensis EP146       TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Flananensis EP151       TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Flananensis EP107       TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Flananensis EP154       TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Flananensis EP152       TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Flananensis EP153       TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Flananensis EP147       TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Flananensis EP145       TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
Flananensis EP150       TAGTAACAATCCGCTCGGTTAACACCTCTATACCAACCTTTGCCCTCTCCGCACCATTAG
GENBANK Fflava          TAGTAACAATCCGCTCAGTTAACACCTCTATACCAGCCTTTGCCCTCTCCGCACCATTAG
AY613793
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Faskewi EP138           CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Faskewi EP134           CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Faskewi EP139           CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Faskewi EP136           CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Faskewi EP130           CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Flananensis EP146       CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Flananensis EP151       CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Flananensis EP107       CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Flananensis EP154       CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Flananensis EP152       CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Flananensis EP153       CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Flananensis EP147       CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Flananensis EP145       CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
Flananensis EP150       CCATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
GENBANK Fflava          CTATTATGTGAACTGTTGTCATCTTAGCAGAAACAAACCGAGCCCCATTTGACTTTGCTG
AY613793
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Appendix C (Continued)

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Faskewi EP138           AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Faskewi EP134           AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Faskewi EP139           AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Faskewi EP136           AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Faskewi EP130           AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Flananensis EP146       AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Flananensis EP151       AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Flananensis EP107       AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Flananensis EP154       AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Flananensis EP152       AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Flananensis EP153       AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Flananensis EP147       AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Flananensis EP145       AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
Flananensis EP150       AAGGGGAATCAGAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
GENBANK Fflava          AAGGAGAGTCASAACTAGTCTCTGGATTTAATATTGAGTACGGCGGAGCCGGCTTTGCTT
AY613793
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Faskewi EP138           TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Faskewi EP134           TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Faskewi EP139           TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Faskewi EP136           TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Faskewi EP130           TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Flananensis EP146       TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Flananensis EP151       TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Flananensis EP107       TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Flananensis EP154       TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Flananensis EP152       TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Flananensis EP153       TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Flananensis EP147       TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Flananensis EP145       TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
Flananensis EP150       TCCTCTTTATAGCCGAATACAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
GENBANK Fflava          TCCTCTTTATAGCCGAATATAGTAACATCTTAATAATAAGACTCCTTACTGCCTGTATAC
AY613793
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Appendix D: Nucleotide alignment of the *COX1* gene

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FaskewiGENBANK          CTTTATGATCTGGTTTGATTGGGTTGGCTTTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
JN180998
FaskewiGENBANK          CTTTATGATCTGGTTTGATTGGGTTGGCTTTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
KT285626
FlananensisEP107        CTTTATGATCTGGTTTGATTGGGTTGGCTTTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
FlananensisEP150        CTTTATGATCTGGTTTGATTGGGTTGGCTTTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
FlananensisEP151        CTTTATGATCTGGTTTGATTGGGTTGGCTTTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
FlananensisEP147        CTTTATGATCTGGTTTGATTGGGTTGGCTTTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
FlananensisEP106        CTTTATGATCTGGTTTGATTGGGTTGGCTTTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
FlananensisEP146        CTTTATGATCTGGTTTGATTGGGTTGGCTTTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
FlananensisEP145        CTTTATGATCTGGTTTGATTGGGTTGGCTTTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
FflavaGENBANK           CTTTATGATCTGGTTTGATTGGATTGGCTCTAAGTCTTTTGATTCGAGCTGAGTTAGGGC
KT285636


FaskewiGENBANK          AGCCAGGAAGGTTGTTGGGGGATGATCAGTTGTATAATGTGATTGTGACGGCGCATGCTT
JN180998
FaskewiGENBANK          AGCCAGGAAGGTTGTTGGGGGATGATCAGTTGTATAATGTGATTGTGACGGCGCATGCTT
KT285626
FlananensisEP107        AGCCAGGAAGGTTGTTGGGGGATGATCAGTTGTATAATGTGATTGTGACGGCGCATGCTT
FlananensisEP150        AGCCAGGAAGGTTGTTGGGGGATGATCAGTTGTATAATGTGATTGTGACGGCGCATGCTT
FlananensisEP151        AGCCAGGAAGGTTGTTGGGGGATGATCAGTTGTATAATGTGATTGTGACGGCGCATGCTT
FlananensisEP147        AGCCAGGAAGGTTGTTGGGGGATGATCAGTTGTATAATGTGATTGTGACGGCGCATGCTT
FlananensisEP106        AGCCAGGAAGGTTGTTGGGGGATGATCAGTTGTATAATGTGATTGTGACGGCGCATGCTT
FlananensisEP146        AGCCAGGAAGGTTGTTGGGGGATGATCAGTTGTATAATGTGATTGTGACGGCGCATGCTT
FlananensisEP145        AGCCAGGAAGGTTGTTGGGGGATGATCAGTTGTATAATGTGATTGTGACGGCGCATGCTT
FflavaGENBANK           AGCCCGGTAGGTTGTTGGGGGATGATCAATTGTATAATGTGATTGTGACGGCGCATGCTT
KT285636
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Appendix D (Continued)

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FaskewiGENBANK          TTATAATAATTTTCTTTTTGGTGATACCTATGATGATTGGAGGTTTTGGTAATTGGCTTA
JN180998
FaskewiGENBANK          TTATAATAATTTTCTTTTTGGTGATACCTATGATGATTGGTGGTTTTGGTAATTGGCTTA
KT285626
FlananensisEP107        TTATAATAATTTTCTTTTTGGTGATACCTATGATGATTGGAGGTTTTGGTAATTGGCTTA
FlananensisEP150        TTATAATAATTTTCTTTTTGGTGATACCTATGATGATTGGAGGTTTTGGTAATTGGCTTA
FlananensisEP151        TTATAATAATTTTCTTTTTGGTGATACCTATGATGATTGGAGGTTTTGGTAATTGGCTTA
FlananensisEP147        TTATAATAATTTTCTTTTTGGTGATACCTATGATGATTGGAGGTTTTGGTAATTGGCTTA
FlananensisEP106        TTATAATAATTTTCTTTTTGGTGATACCTATGATGATTGGAGGTTTTGGTAATTGGCTTA
FlananensisEP146        TTATAATAATTTTCTTTTTGGTGATACCTATGATGATTGGAGGTTTTGGTAATTGGCTTA
FlananensisEP145        TTATAATAATTTTCTTTTTGGTGATACCTATGATGATTGGAGGTTTTGGTAATTGGCTTA
FflavaGENBANK           TTATAATAATTTTCTTTTTGGTGATACCTATGATAATTGGTGGTTTTGGTAATTGGCTTA
KT285636


FaskewiGENBANK          TTCCTCTTATGATTGGGGCTCCGGATATGGCTTTTCCTCGATTAAATAATCTAAGGTTTT
JN180998
FaskewiGENBANK          TTCCTCTTATGATTGGGGCTCCGGATATGGCTTTTCCTCGATTAAATAATCTAAGGTTTT
KT285626
FlananensisEP107        TTCCTCTTATGATTGGGGCTCCGGATATGGCTTTTCCTCGATTAAATAATCTAAGGTTTT
FlananensisEP150        TTCCTCTTATGATTGGGGCTCCGGATATGGCTTTTCCTCGATTAAATAATCTAAGGTTTT
FlananensisEP151        TTCCTCTTATGATTGGGGCTCCGGATATGGCTTTTCCTCGATTAAATAATCTAAGGTTTT
FlananensisEP147        TTCCTCTTATGATTGGGGCTCCGGATATGGCTTTTCCTCGATTAAATAATCTAAGGTTTT
FlananensisEP106        TTCCTCTTATGATTGGGGCTCCGGATATGGCTTTTCCTCGATTAAATAATCTAAGGTTTT
FlananensisEP146        TTCCTCTTATGATTGGGGCTCCGGATATGGCTTTTCCTCGATTAAATAATCTAAGGTTTT
FlananensisEP145        TTCCTCTTATGATTGGGGCTCCGGATATGGCTTTTCCTCGGTTAAATAATCTAAGGTTTT
FflavaGENBANK           TTCCTCTTATGATTGGAGCTCCTGATATGGCTTTTCCTCGATTGAATAATTTGAGGTTTT
KT285636
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Appendix D (Continued)

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FaskewiGENBANK          GGT—TACTTGTGCCTGCTCTTTTTTTATTGCTAAGATCTTCTTTAGTGGAGAGGGGTGT
JN180998
FaskewiGENBANK          GGT—TACTTGTGCCTGCTCTTTTTTTATTGCTAAGATCTTCTTTGGTGGAGAGGGGTGT
KT285626
FlananensisEP107        GGT--TACTTGTGCCTGCTCTTTTTTTATTGCTAAGATCTTCTTTGGTGGAGAGGGGTGT
FlananensisEP150        GGT--TACTTGTGCCTGCTCTTTTTTTATTGCTAAGATCTTCTTTGGTGGAGAGGGGTGT
FlananensisEP151        GGT--TACTTGTGCCTGCTCTTTTTTTATTGCTAAGATCTTCTTTGGTGGAGAGGGGTGT
FlananensisEP147        GGT--TACTTGTGCCTGCTCTTTTTTTATTGCTAAGATCTTCTTTGGTGGAGAGGGGTGT
FlananensisEP106        GGT--TACTTGTGCCTGCTCTTTTTTTATTGCTAAGATCTTCTTTAGTGGAGAGGGGTGT
FlananensisEP146        GGT--TACTTGTGCCTGCTCTTTTTTTATTGCTAAGATCTTCTTTAGTGGAGAGGGGTGT
FlananensisEP145        GGT--TACTTGTGCCTGCTCTTTTTTTATTGCTAAGATCTTCTTTGGTGGAGAGGGGTGT
FflavaGENBANK           GGT—TACTTGTGCCTGCTCTTTTTTTGTTGTTAAGATCTTCTTTGGTGGAGAGGGGTGT
KT285636


FaskewiGENBANK          TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAATATTGCTCATTCTGGAGCTTC
JN180998
FaskewiGENBANK          TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAATATTGCTCATTCTGGAGCTTC
KT285626
FlananensisEP107        TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAATATTGCTCATTCTGGAGCTTC
FlananensisEP150        TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAATATTGCTCATTCTGGAGCTTC
FlananensisEP151        TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAATATTGCTCATTCTGGAGCTTC
FlananensisEP147        TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAATATTGCTCATTCTGGAGCTTC
FlananensisEP106        TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAATATTGCTCATTCTGGAGCTTC
FlananensisEP146        TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAATATTGCTCATTCTGGAGCTTC
FlananensisEP145        TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAATATTGCTCATTCTGGAGCTTC
FflavaGENBANK           TGGGACTGGTTGAACGGTTTATCCGCCGTTGTCTGGGAACATTGCTCATTCTGGAGCTTC
KT285636
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Appendix D (Continued)

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FaskewiGENBANK          AGTGGATTTGGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATTTTGGGGGCTAT
JN180998
FaskewiGENBANK          AGTGGATTTGGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATTTTGGGGGCTAT
KT285626
FlananensisEP107        AGTGGATTTGGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATTTTGGGGGCTAT
FlananensisEP150        AGTGGATTTGGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATTTTGGGGGCCAT
FlananensisEP151        AGTGGATTTGGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATTTTGGGGGCCAT
FlananensisEP147        AGTGGATTTGGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATTTTGGGGGCTAT
FlananensisEP106        AGTGGATTTGGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATTTTGGGGGCTAT
FlananensisEP146        AGTGGATTTGGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATTTTGGGGGCTAT
FlananensisEP145        AGTGGATTTGGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATTTTGGGGGCTAT
FflavaGENBANK           AGTGGATTTAGCTATTTTTTCTTTGCATCTTGCTGGTGCATCTTCTATCTTGGGGGCTAT
KT285636


FaskewiGENBANK          TAACTTTATTTCTACTGTAGGTAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
JN180998
FaskewiGENBANK          TAACTTTATTTCTACTGTAGGCAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
KT285626
FlananensisEP107        TAACTTTATTTCTACTGTAGGTAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
FlananensisEP150        TAACTTTATTTCTACTGTAGGTAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
FlananensisEP151        TAACTTTATTTCTACTGTAGGTAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
FlananensisEP147        TAACTTTATTTCTACTGTAGGTAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
FlananensisEP106        TAACTTTATTTCTACTGTAGGTAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
FlananensisEP146        TAACTTTATTTCTACTGTAGGTAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
FlananensisEP145        TAACTTTATTTCTACTGTAGGTAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
FflavaGENBANK           TAACTTTATTTCTACTGTGGGGAATATGCGGTCTCCAGGATTGGTTGCTGAGCGAATTCC
KT285636
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Appendix D (Continued)

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FaskewiGENBANK          GTTATTCGTGTGGGC---------------------------------------------
JN180998
FaskewiGENBANK          GTTATTCGTGTGGGCTGTAACGGTAACAGCGGTTTTGTTGGTTGCTGCGTTGCCTGTTTT
KT285626
FlananensisEP107        GTTATTCGTGTGGGCTGTAACGGTAACAGCGGTTTTGTTGGTTGCTGCGTTGCCTGTTTT
FlananensisEP150        GTTATTCGTGTGGGCTGTAACGGTAACAGCGGTTTTGTTGGTTGCTGCGTTGCCTGTTTT
FlananensisEP151        GTTATTCGTGTGGGCTGTAACGGTAACAGCGGTTTTGTTGGTTGCTGCGTTGCCTGTTTT
FlananensisEP147        GTTATTCGTGTGGGCTGTAACGGTAACAGCGGTTTTGTTGGTTGCTGCGTTGCCTGTTTT
FlananensisEP106        GTTATTCGTGTGGGCTGTAACGGTAACAGCGGTTTTGTTGGTTGCTGCGTTGCCTGTTTT
FlananensisEP146        GTTATTCGTGTGGGCTGTAACGGTAACAGCGGTTTTGTTGGTTGCTGCGTTGCCTGTTTT
FlananensisEP145        GTTATTCGTGTGGGCTGTAACGGTAACAGCGGTTTTGTTGGTTGCTGCGTTGCCTGTTTT
FflavaGENBANK           GTTGTTCGTGTGGGCTGTAACGGTAACGGCGGTTTTGTTGGTTGCTGCGTTGCCTGTTTT
KT285636


FaskewiGENBANK          ------------------------------------------------------------
JN180998
FaskewiGENBANK          AGCTGGTGCC-ATTACGATGTTGCTTACTGATCGTAATATTAACACGTCTTTTTTTGATC
KT285626
FlananensisEP107        AGCTGGTGCC-ATTACGATGTTGCTTACTGATCGTAATATTAATACGTCTTTTTTTGATC
FlananensisEP150        AGCTGGTGCC-ATTACGATGTTGCTTACTGATCGTAATATTAACACGTCTTTTTTTGATC
FlananensisEP151        AGCTGGTGCC-ATTACGATGTTGCTTACTGATCGTAATATTAACACGTCTTTTTTTGATC
FlananensisEP147        AGCTGGTGCC-ATTACGATGTTGCTTACTGATCGTAATATTAATACGTCTTTTTTTGATC
FlananensisEP106        AGCTGGTGCC-ATTACGATGTTGCTTACTGATCGTAATATTAACACGTCTTTTTTTGATC
FlananensisEP146        AGCTGGTGCC-ATTACGATGTTGCTTACTGATCGTAATATTAACACGTCTTTTTTTGATC
FlananensisEP145        AGCTGGTGCC-ATTACGATGTTGCTTACTGATCGTAATATTAACACGTCTTTTTTTGATC
FflavaGENBANK           AGCTGGTGCC-ATTACGATGTTGCTTACTGATCGTAATATTAACACGTCTTTTTTTGATC
KT285636
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Appendix E: Nucleotide alignment of the *16S* gene

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EP130                    AATGCCTGCCCAGTGAAAAACTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
EP136                    AATGCCTGCCCAGTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
EP138                    AATGCCTGCCCAGTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
EP139                    AATGCCTGCCCAGTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
EP140                    AATGCCTGCCCAGTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
EP145                    AATGCCTGCCCAGTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
EP150                    AATGCCTGCCCAGTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
EP151                    AATGCCTGCCCAGTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
EP152                    AATGCCTGCCCAGTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
EP153                    AATGCCTGCCCAGTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
GenBank_                ----------GTGAAAATTTTTAAACGGCCGCGTTAGCGTGAGCGTGCTAAGGTAGCG
AY238481_F_flava

EP130                    TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCTGTACCCT
EP136                    TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCTGTACCCT
EP138                    TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCTGTACCCT
EP139                    TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCTGTACCCT
EP140                    TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCAGTACCCT
EP145                    TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCTGTACCCT
EP150                    TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCTGTACCCT
EP151                    TTTTAAATAGCCTTTTAATTGGGGGCCAGTGAATGGCAAGACTAGGAATACCTGTACCCT
EP152                    TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCTGTACCCT
EP153                    TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCTGTACCCT
GenBank_                TAATAAATAGCCTTTTAATTGGAGGCCAGTGAATGGCAAGACTAGGAATACCT-TACCCT
AY238481_F_flava
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Appendix E (Continued)

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EP130                   TTATGAAAAAAAAACTTTTCACCTGAGTGAAAAGACTCAGATAGCAAAGGAAGACGAAAA
EP136                   TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGATAGCGAAGGAAGACGAAAA
EP138                   TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGATAGCGAAGGAAGACGAAAA
EP139                   TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGATAGCGAAGGAAGACGAAAA
EP140                   TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGATAGCGAAGGAAGACGAAAA
EP145                   TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGATAGCGAAGGAAGACGAAAA
EP150                   TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGATAGCGAAGGAAGACGAAAA
EP151                   TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGATAGCGAAGGAAGACGAAAA
EP152                   TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGATAGCGAAGGAAGACGAAAA
EP153                   TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGATAGCGAAGGAAGACGAAAA
GenBank_                TTATGAAAAAAAAACTTTTCATCTGAGTGAAAAGACTCAGA-AGCGAAGGAAGACGAAAA
AY238481_F_flava
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EP130                   GACCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCACAAACACAAAAGACAAAAGG
EP136                   GACCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCATAAACACAAAAGACAAAAGG
EP138                   GACCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCATAAACACAAAAGACAAAAGG
EP139                   GACCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCATAAACACAAAAGACAAAAGG
EP140                   GACCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCATAAACACAAAAGACAAAAGG
EP145                   GACCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCATAAACACAAAAGACAAAAGG
EP150                   GACCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCATAAACACAAAAGACAAAAGG
EP151                   GAGCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCTTAAACACAAAAGACAAAAGG
EP152                   GACCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCATAAACACAAAAGACAAAAGG
EP153                   GACCCCGCGGAACTTTACCTTTTCCAGCCTTAGCTGCCCATAAACACAAAAGACAAAAGG
GenBank_                GACCCCGCGGAACTTTACCTTTTCCAGCCCTAGCTGCCCACAAACACAAAAGGCAAAAGG
AY238481_F_flava
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Appendix E (Continued)

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EP130                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAATATTCAT
EP136                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAACATCCAT
EP138                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAACATCCAT
EP139                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAACATCCAT
EP140                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAACATCCAT
EP145                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAACATCCAT
EP150                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAACATCCAT
EP151                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAACATCCAT
EP152                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAACATCCAT
EP153                   TTTGATTGGGGCAATCTCGGAACAACCAAGCTTCCGATTCTCCTTAAAT-AAACATCCAT
GenBank_                TTTGAT-GGGGCAATCTCGGAACAACCAAGCTTCCGATTCTACTTAAGTGAAACATCCAT
AY238481_F_flava
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EP130                   AACCTGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
EP136                   AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
EP138                   AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
EP139                   AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
EP140                   AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
EP145                   AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
EP150                   AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
EP151                   AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
EP152                   AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
EP153                   AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATAACAGCGTAATCCAGCTCAAGAG
GenBank_                AACCCGATAAGGACAAAAAAGAAGTTACCCCGGGGATA-CAGCGTAATCCAGCTCAAGAG
AY238481_F_flava
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Appendix E (Continued)

```
EP130                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATTA
EP136                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATCA
EP138                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATCA
EP139                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATCA
EP140                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATCA
EP145                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATCA
EP150                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATCA
EP151                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATCA
EP152                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATCA
EP153                    -TACACATCGAAAGCTGGGTTTGCG-ACCTCGATGTTG--GCTTAAGGACATCCACATCA
GenBank_AY238481_F_flava CTACACATCGAAAGCTGGGTTTGCGCACCTCGATGTTGCCGCTTAAGGACATCCAC----
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Appendix F: Nucleotide alignment of the *ITS1* gene

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EP130                   GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP134                   GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP135                   GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP136                   GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP139                   GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP145                   GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP146                   GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP150                   GATCATTTCCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP152                   GATCATTTCCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP153                   GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP107                   GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
EP106                   GATCATTTCCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
Fusconaiaflava          GATCATTACCGAAAAGATTTAAAAAGCGCTGGCCGTTTTTTAGATCGAGAGACACAAAAG
ITS_DQ383442
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EP130                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP134                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP135                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP136                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP139                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP145                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP146                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP150                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP152                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP153                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP107                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
EP106                   CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
Fusconaiaflava          CGCATCCGGAAAACGAGAAAAAGACTGGGTTGCGGAGGTGGCTAGATCGCCTCCTTCCCG
ITS_DQ383442
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Appendix F (Continued)

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EP130                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP134                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP135                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP136                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP139                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP145                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP146                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP150                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP152                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP153                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP107                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
EP106                   GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
Fusconaiaflava          GTCTATAAACCTGTGTAGATCCATGGCCGCCGGTCGGGGGGTACCTAAGTCCGAAGTAGGC
ITS_DQ383442
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EP130                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP134                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP135                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP136                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP139                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP145                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP146                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP150                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP152                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP153                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP107                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
EP106                   CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
Fusconaiaflava          CCGCAATGCTTCAAGCGGGGCATGTTCGACGAGAGCTTTGGTACAGCGATGGTTTAAAGA
ITS_DQ383442
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Appendix F (Continued)

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EP130                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP134                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP135                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP136                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP139                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP145                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP146                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP150                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP152                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP153                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP107                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
EP106                   GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
Fusconaiaflava          GAGAGCTTCCAGCTACGCCCCCGCCACACATTCTTTTCCGCCCCGTGCGGTTTTCTCTTT
ITS_DQ383442
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EP130                   GCGTTTGACACTTGTAACCAAAAGTCGACGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP134                   GCGTTTGACACTTGTAACCAAAAGTCGACGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP135                   GCGTTTGACACTTGTAACCAAAAGACGGCGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP136                   GCGTTTGACACTTGTAACCAAAAGACGGCGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP139                   GCGTTTGACACTTGTAACCAAAAGTCGACGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP145                   GCGTTTGACACTTGTAACCAAAAGTCGACGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP146                   GCGTTTGACACTTGTAACCAAAAGTCGACGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP150                   GCGTTTGACACTTGTAACCAAAAGACGGCGGCTCGATTTGGCCCGGTTGGCTCCGTTTCT
EP152                   GCGTTTGACACTTGTAACCAAAAGACGGCGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP153                   GCGTTTGACACTTGTAACCAAAAGTCGACGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP107                   GCGTTTGACACTTGTAACCAAAAGACGGCGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
EP106                   GCGTTTGACACTTGTAACCAAAAGACGGCGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
Fusconaiaflava          GCGTTTGACACTTGTAACCAAAAGTCGACGGCTCGATTTGGCCAGGTTGGCTCCGTTTCT
ITS_DQ383442
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Appendix F (Continued)

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EP130                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP134                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP135                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP136                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP139                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP145                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP146                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP150                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP152                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP153                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP107                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
EP106                   CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
Fusconaiaflava          CTTGTCCATTTTGGGCCCCGTCGATGCAAGGGATCGATCCGTCTCGTTCGTTGCCGGACG
ITS_DQ383442
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EP130                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP134                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP135                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP136                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP139                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP145                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP146                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP150                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP152                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP153                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP107                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
EP106                   GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
Fusconaiaflava          GCCAATCGATCCTAATCCTTGCAAGCTCCCAGCTCGGGGGGGCGAGAAAGGAAATTACTCT
ITS_DQ383442
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