MORPHOLOGICAL CHARACTERIZATION AND GENETIC DIVERSITY OF ECUADORIAN CAPULI *PRUNUS SEROTINA* SUBSP. *CAPULI*

by

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(Under the Direction of Dario Chavez)

ABSTRACT

Found in Ecuador and Mexico, *Prunus serotina* subsp. *capuli*, commonly called capuli, has important medicinal properties, its fruit is used for human consumption, and it is commonly known due to its high-quality timber used to build high-quality furniture and instruments. No commercial varieties of capuli are available in the world. In Ecuador, all fruit and timber are harvested from wild stands of this subspecies. Few studies have characterized these subspecies. The main goal of this research was to characterize *P. serotina* subsp. *capuli* morphological and genetic diversity. Significant variation was reported among different variables related to capuli leaves, flowers, branches, fruit, and endocarp in this study. Its genome size and ploidy level was confirmed to be tetraploid across its geographical range in Ecuador. This research is a foundation step directed towards selection of valuable capuli genotypes to initiate a breeding program for commercial capuli production in the U.S.A. and Ecuador.

INDEX WORDS: capuli, genetic diversity, ploidy level, morphological characteristics

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B.Sc., Punjab Agricultural University, India, 2018

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2021

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May 2021

ACKNOWLEDGEMENTS

First, I want to thank God for everything in my life and all the blessings. Also, I would like to thank my parents, sisters, brothers and Dinesh for encouraging and always supporting me.

I would like to thank specially, Dr. Dario J. Chavez for providing me with this opportunity to study in the UGA and complete my master's degree under his guidance. This work would not have been possible without his support, guidance, and trust in me. I would also like to thank our collaborators from ESPOCH University, Ecuador for all the work and data collection.

I would also like to thank my committee members: Dr. Rachel Itle, Dr. Carol Robacker and Dr. Jason Wallace for agreeing to be part of this project and for giving the required feedback and direction. I would like to also like to thank my friends in Athens and Griffin for their constant support and help. I would also like to acknowledge help of my lab members and friends in the graduate school for their constant motivation and help.

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CHAPTER 1

LITERATURE REVIEW OF *PRUNUS SEROTINA* SUBSP. *CAPULI*

Introduction

Prunus serotina (Black cherry) is the largest tree among all the native cherries growing in the U.S. This species can be found in North America (Hough, 1960). In 1788, Ehrhart named Black cherry as *P. serotina*. Black cherry is widely distributed in eastern and southern U.S., eastern Canada from New Brunswick to Quebec, and Central and South America (Hough, 1960; Marquis, 1990). Other common names are wild black cherry, rum cherry, mountain black cherry (Marquis, 1990).

Black cherry wood is used to build scientific instruments, handles and also high-quality caskets (Handbook, 1955). In North America, black cherry is found growing as a wild species in secondary forests, hedgerows, and logging sites. Its fruit are small, nonfleshy, astringent and with no commercial value (McVaugh, 1951). The fruits are used for making jellies, preservatives, pies, and wines (Marquis, 1990). The wood is used for timber production, and the bark is used for treating coughs (Marquis, 1990). This species was also introduced in Central Europe in the 17th century as an ornamental species but now it has been considered as invasive species in Western Europe (Camenen et al., 2016; Segura et al., 2018; Starfinger et al., 2003).

Prunus serotina has been classified into five subspecies: *P. serotina* subsp. *serotina*, *P. serotina* subsp. *hirsutus*, *P. serotina* subsp. *capuli*, *P. serotina* subsp. *eximia,* and *P. serotina* subsp. *virens* (McVaugh, 1951). The subspecies classification was based on individual geographical distribution and morphological characteristics. Among the morphological

characteristics, leaf shape and size, degree of pubescence, teeth frequency on the leaf margins, and size of inflorescence, and other floral parts have been used (McVaugh, 1951). *Prunus serotina* subsp. *serotina* is present in central and eastern U.S.A., Sierra Madre Oriental mountain range and west central and central Mexico making it the most widely distributed subspecies. On the other hand, *P.serotina* subsp. *eximia* is limited in distribution and indiginous to the Edwards Plateau in Central Texas. *P.serotina* subsp. *hirsuta* is found growing in states like Alabama, Georgia, and Florida. *Prunus serotina* subsp. *virens* is further divided into *P.serotina* ssp. *virens* var. *virens* and *P.serotina* ssp. *virens* var *rufula.* Both varieties are present in New Mexico, Arizona, Texas and Mexican states like Jalisco, Guanajuato, Durango, Sonora and Chihuahua (McVaugh, 1951; Segura et al., 2018). On the other hand, *P.serotina* subsp. *capuli* is endemic to Central and South America (Fresnedo-Ramírez et al., 2011).

Prunus serotina subsp. *capuli* is a domesticated tree species also known as "capuli". This subspecies is also sometimes referred as *Prunus salicifolia* or *Cerasus longifolia* in literature (McVaugh, 1951). The capuli was always an important fruit in Central Mexico even before Spanish arrival. In Ecuador, before Spanish coming to South America, people of the province of Cañar used to worship capuli plants, according to Historian Gonzales Suarez (Popenoe and Pachano, 1923). The earliest records of this tree in Central and South America were provided by Francisco Hernandez, a Public Health Official to the King of Spain, who was sent to Mexico to study useful medicinal plants growing there (Popenoe and Pachano, 1923). Capuli is also known by other names like capulin, tup (Guatemala), sabana (Columbia) and cerezo (Spanish name) in South America. The tree is known to be present in countries like Mexico, Guatemala, Peru, Colombia, and Ecuador.

In Ecuador, the tree is widespread in the Andes region where the best forms of capuli fruit can be found (Popenoe and Pachano, 1923). The trees grow from the province of Carchi to the North to the province of Loja to the South. It can be commonly found in the woods, pastures, roadsides, and backyards of houses (McVaugh, 1951). The capuli fruits in Ecuador are not only larger in size but also very juicy and tasty. They have a pinch of bitterness derived from the skin and are used for human consumption. The fruit plays a significant role in the community as it can be found in local dishes (Popenoe and Pachano, 1923). "Jucho" is a traditional Ecuadorian dessert made from capuli and peaches (Vasco et al., 2009). They are sold fresh in the agricultural markets, but plants are not cultivated in large numbers. Also, wood from the capuli tree is used for making furniture (Popenoe and Pachano, 1923).

Morphological characteristics of *P. serotina* subsp. *capuli*

Natural variation is found within plants for their form and structure. Plant morphology is the study of development, form and structure of plants (Raven et al., 2005). The morphological characterization of different plant parts (trunk, branches, leaves, flowers, fruit, etc.) of a representative group of individuals within and across species constitute an important technique to understand the diversity of horticultural crops (Blazek, 2004).

Prunus serotina is classified into 5 subspecies (McVaugh, 1951). The classification is based on the leaves, flowers and fruits characteristics (Fresnedo-Ramírez et al., 2011). The flowers of *P. serotina* are white, perfect and insect pollinated. The flowers are borne on racemes (Marquis, 1990). The leaves of *P. serotina* subsp. *serotina* are 4 cm wide and 9 cm long. Flowers have 4.5 mm long pedicel with around 35 flowers on the raceme. *P. serotina* subsp. *hirsuta* raceme has around 45 flowers with 2.5-5 mm long pedicel. The leaves are 4.5 cm wide and 8.5 cm long. *Prunus serotina* subsp. *virens* has leaves 2-3 cm wide and 4.5- 7 cm long. Raceme is

10 cm long. The flowers pedicels are 3.5 mm long and there are 30 flowers in the raceme. *Prunus serotina* subsp. *eximia* leaves are 3-4.5 cm in width and 7-9 cm in length. The petiole length is 1.5-2 cm. The flowering branch in subspecies *eximia* is about 12 cm long and has 40 flowers. *Prunus serotina* subsp. *capuli* has a 15 cm long raceme with around 35 flowers. The pedicel is 4 mm long. The leaves are around 2.5-4 cm in width and 8-12 cm long (McVaugh, 1951).

Prunus serotina seedlings have a taproot system and reach a height of 5 to 10 cm within one month of germination. They grow very poorly in low light conditions and are sensitive to competition. Also, average annual growth diameter of black cherry aged 10-40 years is 0.65 cm (Marquis, 1990). Popenoe and Pachano (1923) described Ecuadorian capuli fruits as light maroon to deep purplish in color, depressed- globose in form and with pale brownish flesh and thin skin with sweet, very juicy flavor. The diameter of the fruit can be as long as 2.54 cm. On the other hand, according to McVaugh (1951), fruit diameter can be up to 2-2.5 cm. Vasco et al. (2008) reported Ecuadorian capuli to be 10-30 mm in diameter.

Bonner (1975) studied the physical and chemical characteristics of black cherry fruits growing in Central Mississippi, USA. Fruits were collected every 2 weeks until late July for 3 years. Fruit production was larger in first year and decreased in the later years. Moisture content was high in May and then it decreased in early June and then again it increased as the mesocarps become succulent. The average fruit diameter ranged from 7-10 mm in 3 years. Maturity of the fruits was indicated by change in color from green to dark red to dark purple. The level of crude fat, protein – nitrogen and calcium content started to increase in early June after a decrease in early May and then again decreased in late June. Carbohydrates constituted 20.8%, proteins 7.8% and crude fat only 4.9% of the dry weight of the fruit.

Malusà and Romisondo (1992) studied fruit characteristics such as weight and diameter, coupled with trunk diameter and brix of five-year-old open pollinated seedlings of capuli growing in Italy. Trunk diameter varied from 6.4 cm to 85.9 cm with the mean of 45.9 cm. They studied the fruit characteristics differently for fruits to be used as table purpose and processing purpose. The fruit weight varied from 0.8 g to 2.3 g for table use fruits and 0.9 to 2.8 g for processing purpose. The fruit diameter ranged from 10.5-17 mm for table and 10.5-16.5 mm for processing purpose. Similarly, soluble solids range for processing purpose fruits (16-27.5%) were higher than those of table purpose (13-21%).

Avendaño-Gómez et al. (2015) studied the morphological variation found in capuli trees growing in Tlaxcala, México. The average length of the fruit was 1.74 ± 0.18 cm and average diameter was 1.54 ± 0.22 cm. Endocarp thickness was 0.14 ± 0.04 mm, seed length 0.94 ± 0.08 cm and seed diameter to be 0.78 ± 0.13 cm. Raceme length was found to be around 9.66 ± 2.36 cm and approx. 30 flowers were reported per raceme in cultivated forms of capuli.

Fresnedo-Ramírez et al. (2011) studied 22 quantitative and 17 qualitative morphological characteristics in seven *P. serotina* populations collected from four states of Mexico. Two groups representing central and western forms were distinguished for capulin based on the geography of the regions. The differentiating morphological differences were productivity, internode length in young stems, thickness of young stems, fruit diameter, stamen length, petiole length of second basal leaf of flowering branch, seed diameter, etc.

Guzmán F.A. et al. (2018) studied morphological variation related to the distribution of the *P. serotina* subspecies present in USA and México. Low morphological variation related to climate was observed. Characteristics affected by climate change include variation in stamen length, fruit pedicel, and second floral leaf. Subspecies *eximia*, *hirsuta*, and *serotina* are found in

cold and humid environment. Subspecies *virens* can be found in dry environment. On the other hand, subspecies *capuli* was present in humid climate and had no specific pattern of distribution.

Nutraceutical properties of *P. serotina* subsp. *capuli*

Capuli fruits are an important part of the diet of communities where these species are found. It is an important fruit due to its large size, taste, and nutraceutical value. Roasted seeds of capuli are used as snacks along with the consumption of fresh or dried fruit, or marmalade form in Mexico (Ordaz-Galindo et al., 1999). Tea and syrups made from capuli leaves are used in treatment of hypertension, stomach problems, diarrhea, cold and malaria, and cough (Martínez, 1959). The seeds are also used in making concentrated liquor for treatment of cough. In Ecuador, capuli fruit are eaten fresh or preserved. The bark of the black cherry is also used by the indigenous people from Canada boreal forests for treatment of diabetes related symptoms (McCune and Johns, 2007).

Vasco et al. (2008) analyzed the total phenolic and antioxidants values for 17 major fruits available in Ecuador including capuli. Capuli fruit is eaten whole or peeled in Ecuador. Capuli's peel and pulp were analyzed separately in the experiment. The Folin- Ciocalteu method was used for the total phenolic compounds; samples were classified from the highest to lowest total soluble phenol content. Capuli cherry peel was placed in the group with the highest phenols (1494 mg GAE/100g FW) along with the Andean blackberry (2167 mg GAE/100 g FW) and Banana passion fruit (1010 mg GAE/100 g FW), whereas capuli pulp had medium phenolic content of 331 mg GAE/100g FW. Among all 17 fruits, capuli peel was found to have the highest antioxidant capacity.

Vasco et al. (2009) studied the phenolic compound content of capuli fruit from Ecuador using HPLC. The compounds present were (-)-Epicatechin and (+)-catechin, both quercetin

glycoside compounds. Cyanidin-3-O-glucoside and Cyanidin-3-O-rutinoside were the most important anthocyanins found.

Luna-Vazquez et al. (2013) studied the nutraceutical values of capuli from Mexico. It was found that capuli fruit had four times higher protein values than plums and grape. Moreover, protein levels in capuli fruit were higher than the protein content reported in the literature for plum, apricot, peach, and grapes (US Department of Agriculture, 2016). Also, important nutrients like Na, K, Ca, Mg and P found in capuli were significantly higher than found in plum and grape. Total phenolic content and flavonoids for capuli peel were larger than that of its flesh. Overall, capuli fruit has higher phenol and flavonoid content than plum and grape. Also, antioxidant capacity was higher for capuli than plum and grape. Luna-Vazquez et al. (2013) found that whole fruit and peel extracts of capuli showed significant smooth muscle relaxation on rat aorta and reduction of the systolic blood pressure in hypertensive rats. The antihypertensive and vasodilator properties could be due to collective effect of phenolic compounds like chlorogenic acid, cyanidin-3-O-rutinoside, proanthocyanidins and quercetine glycosides present in the capuli fruit extract.

Antioxidant capacity of capuli at different stages of ripening was explained by Guerra-Ramìrez et al. (2019). Fruit growth analysis was also done at 0, 30, 45, 60, 75, 90 and 97 days after anthesis (DAA). They concluded from the experiment that 93 DAA is the best stage for harvesting capuli fruit as flavonoids and phenols are at maximum level in the fruit.

Aguerrebere et al. (2011) studied the characteristics of *P. serotina* seed oil. The oil is abundant in oleic acid (35%), α-elostearic acid (27%), linoleic acid (27%), palmitic acid (4%), stearic acid (4%) and β-elostearic acid (1%). The presence of α-elostearic acid confers important therapeutic properties to the oil as presence of this acid has been associated with suppression of

growth in cancer cells and decrease in the levels of serum lipids in mammals. It has also been proposed as chemotherapeutic agent against breast cancer (Koba et al., 2002; Moon et al., 2010).

Garcia-Aguilar et al. (2015) studied the nutritional and volatile compounds in black cherry seeds. The protein content in the raw (37.95%) and toasted (36.55%) black cherry seeds was found to be higher than almonds and peanuts. The fat content in the raw (40.37%) and toasted seeds (39.97%) was found to be lower than almonds (49.64%), but not significantly different than peanuts (41.12%). Black cherry seed oil is rich in oleic (35%), linoleic (27%) and α-eleostearic acid (27%) (Aguerrebere et al., 2011). Oleic and linoleic acids are the main oils present in almonds, while oleic acid is the most abundant one in peanuts (Venkatachalam and Sathe, 2006). Also, black cherry seeds have higher content of Ca, Fe, Mg, K and Na than peanuts and Fe, K and Na content is higher than almonds as well. K content in black cherry is higher than both peanuts and almonds suggesting that it can be a good complementary source for human consumption. The main volatile compounds found in black cherry seeds were benzaldeyde and 2,3-butanediol in raw and toasted seeds. These are responsible for flavor and odor of toasted seeds. Benzaldehyde is also present in the black cherry leaves and has an important vasodilator effect, suggesting an important use of capuli seeds for beneficial effects on the cardiovascular system (Ibarra-Alvarado et al., 2009).

Genome size and genetic diversity in *P. serotina*

Genome size

The DNA content of the haploid chromosome complement of an organism is called the C value (Swift, 1950). Knowledge of the genome size and ploidy level of plants is important for carrying out population level studies (Fay et al., 2005). Chromosome counting in a cell is the clear way of determination of the ploidy level for an organism, but the process can be very time

consuming and difficult (Stebbins, 1971). The nuclear DNA content correlates with the ploidy level of the organism therefore, indirect estimation of ploidy level is provided by flow cytometric measurements which are based on the detection of fluorescent signals from stained particles in a liquid suspension (Dolezel et al., 2007; Suda and Travnicek, 2006).

Considerable variation for ploidy level is present in the genus *Prunus*. The basic chromosome number is x = 8 (Darlington, 1928). The chromosome number of *P. serotina* was previously reported to be 2n = 32 by Kobel (1927) and Stairs and Hauck (1968). Sweet cherry and peach are diploid species (2n = 2x = 16), whereas sour cherries and black cherries are tetraploid (2n = 4x = 32) in nature (Stairs and Hauck, 1968). The 2C value for sweet cherry was found to be 0.67 pg, significantly less than the *P. serotina* (1.0 pg) (Dickson et al., 1992). However, tetraploid, pentaploid and hexaploid individuals were reported for *P. serotina*, which established an uncertainty about the true *P. serotina* ploidy level. Pairon and Jacquemart (2005) favored the allotetraploid origin of the black cherry by studying disomic inheritance of six microsatellite markers in black cherry. Guzmán F. et al. (2018) confirmed the genomic size of *P. serotina* subsp. *capuli* to be 1.0 pg and tetraploid while studying the incompatibility of capuli as a rootstock for sweet cherry.

SSRs and genetic diversity

There are three genomes present in the eukaryotic cells: chloroplast, mitochondrial and nuclear DNA. The chloroplast DNA consist of genic DNA and it is between 135 to 160 kb in size (Clegg, 1993; Sato et al., 1999). The mitochondrial DNA in plants consist of genic DNA and introns with a size of 370 to 490 kb (Kubo et al., 2000; Notsu et al., 2002; Unseld et al., 1997). The nuclear genomic DNA consists of a large number of non-genic DNA made of repeating units with small number of genes scattered around in the genome (Doolittle and Sapienza, 1980;

Graur and Li, 2000; Orgel and Crick, 1980). Repeated DNA elements take up the major space in the eukaryotic genome and can be interspersed (present at multiple locations) or tandem (present at fewer locations) (Weising et al., 2005).

Tandem repetitive DNA is divided into: Satellite DNA, minisatellites and microsatellites. Satellite DNA is present in the subtelomeric or centromeric region in the chromosome and is made of a very large number of repetitions of a DNA sequence (commonly 100-300bp long). Minisatellites consists of 10-60 bp of medium- sized long DNA sequences (Weising et al., 2005). Microsatellites are made of very short DNA sequences (1-6 bp) with a lesser rate of repetition at a locus. They are also called simple sequences, simple repetitive sequences (SRS), simple sequence repeats (SSRs) and simple tandem repeats (STRs) (Litt and Luty, 1989; Tautzl and Renz, 1984; Weising et al., 2005). SSRs are popularly used molecular markers because they are abundant, codominant in nature, highly polymorphic and easy to detect (Holton, 2001).

SSRs can be used in the DNA fingerprinting of the same species and also in other closely related species or species of same genus. This is known as the transferability. This can be used in the diversity analysis and DNA fingerprinting (Hendre et al., 2008; Peakall et al., 1998; Zhang et al., 2005). Synteny is present in the case of *Prunus* and other species within the Rosaceous family (Dirlewanger et al., 2004). Peach SSR markers are transferable to other Prunus species like cherry (Dirlewanger et al., 2002; Wunsch and Hormaza, 2002), apricot (Hormaza, 2002), etc. The use of SSRs markers across different *Prunus* species helps in construction of linkage maps and performing genetic diversity studies (Mnejja et al., 2010).

Downey and Iezzoni (2000) used markers developed from peach (*P. persica*), sweet cherry (*P. avium*) and sour cherry (*P. cerasus*) to study the genetic diversity of black cherry accessions from Michigan, México and Ecuador. A chloroplast primer pair from sour cherry and

four primer pairs out of eight were amplified which identified 54 alleles among the accessions of black cherry. Similarly, Pairon and Jacquemart (2005) used eight SSR primer pairs, four were previously used by Downey and Iezzoni (2000) and the other four were developed from a peach fruit cDNA library by Yamamoto et al. (2002) to study the presence of disomic inheritance of SSR markers in *P. serotina* using controlled crosses to support their allopolyploid nature.

Pairon et al. (2008) determined genome specific primers in *Prunus serotina*, where 67 microsatellites primers from cultivated *Prunus* species were tested. The five genome specific primers from *P. avium* and *P. persica*, showing diploid inheritance in the controlled crosses, were selected. Beck et al. (2014) determined the genetic variation present in populations of *P. serotina* subsp. *serotina* in Kansas and its limits by using 5 SSRs markers. The authors reported a reduced genetic variability in the edge populations as compared to the interior ones. Intriago-Baldeón et al. (2013) studied the genetic diversity of *P. serotina* subsp. *capuli* from 3 provinces of Ecuador: Pichincha, Cañar and Azuay, using 8 SSRs markers. A total of 49 alleles were found for the 88 individuals used in this study. Samples from the northern province of Pichincha and the southern provinces of Cañar and Azuay were different from each other based on these genetic analyses. Biological factors such as seed dispersal by birds and mammals, and seed spread due to regional trade and garden sowing, was proposed as a reason for this differentiation between populations. Furthermore, the authors proposed a more detailed study using capuli individuals from all the provinces of Ecuador to better understand capuli's genetic diversity.

In 2015, Guadalupe et al. (2015) conducted a study to evaluate the genetic diversity and population structure of the *P. serotina* subsp. *capuli* found in eight provinces of Ecuador using twelve SSR markers derived from peach, sweet cherry, and sour cherry. The study revealed that there is a moderate degree of diversity among the Ecuadorian capuli. However, the degree of

allelic richness present in the Ecuadorian capuli is less than *P. serotina* in N. American populations. Also, it was observed that the degree of genetic diversity within each province was reduced. However, the use of a limited number of SSR markers needs to be addressed in further experiments.

In México, Guzman et al. (2018) conducted a study on 18 natural populations of black cherry representing subsp. *capuli*, *eximia*, *serotina* and *virens* from Mexico and Texas, U.S. using 16 SSR markers derived from different *Prunus* spp. A total of 246 alleles were detected. Less than 5% of the unique private alleles were present in the population suggesting gene flow among the species. This study demonstrated that there is adequate genetic diversity among the two groups (Texas and Mexican). Furthermore, the genetic diversity results can be used for the identification of the accessions needed for tree conservation.

Genetic diversity studies in other *Prunus* species

Aranzana M. J. et al. (2010) genotyped 224 peach cultivars from America, Europe and Spain using 50 SSRs markers covering the whole peach genome. Genetic variability was calculated and a population structure analyses identified 3 main groups: melting peaches, non-melting peaches and melting nectarines. A total of 6.36 alleles per locus were identified and 2.08 was the average effective number of alleles. Chavez et al. (2014) also studied genetic diversity and population structure of 195 peach genotypes consisting of University of Florida cultivars and advanced selections, landraces, high chilling cultivars and other *Prunus* species using 36 SSR markers. Genotypes were grouped acc. to melting and non-melting flesh characteristics. A total of 423 alleles were amplified.

Ercisli et al. (2011) studied the genetic diversity of wild sweet cherry accessions growing in Turkey using 10 SSR primers from plum, apricot, peach and sweet cherry. A total of 46 alleles

were amplified with an average number of alleles per primer of 4.6. Seven groups were generated using these markers. The genetic diversity study showing high genetic diversity has potential for its use in future cherry breeding programs. Hormaza (2002) studied genetic diversity of 48 genotypes from different regions with 37 SSR primers of different *Prunus* species. 31 primers showed amplification and 82 alleles were detected in total. The genotypes were also classified into two main groups by UPGMA cluster analysis based on their geographical origin.

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CHAPTER 2

FRUIT CHARACTERIZATION OF *PRUNUS SEROTINA* SUBSP. *CAPULI*[1]

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Abstract

Prunus serotina (black cherry) is native to N. America. The tree is popularly used for making furniture as wood is hard and durable. *P. serotina* fruits in N. America are small, taste bitter and not suitable for human consumption. *P. serotina* is divided into five subspecies. *P. serotina* subsp. *capuli* (capuli) is one of the unique subsp. which is native to Central and South America. The fruits are big, sweet and consumed by the people in various forms. The Ecuadorian Andes region provides the best forms of the capuli. The objective of this study was to characterize a collection of different genotypes of capuli's fruit and endocarp found growing in the wild in Chimborazo, Tungurahua, and Cotopaxi provinces of Ecuador. Significant variability was found for the different fruit and endocarp characteristics studied. Multivariate analysis showed that all fruit and endocarp size characteristics except brix were positively correlated with each other within a season. No clear population differentiation was seen among the genotypes from different provinces when Principal Component Analysis was done suggesting lack of clear morphological differentiation.

Introduction

Prunus serotina, commonly known as black cherry, is native to the United States (Hough, 1960). It is commercially important due to its high-quality timber used for making scientific and professional instrument boxes, furniture and cabinets (Cassens, 2007). *Prunus serotina* tree grows in cool moist temperate climates with optimum and evenly dispersed rainfall (Hough, 1960). *Prunus serotina* is further classified into five different subspecies: *P. serotina* subsp. *serotina*, *P. serotina* subsp. *virens, P. serotina* subsp. *hirsuta*, *P. serotina* subsp. *eximia,* and *P. serotina* subsp. *capuli* (McVaugh, 1951). These subspecies have a broad distribution range throughout North, Central and South America, each occupying distinct geographical regions and

each having different morphological characteristics. Among the five subspecies, *P. serotina* subsp. *capuli* or capuli, is a unique subspecies with presence in Mexico, Ecuador, Colombia, and Guatemala with superior forms found in the Ecuadorian Andes (McVaugh, 1951).

Capuli fruit has a depressed globose shape with a light maroon to deep purplish color. Its flesh is pale brownish green, melting and juicy. Fruits have a sweet vinous flavor with a taste of bitterness because of their skin (Popenoe and Pachano, 1923). Fruits are abundantly available in Ecuadorian markets from December to February and are mostly eaten fresh. They can be also stewed, preserved, or made into wine or jam (Vasco et al., 2009). Research has shown that capuli fruit has a high number of phenolic compounds with antihypertensive and antioxidant effects. Its consumption can be beneficial in preventing health issues like hypertension and cardiovascular diseases (Luna-Vazquez et al., 2013). However, despite its significant health benefits, there are no commercial capuli cultivars or breeding programs in the world.

Research regarding capuli fruit morphological characteristics is fundamental for the establishment of future capuli breeding programs. Fruit size and firmness are among the most important morphological characteristics for evaluating a fruit's economic value (Christensen, 1974). For example, fruit size and diameter are one of the main fruit morphological characteristics on which sweet cherries are commercially graded in the United States (Whiting et al., 2005). Similar grading and commercialization exist for other fruits and in other countries in which fruit size is one of the most important characteristics. Christensen (1974) studied sweet cherry cultivars from Denmark and found the average sweet cherry size to be 6.6 g per fruit. Khadivi et al. (2019) studied the morphological characteristics of 45 sweet cherries, 62 sour cherries and 39 duke cherries cultivars from Iran. Fruit length varied from 18.9 to 28.5 mm for sweet cherries, 11.2 mm to 14.6 mm for sour cherries and 15.4 to 18.6 mm for duke cherries

cultivars. For fruit weight, sweet cherries varied from 4.4 to 8.9 g, sour cherries 1.4 to 2.7 g and 4.4 to 6.0 g for duke cherries cultivars.

In North America, the average diameter of *P. serotina* fruits growing in Central Mississippi varied from 7-10 mm (Bonner, 1975). Marquis (1990) also reported the diameter of the black cherry fruit to be around 10 mm. Limited studies have been published regarding capuli fruit morphology. The presence of superior fruit genotypes of *P. serotina* subsp. *capuli* in the Ecuadorian Andes creates a unique opportunity for future breeding and selection. This study aims to characterize a collection of different genotypes of capuli's fruit and endocarp found growing in the wild in Chimborazo, Tungurahua, and Cotopaxi provinces of Ecuador. Capuli fruit characterization will help in understanding more about this species natural morphological variation, which will further aid in the establishment of future capuli breeding programs for superior fruit commercial attributes.

Materials and methods

Plant material: Fruit of 24 *P. serotina* subsp. *capuli* genotypes were collected in 2016 and 2019 from three Ecuadorian provinces (Chimborazo, Cotopaxi and Tungurahua) in the Andes region. Plant material collection was performed in accordance to the Minister of Environment of Ecuador plan for access of genetic resources –collection permit MAE-DNB-CM-2019-0107P37-16-00098. Each genotype was given a unique ID based on their collection site (Table 2.1). Genotypes were selected to represent a broad geographical range in the region (Fig. 2.1).

Fruit and Endocarp Measurements: Ripe fruits were collected in Ziploc bags and stored in a cooler with ice after collection in February of 2016 and 2019 seasons (Fig. 2.2). Samples then were transported to the Escuela Politécnica del Chimborazo (ESPOCH) in Riobamba,

Ecuador. Fruits were then evaluated for the following characteristics: weight of ten fruit (g), fruit diameter (mm) (measurement along the equatorial plane of the fruit at the widest point), fruit height (mm) (measurement from stem to the tip end of the fruit), and °brix. The flesh was separated from the endocarp after fruit measurements were taken. Endocarps were washed and left to dry for 48 h in paper towels. Endocarp diameter (mm) (measurement along the equatorial plane of the endocarp at the widest point) and height (mm) (measurement from stem to the tip end of the endocarp) were measured (Fig. 2.3). All measurements of size were done using a digital caliper (Traceable® Carbon Fiber Calipers, Control Company, USA). Weights were taken using a generic brand digital balance. °Brix was measured using a handheld refractometer from juice obtained from the flesh (Atago CO., LTD., Tokyo, Japan).

Statistical Analyses

Analyses of variance were performed on all fruit and endocarp parameters with genotype, year, and genotype x year as main effects in SAS 9.4 (SAS Institute Inc.; Cary, NC) using PROC GLIMMIX. Means were separated using LSD test with a significance level of $P \leq 0.05$. Multivariate analyses were performed for all parameters within the 2016 and 2019 seasons. PCA (Principal Component Analysis) was performed for both the years 2016 and 2019 with JMP®, Version 14 (SAS Institute Inc., Cary, NC).

Results and discussions

Prunus serotina subsp. *capuli* is known in Ecuador as “capuli”. It plays an important part in the life of the Ecuadorian people and other Andean countries. There are no known commercial types available in Ecuador with most of the fruit production obtained from wild individuals. This research was performed to characterize capuli fruit morphology as present in their natural habitat (trees growing in the wild in Ecuador). Our goal was to understand the natural variation for fruit

and to identify superior individuals that would be potentially used in future breeding and selection.

Fruit and endocarp measurements: Fruit and endocarp characteristics were evaluated for 24 capuli genotypes collected in Ecuador in 2016 and 2019 seasons (Table 2.2-2.4). There were statistically significant differences ($P \leq 0.05$) when comparing data across seasons for fruit diameter, fruit height, ten fruit weight, °brix, endocarp diameter and endocarp height. Differences among genotypes were identified for all variables evaluated ($P \leq 0.05$). No statistically significant differences were identified among replicates for all variables ($P > 0.05$) except for endocarp height. Due to the variation present across years, hereafter, all the analyses were performed in within year comparisons.

Fruit diameter varied from 12.8 to 19.1 mm for 2016 and 12.4 to 21.7 mm for 2019. The largest fruit diameter was observed for PserTU77 (19.1 mm) in 2016 and PserTU53 (21.7 mm) in 2019. The smallest fruit diameter was reported for PserCH94 (12.8 mm) in 2016 and PserCO13 (12.4 mm) in 2019 (Table 2.2). For fruit height, values varied between 11.4 to 17.7 mm (2016) and 12.2 to 19.7 mm (2019). Fruit from PserTU43 had the highest height value for 2016 (17.7 mm), and PserTU41 and PserTU53 had highest fruit height for 2019 (19.7 mm) (Table 2.2).

Limited studies have been published regarding capuli fruit morphology. McVaugh (1951) characterized the fruit size of capuli. He reported a diameter around 20-25 mm. Vasco et al. (2008) reported that fruit of Ecuadorian capuli to be 10-30 mm in diameter. Similarly, Avendaño-Gómez et al. (2015) reported the average length of capuli fruit found in Tlaxcala, Mexico to be 1.7 ± 0.2 cm and diameter of 1.5 ± 0.2 cm. The results from the research presented (Table 2.2) were consistent for *P. serotina* subsp. *capuli*. In the case of its sister species, Bonner

(1975) reported the average diameter of North American black cherry fruit to be 7-10 mm. Capuli fruit was generally larger than fruit data reported for *P. serotina* subsp. *serotina* (Avendaño-Gómez et al., 2015; Bonner, 1975).

Variation in fruit size and morphology is also observed in other *Prunus* species. Miloševic and Miloševic (2012) reported dimensions for sour cherry cultivars 'Oblačinska' and 'Cigančica': fruit length (14.85 ± 0.12 mm and 14.27 ± 0.19 mm, respectively) and fruit width (15.50 ± 0.09 mm and 15.23 ± 0.15 mm, respectively).

As previously described, fruit size is an important characteristic used to evaluate a produce value for marketing and commercialization (Christensen, 1974). For example, this is an important standard used for the commercialization of sweet cherries. Different countries have different size standards. Spain has standards in which cherries having a 25 mm diameter are considered in "extra category" whereas, in Summerland (Canada) the ideal cherry diameter is 29-30 mm (Pérez-Sánchez et al., 2010). In the U.S., 9 rows or 30 mm cherries were preferred more by consumers (Turner et al., 2008). Also, Kappel et al. (1996) described an ideal sweet cherry for the North American market of 11-12 g of weight and 29-30 mm of diameter.

Ten fruit weight for *P. serotina* subsp. *capuli* varied from 11.7 to 43.7 g (2016) and 12.0 to 50.3 g (2019). The highest value for ten fruit weight for 2016 was found for PserTU48 (43.7g) and PserTU53 (50.3g) for 2019. The smallest ten fruit weight was found consistently for PserCO13 15.4 g for 2016 and 12 g for 2019 (Table 2.2). Individual fruit weight was recorded for the largest and smallest fruit in each fruit lot (data not shown). The highest individual fruit weight was observed for PserTU48 and PserTU53 of 5.82 g and 3.92 g (2016), and 4 g and 5g (2019), respectively. These individual weights were consistent with the ten-fruit weight average. Vasco et al. (2008) studied the weight of capuli fruits from Ecuador and reported an individual

fruit weight to vary 2-8 g. Khadivi et al. (2019) reported the average fruit weight of 45 sweet cherry (4.4 to 8.9 g), 62 sour cherry (1.4 to 2.7 g) and 39 duke cherry (4.4 to 6.0 g) cultivars from Iran. Similarly, fruit weight for sour cherry cultivars 'Oblačinska' and 'Cigančica' were reported between 3.48 ± 0.11 g and 2.66 ± 0.09 g, respectively (Miloševic, 2012). The capuli fruit weight reported in this study was below the average size of commercial sweet cherry cultivars described above, but it was higher and comparable to the fruit data for sour cherry and duke cherry cultivars, respectively.

Chimborazo genotypes had the highest average °brix values with genotypes PserCH142 (27.6) for 2016 and PserCH101 (27.1) for 2019 (Table 2.2). The lowest °brix values were 14.1 (2016) and 13.4 (2019) for PserCH113 and PserTU77, respectively (Table 2.2). Vasco et al. (2008) studied the average °brix of capuli fruits from Ecuador and reported values between 16.3 - 22.2. Our results are consistent with Vasco et al. (2008) research. Kappel et al. (1996) reported the minimum °brix value of 15 for sweet cherry cultivars. Khadivi et al. (2019) reported the range of TSS (total soluble solids) values of sweet cherries from 15.6% to 20.88%, sour cherries from 15 to 28%, and duke cherries from 17.13% to 22.53% in Iran. Crisosto et al. (2003) studied the importance of TSS, TSS: TA (titratable acidity) and skin color with regard to consumer acceptance for 'Bing' and 'Brooks' cherry cultivars. The authors pointed out that TSS plays an important role in consumer acceptance. Consumer acceptability is increased with high TSS levels and proposed a minimum TSS of 16% for cherries in the American market. Our study reported average brix values higher than those reported in sweet cherries, sour cherries and duke cherries cultivars.

In the case of endocarp morphological characteristics, the largest endocarp diameter was found in the genotype PserTU41 and PserTU77 (10.4 mm) in 2016 and PserTU53 (12.2 mm) in

2019 (Table 2.3) (Fig 2.3). The highest endocarp height was reported for PserTU43 (13.6 mm) in 2016 and PserTU53 (12.2 mm) in 2019 (Table 2.3). Avendaño-Gómez et al. (2015) studied the endocarp of capuli from Tlaxcala, Mexico under a cultivated management system. The authors observed an endocarp thickness of 0.14 ± 0.04 mm, seed length 0.94 ± 0.08 cm, and seed diameter of 0.78 ± 0.13 cm.

In other *Prunus* species, Khadivi et al. (2019) studied Iranian sweet, sour and duke cherries. Stone length ranged from 10.57 to 12.40 mm (sweet cherries), 7.73 to 10.18 mm (sour cherries) and 9.26 to 11.78 mm (duke cherries); whereas stone width ranged from 8.50 to 10.35 mm (sweet cherries), 8.94 to 10.51 mm (duke cherries) and 7.17 to 10.30 mm (sour cherries). The endocarp data collected in our research is comparable to the seed and endocarp collected of capuli from Mexico and other cherries.

Overall means for all the variables within a year were calculated separately according to their province of origin (Table 2.4). Significant differences were present across the different provinces while comparing overall means for the variable fruit height and endocarp diameter for both years. °Brix values were the highest for Chimborazo province genotypes for year 2019 but no significant differences were noticed for year 2016. Ten fruit weight was significantly highest for Tungurahua genotypes for the year 2016. No significant differences were present among provinces for ten fruit weight for the year 2019. The fruit diameter and endocarp height were significantly higher for Tungurahua in comparison to the other provinces for 2016, but no significant differences were present across provinces for year 2019.

In the current study, fruit collected from the wild in Ecuador represented a large variation for fruit diameter, fruit height, fruit weight, °brix, endocarp diameter, and endocarp height (Tables 2.2 to 2.4). This phenotypic variation present in the wild Ecuadorian capuli can be used as a

foundation for the future capuli breeding programs. *Prunus serotina* in N. America is mostly used for furniture purposes, but its fruit lack flavor and size, which are important commercial fruit attributes. The best forms of capuli are found in Ecuador (Popenoe and Pachano, 1923). Although capuli is important to the Ecuadorian people, no commercial varieties of capuli are available in the Ecuadorian market. In Mexico, seeds of capuli are also used as snacks in addition to eating fresh or dried fruit or making other products (Raya-Pérez et al., 2012). Capuli holds the potential for being a multipurpose commercial product where both the fruit and seeds can be utilized for different purposes.

Multivariate analysis for 2016 and 2019: The edible portion in cherries is the epicarp and mesocarp i.e. skin and flesh of the fruit. Beneath these, there is the stony endocarp which is inedible (Tukey and Young, 1939). In the case of sweet cherries, consumers prefer cherries with a small endocarp and large fruit pulp (Pérez-Sánchez et al., 2010). This study focused on characterizing the fruit and endocarp variables and their relationships.

Results of multivariate analysis are shown in Tables 2.5 and 2.6 for the years 2016 and 2019, respectively. A positive strong correlation was found between fruit weight and diameter within both years ($r_{fruitwt\ vs\ fruitdia\ 2016,2019} = 0.89$, $P \leq 0.0001$). Likewise, fruit diameter and endocarp diameter were positively correlated for both years ($r_{fruitdia\ vs\ endocarpdia\ 2016} = 0.78$, $r_{fruitdia\ vs\ endocarpdia\ 2019} = 0.79$; $P \leq 0.0001$). Endocarp height and fruit height were positively correlated ($r_{fruitht\ vs\ endocarpht\ 2016} = 0.78$, $r_{fruitht\ vs\ endocarpht\ 2019} = 0.61$; $P \leq 0.05$). All fruit and endocarp size characteristics were positively correlated with each other within a season. °Brix was not correlated with any of the size variables for fruit and endocarp in both years (Tables 2.5 and 2.6). Large fruited capuli genotypes were consistently characterized by a large endocarp. This will

make the selection for a large fruited and small endocarp genotype difficult. On the other hand, selection for °brix can be made independently of other variables.

Other studies have reported a presence of a positive correlation between fruit and endocarp size variables. Rakonjac et al. (2010) reported that all the fruit size variables in 'Oblačinska' sour cherry accessions were positively related to each other . Demirsoy and Demirsoy (2004) also found a positive polynomial relationship between fruit weight and fruit diameter in sweet cherry. Khadivi-Khub (2014) found a positive correlation between fruit vs. stone weight, fruit weight vs. fruit length, and fruit width vs. diameter when evaluating 70 cherry genotypes. Also, significant negative correlations between TSS vs. fruit were reported in cherry genotypes by Khadivi et al. (2019).

Principal Component Analysis (PCA): Principal component analysis aims to reduce the number of parameters to differentiate relationships between variables and genotypes. This technique helps in dividing the original variables in the dataset into smaller groups. The groups in a PCA are not related to each other except the variables within each group (Iezzoni and Pritts, 1991). The results from the PCA identified that PC1 and PC2 components accounted for 83.7% of the total variation of the studied variables for *P. serotina* subsp. *capuli* (Table 2.7). In PC1, variables with the highest factor loadings are ten fruit weight, fruit diameter, fruit height and endocarp diameter and height. In PC2, °brix have the highest factor loadings. These results are confirmed in Fig. 2.4, where °brix variable is observed on a different quadrant from all the other variables. Genotypes with the highest PC1 scores are those with overall high values for fruit and endocarp variables such as PserTU48 and PserTU53. On the other side, genotypes with high PC2 scores are the ones having high brix values such as genotypes PserCH142, PserCH110 and PserCH108. No clear-cut groups were observed according to their province of origin in the PCA

based on the morphological characteristics studied (Fig. 2.4). The results suggested that there might be not distinct morphological differences among the populations from Chimborazo, Cotopaxi and Tungurahua. Guadalupe et al. (2015), while studying the genetic diversity and population structure of capuli from 8 Ecuadorian provinces, reported the lack of clear population differentiation among the capuli from the different provinces. They proposed the reason for homogenization of Ecuadorian capuli could be out crossing and self-incompatibility in capuli.

The results of this study can be used as a reference for *P. serotina* subsp. *capuli*. In addition, the information presented is important for future capuli breeding and management programs in Ecuador and the U.S. Capuli fruits have high nutritional value. They are a good source of antioxidants, protein, and minerals, which can be helpful in the prevention of cardiovascular diseases (Luna-Vazquez et al., 2013). Also, black cherry wood is rated highly for making furniture, woodwork, and cabinets (Liu and Pijut, 2008). The present study shows the feasibility and great potential to select and further breed a capuli with superior commercial characteristics. The material studied in this research was collected in the wild without any advanced breeding and selection, and commercial management. Capuli holds a lot of commercial potential in Ecuador and could be an interesting species to be evaluated in the U.S. Capuli holds a promising future similar to other stone fruits and has the potential to be used locally and internationally.

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Table 2.1. List of *Prunus serotina* subsp. *capuli* genotypes collected for fruit evaluation in the Andes region of Ecuador

Collection (No.)	Genotype ID[z]	Country	Province	Latitude	Longitude
1	PserCO01	Ecuador	Cotopaxi	1°7'36.1"S	78°35'21.2"W
13	PserCO13	Ecuador	Cotopaxi	0°53'2"S	78°39'15.7"W
14	PserCO14	Ecuador	Cotopaxi	0°53'22.9"S	78°37'30.7"W
16	PserCO16	Ecuador	Cotopaxi	0°53'44.7"S	78°37'5"W
21	PserCO21	Ecuador	Cotopaxi	0°59'2.5"S	78°36'6.3"W
22	PserCO22	Ecuador	Cotopaxi	0°59'2.1"S	78°36'5.6"W
26	PserCO26	Ecuador	Cotopaxi	1°5'50.9"S	78°36'10.2"W
31	PserCO31	Ecuador	Cotopaxi	1°6'23.3"S	78°36'28.1"W
41	PserTU41	Ecuador	Tungurahua	1°24'8.9"S	78°38'2.2"W
43	PserTU43	Ecuador	Tungurahua	1°23'26"S	78°37'25.8"W
48	PserTU48	Ecuador	Tungurahua	1°21'5"S	78°36'45.2"W
53	PserTU53	Ecuador	Tungurahua	1°18'49.9"S	78°38'19"W
57	PserTU57	Ecuador	Tungurahua	1°17'49.3"S	78°37'14.1"W
67	PserTU67	Ecuador	Tungurahua	1°18'49"S	78°32'46.9"W
70	PserTU70	Ecuador	Tungurahua	1°20'2.3"S	78°33'51.8"W
71	PserTU71	Ecuador	Tungurahua	1°19'23.1"S	78°34'19.1"W
75	PserTU75	Ecuador	Tungurahua	-	-
77	PserTU77	Ecuador	Tungurahua	1°21'11.3"S	78°34'58.5"W
94	PserCH94	Ecuador	Chimborazo	1°34'59.7"S	78°32'17.4"W
101	PserCH101	Ecuador	Chimborazo	1°37'3.3"S	78°35'49.3"W
108	PserCH108	Ecuador	Chimborazo	1°35'45.3"S	78°40'56.8"W
110	PserCH110	Ecuador	Chimborazo	1°35'21.2"S	78°41'17.3"W
113	PserCH113	Ecuador	Chimborazo	-	-
132	PserCH132	Ecuador	Chimborazo	1°41'47"S	78°38'39"W
142	PserCH142	Ecuador	Chimborazo	1°38'46.4"S	78°42'27.2"W

[z]ID= first letter represented the genus (P=*Prunus*), next three letters represented the species (ser=*serotina*), and the following letters represented the province of origin in Ecuador and collection number (CO01=Cotopaxi collection 01).

Table 2.2. Fruit characteristics of *Prunus serotina* subsp. *capuli* genotypes collected in the Andes region of Ecuador in 2016 and 2019 seasons.

Genotype ID	Fruit diameter (mm)		Fruit height (mm)		Ten fruit weight (g)		°Brix	
	2016	2019	2016	2019	2016	2019	2016	2019
PserCH101[z]	13.0 ij[y]	14.4 d-f	12.3 kj	12.2 h	12.8 kl	17.7 gh	20.0 b-f	27.1 a
PserCH108	16.0 c-h	15.5 de	14.6 c-i	14.4 d-g	28.5 d-f	21.3 e-g	25.3 ab	21.5 b-f
PserCH110	15.1 e-j	14.5 d-f	13.7 g-j	12.5 gh	23.0 g-i	21.0 e-g	19.2 d-g	25.5 ab
PserCH113	15.7 c-i	17.0 cd	14.5 d-i	13.5 d-g	24.1 f-i	29.0 cd	14.1 g	24.9 a-c
PserCH132	16.1 b-g	14.4 d-f	14.1 e-j	12.7 f-h	21.8 h-j	21.3 e-g	16.2 e-g	21.6 a-f
PserCH142	15.4 d-j	14.6 d-f	13.3 h-k	13.4 d-h	19.9 h-j	22.0 e-g	27.6 a	23.4 a-d
PserCH94	12.8 j	16.4 c-e	11.4 k	14.7 c-f	11.7 l	22.3 d-g	18.5 d-g	21.8 a-e
PserCO01	15.8 c-h	16.0 c-e	14.6 c-i	13.6 d-h	25.4 e-i	24.0 d-g	19.4 d-g	16.2 f-h
PserCO13	14.0 f-j	12.4 f	13.8 f-j	12.4 gh	15.4 j-l	12.0 h	20.6 b-f	21.5 b-f
PserCO14	15.9 c-g	16.2 c-e	15.5 b-f	13.7 d-h	24.3 e-i	19.0 fg	25.1 a-c	17.2 e-h
PserCO16	13.3 h-j	19.8 ab	12.5 i-k	16.7 bc	19.2 i-k	49.3 a	23.5 a-d	14.3 gh
PserCO21	18.3 a-c	15.1 d-f	15.9 a-f	13.4 d-h	24.7 e-i	27.0 de	17.2 e-g	20.6 b-f
PserCO22	16.6 a-g	16.1 c-e	15.1 c-g	13.0 e-h	29.8 c-f	27.0 de	18.0 d-g	21.4 b-f
PserCO26	14.9 e-j	15.0 d-f	14.7 c-f	14.1 d-h	26.1 e-h	29.0 cd	18.4 d-g	18.2 d-h
PserCO31	16.8 a-f	18.7 bc	16.6 a-d	15.2 b-d	34.6 cd	37.3 b	17.7 e-g	19.5 c-g
PserTU41	17.7 a-e	13.7 ef	16.7 a-c	19.7 a	30.3 c-f	21.0 e-g	19.4 d-g	20.8 b-f
PserTU43	17.9 a-d	15.2 de	17.7 a	13.3 d-h	36.4 bc	20.3 e-g	17.3 e-g	20.0 b-f
PserTU48	18.8 ab	16.4 cd	17.3 ab	17.0 b	43.7 a	33.8 bc	18.5 d-g	17.9 e-h
PserTU53	16.3 b-g	21.7 a	16.2 a-e	19.7 a	30.9 c-e	50.3 a	15.2 fg	19.1 d-g
PserTU57	14.5 f-j	16.3 c-e	13.5 g-k	14.9 b-e	20.4 h-j	26.3 de	21.0 b-e	21.6 a-f
PserTU67	17.9 a-d	16.1 c-e	15.5 b-g	14.0 d-h	33.5 cd	25.3 d-f	23.4 a-d	18.2 d-h
PserTU70	15.7 d-i	16.4 cd	14.6 d-i	14.0 d-h	23.0 g-i	24.3 d-g	18.5 d-g	14.2 gh
PserTU71	14.0 g-j	15.9 de	14.0 f-j	13.5 d-h	19.8 h-j	20.7 e-g	20.1 b-f	19.2 d-g
PserTU77	19.1 a	14.8 d-f	17.5 ab	13.0 f-h	41.4 ab	18.7 f-h	19.6 d-g	13.4 h

[z]Genotype = first letter represented the genus (P=*Prunus*), next three letters represented the species (ser=*serotina*), and the following letters represented the province of origin in Ecuador and collection number (CH101=Chimborazo collection 101).
[y]Different letters within a column indicate significant difference between genotypes using LSD test at $P \leq 0.05$.

Table 2.3. Endocarp characteristics of *Prunus serotina* subsp. *capuli* genotypes collected in the Andes region of Ecuador in 2016 and 2019 seasons.

	Endocarp diameter (mm)				Endocarp height (mm)			
Genotype ID	2016		2019		2016		2019	
PserCH101[z]	8.0	ij[y]	8.0	jk	9.4	h	8.5	j
PserCH108	9.5	b-e	9.0	c-h	10.2	e-h	11.3	a-c
PserCH110	8.3	g-j	8.3	g-j	10.8	b-f	9.7	e-i
PserCH113	8.2	h-j	8.9	c-h	9.7	gh	10.2	d-g
PserCH132	8.9	d-i	8.4	f-k	10.0	e-h	9.5	g-j
PserCH142	9.5	b-e	9.2	c-f	9.9	f-h	10.7	b-e
PserCH94	7.7	j	8.5	e-j	8.1	i	10.2	e-g
PserCO01	9.2	b-f	9.6	bc	10.7	c-g	10.6	b-f
PserCO13	7.7	j	7.5	k	9.7	gh	9.3	g-j
PserCO14	9.0	c-g	8.7	d-j	10.5	d-h	8.7	ij
PserCO16	8.1	ij	10.4	b	10.2	e-h	11.6	ab
PserCO21	8.5	f-j	8.3	f-k	9.4	h	9.5	g-j
PserCO22	8.6	e-i	8.0	i-k	9.7	f-h	9.3	g-j
PserCO26	8.9	d-i	9.2	c-g	9.9	f-h	9.9	d-g
PserCO31	9.5	a-d	9.3	c-e	11.8	b	9.3	g-j
PserTU41	10.4	a	10.4	b	11.7	bc	9.9	d-g
PserTU43	9.8	a-c	7.6	k	13.6	a	8.7	ij
PserTU48	10.1	ab	10.4	b	11.1	b-e	10.8	b-d
PserTU53	9.1	c-g	12.2	a	11.6	b-d	12.2	a
PserTU57	8.6	e-i	8.9	c-i	9.7	gh	9.6	f-j
PserTU67	9.2	b-f	9.0	c-h	10.3	e-h	10.2	d-g
PserTU70	9.3	b-f	9.2	c-f	9.4	h	10.2	c-g
PserTU71	8.7	d-i	9.4	cd	10.6	d-g	9.5	f-j
PserTU77	10.4	a	8.2	h-k	11.9	b	9.0	h-j

[z]Genotype = first letter represented the genus (P=*Prunus*), next three letters represented the species (ser=*serotina*), and the following letters represented the province of origin in Ecuador and collection number (CH101=Chimborazo collection 101).

[y]Different letters within a column indicate significant difference between genotypes using LSD test at $P \leq 0.05$.

Table 2.4. Fruit and endocarp characteristics of *P. serotina* subsp. *capuli* according to its provinces of origin in Ecuador.

Year	Province	Fruit diameter (mm)	Fruit height (mm)	Ten fruit weight (g)	Endocarp diameter (mm)	Endocarp height (mm)	°Brix
2016	Chimborazo	14.9 b[z]	13.4 c	20.3 b	8.6 b	9.7 b	20.1 a
	Cotopaxi	15.7 b	14.8 b	24.9 b	8.7 b	10.2 b	20.0 a
	Tungurahua	16.9 a	15.9 a	31.1 a	9.5 a	11.1 a	19.2 a
2019	Chimborazo	15.3 a	13.3 b	22.1 a	8.6 b	10.0 a	23.7 a
	Cotopaxi	16.2 a	14.0 b	28.1 a	8.9 b	9.8 a	18.6 b
	Tungurahua	16.3 a	15.5 a	27.5 a	9.5 a	10.0 a	18.2 b

[z]Different letters within a column and within a year indicate significant difference between genotypes using LSD test at $P \leq 0.05$.

Table 2.5. Multivariate analysis of fruit and endocarp characteristics of *Prunus serotina* subsp. *capuli* genotypes collected in the Andes region of Ecuador in 2016.

	Ten fruit weight (g)	Fruit diameter (mm)	Fruit height (mm)	°Brix	Endocarp diameter (mm)	Endocarp height (mm)
Ten fruit weight (g)	1.00[x] <.0001[y]					
Fruit diameter (mm)	0.89 <.0001	1.00 <.0001				
Fruit height (mm)	0.92 <.0001	0.92 <.0001	1.00 <.0001			
°Brix	-0.18 0.4068	-0.19 0.3848	-0.27 0.1968	1.00 <.0001		
Endocarp diameter (mm)	0.82 <.0001	0.78 <.0001	0.79 <.0001	0.08 0.7181	1.00 <.0001	
Endocarp height (mm)	0.73 <.0001	0.59 0.0023	0.78 <.0001	-0.14 0.517	0.70 0.0001	1.00 <.0001

[x]Correlation coefficient *r*.
[y]*P*-value.

Table 2.6. Multivariate analysis of fruit and endocarp characteristics of *Prunus serotina* subsp. *capuli* genotypes collected in the Andes region of Ecuador in 2019.

	Ten fruit weight (g)	Fruit diameter (mm)	Fruit height (mm)	°Brix	Endocarp diameter (mm)	Endocarp height (mm)
Ten fruit weight (g)	1.00[x] <.0001[y]					
Fruit diameter (mm)	0.89 <.0001	1.00 <.0001				
Fruit height (mm)	0.66 0.0004	0.82 <.0001	1.00 <.0001			
°Brix	-0.30 0.1486	-0.32 0.1234	-0.27 0.1953	1.00 <.0001		
Endocarp diameter (mm)	0.76 <.0001	0.79 <.0001	0.85 <.0001	-0.32 0.1305	1.00 <.0001	
Endocarp height (mm)	0.69 0.0002	0.61 0.0016	0.61 0.0014	-0.23 0.2747	0.78 <.0001	1.00 <.0001

[x]Correlation coefficient *r*.

[y]*P*-value.

Table 2.7. Eigenvectors and proportion of the total variability for each principal component axes for fruit and endocarp characteristics of *Prunus serotina* subsp. *capuli* genotypes collected in the Andes region of Ecuador in 2016 and 2019.

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Ten fruit wt	0.45446	0.04332	-0.24181	0.64872	-0.11152	-0.54758
°Brix	-0.15948	0.97704	-0.07358	0.02714	0.11666	-0.01418
Fruit dia(mm)	0.45266	0.01889	-0.50161	0.11916	0.20149	0.69881
Fruit ht(mm)	0.45598	0.01321	-0.02192	-0.55327	0.58087	-0.3846
Endocarp dia(mm)	0.44599	0.17733	0.01862	-0.43673	-0.76052	0.01344
Endocarp ht(mm)	0.39536	0.10743	0.82685	0.25959	0.13253	0.25204
Total Eigenvalue	4.0911	0.9329	0.4765	0.2483	0.1892	0.0621
Variance%	68.184	15.549	7.941	4.138	3.153	1.035
Cumulative%	68.184	83.734	91.675	95.812	98.965	100

Fig. 2.1. Map representing collection sites of *Prunus serotina* subsp. *capuli* genotypes collected from 3 different provinces of Ecuador in 2016 and 2019.

Fig. 2.2. Fruits of *Prunus serotina* subsp. *capuli* genotype PserTU48 collected in the Andes region of Ecuador in 2016. A one cent coin is in the center as a size reference.

Fig. 2.3. Endocarps of *Prunus serotina* subsp. *capuli* genotype PserTU77 collected in the Andes region of Ecuador in 2016. A one cent coin is in the center as a size reference.

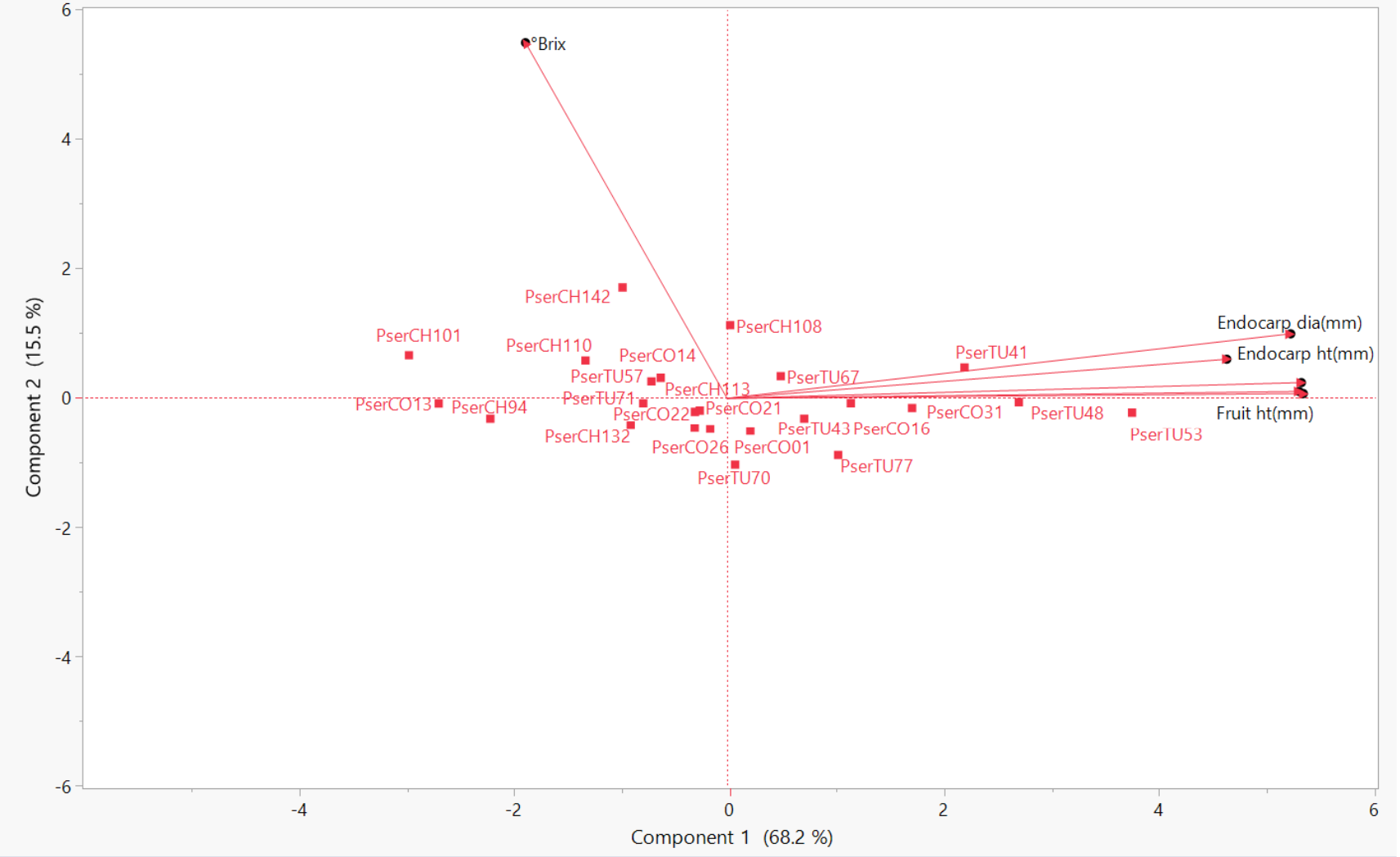

Fig 2.4. Principal component analysis (PCA) based on the fruit and endocarp characteristics of *Prunus serotina* subsp. *capuli* genotypes collected in the Andes region of Ecuador in 2016 and 2019.

CHAPTER 3

MORPHOLOGICAL CHARACTERIZATION OF *PRUNUS SEROTINA* SUBSP. *CAPULI*[2]

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Abstract

Prunus serotina (black cherry) is native to America with five subspecies found: *serotina, eximia, hirsuta, virens*, and *capuli*. *Prunus serotina* subsp. *serotina* is native to North America. It is characterized by small, astringent fruit, unsuitable for human consumption. *Prunus serotina* subsp. *capuli* is found in Central and South America with superior fruit specimens found in Ecuador. These specimens have large, juicy, and tasty fruits used for human consumption. Also, they have important nutraceutical properties and are available in produce markets. However, no commercial varieties of capuli are currently available. The objective of this research was to characterize morphological traits (tree, leaf and flower) of plants grown from OP seeds of 44 capuli accessions collected from three provinces of Ecuador (Cotopaxi, Chimborazo and Tungurahua). Tree measurements included number of primary branches and growth habit. Leaf measurements included petiole length, leaf area, leaf height, leaf width, leaf apex angle and leaf basal angle. Flower measurements included pedicel length, flower width, and flower length. Raceme length, number of racemes per branch and number of flowers per raceme were also characterized. ANOVA were performed with significant differences observed among accessions and years for all variables measured. Multivariate analysis was done, and significant positive correlations were observed within variables of same phenotypic category (flower, leaf, or tree characteristics), but not across the phenotypic variables of different categories. PCA and cluster analysis were also performed. No clear differences were observed across regions that may support the presence of different populations.

Introduction

Prunus serotina is the largest native cherry tree in the United States. It belongs to the family Rosaceae, subfamily Spiraeoideae and tribe Amygdaleae (Potter et al., 2007). The tree

can grow to be 100 feet in height and 5 feet in diameter. It can grow 2.5 inches in diameter per decade between 13 and 33 years of age (Hough, 1960). *Prunus serotina* is constituted by 5 subspecies: *P. serotina* subsp. *serotina*, *P. serotina* subsp. *hirsutus*, *P. serotina* subsp. *virens*, *P. serotina* subsp. *eximia* and *P. serotina* subsp. *capuli* (McVaugh, 1951). *Prunus serotina* subsp. *serotina* is commonly found growing in Mexico, Perú, Colombia, Guatemala and Ecuador, with its best fruit forms growing in the Ecuadorian Andes region (Popenoe and Pachano, 1923).

Prunus serotina subsp. *capuli* have been used from olden times for the treatment of diseases such as diarrhea and cough (Villamar, 1994). The essential oils from leaves have vasorelaxant properties (Ibarra-Alvarado et al., 2009). Other benefits of the capuli tree include its use within an agroforestry system to prevent erosion and as a wind barrier (National Research Council, 1989).

McVaugh (1951) reported that basic differences among the different subspecies of *P. serotina* are due to the differences in leaves, inflorescences, and flower size. *Prunus serotina* subsp. *serotina* leaves are glabrous approximately 4 cm in width and 9 cm in length. The leaf petiole is about 1.5 cm long and its flowering branch is a 12 cm long raceme (McVaugh, 1951).

Capuli fruits are round and glossy and are abundantly available in Andean markets (National Research Council, 1989). The capuli tree in Ecuador can reach up to 15 m in height. Capuli leaves have a dark green upper surface and pale green lower surface. They are mostly oblong lanceolate in shape with fine serrations. They can be 7 to 14 cm long. Flowers are white, held on racemes. The racemes can be 10 to 25 cm long (Popenoe and Pachano, 1923). Flowers are 35 mm in length and petals are around 3.5 mm in length and width. The anthers and style are around 0.8 mm and 1.7 mm in length (McVaugh, 1951).

The morphological characterization of different plant parts (trunk, branches, leaves, flowers, fruit, etc.) of a representative group of individuals within and across species constitute an important technique to understand the diversity of horticultural crops (Blazek, 2004). Phenotyping helps to characterize diverse germplasm (Ganopoulos et al., 2015). The data obtained through phenotyping can be then analyzed using data reduction techniques such as PCA (Principal Component Analysis). PCA helps in identifying patterns in data by regrouping correlated variables into the small sets of original variables (Iezzoni and Pritts, 1991).

The aim of this research was to understand the morphological variation present in OP (open pollinated) seedlings from 44 *P. serotina* subsp. *capuli* accessions collected from 3 different provinces of Ecuador (Cotopaxi, Chimborazo, and Tungurahua) growing in Griffin, Georgia, USA. Our objective was to understand if different morphological characters can differentiate unique populations of *P. serotina* subsp. *capuli* present in Ecuador. The information obtained in this study will aid the preservation and conservation of these species. In addition, it will provide basic morphological data for future capuli breeding programs in Ecuador and the U.S.

Materials and methods

Plant material: Fruit from 44 genotypes of *P. serotina* subsp. *capuli* growing in 3 provinces (Chimborazo, Cotopaxi and Tungurahua) in the Andes region of Ecuador were collected in 2016. Each genotype was given a unique ID based on their collection site. Plant collection permit MAE-DNB-CM-2019-0107 from the Minister of Environment of Ecuador was used for access and use of these genetic resources. Genotypes were selected to represent a broad geographical range in the region. At least 100 ripe fruits per plant were collected in plastic bags and stored in a cooler with ice after collection. Endocarps were washed and left to dry for 48 h in

paper towels. Seed lots of 50 seed per accession then were transported to the Peach Research and Extension program at the University of Georgia, Griffin Campus, Griffin, GA, USA following procedures from the USDA seed importation permit P37-16-00098. Leftover seed per accession were kept by collaborators at the Escuela Politécnica del Chimborazo (ESPOCH) and currently are grown in a germplasm collection on-site.

Seeds were imbibed in water for 4 d, with water being replaced every 24 h. Seeds then were transferred to a Captan solution for 24 h. For stratification and germination, seeds were placed in perlite moistened with Captan in a refrigerator at 4 °C. Once seed started to germinate, seeds were planted in 50 cell trays (A.M. Leonard, Piqua, OH) filled with a 3:1 germination mix media and perlite. Osmocote slow-release fertilizer (15-9-12) 5-6 month was added to the media prior planting (approximately ½ cup per 4 gallons of media). Seedlings were grown and maintained in a greenhouse until being transplanted in the 2017 season. The seedlings were labelled throughout the process and seedling lots /accessions were given the same ID as their mother plants (Table 3.1). Accessions were transplanted directly to the field at the Dempsey Research Farm, Griffin, GA. They were planted in a high-density nursery.

Morphological characters: Leaves, racemes and flowers were collected and characterized in summer 2019 and 2020. A total of 44 accessions (seedling lots) and five genotypes per accession were used throughout the study. Table 3.2 lists of all the studied variables. Number of 1st order branches were measured for each genotype within each accession. The measurements were taken in December 2019 and December 2020. The number of 1st order branches was counted from the base of the plant to the top. Plant growth habit was evaluated using a subjective scale of 1(leaning) to 9 (upright) in December 2019 and December 2020 (Fig. 3.1).

Capuli flowers are borne on a raceme (Figs. 3.2 and 3.3). The total number of racemes per branch for three different branches within each genotype per accession were counted. Racemes and flowers open early April, and they were collected in the first and second week of April for both years, 2019 and 2020. For flower and raceme measurements, three racemes were randomly collected per genotype per accession. Raceme length (mm) was measured for all collected samples. The number of leaves and flowers on each raceme were counted. Further, three flowers from each raceme were used to measure flower length (mm), flower width (mm) and pedicel length (mm) (Fig. 3.4). A digital caliper was used for all these measurements.

For leaves, a total of 5 leaves per accession were phenotyped. Samples were collected and stored in a cooler with ice until transported to UGA Griffin campus, Griffin, GA. Samples were then stored in a fridge at 4 °C for 24 h before processing. A flat bed scan brand Epson Perfection V600 with Epson Scan software (Epson America Inc., Los Alamitos, CA, USA) was used to scan leaves. Image Scanner settings were 48-bit color with a 800 dpi resolution. ImageJ software v1.52p (NIH, Bethesda, MD, USA) was used to process and to measure the scanned images for leaf area (mm^2), leaf width (mm), leaf height (mm), petiole length (mm), apex angle (°) and basal angle (°) (Fig. 3.5). A graph paper reference scale of 5 mm was used for scanning.

Data was taken from the same plants of each accession when possible, every year but due to high density planting, some trees were weak or could not survive and died at the end of the second year of data collection.

Accession PserTU57/53 either belongs to accession PserTU57 or PserTU53 but due to uncertainty at the time of planting, it was planted separately at the end of the last row in the nursery. To avoid any data loss, we measured this accession for all variables and the data was

included in the mean separation as well. However, this accession was not included in the multivariate, Principal Component Analysis (PCA) and cluster analysis.

Statistical analysis

Analyses of variance were performed on all measurements with accession, year, and accession x year as main effects in SAS 9.4 (SAS Institute Inc.; Cary, NC) using PROC GLIMMIX. Means were separated using LSD test with a significance level of $P \leq 0.05$. Multivariate, Principal component analysis (PCA) and cluster analysis were performed with all the variables in JMP v.14 (SAS Institute Inc.; Cary, NC).

Results and discussions

Prunus serotina is classified into 5 subspecies with unique leaf, flowers and fruit morphological characteristics (Fresnedo-Ramírez et al., 2011). McVaugh (1951) reported these unique characteristics per subspecies to better differentiate these subspecies as follows: 1) Leaves of *P. serotina* subsp. *serotina* are 4 cm wide and 9 cm long. Its flowers have a 4.5 mm long pedicel with approx. 35 flowers per raceme. The flowering branch in 2) *Prunus serotina* subsp. *hirsuta* has around 45 flowers per raceme with a 2.5-5 mm long pedicel. Its leaves are 4.5 cm wide and 8.5 cm long. 3) *Prunus serotina* subsp. *virens* has leaves 2-3 cm wide and 4.5-7 cm long with a 5-7 mm long petiole. Flower pedicels are 3.5 mm long with approx. 30 flowers per raceme. 4) *Prunus serotina* subsp. *eximia* leaves are 3-4.5 cm in width and 7-9 cm in length. Its petiole is 1.5-2 cm in length. The flowering branch of subspecies *eximia* is about 12 cm long and has 40 flowers. 5) *Prunus serotina* subsp. *capuli* has a 15 cm flowering branch with approx. 35 flowers. Its pedicel is 4 mm long. The second floral leaf in capuli is about 6 cm long and 2.2 cm in width (McVaugh, 1951).

Diverse morphological characteristics have been used to differentiate the subspecies of *P. serotina.* The main objective of this research was to better characterize the morphological variation of *P. serotina* subsp. *capuli* using accessions collected from Ecuador in 2016 and currently growing at the University of Georgia, Griffin Campus, Griffin, GA, USA. Our hypothesis was that no leaf, flower, and plant morphological variation was present within the accessions collected in Ecuador that could differentiate unique populations of *P. serotina* subsp. *capuli*.

Tables 3.3-3.5 represent the ANOVA tables for the different morphological characters analyzed (plant, leaf, and flower) in this research. Statistical differences were observed among all accessions for all variables analyzed ($P \leq 0.05$). A year effect was significant for all the studied variables except for leaf basal angle. Interaction of accession and year effects were significantly different for all variables except for number of 1st order branches, growth habit, leaf apex angle, and leaf basal angle. Hereafter, to keep a uniformity in analyses and for easy data representation, within year mean comparisons across all the variables will be presented.

Plant measurements: Table 3.6 reports the data collected for tree growth habit and branching variables. The number of 1st order branches count was found to be the largest for PserCH113 with 67.4 in 2019 and 41.8 in 2020. The least number of 1st order branches was found in PserCH86 (21.6) for 2019 and PserCO21(15.8) for 2020 (Table 3.6). PserCO16 and PserTU53 had the highest score for plant growth habit, which represents upright growth, for 2019 and 2020, respectively. On the other hand, accessions PserTU48 were found to be more leaning/less upright in their growth for 2019. PserCO09, PserCO31 and PserCO21 and 2020 were less upright for 2020.

Capuli is an important tree used for timber and agroforestry purposes (Ramírez and Davenport, 2014). The trees which are upright and reduced number of 1st order branches would be more desirable for use as timber species. This would ease their use for making furniture. PserCH99, PserCH153 are accessions having mixture of these characteristics which can be further used as selections for timber purpose.

Marquis (1990) also mentioned black cherry to be extremely sensitive to shade and competition. He mentioned the need of open canopy space during initial years of growth otherwise they start to have reduced growth and even die. The reason for the reduced number of primary branches could be due to the error in the counting or increased competition for the plants or both. Most of the primary branches in the accessions were located only at the very top of the tree in the second year. This was also observed in *Impatiens pallida* where most of the primary branches in the crowded population stand was located at the top of the tree (Weiner et al., 1990).

Flower and raceme measurements: Table 3.7 presents the results of the flower measurements for the years 2019 and 2020. The flower length varied from 11.2 mm to 5.7 mm in 2019 and 9.1 mm to 5.2 mm in 2020. PserCH112 and PserCH127 had the highest flower length in 2019 and 2020, respectively. The flower width varied from 5.0 mm to 2.9 mm in 2019 and 4.3 mm to 2.2 mm in 2020. PserCO31 and PserTU81 had the highest flower width in 2019 and 2020, respectively. Pedicel length varied from 5.6 mm to 2.0 mm in 2019 and 4.1 mm to 2.0 mm in 2020. PserCH112 and PserCO22 had the highest pedicel length in 2019 and 2020, respectively.

Results of the raceme measurements for the year 2019 and 2020 are reported in Table 3.8. The number of racemes per branch in 2019 varied from 0.7 to 18.5 and 3.4 to 11.4 in 2020. PserCO13 and PserCO31 had the highest number of racemes per branch in 2019 and 2020, respectively. The range of number of flowers per raceme varied from 12.9 to 30.7 in 2019 and

12.0 to 23.2 in 2020. PserTU75 and PserCO16 had the highest number of flowers per raceme in 2019 and 2020, respectively. The maximum number of basal leaves per raceme were 6.4 and 6 in 2019 and 2020, respectively. PserCO16 and PserTU41 had the highest number of leaves per raceme in 2019 and 2020, respectively. The raceme length ranged from 8.1 cm to 20.8 cm in 2019 and 9.7 cm to 17.8 cm in 2020. PserTU41 had the highest raceme length in 2019 and 2020. McVaugh (1951) reported that branch bearing flowers on a capuli tree are usually 15 cm long with 35 flowers, somewhat consistent with the data range for this research. Also, he stated that racemes bear usually 1-4 leaves on their lower end and 30-36 flowers on the distal end. Avendaño-Gómez et al. (2015) reported around average 30 flowers on the inflorescence and raceme length 9.66 ± 2.36 cm in cultivated forms of capuli in Tlaxcala, Mexico. The raceme length and number of flowers on raceme reported in our data is comparable to those found by McVaugh (1951) and Avendaño-Gómez et al. (2015).

Fruit count and yield is related to the number of flowers and flowering buds in the sour cherry (Iezzoni and Mulinix, 1992). PserCO13, PserCO16 and PserCO31 are some of the accessions with higher flower count on a raceme and racemes on a branch. With the proper spacing and growth environment, the potential of these accessions could be further evaluated in regard to high yield and fruit count for the capuli fruits in Southeastern US.

McVaugh (1951) used flower and inflorescence sizes along with leaf characters as the main descriptors to classify individual subspecies of *Prunus serotina*. Subspecies *capuli* were reported to have a 15 cm long raceme with approx. 35 flowers. Its flower pedicel was reported to be around 4 mm long. The leaves were 2.5- 4 cm wide and 8-12 cm long with a petiole of 1-2 cm long. The second floral leaf in capuli was about 6 cm long and 2.2 cm wide. Our observations are consistent with McVaugh's (1951) report. In other species, similar studies have been used for

morphological characterization. For example, Rodrigues et al. (2007) reported flower diameter in sweet cherries ranging from 2.9 to 3.7 cm and in sour cherries from 2.5 to 3 cm, all from Portugal.

Leaf measurements: Results of the leaf measurements are listed in Table 3.9. The leaf width ranged from 19.4 mm to 30.4 mm (2019) and 16.1 mm to 28.1 mm (2020). The leaf height ranged from 78.6 mm to 112.9 mm (2019) and 51.2 mm to 100.4 mm (2020). Leaf area ranged from 931.6 mm^2 to 2022.7 mm^2 (2019) and 460.3 mm^2 to 1569.7 mm^2 (2020). The largest leaf area, width and height in 2019 was reported for PserTU41 and in 2020 for PserCO31. Petiole length ranged from 6.1 mm to 14.3 mm (2019) and 4.7 mm to 10.4 mm (2020). Leaf apex angle ranged from 17.5° to 32.2° in 2019 and 19° to 56.5° in 2020. Leaf basal angle ranged from 75.6° to 110.3° in 2019 and 72.7° to 109.7° in 2020. PserCO13 and PserCH132 had the highest leaf apex angle in 2019 and 2020. PserCH138 and PserCO31 had the highest leaf basal angle in 2019 and 2020, respectively.

McVaugh (1951) reported the second floral leaf in capuli to be 10-36 mm in width and 25-95 mm in length with petioles 5-20 mm long. This report was consistent with our data. Similar studies have been done with other *Prunus* species. Khadivi-Khub A. and Anjam (2014) observed that the leaf length in wild *P. scoparia* species from Iran varied from 55.2 mm to 15.10 mm while leaf width varied from 1.60 to 9.2 mm. Similarly, in almonds from Iran, the leaf length varied from 8.56 to 3.90 cm and width from 2.88 cm to 1.38 cm. Also, petiole length varied from 0.91 to 3.39 cm (Khadivi-Khub Abdollah and Etemadi-Khah, 2014).

Other studies have shown variation for leaf characteristics. For example, leaf width varied from 43.20 to 62.20 mm (sweet cherries), 22.08 to 46.16 mm (sour cherries) and 34.50 to 51.90 mm (duke cherries) from Iran (Khadivi et al., 2019). In the same study, leaf length varied

from 99 to 130.30 mm (sweet cherries), 39.07 to 81.03 mm (sour cherries) and 80.50 to 104.80 mm (duke cherries). For petiole length, sweet cherries ranged from 21.90 to 39.50 mm, sour cherries 6.56 to 17.93 mm and 10.60 to 17.80 mm in duke cherries (Khadivi et al., 2019). Rodrigues et al. (2007) studied the morphological characterization of nine sweet and eight sour cherry cultivars in a germplasm bank in Portugal. The apical angle in the study ranged from 60° to 72° and basal angle ranged from 62° to 106.24°. The sweet cherries leaf length varied from 12 to 16 cm and width from 6 to 7 cm. In case of sour cherries, the length varied from 7 to 12 cm and width from 4 to 6 cm.

PserCO31 and PserTU41 were the accessions with high leaf area, width and height. The capuli leaves have vasorelaxant effects (Ibarra-Alvarado et al., 2009). The leaves of the capuli are also used in the form of tea and syrups for treatment of diseases like hypertension, diarrhea and malaria from earlier times (Martínez, 1959). Also, capuli leaves plays a role in the cure of tonsillitis (Ramírez and Davenport, 2014). Although, further scientific research is required for the nutritional analysis of the capuli leaves. Still, selecting trees with larger leaf parameter values will be a foundational base for breeding programs with nutrition objective in mind.

Multivariate analysis: Results of the multivariate analysis is reported in Table 3.10. Accession PserTU57/53 which either belongs to PserTU57 or PserTU53 was not included. Raceme characteristics were positively and significantly correlated to each other ($r_{FC\ vs\ BLN}$ = 0.40, $r_{RL\ vs\ FC}$ = 0.68, $P \leq 0.05$). Flower width and pedicel length were positively correlated with flower length ($r_{FW\ vs\ FL}$ = 0.65, $r_{FPL\ vs\ FL}$ = 0.81, $P \leq 0.05$). Flower length and pedicel length were weakly positively correlated with raceme length ($r_{FL\ vs\ Rl}$ = 0.45, $r_{FPL\ vs\ RL}$ = 0.45; $P \leq 0.05$). Leaf measurements such as petiole length, leaf width, height and area were positively correlated with each other ($r_{LA\ vs\ LPL}$ = 0.44, $r_{LA\ vs\ LW}$ = 0.84, $r_{LH\ vs\ LW}$ = 0.55; $P \leq 0.05$). Similar results were

detected for leaf variables by other authors in other *Prunus* species (Khadivi et al., 2019; Rakonjac V. et al., 2010; Rakonjac V. et al., 2014). Leaf apex angle was weakly negatively correlated with leaf petiole length ($r_{LAA\ vs\ LPL}$ = -0.31, $P \leq 0.05$) and leaf height ($r_{LAA\ vs\ LH}$ = -0.48; $P \leq 0.05$). Growth habit and number of primary branches were not related significantly with any other variable. Overall, there were significant positive correlations observed within variables of same phenotypic category (flower, leaf, or raceme characteristics), but not across the phenotypic variables of different category.

Principal Component Analysis and Dendrogram: Fig 3.6 and Table 3.11 explains the result of Principal component analysis. Accession PserTU57/53 was not included. The first principal component (PC1) explains only 29.5% of the total variation and the second principal component (PC2) explains only 14%. The variables raceme length, flower length, leaf area and leaf height were related to PC1. A scatter plot was prepared for PC1 and PC2 showing the relationships between the accessions based on studied morphological variables (Fig 3.6). An increase in the value for raceme length, flower length, leaf area and leaf height were seen in the accessions from left to right on the PC1. The decrease in the value of the number of primary branches was observed in the accessions along with the negative to positive line of PC2. Also, the increase in the leaf area, leaf width and basal angle was seen along with the negative to the positive slope of PC2. Khadivi et al. (2019) also reported similar results while studying morphological characteristics of sweet cherry, sour cherry and duke cherry from Iran where PC1 was related with leaf height and PC2 with tree growth vigor and branching.

A dendrogram was made based on Ward's minimum variance method using studied morphological traits (Fig 3.7). The dendrogram classified the accessions according to the groups which are most similar. The accessions were differentiated into five main clusters (Fig 3.7). The

first cluster included eight accessions like PserCH101, PserCH112, PserCO14 and PserTU67 which are associated with higher flower measurement values. The second cluster included accessions related to higher raceme measurement values like PserCH114, PserCH137, PserCH135, PserCO01 and PserTU75. The third cluster includes accessions like PserCO31, PserCO16 and PserTU41. This cluster was related with the accessions having higher values of leaf measurements and basal angle. The four and fifth cluster included accessions like PserCH104, PserCH119, PserCO22, PserCO08, PserTU71, PserTU81. They were clustered mainly based on variables like apex angle, growth habit and number of primary branches. The clusters identified were not consistent with the provinces of origin for the accessions used in this study. The results from our study could be due to the self-incompatibility and outcrossing nature of these species. Rakonjac V. et al. (2014) also found similar results while studying morphological variability in wild cherry from Central Serbia.

Characterization and variability of the morphological data is important for selection of the desired accessions in any new breeding program. Significant variability for the different characteristics studied were found among the different capuli accessions. The results of this study can be useful for future capuli breeding programs. The capuli as a tree is useful in several ways and have numerous benefits. Apart from growing capuli for its high-quality fruit, the use of its timber and other medicinal benefits is known (Ibarra-Alvarado et al., 2009). Our study confirmed a diverse morphological variation present for leaf, flower, and plant characteristics within the accessions collected in Ecuador. No unique populations of *P. serotina* subsp. *capuli* could be differentiated using these morphological characteristics. Capuli holds a commercial potential in Ecuador, and it is an interesting species to be evaluated in the U.S. The proper use of

scientific information and our preliminary data generated supports capuli as an interesting species that could be used locally and internationally.

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Table 3.1. List of *Prunus serotina* subsp. *capuli* accessions collected in the Andes region of Ecuador growing in University of Georgia, Griffin, USA.

Collection (No.)	Accession ID[z]	Country	Province	Latitude	Longitude
1	PserCO01	Ecuador	Cotopaxi	1°7'36.1"S	78°35'21.2"W
8	PserCO08	Ecuador	Cotopaxi	1°0'54"S	78°36'31"W
9	PserCO09	Ecuador	Cotopaxi	0°58'38.9"S	78°38'16.1"W
13	PserCO13	Ecuador	Cotopaxi	0°53'2"S	78°39'15.7"W
14	PserCO14	Ecuador	Cotopaxi	0°53'22.9"S	78°37'30.7"W
16	PserCO16	Ecuador	Cotopaxi	0°53'44.7"S	78°37'5"W
21	PserCO21	Ecuador	Cotopaxi	0°59'2.5"S	78°36'6.3"W
22	PserCO22	Ecuador	Cotopaxi	0°59'2.1"S	78°36'5.6"W
26	PserCO26	Ecuador	Cotopaxi	1°5'50.9"S	78°36'10.2"W
31	PserCO31	Ecuador	Cotopaxi	1°6'23.3"S	78°36'28.1"W
41	PserTU41	Ecuador	Tungurahua	1°24'8.9"S	78°38'2.2"W
43	PserTU43	Ecuador	Tungurahua	1°23'26"S	78°37'25.8"W
48	PserTU48	Ecuador	Tungurahua	1°21'5"S	78°36'45.2"W
53	PserTU53	Ecuador	Tungurahua	1°18'49.9"S	78°38'19"W
57	PserTU57	Ecuador	Tungurahua	1°17'49.3"S	78°37'14.1"W
67	PserTU67	Ecuador	Tungurahua	1°18'49"S	78°32'46.9"W
70	PserTU70	Ecuador	Tungurahua	1°20'2.3"S	78°33'51.8"W
71	PserTU71	Ecuador	Tungurahua	1°19'23.1"S	78°34'19.1"W
75	PserTU75	Ecuador	Tungurahua	1°18'54.6"S	-
77	PserTU77	Ecuador	Tungurahua	1°21'11.3"S	78°34'58.5"W
81	PserTU81	Ecuador	Tungurahua	1°25'53.6"S	78°30'57.1"W
86	PserCH86	Ecuador	Chimborazo	1°31'45.1"S	78°30'0"W
90	PserCH90	Ecuador	Chimborazo	1°32'45.1"S	78°31'3.2"W
94	PserCH94	Ecuador	Chimborazo	1°34'59.7"S	78°32'17.4"W
98	PserCH98	Ecuador	Chimborazo	1°37'44.9"S	78°34'57.7"W
99	PserCH99	Ecuador	Chimborazo	1°37'44.9"S	78°34'57.7"W
101	PserCH101	Ecuador	Chimborazo	1°37'3.3"S	78°35'49.3"W
104	PserCH104	Ecuador	Chimborazo	1°36'24"S	78°38'25.2"W
108	PserCH108	Ecuador	Chimborazo	1°35'45.3"S	78°40'56.8"W
110	PserCH110	Ecuador	Chimborazo	1°35'21.2"S	78°41'17.3"W
112	PserCH112	Ecuador	Chimborazo	-	-
113	PserCH113	Ecuador	Chimborazo	-	-
114	PserCH114	Ecuador	Chimborazo	1°38'1.3"S	78°40'40.7"W
119	PserCH119	Ecuador	Chimborazo	1°39'32.9"S	78°42'5.2"W
123	PserCH123	Ecuador	Chimborazo	1°39'36.1"S	78°44'9.6"W
127	PserCH127	Ecuador	Chimborazo	1°42'19"S	78°41'31.6"W
132	PserCH132	Ecuador	Chimborazo	1°41'47"S	78°38'39"W
135	PserCH135	Ecuador	Chimborazo	1°43'38.1"S	78°38'59.3"W

Table 3.1. *Continued.*

Collection (No.)	Accession ID[z]	Country	Province	Latitude	Longitude
137	PserCH137	Ecuador	Chimborazo	1°43’32.3”S	78°39’46.3”W
138	PserCH138	Ecuador	Chimborazo	1°39’14”S	78°40’48.6”W
142	PserCH142	Ecuador	Chimborazo	1°38’46.4”S	78°42’27.2”W
145	PserCH145	Ecuador	Chimborazo	1°37’25.2”S	78°43’30.8”W
147	PserCH147	Ecuador	Chimborazo	1°38’51.2”S	78°41’46.6”W
153	PserCH153	Ecuador	Chimborazo	1°38’49.2”S	78°35’14.8”W

[z]ID= first letter represented the genus (*Prunus*=P), next three letters represented the species (*serotina*=ser), and the following letters represented the province of origin in Ecuador and collection number (Chimborazo collection 01=CH01).

Table 3.2. List of variables and their units used for the morphological characterization of *P. serotina* subsp. *capuli* accessions grown in Griffin, GA.

Variable	Abbreviation	Units
Flower count on a raceme	FC	Number
Flower length	FL	Mm
Flower pedicel length	FPL	mm
Flower width	FW	mm
Leaf apex angle	LAA	degrees
Leaf area	LA	mm^2
Leaf basal angle	LBA	degrees
Leaf height	LH	Mm
Leaf petiole length	LPL	Mm
Leaf width	LW	Mm
No. of basal leaves on a raceme	BLN	Number
No. of 1^{st} order branches on the tree	NPB	Number
No. of racemes on a branch	NRB	Number
Raceme length	RL	cm
Tree Growth habit	GH	Scale (9-1)

Table 3.3. ANOVA for branching and tree growth variable for OP seedlings of *P. serotina* subsp. *capuli* accessions grown at the University of Georgia, Griffin Campus, Griffin, GA in seasons 2019 and 2020.

Effect	No. 1st order branches	Growth habit
Accession	0.0121	0.0003
Year	<.0001	<.0001
Accession * Year	0.3124	0.0539

Table 3.4. ANOVA for raceme and flower variables for OP seedlings of *P. serotina* subsp. *capuli* from Ecuador grown at the University of Georgia, Griffin Campus, Griffin, GA in seasons 2019 and 2020.

Effect	No. of Racemes per branch	Raceme length	No. of Basal leaves per raceme	No. of Flowers per raceme	Flower length	Flower width	Flower pedicel length
Accession	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Year	0.0072	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Accession * Year	<.0001	<.0001	<.0001	0.0013	<.0001	<.0001	<.0001

Table 3.5. ANOVA for leaf variables for OP seedlings of *P. serotina* subsp. *capuli* from Ecuador grown at the University of Georgia, Griffin Campus, Griffin, GA in seasons 2019 and 2020.

Effect	Leaf area	Leaf width	Leaf height	Petiole length	Apex angle	Basal angle
Accession	0.0004	0.0017	<.0001	0.0484	0.0004	0.0003
Year	<.0001	<.0001	<.0001	<.0001	<.0001	0.1474
Accession * Year	<.0001	0.0019	<.0001	0.0272	0.1520	0.0540

Table 3.6. Branching and tree growth variable for OP seedlings of *P. serotina* subsp. *capuli* accessions grown at the University of Georgia, Griffin Campus, Griffin, GA in seasons 2019 and 2020.

Accession ID[z]	No. 1st order branches		Growth habit[x]	
	2019	2020	2019	2020
PserCH101	45.4 a-c	38.4 a	5 a	7.4 ab
PserCH104	37.6 a-c	26.6 a	5.8 a	6.6 ab
PserCH108	28.6 bc	25.4 a	5.4 a	5 ab
PserCH110	30.8 bc	21.8 a	6.2 a	7 ab
PserCH112	47.2 a-c	25.6 a	4.2 a	4.6 ab
PserCH113	67.4 a	41.8 a	6.6 a	6.6 ab
PserCH114	45.4 a-c	26 a	5.8 a	5.8 ab
PserCH119	45 a-c	22.3 a	6.3 a	5.7 ab
PserCH123	38.7 a-c	16.7 a	5 a	6 ab
PserCH127	40.8 a-c	24.8 a	5 a	5.8 ab
PserCH132	41.8 a-c	24 a	4.2 a	5.8 ab
PserCH135	36 a-c	20.4 a	5.4 a	5 ab
PserCH137	36.6 a-c	28 a	6.2 a	6.2 ab
PserCH138	44.8 a-c	31.8 a	4.6 a	4.6 ab
PserCH142	50 a-c	39.8 a	6.2 a	6.2 ab
PserCH145	51.4 a-c	36.2 a	6.6 a	7.4 ab
PserCH147	35.4 a-c	21.8 a	4.6 a	6.2 ab
PserCH153	28.8 bc	18.6 a	5.4 a	6.6 ab
PserCH86	40.6 a-c	21.6 a	5.4 a	7.4 ab
PserCH90	41.2 a-c	25 a	5 a	6.2 ab
PserCH94	53.6 a-c	28.2 a	4.6 a	6.2 ab
PserCH98	43.8 a-c	26 a	6.6 a	7 ab
PserCH99	21.6 c	21.8 a	6.6 a	7.4 ab
PserCO01	47.2 a-c	33 a	4.6 a	5.4 ab
PserCO08	40 a-c	20.2 a	5.4 a	6.2 ab
PserCO09	38.8 a-c	25.4 a	4.2 a	3.4 ab

Table 3.6. *Continued.*

Accession ID[z]	No. 1st order branches		Growth habit[x]	
	2019	2020	2019	2020
PserCO13	55.4 ab	32.8 a	4.6 a	4.6 ab
PserCO14	37.6 a-c	27 a	6.6 a	5 ab
PserCO16	41.2 a-c	29 a	7.4 a	6.6 ab
PserCO21	30.8 bc	15.8 a	5 a	3.4 ab
PserCO22	31.8 bc	30.6 a	6.2 a	6.2 ab
PserCO26	37.6 a-c	18.6 a	4.6 a	5.8 ab
PserCO31	47 a-c	32 a	3.4 a	3.4 ab
PserTU41	29.9 bc	20 a	5.2 a	5 ab
PserTU43	50 a-c	25.2 a	5 a	6.2 ab
PserTU48	30 bc	19 a	3 a	4.2 ab
PserTU53	49.7 a-c	27.7 a	5 a	9 a
PserTU57	33.8 bc	28.6 a	3.8 a	5 ab
PserTU57/53	52.6 a-c	33.2 a	3.4 a	3 b
PserTU67	39.8 a-c	22.6 a	6.6 a	7 ab
PserTU70	42.6 a-c	35 a	5 a	7 ab
PserTU71	38.2 a-c	27.6 a	5.8 a	7 ab
PserTU75	40.2 a-c	24.8 a	4.6 a	5 ab
PserTU77	41 a-c	20.7 a	5 a	7.7 ab
PserTU81	40.6 a-c	24.6 a	5.4 a	6.6 ab

[z]ID= first letter represented the genus (*Prunus*=P), next three letters represented the species (*serotina*=ser), and the following letters represented the province of origin in Ecuador and collection number (Chimborazo collection 01=CH01).
[y]Different letters within a column indicate significant difference between genotypes using LSD test at $P \leq 0.05$.
[x]Growth habit scale: 1(leaning) -5(neither upright nor leaning)- 9 (upright).

Table 3.7. Flower variables for OP seedlings of *P. serotina* subsp. *capuli* accessions grown at the University of Georgia, Griffin Campus, Griffin, GA in seasons 2019 and 2020.

Accession ID[z]	Flower length (mm)		Flower width (mm)		Flower pedicel length (mm)	
	2019	2020	2019	2020	2019	2020
PserCH101	9.1 b-e[y]	8.6 a	4.1 c-g	3.8 a-c	4.6 b-d	3.5 a-c
PserCH104	6.2 kl	7.4 ab	3.1 j	3.6 a-c	2.2 j-l	2.8 a-f
PserCH108	7.1 i-l	7.5 ab	3.3 ij	3.8 a-c	2.6 i-l	2.7 a-f
PserCH110	9.1 b-f	6.8 ab	4.2 b-f	3.4 bc	3.2 f-k	2.5 a-f
PserCH112	11.2 a	8.0 a	4.7 ab	3.3 c	5.6 a	3.2 a-e
PserCH113	10.4 ab	9.1 a	4.5 a-d	4.3 a	4.1 b-g	3.5 a-c
PserCH114	8.9 c-g	7.2 ab	4.6 a-d	3.4 c	3.6 c-h	3.2 a-e
PserCH119	7.2 g-l	- -	4.3 b-f	- -	2.1 j-l	- -
PserCH123	8.1 e-k	5.5 ab	4.1 b-g	2.5 cd	2.7 h-l	2.7 a-f
PserCH127	8.7 d-g	9.1 a	3.9 d-g	4.2 ab	3.1 h-k	3.5 a-c
PserCH132	9.7 a-e	8.2 a	4.2 b-g	3.7 a-c	3.5 e-i	3.1 a-e
PserCH135	9.5 b-e	8.0 a	4.8 ab	3.9 a-c	3.3 e-i	2.9 a-f
PserCH137	8.6 d-g	7.9 a	4.7 ab	3.7 a-c	3.6 d-i	3.0 a-f
PserCH138	9.1 b-f	7.9 a	4.5 a-d	3.7 a-c	3.0 h-k	2.6 a-f
PserCH142	- -	7.7 ab	- -	3.4 c	- -	3.3 a-d
PserCH145	5.7 l	7.1 ab	3.0 j	3.5 bc	2.0 l	2.2 c-f
PserCH147	9.3 b-e	7.2 ab	3.7 f-i	3.6 a-c	4.2 b-e	2.7 a-f
PserCH153	8.4 d-i	8.4 a	4.2 b-g	3.8 a-c	2.9 h-l	3.3 a-d
PserCH86	7.1 h-l	7.9 a	2.9 j	3.6 a-c	3.2 h-k	2.7 a-f
PserCH90	- -	6.1 ab	- -	3.4 c	- -	2.2 d-f
PserCH94	9.3 b-e	7.9 a	4.8 ab	3.2 c	3.7 c-h	3.5 a-c
PserCH98	6.5 j-l	7.5 ab	3.3 ij	3.7 a-c	2.4 i-l	2.8 a-f
PserCH99	- -	7.9 a	- -	3.7 a-c	- -	2.8 a-f
PserCO01	8.2 e-j	6.8 ab	3.9 e-h	3.7 a-c	2.5 i-l	2.1 ef
PserCO08	8.6 d-h	7.8 ab	3.3 h-j	3.3 c	3.9 c-h	3.5 a-c
PserCO09	8.4 d-i	7.8 a	4.0 d-g	3.6 a-c	3.3 e-j	2.5 a-f

Table 3.7. *Continued.*

Accession ID[z]	Flower length(mm)		Flower width(mm)		Flower pedicel length(mm)	
	2019	2020	2019	2020	2019	2020
PserCO13	9.3 b-e	5.2 b	4.6 a-c	2.2 d	3.9 c-h	2.5 a-f
PserCO14	10.2 a-c	8.4 a	4.1 b-g	3.6 a-c	4.6 bc	3.8 ab
PserCO16	8.2 e-j	8.1 a	4.6 a-c	3.9 a-c	2.6 i-l	2.9 a-f
PserCO21	8.3 e-j	9.1 a	4.4 b-e	3.9 a-c	2.9 h-l	3.4 a-c
PserCO22	6.8 j-l	8.5 a	2.9 j	3.3 c	3.1 g-k	4.1 a
PserCO26	7.7 f-k	8.4 a	3.2 ij	3.8 a-c	3.2 f-j	3.6 ab
PserCO31	9.0 b-f	8.1 a	5.0 a	4.1 a-c	3.2 g-k	2.9 a-f
PserTU41	9.4 b-e	8.8 a	3.9 d-h	3.6 a-c	5.2 ab	3.9 ab
PserTU43	8.5 d-i	8.2 a	4.0 d-g	3.7 a-c	3.5 e-i	3.1 a-e
PserTU48	7.6 g-k	8.7 a	3.1 j	4.2 ab	3.4 e-i	3.7 ab
PserTU53	8.3 d-j	6.6 ab	4.2 b-g	3.1 cd	3.6 c-i	2.8 a-f
PserTU57	6.8 j-l	8.5 a	3.1 j	3.9 a-c	2.7 h-l	3.4 a-c
PserTU57/53	9.1 b-e	8.2 a	3.9 d-g	3.6 a-c	3.5 e-i	2.9 a-f
PserTU67	10.2 a-d	9.1 a	4.2 b-f	3.9 a-c	4.6 bc	3.9 ab
PserTU70	9.9 a-e	8.7 a	4.1 c-g	3.6 bc	4.2 b-f	3.1 a-e
PserTU71	6.9 j-l	7.0 ab	3.4 g-j	3.6 a-c	2.7 h-l	2.0 f
PserTU75	8.9 c-g	6.9 ab	4.3 b-f	3.7 a-c	3.3 e-j	2.5 b-f
PserTU77	6.4 j-l	7.6 ab	3.4 g-j	3.8 a-c	2.0 kl	2.5 b-f
PserTU81	6.3 kl	8.7 a	3.1 j	4.3 a	2.3 i-l	2.9 a-f

(-) Denotes no data for that accession for that year.

[z]ID= first letter represented the genus (*Prunus*=P), next three letters represented the species (*serotina*=ser), and the following letters represented the province of origin in Ecuador and collection number (Chimborazo collection 01=CH01).

[y]Different letters within a column indicate significant difference between genotypes using LSD test at $P \leq 0.05$.

Table 3.8. Raceme variables for OP seedlings of *P. serotina* subsp. *capuli* accessions grown at the University of Georgia, Griffin Campus, Griffin, GA in seasons 2019 and 2020.

Accession	No. of racemes per branch		Flower count		No. of Basal leaves on raceme		Raceme length (cm)	
ID[z]	2019	2020	2019	2020	2019	2020	2019	2020
PserCH101	2.3 h	5.3 a-d[y]	15.5 fg	17.3 a	2.9 d-f	4.4 a	13.7 e-h	14.2 ab
PserCH104	2.5 h	4.7 a-d	17.3 fg	17.9 a	5.1 a-d	5.0 a	13.3 e-h	13.5 ab
PserCH108	3.4 hg	9.1 a-d	19.4 b-g	14.6 a	4.4 b-e	4.5 a	15.2 a-h	11.9 ab
PserCH110	1.3 h	5.4 a-d	18.3 e-g	18.6 a	2.5 ef	4.5 a	16.1 a-f	15.4 ab
PserCH112	3.1 h	6.1 a-d	19.7 b-g	16.1 a	2.2 f	5.5 a	14.5 b-h	13.4 ab
PserCH113	9.9 b-f	6.0 a-d	24.9 a-f	20.1 a	4.4 b-e	4.6 a	19.2 a-e	15.9 ab
PserCH114	9.9 b-e	6.8 a-d	25.3 a-e	18.5 a	5.3 a-c	5.1 a	16.4 a-f	14.8 ab
PserCH119	1.4 h	4.7 a-d	12.9 g	12.0 a	2.0 f	5.0 a	8.1 h	13.1 ab
PserCH123	0.7 h	- -	19.2 b-g	- -	3.2 c-f	- -	15.2 a-h	- -
PserCH127	3.2 h	8.5 a-d	20.7 b-g	17.1 a	3.9 b-f	4.5 a	17.4 a-f	15.1 ab
PserCH132	1.9 h	4.5 b-d	16.4 fg	14.8 a	2.7 d-f	4.3 a	12.0 f-h	12.3 ab
PserCH135	13.3 a-c	7.8 a-d	26.7 a-c	23.2 a	5.5 a-c	4.2 a	19.4 a-d	12.8 ab
PserCH137	8.1 c-h	7.8 a-d	28.1 ab	22.2 a	5.7 a-c	5.8 a	18.2 a-e	15.9 ab
PserCH138	2.3 h	4.3 cd	16.8 fg	18.1 a	2.1 f	5.2 a	10.4 gh	14.7 ab
PserCH142	- -	7.8 a-d	- -	19.0 a	- -	5.1 a	- -	13.6 ab
PserCH145	4.1 f-h	4.8 a-d	18.7 d-g	17.3 a	4.7 a-d	5.0 a	14.0 e-h	11.8 ab
PserCH147	6.0 d-h	4.8 a-d	24.7 a-f	15.9 a	4.3 b-f	4.6 a	16.1 a-f	10.7 ab
PserCH153	1.9 h	8.3 a-d	20.6 b-g	19.0 a	2.8 d-f	4.8 a	14.4 b-h	14.6 ab
PserCH86	3.7 gh	5.4 a-d	24.9 a-f	19.1 a	4.4 b-e	4.3 a	14.3 c-h	14.6 ab
PserCH90	- -	5.5 a-d	- -	14.9 a	- -	4.6 a	- -	10.1 b
PserCH94	2.9 h	5.8 a-d	14.9 fg	15.3 a	2.3 f	5.0 a	11.2 f-h	12.5 ab
PserCH98	6.3 c-h	4.8 a-d	19.8 b-g	12.7 a	5.1 a-d	4.1 a	16.1 a-f	9.7 b
PserCH99	- -	5.4 a-d	- -	21.7 a	- -	4.9 a	- -	14.9 ab
PserCO01	9.1 b-g	6.7 a-d	20.9 b-g	22.2 a	3.7 b-e	4.3 a	15.2 a-h	13.5 ab
PserCO08	5.5 e-h	7.8 a-d	21.0 b-g	14.0 a	5.1 a-d	5.0 a	17.1 a-f	12.1 ab

Table 3.8. *Continued.*

Accession	No. of racemes per branch		Flower count		No. of Basal leaves on raceme		Raceme length (cm)	
ID[z]	2019	2020	2019	2020	2019	2020	2019	2020
PserCO09	10.6 b-e	8.1 a-d	21.9 a-g	19.7 a	4.7 a-d	4.4 a	19.7 ab	15.0 ab
PserCO13	18.5 a	8.3 a-d	20.3 b-g	13.7 a	5.0 a-d	4.0 a	15.6 a-g	10.6 ab
PserCO14	10.7 b-e	5.7 a-d	24.5 a-f	19.1 a	3.9 b-f	4.5 a	16.3 a-f	14.2 ab
PserCO16	13.2 a-c	10.0 a-c	26.5 a-d	23.2 a	6.4 a	5.0 a	18.2 a-e	12.6 ab
PserCO21	11.7 b-d	7.6 a-d	22.6 a-f	21.5 a	4.7 a-d	4.2 a	15.1 a-h	16.1 ab
PserCO22	6.1 d-h	5.2 a-d	19.2 b-g	18.3 a	5.2 a-c	4.8 a	15.8 a-g	15.9 ab
PserCO26	3.1 h	7.2 a-d	22.4 a-f	18.1 a	4.8 a-d	4.7 a	14.3 b-h	14.9 ab
PserCO31	14.4 ab	11.4 a	24.1 a-f	19.8 a	4.6 a-d	4.3 a	17.2 a-f	14.0 ab
PserTU41	2.1 h	5.2 a-d	21.1 b-g	18.1 a	5.6 a-c	6.0 a	20.8 a	17.8 a
PserTU43	7.6 c-h	8.3 a-d	24.7 a-f	15.9 a	4.1 b-f	4.8 a	15.8 a-f	15.0 ab
PserTU48	4.0 gh	4.7 a-d	18.5 e-g	20.4 a	5.9 ab	4.3 a	18.7 a-e	16.1 ab
PserTU53	6.2 c-h	9.3 a-d	25.0 a-f	19.2 a	5.2 a-d	5.3 a	17.8 a-f	11.4 ab
PserTU57	3.8 gh	3.4 d	19.1 c-g	16.7 a	4.1 b-f	3.6 a	14.8 a-h	11.8 ab
PserTU57/ 53	6.1 d-h	10.6 ab	22.4 a-f	20.1 a	5.3 a-c	5.5 a	15.9 a-f	13.6 ab
PserTU67	1.7 h	8.3 a-d	23.5 a-f	18.3 a	4.5 a-d	5.9 a	19.6 a-c	14.6 ab
PserTU70	2.5 h	5.7 a-d	21.4 b-g	18.7 a	3.6 c-f	4.5 a	14.2 d-h	12.2 ab
PserTU71	6.6 c-h	6.4 a-d	19.3 b-g	18.9 a	4.4 b-e	4.9 a	17.1 a-f	13.2 ab
PserTU75	5.3 e-h	4.3 b-d	30.7 a	20.9 a	4.3 b-f	4.3 a	20.8 a	13.6 ab
PserTU77	2.2 h	5.7 a-d	21.8 a-g	15.8 a	3.6 b-f	5.1 a	12.9 e-h	13.4 ab
PserTU81	2.7 h	4.7 a-d	16.2 fg	12.7 a	4.3 b-f	5.7 a	14.5 b-h	13.9 ab

(-) Denotes no data for that accession for that year.

[z]ID= first letter represented the genus (*Prunus*=P), next three letters represented the species (*serotina*=ser), and the following letters represented the province of origin in Ecuador and collection number (Chimborazo collection 01=CH01).

[y]Different letters within a column indicate significant difference between genotypes using LSD test at $P \leq 0.05$.

Table 3.9. Leaf variables for OP seedlings of *P. serotina* subsp. *capuli* accessions grown at the University of Georgia, Griffin Campus, Griffin, GA in seasons 2019 and 2020.

Accession ID[z]	Leaf width (mm)		Leaf height (mm)		Leaf petiole length (mm)		Leaf area (mm^2)		Apex angle (°)		Basal angle (°)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
PserCH101	24.0 ab[y]	26.2 ab	86.4 ab	85.1 ab	8.0 ab	9.8 a	1158.1 b	1161.4 ab	21.1 a	22.2 bc	87.0 a	89.3 ab
PserCH104	23.8 ab	16.1 c	97.5 ab	63.0 bc	10.9 ab	6.7 a	1226.4 b	591.0 b	20.2 a	31.3 a-c	75.6 a	90.7 ab
PserCH108	26.4 ab	21.9 a-c	91.5 ab	63.1 bc	6.3 b	5.9 a	1400.6 ab	786.5 b	24.6 a	38.9 a-c	98.3 a	84.9 ab
PserCH110	24.0 ab	21.2 a-c	93.2 ab	74.7 a-c	11.3 ab	5.6 a	1185.3 b	856.8 ab	23.0 a	20.8 bc	86.1 a	73.4 b
PserCH112	26.9 ab	19.6 a-c	104.1 ab	67.5 bc	12.9 ab	5.5 a	1480.5 ab	707.9 b	24.7 a	23.7 bc	81.2 a	92.9 ab
PserCH113	22.7 ab	21.6 a-c	95.2 ab	76.3 a-c	7.5 ab	8.3 a	1207.5 b	905.6 ab	22.4 a	27.8 bc	81.2 a	85.5 ab
PserCH114	25.4 ab	17.3 bc	94.6 ab	63.6 bc	9.0 ba	6.4 a	1294.3 ab	599.3 b	17.9 a	28.2 bc	79.7 a	72.7 b
PserCH119	24.2 ab	18.7 a-c	92.5 ab	51.2 c	10.2 ab	5.0 a	1206.7 b	460.3 b	20.6 a	29.8 a-c	86.1 a	89.1 ab
PserCH123	24.3 ab	20.2 a-c	86.6 ab	72.1 a-c	7.9 ab	7.4 a	1176.2 b	835.1 ab	25.2 a	36.4 a-c	80.2 a	92.5 ab
PserCH127	23.2 ab	23.0 a-c	97.7 ab	70.9 bc	10.7 ab	9.3 a	1205.3 b	658.2 b	21.3 a	23.2 bc	79.9 a	78.3 ab
PserCH132	22.5 ab	22.6 a-c	82.2 b	58.6 bc	7.8 ab	5.6 a	1035.9 b	726.7 b	26.6 a	56.5 a	80.9 a	91.5 ab
PserCH135	25.6 ab	19.6 a-c	87.8 ab	68.8 bc	7.4 ab	6.0 a	1243.2 b	716.6 b	29.1 a	27.6 bc	97.4 a	86.2 ab
PserCH137	23.4 ab	20.1 a-c	94.4 ab	80.9 a-c	8.9 ab	6.1 a	1176.0 b	913.4 a	20.6 a	25.6 bc	75.7 a	75.7 ab
PserCH138	25.2 ab	22.4 a-c	86.2 ab	72.5 a-c	10.6 ab	6.4 a	1169.3 b	904.2 ab	25.1 a	26.1 bc	110.3 a	89.5 ab
PserCH142	29.3 ab	24.9 a-c	88.2 ab	71.1 bc	8.9 ab	7.5 a	1439.5 ab	881.4 ab	27.2 a	23.7 bc	98.2 a	93.3 ab
PserCH145	21.9 ab	18.5 a-c	80.7 b	64.7 bc	8.5 ab	5.0 a	949.2 b	697.7 b	22.0 a	39.7 a-c	104.3 a	89.2 ab
PserCH147	24.6 ab	17.5 bc	85.6 ab	57.5 bc	9.6 ab	5.5 a	1124.5 b	606.5 b	21.7 a	32.9 a-c	93.8 a	84.6 ab
PserCH153	25.0 ab	18.6 a-c	98.8 ab	71.9 a-c	9.5 ab	6.2 a	1379.1 ab	728.4 b	21.5 a	21.8 bc	90.4 a	80.8 ab
PserCH86	23.5 ab	21.1 a-c	88.8 ab	73.2 a-c	9.4 ab	6.4 a	1105.1 b	874.2 ab	22.7 a	25.8 bc	84.3 a	87.6 ab
PserCH90	23.5 ab	22.3 a-c	89.8 ab	64.2 bc	10.0 ab	6.8 a	1154.2 b	769.5 b	25.7 a	31.4 a-c	90.7 a	99.4 ab
PserCH94	21.1 ab	19.9 a-c	78.6 b	73.3 a-c	7.4 ab	8.4 a	931.6 b	725.8 b	17.8 a	19.9 c	89.2 a	81.6 ab
PserCH98	26.4 ab	21.0 a-c	85.2 ab	67.7 bc	9.1 ab	7.3 a	1309.3 ab	825.2 ab	27.6 a	24.6 bc	95.8 a	98.0 ab
PserCH99	24.8 ab	24.9 a-c	98.5 ab	77.0 a-c	10.9 ab	7.3 a	1333.2 ab	999.4 ab	22.0 a	20.9 bc	91.4 a	104.2 ab
PserCO01	25.3 ab	20.1 a-c	93.5 ab	62.1 bc	7.5 ab	5.6 a	1277.5 ab	593.8 b	20.0 a	29.8 a-c	89.6 a	77.7 ab
PserCO08	27.6 ab	17.9 bc	102.1 ab	65.5 bc	12.0 ab	4.7 a	1523.8 ab	639.8 b	20.0 a	19.0 c	96.0 a	90.5 ab
PserCO09	22.8 ab	22.3 a-c	94.8 ab	83.3 a-c	8.2 ab	6.2 a	1241.7 b	1036.3 ab	21.1 a	20.0 c	80.9 a	99.3 ab
PserCO13	22.6 ab	17.2 bc	81.9 b	58.6 bc	8.5 ab	4.8 a	999.5 b	556.5 b	32.2 a	23.2 bc	84.7 a	87.8 ab

Table 3.9. *Continued.*

Accession ID[z]	Leaf width (mm)		Leaf height (mm)		Leaf petiole length (mm)		Leaf area (mm^2)		Apex angle (°)		Basal angle (°)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
PserCO14	25.3 ab	19.3 a-c	96.0 ab	75.7 a-c	9.5 ab	7.3 a	1340.4 ab	733.8 b	17.5 a	23.0 bc	85.8 a	80.8 ab
PserCO16	24.6 ab	25.4 a-c	97.1 ab	75.7 a-c	9.2 ab	7.8 a	1322.6 ab	1061.0 ab	23.1 a	23.8 bc	82.0 a	105.8 ab
PserCO21	25.6 ab	24.0 a-c	94.1 ab	81.1 a-c	12.5 ab	7.4 a	1295.3 ab	1090.1 ab	23.3 a	27.3 bc	93.4 a	85.6 ab
PserCO22	23.8 ab	21.3 a-c	87.0 ab	67.1 bc	8.8 ab	5.6 a	1118.8 b	762.2 b	29.1 a	28.4 bc	88.4 a	89.4 ab
PserCO26	22.0 ab	17.6 bc	85.4 ab	67.5 bc	6.1 b	4.2 a	1083.0 b	698.8 b	28.6 a	24.7 bc	88.6 a	83.2 ab
PserCO31	24.2 ab	28.1 a	97.0 ab	100.4 a	10.4 ab	9.1 a	1279.6 ab	1569.7 a	22.9 a	24.0 bc	92.9 a	109.7 a
PserTU41	30.4 a	19.5 a-c	112.9 a	68.7 bc	11.8 ab	6.6 a	2022.7 a	759.4 b	22.9 a	27.2 bc	93.3 a	74.2 ab
PserTU43	26.9 ab	22.7 a-c	98.0 ab	73.5 a-c	10.8 ab	7.9 a	1409.3 ab	882.2 ab	23.1 a	30.4 a-c	97.2 a	94.0 ab
PserTU48	25.8 ab	21.1 a-c	100.1 ab	62.3 bc	10.3 ab	5.0 a	1401.9 ab	794.9 b	30.9 a	44.9 ba	98.5 a	104.1 ab
PserTU53	26.3 ab	16.3 bc	105.3 ab	63.5 bc	14.3 a	5.1 a	1411.2 ab	608.9 b	22.2 a	26.8 bc	96.9 a	84.9 ab
PserTU57	21.2 ab	19.6 a-c	88.8 ab	66.3 bc	9.6 ab	6.1 a	991.5 b	725.4 b	18.4 a	24.9 bc	89.4 a	90.0 ab
PserTU57/53	25.4 ab	24.7 a-c	102.8 ab	83.4 a-c	12.1 ab	8.75 a	1362.3 ab	1026.7 ab	22 a	20.6 bc	103.1 a	92.8 ab
PserTU67	23.0 ab	22.3 a-c	80.3 b	78.3 a-c	8.1 ab	8.0 a	1025.7 b	962.9 ab	26.6 a	22.3 bc	97.5 a	90.0 ab
PserTU70	22.5 ab	20.4 a-c	84.3 ab	63.1 bc	11.3 ab	8.7 a	1034.7 b	677.1 b	25.4 a	28.3 bc	103.8 a	80.4 ab
PserTU71	19.4 b	22.5 a-c	79.9 b	79.9 a-c	7.2 ab	10.4 a	873.8 b	940.2 ab	24.0 a	24.6 bc	82.7 a	86.4 ab
PserTU75	20.8 ab	23.6 a-c	79.0 b	81.6 a-c	9.2 ab	5.7 a	917.6 b	1102.9 ab	22.4 a	22.7 bc	83.9 a	76.3 ab
PserTU77	28.0 ab	20.9 a-c	101.3 ab	69.9 bc	9.8 ab	6.9 a	1610.3 ab	656.5 b	21.6 a	23.9 bc	88.8 a	81.7 ab
PserTU81	25.8 ab	22.8 a-c	86.8 ab	69.1 bc	9.5 ab	5.1 a	1223.7 b	915.0 ab	31.1 a	29.0 bc	102.7 a	94.2 ab

(-) denotes no data for that accession for that year

[z]ID= first letter represented the genus (*Prunus*=P), next three letters represented the species (*serotina*=ser), and the following letters represented the province of origin in Ecuador and collection number (Chimborazo collection 01=CH01).

[y]Different letters within a column indicate significant difference between genotypes using LSD test at $P \leq 0.05$.

Table 3.10. Multivariate analysis of all flower, leaf and plant growth characteristics averaged across seasons 2019 and 2020 for OP seedlings of *P. serotina* subsp. *capuli* accessions grown at the University of Georgia, Griffin Campus, Griffin, GA.

	NRB*	FC	BLN	RL	FL	FW	FPL	LPL	LA	LW	LH	LAA	LBA	NPB	GH
NRB*	1[x]														
	<.0001[y]														
FC	0.491	1													
	0.0007	<.0001													
BLN	0.4029	0.3998	1												
	0.0067	0.0072	<.0001												
RL	0.2491	0.6801	0.4428	1											
	0.103	<.0001	0.0026	<.0001											
FL	0.1334	0.3139	-0.0507	0.4494	1										
	0.3881	0.038	0.7438	0.0022	<.0001										
FW	0.3144	0.3405	-0.1228	0.2585	0.6483	1									
	0.0377	0.0237	0.4273	0.0902	<.0001	<.0001									
FPL	0.0457	0.1858	0.1293	0.453	0.8132	0.2096	1								
	0.7683	0.2273	0.4027	0.002	<.0001	0.172	<.0001								
LPL	0.0326	0.0702	0.039	0.0919	0.2534	0.2581	0.2022	1							
	0.8334	0.6505	0.8014	0.5528	0.0969	0.0908	0.188	<.0001							
LA	0.2215	0.2727	0.3034	0.4769	0.2468	0.3124	0.2221	0.4385	1						
	0.1484	0.0733	0.0453	0.0011	0.1063	0.039	0.1474	0.0029	<.0001						
LW	0.1502	0.0777	0.1598	0.2029	0.182	0.2554	0.0959	0.3978	0.8373	1					
	0.3305	0.6159	0.3	0.1866	0.237	0.0943	0.5356	0.0075	<.0001	<.0001					
LH	0.2851	0.4313	0.2324	0.5671	0.3162	0.4123	0.2386	0.5695	0.8428	0.553	1				
	0.0606	0.0035	0.129	<.0001	0.0365	0.0054	0.1188	<.0001	<.0001	<.0001	<.0001				
LAA	-0.1702	-0.2331	0.0079	-0.1459	-0.1568	-0.2086	-0.1293	-0.373	-0.1791	0.0036	-0.4816	1			
	0.2693	0.1278	0.9595	0.3446	0.3093	0.1741	0.4028	0.0126	0.2448	0.9816	0.0009	<.0001			
LBA	0.0913	-0.2039	0.1602	-0.2159	-0.1076	-0.0848	-0.1298	0.128	0.296	0.4426	0.0105	0.3037	1		
	0.5554	0.1844	0.2989	0.1593	0.4869	0.584	0.4009	0.4078	0.0511	0.0026	0.9461	0.045	<.0001		
NPB	0.2737	-0.0798	-0.0735	-0.2173	0.0822	0.142	0.0365	0.0414	-0.1795	-0.0617	-0.1338	-0.062	0.0384	1	
	0.0722	0.6066	0.6354	0.1566	0.5957	0.3577	0.814	0.7897	0.2436	0.6906	0.3867	0.6892	0.8047	<.0001	
GH	-0.1916	0.0082	0.2472	-0.1222	-0.2058	-0.2104	-0.156	0.1116	-0.1508	-0.0632	-0.1019	-0.1824	-0.0975	0.138	1
	0.2127	0.958	0.1057	0.4295	0.1802	0.1704	0.312	0.4707	0.3284	0.6834	0.5106	0.236	0.5291	0.3719	<.0001

[x]Correlation coefficient *r*.

[y]*P* value.

*Abbreviations used: Flower count on a raceme (FC), Flower length (FL), Flower pedicel length (FPL), Flower width (FW), Leaf apex angle (LAA), Leaf area (LA), Leaf basal angle (LBA), Leaf height (LH), Leaf petiole length (LPL),

Leaf width (LW), No. of basal leaves on a raceme (BLN), No. of 1st order branches on the tree (NPB), No. of racemes on a branch (NRB), Raceme length (RL), Tree Growth habit (GH).

Table 3.11. Eigenvectors for first 6 PCs for OP seedlings of *P. serotina* subsp. *capuli* accessions morphological characters grown at the Dempsey Farm in Griffin Campus, Griffin, GA.

Morphological characters	PC1	PC2	PC3	PC4	PC5	PC6
No. of racemes on a branch	0.21292	-0.0446	0.27005	0.11098	0.56609	-0.19682
No. of flowers on a raceme	0.29371	-0.2265	0.37955	0.05303	0.0631	-0.14623
No. of basal leaves on raceme	0.17201	0.11647	0.55462	0.0876	0.06096	0.35291
Raceme length	0.34516	-0.18411	0.26395	0.18391	-0.23239	0.03786
Flower length	0.29935	-0.327	-0.33695	0.15956	0.01359	0.27594
Flower width	0.27841	-0.17966	-0.28584	0.01465	0.25812	-0.23867
Flower pedicel length	0.24457	-0.2934	-0.21033	0.17248	-0.13958	0.51504
Leaf petiole length	0.24455	0.18178	-0.22981	-0.40795	-0.02979	0.12955
Leaf area	0.38066	0.34956	-0.03158	0.02834	-0.11003	-0.05686
Leaf width	0.27993	0.45134	-0.14257	0.05275	0.01359	0.0458
Leaf height	0.41128	0.13824	0.00089	-0.19216	-0.11402	-0.19284
Apex angle	-0.18333	0.1584	-0.00793	0.57772	0.01419	0.18739
Basal angle	0.01394	0.50877	-0.08906	0.24048	0.23668	0.20463
No. of primary branches	-0.0318	-0.10269	-0.12151	-0.17766	0.6698	0.25694
Growth habit	-0.07557	0.03755	0.26339	-0.5078	-0.02278	0.46176
Eigenvalue	4.4191	2.0958	1.759	1.5344	1.398	1.1155
%Variance	29.461	13.972	11.727	10.229	9.32	7.437
Cumulative%	29.461	43.432	55.159	65.389	74.709	82.145

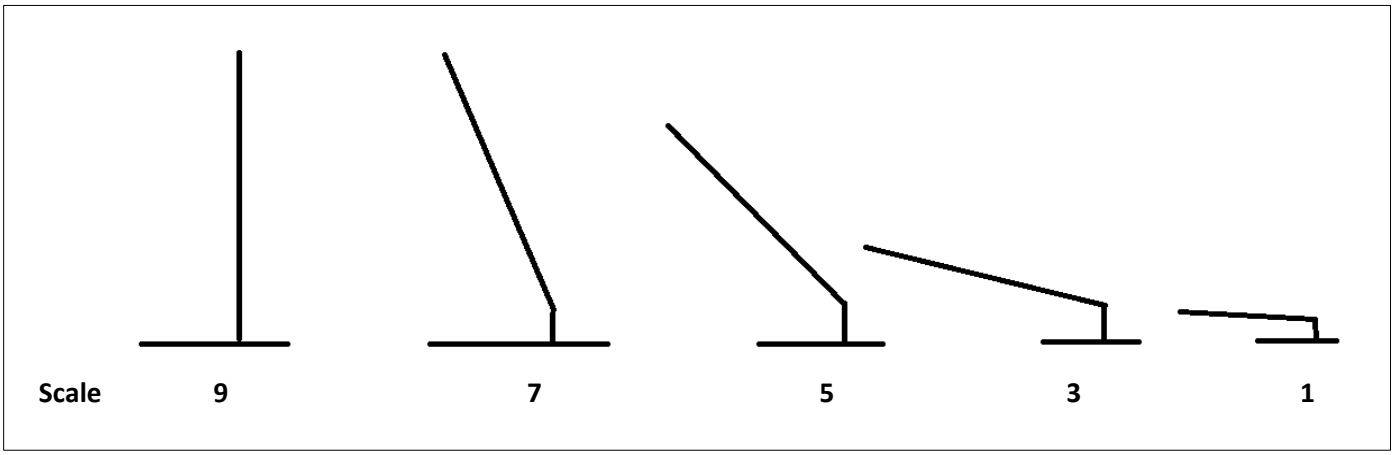

Fig 3.1. Graphical representation of the plant growth habit scale.

Fig. 3.2. A Capuli raceme with flowers.

Fig. 3.3. Racemes on the branch of a capuli tree.

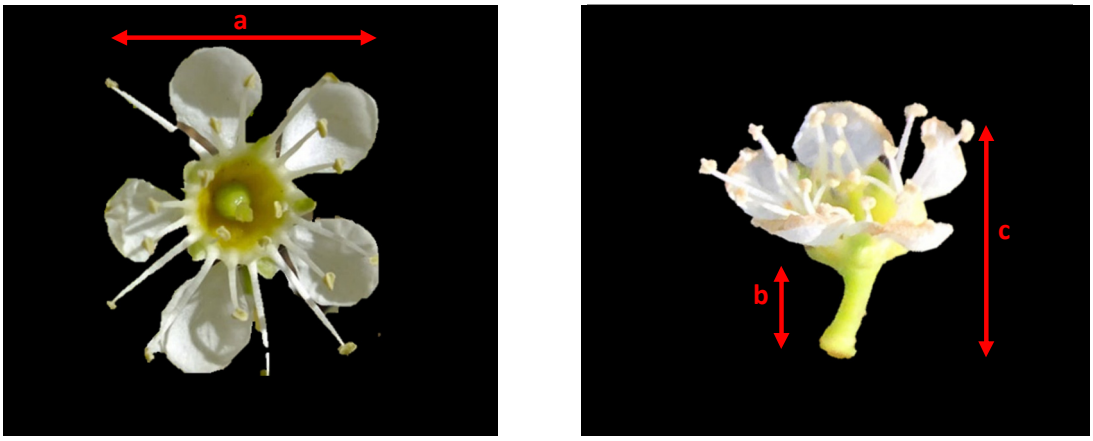

Fig 3.4. Open flower of *P. serotina* subsp. *capuli*. Flower measurements were taken for a) Flower width, b) Flower pedicel length and c) Flower length (mm).

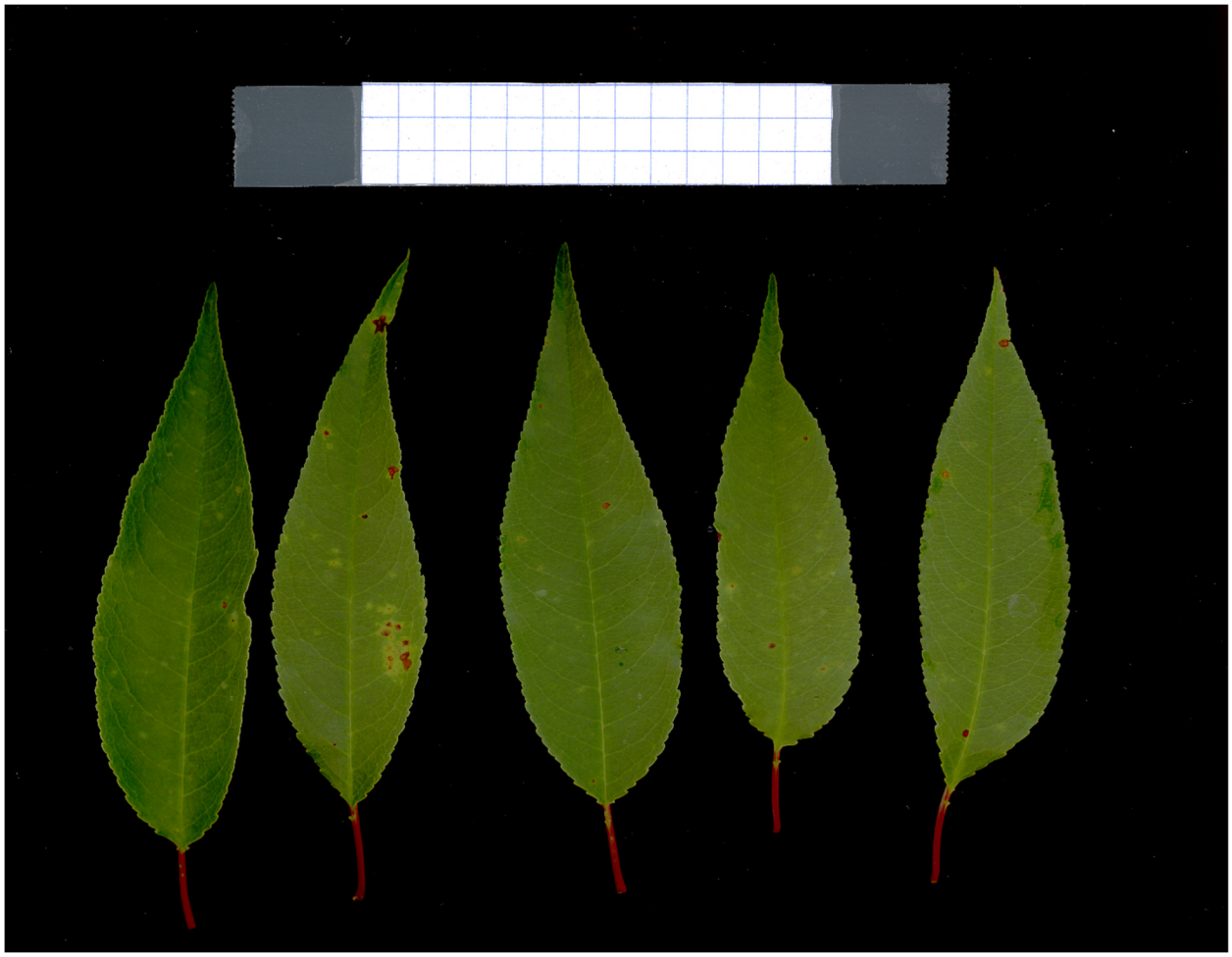

Fig 3.5. *Prunus serotina* subsp. *capuli* leaves. Measurements taken using ImageJ software. A graph paper was used as a scale reference - 5 mm.

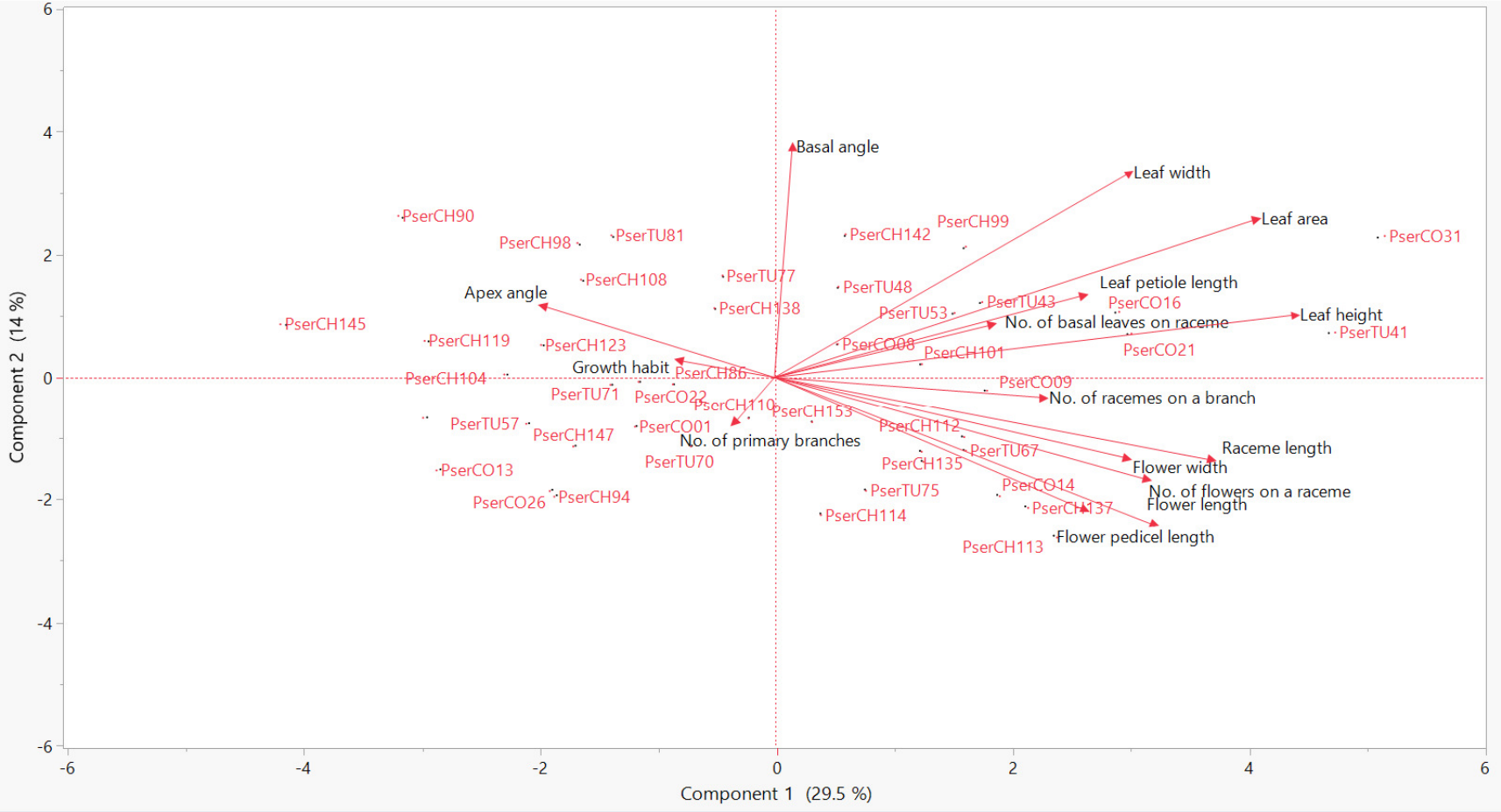

Fig 3.6. Principal component analyses for plant, leaf, and flower morphological characters of *P. serotina* subsp. *capuli* accessions grown at the University of Georgia Dempsey Farm, Griffin, GA.

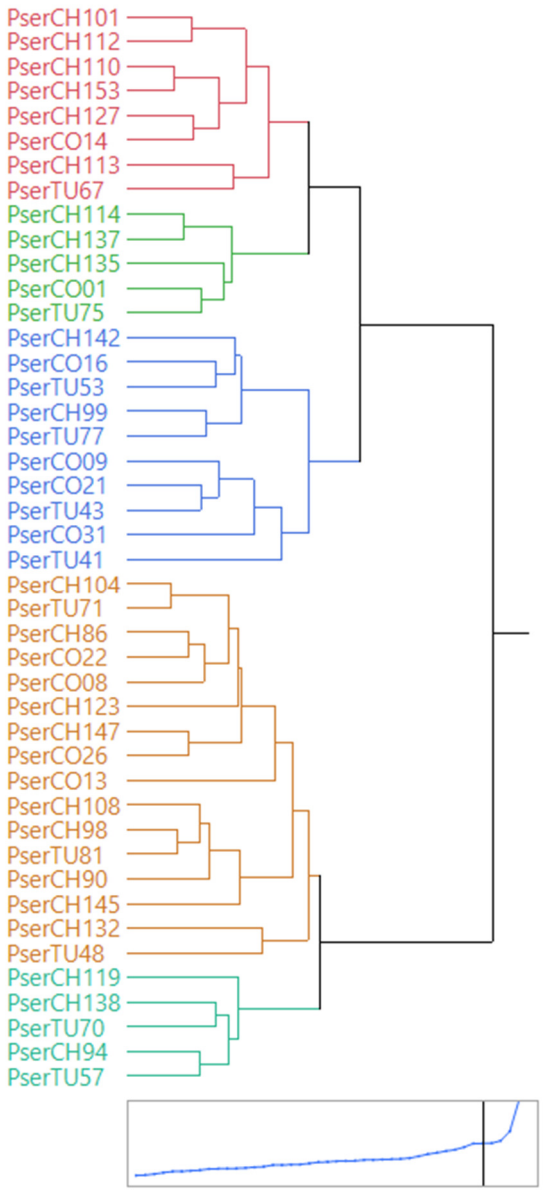

Fig 3.7. Dendrogram based on the Ward's minimum variance method for the studied *P.serotina* subsp. *capuli* accessions based on morphological characters.

CHAPTER 4

GENOME SIZE AND CHROMOSOME COUNT OF *PRUNUS SEROTINA* SUBSP. *CAPULI*[3]

[3]Pathania, S. and D.J. Chavez. To be submitted to *HortScience*

Abstract

The genus *Prunus* belong to the Rosaceae family which includes economically important fruit crops like apple, sweet cherries, peaches and almonds. *Prunus serotina*, also called black cherry, is an important timber species in the US. *P. serotina* subsp. *capuli* is a unique subsp. out of the five subsp. of *P. serotina* due to its large and tasty fruits. The goal of this study was to determine the genome size and chromosome number of *Prunus serotina* subsp. *capuli* OP seedlings originated from three provinces of Ecuador (Chimborazo, Cotopaxi and Tungurahua) and currently growing at the UGA Griffin campus, GA. Genome size of the capuli was estimated to be around 2C DNA = 1.0 pg with average 2C DNA content = 1.04 pg and ploidy to be tetraploid using flow cytometer. No significant differences were found for the genome size of capuli from different provinces. Chromosome counts of capuli confirmed 2n = 32 through meiotic analysis of flower buds.

Introduction

The Rosaceae family is important economically and is the 19th largest family of plants (Stevens, 2001). It includes approximately 90 genera and 3000 species (Potter et al., 2003). The genus *Prunus* belongs to the family Rosaceae, subfamily Spiraeoideae and tribe Amygdaleae (Potter et al., 2007). Important fruit crops like peach, cherries, plum and apricot are found in this genus. Sweet cherry (*Prunus avium*), sour cherry (*Prunus cerasus*), duke cherry (hybrid between sour and sweet cherry), and black cherry (*Prunus serotina*) are all commonly called cherries (Hummer and Janick, 2009). The total world production for cherries and sour cherries in 2019 was 2.5 million tons and 1.4 million tons, respectively (FAO, 2019).

Prunus serotina (black cherry) is the largest native cherry tree. The black cherry tree is also known as wild black cherry, rum cherry and mountain black cherry (Marquis, 1990). It is

recognized for its commercial value as its wood is used for making furniture (Hough, 1960). The wood has a beautiful red color and is ranked high among valued fine hardwood (Hummer and Janick, 2009). *Prunus serotina* is divided into five subsp.: *P. serotina* subsp. *serotina*, *P. serotina* subsp. *hirsutus*, *P. serotina* subsp. *virens*, *P. serotina* subsp. *eximia* and *P. serotina* subsp. *capuli* (McVaugh, 1951). *Prunus serotina* subsp. *capuli,* also called capuli cherry, is native to Central and South America (McVaugh, 1951).

The genus *Prunus* chromosome base number is x = 8 (Darlington, 1928). *Prunus persica* (peach) and *Prunus avium* (sweet cherry) are diploid (2n= 2x=16). *Prunus fruticosa* (ground cherry) is tetraploid (2n=4x=32). *Prunus cerasus* (sour cherry) is a species formed by the natural hybridization between *P. avium* and *P. fruticosa*. It is an allotetraploid species with 2n = 4x = 32 (Baird et al., 1994; Dirlewanger et al., 2009; Pozzi and Vecchietti, 2009). The ploidy level for *P. serotina* has been reported to be tetraploid (2n = 32) (Stairs and Hauck, 1968). However, other ploidy levels – 4x, 5x and 6x have also been described in literature by Dickson et al. (1992).

Counting the number of chromosomes is the standard method for ploidy determination of a species but it is time consuming (Stebbins, 1971). Flow cytometry can be used for estimation of genome size content in plants. It measures the fluorescence intensity of the nuclei stained with DNA specific fluorochrome to give an estimate of the DNA content (Dolezel et al., 2007). C-value refers to the DNA content in an organism with an unreplicated haploid chromosome complement (Swift, 1950).

The goal of this study was to determine the genome size and chromosome number of *Prunus serotina* subsp. *capuli* OP seedlings originated from three provinces of Ecuador and currently growing at the UGA Griffin campus, GA. The knowledge of the genome size and chromosome count of capuli will aid in future breeding of capuli.

Materials and methods

Plant material: Fruit from 44 genotypes of *P. serotina* subsp. *capuli* growing in 3 provinces (Chimborazo, Cotopaxi and Tungurahua) in the Andes region of Ecuador were collected in 2016. Each genotype was given a unique ID based on their collection site. Plant collection permit MAE-DNB-CM-2019-0107 from the Minister of Environment of Ecuador was used for access and use of these genetic resources. Genotypes were selected to represent a broad geographical range in the region. At least 100 ripe fruits were collected in plastic bags and stored in a cooler with ice after collection. Endocarps were washed and left to dry for 48 h in paper towels. Seed lots of 50 seed per accession were transported to the Peach Research and Extension program at the University of Georgia, Griffin Campus, Griffin, GA, USA following procedures from the USDA seed importation permit P37-16-00098. Leftover seed per accession were kept by collaborators at the Escuela Politécnica del Chimborazo (ESPOCH).

Seeds were imbibed in water for 4 d, with water being replaced every 24 h. Seeds then were transferred to a Captan solution for 24 h. For stratification and germination, seeds were placed in perlite moistened with Captan in a refrigerator at 4 °C. Once seed started to germinate, seeds were planted in 50- cell trays (A.M. Leonard, Piqua, OH) filled with a 3:1 germination mix media and perlite. Osmocote slow-release fertilizer (15-9-12) 5-6 month was added to the media prior planting (approximately ½ cup per 4 gallons of media). Seedlings were grown and maintained in a greenhouse until being transplanted in the 2017 season. The seedlings were labelled throughout the process and seedling lots /accessions were given the same ID as their mother plants (Table 4.1). OP seedlings were then transplanted directly to the field at the Dempsey Research Farm, Griffin, GA. They were planted in a high-density nursery.

Flow Cytometer: The nuclear DNA content was estimated using young expanding leaves from OP seedlings from accessions collected in Ecuador and growing at the University of Georgia, Griffin Campus, Griffin, GA. The first samples were collected in early February when *P. serotina* subsp. *capuli* accessions began to break vegetative buds and leaf development occurred (Ramírez and Davenport, 2016). Initially, five genotypes per accession were analyzed. When no differences were observed among the five genotypes, then replicates were reduced to three per accession. Around mid -March, the experiment was stopped due to Covid-19 lockdown. It was then resumed in late June. Afterward, further pooling of the three genotypes into one sample were made to save time and resources for the remaining accessions. The samples with N = 1 and 2 were the ones which were pooled. Pooled samples with threshold of < 3000 G_1 – phase nuclei were run again and if needed individually (Table 4.2). The leaves were collected in the morning and were stored in a cooler with ice. They were transported to the lab and kept in ice until processing. Commercial peach (*Prunus persica*) rootstock cultivar 'Guardian ™' (2C = 0.6 pg) was used as internal standard (Baird et al., 1994). Equal parts of the sample and the reference standard were chopped following the procedure included with the Cystain™ PI Absolute P kit (Sysmex Corporation, Japan) as follows. Staining solution was prepared using 20 mL staining buffer, 120 µL propidium iodide and 60 µL RNAse stock solution for 10 samples. Approx. 0.5 cm^2 or less of leaf tissue of the sample and the reference standard (peach) were added to a petri dish. A total of 500µl nuclei extraction buffer were added to the petri dish. A sharp razor blade was then used to finely chop both samples for 30-60 sec. Then samples were incubated at room temperature (20-25°C) for 30 - 90 s. Samples were then filtered through a 50 µm CellTrics™ filter (Sysmex Corporation, Japan) into a sample tube. Two mL of staining solution were added to the solution. The solution was incubated for 60 minutes protected from light at room

temperature (20-25°C). The filtered solution was immediately analyzed in the CyFlow® Ploidy Analyzer with the CyView™ v.1.6 software (Sysmex Corporation, Japan) for genome size determination. The histogram obtained for each sample run contained the following measurements: Part (number of particles in the detected peak area), mean (arithmetic mean of the signal) and coefficient of variation (CV%).

The formula used to calculate DNA content of each capuli run was:

$$Capuli\ 2C\ DNA = \frac{Mean\ fluorescene\ of\ capuli}{Mean\ fluorescence\ of\ peach} \times standard\ 2C\ DNA\ peach$$

Chromosome count: Racemes from OP seedlings from representative accessions of *P. serotina* subsp. *capuli* were collected during late February and March 2020. Racemes were collected just after growth became apparent and flowers were undifferentiated. Racemes were placed in a Farmer's fixative for 24 h. They were later transferred to 70% ethanol and were kept at 4°C. For chromosome count, anthers from individual flower buds were dissected and placed on a glass slide. A drop of acetocarmine stain was added to the slide with anthers carefully placed then a coverslip was carefully added. A gentle tapping was done to squash the anthers with a metal pen. Slides were sealed and they were visualized using the Zeiss Axio Vert.A1 microscope (Carl Zeiss Microscopy, Jena, Germany) at 40X and 100X magnification.

Statistical Analysis

Mean 2C DNA values were calculated for all samples. Standard deviation was calculated whenever two or more replicates were run per accession. Analysis of variance using PROC GLM was used to identify effects per accession and province in SAS 9.4 (SAS Institute Inc.; Cary,

NC). A mean 2C DNA value per provinces was also calculated. Mean comparisons were done using Tukey's HSD test with a significance level of $P \leq 0.05$.

Results and discussions

A total of 3000-5000 G_1 – phase nuclei were analyzed per sample for each accession, with some samples going below the threshold of < 3000 G_1 – phase nuclei. A detailed description of the results can be found in Table 4.2. The coefficient of variation was less than 5% for all samples. No data could be collected for accessions PserCH123 and PserCH138 due to no leaves present on the tree at the time of the experiment. Peach (*P. persica*) was used as the diploid (2*x*=16) reference for the experiment. The 2C DNA content of peach was previously reported as 0.60 pg DNA (Baird et al., 1994). Fig. 4.1 represents the histogram of DNA content with two peaks associated with nuclei present in the G1 of the standard Peach and *P. serotina* subsp. *capuli* sample. In Fig. 4.1, peak a) Represents the fluorescence of the Peach standard and peak b) Represents the relative fluorescence of the capuli sample (PserCH137).

Our results showed that the genome size values for *P. serotina* subsp. *capuli* accessions from Ecuador ranged from 1.01 to 1.06 pg with the average value of 1.04 pg (Table 4.2). Our results supported that these accessions are tetraploid. No significant differences were found among the accessions and their province of collection when the analysis of the variance was performed. The ploidy level of all the accessions from all the provinces were the same. Mean DNA content values as presented based on the three provinces of origin were also calculated (Table 4.3). The means of the three provinces: Cotopaxi, Chimborazo, and Tungurahua, were not significantly different from each other. The mean 2C DNA values for all the provinces were 1.04 pg.

Chromosome count: The meiotic analysis of *P. serotina* subsp. *capuli* genotypes confirmed a tetraploid level (2n = 32) (Fig. 4.2). Not all the accessions were examined for the meiotic analysis. Accessions were randomly chosen when the anthesis started and examined under the microscope. All stages of meiosis were found while examining the cells. Our study focused on confirming the chromosome count of the capuli. Most of the PMCs targeted were in Prophase I, Metaphase I, and Metaphase II of meiosis, where we can see all the 32 chromosomes or 16 bivalents or 16 univalents (Table 4.4). Our results are consistent with Stairs and Hauck (1968). They reported the chromosome count of the *P. serotina* to be 2n=32.

The internal standardization involving analysis of both plant sample (whose genome size is to be determined) and standard plant material is necessary for the genome size calculation (Dolezel et al., 2007; Roux et al., 2003). Both the sample and standard plant could differ in AT/GC ratio. Propidium iodide (PI) inserts into the DNA without any prejudice for nucleotide bases whereas DAPI prefers AT nucleotides. Therefore, nuclei staining with PI is advised for genome size calculation (Dolezel et al., 2007). Félix et al. (2018) estimated the genome size of the capuli from Mexico to be around 1C = 0.5 pg. This value is same as reported by Dickson et al. (1992) for *P. serotina* species. In our study, we reported similar values. Therefore, it can be proposed that there are no differences in the capuli ploidy level in Ecuador and with respect to the geographic location. The average genome size value found in our study for capuli was 2C= 1.0 pg. We propose capuli to be tetraploid. Other ploidy levels, 5x and 6x were reported by Dickson et al. (1992). However, no other ploidy levels or deviation from 4x ploidy was seen in the capuli accessions during our experiment.

In Fig. 4.1, the G_1 peak representing the capuli accessions was not exactly the double of the G_1 peak of the peach diploid standard. The reason could be the differences in the genome size

of the peach and capuli. Genome size differences in the plants have been attributed to the amount of repetitive DNA (Flavell et al., 1974). Activity of the retrotransposons is one of the main factors for different genome sizes found in the same plant family (Bennetzen et al., 2005). Bonos et al. (2002) reported the ploidy level in six *Agrotis* species, where tetraploid species DNA content was not exactly double of diploid species DNA content. The authors proposed that this could be due to genome size deviation among the species or removal of the repetitive DNA from the higher ploidy level plant.

No information about the ancestors or the parent species of capuli have been previously reported (Shi et al., 2013). Shi et al. (2013) conducted the phylogenetic analyses to understand the genetic relationships among the species included under genus *Prunus*. *Prunus serotina* was placed in a different subgenus from the peach (*P. persica*).

Knowledge of ploidy level in plant species is necessary for breeding and selection, and for genetic studies (Egesi et al., 2002). The results of this experiment confirmed that *P. serotina* subsp. *capuli* from Ecuador to be tetraploid with a genome size of 2C = 1.0 pg. It was also confirmed that *P. serotina* subsp. *capuli* to be tetraploid with 2n = 32 through meiotic analysis. This is the first experiment where genome size and chromosome count of the *P. serotina* subsp. *capuli* from Ecuador is reported.

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Table 4.1. List of *Prunus serotina* subsp. *capuli* accessions collected in the Andes region of Ecuador growing in UGA, Griffin campus, Griffin, GA.

Collection (No.)	Accession ID[z]	Country	Province	Latitude	Longitude
1	PserCO01	Ecuador	Cotopaxi	1°7'36.1"S	78°35'21.2"W
8	PserCO08	Ecuador	Cotopaxi	1°0'54"S	78°36'31"W
9	PserCO09	Ecuador	Cotopaxi	0°58'38.9"S	78°38'16.1"W
13	PserCO13	Ecuador	Cotopaxi	0°53'2"S	78°39'15.7"W
14	PserCO14	Ecuador	Cotopaxi	0°53'22.9"S	78°37'30.7"W
16	PserCO16	Ecuador	Cotopaxi	0°53'44.7"S	78°37'5"W
21	PserCO21	Ecuador	Cotopaxi	0°59'2.5"S	78°36'6.3"W
22	PserCO22	Ecuador	Cotopaxi	0°59'2.1"S	78°36'5.6"W
26	PserCO26	Ecuador	Cotopaxi	1°5'50.9"S	78°36'10.2"W
31	PserCO31	Ecuador	Cotopaxi	1°6'23.3"S	78°36'28.1"W
41	PserTU41	Ecuador	Tungurahua	1°24'8.9"S	78°38'2.2"W
43	PserTU43	Ecuador	Tungurahua	1°23'26"S	78°37'25.8"W
48	PserTU48	Ecuador	Tungurahua	1°21'5"S	78°36'45.2"W
53	PserTU53	Ecuador	Tungurahua	1°18'49.9"S	78°38'19"W
57	PserTU57	Ecuador	Tungurahua	1°17'49.3"S	78°37'14.1"W
67	PserTU67	Ecuador	Tungurahua	1°18'49"S	78°32'46.9"W
70	PserTU70	Ecuador	Tungurahua	1°20'2.3"S	78°33'51.8"W
71	PserTU71	Ecuador	Tungurahua	1°19'23.1"S	78°34'19.1"W
75	PserTU75	Ecuador	Tungurahua	1°18'54.6"S	-
77	PserTU77	Ecuador	Tungurahua	1°21'11.3"S	78°34'58.5"W
81	PserTU81	Ecuador	Tungurahua	1°25'53.6"S	78°30'57.1"W
86	PserCH86	Ecuador	Chimborazo	1°31'45.1"S	78°30'0"W
90	PserCH90	Ecuador	Chimborazo	1°32'45.1"S	78°31'3.2"W
94	PserCH94	Ecuador	Chimborazo	1°34'59.7"S	78°32'17.4"W
98	PserCH98	Ecuador	Chimborazo	1°37'44.9"S	78°34'57.7"W
99	PserCH99	Ecuador	Chimborazo	1°37'44.9"S	78°34'57.7"W
101	PserCH101	Ecuador	Chimborazo	1°37'3.3"S	78°35'49.3"W
104	PserCH104	Ecuador	Chimborazo	1°36'24"S	78°38'25.2"W
108	PserCH108	Ecuador	Chimborazo	1°35'45.3"S	78°40'56.8"W
110	PserCH110	Ecuador	Chimborazo	1°35'21.2"S	78°41'17.3"W
112	PserCH112	Ecuador	Chimborazo	-	-
113	PserCH113	Ecuador	Chimborazo	-	-
114	PserCH114	Ecuador	Chimborazo	1°38'1.3"S	78°40'40.7"W
119	PserCH119	Ecuador	Chimborazo	1°39'32.9"S	78°42'5.2"W
127	PserCH127	Ecuador	Chimborazo	1°42'19"S	78°41'31.6"W
132	PserCH132	Ecuador	Chimborazo	1°41'47"S	78°38'39"W
135	PserCH135	Ecuador	Chimborazo	1°43'38.1"S	78°38'59.3"W
137	PserCH137	Ecuador	Chimborazo	1°43'32.3"S	78°39'46.3"W

Table 4.1. *Continued*

Collection (No.)	Accession ID[z]	Country	Province	Latitude	Longitude
142	PserCH142	Ecuador	Chimborazo	1°38'46.4"S	78°42'27.2"W
145	PserCH145	Ecuador	Chimborazo	1°37'25.2"S	78°43'30.8"W
147	PserCH147	Ecuador	Chimborazo	1°38'51.2"S	78°41'46.6"W
153	PserCH153	Ecuador	Chimborazo	1°38'49.2"S	78°35'14.8"W
138	PserCH138	Ecuador	Chimborazo	1°39’14”S	78°40’48.6”W
123	PserCH123	Ecuador	Chimborazo	1°39’36.1”S	78°44’9.6”W

[z]ID= first letter represented the genus (*Prunus*=P), next three letters represented the species (*serotina*=ser), and the following letters represented the province of origin in Ecuador and collection number (Chimborazo collection 01=CH01).

Table 4.2. Genome size of *P. serotina* subsp. *capuli* accessions collected from Ecuador and growing at the University of Georgia, Griffin Campus, Griffin, GA.

Accession ID	N	2C DNA content (pg)[+]	SD	Estimated ploidy
PserCH101[z]	1	1.05		4*x*
PserCH104	1	1.03		4*x*
PserCH108[*]	2	1.05	0.002	4*x*
PserCH110	1	1.02		4*x*
PserCH112	1	1.03		4*x*
PserCH113	5	1.05	0.006	4*x*
PserCH114	5	1.05	0.014	4*x*
PserCH119[*]	1	1.02		4*x*
PserCH127	1	1.02		4*x*
PserCH132[*]	1	1.04		4*x*
PserCH135	5	1.06	0.010	4*x*
PserCH137[*]	3	1.05	0.016	4*x*
PserCH142[*]	2	1.02	0.015	4*x*
PserCH145[*]	2	1.03	0.020	4*x*
PserCH147	1	1.04		4*x*
PserCH153	1	1.05		4*x*
PserCH86	1	1.01		4*x*
PserCH90	1	1.06		4*x*
PserCH94	3	1.03	0.018	4*x*
PserCH98[*]	2	1.03	0.010	4*x*
PserCH99	1	1.03		4*x*
PserCO01	5	1.05	0.020	4*x*
PserCO08	2	1.05	0.001	4*x*
PserCO09	3	1.05	0.004	4*x*
PserCO13	3	1.04	0.023	4*x*
PserCO14	3	1.05	0.015	4*x*
PserCO16[*]	5	1.05	0.016	4*x*
PserCO21	5	1.04	0.005	4*x*
PserCO22	2	1.03	0.001	4*x*
PserCO26	1	1.03		4*x*
PserCO31	5	1.04	0.007	4*x*
PserTU41	1	1.02		4*x*
PserTU43[*]	2	1.04	0.003	4*x*
PserTU48	1	1.03		4*x*
PserTU53	1	1.04		4*x*
PserTU57[*]	3	1.04	0.021	4*x*
PserTU67	1	1.04		4*x*
PserTU70	3	1.03	0.005	4*x*
PserTU71	1	1.03		4*x*
PserTU75	3	1.04	0.006	4*x*
PserTU77	1	1.04		4*x*
PserTU81[*]	3	1.04	0.015	4*x*

[z]ID= first letter represented the genus (*Prunus*=P), next three letters represented the species (*serotina*=ser), and the following letters represented the province of origin in Ecuador and collection number (Chimborazo collection 01=CH01).

[+] Peach was used as a reference standard. 2C DNA = (Mean fluorescence of the capuli sample/Mean fluorescence of peach sample) * standard 2C DNA content of the Peach.

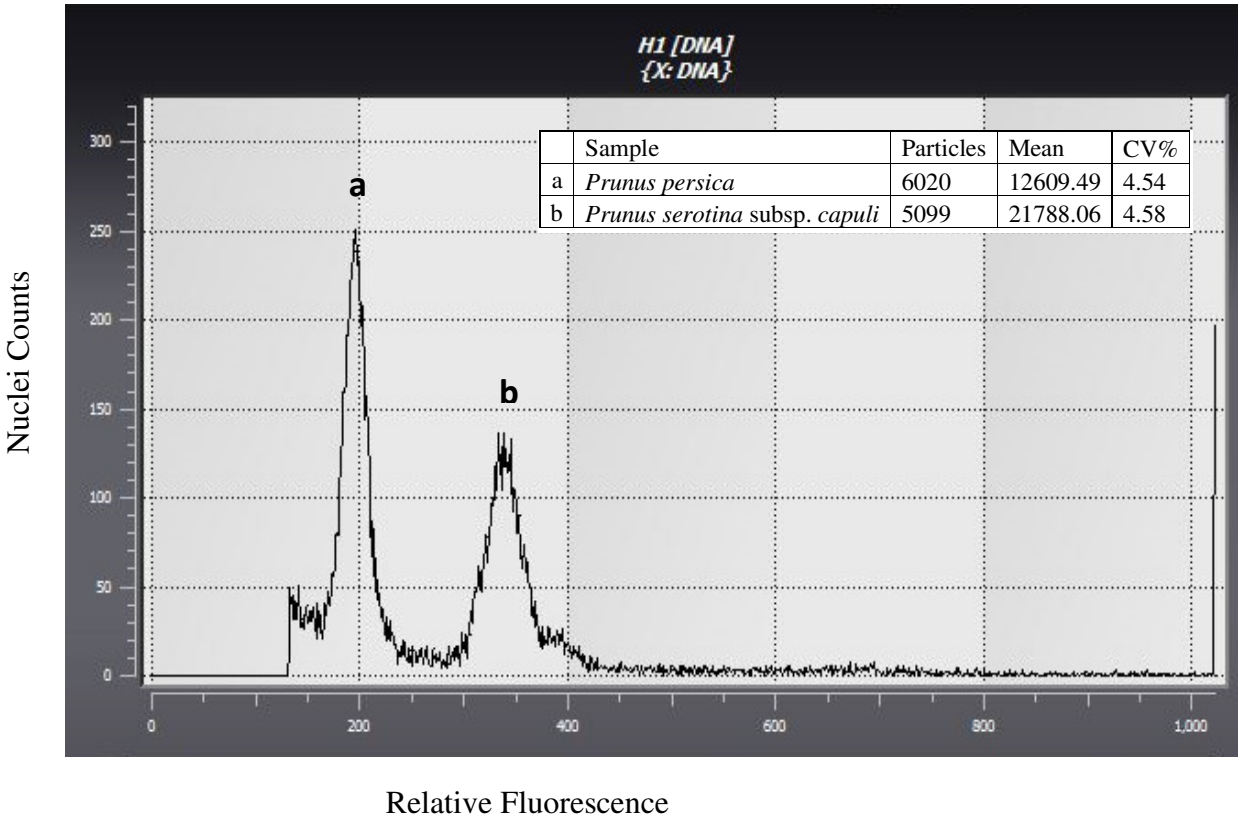
*Samples with run < 3000 nuclei. Otherwise, samples run had 3000-5000 nuclei.

Table 4.3. Genome size of the accessions based on their province of origin in Ecuador.

Province of origin	Accessions (no.)	2C DNA content (pg)	Estimated ploidy level
Cotopaxi	10	1.04	4*x*
Chimborazo	21	1.04	4*x*
Tungurahua	11	1.04	4*x*

Table 4.4. Meiotic analysis of PMCs of *P. serotina* subsp. *capuli* OP seedlings from accessions collected from Ecuador and growing at the University of Georgia, Griffin Campus, Griffin, GA.

Accession	PMCs (no.)			Estimated ploidy level
	Prophase I	Late diakinesis and metaphase I	Prophase II	
	Configuration 32I	Configuration 16II	Assortment 16I	
PserCH112		4	1	4*x*
PserCH127			1	4*x*
PserCH142	2	2		4*x*
PserCH153		2		4*x*
PserCH94	1	1	1	4*x*
PserCO08		1		4*x*
PserCO14		1		4*x*
PserCO22	2	2		4*x*
PserTU53			1	4*x*
PserTU70		3	3	4*x*



	Sample	Particles	Mean	CV%
a	*Prunus persica*	6020	12609.49	4.54
b	*Prunus serotina* subsp. *capuli*	5099	21788.06	4.58

Fig 4.1. Nuclei counts as a function of fluorescence intensity (log scale). a. *Prunus persica*. b. *Prunus serotina* subsp. *capuli* accession PserCH137.

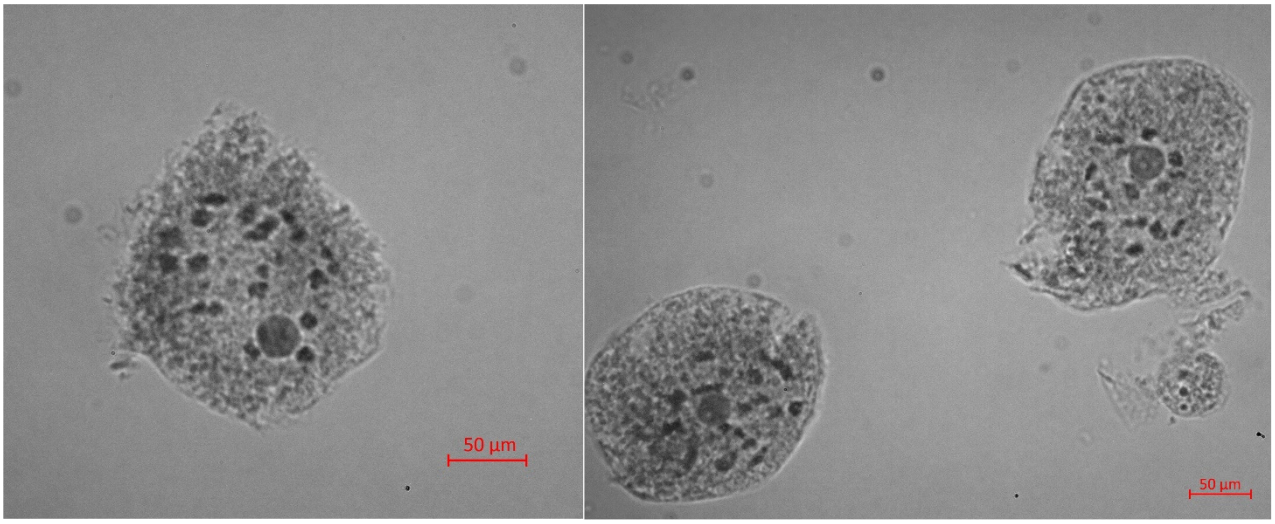


Fig. 4.2. Images representing meiotic analysis of pollen mother cells (PMCs) of *Prunus serotina* subsp. *capuli* PserCH112. PMCs in Prophase I - 16 II (2n =32).

CHAPTER 5

GENETIC DIVERSITY OF *PRUNUS SEROTINA* SUBSP. *CAPULI* ACCESSIONS FROM THREE PROVINCES OF ECUADOR[4]

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Abstract

Prunus serotina (black cherry) is native to North America and is an important timber species. McVaugh (1951) classified *P. serotina* into 5 subsp.: *serotina, eximia, hirsuta, virens and capuli*. *P. serotina* subsp. *capuli* is indigenous to Central and South American countries. The best forms of it are found in Ecuador. Fruits plays an important part in the life of the people, but no commercial varieties exist. The objective of this study was to determine the genetic diversity of representative genotypes of capuli collected in the wild and grown at the University of Georgia Griffin campus from three different provinces of Ecuador: Cotopaxi, Tungurahua and Chimborazo using 36 SSRs markers distributed across peach genome. Observed heterozygosity (Ho) was similar for Chimborazo and Tungurahua (0.4) and less for Cotopaxi (0.3). Wright's Fixation Index (F) was highest for Cotopaxi with 0.33, and 0.2 for Chimborazo and Tungurahua (0.2). AMOVA analysis revealed that most of the genetic diversity is present within the capuli populations. Structure analysis for capuli showed admixture between capuli groups with no clear distinction between any of the populations. The genetic variation along with morphological variation found in this study can be exploited for a future capuli breeding program. Unique morphological and genetic characteristics found in Chimborazo and Tungurahua genotypes demonstrates the potential of this material as diverse capuli germplasm which can be used both in Southeastern USA and Ecuador.

Introduction

Prunus serotina is the largest native cherry tree in America (Marquis, 1990). *Prunus serotina* is present in Canada, USA, Central and South America (Marquis, 1990). *Prunus serotina* is classified in 5 subspecies: *P. serotina* subsp. *serotina*, *P. serotina* subsp. *eximia*, *P. serotina* subsp. *hirsuta*, *P. serotina* subsp. *virens*, *P. serotina* subsp. *capuli* (McVaugh, 1951).

Black cherry has been reported to be an allotetraploid, but diploid, pentaploid and hexaploid plants have been also reported (Dickson et al., 1992; Pairon et al., 2008).

Prunus serotina subsp. *capuli* is indigenous to Central and South American countries. The best forms of it are found in Ecuador (Popenoe and Pachano, 1923). It is found growing in the woods, roadsides, pastures and backyards of the houses (McVaugh, 1951). The capuli is a multipurpose tree used for its fruits, seeds and leaves. Leaves are used for treating respiratory and nervous disorders. Also, capuli wood is used for making furniture (Avendaño-Gómez et al., 2015; Martínez, 1959; Popenoe and Pachano, 1923). Fruits of capuli has higher protein and antioxidant capacity than plums and grapes (Luna-Vazquez et al., 2013). Also, seeds have higher protein content than almonds and peanuts (Garcia-Aguilar et al., 2015).

Molecular markers are known DNA sequences which controls particular trait or gene. They can detect polymorphism among different individuals due to insertion, deletions and translocation at that loci (Mondini et al., 2009). Among all molecular markers, SSRs or microsatellites are the marker of choice because of their co-dominant nature, their abundance in the genome and large number of alleles per locus (Kalia et al., 2010).

Synteny is present in the case of the *Prunus* genome (Dirlewanger et al., 2004). SSR markers developed in one *Prunus* species can be used in other *Prunus* species for genetic diversity studies (Hormaza, 2002). Peach SSR markers are transferable to other *Prunus* species like cherry (Dirlewanger et al., 2002; Wunsch and Hormaza, 2002), apricot (Hormaza, 2002), etc.

Downey and Iezzoni (2000) used SSR markers developed from peach (*P. persica*), sweet cherry (*P. avium*) and sour cherry (*P. cerasus*) to study the genetic diversity of black cherry accessions from Michigan, Mexico and Ecuador. Similarly, Pairon et al. (2008) found genome

specific primers to the ancestral progenitors' genomes of *Prunus serotina*. The authors utilized 67 SSRs from cultivated *Prunus* species with 26 amplifying across samples and only five being genome specific primers from *P. avium* and *P. persica* with disomic inheritance. The study reported the capability of SSRs in differentiating accessions and providing the basis for genetic diversity analysis.

Intriago-Baldeón et al. (2013) studied the genetic diversity of *P. serotina* subsp. *capuli* from 3 provinces of Ecuador: Pichincha, Cañar and Azuay using 8 SSRs markers. Similarly, Guadalupe et al. (2015) found moderate degree of genetic variation while studying genetic diversity in capuli from eight provinces of Ecuador. Guzman et al. (2018) studied the genetic structure of subsp. *virens*, *eximia*, *serotina* and *capuli* using SSRs from different *Prunus* species.

The objective of this study was to determine the genetic diversity of representative genotypes of capuli collected in the wild and grown at the University of Georgia, Griffin campus from three different provinces of Ecuador: Cotopaxi, Tungurahua and Chimborazo. This information can be used to differentiate capuli populations, if present, which can further aid in the selection of superior genotypes for the future breeding programs.

Materials and methods

Germplasm collection: Fruit from 44 genotypes of *P. serotina* subsp. *capuli* growing in the wild in 3 provinces (Chimborazo, Cotopaxi and Tungurahua) in the Andes region of Ecuador were collected in 2016. Each genotype was given a unique ID based on their collection site (Table 5.1). Plant collection permit MAE-DNB-CM-2019-0107 from the Minister of Environment of Ecuador was used for access and use of these genetic resources. Genotypes were selected to represent a broad geographical range in the region. At least 100 ripe fruits were collected in plastic bags per genotype and stored in a cooler with ice. Endocarps were washed

and left to dry for 48 h in paper towels. Seed lots of 50 seed per accession then were transported to the Peach Research and Extension program at the University of Georgia, Griffin Campus, Griffin, GA, USA following procedures from the USDA seed importation permit P37-16-00098. Leftover seed per accession were kept by collaborators at the Escuela Politécnica del Chimborazo (ESPOCH).

Seeds were imbibed in water for 4 d, with water being replaced every 24 h. Seeds then were transferred to a Captan solution for 24 h. For stratification and germination, seeds were placed in perlite moisten with Captan in a fridge at 4 °C. Once seed started to germinate, seeds were planted in 50 cell trays (A.M. Leonard, Piqua, OH) filled with a 3:1 germination mix media and perlite. Osmocote slow-release fertilizer (15-9-12) 5-6 month was added to the media prior planting (approximately ½ cup per 4 gallons of media). Seedlings were grown and maintained in a greenhouse until being transplanted in the 2017 season. The seedlings were labelled throughout the process and seedling lots /accessions were given the same ID as their mother plants (Table 5.1). Accessions were transplanted directly to the field at the Dempsey Research Farm, Griffin, GA. They were planted in a high-density nursery.

Genetic diversity: One genotype per accession, for a total of 44 genotypes, were used to study the genetic diversity of *P. serotina* subsp. *capuli*. Ecuadorian capuli (PserEC03) genotype growing at the USDA Fruit and Tree Nut Research, Byron, GA was also included. Additionally, local *P. serotina* genotypes from Florida and Georgia were also added in the experiment (PserFL01-PserFL03, PserGA01 and PserGA02). Finally, sample representatives of *P. serotina* from USA were included from the USDA *Prunus* germplasm GRIN (Germplasm Resources Information Network) [DPRU 2674 and DPRU 3138 (Iowa, US) for *P. serotina*] and *P. laurocerasus* [DPRU 2849 (Georgia)].

DNA extraction: All leaf samples were collected and kept at 4°C. Before extraction, 50 mg of leaf tissue per sample was weighed into 2 mL tubes. These tubes were kept at -80°C before extraction. *DNeasy ® Plant Pro Kit* was used for DNA extraction following manufacturing protocols (Qiagen Inc. Valencia, California). A final volume of 100 µL of DNA per sample was obtained. Visual confirmation of DNA presence per sample was performed using gel electrophoresis with a 1.5% agarose gel. Lambda DNA standards (Promega Corporation, Madison, WI, USA) were used for visual confirmation. DNA quantification was done by using NanoDrop™ 2000c Spectrophotometer (Thermo Scientific, Waltham, MA, USA). DNA concentration for all the samples was standardized to 20 ng/uL.

SSR markers: A total of 36 SSR markers distributed across the peach genome (~10.5 cM between markers) were selected from the *Prunus* 'Texas' almond × 'Earlygold' peach (T × E) reference map (Dirlewanger et al., 2004; Jung et al., 2008). These SSR markers were used to determine the genetic diversity among *P. serotina* subsp. *capuli* individuals. Forward markers were modified using a 5' fluorophore label 6-FAM (standard) [6-FAM] or HEX [5HEX] (Eurofins MWG Operon, Huntsville, AL, USA) for multiplex product fragment analysis (Table 5.3).

PCR products were amplified in a 16 µL volume reaction containing 2 µL of 20 ng/µL DNA template, 2.25 µL 10X ThermoPol Reaction Buffer [10mM KCl, 10mM $(NH_4)_2SO_4$, 20mM Tris-HCl, 2mM $MgSO_4$, 0.1% Triton X-100, pH 8.8 @ 25ºC], 1 µL 2.5 mM dNTPs, 0.2 µL Taq DNA Polymerase, 6.55 µL DNA grade water, and 4 µL 5µM (2 µL forward and 2 µL reverse) primers. PCR parameters were: 3 min at 94°C followed by 40 cycles of 30 s denaturing at 94°C, 30 s at primer's specific annealing temperature [Ta(°C)], and 1 min of elongation at 72°C, ending with 7 min at 72°C. PCR reactions were performed using Master® Nexus PCR (Eppendorf, Germany). PCR products were separated on 3% (m/v) agarose gel for confirmation. Gels were

stained with ethidium bromide and recorded using digital gel documentation system FOTO/Analyst™ Investigator Eclipse UV (Thermo Scientific, Waltham, MA, USA).

Gel images and PCR amplification intensities were used to determine PCR dilution ratios for fragment analysis on an ABI3730 sequencer (Applied Biosystems®, Grand Island, NY, USA) at the Genomics Center at the University of Georgia, Athens, GA. Fragment analysis of chromatographs were visualized and allele calling for each marker was done using GeneMarker® v.1.6 software (SoftGenetics, LLC, State College, PA, USA) using 600 LIZ® size standard (Applied Biosystems®, Grand Island, NY, USA). Alleles sizes were scored as diallelic based on all the alleles identified across all 56 samples per marker. A binary matrix was produced for all data. Locus were alphabetically coded (A, B, C, etc.) in increasing order.

Data Analysis

Genetic diversity analyses were performed using GenAlEx v.6.5 software (Peakall and Smouse, 2012). Genetic variation was characterized for each locus by the number of observed alleles (*A*), effective number of alleles (*Ae*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Wright's fixation index [*F=(He-Ho)/He=1-(Ho/He)*], and percentage of polymorphic loci. AMOVA (Analysis of molecular variance) and Nei's genetic distance (Nei and Takezaki, 1983) were also calculated. An Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Sokal and Michener, 1958) cluster and Principal component analysis (PCA) from Nei's genetic distance were performed using DARwin software v.6.0.21 (Perrier and Jacquemoud-Collet, 2006).

Population structure analyses were performed for all the genotypes and separately for the capuli samples only using Structure v.2.3.4 software (Pritchard et al., 2003). Structure simulation parameters were run under the admixture model assumption with correlated alleles using four reps

per run, for one to ten K subgroups for all runs with 10^5 interactions after a burn-in of 10^4 interactions. Structure Harvester software (Earl and Vonholdt, 2011) was used to implement the Evanno method to analyze the population structure results (Evanno et al., 2005).

Results and discussions

The main aim goal of this research was to estimate the genetic diversity of *P. serotina* subsp. *capuli* from three different provinces of Ecuador (Chimborazo, Cotopaxi and Tungurahua) and to determine if structured populations are present in Ecuador. OP seed from genotypes growing in the wild in Ecuador were germinated and grown at the UGA Griffin campus, Griffin, GA, US.

We divided our analyses in two to understand the overall genetic diversity and population structure present in accessions: i) all 56 samples including *P. serotina* subsp. *capuli*, *P. serotina* and *P. laurocerasus*, and ii) 46 capuli individuals.

A total of 36 SSRs markers were used but 5 markers BPPCT023, BPPCT027, BPPCT029, CPPCT022 and BPPCT038 were dropped in the analysis due to poor amplification issues or if no consistency was found for capuli samples during allele calling.

Genetic diversity parameters for all 56 individuals

A total of 576 alleles were observed for all 56 genotypes analyzed for all 31 primers selected from the *Prunus* 'Texas' almond × 'Earlygold' peach (T × E) reference map (Dirlewanger et al., 2004; Jung et al., 2008). Results of genetic diversity parameters for all 56 genotypes are found in Table 5.4. Average number of different alleles per locus (Na) was 9.29 and average number of effective alleles per locus (Ne) was 3.63. BPPCT039A and CPPCT005A has the highest number of alleles per locus: 23 and 21 respectively. UDP96-001C has the least number of alleles (2) per locus. BPPCT006D has the highest number of effective alleles, Ne (10.46), and CPPCT015D has the least, Ne (1.14).

Marker BPPCT030A has the highest observed heterozygosity, Ho (1.0). Markers UDP96-003C and CPPCT015C had the lowest Ho (0.0). BPPCT006D and BPPCT006A has the highest expected heterozygosity, He (0.90 and 0.87). CPPCT015C and CPPCT015D has the least value of expected heterozygosity, He (0.14 and 0.12). Average Wright's fixation index (F) was 0.36 per locus suggesting some genetic differentiation in the overall samples analyzed.

Moderate amount of heterozygosity was observed in this study, Ho= 0.41 which could be attributed to the out-crossing nature of the capuli species. High number of total alleles, 576 were observed in this study. Guadalupe et al. (2015) also observed He= 0.71 for Ecuadorian capuli collected from 8 different provinces. They studied genetic diversity of Ecuadorian capuli for 217 individuals using 12 SSR primers from peach, sweet cherry and sour cherry. A total of 49 alleles were observed across all the samples with presence of 4 -10 alleles per locus. Guzman et al. (2018) estimated total 246 alleles from 178 individuals representing four different subsp. of *P. serotina* (*eximia*, *virens*, *serotina*, *capuli*) with 16 microsatellites. They found average allele per locus was 15.4. Also, in their study marker BPPCT-039 generated higher number of alleles (24).

Genetic diversity parameters for only 46 genotypes of P. serotina subsp. capuli

Results for genetic diversity parameters calculated for only capuli samples are reported in Table 5.5. The average number of different alleles (Na) calculated for Chimborazo, Cotopaxi and Tungurahua were 4.66, 3.53 and 4.44, respectively. Number of effective alleles (Ne) observed was highest for Chimborazo (2.82), followed by Tungurahua (2.76) and Cotopaxi (2.46). Observed heterozygosity (Ho) was similar for Chimborazo and Tungurahua (0.4) and less for Cotopaxi (0.3). Expected heterozygosity (He) was similar for all three provinces around 0.5. Wright's Fixation Index (F) was highest for Cotopaxi with 0.33, and 0.2 for Chimborazo and Tungurahua (0.2).

Genetic variation is present equally in comparison between locations, with Cotopaxi showing a high degree of fixation.

Number of private alleles that were unique to a single population were also calculated for each population. The highest number of private alleles was identified for Tungurahua (0.94), Chimborazo (0.90) and the least for Cotopaxi (0.31). This could mean that individuals from Chimborazo and Tungurahua genotypes could provide for a unique valuable breeding material for the start of a capuli breeding program.

Downey and Iezzoni (2000) used 4 SSR markers from peach, sweet cherry and sour cherry, identifying 54 alleles in the black cherry samples collected from Michigan, Mexico and Ecuador. Intriago-Baldeón et al. (2013) observed average 6.13 alleles per locus for eight SSR markers on 88 individuals collected from Pichincha, Cañar, and Azuay provinces in Ecuador and found moderate genetic diversity in their study.

Analysis of molecular variance (AMOVA)

AMOVA (Analysis of molecular variance) was analyzed separately for all the genotypes (*P. serotina* subsp. *capuli* for all three provinces, *P. laurocerasus* and *P. serotina*) and only capuli genotypes from all 3 provinces from Ecuador. Most of the genetic variation was found within the populations (86%) than among populations (14%) for AMOVA calculated for all the 56 genotypes (Table 5.6). AMOVA for only capuli populations revealed 97% of the variation was within the three populations of capuli and only 3% variation was found among the populations (Table 5.7). This suggests us that there are no differences among populations and that most of the genetic diversity is present within the populations. Guzman et al. (2018) also found similar results while studying genetic variation in four different subspecies of *P. serotina* with 79% variation present within the populations.

UPGMA hierarchical clustering and Principal Component Analysis

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clusters were developed using DARwin software with Nei's genetic distance matrix. UPGMA hierarchical clustering when done for all the 56 individuals together. Samples were separated into clusters with *P. laurocerasus* samples rooting the whole cladogram and a cluster represented by *P. serotina* samples from USA (PserFL01-PserFL03, PserGA01, PserGA02, and DPRU 3138). The largest cluster was constituted by *P. serotina* subsp. *capuli* samples (Fig 5.1).

UPGMA cluster analyses of 46 *P. serotina* subsp. *capuli* genotypes classified samples into numerous clusters without any clear differentiation of samples according to their site of origin. One interesting result was to observe PserEC03 clustered with DPRU2674. It was determined that PserEC03 was requested from the GRIN system decades ago and can be traced back to the original DPRU2674 accession from GRIN after checking in the USDA in Byron records.

PCA was consistent with the cluster analysis results (Fig 5.2). The genotypes were divided into 3 clusters. *P. laurocerasus* individuals were placed farthest from all *P. serotina* and *P. serotina* subsp. *capuli* individuals. All *P. serotina* samples belonging to USA were placed together in the cluster next to the *P. serotina* subsp. *capuli* individuals.

Structure results

Structure analysis for all 56 genotypes yielded K=3 with the highest delta K (ΔK) value followed by K=7 (Figs. 5.3 and 5.4) using Evanno method (Evanno et al., 2005) as implemented in the Structure Harvester software (Earl and Vonholdt, 2011). Structure K=3 differentiated three main groups: i) *P. serotina* subsp. *capuli* from Ecuador, ii) *P. serotina* from USA, and iii) *P. laurocerasus*. These results were similar to UPGMA and PCA results. The population structure results with K=7 classified samples into five groups: i) *P. serotina* subsp. *capuli* from Ecuador, ii)

P. serotina from Florida (PserFL01-03), iii) *P. serotina* from Georgia (PserGA01,02), iv) *P. serotina* from Iowa (DPRU 3138), and v) *P. laurocerasus* (not shown). The genus *Prunus* is divided into 5 subgenera. Among those, *P. laurocerasus* belongs to subgenera *Laurocerasus*. On the other hand, *P. serotina* belongs to subgenera *Padus* (Rehder, 1927). Our results are consistent with the taxonomic classifications. *Prunus laurocerasus* was used in this research as an outgroup species to help us during analysis.

In structure results involving only 46 *P. serotina* subsp. *capuli* genotypes, K= 7 had the highest delta K (ΔK) value followed by K=5 (Figs. 5.5 and 5.6). The structure results again did not divide the capuli according to their collection location. Capuli individuals were not differentiated into clear structure groups and showed admixture between groups with no clear distinction between any of the populations. Similar, admixture was seen in the results with K=5. One interesting observation was the presence of individuals from a similar grouping for Cotopaxi individuals. In Fig. 5.5, individuals from Cotopaxi are represented by red grouping. In the genetic diversity analyses, it was previously reported that the Cotopaxi individuals had a lower number of private alleles, in addition to a higher fixation index in comparison with the other samples from the two additional provinces. It may be important to check the genetic makeup of a larger number of samples representative of this province in comparison with the other provinces as well to check the level of heterozygosity within OP seedlings from one accession. It may help understanding the level of outcrossing and any genetic differences among and within locations.

The structure results reported in our research were similar to previous studies. Guadalupe et al. (2015) suggested absence of population differentiation at provincial level in the Ecuadorian capuli collected from 8 provinces of Ecuador. The range of pairwise Fst values were 0.007 to 0.032 between provinces. They proposed that self- incompatibility and outcrossing as the main reason

for this population characteristics. Also, genetic distance value was higher for province Cotopaxi than Imbabura, Pichincha and Tungurahua. Intriago-Baldeón et al. (2013) also observed similar results where 88 individuals collected from 3 three provinces of Ecuador did not clustered according to their place of origin in UPGMA analysis. Admixture of individuals was also reported.

Overall, moderate genetic diversity was observed in the capuli populations analyzed in this study. Presence of unique alleles and variation within each capuli population from Chimborazo, and Tungurahua makes a highly diverse material. There also no clear differentiation between the capuli accessions according to their region of origin. Capuli can not only be used as a horticultural crop for its fruits but its seeds, leaves and wood can also be utilized (Avendaño-Gómez et al., 2015; Garcia-Aguilar et al., 2015; Popenoe and Pachano, 1923). The genetic variation along with the morphological variation found can be exploited in a breeding program. Unique morphological and genetic characteristics, in addition to capuli being a multipurpose tree, demonstrates the potential of it as a commercial product both in Southeastern USA and Ecuador.

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Table 5.1. List of *Prunus serotina* subsp. *capuli* accessions collected in the Andes region of Ecuador grown in UGA Griffin, GA, US.

Collection (No.)	Accession ID[z]	Country	Province	Latitude	Longitude
1	PserCO01	Ecuador	Cotopaxi	1°7’36.1” S	78°35’21.2” W
8	PserCO08	Ecuador	Cotopaxi	1°0’54” S	78°36’31” W
9	PserCO09	Ecuador	Cotopaxi	0°58’38.9” S	78°38’16.1” W
13	PserCO13	Ecuador	Cotopaxi	0°53’2” S	78°39’15.7” W
14	PserCO14	Ecuador	Cotopaxi	0°53’22.9” S	78°37’30.7” W
16	PserCO16	Ecuador	Cotopaxi	0°53’44.7” S	78°37’5” W
21	PserCO21	Ecuador	Cotopaxi	0°59’2.5” S	78°36’6.3” W
22	PserCO22	Ecuador	Cotopaxi	0°59’2.1” S	78°36’5.6” W
26	PserCO26	Ecuador	Cotopaxi	1°5’50.9” S	78°36’10.2” W
31	PserCO31	Ecuador	Cotopaxi	1°6’23.3” S	78°36’28.1” W
41	PserTU41	Ecuador	Tungurahua	1°24’8.9” S	78°38’2.2” W
43	PserTU43	Ecuador	Tungurahua	1°23’26” S	78°37’25.8” W
48	PserTU48	Ecuador	Tungurahua	1°21’5” S	78°36’45.2” W
53	PserTU53	Ecuador	Tungurahua	1°18’49.9” S	78°38’19” W
57	PserTU57	Ecuador	Tungurahua	1°17’49.3” S	78°37’14.1” W
67	PserTU67	Ecuador	Tungurahua	1°18’49” S	78°32’46.9” W
70	PserTU70	Ecuador	Tungurahua	1°20’2.3” S	78°33’51.8” W
71	PserTU71	Ecuador	Tungurahua	1°19’23.1” S	78°34’19.1” W
75	PserTU75	Ecuador	Tungurahua	1°18’54.6” S	-
77	PserTU77	Ecuador	Tungurahua	1°21’11.3” S	78°34’58.5” W
81	PserTU81	Ecuador	Tungurahua	1°25’53.6” S	78°30’57.1” W
86	PserCH86	Ecuador	Chimborazo	1°31’45.1” S	78°30’0” W
90	PserCH90	Ecuador	Chimborazo	1°32’45.1” S	78°31’3.2” W
94	PserCH94	Ecuador	Chimborazo	1°34’59.7” S	78°32’17.4” W
98	PserCH98	Ecuador	Chimborazo	1°37’44.9” S	78°34’57.7” W
99	PserCH99	Ecuador	Chimborazo	1°37’44.9” S	78°34’57.7” W
101	PserCH101	Ecuador	Chimborazo	1°37’3.3” S	78°35’49.3” W
104	PserCH104	Ecuador	Chimborazo	1°36’24” S	78°38’25.2” W
108	PserCH108	Ecuador	Chimborazo	1°35’45.3” S	78°40’56.8” W
110	PserCH110	Ecuador	Chimborazo	1°35’21.2” S	78°41’17.3” W
112	PserCH112	Ecuador	Chimborazo	-	-
113	PserCH113	Ecuador	Chimborazo	-	-
114	PserCH114	Ecuador	Chimborazo	1°38’1.3” S	78°40’40.7” W
119	PserCH119	Ecuador	Chimborazo	1°39’32.9” S	78°42’5.2” W
123	PserCH123	Ecuador	Chimborazo	1°39’36.1” S	78°44’9.6” W
127	PserCH127	Ecuador	Chimborazo	1°42’19” S	78°41’31.6” W
132	PserCH132	Ecuador	Chimborazo	1°41’47” S	78°38’39” W
135	PserCH135	Ecuador	Chimborazo	1°43’38.1” S	78°38’59.3” W

Table 5.1. *Continued*.

Collection (No.)	Accession ID[z]	Country	Province	Latitude	Longitude
137	PserCH137	Ecuador	Chimborazo	1°43’32.3” S	78°39’46.3” W
138	PserCH138	Ecuador	Chimborazo	1°39’14” S	78°40’48.6” W
142	PserCH142	Ecuador	Chimborazo	1°38’46.4” S	78°42’27.2” W
145	PserCH145	Ecuador	Chimborazo	1°37’25.2” S	78°43’30.8” W
147	PserCH147	Ecuador	Chimborazo	1°38’51.2” S	78°41’46.6” W
153	PserCH153	Ecuador	Chimborazo	1°38’49.2” S	78°35’14.8” W

[z]ID= first letter represented the genus (*Prunus*=P), next three letters represented the species (*serotina*=ser), and the following letters represented the province of origin in Ecuador and collection number (Chimborazo collection 01=CH01).

Table 5.2. List of the genotypes of different *Prunus* species used in this experiment.

Genotype ID[z]	Population ID[x]	Subspecies
PserCH101-3	1	*P. serotina* subsp. *capuli*
PserCH104-1	1	*P. serotina* subsp. *capuli*
PserCH108-4	1	*P. serotina* subsp. *capuli*
PserCH110-3	1	*P. serotina* subsp. *capuli*
PserCH112-1	1	*P. serotina* subsp. *capuli*
PserCH113-4	1	*P. serotina* subsp. *capuli*
PserCH114-3	1	*P. serotina* subsp. *capuli*
PserCH119-3	1	*P. serotina* subsp. *capuli*
PserCH127-4	1	*P. serotina* subsp. *capuli*
PserCH132-5	1	*P. serotina* subsp. *capuli*
PserCH135-11	1	*P. serotina* subsp. *capuli*
PserCH137-4	1	*P. serotina* subsp. *capuli*
PserCH138-2	1	*P. serotina* subsp. *capuli*
PserCH142-4	1	*P. serotina* subsp. *capuli*
PserCH145-1	1	*P. serotina* subsp. *capuli*
PserCH147-5	1	*P. serotina* subsp. *capuli*
PserCH153-4	1	*P. serotina* subsp. *capuli*
PserCH86-2	1	*P. serotina* subsp. *capuli*
PserCH90-3	1	*P. serotina* subsp. *capuli*
PserCH94-1	1	*P. serotina* subsp. *capuli*
PserCH98-3	1	*P. serotina* subsp. *capuli*
PserCH99-3	1	*P. serotina* subsp. *capuli*
PserCo01-5	2	*P. serotina* subsp. *capuli*
PserCo08-5	2	*P. serotina* subsp. *capuli*
PserCo09-2	2	*P. serotina* subsp. *capuli*
PserCo13-4	2	*P. serotina* subsp. *capuli*
PserCo14-3	2	*P. serotina* subsp. *capuli*
PserCo16-5	2	*P. serotina* subsp. *capuli*
PserCo21-9	2	*P. serotina* subsp. *capuli*
PserCo22-2	2	*P. serotina* subsp. *capuli*
PserCo26-4	2	*P. serotina* subsp. *capuli*
PserCo31-9	2	*P. serotina* subsp. *capuli*
PserTU41-1	3	*P. serotina* subsp. *capuli*
PserTU43-3	3	*P. serotina* subsp. *capuli*
PserTU48-4	3	*P. serotina* subsp. *capuli*
PserTU53-5	3	*P. serotina* subsp. *capuli*
PserTU57/53-1	3	*P. serotina* subsp. *capuli*
PserTU57-3	3	*P. serotina* subsp. *capuli*
PserTU67-7	3	*P. serotina* subsp. *capuli*
PserTU70-18	3	*P. serotina* subsp. *capuli*
PserTU71-4	3	*P. serotina* subsp. *capuli*
PserTU75-4	3	*P. serotina* subsp. *capuli*

Table 5.2. *Continued.*

Genotype ID[z]	Population ID[x]	Subspecies
PserTU77-3	3	*P. serotina* subsp. *capuli*
PserTU81-3	3	*P. serotina* subsp. *capuli*
PserEC03	3	*P. serotina* subsp. *capuli*
DPRU2674	3	*P. serotina* subsp. *capuli*
PserFL01	4	*P. serotina*
PserFL02	4	*P. serotina*
PserFL03	4	*P. serotina*
PserGA01	4	*P. serotina*
PserGA02	4	*P. serotina*
DPRU3138	4	*P. serotina*
DPRU3138-2A	4	*P. serotina*
DPRU3138-8A	4	*P. serotina*
DPRU2849-1	5	*P. laurocerasus*
DPRU2849-2	5	*P. laurocerasus*

[z]ID= first letter represented the genus (*Prunus*=P), next three letters represented the species (*serotina*=ser), and the following letters represented the province of origin in Ecuador and collection number (Chimborazo collection 01=CH01).

[x] Population ID based on origin - 1: Chimborazo, 2: Cotopaxi, 3: Tungurahua, 4: Florida and Georgia, DPRU accessions were collected from USDA GRIN.

Table 5.3. Simple sequence repeat (SSR) markers selected from the *Prunus* 'Texas' almond × 'Earlygold' peach (T × E) reference map.

Chr	Position (cM)[z]	Position (Mbp)	Marker	Fluorophore	Ta(°C)[y]	Forward sequence	Reverse sequence	Reference
1	9	S1:22.79	CPSCT008	HEX	62	TGGATCCAATCCAAGAGTCTG	GCAGCAAGTTGTTCTTGGTTC	Mnejja et al. (2005)
1	25.8	S1:11.67	CPDCT038	HEX	62	ATCACAGGTGAAGGCTGTGG	CAGATTCATTGGCCCATCTT	Mnejja et al. (2005)
1	33.9	S1:30.53	CPPCT026	HEX	55	AGACGCAGCACCCAAACTAC	CATTACATCACCGCCAACAA	Aranzana et al. (2002)
1	47.3	S7:14.62	BPPCT027	HEX	57	GGACGGACAGAAATGAAGGT	CCTTAACCCACGCAACTCC	Dirlewanger et al. (2002)
1	77.4	S1:45.69	BPPCT028	HEX	57	TCAAGTTAGCTGAGGATCGC	GAGCTTGCCTATGAGAAGACC	Dirlewanger et al. (2002)
2	9.6	S2:10.87	UDP98-025	FAM	57	GGGAGGTTACTATGCCATGAAG	CGCAGACATGTAGTAGGACCTC	Yamamoto et al. (2005)
2	25	S2:17.93	BPPCT013	HEX	57	ACCCACAAATCAAGCATATCC	AGCTTCAGCCACCAAGC	Dirlewanger et al. (2002)
2	38	S2:22.25	BPPCT030	HEX	57	AATTGTACTTGCCAATGCTATGA	CTGCCTTCTGCTCACACC	Dirlewanger et al. (2002)
2	48.6	S2:26.35	CPSCT034	HEX	62	AGGTGGACAATAGCCGTGAT	TTTCCAGACCCTGAGAAAGC	Mnejja et al. (2005)
3	18	S3:5.80	BPPCT039	FAM	57	ATTACGTACCCTAAAGCTTCTGC	GATGTCATGAAGATTGGAGAGG	Dirlewanger et al. (2002)
3	36.4	S3:16.94	CPDCT025	HEX	62	GACCTCATCAGCATCACCAA	TTCCCTAACGTCCCTGACAC	Mnejja et al. (2005)
3	46.4	S3:21.67	CPDCT027	HEX	62	TGAGGAGAGCACTGGAGGAG	CAACCGATCCCTCTAGACCA	Mnejja et al. (2005)
4	10.4	S1:6.34	CPPCT005	FAM	52	CATGAACTCTACTCTCCA	TGGTATGGACTCACCAAC	Aranzana et al. (2002)
4	28.3	S4:8.76	UDP96-003	FAM	57	TTGCTCAAAAGTGTCGTTGC	ACACGTAGTGCAACACTGGC	Yamamoto et al. (2005)
4	34.1	S4:6.13	EPDC3832	FAM	57	CTTTTGAAGGCCCAATACCA	ATCACTGCTTCGCCTTCATT	Cipriani et al. (1999)
4	45.4	S4:14.73	BPPCT023	HEX	57	TGCAGCTCATTACCTTTTGC	AGATGTGCTCGTAGTTCGGAC	Dirlewanger et al. (2002)
4	52.7	S4:12.77	EPPISF032	HEX	57	TCCCCCACAGATATTTCAGC	GTCGAGGAGAGAGGGCTTTT	Vendramin et al. (2007)
5	5.2	S5:4.38	BPPCT026	FAM	57	ATACCTTTGCCACTTGCG	TGAGTTGGAAGAAAACGTAACA	Dirlewanger et al. (2002)
5	20.1	S5:11.74	BPPCT017	HEX	57	TTAAGAGTTTGTGATGGGAACC	AAGCATAATTTAGCATAACCAAGC	Dirlewanger et al. (2002)
5	32.9	S5:14.66	BPPCT038	FAM	57	TATATTGTTGGCTTCTTGCATG	GAGCTTGCCTATGAGAAGACC	Dirlewanger et al. (2002)
5	44	S5:16.63	BPPCT014	HEX	57	TTGTCTGCCTCTCATCTTAACC	CATCGCAGAGAACTGAGAGC	Dirlewanger et al. (2002)
6	8.7	S6:0.49	CPPCT008	FAM	59	GAGCTCTCACGCATTAGTTT	TTTGACTGCATAACAAAACG	Aranzana et al. (2002)
6	17.5	S6:7.04	UDP96-001	HEX	57	AGTTTGATTTTCTGATGCATCC	TGCCATAAGGACCGGTATGT	Yamamoto et al. (2005)
6	30.1	S6:10.28	BPPCT008	FAM	57	ATGGTGTGTATGGACATGATGA	CCTCAACCTAAGACACCTTCACT	Dirlewanger et al. (2002)
6	35.8	S6:16.35	CPPCT015	FAM	50	TGGAGTGCCAATACTATTTA	CATATGCATGGTTATGGT	Aranzana et al. (2002)
6	41	S6:26.46	EPPISF002	FAM	56	CGACGTGTGACCAAAGGAC	GCAACTCCATCCACATTTCTC	Vendramin et al. (2007)
6	56.4	S6:21.13	BPPCT025	FAM	57	TCCTGCGTAGAAGAAGGTAGC	CGACATAAAGTCCAAATGGC	Dirlewanger et al. (2002)
6	72	S6:24.75	UDP98-412	HEX	57	AGGGAAAGTTTCTGCTGCAC	GCTGAAGACGACGATGATGA	Yamamoto et al. (2005)
7	9.5	S7:6.68	CPSCT004	FAM	62	GCTCTGAAGCTCTGCATTGA	TTTGAAATGGCTATGGAGTACG	Mnejja et al. (2005)
7	18.6	S7:10.22	CPPCT022	FAM	50	CAATTAGCTAGAGAGAATTATTG	GACAAGAAGCAAGTAGTTTG	Aranzana et al. (2002)
7	29.6	S7:14.62	BPPCT029	FAM	57	GGACGGACAGAAATGAAGGT	CCTTAACCCACGCAACTCC	Dirlewanger et al. (2002)
7	38.9	S7:16.70	CPPCT033	HEX	50	TCAGCAAACTAGAAACAAACC	TTGCAATCTGGTTGATGTT	Aranzana et al. (2002)
7	47.8	S7:18.11	PMS2	FAM	55	CACTGTCTCCCAGGTTAAACT	CCTGAGCTTTTGACACATGC	Yamamoto et al. (2005)
8	14.1	S8:5.97	BPPCT006	HEX	57	GCTTGTGGCATGGAAGC	CCCTGTTTCTCATAGAACTCACAT	Dirlewanger et al. (2002)
8	20.8	S8:11.33	UDP96-019	HEX	57	TTGGTCATGAGCTAAGAAAACA	TAGTGGCACAGAGCAACACC	Yamamoto et al. (2005)
8	54.7	S8:20.22	EPDCU3117	FAM	57	CAGAGGGAACAGTGTGAGCA	TGTTGTTGTCGACCCTGAAA	Jung et al. (2008)

[z]Position for each marker based on their genetic distance (cM) as reported on the TxE reference map and their physical distance (Mbp) as found on the reference Peach v1.0 genome (Verde et al., 2013). The letter S followed by a number represents the scaffold number on the reference genome.

[y]Ta = annealing temperature.

Table 5.4. Summary statistics of 31 SSR markers for 56 genotypes of *P. serotina* subsp. *capuli* and other *Prunus* genotypes[z].

Locus	Na[y]	Ne	Ho	He	UHe	F
BPPCT006A	14.00	7.44	0.59	0.87	0.87	0.32
BPPCT006B	9.00	4.57	0.55	0.78	0.79	0.30
BPPCT006C	8.00	3.07	0.26	0.68	0.68	0.62
BPPCT006D	17.00	10.46	0.57	0.90	0.91	0.37
BPPCT008B	8.00	3.33	0.63	0.70	0.71	0.10
BPPCT013A	9.00	1.76	0.11	0.43	0.44	0.75
BPPCT013B	13.00	5.38	0.70	0.81	0.82	0.14
BPPCT013C	3.00	2.46	0.00	0.59	0.63	1.00
BPPCT014A	7.00	3.71	0.68	0.73	0.74	0.07
BPPCT017B	8.00	1.28	0.14	0.22	0.21	0.34
BPPCT017C	16.00	5.70	0.73	0.82	0.83	0.11
BPPCT025A	13.00	4.01	0.67	0.75	0.76	0.10
BPPCT025B	14.00	2.68	0.58	0.63	0.63	0.07
BPPCT026A	11.00	4.08	0.45	0.76	0.76	0.41
BPPCT028A	15.00	4.28	0.66	0.77	0.77	0.14
BPPCT030A	7.00	2.31	1.00	0.57	0.57	-0.76
BPPCT039A	23.00	9.43	0.86	0.89	0.90	0.04
BPPCT039C	10.00	1.59	0.32	0.37	0.38	0.15
CPDCT025A	19.00	5.41	0.77	0.82	0.82	0.06
CPDCT027A	14.00	2.81	0.56	0.64	0.65	0.13
CPDCT038B	7.00	1.59	0.09	0.37	0.37	0.75
CPPCT005A	21.00	5.15	0.60	0.81	0.81	0.26
CPPCT008B	14.00	7.05	0.80	0.86	0.87	0.07
CPPCT015C	3.00	1.16	0.00	0.14	0.14	1.00
CPPCT015D	3.00	1.14	0.09	0.12	0.12	0.25
CPPCT015E	4.00	2.18	0.39	0.54	0.55	0.28
CPPCT026A	18.00	5.85	0.42	0.83	0.84	0.49
CPPCT026B	9.00	4.01	0.14	0.75	0.76	0.82
CPPCT033A	7.00	2.47	0.47	0.60	0.60	0.21
CPPCT033B	7.00	1.45	0.16	0.31	0.31	0.47
CPSCT004A	6.00	2.03	0.21	0.51	0.51	0.58
CPSCT008A	11.00	2.73	0.82	0.63	0.64	-0.30
CPSCT008B	3.00	1.29	0.17	0.23	0.23	0.25
CPSCT034A	5.00	1.81	0.22	0.45	0.45	0.51
CPSCT034B	16.00	7.00	0.28	0.86	0.87	0.68
CPSCT034C	4.00	2.17	0.36	0.54	0.55	0.33
CPSCT034D	10.00	6.90	0.77	0.86	0.89	0.10
EPDC3832A	4.00	1.53	0.09	0.34	0.35	0.73
EPDC3832B	3.00	2.38	0.47	0.58	0.59	0.19
EPDC3832C	4.00	2.31	0.33	0.57	0.57	0.43
EPDC3832D	4.00	3.53	0.27	0.72	0.73	0.63

Table 5.4. *Continued*

Locus	Na[y]	Ne	Ho	He	UHe	F
EPDC3832E	13.00	4.70	0.30	0.79	0.80	0.62
EPDC3832F	8.00	6.75	0.89	0.85	0.88	-0.04
EPDCU3117A	12.00	2.67	0.45	0.63	0.63	0.29
EPPISF002A	10.00	3.94	0.38	0.75	0.76	0.49
EPPISF002B	19.00	10.13	0.27	0.90	0.91	0.71
EPPISF002C	3.00	2.39	0.43	0.58	0.63	0.26
EPPISF032A	8.00	2.78	0.54	0.64	0.65	0.16
PMS2A	5.00	1.47	0.05	0.32	0.32	0.83
UDP96-001A	3.00	2.26	0.28	0.56	0.56	0.50
UDP96-001B	4.00	2.92	0.48	0.66	0.66	0.27
UDP96-001C	2.00	1.63	0.44	0.39	0.39	-0.13
UDP96-001D	3.00	2.40	0.19	0.58	0.60	0.68
UDP96-003B	11.00	4.37	0.41	0.77	0.78	0.47
UDP96-003C	5.00	2.70	0.00	0.63	0.65	1.00
UDP96-019A	16.00	5.03	0.71	0.80	0.81	0.11
UDP96-019B	16.00	4.72	0.67	0.79	0.80	0.15
UDP98-025B	11.00	3.66	0.56	0.73	0.73	0.23
UDP98-412A	4.00	1.36	0.03	0.27	0.27	0.88
UDP98-412B	7.00	1.93	0.42	0.48	0.49	0.13
UDP98-412C	9.00	3.20	0.17	0.69	0.70	0.76
UDP98-412D	6.00	2.53	0.10	0.60	0.62	0.84
Average	9.29	3.63	0.42	0.62	0.63	0.36

[z]*Prunus* genotypes used of *P. serotina* and *P. laurocerasus* as outgroup samples.

[y]Na = No. of different alleles, Ne = No. of effective alleles, Ho = Observed heterozygosity, He = Expected heterozygosity, UHe = Unbiased Expected Heterozygosity, F = Wright's fixation index [F=(He-Ho)/He=1-(Ho/He)].

Table 5.5. Average statistics of 31 SSR markers for 46 genotypes of *P. serotina* subsp. *capuli* growing in UGA Griffin campus collected from Chimborazo, Cotopaxi and Tungurahua provinces in Ecuador.

Population	Genotypes No.	Na[z]	Ne	Ho	He	F	Polymorphic loci (%)	No. of private alleles
Chimborazo	22	4.66	2.82	0.41	0.54	0.24	95.16%	0.90
Cotopaxi	10	3.53	2.46	0.33	0.48	0.33	87.10%	0.31
Tungurahua	14	4.44	2.76	0.42	0.53	0.23	91.94%	0.94
Mean	46	4.21	2.68	0.39	0.51	0.27	91.40%	0.72

[z]Na = No. of different alleles, Ne = No. of effective alleles, Ho = Observed heterozygosity, He = Expected heterozygosity, F = Wright's fixation index [F=(He-Ho)/He=1-(Ho/He)].

Table 5.6. Analysis of Molecular Variance (AMOVA) for 56 genotypes of *P. serotina* subsp. *capuli* and other *Prunus* genotypes[z].

Source	df	SS	MS	Estimated Variance	%
Among Pops	4	531.205	132.801	8.028	14%
Within Pops	51	2572.813	50.447	50.447	86%
Total	55	3104.018		58.475	100%

[z]*Prunus* genotypes used of *P. serotina* and *P. laurocerasus* as outgroup samples.

Table 5.7. Analysis of Molecular Variance (AMOVA) for 3 populations of *P. serotina* subsp. *capuli* genotypes growing in UGA Griffin campus collected from Chimborazo, Cotopaxi and Tungurahua provinces in Ecuador.

Source	df	SS	MS	Estimated Variance	%
Among Pops	2	133.612	66.806	1.268	3%
Within Pops	43	2080.670	48.388	48.388	97%
Total	45	2214.283		49.656	100%

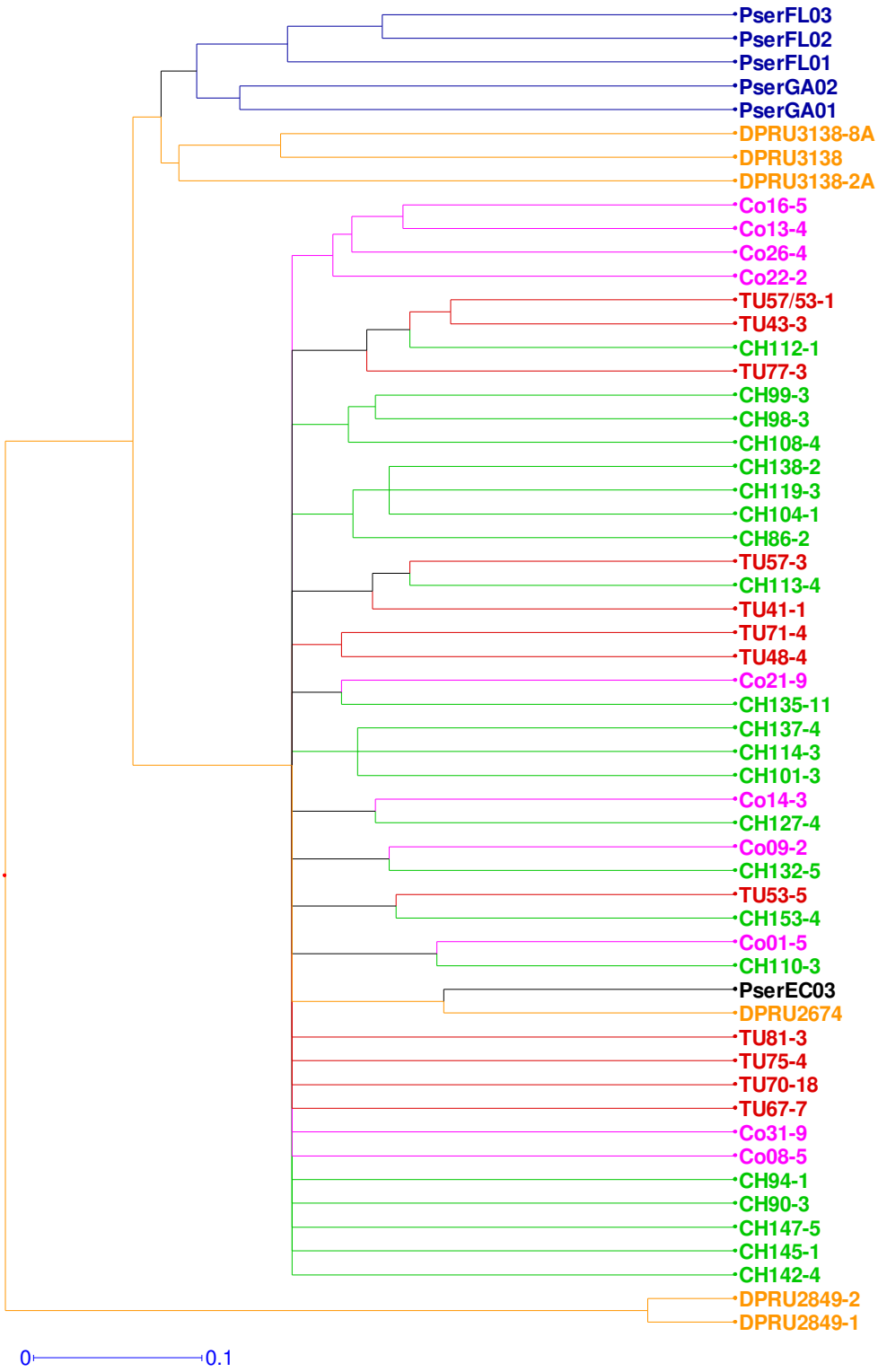

Fig 5.1. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical cluster analysis based on Nei's genetic distance matrix of 31 simple sequence repeat (SSR) markers for

56 genotypes including *P. serotina* subsp. *capuli* from Ecuador, *P. serotina* from US and *P. laurocerasus*.

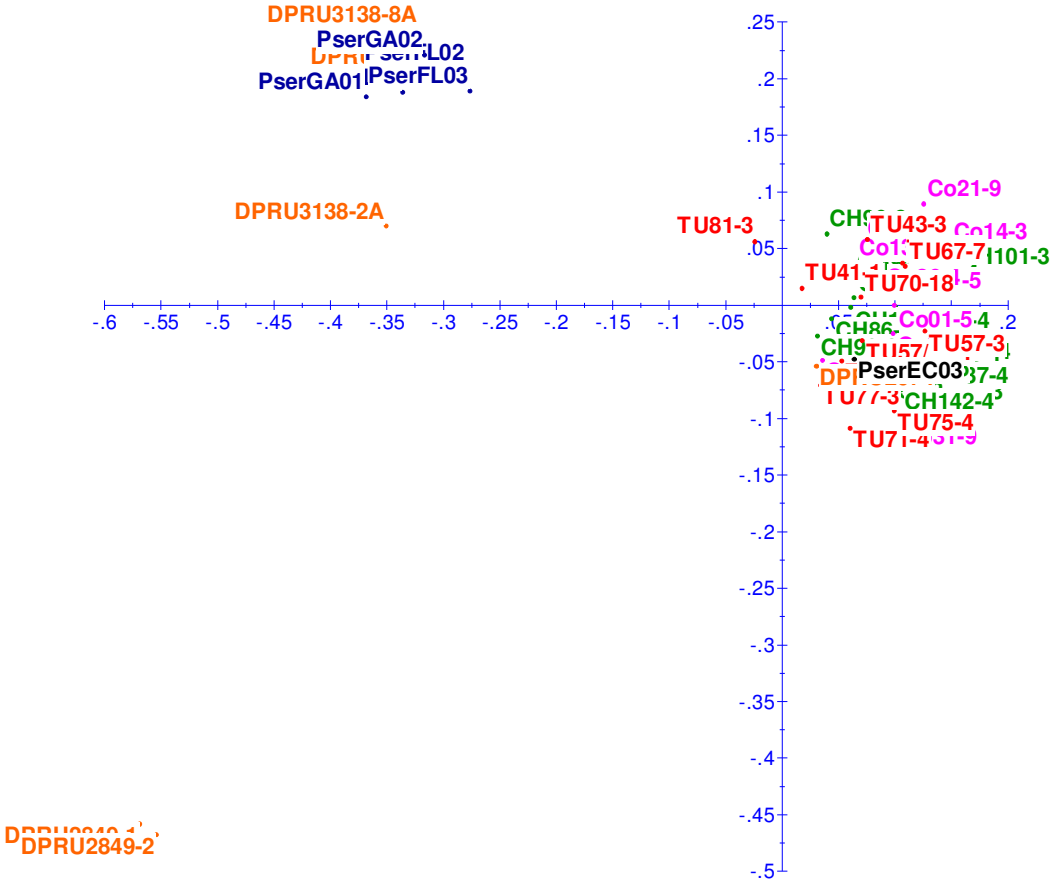

Fig 5.2. Principal component analysis based on 31 simple sequence repeat (SSR) markers for 56 genotypes including *P. serotina* subsp. *capuli* from Ecuador, *P. serotina* from US and *P. laurocerasus*.

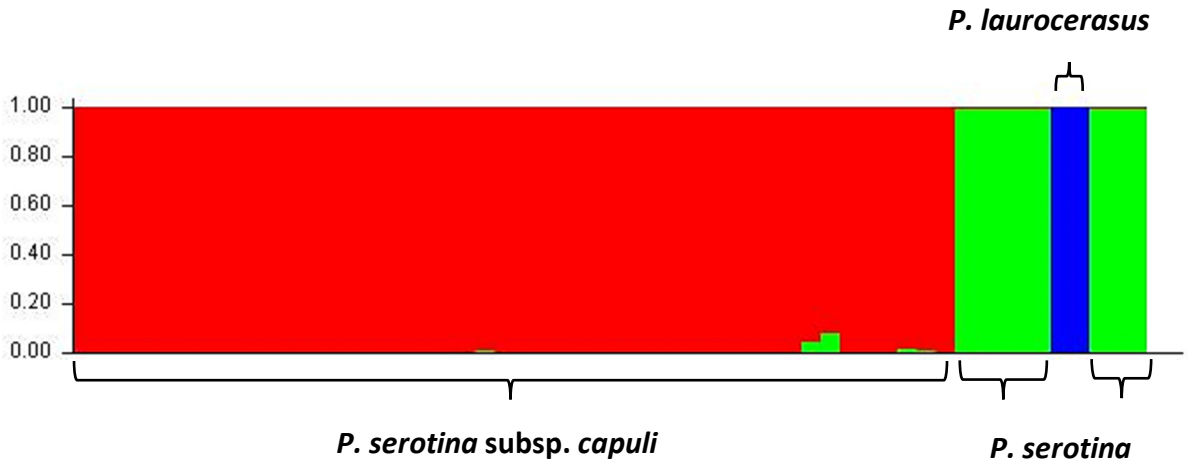

Fig 5.3. Population structure result for K=3 of 31 simple sequence repeat (SSR) markers for all 56 genotypes: *P. serotina* subsp. *capuli*, *P. serotina* and *P. laurocerasus*.

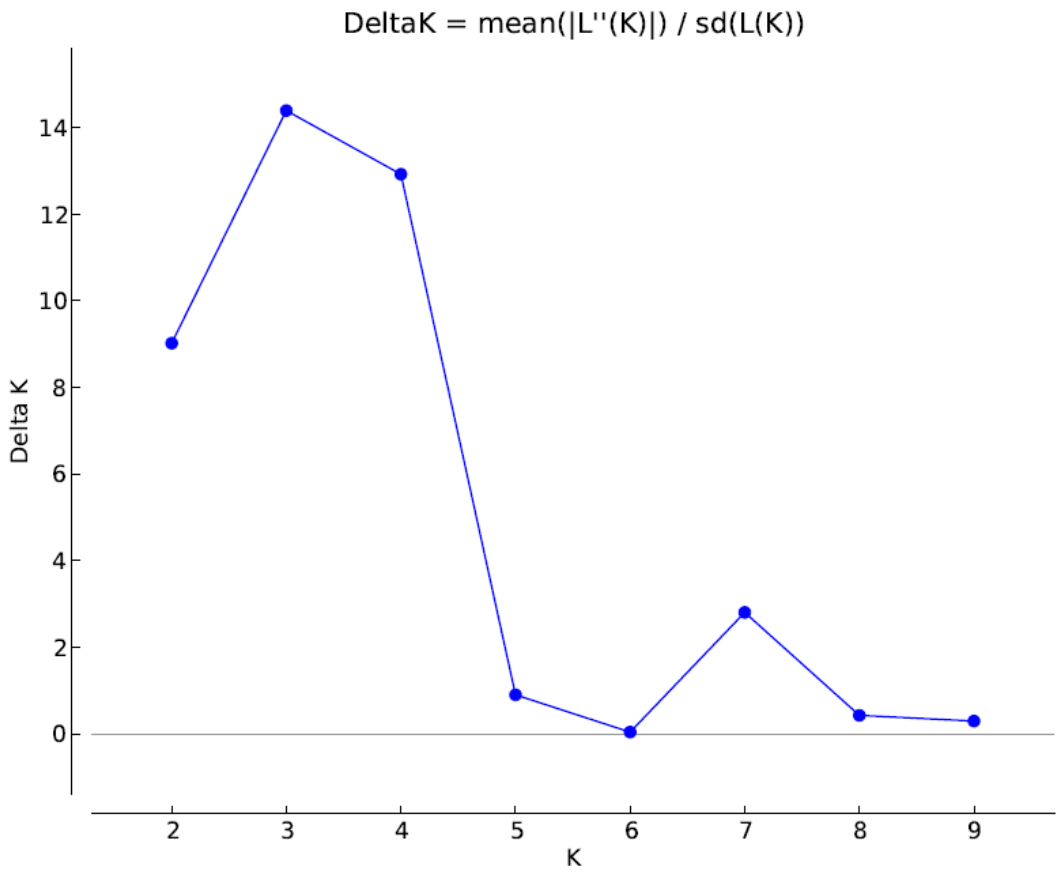

Fig 5.4. Analysis of the population structure results for 56 genotypes of *P. serotina* subsp. *capuli*, *P. serotina* and *P. laurocerasus* using the Evanno method (Evanno et al., 2005) implemented by the Structure Harvester software (Earl and Vonholdt, 2011). Second order change in the log-likelihood delta K (ΔK).

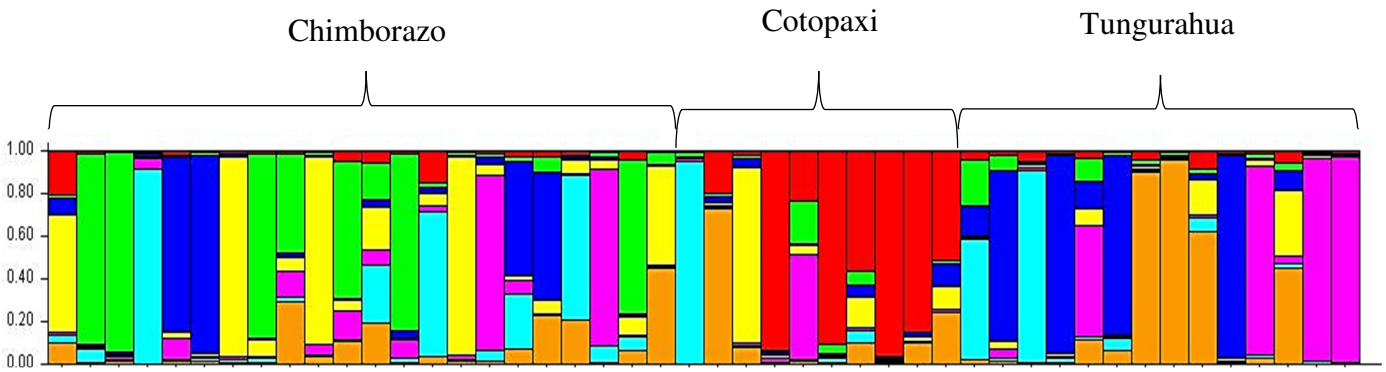

Fig 5.5. Population structure result for K=7 of 31 simple sequence repeat (SSR) markers for 46 genotypes of *P. serotina* subsp. *capuli* genotypes growing in UGA Griffin campus collected from Chimborazo, Cotopaxi and Tungurahua provinces in Ecuador.

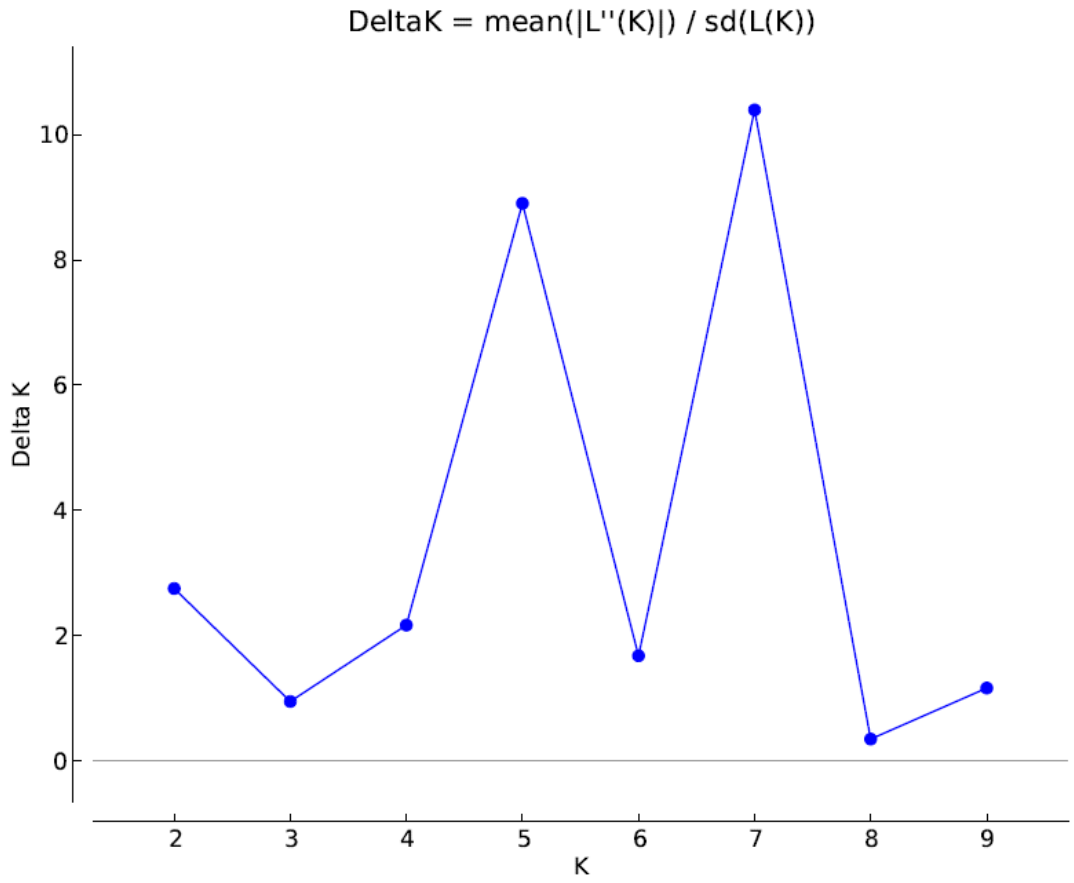

Fig 5.6. Analysis of the population structure results for 46 genotypes of *P. serotina* subsp. *capuli* using the Evanno method (Evanno et al., 2005) implemented by the Structure Harvester software (Earl and Vonholdt, 2011). Second order change in the log-likelihood delta K (ΔK).