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Persea (avocados) phylogenetic analysis based on morphological characters: hypothesis of species relationships

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Abstract

The genus *Persea* consists of two subgenera, *Persea* (known as avocados) and *Eriodaphne* (known as *aguacatillos*, avocado-like species). The present study aimed to determine whether the genus *Persea* is a monophyletic group and whether the division into two subgenera is an artificial one. In conjunction with these goals, a hypothesis of the phylogenetic relationships among *Persea* species is proposed. Our results suggest that *Persea* is not a monophyletic group. Two clades of *Persea* can be recognized. The results suggest that *Eriodaphne* and *Persea* should be considered to be independent genera. Various leaf and floral characters contributed for separation into groups. The definition of *Eriodaphne* is based on fruit color, sessile glands at the base of the stamens, vein prominence and leaf shape. *Persea* is more closely related to genera *Nectandra* and *Ocotea* than *Eriodaphne*. The 11 species included in *Persea* are recognized as species. The group is defined by fruit flavor, mature leaf color, number of tertiary divergent veins, and pubescent bracts in the inflorescence. Within this group, a clade of six species in which *P. guatemalensis* is included is recovered by seed shape, venation pattern relief, and number of fruits per cluster. Moreover, the seed shape supports the separation of *P. floccosa* and *P. zentmyerii*. A molecular character analysis is necessary to support the *Persea* clades proposed herein. Nevertheless the phylogenetic relationships revealed by this study provide new bases for the selection and conservation of the species *Persea*.

Introduction

The name *Persea* was first proposed by Clusius (1601), who took it from the Greek mythology. The type species, *Persea americana*, was described by Miller in his Gardener's Dictionary in 1768 (van der Werff 2002). *Persea* is currently recognized as having two subgenera, *Persea* and *Eriodaphne* (Kopp 1966; van der Werff 2002). The subgenus *Eriodaphne* is numerous, variable and clearly differentiated. Its species' main characteristic is the small fruit size (less than 2 cm). For this reason, it is considered to be the subgenus of *aguacatillos*, avocado-like trees (Bergh and Ellstrand 1987; Storey et al. 1987; Bergh 1992; Zentmyer and Schieber 1992). The importance of *Eriodaphne* is that some species of this subgenus are immune to avocado root rot, a disease caused by *Phytophthora cinnamomi* Rands., but their gametes are not compatible with those of *P. americana* Mill. (Litz 2001; Zentmyer and Schieber 1992). *Eriodaphne* includes *Persea borbonia* Spreng., *P. cinerascens*

S.F.Blake, *P. donnell-smithii* Mez, *P. excelsa* (Blume) Kosterm., *P. indica* Spreng., *P. lingue* Nees, *P. palustris* Sarg., *P. rigens* C.K.Allen, *P. silvatica* van der Werff, *P. skutchii* C.K.Allen, and *P. standleyi* C.K.Allen (Kopp 1966; Williams 1977; Bergh and Ellstrand 1987; Barrientos and López 2000).

The majority of recognized members of the subgenus *Persea* grow from central Mexico and Guatemala through most of Central America, which supports the theory of the origin of the genus *Persea*, and likely the entire subgenus *Persea* (Bergh and Ellstrand 1987; Storey et al. 1987; Schroeder 1990; Ben Ya'acov 1992). According to Kopp (1966), the origin of current forms of *Persea* is unclear, due to their close connection with representatives of ancient civilizations in the Mexico–Guatemala–Honduras region, who esteemed their fruits. Fruit size does not help distinguish between wild forms from cultivated forms and it is claimed that there is no clear botanical or ecological difference between cultivated and wild avocados (Ben Ya'acov 1992). Ben Ya'acov (1992) and Bergh and Ellstrand (1987) supported this theory when stating that the large fruit of *P. americana* Mill. var. *guatemalensis* (L.O.Williams) Scora is considered primitive. The subgenus *Persea* includes *P. americana* Mill., *P. drymifolia* Schltdl. et Cham., *P. floccosa* Mez, *P. gigantea* L.O.Williams, *P. guatemalensis* Lundell, *P. nubigena* L.O.Williams, *P. parvifolia* Spreng., *P. primatogena* L.O.Williams et A.Molina, *P. schiedeana* Nees, *P. steyermarkii* C.K.Allen, *P. tolimanensis* Zentmyer et Schieber, and *P. zentmyerii* Schieber et Zentmyer. Some authors consider that some species should rather be considered as *P. americana* subspecies: *P. parvifolia*, *P. primatogena* and *P. tolimanensis* (Schieber and Zentmyer 1981; Storey et al. 1987; Zentmyer 1991; Bergh 1992) or races [*P. americana* var. *americana* (= West Indian race), *P. americana* Mill. var. *drymifolia* (Schltdl. et Cham.) S.F.Blake (= Mexican race), *P. americana* var. *guatemalensis* (= Guatemalan race)] (Williams 1977; Bergh and Ellstrand 1987; Scora and Bergh 1990; Zentmyer and Schieber 1992; Bergh 1995). A synthesis of species, varieties, and races is given in Table 1. Work performed with isoenzymes (Bergh and Bufler 1987) supports the taxonomic conclusion that the three avocado races are closely related, the most distant being the Mexican race, as proposed by Kopp (1966), and that the Guatemalan race is not a subspecies as proposed by Williams (1977). Other authors concluded that the three avocado races do not differ from each other enough to be considered separate subspecies and are therefore botanical varieties (Popenoe 1941; Kopp 1966). Various morphological, isoenzymatic and physiological studies proposed that the Mexican and Guatemalan races are more similar and quite remote from the West Indian race (Scora et al. 1970–1973, 1975; Rhodes et al. 1971; García and Tsunewaki 1977; Garcia and Ichikawa 1979; Bergh and Bufler 1987). More recently, Bufler and Ben-Ya'acov (1992) analyzed variation in rDNA length repetitions and restricted sites for the three races of *P. americana*, establishing a closer relationship between the Guatemalan and West Indian races, but the three races were virtually equidistant according to an RAPD rDNA analysis (Bufler and Fiedler 1996).

It has been suggested that *Persea nubigena*, *P. steyermarkii* (Schieber and Zentmyer 1978, 1981), *P. tolimanensis* and *P. zentmyerii* (Schieber and Bergh 1987) are ancestors of *P. guatemalensis*. Bergh et al. (1973) pointed out a close affinity between *P. nubigena* and *P. americana* var. *guatemalensis*, whilst Furnier et al. (1990) have shown that *P. steyermarkii* and *P. nubigena* are closely related to *P. americana* var. *guatemalensis*. There is evidence to suggest that *P. nubigena*, *P. steyermarkii*, *P. tolimanensis* and *P. zentmyerii* are ancestors of *P. americana* var. *guatemalensis* (Schieber and Bergh 1987). *Persea floccosa* has been considered to be an ancestor of *P. americana* var. *drymifolia* (Scora and Bergh 1990). Garcia and Ichikawa (1979) believed that *P. americana* var. *americana* may have emerged as a hybrid of *P. americana* var. *drymifolia* and *P. schiedeana*. *Persea schiedeana* has been suggested to be an ancestor of *P. americana* var. *americana* (Bergh et al. 1973). Williams (1977) assumed that it originated by natural selection from *P. americana* var. *drymifolia*. None of these hypotheses has been tested by a phylogenetic analysis.

The genus *Beilschmiedia*, whose morphology and fruits are similar to those of *Persea americana*, particularly *B. anay*, is considered a closely related genus (Borys et al. 1993). Kostermans (1952) suggested that the genus *Persea* is related to the genera *Machilus, Nothaphoebe* and *Alseodaphne*. Other genera related to *Persea* are *Nectandra, Ocotea* and *Phoebe* with similar flower structure as *Beilschmiedia* (Standley and Steyermark 1946).

Table 1. Important taxonomical literature in subgenus *Persea* (synopsis).

Species		Variety/Subspecies	Race
P. americana	Miller 1768, van der Werff 2002	Kopp 1966; Williams 1977, Bergh and Ellstrand 1987, Pliego-Alfaro and Bergh 1992, Bergh 1993, Gama 1994, Scora et al. 2002	Popenoe 1941, Popenoe and Williams 1947, Bergh 1961, Bergh 1975, Zentmyer et al. 1987, Barrientos and López 2000, Scora et al. 2002
P. drymifolia	Schltdl. and Cham. 1831	Blake 1920, Kopp 1966, Smith 1966, Williams 1977, Bergh et Ellstrand 1987, Pliego-Alfaro and Bergh 1993, Bergh 1992, Gama 1994, van der Werff 2002, Scora et al. 2002	Popenoe 1941, Popenoe and Williams 1947, Bergh 1961, Bergh 1975, Zentmyer et al. 1987, Barrientos and López 2000, Scora et al. 2002
P. floccosa	Mez 1889, Williams 1977	Bergh and Ellstrand 1987, Scora et Bergh 1990, Pliego-Alfaro and Bergh 1992, Bergh 1993, Gama, 1994, van der Werff, 2002, Scora et al. 2002	
P. gigantea	Williams 1953		
P. guatemalensis	Lundell 1973	Williams 1977, Bergh and Ellstrand 1987, Pliego-Alfaro and Bergh 1992, Bergh 1993, Gama 1994, van der Werff 2002, Scora et al. 2002	Popenoe 1941, Bergh 1961, Popenoe and Williams 1947, Bergh 1975, Zentmyer et al. 1987, Barrientos and López 2000, Scora et al. 2002
P. nubigena	Williams 1950	Kopp 1966, Bergh et al. 1973, Williams 1977, Bergh and Ellstrand 1987, Pliego-Alfaro and Bergh 1992, Bergh 1993, Gama 1994, van der Werff 2002, Scora et al. 2002	
P. parviflora	Spreng. 1825, Williams 1977, Scora et al. 2002		
P. primatogena	Williams 1977		
P. schiedeana	Nees von 1836, Williams 1977, van der Werff 2002, Scora et al. 2002.	Meissner 1864	
P. steyermarkii	Allen 1945, Williams 1977	Schieber and Zentmyer 1978, Scora and Bergh 1990, Bergh and Ellstrand 1987, Pliego-Alfaro and Bergh 1992, Bergh 1993, Gama 1994, van der Werff 2002, Scora et al. 2002	
P. tolimanensis	Zentmyer and Schieber 1990	Bergh and Ellstrand 1987, Pliego-Alfaro and Bergh 1992, Bergh 1993, Scora et al. 2002	
P. zentmyerii	Schieber and Zentmyer 1972, Schieber and Bergh 1987	Schieber and Bergh 1987, Bergh and Ellstrand 1987, Pliego-Alfaro and Bergh 1992, Bergh 1993, Scora et al. 2002	

Both *Persea* and *Beilschmiedia* have a segmented calyx and 2–4 valves on the anthers; and they differ by the length and number of staminodes. *Phoebe* and *Persea* both have a persistent perianth, a feature that may or may not be present in *Persea*. *Nectandra* differs from *Persea* in that its anthers are more joined and are located at the same height. In *Ocotea*, the anthers are in pairs (Standley and Steyermark 1946), unlike the other genera mentioned. The present study thus aimed to determine whether the genus *Persea* is a monophyletic group and whether the subgenera are monophyletic. In addition, we give a hypothesis of phylogenetic relationships among fifteen *Persea* species as a method of predicting a better selection of genotypes and testing the hypothesis of relationships proposed by several authors.

Materials and methods

Taxa studied

Materials from the Fundacion Salvador Sanchez Colin CICTAMEX, S.C high altitude *Persea* germplasm bank located at the La Cruz Experimental Center at Coatepec Harinas in the State of Mexico were studied. Fifteen *Persea* species were examined. Outgroups were represented by the genera *Beilschmiedia*, *Nectandra*, *Ocotea* and *Phoebe*. Data were recorded during 2002 and 2003. Individuals representing the species have been grown under similar conditions in the experimental center for more than 15 years.

Characters

For each species, 15–20 leaves, flowers, fruits, and seeds were measured with a ruler or a vernier caliper. The assessed characters were recorded according to the avocado descriptors (CICTAMEX 1990). A data matrix was constructed of 40 characters, out of which 19 relate to vegetative and 21 to reproductive structures (see Appendix 1 for character explanation).

Phylogenetic analysis

The phylogenetic analyses were conducted using the parsimony algorithm of the Nona program (Goloboff 1993) in conjunction with a WinClada shell (Nixon 2002). The final result presented here was obtained with the following settings: the heuristic search was conducted with 1000 stepwise repetitions and TBR branch swapping with activated MULTIPARS. All characters were weighted equally. Bootstrap and jackknife support values for the nodes were calculated with 1000 replicates, 1000 search replicates (mult*1000), with TBR branch swapping and maximum number of trees set at 10,000.

Results and discussion

The consensus tree, produced from the five most parsimonious trees with a length of 181 steps, a consistence index of 39, and a retention index of 60 (Figure 1) showed that *Phoebe* is the basal taxon sister to subgenus *Eriodaphne* and the remainder species. The strict consensus tree allows us to understand character transformation, to identify the unique combination of features that support the recognition of species, and to select those characters that support sister relationships as traits that may be used in future genetic improvement programs. The cladogram showed that *Persea* is recovered as a paraphyletic genus. Two well-defined clades can be recognized, and have been determined as subgenera *Eriodaphne* and *Persea* (Kopp 1966; Williams 1977; Bergh and Ellstrand 1987; Scora and Bergh 1990). The results suggest that the subgenera *Eriodaphne* and *Persea* should be considered as independent genera. These findings support the reported sexual incompatibility among species of both subgenera, suggested elsewhere (Bergh and Ellstrand 1987; Litz 2001). However, in order to avoid confusion, we will continue naming them as subgenera, because we do not pretend to propose a new taxonomic circumscription herein, since not all species have been included in this study.

The studied species (*Persea borbonia*, *P. cinerascens*, *P. indica* and *P. palustris)* of the subgenus *Eriodaphne* (Kopp 1966; Williams 1977; Bergh and Ellstrand 1987; Scora and Bergh 1990) were grouped in a clade sister to the rest to the species (Figure 1). The clade is supported by four characters: enhanced venation pattern (13), lanceolate leaves (14), sessile glands at the base of the stamens (26), and black colored fruits (30). *Persea indica*

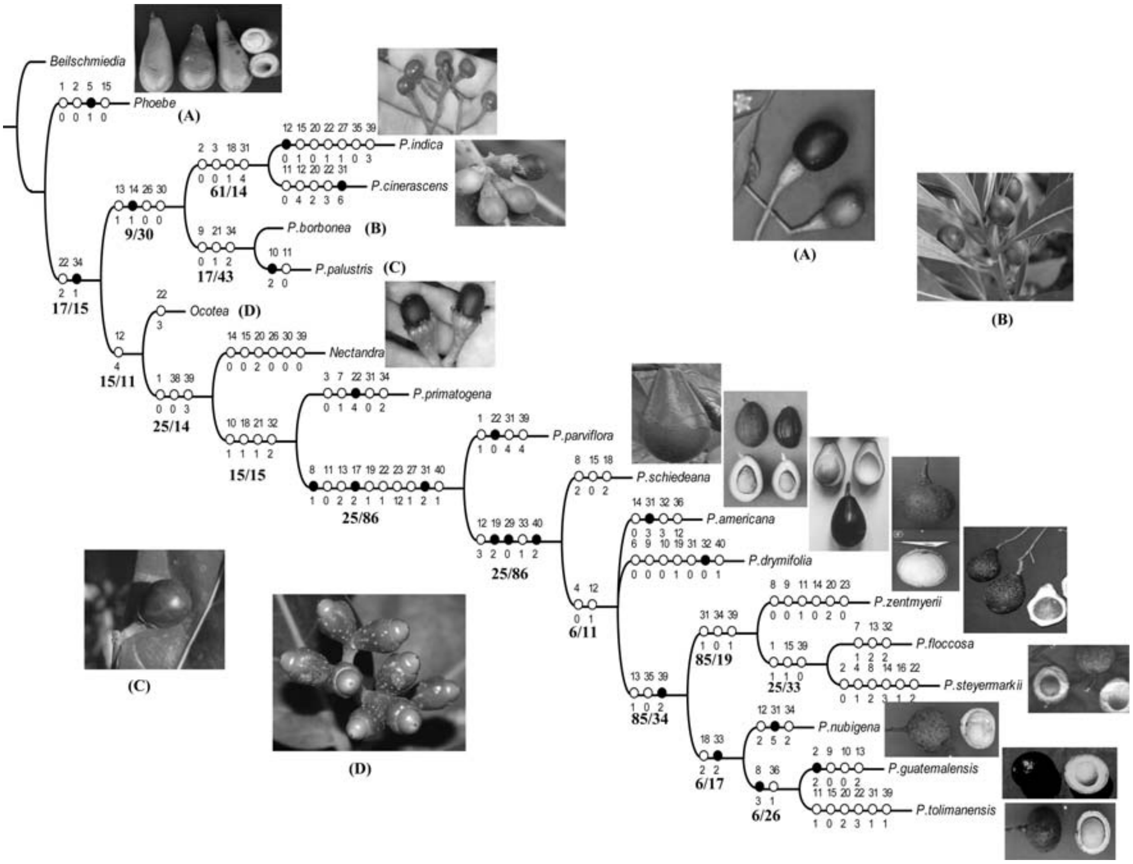

Figure 1. Strict consensus of the five equally parsimonious trees for *Persea* taxa based on morphological characters. Length = 181, consistency index = 39 and retention index = 60. Synapomorphies are black circles and homoplasies are open circles. Bootstrap/jackknife percentages are given below branches.

and *P. cinerascens* are sister taxa sharing four characters, while pubescent leaf color (12), in addition to other five characters, support the difference between *P. indica* and *P. cinerascens*, which is distinctive for its oblong fruits (31). The mentioned autapomorphies support the recognition of both species. Claims made by various authors (Kopp 1966; Williams 1977; Bergh and Ellstrand 1987) regarding *P. borbonia* and *P. palustris* being a single species, could not be sustained by the results of our study. *Persea borbonia* sister relationship with *P. palustris* is sustained by the combination of three characters: reddish young leaves (9), pubescence in inflorescence bract surfaces (21), and green pulp fruit (34). No autapomorphic character is recognized for *P. borbonia*, but *P. palustris* is characterized by its mature red leaves (10) with pubescence (11), which allowed the two different species to be recognized.

The subgenus *Persea* was recovered as monophyletic based on a unique combination of leaf surface color (10), number of tertiary veins (18), pubescent inflorescence bracts (21), and neutral fruit flavor (32). Except for pubescent inflorescence bracts, none of the characters mentioned were proposed by other authors as morphological features that distinguish both subgenera (Allen 1945; Kopp 1966; Williams 1977). Divergence angle leaf veins (8), leaf width (17) and fruit shape (31) were recognized as the synapomorphies for the clade made up of ten *Persea* species. This clade has a high bootstrap value (84) and *P. primatogena* is the sister taxon (Figure 1). The combination of enhanced venation relief (13), fruits with exocarp thicker than 4 mm (35), and round seeds (39) supports the group of species considered in the literature as ancestral: *P. floccosa*, *P. guatemalensis*, *P. nubigena*, *P. steyermarkii*, *P. tolimanensis*, and *P. zentmyerii* (Bergh and Ellstrand 1987; Scora and Bergh 1990). *Persea americana* has been considered a polymorphic species which could include several species, among them *Persea drymifolia*, *P. floccosa*, *P. guatemalensis*, *P. nubigena*, *P. steyermarkii*, *P. tolimanensis*, and *P. zentmyerii*. These species include the three commercially important varieties or races of avocado (*P. americana*, *P. drymifolia* and *P. guatemalensis*; West Indian, Mexican and Guatemalan, respectively). Schieber and Bergh (1987) suggest that all the subgenus *Persea* species, with the exception of these last three, can be considered as ecotypes. However, our results indicate that each of these eight species can be recognized by a unique combination of morphological characters. In contrast to previous proposals (Popenoe 1941; Kopp 1966; Williams 1977; Davies et al. 1998; Fiedler et al. 1998), we determined that the three avocado races differ from each other enough to be considered *Persea* species, as suggested elsewhere (Bergh and Ellstrand 1987; Scora and Bergh 1990). Zentmyer and Schieber (1992) consider that *P. nubigena*, *P. steyermarkii*, and *P. zentmyerii*, are species different from *P. americana*. These hypotheses are supported by the results of the present study; rather, *P. drymifolia* and *P. americana* are grouped in a polytomy with the clade made up of the six species *P. floccosa*, *P. guatemalensis*, *P. nubigena*, *P. tolimanensis*, *P. steyermarkii* and *P. zentmyerii* (Figure 1).

The clade grouping the subgenus *Persea* species has *Nectandra* as sister taxon and *Ocotea* as sister of both. The relationship between them suggests that the separation of the *Persea* subgenus is poorly supported with the lowest bootstrap and jackknife values (Figure 1). The inclusion of more *Nectandra* and *Ocotea* species should confirm that they are sister taxa of the *Persea* subgenus. In this study, *P. primatogena* was grouped by the unique combination of five characters including flower color (22) and is the sister taxon of all other species of subgenus *Persea*. It is evident that speciation of these species stemmed from *P. drymifolia*, and that this group including the core of subgenus *Persea* is defined by seed diameter (40) and fruit shape (31), in addition to two floral characters (23, 27) and five vegetative ones (Figure 1). The associated changes in fruit shape and seed diameter shared by the members of this group as evolutionary novelties are predicted as inherited by the offspring and should be considered in strategies of genetic improvement programs.

The sister taxa relationship of *P. floccosa*, *P. steyermarkii* and *P. zentmyerii* is defined by elliptic-globose fruits (31), yellow pulp (34) and oblate seeds (39) (Figure 1). The clade made up of *P. guatemalensis*, *P. nubigena* and *P. tolimanensis* shares the presence of 6–10 divergent veins in leaf (18) and fruit diameter larger than 5 cm (33). These results suggest that *P. nubigena* is sister to *P. guatemalensis* and *P. tolimanensis*, a relationship that has not been suggested previously (Figure 1). The pairs of species, *P. nubigena*–*P. tolimanensis* and *P. steyermarkii*–*P. zentmyerii*, have been proposed as ancestors of *P. guatemalensis*, but the present study does not support these assertions. *Persea tolimanensis* was determined as being more closely related to *P. guatemalensis*. They are sister taxa and share leaf veins divergence angle (8) and number of fruits per cluster (36). *Persea steyermarkii* was not included in this clade, and none of these species was determined as being ancestor of *P. guatemalensis*. They may be considered the result of speciation of an ancestor shared with *P. drymifolia* and the interaction of changes in their environment. The results of the present study support the conclusion by Bergh et al. (1973) that *P. guatemalensis* and *P. nubigena* are closely related. The proposal that *P. steyermarkii* (Schieber and Bergh 1987; Furnier et al. 1990) is related to *P. guatemalensis* is not supported by the morphological characters examined in the present study, as *P. steyermarkii* is the sister taxon of *P. floccosa*, considered by some authors to be an ancestor of *P. drymifolia* Scora and Bergh 1990). It is clear that the morphological analysis needs to be complemented by an analysis of molecular characters, such as ITS, to provide a better resolution within *Persea* clades and to support the relationships proposed in this study. The molecular study is currently underway to gain further insight into the species relationships.

The phylogenetic relationships revealed by this study provide the basis for the use, selection and conservation of *Persea* species, particularly those related to *P. americana*. If these relationships are supported by other sources of data such as ITS, various taxonomic changes could take place. Among them, the recognition of various species currently considered ecotypes of *P. americana*, and the elevation of the subgenera to genera. The new hypotheses of relationships among species of *Persea* should be considered in the breeding program. Several of the morphological characters (leaves, number of fruits per cluster, fruit shape, pulp color, and seed size) that are considered as evolutionary novelties for various clades, should be included in genetic improvement programs of *Persea* germplasm.

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Appendix

Appendix 1. List of characters and character states used in this study based on field observations.

Tree

01 Habit. Trees were plants taller than 10 m height (0), medium-sized trees were plant 5–9 m height (1), and shrubs, plants lacking a trunk with branches developing near soil (2)

02 Bark color in branches. The bark has three distinctive color in the material studied. White (0), brown (1), and green (2)

03 Bark texture. Bark texture allows distinguishing species in the field and two character states were recognized in the material studied. Smooth (0) and rough (1)

04 Pubescent bracts near apex. According to Allen (1945) the bracts in species of subgenus *Eriodaphne* are pubescent (0) and glabrous (1) in species of subgenus *Persea*

Leaf

05 Domatia. The occurrence of domatia in the interception of the primary vein and the secondaries is distinctive of some genera. They are absent in *Persea* (0) and present (1) in *Phoebe*

06 Scent to anise. Most members of the family Lauraceae have a distinctive anise scent (0) related to the occurrence of oil cells in leaves and fruits, however some species lack the scent (1)

07 Leaf texture. This character has been used by several authors to distinguish among species of subgenus *Persea*. Two character states are recognized based on their texture: Coriaceous (0) and chartaceous (1)

08 Angle of divergence of secondary veins in leaves. This character has been used to distinguish species of *Persea* (Standley and Steyermark 1946) and five character states we defined based on ANOVA analyses for the material studied: < 30° (0) in, 30–40° (1), 41–50° (2), 51–60° (3), and > 61° (4).

09 Young leaf color. When the blade is expanding the new leaves may possess two distinctive colors: Red (0) and green (1)

10 Mature leaf color. When the blade reaches maturity three different colors distinguish most species studied. The typical green (0), green-yellowish (1), and red (2)

11 Pubescent leaves. We recognized leaf as pubescent (0) in various species and glabrous (1)

12 Pubescent leaves color. When pubescence is present it acquires different colors distinguishing various genera of the family Lauraceae and species of *Persea* (Standley 1920). Four character states are recognized for the material studied. Yellow (0), brown (1), white (2), and red (3)

13 Venation pattern reliefs. The venation pattern relief was pointed out by Standley and Steyermatk (1946) as a character that differentiates species. Three character states for the venation relief were recognized in the species studied. Leveled (0), enhanced (1), and depressed (2)

14 Leaf shape. This character was assigned based on Hickey (1973) proposal and four character states recognized for the material studied. Elliptic when length/width ratio is 2:1 (0), lanceolate when ratio was 3:1 (1), oblong when ratio was 2:1 (2), and ovate when ratio 1.5:1 (3).

15 Phylotaxy. Phylotaxy in the family Lauraceae has been recognized by Standley (1920). The most common arrangement of leaves is alternate (0), but opposite (1) and alternating distichously (2) were also found in the material studied.

16 Leaf lengths. Variation in leaf length based on ANOVA analysis allows to recognize four character states for the species studied. < 5 cm (0), 5–15 cm (1), 16–30 cm (2), and > 30 cm (3)

17 Leaf widths. Variation in leaf width based on ANOVA analysis allows to recognize four character states for the species studied. < 1 cm (0), 1–5 (1), 6-10 cm (2), and > 10 (3)

18 Number of tertiary divergent veins. Various authors recognized that number of divergent veins is a character that distinguishes among species (Williams 1977), based on ANOVA analyses three character states were defined. < 1 (0), 1–5 (1), 6–10 (2), and > 10 (3)

19 Petiole lengths. The petiole length has been used by several authors to distinguish among species; in addition to this assertion ANOVA analysis allows the recognition of four character states. < 0.5 cm (0), 0.5–1 cm (1), 1.5–6 cm (2), and > 6 cm (3)

Appendix 1. Continued.

Flower

20 Inflorescence position. Three character states were defined for inflorescence position in the material studied. Terminal (0), axillary (1), and subaxillary (2)

21 Pubescent inflorescence bract. Williams (1977) recognized inflorescence bracts as pubescent in the adaxial surface (0) in species of subgenus *Persea* and pubescent both surfaces (1) in members of subgenus *Eriodaphne*

22 Flower color. In the species studied four flower colors were recognized. White (0), green-yellowish (1), green (2), and yellow (3)

23 Flower size. Lorea and van der Werff (1997) recognized that flower size distinguish various species of *Persea* in addition to both subgenera. ANOVA analysis supports this assertion and four character states were recognized. <1 mm (0), 1–5 mm (1), 6–10 mm (2), and >10 mm (3)

24 Tepal position. Kopp (1966) recognized tepal position as reflexed (0) in species of subgenus *Persea* and no reflexed (1) in species of subgenus *Eriodaphne*

25 Tepal persistency. Kopp (1966) recognized the persistency of tepals as deciduous (0) in species of subgenus *Persea* and persistent (1) in species of subgenus *Eriodaphne*

26 Glands at the base of the stamens of the third whorl. Kopp (1966) recognized the stipitate glands (0) in species of subgenus *Persea* and sessile glands (1) in species of subgenus *Eriodaphne*

27 Staminode shape. Authors (Lorea and van der Werff 1997; van der Werff 2002) recognized the not arrow tip (0) in various species and the arrow tip (1) in most species of subgenus *Eriodaphne*

28 Number of fertile stamens. This character defines a number of Lauraceae genera. Most authors recognized that *Persea* has nine fertile stamens and *Lindera* 6, associated to this character is the number of thecas per anther. Equal to nine stamens (0), >9 (1), and <9 (2)

29 Pubescent pistil. Kopp (1966) recognized pistil as pubescent (0) in species of subgenus *Persea* and glabrous (1) in species of subgenus *Eriodaphne*

Fruit

30 Fruit color. When fruits reach maturity species have four distinctive colors. Black (0), green (1), brown (2), and purple (3)

31 Fruit shape. Although it is considered that fruit shape varies markedly in relation to inbreeding, different shapes were recognized in the material studied. Elliptic (0), elliptic-globose (1), round (2), pyriform (3), ovoid-spherical (4), ovoid (5), and oblong (6)

32 Fruit flavor. The rich, slightly nutty taste of some avocados is generally preferred over bland flavors. The spicy or anise-like flavor of Mexican types such *P. drymifolia* are consider more desirable by some consumers. In some cases flavor is signal of an edible fruit by humans or not. Based on a test four states were recognized. Anise (0), bitter (1), neutral (2), and sweet (3)

33 Fruit diameter. This character is used in improvement genetic programs and ANOVA analysis found three distinctive group. <3 cm (0), 3.5–5 cm (1), and >5 cm (2)

34 Pulp color. The pulp color is a consistent feature that distinguishes among species of *Persea*. Three character states were recognized. Yellow (0), green-yellowish (1), and green (2)

35 Exocarp thickness. The leathery easy-peeling type or thicker exocarp avocados are usually preferred for edible fruits. Thin-exocarp fruit is prone to damage and a exocarp that is too thick prevents determination of ripening time. Authors (Bergh 1992) used this character for genetic improvement. >4 mm (0) and <4 mm (1)

36 Number of fruits per cluster. The number or fruits per inflorescence is a character that differentiates species and is an important character for genetic improvement programs. Based on number of fruits variation three states were recognized. >4 (0), 2–4 (1), and <2 (2)

37 Fruit length. According to various authors (Bergh 1993; Gamma 1994; Bergh and Ellstrand 1987) fruit length varies among species and based on ANOVA analysis three characters were recognized. Moreover, this is a feature of interest in genetic improvement programs because of its variation pattern. <10 cm (0), 10–15 cm (1), and >15 cm

38 Cupule. Group of persistent bracts that surround the mature fruit. This feature distinguishes most species of both subgenera (Kopp 1966; van der Werff 2002). The cupule is absent (0) in *Nectandra* and most species of subgenus *Persea*, and present (1) in *Phoebe* and most species of*Eriodaphne*.

Seed

39 Seed shape. The seed shape is considered a high stable character associated in most species with fruit shape and five character states were recognized. Ellipsoid (0), oblate (1), round (2), ovoid (3), and sub-oblong (4)

40 Seed diameter. In most genetic improvement programs the relationship between small seed size and fruit size is desire. Based on ANOVA analysis four characters states were recognized for seed diameter. <1 cm (0), 1.1–2 cm (1), 2.1–5 cm (2), and >6 cm (3)

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