

## ADDRESSING THE “HARDEST PUZZLE IN AMERICAN POMOLOGY:” PHYLOGENY OF *PRUNUS* SECT. *PRUNOCERASUS* (ROSACEAE) BASED ON SEVEN NONCODING CHLOROPLAST DNA REGIONS<sup>1</sup>

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*Prunus* subg. *Prunus* sect. *Prunocerasus* (Rosaceae) is a North American taxon with 17 commonly recognized taxa. To test the hypothesis of monophyly for the section we sequenced the *trnG* and *rpL16* introns and the *trnH-psbA* and *trnS-trnG* intergenic spacers for at least two representatives of each of the five subgenera in *Prunus*. Additionally we sampled heavily among *Prunus* subg. *Prunus* sections *Prunus* and *Armeniaca* and *Prunus* subg. *Amygdalus* because these groups are putatively most closely related to *Prunocerasus*. Once monophyly of sect. *Prunocerasus* was shown we added the sequences of *trnL* and *rpS16* introns and the *trnL-trnF* spacer in an attempt to increase resolution within the section. The species of sect. *Prunocerasus* showed an initial split with *P. subcordata*, the only species from western North America, sister to the rest of the group. The remaining species fell into three primary clades. Within each of the three primary clades there was little phylogenetic resolution. Lastly, we present evidence that *P. texana*, previously classified in subg. *Amygdalus*, may be a plum or at least contain a *Prunocerasus* chloroplast. This is the first phylogenetic hypothesis presented for sect. *Prunocerasus*, and the clades recovered contrast sharply with previously defined groups based on morphological characters.

**Key words:** Amygdaloideae; *Amygdalus*; chloroplast DNA; *Euprunus*; Plum; *Prunocerasus*; *Prunus*; Rosaceae.

*Prunus* L. (Rosaceae) is a morphologically diverse holarctic genus of about 200 species of small trees or shrubs with pentamerous flowers and endozoochorous monocarpellate drupes (Rehder, 1940; Robertson, 1974). Many species are economically significant, especially those that are used for ornamentals and food (plums/prunes, peaches, apricots, cherries, and almonds). The Eurasian plums of sect. *Prunus* (see Supplementary Data accompanying the online version of this article) are those most heavily used in agriculture, but they have proven difficult to grow in many parts of North America because they are not well adapted to drought, frost, and particularly in the eastern and southern states, fungal, bacterial, and viral diseases (Fogle, 1978). Native North American species of sect. *Prunocerasus* are currently not cultivated on a large scale, although they have been in the past. Species such as *P. americana* Marshall, *P. hortulana* L.H.Bailey, *P. angustifolia* Marshall, and *P. subcordata* Benth. were (Waugh, 1899, 1901) and more recently, *P. maritima* Marshall (Dunn, 2002; Karp, 2003; R. Uva, Cornell University, personal communication) and *P. angustifolia* (Reid and Gast, 1993) are under study in agricultural trials. Additionally, nearly all *Prunus* sect. *Prunocerasus* species hybridize, many naturally, not only with themselves but also with section *Prunus*, and thus the potential for better-adapted agricultural and horticultural varieties may be unlimited (Wight, 1915). Over 2800 varieties of European plums have been cultivated (Fogle, 1978) and as there are several

more North American plum species than there are European plum species, the potential for varieties is much greater (Hedrick et al., 1911).

Several species within sect. *Prunocerasus* are of conservation concern. Of greatest concern is *P. geniculata* R.M. Harper that has a global distribution of only four counties in central Florida and is Federally Endangered; despite being federally protected it is currently being destroyed by suburban development (J. Shaw, personal observation). Seven other species in sect. *Prunocerasus* are legally protected at the state level, as shown in the Supplementary Data accompanying the online version of this article.

The most widely accepted classification of *Prunus* is that of Rehder (1940). He divided *Prunus* into five subgenera (*Prunus* [= *Prunophora*], *Amygdalus*, *Cerasus*, *Padus*, and *Laurocerasus*; see Supplementary Data accompanying the online version of this article) and divided subg. *Prunus* into three sections: *Prunus* = (*Euprunus*) (Eurasian plums), *Prunocerasus* (North American plums), and *Armeniaca* (apricots). Subgenus *Emplectocladus* was not recognized by Rehder (1940), he placed *P. fasciculata* A.Gray in subg. *Amygdalus*; however, earlier Mason (1913) believed it to be closely related to sect. *Armeniaca*, and more recently Bortiri et al. (2001) proposed *P. fasciculata* as sister to an extended subg. *Prunus*.

Among the first investigators of our native plums was Waugh (1899, 1901), who noted their extensive hybridization and intergradation. He divided the North American plums into four informally named series: Americana, Chickasaw, Hortulana, and Maritima. The Americana Series, centered on *P. americana* var. *americana*, graded into *P. nigra* Aiton to the northeast and *P. americana mollis* Torr. & A.Gray (*P. americana* var. *lanata* Sudw.) to the southwest. The Chickasaw Series only included *P. angustifolia* and a variety. The Hortulana Series was described as “a great congeries of hybrids” (Waugh, 1899, p. 233), whose morphological characters “run into each other in the most puzzling and intricate manner” (Waugh, 1899, p. 233). In this series he noted that *P. hortulana*

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var. *mineri* L.H.Bailey grades into *P. americana* to the north and into *P. hortulana* var. *waylandii* L.H.Bailey to the south and then also into *P. munsoniana* Wight and Hedrick and eventually *P. angustifolia* “so connectedly as to leave not the slightest break between them” (Waugh, 1899, p. 233). Waugh (1901) also thought that *P. hortulana* and its relatives were of hybrid origin (*P. americana* × *P. angustifolia*). Lastly, he noted that members of this series also grade into *P. maritima* Marshall and *P. injucunda* Small (= *P. umbellata* Elliot var. *injucunda* [Small] Sarg.). His Maritima Series included *P. maritima* and *P. gravesii* Small (= *P. maritima* var. *gravesii* [Small] G.J.Anderson). He also put *P. injucunda* into this section and noted that he could not distinguish the type material from *P. maritima*. He believed *P. gracilis* Engelm. and A.Gray to be a good species and thought it to be closely related to *P. injucunda*. Lastly, Waugh (1899) thought *P. gracilis* to be closely related to *P. glandulosa* (Hook.) Torr. & Gray (not *P. glandulosa* Thunb.), which is a synonym of *P. texana* Dietr. In contrast Wight (1915) placed this taxon in the genus [subg.] *Amygdalus*, and Correll and Johnston (1970) refer to it as Peach Bush.

Wight (1915) provided the most comprehensive treatment within sect. *Prunocerasus* (hereafter referred to as *Prunocerasus*) and informally recognized six groups: Americana, Angustifolia, Gracilis, Hortulana, Maritima, and Subcordata (see Supplementary Data accompanying the online version of this article). Similar to Waugh (1899), he placed *P. americana*, *P. nigra*, and *P. mexicana* S.Watson together in the Americana group. He also agreed with Waugh (1899) that *P. angustifolia* and *P. munsoniana* are closely related and placed them together in the Angustifolia group. Again not contradicting Waugh (1899), Wight (1915) saw a relationship between *P. umbellata* var. *umbellata* Elliot, *P. umbellata* var. *injucunda*, and *P. maritima*, but in contrast to Waugh (1899), Wight (1915) placed *P. gracilis* in its own group and never mentioned *P. texana*. He also placed *P. subcordata* in its own group. *Prunus geniculata* was not included in his work because it was described around the same time as Wight’s (1915) work.

Morphological taxonomy has long been difficult within *Prunocerasus* because species boundaries are blurred by interspecific similarities and intraspecific variation and likely by interspecific hybridization (Waugh, 1899, 1901; Hedrick et al., 1911; Wight, 1915; Rehder, 1940). There are 17 commonly recognized taxa in *Prunocerasus* (see Supplementary Data); several of which are only differentiated by a few continuous (and often highly variable) characters that different authors may or may not have considered important. This has resulted in a lot of taxonomic confusion. For example, Fernald (1950, p. 877) described *P. americana* var. *americana* as “passing insensibly into *P. americana* var. *lanata*” (which was described as *P. lanata* Sudw.) whereas Gleason and Cronquist (1991) placed *P. americana* var. *lanata* as a synonym of *P. mexicana* (characterized by densely pubescent leaves). Robertson (1974, p. 659) noted that “*P. americana* and *P. mexicana* need to be studied more to ascertain their distinctiveness and distribution.” Additionally, *P. umbellata* has been variously subdivided into *P. injucunda*, which has pubescent leaves and twigs, and *P. mitis*, which has glabrous twigs (Radford et al., 1968); both of these putative species differ from *P. umbellata*, which is completely glabrous. Sargent (1902) saw *P. tarda* Sarg. as a distinct species, but Wight (1915, p. 54) noted “*P. umbellata* ssp. *injucunda* merges imperceptibly

into *P. umbellata* ssp. *tarda*.” Duncan and Duncan (1988) believed that *P. umbellata* only differs from *P. alleghaniensis* Porter in distribution. To add to the confusion, Waugh (1899, p. 234), after studying the type material of *P. injucunda*, noted it as “easily being referable to *P. maritima*.” Other examples of convoluted taxonomic disagreement abound and excessive “splitting” within this group was noted as early as 1915 (Wight, 1915). Hedrick et al. (1911, p. 5) went so far as to say that *Prunocerasus* is “plastic in all physical characters” and Bailey (1892, p. 90) referred to *Prunocerasus* as “the hardest puzzle in American pomology.” Because variable morphological characters have resulted in ambiguous and conflicting species delimitation and classification, many taxa within the section have been variously accepted, ranked, lumped, and split. This has not only created a large amount of taxonomic confusion, but bewildering nomenclatural synonymy. Clearly, morphological data alone are unable to unequivocally define species boundaries and relationships within *Prunocerasus*.

Several authors have employed molecular tools in addressing broader phylogenetic questions in *Prunus* and all of them have included some representatives from *Prunocerasus* (Mowrey and Werner, 1990; Bortiri et al., 2001, 2002; Lee and Wen, 2001). Because of what appears to be relatively low divergence within *Prunus*, these studies are only taxonomically suggestive and lack the support or resolution upon which to confidently base a hypothesis of monophyly for *Prunocerasus*. Additionally, some of these earlier studies have suggested, although with weak support, that *Prunocerasus* may not be monophyletic (Mowrey and Werner, 1990; Lee and Wen, 2001; Bortiri et al., 2002).

The earliest work to employ molecular tools (isozymes) suggested that *Prunocerasus* is polyphyletic with *P. americana*, *P. munsoniana*, *P. hortulana*, *P. subcordata*, and *P. angustifolia* belonging to a clade and *P. maritima* and *P. umbellata* belonging to another clade, with the latter clade more closely related to sect. *Prunus* and subg. *Cerasus* than to *Prunocerasus* (Mowrey and Werner, 1990). Based on these relationships, Mowrey and Werner (1990) suggested that there were at least two immigrations of plums into North America. More recently, Lee and Wen (2001) included four species of *Prunocerasus* in a nuclear ribosomal internal transcribed spacer (ITS) analysis of *Prunus*. Their data had weak resolution in this part of the tree and were unable to resolve *P. armeniaca* L. of sect. *Armeniaca* from representatives of *Prunocerasus*. Bortiri et al. (2001) combined data from the cpDNA *trnL-trnF* spacer and ITS sequences to address phylogenetic relationships within *Prunus*. Their data support monophyly of some eastern North American *Prunocerasus* representatives, but *P. subcordata*, of northwest North America, was not resolved from species outside *Prunocerasus*. Finally, Bortiri et al. (2002), in a second broad analysis of *Prunus* using the single copy nuclear gene *s6pdh*, showed that *P. subcordata* is sister to other sampled *Prunocerasus* species and that *P. armeniaca* may be closely related to *Prunocerasus* (in agreement with Lee and Wen, 2001). While these studies conditionally support a close relationship among *Prunocerasus* species, none of them unequivocally support or refute the monophyly of *Prunocerasus* and none of them amply sample within *Prunocerasus* to allow relationships within the section to be inferred.

In summary, *Prunus* subg. *Prunus* sect. *Prunocerasus* is a valuable, somewhat vulnerable, and complicated group of organisms that are “misunderstood.” Understanding the classi-

fication and evolutionary history of the North American plums is of economic importance as well as conservation and taxonomic interest. To date no rigorous phylogenetic analysis addressing the monophyly of, or relationships among, species within *Prunocerasus* has been conducted. Given the lack of resolution in the aforementioned foundational phylogenetic studies, the longstanding taxonomic difficulty of this group, and the potential applicability of known evolutionary relationships to agriculture, horticulture, and conservation, the goals of this research were to (1) test the monophyly of *Prunus* subg. *Prunus* sect. *Prunocerasus* and its relationship to other sections of subg. *Prunus* and (2) to produce a phylogenetic hypothesis of the relationships within *Prunocerasus* based on cpDNA sequences.

## MATERIALS AND METHODS

**Plant material and outgroup choice**—We sampled 17 species and subspecific taxa of *Prunocerasus* that represent all of the commonly accepted taxa within the following works: Small (1933), Rehder (1940), Bailey and Bailey (1941), Fernald (1950), Blackburn (1952), Gleason (1952), Steyermark (1963), Radford et al. (1968), Correll and Johnston (1970), Duncan and Duncan (1988), Godfrey (1988), Wunderlin (1988), Gleason and Cronquist (1991), Smith (1994), and Wofford and Chester (2002). Samples of the federally endangered *P. geniculata* were obtained from scientists at the Archbold Biological Station in Lake Placid, Florida, and two collections of *P. subcordata* from California were provided by colleagues at Oregon State University. Additionally, we sampled several representative species from each of the other two sections in subg. *Prunus* along with representatives from each of the other four subgenera: *Amygdalus*, *Cerasus*, *Padus*, and *Laurocerasus* (see Supplementary Data). Because none of the taxa within *Prunus* has unequivocally been shown to be sister to the rest of the genus (Mowrey and Werner, 1990; Bortiri et al., 2001, 2002; Lee and Wen, 2001), *Physocarpus opulifolius* (L.) Maxim. was used as an outgroup (see Bortiri et al., 2001). Ingroup sampling of *Prunocerasus* was from wild-collected populations except for one case of a herbarium specimen and outgroup samples were both from wild-collected populations and from the *Prunus* germplasm facility at the University of California, Davis, USA (DPRU).

**DNA sequences**—For all 43 taxa in this investigation the *rpL16* and *trnG<sup>UUC</sup>* introns as well as the *trnS<sup>GCU</sup>-trnG<sup>UUC</sup>* and *trnH<sup>GUG</sup>-psbA* intergenic spacers were polymerase chain reaction (PCR) amplified and sequenced. These regions were chosen because they were shown to be highly informative noncoding cpDNA regions (Shaw et al., unpublished data). For the ingroup analyses, sequence data were obtained from seven separate chloroplast loci including four introns: *trnL<sup>UAA</sup>*, *trnG<sup>UUC</sup>*, *rpS16*, *rpL16* and three intergenic spacers: *trnL<sup>UAA</sup>-trnF<sup>GAA</sup>*, *trnH<sup>GUG</sup>-psbA*, and *trnS<sup>GCU</sup>-trnG<sup>UUC</sup>*. The *trnL* and *rpS16* introns along with the *trnL-trnF* intergenic spacer were amplified and sequenced for the seventeen ingroup taxa plus *P. mahaleb* L. before J. Shaw et al. (unpublished data) showed that they are comparatively less variable than other noncoding cpDNA choices. Primer pairs for the aforementioned regions are listed in Table 1; primers for the *trnL* intron and the *trnL-trnF* intergenic spacer are from Taberlet et al. (1991), primers for *rpL16* are from Small et al. (1998), and the remaining primers in Table 1 are from J. Shaw et al. (unpublished data).

**Laboratory procedures**—DNA was extracted from leaves using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). Polymerase chain reaction (PCR) was performed using Eppendorf Mastercycler gradient or Mastercycler personal thermal cyclers in 50  $\mu$ L volumes with the following reaction components: 1  $\mu$ L template DNA (~10–100 ng), 1 $\times$  *ExTaq* buffer (PanVera/TaKaRa, Madison, Wisconsin, USA), 200  $\mu$ mol/L each dNTP, 3.0 mmol/L MgCl<sub>2</sub>, 0.1 mmol/L each primer, and 1.25 units *ExTaq* (PanVera/TaKaRa). Reactions included bovine serum albumin at a final concentration of 0.2  $\mu$ g/ $\mu$ L, which improved amplification from difficult templates. For amplification of the *trnS-trnG-trnG* region, 10 mmol/L of tetramethyl am-

monium chloride (TMACl) was added to the reaction mixture because it is thought to help PCR through polynucleotide runs (see Oxelman et al., 1997), and J. Shaw et al. (unpublished data) have shown that a poly-A/T run exists ~150 base pairs (bp) upstream from the 3' *trnG* exon across all lineages of phanerogams.

The PCR and sequencing primers are given in Table 1, and all PCR protocols described below were preceded by template DNA denaturation at 80°C for 5 min. The PCR cycling conditions for *rpL16* were: 30 cycles of denaturation at 95°C for 1 min, primer annealing at 50°C for 1 min, followed by a ramp of 0.3°C/s to 65°C, and primer extension at 65°C for 4 min. A final extension step consisted of 5 min at 65°C. The PCR cycling conditions for *trnL+trnL-trnF* were: 30 cycles of denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min, primer extension at 72°C for 2 min. A final extension step consisted of 5 min at 72°C. The PCR cycling conditions for *rpS16* were: 30 cycles of denaturation at 94°C for 30 s, primer annealing at 53°C for 30 s, primer extension at 72°C for 2 min. A final extension step consisted of 5 min at 72°C. The PCR cycling conditions for *trnH-psbA* were: 30 cycles of denaturing at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min. The PCR cycling conditions for *trnS-trnG-trnG* were by the touchdown method: 15 cycles of denaturation at 96°C for 1 min, primer annealing at 76°C (–0.4°C/cycle) for 45 s, primer extension at 72°C for 2 min, and then 30 cycles of denaturation at 96°C for 1 min, primer annealing at 69.5°C for 45 s, primer extension at 72°C for 2 min. A final extension step consisted of 5 min at 72°C.

The PCR products were checked on 1% agarose gels before being cleaned with either the QIAquick PCR Purification Kit (Qiagen) or ExoSAP-IT (USB, Cleveland, Ohio, USA). DNA sequencing was performed with the same primers used in amplification (Table 1) with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit, v. 2.0 or 3.1 (Perkin-Elmer/Applied Biosystems, Foster City, California, USA), electrophoresed and detected on an ABI Prism 3100 automated sequencer. Sequencher 3.1.1 (Gene Codes, 1998) was used to edit and assemble complementary DNA strands and check for agreement between them. In no cases did the adjoined complementary strands disagree. All sequences have been deposited in GenBank; accession numbers (AY500595–AY500780) are given in the Supplementary Data accompanying the online version of this article.

**Data analysis and phylogenetic assessment**—For both data sets, alignment of DNA sequences was initially performed with ClustalX (Thompson et al., 2001), with subsequent manual adjustment by eye in MacClade v. 4.0 (Sinauer, Sunderland, Massachusetts, USA). Variable positions in the data matrices were double checked against the original chromatogram files to make sure that all base calls were true at all variable positions. In all cases, alignment of potentially informative positions was unambiguous. Because indels have been shown to provide approximately one-third of the potentially phylogenetically informative information in a cpDNA data set (Shaw et al., unpublished data), potentially phylogenetically informative indels were coded as additional binary characters. However, a few indels, such as those within polynucleotide runs, were omitted from the data set as they may be PCR artifact and not reflective of the phylogenetic history of the group.

Analysis of phylogenetic relationships was conducted using the optimality criterion of maximum parsimony. Searches for most-parsimonious trees were executed in PAUP\* v. 4.0b10 (Swofford, 2002). Parsimony analyses were carried out by a heuristic search with tree bisection-reconnection (TBR) branch swapping and 1000 random sequence addition replicates. Bootstrap support (Felsenstein, 1985) was estimated with 1000 replications of heuristic search and simple taxon addition with the constraint of 1 000 000 rearrangements per replicate. Both the consistency and retention indices (CI and RI, respectively) were used to assess the amount of homoplasy present in the data set. Additionally, Bayesian analysis was employed as alternative means of phylogenetic assessment. Bayesian analyses of the data were performed using MrBayes 2.01 (Huelsenbeck and Ronquist, 2001) to generate posterior probability distribution using Markov chain Monte Carlo (MCMC) methods. No a priori assumptions about tree topology were made. The statistical model of DNA substitution, Felsenstein, 1981 (F81), was that estimated as the best-fitting maximum likelihood model using MrModeltest 1.1b (Nylander, 2002),

which is a simplified version of MODELTEST 3.06 (Posada and Crandall, 1998). The Markov chain Monte Carlo (MCMC) process was set to run  $1 \times 10^6$  generations with four chains. Burn-in was estimated visually by plotting log-likelihood values in Microsoft Excel to determine the number of generations that had run before likelihood values reached an asymptote. To calculate the posterior probability of each bipartition a 50% majority-rule consensus tree was constructed from the remaining trees using PAUP\*.

## RESULTS

**Characterization of the cpDNA data sets**—For the broad analyses, sequence data were obtained from four different non-coding cpDNA regions: *trnH-psbA*, *rpL16*, *trnG*, and *trnS-trnG* (Table 1). As expected, given the complete linkage among cpDNA regions, data derived from different cpDNA regions were congruent and thus were combined into a single data set.

The combined cpDNA data set for the broad investigation of *Prunus* consisted of 3270 aligned nucleotide positions with 231 variable positions, 134 of which were parsimony informative (including multi-bp indels coded as binary characters). Characteristics of individual cpDNA regions are summarized in Table 1. In the maximum parsimony analysis, the heuristic search found 25 171 most parsimonious trees of 422 steps and high consistency and retention indices, CI = 0.92 and RI = 0.94, which indicates that there was little homoplasy within the data set. Bootstrap values are shown above the branches in Fig. 1. The topology of the tree generated with the Bayesian method (Fig. 2) was consistent with the parsimony tree although in two groups the Bayesian analysis showed more resolution (sections *Prunus* and *Amygdalus*). Support values for equivalent tree branches were consistently higher in the Bayesian analyses than in the parsimony analyses.

Members of sect. *Microcerasus* contain a relatively high number of homoplasious characters compared to other taxa in this study. Additional Bayesian and bootstrap analyses (not shown) with the same parameters described above were performed on the broad data set, but excluding members of sect. *Microcerasus* (*P. pumila* L., *P. glandulosa* Thunb., and *P. tomentosa* Thunb.). Without these species, bootstrap values and posterior probabilities for several of the clades increased. Resolution within the subg. *Prunus*-subg. *Amygdalus* clade also increased in the bootstrap analysis as there was 64% bootstrap support for a monophyletic sect. *Prunus* clade (the same clade shown in the Bayesian analysis in Fig. 2).

Because the results of the broad analysis showed that *Prunocerasus* is a monophyletic assemblage, the *trnL-trnF* intergenic spacer and the *trnL* and *rpS16* introns were added to the aforementioned four cpDNA regions and separate analyses were performed on sect. *Prunocerasus* + *P. mahaleb* (an outgroup species). The ingroup data set, made up of the seven cpDNA regions, consisted of 4375 aligned nucleotide positions, had 88 variable positions, 37 of which were parsimony informative (including multi-bp indels coded as binary characters). Characteristics of individual cpDNA regions are summarized in Table 1. Maximum parsimony analysis found one most parsimonious tree of 128 steps with high consistency and retention indices, CI = 0.98 and RI = 0.98, which indicate a low amount of homoplasy within the data set. The topology of the *Prunocerasus*-only tree is nearly identical to the topology found in the broad analysis and thus is mapped onto the trees generated in the broad analyses. The additional resolution in the American clade provided by this analysis is represented in Figs. 1–3 by dotted lines. Bootstrap values for the *Pruno-*

*cerasus*-only analysis are marked below the branches in Fig. 1. Similar to the results of the broad analysis, the topology of the tree generated with Bayesian methods was topologically identical to the parsimony tree. Posterior probabilities for the *Prunocerasus*-only analysis are marked below the branches in Fig. 2. Again, support values for all of the tree branches were consistently higher in the Bayesian than in the parsimony analyses.

**Phylogenetic results: broad analyses of *Prunus***—The broad phylogenetic analyses using the four combined cpDNA data sets provided unequivocal support for a monophyletic sect. *Prunocerasus* with a bootstrap value of 83% and a posterior probability of 1.0. The Bayesian analysis showed sect. *Prunus* as monophyletic with *P. glandulosa* of subg. *Cerasus* sect. *Microcerasus* sister to it with a weak posterior probability of 0.68. The sect. *Prunus* clade was only supported by two nucleotide substitutions, one of which is likely homoplasious because it disagrees with several other characters in the data set and is possibly the reason why *P. glandulosa* appeared to be sister to the rest of sect. *Prunus*. Within sect. *Prunus*, the European-West Asian plums and the Asian plums formed two distinct clades that were supported by the bootstrap analysis; however, in the bootstrap analysis there was no support for these two clades being sister to each other (because of the apparent homoplasy of *P. glandulosa*). Removal of sect. *Microcerasus* (which includes *P. glandulosa*) from the analysis raised the posterior probability for the sect. *Prunus* clade from 0.68 to 0.98; the results of the minus-*Microcerasus* bootstrap analysis, which before revealed two separate clades that were unresolved from the backbone of the Pruno-Amygdaloid clade (subgenera *Prunus* and *Amygdalus*), reveals a single sect. *Prunus* clade supported by a bootstrap value of 64%. The Bayesian analysis showed support, although weak, for a monophyletic subg. *Amygdalus* as well as for the distinctiveness of the two sections that comprise it: *Amygdalus* (= *Euamygdalus*) and *Chamaeamygdalus*. As in sect. *Prunus*, the deeper node was not supported in the bootstrap analysis, leaving only support for a monophyletic sect. *Amygdalus* (the placement of *P. tenella* Batsch, the sect. *Chamaeamygdalus* representative was unresolved in the bootstrap analysis).

Both analyses show strong support for a monophyletic Pruno-Amygdaloid clade, with a bootstrap value of 97% and a posterior probability of 1.0. *Prunus fasciculata* of subg. *Emplectocladus* and subg. *Cerasus* sect. *Mahaleb* were consecutively sister to the Pruno-Amygdaloid clade with high support. Subgenus *Cerasus* sect. *Microcerasus*, *P. pumila*, *P. tomentosa*, and *P. glandulosa* appeared not to be a monophyletic group (see Discussion), but its constituent species were strongly supported within the Pruno-Amygdaloid clade. Lastly, subgenera *Padus* and *Laurocerasus* appeared to be closely related, paraphyletic, and sister to the remaining members of *Prunus*.

**Phylogenetic results: section *Prunocerasus***—Within the *Prunocerasus* clade, the backbone of the tree indicated an initial split in the section between the northwestern species, *P. subcordata*, and the remaining species. *Prunus texana*, classified in subg. *Amygdalus* (Wight, 1913), was positioned with strong support within sect. *Prunocerasus* as sister to the remaining species (inside of *P. subcordata*). Within these remaining species the data distinguished three major clades: an “American clade,” a “Beach clade,” and a “Chickasaw clade” (see Fig. 3). The American clade was supported with

TABLE 1. Primer and region information.

cpDNA region	Primers	Primer source	Aligned length	Number of informative indels	Number of informative nucleotide substitutions	Number of informative inversions	Total informative characters
<i>Prunocerasus</i> analyses:							
<i>trmH-psbA</i> intergenic spacer	psbA <i>trmH2R</i> (GUG)	Shaw et al., unpublished data	363	3	1	0	4
<i>rpL16</i> intron	F71 R1516	Small et al., 1998	996	7	6	0	13
<i>trmS-trmG</i> intergenic spacer	<i>trmS</i> (GCU) 3' <i>trmG</i> (UUC)	Shaw et al., unpublished data	703	2	4	0	6
<i>trmG</i> intron	included in <i>trmS-trmG</i> above		711	0	4	0	4
<i>trmL-trmF</i> intergenic spacer	TabC TabF	Taberlet et al., 1991	397	0	3	0	3
<i>trmL</i> intron	included in <i>trmL-trmF</i> above		522	0	1	0	1
<i>rpS16</i> intron	rpS16F rpS16R	Shaw et al., unpublished data	683	2	4	0	6
Combined			4375	14	23	0	37
<i>Prunus</i> analyses:							
<i>trmH-psbA</i> intergenic spacer			516	13	11	0	24
<i>rpL16</i> intron			1105	10	21	0	31
<i>trmS-trmG</i> intergenic spacer			903	14	28	1	43
<i>trmG</i> intron			746	4	32	0	36
Combined			3270	41	92	1	134

Primer information same as above.

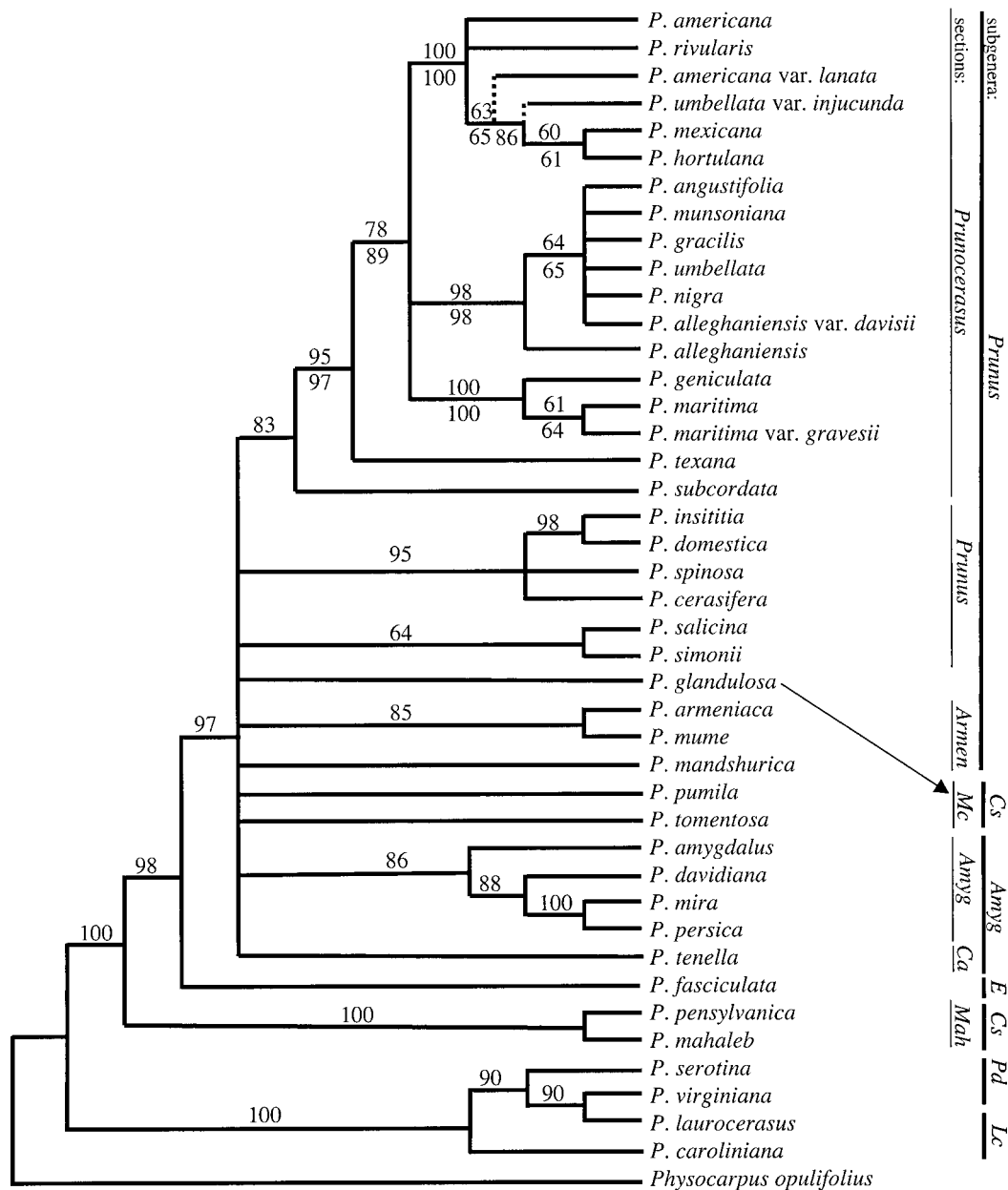


Fig. 1. Strict consensus of 25 171 equally parsimonious trees resulting from maximum parsimony analysis using the combined data from the *rpL16* and *trnG* introns as well as the *trnS-trnG* and *trnH-psbA* intergenic spacers (3270 aligned nucleotide positions, 134 are parsimony informative; tree length = 422 steps; consistency index [CI] = 0.92; retention index [RI] = 0.94). Bootstrap values greater than 50% are above the branches. Branches shown with dotted lines are not resolved in the broad analysis but show up in the *Prunocerasus*-only analysis with the addition of the *trnL-trnF* intergenic spacer and the *trnL* and *rpS16* introns to the aforementioned four cpDNA regions. A separate, *Prunocerasus*-only maximum parsimony analysis of the seven cpDNA regions found one most parsimonious tree of 128 steps (4375 aligned nucleotide positions, 37 are parsimony informative; tree length = 128 steps; CI = 0.98; RI = 0.98). Bootstrap values for the *Prunocerasus*-only analysis greater than 50% are shown below the branches. Classification into subgenera and sections follows Rehder (1940). Cs = *Cerasus*, Amyg = *Amygdalus*, E = *Emplectocladus*, Pd = *Padus*, Lc = *Laurocerasus*, Armen = *Armeniaca*, Mc = *Microcerasus*, Ca = *Chamaeamygdalus*, Mah = *Mahaleb*.

bootstrap value of 100% and a posterior probability of 1.0. Within this clade relationships were weakly resolved because of the lack of sequence divergence but showed support for *P. hortulana* and *P. mexicana* as being sister taxa. The Chickasaw clade was highly supported with a bootstrap value of 98% and a posterior probability of 1.0. Within this clade, there was little resolution because the sequences are nearly identical. Of the 4375 total aligned nucleotides used in this study, the sequences of *P. angustifolia*, *P. munsoniana*, *P. umbellata*, and

*P. alleghaniensis* var. *davisii* (Wight) Sarg. were identical, while *P. gracilis* and *P. alleghaniensis* and *P. nigra* each contained a single nucleotide difference. Within this clade however, there was weak support for *P. alleghaniensis* being sister to the rest of the clade. The Beach clade was also highly supported with a bootstrap value of 100% and a posterior probability of 1.0, and there was support for *P. geniculata* being sister to *P. maritima* var. *maritima* plus *P. maritima* var. *gravesii*.

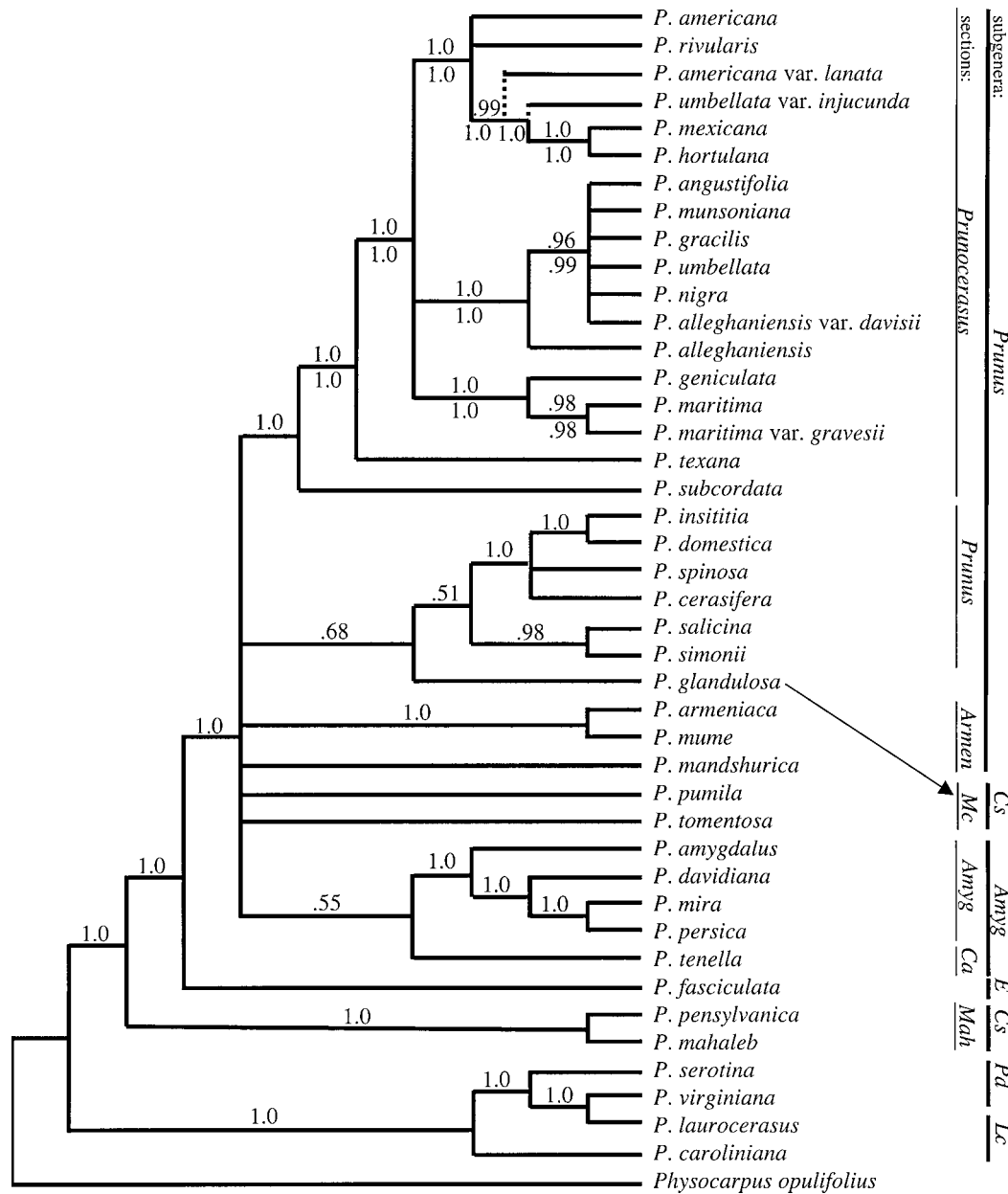


Fig. 2. Bayesian analysis tree for *Prunus* using the combined data from the *rpL16* and *trnG* introns as well as the *trnS-trnG* and *trnH-psbA* intergenic spacers (3270 aligned nucleotide positions, 134 are parsimony informative). Numbers above the branches are posterior probabilities derived in the broad analysis. Branches shown with dotted lines are not resolved in the broad analysis but show up in the *Prunocerasus*-only analysis with the addition of the *trnL-trnF* intergenic spacer and the *trnL* and *rpS16* introns to the aforementioned four cpDNA regions (4375 aligned nucleotide positions, 37 are parsimony informative). Posterior probabilities for the *Prunocerasus*-only analysis are below the branches. Classification into subgenera and sections follows Rehder (1940).

DISCUSSION

**Molecular evolution of the cpDNA regions**—As phylogenetic studies move to focus on lower-level taxonomic groups, it has become apparent that a multi-locus approach is necessary to obtain a sufficient number of phylogenetically informative characters, especially when using the relatively slowly evolving chloroplast genome. Many recent investigations have used several noncoding cpDNA regions to obtain sufficient characters for phylogenetic resolution (e.g., Kusumi et al., 2000; Bayer et al., 2002; Cronn et al., 2002; Goldblatt et al., 2002; Hartmann et al., 2002; Schönenberger and Conti, 2003;

Yamane et al., 2003). At low taxonomic levels, some noncoding cpDNA regions may show sufficient variation for phylogenetic resolution while others do not (e.g., McCauley, 1995; Demesure et al., 1996; Dumolin-Lapegue et al., 1997; Ohsako and Ohnishi, 2000). The seven chloroplast regions chosen for this phylogenetic investigation, the *rpS16*, *rpL16*, *trnL*, and *trnG* introns as well as the *trnL-trnF*, *trnS-trnG*, and *trnH-psbA* intergenic spacers, showed dissimilar characteristics with respect to the amount of variability present within a particular region (Table 1). Comparison of the *trnH-psbA* and *trnS-trnG* spacers and *trnG* and *rpL16* introns in the broad analysis showed that the *trnS-trnG* intergenic spacer provided the

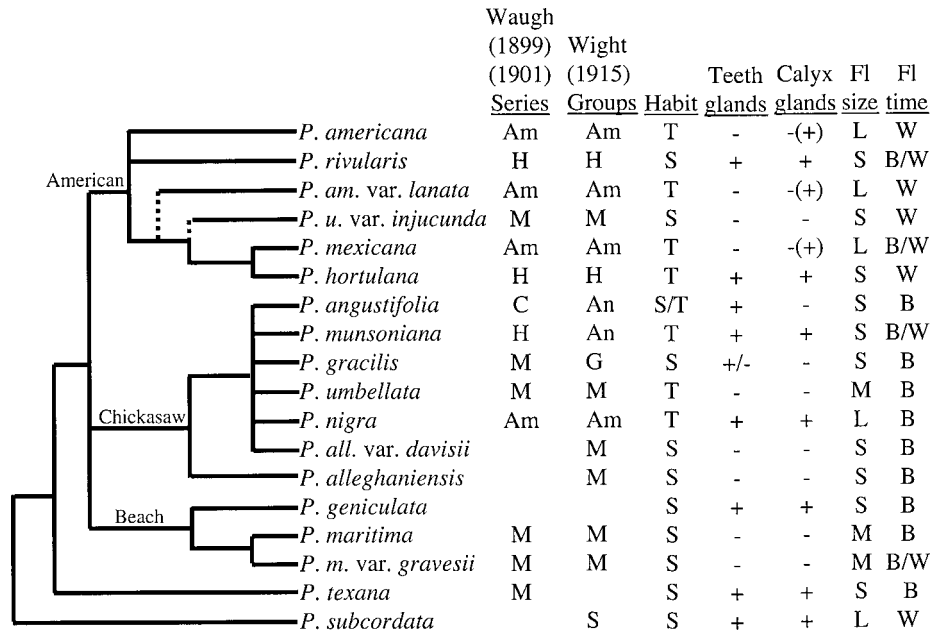


Fig. 3. Phylogenetic relationships of *Prunocerasus* shown with the classifications of Waugh (1899, 1901) and Wight (1915) in addition to five morphological characters classically used to delimit taxonomic boundaries within the section. The three major clades, the American, Chickasaw, and Beach, are shown on the respective branches.

Abbreviations for classification by Waugh (1899, 1901) and Wight (1915): Am = Americana, An = Angustifolia, C = Chickasaw (same as An), H = Hortulana, M = Maritima, G = Gracilis, and S = Subcordata. Abbreviations for morphological characters: Habit: T = tree, S = shrub; Teeth glands: + = glands present on each tooth of the leaf blade, - = glands absent on each tooth of the leaf blade; Calyx glands: + = glands present on margin of the calyx lobes, - = glands absent on margin of the calyx lobes; Flower size (Fl size): flowers large (L) = flowers greater than 15 mm wide, flowers small (S) = flowers less than 15 mm wide, flowers midsize (M) = flowers ~15 mm wide; Time of flowering (Fl time): W = flowers emerging with the leaves, B = flowers present before leaves emerge.

greatest number (43) of informative characters in the data set, followed by the *trnG* (36), and *rpL16* (31) introns. However, from a cost-benefit point of view, because the *trnS-trnG* spacer and the *trnG* intron were amplified and sequenced together with the same two primers, this *trnS-trnG-trnG* region was by far the most valuable as it provided 79 out of 134 informative characters in the broad analysis (outlined in Table 1).

In the narrow phylogenetic investigation of only *Prunocerasus*, the *rpL16* region provided more informative characters than did *trnS-trnG-trnG* (13 and 10, respectively). These two regions were the most informative ones used in this investigation and the addition of the *rpS16* and *trnL* introns and the *trnL-trnF* and *trnH-psbA* spacers provided little more resolution but did help to raise support for some nodes.

**Relationships within *Prunus***—The combined cpDNA data sets provide insight into the phylogenetic relationships within *Prunus*. It is clear that *Prunocerasus* is a monophyletic assemblage of taxa, and this is discussed below. We, in agreement with Bortiri et al. (2001), show support for a monophyletic sect. *Prunus* inside of which there is support for both Asian and European-West Asian lineages. Interestingly, although the data set contains several characters uniting sect. *Prunus*, *P. domestica* L. and *P. insititia* L. have identical sequences and share three autapomorphic positions with respect to *P. spinosa* L. and *P. cerasifera* Ehrh. (*P. insititia* has been described as a subspecies, variety, and nothomorph of *P. domestica*). Subgenus *Amygdalus* is a monophyletic assemblage and we further show support for members of sect. *Amygdalus* grouping together with the single representative of sect. *Chamaeamygdalus* sister to the rest of the species in that subgenus, again

in agreement with Bortiri et al. (2001). Among the monophyletic groups, *Prunocerasus*, sect. *Prunus*, and subg. *Amygdalus*, there is no support for relationships among them, except that they all belong to a monophyletic polytomy. Other investigators have shown this close relationship between subg. *Prunus* and subg. *Amygdalus* (Watkins, 1976; Mowrey and Werner, 1990; Badenes and Parfitt, 1995; Bortiri et al., 2001; Lee and Wen, 2001; Jung et al., 2002), which we refer to as the Pruno-Amygdaloid clade. Interestingly, species of subg. *Cerasus* sect. *Microcerasus* are well supported within the Pruno-Amygdaloid clade, a position that has been shown previously (Mowrey and Werner, 1990; Bortiri et al., 2001; Lee and Wen, 2001). The Pruno-Amygdaloid clade, including several unresolved *Microcerasus* species, is strongly supported here by a 1.0 posterior probability and 100% bootstrap support. Sister to the Pruno-Amygdaloid clade is *P. fasciculata*, which like many taxa within the genus has been subjected to fluctuating nomenclature. Rehder (1940) placed it in subg. *Amygdalus* while Mason (1913) had it in subg. *Emplectocladus*; in agreement with Bortiri et al. (2001), our data place *P. fasciculata* as sister to the Pruno-Amygdaloid clade. Outside of *P. fasciculata*, representative species of subg. *Cerasus* sect. *Mahaleb* form a clade sister to the Pruno-Amygdaloid clade + *P. fasciculata*. Outside of sect. *Mahaleb* we show paraphyly of subgenera *Padus* and *Laurocerasus*; this is in agreement with both Bortiri et al. (2001) and Lee and Wen (2001). However, Lee and Wen (2001) showed *P. virginiana* L. and *P. serotina* Ehrh. sister, with *P. laurocerasus* L. outside of several other *Padus* and *Maddenia hypoleuca* Koehne. They also showed *P. caroliniana* Aiton (and *P. grayana* Maxim., which is not included

here) as sister to all of *Prunus*. This is in contrast to Bortiri et al. (2001) who showed *P. serotina* and *P. mahaleb* as sister taxa and *P. virginiana* and *P. laurocerasus* as more closely related to each other than either is to *P. serotina* + *P. mahaleb*. Our data do not support the relationship between *P. serotina* and *P. mahaleb*.

Taxa of subg. *Cerasus* sect. *Microcerasus* (*P. pumila*, *P. tomentosa*, and *P. glandulosa*) have been difficult to place phylogenetically (Bortiri et al., 2001; Lee and Wen, 2001) although they appear to be associated with the Pruno-Amygdaloids. Mowrey and Werner (1990) showed sect. *Microcerasus* as a polyphyletic group with *P. pumila* and others nested between sections *Prunocerasus* and *Prunus*, while *P. glandulosa* was sister to the cherries and *P. tomentosa* was sister to the remaining Pruno-Amygdaloid group. Lee and Wen (2001) also showed sect. *Microcerasus* as a polyphyletic group with *P. besseyii* L.H.Bailey, a variety of *P. pumila* (Rohrer, 2000), in three very different parts of their trees in three different analyses. Additionally, they suggested that *P. tomentosa* is either related to sect. *Prunocerasus*, subg. *Amygdalus*, or sister to some Pruno-Amygdaloids in their three different analyses. Lastly, Bortiri et al. (2001) have shown sect. *Microcerasus* as a paraphyletic group with *P. besseyii* sister to members of sect. *Penarmeriaca* (not sampled in this study); they also showed *P. glandulosa* and *P. tomentosa* as unresolved within the Pruno-Amygdaloids. Possibly the most striking result of our broad analysis is the amount of apparent homoplasy among species of sect. *Microcerasus*. *Prunus tomentosa*, *P. pumila*, and especially *P. glandulosa* share molecular characters with several of the other groups that are resolved in our analysis. For example, *P. glandulosa* shares a 5-bp insertion with *P. mume* Siebold & Zucc. + *P. armeniaca*, a nucleotide substitution with *P. mume* + *P. mahaleb* + *P. pensylvanica* L., a substitution with sect. *Prunus*, another with *P. subcordata* and the American clade of sect. *Prunocerasus*, and at two other separate positions it shares substitutions with either *P. geniculata* or *Physocarpus opulifolius*. The homoplasy within, or at least paraphyly of, sect. *Microcerasus* has been noted previously (Mowrey and Werner, 1990; Bortiri et al., 2001; Lee and Wen, 2001).

Bortiri et al. (2001) noted that *Microcerasus* is not related to the rest of subg. *Cerasus*, a finding that we report here as well; they also speculated that this section appears not to constitute a natural group. Waugh (1901) was ambiguous with respect to the placement of *P. pumila* var. *pumila* and *P. pumila* var. *besseyii* with the cherries or with the plums, perhaps indicating morphological intermediacy. Based on hybridization studies, Watkins (1976) believed in a closely related Pruno-Amygdaloid group and noted that members of subg. *Cerasus* would not hybridize directly with them but genetic transfer was possible via the “*Microcerasus* bridge.” Members of *Microcerasus* hybridize with both the Pruno-Amygdaloids and subg. *Cerasus*. Based on this, and the apparent extensive homoplasy within sect. *Microcerasus*, we hesitantly speculate that it is possible that this group represents an assemblage of taxa that carries the results of ancient hybridization like pleisiomorphic, homoplasious scars—as might be possible if sect. *Microcerasus* represents a polyphyletic assemblage of hybrids with much of the maternal parentage being via several different ancient Pruno-Amygdaloids.

**Relationships within *Prunus* section *Prunocerasus***—This study provides the first phylogenetic hypothesis for the species

of *Prunus* sect. *Prunocerasus*. We report that the North American plums, sect. *Prunocerasus*, are monophyletic and include *P. texana*, which has previously been classified as a peach (subg. *Amygdalus*). *Prunus subcordata*, the only species within sect. *Prunocerasus* of western North America, is sister to the rest of the section. Inside of *P. subcordata*, we show strong support for the inclusion of *P. texana*, which may be a North American plum or possibly may have captured a *Prunocerasus* chloroplast. Sister to *P. texana* we report the existence of three strongly supported clades here designated the “American Clade,” the “Chickasaw Clade,” and the “Beach Clade” (Fig. 3). The American clade includes *P. americana* var. *americana*, *P. americana* var. *lanata*, *P. mexicana*, *P. rivularis* Scheele, *P. hortulana*, *P. umbellata* var. *injucunda*; the Chickasaw clade includes *P. angustifolia*, *P. munsoniana*, *P. gracilis*, *P. nigra*, *P. umbellata* var. *umbellata*, *P. alleghaniensis* var. *alleghaniensis*, and *P. alleghaniensis* var. *davisii*; and the Beach clade includes *P. geniculata*, *P. maritima* var. *maritima*, and *P. maritima* var. *gravesii*.

Classically, species within *Prunocerasus* have been defined and grouped based on relatively few qualitative characters; the lack of invariant quantitative characters in the section has led to much taxonomic confusion. Characters such as the presence or absence of glands terminating the leaf teeth or marginal on the calyx lobes, leaf or calyx pubescence, whether or not the flowers appear before or with the leaves, habit (tree or shrub), ability to root sprout, petal length (greater or less than 7.5 mm long), and geography have been the main characters used for species delimitation and grouping.

Taxa such as *P. americana* var. *americana*, *P. americana* var. *lanata*, and *P. mexicana* have been largely separated using the characters of leaf and calyx pubescence; these three taxa in addition to *P. nigra* are often grouped because they are large trees with large flowers, comparatively. Additionally, pubescence is the main character separating some other closely related taxa like *P. umbellata* var. *umbellata* and *P. umbellata* var. *injucunda*. Small (1898, p. 150) described *P. injucunda* as a distinct species and noted that it is easily confused with *P. umbellata* from which it differs by having “a more rigid habit” and tomentose leaves and twigs. Additionally, it is difficult to separate *P. umbellata* and *P. umbellata* var. *injucunda* from *P. alleghaniensis* and *P. alleghaniensis* var. *davisii* and to a lesser extent *P. maritima* without some knowledge of their geography as these species are all morphologically overlapping. *Prunus alleghaniensis* and *P. umbellata* differ only in distribution according to Duncan and Duncan (1988), and Waugh (1899) reported that he could not morphologically distinguish the type material of *P. injucunda* from *P. maritima*. Lastly, *P. alleghaniensis* and *P. maritima* are also morphologically very close; to paraphrase T. C. Porter’s (1877) description of *P. alleghaniensis*: it is nearly allied to *P. maritima*, but its remoteness from the seaboard, habitat on bluffs and mountains, narrower and longer more acuminate leaves, smaller fruit, and character of the stone, entitle it to the rank as a distinct species. Lastly, *P. angustifolia*, *P. munsoniana*, *P. hortulana*, and *P. rivularis* can easily be confused. Their leaf margins are all distinctly serrate glandular and with the exception of *P. angustifolia*, their calyx lobes are glandular also. Furthermore, there seem to be many intermediates among them (Waugh, 1899; J. Shaw, personal observation).

Within *Prunocerasus* there are a few morphologically coherent “groups” that can relatively easily be delimited; it is within these “groups” that species determination is difficult.

For example, it may be difficult to distinguish *P. umbellata* from *P. alleghaniensis*, which are members of Wight's (1915) Maritima group; however, it is much easier to separate *P. alleghaniensis* from *P. americana*, which are members of Wight's (1915) Maritima and Americana groups, respectively. The gaps between these morphologically coherent groups are what previous authors like Waugh (1899) and Wight (1915) used to divide the North American plums into four series/six groups, respectively. Neither of these two authors clearly defined the delimiting characteristics of their series/groups although it is clear to us that their groups are based on overall similarity using the suite of aforementioned morphological characters (unless cited otherwise, in the following discussion Waugh = Waugh, 1899 and Wight = Wight, 1915). Waugh believed each series to be a group of hybrids sharing overall similarity. Into the Americana series, he placed *P. americana*, *P. americana mollis* (*P. americana* var. *lanata* and/or *P. mexicana*), and *P. nigra*. He thought *P. americana* to be a species of the central eastern United States that becomes increasingly glandular on the leaves and calyx lobes to the northeast, grading to *P. nigra*, and less glandular and more pubescent on the leaves and calyx lobes to the southwest, grading to *P. americana* var. *lanata* or *P. mexicana*. Waugh's Maritima series includes several forms of *P. maritima* in addition to *P. maritima* var. *gravesii*, *P. umbellata* var. *injucunda*, *P. gracilis*, and interestingly *P. texana*, all of which are smaller in stature and have smaller flowers than members of the Americana series. His Chickasaw series only includes *P. angustifolia*, and his Hortulana series is centered on *P. hortulana*, its varieties, and questionably *P. munsoniana*. Waugh described the Hortulana series as a hybrid swarm created through various hybridizations between the other series (see Waugh, 1901), which intergraded with them where their ranges overlap. Although he did not include as many species as Wight, the series he described were very similar to the groups proposed by Wight whose delimitation of groups is shown in Fig. 3.

Our cpDNA-based phylogenetic hypothesis for sect. *Prunocerasus* is strongly supported and largely in disagreement with the previous morphological classifications described above (Waugh, 1899; Wight, 1915). While the groups that Waugh and Wight proposed make sense based on morphological similarity, and morphologically we do not disagree with them, they are not supported phylogenetically (see Fig. 3 and the Supplementary Data). Figure 3 shows both a comparison of previous classifications and the homoplasious distribution of the common morphological characters compared to the cpDNA phylogeny. The commonly used morphological characters such as habit, leaf teeth glands, calyx lobe glands, flower size, and time of flowering are all homoplasious with respect to the cpDNA phylogeny.

We show that, although many of the species within Waugh's series or Wight's groups are hardly morphologically distinguishable, they are split among three of the major phylogenetic clades of sect. *Prunocerasus*. Because the groups that Waugh and Wight proposed are similar, and because Wight recognized more taxa, the following discussion is based on Wight's classification (see Supplementary Data or Fig. 3).

Our data place *P. americana*, *P. americana* var. *lanata*, and *P. mexicana* together in the American clade, in agreement with Wight; however, *P. nigra*, which both Waugh and Wight believed to be related to *P. americana*, is here placed in the Chickasaw clade. Additionally, although *P. americana* and *P. mexicana* are both in the American clade, they are separated

by species that both Waugh and Wight placed in other groups and are therefore apparently not as closely related as Waugh and Wight had thought.

The morphologically coherent Maritima group of Wight is not supported here. Members of this group are nearly evenly split among the American, Chickasaw, and Beach clades. The close relationship between *P. maritima* and *P. geniculata* is unexpected with respect to morphology. *Prunus geniculata* is the most easily distinguishable species in the section and impossible to confuse with any of the other taxa. Morphologically, it resembles either a dwarfed *P. angustifolia* or *P. texana*. The only apparent commonality between *P. geniculata* and *P. maritima* is that both species are found in sandy soils, although they are separated by hundreds of miles in central Florida and the east coast north of Maryland through to Canada, respectively.

Within the Chickasaw clade we report a noticeable lack of genetic divergence between taxa; the sequences of members of this clade are nearly identical. While morphologically, some of the taxa are nearly identical (*P. angustifolia* and *P. munsoniana* or *P. umbellata* and *P. alleghaniensis*), *P. gracilis* and *P. nigra* are quite easily distinguished from the rest of the taxa in the clade. Additionally, it is quite easy to differentiate between *P. angustifolia* or *P. munsoniana* and *P. umbellata* or *P. alleghaniensis*. The lack of genetic divergence within the Chickasaw clade suggests that either (1) the species in this clade are the result of recent, rapid radiation, (2) frequent hybridization between these species has resulted in chloroplast sharing among species, or (3) several of the members of this clade are of hybrid origin and share a common chloroplast lineage. Based on the morphological incongruence between taxa within this clade, we suspect that reasons number two and/or three may be the most likely (see discussion below).

Wight noted that *P. hortulana* and *P. rivularis* are closely related and although the close relationship is also not supported here since these two species are separated by several other species, both species belong to the American clade. Morphologically, these two species are most similar to *P. munsoniana* and *P. angustifolia* of the Chickasaw clade. This is yet another example of morphologically very similar species being separated by less similar ones.

Members of each of the American and Chickasaw clades are unequivocally closely related with respect to evidence provided by the maternally inherited cpDNA molecule. Yet, morphologically, each of the two clades contains taxa that appear to be more closely related to taxa of the other clade, based on morphological evidence.

Although strongly supported, our cpDNA-based phylogenetic hypothesis for *Prunocerasus* is largely in disagreement with the previous morphological classifications described above (Waugh, 1899; Wight, 1915). This appears to be due to homoplastic morphological characters classically used to delineate the taxa within *Prunocerasus* (see Fig. 3). We suspect that the apparent homoplasy, with respect to morphological characters, may be indicative of hybrid origin for several of the species of the section as (1) origin via hybridization has been shown to occur within the genus (Watkins, 1976; Brettin et al., 2000; Mohanty et al., 2000), (2) much of *Prunocerasus* is capable of natural hybridization (Hedrick et al., 1911; Wight, 1915; Flory, 1938; Rehder, 1940), (3) *Prunocerasus* is a group of obligate outcrossers, and (4) other researchers have proposed species within the section to be of hybrid origin (Waugh, 1899, 1901; Steyermark, 1963). For example, the

close relationship between *P. hortulana* and *P. mexicana* reported here is unusual because: (1) *P. mexicana* and *P. hortulana* do not share many of the characters classically used to distinguish species within the section and are relatively easily discernible, and (2) *P. hortulana* and *P. rivularis* are very morphologically similar as are *P. mexicana* and *P. americana* var. *lanata*, yet relationships shown in our phylogenetic hypothesis do not agree with morphological similarity. Then again, Steyermark (1963) noted that some individuals of *P. hortulana* appear to be hybrids between it and *P. mexicana*. Based on our cpDNA-based phylogenetic evidence being incongruous with morphology and the idea of speciation via hybridization being common within the genus we reason that some members of the American and Chickasaw clades are of hybrid origin. Furthermore, an analysis of nuclear encoded ITS in addition to preliminary data from a granule bound starch synthase gene (GBSSI-2) reveals that several of the taxa in each of these two clades section contain polymorphic nucleotide positions and indels (J. Shaw, unpublished data). Further evidence from the nuclear genome as well as analysis of cpDNA from additional accessions from each of the putative taxa is being pursued to resolve this uncertainty.

**A biogeographical note**—*Prunus* is thought to have originated in Central Asia (Watkins, 1976). The work of Bortiri et al. (2001) does not refute an Asian origin for the genus but it does not unambiguously support it either. Because there is no clear sister taxon to *Prunus* and both Old and New world taxa are possible candidates (Bortiri et al., 2001), the geographic origin of the genus is equivocal. If *Prunus* originated in Asia, then based on *P. subcordata* being the only western species and phylogenetically sister to the rest of *Prunocerasus*, we would suggest that the common ancestor of *Prunocerasus* arrived in North America via Beringia. But more work is needed within the genus for us to root a phylogeographic hypothesis. In any case, our data refute the hypothesis of Mowrey and Werner (1990) that suggested *Prunocerasus* is paraphyletic and arrived in North America via two immigration events.

**Conclusions and future directions**—Four main conclusions can be drawn from the data and analyses presented in this paper. First, phylogenetic analysis of cpDNA sequences demonstrates that *Prunus* subg. *Prunus* sect. *Prunocerasus* is a monophyletic assemblage. Second, phylogenetic analysis of this noncoding cpDNA data set provides a maternally inherited framework on which we can further develop a hypothesis of relationships for the species within *Prunocerasus*. Third, our data counter the hypothesis of Mowrey and Werner (1990) that *Prunocerasus* is polyphyletic and experienced more than one immigration event to North America. Finally, the results presented in this paper highlight the continuing need, especially at low taxonomic levels, to assay the relative utility of multiple cpDNA regions before investigation instead of just using commonly employed tools.

Future investigations of this section will include: (1) sampling cpDNA sequences from additional populations of each putative species to determine how much variation there is within species and more importantly if chloroplasts are monophyletic within each putative species and (2) the addition of a nuclear marker to this analysis to further illuminate relationships, especially those that may be reticulating, within sect. *Prunocerasus*.

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