

Phylogenetic inferences in *Prunus* (Rosaceae) using chloroplast *ndhF* and nuclear ribosomal ITS sequences

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Abstract Sequences of the chloroplast *ndhF* gene and the nuclear ribosomal ITS regions are employed to reconstruct the phylogeny of *Prunus* (Rosaceae), and evaluate the classification schemes of this genus. The two data sets are congruent in that the genera *Prunus* s.l. and *Maddenia* form a monophyletic group, with *Maddenia* nested within *Prunus*. However, the *ndhF* data set is incongruent with the ITS data supporting two major groups within *Prunus*: one consisting of subgenera *Laurocerasus* (including *Pygeum*) and *Padus* as well as the genus *Maddenia* and another of subgenera *Amygdalus*, *Cerasus*, and *Prunus*. The ITS data, on the other hand, support a clade composed of subgenera *Amygdalus* and *Prunus* and *Prunus* sect. *Microcerasus* in addition to a paraphyletic grade of subgenera *Laurocerasus* and *Padus* (and the genus *Maddenia*) taxa. In general, the subgeneric classifications of *Prunus* s.l. are not supported. The ITS and *ndhF* phylogenies differ mainly in interspecific relationships and the relative position of the *Padus/Laurocerasus* group. Both ITS and *ndhF* data sets suggest that the formerly recognized genus *Pygeum* is polyphyletic and that the distinction of the subgenera *Padus* and *Laurocerasus* is not supported. The biogeographic interactions of the temperate and tropical members in the *Padus/Laurocerasus/Maddenia* alliance including *Pygeum* are shown to be highly dynamic and complex.

Key words ITS, *ndhF*, phylogeny, *Prunus*, Rosaceae.

Prunus L. (Rosaceae) consists of approximately 250 species distributed across the Northern Hemisphere and into the sub-tropics and tropics, including a large number of economically significant species such as cherries, peaches, plums, apricots, almonds and a wide variety of ornamentals and timber species (Lee & Wen, 2001). Many taxa are important fruit crops, and several have been used as such since prehistoric times (Komarov, 1971; Schery, 1972; Watkins, 1995). The genus is usually included in the subfamily Amygdaloideae Arn., which is also known as the Prunoideae Focke (see Robertson, 1974). The Amygdaloideae has traditionally contained four genera: *Prunus* s.l., *Prinsepia* Royle, *Maddenia* Hook. f. & Thoms., and *Oemleria* Rehb. (= *Osmaronia* Greene, *Nuttallia* Torrey & Gray; see Landon, 1975) and is distinguished from other rosaceous subfamilies by its simple leaves, drupaceous fruits, superior ovaries, and $x=8$ chromosome number (Robertson, 1974; Ghora &

Panigrahi, 1995). While most treatments follow this concept of the subfamily (e.g., Rehder, 1940; Robertson, 1974; Ghora & Panigrahi, 1995), several other workers raised the subfamily to family rank, either as Amygdalaceae or Drupaceae, though this treatment is less common (e.g., Rydberg, 1900, 1917; Berry, 1930; Small, 1933; Dahlgren, 1983; Mai, 1984). The genus *Exochorda* Lindl., often placed in the subfamily Spiraeoideae Arn. because of its five carpels that produce capsular fruits, has sometimes been allied with the Amygdaloideae, based on several lines of evidence (Stebbins, 1958; Goldblatt, 1976; Zhang, 1992; Morgan et al., 1994; Lee & Wen, 2001). Other genera formerly included in this subfamily include *Pygeum* Gaertner [which was merged into *Prunus* subgen. *Laurocerasus* Duhamel (Kalkman, 1965)], *Plagiospermum* Oliver [which was shown to be synonymous with the genus *Prinsepia* (Rehder, 1915)], and the various segregate genera from *Prunus* s.l. (Lee & Wen, 2001). Recently, Potter et al. (2007) proposed a new classification of Rosaceae based on molecular phylogenetic analyses, in which they

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recognized three subfamilies: Rosoideae, Dryadoideae and Spiraeoideae. The newly defined Spiraeoideae includes all genera previously assigned to Amygdaloideae and Maloideae. Monophyly of the traditional Amygdaloideae was not supported. Instead, *Prunus* s.l., along with *Maddenia* and *Pygeum*, is treated in the tribe Amygdaleae of the subfamily Spiraeoideae, while *Exochorda*, *Oemleria*, and *Prinsepia* are placed in tribe Osmaronieae of the same subfamily.

Classification within the genus *Prunus* s.l. has been varied. *Prunus* is distinguished from the other three genera in the traditionally defined subfamily Amygdaloideae by the combination of its single carpel (rarely 2), five sepals (occasionally more), bisexual flowers (rarely andromonoecious, see Wolfe & Drapalik, 1999), and a solid stem pith. Tournefort (1700) offered the first classification of *Prunus* s.l. by proposing six genera based on fruit morphology: *Amygdalus* L., *Armeniaca* Miller, *Cerasus* Miller, *Laurocerasus*, *Persica* Miller, and *Prunus* (s.s.). Linnaeus (1753) reduced these six genera to two by merging *Persica* into *Amygdalus* and putting the rest (including another genus *Padus* Miller) into *Prunus*. Since then, many other classifications have been proposed for *Prunus* s.l., recognizing as many as seven or more distinct genera (De Candolle, 1825; Hutchinson, 1964; Browicz, 1969), or one broadly defined genus (Rehder, 1940). Many classifications treated *Prunus* inclusively with several subgenera or sections, following Bentham and Hooker (1865), and Focke (1894) (e.g., Koehne, 1911; Rehder, 1940; Fernald, 1950; Robertson, 1974; Ghora & Panigrahi, 1995). On the other hand, several workers divided *Prunus* into multiple genera (e.g., Hutchinson, 1964; Browicz, 1969; Komarov, 1971; Yü et al., 1986). Rehder's (1940) treatment of *Prunus* in the inclusive sense with five subgenera [*Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus*, and *Prunophora* Neck. (= *Prunus* s.s.)] and twelve sections is favored by several workers (Bate-Smith, 1961; Robertson, 1974; Ghora & Panigrahi, 1995; Lersten & Horner, 2000). Krüssmann (1978) recognizes section *Microcerasus* of subgenus *Cerasus* as a distinct subgenus, *Lithocerasus* Ingram, resulting in a total of six subgenera and 14 sections recognized within the genus. For a detailed history of classification within *Prunus* s.l., see McVaugh (1951), Kalkman (1965), Ghora and Panigrahi (1995), and Lee and Wen (2001).

Several recent phylogenetic studies of *Prunus* have been conducted. One of the earliest works was done by Mowrey and Werner (1990) who examined isozyme profiles of 34 species from subgenera

Prunus, *Amygdalus*, *Cerasus* and *Lithocerasus* (section *Microcerasus* of subgen. *Cerasus* sensu Rehder). They found support for the subgenera *Prunus*, *Amygdalus*, and *Cerasus*. The most noteworthy exception of their hypothesis, with respect to the classically recognized subgeneric groupings, was that several species of subgen. *Lithocerasus* were nested within subgen. *Prunus*.

Zhang (1992) sampled wood anatomy in the Rosaceae, including 83 samples of *Prunus* s.l. He found that the genera (subgeneric levels in other treatments) that comprise *Prunus* s.l. form a monophyletic group nested within the other amygdaloid genera. Within *Prunus* s.l., sect. *Armeniaca* of subgen. *Prunus* (sensu Rehder) and subgen. *Amygdalus* are suggested to be the most derived, subgen. *Cerasus* is sister to that, then a clade of *Padus* and some members of *Laurocerasus* ("group A"), then *Prunus* s.s., while the *Pygeum* group and the remainder of *Laurocerasus* ("group B") are considered the least advanced groups.

Chloroplast DNA restriction sites were used to construct the phylogeny of eight cultivated members of *Prunus* (Badenes & Parfitt, 1995). While too few species were sampled to study *Prunus* classification, this study suggested that subgenera *Amygdalus* and *Prunus* are more closely related to one another than either is to subgenus *Cerasus*.

Lersten and Horner (2000) examined leaf crystals in several members of *Prunus* s.l., and suggested that the *Prunophora* (= *Prunus* s.s.) and *Amygdalus* subgenera are related and apparently are the most advanced subgenera. The *Cerasus* and *Laurocerasus* subgenera are intermediate and very diverse, while *Padus* is suggested to be the least advanced subgenus and the furthest from subgenera *Prunus* and *Amygdalus*.

Lee and Wen (2001) employed nuclear ribosomal ITS sequences to construct the phylogeny of *Prunus*. The ITS data suggest a close relationship between subgenera *Prunus* and *Amygdalus*. They reported that subgenera *Padus* and *Laurocerasus* are closely related, and form a basally branching paraphyletic group. They also found that *Maddenia* is nested within the *Padus/Laurocerasus* group, and that subgenera *Cerasus* and *Padus* are both polyphyletic. Bortiri et al. (2001) used the nuclear ribosomal ITS and the chloroplast *trnL-F* spacer region to construct the phylogeny of *Prunus*. Even though the resolution of the *trnL-F* tree was relatively low, the combined analysis showed a congruent phylogeny as seen in Lee and Wen (2001). To further resolve some deep nodes Bortiri et

al. (2002) employed sequences of the *s6pdh* (sorbitol 6-phosphate dehydrogenase gene). The resolution remained low and the authors proposed a rapid radiation in the early history of the genus. Shaw and Small (2004) focused on *Prunus* sect. *Prunocerasus* using seven noncoding chloroplast DNA regions and also sampled widely throughout the other subgenera of the genus. Their data yielded a well-resolved phylogenetic hypothesis showing support for subgen. *Prunus* sect. *Prunus*, subgen. *Prunus* sect. *Prunocerasus*, and subgen. *Amygdalus*. Three species of subgen. *Cerasus* sect. *Microcerasus* were nested within the pruno-amygdaloid clade that was sister to other species of subgen. *Cerasus*. All molecular analyses of the above mentioned studies have only included a few taxa from tropical regions.

This study aims to provide further insights into the phylogenetic relationships of *Prunus* using sequences of the chloroplast *ndhF* gene, and the nuclear ribosomal ITS regions. We expanded our sampling of tropical members of *Prunus*, especially the unsampled *Pygeum* group. Our primary goal of the study is to examine how the previously poorly sampled subgen. *Laurocerasus* including the *Pygeum* group is related to its putative closest relative subgen. *Padus* and how the two putative subgenera are related to the rest of the genus. Questions to be addressed include: (1) Is the generic status of *Pygeum* supported? (2) What are the phylogenetic relationships among the subgenera within the genus *Prunus* s.l.? (3) Are any of the current classifications of *Prunus* s.l., supported by molecular evidence? (4) Is the chloroplast *ndhF* data set congruent with the other molecular data sets described above (nDNA ITS and *s6pdh* and cpDNA *trnL-F* and other regions included in Shaw and Small, 2004)? and (5) What are the relationships between the temperate and the tropical members of *Prunus* s.l.?

1 Material and Methods

Our sampling included 59 (*ndhF*) or 51 (ITS) accessions of *Prunus* s.l. The samples covered all five subgenera of *Prunus* s.l. (*Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus*, and *Prunus*; sensu Rehder, 1940), and most of the subgeneric sections, as well as the genus *Maddenia* and six species formerly classified in *Pygeum*. The outgroups included species of tribe Osmaronieae (*Exochorda*, *Oemleria*, and *Prinsepia*), which were formerly classified with *Prunus* in Amygdaloideae and species of two other genera of Spiraeoideae, *Physocarpus* Maxim. and *Lyonothamnus* A. Gray.

Total DNA was extracted from leaf material with the CTAB method of Doyle and Doyle (1987). DNA amplifications were performed in 100- μ L reactions following Wen and Zimmer (1996). Most of the ITS PCR products were purified using millipore columns (Ultrafree-MC Filter Unit, 30,000 NMWL, Millipore, Bedford, Massachusetts, USA), while all of the *ndhF* PCR products and a portion of the ITS products were purified using Wizard® purification preps (Cat. #A7170, Promega, Madison, WI, USA). Sequences were generated by automated sequencing (ABI PRISM® 377XL, Perkin-Elmer) and Big Dye chemistry. Two primers (C26A and N18L18 in Wen & Zimmer, 1996) were used to obtain the entire ITS and 5.8S regions from both directions. The *ndhF* region was sequenced using several published primers (*ndhF*-274R, *ndhF*-536R, *ndhF*-803R, *ndhF*-972R, *ndhF*-1318R, *ndhF*-1318, *ndhF*-2110R; Olmstead & Sweere, 1994) and one primer (*ndhF*-1577pr: CGTTTATTAGTATTGCTCGKTTTG) that was designed in this study. All the sequences were deposited in GenBank (see Table 1 for accession numbers).

Phylogenetic analyses were performed using the maximum parsimony (Swofford et al., 1996) and the Bayesian inference methods. Parsimony analysis was performed with tree bisection-reconnection branch swapping, MulTrees on, and simple taxon addition in PAUP* version 4.0b10 (Swofford, 2003). Parsimony bootstrap support for each clade was estimated as above from 500 heuristic search replicates, with 100 random taxon addition replicates saving all optimal trees at each step.

The optimal model of molecular evolution was determined by the Akaike Information Criterion (AIC) using Modeltest ver. 3.7 (Posada & Crandall, 1998; Posada & Buckley, 2004). In each case the optimal model was the General Time Reversible model, with rate heterogeneity modeled by assuming that some proportion of sites are invariable and that the rate of evolution at other sites is modeled using a discrete approximation to a gamma distribution [GTR+I+ Γ]. Bayesian inferences were implemented in MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001) with the model estimated above and each gene was partitioned. We used one cold and three heated chains, with random initial trees. Trees were generated for 2,000,000 generations, with sampling every 100 generations. Following a burn-in period of the first 2000 generations, 19,800 trees were sampled from the posterior distribution to calculate the posterior probabilities (PP).

Congruence among the two different data sets

Table 1 Taxa of *Prunus* and outgroups sampled for this study, and GenBank accession numbers (the classification system of *Prunus* follows Rehder, 1940; and US, CS, and F in parentheses are acronyms of the US National Herbarium, Colorado State University Herbarium, and Field Museum of Natural History Herbarium, respectively)

Taxon	Source and voucher ¹⁾	GenBank accession (ITS; <i>ndhF</i>)
Subgen. 1. <i>Prunus</i>²⁾		
Sect. 1. <i>Prunus</i>		
<i>P. insititia</i> L. 7307	USA, Illinois, cult. Morton Arboretum: <i>Wen 7307</i> (US)	EU669097; EU669166
<i>P. maritima</i> Marsh. 7311	USA, Illinois, cult. Morton Arboretum: <i>Wen 7311</i> (US)	EU669098; EU669168
<i>P. murrayana</i> Palmer 7283	USA, Texas, Brewster Co.: <i>Wen 7283</i> (US)	EU669099; EU669099
<i>P. salicina</i> Lindl.	China, Zhejiang Prov.: <i>Wen 3020</i> (CS)	AF179486, AF179487; EU669143
<i>P. spinosa</i> L. 7308	USA, Illinois, cult. Morton Arboretum: <i>Wen 7308</i> (US)	EU669100; EU669167
<i>Prunus</i> sp. 8080	China, Chongqing Shi: <i>Wen 8080</i> (US)	–; EU669171
Sect. 2. <i>Prunocerasus</i> Koehne		
<i>P. americana</i> Marsh. 4021	USA, Colorado, Larimer Co.: <i>Lee & Wen 4021</i> (CS)	AF179488; EU669150
<i>P. americana</i> Marsh. 4061	Canada, Alberta: <i>Whitcher s.n.</i> (CS)	AF179489; EU669125
<i>P. americana</i> Marsh. 5011	USA, Colorado, Larimer Co.: <i>Lee & Wen 5011</i> (US)	–; EU669137
<i>P. angustifolia</i> Marsh.	USA, Florida, Jackson Co.: <i>Gholson s.n.</i> (CS)	AF179490; EU669131
<i>P. nigra</i> Ait.	USA, Colorado, cult. CS TS88139: <i>Lee & Wen 4024</i> (CS)	AF179491, AF179492; EU669128
<i>P. umbellata</i> Ell.	USA, Florida, Jackson Co.: <i>Gholson s.n.</i> (CS)	AF179493; EU669120
Sect. 3. <i>Armeniaca</i> (Lam.) Koch.		
<i>P. armeniaca</i> L. var. <i>mandshurica</i> Maxim.	USA, Colorado, cult. CS TS81501: <i>Lee & Wen 4025</i> (CS)	AF179494, AF179495; EU669149
<i>P. mume</i> (Sieb.) Sieb. & Zucc.	China, Zhejiang Prov.: <i>Wen 3043</i> (CS)	AF179496, AF179497; EU669141
Subgen. 2. <i>Amygdalus</i> (L.) Focke		
<i>P. andersonii</i> Gray	USA, Nevada, Douglas Co.: <i>M. Beck s.n.</i> (CS)	EU669083; EU669151
<i>P. davidiana</i> (Carr.) Franch.	USA, Missouri, cult. MBG 1981-1933-1: <i>H. M. Davis s.n.</i> (CS)	EU669084; EU669142
<i>P. dulcis</i> (Mill.) Webb.	USA, Missouri, cult. MBG 1983-0585: <i>H. M. Davis s.n.</i> (CS)	EU669085; EU669146
<i>P. fasciculata</i> (Torr.) Gray	USA, California, Riverside Co.: <i>M. Beck s.n.</i> (CS)	EU669086; EU669153
<i>P. fremontii</i> Wats.	USA, California, Riverside Co.: <i>M. Beck s.n.</i> (CS)	EU669087; EU669152
<i>P. havardii</i> Mason	USA, Colorado, cult. CS: <i>Wen s. n.</i> (US)	EU669096; EU669165
<i>P. persica</i> (L.) Batsch.	China, Zhejiang Prov.: <i>Wen 3017</i> (CS)	AF179562; EU669129
<i>P. tenella</i> Batsch.	USA, Colorado, cult. CS TS93054: <i>Lee & Wen 4011</i> (CS)	AF179560, AF179561; EU669119
<i>P. triloba</i> Lindl.	USA, Colorado, cult. CS s.n.: <i>S. Berggren s.n.</i> (CS)	EU669088; EU669140
Subgen. 3. <i>Cerasus</i> Pers.		
Sect. 1. <i>Microcerasus</i> Webb.		
<i>P. besseyi</i> Bailey	USA, Colorado, cult. CS TS85155: <i>Lee & Wen 4023</i> (CS)	AF179498, AF179499; EU669121
<i>P. glandulosa</i> Thunb.	USA, Colorado, cult. Ft. Collins: <i>S. Berggren s.n.</i> (CS)	EU669089; EU669147
<i>P. tomentosa</i> Thunb.	USA, Colorado, cult. CS TS81261: <i>Lee & Wen 4010</i> (CS)	AF179500; EU669122
Sect. 2. <i>Pseudocerasus</i> Koehne		
<i>P. campanulata</i> Maxim.	USA, Washington DC, cult. USNA 58776: <i>Lee & Wen 4014</i> (CS)	AF179501, AF179502; EU669123
<i>P. incisa</i> Thunb.	USA, Washington DC, cult. USNA 58816: <i>Lee & Wen 4071</i> (CS)	AF179504, AF179505; EU669145
<i>P. mahaleb</i> L.	USA, Colorado, cult. CS TS83156: <i>Lee & Wen 4015</i> (CS)	AF179523, AF179524; EU669134
<i>P. nipponica</i> Matsum. var. <i>nipponica</i>	USA, Washington DC, cult. USNA 45734: <i>Lee & Wen 4077</i> (CS)	AF179507, AF179508; EU669144
<i>P. pensylvanica</i> L. f. 7298	USA, Wisconsin: <i>Wen 7298</i> (US)	EU669090; EU669138
<i>P. subhirtella</i> Miq. var. <i>subhirtella</i>	USA, Washington DC, cult. USNA 61383: <i>Lee & Wen 4080</i> (CS)	AF179519; EU669135
Sect. 3. <i>Phyllomahaleb</i> Koehne		
<i>P. maximowiczii</i> Rupr. 4079	USA, Washington DC, cult. USNA 62ER: <i>Lee & Wen 4079</i> (CS)	AF179526; EU669124
Sect. 4. <i>Lobopetalum</i> (Koehne) T. T. Yü & C. L. Li		
<i>P. dielsiana</i> Schneid. 8091	China, Chongqing Shi: <i>Wen 8091</i> (US)	–; EU669172
Subgen. 4. <i>Padus</i> (Moench) Koehne		
<i>P. grayana</i> Maxim.	USA, Washington DC, cult. USNA 46329: <i>Lee & Wen 4073</i> (CS)	AF179531; EU669136
<i>P. maackii</i> Rupr. 4009	USA, Colorado, cult. CS TS78092: <i>Lee & Wen 4009</i> (CS)	AF179532, AF179533; EU669139

Table 1 (continued)

Taxon	Source and voucher ¹⁾	GenBank accession (ITS; <i>ndhF</i>)
<i>P. napaulensis</i> K. Koch. 6470	China, Yunnan: <i>Wen 6470</i> (US)	EU669106; EU669159
<i>P. padus</i> L. var. <i>commutata</i> Dipp.	USA, Colorado, cult. CS TS82097: <i>Lee & Wen 4027</i> (CS)	AF179527; EU669132
<i>P. phaeosticta</i> Maxim.	China, Taiwan: <i>H-Y Liu s.n.</i> (F)	EU669095; EU669163
<i>P. serotina</i> Ehrh. 7229	USA, Illinois: <i>Wen 7229</i> (US)	EU669104; EU669160
<i>P. vana</i> J. F. Macbr.	Ecuador: <i>Talfur & Villacres 123</i> (F)	EU669105; EU669148
<i>P. virginiana</i> L. var. <i>virginiana</i>	USA, Colorado, Larimer Co.: <i>Lee & Wen 4022</i> (CS)	AF179536, AF179537; EU669126
<i>P. virginiana</i> var. <i>demissa</i> (Nutt.) Murr.	USA, Colorado, cult. CS: <i>S. Berggren s.n.</i> (CS)	EU669101; EU669127
Subgen. 5. <i>Laurocerasus</i> Koehne		
<i>P. caroliniana</i> Aiton	USA, Florida, Gadsden Co.: <i>Gholson 9-20-98</i> (CS)	AF179540; EU669130
<i>P. ilicifolia</i> (Nutt.) Walp.	USA, California, Santa Barbara: <i>D. A. Young s.n.</i> (CS)	AF179543, AF179544; EU669133
<i>P. laurocerasus</i> L. 5001	Cult. AA 889-72-D: <i>Lee & Wen 5001</i> (CS)	AF179545, AF179546; EU669118
<i>P. undulata</i> (D. Don) Roem. 6452	China, Yunnan: <i>Wen 6452</i> (US)	EU669108; EU669155
<i>P. zippeliana</i> Miq. 6030	Vietnam, Lao Cai: <i>Wen 6030</i> (US)	–; EU669170
<i>Prunus</i> sp. 5928	Vietnam, Lao Cai: <i>Wen 5928</i> (US)	–; EU669157
<i>Prunus</i> sp. 6812	Costa Rica, Puntarenas Prov., Monteverde area: <i>Wen 6812</i> (US)	EU669092; EU669161
<i>Prunus</i> sp. 6846	Costa Rica, Puntarenas Prov., Monteverde area: <i>Wen 6846</i> (US)	EU669093; EU669162
<i>Prunus</i> sp. 7042	Costa Rica, San Jose Prov.: <i>Wen 7042</i> (US)	EU669094; EU669164
<i>Prunus</i> sp. 8419	Malaysia, Paso: <i>Wen 8419</i> (US)	–; EU669156
<i>Pygeum</i> group		
<i>Pygeum stipulaceum</i> King 8418	Malaysia, Paso: <i>Wen 8418</i> (US)	EU669103; EU669175
<i>Pygeum topengii</i> Merr. 5813	China, Guangdong: <i>Wen 5813</i> (US)	EU669110; EU669154
<i>Prunus africana</i> (Hook. f.) Kalkman 6226	USA, New York, cult. Cornell Univ.: <i>Wen 6226</i> (US)	EU669109; EU669158
<i>P. grisea</i> (Blume Ex Müll. Berol.) Kalkman 8262	Philippines, Los Banos: <i>Wen 8262</i> (US)	EU669102; EU669173
<i>P. arborea</i> (Blume) Kalkman 8431	Malaysia, Paso: <i>Wen 8431</i> (US)	–; EU669174
<i>P. malayana</i> Kalkman 8366	Malaysia, Pahang, Cameron Highlands: <i>Wen 8366</i> (US)	EU669107; EU669176
<i>Exochorda giraldii</i> Hesse var. <i>wilsonii</i> (Rehder) Rehder	USA, Massachusetts, cult. AA 11626-C: <i>Lee & Wen 5003</i> (CS)	AF179555, AF179556; EU669114
<i>Maddenia hypoleuca</i> Koehne	USA, Massachusetts, cult. AA 665-65-A: <i>Lee & Wen 5005</i> (CS)	AF179549, AF179550; EU669117
<i>Oemleria cerasiformis</i> (Hook. & Arn.) Landon	USA, Massachusetts, cult. AA 275-85-A: <i>Lee & Wen 5002</i> (CS)	AF179553, AF179554; EU669115
<i>Prinsepia uniflora</i> Batal.	USA, Colorado, cult. CS TS81293: <i>Lee & Wen 4086</i> (CS)	AF179559; EU669116
<i>Physocarpus monogynus</i> (Torr.) Coult.	USA, Colorado, Larimer Co.: <i>Owens 205</i> (CS)	–; EU669112
<i>Lyonothamnus floribundus</i> Gray	USA, California, cult. SBBG 63-048: <i>D. A. Young s.n.</i> (CS)	AF179558; EU669111
<i>Holodiscus discolor</i> (Pursh) Maxim. 7257	USA, Texas, Jeff Davis Co.: <i>Wen 7257</i> (US)	EU669091; EU669113

1) Abbreviations: AA=Arnold Arboretum, CS=Colorado State University Arboretum, MBG=Missouri Botanical Garden, SBBG=Santa Barbara Botanical Garden, USNA=United States National Arboretum. 2) Subgeneric and sectional name *Prunus* was used instead of *Prunophora* and *Euprunus*, respectively, following the International Code of Botanical Nomenclature (McNeill et al., 2006).

was tested using the incongruence length difference (ILD) test in PAUP* using 1000 data bipartitions and analyzing a maximum of 10,000 trees for each (Farris et al., 1995).

2 Results

2.1 Phylogenetic analysis of *ndhF* data

With gaps treated as missing data, the parsimony analysis of the *ndhF* data generated 196200 maximally parsimonious trees (MPT's) with a length of 815 steps, a consistency index (CI) of 0.71, a COI excluding uninformative characters of 0.56, and a retention

index (RI) of 0.86. The strict consensus tree is shown in Fig. 1.

The genus *Maddenia* is nested within *Prunus* s.l. and shows a close relationship with some taxa of subgenera *Laurocerasus* (including some species of the *Pygeum* group) and *Padus*. The subgenera *Padus* and *Laurocerasus* (along with *Maddenia*) form a monophyletic group with Bayesian posterior probability (PP) 99%, but bootstrap value (BV) less than 50% (Fig. 1). This large *Laurocerasus-Pygeum-Padus-Maddenia* clade contains two major subclades, each with taxa of the subgenera *Laurocerasus*, *Pygeum* and *Padus*. The monophyly of each of those three

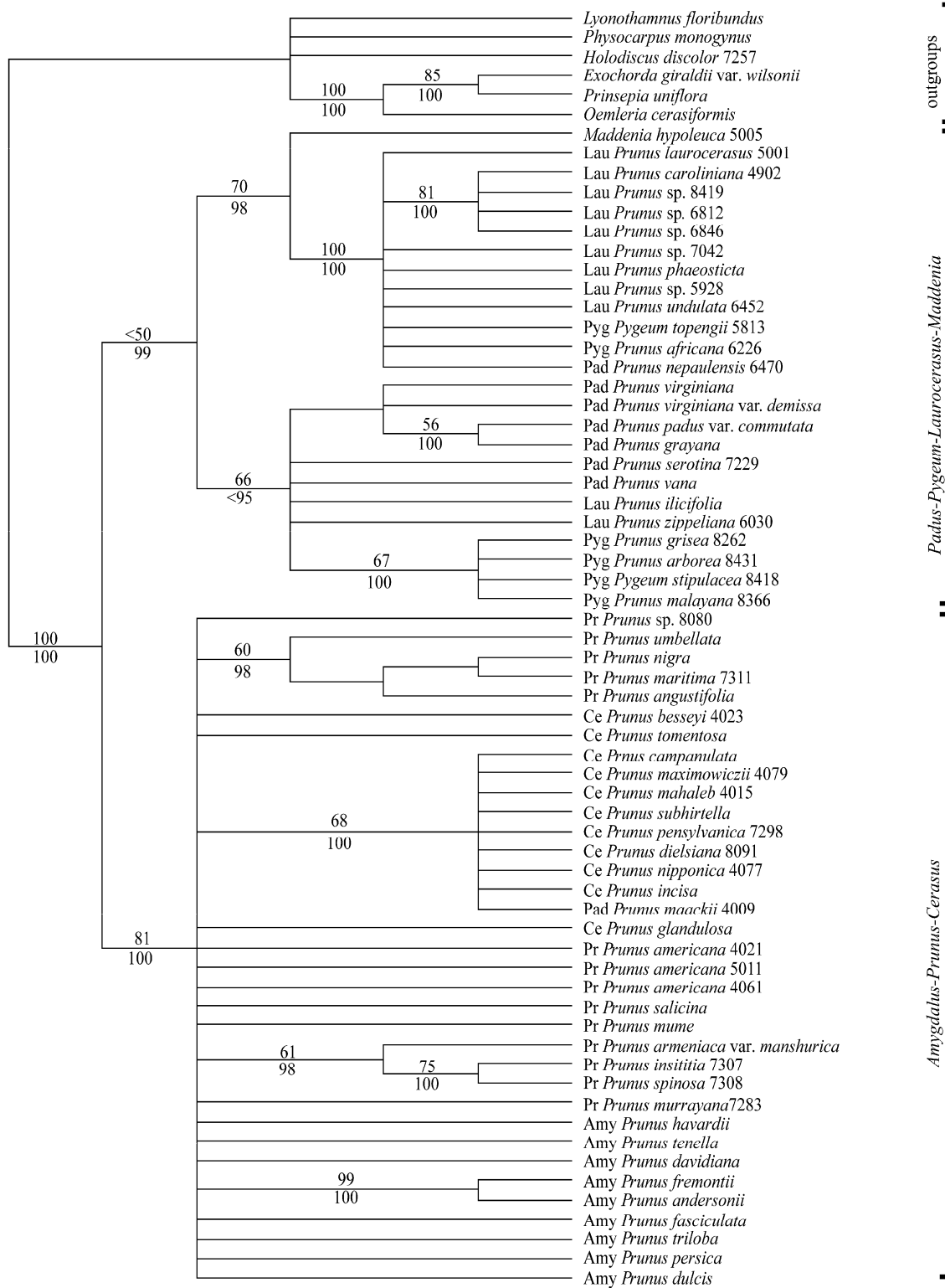


Fig. 1. Strict consensus of 196200 maximally parsimonious trees of the *ndhF* sequence data ($CI=0.71$, CI excluding uninformative characters= 0.56 , and $RI=0.86$). Numbers above the lines are bootstrap values, and the numbers below the branches are Bayesian posterior probabilities. The abbreviations Amy, Ce, Lau, Pad, and Pr stand for subgenera *Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus*, and *Prunus*, respectively; and Pyg represents the *Pygeum* group.

subgroups is thus not supported by the *ndhF* data. Four Asian species formerly classified in *Pygeum* form a clade, but neither the Asian *Pygeum topengii* nor the African *Prunus africana* (also formerly classified in *Pygeum*) is included in that clade. Subgenera *Amygdalus*, *Cerasus*, and *Prunus* form another strongly supported monophyletic group (PP=100, BV=81), with *Prunus maackii* and the core members of *Cerasus* forming a subclade (PP=100, BV=68; Fig. 1). The monophyly of subgenus *Cerasus* is, however, not supported because *Prunus besseyi*, *P. tomentosa*, and *P. glandulosa* are unresolved in the *Amygdalus-Prunus-Cerasus* clade.

2.2 Phylogenetic analysis of ITS data

Treating gaps as missing data, the parsimony analysis of the ITS data generated 49200 MPT's with a length of 791 steps, a CI of 0.56, a CI excluding uninformative characters of 0.45, and an RI of 0.70. The strict consensus tree is shown in Fig. 2.

As in the *ndhF* analysis, the ITS analysis indicates that *Maddenia* is nested within *Prunus*, and more specifically within a group consisting of members of the *Padus* and *Laurocerasus* subgenera (Fig. 2). Neither subgenus *Padus* nor subgenus *Laurocerasus* is monophyletic, and most of the species of *Laurocerasus* (including *Pygeum*) form a paraphyletic grade within which the rest of the genus is nested. Here, the four sampled Asian species of *Pygeum* form a well-supported monophyletic group, but *Prunus africana* is not included in that clade. Subgenus *Cerasus* is poorly resolved and clearly not monophyletic. *Prunus besseyi*, *P. glandulosa*, and *P. tomentosa* of subgenus *Cerasus* group with taxa of subgenera *Amygdalus* and *Prunus* (Fig. 2). The *Prunus* and *Amygdalus* subgenera are closely related, and they form a monophyletic group with low support with the aforementioned three taxa from subgenus *Cerasus* (Fig. 2).

A partition homogeneity test (Farris et al., 1995) performed in PAUP* (Swofford, 2003) resulted in a p-value of 0.01, indicating significant incongruence between the two data sets, which therefore were not combined for further analysis.

3 Discussion

3.1 *Maddenia* nested within *Prunus*

Maddenia, a small genus of five species in the Himalayas and China (Rehder, 1940), was shown to be nested within *Prunus* s.l. In each analysis, it was found embedded in a clade composed of members of the subgenera *Laurocerasus* and *Padus* (Figs. 1, 2).

Maddenia shares several characters with these subgenera, including racemose inflorescences and generally monocarpellate flowers, but has been given generic status based on its dioecious flowers that have ten sepals and no petals (as opposed to five in *Prunus*). These characters may not clearly delimit *Maddenia* from *Prunus*. Some species in *Prunus* subgenus *Laurocerasus* (specifically, former members of the genus *Pygeum* that were merged into subgenus *Laurocerasus*) have ten perianth parts that are indistinguishable or only slightly distinguishable as petals or sepals (see Kalkman, 1965). Furthermore, Sterling (1964) points out that the occurrence of dioecy among species of *Maddenia* is inconsistent, and that the fruits of *Maddenia* and the *Pygeum* group share several characters. The close alliance of *Maddenia* with the *Laurocerasus* and *Padus* group seems noteworthy in that at least one species in subgenus *Laurocerasus*, *Prunus caroliniana*, frequently shows andromonoecy (Wolfe & Drapalik, 1999), a breeding system believed to be a precursor of dioecy (Bertin, 1982; Solomon, 1986). If *Maddenia* is indeed derived from a common ancestor of some members of the *Laurocerasus/Padus* group, this is a specific example of a dioecious species arising from bisexual and andromonoecious ancestors. The evolution of breeding systems potentially exemplified in the *Maddenia-Prunus* alliance deserves further study.

3.2 Relationships within *Prunus* s.l.

Subgenera *Laurocerasus* (including *Pygeum*) and *Padus* and the genus *Maddenia* form a monophyletic group that is sister to the rest of *Prunus* s.l. in the *ndhF* tree (Fig. 1). These relationships are also supported by cpDNA *trnL-trnL-trnF+trnS-trnG-trnG+psbA-trnH* sequences (JS, JW and DP unpublished study). These same taxa form a paraphyletic group in the ITS tree (Fig. 2). Lersten and Horner's (2000) leaf crystal data suggests that subgenus *Padus* probably is a less advanced group within *Prunus* s.l., with subgenus *Laurocerasus* next (see also Kalkman, 1965), but the data presented here do not support this view as members of each subgenus are intermixed among two sister clades. In any case, the *ndhF* parsimony data (Fig. 1) presented here suggest a *Padus/Laurocerasus/Maddenia* alliance (PP=99, BV<50).

The subgenera *Laurocerasus* (including *Pygeum*) and *Padus* share racemose inflorescences, small flowers, and small floral bracts, but have been delimited because members of subgenus *Laurocerasus* generally have evergreen leaves, naked peduncles, and axillary inflorescences (flowers are in new terminal racemes within subgenus *Padus*) (see Rehder, 1940;

small flowers with cup-shaped hypanthia (Rehder, 1940). *Prunus maackii*, which Rehder (1940) chose to put in subgenus *Padus*, forms natural hybrids with *Prunus maximowiczii*, a species he placed in subgenus *Cerasus* that has a short, 5–6 flowered raceme.

Subgenus *Cerasus* section *Microcerasus* of *Prunus* s.l. was sampled with three species (*P. besseyi*, *P. glandulosa*, and *P. tomentosa*), which were nested within groups composed of subgenera *Amygdalus* and *Prunus* in the analyses (Figs. 1, 2). This corroborates the data seen in Lee and Wen (2001), Bortiri et al. (2001) and Shaw and Small (2004). Section *Microcerasus* shows axillary buds in threes along with short pedicellate flowers, a trait more like members of subgenus *Amygdalus* than subgenus *Cerasus*, but they do lack the typical bloom or pubescence found on the fruits of species in subgenera *Prunus* and *Amygdalus*. Lersten and Horner (2000) noticed that the leaf crystals in several *Microcerasus* species showed similarities with subgenera *Prunus* (= *Prunophora*) and *Amygdalus*, and isozyme studies revealed several members of *Microcerasus* that grouped with members of subgenera *Prunus* and *Amygdalus* (Mowrey & Werner, 1990). It also has been shown that *Microcerasus* species can form hybrids with cultivated members of subgenera *Prunus* and *Amygdalus*, while other members of subgenus *Cerasus* do not (Watkins, 1995).

The *Microcerasus* group has generally been treated as part of subgenus *Cerasus* (Rehder, 1940; Ghora & Panigrahi, 1995), but Focke (1894) chose to raise the group to subgeneric status (=Untergattung) equal to the other traditional subgenera (like *Cerasus*). Krüssmann (1978) followed a similar approach by treating this taxon as subgenus *Lithocerasus* with three sections. This subgeneric treatment circumscribes subgenus *Cerasus* section *Microcerasus* sensu Rehder. Our analysis does not suggest that the *Microcerasus* group deserves subgeneric status equal to the other group within *Prunus* s.l., since *Microcerasus* is found in several places within the *Prunus*/*Amygdalus* alliance, but the workers who treated this group as a separate subgenus recognize that some of these species do not show a close relationship with subgenus *Cerasus*. Both data sets from this study echo that assertion. Mowrey and Werner (1990) suggest that *Lithocerasus* (=section *Microcerasus* sensu Rehder) is not supported as a natural group, since members are found nested in several different other groups in their data (this is also supported by unpublished cpDNA *trnL-trnL-trnF+trnS-trnG-trnG+psbA-trnH* sequences of JS, JW and DP). More samples need to be exam-

ined to determine the phylogenetic position of other members of section *Microcerasus*.

Members of these two subgenera (*Prunus* and *Amygdalus*) are intermixed in the ITS trees (Fig. 2), and unresolved in the *ndhF* tree (Fig. 1), the former supporting the conclusion neither subgenus is monophyletic and the later not refuting this claim (also see Bortiri et al. 2001, 2002; Lee & Wen, 2001; Shaw & Small, 2004). Kalkman (1965) points out that all the groups (subgenera) within *Prunus* s.l. “are not very sharply delimited” morphologically and the molecular data from this study seem to support that assertion in the *Padus/Laurocerasus* group. Furthermore, Watkins (1995) also notes that hybridization is common within *Prunus* s.l., even between the traditional subgenera (Rehder, 1940). The *Prunus*/*Amygdalus* groups show close ties with each other, including morphological similarities, such as sulcate, bloomy fruits, furrowed and/or rough-pitted stones, and flowers in fascicles or umbels.

Within the *Amygdalus*/*Prunus* group, both data sets strongly support the close relationship of *Prunus andersonii*, the “desert peach” (Wilken, 1993) and *P. fremontii*, the “desert apricot” (Figs. 1, 2; also see Bortiri et al., 2001, 2002). Both are thorny shrubs (*P. fremontii* can be a small tree) from dry areas in the western United States that share puberulent, dry fruits and small hypanthia (Munz, 1959; Wilken, 1993).

3.3 Glimpse into the diversification of tropical members of *Prunus* s.l.

Subgenus *Laurocerasus* has been delimited by characters generally found in tropical habitats, such as evergreen leaves and bractless racemes (Rehder, 1940). The data from this study suggest the history of subgenus *Laurocerasus* is probably more complex, and grouping of these species into a distinct subgenus is not supported. In our analysis, subgenus *Laurocerasus* including *Pygeum* is intermixed with members of the primarily temperate subgenus *Padus* (Figs. 1, 2). Subgenus *Laurocerasus* is disjunct across several tropical and subtropical areas of the world including Africa, southeast Asia, Central America, and South America; and separate evolutionary events may have given rise to members of this group. More robust phylogenies based on more extensive sampling of both taxa and characters will be required to thoroughly test this hypothesis. Zhang (1992) noted that subgenus *Laurocerasus* is a diverse group (even with the exclusion of *Pygeum*; cf. Kalkman, 1965), and he found two disparate clades based on wood anatomy. Lersten and Horner (2000) noted that leaf crystals in subgenus *Laurocerasus* are very diverse, unlike other

subgenera studied. Lee and Wen (2001) also found several paraphyletic branches of which members of subgenera *Laurocerasus* and *Padus* were found. Subgenus *Laurocerasus* is likely a complex group of species that does not represent a single, natural relationship. This group needs further work to understand the diversifications among these species.

4 Conclusions

Our analysis suggests that (1) *Pygeum* is polyphyletic and its generic status is not supported; (2) the subgeneric status of *Padus* and *Laurocerasus* is not supported; (3) the relationships among subgenera *Prunus*, *Amygdalus*, and *Cerasus* are unresolved because of lack of information in the data sets; (4) we need more data to more adequately test all of the proposed or current classification schemes of *Prunus*; (5) the *ndhF* tree is congruent with other published cpDNA phylogenies, however, it is NOT congruent with the ITS data presented here and published previously (Lee & Wen, 2001; Bortiri et al., 2001); and (6) the biogeographic interactions among tropical and temperate members in the *Padus/Laurocerasus/Maddenia* alliance including *Pygeum* are shown to be dynamic and complex. The diversification of *Prunus* may have involved reticulate evolution, polyploidy, and other molecular processes. Future analyses require more chloroplast as well as single- or low-copy nuclear markers.

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