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

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9(Department of Plant Sciences, MS 2, University of California, Davis, CA 95616, USA)

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* Reh. (=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 *Exocordia* 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* Gaertnter [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...
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 Osmanorinieae 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 prunamygdaloid 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 Osmaroneae (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-μl 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)

<table>
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<th>Taxon</th>
<th>Source and voucher</th>
<th>GenBank accession (ITS; ndhF)</th>
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<td><em>P. insititia</em> L. 7307</td>
<td>USA, Illinois, cult. Morton Arboretum: Wen 7307 (US)</td>
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<td><strong>Subgen. 2. Prunocerasus Koehne</strong></td>
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<td><strong>Subgen. 3. Armeniaca (Lam.) Koch.</strong></td>
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<td><em>P. armeniaca</em> L. var. mandshurica Maxim.</td>
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<td><em>P. mume</em> (Sieb.) Sieb. &amp; Zucc.</td>
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<td><em>P. andersonii</em> Gray</td>
<td>USA, Nevada, Douglas Co.: M. Beck s.n.(CS)</td>
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<td><em>P. davidiana</em> (Carr.) Franch.</td>
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<td><em>P. dulcis</em> (Mill.) Webb.</td>
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<td><em>P. havardii</em> Mason</td>
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<td><em>P. besseyi</em> Bailey</td>
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<td><em>P. glandulosa</em> Thunb.</td>
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<td><strong>Subgen. 2. Pseudocerasus Koehne</strong></td>
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<td><em>P. campanulata</em> Maxim.</td>
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<td><em>P. phaeoictica</em> Maxim.</td>
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<td><em>P. serotina</em> Ehrh. 7229</td>
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<td>*P. vanz J. F. Macbr.</td>
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<td><em>P. virginiana</em> L. var. virginiana</td>
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Subgen. 5. *Laurocerasus* Koehne

*P. caroliniana* Aiton

USA, Florida, Gadsden Co.: *Gholson* 9-20-98 (CS) | AF179540; EU669130 |

*P. ilicifolia* (Nutt.) Walp.

USA, California, Santa Barbara: *D. A. Young* s.n. (CS) | AF179543, AF179544; EU669133 |

*P. laurocerasus* L. 5001

Cult. AA 889-72-D: *Lee & Wen* 5001 (CS) | AF179545, AF179546; EU669118 |

*P. undulata* (D. Don) Roem. 6452 | China, Yunnan: *Wen* 6452 (US) | EU669108; EU669155 |

*P. zippeliana* Miq. 6030 | Vietnam, Lao Cai: *Wen* 6030 (US) | –; EU669170 |

*Prunus* sp. 5928

Vietnam, Lao Cai: *Wen* 5928 (US) | –; EU669157 |

*Prunus* sp. 6812

Costa Rica, Puntarenas Prov., Monteverde area: *Wen* 6812 (US) | EU669092; EU669161 |

*Prunus* sp. 6846

Costa Rica, Puntarenas Prov., Monteverde area: *Wen* 6846 (US) | EU669093; EU669162 |

*Prunus* sp. 7042

Costa Rica, San Jose Prov.: *Wen* 7042 (US) | EU669094; EU669164 |

*Prunus* sp. 8419

Malaysia, *Wen* 8419 (US) | –; EU669156 |

**Pygeum group**

*Pygeum stipulaceum* King 8418 | Malaysia, *Wen* 8418 (US) | EU669103; EU669175 |

*Pygeum tomentell Merr.* 5813 | China, Guangdong: *Wen* 5813 (US) | EU669110; EU669154 |

*Prunus africana* (Hook. f.) Kalkman 6226 | USA, New York, cult Cornell Univ.: *Wen* 6226 (US) | EU669109; EU669158 |

*P. grisea* (Blume Ex Müll. Berol.) Kalkman 8262 | Philippines, Los Banos: *Wen* 8262 (US) | EU669102; EU669173 |

*P. arboarea* (Blume) Kalkman 8431 | Malaysia, *Wen* 8431 (US) | –; EU669174 |

*P. malayana* Kalkman 8366 | Malaysia, Pahang, Cameron Highlands: *Wen* 8366 (US) | EU669107; EU669176 |

*Exocorda giraldii* Hesse var. wilsonii (Rehder) Rehder

USA, Massachusetts, cult. AA 11626-C: *Lee & Wen* 5003 (CS) | AF179555, AF179556; EU669114 |

*Maddenia hypoleuca* Koehne

USA, Massachusetts, cult. AA 665-65-A: *Lee & Wen* 5003 (CS) | AF179549, AF179550; EU669117 |

*Oemleria cerasiformis* (Hook. & Arn.) Landon

USA, Massachusetts, cult. AA 275-85-A: *Lee & Wen* 5002 (CS) | AF179553, AF179554; EU669115 |

*Prinsepia uniflora* Batal.

USA, Colorado, cult. CS TS81293: *Lee & Wen* 4027 (US) | AF179559; EU669116 |

*Physocarpus monogynus* (Torr.) Coult.

USA, Colorado, Larimer Co.: *Owens* 205 (CS) | –; EU669112 |

*Lyonothamnus floribundus* Gray

USA, California, cult. SBBG 63-048: *D. A. Young* s.n. (CS) | AF179558; EU669111 |

*Holodiscus discolor* (Pursh) Maxim. 7257 | USA, Texas, Jeff Davis Co.: *Wen* 7257 (US) | EU669091; EU669113 |

1) Abbreviations: AA=Arnold Arboretum, CS=Colorado State University Arboretum, MBG=MISSISSIPPI National Arboretum, SBBG=Santa Barbara Botanical Garden, USNA=United States National Arboretum. 2) Subgeneric and sectional name *Prunus* was used instead of *Prunaphora* 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 bifurcations 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-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;
Hutchinson, 1964). Since this study suggests that members of the two subgenera are intermixed; thus, the evolution of the delimiting characters of these two subgenera (particularly evergreen leaves) may have occurred several times.

Taxa sampled from subgenus *Cerasus* form a monophyletic group in the *ndhF* tree (Fig. 1) with two exceptions: the inclusion of *Prunus maackii*, traditionally placed in subgenus *Padus*, and the absence of the representatives of section *Microcerasus* (sensu Rehder, 1940). In the *ndhF* data, the core *Cerasus* group is shown to be a monophyletic lineage branch-

**Fig. 2.** Strict consensus of 49200 maximally parsimonious trees of the nuclear ribosomal ITS sequence data (*CI*=0.56, *CI* excluding uninformative characters=0.45, and *RI*=0.70). 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.
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 examined 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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