Prunus sunhangii: A new species of Prunus from central China

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A R T I C L E   I N F O

Article history:
Received 5 November 2018
Received in revised form 20 January 2019
Accepted 21 January 2019
Available online 27 January 2019

(Edited: Sergey Volis)

Keywords:
Phylogenetic analyses
Taxonomy
Cerasus
Section Serrula

A B S T R A C T

A new species of Rosaceae from Central China, Prunus sunhangii D. G. Zhang & T. Deng, sp. nov., is described and illustrated. The new species is placed in Prunus subgenus Cerasus by flower and fruit characteristics. It is most similar to Prunus cerasoides, but differs by having longitudinally 2-lobed apical petals, an acuminate leaf apex, 17–25 stamens, white petals, dark black drupes, brown hypanthium, and different phenology. The phylogenetic placement of this species was assessed based on morphological and molecular data. Molecular analysis (cpDNA + ITS) corroborated its placement in subgenus Cerasus, specifically Prunus section Serrula.

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1. Introduction

Rosaceae is a globally distributed family found mostly in the northern hemisphere that comprises approximately 2950 species in 91 genera (Christenhusz and Byng, 2016). Up to 55 genera and 950 species of Rosaceae, of which 55% are endemics, are listed in the Flora of China (Lingdi et al., 2003). Rosaceae has been divided into 11 sections by Li and Bartholomew (2003). These species have been grouped into three subgenera: Amygdaloideae, Rosaeae, Maloideae and Prunoideae (Potter et al., 2007). The genus Prunus L belongs to subfamily Pomoideae.

The genus Prunus L. senso lato (s.l) consists of 430 species (Chen et al., 2013). Species within the genus Prunus L are mainly trees or shrubs. The genus is defined based on a combination of characters, including the presence of leaf glands, having a superior ovary, a solitary carpel with two pendulous anatropous ovules per carpel, and drupes for fruits, which have succulent mesocarp that is fleshy, or dry, and does not split, or more rarely, splits when ripe (Rehder, 1940; Li and Bartholomew, 2003; Chin et al., 2013; Zhao et al., 2016). In Shi et al. (2013) used molecular analysis to divide the genus Prunus senso lato into three subgenera: subg. Padus, subg. Cerasus, and subg. Prunus. Cerasus is the most important subgenus of the genus Prunus. It consists of 45 species in China (Yu and Li, 1986; Li and Bartholomew, 2003; Chen et al., 2013). These species have been grouped into 11 sections by Li and Bartholomew (2003). The subg. Cerasus is characterized by having inflorescences with usually conspicuous bracts, solitary flowers or several flowers in short racemes or corymb, and by having smooth drupes that are glabrous but not glaucous, with endocarp that is not compressed or is slightly compressed (Rehder, 1940; Yu and Li, 1986; Li and Bartholomew, 2003).

During field studies in Hunan province (Yongshun County, Xiaoxi National Nature Reserve and Suining County, Huangsang National Nature Reserve) and Hubei Province (Wufeng County) conducted by Tao Deng, Xiao-Shuang Zhang, Meng-Hua Zhang, Dai-Gui Zhang between 2013 and 2015, several specimens of an unknown species of Prunus were found. The new taxon was determined to be most morphologically similar to Prunus cerasoides Buch.-Ham. ex D.Don of Prunus subg. Cerasus sect. Serrula based
Table 1
Diagnostic morphological characters comparing *Prunus sunhangii* with related species.

| Characters      | *Prunus sunhangii*       | *Prunus cerasoides*     |
|-----------------|--------------------------|-------------------------|
| Leaf margin     | apex acuminate           | apex acuminate to long acuminate |
| Petals          | white                    | white or pink           |
| Sepals margin   | laxly dentate            | entire                  |
| Petals apex     | longitudinally 2-lobed    | emarginate              |
| Stamens         | 17–25                    | 32–34                   |
| Hypanthium color| brown                    | red to dark red         |
| Drupe           | black                    | purplish black          |
| Flowering       | March–April              | October–December        |
| Fruiting        | April–May                | February–March          |

Fig. 1. Living images of *Prunus sunhangii* D. G. Zhang & T. Deng *sp. nov.* (A) Habitat; (B) Stem; (C) Leaf, showing abaxial indumentums; (D) Inflorescence; (E–F) Opening white flower: front and back side; (G) Ripe fruit. Scale bars: 1 cm in C, D, E, F, G.
on the presence of characteristic features such as having 1–4 flowered, umbellate or subumbellate inflorescences, petals apex divided. The results of morphological and phylogenetical analyses support the status of the taxon as a new species, which is described below.

2. Material and methods

2.1. Morphological analyses

Morphological observations and measurement of leaves, calyx, flowers and fruits were randomly made on flowering and fruiting plants. A total of 9 diagnostic characters were compared (Table 1). Specimens were investigated in herbaria from KUN and JIU.

2.2. Taxon sampling and outgroup selection

The voucher information of the studied taxa is listed in Appendix A1. In total, 12 species were sampled to represent Prunus subg. Cerasus, and one outgroup Prunus henryi (Prunus subg. Laurocerasus Koehne, Chin et al., 2014).

2.3. DNA extraction, amplification, and sequencing

Genomic DNA was isolated using TaKaRa Universal Genomic DNA Extraction Kit Ver. 3.0 (TaKaRa, Osaka, Japan) according to the manufacturer’s protocol. The selected DNA regions were amplified, following standard polymerase chain reaction (PCR) protocols (Kusukawa et al., 1990), and were sequenced using primers for the ITS protocols as described by White et al. (1990). Thermocycling conditions were 4 min at 94 °C, followed by 35 cycles at 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1.5 min, with a final extension at 72 °C for 7 min. The cpDNA regions were amplified using primers trnG-S (Hamilton and Baulcombe, 1999), rps16 (Shaw and Small, 2004), trnL–trnF (Taberlet et al., 1991) and psbA–trnH (Sang et al., 1997). The thermocycling conditions for all genes were 4 min at 94 °C, followed by 35 cycles at 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1.5 min, with a final extension at 72 °C for 7 min.

2.4. Phylogenetic analyses

The sequences were edited in Sequencher™ 4.12 (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned using the program

Fig. 2. Illustration of Prunus sunhongii D. G. Zhang & T. Deng sp. nov. (A) Fruiting shoot; (B) Leaf, showing abaxial indumentums; (C) Flower, showing with pistil and stamens; (D–E) Fruit, showing with pedicel.
Fig. 3. Bayesian 50% majority-rule consensus tree of Prunus subg. Cerasus and closely related taxa, inferred from combined sequences data from plastid (trnG-S, psbA-trnH, rps16, trnL-F) and nuclear (ITS) markers. Bayesian posterior probabilities/maximum likelihood values are given above the branches. The new species is shown in bold.

Fig. 4. Distribution of Prunus sunhangii.
Mega 7.0 (Hall, 1999). A homogeneity test was conducted in PAUP v4.0 (Swofford, 2013) to check for a congruence of ITS and Chloroplast trees ($P = 0.87; P > 0.5$). The homogeneity test revealed a positive correlation for combined datasets. The best-fitting substitution models for Bayesian inference were selected using MrModeltest 2.3 (Nylander et al., 2004). Bayesian inference (BI) employed MrBayes v.3.2.6 and Maximum likelihood (ML) employed RAxML v.8.2.10 at the Cipres Portal (https://www.phylo.org/portal2).

Bayesian analysis was performed with four Markov chains each initiated with a random tree and two independent runs each for 10,000,000 generations, sampled every 100th generation. When the log-likelihood scores stabilized, the first 20% of trees were discarded as burn-in, and the retained trees were imported into PAUP to produce a 50% majority-rule consensus tree.

3. Results

3.1. Taxonomic treatment

Prunus sunhangii D. G. Zhang & T. Deng, sp. nov (Figs. 1 and 2).

Type: CHINA, Hunan, Yong Shun, XiaoXi National Nature Reserve, along the roadside, 28°46′27.10″N, 110°15′21.18″E, 319 m, 17 March 2016, D.G. Zhang, T. Deng 107 (Type KUN!, Holotype KUN!)

3.2. Diagnosis

P. sunhangii is easily differentiated from P. cerasoides by its apically longitudinally 2-lobed petals and other features: white flowers, black drupe, stamens number (17–25), brown hypanthium and with phenology.

3.3. Description

Trees 20–25 m tall, stems 40 cm in diam. Bark gray, longitudinally fissured. Young branchlets green, gray pilose. Stipules brown, linear, shorter than petiole, margin glandular dentate. Petiole, 0.9–1.2 cm long, densely white pubescent leaf blades ovate-lanceolate, 7–12 × 3–4 cm, abaxially pale green and appressed white pilose or more densely so along midvein, adaxially dark green and glabrous or appressed pilose on veins, margin sharply biserrate, apex acuminate. Secondary veins 12–19 on each side, straight and parallel. Inflorescences umbellate, 3- or 5-flowered rare 2, involucr bracts obovate, outside pilose, soon deciduous.

Fig. 5. Predicted range of Prunus sunhangii based on bioclimatic modeling. Green areas denote protected areas and dots - species occurrences.
after flowering. Flowers opening at same time as leaves. Peduncle short 0.19–0.35 cm. Pedicels 0.68–1.90 cm, densely pilose. Hypan-
thyrum brown, urceolate, 3.2–5.5 × 2.0–3.9 mm, outside densely pubescent. Sepals ovate or oblong-triangle, 1.9–3.0 × 1.0–2.0 mm, nearly as long as hypanthyrum or slightly shorter, open and flat, margin laxly dentate, apex acute. Petals white, apically longitudinally 2-lobed (rare emarginate), 0.75–1.21 × 0.47–0.80 cm. Stamens 17–25. Drupe black, ovoid.

**Phenology** — Flowering from March to April, fruiting from April to May.

**Distribution and habitat** — *P. sunhangii* is restricted to the Wuling Mountains in South China, which is a center of the *Metasequoia* Flora (Chen et al., 2017). Plants were growing on limestone soil, scattered along a slope adjoining the valley at 300–600 m in Hunan province and at 1000–1200 m in Hubei province (Fig. 4).

**Etymology** — The epithet of the new species refers to the Chinese botanist Prof. Hang Sun, who made a significant contribution to our knowledge of the flora of China.

**Vernacular name** - Chinese mandarin: Sün Háng Ying (孙航樱).

**Morphological evidence** — As shown in Figs. 1 and 2, the new species is morphologically similar to *P. cerasoides* and appears to belong to the same section, *Serrula* (Koehne) Yu et Li (Yu and Li, 1986), but differs from the latter by petal shape and color, hypanthyrum color, number of stamens, phenology and other traits (Table 1).

**Molecular phylogenetic evidence** — The aligned combined data matrix included 4209 bp (ITS-799; cpDNA-3410). The best fit model of nucleotide substitutions for the combined ITS and cpDNA datasets was found to be the generalized time reversible model GTR + G (the hierarchical likelihood ratio test, MrModeltest). The phylo-
genetic tree generated by these data placed *P. sunhangii* in the same clade with *P. cerasoides* (PP = 1, ML = 100) (Fig. 3).

**Distribution evidence** — *P. cerasoides* is found in China (South Xizang, North-West Yunnan), Bhutan, North India, Kashmir, North Laos, Myanmar, Nepal, Sakkim, North Thailand, North Vietnam. *P. cerasoides* is not found in the provinces of Hunan and Hubei (Wuling Mountains), where *P. sunhangii* was found.

**Conservation Significance**: *P. sunhangii* is an endemic species known from 4 localities (Fig. 4). The area of occupancy (AOO) of the new species is approximately 75 km². Therefore, *P. sunhangii* should be assigned a risk of extinction of “Endangered” [criterion B2 (a)] following the IUCN Red List (International Union for Conservation of Nature, 2016). We used species distribution modeling to predict the geographic distribution of suitable habitat for *P. sunhangii* under current climatic conditions. The MAXENT (Phillips et al., 2006; Phillips and Dudik, 2008) was used to generate an estimate of probability of presence of the species from 0 to 1, where 0 is the lowest and 1 the highest probability. In our analyses, we set number of iterations to 500 and used ten replicates under the ‘crossvalidate’ option. The summary map was generated by averaging Maxent outputs (Fig. 5).

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Acknowledgements**

We are grateful to Peter Fritsch and two anonymous reviewers for constructive comments. This study was supported by the Major Program of the National Natural Science Foundation of China (31590823), the National Key R & D Program of China (2017YFC0505200), the Strategic Priority Research Program of Chinese Academy of Sciences (XDA20050203), the National Natural Science Foundation of China (3170165), the Fund for Reserve Talents of Young and Middle-aged Academic and Technical Leaders of Yunnan Province (2014HB027), the CAS “Light of West China” Program, the Comprehensive Scientific Investigation of Biodiversity from the Wuling Mountains (2014FY10100), and the survey on baseline resources of Wufeng Houhe National Nature Reserve in Hubei Province.

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