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NOTE

Prunus sunhangii is a new synonym of *P. itosakura* var. *ascendens*

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ABSTRACT: *Prunus sunhangii* was recently described as an independent species related to *P. cerasoides* with molecular evidence from ITS and four cpDNA sequences (*psbA-trnH*, *rpS16*, *trnL-trnF* and *trnS-trnG*). However, while repeating the phylogenetic analysis of *P. sunhangii*, we found some flaws in the previous analysis, including the extremely long branch of the outgroup and the poor sequence quality, which may affect the reliability of the result. Therefore, a new phylogenetic analysis was performed to verify the identity of *P. sunhangii* with a new data matrix and morphological comparisons. The results showed that *P. sunhangii* forms a monophyletic clade with *P. itosakura* var. *ascendens* with high support and *P. sunhangii* is similar with *P. itosakura* var. *ascendens* in every morphological aspect. We concluded that *P. sunhangii* is conspecific with *P. itosakura* var. *ascendens* and should be synonymized with the latter species.

KEY WORDS: *Cerasus*, China, cpDNA, Hunan, ITS, *Prunus sunhangii*, sequences, synonym, taxonomy.

INTRODUCTION

Prunus sunhangii D. G. Zhang & T. Deng was recently described based on specimens collected from north-western Hunan, China (Zhang *et al.*, 2019). With the phylogenetic evidence of the ITS and four cpDNA sequences (*psbA-trnH*, *rpS16*, *trnL-F* and *trnS-G*), *P. sunhangii* was recognized to be related to *P. cerasoides* D. Don but different in white and 2-lobed petals, brown hypanthia, fewer stamens, black fruits, and the phenology. However, according to the description and photos provided by Zhang *et al.* (2019), *P. sunhangii* is very similar to *P. itosakura* Siebold var. *ascendens* (Makino) Makino, especially in several key characteristics of the latter, such as the umbellate inflorescence with sessile peduncle and the tubular-urceolate hypanthium. Curiously, the phylogenetic analysis of Zhang *et al.* (2019) shows that, *P. sunhangii* is genetically distant from *P. itosakura* var. *ascendens* but close to *P. cerasoides*, which is obviously different in morphology. To solve the puzzle, we performed the Bayesian analysis to reconstruct the phylogenetic tree with the same set of data from Zhang *et al.* (2019). The result is basically consistent with that of Zhang *et al.* (2019); however, the basal branch of the outgroup - *Pygeum henryi* Dunn (which is mistyped as *Prunus henryi* Kohene in Zhang *et al.*, (2019)) is extremely long (Figure 1), and not shown in the cladogram proposed by Zhang *et al.* (2019). We further checked the dataset and found that the sequences of *P. cerasoides* and *P. cerasus* L. are poor in quality with several missing data in ITS and *trnL-F* of *P. cerasus*, and many degenerate bases in the ITS sequence of *P. cerasoides*. Moreover, we also found that the ITS sequence of *P. itosakura* var. *ascendens* is derived from a hybrid cultivar, *P. subhirtella* 'autumnalis'. These flaws in the dataset may cause unreliable phylogenetic inference, causing the misjudgment of the recognition of *P. sunhangii*.

Therefore, it is necessary to verify the identity of *P. sunhangii*. For this purpose, we performed a phylogenetic analysis based on a new data matrix to examine the phylogenetic position of *P. sunhangii* and compared morphologies between *P. sunhangii* and *P. itosakura* var. *ascendens*.

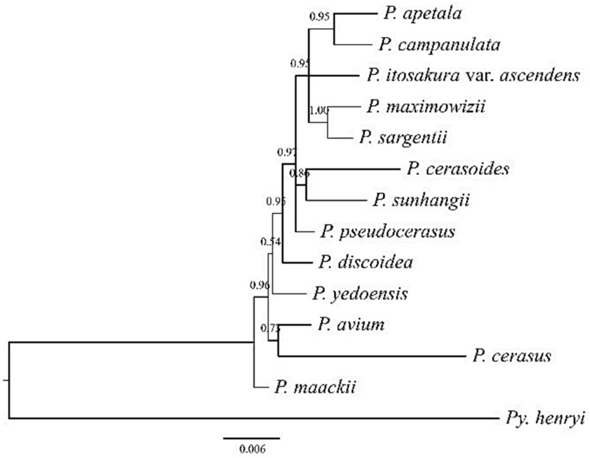

Fig. 1. Bayesian consensus tree based on the original dataset (ITS, *psbA-trnH*, *rps16*, *trnL-F*, *trnG-S*) of Zhang *et al.* (2019). The numbers above the branches indicated the Bayesian posterior probabilities.

Table 1. Samples used in the phylogenetic analysis with their voucher information and GenBank accession numbers.

Sample	Voucher (Herbarium)	Locality	*ITS*	*psbA-trnH*	*rps16*	*trnL-F*	*trnG-S*
Prunus avium (L.) L.	*Wu & Shi P2020904* (IBSC)	Sichuan (cultivated)	OL662909	OL739702	OL739713	OL739720	OL739734
Prunus campanulata Maxim.	*Wu et al. PP201809716* (CANT)	Yunnan	OL662911	OL739700	OL739710	OL739722	OL739729
Prunus cerasoides Buch.-Ham. ex D.Don	*Anonymous PKM20141020* (CANT)	Yunnan (cultivated)	OL662907	OL739701	OL739708	OL739721	OL739735
Prunus discoidea (T.T.Yu & C.L.Li) Z.Wei & Y.B.Chang	*Wu & Zhao PP201758* (CANT)	Fujian	OL662910	OL739698	OL739709	OL739723	OL739732
Prunus maackii Rupr.	*Wu & Shi P20201516* (IBSC)	Liaoning	OL662906	OL739699	OL739711	OL739724	OL739730
Prunus maximowiczii Rupr.	*Wu & Shi P20201507* (IBSC)	Liaoning	OL662905	OL739697	OL739712	OL739718	OL739733
Prunus pseudocerasus Lindl.	*Wu & Zhao PP2018052* (CANT)	Hubei	OL662908	OL739696	OL739706	OL739719	OL739731
Prunus sargentii Rehder	*Li Y2040* (CANT)	Beijing (cultivated)	OL662912	OL739695	OL739703	OL739717	OL739728
Prunus itosakura Siebold var. *ascendens* (Makino) Makino sample 1	*Wu & Lin P2020217* (IBSC)	Chongqing	OL662903	OL739693	OL739705	OL739715	OL739726
Prunus itosakura Siebold var. *ascendens* (Makino) Makino sample 2	*Wu & Lin P2020202* (IBSC)	Chongqing	OL662902	OL739692	OL739704	OL739714	OL739725
Prunus sunhangii D.G.Zhang & T.Deng sample 1	*Chen PS1* (CANT)	Hunan	OL662904	OL739694	OL739707	OL739716	OL739727
Prunus sunhangii D.G.Zhang & T.Deng sample 2	Deng 107 (KUN)	Hunan	MK411814	MK411810	MK411812	MK411811	MK411813

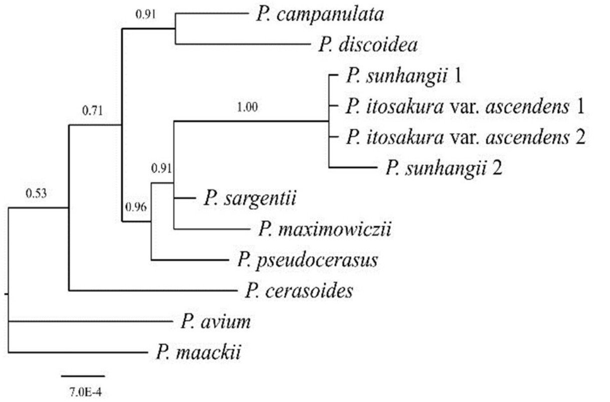

Fig. 2. Bayesian consensus tree of the new dataset (ITS, *psbA-trnH*, *rps16*, *trnL-F*, *trnG-S*) generated in this study. The numbers above the branches indicated the Bayesian posterior probabilities.

MATERIALS AND METHODS

To clarify the phylogenetic position of *P. sunhangii*, we generated a new data matrix that integrated the ITS and four cpDNA sequences (*psbA-trnH*, *rpS16*, *trnL-F* and *trnS-G*) of *P. sunhangii* from Zhang *et al.* (2019) with additional sequences from the complete ribosomal DNA sequences and complete chloroplast genomes of 11 samples of 10 taxa collected by ourselves. Detailed information on the vouchers and GenBank accession numbers are given in Table 1. Nucleotide sequences were aligned by using MAFFT 7 (Katoh *et al.*, 2002), and then the alignments were cleaned with Gblocks 0.91b (Talavera and Castresana, 2007) and concatenated by PhyloSuite v.1.2.2 (Zhang *et al.*, 2020). Subsequently, the partitioning scheme and the best-fitting models (ITS: K81UF+I+G; *psbA-trnH*: HKY+I; *rps16*, *trnL-F*, *trnS-G*: TIM+I) were estimated by PartitionFinder v.2.1.1 (Lanfear *et al.*, 2017) under the corrected Akaike Information Criterion (AICc). Partitioned Bayesian analysis was performed by MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003, Ronquist *et al.*, 2012). Two independent Markov Chain Monte Carlo (MCMC) runs with four simultaneous chains were performed for 10 million generations, with topologies sampled every 1000 generations. *Prunus maackii* Rupr. Was selected as the outgroup.

Morphological comparisons were performed based on specimens, individuals in the field, photos, and descriptions in the literature. Specimens (listed in Appendix) deposited at CSFI, HHGB, IBSC, JIU, K, KUN, L, LBG, N, NTUF, P and PE were carefully examined by visiting herbaria or through the database online.

RESULTS

The combined data matrix of ITS and cpDNA (*psbA-trnH*, *rpS16*, *trnL-F* and *trnS-G*) has 3874 characters (ITS-598, cpDNA-3346) in length, of which 70 are variable sites with 23 parsimony-informative sites. The Bayesian inference tree showed a monophyletic clade with 100% posterior probability in *P. sunhangii* and *P. itosakura* var. *ascendens*, while *P. cerasoides* is distant from this clade (Figure 2).

The morphology of *P. sunhangii* is similar to *P. itosakura* var. *ascendens*, especially in tubular-urceolate

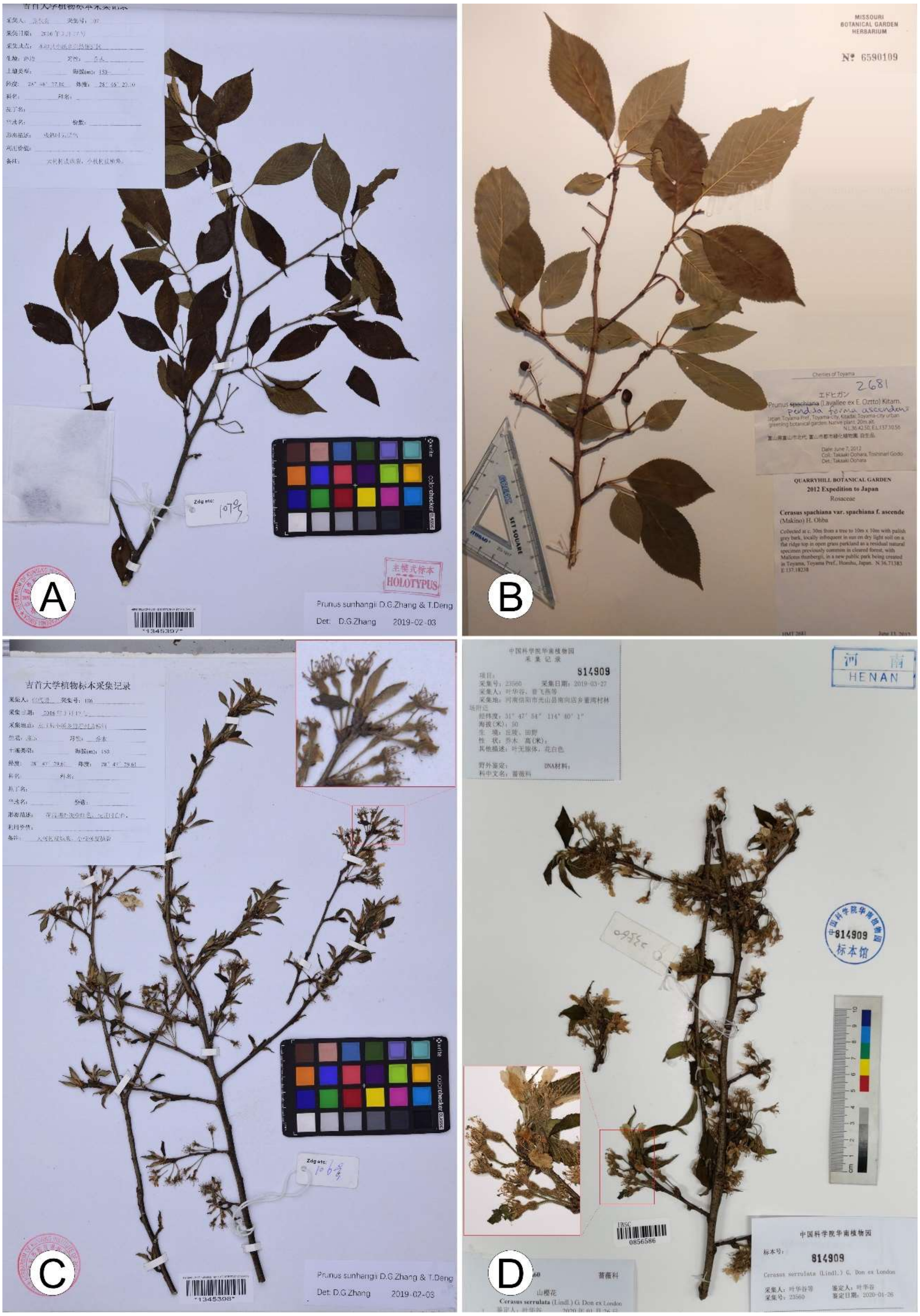

Fig. 3. Specimens of ***Prunus itosakura*** var. ***ascendens***; **A.** *D.-G. Zhang & T. Deng 107* (KUN 1345397) from Hunan, China, holotype of ***P. sunhangii***; **B.** *O. Takaaki & G. Toshinari s.n.* (MO 6590109) from Toyama, Japan; **C.** *D.-G. Zhang 106* (KUN 1345398) from Hunan, China, flower branch of ***P. sunhangii***; **D.** *H.-G. Ye et al. 23560* (IBSC 0856586) from Henan, China.

Table 2. Morphological comparison of ***Prunus sunhangii*** and ***P. itosakura*** var. ***ascendens***.

	P. sunhangii (based on Zhang *et al.* 2019)	***P. itosakura*** var. ***ascendens***
Bark	gray, longitudinally fissured	gray to grayish brown; old trunk black and longitudinally fissured
Branchlet	green, gray pilose	green to grayish brown, pilose
Stipule	brown, linear	green to brownish-green, linear
Petiole	0.9–1.2 cm, densely pubescent	0.4–1.3 cm, white pubescent, densely when young
Leaf blade	ovate-lanceolate, 7–12 × 3–4 cm, abaxial surface appressed white pilose, adaxial surface glabrous or appressed pilose on veins	narrowly obovate, elliptic, or ovate-lanceolate, 5–12 × 2–4 cm, abaxial surface appressed white pilose, adaxial surface glabrous or appressed pilose on veins
Leaf margin	sharply biserrate	sharply biserrate
Secondary veins	secondary veins 12–19 pairs, straight and parallel	secondary veins 10–16 (18) pairs, straight and nearly parallel
Inflorescence	umbellate, (2) 3- or 5-flowered, with short peduncle	umbellate, (1) 2- or 5-flowered, with sessile peduncle
Pedicel	0.68–1.90 cm, densely pilose	0.6–2 cm, pilose to densely pilose
Hypanthium	urceolate, outside densely pubescent	tubular to urceolate, outside more or less pubescent
Sepal	ovate or oblong-triangle, margin laxly dentate	ovate, oblong-ovate or oblong-triangle, margin laxly dentate
Petal	white, apically longitudinally 2-lobed	white to pinkish-white, obovate to oblong, apex concave
Stamens	17–25	17–25
Fruit	black, ovoid	black, ovoid to oblong

hypanthia, umbellate inflorescences with sessile peduncle, pubescent branchlets, petioles and pedicels, and nearly parallel secondary leaf veins (Figure 3). More detailed comparisons are given in Table 2.

DISCUSSION

Prunus itosakura var. *ascendens* and its cultivars are known as spring cherries and are widely cultivated for their attractive blossoms. It is distinct in umbellate inflorescences with sessile peduncle, tubular-urceolate hypanthia, and the ascending to appressed pubescence on young branchlets, petioles, pedicels, and hypanthia (Ohwi, 1965). Morphological comparisons confirm that *P. sunhangii* is identical to *P. itosakura* var. *ascendens* in these diagnostic features. Other characters of *P. sunhangii* also fall into the morphological variation range of *P. itosakura* var. *ascendens*. Additionally, the reconstructed phylogeny shows that *P. sunhangii* and *P. itosakura* are not separated and form a monophyletic group, suggesting they should be conspecific.

Considering the phylogeny and morphology, we concluded that it is premature to place *P. sunhangii* in the rank of species and should be synonymous with *P. itosakura* var. *ascendens*.

Although *P. itosakura* var. *ascendens* is a well-known flowering cherry, it is still unfamiliar to the Chinese (Wang, 2000). It was merged into *P. subhirtella* Miq. and stated to be native to Japan and introduced and cultivated in China in "*Flora Reipublicae Popularis Sinicae*" (FRPS, Yu and Li, 1986) and "*Flora of China*" (FOC, Li and Bartholomew, 2003). However, *P. subhirtella* was suggested (Katsuki, 2009; Kuitert, 1999; Makino, 1940; Ohwi, 1965) and currently proved (Kato *et al*., 2014) as a cultivated hybrid, with *P. itosakura* var. *ascendens* being one parent and either *P. incisa* Thunb. or *P. apetala* (Siebold & Zucc.) Franch. & Sav. the other parent. Moreover, *P. itosakura* var. *ascendens* is also native to China in many provinces, such as Anhui and Hubei (Wang, 2000). We think that the neglect of morphological comparisons of *P. itosakura* var. *ascendens* and *P. sunhangii* in Zhang *et al*. (2019) may partly stem from the inaccurate taxonomy in FRPS and FOC, suggesting that the taxonomy of Chinese cherries still requires further study.

TAXONOMIC TREATMENT

Prunus itosakura Siebold var. ***ascendens*** (Makino) Makino Bot. Mag. Tokyo 22: 114. 1908.

≡ *Prunus pendula* Maxim. var. *ascendens* Makino, Bot. Mag. Tokyo 7: 103. 1893

≡ *Prunus subhirtella* Miq. var. *ascendens* (Makino) E. H. Wilson, Cherries Japan: 10. 1916. ***Type***: Japan, Kochi, Nanokawa, Tosa, *K. Watanabe s.n.* (MAK, digital image!).

= *Prunus sunhangii* D. G. Zhang & T. Deng, Plant Diversity 41: 23. 2019, ***syn. nov***. ***Type***: China, Hunan, Yongshun, XiaoXi National Nature Reserve, along the roadside, 28°46′27.11″N, 110°15′21.18″E, elev. 319m, 17 March 2016, *D.-G. Zhang & T. Deng 107* (Holotype, KUN barcode 1345397, digital image!).

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Appendix: Specimens for morphological comparison (the herbarium acronyms and barcode numbers were provided in the parentheses).

China: **Anhui**: **Qingyang**, *R.-C. Ching 2630* (IBSC 0295486); **Huangshan**, *Anonymous 125* (PE 00773248). **Chongqing**: **Chengkou**, *R. P. Farges s.n.* (P P03358236,P P03358237, P P03358238, P P03358240, P P03358243, P P03358245, P P03358246, P P03358133 in part, P P03358134 in part, P P03358136 in part, P P03358142 in part, P P03358143 in part, P P03358212 in part, P P03358213, P P03358214, P P03358223, P P03358225, P P03358228, P P03358229, P P03358230, P P03358231, P P03358235, P P03358241, P P03358244), *R. P. Farges 998* (P P03358226, P P03358227, P P03358232, P P03358233, P P03358234). **Henan: Guangshan**, *H.-G Ye & F.-Y. Zeng 23560* (IBSC 0856586), *24208* (IBSC 0857163); **Hubei**: **Changyang**, *A. Henry 7804* (K K000737031), *E. H. Wilson 2833* (P P03358242), *E. H. Wilson Veitch Exped.70* (P P03358224). **Hunan**: **Suining**, *J.-J. Zhou & D. Zhou 1403097* (CSFI CSFI026587); **Yongshun**, *G.-X. Chen PS-1* (IBSC), *PS-2* (IBSC), *Z.-H. Shen 1027* (IBSC 0295396), *D.-G. Zhang YD11032* (JIU JIU61402), *zdg10222* (JIU JIU04068); **Yuanling**, *Z.-H. Shen 065* (CSFI CSFI011579). **Jiangsu**: **precise location unknown**, *W.-C. Cheng 4688* (PE 00806165, PE 00806166); **Jurong**, *L.F. Tsu 425* (N 126073233). **Jiangxi**: **Jiujiang**, *M.-J. Wang 01494* (LBG 00010594); **Yichun**, *H.-G. Ye & F.-Y. Zeng LXP10-2924* (IBSC 0771591). **Zhejiang**: **precise location unknown**, *Anonymous 450* (HHGB HZ016977); **Hangzhou**, Q.-G. *Zhu &Q.-W. Liu 536* (PE 00773246), *455* (PE 00773248); **Lin'an**, *B.-L. Qiu 429* (HHBG HZ016976), *X.-Y. He 21085* (PE 00806158), *21951* (IBSC 0295484), *21090* (IBSC 0295485, PE 00001596), *Anonymous s.n.* (PE 00806159).

Japan： **precise location unknown**, *Tschonoski s.n.* (P P03359619、P P03359621), *Savatier s.n.* (P P03359626).

South Korea: **precise location unknown**, *U. Faurie 1546* (P P03359622).

Supplementary materials are available from Journal Website.