Prunus sunhangii: A new species of *Prunus* from central China

Xiaoshuang Zhang [a, 1], Zhilin Jiang [b, c, 1], Ziyoviddin Yusupov [d, e, 1], Menghua Zhang [a], Daigui Zhang [a], Komiljon Tojibaev [e], Ying Meng [a, **], Tao Deng [a, d, *]

[a] *Key Laboratory of Plant Resources Conservation and Utilization, Jishou University, Jishou 416000, Hunan, China*
[b] *Puer University, Puer 665000, Yunnan, China*
[c] *Institute of Comparative Study of Traditional Materia Medica, Institutes of Integrative Medicine, Fudan University, Shanghai, 200040, China*
[d] *Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, China*
[e] *Central Herbarium of Uzbekistan, Institute of Botany, Academy Sciences of Uzbekistan, Tashkent 100125, Uzbekistan*

ARTICLE INFO

ABSTRACT

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 (*cp*DNA + ITS) corroborated its placement in subgenus *Cerasus*, specifically *Prunus* section *Serrula*.

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 four subfamilies: Amygdaloideae, Rosaceae, Maloideae and Prunoideae (Potter et al., 2007). The genus *Prunus* L. belongs to subfamily Prunoideae.

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 corymbs, 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
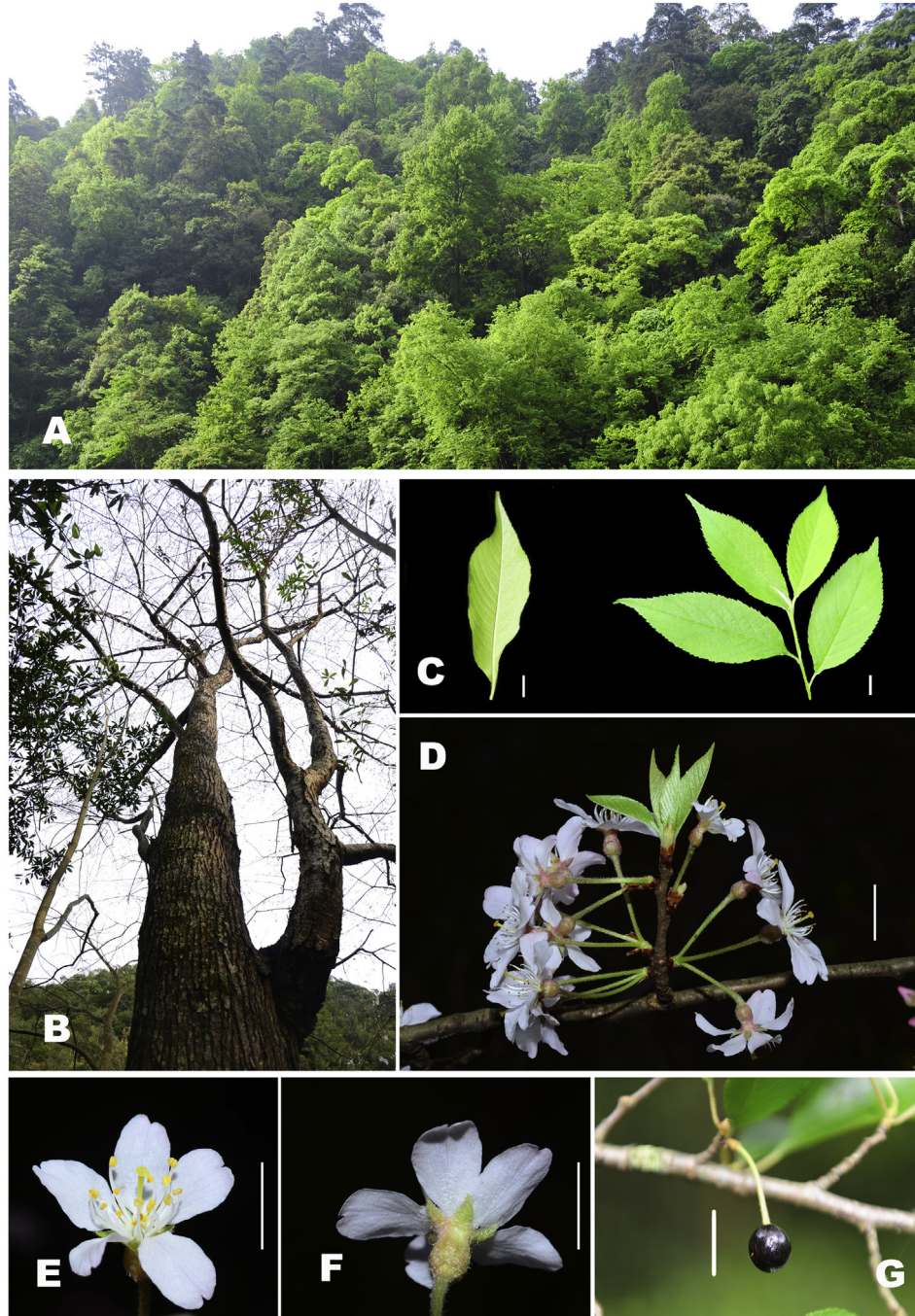
Fig. 1. Living images of ***Prunus sunhangii*** D. G. Zhang & T. Deng ***sp. nov.*** (**A**) Habitat; (**B**) Stem; (**C**) Leaf, showing abaxial indumentums; (**D**) Inflorescence; (**E**−**F**) Opening white flower: front and back side; (**G**) Ripe fruit. Scale bars: 1 cm in C, D, E, F, G.

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. *Laurocerassus* 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 (DV811A) (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
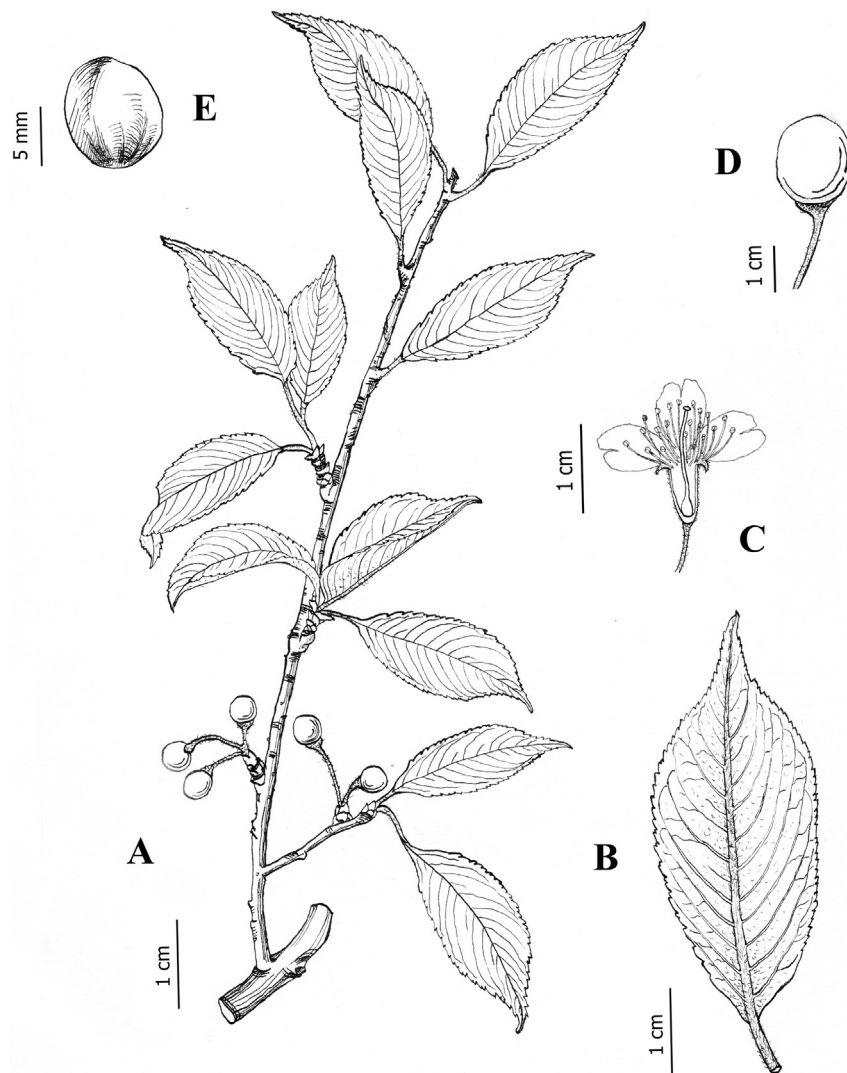

Fig. 2. Illustration of *Prunus sunhangii* D. G. Zhang & T. Deng **sp. nov.** (**A**) Fruiting shoot; (**B**) Leaf, showing abaxial indumentums; (**C**) Flower, showing with pistil and stamens; (**D–E**) Fruit, showing with pedicel.
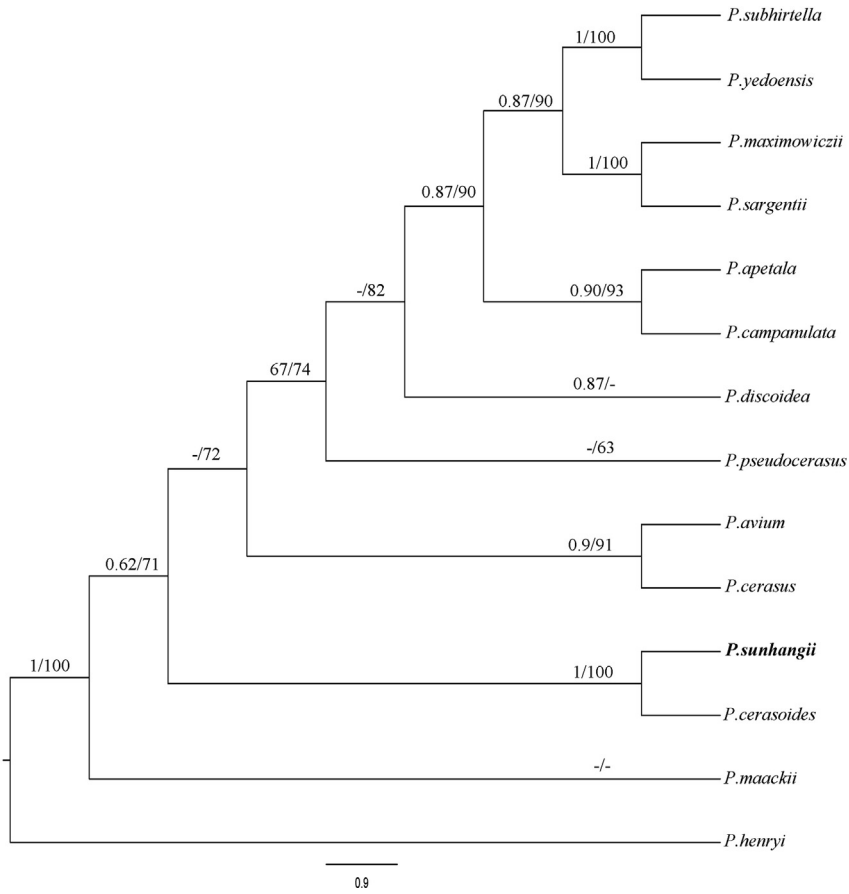
Fig. 3. Bayesian 50% majority-rule consensus tree of *Prunus* subg. *Cerasus* and closely related taxa, inferred from combined sequences data from plastid (*trnG-S*, *psbA-trnH*, *rps16*, *trnL-F*) and nuclear (ITS) markers. Bayesian posterior probabilities/maximum likelihood values are given above the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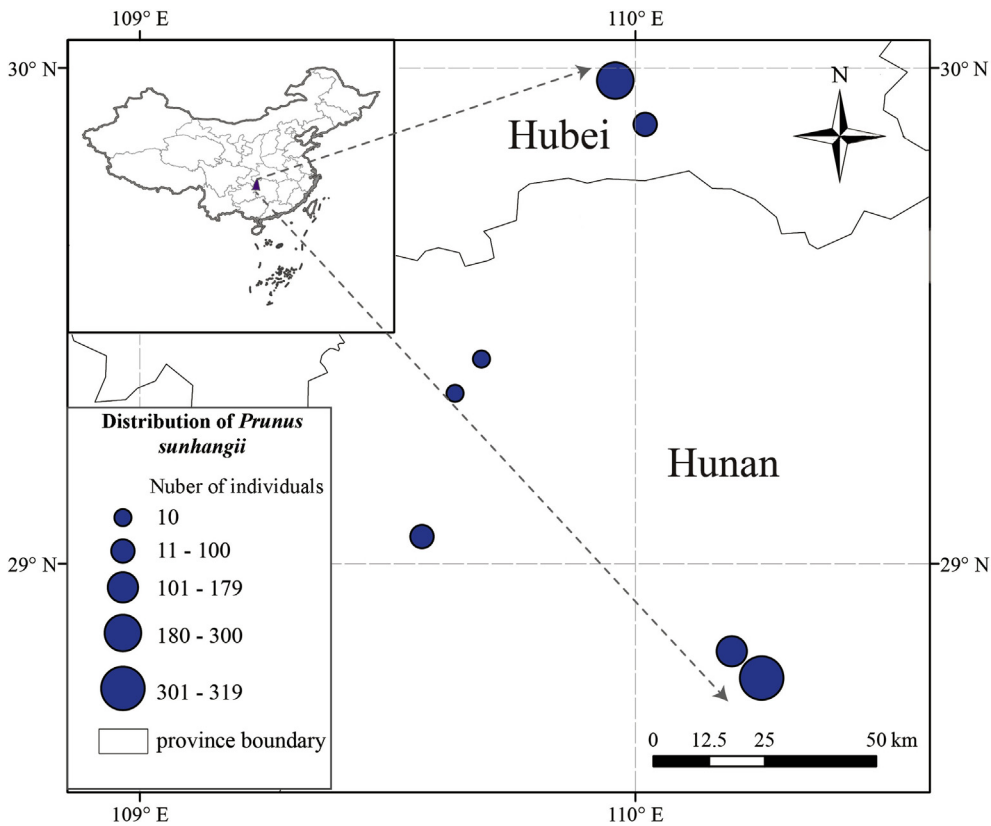

Fig. 4. Distribution of *Prunus sunhangii*.

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, involucre bracts obovate, outside pilose, soon deciduous
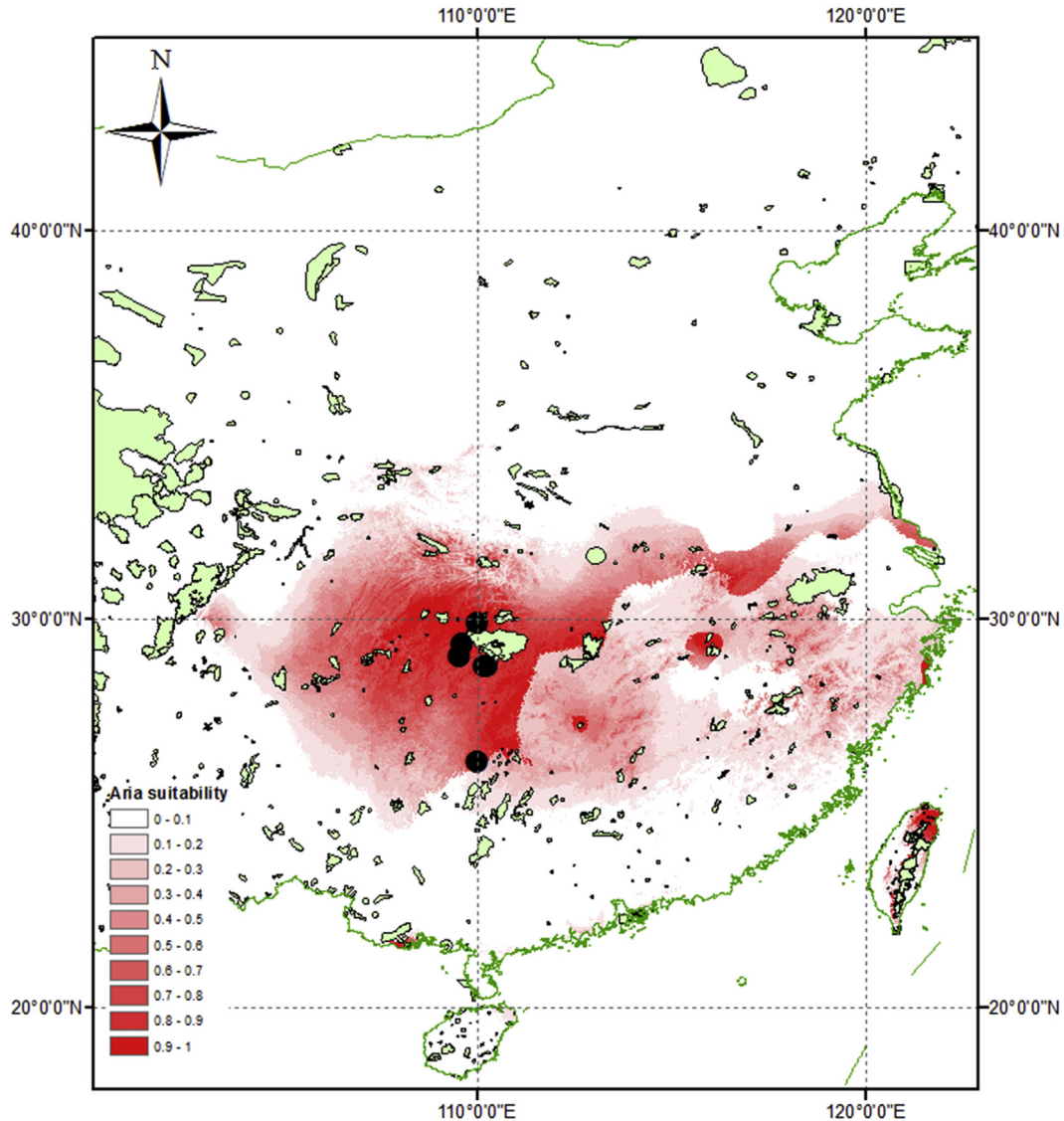

Fig. 5. Predicted range of *Prunus sunhangii* based on bioclimatic modeling. Green areas denote protected areas and dots - species occurrences.

after flowering. Flowers opening at same time as leaves. Peduncle short 0.19–0.35 cm. Pedicels 0.68–1.90 cm, densely pilose. Hypanthium 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 hypanthium 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

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 that varies 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.

Appendix A1

Taxa studied for DNA sequences. Voucher information is given for newly generated sequences and corresponding citation for sequences obtained from previous publications. GenBank accession numbers are given for all ITS, *psbA-trnH*, *trnG-trnS*, *rps16* and *trnL-trnF* sequences included in this study.

Taxa	ITS	*psbA-trnH*	*trnG-trnS*	*rps16*	*trnL-trnF*
Prunus apetala (Siebold & Zucc.) Franch & Sav.	AF411509	AB254601	–	AB254554	AF429902
Prunus avium (L.) L.	FJ899097	FN675831	HQ244223	JQ776739	KT875773
Prunus campanulata Maxim.	AF318658	AB254632	–	AB254585	AF429903
Prunus cerasoides Buch.-Ham. ex D.Don	KF186459	AB254636	HQ244236	AB254589	JQ034182
Prunus cerasus L.	FJ899099	FN675832	HQ244241	–	FJ899125
Prunus discoidea (T.T.Yu & C.L.Li) Z.Wei & Y.B.Chang	KT887495	HQ427051	–	JQ776741	JQ034176
Prunus henryi (C.K.Schneid.) Koehne	JQ926620	HQ188785	HQ244268	–	HQ244039
Prunus maackii Rupr.	AY864831	HQ188801	AY871251	JQ776783	JQ034175
Prunus maximowiczii Rupr.	KT887510	AB254637	–	AB254595	AF429915
Prunus pseudocerasus Lindl.	KF241133	AB254631	HQ244332	AB254584	AF318684
Prunus sargentii Rehder	AY052507	AB254623	–	AB254577	AF429918
Prunus subhirtella Miq	AF179520	AB254614	–	AB254567	AF429917
Prunus × yedoensis Matsum.	KT887511	EF590735	–	JQ776749	AF429936
Prunus sunhangii D. G. Zhang et T. Deng	MK411814	MK411810	MK411813	MK411812	MK 411811

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: Sūn Háng Yīng (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, hypanthium 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 phylogenetic 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, Sikkim, 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

Acknowledgements

References

Chen, Z.L., Chen, W.J., Chen, H., Zhou, Y.Y., Tang, M.Q., Fu, M.Q., Jin, X.F., 2013. Prunus pananensis (Rosaceae), a new species from Pan'an of central Zhejiang, China. PLoS One 8 (1), e54030.

Chen, Y.S., Deng, T., Zhou, Z., Sun, H., 2018. Is the East Asian flora ancient or not? Nat. Sci. Rev. 5, 920–932.

Chin, S.W., Shaw, J., Haberle, R., Wen, J., Potter, D., 2014. Diversification of almonds, peaches, plums and cherries—molecular systematics and biogeographic history of *Prunus* (Rosaceae). Mol. Phylogenet. Evol. 76, 34–48.

Chin, S.W., Lutz, S., Wen, J., Potter, D., 2013. The bitter and the sweet: inference of homology and evolution of leaf glands in Prunus (Rosaceae) through anatomy, micromorphology, and ancestral—character state reconstruction. Int. J. Plant Sci. 174, 27–46.

Christenhusz, M.J., Byng, J.W., 2016. The number of known plants species in the world and its annual increase. Phytotaxa 261 (3), 201–217.

Hall, A.T., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic Acids Symposium, 41. Oxford University Press, pp. 95—98.

Hamilton, A., Baulcombe, D., 1999. A species of small antisense RNA in post-transcriptional gene silencing in plants. Science 286 (5441), 950—952.

International Union for Conservation of Nature, 2016. Guidelines for Using the IUCN Red List Categories and Criteria.

Kusukawa, N., Uemori, T., Asada, K., Kato, I., 1990. Rapid and reliable protocol for direct sequencing of material amplified by the polymerase chain reaction. Biotechniques 9, 66—68.

Li, C., Bartholomew, B., 2003. *Cerasus* mill. In: Wu, C.Y., Raven, P.H. (Eds.), Flora of China, vol. 9. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, pp. 404—420.

Lingdi, L., Cuizhi, G., Chaoluan, L., Alexander, C., Bartholomew, B., Brach, A.R., Spongberg, S.A., 2003. Rosaceae. *Flora of China*. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, pp. 46—434.

Nylander, J.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53 (1), 47—67.

Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190, 231—259.

Phillips, S.J., Dudik, M., 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31, 161—175.

Potter, D., Eriksson, T., Evans, R.C., Oh, S.H., Smedmark, J.E.E., Morgan, D.R., et al., 2007. Phylogeny and classification of Rosaceae. Plant Systemat. Evol. 266, 5—43.

Rehder, A., 1940. Manual of Cultivated Trees and Shrubs Hardy in North America Exclusive of the Subtropical and Warmer Temperate Regions, second ed. Mac-Millan, New York.

Sang, T., Crawford, D.J., Stuessy, T.F., 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Am. J. Bot. 84 (9), 1120—1136.

Shaw, J., Small, R.L., 2004. Addressing the "hardest puzzle in American pomology:" Phylogeny of *Prunus* sect. *Prunocerasus* (Rosaceae) based on seven noncoding chloroplast DNA regions. Am. J. Bot. 91 (6), 985—996.

Shi, S., Li, J., Sun, J., Yu, J., Zhou, S., 2013. Phylogeny and classification of *Prunus sensu lato* (Rosaceae). J. Integr. Plant Biol. 55 (11), 1069—1079.

Swofford, D.L., 2013. PAUP. Phylogenetic Analysis Using Parsimony (And Other Methods), Version 4, vol. 325. INSECTA MUNDI, Sinauer, Sunderland, Massachusetts.

Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol. Biol. 17, 1105—1109.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols-A Guide to Methods and Applications. Academic Press, San Diego, California, pp. 315—322.

Yu, T.T., Li, C.L., 1986. Cerasus mill. In: Yu, T.T. (Ed.), Flora Reipublicae Popularis Sinicae, vol. 38. Science Press, Beijing, pp. 41—89.

Zhao, L., Jiang, X.W., Zuo, Y.J., Liu, X.L., Chin, S.W., Haberle, R.,R., Potter, D., Chang, Wen, J., 2016. Multiple events of allopolyploidy in the evolution of the racemose lineages in Prunus (Rosaceae) based on integrated evidence from nuclear and plastid data. PLoS One 11 (6), e0157123.