

Morphological and molecular status of *Daphne wolongensis* Brickell et Mathew as genetic resource for horticulture

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Abstract

Daphne wolongensis described on the basis of a few known individuals was investigated in the wild, in Baoping Valey, Sechuan. Its status of valid species was verified morphologically and genetically. Three newly found populations were compared to the closely related species *Daphne retusa*, *D. tangutica*, *D. longilobata*, *D. acutiloba*, *D. sureil*, to clones available in cultivation and selected cultivars. The high bootstrap values indicate a good level of genetic differentiation between each of the studied species. The hypothesis whether *D. wolongensis* is a hybridogenous species was rejected, it is a well-defined independent species. Based on morphological and genetic data, it seems likely that another species, *D. limprichtii* can be a mountain form of *D. tangutica*. Variability of populations in Wolong gives a good opportunity to select genotypes with a higher or better performance of combination of traits. From 51 samples collected in Wolong it was possible to select the top ten different types as genetic resources for breeding. *Daphne wolongensis*, in the visited sites of Wolong area, occupies less than 1 km². Together with isolated finds, the number of found individuals is less than 500 and the area is not larger than 10 km², thus it falls to the IUCN category of “Critically Endangered” plants.

Keywords: *Daphne wolongensis*, distribution, morphology, relationships, genetics, AFLP, related species, Rehdera section

Introduction

The genus *Daphne* L. (*Thymelaeaceae*) comprises approximately 95 species which are distributed in Southeast Asia, extending from Japan and Korea through India, Nepal and Bhutan to Europe (Hong 2007, Wang et al. 2007). The distribution is limited to the northern hemisphere only. Flora of China lists 69 species, of which 41 are endemic. Flora Europaea lists 18 species (Webb 1978). The genus *Daphne* is very desirable for ornamental horticulture, 34 species are described for horticultural use, 18 interspecific natural and artificial hybrids and over 100 cultivars and selected forms are in cultivation (Brickell & Matthews 1976, White 2006).

Daphne wolongensis is a relatively new species described on the basis of one cultivated plant raised from seed. The initial information on this species was published by Martyn Rix (Rix, 1992) in the description of his journey to Sechuan. He published a picture with a legend “*Daphne tangutica* - unusual form”. This original plant was found near Panda Research Station. A British nurseryman, Robin White, raised two plants from collected branches from a Chinese nursery (Kaichen) in 2001. He selected one plant for propagation in his nursery and named it ‘Guardsman’ according to its upright habit. He was not able to determine it and stated that it is related to *D. retusa* and *D. tangutica*. Another collection of this *Daphne* came from Mao Lou, Sunang Valley (collected by Ben Wilson) and it was very similar to the Kaichen plant. The third collection was done by Stella and David Rankin

from the region Wolong and it had similar characteristics. David Rankin sent a picture of the plant from the region Wolong to Chris Brickell in 2005. Chris understood that it is a new species, selected the third one as a type and described this *Daphne* as a new species (Brickell and Mathew, 2007). *Daphne wolongensis* differed in habit, leaf shape and with the axillary fasciculate inflorescences. Botanists and horticulturists reported the occurrence of only individual plants along the main road and declared it as an extremely rare species. Those fewer than 10 finds remained the only known plants of the species and became the only sources of material for horticulture and got cultivar names: Guardsman, MiyaLou, Kevock Star, China Pink.

The taxonomy of the genus *Daphne*, with respect to Chinese related species, was seldom studied. In their study, Brickell and Mathew (1976) followed the generic classification of Keisler (1898) adopting 5 sections. The section *Daphnantes* C.A.Meyer is subdivided to 6 subsections. The subsection *Daphnanthoides* comprises 14 related species; *D. acutiloba*, *D. bholua*, *D. gruenigiana*, *D. kiusiana*, *D. longilobata*, *D. luzonica*, *D. miyabenana*, *D. odora*, *D. papyracea*, *D. retusa*, *D. shillong*, *D. sureil*, *D. taiwaniana* and *D. tangutica*.

Halda (1990) divides the genus *Daphne* into 5 subgenera and 5 sections. The section *Rehdera* comprises 14 similar species including *D. acutiloba*, *D. limprichtii*, *D. odora*, *D. tangutica*, while *D. bholua* is separated to an independent subsection.

The author could not believe that *Daphne wolongensis* is limited to only isolated plants along the road in the Wolong region. Therefore, he investigated the region over three years. The main aim of the study was to test whether *D. wolongensis* can be an independent “good” species on both phenotype and genotype levels and what affinities can be found to related species. Another aim was to look for variation useful for breeding and ornamental horticulture. For genotype description, the method of AFLP was used.

Material and Methods

Herbarium material was checked in Chinese Kunming (KMG) and Chengdu (CHG) herbaria. European herbaria (K, BM, E, W) were accessed online for available related species (*D. retusa*, *D. tangutica*, *D. longilobata*). The distribution area of *D. wolongensis*, the Baoxing Valley around Wolong village, was visited in 2011, 2012 and 2013. *Daphne wolongensis* specimens were collected for herbarium, biometrics and DNA analyses. Spare branches were grafted for cultivation in order to have living voucher specimens. Other related *Daphne* species, mainly from the section *Rehdera*, were collected for comparison. Morphological characters were measured on fresh plants *in situ*, in cultivation and on herbarium specimens. Measurements of floral parts and indumentum were taken under a stereomicroscope.

Fresh leaves were collected, frozen and stored for DNA analyses. Closely related species *D. tangutica*, *D. retusa*, and *D. limprichtii* were added for comparison as well as other more distantly related species *D. calcicola*, *D. sureil*, *D. acutiloba*.

Daphne wolongensis samples were collected from three populations in Baoxing Valley, in the Wolong region, and marked W1, W2 and W3 (Table 2). The plants were numbered for measurement and further study (W1: 1 to 23, W2: 1 to 22 and W3: 1 to 22). In total, 67 individuals were analysed *in situ* and further in laboratory.

Related species, *D. retusa*, *D. tangutica*, *D. limprichtii*, were investigated in wild populations in Yunnan and Sechuan regions and *D. longilobata* in Eastern Tibet (Table 1). For conservation assessments, the IUCN red list categories and criteria (IUCN 2014) have been applied.

DNA Extraction

DNA was extracted from leaves according to the optimised protocol using CTAB extraction buffer (2 M NaCl, 0.2 M Tris, 50 mM EDTA, 2 % CTAB, pH 7.5) and 10 mg PVP per each sample. DNA was precipitated by one volume of absolute ethanol and diluted in an appropriate volume of TE

buffer. DNA was run in 0.8 % agarose gels to verify the quality and the concentration. λ HindIII (Fermentas, Vilnius, Lithuania) was used to determine the size and the concentration of DNA.

AFLP Analysis

AFLP markers were generated with the Applied Biosystems kit for plant genomes (Applied Biosystems, Foster City, CA, USA). DNA digestion was carried out using the restriction enzymes *EcoRI* and *MseI* (Vos et al. 1995). Eighty combinations of primers were tested, and eighteen pairs were selected for further analyses. These combinations of primer pairs were chosen because they generated a high number of scorable fragments with a range of sizes (100 – 500 bp). The selective amplification, with *MseI* primers and fluorescently marked *EcoRI* primers, was performed as a multiplex PCR in a Labcycler (SensoGuest GmbH, Göttingen, Germany) with a reaction mixture of 10 μ l containing the following: 0.2 mM dNTP, 1 μ M *MseI* primer, 3 x 0.5 μ M *EcoRI* primers, 1 U *Taq* polymerase (Qiagen GmbH, Germany), 1x buffer with 10 mM $MgCl_2$ and 1 μ l diluted (1:20) preselective amplification reaction. Amplification products were separated by capillary electrophoresis in an ABI PRISM 310 (Applied Biosystems, Foster City, CA, USA) and analysed using GeneScan and Genotyper software (Applied Biosystems, Foster City, CA, USA). Based on the presence or absence of AFLP amplification, a binary matrix was built and used for data analysis.

Data Analysis

A matrix of distances between all genotypes was calculated using the Jaccard dissimilarity coefficient in the DARwin software (<http://darwin.cirad.fr/darwin>; Perrier and Jacquemoud-Collet, 2006). For clustering, an unweighted neighbour-joining method was used. The support for the phenogram branches was obtained using 2000 bootstrap re-samplings.

To find a population structure, Bayesian statistics implemented in the software Structure version 2.3.4 was used (Pritchard et al., 2000). Ten independent runs of 1 - 20 groups ($K = 1 - 20$) were performed using 100,000 Markov chain iterations after a burn-in period of 10,000 iterations. The number (K) of clusters into which the sample data (X) were fitted with posterior probability $Pr(X|K)$ was estimated using a model with admixture and correlated allele frequency (Falush et al., 2003). The optimal value of K was estimated based on $\ln(K)$ and on the ΔK calculation, which considers the rate of change in the $\ln P(D)$ values among successive K runs to account for patterns of dispersal that are not homogeneous among populations (Evanno et al., 2005).

An exact test for population differentiation was calculated using the Tools for Population Genetic Analyses (TFPGA; version 1.3; Miller 1997) with 10,000 permutation steps.

Results

Distribution and habitat of *D. wolongensis*

Herbarium material was checked in Kunming (KMG) and Chengdu (CHG) herbaria. No collections of *D. wolongensis* were found there. European herbaria accessed on line did not yield any *D. wolongensis*. The only exsiccate specimen was found in RHS Wisley herbarium (WYS) marked as Stella & David Rankin SDR 2, a holotype from Wolong, beside the road near Wolong, 2000 m. Its isotype is in C. Brickell herbarium as well as the original cultivated plant named Guardsman.

In the Baoxing Valley, only a few plants were reported, by different authors and visitors, along the main road. The author visited the valley along the river and road in 2011 and 2012 and checked various plant communities. No plants were found along the River Wolong. Plants reported along the road were mostly damaged by an earthquake in 2008 or by the construction of a new road thereafter.

Visiting tributaries of the river revealed three strong populations of the species in 2013. Therefore, the previously found isolated plants along the road were just sporadic seedlings. All three populations were located mainly on riverbanks, in coarse sandy deposits, in stony soil, among boulders, amongst dense willow shrubs and in surrounding open leafy forest. The site marked Wolong 1 (W1, Table 2),

occurring along a rivulet, had a population of *Daphne wolongensis* over a distance of about 300 m, with about 100 - 150 plants of different ages. The site Wolong 2 (W2) was found in a deep, steep and shady gorge alongside a fast flowing stream. The site was less than 100 m long and the number of plants there was approximately 100. The site marked Wolong 3 (W3) was situated in a shallower valley in forest, over a distance of about 1 km and the number of individuals was 150 - 300. All three plant populations were associated with the valley bottom, not climbing the rocky sides.

Closely related species of the section *Rehdera* differ ecologically. While *D. wolongensis* is a typical species for marginal communities of water gullies mainly in wet habitats, *D. retusa*, and *D. tangutica* grow in subalpine shrublands and pastures, usually on slopes with good drainage. *D. limprichtii* is a high alpine species growing in grasslands. *D. longilobata* is a typical forest species occupying rather wet habitats.

Morphological diversity of *D. wolongensis*

The plants within found populations were very variable in morphological characters (Table 2). The shrub height was in the range (50-) 100-180 cm. The mean leaf length in W1 plants was 47 (27 to 70) mm long, in W2 plants was 48.8 (30 to 90) mm long and in W3 plants was 54.3 (40 to 72) mm long. The largest leaves were noticed within the population W3 and the most variable were in the population W2. The leaf width was between 8 and 16 mm for W1 (mean 12.6 mm), 8-22 mm for W2 (mean 13.02 mm) and 8-18 mm for W3 (mean 11.78 mm). The length/width index was 2.2 to 8 mm for W1, 2.2 to 7.8 mm for W2 and 2.6 to 7 mm for W3. The leaf shape was linear lanceolate, oblanceolate to oblong with acute to acuminate apex, the latter in longer leaves. The base is cuneate. The margin is more or less revolute, more so in those of smaller leaves. The plants with larger leaves usually had thinner lamina than smaller leaves that had thicker dark green lamina and a more leathery structure. The smaller leaf forms resemble the related *D. retusa*. The flowers are borne in rich fascicles in axillar inflorescences and even richer in terminal inflorescences, equally in all three populations. The bracts varied in shape from ovate to lanceolate, 8-12 mm long, glabrous, occasionally ciliate on margin. The flower colour is pink for all three populations, there are plants with darker flowers, and some nearly white. The perianth tube formed by the calyx is narrowly cylindrical, 9-12 mm long, glabrous, lobes 4, ovate, 6-9 mm long and 4-8 mm wide, with acuminate apex. The variation in flower parts is analogic in all three populations. Fruits are very juicy, nearly globose 8-12 mm long, the largest in population W2. Seeds are subglobose 5-7 x 4-6 mm, smaller in population W1.

Differences among related species

Morphological diversity of *D. wolongensis* is much higher than in any of the related investigated species. Generally, most of the characters stay between *D. retusa* and *D. tangutica*. *D. wolongensis* differs from *D. retusa*, *D. tangutica*, *D. limprichtii* and *D. longilobata* by a combination of morphological traits (Table 3). Most of those traits overlap in scales. There are four differentiating traits for *D. wolongensis*: presence of axillar inflorescences together with terminal, young branch and peduncle indumentum being densely hairy to villous, shape and size of bracts and leaf thickness. The bracts are concave and large compared with other listed species, where the bud scales are long acuminate. The leaf thickness ranges between *D. longilobata* and other species and make the leaf appearance thinly leathery, but not papery. Outer winter bud scales are ovate and deeply concave compared with other species.

The species differ also in habitat and altitude (Table 1).

Genetic diversity of *D. wolongensis* and related species

Five hundred and twenty-two polymorphic AFLP markers were scored using 18 primer combinations across 38 *Daphne* spp. samples that were at our disposal. This corresponds to an average of 34.8 polymorphic bands per primer combination.

Cluster analysis (Figure 1) showed three main clusters. The first one involves genotypes of *D. acutiloba*, *D. calcicola*, and *D. sureil*. The second cluster contains items of *D. retusa*, *D. tangutica* and

D. limprichtii. The largest cluster is made of genotypes of *D. wolongensis* including varieties derived from this species. The high bootstrap values indicate a good level of genetic differentiation between each of the studied species. This fact was supported by structure analysis (Figure 2). Four genetic populations ($K = 4$) were identified within the data representing the species: *D. wolongensis* (K3), *D. retusa* (K1), *D. tangutica* (K4), and *D. acutiloba* with *D. calcicola* (K2). *D. limprichtii* is likely to be a mixture of *D. tangutica* and *D. retusa* gene pools. Based on an exact test of population differentiation ($\chi^2 = 4248.3$, $df = 1044$, $p < 0.01$) null hypothesis about identity of *D. wolongensis* and other analysed species was rejected. *D. wolongensis*, on genotype level, is a well-defined separate species.

There is a low level of genetic diversity within the three tested populations W1, W2, and W3 (Table 5). Only the variety China Pink was identified as pure *D. wolongensis*. The other analysed varieties were derived from *D. wolongensis* by hybridization with other *Daphne* species.

Based on morphological and genetic data, it seems likely that *D. limprichtii* can be a mountain form of *D. tangutica*. On the other hand, *D. wolongensis* is a well-defined “good” species.

Diversity of *D. wolongensis* for breeding and horticulture

The present cultivars Kevock Star, China Pink and Miya Low are similar among themselves and their morphological characters are within the variation of populations W1, W2 and W3. The cultivar Guardsman differs from the other cultivars by a denser upright crown, higher number of axillar flowers and deeper rose colour of buds and florets. (Table 2).

Variability of populations in Wolong gives a good opportunity to select genotypes with a higher or better performance of combination of traits. From 51 samples collected in Wolong it was possible to select the top ten different types (Table 5). For horticultural use it is desirable to select the following characters: compact plant habit, shape and colour of leaves, richness of flowering, size and colour of flowers, nectaria producing pleasant odour, production of large fruits. The ten selected genotypes offer good diversity in characters for breeding and to enlarge the cultivated germplasm which is presently available. The main disadvantage of growing in a continental climate is a low winter hardiness of the species, because all three populations are from around 2000 m where frosts are only light and occasional.

Conservation status

Daphne wolongensis, in the visited sites of Wolong area, occupy less than 1 km². Together with isolated finds, the area is not larger than 10 km². The number of individuals is approximately 500. It makes the species fall in the IUCN category of “Critically Endangered” plants (CE, IUCN, 2014). The region falls in the Chinese-Japanese Regions of plant diversity according to Vavilov (Zeven, de Wet, 1982) and Sino-Himalayan diversity hotspot (Myers et al, 2000). Currently *D. wolongensis* has no conservation coverage. Therefore, an effort to protect the new species in its habitat is urgent.

Discussion

D. wolongensis is not listed in Flora of China, having been described later, after its publication. Apart from its original description (Brickell and Mathew, 2007), there is no botanical treatment available in literature. There is only one source of horticultural information (Rix, 1992). The lack of treatment is most likely due to its remote distribution area.

D. wolongensis is highly variable and intermediate in morphological characters between *D. tangutica* and *D. retusa*, but it is genetically clearly independent. There are unique characters that distinguish it from both the above mentioned and other species. Combinations of traits distinguish related species of the *Rehdera* section satisfactorily.

The hypothesis whether *D. wolongensis* is a hybridogenous species was rejected, based on detailed morphological evaluation and genetic study. However, hybridogenous origin can be considered in the evolution within the *Rehdera* section. There are species with a large area of distribution like *D. tangutica* and *D. retusa*, and very localized species like *D. wolongensis* and *D. thanguensis*. Both narrow endemic species are related to *D. tangutica*. The recently described *D. thanguensis* differs from *D. tangutica* by having leaves with revolute margin and a tuft of hairs at the apex, ebracteate inflorescence and flowers, calyx lobes with a tuft of hairs at the apex and annular, slightly undulate

hypogynal disk (Ghosh et al, 2018). Both species *D. wolongensis* and *D. thanguensis* evolved in the distribution area of *D. tangutica* and *D. retusa*, but without their current presence, thus isolated.

This contribution uncovers a real distribution of the species, uncovers variation in characters in the wild and brings material for breeding. Until recently, only progenies of several collected specimens have been available, cultivars were made on the basis of occasional finds. Desirable germplasm for hybridisation with other species is now available, enabling the introduction of new characters, colours and plant shape. It will be an enrichment of cultural plants from CWR.

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Tables

Table 1. Investigated species and localities/sources of *Daphne* section *Rehdera*. Material from UK was provided by Mr. Robin White, Blackthorn

species	locality/source	altitude	ecology	no of samples
<i>D. wolongensis</i>	W1, Wolong, Baoxing Valley, Sechuan	2060	open forest along rivulet	23
<i>D. wolongensis</i>	W2, Wolong, Baoxing Valley, Sechuan	2084	shady gorge along quick stream	22
<i>D. wolongensis</i>	W3, Wolong, Baoxing Valley, Sechuan	2100	forest along small river	22
<i>D. wolongensis</i>	Coll Martyn Rix, ex UK			1
<i>D. wolongensis</i>	MiyaLow, ex UK			1
<i>D. wolongensis</i>	China Pink ex ChenYi, ex UK			1
<i>D. wolongensis</i>	Kevoock Star, ex UK			
<i>D. acutiloba</i>	CDC 626, ex UK			1
<i>D. retusa</i>	Zheduo Shan, Kanding, Sechuan	4100	subalpine shrubland	1
<i>D. retusa</i>	Huanglong, Min Shan, Sechuan	4300	alpine shrubland	1
<i>D. retusa</i>	ex UK			1
<i>D. tangutica</i>	Zhongdian, Yunnan	3600	open forest and shrubland	1
<i>D. tangutica</i>	form A, ex UK			1
<i>D. tangutica</i>	form B, ex UK			1
<i>D. tangutica</i>	Coll B4, Keith Rushforth, ex UK			1
<i>D. limprichtii</i>	Jiuzhai, Min Shan, Sechuan	4100	alpine shrubland	1
<i>D. longilobata</i>	Baimucun, Lunang, Tibet	3600	deciduous forest	10
<i>D. sureil</i>	ex UK			1
<i>D. calcicola</i>	Zhongdian, NapaHai, Yunnan	3644	evergreen oak forest (<i>Q. aquifolioides</i>)	1

Table 2. Morphological characters - differentiation of *D. wolongensis* within species (populations W1-W3, cultivars)

Character	D.wolongensis W1	D.wolongensis W2	D.wolongensis W3	D. w. Kewock Star	D. w. China Pink	D. w. Guardsman	D. w. MiyaLow
Plant size	variable	variable	variable	medium	small	large erect	medium
plant habit	spawling to upright	spawling to upright	spawling to upright	spawling densely	spawling densely	densely upright densely	lower, rounded
young branch indumentum	densely pubescent up to villous among leaves	densely pubescent, denser among leaves	densely pubescent, denser among leaves	pubescent, caducous sparsely	pubescent, caducous sparsely	pubescent, caducous	denser among leaves
year branches leaves	sparsely pubescent or glabrous linear lanceolate, oblanceolate to oblong	sparsely pubescent or glabrous linear lanceolate, oblanceolate to oblong	sparsely pubescent or glabrous linear lanceolate, oblanceolate to oblong	pubescent or glabrous ovate lanceolate	pubescent or glabrous ovate lanceolate	nearly glabrous narrowly ovate	sparsely pubescent or glabrous oblong lanceolate alternate,
leaf position	±clustered, lower few, remote	±clustered, lower few, remote	±clustered, lower few, remote	±clustered, lower remote	±clustered, lower remote	±clustered, lower many, remote	±clustered, lower remote
leaf size (mm)	27-80 × (8-)10-20	24-90 × 7-22	32-70 × 8-18	35-60 × 12-18	45-55 × 15-18	38-50 × 10-12	60-65 × 20-24
leaf appearance	thinly leathery	thinly leathery	thinly leathery	thinly leathery	thinly leathery	medium leathery	thinly leathery to papery (0.12-)0.20-0.30(- 0.35)
leaf thickness	(0.14-)0.20-0.34	(0.14-)0.20-0.36	(0.12-)0.20-0.30(-0.35)	0.23-0.27	0.23-0.29	0.33-0.40	cuneate
base	widely cuneate	widely cuneate	widely cuneate	widely cuneate	widely cuneate	cuneate	acute
apex	acute to acuminate	acute to acuminate	acute to acuminate	acute	acute flat to partly	acute to rounded	acute
margin	±revolute	±revolute	±revolute	partly revolute terminal and axillar	revolute terminal and axillar	revolute terminal and rich axillar	flat terminal and axillar
Inflorescences flowers - number	terminal and axillar 5-15 terminal, 2-5 in axils	terminal and axillar 5-15 terminal, 2-5 in axils	terminal and axillar 5-15 terminal, 2-5 in axils	5-15 terminal, 2- 5 in axils densely	5-15 terminal, 2- 5 in axils densely	5-15 terminal, 4-8 in axils	5-15 terminal, 2-5 in axils
peduncle, flower bud colour	densely pubescent to villous rose to pink	densely pubescent to villous rose to pink	densely pubescent to villous rose to pink	pubescent to villous rose	pubescent to villous light rose, whitish	pubescent deep rose	densely pubescent to villous rose to pink
calyx tube lobes	pink to rose or white pink to rose	pink to rose or white pink to rose	pink to rose or white pink to rose	pink pure pink	pink light pink	rose rose to redish	pink to rose pink to rose

tube length	8-11	8-11	8-10	9-10	9-10	9	9-10
lobe size	(6-)7-11 × (4-)5-8	(5-)6-11 × (4-)5-7	5-7 × 5-6	9-10 × 6-8	8-10 × 6-7	6-8 × 3-4	10-12 × 7-8
lobe shape	outer ovate, inner lanceolate, ±bent	outer ovate, inner lanceolate, ±bent	outer ovate, inner lanceolate, ±bent	lanceolate, straight folded acute, slightly bent down	ovate, a bit folded acute, slightly bent down	uniform, elliptic acuminate, bent up	ovate 3-grooved
lobe apex	usually acute	usually acute	usually acute	usually acute	usually acute	usually acute	usually acute
Drupe	carmine red	carmine red	carmine red	carmine red	carmine red	carmine red	carmine red

Table 3. Morphological characters - differentiation of *D. wolongensis* to related species

Character	<i>D. wolongensis</i>	scale	<i>D. tangutica</i>	scale	<i>D. retusa</i>	scale	<i>D. limprichtii</i>	scale	<i>D. longilobata</i>	scale
Plant size	(50-) 100-180	3	(50-)100-250	3	40-150	2	20-80	1	100-150	3
branches	pale green, turning grayish brown	1	yellowish green, turning grayish red or brown	2	green turning dark brown	3	pale yellowish green, turning brownish gray	2	pale green turning purple-brown	3
young branch	densely pubescent up to villous	1	sparsely pubescent, glabrescent	2	strigose	3	shortly pubescent	2	pubescent	2
indumentum	among upper leaves	1		1	(1-)3-5	3	1(-3)	2	1	1
terminal winter buds	1	1	1	1						
outer bud scale										
size	4.5-9 x 1.5-3		12 x 2-2.5		15 x 5		9-12 x 3-5		8 x 1.5	
bud scale colour	pale green	1	pale green	1	purplish brown	2	pale green	1	pale green	1
leaves	linear lanceolate, oblanceolate to oblong	1	lanceolate to oblanceolate	1	oblong-lanceolate, elliptic	2	lanceolate or oblong-oblanceolate	1	lanceolate or oblanceolate	1
leaf position	alternate, ±clustered, lower remote	1	alternate, ±clustered	2	densely clustered	3	alternate, ±clustered	2	alternate remote	4
leaf size (mm)	27-90 × 8-22	2	2-10 × 0.5-2.2(-3)	2	1.4-4(-7) × 0.6-1.4	1	1.5-3.2 × 0.5-0.8	2	1.5-4.5(-9) × 0.6-1.1(-2)	2
leaf appearance	thinly leathery	2	leathery	3	thick leathery	4	thinly leathery	2	papery	1
leaf thickness	(0.12-)0.20-0.30(-0.36)	2	0.35-0.48	3	0.40-0.53	4	0.37-0.49	3	0.18-0.25	1
surfaces	glabrous	1	glabrous, abaxially sparsely puberulous	2	glabrous	1	glabrous	1	glabrous	1
base	widely cuneate	3	cuneate or decurrent	2	cuneate or attenuate	2	narrowly cuneate	1	cuneate	1
apex	acute to acuminate	1	obtuse, rarely retuse	2	retuse and	3	rounded - retuse	2	obtuse-rounded,	2

				apiculate, or obtuse				rarely apiculate		
margin	±revolute	2	revolute	2	strongly revolute	3	revolute	2	flat, sometimes revolute	1
Inflorescences	terminal capitate	1	terminal capitate	1	terminal capitate	1	terminal capitate	1	terminal shortly racemose	2
flowers										
arrangement	terminal and axillar	2	only terminal	1	only terminal	1	only terminal	1	only terminal	1
flowers - number	5-15 terminal, 2-5 in axils	3	3-12-	2	several 3-5	1	5-7	2	3-5(-6)	1
peduncle,	densely pubescent to villous	3	pubescent	1	densely strigose	4	sparsely tomentose	2	pubescent	1
pedicel	0.5-2 mm	2	0.5-1 mm	1	nearly absent	1	1 mm	2	1-3 mm	2
bracts	ovate, spatulate, bowl form, with prominent keel	2	ovate-lanceolate, narrow	1	ovate-oblong or obovate-oblong	1	ovate to lanceolate	1	narrowly ovate	1
bract size	5-11 × 6-7	1	5-9 × 3-4	1	5-8 × 3-4	1	4.5-6(-9) × 3-4(-5)	1	4.5-6(-9) × 3-4(-5)	1
flower bud colour	deep rose	3	white, pink, rose	2	purple	4	white, pinkish to rose	2	greenish white pale greenish white, purple base	1
calyx tube	pink to rose or white	2	white to pink, purplish	1	purple to pale	3	white	1	white - pale cream	1
lobes	pink to rose	3	pink to white	2	pink or white	2	white - pale pink	1	7-10(-14) mm	1
tube length	(7-)8-10(-11)	1	9-13(-15)	1	8-12	1	10-11(-14)	1	1.2-1.8 mm	1
tube width	1.8-2.2 mm	3	1.5-2 mm	2	2.5-3 mm	4	1.5-2 mm	2	5-7 × 2-2.5(-3.5) mm	1
lobe size	(5-)7-11(-12) × (4-)5-7	3	5-8(-10) × (3-)4-5(-6)	2	7-10 × 4-8	3	5-8 × 3-5	2	narrow lanceolate	1
lobe shape	ovate-lanceolate or ovate	2	ovate or ovate-elliptic	2	broadly ovate or ovate-elliptic	3	ovate-lanceolate	2	long acuminate	1
lobe apex	usually acute	2	obtuse to acute	2	obtuse to rounded	3	obtuse to retuse	2	pale yellow	1
anthers	orange-yellow, prominent	3	pale yellow	1	yellow	2	pale yellow	1	1.5 mm	2
anthers	0.8-1.5	1	1-1.2 mm	1	1-1.5 mm	2	1 mm	1	scarlet-red	1
Drupe	carmine red	2	red to scarlet	1	red	2	red to scarlet	1	ovoid-globose	2
drupe shape	subglobose to globose	2	subglobose or ovoid	1	subglobose or ovoid	1	ovoid	1	8-12 × 8-11	2
drupe size	8-12 x 7-11	2	8-9 x 8	1	9-13 × 8-11	2	9-10 x 8-9	1	forest, among rocks	1
habitat	forest, shrubs along rivers	2	forest	1	Shrubby grassland	3	Among boulders, sunny	3	1600-3500	
altitude	2000-2100	2	1000-3800	1	3000-3900	3	3000-4400	3		

Table 4. Morphological trait mean values of *D. wolongensis* populations and related species of the *Rehdera* section

Species/population	lv_length	lv_width	lv_index	lv_thickness	fl_tube_length	lobe_length	lobe_width	bract_length	bract_width	bud_scale_length	bud_scale_width	seed_length	seed_width	seed_index	fruit_length	fruit_width	fruit_index
<i>D. wolongensis_W1</i>	48.871	13.317	3.792	0.254	9.385	8.590	5.359	9.600	5.900	5.973	2.111	5.665	4.982	1.131	9.936	8.473	1.195
<i>D. wolongensis_W2</i>	52.391	13.533	3.912	0.274	9.732	8.254	5.518	9.000	5.923	5.922	2.147	6.054	5.546	1.096	10.392	8.808	1.183
<i>D. wolongensis_W3</i>	53.470	12.121	4.626	0.243	9.600	6.467	5.300	8.500	6.000	6.139	2.256	6.050	5.371	1.128	8.807	8.286	1.063
<i>D. wolongensis</i> average	51.316	13.110	4.040	0.257	9.591	8.129	5.432	9.045	5.932	5.989	2.154	5.914	5.318	1.118	9.676	8.518	1.142
<i>D. tangutica</i>	48.647	12.529	1.804	0.421	7.400	6.400	4.000	6.167	3.917	11.222	3.111	6.467	5.667	1.142	9.133	8.133	1.123
<i>D. limprichtii</i>	42.308	12.000	3.561	0.417	8.500	6.500	4.333	5.250	3.333	9.962	3.369	6.408	5.592	1.148	9.514	8.443	1.129
<i>D. retusa</i>	35.889	16.011	2.368	0.468	9.211	8.079	4.763	5.756	3.556	9.935	5.071	7.178	5.853	1.229	11.179	9.595	1.165
<i>D.longilobata</i>	44.667	11.444	3.913	0.221	7.800	5.800	1.960	5.240	3.620	7.600	1.300	7.556	6.000	1.277	10.625	10.000	1.063

Table 5. Selected genotypes with a good performance for further use in breeding

Druh	Populace	Původ	Q1	Q2	Q3	Q4	K
<i>Daphne wolongensis</i>	W1	China	0.004	0.001	0.994	0.001	3
<i>Daphne wolongensis</i>	W1	China	0.002	0.001	0.996	0.001	3
<i>Daphne wolongensis</i>	W1	China	0.001	0.001	0.997	0.002	3
<i>Daphne wolongensis</i>	W1	China	0.007	0.014	0.977	0.003	3
<i>Daphne wolongensis</i>	W1	China	0	0	0.999	0	3
<i>Daphne wolongensis</i>	W1	China	0.003	0.002	0.995	0.001	3
<i>Daphne wolongensis</i>	W1	China	0	0	0.999	0	3
<i>Daphne wolongensis</i>	W2	China	0	0	0.999	0	3
<i>Daphne wolongensis</i>	W2	China	0.001	0	0.998	0.001	3
<i>Daphne wolongensis</i>	W2	China	0.002	0.002	0.995	0.001	3
<i>Daphne wolongensis</i>	W2	China	0	0	0.999	0	3
<i>Daphne wolongensis</i>	W2	China	0.001	0.001	0.998	0	3
<i>Daphne wolongensis</i>	W2	China	0.001	0.001	0.986	0.012	3
<i>Daphne wolongensis</i>	W2	China	0.001	0.001	0.998	0	3
<i>Daphne wolongensis</i>	W2	China	0.003	0.001	0.994	0.002	3
<i>Daphne wolongensis</i>	W2	China	0.001	0.001	0.998	0	3
<i>Daphne wolongensis</i>	W2	China	0.001	0	0.997	0.001	3
<i>Daphne wolongensis</i>	W2	China	0.001	0	0.999	0	3
<i>Daphne wolongensis</i>	W3	China	0.001	0.001	0.998	0	3
<i>Daphne wolongensis</i>	W3	China	0	0	0.999	0	3
<i>Daphne wolongensis</i>	W3	China	0.001	0.001	0.997	0	3
<i>Daphne wolongensis</i>	W3	China	0.006	0.071	0.923	0	3
<i>Daphne wolongensis</i>	W3	China	0	0	0.999	0	3
<i>Daphne wolongensis</i>	W3	China	0	0	0.999	0	3
<i>Daphne wolongensis</i>	Coll Martyn	UK	0.001	0.111	0.691	0.196	3
<i>Daphne wolongensis</i>	China pink	UK	0.001	0.001	0.998	0	3
<i>Daphne wolongensis</i>	Miyalow	UK	0.26	0.089	0.65	0.001	3
<i>Daphne tangutica</i>	form A	UK	0.002	0	0.001	0.997	4
<i>Daphne tangutica</i>	form B	UK	0.002	0.001	0	0.997	4
<i>Daphne tangutica</i>	Coll B4	UK	0.001	0.001	0.001	0.997	4
<i>Daphne sureil</i>	White fls	UK	0.001	0.996	0.001	0.001	2
<i>Daphne calcicola</i>	Napa Hai	China	0.001	0.999	0	0	2
<i>Daphne acutiloba</i>		UK	0.003	0.99	0.002	0.005	2
<i>Daphne retusa</i>	Sechuan	China	0.903	0.002	0.002	0.093	1
<i>Daphne retusa</i>	Hualong	China	0.782	0.002	0.001	0.215	1+4
<i>Daphne retusa</i>		UK	0.476	0.008	0.067	0.449	1+4
<i>Daphne limprichtii</i>	Sechuan	China	0.64	0.083	0.175	0.102	1+3+4

Table 6. Selected genotypes of *D. wolongensis* with a good performance for further use in breeding

	lv_length	lv_width	lv_index	lv_thickness	fl_tube_length	lobe_length	lobe_width	bract_length	bract_width	bud_scale_length	bud_scale_width	seed_length	seed_width	seed_index	fruit_length	fruit_width	fruit_index	note
<i>D. wolongensis</i>																		
W1-5	66.14	16.57	4.03	0.25	9.67	9.00	4.33			6.00	1.90	5.73	4.87	1.18	9.67	7.00	1.38	vigorous healthy plant, large pink fls
W1-9	30.57	11.00	2.80	0.31	8.86	7.29	4.29			6.25	2.15	5.85	5.30	1.10	11.50	10.50	1.10	darker pink fls, odoriferous
W1-15	44.63	15.00	3.01	0.25	8.50	9.00	4.83	9.60	5.90	6.20	2.12	5.40	4.82	1.12	9.26	8.84	1.05	red tube, pink lobes
W1 average (22 pl)	48.87	13.32	3.79	0.25	9.38	8.59	5.36	9.60	5.90	5.97	2.11	5.67	4.98	1.13	9.94	8.47	1.20	
W2-5	26.20	7.80	3.36	0.25	9.20	6.80	5.80	8.00	5.00									compact plant, white flowers
W2-7	39.14	13.29	2.83	0.32	10.43	9.43	6.14	10.00	6.00	6.00	2.00							large fls, widely ovate lobes, very odoriferous
W2-8	72.00	19.11	3.78	0.29	9.89	7.44	5.44	10.33	6.00	5.50	1.50							large fls, red tubes
W2-14	58.45	15.18	3.87	0.29	10.00	11.75	6.25			6.29	2.17							very large fls
W2-10	54.60	14.60	3.76	0.29	9.30	6.82	5.20	8.00	6.00	6.00	3.00	7.27	6.80	1.07	9.37	8.83	1.06	compact habit, pale pinkish fls
W2 average (17 pl)	52.39	13.53	3.91	0.27	9.73	8.25	5.52	9.00	5.92	5.92	2.15	6.05	5.55	1.10	10.39	8.81	1.18	
W3-2	52.75	15.63	3.46	0.26	10.00	7.00	5.40			6.19	2.14							compact habit, large fls
W3-3	53.50	15.50	3.50	0.29	8.75	5.25	5.13	8.50	6.00	6.00	2.38	6.20	5.60	1.11	9.25	8.63	1.07	very odoriferous
W3 average (12 pl)	53.47	12.12	4.63	0.24	9.60	6.47	5.30	8.50	6.00	6.14	2.26	6.05	5.37	1.13	8.81	8.29	1.06	

Figure 1 – Dendrogram constructed using unweighted neighbour joining clustering method based on genetic distance matrix computed by means of Jaccard coefficients with 2000 bootstraps

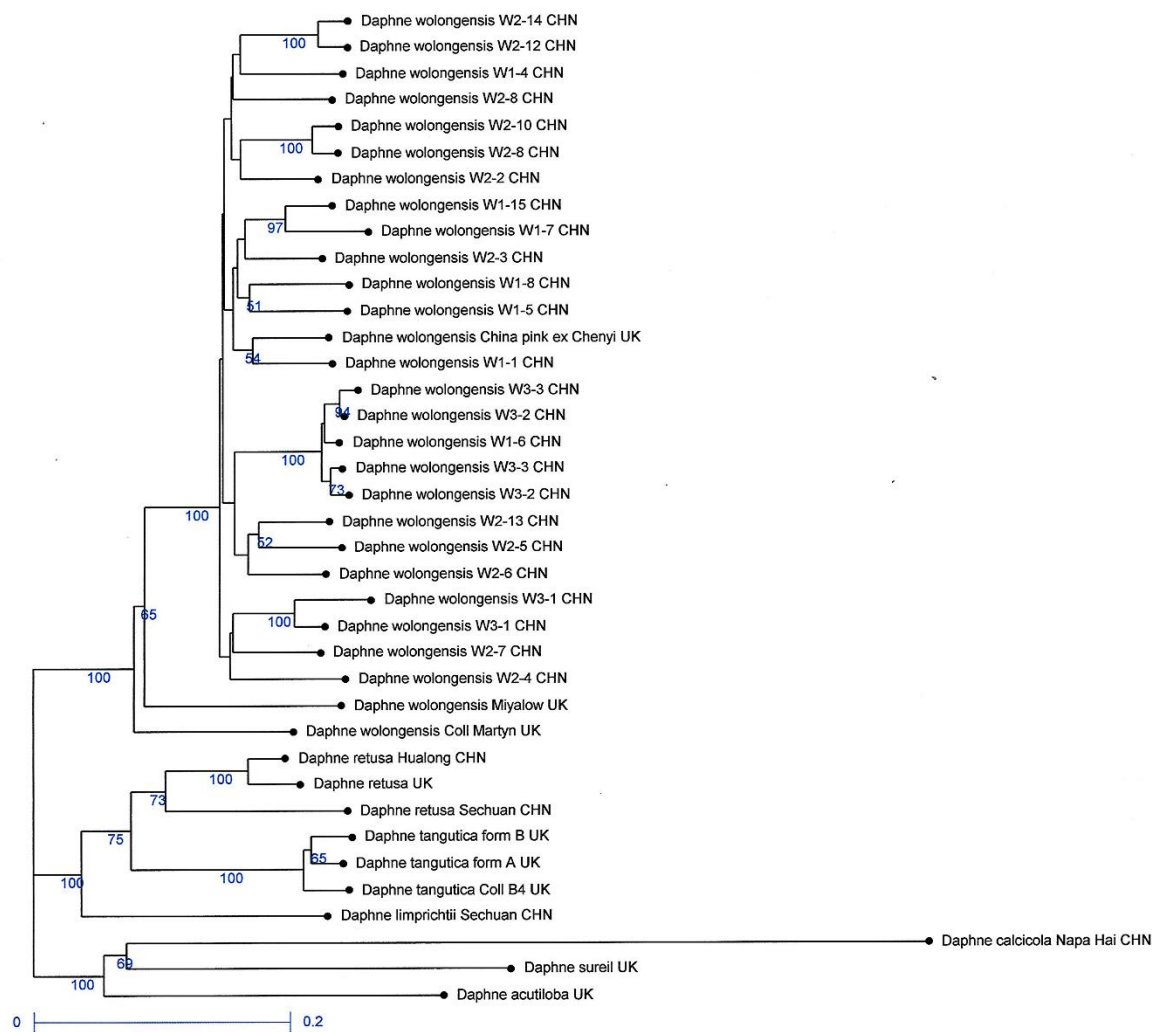
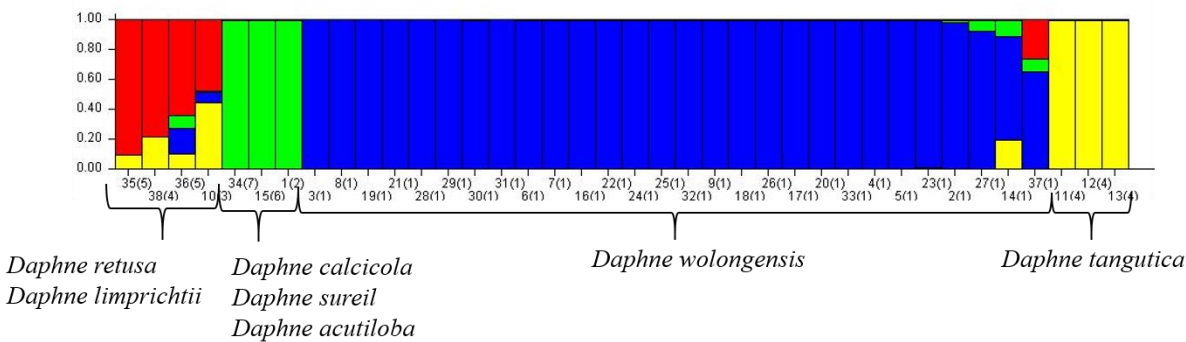


Figure 2 Cluster analysis of *Daphne* spp. based on a Bayesian approach. Each genotype is represented by a bar divided into K colours, where K is the number of clusters assumed in the analysis: K1 – red colour; K2 – green colour; K3 – blue colour; K4 – yellow colour



Pictures (for contribution and or supplementary materials)

Daphne wolongensis site W1

Daphne wolongensis site W2

Daphne wolongensis site W3

W1_2

W1_5

W1_9

W2_2

W2_5

W2_7

W2_10

W3_2

Daphne retusa

Daphne tangutica

Daphne longilobata

Daphne wolongensis 'Kevoek Star'