 doi:10.20944/preprints202009.0243.v2

# 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 Baoxing Valley, 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 51samples 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 km$^2$. Together with isolated finds, the number of found individuals is less than 500 and the area is not larger than 10 km$^2$, 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

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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 *Daphnanthes* C.A.Meyer is subdivided to 6 subsections. The subsection *Daphnanthoides* comprises 14 related species; *D. acutiloba, D. bholua, D. grueningiana, 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

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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 *Eco*RI and *Mse*I (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 *Mse*I primers and fluorescently marked *Eco*RI primers, was performed as a multiplex PCR in a Labcycler (SensoGuest GmbH, Göttingen, Germany) with a reaction mixture of 10 µl containing the following: 0.2 mM dNTP, 1 µM MseI primer, 3 x 0.5 µM *Eco*RI primers, 1 U *Taq* polymerase (Qiagen GmbH, Germany), 1x buffer with 10 mM $MgCl_2$ and 1 µl 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 lnP(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),

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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 fascicules 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) nul 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 51samples 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 km$^2$. Together with isolated finds, the area is not larger than 10 km$^2$. 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.

**Acknowledgements**

This research was supported by project MZE-RO0418 and by National Programme for Plant Genetic Resources (Grant No. 206553/2011-17253).

The authors would like to thank Hana Udavska for technical assistance, Mr. Robin White, UK for providing additional materials and Mr. David Sharp for English editing.

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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 |
|---|---|---|---|---|
| *D. wolongensis* | W1, Wolong, Baoxing Valley, Sechuan | 2060 | open forest along rivulet | 23 |
| *D. wolongensis* | W2, Wolong, Baoxing Valley, Sechuan | 2084 | shady gorge along quick stream | 22 |
| *D. wolongensis* | W3, Wolong, Baoxing Valley, Sechuan | 2100 | forest along small river | 22 |
| *D. wolongensis* | Coll Martyn Rix, ex UK | | | 1 |
| *D. wolongensis* | MiyaLow, ex UK | | | 1 |
| *D. wolongensis* | China Pink ex ChenYi, ex UK | | | 1 |
| *D. wolongensis* | Kevock Star, ex UK | | | |
| *D. acutiloba* | CDC 626, ex UK | | | 1 |
| *D. retusa* | Zheduo Shan, Kanding, Sechuan | 4100 | subalpine shrubland | 1 |
| *D. retusa* | Huanglong, Min Shan, Sechuan | 4300 | alpine shrubland | 1 |
| *D. retusa* | ex UK | | | 1 |
| *D. tangutica* | Zhongdian, Yunnan | 3600 | open forest and shrubland | 1 |
| *D. tangutica* | form A, ex UK | | | 1 |
| *D. tangutica* | form B, ex UK | | | 1 |
| *D. tangutica* | Coll B4, Keith Rushforth, ex UK | | | 1 |
| *D. limprichtii* | Jiuzhai, Min Shan, Sechuan | 4100 | alpine shrubland | 1 |
| *D. longilobata* | Baimucun, Lunang, Tibet | 3600 | deciduous forest | 10 |
| *D. sureil* | ex UK | | | 1 |
| *D. calcicola* | Zhongdian, NapaHai, Yunnan | 3644 | evergreen oak forest (*Q. aquifolioides*) | 1 |

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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 | sprawling to upright | sprawling to upright | sprawling to upright | sprawling | sprawling | densely upright | lower, rounded |
| young branch indumentum | densely pubescent up to villous among leaves | densely pubescent, denser among leaves | densely pubescent, denser among leaves | densely pubescent, caducous | densely pubescent, caducous | densely pubescent, caducous | denser among leaves |
| year branches | sparsely pubescent or glabrous | sparsely pubescent or glabrous | sparsely pubescent or glabrous | sparsely pubescent or glabrous | sparsely pubescent or glabrous | nearly glabrous | sparsely pubescent or glabrous |
| leaves | linear lanceolate, oblanceolate to oblong | linear lanceolate, oblanceolate to oblong | linear lanceolate, oblanceolate to oblong | ovate lanceolate | ovate lanceolate | narrowly ovate | oblong lanceolate |
| leaf position | ±clustered, lower few, remote | ±clustered, lower few, remote | ±clustered, lower few, remote | ±clustered, lower remote | ±clustered, lower remote | ±clustered, lower many, remote | alternate, ±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 |
| 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 | (0.12-)0.20-0.30(-0.35) |
| base | widely cuneate | widely cuneate | widely cuneate | widely cuneate | widely cuneate | cuneate | cuneate |
| apex | acute to acuminate | acute to acuminate | acute to acuminate | acute | acute | acute to rounded | acute |
| margin | ±revolute | ±revolute | ±revolute | partly revolute | flat to partly revolute | revolute | flat |
| Inflorescences | terminal and axillar | terminal and axillar | terminal and axillar | terminal and axillar | terminal and axillar | terminal and rich axillar | terminal and axillar |
| flowers - number | 5-15 terminal, 2-5 in axils | 5-15 terminal, 2-5 in axils | 5-15 terminal, 2-5 in axils | 5-15 terminal, 2-5 in axils | 5-15 terminal, 2-5 in axils | 5-15 terminal, 4-8 in axils | 5-15 terminal, 2-5 in axils |
| peduncle, flower bud | densely pubescent to villous | densely pubescent to villous | densely pubescent to villous | densely pubescent to villous | densely pubescent to villous | pubescent | densely pubescent to villous |
| colour | rose to pink | rose to pink | rose to pink | rose | light rose, whitish | deep rose | rose to pink |
| calyx tube | pink to rose or white | pink to rose or white | pink to rose or white | pink | pink | rose | pink to rose |
| lobes | pink to rose | pink to rose | pink to rose | pure pink | light pink | rose to redish | pink to rose |

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|---|---|---|---|---|---|---|---|
| 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 | ovate, a bit folded | uniform, elliptic | ovate 3-grooved |
| lobe apex | usually acute | usually acute | usually acute | acute, slightly bent down | acute, slightly bent down | acuminate, bent up | acute, revolute |
| 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** | ***D.wolongensis*** | **scale** | ***D. tangutica*** | **scale** | ***D. retusa*** | **scale** | ***D. limprichtii*** | **scale** | ***D. longilobata*** | **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 indumentum | densely pubescent up to villous among upper leaves | 1 | sparsely pubescent, glabrescent | 2 | strigose | 3 | shortly pubescent | 2 | pubescent | 2 |
| terminal winter buds | 1 | 1 | 1 | 1 | (1-)3-5 | 3 | 1(-3) | 2 | 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 greeen | 1 | pale greeen | 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 - small | 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 |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 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, spathulate, 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 | 1 |
| calyx tube | pink to rose or white | 2 | white to pink, purplish | 1 | purple to pale | 3 | white | 1 | pale greenish white, purple base | 1 |
| lobes | pink to rose | 3 | pink to white | 2 | pink or white | 2 | white - pale pink | 1 | white - pale cream | 1 |
| tube length | (7-)8-10(-11) | 1 | 9-13(-15) | 1 | 8-12 | 1 | 10-11(-14) | 1 | 7-10(-14) 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 | 1.2-1.8 mm | 1 |
| lobe size | (5-)7-11(-12) × (4-)5-7 | 3 | 5-8(-10) × (3-)4-5(-6) | 2 | 7-10 x 4-8 | 3 | 5-8 × 3-5 | 2 | 5-7 × 2-2.5(-3.5) mm | 1 |
| lobe shape | ovate-lanceolate or ovate | 2 | ovate or ovate-elliptic | 2 | broadly ovate or ovate-elliptic | 3 | ovate-lanceolate | 2 | narrow lanceolate | 1 |
| lobe apex | usually acute | 2 | obtuse to acute | 2 | obtuse to rounded | 3 | obtuse to retuse | 2 | long acuminate | 1 |
| anthers | orange-yellow, prominent | 3 | pale yellow | 1 | yellow | 2 | pale yellow | 1 | pale yellow | 1 |
| anthers | 0.8-1.5 | 1 | 1-1.2 mm | 1 | 1-1.5 mm | 2 | 1 mm | 1 | 1.5 mm | 2 |
| Drupe | carmine red | 2 | red to scarlet | 1 | red | 2 | red to scarlet | 1 | scarlet-red | 1 |
| drupe shape | subglobose to globose | 2 | subglobose or ovoid | 1 | subglobose or ovoid | 1 | ovoid | 1 | ovoid-globose | 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 | 8-12 x 8-11 | 2 |
| habitat | forest, shrubs along rivers | 2 | forest | 1 | Shrubby grassland | 3 | Among boulders, sunny | 3 | forest, among rocks | 1 |
| altitude | 2000-2100 | 2 | 1000-3800 | 1 | 3000-3900 | 3 | 3000-4400 | 3 | 1600-3500 | 1 |

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

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| 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_with | fruit_index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *D. wolongensis*_W1 | 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 |
| *D. wolongensis*_W2 | 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 |
| *D. wolongensis*_W3 | 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 |
| *D. wolongensis* 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 |
| *D. tangutica* | 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 |
| *D. limprichtii* | 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 |
| *D. retusa* | 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 |
| *D.longilobata* | 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 |

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

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

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

| *D. wolongensis* | 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_with | fruit_index | note |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | |

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

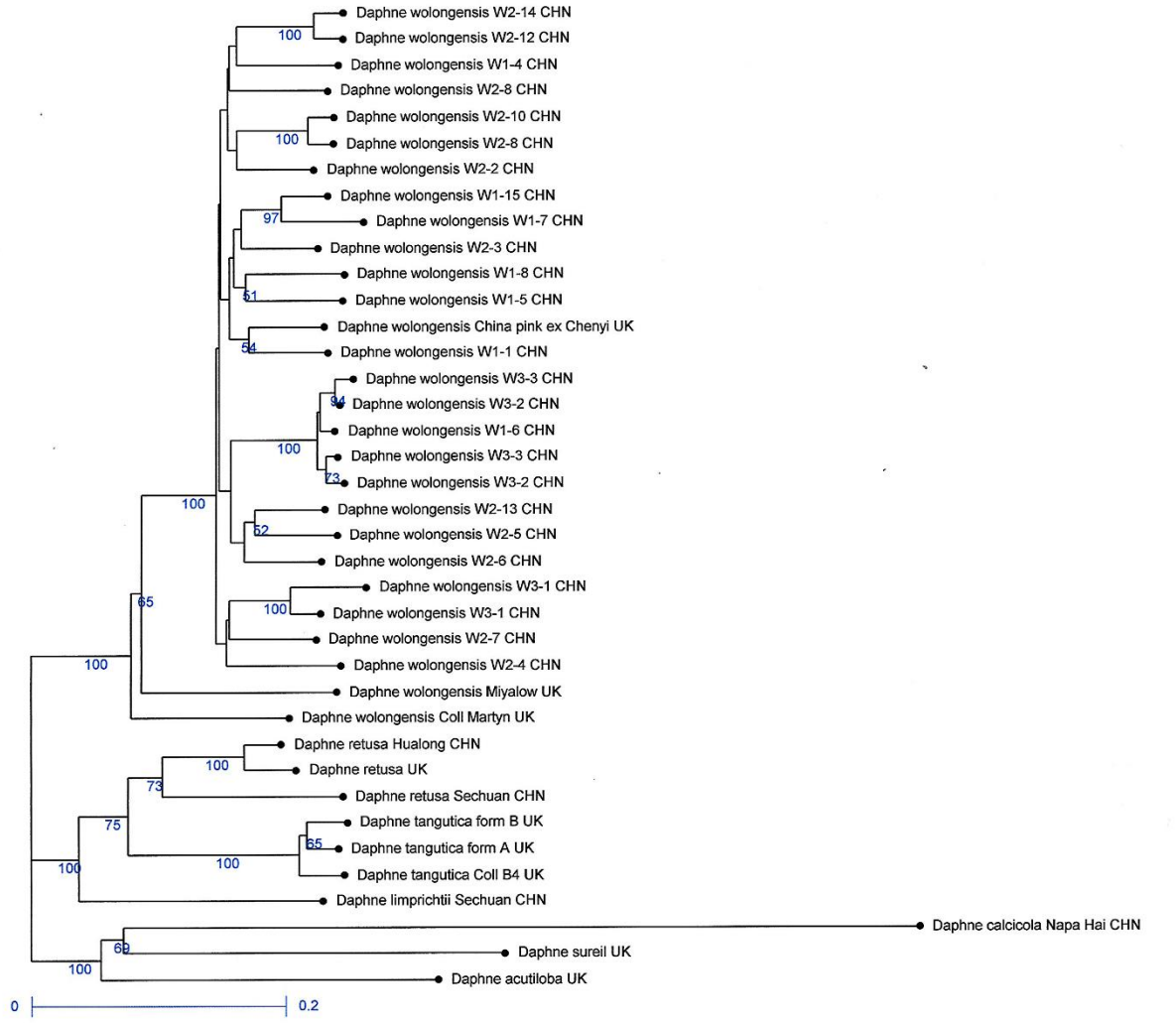
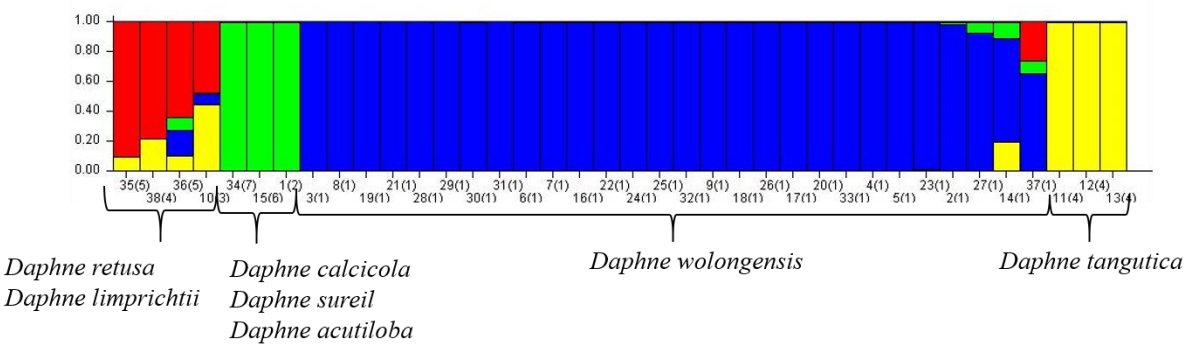



Figure 2 Cluster analysis of *Daphne* spp. based on a Bayesian approach. Each genotype is represented by a bar divided into K colours, where K is the number of clusters assumed in the analysis: K1 – red colour; K2 – green colour; K3 – blue colour; K4 – yellow colour



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**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 ´Kevock Star´