

Appendices

The Appendices include five tables and five figures.

Table S1. Sample locations of *Halenia elliptica* var. *grandiflora* and *H. elliptica* var. *elliptica* for reconstructing the phylogenetic relationships within *Halenia*.

| Population | Location | Latitude | Longitude | Altitude | Sample size |
|---|---|-------------|---------------|----------|-------------|
| <i>H. elliptica</i> var. <i>grandiflora</i> | | | | | |
| XGLL | Shangri-La Botanical Garden, Shangri-La, Yunnan, China | 27°54'36.0" | 99°38'24.0" | 3330 | 12 |
| XZD | Xiaozhongdian, Shangri-La, Yunnan, China | 27°27'21.3" | 99°48'46.7" | 3282 | 12 |
| KM | Kunming, Yunnan, China | 25°09'51.1" | 102°43'25.99" | 2187 | 12 |
| LJ | Lijiang, Yunnan, China | 27°00'03.2" | 100°11'56.7" | 2669 | 12 |
| <i>H. elliptica</i> var. <i>elliptica</i> | | | | | |
| KD | Kangding, Sichuan, China | 30°00'12.9" | 101°56'28.2" | 3012 | 12 |
| DQ | Deqin, Yunnan, China | 28°24'07.4" | 98°59'11.2" | 4034 | 12 |
| GL | Guoluo, Qinghai, China | 33°16'26.4" | 100°40'09.6" | 3926 | 12 |

Table S2. Principle component analysis (PCA) of 21 morphological traits and their contribution to PC 1, PC 2 and PC 3.

| | Principle component | PC 1 | PC 2 | PC 3 |
|--|--|--------|--------|--------|
| Initial | Eigenvalues | 11.38 | 3.50 | 1.11 |
| Eigenvalues | % of Variance | 54.19 | 16.65 | 5.31 |
| Contribution of morphological traits to different components | Initial flowering date of plant | 0.97 | 0.04 | - 0.02 |
| | Plant height (cm) | - 0.56 | 0.62 | 0.14 |
| | Total flower number | - 0.27 | 0.79 | 0.23 |
| | Number of lateral branches | - 0.04 | 0.84 | 0.03 |
| | Length of the third leaf on the main branch from the bottom (cm) | - 0.22 | 0.88 | - 0.30 |
| | Width of the third leaf on the main branch from the bottom (cm) | < 0.01 | 0.93 | 0.13 |
| | Length/width ratio of leaf | - 0.44 | 0.07 | - 0.82 |
| | Flower openness (cm) | 0.83 | 0.12 | 0.00 |
| | Petal height (cm) | 0.85 | 0.10 | - 0.03 |
| | Petal width at the base (cm) | 0.76 | - 0.03 | 0.03 |
| | Spur length (cm) | 0.93 | 0.04 | - 0.01 |
| | Width at the base of spur (cm) | 0.89 | 0.06 | - 0.01 |
| | Distance from the top of spur to the top of petal (cm) | - 0.75 | -0.03 | 0.07 |
| | Distance from the top to the base of spur (cm) | 0.76 | 0.02 | 0.20 |
| | Pistil height (cm) | 0.94 | 0.07 | - 0.08 |
| | Stamen height (cm) | 0.91 | 0.08 | - 0.03 |
| | Pollen number | 0.94 | 0.04 | 0.03 |
| | Ovule number | 0.86 | 0.11 | - 0.04 |
| | Pollen/ovule ratio | 0.75 | - 0.08 | 0.11 |
| | Seed number | 0.83 | 0.22 | - 0.04 |
| | Seed weight (mg) | - 0.67 | - 0.15 | 0.43 |

Table S3. Comparisons of seed number per fruit from flowers subjected to different treatments between the two varieties of *Halenia elliptica* based on generalized linear models, with treatment, varieties and their interaction as fixed factors.

| Source | Wald χ^2 | df | P |
|----------------------------|---------------|----|--------|
| Treatment | 406.22 | 4 | < 0.01 |
| Variety | 72.41 | 1 | < 0.01 |
| Treatment \times variety | 244.90 | 4 | < 0.01 |

Table S4. Variable sites of aligned sequences of the internal transcribed spacer (ITS) 1 + 2 and *rpl16* of the two varieties of *Halenia elliptica*. Sequences are numbered from 5' - to the 3'. Except for *H. elliptica* var. *elliptica*_MJG*, which was taken from GenBank, sequences of *H. elliptica* var. *elliptica* and *H. elliptica* var. *grandiflora* were newly generated for this study (Fig. S2).

| Sequences | Nucleotide position | | | | | | | | | | | | | | | |
|--|---------------------|---|---|---|---|------|---|---|---|---|--------------|---|---|---|---|---|
| | ITS1 | | | | | ITS2 | | | | | <i>rpl16</i> | | | | | |
| | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 1 | 1 | 1 |
| | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 0 | 1 | 2 |
| | 1 | 1 | 5 | 0 | 1 | 3-3 | 7 | 8 | 8 | 8 | 5 | 9 | 9 | 1 | 4 | 3 |
| | 5 | 6 | 9 | 6 | 6 | 3 9 | 8 | 0 | 4 | 9 | 9 | 2 | 9 | 5 | 4 | |
| <i>H. elliptica</i> var. <i>elliptica</i> _MJG* | S | - | A | G | A | - | G | G | T | C | G | Y | G | M | | |
| <i>H. elliptica</i> var. <i>elliptica</i> _GL6 | G | C | A | G | A | ★ | G | G | T | C | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>elliptica</i> _GL7 | G | C | A | G | A | ★ | G | G | T | C | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>elliptica</i> _DQ11 | G | C | G | G | A | ★ | G | G | T | C | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>elliptica</i> _DQ10 | G | C | G | G | A | ★ | G | G | T | C | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>elliptica</i> _KD2 | G | C | G | G | R | ★ | G | G | C | C | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>elliptica</i> _KD8 | G | C | G | G | R | ★ | G | G | C | C | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>grandiflora</i> _XGLL1 | G | C | G | R | A | ★ | R | G | Y | Y | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>grandiflora</i> _XGLL2 | G | C | G | R | A | ★ | R | G | Y | Y | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>grandiflora</i> _LJ | G | C | G | A | A | ★ | A | S | C | C | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>grandiflora</i> _LJ5 | G | C | G | A | A | ★ | A | S | C | C | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>grandiflora</i> _XZD4 | G | C | G | A | A | ★ | A | G | C | T | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>grandiflora</i> _XZD9 | G | C | G | A | A | ★ | A | G | C | T | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>grandiflora</i> _KM1 | G | C | G | A | A | ★ | A | G | C | C | T | T | T | A | | |
| <i>H. elliptica</i> var. <i>grandiflora</i> _KM3 | G | C | G | A | A | ★ | A | G | C | C | T | T | T | A | | |

Note: Dash represents missing nucleotides; ★ AAAACGA; S = C/G; R = A/G; Y = T/C; M=A/C.

Table S5. Comparisons of SNPs/InDels between *H. elliptica* var. *elliptica* and *H. elliptica* var. *grandiflora*, and between *H. elliptica* var. *elliptica* and other species of Gentianaceae.

| Species pair | SNPs | InDels | Total |
|---|------|--------|-------|
| <i>H. elliptica</i> var. <i>elliptica</i> vs. <i>H. elliptica</i> var. <i>grandiflora</i> | 84 | 52 | 32 |
| <i>H. elliptica</i> var. <i>elliptica</i> vs. <i>H. elliptica</i> (published) | 90 | 56 | 34 |
| <i>H. elliptica</i> var. <i>elliptica</i> vs. <i>H. corniculata</i> | 1215 | 1002 | 213 |
| <i>H. elliptica</i> var. <i>elliptica</i> vs. <i>Swertia bimaculata</i> | 2095 | 1694 | 401 |
| <i>H. elliptica</i> var. <i>elliptica</i> vs. <i>Veratrilla baillonii</i> | 3229 | 2725 | 504 |
| <i>H. elliptica</i> var. <i>elliptica</i> vs. <i>Gentiana caelesis</i> | 6179 | 5276 | 903 |

Figure S1. A schematic explanation of the measurements of spur width (1), spur length (2), the distance from the top to the base of the spur (3), and the distance from the top of the spur to the top of the petal (4). The last two types of distance were used to evaluate spur curvature of the two varieties of *Halenia elliptica*.



Figure S2. Microphotographs of chromosomes of *Halenia elliptica* var. *grandiflora*.
Scale bar = 10 μ m.

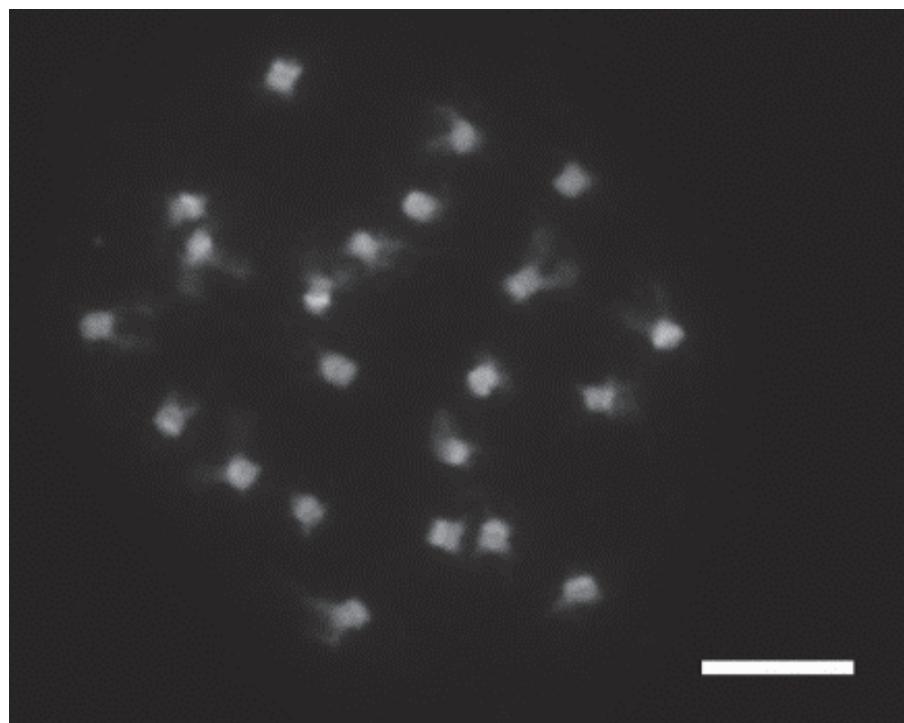


Figure S3. Maximum-likelihood tree of *Halenia* based on ITS and *rpl16* following earlier published data (von Hagen KB, Kadereit JW. 2003. Evolution 57: 2507-2518). Support values (ML Bootstrap percentage/Bayesian posterior probability) are provided at nodes. Except for *H. elliptica* var. *elliptica*_MJJ*, which was taken from GenBank, all sequences of *H. elliptica* were newly generated for this study. *H. elliptica* var. *elliptica* is shaded dark grey whereas *H. elliptica* var. *grandiflora* is shaded light grey.

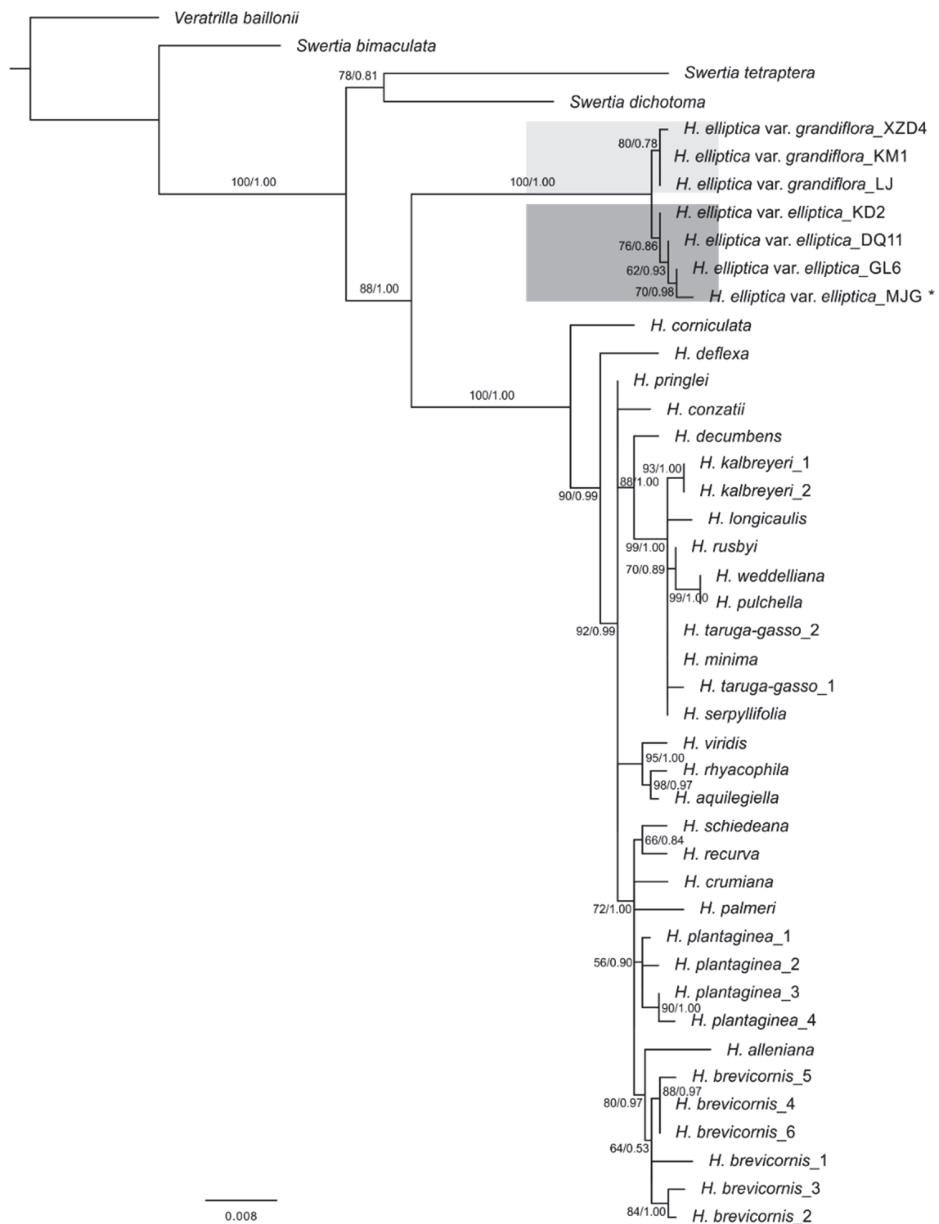


Figure S4. Nucleotide additivity at four sites in the ITS region detected in the XGLL population, which was identified as *Hallenia elliptica* var. *grandiflora* based on morphology, but could be a hybrid swarm between *H. e.* var. *grandiflora* and *H. e.* var. *elliptica*.

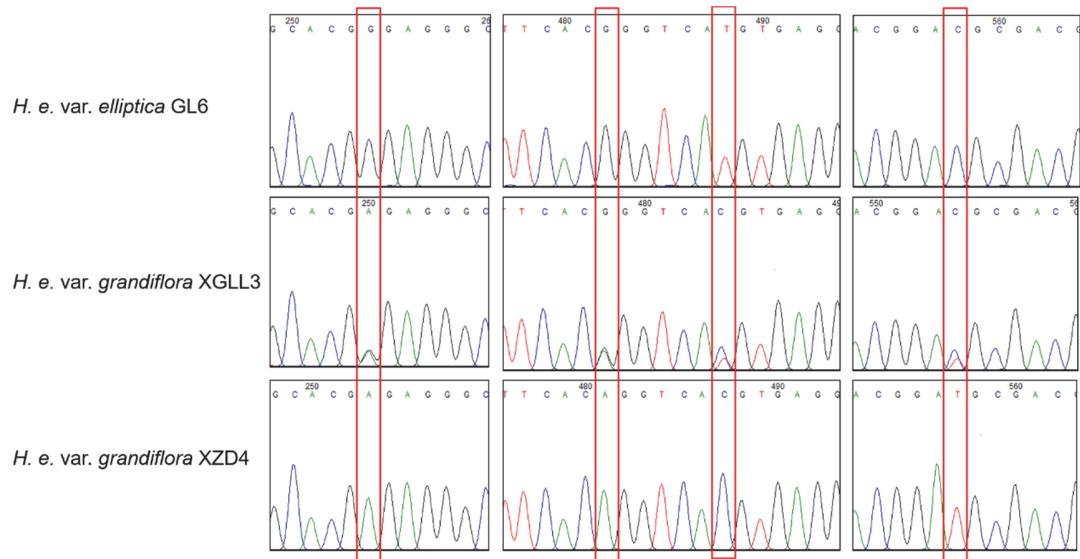


Figure S5. Chloroplast genomes of the two *Halenia elliptica* varieties. The outer circle represents *H. elliptica* var. *elliptica* and the inner circle *H. elliptica* var. *grandiflora*. Using *H. elliptica* var. *elliptica* as a reference, SNP and InDel types were labelled as colored circles and squares, respectively.

