

Description of new taxa of *Halenia* Borkh. (Gentianaceae) from Colombia and Venezuela with significance for testing a key innovation hypothesis

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Abstract

As a result of an ongoing complete taxonomic revision of *Halenia* (Gentianaceae), the new taxa *Halenia perijana* K.B. Hagen and *H. major* subsp. *meridensis* K.B. Hagen are described from Colombia and Venezuela, respectively. Flower morphology suggests that both taxa belong to the *Halenia viridis* group. *H. perijana* is vegetatively well separated from its probable closest relatives, and the only member of *Halenia* from a small and rather isolated mountain range. The new subspecies *meridensis* has small remnants of nectary spurs, and probably links the unspurred *viridis* group to its prominently spurred Central American sister group. Previous molecular clock approaches showed that the *viridis* group arrived in South America approximately at the same time as the *weddelliana* group, the distantly related second major South American group, which contains species with prominent spurs. Based on the new extensive knowledge of all species, the distribution patterns of both groups in Colombia and Venezuela are compared. The existence of marked differences—*weddelliana* group species grow in sympatry more often than species of the *viridis* group—is consistent with a key innovation effect of the presence/absence of nectary spurs, i.e., the reduction of pollinator overlap mediated by the presence of nectary spurs may allow sympatric speciation or remigration after a shorter phase of allopatric differentiation. However, this new evidence is relatively weak due to lack of statistical support and several other unresolved problems.

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Introduction

The genus *Halenia* Borkh. is part of the subtribe Swertiinae from the Gentianaceae-Gentianeae, and the only member of its family with nectary spurs (Struwe et al. 2002). Its closest relatives are part of the highly polyphyletic genus *Swertia* L. (Chassot et al. 2001; von Hagen and Kadereit 2002), and much less diverse than *Halenia* (Fig. 1). These findings fit a general pattern in angiosperms, that between spurred and unspurred sister

taxa the latter are significantly less diverse than the former (e.g. Hodges 1997). For this reason, nectary spurs have been termed a key innovation. It has been proposed that nectary spurs reduce the potential spectrum of pollinators. They may also narrow the overlap of pollinators between species and thereby reduce gene flow between emergent species (Nilsson 1988; Grant 1993; Lunau 2004). In theory this could trigger an increase in the diversification rate. However, in a recent phylogenetic analysis of *Halenia* and related lineages based on nuclear and chloroplast sequences it was found that a significant increase in diversification

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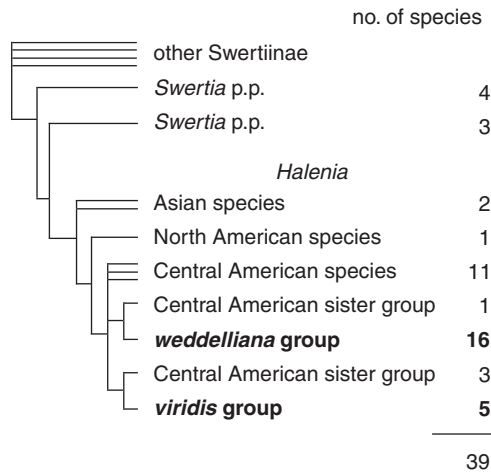


Fig. 1. Important relationships of the *Halenia viridis* and *H. weddelliana* groups based on a combined ITS and *rp16* intron phylogeny (simplified from Hagen and Kadereit 2003). Species numbers behind group names are taken from an ongoing revision of *Halenia* (Hagen in preparation), which will reduce the number of accepted species by about 50%. The two groups from South America compared in the present study are shown in boldface. Some complicated phylogenetic relationships which are not important in the present study are shown as polytomies.

rate in *Halenia* was correlated with migration to new habitats within *Halenia* rather than with the first ‘invention’ of nectary spurs at the base of *Halenia* (von Hagen and Kadereit 2003). This was concluded from an analysis of branch lengths and clade diversities within *Swertia* p.p. and *Halenia* (Fig. 1). For example, the distances between lower nodes of the *Halenia* tree were rather large, and the basally branching northern-hemisphere lineages of *Halenia* were as species-poor as the closely related spurless *Swertia* p.p. lineages. In conclusion, the scenario of nectary spurs as a key innovation was rejected for *Halenia* as a whole.

A closely related problem was that *Halenia* also included several species lacking nectary spurs. These are probably all part of the *viridis* group (Fig. 1), whereas all species with nectary spurs that were sampled in a molecular analysis were part of the only distantly related *weddelliana* group (von Hagen and Kadereit 2003). This separation conforms with the traditional sectional circumscriptions of Gilg (1916) and Allen (1933) for the South American species. *Halenia brevicornis* (Kunth) G. Don is not related to the other South American species, and is not considered any further here.

Biogeographical and molecular clock analyses revealed that the two major lineages colonized South America independently, coming from Central America approximately 1 mya (von Hagen and Kadereit 2003). Therefore, this seems to be a second suitable system in

which the effect of presence/absence of nectary spurs on diversification patterns could be tested on closely related groups of similar age. Moreover, both groups have thrived under similar evolutionary conditions partly in the same geographical region, which constitutes an advantage over the *Swertia/Halenia* case. To maintain this advantage the present study sticks to Colombian and Venezuelan species because both *Halenia* groups co-occur in this region only. Unfortunately, the sampling of species and, even more important, the phylogenetic resolution of the available marker systems (ITS and *rp16* intron) were insufficient to evaluate a possible key innovation effect in these groups with the same methods as applied to the *Swertia/Halenia* problem (von Hagen and Kadereit 2003).

In the absence of a suitable phylogenetic framework I here suggest that an analysis of the geographical distribution patterns within *Halenia* could substitute for statistical tests based on branch lengths and clade diversities. My reasoning is this: If nectary spurs promote speciation by reducing gene flow between emergent species, this could happen in sympatry or at least considerably facilitate remigration without hybridization after a shorter time of allopatric differentiation. Consequently, a difference between groups with and without nectary spurs could be that the individual distribution ranges within the former group overlap much more often than in the latter. However, this pattern is supposed to fade through time the more post-speciation changes of distribution ranges accumulate.

To test this simple hypothesis the geographical distribution of all species has to be known. The latest comprehensive revision of *Halenia* was published by Allen in 1933, when for a number of taxa the available plant material still was too scanty. In addition, many new species have been described since (e.g. from Colombia and Venezuela: Cuatrecasas 1933; Allen 1942; Allen 1944), and there are only two modern but regional accounts of the genus (Wilbur 1984a, b for Central America; Pringle 1995 for Ecuador). Currently there are 80 species accepted in the literature, out of about 120 names described (plus many subspecies/ variations). In essence, however, the number, circumscription, and geographical distribution of most South American species is unknown. I, therefore, have been working on a new, complete taxonomic revision of the genus, based on material from all relevant major and some local herbaria. In the final analysis, the number of accepted species in *Halenia* probably will be reduced to 39 (not counting subspecies) (see also Fig. 1). In the present study I describe two new taxa as a first result of the revision. In addition, the knowledge of all species from Colombia and Venezuela is used to compare distributional patterns of the two major clades, with results significant to key-innovation theory in *Halenia*.

Material and methods

Plant material

Material from the following herbaria was used: AAU, B, BR, E, F, FR, G, GH, GOET, HBG, JE, K, L, LBP, MEXU, MICH, MO, NY, P, PENN, PH, QCA, QCNE, S, TEX, TUB, U, US, VEN.

Flowers were softened and prepared for microscope examination using hot water. Microscopic measurements were made with an AxioCam and the software AxioVision 3.1 (Carl Zeiss AG, Germany) with semi-automated scaling.

Comparison of geographical distributions

For the preparation of distribution maps locality dots were plotted on a map by hand. Maps were digitized using a DrawingBoard III (CalComp Inc., Maryland); final output was made using ArcGIS 8.3 (ESRI, California) and the maps included therein.

Table 1 provides a list of the relevant species and their synonyms. Detailed explanations and a new determination key will be published as part of the complete revision (Hagen, in preparation). This especially concerns *H. kalbreyeri* Gilg, *H. hypericoides* (Kunth) G. Don, and *H. gracilis* (Kunth) G. Don (all described from Colombia) which my revision treats significantly differently from earlier classifications (Gilg 1916; Allen 1933; Pringle 1995).

To compare the amount of overlap of distribution ranges within a group of species I have developed a very simple descriptive value, which is calculated using pairwise comparisons of species distributions within one clade. The number of species pairs with geographical overlap divided by the total number of all possible pairwise combinations results in a value ranging between 0 for complete vicariance and 1 for sympatry of all possible species pairs within one group.

To test statistically whether the relative frequency of sympatric vs. vicariant species pairs was significantly different between both groups, a Monte Carlo procedure was applied (see Acknowledgments below). A triangle matrix including all 12 species was prepared, in which geographical overlap was recorded as 1 and vicariance as 0 for all possible species pairs. The values among all pairs in the *viridis* group only (first five taxa) were summarized, then those among members of the *weddelliana* group (last seven taxa). The difference between both values was calculated. Next, taxon order was randomized (using the shuffle option in MacClade 4.03 [Maddison and Maddison 2001], substituting taxa for characters), the values of the first five and last seven taxa of the resulting matrix were summarized, and the difference was calculated again. This randomization of

Table 1. List of synonyms for *Halenia* species from Colombia and Venezuela

Accepted names	Synonyms
<i>H. adpressa</i> C.K. Allen	<i>H. pauana</i> Cuatrec., <i>H. venezuelensis</i> C.K. Allen
<i>H. asclepiadea</i> (Kunth) G. Don	<i>Swertia quadricornis</i> Willd. ex Roem. and Schult., <i>H. cuatrecasatii</i> C.K. Allen, <i>H. occulta</i> C.K. Allen
<i>H. brevicornis</i> (Kunth) G. Don	Many synonyms, but not part of present study
<i>H. elata</i> Wedd.	<i>H. campanulata</i> Cuatrec., <i>H. dasyantha</i> Gilg, <i>H. foliosa</i> Gilg, <i>H. hygrophila</i> Gilg, <i>H. schultzei</i> Gilg, <i>H. tolimae</i> Gilg, <i>H. verticillata</i> Gilg
<i>H. gentianoides</i> Wedd.	—
<i>H. gigantea</i> C.K. Allen	<i>H. elegans</i> C.K. Allen
<i>H. gracilis</i> (Kunth) G. Don	several synonyms, but does not occur in Colombia in my view (Hagen in prep.)
<i>H. hypericoides</i> (Kunth) G. Don	doubtful species of unclear status (Hagen in prep.)
<i>H. insignis</i> C.K. Allen	<i>H. nivalis</i> C.K. Allen
<i>H. kalbreyeri</i> Gilg	—
<i>H. major</i> Wedd.	<i>H. karstenii</i> Gilg, <i>H. macrantha</i> Gilg, <i>H. stellarioides</i> Gilg
<i>H. perijana</i> K.B. Hagen	—
<i>H. purdieana</i> Wedd.	<i>H. garcia-barrigae</i> C.K. Allen, <i>H. hoppii</i> Reimers
<i>H. viridis</i> (Griseb.) Gilg	<i>H. inaequalis</i> Wedd., <i>H. parallela</i> C.K. Allen, <i>H. subinvoluta</i> Gilg
<i>H. weddelliana</i> Gilg	<i>H. antigonorhica</i> Gilg, <i>H. meyeri-johannis</i> Gilg, <i>H. phyllophora</i> Cuatrec.

group identities was repeated many times. The numerator of the final test value is the sum of how many times the difference between the five- and seven-taxon groups exceeds or is equal to the original difference plus one (for the observed difference); the denominator is the number of randomizations plus one (for the observed group identities). The same procedure was repeated with a table of 13 taxa (treating subsp. *meridensis* separately).

Results

New taxa

Halenia perijana K.B. Hagen, sp. nov.

Figs. 2a–d, 3a–h.

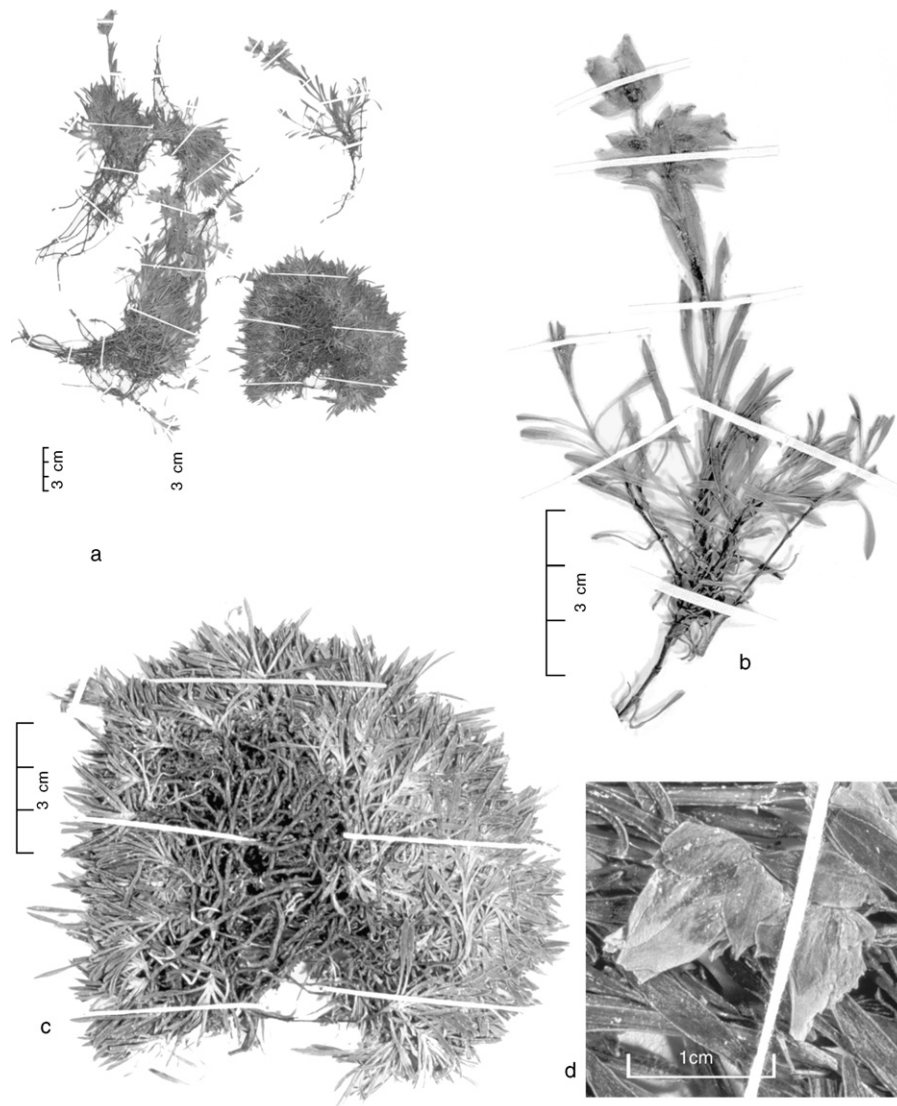


Fig. 2. Photographs of holotype of *Halenia perijana*: (a) complete sheet; (b) one flowering stem; (c) dense assemblage of sterile rosettes; and (d) two flowers with broadly elliptical calyx lobes.

Etymology: Named *H. perijana* because its distribution is limited to the Serranía de Perijá.

Type: COLOMBIA, Depto. Magdalena, Mpio. La Paz, frontera Colombo-Venezolana, Serranía de Perijá, cima del cerro El Avión, alt. 3600 m. 3 Marzo 1959, *R. Romero 7376* (holotype: US [no. 3.085.783], no other types known).

Diagnosis: Herba perennis. Caules ramosi et repentes, e basi circa 5 cm nudi. Folia ad apicem caulis rosulata, anguste oblanceolata, 2–2.5 cm longa 1.5–2.5 mm lata. Paucae rosulae simplicibus caulibus floriferis. Caulis florifer usque ad 12 cm altus, foliis paribus oppositis 2–4. Inflorescentia brevis, floribus 2–5 terminalibus et axillaribus, pedicellatis 0.2–1 cm longis. Flos tetramerus 1–1.5 cm longus, calyce et corolla viridi. Lobi calycini late elliptici et breve acuminati, inter sese margine superposite. Corolla 1–1.5 cm longa e basi

usque ad 3 mm sympetala. Lobi corollae elliptici. Nectaria epipetala 4, elliptica 2.6 mm longa 1.6 mm lata. Stamina 4 exserta ad sinus corollae affixa. Antherae oblongae 2.7 mm longae 2 mm latae. Pistillum anguste obclavatum sine stylo manifesto, lobis corollae brevior. Stigmata 2 papillosa, anguste elliptica, 1 mm longa.

Habitat in montibus altis Serranía de Perijá in Colombia boreo-orientali.

Differt haec species ab *Halenia elata*, *H. gentianoides*, *H. major* et *H. viridis* caulis ramosis repentibus rosulatis, foliis multis brevioribus et angustioribus. Differt ab *H. adpressa* et *H. purdieana* corolla omnino ecalcerata, pagina nectarii majora, lobis calycinis late ellipticis inter sese margine superposite.

Description: Perennial herb, 8–12 cm high. Lower parts of stems short, densely branched, creeping, leaves lost. Stems terminated by crowded rosette leaves; few

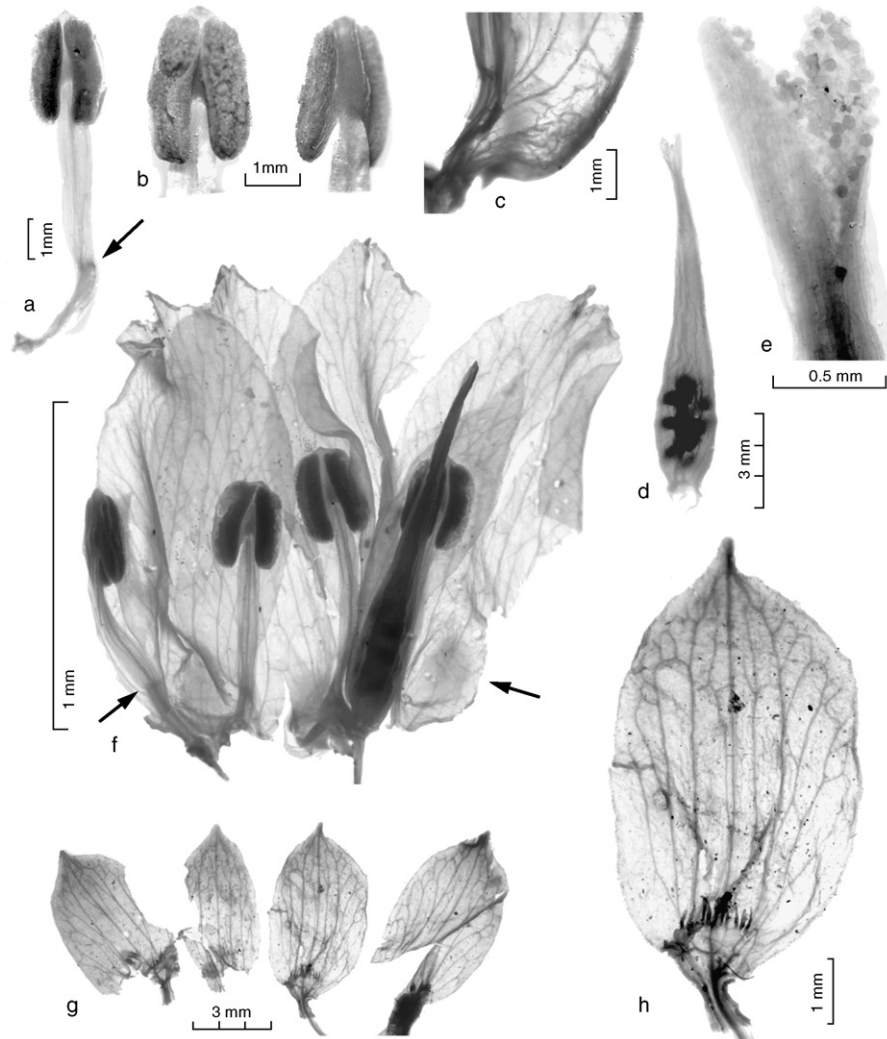


Fig. 3. Microscopical details of a *Halenia perijana* flower: (a) complete stamen; arrow shows point of insertion in corolla tube; (b) frontal and dorsal views of still introrse dorsifixed pollen sacks and connective; (c) lower part of corolla tube in lateral view; darker shade in convex part and concentration of vascular bundles mark the position of one nectary; (d) gynoeceum with inversely club-shaped ovary, indistinguishable style, and stigmatic lobes; dark black structures are ovules; (e) narrowly elliptical stigmatic lobes covered with pollen; (f) opened tetramerous corolla with elliptical corolla lobes, stamens, and gynoeceum; left arrow points to insertion of filament in corolla, right arrow to one epipetalous nectary; (g) four broadly elliptical calyx lobes; fissures are artifacts from preparation; and (h) single calyx lobe seen from adaxial side; dark, finger-like structures near base are calycine collectors.

form elongate sterile shoots, few form unbranched flowering shoots. Rosettes with 4–6 pairs of leaves which are narrowly oblanceolate, 2–2.5 cm long, 1.5–2.5 mm broad. Flowering and sterile stems with 2–4 pairs of leaves above rosette. Stem leaves less narrowly oblanceolate than rosette leaves, 1.5–2 cm long, about 2.5 mm broad, longer than internodes. Lamina of all leaves tapering very gradually into a petiole. Flowers in few flowered terminal and axillary clusters. Flower buds nodding before anthesis; orientation of open flowers probably upwards or horizontal. Flowers almost sessile or with pedicel of up to 1 cm length, tetrameric. Calyx lobes green, elliptical to broadly elliptical, slightly acuminate, 3.5–4.5 mm broad,

6–8 mm long, overlapping each other in lower part. At base of inner sides of calyx lobes up to 10 linear calycine collectors of 0.3–0.5 mm length, arranged in horizontal line (such collectors may be compared with extrafloral nectaries; [Struwe et al. 2002](#)). Sympetalous corolla green, 1–1.5 cm long in total, connate part about 3 mm. Free parts of lobes elliptical in shape. The four epipetalous nectaries form elliptical patches with most of surface in connate part of corolla (middle between lower parts of filaments) and smaller portion in free part of corolla; vertical axis up to 2.6 mm long, horizontal axis about 1.6 mm. In contrast to many other species of *Halenia*, nectaries do not form spurs or pouches on outside of petals. Lower part of filament (3 mm)

attached to corolla. Flattened filaments (0.8 mm broad) diverge from corolla exactly in sinuses between corolla lobes. Free part of filament about 4.8 mm long. Closed anthers oblong, 2.7 mm long, 2 mm broad, dorsifixed at about 1/3 of their length. Connective slightly pointed at top. Orientation of pollen sacks introrse before anthesis (probably changes to extrorse after anthesis as in the other *Halenia* species); sacks open with longitudinal slits. Sessile pistil slightly shorter than petals and inversely club-shaped, 2 mm broad in broadest part near base, tapering gradually to smallest part (0.3 mm) just below stigma; style not distinguishable. Stigma bipartite (V-shaped in side view), each lobe narrowly elliptical, 1 mm long, 0.4 mm broad. Stigmatic surface

papillate, covered with pollen on both flowers examined in microscopic detail. Fruits and seeds not observed. Only known from type locality.

***Halenia major* Wedd. subsp. *meridensis* K.B. Hagen subsp. nov.**

Fig. 4a–i, k.

Etymology: Named after its distribution in the Cordillera de Mérida of Venezuela.

Type: VENEZUELA, Edo. Mérida, Cuenca Santo Domingo, along Highway 7, 2700 m. Occasional sprawling herb in forest along stream, 20 Sept. 1972, R.E. Weaver 2603 (holotype: GH; isotypes in GH, MO 3479627, NY).

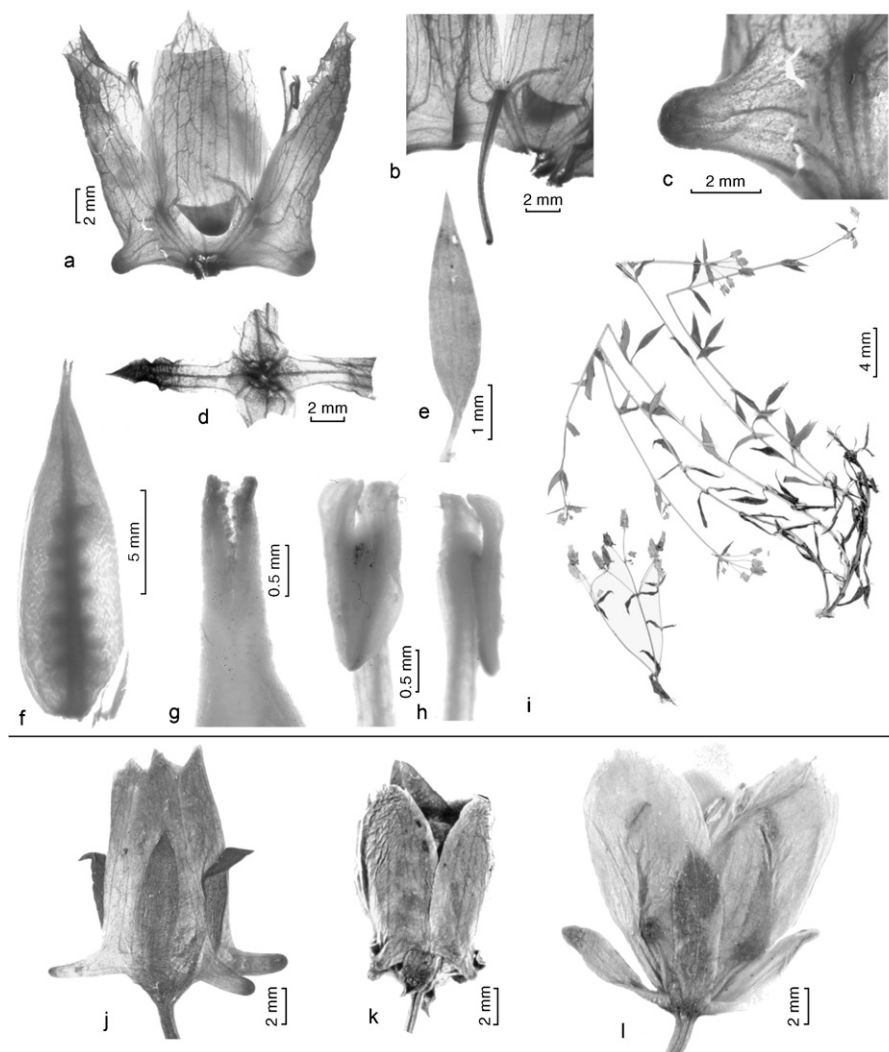


Fig. 4. Photographs and microscopical details of holotype of *Halenia major* subsp. *meridensis* (a–i, k), and comparison with related taxa (j, l): (a) side view of corolla, calyx removed; (b) insertion of filament in corolla, anther fallen off; note that filament inserts in sinus between corolla lobes; (c) single nectary spur; fissures are from preparation; (d) calyx lobes, only one lobe not broken off during preparation; (e) single leaf from upper half of stem; (f) gynoeceum with ovules shining through; (g) narrowly elliptical stigmatic lobes covered with pollen; (h) frontal and dorsal views of extrorse dorsifixed pollen sacks and connective; (i) complete sheet of holotype; (j) flower of *H. rhyacophila* C.K. Allen from the prominently spurred Central American sister group of the *viridis* group; (k) flower of *H. major* subsp. *meridensis*; note intermediate length and horizontal orientation of nectary spurs; and (l) typically unspurred flower of *H. major* subsp. *major*.

Additional specimens seen: (1) VENEZUELA, without exact locality, Jun–Sep 1958, *W. Schwabe* (B). (2) VENEZUELA, Mérida, 17 km from Apartaderos along road to Barinas, 2650 m, 2 Sep 1964, *F.J. Breteler* 4170 (F 1800234, K, MO 2474185, NY, S, US 00402075, VEN 251191). (3) VENEZUELA, Mérida, trail leading from La Negrita to Boquerón of Quebrada de las Cañas, 2990–3300 m, 31 Oct 1978, *J.L. Luteyn* et al. 6110 (NY, VEN 000162215). (4) VENEZUELA, Mérida, 81 km from Merida along road to Barinas, in valley of small river, 2650 m, 21 Nov 1963, *F.J. Breteler* 3378 (NY, US 00402073 and 00402074).

Diagnosis: Herba probabiliter perennis. Caulis principalis saepe repens, 2–6 caulibus floriferis 40–70 cm longis ex axillis foliorum. Folia lanceolata usque ad anguste lanceolata, petiolis 0.5–2 cm longis. Inflorescentia subumbellata. Flores virides tetrameri, pedicellis 2.5–4 cm longis. Lobi calycini anguste oblongi elliptici et acuminati. Corolla 13–17 mm longa, e basi usque ad 4 mm sympetala. Calcaria 1–2.5 mm longa, horizontaliter divergentes. Stamina 4 exserta ad sinus corollae affixa. Pistillum obclavatum sine stylo. Stigmata 2, papillosa et anguste lanceolata, 1.2 mm longa.

Habitat in sylvis et prope rivulos in montibus altis Cordillera de Merida in Venezuela occidentali.

Hoc taxon *Halenia major* subsp. *major* maxime simile characteribus vegetativis, sed differt calcaribus horizontalibus brevibus sed manifestis plerumque 1.0–2.5 mm longis. Calcaria subspeciei *major* plerumque nulla ad 1 mm longa.

Description: (Probably) perennial herb, 40–70 cm high. Main stem often decumbent, with withered remains of

leaves. Often several flowering stems ascend from decumbent main stem. Stems loosely leaved; internodes shorter than leaves in lower parts, up to twice as long as leaves in higher parts. Leaves 3.5–5 cm long, petiolate (0.5–2 cm) in lower parts of stem, almost sessile below inflorescence. Shape of leaf blade lanceolate to narrowly lanceolate. Terminal and axillary flowers form a subumbellate inflorescence with about 3–12 erect tetramerous flowers. Pedicels (0.5–) 2.5–4 cm long. Calyx divided almost to base; its lobes narrowly oblong, acuminate, 2–3 mm broad, 10 mm long. Calycine colletes not observed. Corolla greenish, 13–17 mm long, divided up to 3/4 of its length. Corolla lobes elliptical. The four nectaries form short protuberances sticking out almost horizontally 1–2.5 mm from abaxial side in connate part of corolla. Lower parts of flattened filaments diverge from corolla in sinuses between corolla lobes. Free parts of filaments about 5 mm long. Empty anthers oblong, 2.5 mm long, 1.5 mm broad, dorsifixed at about 1/3 of their length, in extrorse orientation. Sessile pistil broadly club-shaped, 5 mm broad, 1.6 cm long. Bipartite stigma V-shaped in side view, each side narrowly lanceolate, 1.2 mm long, 0.4 mm broad. Fruits and seeds not observed.

Distribution of Colombian and Venezuelan species

The distribution ranges of all species in the *viridis* group are shown in Fig. 5a. For members of the *weddelliana* group from northern South America see

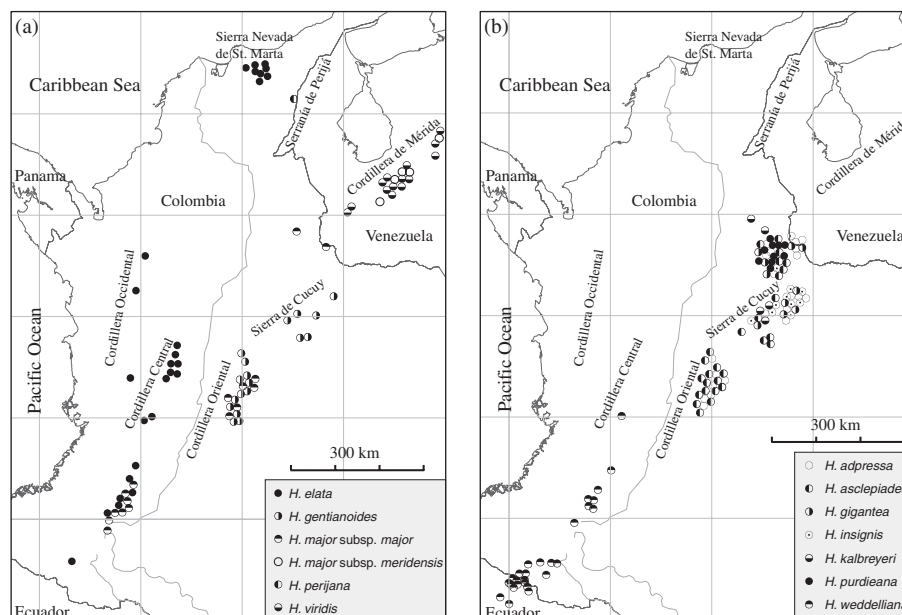


Fig. 5. Distribution of *Halenia* in Colombia and Venezuela, excluding only the distantly related *H. brevicornis* (Kunth) G. Don. Some dots were slightly spread for better visibility: (a) predominantly vicariant geographical distribution of the unspurred *viridis* group; only *Halenia major* occurs in sympatry with other species; and (b) strong geographical overlap within the spurred *weddelliana* group.

Fig. 5b. All species of *Halenia* present in Colombia and Venezuela are included in these figures, except for the only distantly related *H. brevicornis*.

The evaluation of geographical overlap in the *viridis* group resulted in 10 possible species pairs and an actual overlap of three pairs (all of which involve *Halenia major*) thus in a value of 0.30. In the *weddelliana* group overlap was found in 14 (involving all species except *H. weddelliana*) of the possible 21 species pairs, resulting in a value of 0.66. The difference between both lineages was even more pronounced when subsp. *meridensis* was regarded as a separate species. This yielded a value of only $3/15 = 0.20$ for the *viridis* group.

Monte Carlo simulation was extremely tedious, because all calculations had to be done manually. Only 99 randomizations per dataset could be performed in a reasonable time frame, which sets the minimum probability of error at $P > 0.01$. However, absolute values were rather high, i.e. $P = 0.14$ for the 12-taxon matrix and $P = 0.10$ for the 13-taxon matrix, thus can be interpreted without great qualms. The high values mean that in the 12-taxon case the difference in range overlap between the major groups could have arisen by chance alone. In the 13-taxon case the exact probability will be somewhere around the $P = 0.1$ threshold, but even after many more randomizations a precise result probably would never come close to the more stringent and commonly used $P = 0.05$ level of significance. In conclusion, a difference in range overlap between the groups is obvious but does not receive even intermediate support from statistical testing.

Discussion

The status and relationships of *H. perijana*

H. perijana clearly belongs to the *viridis* group of species, because it has the rather typical large nectary patches which do not form spurs on the abaxial side of the petal lobes. It also has broadly elliptic corolla lobes, a character found in some other species of this group but not outside of it. The new species differs from probable relatives by its especially broad and clearly overlapping corolla lobes, and vegetatively by the small size of the plants, the narrow shape and small size of the leaves, and most importantly by the presence of multiple dense rosettes at the tips of short and decumbent stems. There are few other species of *Halenia* in northern South America with similar vegetative characters (e.g., *H. purdieana* Wedd. var. *congesta* C.K. Allen and *H. adpressa* C.K. Allen sometimes have multiple rosettes and short, narrow leaves), but these have the typical flower structure of the distantly related *weddelliana* group, with slender spurs, smaller surface of nectaries

and narrow calyx lobes, and therefore cannot be mistaken for *H. perijana*. In conclusion, *H. perijana* seems well separated vegetatively, and is the fifth species of the spurless *viridis* group.

A major problem with the new species is that only a single collection with four mounted plants, and only herbarium material, was available, as all attempts to gather more material from local herbaria were frustrated. Due to the critical political situation in Colombia, it may be doubted that more *Halenia* material will be collected in the near and intermediate future from the extremely remote area where *H. perijana* was found (Santiago Madriñan, Bogotá, pers. comm.). I also have to state that in other cases of single, aberrant collections, e.g. from Peru, I refrained from describing new taxa, because the differences to other species were often relatively minor, and because morphological variability and potential overlap with probably related taxa should be considered before a new species is proposed. In the case of *H. perijana*, the probable relatives are well known and the morphological gap to them is comparatively large. Moreover, no other species of *Halenia* has ever been collected in the rather isolated mountains of the Serranía de Perijá (Fig. 5a), and the species composition of neighbouring mountain ranges seems sufficiently known. In summary, due to the clear morphological and distributional differences to other species and the continuing inaccessibility of more specimen I felt justified to publish the above description.

The status and relationships of *Halenia major* subsp. *meridensis*

The new subspecies *meridensis* is similar or identical to the type subsp. *major* in most vegetative characters. A minor difference is that several flowering stems arise from one decumbent main stem more often in subsp. *meridensis* (Fig. 4i) than in subsp. *major*. The former differs more clearly by its short but distinct nectary spurs (1.0–2.5 mm long, 1.0–2.0 broad at the tips, 2.0–3.0 mm broad at the base; Fig. 4a and c). In contrast, the nectaries of the type subspecies either do not stick out from the abaxial side of the petals or form plump pouches of up to 1 mm length and 2 mm width on the abaxial side (Fig. 4l). Overlap in this character between the subspecies is found very rarely only. Specimen with an average spur length of 1.8 mm occur in the Sierra de Mérida in Venezuela only (new subspecies), whereas plants exhibiting the opposite state (spurs more or less absent) occur in three different areas of Colombia. A subspecific rank seems appropriate because of the small and slightly overlapping difference which, however, is correlated very well with a geographically vicariant distribution.

Halenia major clearly belongs to the *viridis* group because of a deeply divided corolla, elliptical to broadly elliptical corolla lobes, and nectaries with a relatively large surface which usually do not distinctly stick out from the abaxial side of the corolla (see, e.g., description of *H. perijana*; Fig. 3f). Much in contrast, the sister group of the spurless *viridis* group from Central America contains a total of three species with rather prominent nectary spurs. This sister-group relationship found in the recent phylogenetic analysis was surprising and hard to explain. Most conspicuously, two species of the sister group (*H. euryphylla* and *H. rhyacophila*) have spurs that stick out horizontally from the petals (Fig. 4j). This is different from most South American species of *Halenia*, from the third species of the Central American sister group, and also from much of the remainder of *Halenia* where spurs often point more or less backwards. The new subsp. *meridensis* possesses short remnants of spurs which also spread out horizontally as in the related Central American species (Fig. 4a, j and k). In conclusion, it seems perfectly reasonable to suppose that subsp. *meridensis* has conserved an intermediate (thus plesiomorphic) stage of spur evolution linking the spurred Central American relatives to the usually unspurred *viridis* group (e.g. Fig. 3f) and to the type subspecies of *H. major* (Fig. 4l). In fact, this is the first and only morphological evidence that supports the unexpected relationship found in the molecular phylogenetic analysis. If all this is true then *Halenia major* could be regarded as the first branching or oldest species of the *viridis* group, whereas the other species would be more closely related to each other.

Comparison of distribution patterns and consequences for the ‘spurs as a key innovation’ theory in *Halenia*

The distribution of both major lineages of *Halenia* in Colombia and Venezuela (Fig. 5a and b) is confined to high alpine habitats (above 2500 m). Most often the plants occur in the paramo zone above the regular treeline. It appears that the *viridis* group is more widespread in northern South America than the *weddelliana* group mainly because it has additionally colonized the Sierra de Sta. Marta, the Serranía de Perijá, and the Cordillera de Mérida, which are not connected to the main chain of the Andes or only with chains of lesser height. Nevertheless, the *weddelliana* group as a whole has a more widespread distribution (not shown) because it also occurs further to the South (nine additional species from Ecuador to northern Argentina). It should be noted here that some southern members of the *weddelliana* group also grow in close regional sympatry, e.g. *H. weddelliana* with *H. gracilis* and *H. longicaulis* in Ecuador, or *H. umbellata* with

H. caespitosa in Peru (Hagen in preparation). This conforms with the findings of the present study in general, but it cannot be compared with the *viridis* group which is absent from these regions.

Some notable distributional features of the *viridis* group are: (1) There is a large disjunction, within *H. elata* bridging a long stretch of tropical lowland. (2) *Halenia major* is widespread, with several disjunct partial ranges which overlap with three species. (3) The most obvious beginning of specific differentiation in the *viridis* group (subsp. *major* and *meridensis*) happens in allopatry; this and the disjunct distribution of subsp. *major* might be attributed to post- or interglacial habitat separation (see below). (4) The other four species are geographically vicariant to each other.

Some notable distributional features of the *weddelliana* group are: (1) three species, *H. adpressa*, *H. asclepiadea* and *H. weddelliana*, are much more widespread than the others. (2) Only *H. weddelliana* has no geographical overlap with other species in Colombia (although there is partial overlap with other species in Ecuador, see above). (3) Most importantly, the distribution of the other six species shows broad geographical overlap between almost all possible species combinations (except for the pair *H. insignis*/*H. purdieana*); species overlap is especially high in and north of the Sierra de Cucuy; in special cases very detailed herbarium labels even allow the conclusion that some species occur in the same locality.

Quantification of the difference in geographical overlap between the spurred and the unspurred lineage produced a noticeable difference, but statistically this was marginally not significant. Nevertheless, most findings itemized above (especially points 3 and 4 for the *viridis* group and point 3 for the *weddelliana* group) conform with predictions from key-innovation theory as explained in the Introduction. In conclusion, I count this as weak evidence in favour of the theory. It may be repeated here that current geographical overlap does not necessarily mean that speciation happened in sympatry. It is equally likely that only remigration after initial allopatric differentiation was facilitated, because presentation of nectar in spurs may also reduce introgression upon secondary contact (as Nilsson 1988 observed for deep corolla tubes).

The apparently contradicting distribution of *H. major* overlapping with other unspurred species probably can be incorporated in this theory without much difficulty. Firstly, *H. major* could have enlarged its distribution range multiple times, because high mountain ranges presently separated by lower regions probably were better connected by a shift of vegetation zones during cool phases of the Quaternary (Simpson 1975; Burnham and Graham 1999). Secondly, I suggest that a difference in distribution patterns can be supposed to vanish with increasing age of the species under examination, because

additional means of genetic isolation (e.g. chromosomal rearrangements) will necessarily evolve in lineages with or without nectary spurs the more time has passed since division from the last common ancestor (Levin 2002). After a sufficient time of separation, also species without nectary spurs will not merge in secondary sympatry. *Halenia major* simply seems to be the first branching or oldest lineage of the *viridis* group, because it is the only species with suspicious remnants of nectary spurs in parts of its distribution. Therefore, it was the most likely candidate anyway for secondary deviation from originally potential strict vicariance in the *viridis* group. These considerations also lead to an interesting prediction about the effect of spurs. I have suggested that in older lineages nectary spurs are probably just one of several means to keep gene pools apart. In turn, this means that the less time has passed since gene pools have become separated the higher should be the relative amount of reproductive isolation imposed by nectary spurs compared with other traits; this prediction, however, will be difficult to test.

Problems and prospects

The conclusions of the preceding paragraph require several assumptions which may not be fully justified. An important precondition was that both groups are suitable for comparison, thus have the same age, evolved in the same region and, speaking rather unspecifically, have been subject to the same phylogenetic constraints. Obviously, sister groups would be preferable for comparison because they have the same age and constraints by definition. In the present case, similar ages were concluded from a statistically well supported molecular clock approach only (ca. 0.95 my for the *viridis* group, ca. 0.83 my for the *weddelliana* group; von Hagen and Kadereit 2003). Discovery that the *weddelliana* group is significantly older than the *viridis* group would destroy much of the above reasoning, because the group would have had more time for the accumulation of post-speciation changes of distribution ranges. Conversely, significantly higher age of the *viridis* group would strengthen the conclusions drawn above. Another potential problem is that features less prominent than presence/absence of nectar spurs could account for the patterns observed. One such character is ploidy level, though all of the few *Halenia* species examined have the same haploid number of $n = 11$ (summarized in Struwe et al. 2002).

Although the distribution maps are based on as much material as available, it is not exactly clear what the observed geographical overlap means, because in several areas sampling density is not very high and the labels on herbarium sheets often are not very detailed. It is, therefore, impossible to determine whether different

species truly grow side by side or whether there are slight differences in ecological preferences which would prevent gene flow in regionally sympatric species also without any key evolutionary effect. This ‘herbarium point of view’ of the present study could be altered by field work only but, as stated before, the political situation in Colombia does not allow such studies.

Observation of the unknown pollinators, or finding evidence for hybridization, would also help to support or refute the findings of this study. However, both methods again would require field work or additional greenhouse experiments for the latter approach, and *Halenia* is very difficult to cultivate.

The circumscriptions of the *viridis* and *weddelliana* groups might change once plant material for detailed molecular analysis becomes available. Our repeated attempts to extract more suitable DNA samples (mostly material from 1960–75) have been unsuccessful, thus we have to stick to the existing, congruent results based on the traditional circumscriptions and relatively few molecular samples (eight species, mostly from Ecuador, included in von Hagen and Kadereit 2003). The numerical procedures to compare geographical overlap between groups using all possible species pairs also could be enhanced considerably by more complete sampling. With sufficient phylogenetic resolution at hand, it would be better to use values derived only from sister species and sister lineages within the spurred and unspurred lineages, respectively, because this would be linked more closely to the true underlying modes of speciation.

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