



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Morphological and anatomical evolution of species of *Valeriana* (Caprifoliaceae) during the uplifting of the Andean range

Citation for published version:

da Silva Cruz, R, Klimeš, A, Doležal, J, Sklenář, P & Klimešová, J 2023, 'Morphological and anatomical evolution of species of *Valeriana* (Caprifoliaceae) during the uplifting of the Andean range', *Botanical journal of the linnean society*. <https://doi.org/10.1093/botlinnean/boad011>

Digital Object Identifier (DOI):

[10.1093/botlinnean/boad011](https://doi.org/10.1093/botlinnean/boad011)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Botanical journal of the linnean society

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



1 **Morphological and anatomical evolution of species of *Valeriana* (Caprifoliaceae) during**
2 **the uplifting of the Andean range**

3 Rafael Cruz^{1,2*}, Adam Klimeš^{2,3}, Jiří Doležal², Petr Sklenář⁴, and Jitka Klimešová^{2,4}

4 (1) Institute of Molecular Plant Sciences, University of Edinburgh, Max Born Crescent, EH9
5 3BF, Edinburgh, United Kingdom

6 (2) Institute of Botany, Czech Academy of Sciences, Dukelská 135, 37901 Třeboň, Czech
7 Republic

8 (3) Department of Biological Sciences, University of Bergen, Bergen, Norway

9 (4) Department of Botany, Charles University, Benátská 2, 12801 Prague 2, Czech Republic

10 * Corresponding author: rafaeldscruz@gmail.com

11 Manuscript received _____; revision accepted _____.

12

13 Running title: Morphological evolution of *Valeriana* (Caprifoliaceae)

14 ABSTRACT:

15 Andean species of *Valeriana* are frequently pointed to as an example of island woodiness,
16 i.e., plants of herbaceous ancestors usually evolve woodier forms on islands. We investigated
17 the phenomenon through morphoanatomical and phylogenetic analyses. Plants were collected
18 in the Páramos of Ecuador and had their vegetative morphology described. Stems were
19 sectioned for histological analyses of their anatomy. We mapped the morphoanatomical data
20 plus the maximum reported size for these species and other of the genus, on phylogenetic
21 trees reconstructed based on previously published sequences. Bigger than their Holarctic
22 counterparts, the ancestor of the South American *Valeriana* was likely to have had a
23 maximum of 132 cm, and then after evolved to bigger and smaller sizes in a pattern similar to
24 Brownian motion, as supported by phylogenetic signal values. We classified the collected
25 plants into six growth forms (rosette herbs, semirosette herbs, elevated rosettes, elevated
26 semirosettes, shrubs, and lianas), that are not directly related to variable levels of woodiness,
27 as pointed by our histological analyses. However, the production of unlignified parenchyma
28 in the wood is very frequent, except in older regions of shrubs. The ancestor of the South
29 American *Valeriana* is also very likely to have unlignified parenchyma in its wood.

30

31 KEY WORDS: cambial variant - habits - growth forms - island woodiness - plant anatomy -
32 reconstruction of ancestral character states - tropical alpine - unlignified parenchyma - wood
33 anatomy

34 INTRODUCTION

35 Darwin (1859) was one of the first to recognize that representatives of predominantly
36 herbaceous groups are usually more woody on islands, a phenomenon known as insular
37 woodiness described by several authors (Carlquist, 1965, 1974; Dulin & Kirchoff, 2010).
38 Insular woodiness may be identified as relictual or secondary (Carlquist, 1974; Dulin &
39 Kirchoff, 2010), depending on whether the plants are descendants from a continental woody
40 ancestor or the woodiness on the island evolved from a herbaceous ancestor, respectively.
41 Thus, relictual woodiness should be interpreted as a plesiomorphic state and secondary
42 woodiness should be interpreted as an apomorphic state (Dulin & Kirchoff, 2010). Nürk et al.
43 (2019) show that secondary woodiness can be a key innovation for plants that can drive
44 diversification in island ecosystems. At least 175 evolutionary transitions occurred in islands,
45 indicating that secondary woodiness represents a remarkable aspect of different insular floras
46 (Zizka *et al.*, 2022).

47 The reasons for the secondary origin of woodiness are explained by several hypotheses,
48 among others: climatic mitigation and seasonal release, absence of large herbivores, ecological
49 shift in the transition to forest habitats on some islands, gaining competitive advantage by
50 growing taller and increasing longevity (reviewed in detail by Carlquist, 1974 and by Dulin
51 and Kirchoff, 2010). Zizka et al. (2022) identified reduced herbivory, increased drought, and
52 isolation as the main factors correlated with woodiness in continental islands. Biddick and
53 Burns (2021) propose a mechanism independent of natural selection and explain evolutionary
54 changes in plant size from the islands of the Southwest Pacific by an evolutionary drift, with
55 larger sizes related to woody growth forms. The listed factors are not mutually exclusive and
56 may work together or independently in different taxonomical groups as was suggested for the
57 flora of Canary Island, where 38 independent shifts toward insular woodiness occurred in
58 plants of 15 families (Lens *et al.*, 2013). One of the genera studied by Lens *et al.* (2013) is

59 *Pterocephalus* Vaill. ex Adans. (Caprifoliaceae), an oriental group from the same family of the
60 valerians, plants that are reported as a good example of island woodiness in South America
61 (Carlquist, 1974; Nürk *et al.*, 2019). However, a work similar to Lens *et al.* (2013) is still
62 missing for plants in the New World.

63 The subfamily Valerianoideae Raf. (Caprifoliaceae), formerly known as Valerianaceae
64 Batsch, comprises five genera and ca. 400 species, present mainly in the northern hemisphere
65 and the mountains of South America (Weberling & Bittrich, 2016). Valerianoideae represents
66 the most extensive radiation in Dipsacales, mainly in South America (Bell & Donoghue,
67 2005a,b) which hosts about 250 species of this group (Bell *et al.*, 2012). Eriksen (1989) points
68 out that the largest genus, *Valeriana* L., has most species in South and South America, with a
69 center of diversity along the Andes. The work of Eriksen (1989) itself is one of the greatest
70 contributions to the knowledge of the species from South America, detailing the occurrence
71 and morphology of several species from Ecuador. This diversity is often overlooked, since
72 Valerianoideae has often been considered annuals and herbaceous, based on the morphology
73 of plants from the Northern hemisphere (Stevens, 2001; Lee *et al.*, 2021). However, more
74 detailed plant lists point to the presence of perennial growth forms, such as shrubs and scandent
75 plants, in the Andean *Valeriana* L. (Eriksen, 1989; Bell *et al.*, 2012; Weberling & Bittrich,
76 2016).

77 The colonization of the high Andes is comparable to the colonization of a newly created
78 island system (Vuilleumier, 1970; Smith & Young, 1987; Dulin & Kirchoff, 2010; Nürk *et al.*,
79 2019), as the mountain range is isolated and has similar patterns of diversification compared to
80 the oceanic islands, potentially driving species radiations (Nürk *et al.*, 2019). It is therefore
81 not surprising that *Valeriana* in the Andes, with its more woody representatives, is described
82 as an example of island woodiness (Carlquist, 1974; Nürk *et al.*, 2019). One rapid radiation of
83 Andean *Valeriana* possibly occurred in the Páramo region with a calculated diversification rate

84 of 0.8-1.34 species per Myr (million years), a period related to the uplift of the Andes (Bell &
85 Donoghue, 2005b).

86 The Páramo is a neotropical high mountain ecosystem that concentrates around 6.7%
87 of the endemic plant species of the world (Chuncho Morocho & Chuncho, 2019). The
88 vegetation comprises mainly a gramineous covering, stemmed and acaulescent rosette plants,
89 and cushion plants usually associated with peat bog, occurring above forests and below
90 regional snowlines (Lauer, 1981; Rada *et al.*, 2019). Its origin is related to a rapid rise of the
91 Andes accentuated between 2 and 5 Ma (Gregory-Wodzicki, 2000; Llambí & Soto-W, 2012),
92 occurring mostly in elevations between 3200 and 4000 m above sea level, but sometimes
93 reaching altitudes exceeding 4700 m (Lauer, 1981). This environment reaches freezing
94 temperatures during the night, and high temperatures during the day, reaching 30°C in some
95 locations, varying according to the elevation, with a yearly average temperature between 2°
96 and 10°C, and a yearly precipitation that may vary between 500 mm in dry regions and more
97 than 4000 mm in wet regions (Lauer, 1981; Luteyn, 1999; Llambí & Soto-W, 2012). The
98 regions that receive humidity from the Amazon and the Orinoco basins, as well as from the
99 tropical convergence zone, are very humid and have low pluvial seasonality (e.g., páramos of
100 Colombia and northwest Ecuador). However, the North of the Andes of Venezuela, Colombia,
101 and Cota Rica has a well-marked dry season (Llambí & Soto-W, 2012).

102 Phylogenetic studies of South American *Valerianoideae* (Bell *et al.*, 2012, 2015) and
103 their relationships with other Caprifoliaceae (Lee *et al.*, 2021) based on molecular data
104 reinforce the relatedness and recent diversification of South American *Valeriana*. Growth
105 forms of South American *Valeriana* species are generally mentioned in the descriptive
106 literature, although sometimes only in a general way. For this reason, growth forms frequently
107 need to be deducted from illustrations without a textual description (Meyer, 1951; Eriksen,
108 1989 are examples of descriptions with good supporting pictures), and sometimes not

109 consistent between sources. Wood anatomy studies of these South American plants are mostly
110 lacking, with a few exceptions (Lörcher & Weberling, 1982, 1985; Bach *et al.*, 2014, 2020)
111 which report various patterns of anomalous growth with successive cambia and a large non-
112 fibrous phloem and parenchyma production. These descriptions show high contrast with
113 Valerianoideae species in the Northern Hemisphere, which are characterized by the regular
114 eustelic organization with a single cambium producing inner secondary xylem and outer
115 secondary phloem, with xylem parenchyma scant or absent (Solereeder, 1908; Metcalfe &
116 Chalk, 1957). A detailed study on the anatomy of South American Valerianoideae is necessary
117 since wood anatomy has been proven to be an important source of data for studying evolution
118 (Carlquist, 1980; Onyenedum & Pace, 2021), including its relatedness with specific growth
119 forms (Angyalossy *et al.*, 2012; Pace & Angyalossy, 2013).

120 In this study, we propose the use of *Valeriana* as a model group to improve the
121 knowledge about the evolution of different growth forms, including woody ones, in insular
122 environments. Therefore, to better understand the structural changes related to the rapid
123 diversification of Valerianoideae, we present an investigation of how morphology evolved in
124 *Valeriana* from equatorial South America, mainly centered on three topics: (i) how the size of
125 the aboveground part of the plants evolved in the group, (ii) a detailed study of the growth
126 forms found in representatives of the Páramos, (iii) a detailed anatomical study of these
127 representatives, verifying if the different growth forms are explainable by differential levels of
128 secondary growth. At first, we hypothesized to find an overall increase of lignified tissues and
129 taller plants along the phylogeny of the group, as predicted by the phenomenon of island
130 woodiness. However, our analyses revealed the presence of many complex patterns of wood
131 anatomy related to the presence of non-lignified tissues and the evolution of taller and shorter
132 plants from a relatively tall ancestor.

133

134 MATERIAL AND METHODS

135 **Plant collection and morphological analyses**— Species were sampled in natural stands of
136 Páramo in Ecuador (Table I). Each specimen was dug out of the soil with care to obtain all
137 belowground coarse organs (roots and stems). In the laboratory, plants were evaluated for their
138 morphology and anatomical samples were obtained as stem sections from the oldest and
139 youngest parts of the perennial stem, labeled and stored in 50% ethanol.

140 All plants were perennial having persisting stems aboveground and/or belowground. In order
141 to classify the diversity of growth forms in different categories, we generated the following
142 identification key, based on previous descriptions of *Valeriana* in the Páramo (Eriksen, 1989),
143 and the criteria for basic architectural models described by Klimešova (2018), in order to
144 minimize inconsistencies, although some individuals may slightly deviate from the
145 classification. The growth form was assessed according to the following key, using exclusively
146 macromorphological structures:

- 147 1. Non-persisting aboveground stems, usually green, perennial structures present only in
148 the belowground stem 2
149 Aboveground stem persisting 3
- 150 2. Foliage leaves exclusively in a rosulate arrangement, flowering shoots of axillary or
151 terminal position Rosette herb
152 Foliage leaves in a rosulate arrangement, but not exclusively, foliage leaves also present
153 in a flowering erect shoot of apical position Semirosette herb
- 154 3. Foliage leaves exclusively in a rosulate arrangement elevated by a single trunk-like
155 perennial stem aboveground, with axillary flowering shoots Elevated rosette
156 Foliage leaves not exclusively in a rosulate arrangement, multi-stemmed perennial
157 regions, presence of apical flowering shoots 4

- 158 4. Flowering shoots of apical origin not persisting, new branches grow sympodially from
 159 axillary parts.....Elevated semirosette
 160 Flowering shoots of apical origin loses only inflorescences, keeping persisting
 161 vegetative axes 5
 162 5. Erect plant with rigid stems, ramified near the base..... Shrub
 163 Leaning to climbing plant with voluble stem Liana

164 **Anatomical analyses**— From the material fixed in 50% ethanol, we isolated the youngest and
 165 oldest regions of perennial stems. The material was directly sectioned transversally in a sliding
 166 microtome and double-stained with Astra-blue and safranin (Bukatsch, 1972). Permanent
 167 slides were observed and photographed in an Olympus BX53 microscope with an Olympus
 168 DP73 camera attached.

169 **Phylogenetic analyses**— Sequence data were recovered from data available in the two more
 170 recent phylogenies of South American Valerianoideae and related species to be used as
 171 outgroups (Bell *et al.*, 2012, 2015). The descriptive literature for all the plants was extensively
 172 searched for maximum sizes reported for aboveground plants (height or total length in the case
 173 of climbing plants). As a conservative approach, we rounded down to five meters in cases
 174 where the descriptions state “several meters”. Synonyms were checked on Tropicos.org
 175 (Missouri Botanical Garden, 2021). One voucher per species was selected based on the
 176 occurrence of most of the analyzed molecular markers and the availability of size data. By the
 177 end, we generated a matrix with 130 vouchers and six markers: the nuclear ribosomal ITS, and
 178 cpDNA sequences of trnG, psbM–trnD, accD, ndhJ, and ycf5. Vouchers, sequence accession
 179 numbers, and references for the maximum reported sizes are available in Supplementary
 180 Material (Appendix 1). Sequences were aligned with MAFFT ver. 7 (Kato & Standley, 2013)
 181 and manually adjusted and concatenated in Geneious ver. R10 (Kearse *et al.*, 2012), producing
 182 a matrix of 130 taxa and 3235 characters (available as Supplementary Material as Appendix

183 2). The best partition scheme and model of evolution were selected with PartitionFinder ver. 2
184 (Lanfear *et al.*, 2016), which detected three partitions (first: ndhJ+accD+ycf5+psbMtrnD;
185 second: ITS; and third: trnG) and GTR+ Γ +I as the best model for all of them. A maximum-
186 likelihood tree (ML) was produced with these parameters in RAxML ver. 8 (Stamatakis, 2014)
187 on Cipres (Miller *et al.*, 2010). For support analysis, we ran 1000 bootstrap replications. This
188 tree was used to create an ultrametric tree using the RelTime method (Tamura *et al.*, 2012,
189 2018), implemented in MEGA ver. 11 (Kumar *et al.*, 2018; Mello, 2018), using the values
190 calculated for the origin of *Valerianoideae*, 50–58 Myr old to calibrate the analysis (Lee *et al.*,
191 2021) and the model GTR+ Γ +I. For ancestral character reconstruction of plant size, we used
192 the function “fastANC”, a function that performs (reasonably) fast estimation of the ML
193 ancestral states for continuous traits, of the package phytools, ver. 1.0-3 (Revell, 2012) for R
194 ver. 4.1.3 (R Core Team, 2020), and K-values for phylogenetic signal (Blomberg *et al.*, 2003)
195 were calculated with the function “multiPhylosignal” of the package picante 1.8.2 (Kembel *et*
196 *al.*, 2010). A phylogeny with fewer terminals and more molecular markers was produced for
197 plants with available anatomical data, some of them without size data available, in order to
198 reconstruct the evolution of growth forms and anatomy in these plants. We used all vouchers
199 included in the previous phylogenetic analyses (Bell *et al.*, 2012, 2015) for *Valeriana*
200 *officinalis* (outgroup), *Valeriana adscendens*, *Valeriana convallarioides*, *Valeriana hirtella*,
201 *Valeriana microphylla*, *Valeriana pilosa*, *Valeriana plantaginea*, *Valeriana rigida*, *Valeriana*
202 *tatamana*. ITS, Agt1, Chlp, Hmgs, accD, Matk-trnK intron, ndhJ, psbM-trnD, rpoC1, trnG,
203 ycf5, trnL-F, and psbA-trnH intergenic space were aligned with MAFFT ver. 7 (Katoh &
204 Standley, 2013) and manually adjusted and concatenated in Geneious ver. R10 (Kearse *et al.*,
205 2012). A ML tree was produced with RAxML ver. 8 (Stamatakis, 2014) based on the best
206 partition scheme and models selected with PartitionFinder ver. 2. The habit and anatomy
207 evolution was optimized in this tree with Mesquite ver. 3.7 (Maddison & Maddison, 2019) with

208 the maximum likelihood criterium and the Markov k-state 1 parameter model. These data are
209 compiled as supplementary material (Appendix 3).

210

211 RESULTS

212 In the broader phylogenetic analysis (Fig. 1, Appendix 4), we reconstructed that the
213 common ancestor of the South-American clade lived ca 14.51 (9.12 – 22.68, 95% confidence
214 interval) Myr and reached 132 cm tall of maximum size (Fig. 1, Appendix 5). Inside this group,
215 the size of different lineages decreased or increased, so several processes of gigantism and
216 dwarfism occurred secondarily in the group. We calculated a K-value of 0.92 for the
217 phylogenetic signal for the South America clade, only slightly larger than 0.87 for the core
218 *Valeriana* clade (the sister-group of *Centranthus*), but very distinct from 0.26 calculated for all
219 Valerianoideae (P-values = 0.001 for all the analyses). Thus, the size of *Valeriana*, especially
220 species of the South-American clade, evolved in a pattern similar to what is expected in a
221 Brownian pattern (values close to 1.0), when compared with their relatives elsewhere.

222 According to their architecture, the collected plants were classified into different
223 growth forms. All plants have a perennial woody region at the base and a transient part at the
224 tip of the shoots. The perennial region may be located aboveground or belowground, and the
225 transient region is always aboveground, bearing leaves and inflorescences. For the six
226 identified morphologies, as represented in Fig. 2, we have additional observations for the
227 analyzed specimens:

- 228 • Rosette herbs always present a perennial belowground stem. *Valeriana alypifolia* was
229 reported to have perennial stems aboveground forming a cushion (Eriksen, 1989), but
230 we have not observed this growth form.

- 231 • Semirosette herbs have a perennial belowground stem, with sympodial branching.
232 Some specimens were observed having part of perennial stem aboveground in some
233 localities.
- 234 • Elevated rosettes have the main stem covered by remnants of old leaves, and may be
235 massive, resembling a small *Espeletia* (a genus of Asteraceae that is a notable part of
236 the Páramos landscapes, a silhouette of this plant is shown as a phorophyte for the liana
237 on Fig. 2).
- 238 • Elevated rosettes, elevated semirosettes, shrubs, and lianas do not have belowground
239 stems.
- 240 • Lianas are highly branched at distinct levels.

241 Concerning the anatomy, all analyzed regions are stems (Fig 3), with the typical
242 endarch xylem (protoxylem occurring towards the center, Figs. 4a-b). The elevated semirosette
243 *Valeriana tatamana* is the only plant that does not form a cambium in the young regions. All
244 other plants show diverse levels of woodiness (i.e., secondary growth, with a cambium
245 producing secondary xylem) in the examined regions, even when the plants were recognized
246 as herbs in their growth form. The youngest part of the perennial stem presents the typical
247 conformation of a eudicot with a fibrous wood, i.e., secondary xylem mostly composed of
248 fibers, tracheary elements, and other lignified cells (Gibson, 1973; Melo-De-Pinna, 2009), with
249 few unlignified (and mainly parenchymatic) cells (Fig 4a). Unlignified parenchyma occurs in
250 the leaf gaps, the portions that interrupt the vascular cylinder in the divergence of leaf vascular
251 traces at the nodal regions of the stem (Figs. 4c-d). Considering that the nodes are condensed
252 at the rosulate regions, these gaps are common. They are not anatomically part of the wood,
253 since they are a product of primary and non-vascular growth (derived from the shoot apical
254 meristem), not the vascular cambium. The installation of the cambium starts in the fascicular
255 region, followed by the interfascicular cambium (Fig. 4e).

256 In the oldest part of the perennial stems, the initial wood usually keeps this same
257 structure, suggesting the maintenance of the initial program of secondary development through
258 the organ. However, in most species, after this initial wood, a process of parenchyma
259 proliferation is evident during the late development of the secondary xylem. In these species,
260 the axial or radial structure of the wood comprises large portions of unlignified parenchyma.
261 Based on these structural changes and the differences between them, we detected five different
262 anatomical organizations related to their secondary growth, described below, and illustrated in
263 Fig. 3:

- 264 • Type 1: cambium mostly producing lignified cells centripetally (fibrous wood), with
265 absent or very low proportions of unlignified axial parenchyma.
- 266 • Type 2: cambium producing axial and radial system with portions of lignified
267 parenchyma alternating with unlignified parenchyma.
- 268 • Type 3: cambium producing unlignified parenchyma, mainly in rays.
- 269 • Type 4: successive cambia and inner phloem, with many portions of unlignified
270 parenchyma.

271 We also registered the condition where no cambial activity is present, wood thus absent,
272 with well-developed primary xylem and lignification on the pith. The vascular bundles are
273 closed, i.e., without procambial cells that may give origin to a fascicular cambium.

274 Type 2 is present in all semirosette herbs, as well as in representatives of the other
275 growth forms. In this type, portions of the cambium change their activity from producing
276 lignified cells to production of unlignified parenchyma and vice versa (Fig. 5). These plants
277 may either have a reduced cortical region, as in *Valeriana pilosa* (Fig. 5a) or a prominent
278 cortical region, as in *Valeriana plantaginea* (Fig. 5b).

279 Most shrub species produce type 1 wood for a longer time, later changing to type 3
280 (Fig. 6). The exceptions are *Valeriana imbricata* that changes from type 1 to type 2 when older,
281 and *Valeriana* sp. nov. that has type 2 wood in both young and old stems. In the majority of
282 these plants, a considerable number of lignified elements are produced at first, possibly being
283 responsible for the establishment of the structural function of the xylem, before the formation
284 of unlignified portions of radial parenchyma. This change is abrupt in *V. buxifolia* (Fig. 6a),
285 forming large portions of unlignified parenchyma. These portions are less prominent in *V.*
286 *hirtella* and *V. microphylla* (Figs. 6b-c), which have narrower rays. The phloem in these species
287 may present similar activity (Fig. 6d), although its more detailed structure could not be
288 recovered in most samples. In *Valeriana buxifolia*, non-lignification is restricted to the rays
289 (Fig. 6e).

290 Apart from the general patterns, some of the analyzed species deserve special attention
291 in their description since they differ significantly from all the others in their anatomy.

292 The liana *Valeriana tomentosa* (Fig. 7) has its old stems nested within the type 3
293 anatomy, but with an interesting variation in the lignified portions of the wood. In some
294 branches, the structure is similar to the other type 3 species (Figs. 7a-b). In other branches,
295 there are many unlignified parenchymatic cells within portions with lignified elements,
296 although the cambium also produces portions with exclusively unlignified cells (Figs. 7c-d).

297 The elevated rosette *Valeriana convallarioides* (Fig. 8) presents a cambial variant in all
298 the analyzed stages. Even the young region (Fig. 8a) of the perennial stems has a complex
299 production of secondary tissue with many unlignified cells. The inner part of the vascular
300 cylinder has a cambium (Fig. 8b) that produces small islands of phloem (Fig. 8c), surrounded
301 by the parenchymatic tissue. There are also several successive and not contiguous cambia

302 producing external phloem and internal xylem, both with large portions of unlignified
303 parenchyma (Fig. 8d).

304 The elevated semirosette *Valeriana tatamana* (Fig. 9) has its young portion completely
305 devoid of cambial cells (Fig. 9a). The primary vascular bundles of this region are closed, i.e.,
306 do not have procambial cells capable of producing fascicular cambium. This plant, however,
307 has a well-marked lignification of the outer region of the pith, as well as big portions of the
308 primary xylem (Fig. 9b).

309 Additional small anatomical descriptions are compiled in Fig. 10. Some interesting
310 features are the lignified elements of wood formed only by tracheids in *Valeriana rigida* and
311 the absence of fibers in *Valeriana tomentosa*. Even though, we describe the lignified part of
312 the wood in these plants as fibrous (in accord with Gibson, 1973).

313 In the more restricted phylogenetic analysis (Appendix 3), species that are represented
314 by more than one voucher form a single clade, except *V. pilosa* and *V. plantaginea*. These
315 plants are sustained by short branches and low support values, indicating that the four analyzed
316 vouchers are closely related and possibly the same species. In the reconstruction of ancestral
317 character states (Fig. 11), we found an ambiguous result for growth form in the most recent
318 ancestral taxon of the Ecuadorian *Valeriana*, i.e., it may have been of any of the analyzed states.
319 However, for the anatomy, type 2 (0.41) or type 3 (0.45), forms with unlignified parenchyma
320 in the xylem, were reconstructed as being as more likely to have been present in the old region
321 of the perennial stem of this ancestor. The common ancestral of *V. convallarioides* and *V. rigida*
322 is more likely to have had the type 2 anatomy (0.44). Thus, the type 4 present in *V.*
323 *convallarioides* is probably derived from type 2, by the origin of additional cambia and the
324 formation of inner phloem. The remaining *Valeriana* forms a clade, with an ancestor likely to
325 have type 2 (0.39) or type 3 (0.50) anatomy.

326

327 DISCUSSION

328 As for our phylogenetic analysis, it mostly agrees with previous studies (Bell *et al.*,
329 2012, 2015). However, the relationship between *V. plantaginea* and *V. pilosa* needs to be
330 discussed in detail. According to Eriksen (1989), *V. plantaginea* can approach *V. pilosa* in
331 appearance at low altitudes and on waterlogged soils. In the provided identification key, the
332 plants differ only in bracts and the rough appearance of *V. plantaginea* versus the slender
333 appearance of *V. pilosa*. As the two species do not differ in many aspects (including anatomy
334 and general morphology in some environments) and are so interconnected in their phylogenetic
335 position, special attention should be paid to these two species in future taxonomic revisions of
336 the group.

337 We found that speciation of *Valeriana* in Páramos resulted in a more variable structural
338 organization in plants, including extremes sizes for the whole genus (from cushion plants to
339 several-meters lianas). Instead of the expected increase in sizes from a small ancestor, we
340 detected the evolution of shorter and much taller forms from an already tall ancestor, when
341 compared with its counterparts in other regions. Moreover, we did not find a simple relation
342 between growth form and anatomy. Instead of an increase in lignified tissues, we registered a
343 very diverse organization of unlignified tissues in the wood of plants with different habits. The
344 functional role of lignification in providing support for the plant could only be associated with
345 most shrub species. These species produce young shoots with the rigid tissue necessary for
346 upright growth but produce less rigid tissues when older. The developmental alternation
347 between lignified and unlignified tissues may be the expression of a trade-off between storage
348 (better provided by the unlignified parenchyma) and structural rigidity (better provided by the
349 lignified cells).

350

351 *Plant size and growth forms*

352 The South American *Valeriana* has undergone a rapid diversification over the past 10
353 million years (Bell *et al.*, 2012), among other clades that represent at least three introductions
354 on the South American continent (Bell & Donoghue, 2005b; Bell *et al.*, 2012, 2015). Our main
355 finding about the evolution of plant size is that the Andean plants' ancestor was likely to have
356 been taller than their relatives before the rapid rise of the Andes. Even if these numbers are
357 underestimated (since we rounded down maximum sizes to five meters), these plants were still
358 estimated to have been taller than the ancestors of all other clades of the subfamily. And since
359 this possible ancestor of up to 132 cm in height, several events of increase and decrease in size
360 probably happened. *Valeriana* is often cited as an example of island woodiness (Carlquist,
361 1974; Nürk *et al.*, 2019), however, the presence of very small herbaceous plants (few
362 centimeters, including cushion-like forms) along tall woody plants is often not considered in
363 these studies. Our results revealing random evolutionary drift in plant size (K-values close to
364 1.0) are similar to findings by Biddick & Burns (2021) and are, rather than the classical example
365 of island woodiness, an example of a broader "island rule" that explains variations in size
366 (Biddick *et al.*, 2019). According to this rule, small species become larger and larger species
367 become smaller in islands, and Biddick & Burns (2021) point evolutionary drift as a cause for
368 this phenomenon.

369 The species radiation in *Valeriana* into the Páramos does not appear to have been
370 controlled by selection for larger and more lignified growth forms, but rather by release from
371 restrictions that prohibited independent variation of anatomical and morphological features.
372 For example, some plants may have been restricted in a more seasonal or hostile environment
373 in the past, needing adaptations such as root contraction to keep the vegetative apical meristem

374 close to the soil surface (Wilson & Anderson, 1979; Stevenson, 1980; Cyr *et al.*, 1988),
375 shortening of internodes to produce basal rosettes and prolongation of internodes when apical
376 meristems transfer to the flowering phase (Kawamura *et al.*, 2011; McKim, 2020). Such
377 restriction may be necessary to reconcile every developmental step in the life of the plant
378 growing in an environment under selective pressure but is removed when entering a less
379 seasonal or hostile environment. All of these steps can be triggered by a combination of external
380 and internal factors in seasonal climates such as day length, temperature changes, and plant
381 size. Plants coming to a less seasonal Páramo, for example, could have been released from
382 some of these external constraints so that in different genotypes or epitypes (these signals can
383 be controlled genetically or epigenetically; Grimanelli and Roudier, 2013) there could be
384 greater variability in growth forms. The events under this hypothesis, together with the
385 stochastic model described by Biddick & Burns (2021), could have allowed these plants to
386 independently gain incredible diversity in plant size, growth form, and anatomy found in the
387 group, as well in other plant groups, as happens with *Lupinus* L. in the Andes (Hughes &
388 Eastwood, 2006).

389 The diversity of habits among South American representatives of *Valeriana*, already
390 documented by some authors (Eriksen, 1989; Weberling & Bittrich, 2016) and organized by
391 us in six different forms, can be perceived as variable and depending mainly on the position of
392 perennial organs and their branching patterns. The ambiguous result, in our reconstruction, for
393 the growth form of the ancestor may have been caused by a rapid origin of these different forms
394 during a brief period of speciation in the group. Other possibilities may be our limited sampling
395 of the species within this big family, since the found likelihood values are not conclusive and
396 this diversification pattern may change if more data and sampling is available. In addition,
397 more cryptical reasons for differences in growth forms may occur. In the case of *V. alypifolia*,

398 for example, we only rosette herbs, however bigger growth forms are described for the species
399 and they may be related to hybridization with *V. microphylla* (Eriksen, 1989).

400 During the diversification of the South American *Valeriana*, the Andean region did not
401 reach elevations above 3000 m, although extreme adaptations to frost may have occurred in
402 some areas above 2300 m (van der Hammen & Cleef, 1986; Boschman, 2021). The Páramos
403 palynological registry was rich in Poaceae and Asteraceae at the time, suggesting that open
404 vegetation predominated (van der Hammen & Cleef, 1986; Gregory-Wodzicki, 2000). Past and
405 recent climatic variability of the Andean Páramo, i.e., seasonality in the southern parts and
406 diurnal variations in the equatorial parts (Rada *et al.*, 2019) may be the reason why the
407 ecosystem hosts a large variety of growth forms that can cope with harsh conditions.
408 Densifying the axis of the shoots into a rosette can protect young plant parts near the soil and
409 protect the apical meristem surrounded by leaves and underground axillary buds from frost in
410 winter (temperate regions) or at night (elsewhere). These small plants are more decoupled from
411 air temperatures and usually keep their leaves warmer, while large stature is more coupled with
412 the atmosphere temperature (Sklenář *et al.*, 2016).

413

414 *Plant anatomy and growth forms*

415 Although we hypothesized to find an overall increase of lignified tissues and taller
416 plants along the phylogeny of the group, our analyses revealed the presence of many complex
417 patterns of wood anatomy not coordinated with the growth forms. The most basic anatomical
418 pattern that appears to be preserved in the youngest regions of the perennial stems of the studied
419 species is a fibrous wood with small vessels and little or no parenchyma. This pattern is very
420 similar to that described for Valerianaceae by Metcalfe & Chalk (1957), based on species in

421 the Holarctic region, and can be interpreted by us as relating to support but of little importance
422 for storage in young tissues.

423 Previous studies examining the anatomy of the South American *Valeriana* (Lörcher &
424 Weberling, 1982, 1985; Bach *et al.*, 2014) found different patterns of anomalous growth, with
425 successive cambia and large production of non-fibrous phloem and parenchyma, as we have
426 found. We found successive cambia and inner phloem in *V. convallarioides* as described for *V.*
427 *condamoana*, *V. nivalis*, *V. pycnantha*, and *V. globularis* (Lörcher & Weberling, 1982); *V.*
428 *thalictroides* and *V. micropterina* (Lörcher & Weberling, 1985); and *V. carnosae* and *V.*
429 *clarinifolia* (Bach *et al.*, 2014). Maintaining the internal parts of the phloem may be useful to
430 protect the tissue from adverse environments, such as freezing temperatures or herbivores,
431 including aphids, which have been previously reported to attack the genus (Mooney *et al.*,
432 2012; Sasaki, 2021). Because all these species have a rosulate leaf arrangement, these
433 anatomical variants may complement the protection provided by their external morphology.
434 These cambial variants do not represent disordered growth and have a polyphyletic origin in
435 the angiosperms (Carlquist, 2001), and are particularly common in lianas (Angyalossy *et al.*,
436 2012). Surprisingly, no cambial variants have been described for *Valeriana* lianas in this or
437 previous studies.

438 The scandent species *V. Tomentosa* analyzed by us shows the regular activity of a single
439 cambium, producing outer phloem and inner xylem with a very persistent aboveground stem.
440 The presence of many parts of unligified parenchyma may be justified because this habit does
441 not require supportive tissue since it grows on a phorophyte or other support. The presence of
442 unligified, soft elements blending with rigid elements is quite common in lianas (Angyalossy
443 *et al.*, 2012; Bastos *et al.*, 2016) and is related to the flexibility and torsion resistance of these
444 plants. However, this may be a secondary function, as the non-lianescent ancestor of this group

445 already had these parts of unlignified wood. Thus, these large amounts of soft tissues may have
446 had another primary function during evolution, as storage tissue, for example.

447 Using different strategies of development, the older parts in most of the studied species
448 produce non-lignified elements in their secondary xylem. The vascular parenchyma is a storage
449 tissue (Evert, 2006; Appezzato-da-Glória, 2015) where unlignified cells not only store
450 carbohydrates but are readily permeable to their water-soluble forms. We can thus expect that
451 the main function of the non-fibrous *Valeriana* wood (types 2-4) is storage. However, the
452 occurrence of unlignified parenchyma in axial wood of angiosperms appears to be very limited,
453 including Cactaceae and Portulacaceae from very seasonal Brazilian semi-arid areas (Wheeler
454 *et al.*, 1981, 2020; Melo-De-Pinna, 2009), which allows these plants store water and possibly
455 carbohydrates. A study of species of *Senecio* L. (Asteraceae) from the equatorial Andes,
456 revealed that in representatives of Páramo, hydraulic safety is preferable to efficient
457 transportation, reflected by smaller tracheary elements, that are more resistant to cavitation
458 (Soukup *et al.*, 2021). *Valeriana* also has small tracheary elements, and the presence of storage
459 tissue for water and solutes may help the conductivity regulation.

460

461 CONCLUSION

462 The genus *Valeriana* from Andean Páramos is a good example of insular rule where
463 descendants of common ancestors are characterized by a large array of sizes, growth forms,
464 and anatomy that are not perfectly coordinated together. Next studies should focus on
465 additional regions of the South American Páramo and *Valeriana* of the cushion growth form
466 (very small plants that form compact mats) which includes many described species that were
467 missing among our samples. Moreover, a comparison of the growth forms and anatomy in more

468 species may enlighten how the transition between so disparate patterns may have occurred over
469 such a short period.

470

471 ACKNOWLEDGEMENTS

472 We thank Dr. Bente Eriksen (University of Gothenburg) for the determination of plants and
473 Dr. Charles Bell (University of New Orleans) for providing the original alignments of his
474 phylogenetic studies. AK, PS, and JK thank the funding from the Grant Agency of the Czech
475 Republic (No. 17-12420S) for fieldwork in Páramos. RC and JK acknowledge support from
476 the Praemium Academiae award from the Czech Academy of Sciences of the Czech Republic
477 and the long-term research development project of the Czech Academy of Sciences (No. RVO
478 67985939). JD acknowledges support from the MSMT INTER-EXCELLENCE project (No.
479 LTAUSA18007). The work was supported by research permits issued by the Ministerio del
480 Ambiente, Quito, Ecuador (No. 09-IC-FLO-DNB/MAE).

481

482 AUTHOR CONTRIBUTIONS

483 JK, AK and PS collected the plants in Ecuador, JK analyzed the plants morphologically. PS
484 identified the plants. RC and JD conducted the anatomical analyses. RC conducted
485 phylogenetic analyses. All authors discussed the data and results, and contributed to the final
486 text of the manuscript.

487

488 DATA AVAILABILITY STATEMENT

489 Phylogenetic data, including accession numbers, alignments, and matrices are available as
490 supporting material.

491 Table I. Collection details of the specimens collected in Ecuador between October and November of 2018. Detailed illustrations of plant
 492 morphology are available upon request.

| Species | Locality and elevation (m) | Voucher for population |
|--|----------------------------|--|
| <i>Valeriana adscendens</i> Turcz. | Pato Cocha, 3500 | PRC, Sklenár P., Ptáček J., Klimešova J., Klimeš A. 15545 |
| <i>Valeriana adscendens</i> Turcz. | Guamaní, 4350 | Spirit collection of Institute of Botany, Třeboň, Czech Republic |
| <i>Valeriana alypifolia</i> Kunth | Antisana, 4420 | PRC, P. Sklenár & E. Duskova 12434 |
| <i>Valeriana buxifolia</i> F.G. Mey. | Yacuri, 3400 | PRC, Sklenár P., Ptáček J., Klimešova J., Klimeš A. 15691 |
| <i>Valeriana convallarioides</i> (Schmale) B.B. Larsen | Yacuri, 3780 | PRC, Sklenár P., Ptáček J., Klimešova J., Klimeš A. 15686 |
| <i>Valeriana hirtella</i> Kunth | Tinajillas, 3430 | PRC, Sklenár P., Ptáček J., Klimešova J., Klimeš A. 15507 |
| <i>Valeriana imbricata</i> Killip | Cordoncillo, 3150 | PRC, Sklenár P., Ptáček J., Klimešova J., Klimeš A. 15825 |
| <i>Valeriana microphylla</i> Kunth | Guamaní, 4350 | Spirit collection of Institute of Botany, Třeboň, Czech Republic |
| <i>Valeriana pilosa</i> Ruiz & Pav. | Guamaní, 4350 | Spirit collection of Institute of Botany, Třeboň, Czech Republic |
| <i>Valeriana plantaginea</i> Kunth | Guamaní, 4350 | Spirit collection of Institute of Botany, Třeboň, Czech Republic |
| <i>Valeriana rigida</i> Ruiz & Pav. | Antisana, 4420 | PRC, Zeisek, V., Irazabel, J. V1 |
| <i>Valeriana tatamana</i> Killip | Pato Cocha, 3500 | PRC, Sklenár P., Ptáček J., Klimešova J., Klimeš A. 15659 |
| <i>Valeriana tatamana</i> Killip | Yacuri, 3780 | PRC, Sklenár P., Ptáček J., Klimešova J., Klimeš A. 15733 |
| <i>Valeriana tomentosa</i> Kunth | Tinajillas, 3315 | PRC, Sklenár P., Ptáček J., Klimešova J., Klimeš A. 15839 |
| <i>Valeriana</i> sp. nov. | Yacuri, 3400 | PRC, Sklenár P., Ptáček J., Klimešova J., Klimeš A. 15684 |

493

494

495 **Figure 1.** Maximum likelihood phylogeny of *Valerianoideae*, with branches colored
496 according to maximum likelihood estimation for plant size of ancestors. Occurrences are
497 detailed beside species names and, according to the legend. The ancestral of the South
498 American clade is pointed, with estimated age and size.

499

500 **Figure 2.** Diversity of growth forms of the analyzed species of *Valeriana*, assessed by the
501 identification key found in the methods.

502

503 **Figure 3.** Diversity of anatomical vascular structure in perennial stems of the analyzed
504 species of *Valeriana*, comparing young and old regions. Pairs of circles show combination of
505 types in individual species, upper circle: young part of the perennial stem; lower circle: old
506 part of the perennial stem. Numbers between circles points with up/down arrows the types of
507 anatomical variation found in these plants.

508

509 **Figure 4. a-e.** Structure of perennial stems of *Valeriana*. **a.** *Valeriana adscendens*, with
510 initial secondary growth. Protoxylem poles of the bundles pointed by arrowheads, nearest to
511 the pith (pi). **b.** *Valeriana tomentosa*. Detail of the xylem in a bundle, with protoxylem poles
512 (black arrowheads) in the opposite side of the cambium (white arrowhead) in the beginning
513 of its activity. **c-d.** *Valeriana alypifolia*. **c.** Presence of many parenchymatic gaps (white
514 arrowheads) related to the leaf traces (some of them pointed by black arrowheads) in the
515 condensed nodes of the rosette in the young region. **d.** Persistence of the leaf gaps structure in
516 older region (arrowheads). **e.** *Valeriana adscendens*. The activity of the fascicular cambium

517 (white arrowhead) precedes the installation of the fascicular cambium (black arrowhead).

518 **Bars:** a=100 μm ; b=40 μm ; c=300 μm ; d=200 μm , e=150 μm .

519

520 **Figure 5. a-b.** Old region of perennial stems with the formation of portions of unlignified
521 parenchyma alternating with lignified elements (type 2). **a.** *Valeriana pilosa*. The axial
522 portion of the cambium interchanges its activity (the beginning of the region marked by
523 arrowheads) alternating portions of unlignified parenchyma (white arrowheads) and lignified
524 cells (black arrowheads). **b.** *Valeriana plantaginea*, a semirosette herb with type 2 anatomy in
525 the secondary xylem (2nd xy), with a considerable portion of phloem (ph) and cortical (co)
526 tissue. **Bars:** a=100 μm ; b=400 μm .

527

528 **Figure 6. a-e.** Anatomy of the old perennial stems of *Valeriana* shrubs. **a.** *Valeriana*
529 *buxifolia*. Abrupt transition from type 1 to type 3, forming large portions of unlignified
530 parenchyma in the rays (arrowheads). **b.** *Valeriana hirtella*. Formation of narrower portions
531 of unlignified parenchyma (arrowheads). **c-d.** *Valeriana microphylla*. **c.** Xylem. The
532 formation of unlignified parenchyma (black arrowheads) begins after some production of an
533 amount of fibrous wood, as indicated by the presence of growth rings (some of them pointed
534 by white arrowheads). **d.** Cambial activity. The secondary phloem begins also produce large
535 portions of unlignified parenchyma after the production of fibers (arrowhead). **e.** Large non-
536 lignified rays in tangential section of *Valeriana buxifolia*. **Bars:** a, c=300 μm ; b=400 μm ;
537 d=100 μm ; e=800 μm .

538

539 **Figure 7. a-d.** Anatomy of the old perennial stems of the liana *Valeriana tomentosa*. **a.**
540 General aspect of the type 3 anatomy in branches with strong lignification. **b.** Details of the
541 fibrous portion of the wood. **c.** General aspect of the type 3 anatomy in branches with weak
542 lignification. **d.** Details of the fibrous portion of the wood with many unlignified
543 parenchymatic cells among them. **Bars:** a, c=400 μm ; b, d=40 μm .

544

545 **Figure 8. a-d.** Cambial variant in the elevated rosette *Valeriana convallarioides*. **a.** Young
546 region of the perennial stem with a well established secondary growth, between the cortex (co)
547 and pith (pi). **b-d.** Cross-sections of the older region of perennial stem. The left side of the
548 images are turned toward the inner region of the stem, and the right side to the outer region.
549 **b.** Formation of inner phloem (ip) close to the pith (pi) by inner portions of the cambium
550 (arrowheads). **c.** Detail of the inner islands of phloem (some of them pointed by arrowheads).
551 **d.** Successive cambia, not contiguous, marked with arrowheads. **Bars:** a=500 μm ; b, d =400
552 μm ; c =40 μm .

553

554 **Figure 9. a-b.** Anatomy of the young perennial stems of the elevated semirosette *Valeriana*
555 *tatamana*. **a.** Transition (arrowhead), in a vascular bundle, between primary xylem (below)
556 and primary phloem (above), with no cambial cells. **b.** Vascular bundles (arrowheads), and a
557 large portion of pith with lignified cells (lp). **Bars:** a=40 μm ; b =400 μm .

558

559 **Figure 10.** Additional anatomical description for the analyzed *Valeriana* perennial stems.
560 Non-applicable characters are marked with an en dash (–) in plants with a superscript number,
561 for 1. *Valeriana rigida* (vessels and fibers absent, tracheal elements restrict to tracheids), 2.

562 *Valeriana tatamana* (no secondary growth in the young region), 3. *Valeriana tomentosa*
563 (fibers absent).

564

565 **Figure 11.** Optimization of the habit (left) and type of anatomy in the older region of the
566 perennial stems (right) on the ML tree, using the Mk1 substitution model for categorical
567 characters. Superscript numbers mean the article with the original sequences for these
568 vouchers with ¹ for Bell et al. (2012) and ² for Bell et al. (2015). Ecuadorian species are
569 marked with a green box.

570 REFERENCES

- 571 **Angyalossy V, Angeles G, Pace MR, Lima AC, Dias-Leme CL, Lohmann LG, Madero-Vega C.**
572 **2012.** An overview of the anatomy, development and evolution of the vascular system of lianas. *Plant*
573 *Ecology and Diversity* 5: 167–182.
- 574 **Appezato-da-Glória B. 2015.** *Morphology of plant underground systems*. 3i Editora.
- 575 **Bach H, Valera B, Fortunato R, Wagner M. 2020.** Morfología y anatomía de ‘valerianas’ utilizadas
576 en medicina tradicional argentina. *Plataformas tecnológicas y comerciales para aromáticas*
577 *cultivadas, nativas y medicinales*. Ediciones INTA, 75–80.
- 578 **Bach HG, Varela BG, Fortunato RH, Wagner ML. 2014.** Pharmacobotany of two Valeriana
579 species (Valerianaceae) of Argentinian Patagonia known as ‘Ñancolahuen’. *Latin American Journal*
580 *of Pharmacy* 33: 891–896.
- 581 **Bastos CL, Tamaio N, Angyalossy V. 2016.** Unravelling roots of lianas: A case study in
582 Sapindaceae. *Annals of Botany* 118: 733–746.
- 583 **Bell CD, Calderon G, Gonzalez L, Scholz A, Liede-Schumann S. 2015.** Resolving Relationships
584 within Valerianaceae (Dipsacales): New Insights and Hypotheses from Low-Copy Nuclear Regions.
585 *Systematic Botany* 40: 327–335.
- 586 **Bell CD, Donoghue MJ. 2005a.** Dating the Dipsacales: Comparing models, genes, and evolutionary
587 implications. *American Journal of Botany* 92: 284–296.
- 588 **Bell CD, Donoghue MJ. 2005b.** Phylogeny and biogeography of Valerianaceae (Dipsacales) with
589 special reference to the South American valerians. *Organisms Diversity and Evolution* 5: 147–159.
- 590 **Bell CD, Kutschker A, Arroyo MTK. 2012.** Phylogeny and diversification of Valerianaceae
591 (Dipsacales) in the southern Andes. *Molecular Phylogenetics and Evolution* 63: 724–737.
- 592 **Biddick M, Burns KC. 2021.** A simple null model predicts the island rule. *Ecology Letters* 24: 1646–
593 1654.

594 **Biddick M, Hendriks A, Burns KC. 2019.** Plants obey (and disobey) the island rule. *Proceedings of*
595 *the National Academy of Sciences of the United States of America* 116: 17632–17634.

596 **Blomberg SP, Garland T, Ives AR. 2003.** Testing for phylogenetic signal in comparative data:
597 behavioral traits are more labile. *Evolution* 57: 717–745.

598 **Boschman LM. 2021.** Andean mountain building since the Late Cretaceous: A paleoelevation
599 reconstruction. *Earth-Science Reviews* 220: 103640.

600 **Bukatsch F. 1972.** Bemerkungen zur Doppelfärbung Astrablau-Safranin. *Mikrokosmos* 61: 255.

601 **Carlquist S. 1965.** *Island Life*. New York: The Natural History Press.

602 **Carlquist S. 1974.** *Island Biology*. New York and London: Colombia University Press.

603 **Carlquist S. 1980.** Further Concepts in Ecological Wood Anatomy, with Comments on Recent Work
604 in Wood Anatomy and Evolution. *Aliso* 9: 499–553.

605 **Carlquist S. 2001.** Cambial Variants (Anomalous Secondary Growth). In: Carquist S, ed.
606 *Comparative wood anatomy*. Berlin: Springer-Verlag, 271–295.

607 **Chuncho Morocho C, Chuncho G. 2019.** Páramos del Ecuador, importancia y afectaciones: Una
608 revisión. *Bosques Latitud Cero* 9: 71–83.

609 **Cyr RJ, Lin BL, Jernstedt JA. 1988.** Root contraction in hyacinth. II. Changes in tubulin levels,
610 microtubule number and orientation associated with differential cell expansion. *Planta* 174: 446–452.

611 **Darwin C. 1859.** *On the Origin of Species*. New York: Race Point.

612 **Dulin MW, Kirchoff BK. 2010.** Paedomorphosis, Secondary Woodiness, and Insular Woodiness in
613 Plants. *Botanical Review* 76: 405–490.

614 **Eriksen B. 1989.** 186. Valerianaceae. *Flora of Ecuador* 34: 1–60.

615 **Evert RF. 2006.** *Esau's Plant Anatomy - Meristems, Cells, and Tissues of the Plant Body - Their*
616 *Structure, Function, and Development*. Hoboken: John Wiley & Sons.

617 **Gibson AC. 1973.** Comparative Anatomy of Secondary Xylem in Cactoideae (Cactaceae). *Biotropica*
618 5: 29.

619 **Gregory-Wodzicki KM. 2000.** Uplift history of the Central and Northern Andes: A review. *Bulletin*
620 *of the Geological Society of America* 112: 1091–1105.

621 **Grimanelli D, Roudier F. 2013.** Epigenetics and Development in Plants. *Current Topics in*
622 *Developmental Biology* 104: 189–222.

623 **van der Hammen T, Cleef AM. 1986.** Development of the High Andean Páramo Flora and
624 Vegetation. In: Vuilleumier F,, In: Monasterio M, eds. *High Altitude Tropical Biogeography*. Oxford
625 University Press, 153–201.

626 **Hughes C, Eastwood R. 2006.** Island radiation on a continental scale: Exceptional rates of plant
627 diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences of the*
628 *United States of America* 103: 10334–10339.

629 **Katoh K, Standley DM. 2013.** MAFFT multiple sequence alignment software version 7:
630 improvements in performance and usability. *Molecular biology and evolution* 30: 772–80.

631 **Kawamura K, Hibrand-Saint Oyant L, Crespel L, Thouroude T, Lalanne D, Foucher F. 2011.**
632 Quantitative trait loci for flowering time and inflorescence architecture in rose. *Theoretical and*
633 *Applied Genetics* 122: 661–675.

634 **Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A,**
635 **Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012.** Geneious Basic:
636 An integrated and extendable desktop software platform for the organization and analysis of sequence
637 data. *Bioinformatics* 28: 1647–1649.

638 **Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP,**
639 **Webb CO. 2010.** Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26: 1463–
640 1464.

641 **Klimešová J. 2018.** *Temperate herbs: an architectural analysis*. Czech Academy of Sciences.

642 **Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018.** MEGA X: Molecular evolutionary genetics
643 analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549.

644 **Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016.** PartitionFinder 2: New
645 Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological
646 Phylogenetic Analyses. *Molecular Biology and Evolution*: msw260.

647 **Lauer W. 1981.** Ecoclimatological Conditions of the Paramo Belt in the Tropical High Mountains.
648 *Mountain Research and Development* 1: 209.

649 **Lee AK, Gilman IS, Srivastav M, Lerner AD, Donoghue MJ, Clement WL. 2021.** Reconstructing
650 Dipsacales phylogeny using Angiosperms353: issues and insights. *American Journal of Botany* 108:
651 1122–1142.

652 **Lens F, Davin N, Smets E, del Arco M. 2013.** Insular woodiness on the Canary Islands: A
653 remarkable case of convergent evolution. *International Journal of Plant Sciences* 174: 992–1013.

654 **Llambí LD, Soto-W A. 2012.** Ecología de Páramos. In: Llambí LD,, In: Soto-W A,, In: Célleri R,, In:
655 De Bievre B,, In: Ochora B,, In: Borja P, eds. *Páramos Andinos: Ecología, hidrología y suelos de*
656 *páramos*. Quito: Monsalve Moreno, 292.

657 **Lörcher H, Weberling F. 1982.** Zur Achsenverdickung hochandiner Valerianaceen. *Berichte der*
658 *Deutschen Botanischen Gesellschaft* 95: 57–74.

659 **Lörcher H, Weberling F. 1985.** Zur Achsenanatomie hochandiner Valeriana-Arten (Valeriana
660 micropterina Wedd., V. thalictroides Graebn.). *Flora* 176: 197–212.

661 **Luteyn JL. 1999.** *Páramos: A checklist of Plant Diversity, Geographical Distribution and Botanical*
662 *Literature*. Bronx, New York: The New York Botanical Garden.

663 **Maddison WP, Maddison DR. 2019.** Mesquite: a modular system for evolutionary analysis.

664 **McKim SM. 2020.** Moving on up – controlling internode growth. *New Phytologist* 226: 672–678.

665 **Mello B. 2018.** Estimating timetrees with MEGA and the timetree resource. *Molecular Biology and*

666 *Evolution* 35: 2334–2342.

667 **Melo-De-Pinna GF. 2009.** Non-lignified parenchyma in Cactaceae and Portulacaceae. *Botanical*
668 *Journal of the Linnean Society* 159: 322–329.

669 **Metcalf CR, Chalk L. 1957.** *Anatomy of the Dicotyledons. Leaves, stem, and wood in relation to*
670 *taxonomy with notes on economic uses.* Oxford: University Press.

671 **Meyer FG. 1951.** Valeriana in North America and the West Indies (Valerianaceae). *Annals of the*
672 *Missouri Botanical Garden* 38: 377.

673 **Miller MA, Pfeiffer W, Schwartz T. 2010.** Creating the CIPRES Science Gateway for inference of
674 large phylogenetic trees. *2010 Gateway Computing Environments Workshop (GCE)*: 1–8.

675 **Missouri_Botanical_Garden. 2021.** Tropicos.org.

676 **Mooney KA, Fremgen A, Petry WK. 2012.** Plant sex and induced responses independently
677 influence herbivore performance, natural enemies and aphid-tending ants. *Arthropod-Plant*
678 *Interactions* 6: 553–560.

679 **Nürk NM, Atchison GW, Hughes CE. 2019.** Island woodiness underpins accelerated disparification
680 in plant radiations. *New Phytologist* 224: 518–531.

681 **Onyenedum JG, Pace MR. 2021.** The role of ontogeny in wood diversity and evolution. *American*
682 *Journal of Botany* 108: 2331–2355.

683 **Pace MR, Angyalossy V. 2013.** Wood Anatomy and Evolution: A Case Study in the Bignoniaceae.
684 *International Journal of Plant Sciences* 174: 1014–1048.

685 **R Core Team. 2020.** R: A language and environment for statistical computing.

686 **Rada F, Azócar A, García-Núñez C. 2019.** Plant functional diversity in tropical Andean páramos.
687 *Plant Ecology and Diversity* 12: 539–553.

688 **Revell LJ. 2012.** phytools: An R package for phylogenetic comparative biology (and other things).
689 *Methods in Ecology and Evolution* 3: 217–223.

690 **Sasaki D. 2021.** First record of *Myzus (Nectarosiphon) persicae* (Sulzer, 1776) (Hemiptera:
691 Aphididae) occurring on *Valeriana fauriei* (Caprifoliaceae). *Russian Entomological Journal* 30: 247–
692 251.

693 **Sklenář P, Kučerová A, Macková J, Romoleroux K. 2016.** Temperature Microclimates of Plants in
694 a Tropical Alpine Environment: How Much does Growth Form Matter? *Arctic, Antarctic, and Alpine*
695 *Research* 48: 61–78.

696 **Smith AP, Young TP. 1987.** Tropical Alpine Plant Ecology. *Annual Review of Ecology and*
697 *Systematics* 18: 137–158.

698 **Solereder H. 1908.** *Systematic Anatomy of the Dicotyledons*. Oxford: Clarendon Press.

699 **Soukup A, Pecková E, Ježková B, Sklenář P. 2021.** Structural adaptations in plants from the humid
700 equatorial Andes indicate a trade-off between hydraulic transport efficiency and safety. *American*
701 *Journal of Botany* 108: 2127–2142.

702 **Stamatakis A. 2014.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of large
703 phylogenies. *Bioinformatics* 30: 1312–1313.

704 **Stevens PF. 2001.** Angiosperm Phylogeny Website. Version 14, July 2017.

705 **Stevenson D. 1980.** Observations on root and stem contraction in cycads (Cycadales) with special
706 reference to *Zamia pumila* L. *Botanical Journal of the Linnean Society* 81: 275–281.

707 **Tamura K, Battistuzzi FU, Billings-Ross P, Murillo O, Filipski A, Kumar S. 2012.** Estimating
708 divergence times in large molecular phylogenies. *Proceedings of the National Academy of Sciences of*
709 *the United States of America* 109: 19333–19338.

710 **Tamura K, Tao Q, Kumar S. 2018.** Theoretical foundation of the reftime method for estimating
711 divergence times from variable evolutionary rates. *Molecular Biology and Evolution* 35: 1770–1782.

712 **Vuilleumier F. 1970.** Insular Biogeography in Continental Regions. I. The Northern Andes of South
713 America. *The American Naturalist* 104: 373–388.

714 **Weberling F, Bittrich V. 2016.** Valerianaceae. *The Families and Genera of Vascular Plants -*
715 *Volume XIV Aquifoliales, Boraginales, Bruniales, Dipsacales, Escalloniales, Garryales,*
716 *Parachyphiales, Solanales (except convolvulaceae), Icacinaceae, Metteniusaceae, Vahliaceae.*385–
717 401.

718 **Wheeler EA, Bass P, Gasson PE. 1981.** *IAWA list of microscopic features for hardwood*
719 *identification.*

720 **Wheeler EA, Gasson PE, Baas P. 2020.** Using the InsideWood web site: Potentials and pitfalls.
721 *IAWA Journal* 41: 412–462.

722 **Wilson K, Anderson GJH. 1979.** Further observations on root contraction. *Annals of Botany* 43:
723 665–675.

724 **Zizka A, Onstein RE, Rozzi R, Weigelt P, Kreft H, Steinbauer MJ, Bruelheide H, Lens F. 2022.**
725 The evolution of insular woodiness. *Proceedings of the National Academy of Sciences of the United*
726 *States of America* 119: 1–11.

727