The effect of elevational gradient on alpine gingers (*Roscoea alpina* and *R. purpurea*) in the Himalayas

Babu Ram Paudel [Corresp., 1, 2, 3] , **Adrian G Dyer** [4] , **Jair E Garcia** [4] , **Mani Shrestha** [4]

[1] Yunnan Key Laboratory of Plant Reproductive Adaption and Evolutionary Ecology, Yunnan University, Kunming, Yunnan, China

[2] Laboratory of Ecology and Evolutionary Biology, State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming, Yunnan, China

[3] Department of Botany, Prithvi Narayan Campus, Tribhuvan University, Pokhara, Gandaki, Nepal

[4] School of Media and Communication, RMIT University, Melbourne, Victoria, Australia

Corresponding Author: Babu Ram Paudel
Email address: babu@xtbg.ac.cn

There is currently enormous interest in how morphological and physiological responses of herbaceous plants may be affected by changing elevational gradient. Mountain regions provide an excellent opportunity to understand how closely related species may adapt to the conditions that rapidly change with elevation. We investigated the morphological and physiological responses of two Himalayan alpine gingers (*Roscoea alpina* and *R. purpurea*) along two different vertical transects of 400 m, *R. purpurea* between 2174- 2574 m a.s.l and *R. alpina* between 2675-3079 m a.s.l . We measured the variables of plant height, leaf length, leaf area, specific leaf area, and stomata density at five plots, along the vertical transect at an elevational gap of ca. 100 m. Results revealed that with increased elevation plant height, and leaf area decreased while stomata density increased, whereas changes in specific leaf area, were not correlated with the elevation. Our results reveal that these alpine gingers undergo local adaptation by modifying their plant height, leaf area and stomata density in response to the varying selection pressure associated with the elevational gradient. Thus, the findings of this research provide valuable information on how a narrow range of elevational gradient affects the herbaceous plants at the alpine habitat of the Himalayas.

1   RESEARCH ARTICLE

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3   *purpurea*) in the Himalayas

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5   Babu Ram Paudel[1,2,3*], Adrian G. Dyer[4], Jair E. Garcia[4], and Mani Shrestha[4]

6   [1]Yunnan Key Laboratory of Plant Reproductive Adaption and Evolutionary Ecology, Yunnan

7   University, Kunming, China.

8   [2] Laboratory of Ecology and Evolutionary Biology, State Key Laboratory for Conservation and

9   Utilization of Bio-Resources in Yunnan, Yunnan University, Kunming,650091, Yunnan, P.R.

10   China

11   [3]Department of Botany, Prithvi Narayan Campus, Tribhuvan University, Pokhara, Nepal

12   [4] School of Media and Communication, RMIT University, Melbourne, Victoria 3001, Australia

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14   *For correspondence: brp2033@gmail.com

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Abstract

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28 herbaceous plants may be affected by changing elevational gradient. Mountain regions provide

29 an excellent opportunity to understand how closely related species may adapt to the conditions

30 that rapidly change with elevation. We investigated the morphological and physiological

31 responses of two Himalayan alpine gingers (*Roscoea alpina* and *R. purpurea*) along two

32 different vertical transects of 400 m, *R. purpurea* between 2174- 2574 m a.s.l and *R. alpina*

33 between 2675-3079 m a.s.l. We measured the variables of plant height, leaf length, leaf area,

34 specific leaf area, and stomata density at five plots, along the vertical transect at an elevational

35 gap of ca. 100 m. Results revealed that with increased elevation plant height, and leaf area

36 decreased while stomata density increased, whereas changes in specific leaf area, were not

37 correlated with the elevation. Our results reveal that these alpine gingers undergo local

38 adaptation by modifying their plant height, leaf area and stomata density in response to the

39 varying selection pressure associated with the elevational gradient. Thus, the findings of this

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41 herbaceous plants at the alpine habitat of the Himalayas.

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45 Short title: Response of alpine gingers with the elevational gradient in the Himalayas

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Introduction

The elevational gradient is one of the key environmental factors that affect growth, morphology and physiology of plants (Cordell et al., 1998; Hultine & Marshall, 2000; Qiang et al., 2003). The elevational gradient in alpine regions provides a sharp environmental change across relatively short spatial distances because small changes in elevation can lead to a large shift in temperature, humidity, exposure, and concentration of atmospheric gases (Hovenden & Vander Schoor, 2004). Thus, alpine environments can provide useful natural avenues to investigate the response of plants to a suite of climatic conditions that are representative of the broader latitudinal range (Montesinos-Navarro et al., 2011). With the increase in elevation, there is typically an increase in both precipitation and light intensity including changes in distributions of short wavelength UV-A (315-400 nm) and UV-B (280-315 nm) radiation (Diffey, 1991; Rozema et al., 1997) whilst temperature and concentration of carbon dioxide and oxygen decrease (Friend & Woodward, 1990). These environmental variations may potentially alter the morphology and physiology of plants to endure the different stresses linked with changing elevation (Hovenden & Brodribb, 2000; Körner, 2007).

The alpine environment is potentially affected by climate change associated with global warming, and thus alpine plants may face rapidly changing environmental conditions that likely impose different stress levels on plants (Beniston, 2003; Byars, Papst & Hoffmann, 2007). Thus, based on the adaptative plasticity, the plant species exhibit local adaptation by altering the morphological and/or physiological traits over the range of elevational gradient (Hirano, Sakaguchi & Takahashi, 2017). For example, local adaptations of plants in response to variable climatic conditions at different elevations may result in variation of plant height and leaf length (Wang & Gao, 2004). Variation in carbon assimilation, energy balance and water relations along

70   the elevational gradient could result in variation of leaf morphological and physiological traits

71   such as leaf area, specific leaf area (SLA) and stomata density (Ackerly et al., 2002). Therefore,

72   the study of the variation in the growth forms, morphology and physiology of a plant species along

73   an elevational gradient could provide valuable insights on how plants may respond to

74   environmental stress imposed by rapid changes in climatic conditions (Premoli & Brewer, 2007;

75   Körner, 2007; Bresson et al., 2011).

76   Although several previous studies have documented the effects of elevational gradient on

77   the growth, morphology and physiology of the plants, most of the studies are focused on tree

78   species (Cordell et al., 1998; Hultine & Marshall, 2000; Li et al., 2008). Recently a few studies

79   have been conducted to understand how the elevational gradients affect the herbaceous plants

80   (Gonzalo-Turpin & Hazard, 2009; Scheepens, Frei & Stöcklin, 2010; Hulshof et al., 2013; Bastida,

81   Rey & Alcántara, 2015; Takahashi & Matsuki, 2017; Kiełtyk, 2018). These studies particularly

82   focused on the variation of a specific trait, such as vegetative trait, reproductive trait or leaf trait.

83   Currently however, there is a lack of empirical evidence on the adaptative potential of herbaceous

84   plants along the elevational gradient in steep environments such as the Himalayas. As the alpine

85   ecosystem in the Himalayas is likely to experience the adverse effects of the changing climate

86   associated with global warming and anthropogenic disturbances (Beniston, 2003; Byars, Papst &

87   Hoffmann, 2007), understanding the performance of herbaceous plants along the elevational

88   gradient provides important insights for the enhanced prediction of the response of herbaceous

89   plants under altered climatic conditions.

90   The genus *Roscoea*, with 22 known species, is a Himalayan endemic alpine perennial herb

91   and the only alpine member of the predominately-tropical family Zingiberaceae (Cowley, 1982,

92   2007). The genus is distributed between the elevations of ca 1500 to 4500 m a.s.l (Cowley, 2007),

93   thus serving as a key model for how herbaceous plants respond to the potentially stressful

94   environmental conditions associated with increasing elevation. All *Roscoea* species are small

95   herbs with annual leafy shoots produced from a reduced erect rhizome (Cowley, 1982, 2007).

96   Among the *Roscoea* species, *R. alpina Royle* and *R. purpurea Smith* are widely distributed in the

97   Himalayan Mountains from Kashmir (Pakistan) in the west through Nepal, India, Bhutan and

98   Tibet. As these two *Roscoea* species are widely distributed from low to high elevations,

99   characterization of the variation in morphological and physiological traits along the elevational

100   gradient will help to understand how these alpine gingers respond to changes in climatic conditions

101   associated with elevation. In this study, we explore the changes in the morphological (plant height

102   and leaf length) and physiological variables (leaf area, SLA and stomata density) of these alpine

103   gingers along the well-defined elevational gradient in the Himalayan mountain range.

Materials and Methods

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Study species

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106   The two widespread *Roscoea* species used in this study were *R. alpina* and *R. purpurea*

107   (Fig. 1). *Roscoea alpina* is a common species with a wide distribution between the elevations

108   2130-4270 m a.s.l in the Himalayan range from Kashmir (Pakistan) in the west through Bhutan in

109   the east. The annual pseudostem may grow up to 12-20 cm high and presents flowers from the end

110   of May to mid-August (Cowley, 2007). It has 2-3 obtuse sheathing leaves. Leaves are usually 1-2

111   in number and underdeveloped; occasionally the plant may bear up to four well-developed leaves.

112   Leaves are linear, broadly elliptic or lanceolate. Only the first leaf is slightly auriculate and widest

113   at the base while rest of the leaves are widest at the middle, with 17- 25 cm in length. Leaves are

114   usually glabrous but young leaves are occasionally hairy at acute apex. Inflorescences are without

115   exserted peduncle. Flowers are deep purple to white in appearance for a human observer (Fig. 1).

116   A single plant can develop up to five flowers, however only one flower blooms at a time. Obtuse

117   to almost truncate bracts are shorter than the ovary. The calyx is much longer than the bract and

118   bluntly bi-dentate. A long corolla tube is exserted from the calyx (Cowley, 2007).

119   *Roscoea purpurea* is also a widespread member of the Himalayan *Roscoea*, distributed

120   between the elevations 1520-3100 m a.s.l. from Himachal Pradesh (India) in the west through

121   Assam/Bhutan in the east. The annual erect pseudostem is most variable in habit and form and

122   may grow up to 25-38 cm high, bearing 0-2 obtuse to truncate sheathing leaves. Leaves are usually

123   4-8 in number, lanceolate to oblong-ovate and 14-20 cm long with acuminate and sometimes with

124   ciliated apex. Lower leaves are slightly auriculate at the base. The plant flowers from the end of

125   June to early September (Cowley, 2007). The inflorescence is enclosed in upper leaf sheaths with

126   only the upper part of bracts and flowers visible. Flowers are light purple or white with purple

127   markings. Usually, 1-2 flowers open at a time. Bracts longer than calyx with acute apex which is

128   pale green. The sharply bi-dentate and apiculate calyx is usually pale green and sometimes marked

129   with pink. The corolla tube has a mauve or white colouration and is hardly exserted from the calyx

130   (Cowley, 2007).

131   Study sites

132   The research was conducted along an elevational gradient at two sites, Daman and

133   Ghorepani, Central Nepal (Fig. 2). Daman is located in Makawanpur district and forms a part of

134   the Mahabharat mountain range (mountains lower than the Himalayas). This site lies about 70 km

135   south-west of Kathmandu and is midway between Kathmandu and Hetauda. The vegetation type

136   of this site typically comprises a mixed forest of *Pinus*, *Rhododendron* and *Quercus*. The site

137   experiences cool temperate to subalpine climate with warm summers and cold winters that

138   typically incur mild to heavy snowfall from November to February (Pers. Obs. BRP). Ghorepani,

139   located in Myagdi district, is about 270 km west of Kathmandu. The vegetation type of this site

140   comprises a mixed forest of *Pinus* and *Rhododendron*. The site has a subalpine climate and cool

141   weather throughout the year, and heavy snowfall from November to February (Pers. Obs. BRP).

142   The geographical coordinates and the elevations of the study sites are presented in Table 1.

143   Measurement of traits

144   The field sampling was conducted from May to August 2014 and repeated the sampling

145   again in 2017 (May to August). Five sampling plots were selected along a vertical transect from

146   2174 to 2574 m for *R. purpurea* and from 2675 to 3079 m for *R. alpina*. The sampling was done

147   in a counterbalanced random fashion such that two adjacent sampling plots were at an elevation

148   gap of ca 100 m. Plant height and leaf length were measured to examine the morphological

149   variables. Physiological variables included leaf area, specific leaf area (SLA) and stomata density.

150   At each sampling plot, a horizontal transect of 100 m length was laid down and twenty plants were

151   randomly selected along the horizontal transect in such a way that the distance between the

152   adjacent sampling plant was at least 5 m. A standard metric ruler was used to measure plant height

153   (the distance from the ground to the topmost part of the stem). The largest leaf of every sampled

154   plant was removed and leaf length was measured with a ruler. We used a graph paper to trace and

155   quantify the area of each leaf, enabling robust repeatable measurements in remote locations.

156   Specifically, two alternative methods were used to measure the area of the leaf. In 2014, the area

157   of the leaf was measured after wet storage, while in 2017 the area was measured on the freshly

158   plucked leaf. To prevent the leaves from possible shrinkage during wet storage, the leaves were

159   first flattened if necessary and carefully placed in between the folds of a paper. Resulting samples

160   were then placed in a sample box to avoid the external light and heat sources. For both respective

161   leaf collection methods, the leaf (either wet stored or freshly plucked) was placed on a graph paper,

162   its outline was sketched and the number of squares enclosed within the leaf-outline were counted.

163   Complete and greater than half squares were scored, whilst squares less than half a square were

164   excluded. The measurements were repeated several times for each leaf to enable a robust field

165   measurement of leaf area. Twenty leaves at each sampling plot were measured to assess variability.

166   The area of leaf as measured by two alternative methods did not differ significantly (t test, P>0.05),

167   thus data generated from the freshly removed leaf were used for further analysis. All collected

168   leaves were gently pressed between the folds of an absorbent paper for five days to flatten the leaf

169   surface and to absorb any excess moisture. The pressed leaves were subsequently oven dried at the

170   university laboratory for 48 hours at 70^0 C. Dry leaf weight was measured using a digital electronic

171   balance (Fameway International (HK) Limited; accuracy 0.001g). Specific leaf area (SLA) of a

172   leaf was calculated as the ratio of the area of a fresh leaf and its dry weight and expressed in cm^2/g.

173   To determine the stomatal count, transparent nail polish was applied on the middle dorsal

174   surface of a fresh leaf. After a few minutes, when nail polish had dried, a thin layer was peeled

175   from the middle dorsal surface of a leaf. The peeled layers were separately preserved in a 10%

176   glycerine solution for about 72 hours. In the laboratory, the temporary slide of each layer was

177   prepared using safranin as a staining agent. The stained layers were individually mounted on

178   microscope slides, and all stomata observed under a 10-x magnification microscopic field were

179   counted. The stomata counts were repeated at three different microscopic fields to ensure the exact

180   measurement of the stomata density. Area of the microscopic field was calculated using the

181   formula $A= \pi r^2$ where r is the radius of microscopic field and density of stomata was calculated as

182   the number of stomata under a microscopic field divided by the area of the microscopic field. The

183   stomata density was expressed in terms of number per square millimetre.

184   Statistical Analyses

185   An independent sample *t* test was used to test the variation in measured traits between the

186   years. Data from each of the measured morphological variables were summarized as Q-Q plots

187   and tested for normality. Exploratory data analyses revealed that some of the response variables

188   were not normally distributed and were better described by a Gamma distribution as most data

189   consisted of positive values larger than zero (Zuur, Hilbe & Ieno, 2013). Consequently, non-

190   parametric correlation analyses were performed among the five different traits measured for each

191   species implementing Kendall's tau statistic (τ). This coefficient was chosen as it has a known

192   standard error and provides a better estimate with low sample size. After the exploratory

193   analyses, generalised linear regression models (GLM) were applied to test for the potential

194   effects of elevation on the different traits measured for each species. For the five regression

195   models, elevation was used as a predictor and it was assumed that the response variable followed

196   a Gamma distribution. Link function for each model was selected based on a comparison of AIC

197   scores obtained after fitting models implementing different link functions (Zuur, Hilbe & Ieno,

198   2013). Regression analyses were performed using the routine *glm* available as part of the base

199   distribution of the R language and environment for statistical computing (version 3.3.1) (R Core

200   Team, 2015).

201   Results

202   Correlation analyses

203   Our results indicated that all the measured variables did not differ significantly between

204   years ($P>0.05$), thus only 2017 data were used for further analyses. For *R. purpurea*, there was a

205   significant correlation between stomata density and the variables of leaf area ($P < 0.001$) and

206   specific leaf area (SLA) ($P = 0.020$) (Fig. 3). In *R. alpina*, leaf length was correlated with all

207 remaining variables (Fig. 4). Consequently, we separately performed the regression analyses for

208 the two species for each of the measured response variables.

209 ## Variations of traits with elevation

210 Leaf length of *R. alpina* and plant height significantly decreased with increasing elevation

211 (P = 0.001 for leaf length and P= 0.017 for plant height). The same trend was observed for leaf

212 area (P < 0.001), while stomata density increased with elevation (P = 0.005). SLA values for this

213 species were not significantly correlated with elevation (P = 0.114) (Fig. 5).

214 Plant height and leaf area significantly decreased, while stomata density increased, with

215 increasing elevation in *R. purpurea* (P = 0.044, P = 0.001 and, P = 0.002 for plant height, leaf area

216 and stomata density respectively). However, we did not find a significant relationship of elevation

217 either with leaf length (P = 0.471) or with SLA (P = 0.555) (Fig. 5). Details on the regression

218 analysis including coefficients and associated 95% confidence intervals are provided in Table 2

219 and Table 3.

220

221 # Discussion

222 ## Variations of morphological traits with elevation

223 In the current study, we found a significant decrease in plant height of both species of

224 *Roscoea* (*R. alpina* and *R. purpurea*) with increased elevation. Reduction of plant height in these

225 alpine gingers with increased elevation is consistent with several previous findings reported for

226 tree species (Körner, 1998; Cordell et al., 1998; Kronfus & Havranek, 1999; Paulsen, Weber &

227 Korner, 2000; Kogami et al., 2001; Li, Yang & Kräuchi, 2003; Shi et al., 2006) and herbaceous

228 species (Takahashi & Matsuki, 2017; Kiełtyk, 2018). Similarly, a decrease of leaf length of *R.*

229 *alpina* with the increased elevation in the current study is consistent with the previous findings

230   (Hansen-Bristow, 1986; Schoettle, 1990; Kajimoto, 1993; Kao & Chang, 2001; Kiełtyk, 2018).

231   Based on the present result, we conclude that the elevational gradient has a significant effect on

232   the growth form of these alpine gingers. At the lower elevation, environmental conditions are

233   likely to be more favourable for optimum plant growth. The reduction of plant height and leaf

234   length of these two alpine gingers with increasing elevation reflects the morphological adaptation

235   to increased environmental stresses such as low concentration of carbon dioxide, decreased

236   temperature, higher solar radiation and/or low water availability (Wang & Gao, 2004; Davis, Shaw

237   & Etterson, 2005; Guerin, Wen & Lowe, 2012). The observed relatively smaller plants with shorter

238   leaf characteristics of these gingers at higher elevation thus may reflect local adaptation at a higher

239   altitude to enable reduction of transpiration and maintain efficient utilization of water (Ackerly et

240   al., 2002; Royer et al., 2008; Peppe et al., 2011; Guerin, Wen & Lowe, 2012).

241   Variation of physiological traits with Elevation

242   Leaf traits variation with elevation:

243   Our findings revealed that variation in leaf area showed a significant but negative

244   correlation with elevation, while the correlation between SLA and elevation was non-significant.

245   Consistent with our result, Kouwenberg, Kurschner and McElwain (2007) found a decreasing

246   trend in the leaf area of *Quercus kelloggii* with increasing elevation. Our result on the variation of

247   leaf characters (leaf area and SLA) with the elevation is partially consistent with the previous

248   findings reported by Hultine and Marshall (2000); Scheepens, Frei and Stöcklin (2010); Hulshof

249   et al., (2013); Bastida, Rey and Alcántara, (2015), while the findings of Gonzalo-Turpin and

250   Hazard (2009) indicate a different effect. Previous studies have suggested that the environment at

251   higher elevations is characterized by higher solar radiation, lower water availability and lower

252   stomatal conductance (Parkhurst & Loucks, 1972; Givnish & Vermeij, 1976; Ackerly et al., 2002).

253   Under such potentially stressful environmental conditions, small leaf size provides optimum

254   adaptation to the plants by reducing boundary layer resistance and maintaining favorable leaf

255   temperature and high photosynthetic water use efficiency (Renzhong et al., 2001). Thus, decreased

256   leaf area of these alpine gingers with increased elevation may reflect an adaptation for the

257   increased environmental stress and may be favourable to reduce water loss and maintain efficient

258   use of absorbed water (Renzhong et al., 2001). In addition, some authors have implicated

259   increasing UVB radiation levels as having a damaging effect on certain plant structures (Jansen,

260   Gaba & Greenberg, 1998; Rozema, Aerts & Cornelissen, 2002); and there is some evidence of this

261   affecting plant growth in some lowland terrestrial species (Rozema, Aerts & Cornelissen, 2002).

262   These topics may be of high value to explore in alpine environments where there are likely large

263   changes in UV levels. SLA is closely associated with leaf thickness, which mediates the trade-off

264   between light capture, water loss and diffusion of carbon dioxide (Oberle & Schaal, 2011). Higher

265   SLA leaves are thicker and contain more photosynthetic enzymes and there is more demand for

266   carbon dioxide per unit area. Thus, stomata density increases to supply the higher demand for

267   carbon dioxide. Consequently, the increase in SLA may be an advantage for carbon dioxide uptake.

268   Non-linear change of SLA of both species along the elevational gradient may indicate that

269   environmental factors associated with altitude alone cannot regulate the trade –off between light

270   capture, water loss and diffusion of carbon dioxide in these alpine gingers. The smallest SLA of

271   *R. alpina* at 2674 m a.s.l and *R. purpurea* at 2374 m a.s.l may indicate limited carbon gain and

272   supply due to poor availability of resources and may be associated with the least productive zone

273   of these species where retention of captured resources and protection from desiccation is of high

274   priority (Wilson, Thompson & Hodgson, 1999).

275   Variation in Stomata density with Elevation

276   We found a significant increase in stomata density of both species (*R. alpina* and *R.*

277   *purpurea*) with increased elevation. Many authors have made comprehensive efforts to relate the

278   variation in stomata density along elevation gradients and have obtained different results.

279   Consistent to our current results, Körner and Cochrane (1985); Friend and Woodward (1990);

280   Hovenden and Brodribb (2000); and Kouwenberg, Kurschner and McElwain (2007) have found

281   that stomata density increased linearly with elevation. Li et al. (2006) found that stomata density

282   of *Quercus aquifolioides* increased linearly up to the height of 2800 m a.s.l., whilst above that

283   height, it decreased linearly. Schoettle and Rochelle (2000) found that the stomata density of *Pinus*

284   *flexilis* decreased linearly with altitude whilst Woodward (1986) did not observe any significant

285   change in stomata density of *Vaccinium myrtillis* considering altitudes from 200 to 1100 m asl. The

286   significant increase in stomata density with increasing elevation in our findings may be associated

287   with lower availability of carbon dioxide, higher UV-B and long wave radiation, all reducing

288   photosynthetic efficiency by decreasing stomatal absorption and conductance (Kouwenberg,

289   Kurschner & McElwain, 2007; Körner, 2007). To adapt to such a harsh environmental conditions

290   and maintain vitalities, stomata density of these gingers may have increased. The increase in

291   stomata density provides compensation against the reduced stomatal conductance and carbon

292   dioxide partial pressure to maintain photosynthetic efficiency (Kao & Chang, 2001; Kouwenberg,

293   Kurschner & McElwain, 2007; Körner, 2007).

294   Our results indicate two major patterns in the vegetative traits of these alpine gingers with

295   increased elevation: a significant decrease of leaf area and a significant increase of stomata density.

296   These variations provide compensation to cope with the change in the concentration of

297   atmospheric carbon dioxide, temperature, humidity and light at higher altitudes (Van de Water,

298 Leavitt & Betancourt, 1994; Hultine & Marshall, 2000; Qiang et al., 2003). A non-significant

299 correlation between SLA and stomata density may suggest that leaf thickness have little role in

300 regulating the carbon dioxide uptake and transpiration in these two alpine gingers. A negative

301 correlation of stomata density with leaf area has previously indicated that with the increase of

302 stomata density at a higher elevation, narrowing of leaves may reduce excess transpiration (Herms

303 & Mattson, 1992; Etterson & Shaw, 2001). The closely correlated variation in these two traits thus

304 maintains a likely trade-off between photosynthesis and transpiration and provides local adaptation

305 to the specific conditions, at different elevations.

306 Conclusions

307   Growth, morphology and physiology of *R. alpina* and *R. purpurea* were found to have a

308 significant association with altitude. These alpine gingers exhibit optimum growth at their

309 respective lowermost distribution range, and their growth response retards with increasing

310 elevation. Based on the present result, it can be concluded that these alpine gingers favour shorter

311 height, smaller leaf and higher stomata density at a higher elevation to adapt with the stressful

312 factors associated with the change in elevational gradients. Variation in those traits at different

313 elevations may reflect the response to the combined selection pressure of different abiotic and

314 biotic factors that may generate different micro-environmental conditions at the respective

315 elevation. Decreased growth forms and leaf area of these alpine gingers at a higher altitude may

316 indicate a selection response to reduce water loss from the plant body during transpiration while

317 increased stomata density may indicate the adaptation to cope with the decreased concentration of

318 carbon dioxide. The closely correlated modification of these traits at different elevations may have

319 played a significant role in providing local adaptation to these alpine gingers.

320

321 ## Acknowledgements

326 ## References

327 Ackerly DD, Knight CA, Weiss SB, Barton K, Starmer KP. 2002. Leaf size, specific leaf area

328   and microhabitat distribution of chaparral woody plants: contrasting patterns in species

329   level and community level analyses. *Oecologia* 130:449–457. DOI:

330   10.1007/s004420100805.

331 Bastida JM, Rey PJ, Alcántara JM. 2015. Local adaptation to distinct elevational cores

332   contributes to current elevational divergence of two *Aquilegia vulgaris* subspecies. *Journal*

333   *of Plant Ecology* 8:273–283. DOI: 10.1093/jpe/rtu017.

334 Beniston M. 2003. Climatic change in Mountain regions: a review of possible impacts. *Climate*

335   *change* 59:5–31.

336 Bresson CC, Vitasse Y, Kremer A, Delzon S. 2011. To what extent is altitudinal variation of

337   functional traits driven by genetic adaptation in European oak and beech? *Tree physiology*

338   31:1164–74. DOI: 10.1093/treephys/tpr084.

339 Byars SG, Papst W, Hoffmann A a. 2007. Local adaptation and cogradient selection in the alpine

340   plant, *Poa hiemata*, along a narrow altitudinal gradient. *Evolution; international journal of*

341   *organic evolution* 61:2925–41. DOI: 10.1111/j.1558-5646.2007.00248.x.

342 Cordell S, Goldstein G, Mueller-Dombois D, Webb D, Vitousek PM. 1998. Physiological and

343   morphological variation in *Metrosideros polymorpha*, a dominant Hawaiian tree species,

344   along an altitudinal gradient: the role of phenotypic plasticity. *Oecologia* 113:188–196.

345   DOI: 10.1007/s004420050367.

346   Cowley EJ. 1982. A revision of *Roscoea* (Zingiberaceae). *Kew Bulletin* 36:747–777.

347   Cowley EJ. 2007. *The genus Roscoea*. Royal Botanic Garden Kew, UK.

348   Davis MB, Shaw RG, Etterson JR. 2005. Evolutionary responses to changing climate. *Ecology*

349   86:1704–1714.

350   Diffey BL. 1991. Solar ultraviolet radiation effects on biological systems. *Physics in Medicine*

351   *and Biology* 36:299–328.

352   Etterson JR, Shaw RG. 2001. Constraint to aAdaptive evolution in response to global warming.

353   *Science* 294:151–154. DOI: 10.1126/science.1063656.

354   Friend AD, Woodward FI. 1990. Evolutionary and ecophysiological responses of mountain

355   plants to the growing season environment. *Advances in Ecological Research* 20:59–124.

356   Givnish TJ, Vermeij GJ. 1976. Sizes and shapes of liane leaves. *The American naturalist*

357   110:743–778.

358   Gonzalo-Turpin H, Hazard L. 2009. Local adaptation occurs along altitudinal gradient despite

359   the existence of gene flow in the alpine plant species *Festuca eskia*. *Journal of Ecology*

360   97:742–751. DOI: 10.1111/j.1365-2745.2009.01509.x.

361   Guerin GR, Wen H, Lowe AJ. 2012. Leaf morphology shift linked to climate change. *Biology*

362   *Lett.*:1–5.

363   Hansen-Bristow K. 1986. Influence of increasing elevation on growth characteristics at

364   timberline. *Canadian Journal of Botany* 64:2517–2523.

365   Herms DA, Mattson WJ. 1992. The dilemma of plants-to grow or defend. *Q. Rev. Biol.* 67:283–

366   335.

367   Hirano M, Sakaguchi S, Takahashi K. 2017. Phenotypic differentiation of the *Solidago virgaurea*

368   complex along an elevational gradient: insights from a common garden experiment and

369   population genetics. *Ecology and Evolution* 7:6949–6962. DOI: 10.1002/ece3.3252.

370   Hovenden MJ, Brodribb T. 2000. Altitude of origin influences stomatal conductance and

371   therefore maximum assimilation rate in Southern Beech, *Nothofagus cunninghamii. Aust. J.*

372   *Plant Physiol.* 27:451–456.

373   Hovenden MJ, Vander Schoor JK. 2004. Nature vs nurture in the leaf morphology of Southern

374   beech, *Nothofagus cunninghamii* (Nothofagaceae). *New phytologist* 161:585–594.

375   Hulshof CM, Violle C, Spasojevic MJ, Mcgill B, Damschen E, Harrison S, Enquist BJ. 2013.

376   Intra-specific and inter-specific variation in specific leaf area reveals the importance of

377   abiotic and biotic drivers of species diversity across elevation and latitude. *Journal of*

378   *Vegetation Science* 24:921–931. DOI: 10.1111/jvs.12041.

379   Hultine KR, Marshall JD. 2000. Altitude trends in conifer leaf morphology and stable carbon

380   isotope composition. *Oecologia* 123:32–40. DOI: 10.1007/s004420050986.

381   Jansen MAK, Gaba V, Greenberg B. 1998. Higher plants and UV-B radiation: balancing

382   damage, repair and acclimation. *Trends in Plant Science* 3:131–135. DOI:

383   http://dx.doi.org/10.1016/S1360-1385(98)01215-1.

384   Kajimoto T. 1993. Shoot dynamics of *Pinus pumila* in relation to altitudinal and wind exposure

385   gradients on the Kiso mountain range, central Japan. *Tree physiology* 13:41–53.

386   Kao WY, Chang KW. 2001. Altitudinal trends in photosynthetic rate and leaf characteristics of

387   *Miscanthus populations* from central Taiwan. *Aust. J. Bot.* 49:509–514.

388   Kiełtyk P. 2018. Variation of vegetative and floral traits in the alpine plant *Solidago minuta*:

389   evidence for local optimum along an elevational gradient. *Alpine Botany* 128:47–57. DOI:

390   10.1007/s00035-017-0197-7.

391   Kogami H, Hanba YT, Kibe T, Terashima I, Masuzawa T. 2001. CO_2 transfer conductance, leaf

392   structure and carbon isotope composition of *Polygonum cuspidatum* leaves from low and

393   high altitudes. *Plant, Cell and Environment* 24:529–538. DOI: 10.1046/j.1365-

394   3040.2001.00696.x.

395   Körner C. 1998. A re-assessment of high elevation treeline positions and their explanation.

396   *Oecologia* 115:445–459.

397   Körner C. 2007. The use of "altitude" in ecological research. *Trends in ecology & evolution*

398   22:569–74. DOI: 10.1016/j.tree.2007.09.006.

399   Körner C, Cochrane PM. 1985. Stomatal responses and water relations of *Eucalyptus paucitlora*

400   in summer along an elevational gradient. *Oecologia* 66:443–455.

401   Kouwenberg LLR, Kurschner WM, McElwain JC. 2007. Stomatal frequency change over

402   altitudinal gradients: prospects for paleoaltimetry. *Reviews in Mineralogy and Geochemistry*

403   66:215–241. DOI: 10.2138/rmg.2007.66.9.

404   Kronfus H, Havranek WM. 1999. Effects of elevation and wind on the growth of *Pinus cembra*

405   L . in a subalpine afforestation. *Phyton(Austria)* 39:99–106.

406   Li M-H, Xiao W-F, Shi P, Wang S-G, Zhong Y-D, Liu X-L, Wang X-D, Cai X-H, Shi Z-M.

407   2008. Nitrogen and carbon source-sink relationships in trees at the Himalayan treelines

408   compared with lower elevations. *Plant, cell & environment* 31:1377–1387. DOI:

409   10.1111/j.1365-3040.2008.01848.x.

410   Li MH, Yang J, Kräuchi N. 2003. Growth responses of *Picea abies* and *Larix decidua* to

411   elevation in subalpine areas of Tyrol, Austria. *Canadian Journal of Forest Research*

412   33:653–662. DOI: 10.1139/X02-202.

413 Li C, Zhang X, Liu X, Luukkanen O, Berninger F. 2006. Leaf morphological and physiological

414   responses of *Quercus aquifolioides* along an Altitudinal Gradient. *Silva Fennica* 40:5–13.

415 Montesinos-Navarro A, Wig J, Pico FX, Tonsor SJ. 2011. *Arabidopsis thaliana* populations

416   show clinal variation in a climatic gradient associated with altitude. *New phytologist*

417   189:282–294. DOI: 10.1111/j.1469-8137.2010.03479.x.

418 Oberle B, Schaal BA. 2011. Responses to historical climate change identify contemporary

419   threats to diversity in *Dodecatheon*. *Proceedings of the National Academy of Sciences of the*

420   *United States of America* 108:5655–60. DOI: 10.1073/pnas.1012302108.

421 Parkhurst D, Loucks O. 1972. Optimal leaf size in relation to environment. *Journal of Ecology*

422   60:505–537.

423 Paulsen J, Weber UM, Korner C. 2000. Tree growth near treeline : abrupt or gradual reduction

424   with altitude ? *Arct. Antarc. Alp. Res.* 32:14–20.

425 Peppe DJ, Royer DL, Cariglino B, Oliver SY, Newman S, Leight E, Enikolopov G, Fernandez-

426   Burgos M, Herrera F, Adams JM, Correa E, Currano ED, Erickson JM, Hinojosa LF,

427   Hoganson JW, Iglesias A, Jaramillo C a, Johnson KR, Jordan GJ, Kraft NJB, Lovelock EC,

428   Lusk CH, Niinemets U, Peñuelas J, Rapson G, Wing SL, Wright IJ. 2011. Sensitivity of leaf

429   size and shape to climate: global patterns and paleoclimatic applications. *New phytologist*

430   190:724–39. DOI: 10.1111/j.1469-8137.2010.03615.x.

431 Premoli AC, Brewer CA. 2007. Environmental vs. genetically driven variation in

432   ecophysiological traits of *Nothofagus pumilio* from contrasting elevations. *Australian*

433   *Journal of Botany* 55:585. DOI: 10.1071/BT06026.

434 Qiang W, Xun-ling W, Chen T, Hu-yuan F, Li-zhe A, Yuan-qin H, Gang W. 2003. Variations of

435   stomatal density and carbon isotope values of *Picea crassifolia* at different altitudes in the

436   Qilian Mountains. *Trees* 17:258–262. DOI: 10.1007/s00468-002-0235-x.

437 R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for

438   Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

439 Renzhong W, Ripley EA, Yuangang Z, Shaoquan N. 2001. Demography of reproductive and

440   biomass allocation of grassland and dune *Leymus chinensis* on the Songnen Plain, north-

441   eastern China. *Journal of Arid Environments* 49:289–299. DOI: 10.1006/jare.2000.0787.

442 Royer DL, McElwain JC, Adams JM, Wilf P. 2008. Sensitivity of leaf size and shape to climate

443   within *Acer rubrum* and *Quercus kelloggii*. *New Phytologist* 179:808–817. DOI:

444   10.1111/j.1469-8137.2008.02496.x.

445 Rozema J, Aerts R, Cornelissen H (eds.). 2002. *Plants and Climate Change*. Vrije Universiteit,

446   Amsterdem,The Netherlands.

447 Rozema J, Staaij van de J, Björn L., Caldwell M. 1997. UV-B as an environmental factor in

448   plant life: stress and regulation. *Trends in Ecology & Evolution* 12:22–28.

449 Scheepens JF, Frei ES, Stöcklin J. 2010. Genotypic and environmental variation in specific leaf

450   area in a widespread alpine plant after transplantation to different altitudes. *Oecologia*

451   164:141–150. DOI: 10.1007/s00442-010-1650-0.

452 Schoettle AW. 1990. The interaction between leaf longevity and shoot growth and foliar biomass

453   per shoot in *Pinus contorta* at two elevations. *Tree physiology* 7:209–214.

454 Schoettle AW, Rochelle SG. 2000. Morphological variation of *Pinus flexilis* (Pinaceae), a bird-

455   dispersed pine, across a range of elevations. *American Journal of Botany* 87:1797–1806.

456   DOI: 10.2307/2656832.

457 Shi Z, Liu S, Liu X, Centritto M. 2006. Altitudinal variation in photosynthetic capacity,

458   diffusional conductance and 13 C of butterfly bush (*Buddleja davidii*) plants growing at

459   high elevations. *Physiologia Plantarum* 128:722–731. DOI: 10.1111/j.1399-

460   3054.2006.00805.x.

461   Takahashi K, Matsuki S. 2017. Morphological variations of the *Solidago virgaurea* L. complex

462   along an elevational gradient on Mt Norikura, central Japan. *Plant Species Biology* 32:238–

463   246. DOI: 10.1111/1442-1984.12148.

464   Wang R, Gao Q. 2004. Morphological responses of *Leymus chinensis* (Poaceae) to the large-

465   scale climatic gradient along the North-east China Transect (NECT). *Diversity and*

466   *Distributions* 10:65–73. DOI: 10.1111/j.1472-4642.2004.00056.x.

467   Van de Water PK, Leavitt SW, Betancourt JL. 1994. Trends in stomatal density and 13C/12C

468   ratios of *Pinus flexilis* needles during last Glacial-Interglacial Cycle. *Science* 264:239–243.

469   DOI: 10.1126/science.264.5156.239.

470   Wilson PJ, Thompson K, Hodgson JG. 1999. Specific leaf area and leaf dry matter content as

471   alternative predictors of plant strategies. *New phytologist* 143:155–162.

472   Woodward FI. 1986. Ecophysiological studies on the shrub *s* L. taken from a wide altitudinal

473   range. *Oecologia* 70:580–586.

474   Zuur AF, Hilbe JM, Ieno EN. 2013. *A Beginner's Guide to GLM and GLMM with R*. Highland

475   Statistics, Newburgh, UK.

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Figure 1

Figure 1

Study species *Roscoea purpurea* (A) and *R. alpina* (B) in their natural habitat.

Figure 2

Figure 2

Map of the study area: Yellow hexagons represent the study site (Ghorepani) of Roscoea alpina whereas Light-Oliventine triangles represent the study site (Daman) of R. purpurea (See Table 1 for detail). The top map represents the elevational gradient of study locations.
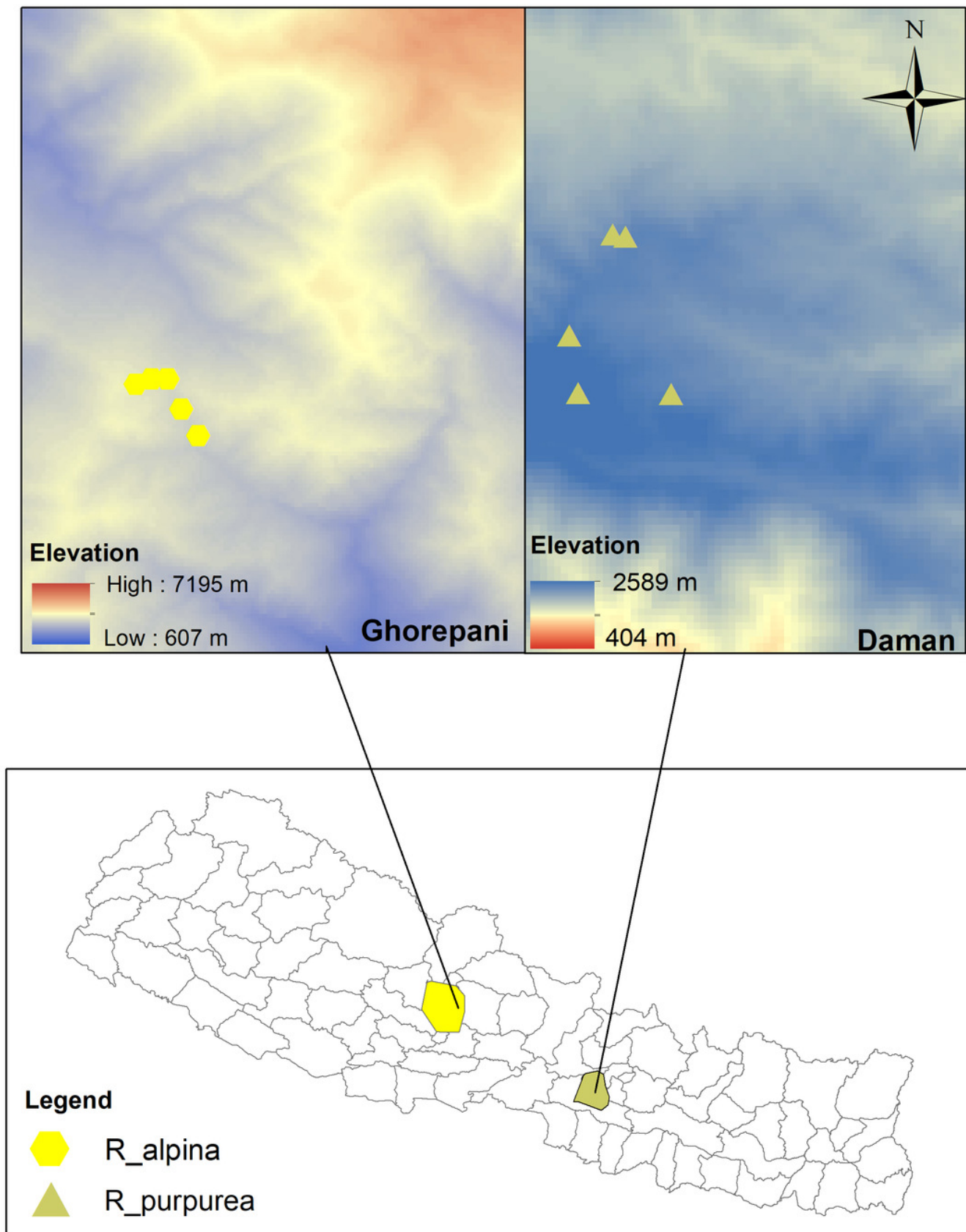
Figure 3

Figure 3

Correlations between the different traits of *R. purpurea* measured at five different elevations.

a) τ (Kendall's tau (τ) = -0.08, $P = 0.41$); Leaf length (cm) vs Plant height (cm)
b) $\tau = 0.13, P = 0.20$; Leaf area (cm^2) vs Plant height (cm)
c) $\tau = 0.07, P = 0.5$; Specific leaf area (cm^2/g) vs Plant height (cm)
d) $\tau = -0.11, P = 0.27$; Stomata density (number/mm^2) vs Plant height (cm)
e) $\tau = 0.19, P = \mathbf{0.05}$; Leaf area (cm^2) vs Leaf length (cm)
f) $\tau = -0.12, P = 0.25$; Specific leaf area (cm^2/g) vs Leaf length (cm)
g) $\tau = -0.02, P = 0.87$; Stomata density (number/mm^2) vs Leaf length (cm)
h) $\tau = 0.08, P = 0.45$; Specific leaf area (cm^2/g) vs Leaf area (cm^2)
i) $\tau = -0.33, P = \mathbf{0.001}$; Stomata density (number/mm^2) vs Leaf area (cm^2)
j) $\tau = 0.24\ P = \mathbf{0.02}$; Stomata density (number/mm^2) vs Specific leaf area (cm^2/g)
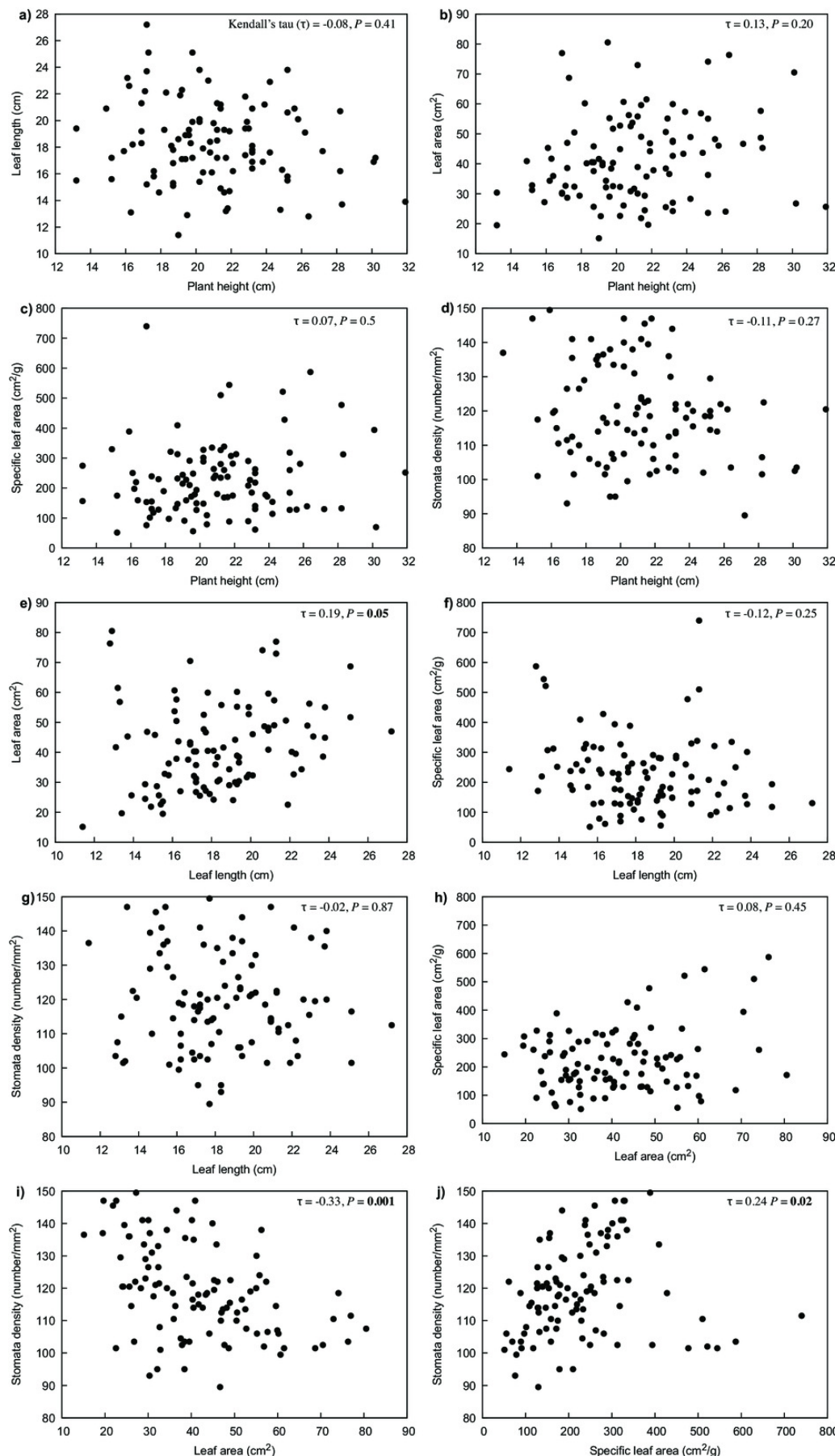
Figure 4

Figure 4

Correlations between the different traits of *R. alpina* measured at five different elevations.
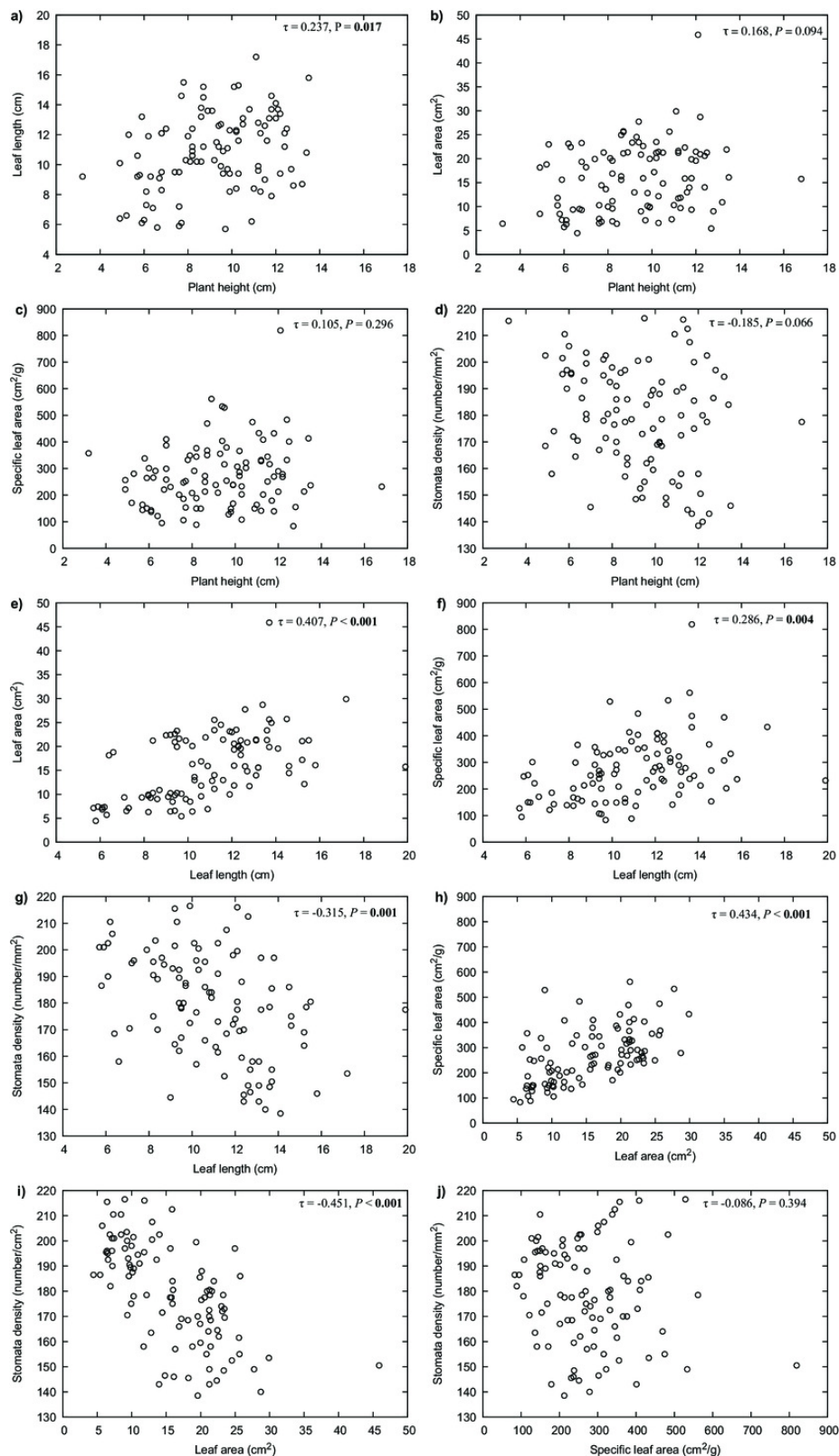
Figure 5

Figure 5

Generalised linear regression models showing the effect of elevation on plant height (fig. a), leaf length (fig. b), leaf area (fig. c), specific leaf area (fig. d) and, stomata density (fig. e) for *R. purpurea* (blue line with filled markers) and *R. alpina* (purple line with empty markers). Markers indicate the mean value of the corresponding trait at each elevation and error bars indicate standard deviation. Solid lines represent the regression function for each trait and species. A significant correlation of elevation on the value for each trait is indicated by two asterisks (**) while a non-significant correlation of elevation is indicated by "NS" above the corresponding regression line.
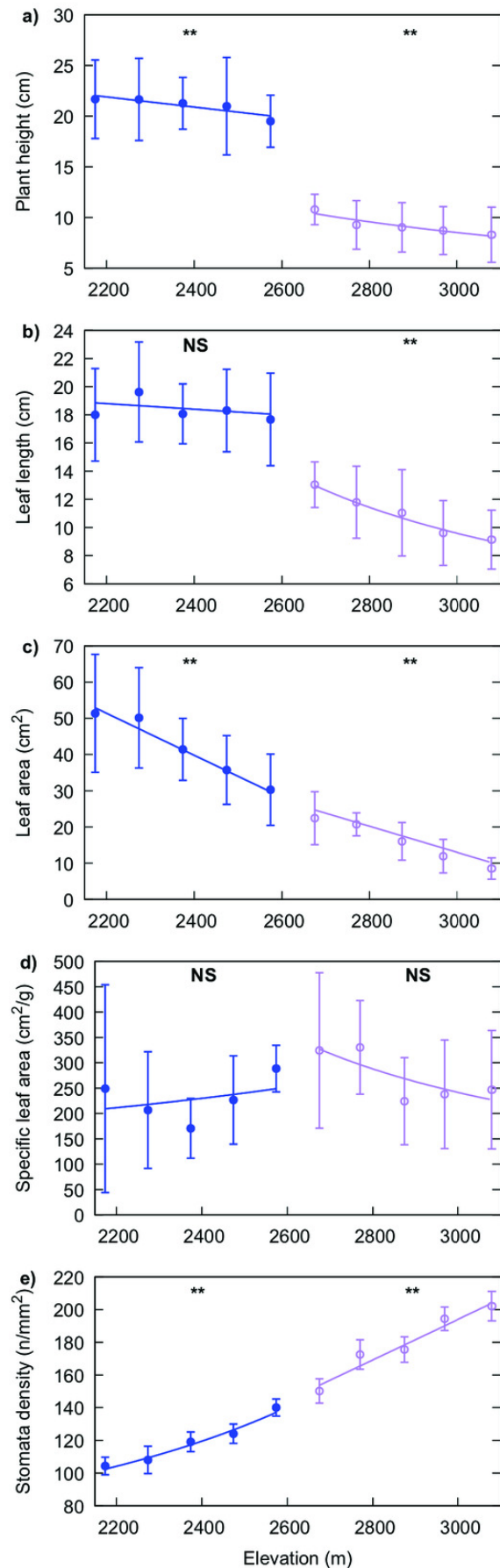
Table 1(on next page)

Table 1

Geographical details of the study sites.

Table 1: Geographical details of study sites.

	Roscoea purpurea			*R. alpina*		
	Latitude	Longitude	Elevation (a.s.l.)	Latitude	Longitude	Elevation (a.s.l.)
	27°36'45.7"	85°5'32"	2174 m	28°23'21.9"	83°42'22.1"	2675 m
	27°36'44.7"	85°5'37.6"	2274 m	28°23'42.2"	83°42'9.2"	2770 m
	27°36'2.1"	85°5'13.4"	2374 m	28°24'4.9"	83°41'58.9"	2874 m
	27°35'37.1"	85°5'57.3"	2474 m	28°24'5.2"	83°41'46.8"	2968 m
	27°35'37.4"	85°5'17.3"	2574 m	28°24'0.9"	83°41'34.7"	3079 m

Table 2(on next page)

Table 2

Results of regression analysis between various traits of *R. alpina* and elevation.

1   **Table 2:** Results of regression analysis between various traits of *R. alpina* and elevation.

2

Traits	Parameters	Coefficients and 95 % Cis			Distribution	link	P
		2.5	50	97.5			
Plant height	m	3.85E-05	6.48E-05	9.12E-05	Gamma	Inverse	
	b	-1.52E-01	-7.71E-02	-1.85E-05			0.017
Leaf length	m	6.99E-05	8.37E-05	9.75E-05	Gamma	Inverse	
	b	-1.87E-01	-1.47E-01	-1.08E-01			**<0.001**
Leaf area	m	-4.00E-02	-3.60E-02	-3.30E-02	Gamma	Identity	
	b	1.09E+02	1.21E+02	1.33E+02			**<0.001**
SLA	m	3.91E-07	3.33E-06	6.31E-06	Gamma	Inverse	
	b	-1.43E-02	-5.85E-03	2.55E-03			0.114
Stomata Density	m	9.10E-02	1.24E-01	1.58E-01	Gaussian	Identity	
	b	-2.75E+02	-1.78E+02	-8.15E+01			0.005

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Table 3(on next page)

Table 3

Results of regression analysis between various traits of *R. purpurea* and elevation.

1   **Table 3:** Results of regression analysis between various traits of *R. purpurea* and elevation.

Traits	Parameters	Coefficients and 95 % Cis			Distribution	link	P
		2.5	50	97.5			
Plant height	m	-7.90E-03	-5.00E-03	-2.10E-03	Gaussian	Identity	0.044
	b	2.59E+01	3.29E+01	3.99E+01			
Leaf length	m	-7.00E-03	-2.00E-03	3.00E-03	Gamma	Identity	0.47
	b	1.15E+01	2.32E+01	3.49E+01			
Leaf area	m	-6.70E-02	-5.80E-02	1.55E+02	Gamma	Identity	**<0.001**
	b	-4.80E-02	1.79E+02	2.03E+02			
SLA	m	-7.55E-06	-1.90E-06	3.71E-06	Gamma	Inverse	0.55
	b	-4.40E-03	8.91E-03	2.26E-02			
Stomata Density	m	-7.31E-02	-6.18E-06	-5.05E-06	Gamma	Inverse	0.002
	b	2.04E-02	2.32E-02	2.59E-02			

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