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

2 The effect of elevational gradient affect on alpine gingers (*Roscoea alpina* and *R.*

3 *purpurea*) in the Himalayas

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

13 *For correspondence: brp2033@gmail.com

Abstract

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.

42

43

44

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

46

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

68   climatic conditions at different elevations may result in variation of plant height and leaf length

69   (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

104

Study species

105

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   ciliate 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* (*Pinus roxburghii*), *Rhododendron* (*R.*

137   *arboreuum, R. campanualatum*) and *Quercus (Q. semecarpifolia, Q. lanata)*. The site experiences

138   cool temperate to subalpine climate with warm summers and cold winters that typically incur mild

139   to heavy snowfall from November to February (Pers. Obs. BRP). Ghorepani, located in Myagdi

140   district, is about 270 km west of Kathmandu. The vegetation type of this site comprises a mixed

141   forest of *Pinus (P. wallichiana), Abies* (*A. spectabilis*) and *Rhododendron (R. arboretum, R.*

142   *barbatum, R campanulatum, R antohopogon* at upper limit). The site has a subalpine climate and

143   cool weather throughout the year, and heavy snowfall from November to February (Pers. Obs.

144   BRP). The geographical coordinates and the elevations of the study sites are presented in Table

145   1.

146   Measurement of traits

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

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

149   2174 to 2574 m a.s.l. for *R. purpurea* and from 2675 to 3079 m a.s.l. for *R. alpina*. The sampling

150   was done in a counterbalanced random fashion such that two adjacent sampling plots were at an

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

152   morphological variables. Physiological variables included leaf area, specific leaf area (SLA) and

153   stomata density. At each sampling plot, a horizontal transect of 100 m length was laid down and

154   twenty plants were randomly selected along the horizontal transect in such a way that the distance

155   between the adjacent sampling plant was at least 5 m. A standard metric ruler was used to measure

156   plant height (the distance from the ground to the topmost part of the stem). The largest leaf of

157   every sampled plant was removed and leaf length was measured with a ruler. We used a graph

158   paper to trace and quantify the area of each leaf, enabling robust repeatable measurements in

159   remote locations. Specifically, two alternative methods were used to measure the area of the leaf.

160   In 2014, the area of the leaf was measured after wet storage, while in 2017 the area was measured

161   on the freshly plucked leaf. To prevent the leaves from possible shrinkage during wet storage, the

162   leaves were first flattened if necessary and carefully placed in between the folds of a paper.

163   Resulting samples were then placed in a sample box to avoid the external light and heat sources.

164   For both respective leaf collection methods, the leaf (either wet stored or freshly plucked) was

165   placed on a graph paper, its outline was sketched and the number of squares enclosed within the

166   leaf-outline were counted. Complete and greater than half squares were scored, whilst squares less

167   than half a square were excluded. The measurements were repeated several times for each leaf to

168   enable a robust field measurement of leaf area. Twenty leaves at each sampling plot were measured

169   to assess variability. The area of leaf as measured by two alternative methods did not differ

170   significantly (*t* test, P>0.05), thus data generated from the freshly removed leaf were used for

171   further analysis. All collected leaves were gently pressed between the folds of an absorbent paper

172   for five days to flatten the leaf surface and to absorb any excess moisture. The pressed leaves were

173   subsequently oven dried at the university laboratory for 48 hours at 70^0 C. Dry leaf weight was

174   measured using a digital electronic balance (Fameway International (HK) Limited; accuracy

175   0.001g). Specific leaf area (SLA) of a leaf was calculated as the ratio of the area of a fresh leaf and

176   its dry weight and expressed in cm^2/g.

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

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

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

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

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

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

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

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

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

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

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

188 Statistical Analyses

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

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

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

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

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

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

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

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

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

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

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

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

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

202 2013). Regression analyses were performed using the routine *glm* available as part of the base

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

204 Team, 2015).

205 Results

206 Correlation analyses

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

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

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

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

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

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

213 Variations of traits with elevation

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

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

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

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

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

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

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

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

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

223 and Table 3.

224

Discussion

Variations of morphological traits with elevation

In the current study, we found a significant decrease in plant height of both species of *Roscoea* (*R. alpina* and *R. purpurea*) with increased elevation. Reduction of plant height in these alpine gingers with increased elevation is consistent with several previous findings reported for tree species (Körner, 1998; Cordell et al., 1998; Kronfus & Havranek, 1999; Paulsen, Weber & Korner, 2000; Kogami et al., 2001; Li, Yang & Kräuchi, 2003; Shi et al., 2006) and herbaceous species (Takahashi & Matsuki, 2017; Kiełtyk, 2018). Similarly, a decrease of leaf length of *R. alpina* with the increased elevation in the current study is consistent with the previous findings (Hansen-Bristow, 1986; Schoettle, 1990; Kajimoto, 1993; Kao & Chang, 2001; Kiełtyk, 2018). Based on the present result, we conclude that the elevational gradient has a significant effect on the growth form of these alpine gingers. At the lower elevation, environmental conditions are likely to be more favourable for optimum plant growth. The reduction of plant height and leaf length of these two alpine gingers with increasing elevation reflects the morphological adaptation to increased environmental stresses such as low concentration of carbon dioxide, decreased temperature, higher solar radiation and/or low water availability (Wang & Gao, 2004; Davis, Shaw & Etterson, 2005; Guerin, Wen & Lowe, 2012). The observed relatively smaller plants with shorter leaf characteristics of these gingers at higher elevation thus may reflect local adaptation at a higher altitude to enable reduction of transpiration and maintain efficient utilization of water (Ackerly et al., 2002; Royer et al., 2008; Peppe et al., 2011; Guerin, Wen & Lowe, 2012).

245   Variation of physiological traits with Elevation

246   Leaf traits variation with elevation:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

279 Variation in Stomata density with Elevation

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

309 to the specific conditions, at different elevations.

310 ## Conclusions

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

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

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

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

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

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

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

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

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

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

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

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

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

324

Acknowledgements

326   We are thankful to the Department of Plant Resources, Thapathali, Kathmandu for

327   providing research permission. We thank Mr Kul Prasad Lamichhane, Kapil Paudel, and Dipesh

328   Baral for assistance in the field. We thank Dr Subodh Adhikari for the constructive comments on

329   the earlier version of the manuscript. We thank Dr Lalina Muir for proof reading the manuscript.

330   We also thank Editor Dr Gabriele Casazza, reviewer Dr Marco Porceddu and an anonymous

331   reviewer for their constructive suggestion.

References

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

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

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

336   10.1007/s004420100805.

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

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

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

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

341   *change* 59:5–31.

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

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

344   31:1164–74. DOI: 10.1093/treephys/tpr084.

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

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

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

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

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

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

351   DOI: 10.1007/s004420050367.

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

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

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

355   86:1704–1714.

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

357   *and Biology* 36:299–328.

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

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

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

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

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

363   110:743–778.

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

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

366   97:742–751. DOI: 10.1111/j.1365-2745.2009.01509.x.

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

368   *Lett.*:1–5.

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

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

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

372   335.

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

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

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

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

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

378   *Plant Physiol.* 27:451–456.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

396   10.1007/s00035-017-0197-7.

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

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

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

400   3040.2001.00696.x.

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

402   *Oecologia* 115:445–459.

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

404   22:569–74. DOI: 10.1016/j.tree.2007.09.006.

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

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

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

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

409   66:215–241. DOI: 10.2138/rmg.2007.66.9.

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

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

412   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.

413   2008. Nitrogen and carbon source-sink relationships in trees at the Himalayan treelines

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

415   10.1111/j.1365-3040.2008.01848.x.

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

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

418   33:653–662. DOI: 10.1139/X02-202.

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

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

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

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

423   189:282–294. DOI: 10.1111/j.1469-8137.2010.03479.x.

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

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

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

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

428   60:505–537.

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

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

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

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

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

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

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

436   190:724–39. DOI: 10.1111/j.1469-8137.2010.03615.x.

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

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

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

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

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

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

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

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

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

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

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

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

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

450   10.1111/j.1469-8137.2008.02496.x.

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

452   Amsterdem,The Netherlands.

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

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

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

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

457   164:141–150. DOI: 10.1007/s00442-010-1650-0.

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

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

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

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

462   DOI: 10.2307/2656832.

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

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

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

466   3054.2006.00805.x.

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

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

469   246. DOI: 10.1111/1442-1984.12148.

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

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

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

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

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

475   DOI: 10.1126/science.264.5156.239.

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

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

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

479   range. *Oecologia* 70:580–586.

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

481   Statistics, Newburgh, UK.

482

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.
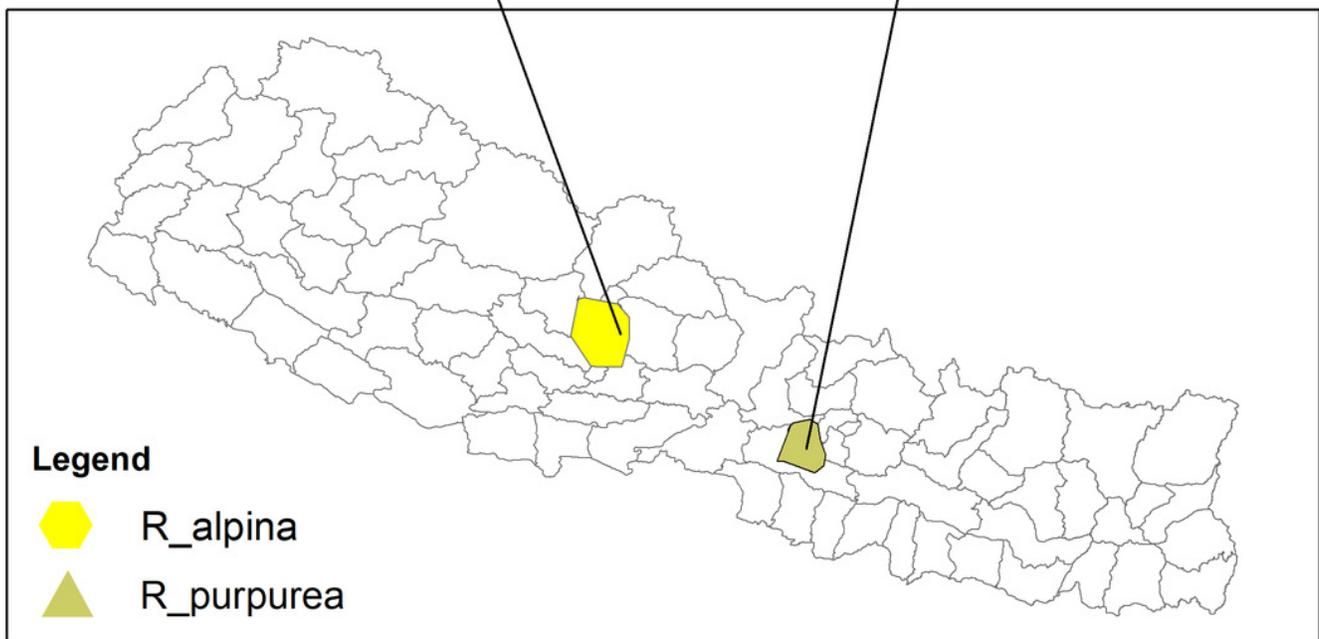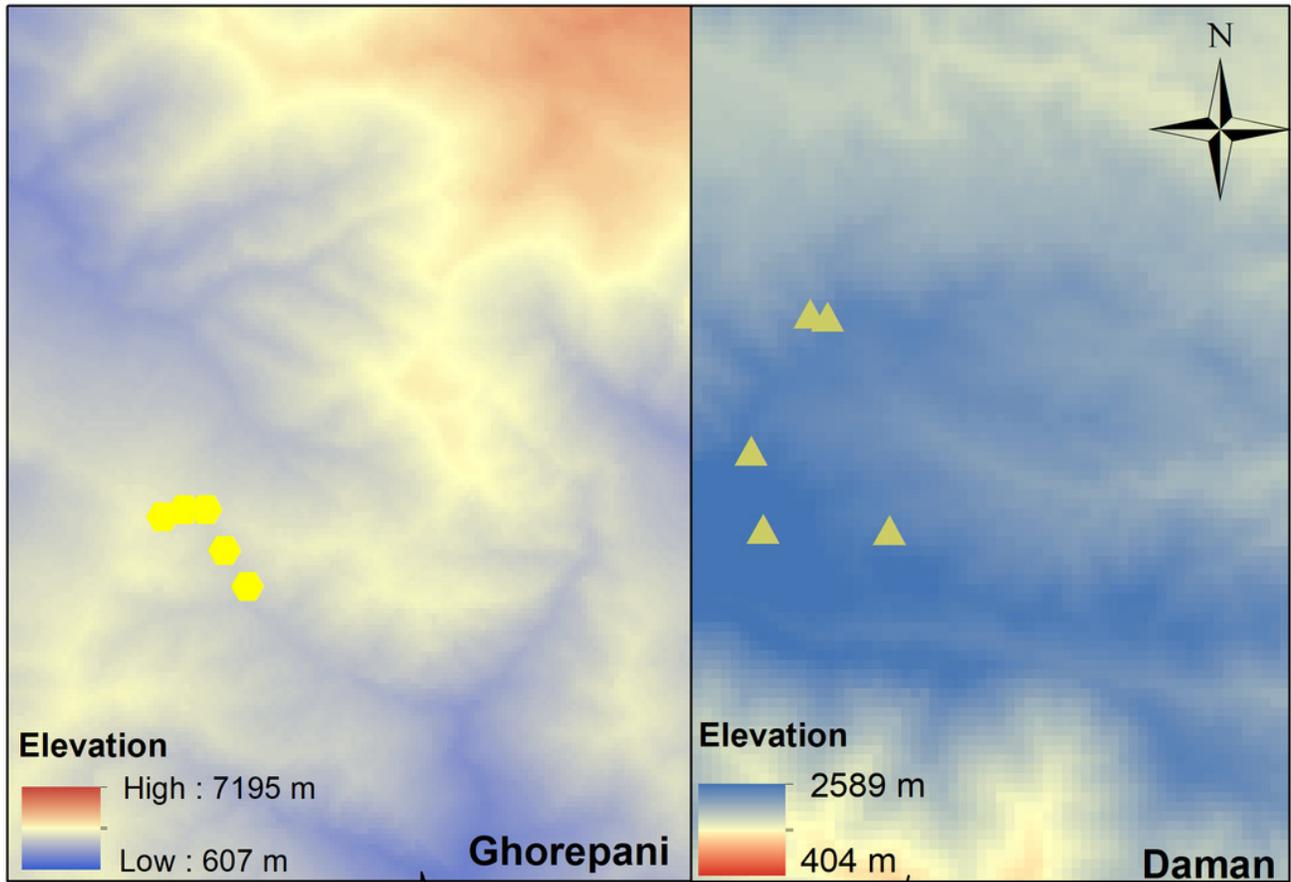
Figure 3

Figure 3

Correlations between the different traits of *R. purpurea* measured at five different elevations.
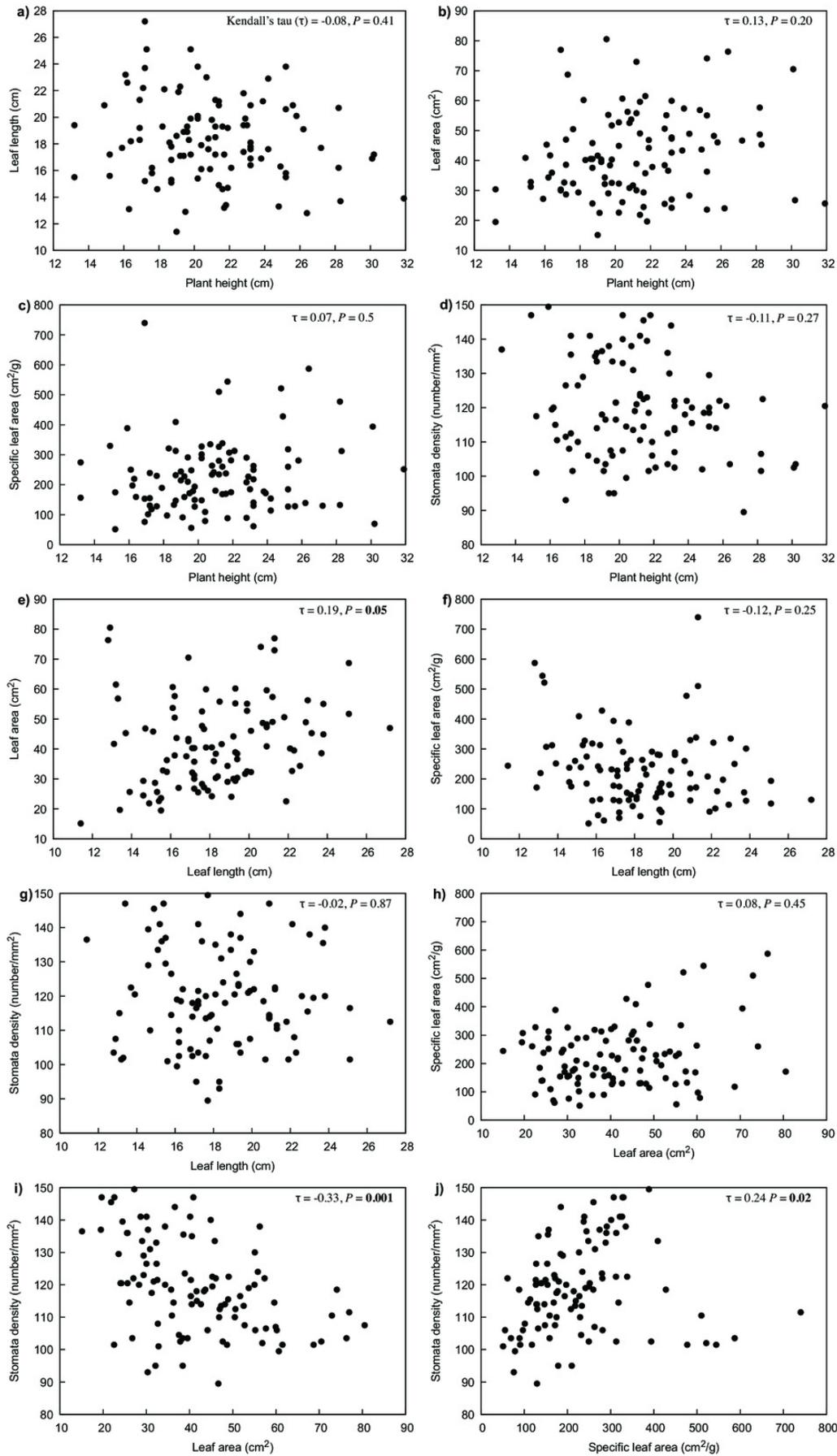
Figure 4

Figure 4

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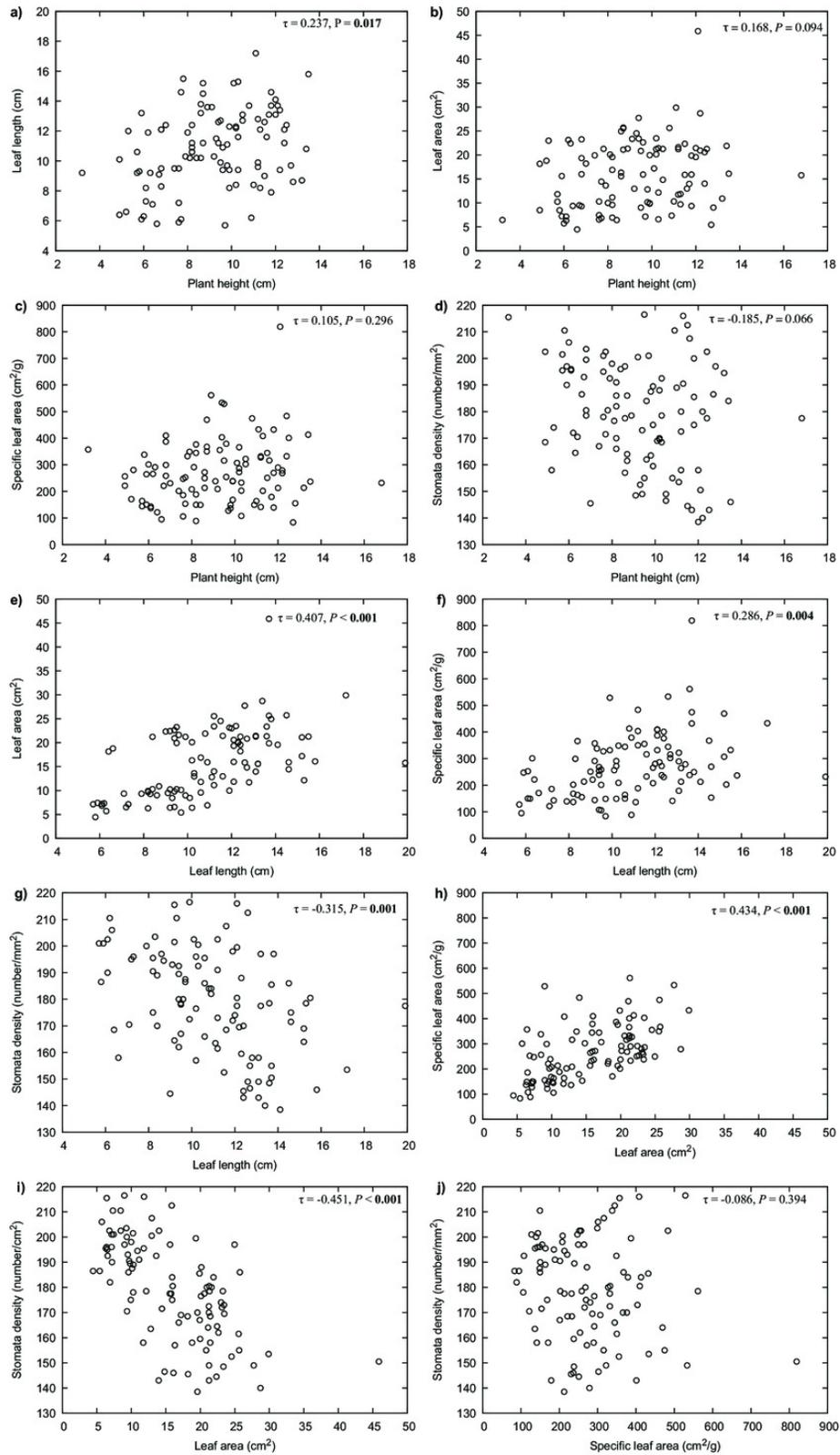
Figure 5

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.
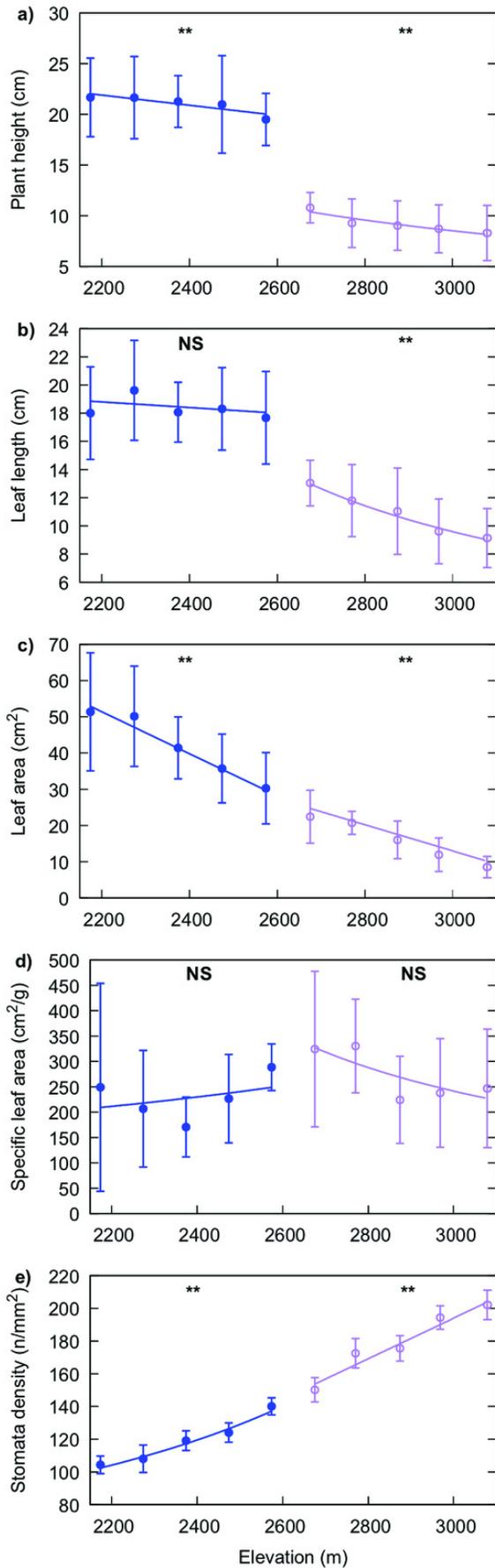
Table 1

Geographical details of the study sites.

1 **Table 1: Geographical details of study sites**.

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

4
5
6
7
8
9
10
11
12

Table 2

Results of regression analysis between various traits of *R. alpina* and elevation. SLA =
Specific Leaf Area.

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

2   Specific Leaf Area.

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

Table 3

Results of regression analysis between various traits of *R. purpurea* and elevation. SLA = Specific Leaf Area.

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

2   = Specific Leaf Area.

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			