



Phenology determines leaf functional traits across *Rhododendron* species in the Sikkim Himalaya

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Abstract

Elevation gradients provide an ideal setting to infer species' functional trait responses to predicted future climate change. In plants, leaf functional traits help assess their capacity to cope with varying resources. Variation in abiotic conditions over short vertical distances can influence plant phenology, particularly leafing and flowering durations, and leaf functional traits at both inter- and intra-specific levels. However, studies examining relationships between leaf functional traits and phenology duration along elevation gradients are limited. We tested the relationship between leaf size, leaf thickness, specific leaf area, and leafing durations in 10 *Rhododendron* species in the Sikkim Himalaya. All the investigated traits varied significantly across species, but intra-specific variation in functional traits was observed only among a few. Leaf size and thickness showed significant negative relationships with elevation and a comparative phylogenetic method exhibited a strong relationship between leaf traits and leafing duration. We observed higher leaf thickness and size in species with longer leafing durations and less overlap in leafing and flowering durations. In contrast, species with shorter leafing durations and relatively more overlap in their flowering and leafing durations showed lower leaf thickness and leaf size. Leaf traits such as leaf thickness and leaf size also exhibited a strong phylogenetic signal across 10 *Rhododendron* species. Overall, from our findings, we infer that along an elevation gradient, the magnitude of leaf trait responses to future increases in temperature may vary depending on species phenology durations and phylogeny.

Keywords Eastern Himalaya · Elevation gradient · Climate change · Functional traits · Phenology · *Rhododendron*

Introduction

Climate change has affected many natural systems (Parmesan 2006). Mountain ecosystems, in particular, are the most vulnerable for they show continued warming when compared to the global average (Diaz et al. 2003; Körner et al. 2019). Among climatic factors, temperature is the most important variable that influences, and often limits plant growth in higher elevations (Körner 1999). Here, changes in temperature are expected to cause local extinctions as well as shifts in range and phenology, besides altering the biological

interactions of many endemic, cold-adapted plant species (Thuiller et al. 2005; Dullinger et al. 2012). Understanding how plants will respond to this unprecedented increase in global temperature is of utmost importance for conservation and the maintenance of future ecosystem functions (Hampe et al. 2005). Over the last 2 decades, plant functional traits have emerged as a reliable predictor of plant responses to climate change (Read et al. 2014; Henn et al. 2018; Heilmeyer et al. 2019). The local environment can impact plant functional traits such as leaf size and act as a selective filter that helps plant with certain leaf size to cope with the abiotic gradients associated with an elevational range (Read et al. 2014). Therefore, studying plant functional traits along elevational gradients can contribute to an enhanced prediction of plant responses to ongoing climate change (Theurillat and Guisan 2001; Felde et al. 2012). Vegetative plant traits, in particular, can help assess plants' abilities to cope with varying resource levels (Cornwell and Ackerly 2009; Kraft et al. 2015). For example, leaf traits such as leaf size and specific leaf area (SLA) are associated with lower water and/

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or nutrient availability, and are recognized as good proxies of broad resource-use strategies (Ackerly et al. 2002). Similarly, high-elevation plants adapt to extreme weather conditions by changing their leaf morphological traits, such as growing smaller and thicker leaves. Also, due to the short growing season in higher elevations, plants here have a shorter phenology in comparison to species found in lower elevations (Inouye and Wielgolaksi 2013; Basnett et al. 2019).

The timing of reproductive events, however, can alter the trade-offs between vegetative and reproductive growth (Elzinga et al. 2007; Bolmgren and Cowan 2008). For instance, flowering and leafing duration may show little overlap in plants that flower early compared to species that flower late and have a shorter time to complete their reproductive cycles. Although the impact of phenology on plant functional traits has been highlighted (McKown et al. 2013; Fajardo et al. 2016), very few studies have examined the relationship between them. Some species may respond to environmental and ecological factors readily, and variation in their phenology and functional traits may be the outcome of phenotypic plasticity, which enables plants to adapt to a changing environment (Pfennigwerth et al. 2017). However, recent studies have highlighted the role of phylogenetic constraints on plant functional traits, particularly phenology and leaf traits, and how evolutionary relationships between species determine their biological characteristics (Davies et al. 2013; Du et al. 2015, 2017). Because of their shared evolutionary history, closely related species share similar genetic constraints that limit their potential evolutionary responses to the environment (Rathcke and Lacey 1985). Thus, closely related species are likely to exhibit similar phylogenetic signals and share similar traits more than expected by chance (Harvey and Pagel 1991).

In the high-elevation regions of the Himalayas, increases in elevation provide gradients of temperature over a relatively short vertical distance (Kattal et al. 2015). Temperature is expected to decrease at the rate of 4.3 °C to 6.1 °C for every 1000 m of elevation gain (Kattal et al. 2015). The temperature range along the elevation gradient here provides an ideal system to study plant responses to temperature (Thakur et al. 2019; Dolezal et al. 2016). So far, most studies in this region have focused on species diversity attributes such as richness and distribution (Telwala et al. 2013; Manish et al. 2017). Studies addressing intra- and inter-specific plant leaf traits and phenology along the elevation gradient are limited.

In the Himalaya, *Rhododendron* (Ericaceae) is an important genus of mountain vegetation that is found from 1500 m above sea level (m a.s.l.) to > 5000 m a.s.l. *Rhododendron* life forms range from being tall trees in wet temperate forests to dwarf shrubs in the higher alpine regions. Apart from the plants' ecological importance in the landscape, they are also known for their local socio-economic and ethnic values. In

the current study, we used elevation as a proxy for temperature, and tested the relationship between plant functional traits and elevation. We also studied the link between plant phenology and functional traits. Furthermore, we analyzed the role of evolutionary history in explaining plant functional traits across locally aggregated species. Specifically, we address these questions: (a) Are there intra-specific and inter-specific variation in leaf size, SLA, and leaf thickness across *Rhododendron* species, and do these traits change with elevation? (b) Is there a phylogenetic signal in leaf traits and is there a relationship between leafing duration and leaf traits, considering the phylogenetic relationships between species? And finally, (c) Is there an overlap in leafing and flowering durations across *Rhododendron* species?

Methods

Study area

The study was carried out between 2013 and 2015 in Kyongnosla Alpine Sanctuary (KAS from here on) situated in the district of East Sikkim (27° N, 88° E) in Sikkim, India. In KAS, *Rhododendron* forms an important component of the sub-alpine and alpine forests distributed along an elevational gradient. Tall trees and shrubs of *Rhododendron* and conifers dominate the sub-alpine region from 3200 to 3800 m a.s.l. The region above 3900 m a.s.l. represents alpine forests, which are dominated by alpine herbs, bushy, stunted conifers, and *Rhododendron* species. The treeline lies between 3800 and 3900 m a.s.l. (Basnett et al. 2019; Fig. S1). Ten iButton (iButtonLink, <https://www.ibuttonlink.com/>) temperature sensors were deployed at every 200 m interval to record soil and atmospheric temperature and were tracked for 3 years. The iButton temperature sensors were covered in silicone capsules before installation, and temperatures were recorded hourly. These sensors were installed in an open space about 4–5 feet above the ground. Bi-monthly mean air temperatures over the 3 years ranged from – 1.87 to 10.96 °C (Basnett et al. 2019; Fig. S2), and the total annual rainfall recorded was 4171 mm (Basnett et al. 2019). Sampling was carried out from 3400 m a.s.l. and upwards to avoid anthropogenic disturbances on the fringes of the Sanctuary.

Leaf traits across *Rhododendron* species along the elevation gradient

We laid three transects of 50 × 20 m² at every 100 m change in elevation starting from 3400 to 4230 m a.s.l. A total of 27 transects were laid along the elevation gradient. All *Rhododendron* species encountered inside the vegetation plots were selected as study species. Species were identified by referring to *Rhododendrons of Sikkim* (Pradhan 2010), the

Flora of China (Wu et al. 2005), and the *Flora of Bhutan* (Grierson and Long 1983). At every 100 m interval, 10–12 relatively young but fully expanded and sun-facing sides of hardened leaves were randomly selected from among healthy adult individuals of each *Rhododendron* species. Leaf measurements comprising length, size, thickness, and SLA were conducted. Leaf collections were wrapped in moist paper and stored in sealed plastic bags for 2–3 h. Upon reaching the field station, leaves were scanned using a scanner, and the scanned image was used to measure leaf size using the Blackspot leaf area calculator (Varma and Osuri 2013). For huge leaves that exceeded the scanner's window, leaves were cut into two halves, and the total size was measured. Following this, the leaves were placed in a hot air oven at 60 °C for at least 72 h after which they were weighed using a digital weighing balance. SLA was measured using the one-sided area of a fresh leaf, divided by its oven-dry mass (Perez-Harguindeguy et al. 2013). A calibrated digital vernier caliper was used to measure leaf length and leaf thickness. Leaf trait measurements were carried out for only 1 year.

Phylogenetic inference

For all species selected for the current study, leaf samples and voucher specimens were collected from KAS. To reconstruct a dated phylogenetic tree for the 10 *Rhododendron* species chosen, we first identified candidate loci that have been successfully used in Ericaceae systematics. Based on this prior information (Kurashige et al. 2001; Liang-Ming et al. 2003; Liu et al. 2012), we selected three loci from the chloroplast and nuclear genomes, which included an intergenic spacer (*psbA-trnH*), internal transcribed spacer (*ITS*), and maturase K gene (*matK*). The total genomic DNA from leaf samples was extracted using a modified CTAB method (Sambrook and Russell 2001). The DNA was subjected to a PCR reaction to sequence the loci *psbA-trnH* and *ITS* (Basnett et al. 2019; see Text S1 for complete laboratory protocol), and for *matK*, we used sequences available on GenBank. We also collected sequence data of 118 species belonging to the family Ericaceae and outgroup Actinidiaceae (Basnett et al. 2019; Table S1). Ericaceae-wide phylogenetic relationships were inferred using the Maximum-Likelihood (ML) and Bayesian methods (see Text S1 for complete analytical details of dated phylogeny construction). Finally, for further analysis, the dated phylogenetic tree of 118 taxa (Basnett et al. 2019; Fig. S3) of Ericaceae was pruned for the 10 *Rhododendron* species of interest.

Leafing and flowering durations of *Rhododendron* species along an elevation gradient

Leafing and flowering durations were measured for all *Rhododendron* species encountered inside the vegetation

plots. To record the leafing and flowering phenology, regular observations were carried out at every 15 day-interval for each *Rhododendron* species occurring at every 100 m interval. Ten trees were randomly selected at every 100 m, and on each tree, a branch tagged to monitor both leaf and flowering phenology. Phenology durations were then calculated based on the differences between the first and last days of the year (DOY), which refers to the percentages of the number of first and last leaves and florets remaining during the course of the study. In the case of a leaf, the last day leaf refers to the time when the plant has fully folded its leaf or was totally immersed inside the snow. Leafing and flowering durations were measured for all 3 years, and the average of these 3-year data was used for analysis.

Statistical analysis

The R package ‘corrplot’ (Wang et al. 2013) was used to determine multicollinearity correlation coefficients for leaf traits. Variables with a Pearson's correlation coefficient $|r| \geq 0.75$ were removed before model building. Considering the elevation gradient as an independent factor, we tested the intra-specific variation in each *Rhododendron* species using One-way Analysis of Variance (ANOVA). Similarly, considering two independent factors (e.g., elevation and species), we conducted a two-way ANOVA test to calculate inter-specific differences in leaf traits among 10 *Rhododendron* species across the elevational gradient. Some of the data on traits followed a normal distribution; data on leaf traits that did not were log-transformed to meet normal distribution assumptions. To understand the relations between elevation and leaf traits, we built a linear mixed model (LMM) using species identity as a random factor, and the model was tested using package ‘lme4’ (Bates et al. 2011).

We used Blomberg's K (Blomberg et al. 2003) to measure the phylogenetic signal across 10 *Rhododendron* species. The average value of traits per species was used to carry out the analysis. Blomberg's K explains the observed degree of similarity among phylogenetically related species compared with expectations obtained from the Brownian motion of model of evolution (Kembel et al. 2010). Blomberg's K value close to one represents trait evolution consistent with the Brownian motion model of evolution, and values close to zero indicate a random distribution of trait values concerning the phylogeny (Blomberg et al. 2003). Blomberg's K significance was tested by comparing the observed K value to a null distribution generated by comparing 1000 randomizations of trait values across the phylogenetic tree tips, and it was measured using the Picante package in R (Kembel et al. 2010). We used a phylogenetic generalized least-squares regression model (PGLS) to test the relationship between leaf durations and leaf traits. PGLS takes into account phylogenetic non-independence in the data (Freckleton et al.

2002), and the analysis was performed in the “Caper” package (Orme 2013) in R. For the response variable, we considered the mean duration of leafing of every species averaged across 3 years; similarly, leaf traits were averaged per species. R -squared (R^2) and lambda parameter (λ) were used as metrics to check how well the model fits the data.

We measured the overlap in leafing and flowering durations among *Rhododendron* species using the Pianka Overlap Index (POI). POI close to 0.0 reflects no overlap, whereas values close to 1.0 reflect more overlap. Data were organized in a data frame in which each row is the duration of leafing/flowering, each column is the timing of those events, and the entries represent the presence and absence of leaf and flowers at that particular time. The test’s statistical significance was measured by comparing with 1000 pseudo-communities that calculated the mean values of a null model. We used the RA3 algorithm; it reshuffles the row values and retains the observed niche breadth of flowers and leaf durations. Higher observed values (OV) compared to the Simulated Values (SV) indicates a higher overlap (Gotelli and Graves 1996). We report Standardized Effect Size (SES), which converts the P value into a standardized deviate. Large positive values of the SES indicate increasingly small upper-tail probabilities, and large negative values of SES indicate increasingly small lower tail probabilities. Non-significant tail probabilities usually fall between -2.0 and $+2.0$. This analysis was performed using the “EcoSim R” package in R (Gotelli et al. 2015).

Results

Intra- and inter-specific variation in leaf traits among rhododendrons

Among the 10 species encountered inside the vegetation plots, *Rhododendron thomsonii*, *R. hodgsonii*, *R. cinnabarinum*, and *R. campylocarpum* occurred between 3400 and 3800 m, whereas *R. campanulatum*, the widest-ranging species, was found from 3400 m until 3915 m. Above 3800 m, we encountered *R. aeruginosum*, *R. setosum*, *R. lepidotum*, *R. wightii*, and *R. anthopogon*. *R. anthopogon*, and *R. lepidotum* were the most wide-ranging high-elevation species (Table S2). Henceforth, *Rhododendron* species growing below the treeline (3800–3900 m) are referred to as low-elevation species, and species growing above the treeline as high-elevation species. A strong correlation of leaf length was observed with leaf thickness ($r^2=0.85$) and leaf size ($r^2=0.95$; Fig. S4). Therefore, we considered leaf thickness, size, and SLA, but removed leaf length from further analyses.

The two-way ANOVA interaction effect between species and elevation significantly confirmed that the variation

observed in leaf traits—SLA (ANOVA, $F_{21, 395}=1.68$, $P<0.01$), leaf size (ANOVA, $F_{21, 395}=2.54$, $P<0.0001$), and leaf thickness (ANOVA, $F_{21, 395}=2.39$, $P<0.0001$) across different species are also dependent on elevation gradient (Fig. 1; Tables 1, S3). Intra-specific variation in SLA and leaf thickness were observed in *R. campanulatum* and *R. anthopogon*. *R. setosum* also showed significant intra-specific variation in leaf thickness. The other three species, *R. campylocarpum*, *R. hodgsonii*, and *R. setosum* showed significant intra-specific variation in leaf size (Tables S4, S5).

Along the elevation gradient, we observed a significant decrease in mean leaf thickness (LMM, estimate = -0.081 ± 0.065 , $t = -2.08$, $P < 0.04$; Fig. 1a) and leaf size (LMM, estimate = -0.099 ± 0.0330 , $t = -3.173$, $P < 0.02$; Fig. 1b), whereas SLA (LMM, estimate = -0.005 ± 0.003 , $t = -1.70$ and $P < 0.09$; Fig. 1c) did not show a significant relationship with elevation.

Influence of phylogeny on leaf traits and relationship with leafing duration

We observed a significant relationship between leaf durations and leaf thickness ($R^2=0.35$, $P=0.043$, $\lambda=0.50$; Fig. 2a) and leaf size ($R^2=0.40$, $P=0.025$, $\lambda=0.51$; Fig. 2b). In general, low-elevation species with longer leafing durations showed higher leaf thickness and leaf size compared to species found in the higher elevations. We observed a negative relationship between SLA and leaf duration, in which higher elevation species shared relatively higher SLA compared to those in the lower elevations. However, the relationship observed was not significant ($R^2=0.12$, $P=0.92$, $\lambda=0.30$, Fig. 2c). For all the three PGLS models, we observed that the estimated Pagels λ was typically much greater than zero (Table S6). In the case of leaf traits, the values of Blomberg’s K across species ranged from 0.355 to 0.886. A significant phylogenetic signal ($P < 0.01$) in mean leaf thickness and leaf size was observed across 10 *Rhododendron* species, while SLA did not show any apparent phylogenetic signal among species (Table 2).

Overlap in flowering and leafing durations in *Rhododendron* species

One of the lower elevation species *R. campanulatum* showed a significant non-overlap in its leaf and flowering durations with observed values being lesser than the Simulated ones. All other species also showed similar trends, but we did not observe a significant difference in the strength of non-overlap in the duration of their two phenology events. However, in contrast to the lower elevation species such as *R. campanulatum*, *R. campylocarpum*, *R. thomsonii*, *R. cinnabarinum*, and *R. hodgsonii*, most of the higher elevation species (e.g., *R. setosum*,

Fig. 1 Relationship of **a** leaf thickness, **b** leaf area, and **c** specific leaf area with elevation. The point represents the individual values per species for each elevation and the solid line with gray loess curves shows trend in leaf traits with elevation measured using linear mixed model

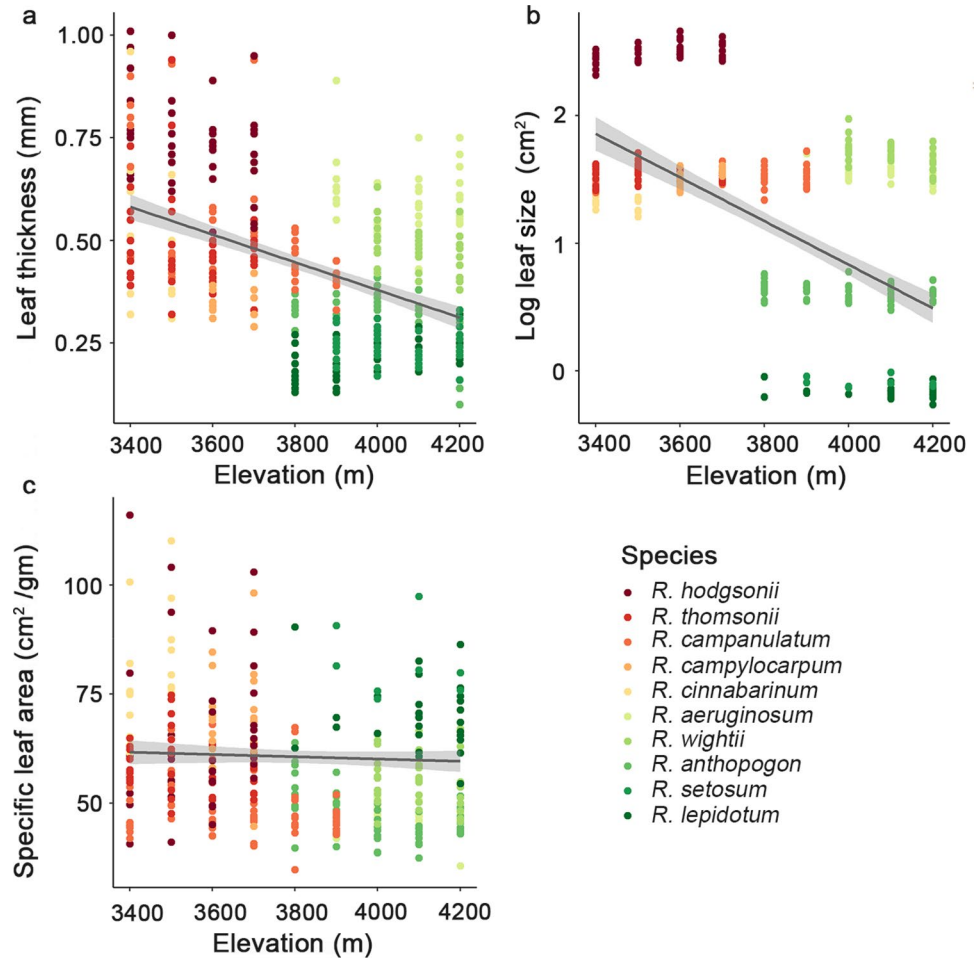


Table 1 Details of elevational range, mean leaf traits (\pm SD), and phenology durations of ten *Rhododendron* species in Kyongnosla Alpine Sanctuary, Sikkim

Species	Elevation (m.a.s.l)	Log leaf size (cm ²)	Leaf thickness (mm)	SLA (cm ² /gm)
<i>R. hodgsonii</i> n=43	3500–3745	314 \pm 51.04	0.74 \pm 0.11	64.26 \pm 16.97
<i>R. thomsonii</i> n=45	3450–3805	35.36 \pm 4.93	0.48 \pm 0.11	59.09 \pm 6.13
<i>R. campanulatum</i> n=60	3400–3915	34.89 \pm 5.46	0.51 \pm 0.14	48.81 \pm 6.95
<i>R. campylocarpum</i> n=19	3602–3787	34.18 \pm 4.85	0.35 \pm 0.03	69.75 \pm 11.22
<i>R. cinnabarinum</i> n=20	3430–3581	22.11 \pm 3.76	0.46 \pm 0.15	77.25 \pm 13.68
<i>R. aeruginosum</i> n=44	3973–4230	38.42 \pm 6.85	0.60 \pm 0.07	46.39 \pm 3.17
<i>R. wightii</i> n=36	4040–4223	51.41 \pm 11.72	0.46 \pm 0.07	55.71 \pm 6.17
<i>R. anthopogon</i> n=54	3830–4230	4.18 \pm 0.06	0.33 \pm 0.31	46.76 \pm 5.54
<i>R. setosum</i> n=48	3990–4235	0.81 \pm 0.07	0.24 \pm 0.04	81.93 \pm 7.68
<i>R. lepidotum</i> n=60	3920–4230	0.73 \pm 0.00	0.25 \pm 00	71.10 \pm 8.45

Fig. 2 Relationship of leaf traits with leaf phenology durations. The point represents the species mean with \pm SE, and the solid line with gray loess curves shows trend in leaf traits with leaf phenology durations measured using phylogenetic generalized least-squares

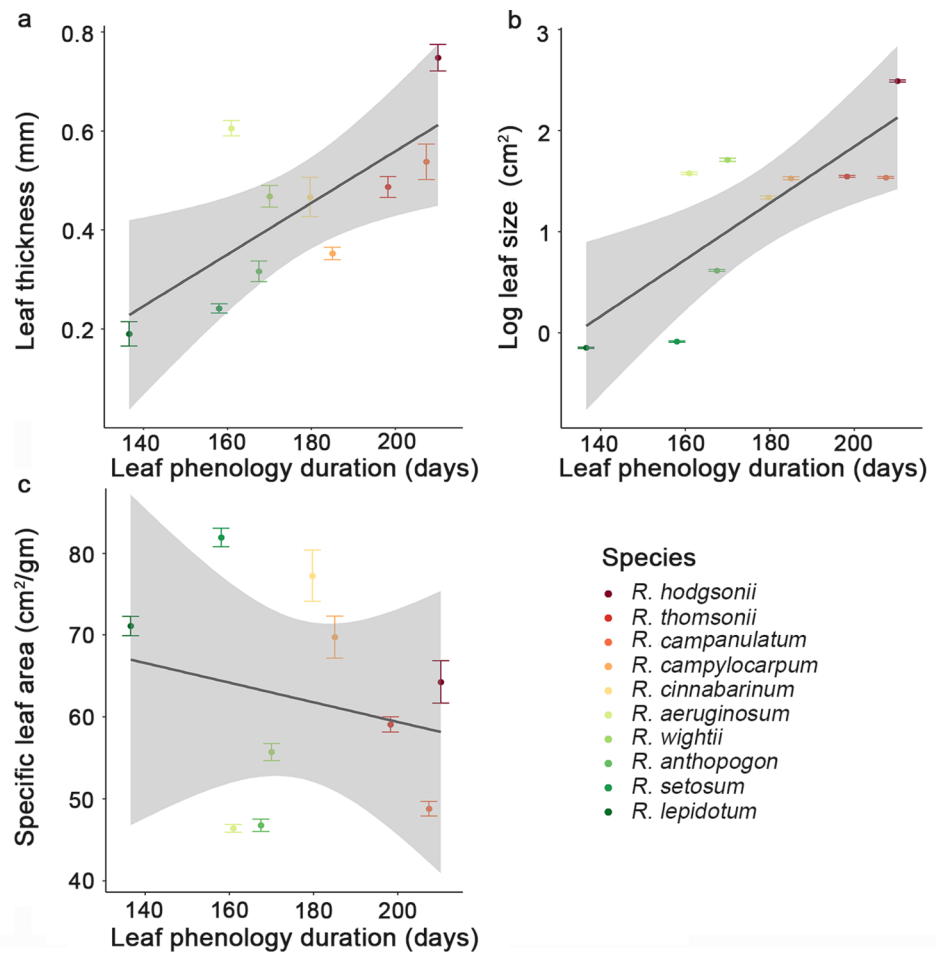


Table 2 Strength and significance of phylogenetic signal in the mean leaf traits of *Rhododendron* species estimated using Bayesian evolutionary analysis sampling tree. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Leaf traits	Blomberg's K	P value
Thickness	0.562	0.02*
Size	0.883	0.002**
Specific leaf area	0.355	0.25

R. lepidotum, *R. anthopogon*, and *R. aeruginosum*) showed relatively less difference in the two phenology durations as indicated by lesser difference in its observed and simulated overlap index. However, *R. wightii*, one of the early flowering species of the higher elevation, showed less overlap in their phenology durations than the rest of the higher elevation species (Table 3, Fig. S5).

Discussion

Our results highlight inter-specific variation in leaf thickness, SLA, and leaf size among 10 *Rhododendron* species in the Sikkim Himalaya. However, intra-specific variation in leaf traits was not consistent across any of these species. A comparative phylogenetic method exhibited a strong relationship between leaf traits and leafing durations. All species with longer leafing durations and lesser overlaps between leafing and flowering durations possessed higher leaf thickness and leaf size. Furthermore, species with short leafing durations and relatively more overlap in their flowering and leafing durations showed lesser leaf thickness and leaf size. Leaf traits such as leaf thickness and leaf size also exhibited a strong phylogenetic signal across the 10 *Rhododendron* species.

Species-specific variation in leaf traits along the elevation gradient

Some of the late-flowering species of high and low elevations such as *R. lepidotum*, *R. setosum*, and *R. cinnabarinum* showed the highest SLA compared to the other species. The

Table 3 Details of overlap in leafing and flowering phenology durations of ten *Rhododendron* species in Kyongnosla Alpine Sanctuary, Sikkim

Species	Leafing duration start–end (DOY)	Flowering duration start–end (DOY)	Observed value (OV)	Simulated value (SV)	Standardized effect size (SES)
<i>R. hodgsonii</i>	167.33–346.33	122.66–166.00	0.50	0.66	–1.37
<i>R. thomsonii</i>	168.58–343.91	131.33–168.58	0.50	0.65	–1.54
<i>R. campanulatum</i>	170.53–349.33	130.00–170.53	0.28	0.58	–3
<i>R. campylocarpum</i>	183.00–352.00	137–183	0.28	0.57	–1.6
<i>R. cinnabarinum</i>	170.00–349.33	152.33–188.33	0.66	0.75	–0.60
<i>R. aeruginosum</i>	167.75–328.66	154.83–196.91	0.43	0.63	–1.82
<i>R. wightii</i>	168.77–326.00	136.33–168.77	0.50	0.67	–1.50
<i>R. anthopogon</i>	168.66–335.26	157.93–189.26	0.64	0.73	–1.50
<i>R. setosum</i>	180–339	168.00–197.00	0.74	0.77	–0.60
<i>R. lepidotum</i>	192.00–334.55	192.00–214.66	0.72	0.77	–0.70

leafing phenology of these species begins in June–July when the region experiences the highest bi-monthly mean temperatures ranging from 10 to 10.5 °C. These are the late leafing species that get only a short window to complete their phenology cycles when compared to the other early leafing species. Therefore, these plants may take full advantage of the favourable months for young buds to quickly break open, and for leaves to mature. SLA is also a key trait that is tightly associated with photosynthesis and relative growth rate (Reich et al. 1992; Cornelissen et al. 1996). Therefore, during these favourable but short windows, the later leafing plants may also benefit from higher temperatures to enhance their photosynthetic capacities to ensure positive carbon gain (Kudo 1996; Sides et al. 2014). This may be a strategy by these species to take maximum advantage of the short favourable months after which the region experiences a continuous drop in temperature, high rainfall, and extended gloomy weather.

We also observed lowest SLA among some of the high-elevation species such as *R. aeruginosum* and *R. anthopogon*. In the high elevations, low SLA is often associated with structural enforcement, where instead of growing, plants invest more resources to enhance structures that help them persist in areas with strong radiations, cold temperatures, and strong winds that increase the high evaporation rates (Kudo 1996). Therefore, for some species in the higher elevations, survival and persistence may be more important than maximizing carbon assimilation (Körner 2012).

The high variation in SLA and leaf thickness among individuals of wide-ranging species like *R. campanulatum*, *R. anthopogon*, and *R. setosum* may reflect morphological/leaf phenotypic plasticity, which enables them to adjust to the several micro-environmental variation along the elevation gradient and, therefore, have greater niche breadth (Sides et al. 2014). A study on *R. campanulatum* in the Nepal Himalaya also found that this wide-ranging species could adjust its leaf traits to environmental conditions (Sharma

et al. 2020). Similarly, a study on *R. maximum* from the South Central Appalachia in the United States has also reported SLA variability along an elevational gradient (Pfenigwerth et al. 2017). Since SLA is directly related to relative growth rate (Wright and Westoby 2000), the observed shifts in SLA within individuals of a species may indicate variation in individual physiology and morphology across elevations which could allow these species to better respond to differing external filters along environmental gradients (Sides et al. 2014). However, whether the intra-specific regulation of leaf traits is due to genetic or plastic variation is a question that will need further investigation.

In agreement with an earlier study on alpine plants in the Alps (Choler 2005), we observed increased leaf thickness in lower elevation species which face early snow melts. This may be an adaptation in defense against herbivores (Bardgett and Wardle 2010). During winters in the KAS, rhododendrons are the only evergreen broadleaf plants available as a food resource for herbivores such as musk deer and goral that are found in these elevational ranges (Srivastava et al. 2018), and increased leaf thickness may be a defense mechanism against these ungulates. In the higher elevation where the snow melts much later, dwarf rhododendrons which grow up to only 2–3 feet in height are completely submerged under snow from November and until May (Basnett et al. 2019). They start folding their leaves from early November on when the harsh weather sets in, and their smaller and thinner leaves may aid easy folding. As water is not a limiting factor, leaf folding—especially among these species—may be a strategy to protect themselves from frost rather than avoiding water loss. We found a significantly high leaf area and leaf thickness in *R. hodgsonii*, a species that occurs as tall trees in the lower elevations. Leaf thickness plays a key role in determining the physical strength of leaves, and thicker leaves are needed to support a larger leaf area (Perez-Harguindeguy et al. 2013). A decrease in LA is usually observed with decreasing water and/or nutrient

availability (Wright et al. 2017). In KAS, *R. hodgsonii* individuals are mostly seen occurring in wet marshy areas as the understory of tall conifers, and their large leaf area may be an adaptation to these water-rich environments.

Influence of leafing durations, flowering durations, and phylogeny on leaf traits of *Rhododendron* species

In the KAS, *Rhododendrons* start flowering from the first week of May and blooms last until the end of July or early August (Basnett et al. 2019). Species that flower between May and June showed less overlap in their leafing and flowering durations. These lower elevation species also get extended periods of growth and reproduction with a reduced overlap of leafing and flowering period. This may also allow them to optimally allocate resources for reproductive and vegetative parts (Korner 2003). Together, these factors may have contributed to the observed, significant increase in leaf thickness, leaf area, and SLA along with the extended period of leafing. The later-flowering *Rhododendron* species, especially in the higher elevations, are known to adopt certain life-history traits such as short flowering times (Basnett et al. 2019), small fruit size (Hart et al. 2016), low seed mass (Guo et al. 2010), and shorter periods of fruit dehiscence (Basnett et al. 2019) which will enable them to survive in the short window with fewer resources. Likewise, more overlap in flowering and leafing durations may also be one of the strategies to complete vegetative and reproductive growth before the start of harsh winter conditions. Small leaf length and thickness along with lower leaf area and SLA may be an adaptation to their short growing window that lasts from June until November. As previously observed, the region experiences a significant drop in temperature followed by heavy snowfall from December until April (Basnett et al. 2019).

We found a strong phylogenetic signal in leaf size and thickness, and this can be due to evolutionary responses to specific environmental cues. In KAS, *Rhododendrons* are exposed to harsh environmental conditions; for more than 5 months, the land surface is covered in snow. During the 3 years of the study, the bi-monthly mean day and night air temperatures ranged from -1.87 to 10.96 °C (Basnett et al. 2019). However, we observed a significant drop in temperature along the elevation gradient, and the growing season is relatively shorter at high elevations compared to the lower elevations. Therefore, the larger leaf area and thickness among the five lower elevation *Rhododendron* species and smaller leaf area and thickness among most of the high-elevation ones may indicate how such leaf trait adaptations may be necessary for these species to survive harsh environmental gradients. Likewise, many other studies on plants and birds have also found a strong

phylogenetic signal in harsh environmental conditions (Cavender-Bares et al. 2004; Graham et al. 2009).

Conclusion

Our study that examined 10 *Rhododendron* species in the Sikkim Himalaya suggests that leafing and flowering durations, as well as phylogeny determine leaf functional traits. The study also highlights the inter- and intra-specific variability in leaf traits among *Rhododendrons*. Overall, our findings indicate that the response to future environmental conditions may vary across species. For example, some of the lower elevation species with longer leafing and flowering durations may be at an advantage with increasing temperature in the future than higher elevation species, which get only a small window to complete their phenology events. Similarly, some of the wide-ranging species such as *R. campanulatum* and *R. anthopogon*, with high intra-specific leaf traits variability, may survive and adapt to the future increases in temperature compared to narrow-ranged species with less intra-specific variability in their functional traits. Our study also highlights that some leaf traits are constrained by phylogeny, suggesting that phylogenetic relatedness is a potential tool to predict *Rhododendron* leaf trait variation in this region. However, more studies considering more extensively distributed species are required to understand the general trend. Future research to examine leaf trait variation across microclimatic as well as biotic gradients across multiple sites could provide further insight into the ecological complexities confounding the use of elevation gradients as climate change proxies.

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Author contributions SB and SD conceived and designed the research. SB carried out the field work, analyzed the data, and wrote the manuscript. SD contributed to writing and all authors approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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