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Morphological and ecophysiological traits shaping
altitudinal distribution of three *Polylepis* treeline species
in the dry tropical Andes

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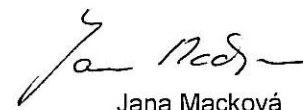
Annotation

Numerous species of the genus *Polylepis* form the highest treeline in the world, with striking dissimilarities in their upper altitudinal limits. The commonly accepted hypothesis is: growth at a treeline is temperature-limited. The aim of this work was to identify other factors influencing altitudinal distribution of *P. tarapacana*, *P. tomentella* and *P. rugulosa* in dry tropical Andes by means of using records of morphological and ecophysiological traits.

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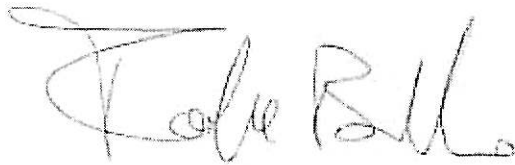
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Hereby I confirm, that Jana Macková significantly contributed to measuring and collecting data in the field, laboratory analyses and manuscript preparation in the following research paper:

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Original article

Morphological and ecophysiological traits shaping altitudinal distribution of three *Polylepis* treeline species in the dry tropical Andes[☆]Petr Macek^{a,c,*}, Jana Macková^{a,b}, Francesco de Bello^d^a Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 31, České Budějovice CZ-370 05, Czech Republic^b Department of Plant Physiology, Faculty of Science, University of South Bohemia, Branišovská 31, České Budějovice CZ-370 05, Czech Republic^c Section of Plant Ecology, Institute of Botany, AS ČR, Dukelská 135, Třeboň CZ-379 01, Czech Republic^d Laboratoire d'Ecologie Alpine, UMR CNRS 5553, Université Joseph Fourier, 38041, Grenoble, France

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ABSTRACT

Numerous species of the genus *Polylepis* form the highest treeline in the world, with striking dissimilarities in their upper altitudinal limits. The commonly accepted hypothesis is that growth at a treeline is limited by temperature. Here, using *in situ* records of various morphological and ecophysiological traits, we aimed to identify other factors influencing altitudinal distribution of three congeneric species from the dry tropical Andes: *Polylepis rugulosa*, *Polylepis tarapacana* and *Polylepis tomentella*. While *P. tarapacana* and *P. tomentella* reach their altitudinal limit at around 5000 m asl, *P. rugulosa* does not thrive above 4300 m, but precipitation is markedly lower in its distribution area. The three species responded to altitude by a change of morphological (e.g. decreased tree height and leaf size) and ecophysiological (e.g. decrease of transpiration rate, nutrient concentration or enrichment in the ¹³C isotope) traits, and this response was generally more pronounced in *P. rugulosa*. In comparison with *P. tarapacana* and *P. tomentella*, *P. rugulosa* displayed higher transpiration rates. Waxes from the abaxial (stomatous) leaf side of *P. rugulosa* were most strongly enriched in ¹³C. Furthermore, leaves of all species studied here had exceptionally low N and P concentrations. Trade-offs linked to changes in leaf area (e.g. bigger leaves, higher photosynthetic capacity but elevated transpiration) seem to drive differentiation and adaptations to altitude among these three congeneric species. We hypothesize that, while the upper distribution limit of *P. tarapacana* and *P. tomentella* is largely driven by low temperature, water is an important additional factor controlling the altitudinal distribution of *P. rugulosa*. Our results suggest that water stress needs to be taken into account among the factors shaping the altitudinal distribution of tropical treeline species.

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1. Introduction

The high altitude forest limit, i.e. treeline, is an obvious and often relatively sharp vegetation boundary, which has been suggested to follow a common thermal limitation within different climatic regions (Körner and Paulsen, 2004; Kessler et al., 2007). Treeline ecotones are expected to be among the systems most susceptible to the expected global climate changes worldwide, with their adaptability being dependent on species' ecophysiological characteristics. Therefore, studies of morphological and ecophysiological traits can give a relevant insight into the potential tree distribution shifts in response to climate change.

While the importance of treeline thermal limitation is recognized (Körner, 2007), little is known about the potential effect of other factors, such as water stress in dry mountain regions of the world (Morales et al., 2004). It has been suggested that high-elevation environments in tropical mountains are more arid than similar elevations in mid-latitude mountains as a result of both higher evaporative demand and lower rainfall (Leuschner, 2000). A particularly interesting area to study this kind of questions is the 'Altiplano' in western Bolivia, a flat semi-desert between the two mountain ranges of Cordillera Occidental and Cordillera Oriental. Unique to this Andean region is the occurrence of the world's highest treeline formed by scattered forest fragments of different congeneric *Polylepis* species (Kessler, 2002; Hoch and Körner, 2005). Populations of three closely related *Polylepis* species grow along a west-east precipitation gradient in almost allopatric distribution, with altitudinal ranges also differing.

The altitudinal distribution ranges of *Polylepis* species are defined by both land-use and climatic factors, with the lower

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altitudinal limit being shaped mainly by human activities and possibly several climatic factors controlling the upper distribution limit (cf. Kessler, 2002; Hoch and Körner, 2005). In particular, striking differences in the upper limit of each of the three *Polylepis* species may originate from different morphological and ecophysiological adaptations to cope with high altitude conditions. The treeline of *Polylepis tarapacana* was reported to be determined by temperature (Hoch and Körner, 2005), but the environmental factors controlling the upper limit of the distribution of *Polylepis rugulosa* and *Polylepis tomentella* remain unexplored. In particular, little is known about the extent by which the distribution of these species could be co-limited by a lack of water.

Water-carbon relations in plants can be assessed both by instantaneous (i.e. gas exchange) and long-term (e.g. carbon isotopic composition) measurements; since both approaches give different insights into these relations, we aimed to combine them in the analysis of these *Polylepis* species. Water balance at high altitudes is affected by several factors associated with altitude: decreasing atmospheric pressure leads to faster diffusion and unequally affects transpiration and CO₂ uptake at leaf level. Furthermore, lower temperatures reduce the saturated water vapour pressure and hence reduce leaf-to-air vapour pressure deficit (VPD). However, when the leaf is warmer than the ambient air, the driving force for water loss may be even stronger than at low altitudes (Smith and Geller, 1979; Körner, 2007). In addition, precipitation tends to decrease with increasing altitude in subtropical and tropical areas (Goldstein et al., 1994; Körner, 2007). These findings, along with increasing irradiation with altitude, imply a possible water stress control on treelines in the subtropical Andes (Morales et al., 2004). Considering that increased water stress, together with increased temperature, is expected in the Altiplano for the next decades (Rada et al., 1997; Cox et al., 2000) it is urgent to assess to which extent tree distribution depends on water limitation.

Water shortage causes stomatal closure and consequently decreases the CO₂ concentration in the leaf (c_i). Since the ratio between internal and ambient CO₂ concentration (c_i/c_a) reflects the balance between net photosynthesis and stomatal conductance, it affects the discrimination (Δ) against the carbon isotope ¹³C in plants. In this sense, Δ calculated from a simplified version of the model by Farquhar et al. (1982) has frequently been used to determine time-integrated leaf water use efficiency (WUE; Rebetzke et al., 2002; Rundel et al., 2003). However, doubts have been raised that ¹³C in bulk leaf material can be used as a correct indicator of WUE (Keitel et al., 2003; Peuke et al., 2006). On the other hand, $\delta^{13}\text{C}$ of leaf organic matter from pools with very rapid turnover (aqueous leaf extracts, soluble carbon in phloem) is a reliable indicator of instantaneous c_i/c_a (Keitel et al., 2003). In this respect, cuticular waxes (with known isotopic fractionation during biosynthesis) seem to be suitable components, since wax turnover is shorter than leaf lifetime, but still long enough to cover variability between seasons (cf. Hauke and Schreiber, 1998; Jetter and Schäffer, 2001; Bringe et al., 2006).

The cuticular waxes are formed mainly from newly assimilated carbon and, similarly to leaf tissue, their synthesis and ¹³C signal are

affected by environmental conditions (Conte et al., 2003). The waxes are created exclusively in epidermal cells (Kunst and Samuels, 2003; Shepherd and Griffiths, 2006) where their precursors are synthesized from photosynthates assimilated in chloroplasts in the vicinity of epidermal cells. Hence, the isotopic composition of abaxial waxes reflects the ¹³C/¹²C ratio of CO₂ in the nearest chloroplasts (provided the constant fractionation during biosynthesis) and could therefore be used for more accurate estimates of seasonal changes of stomatal closure.

We aimed here to identify the main environmental factors influencing altitudinal distribution of three congeneric species from the dry tropical Andes: *P. rugulosa*, *P. tarapacana* and *P. tomentella*. While *P. tarapacana* and *P. tomentella* reach their altitudinal limit at around 5000 m asl, *P. rugulosa*, distributed in areas with markedly lower precipitation, does not thrive above 4300 m. To clarify how these three species might adapt to different environmental conditions, we posed the following main questions: 1) how do morphological or ecophysiological traits change within these three species along the altitudinal gradient? And in particular, 2) to which extent can the trait changes be related to water and/or temperature limitations? Answering these questions will help to shed light on the factors controlling the altitudinal distribution of these three *Polylepis* species.

2. Material and methods

2.1. Study species

The genus *Polylepis* Ruiz & Pav. (Rosaceae, Sanguisorbeae) comprises about 28 woody species distributed along the whole tropical and subtropical Andean range at altitudes mainly between 3000 and 5000 m asl (Simpson, 1979; Kessler, 1995; Schmidt-Lebuhn et al., 2006a). *Polylepis* forests in western Bolivia are formed by endangered species and are important to endemic bird communities. Furthermore, local people depend on them for firewood, construction material, etc. The uppermost natural *Polylepis* stands are found at 4200 m on the more humid eastern Andean slope and even above 5000 m on the arid volcanoes of the Cordillera Occidental (Kessler, 1995, 2002). With respect to precipitation, the distribution limit of *Polylepis* lies at about 100 mm of annual rainfall (Altiplano of Chile, Bolivia and Argentina; Kessler, 1995; Kessler et al., 2007). The lower elevation *Polylepis* distribution limit is generally determined by its poor competitive ability, but can also be affected by human activities (Cierjacks et al., 2007). To assess within-species adaptations to altitude we selected three phylogenetically closely related species, i.e. *P. rugulosa* Bitter, *P. tarapacana* Phil., and *P. tomentella* Wedd. subsp. *tomentella*, originating from the ecologically specialized *Polylepis incana* complex.

2.2. Study sites and sampling design

The study was carried out along gradients of altitude (3180–5010 m asl) and precipitation (160–480 mm year⁻¹) in the puna regions of NW and central Bolivia and NE Chile (Table 1). Since

Table 1

The characterization of *Polylepis* locations compiled from Kessler (1995), Rada et al. (1997), Rundel et al. (2003), Hoch and Körner (2005), Navarro et al. (2005), Muñoz and Bonacic (2006) and personal records.

	<i>P. rugulosa</i>	<i>P. tarapacana</i>	<i>P. tomentella</i> ssp. <i>tomentella</i>
Geographical location	Cordillera Occidental (West slopes)	Cordillera Occidental/western Altiplano	Eastern Altiplano
GPS position	W 69° 31' S 18° 14'	W 68° 57' S 18° 07'	W 66° 28' S 19° 45'
Soil	Volcanic	Volcanic	Detrital sediments
Precipitation range (mm yr ⁻¹)	100–300	140–400	300–900
Wet season duration	Jan–Feb	Dec–Mar	Nov–Apr
Altitudes of locations (m asl)	3180, 3720, 4300	4280, 4640, 5010	4300, 4620, 4900
Slope orientation (degrees)	–flat, 345, 10	340, 20, 350	50, 10, 350

there is little overlap between the geographical distributions of the three species studied, three different locations were selected. The average yearly precipitation was ~200, 316 and >400 mm for sites of *P. rugulosa* (Putre, 3500 m asl; Muñoz and Bonacic, 2006), *P. tarapacana* (Sajama, 4220 m asl, 1975–85 average; Hardy et al., 1998) and *P. tomentella* (estimated from closest climatic stations in Oruro, 3708 m asl and Potosí, 4090 m asl; Leith et al., 1999), respectively. At each of these locations, three altitudes were assessed representing the extreme and intermediate altitudinal distribution of the selected species. At each altitude, five individual trees were sampled and surveys of the surrounding vegetation carried out. Several traits were recorded for each tree either once (tree height, nutrient and isotopic analyses), or repeatedly (at least 5–6 times; leaf traits, gas exchange traits; see below and Table 2). Measurements were supplemented by determining characteristics of environmental conditions (e.g. soil conditions; see below) and the composition of the surrounding vegetation.

To characterize abiotic conditions at each location, we installed relative humidity (RH) and temperature loggers (Comet System, Czech Republic) at the highest altitude of each location (height 1 m above soil surface). The loggers were recording data at 1 h intervals from January 2007 to July 2008. The mean annual temperature calculated from recorded data from February 2007 to January 2008 was 6.2, 3.8 and 3.3 °C for the locations of *P. rugulosa*, *P. tarapacana* and *P. tomentella*, respectively.

2.3. Traits

Various plant and leaf traits were measured to investigate patterns in plant growth, gas exchange, and nutrient and water economy. Tree height was determined with a yard stick. Leaf thickness (mm) of 10 individual leaves per tree was measured using a manual micrometer in the field. Leaf area (LA; mm²) was determined as one-sided projected leaf area using a portable LA meter (AM300, ADC BioScientific Limited, UK). Specific leaf area (SLA; mm² mg⁻¹) was calculated as:

$$SLA = \frac{LA}{DW}$$

where DW (mg) is leaf dry weight (dried out at 70 °C to constant weight). Total leaf nitrogen and phosphorus concentration (mg g⁻¹) was determined using an elemental analyzer (EA11100, ThermoQuest, Italy) and FIA-STAR (FOSS TECATOR), respectively; the same analyzers were used for soil nutrient analyses. Furthermore, soil water content (%), loss on ignition (LOI; %) and water extractable pH of the soils were measured using standard pedologic methods.

Table 2

The values of *F*-statistic resulting from two-way ANOVA with altitude and species as factors. Bold *F*-values represent significant difference. Note the factor species cannot be fully distinguished from site identity. For trait explanations, see the text.

Trait	n	Altitude	Species	Altitude × Species
Tree height	45	19.9***	1.81	4.13**
Leaf area	225	9.61***	164.6***	16.9***
Leaf thickness	450	3.37*	3.95*	3.34*
SLA	225	1.67	1.36	1.44
J _{psii}	270	4.79*	28.8***	1.48
g _i	270	2.78	12.1***	2.23
E	270	18.7***	60.6***	4.83**
¹³ C of leaf tissue	45	0.36	2.37	1.25
Leaf ¹³ C recalculated	45	1.13	13.2***	1.28
¹³ C of abaxial cuticle wax	42	5.41**	0.68	1.40
Leaf tissue N	45	3.42*	0.15	9.14***
Leaf tissue P	45	11.0***	0.25	1.05
Leaf N/P	45	3.17*	0.40	0.94
Leaf C/N	45	2.74	2.73	7.06***

P* ≤ 0.05; *P* < 0.01; ****P* < 0.001.

Gas exchange measurements, e.g. transpiration rate (*E*; mmol m⁻² s⁻¹), total leaf conductance (*g_i*; mmol m⁻² s⁻¹), leaf temperature, relative humidity (RH; %) and irradiance (μmol m⁻² s⁻¹) were obtained with a null-point porometer (Li 1600, Li-Cor, Nebraska, USA) during 5-min intervals. The quantum yield of photosystem II (QY_{PSII}) was measured using a FluorPen instrument (PSI, Brno, Czech Republic). The parameter QY_{PSII} was used to calculate linear electron transport rate (*J*) and hence, overall photosynthetic capacity according to the equation:

$$J_{PSII} = QY_{PSII} \times PFD \times 0.5$$

where QY_{PSII} represents quantum yield of PSII, PFD represents photon flux density (μmol photon m⁻² s⁻¹) and the factor of 0.5 represents the partitioning of energy between PSII and PSI (Maxwell and Johnson, 2000).

The vapour pressure deficit (VPD; kPa) was calculated as:

$$VPD = e_s - e_a,$$

where *e_s* is saturation vapour pressure (kPa) at leaf temperature and *e_a* is actual vapour pressure (kPa) at ambient temperature and RH. Gas exchange and QY_{PSII} were measured daily between 10 a.m. and 4 p.m. at each location over a period of four days under subjectively similar weather conditions.

2.4. Isolation of cuticular membranes

Cuticular membranes (CMs) were isolated by immersing central parts of leaves (after cutting away leaf margins) in an aqueous solution of 2% (v/v) cellulase (Celluclast, Novo Nordisk, Bagsværd, Denmark) and 2% (v/v) pectinase (Trenolin Super DF, Erbslöh, Geisenheim, Germany) in 0.01 M citric acid buffer (Merck, Germany; pH = 3.0, adjusted with KOH). The detailed procedure is described in Schönherr and Riederer (1986). Sodium azide (NaN₃, Fluka, Neu-Ulm, Germany) was added to the enzymatic solution to prevent microbial growth (concentration 0.1 mM). Isolated CMs from adaxial and abaxial leaf sides were separated, carefully washed with deionized water, air-dried and stored at room temperature.

2.5. Wax extraction

Seven to ten CMs isolated from the abaxial leaf side were immersed in 1 ml of chloroform (CHCl₃, Merck, Darmstadt, Germany) for 1 min per sample. Extracted waxes were dried on pure silica sand. The sand was then quantitatively transferred to a tin capsule and prepared for isotope analysis.

2.6. Carbon isotope analysis

The carbon isotope compositions of whole leaves and waxes from the abaxial leaf side were determined using an elemental analyzer (EA1110, ThermoQuest Italy) connected to an isotope ratio mass spectrometer (DeltaXLplus, ThermoFinnigan, Bremen, Germany). The δ¹³C was calculated versus the VPDB (Vienna Pee Dee Belemnite) standard. The standard deviation of δ¹³C determination in standard samples was lower than 0.1‰.

2.7. Data analyses

Principal Component Analysis (PCA) was used to demonstrate visually the main correlations among 1) biotic and abiotic characteristics and 2) recorded traits of *Polylepis* species. Since we were mainly interested in the correlation structure of measured plant traits, the biotic and abiotic conditions were used in the analyses as

supplementary variables with no effect on the analysis. Because the variables were recorded on different scales, they were standardized to their z-score prior to the analyses (option center and standardize corresponds to the PCA on the correlation matrix). PCA was performed with the CANOCO package (ter Braak and Šmilauer, 2002).

Hierarchical (nested) ANOVA was performed with replicate trees nested within altitude and species. There are overlapping effects of species and site that cannot be disentangled in this study; however, the three geographically closest possible locations were selected in order to minimize the differences between the sites. In this design, the replicates of trees can be taken as repetition units, and the statistical results only indicate the significance of differences between the three individual elevation gradients. For post-hoc comparisons, we used Tukey's Honestly Significant Difference (HSD) test. For ANOVA and regression, the program Statistica 8.0 was used (StatSoft, 2008).

3. Results

Climatic records show differences between locations with regard to temperature. The highest site at which we investigated *P. rugulosa* was $\sim 2^\circ\text{C}$ warmer (monthly minima) than the highest study sites for *P. tarapacana* and *P. tomentella*. Monthly averages of minimal daily temperature at different locations over a course of 19 months are shown in Fig. 1a. Although results cannot be generalized outside the measured interval, slight differences among locations were also found in relative humidity (monthly averages),

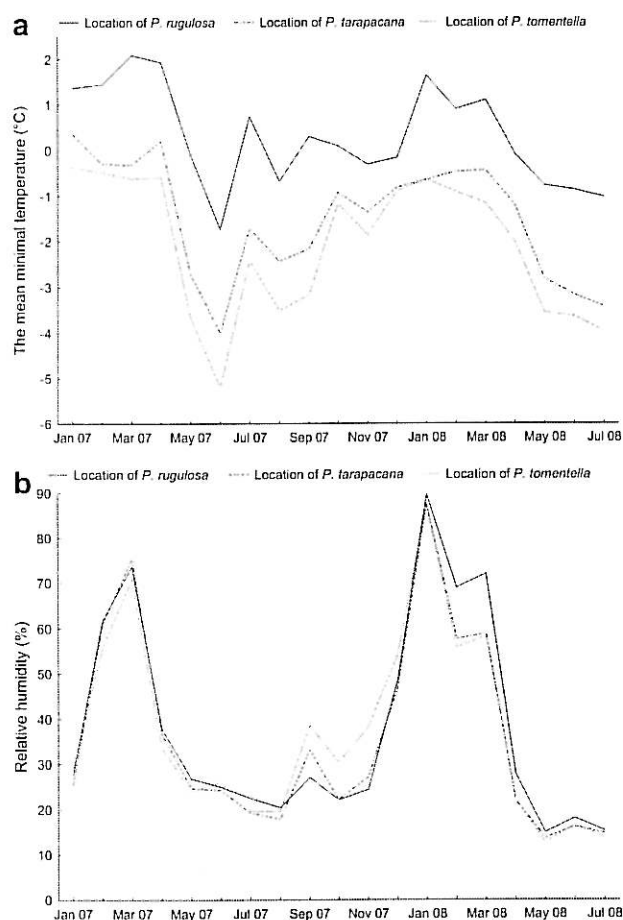


Fig. 1. The mean minimal temperature (a) and the mean relative humidity (b) recorded for 19 months at the highest location of each species.

indicating that the wetter season was shorter at the *P. rugulosa* site than at the other two locations (Fig. 1b); while recorded RH data could serve to estimate the length of the wet season, they could not be linked to precipitation intensity.

Sites at higher altitude were characterized by lower herbaceous and shrub cover but greater cover with cushion plant species. Skeletal soils, elevated nutrients and lower pH were more abundant at higher altitudes, too. For correlations of species traits with community and abiotic characteristics, see Fig. 2. In this PCA analysis, the first and second ordination axes explained 23.4 and 18.9% of variability in measured traits, respectively.

The greatest number of morphological and ecophysiological traits was affected by altitude: generally, the trees were smaller and had smaller but thicker leaves at higher sites (Fig. 3, Table 2). While SLA did not change with altitude at all, it showed a positive relationship with leaf nutrients (P, N and N/C); negative relations with leaf nutrient concentrations were found for leaf thickness (Fig. 2). J_{PSII} and transpiration significantly decreased with increasing altitude, while g_l was rather constant (Table 2, Fig. 3). Cuticular waxes from the abaxial leaf side were more strongly enriched in ^{13}C at higher sites (Table 2). The calculated linear regression from wax isotope composition for *P. rugulosa* and *P. tomentella* was $\delta^{13}\text{C} = -30.71 + 0.0014 \times \text{altitude}$ and $\delta^{13}\text{C} = -34.92 + 0.0021 \times \text{altitude}$, respectively. The hypothetical sea-level values of waxes correspond to -30.71‰ and -34.92‰ for *P. rugulosa* and *P. tomentella*, respectively (note the depletion of lipid surface extract relative to leaf tissue). There was no linear relationship for *P. tarapacana* (Fig. 4). In case of bulk leaf $\delta^{13}\text{C}$, we did not observe a clear correlation between altitude and $\delta^{13}\text{C}$ in our altitudinal gradient (3180–5010 m; all samples pooled). However, when leaf ^{13}C signals were recalculated taking into account an altitudinal effect on $\delta^{13}\text{C}$ (i.e. shift of 1‰ per

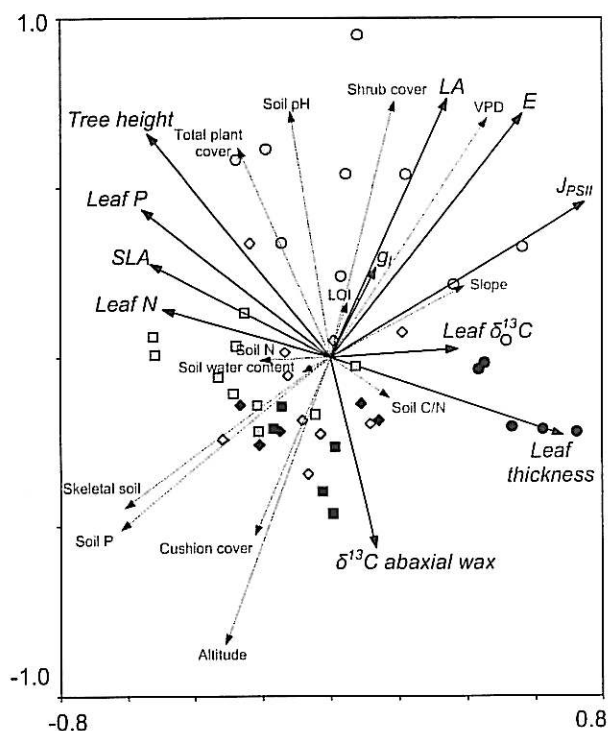


Fig. 2. Correlations among recorded morphological and ecophysiological traits (italics) visualized in a PCA triplot (together with environmental conditions used in the analysis as supplementary variables with no effect on the analysis). Symbols represent species (circles: *P. rugulosa*; squares: *P. tarapacana*; diamonds: *P. tomentella*) with colors denoting altitude (white: low; grey: medium; black: high) within each species range; see text for further explanations.

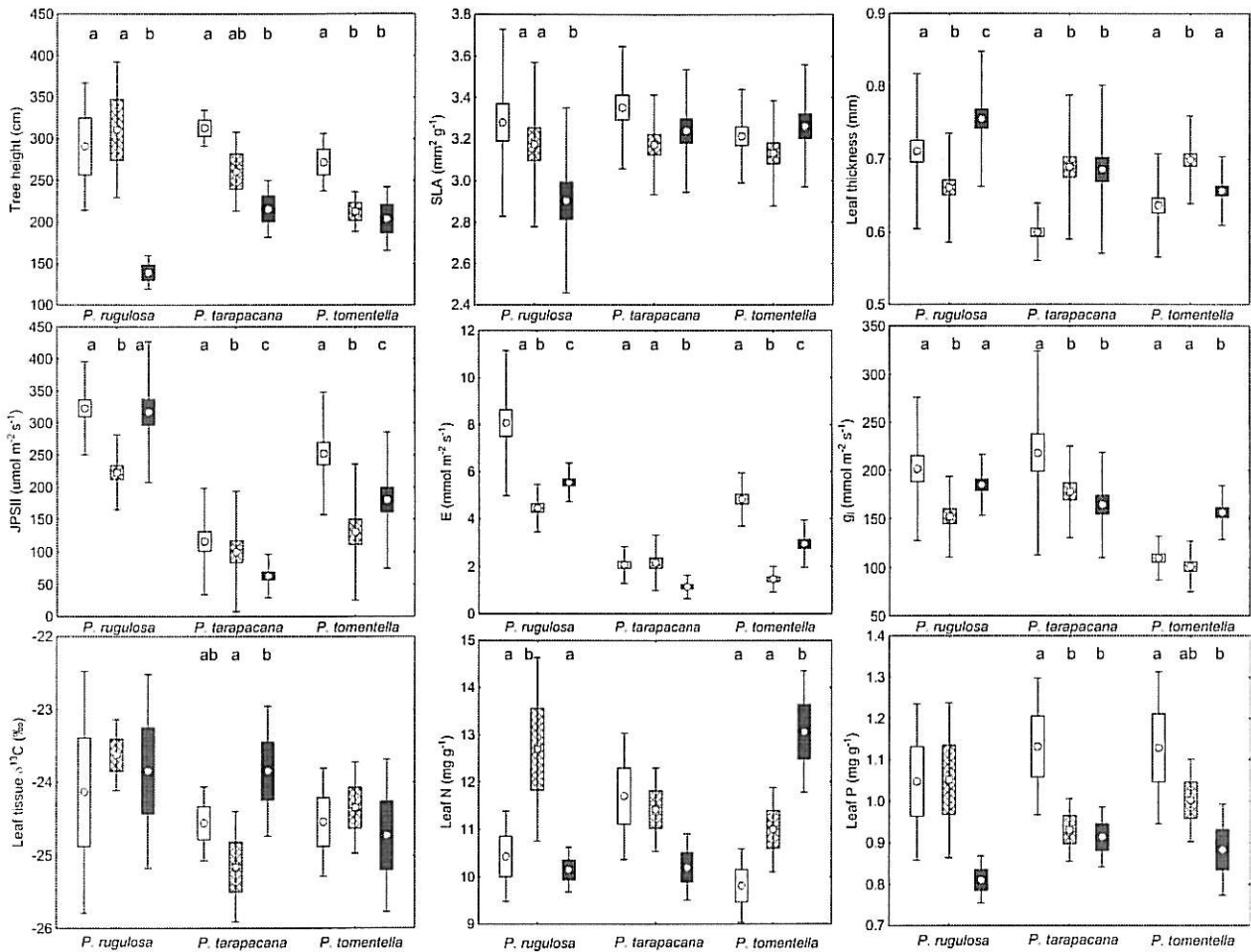


Fig. 3. Comparisons of recorded morphological and ecophysiological traits of three *Polylepis* species over an altitudinal gradient. Mean trait values (box: \pm SE; whisker: \pm SD) are shown for lowest (blank box), medium (hatched box) and highest (filled box) altitudes for each species. For exact altitudes see Table 1. Significant differences among altitudes within each species are represented by different letters.

km; Körner et al., 1988), *P. rugulosa* leaves turned out to be more enriched than those of the other species (Table 2), and the differences were highly significant for dry biomass and branches as well (data not shown).

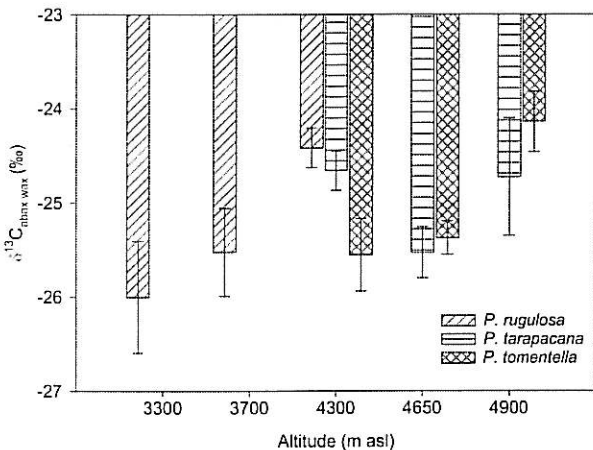


Fig. 4. Carbon isotope composition (mean \pm SE) of waxes isolated from abaxial (stomatous) leaf sides of *Polylepis* species at different altitudes.

The major difference among species was found in LA and transpiration, which also changed with altitude in a different way for the different species (*F*-values in Table 2). The largest leaves were found in *P. rugulosa* at the lowest location, and LA decreased by 43% with altitude within this species, but remained still significantly greater than in the other two species at the same altitude. In contrast, *P. tarapacana* and *P. tomentella* had rather constant leaf size across the altitudinal gradient (Table 3). However, there were no differences in SLA, except for leaves of *P. rugulosa*. Species

Table 3

The mean (\pm SD) leaf area of *P. rugulosa*, *P. tarapacana*, *P. tomentella* at all sampling locations. The area of whole leaf comprising of three leaflets is shown. Low, medium and high altitudes represent relative altitudes for each species. Different letter indicates a significant difference from other values within the same species (Tukey HSD test, $P < 0.001$).

Species	Altitude	Leaf area (mm ²)
<i>P. rugulosa</i>	Low	330.08 \pm 53.5 ^a
<i>P. rugulosa</i>	Medium	370.56 \pm 72.9 ^b
<i>P. rugulosa</i>	High	202.32 \pm 30.1 ^c
<i>P. tarapacana</i>	Low	125.64 \pm 32.2
<i>P. tarapacana</i>	Medium	124.46 \pm 22.9
<i>P. tarapacana</i>	High	132.36 \pm 25.9
<i>P. tomentella</i>	Low	120.44 \pm 30.2 ^a
<i>P. tomentella</i>	Medium	123.72 \pm 15.0 ^a
<i>P. tomentella</i>	High	143.16 \pm 20.8 ^b

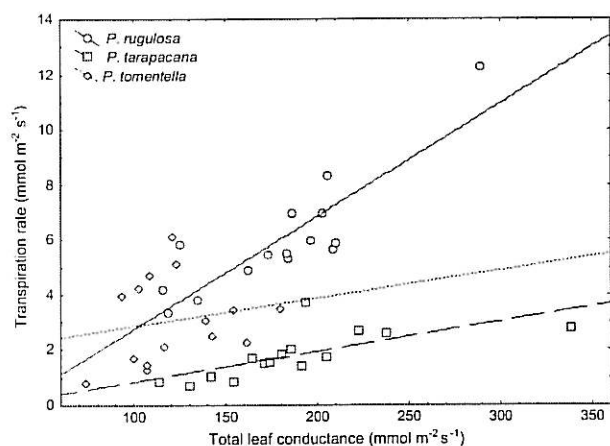


Fig. 5. Relationship between total leaf conductance and transpiration rate of target leaves. Symbols represent average values for each measured tree (circles: *P. rugulosa*; squares: *P. tarapacana*; diamonds: *P. tomentella*). Solid (*P. rugulosa*), punctuated (*P. tarapacana*) and dotted (*P. tomentella*) linear regression lines are shown; $R^2 = 0.75$, $P < 0.001$, $r = 0.86$; $R^2 = 0.48$, $P = 0.004$, $r = 0.69$; $R^2 = 0.04$, $P = 0.501$, $r = 0.19$, respectively.

differed in gas exchange traits as well: *P. rugulosa* had generally higher transpiration rates and J_{PSII} than both other species (Table 2, Fig. 3). For the same g_l values, *P. rugulosa* had higher transpiration rates than other species (Fig. 5).

Since the different species' study sites were distributed along only partially overlapping altitudinal gradients, which affected most measured characteristics as well, we further focused on species comparison at the same altitude to reveal evidence of species differences. In this comparison, most of studied traits were even more clearly expressed (Table 4). The highest values of leaf thickness, J_{PSII} , transpiration, N/P ratio and ^{13}C in waxes were observed at *P. rugulosa*, while its tree height, SLA and P concentration were the lowest.

4. Discussion

The three congeneric *Polylepis* species differ in a set of key morphological and ecophysiological traits. The variation of these traits with altitude confirms general patterns of decrease of plant stature and leaf size with altitude accompanied by a set of ecophysiological responses. However, such trait responses were

Table 4
Mean (\pm SD) values of recorded traits of selected *Polylepis* species growing at the same altitude, but at three different locations. Bold *F*-values (df = 2,12) represent significant difference. Different letter indicates a significant difference from other values (Tukey HSD test, $P < 0.05$). For trait explanations, see the text.

Trait	<i>F</i>	<i>P. rugulosa</i>	<i>P. tarapacana</i>	<i>P. tomentella</i>
Tree height (cm)	59.64***	139.6 \pm 20.3 ^a	313.0 \pm 21.7 ^b	272.0 \pm 34.4 ^b
Leaf thickness (mm)	9.00**	0.76 \pm 0.09 ^a	0.60 \pm 0.04 ^b	0.64 \pm 0.07 ^c
SLA (mm ² mg ⁻¹)	4.28*	2.90 \pm 0.45 ^a	3.35 \pm 0.29 ^b	3.21 \pm 0.22 ^b
J_{PSII} (μ mol m ⁻² s ⁻¹)	12.62**	346.5 \pm 157.4 ^a	96.8 \pm 48.8 ^b	252.2 \pm 95.6 ^c
g_l (mmol m ⁻² s ⁻¹)	7.91**	184.9 \pm 31.6 ^a	218.3 \pm 105.3 ^a	109.7 \pm 22.5 ^b
<i>E</i> (mmol m ⁻² s ⁻¹)	40.76***	5.54 \pm 0.82 ^a	2.07 \pm 0.77 ^b	4.82 \pm 1.12 ^c
^{13}C of leaf tissue (‰)	0.99	-23.8 \pm 1.3	-24.6 \pm 0.5	-24.5 \pm 0.7
^{13}C of abaxial cuticle waxes (‰)	4.25*	-24.4 \pm 0.4 ^a	-24.7 \pm 0.5 ^{ab}	-25.6 \pm 0.9 ^b
Leaf tissue N (mg g ⁻¹)	5.88*	10.2 \pm 0.5 ^{ab}	11.7 \pm 1.3 ^a	9.8 \pm 0.8 ^b
Leaf tissue P (mg g ⁻¹)	7.93**	0.81 \pm 0.06 ^a	1.13 \pm 0.17 ^b	1.13 \pm 0.18 ^b
Leaf N/P	11.45**	12.6 \pm 1.2 ^a	10.4 \pm 1.2 ^b	8.8 \pm 1.3 ^b
Leaf C/N	2.99	50.1 \pm 2.6	46.9 \pm 5.8	54.3 \pm 5.4

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

often found to be different among species (Fig. 3), with a generally stronger response to altitude in *P. rugulosa* than in the other two species. The most remarkable trait variation was found for leaf size: *P. rugulosa* had the largest leaves and their size decreased with altitude. *P. rugulosa* displayed also higher transpiration rates than the other species and transpiration decreased with altitude; its waxes from the abaxial (stomatous) leaf side were most strongly enriched in ^{13}C and tended to become more enriched at higher altitudes. All the above, together with exceptionally low concentrations of N and P in leaves in this species, suggest higher water loss and significant higher water stress in *P. rugulosa*, which is exacerbated with increasing altitude. We hypothesize that while the upper distribution limits of *P. tarapacana* and *P. tomentella* are largely driven by low temperatures, water stress seems to be an additional factor for the altitudinal distribution, especially for *P. rugulosa*.

Our hypothesis is supported by patterns in various morphological and ecophysiological traits. Namely, a decrease of leaf size with altitude is concordant with general trends that cold and high radiation stress, present at high altitudes, but also dry environments, all tend to select for smaller leaves (Cornelissen et al., 2003; Colmenares-Arteaga et al., 2005). In addition, larger leaves are more difficult to cool down during hot days (Smith and Geller, 1979; Rundel et al., 2003). Higher average temperatures found at the *P. rugulosa* location during the whole year suggest less freezing stress, but also possible heat stress and high VPD. A combination of these factors inevitably leads to higher transpiration rates (Fig. 3; Table 4).

Although a relatively high plasticity of leaf area in *P. tarapacana* was documented by González et al. (2002), we found that variation of this trait was of minor importance across the altitudinal distribution range of *P. tarapacana*. In contrast, for *P. rugulosa* the importance of plasticity is indirectly demonstrated by a decrease in leaf size with increasing altitude. However, at the highest site of *P. rugulosa*, this species is most probably reaching the limit of its leaf size: the average length of leaflets at this site was ~ 1.1 cm (results not shown). This size has also been reported in previous studies as the smallest leaflet size of *P. rugulosa* (Kessler and Schmidt-Lebuhn, 2006; Schmidt-Lebuhn et al., 2006b). Hence, it might be impossible for this species to further reduce leaf size.

Furthermore, higher photosynthetic capacity and transpiration rates were found for *P. rugulosa*. This is rather surprising, because this species inhabits the driest locations with precipitation ranging very close to the species' limit; e.g. in 2003, the total precipitation was as low as 84.7 mm (Muñoz and Bonacic, 2006). Furthermore, on most occasions *P. rugulosa* had higher transpiration rates when its leaf conductance was comparable to that of *P. tarapacana* and/or *P. tomentella*, which likely resulted in higher water loss. Elevated values of gas exchange variables could be explained by the timing of our measurements: they were recorded during the wettest months (January and February), when more favorable conditions for growth prevail (Rundel et al., 2003). Precipitation and elevated relative humidity during the wet season together with high irradiation and temperature promote relatively high transpiration rates and high potential photosynthetic capacity. During this time, which is certainly more suitable for growth, *P. rugulosa* opens its stomata and increases its photosynthesis in order to balance relatively lower increments in the dry season. Although this statement is not validated, and needs to be verified by phenological measures and/or further measurement of gas exchange traits during the dry season, an indirect support of highly reduced growth can be found in tree heights. Trees at the highest *P. rugulosa* site were the smallest, compared with trees of *P. tarapacana* and *P. tomentella*, irrespective of altitude. This seems to indicate a trade-off between plant height and stress tolerance (Cornelissen et al., 2003).

A common constraint for interpreting gas exchange measurements is the limited time frame of the measurements. While gas exchange was recorded for a few days, a better insight into plant history and growth conditions can usually be obtained from isotopic C composition of tissues. The commonly accepted altitudinal effect on $\delta^{13}\text{C}$ includes a shift of 1‰ per km (Körner et al., 1988). In our altitudinal gradient (3180–5010 m; all samples pooled), we did not observe a clear correlation between altitude and $\delta^{13}\text{C}$. However, comparisons of recalculated values (1‰ per km) revealed that the three species differ in $\delta^{13}\text{C}$, indicating that *P. rugulosa* is the one most severely stressed by water shortage. However, a leaf is a mixture of materials with different turnover times, formed and transformed before and during the wet season (Peuke et al., 2006); it has been reported that evergreen *Polylepis* species keep a positive carbon balance throughout the year (Rada et al., 1996; Rundel et al., 2003; García-Núñez et al., 2004). Furthermore, the isotopic signal may also be influenced by non-structural carbohydrates (NSC) and reserves (e.g. high amount of NSC and lipids for *P. tarapacana* from high altitude; Hoch and Körner, 2005). Hence, a better indicator of water stress can be obtained from the isotopic signal of cuticular waxes. Wax isotopic composition should reflect the environmental conditions during leaf development. Since we selected only fully developed leaves roughly in "middle age", the $^{13}\text{C}_{\text{wax}}$ corresponds largely to conditions during the dry season. The observed differences in $\delta^{13}\text{C}$ of waxes from the abaxial leaf side indicate that *P. rugulosa* generally kept its stomata less widely open than other *Polylepis* species at the same altitude (Fig. 4).

Last but not least, since nutrient uptake is usually coupled with water uptake, nutrient limitation is frequently noticed under water scarcity. In the case of the *Polylepis* species studied here, nutrient concentration is quite low (means of 1.12% and ~0.99‰ for N and P respectively) indicating nutrient deficiency compared with other alpine plants (Körner, 1989, 2003), and also a possible limitation by water availability. Nutrient deficiency is further supported by rapidly increasing N and P retranslocation with altitude (Macek et al., unpublished results). Koerselman and Meuleman (1996) established that N/P values below 14 indicate N limitation, which is the case for all these *Polylepis* species. Similarly, other leaf traits, such as leaf thickness or SLA, might also indicate water or nutrient limitation (Rao and Wright, 1994; Cornelissen et al., 2003). Thus, the thickest leaves and lowest SLA of *P. rugulosa* are another indirect evidence of water and/or nutrient limitation at the highest altitude.

The combined multiple trait responses to altitude suggest that water–carbon relations represent an important element in tree growth at high altitudes of dry regions. Data presented above point to an increasing effect of water stress on growth of *P. rugulosa* in northern Chile. Similar to our conclusions, Kessler et al. (2007) confirmed predominantly temperature-related limitation of Bolivian *Polylepis* forests, but with a small additional role of water. Our results also show, however, that temperature limitation could be less important when the effect of water limitation becomes increasingly important. This might have a serious effect on *Polylepis* distribution: further increase of temperature due to climate change might result in a decrease of available habitats (cf. Reichstein et al., 2007). However, a test on a greater number of independent locations, including more long-term measures of several abiotic conditions (especially soil temperatures, soil humidity and precipitation patterns) and coupled with measurements of osmotic adjustment or water potential, is needed to clarify to which extent water stress affects the altitudinal distribution of different species occurring at the treeline of these regions. Overall, this study highlights that, apart from general limits imposed by temperature (Körner, 1998; Körner and Paulsen, 2004), water stress can be an additional factor that

most probably shapes the altitudinal distribution of treeline *Polylepis* species.

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