Validation of Non-destructive Techniques for Estimating the Age and Growth of *Polylepis tarapacana* in the High Andes of Northern Argentina

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Abstract

Accurately determining the age of trees is fundamental for ecological and conservation studies, particularly for slow-growing, threatened species in extreme environments. This study evaluates the effectiveness of a non-destructive technique for estimating the age of apical branches and analyzing the growth of *Polylepis tarapacana*, a near-threatened tree species that forms the world's highest forests and shrublands in the Argentine Altiplano. The technique relies on counting the external bud marks (scars) left by annual shoots on apical branches. To validate this method, we collected 48 apical branches from across the species' distribution in Argentina. The age estimated from bud mark counts was correlated with the age determined by annual ring counts on cross-sections. Statistical analysis revealed a highly significant positive correlation between the two methods (Spearman's $\rho = 0.820$, $p < 0.001$), with a coefficient of determination of $R^2 = 0.672$. The overall mean absolute error of the non-destructive technique was 1.11 years. Accuracy varied with age, showing higher relative error in very young branches (1-3 years) but high reliability (relative error of 8.5%) for branches over 13 years old. Furthermore, growth analysis indicated a significant decrease in annual shoot elongation with increasing age, with a reduction observed between the youngest (1-3 years) and oldest (10-12 years) age groups. These results demonstrate that the bud mark counting technique is a statistically valid, accurate, and robust non-destructive method for age estimation in young apical branches (up to ~25 years) of *P. tarapacana*. Its application provides a valuable tool for demographic monitoring and conservation strategies for this high-altitude species, enabling growth assessment without damaging vulnerable populations.

Keywords: Bud mark counting; Determinate growth; Altiplano; Queñoa; High-altitude forest; Near-threatened species

Introduction

Height growth in trees and shrubs results from the expansion of terminal shoots on the main stem and its branches. These shoots typically undergo determinate growth (fixed growth *sensu* Kozlowski and Pallardy (1997) or determinate growth sensu Hallé et al. (1978), where they grow after a period of dormancy, leaving external marks from buds on branches (Kozlowski and Pallardy, 1997). This information is used to estimate ages along apical shoots or branches by counting internodes (Kajimoto et al., 1998; Hoch and Körner, 2005) or by observing bud marks or scars (Kajimoto et al., 1998; Cuevas, 2002; Gea et al., 2004; Martínez Pastur et al., 2007; Soler et al., 2018). By combining estimated age and branch length, it is possible to calculate the annual growth of an individual tree or branch in forest species with determinate growth patterns (Kajimoto et al., 1998; Cuevas, 2002; Gea et al., 2004; Hoch and Körner, 2005; Martínez Pastur et al., 2007; Soler et al., 2018). This estimation can only be performed on young branches, as the method tends to become less precise due to bark formation that obscures the scars on older branches (Kajimoto et al., 1998; Cuevas, 2002). The accuracy of the ages determined by this non-destructive method must be verified by counting the annual rings at each stem base through cutting, polishing, and observation under a magnifying glass (Kajimoto et al., 1998).

Forest growth at its elevation limit is determined by climatic factors, and as elevation increases, its structure changes from trees to scattered shrubs (Hoch and Körner, 2005), reaching its highest elevation in dry regions such as Tibet (Miehe et al., 2003) and especially in the South American Altiplano (Braun, 1997). The upper elevation limit of forests occurs under similar temperature conditions globally, but the functional mechanisms that reduce tree growth are poorly understood (Toivonen et al., 2014).

The Argentine Altiplano is characterized by heterogeneous landscapes, which include low grasslands and scrublands with occasional groves of the only tree genus, *Polylepis* (*Rosaceae*), of which *Polylepis tarapacana* Phill. (commonly called Queñoa) forms scattered monospecific forests at the highest altitudes (3900-4400-5000 m a.s.l.) (Kessler, 1995, López et al., 2022). This species is considered Near Threatened (NT) (IUCN, 2022) due to its use as fuel by local communities (Renison et al., 2010). The various adverse conditions present in the high plateau (high solar radiation, evapotranspiration, high temperature range, among others) are overcome by optimizing the use of its resources through physiological (growth rate, development) and morphological modifications, both internal and external (Cozzi and Moschione, 2011). In the case of threatened and slow-growing species, such as *P. tarapacana*, estimating age requires non-destructive techniques that avoid the collection of branches, saplings, or entire specimens and their subsequent polishing and ring counting. Growth studies exist for *Nothofagus pumilio* (Poepp. & Endl.) Krasser and *Nothofagus antarctica* (G. Forst.) Ørsted where age is estimated by the marks left by buds on the stems of seedlings, achieving high accuracy up to 15-20 years of age (Cuevas, 2002). This technique was also used in *Pinus pumila* (Pall.) Regel saplings, where seedling age is determined by counting the number of nodes corresponding to annual growth along a main stem from the basal portion to the terminal bud (Kajimoto et al., 1998). A similar technique was used by Hoch and Körner (2005) who studied apical growth in *P. tarapacana* trees, using bud marks and the distance between these bud marks on tree shoots to determine apical growth over two consecutive periods. They used this information to study growth along an elevation gradient in Sajama, Bolivia. However, these authors did not corroborate the accuracy of this technique by relating the age determined by annual ring count (age) to the marks.

Previous studies have applied bud mark counting in *P. tarapacana* for growth analysis (Hoch and Körner, 2005) or explored its potential for age estimation with a limited sample (López et al., 2021). However, no study has yet provided a statistically validated calibration of the technique against annual ring counts across a large, geographically representative sample. The present study offers three genuine extensions beyond previous work: (i) sample size and geographic coverage -48 branches from the entire Argentine distribution of the species, compared to 13 branches from a single site in López et al. (2021); (ii) statistical validation -we quantify accuracy, mean absolute error, and relative error across age groups, and test for heteroscedasticity and plot effects; (iii) calibrated predictive model-we provide a regression equation that allows researchers to estimate branch age non-destructively with known confidence intervals.

Therefore, the objective of this study is to evaluate the effectiveness of the age estimation technique using bud marks in *P. tarapacana* for estimating the age of young apical branches. The specific objectives are a) To validate the non-destructive age estimation technique through the correlation between the number of bud marks and the age, b) To quantify the accuracy and error of the bud marks estimation technique in different age groups and c) To analyze growth patterns and temporal variations in shoot elongation.

Materials and Methods

Study Area

In Argentina, the Altiplano is located in the arid northwest, bordering Chile and Bolivia. It consists of a vast plateau with elevations starting at 4000 m above sea level, covering 60% of the territory of the province of Jujuy, which has an arid and cold climate.

The climate in the High-Andes is dry and cold, with significant temperature variations reaching absolute minimum temperatures as low as -20°C. Precipitation is concentrated in the summer months with average amounts varying between 100 mm and 300 mm. The daily temperature range reaches values between 30 and 35°C. During the winter months, the High-Andes experiences strong, dry winds (Paoli, 2014) blowing intensely and continuously from the southwest of the continent, accompanied by frequent snowfall on the high Andean peaks surrounding the plateau. This makes it the most inhospitable arid zone in Argentina (Morales et al., 2012).

Polylepis tarapacana

Polylepis tarapacana develops in the High Andean phytogeographic province, forming sparse and dispersed forests typical of the central Andes (Figure 1.A) (Liberman-Cruz et al., 2021, López et al., 2022; 2023). Is a sympodial tree with a short trunk (Figure 1.B), reaching up to 3 m in height (Morales et al., 2004). Regarding its height growth, the period of greatest shoot elongation occurs after the rainy season (March and April), when the reduction in temperature generates rain and cloud cover (Hoch and Körner, 2005), marking a seasonality in its growth. It is a tree characterized by its very scaly, laminated, reddish-brown bark, which acts as thermal insulation to protect it against frost; small, imparipinnate leaves (Figure 1.B) with 1-3 oval, obovate to circular leaflets, glossy on the upper surface and covered with whitish hairs on the underside, thick and coated in resin; small flowers in clusters; and a twisted trunk. The growth of *P. tarapacana* is significantly controlled by rainfall, which has decreased in recent decades (Morales et al., 2022). These changes may affect the mortality, growth, and establishment patterns of this species (López et al., 2021).

Sampling Site

A total of 48 patches of *P. tarapacana* were selected throughout its entire distribution in Argentina, considering the following criteria: (a) patch size greater than 1 ha, (b) homogeneous cover with a practically constant distance between individuals, and (c) accessibility. The sampled patches span the full latitudinal and elevational range of the species in Argentina (from 4165 to 4842 m a.s.l.; see Figure 2), covering the main known populations in the Sierras de San José, Mina Pirquitas, Coranzuli, Susques, Ramadayoc, and Granada Volcanoes

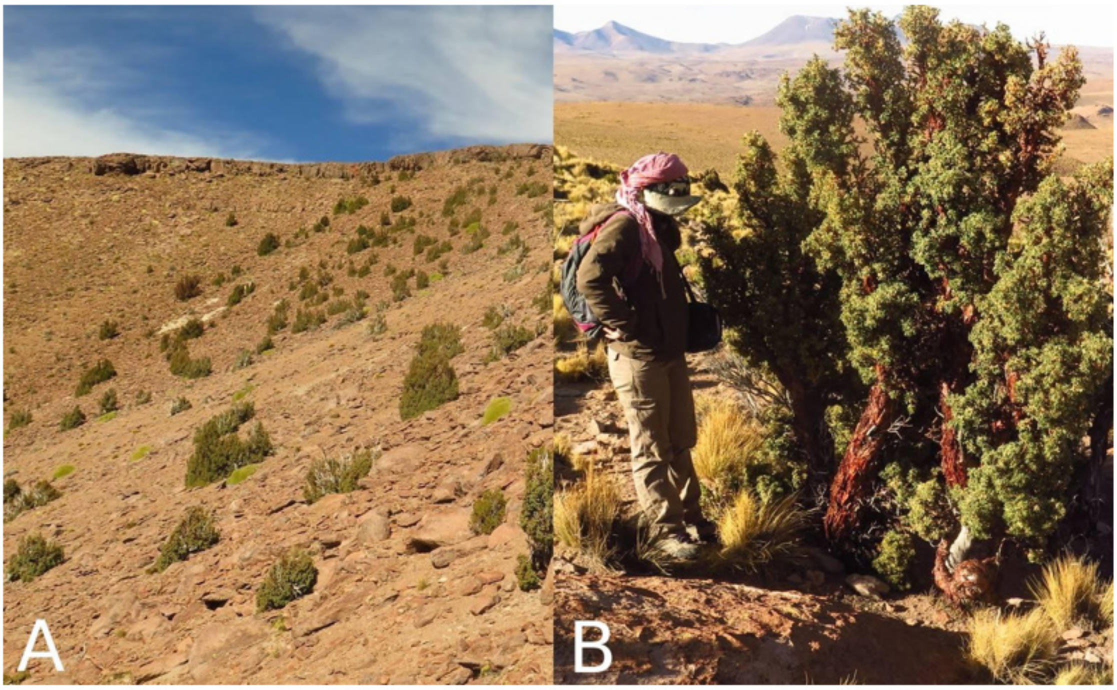

Figure 1 A) Environment where the Polylepis forest and shrubland develops and B) detail of a *Polylepis tarapacana* tree at human scale

(Wawrzyk and Vilá, 2013). This sampling design captures the environmental and structural variability of *P. tarapacana* forests, ensuring representativeness of the species' distribution in Argentina. Samples were collected in November 2019. All 48 branch samples analyzed in this study were collected specifically for this validation and have not been used in any previous publication on bud mark counting or age estimation in *Polylepis tarapacana*. The dataset is independent from the exploratory sample (n=13) reported in López et al. (2021).

Within each patch, a 20 cm long apical branch was collected from the healthy individual closest to the center, provided its height was at least equal to the average height. Each selected branch was stored in a labeled paper envelope to preserve and isolate the sample. This non-destructive sampling method follows established protocols for dendroecological studies in threatened species (Bräuning et al., 2020). The collected material from *P. tarapacana* was analyzed in the laboratory. On each of the 48 branches, the number of visible external bud marks along each 20 cm branch was counted and marked to estimate its age (Figure 3). The use of bud marks for age estimation follows the conceptual approach of López et al. (2021), but the schematic

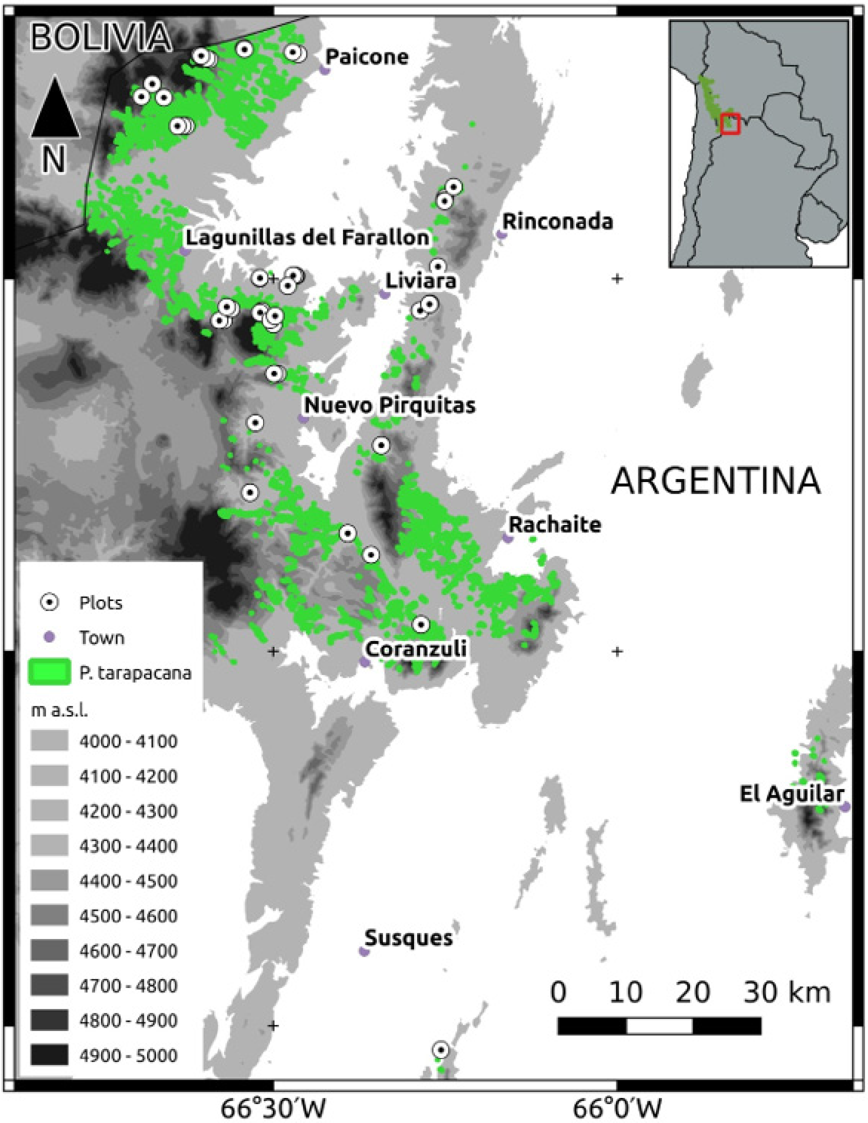

Figure 2 Location map of the study area. Sampled patches (white circles) are shown in the Argentine portion of the Altiplano (Jujuy Province). The known distribution of *Polylepis tarapacana* (queñoa) is indicated in green. Grayscale elevation contours range from >4000 to <5000 m a. s. l. Base map sources: SRTM elevation data and administrative boundaries

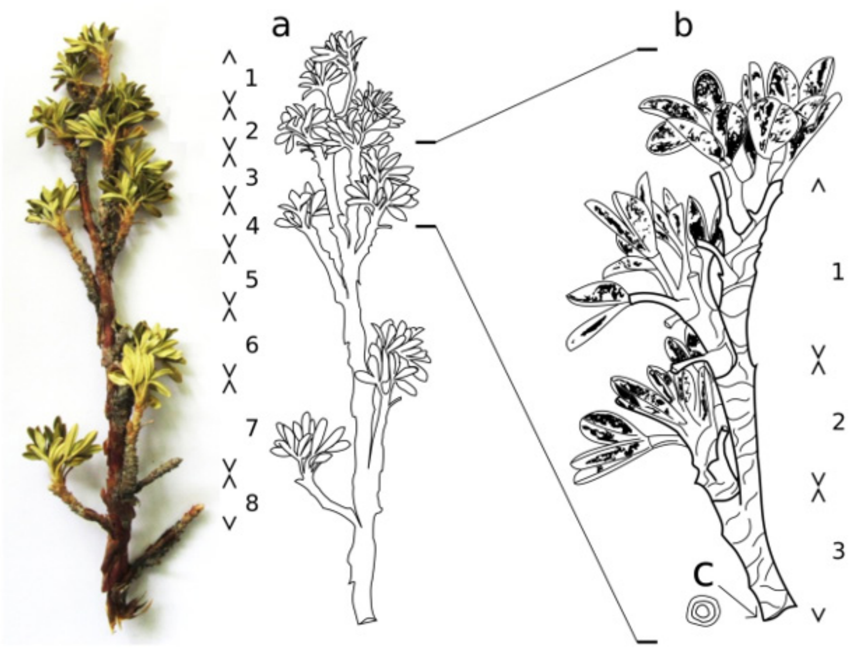

Figure 3 (a) Photograph of a *Polylepis tarapacana* apical branch showing visible bud marks (arrows) indicating annual growth increments. (b) Schematic diagram of the same branch, with numbered segments (1-3) corresponding to successive years of shoot elongation. (c) Cross-section of the branch at the base of segment 3, showing annual growth rings (dashed circles) that correspond to the bud marks

representation in Figure 3 has been redesigned for this study. The correspondence between bud marks (longitudinal) and growth rings (transverse) is indicated by matching colors/numbers.

To validate the bud marks technique, cross-sections of twig subsamples were cut and progressively sanded with 60 to 600 grit sandpaper until an optimal surface for visualizing the growth rings was obtained. Ring counting was performed under a light microscope at 10-40X magnification, considering the band of annual growth defined by the alternating porosity as a complete ring (Morales et al., 2022; Paredes-Villanueva et al., 2023). In *P. tarapacana*, annual growth rings are clearly demarcated by a combination of anatomical features: (i) alternating porosity, with larger vessels in earlywood and smaller vessels in latewood; (ii) marginal parenchyma bands that form a thin line of flattened cells at the ring boundary; and (iii) fiber compression in the latewood. These characteristics are consistent with previous descriptions for *Polylepis* species (Morales et al., 2022; Paredes-Villanueva et al., 2023). Growth pattern analysis was conducted using longitudinal shoot elongation measurements from 48 unique branches.

Statistical Analysis

The analytical workflow consisted of three sequential steps, corresponding to the three specific objectives: a) Validation: For each of the 48 branches, we counted visible bud marks (field and laboratory) and then cut, sanded, and counted annual growth rings under a microscope. We calculated Spearman's rank correlation between bud marks and ring counts, fitted a linear regression model, and tested for plot effects using mixed effects models, b) Accuracy and error by age group: We computed mean absolute error (MAE) and mean relative error (MRE) for each branch, then stratified branches into five age groups (1-3, 4-6, 7-9, 10-12, 13+ years). For each group, we calculated average MAE and MRE, and used LOESS regression to characterise error trends across the age gradient, and c) Growth patterns: From the 48 branches, we measured annual shoot elongation (distance between successive bud marks) for each year of growth, resulting in 1548 individual annual observations. We grouped these observations by branch age and used Kruskal Wallis tests followed by pairwise Wilcoxon comparisons with Bonferroni correction to detect differences in shoot elongation among age groups. We also generated hexagonal density plots with LOESS trends to visualise the relationship between age and growth.

The following subsections provide detailed descriptions of each statistical procedure. The non-destructive age estimation technique was validated by correlating the number of bud marks with the age determined by annual ring count (age). The statistical analysis was structured according to the specific aims: (a) to validate the non-destructive age estimation technique, the Spearman rank correlation coefficient (ρ) between the number of bud marks and the growth ring count was calculated, followed by a simple linear regression model. Spearman's correlation was chosen because the data did not meet the assumption of normality (Shapiro-Wilk: $p < 0.05$). Additionally, a mixed linear model was implemented considering the random plot effect to control for spatial variability. The accuracy of the technique was evaluated using mean absolute error and mean relative error, stratified by age groups. The graph was constructed using hexagonal geometry with a viridis density scale to represent point concentration, including 99% confidence intervals for the regression and a 1:1 reference line for ideal comparison. Aim (b), to quantify the accuracy and error of the scar estimation technique, the mean absolute error and mean relative error were calculated, stratified by age groups (1-3, 4-6, 7-9, 10-12, >13 years). To characterize the error trend across the age gradient, LOESS (Locally Estimated Scatterplot Smoothing) regression with adaptive smoothing parameter and 99% confidence interval was used. The choice of LOESS allowed for the capture of nonlinear patterns without assuming a specific functional form. The analysis included calculating Spearman's rank correlation between age and error. Finally c), to analyze growth patterns and temporal variations in shoot elongation, growth pattern analysis was conducted using longitudinal shoot elongation measurements from 48 unique branches. The data were grouped into four age groups (1-3, 4-6, 7-9, and 10-12). Since the data did not meet the assumptions of normality (Shapiro-Wilk: $p < 0.05$) or homoscedasticity (Levene: $p = 0.170$), the non-parametric Kruskal-Wallis test was used, followed by pairwise comparisons with the Wilcoxon test, applying Bonferroni correction for multiple comparisons. This analysis allows for the identification of significant differences in growth between age groups. The relationship between individual longitudinal shoot elongation and bud marks was analyzed using a hexagonal density plot that included 1548 annual observations. A LOESS trend with a 99% confidence interval was superimposed, allowing for the observation of the growth pattern with number of bud marks, complementing the analysis by grouped periods.

All statistical analyses were performed using R version 4.3.2 (R Core Team 2023). Mixed-effects models were fitted using the 'lme4' package version 1.1-34 (Bates et al. 2015). Graphics were created with 'ggplot2' version 3.4.4 (Wickham 2016). Additional data manipulation was done using 'dplyr' version 1.1.2 (Wickham et al. 2023).

Results

Correlation between bud marks and age determined by annual ring count

The average ring width across all sampled branches was 0.48 ± 0.12 mm (range: 0.21-0.89 mm), consistent with slow growth rates reported for high-elevation *Polylepis* forests. The validation of the non-destructive estimation technique showed a highly significant correlation between the number of bud marks and age determined by annual ring count (Spearman's $\rho = 0.820$, $p < 0.001$), with a coefficient of determination of $R^2 = 0.672$. The linear regression model ($\text{age} = 0.4529 + 0.9157 \times \text{Scars}$, $R^2 = 0.884$, $F(1,521) = 3570.6$, $p < 0.001$) indicated a consistent relationship across the entire sampled age range (1–25 years). Residual diagnostics for the regression model confirmed normality (Shapiro Wilk, p = 0.214), no significant heteroscedasticity (Breusch Pagan test, p = 0.126), and no influential outliers (maximum Cook's distance = 0.023, well below the conventional threshold of 1). The high R^2 value (0.884) indicates that bud marks alone explain 88.4% of the variance in age, with the remaining 11.6% attributable to counting errors, bark concealment, or years with no measurable shoot growth. The overall mean absolute error was 1.11 years, with a relative error of 28.8%. Accuracy varied among age groups: young individuals (1-3 years) showed the highest relative error (81.2%), while mature individuals (13+ years) showed the highest accuracy (relative error 8.5%). The distribution of points revealed a higher density of observations in the intermediate age classes (5-15 years) and a lower representation at the extremes of age. The regression line remained close to the ideal 1:1 ratio, particularly in the 5-20 bud marks range. The 99% confidence interval indicated high accuracy in the estimates (Figure 4A). The technique proved to be particularly reliable for branches older than 5 years, where the absolute error consistently remained below 1.5 years (Figure 4B).

The analysis revealed a positive correlation between age and absolute prediction error ($\rho = 0.154$), indicating a slight trend toward increasing error with age (Figure 4B). The LOESS regression showed a nonlinear trend characterized by a moderate decrease in error at younger ages (1-8 years), followed by a plateau at middle ages (8-18 years), and a slight increase in older individuals (>18 years). The density distribution in the hexagon plot identified major error clusters between 0.5 and 1.5 years for most age groups, with less frequent extreme errors (>2.5 years). The 99% confidence interval for the LOESS curve showed a relatively narrow band, particularly in the 5-20 year age range where the data density was highest. For the Heteroscedasticity Analysis, the Breusch-Pagan test showed no evidence of significant heteroscedasticity (BP statistic = 2.34, p = 0.126) and the error-age correlation (Spearman's $\rho = 0.08$, $p = 0.089$). Thus, the estimation error does not increase significantly with age, demonstrating the robustness of the technique across the age gradient.

Validation with mixed effects models confirmed the relationship between bud marks and age, controlling for random plot effects.

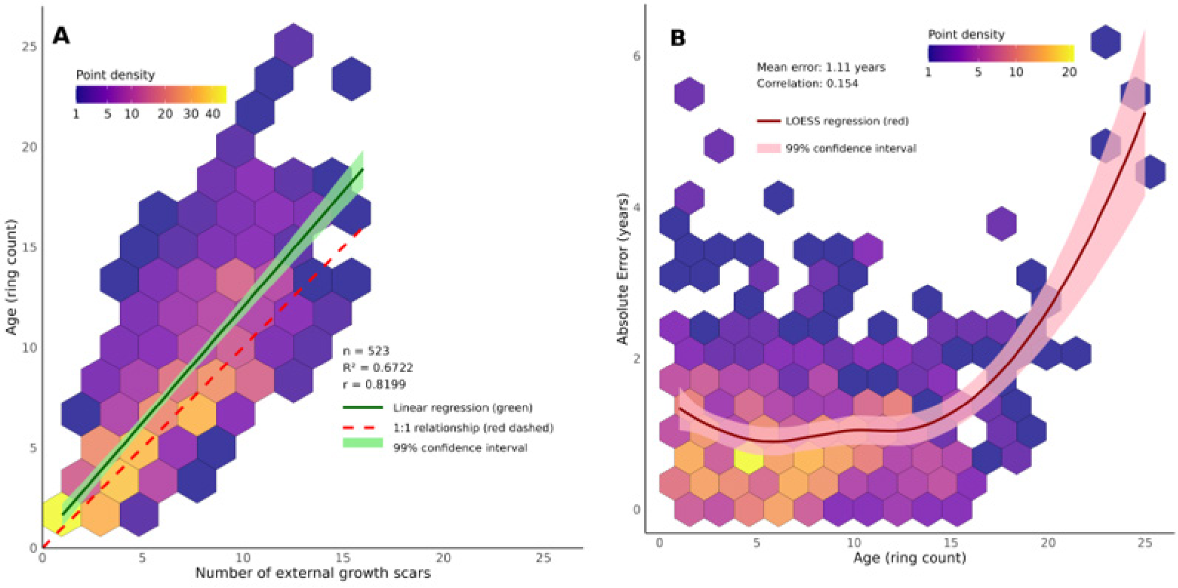

Figure 4 A)Validation of the Non-Destructive Age Estimation Technique using Correlation between bud marks and age determined by annual ring count, B) Analysis of prediction error vs. age

A linear mixed model (LMM) was selected because branches were nested within 48 forest patches (plots), which could introduce non independence among observations. The model was specified as: Age ~ BudMarks + (1 | Plot). We evaluated model assumptions by inspecting residuals for normality (Shapiro Wilk test), homoscedasticity (plot of residuals vs. fitted values), and influential observations (Cook's distance). The Intraclass Correlation (ICC) was 0.048, which implies that only 4.8% of the variation is explained by differences between plots, while 95.2% of the variation is attributable to the linear relationship between bud marks and age. Residue analysis showed a normal distribution of residues (Shapiro-Wilk: p = 0.214), a mean residue of -0.02 (not significantly different from zero), and 95.3% of the residues were within ±1.96 SD (expected: 95%). Based on these results, it can be observed that the plots represent different environmental conditions, and the bud marks-age relationship is very consistent across plots, demonstrating that the technique works well in different plots.

Growth Analysis Results

A significant decrease in shoot elongation was observed across age groups (Kruskal-Wallis: $\chi^2 = 29.794$, $p < 0.001$; Table 1). The youngest branches (1-3 years) grew significantly faster than all older groups ($p < 0.001$ for all pairwise comparisons). No significant differences were detected among branches aged 4-6, 7-9, 10-12, or 13+ years ($p > 0.05$ for all pairwise comparisons), indicating that growth reduction occurs primarily during the first 3-4 years and stabilizes thereafter. The oldest branches (13+ years) showed the lowest mean growth, but this was not statistically different from the 4-6 year group ($p = 0.087$). Rather than a continuous progressive decline, the pattern is best described as an initial rapid reduction in shoot elongation followed by a plateau at lower growth rates (Table 1).

Analysis with Individual Annual Data

Analysis of 1548 individual annual observations confirmed a significant negative correlation between age and annual growth (Spearman's $\rho = -0.324$, $p < 0.001$). The LOESS trend showed a rapid decline during the first 6 years, followed by a stabilization of growth (Figure 5). The consistent downward trend across all temporal windows indicates a progressive reduction in growth performance over time.

Discussion

Our results demonstrate that the bud mark counting technique is a statistically valid method for estimating branch age in *Polylepis tarapacana*, with a highly significant correlation between bud marks and annual ring counts (Spearman's $\rho = 0.820$, $p < 0.001$). The overall mean absolute error of 1.11 years indicates that, for most branches, the non-destructive estimate falls within one year of the true age. This level of accuracy is comparable to that reported for conifer species, where mean errors ranged from 0.18 to 1.25 years for branches up to 21 years old (Urza and Sibold, 2013; Hankin et al., 2018). However, our study provides the first statistically robust validation of this technique for any *Polylepis* species, extending the preliminary findings of López et al. (2021) who suggested potential applicability with a smaller sample (n=13).

The accuracy of the technique varied with age: relative error was highest in very young branches (1-3 years, 81.2%) but decreased substantially to 8.5% in branches older than

TABLE 1

Kruskal-Wallis analysis for individual annual data by age determined by annual ring count group

Age Group	n	Average growth ± SD
1-3	428	2.15 ± 0.89 a
4-6	385	1.78 ± 0.75 b
7-9	312	1.69 ± 0.72 b
10-12	243	1.64 ± 0.74 b
13+	180	1.58 ± 0.68 b

SD = Standard deviation

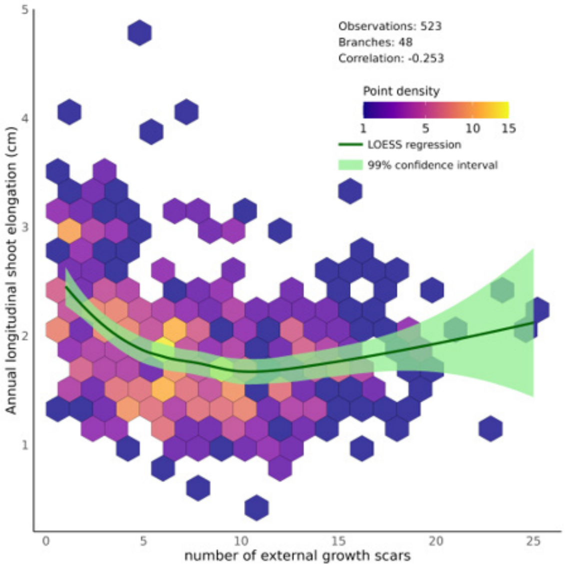

Figure 5 Annual shoot elongation decreases with the number of bud marks in *Polylepis tarapacana*

13 years. This pattern likely reflects the greater proportional impact of counting errors on shorter age estimates, as well as the possibility that some very young branches may have incomplete bud mark expression. Importantly, the absence of significant heteroscedasticity (Breusch-Pagan test, p = 0.126) indicates that estimation error does not increase with age, contradicting the expectation that bark formation progressively obscures older marks (Hett and Loucks, 1976; Thabeet et al., 2009). For *P. tarapacana*, bud marks remained visible and countable up to at least 25 years, suggesting that the species' bark characteristics (scaly, exfoliating bark) may delay mark occlusion compared to other taxa.

This study presented results similar to those observed by Urza and Sibold (2013) and by Hankin et al. (2018) in conifers; in these studies, the mark count underestimated the age of the shoots. In the case of *Polylepis* in general, and *P. tarapacana* in particular, there is evident bark production (Kessler, 2006), which results in concealment directly proportional to the age of the mark. However, there could be a discrepancy between the number of bud marks and the measured age due to the lack of height growth in unfavorable years.

This methodology of counting bud marks on branches was used in studies on *N. pumilio*; in those studies, the technique was validated by Cuevas (2002), and later applied by Gea et al. (2004) and Soler et al. (2018) in growth studies. This technique, validated for young stands of *P. pumila* (Kajimoto et al., 1998), was applied to different species of the Pinaceae family, including Abies alba Mill., *Pinus halepensis* Mill., *Pinus nigra* J. F. Arnold, *Pinus pinea* L., *Pinus pinaster* Aiton, and *Pinus sylvestris* L. (Mutke et al., 2005; Thabeet et al., 2009; Crone et al., 2011; Girard et al., 2011; Vennetier et al., 2013). In the case of *Picea engelmannii* Parry ex Engelm, *Pseudotsuga menziesii* (Mirb.) Franco, *Pinus contorta* Douglas, and *Larix occidentalis* Nutt, the accuracy of the method varies with the species studied and the estimated age (Urza and Sibold, 2013). Average errors ranged from 0.18 to 1.25 years, and standard deviations ranged from 1.83 to 2.70 years for estimates of 2 to 21 years. These authors reported that counting bud marks along the branch underestimated age by an average of 4.1 years, with the bias increasing with the age of the mark. Given this increasing bias, it is crucial to select age-determination methods based on the accuracy required to answer specific ecological questions (Hankin et al., 2018), highlighting the importance of validating these techniques.

Furthermore, field observations showed that the identification of terminal bud marks for *P. engelmannii*, *P. menziesii*, *P. contorta*, and

L. occidentalis is more limited by extremely narrow growth increments than by marks obscured by bark formation (Urza and Sibold, 2013). In the case of *P. menziesii*, the bias increased with sample age more rapidly in slower-growing individuals, suggesting that these individuals may produce unclear annual nodes due to physiological limitations (Hankin et al., 2018). Hoch and Körner (2005) used this methodology in *P. tarapacana* and calculated the annual increment of apical branches using the two-year growth length of the terminal shoot without verifying the accuracy of the technique. Applying the bud mark counting method does not harm the plants, is quick, and can be performed in the field without specialized equipment. Bud marks from 1 to 20 years can be identified, and vertical growth rates can be obtained without direct measurements each year.

These results demonstrate that the bud mark technique is statistically valid for age estimation in young apical branches of *Polylepis tarapacana* (up to approximately 25 years) and maintains acceptable accuracy across all age groups within this range. These findings support the use of this non destructive technique for demographic studies in this species and in other *Polylepis* species with similar bark characteristics, but caution should be exercised when extrapolating to other taxa without independent validation. The results also highlight the need to consider local factors in the management and conservation of *P. tarapacana*. However, our conclusions are limited to the specific sampling design (48 patches across the Argentine distribution) and to branches ≤20 cm in length; further studies would be needed to assess the technique's performance on longer branches or in populations outside Argentina. Regarding growth patterns, the technique detected a significant reduction in shoot elongation between the youngest branches (1-3 years) and older branches (≥4 years). However, this pattern is best characterized as an initial fast growth phase followed by stabilization at lower elongation rates, rather than a continuous age related decline (Figure 5). The observed reduction may reflect a combination of ontogenetic changes in resource allocation (e.g., increased investment in radial growth and reproduction after early establishment) and environmental constraints typical of high altitude ecosystems (Hoch and Körner, 2005). The technique's ability to capture this age related pattern suggests adequate sensitivity for monitoring changes in the species' productivity, but any interpretation of temporal 'decline' should be made cautiously, as our data are cross sectional (different branches of different ages) rather than longitudinal (repeated measurements of the same branches over time).

Conclusion

This study evaluated the effectiveness of the non destructive bud mark counting technique for estimating branch age and analyzing growth in *Polylepis tarapacana*, a near threatened high altitude species. Based on the three specific objectives, we conclude the following: A) Validation of the technique: The bud mark count showed a strong and highly significant correlation with the true age determined by annual ring counts (Spearman's $\rho = 0.820$, $p < 0.001$). The mixed effects model confirmed that this relationship is consistent across different forest patches, with only 4.8% of the variance explained by plot differences. Thus, the non destructive technique is statistically validated for estimating branch age in *P. tarapacana* up to approximately 25 years. B) Accuracy and error by age group: The overall mean absolute error was 1.11 years, with a relative error of 28.8%. Accuracy improved with age: the highest relative error occurred in the 1-3 year group, while the lowest error was in branches older than 13 years. The technique is therefore most reliable for branches older than 5 years, where absolute error consistently remained below 1.5 years. C) Growth patterns and temporal variations: Shoot elongation decreased significantly with branch age. However, the reduction was not a continuous progressive decline but rather an initial rapid decrease during the first 3-4 years, followed by a stabilization at lower growth rates. Branches in the 1-3 year group grew significantly faster than all older groups, among which no significant differences were detected. The oldest branches (13+ years) showed the lowest mean growth, but this was not statistically different from the 4-6 year group.

The bud mark counting technique provides a quick, affordable, and reliable non destructive method for age estimation in young branches (≤25 years) of *P. tarapacana* within the Argentine Altiplano. Its application is particularly valuable for demographic monitoring and conservation strategies in this threatened species, as it avoids destructive sampling of vulnerable high altitude populations. However, users should be aware that accuracy is lower for very young branches (1-3 years, relative error 81.2%) and that the technique does not estimate total tree age. Future research should extend the validation to other *Polylepis* species, test the technique's performance under different environmental conditions and climate variability scenarios, and, if possible, develop longitudinal studies (repeated measurements of the same branches) to confirm the age growth patterns suggested by our cross sectional data.

Acknowledgments

We extend our deepest gratitude to the local communities of the Argentine Altiplano in Jujuy. During a period of severe state defunding of scientific research, your hospitality - providing us with food and shelter - was not just assistance but the essential support that made this work possible. Without your solidarity, this research could not have been conducted. This study was carried out in the absence of specific research grants. The fieldwork and laboratory analyses costs were covered entirely by the authors' personal salaries, demonstrating the critical situation of scientific research in Argentina.

Ethical Considerations

The research followed the sustainable collection principles established by the IUCN for species from vulnerable ecosystems, limiting sampling to non-essential plant material and ensuring the survival of sampled individuals (IUCN, 2022).

Data Availability Statement

The dataset supporting the findings of this study has been deposited in the Open Science Framework (OSF) and is publicly available under a CC BY 4.0 license. The data include all field measurements, growth ring counts, and R scripts used for statistical analyses, ensuring full transparency and reproducibility of the results.

DOI: https://doi.org/10.17605/OSF.IO/XTZV7

References

Bates, D., Maechler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*. **67(1)**: 1-48.

Braun, G. (1997). The use of digital methods in assessing forest patterns in an Andean environment: the *Polylepis* example. *Mountain Research and Development* **17**: 253-262.

Bräuning, A., Homeier, J., Cueva, E., Beck, E. and Günter, S. (2020). Non-destructive sampling for dendrochronological studies in tropical mountain forests. *Dendrochronologia*, **61**: 125707.

Cozzi, E. A. and Moschione, F. (2011). Guía de flora y fauna en la región de Mina Pirquitas. Mina Pirquitas inc. Jujuy. Argentina.

Crone, E. E., McIntire, E. J. B. and Brodie J. (2011). What defines mast seeding?

Spatio-temporal patterns of cone production by whitebark pine. *Journal of Ecology* **99**: 438-444. https://doi.org/10.1111/j.1365-2745.2010.01790.x.

Cuevas, J. G. (2002). Episodic regeneration at the *Nothofagus pumilio* alpine timberline in Tierra del Fuego, Chile. *Journal of Ecology*, **90**: 52-60.

Gea-Izquierdo, G., Martinez Pastur, G., Cellini, J. M. and Lencinas, M. V. (2004). Forty years of silvicultural management in southern *Nothofagus pumilio* primary forests. *Forest Ecology and Management*, **201**: 335-347.

Girard, F., Vennetier, M., Guibal, F., Corona, C., Ouarmim, S. and Herrero, A. (2011). *Pinus halepensis* Mill. crown development and fruiting declined with repeated drought in Mediterranean France. *European Journal of Forest Research* **131**: 919-931. https://doi.org/10.1007/s10342-011-0565-6.

Hallé, F., Oldeman, R. A. A. and Tomlinson, P. B. (1978). Tropical trees and forests. An architectural analysis. Springer-Verlag, Berlín, Germany.

Hankin L. E., Higuera, P. E., Davis, K. T. and Dobrowski, S. Z. (2018). Accuracy of node and bud-scar counts for aging two dominant conifers in western North America. *Forest Ecology and Management*, **427**:365-371. https://doi.org/ 10.1016/j.foreco.2018.06.001.

Hett, J. M. and Loucks, O. L. (1976). Age structure models of balsam fir and eastern hemlock. *Journal of Ecology*, **64**: 1029-1044. https://doi.org/10.2307/2258822.

Hoch, G. and Körner, C. (2005). Growth, demography and carbon relations of *Polylepis* trees at the world's highest treeline. *Functional Ecology* **19**: 941-951.

IUCN. (2022). Guidelines for the conservation and sustainable use of biodiversity in high-altitude ecosystems. *International Union for Conservation of Nature*.

Kajimoto, T., Onodera, H., Ikeda, S., Daimaru, H. and T. Seki. (1998). Seedling establishment of subalpine stone pine (*Pinus pumila*) by nutcracker (*Nucifraga*) seed dispersal on Mt. Yumori, Northern Japan. *Arctic and Alpine Research*, **30**: 408-417.

Kessler, M. (1995). Present and potential distribution of *Polylepis* (Rosaceae) forests in Bolivia. In: Proceedings of the neotropical montane forest biodiversity and conservation symposium. The New York Botanical Garden. New York. USA 281-294.

Kessler, M. (2006). Bosques de *Polylepis*. In: M. Morales, et al. Botánica Económica de los Andes Centrales. Universidad Mayor de San Andrés, La Paz, Bolivia 110-120.

Kozlowski, T. T. and Pallardy, S. G. (1997). Physiology of woody plants. Second edition. Academic, California, USA.

Liberman-Cruz, M., Chará, J. and Silman, M. R. (2021). *Polylepis* forest remnants and their role in the conservation of Andean biodiversity. *Journal of Tropical Ecology*, **37(4)**:178-191.

López, V. L., Crisci, G., Cuyckens, G. A. E. and Cellini, J. M. (2021). Conteo de marcas de yemas para estimar la edad en *Polylepis tarapacana*: Una técnica potencial. *Ecología Austral*, **31(1)**:182-189. https://doi.org/10.25260/EA.21.31.1.0.1145

López, V. L., Huertas Herrera, A., Rosas, Y. M., and Cellini, J. M. (2022). Optimal environmental drivers of high-mountains forest: *Polylepis tarapacana* cover evaluation in their southernmost distribution range of the Andes. *Trees, Forests and People*, **9**:100321. https://doi.org/10.1016/j.tfp.2022.100321

López, V. L., Bottan, L., Martínez Pastur, G., Lencinas, M. V., Cuyckens, G. A. E. and Cellini, J. M. (2023). Characterization of *Polylepis tarapacana* Life Forms in the Highest-Elevation Altiplano in South America: Influence of the Topography, Climate and Human Uses. *Plants*, **12(9)**: 1806. https://doi.org/10.3390/plants12091806

Martínez Pastur, G., Lencinas, M. V., Peri, P. and Arena, M. (2007). Photosynthetic plasticity of *Nothofagus pumilio* seedlings to light intensity and soil moisture. *Forest*

Ecology and Management, **243**:274-282.

Miehe, G., Miehe, S., Koch, K. and Will, M. (2003). Sacred forests in Tibet using geographical information systems for forest rehabilitation. *Mountain Research and Development*, **23**:324-328.

Morales, M. S., Christie, D. A., Villalba, R., Argollo, J., Pacajes, J., Silva, J. S. and Giraldo, J. P. (2022). Tree-ring formation, radial increment and climate signals in *Polylepis tarapacana* along elevational gradients in the Central Andes. *Dendrochronologia*, **73**: 125957.

Morales, M. S., Christie, D. A., Villalba, R., Argollo, J., Pacajes, J., Silva, J. S., Alvarez, C. A. Llancabure, J. C. and Soliz Gamboa, C. C. (2012). Precipitation changes in the South American Altiplano since 1300 AD reconstructed by tree-rings. *Climate of the Past*, **8**:653-666.

Mutke, S., Sievänen, R., Nikinmaa, E., Perttunen, J. and Gil, L. (2005). Crown architecture of grafted Stone pine (*Pinus pinea* L.): shoot growth and bud differentiation. *Trees*, **19**:15-25.

Paoli, H. P., Elena, H. J., Mosciaro, M. J., Ledesma, F. and Noé, Y. E. (2014). Caracterización de las cuencas hídricas de las provincias de Salta y Jujuy. Salta, Argentina: Área de Recursos Naturales, INTA, EEA.

Paredes-Villanueva, K., Sánchez-Salguero, R., Ticse-Otarola, G. and Requena-Rojas, E. (2023). Wood anatomy and dendrochronological potential of *Polylepis* species in the high Andes. *IAWA Journal*, **44(1)**: 45-67.

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Renison, D., Hensen, I., Suárez, R., Cingolani, A. M., Marcora, P. and Giorgis, M. A. (2010). Soil conservation in *Polylepis* mountain forests of Central Argentina: Is livestock reducing our natural capital? *Austral Ecology*, **35**:435-443. https: //doi.org/10.1111/j.1442-9993.2009.02055.x.

Soler, R., Lencinas, M. V., Bustamante, G. and Martínez Pastur, G. (2018). Atributos de la regeneración natural de ñire (*Nothofagus antarctica*) en Tierra del Fuego: beneficios y perjuicios que genera el uso silvopastoril. *Ecosistemas*, **27(3)**:41-47 https://doi.org/10.7818/ECOS.1505

Thabeet, A., Vennetier, M., Gadbin-Henry, C., Denelle, N., Roux, M., Caraglio, Y. and Vilá, B. (2009). Response of *Pinus sylvestris* L. to recent climatic events in the French Mediterranean region. *Trees*, **23**:843-853. https://doi.org/10.1007/s00468-009-0326-z.

Toivonen J. M., Horna, V., Kessler, M., Ruokolainen, K. and Hertel, D. (2014). Interspecific variation in functional traits in relation to species climatic niche oima in Andean *Polylepis* (Rosaceae) tree species: evidence for climatic adaptations. *Functional Plant Biology*, **41**:301-312.

Urza, A. K. and Sibold, J. S. (2013). Nondestructive aging of postfire seedlings for four conifer species in Northwestern Montana. *Western Journal of Applied Forestry* **28(1)**:22-29. https://doi.org/10.5849/wjaf.11-014.

Vennetier, M., Girard, F., Taugourdeau, O., Cailleret, M., Caraglio, Y., Sabatier, S. A., Ouarmim, S., Cody, D. and Thabeet, A. (2013). Climate change impact on tree architectural development and leaf area: in Singh Bharat Raj (ed.). Climate change - realities, impacts over ice cap, sea level and risks. *Tech. Rijeka, Croacia* **103**-126. https://doi.org/10.5772/51510

Wawrzyk, A. C. and Vilá, B. L. (2013). Dinámica del pastoreo en dos comunidades (Lagunillas del Farallón y Suripujio) de la puna de Jujuy, Argentina. Chungara: *Revista de Antropología Chilena*, **45(2)**:349-362.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, USA.

Wickham, H., François, R., Henry, L., Müller, K. and Vaughan, D. (2023). dplyr: A grammar of data manipulation. R package version 1.1.2

Williams, C. E. and Carter Johnson, W.

(1990). Age structure and the maintenance of pinus pungens in Pine-oak forests of Southwestern Virginia. *The American Midland Naturalist* **124(1)**: 130-141. https://doi.org/10.2307/2426086