



The age of monumental trees. A case study of *Juniperus thurifera* L. in Spain

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Abstract

Aim of study: To provide tree-age estimation of monumental *Juniperus thurifera* trees based on dendrochronological methods.

Area of study: “Sabinar de Calatañazor” Nature Reserve (Calatañazor, Soria, Spain), a monumental forest of *Juniperus thurifera* traditionally used for grazing.

Material and methods: Tree-ring width analysis of increment cores and four different methods for estimating the age of each of the trees analysed.

Main results: Our estimates suggest that most of the trees in this Nature Reserve with a radius greater than 30 cm are over 300 years old. Moreover, the discussion on the constraints and accuracy of each of the four tree-age estimation methods employed can be helpful in future studies of age in many monumental trees. A well-replicated local chronology, ranging from 1738 to 2012 (275 years), was also established for its use in reconstruction studies related to management, past events and climate change.

Research highlights: This study involved analyzing many trees with high percentages of rings that had disappeared as a result of rot. In this case, the age estimation models based on the classical hypotheses of constant growth in radius or basal area, as well as a new estimation method based upon biological behavior and considering two growth stages (juvenile and mature), are the ones that provided the most reliable estimates. On the contrary, regression models are less recommendable, due to being less accurate.

Keywords: Dendrochronology; tree-age estimate; rot; growth stages; management and conservation measures.

Abbreviations used: AD: Absolute Deviation; BA: Basal Area; BAI: Basal Area Increase; DBH: Diameter at Breast Height; EPS: Expressed Population Signal; ER: Estimated Radius; ETR: number of Estimated Tree-Rings; ETRW: Estimated Tree-Ring Width; MBA: Missing Basal Area; MBAL: Mean Basal Area Increase; MR: Missing Radius; MTR: number of Missing Tree-Rings; MTRW: Mean Tree-Ring Width; QD: Quadratic Deviation; R: Radius; RCH: Radius at the time of Growth CHange; SL: Sample Length; TR: number of Tree-Rings; TRW: Tree-Ring Width.

Authors' contributions: MG designed the general plan of the paper and performed the dendrochronological analysis. ES performed the statistical analysis. Both authors wrote and revised the manuscript.

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Introduction

Among many other attributes, big monumental trees are of great symbolic and cultural significance, mainly in reference to their longevity. Their age is not always easy to establish, and the degree of accuracy of tree-age estimates can vary depending upon the previous available data on each tree species and upon the methods used. Some examples are the age estimates of the trees named General Sherman (*Sequoiadendron giganteum* (Lindl.) Buchholz) and Tule Tree (*Taxodium mucronatum* Ten.). The Gene-

ral Sherman is considered to be the tree with the largest amount of biomass on earth (83.8 m high and 31 m perimeter). Since the initial attempts, estimations of its age have varied: 3500 years (Douglass, 1946) or 2500 years (Hartesveldt *et al.*, 1975); the most recent calculation places it between 1900 and 2400 years old (Stephenson, 2000). As for the Tule Tree, which has the longest trunk diameter in the world (14 m), estimates of its age have varied from 3000 years old, established in a study conducted in 1892, to the most recent estimates, which indicate an age of between 1400 and 1600 years old (Debreczy &

Rácz, 1997). In the Mediterranean region, the olive tree (*Olea europaea* L.) have traditionally been considered a particularly long-lived species, in which millenarian trees are not uncommon. However, recent studies in the NE of Spain and in the Garden of Gethsemane (Jerusalem), have determined maximum ages between 700 and 900 years, respectively, for monumental trees of more than 8 m perimeter (Arnan *et al.*, 2012; Bernabei, 2015).

In many species forming annual tree rings, the best tree-age estimate is based on the exact correspondence between each ring and the calendar year in which it was formed (Stokes & Smiley, 1968). Disks taken from dead monumental trees provide a complete tree-ring series if the wood is in good condition (Génova *et al.*, 2017), but this technique does not usually apply to living trees, especially if they are valuable or protected specimens growing in parks and natural reserves (Rozas, 2003). The most habitual sampling technique therefore involves extracting increment cores and analyzing these by means of dendrochronological methods, because well-replicated chronologies perfectly estimate tree age (Stokes & Smiley, 1968; Fritts, 1976). Furthermore, evidence has shown that tree coring hardly affects conifer species (Grissino-Mayer, 2003).

Nevertheless, tree-age estimation based on dendrochronological analysis of increment cores presents limitations because the cores are frequently incomplete, and the number of missing rings is unknown. In many large trees, for example, the inner part is missing because the wood has rotted. However, even in cases where the central zone of the tree (the oldest wood) has disappeared or is poorly conserved, the age of an individual can be established with a certain degree of accuracy by means of the analysis of known tree-ring series (Norton *et al.*, 1987; Stephenson, 2000; Rozas, 2003; Clark & Hallgren, 2004). These methods of estimation generally involve extrapolating the mean ring width (or the mean increment of the basal area) in order to estimate the number of rings missing as far as the pith (Norton *et al.*, 1987), or using regression techniques to estimate the age based upon the diameter at breast height (DBH) (Rozas, 2003; Arnan *et al.*, 2012). Both approaches present drawbacks that can give rise to lower accuracy in age estimation. For example, the use of mean values implies a hypothetical concentric radial tree-growth and the regression does not take into account the variability of individual growth patterns (Clark & Hallgren, 2004). Furthermore, tree-ring series from long-lived trees provide extensive records of information, which can be very useful because their annual resolution can enable reconstruction of past events (Arnan *et al.*, 2012).

Juniperus thurifera L. (Spanish juniper) is a long-lived conifer species, endemic to the western Mediterranean region and growing under continental and cold climatic conditions; it is more abundant in North Central Spain and the High Atlas Mountains in Morocco (Costa *et al.*, 1997;

Gauquelin *et al.*, 1999). Dendrochronological studies on Spanish juniper began in Spain with Alcalde & Génova (2006), Olano *et al.* (2008) and Rozas *et al.* (2008), and in recent years have multiplied and extended (Esper *et al.*, 2015; Olano & Rozas, 2015). The oldest estimated age of a specimen in Morocco exceeds 500 (DeSoto *et al.*, 2014); this age is lower in Spain (ca. 400 years old, DeSoto *et al.*, 2012).

The Dehesa de Carrillo (Calatañazor, Soria, Spain) houses a unique forest of *Juniperus thurifera* traditionally used for grazing; in the year 2000 it was declared the “Sabinar de Calatañazor” Nature Reserve by the Castilla y León Regional Government. It is located at 1,000 m a.s.l. on mesozoic sands and conglomerates, and cretaceous limestones; it is characterized by a Mediterranean climate with a high degree of continentality. Amongst its most noteworthy values are monumental trees with perimeters that exceed 4 m and heights of up to 25 m (Alcalde & Génova, 2006).

The present research provides technical age-data to support Spanish juniper management and conservation measures in this Nature Reserve, in order to advance and consolidate previous efforts in this sense. To this end, we employed more better-quality increment cores and applied different methods to estimate tree ages. In addition, the discussion on the constraints and accuracy of each of the methods used can be of great use in future studies on the ages of many monumental trees. Lastly, establishing a well replicated local chronology, along with other long chronologies developed in the western Mediterranean, could be useful in reconstruction studies referring to management, past events and climate change (Olano *et al.*, 2008; Camarero *et al.*, 2014; DeSoto *et al.*, 2014; Esper *et al.*, 2015).

Material and methods

Field sampling and tree-ring data analysis

We selected 25 *J. thurifera* trees growing in the Dehesa de Carrillo (Calatañazor, Soria, Central Spain) with a large perimeter, a straight trunk without bifurcation and a healthy appearance, with the aim of obtaining dendrochronological samples that were as long as possible. All these trees were growing under similar ecological conditions and it is assumed that they were all traditionally pruned to provide young branches for cattle fodder. Two to four cores per tree at a height of 1.3 m were collected with a standard Pressler increment borer (40 cm) during the fieldwork in April 2004 and in July-October 2012 (Fig. 1). In each tree, the perimeter was measured at the same height at which the samples were taken. The cores were air dried and cut in transverse section until tree rings became clearly visible. Tree-ring widths were measured

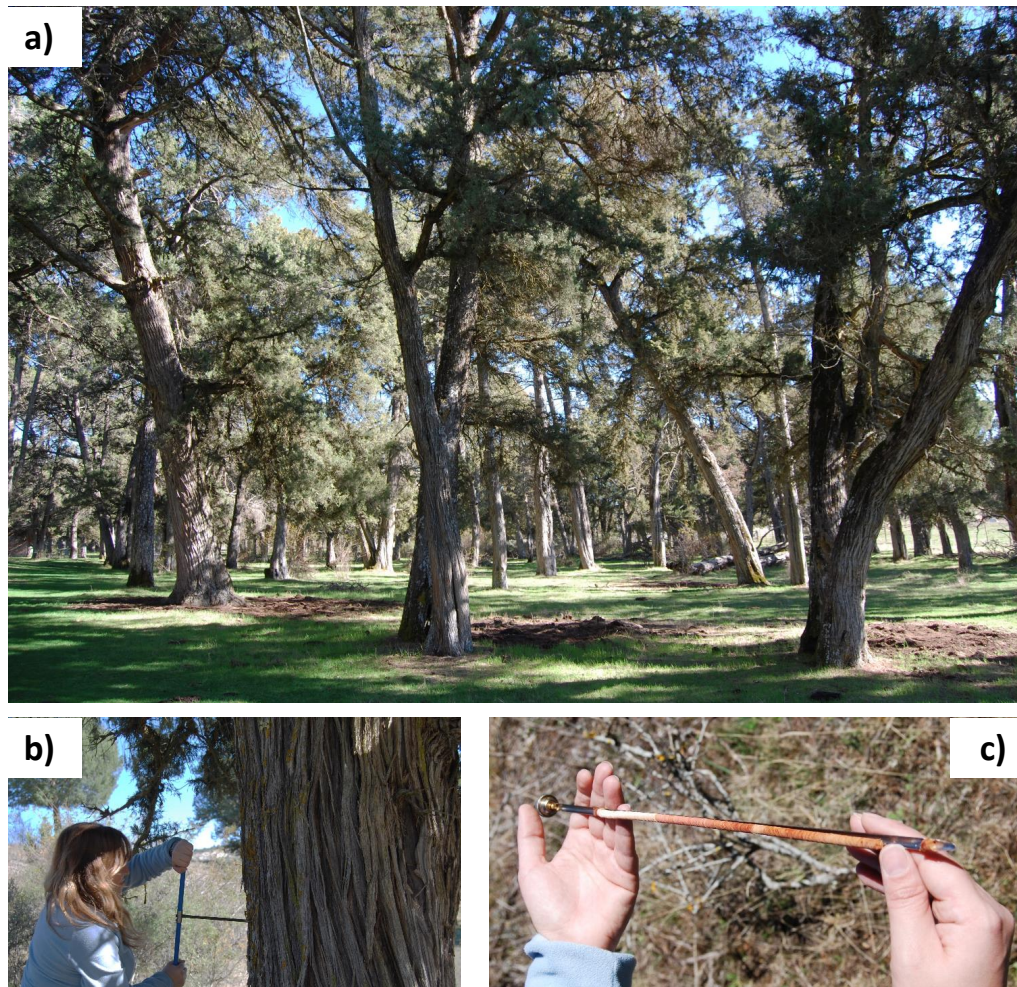


Figure 1. a) General view of the Dehesa de Carrillo forest; b) Dendrochronological sampling with a standard Pressler increment borer; c) One of the samples obtained.

at an accuracy of 1/100 mm with a LINTAB™ measuring device (Rinntech, Heidelberg, Germany). We measured 8,925 tree-ring widths from 65 tree-ring series.

The series of tree-ring widths measured were crossdated with the use of TSAPWin software and the COFECHA program facilities. Crossdating is a technique that ensures that each individual tree-ring is assigned its exact year of formation. TSAPWin software enables the tree-ring series to be verified; it detects false, incomplete, or missing rings and eliminates possible errors in order to pinpoint the correct dated position in time (Rinn, 2003). COFECHA is a quality-control program for checking the crossdating and overall quality of tree-ring chronologies (Holmes, 1999). We employed these crossdating techniques to synchronize the tree-ring width series and elaborate a chronology for each tree.

Lastly, tree-ring widths were converted into indices by standardizing and detrending the raw data for each tree with the program ARSTAN (Cook & Holmes, 1996) to obtain a representative local chronology. Detrending enabled tree-ring widths to be transformed into dimensionless growth indexes by dividing measured ring-width

values by fitted values. Tree-ring width series were double-detrended, a negative exponential function was fitted, followed by a 30-year long spline, and a biweight robust mean was computed. To assess the reliable period of the chronology, we used the Expressed Population Signal (EPS); EPS values above 0.85 are a threshold widely used in dendrochronological analysis (Wigley *et al.*, 1984).

Despite their healthy external appearance, numerous trees presented internal decay, and the samples obtained were therefore incomplete; in addition, some trees had a radius greater than the standard borer used (Table 1). Consequently, to establish tree ages we needed to estimate the tree-ring number in the missing portion. To this end, we used different statistical methods that are explained in the following section.

Tree-age estimation

Tree-age was calculated based on the estimated radius (ER), according to the perimeter value. Due to internal decay and a large perimeter in some trees, many

Table 1. Main dendrochronological characteristics of the studied trees arranged from the smallest to the largest estimated missing tree-radius portion. In the last two columns the data for individual chronologies overcoming the critical correlation (0.32) are shown.

ID	ER (cm)	SL (cm)	TR	MTRW (mm)	Internal rots	MR (cm)	$\frac{MR}{ER} \times 100$	TS	IT
2367	18	16.9	121	1.40	no	1.1	6.12	-----	-----
1377	25	22.9	193	1.19	no	2.1	8.44	1826-2012	0.49
1623	33	29.2	243	1.20	no	3.8	11.41	1769-2011	0.46
844	35	30.7	249	1.23	no	4.3	12.30	1755-2000	0.49
1318	27	22.4	256	0.88	no	4.6	16.86	-----	-----
2257	34	28.0	274	1.02	no	6.0	17.56	1738-2011	0.33
477	32	24.5	216	1.13	no	7.5	23.41	1797-2012	0.53
1944	32	23.9	211	1.13	no	8.1	25.25	1802-2012	0.44
2364	33	20.8	207	1.01	yes	12.2	36.91	1806-2012	0.34
308	27	13.0	120	1.09	yes	14.0	51.69	1884-2003	0.57
2329	28	12.7	220	0.58	yes	15.3	54.72	1793-2012	0.50
1370	35	18.2	211	0.86	yes	16.8	48.09	1800-2010	0.48
854	32	13.4	144	0.93	yes	18.6	58.04	1860-2003	0.45
1369	41	21.1	219	0.96	yes	19.9	48.60	1792-2010	0.43
385	38	17.9	172	1.04	yes	20.1	52.98	-----	-----
107	38	17.2	150	1.14	yes	20.8	54.83	-----	-----
140	37	15.2	150	1.01	yes	21.8	59.03	1854-2003	0.42
1048	35	12.6	199	0.63	yes	22.4	63.95	1805-2003	0.50
980	38	14.9	149	1.00	yes	23.1	60.84	1855-2003	0.51
600	42	18.6	138	1.34	yes	23.4	55.82	1866-2003	0.33
1070	36	10.2	126	0.81	yes	25.8	71.73	1878-2003	0.56
1039	40	12.3	97	1.27	yes	27.7	69.26	1918-2003	0.48
1480	38	10.1	113	0.90	yes	27.9	73.29	1900-2012	0.68
1246	36	8.1	184	0.44	yes	27.9	77.43	-----	-----
590	48	12.4	123	1.01	yes	35.6	74.07	1881-2003	0.37

ER: estimated tree-radius; SL: sample length; TR: number of tree rings measured; MTRW: mean tree-ring width; MR: missing tree-radius portion. Maximum and minimum values are highlighted in bold. TS: time span; IT: intercorrelation (Cofecha).

cores were incomplete and there was therefore a missing tree-radius portion (MR) of variable length in each tree (Table 1). Consequently, we calculated tree-age (number of estimated tree-rings, ETR) by adding the maximum number of tree rings measured (TR) to the estimated number of missing tree-rings (MTR) in MR. As the length of the MR increases, there is a logical decrease in the accuracy of the age estimation (Norton *et al.*, 1987; Rozas, 2003; Altman *et al.*, 2016). In each tree, we evaluated the percentage of uncertainty presented by the sam-

ples obtained in relation to the estimated radius (ER), by means of:

$$\frac{MR}{ER} \times 100$$

In order to check the ETR calculation accuracy, we employed four different methods to estimate MTR, taking into account sample length (SL), TR and tree-ring width (TRW).

Method 1

Habitually, when estimating the number of missing rings in long-lived trees, the radial increment (TRW) or the increment of the basal area (BAI) are considered to be constant in each tree throughout time (Norton *et al.*, 1987; Biondi & Qeadan, 2008). However, the tree-growth tendencies do not generally respond to this pattern, so that direct estimation based upon any of these hypotheses will generally give rise to bias in the estimations. Extrapolation of the TRW tends to overestimate the number of missing rings, whereas application of the constant BAI generally involves underestimation. To balance this over- and underestimation, which result from the common decrease in TRW and the increase in BAI as tree age increases, Altman *et al.* (2016) propose averaging the number of rings estimated with these two techniques. Besides they point out that the maximum error is less when this average TRW-BAI value is used than when using either of the two estimators separately. We employed this proposal, calculating MTR as the mean of these two estimates:

$$MTR = \frac{MTR_{TRW} + MTR_{BAI}}{2} \quad [1]$$

MTR_{TRW} was obtained as the quotient between MR and MTRW (calculated as the quotient between SL and TR):

$$MTR_{TRW} = \frac{MR}{MTRW}; MTRW = \frac{SL}{TR} \quad [2]$$

And, analogously, MTR_{BAI} was obtained as the quotient between the Missing Basal Area (MBA) and the Mean Basal Area Increase (MBAI). MBAI represents the average of the increments in the basal area based on the tree rings present in the sample, *i.e.* the quotient between the estimated area of the section, presuming a shape of a circular crown, and the number of rings that it contains:

$$MTR_{BAI} = \frac{MBA}{MBAI} = \frac{\pi \cdot MR^2}{MBAI}; MBAI = \frac{\pi \cdot (ER^2 - MR^2)}{TR} \quad [3]$$

Method 2

In this method, we introduce a new way of estimating MTR_{TRW} and TRW_{BAI} , so far original, which consists of simultaneously obtaining, through an iterative process, the values of the number of missing rings and the ring widths that make up the series most similar to the actual data. In each step of the process, we considered one of the MTR possibilities ($MTR = 1, 2, 3$, etc.), and the tree-ring width value associated with each possibility, under the hypothesis of constant annual width increment:

$$TRW = \frac{ER}{TR + MTR} \quad [4]$$

This growth model was compared with the data referring to ring width (TRW_k , ring width generated in the year k according to the previous hypothesis) by means of the sum of the squares of the differences between the corresponding TRW and TRW_k values, *i.e.* based on the quadratic deviation (QD) between the model values and the measured values:

$$QD_{TRW} = \sum_{k=MTR+1}^{TR+MTR} (TRW - TRW_k)^2 \quad [5]$$

The value of MTR that minimises the sum of the squares was the estimation of the number of missing rings (MTR_{TRW}).

Analogously, MTR was calculated under the hypothesis of constant annual increment of basal area. For each possible MTR value the corresponding BAI was obtained:

$$BAI = \frac{\pi \cdot ER^2}{TR + MTR} \quad [6]$$

This value was used to calculate the TRW_k by means of the following expression:

$$TRW_k = R_k - R_{k-1} = \sqrt{\frac{k \cdot BAI}{\pi}} - \sqrt{\frac{(k-1) \cdot BAI}{\pi}} \quad [7]$$

R_k being the radius of the section in the year k .

The estimation of MTR_{BAI} was the value that minimises the sum of the squares (quadratic deviation):

$$QD_{BAI} = \sum_{k=MTR+1}^{TR+MTR} (TRW_k - TRW)^2 \quad [8]$$

All these estimation processes were made by algorithms designed with the mathematics software MAPLE (Maple, 2018).

Finally, and following the same criterion as in method 1, the number of missing rings was calculated by means of:

$$MTR = \frac{MTR_{TRW} + MTR_{BAI}}{2} \quad [9]$$

Method 3

To calculate the tree-age, regression models are also used which relate the age to certain easy-to-measure dendrometric variables. These models are particularly useful when the tree cannot be sampled or the cores do not reach the pith or the internal wood is rotten (Clark & Hallgren, 2004). Among the most habitually used models are those that relate age with DBH (Rozas, 2003; Clark & Hallgren, 2004; Arnan *et al.*, 2012; Altman *et al.*, 2016) with the available information, we fitted TR and SL data to a simple linear regression model. We used a regression line without intercept as recommended by some authors (Stephenson & Demetry, 1995; Rozas, 2003), in order for the model to ensure that a 0 radius corresponds to the 0 age. To estimate the model, we discarded all tree-ring

series exhibiting an anomalous growth pattern (outliers). We subsequently adapted the equation obtained to determine the MTR in each tree based upon MR. The model was applied to the variable MR, and not to ER, in order to obtain the most reliable estimates, especially in the thicker trees.

Method 4

The tree-growth pattern usually presents two clearly differentiated stages: a juvenile one, in which the growth rate decreases linearly with age, and a mature period, more adjusted with the environment, and in which the growth rate is constant over time (Cook, 1985). This general pattern is the source of inspiration to propose a new estimation method, which consists of estimating the relationship (λ) between the average of the tree-ring width in the juvenile stage ($MTRW_J$) and in the mature stage ($MTRW_M$), *i.e.*:

$$\lambda = \frac{MTRW_J}{MTRW_M} \quad [10]$$

In our case study, λ was calculated as the average values in trees that provide sufficient information, without rot and that clearly show these two growth stages. λ was obtained, in each tree, as the maximum reached by the function $f(x)$. This function has defined as the quotient between MTRW until reaching x (the ring-width average of the sample from the pith to x) and MTRW after x (the ring-width average of the sample from x to the bark), being x the radius at the possible time of stage change. R_{CH} (Radius at the time of growth CHange) corresponds to the value of x that maximizes relationship (the point at which the function $f(x)$ reaches its maximum).

$$f(x) = \frac{\text{MTRW until reaching } x}{\text{MTRW after } x} \quad [11]$$

Having calculated R_{CH} and λ for the population (as the mean of the individual values), we estimated MTR. MTR_J was the number of missing rings estimated for the juvenile stage (before the radius reaches R_{CH}) and MTR_M was the number of missing rings estimated for the mature stage (after the radius has reached R_{CH}). MTR was estimated according to two possible situations (see Fig. 2):

a) Trees in which $MR > R_{CH}$:

$$MTR = MTR_J + MTR_M, \text{ being } MTR_J = \frac{R_{CH}}{\lambda \cdot MTRW_M} \text{ and } MTR_M = \frac{MR - R_{CH}}{MTRW_M} \quad [12]$$

b) Trees in which $MR \leq R_{CH}$:

$$MTR = \frac{1}{2} \left(\frac{MR}{\lambda \cdot MTRW_M} + \frac{MR}{MTRW_J} \right) \quad [13]$$

When $MR \leq R_{CH}$, there are at least two options to obtain MTR: $\frac{MR}{MTRW_J}$ and $\frac{MR}{\lambda \cdot MTRW_M}$, the average of the two was chosen as the estimator.

Results

Chronology of J. thurifera of the “Sabinar de Calatañazor” Nature Reserve

Most tree-ring width series were crossdated and synchronized, once determined an average of 0.02% missing rings. However, depending on the intercorrelation values of the individual chronologies, we selected those that overcome the critical correlation (99% confidence level, 0.3281; see table 1) to elaborate a local chronology of the “Sabinar de Calatañazor”. This chronology ranges from 1738 to 2012 (275 years, Fig. 3) and the time span in that $EPS > 0.85$ extends 1880-2000. It is the second longest Spanish juniper chronology on the Iberian Peninsula, being the first one from southeast Iberian System, ranged 1681 to 2010 and with EPS values exceeding 0.81 from the mid-eighteenth century (Esper *et al.*, 2015).

Estimated tree-age

The percentage of uncertainty, which assesses the degree of lack of knowledge of the length of the missing radius, ranged from a minimum of 6.12% and a maximum of 77.43% for the samples analysed. It was greater than 20% in over three-quarters of the trees, and very close to or over 50% in more than half the trees (Table 1).

Table 2 shows the estimates furnished by means of the four methods used. Estimations of the number of missing rings by means of methods 1, 2 and 4 provided similar results. In both method 1 and method 2, the number of missing tree rings estimated under the hypothesis of constant radial increment (MTR_{TRW}) exceeds the number of missing tree rings estimated under the hypothesis of constant basal area (MTR_{BAI}), *i.e.* $MTR_{TRW} \geq MTR_{BAI}$. The effect of calculating the mean of both estimates is shown in Fig. 4. The average estimate corresponds to another model, in which the raw data are located, in general, among those corresponding to the models of constant TRW or BAI. This may not be the case when a tree shows abrupt changes in the growth trend (see Fig. 4-B).

In method 3, we have fitted a regression line relating age and radius to the available data, obtaining $TR = 0.925118 \cdot SL$ and adapting it to $MTR = 0.925118 \cdot MR$. The fitted model could likely be reliable, given its high coefficient of determination ($R^2 = 98.31\%$). However, the number of years estimated by this method generally exceeded that obtained by other methods (table 2), and it increased as MR becomes longer. As exception, the number of years

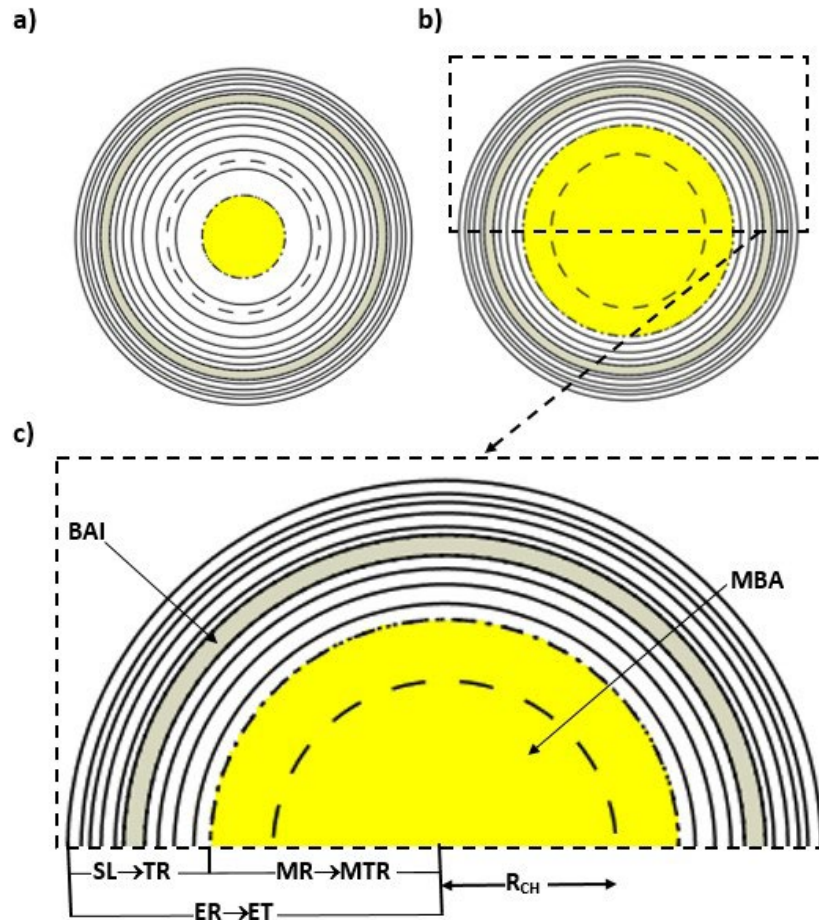


Figure 2. Idealised graph of a trunk cross section with circular rings. The dashed line with dots limit the rotten zone or the one that could not be sampled (also shown by the shaded inner circle). The dashed circumference marks the boundary between the juvenile and mature stages and, therefore, the width reached at the time of change in growth stage. a) Case in which $MR > R_{CH}$, b) Case in which $MR \leq R_{CH}$, c) Enlarged view showing the main variables: SL=Sample Length and TR=number of Tree-Rings; MR=Missing Radius and MTR=number of Missing Tree-Rings; ER=Estimated Radius and ETR number of Estimated Tree-Rings; RCH=Radius at the time of Growth Change; BAI=Basal Area Increase; MBA=Missing Basal Area.

estimated had lower than that estimated with other methods when the average ring-width was very narrow, as it happened with the outlier tree-ring series that were discarded for the estimation of the model (see Fig. 5). Fig. 5 and Table 3 show the confidence intervals for the prediction or for the mean of the age of each tree. Some predictions were based on values that lie outside the range of SL (8.1-30.7 cm), thus increasing the uncertainty of that results (see table 1).

The estimations of R_{CH} and λ (method 4) were obtained from the samples providing sufficient information on the two growth stages considered (juvenile and mature), and in which the time of change from one phase to the other can clearly be determined. In our case study, only the ones displaying no rot meet these requirements (Table 1). Nevertheless, the ring series of trees 1377 (which

does not show a decrease in tree-ring width over time), 2367 (comparatively young), and 844 and 1623 (in which the value of R_{CH} cannot be clearly calculated), could not be used; thus the dataset was limited to four trees (2257, 1318, 477, 1944, see Fig. 6). Both R_{CH} and λ were estimated for each of the tree-ring series selected; results are shown in Table 4. In the calculation of individual R_{CH} we did not consider values of x at the start or the end of the series when they generate maxima in the function $f(x)$ that do not correspond with the radius at the time of growth change (see Fig. 6).

Taking as a reference the average of the tree-age estimates (Table 2), it can be said with great certainty that all the specimens are over 200 years old (except for specimen 2367). Almost half the trees are over 300 years old and another 25% are close to this age. Furthermore, 3 trees

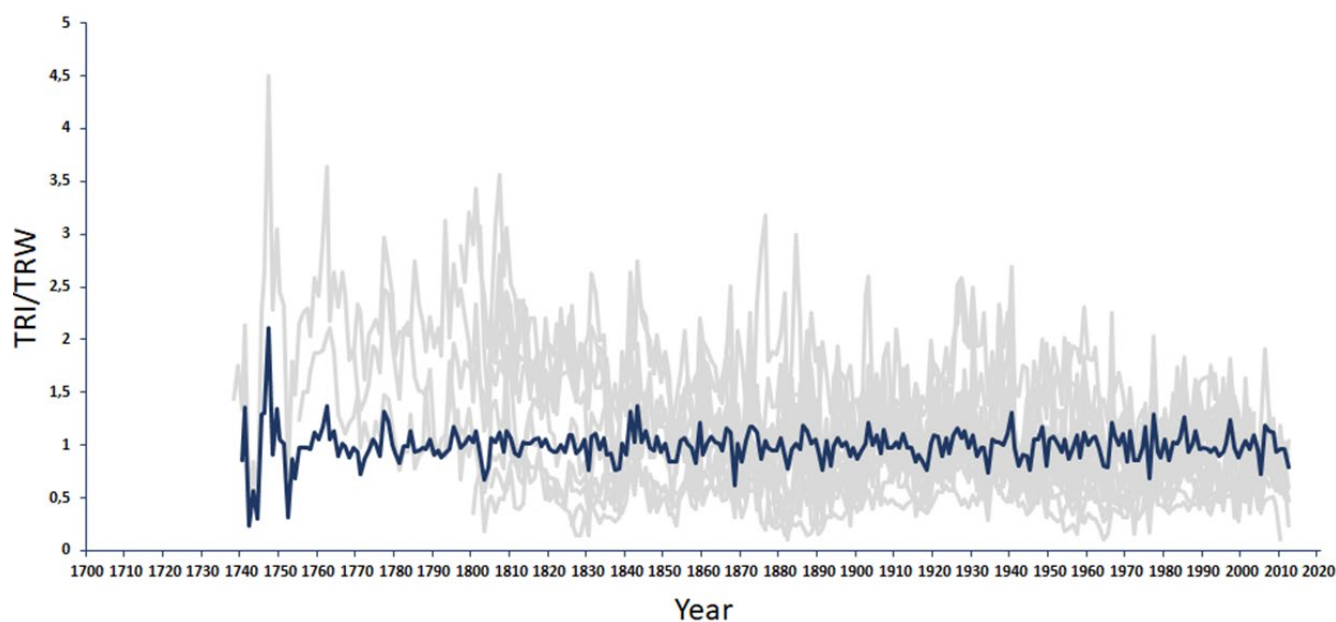


Figure 3. Individual tree-ring chronologies and the standardized residual local chronology highlighted in black. TRI/TRW: tree-ring index and tree-ring width, respectively.

Table 2. Estimated tree-age for each tree analysed with the four methods employed, average and standard deviation (SD)

ID	Method 1	Method 2	Method 3	Method 4	Average $\pm$ SD
2367	125	130	131	126	128 $\pm$ 2.94
1377	203	220	213	207	211 $\pm$ 7.41
1623	260	264	278	259	265 $\pm$ 8.77
844	268	276	289	269	276 $\pm$ 9.68
1318	286	287	298	280	288 $\pm$ 7.50
2257	305	311	329	300	311 $\pm$ 12.66
477	255	255	285	243	260 $\pm$ 17.92
1944	254	255	286	242	259 $\pm$ 18.79
2364	284	282	320	256	286 $\pm$ 26.30
308	206	210	249	182	212 $\pm$ 27.74
2329	400	398	362	360	380 $\pm$ 21.97
1370	340	353	367	322	346 $\pm$ 19.16
854	280	283	316	265	286 $\pm$ 21.49
1369	356	358	403	351	367 $\pm$ 24.18
385	302	308	358	296	316 $\pm$ 28.43
107	273	276	343	269	290 $\pm$ 35.28
140	298	299	352	294	311 $\pm$ 27.58
1048	444	450	406	437	434 $\pm$ 19.57
980	309	309	363	308	322 $\pm$ 27.17
600	256	257	355	258	282 $\pm$ 49.01
1070	353	352	365	356	357 $\pm$ 5.92
1039	251	252	353	258	279 $\pm$ 49.76
1480	334	334	371	342	345 $\pm$ 17.58
1246	637	633	442	651	591 $\pm$ 99.47
590	373	375	452	403	401 $\pm$ 36.81

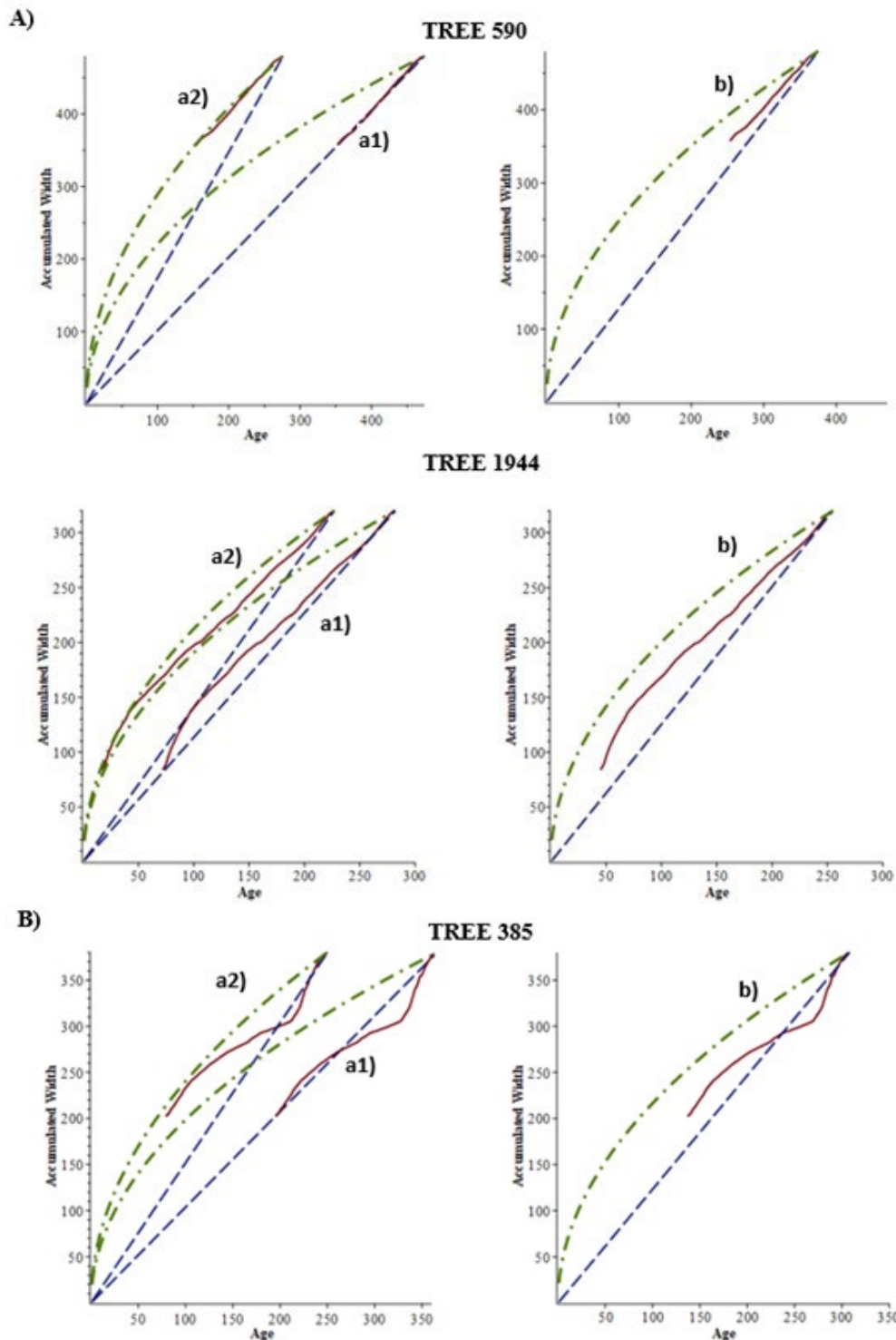


Figure 4. Accumulated radius for the 590 and 1944 (A), and 385 (B) samples: raw (solid line), assuming constant TRW (dashed line) and assuming constant BAI (dashed line with dots). a1) Estimation of MTR with the quadratic deviation between the raw data and the constant TRW model; a2) Estimation of MTR with the quadratic deviation between the raw data and the constant BAI model; b) Averaged estimate. TRW: tree-ring width; BAI: basal area increase.

(1048, 1246 and 590) might be older than 400 years and one of them (1246) very likely exceeds 500 years; they all possess an estimated radius (ER) of over 35 cm. Many other trees, however, present an ER over 35 cm, with average ages estimated at between 275 and 381 years. It be-

comes increasingly speculative to establish age as the ER gets bigger, and the percentage of uncertainty approaches or surpasses 50%, but in general terms, trees with an ER over 30 cm can live up to, or around, 300 years in the study area.

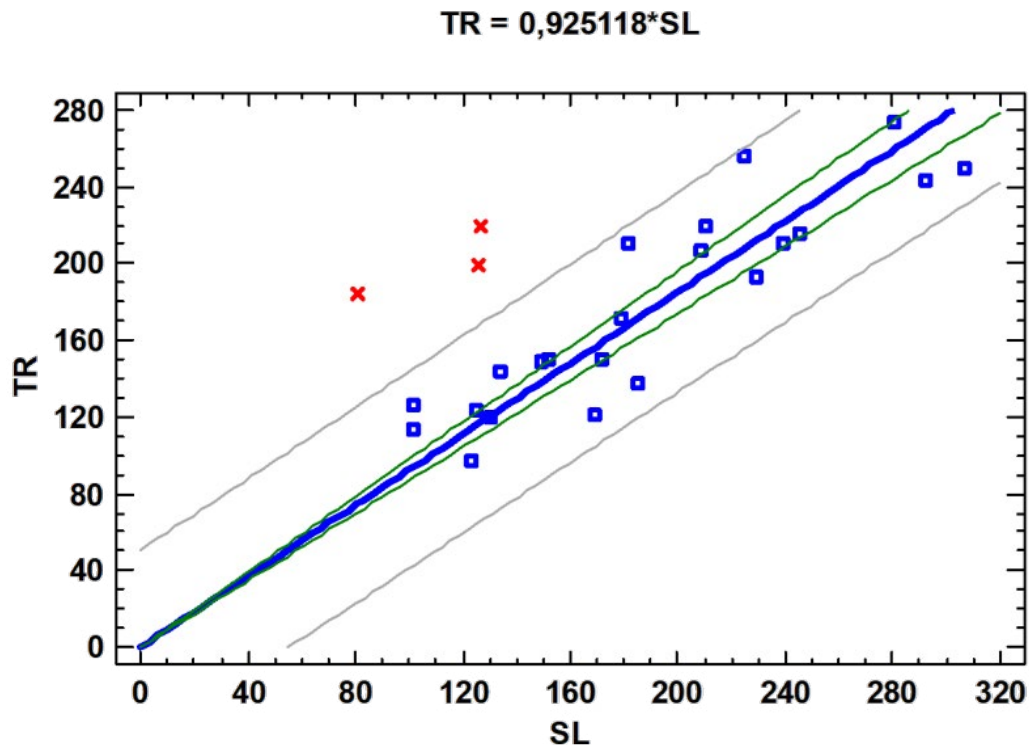


Figure 5. Scatter plot and regression line fitted to the available data. It includes the 95% confidence bands, for the mean and for the prediction of a new observation. The blue squares represent the values used and the red crosses are the outliers not employed in the model ($R^2 = 98.31\%$). SL: sample length; TR: number of tree rings.

Discussion

Tree-age estimation according to the constant growth hypothesis

The hypotheses of constant TRW or BAI give rise to different models that attempt to resemble the raw data as closely as possible, each one from its approach. Calculating the mean of both values involves balancing, at least partially, the inaccuracy occurring in age estimation based upon the TRW (overestimation) or BAI (underestimation) methods. The accuracy of the estimate with the TRW-BAI method decreases with the increase in the longitude of the missing radius in a similar manner to that of the TRW and BAI methods, but the errors (both absolute and percentage) are clearly lower (Altman *et al.*, 2016). Even when the missing radius represents over 50% of the radius (as in more than half the trees, our case study, Table 1), this averaged method appears to be recommendable because it compensates the estimations by excess and by defect.

As opposed to using partial increments in the estimation (TRW and BAI of the 5, 10, 20 or 50 innermost rings) employed by different authors (Norton *et al.*, 1987; Rozas, 2003; Altman *et al.*, 2016), we have used all the available width-data for each tree, in accordance with the recommendation proposed by Altman *et al.* (2016). When

the missing radius represents a very high percentage of the total radius, the innermost rings provide very partial information.

Estimations of the number of missing rings by means of methods 1 and 2 provided very similar results. However, the latter method proved to be more versatile. Whilst in the former method MTR is deduced from MTRW or from MBAI, in method 2 MTR and TRW act as a bi-dimensional value and are estimated jointly. Moreover, the least squares estimation endows the raw data with a more influential role in comparison with their mean values. Method 2 could even be conveniently adapted to incorporate other hypotheses (*e.g.*, assuming a negative exponential growth model) and to contrast different results. An improvement of both methods would be obtained by using estimates based upon the weighted average of the MTR_{TRW} and MTR_{BAI} estimators, with different weights adapted to this or other species.

Tree-age estimation according to regression model

Stephenson (2000) successfully used regression models to estimate the number of missing rings in monumental *Sequoiadendron giganteum*, although this study was based upon a large set of complete samples of stumps of dated age. Moreover, Fraver *et al.* (2011) estimated

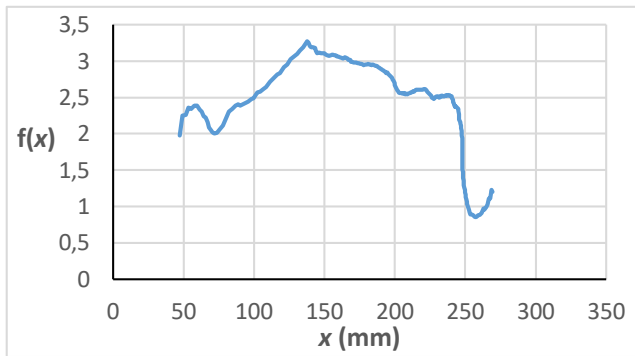
Table 3. Estimated tree-age or number of estimated tree rings (ETR) provided by a linear regression model. The table also shows the confidence intervals (CI) for the mean and the prediction (95% confidence level)

ID	ETR	CI prediction (min-max)		CI mean (min-max)	
2367	131	80	182	131	132
1377	213	162	263	211	214
1623	278	227	329	276	280
844	289	238	340	286	291
1318	298	247	349	296	301
2257	329	278	380	326	333
477	285	234	336	281	289
1944	286	235	337	281	290
2364	320	268	371	313	326
308	249	198	301	241	257
2329	362	310	413	353	370
1370	367	315	418	357	376
854	316	264	368	306	326
1369	403	351	455	392	414
385	358	306	410	347	369
107	343	291	395	331	354
140	352	300	404	340	364
1048	406	354	458	394	418
980	363	310	415	350	376
600	355	302	407	342	368
1070	365	312	418	351	379
1039	353	300	406	338	369
1480	371	318	424	355	386
1246	442	389	495	427	457
590	452	397	506	432	471
1246	442	389	495	427	457
590	452	397	506	432	471

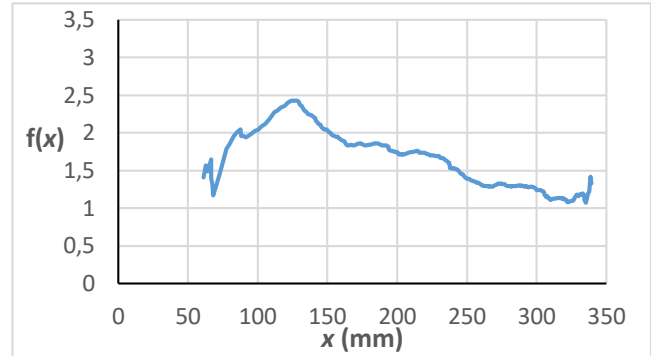
the age by expressing the number of rings according to sampling height and to the width rate of the innermost rings. The relationship between age and size varies greatly according to the age of the tree and its environmental conditions, especially when the trees are bigger and older (Rozas 2003; Altman *et al.*, 2013). For this reason, Rozas (2003) proposed the use of multiple predictive regression models if the age structure is discontinuous. The above-mentioned studies show that the performance of the regression models was enhanced when a relatively large number of tree-ring series reached or almost reached the pith and when other variables were included in the mo-

dels. In our case study, we had samples of 25 monumental trees, even though only 20% of them approaching to the pith. However, further samples could not be obtained, because the study area lies within a protected natural space. In addition, trees are part of a population subjected throughout its history to multiple managements. These conditions are far from the criteria that advise using regression models in estimating age. The increased uncertainty that this method implies makes the estimates obtained less reliable. To improve this regression analysis for future studies, there is a need for further samples, as well as for data referring to other variables from nearby sites.

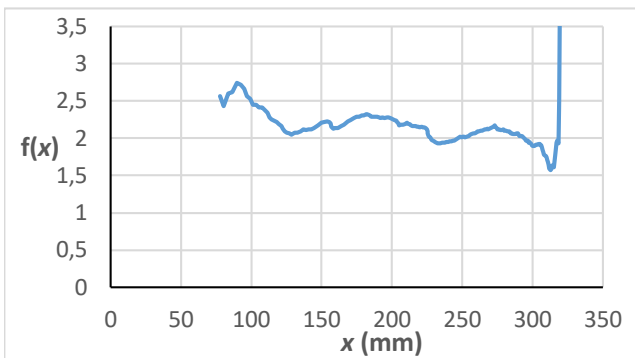
a) Tree 1318



b) Tree 2257



c) Tree 477



d) Tree 1944

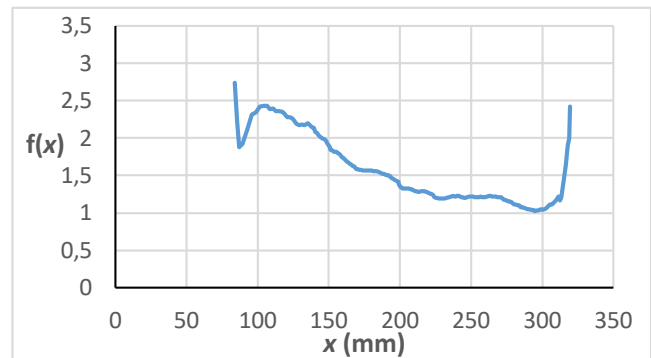


Figure 6. Evolution of $f(x) = \frac{\text{MTRW until reaching } x}{\text{MTRW after } x}$ in the sampled trees used to estimate R_{CH} and λ . λ is the maximum value $f(x)$ observed on the graph, and is reached for $x = R_{CH}$. The x values at the beginning or the end of the graphics were not taken into account in the calculation of the maximum.

Table 4. Estimated tree-age or number of estimated tree rings (ETR) provided by a linear regression model. The table also shows the confidence intervals (CI) for the mean and the prediction (95% confidence level).

ID	R_{CH} (mm)	λ
1318	137.8	3.27
2257	127.8	2.43
477	89.9	2.75
1944	104.2	2.43
Mean	114.9	2.72

λ : relationship between mean ring width in the juvenile and mature stages; R_{CH} : radius at the time of growth change.

Tree-age estimation according to two growth stages

As only four trees are suitable with regard to developing the growth model in two stages, we analysed how this small sample size would affect to the values of R_{CH} and λ in the estimation of ETR. Using the tree-ring series of the four trees selected, we calculated the value of λ corresponding to values of R_{CH} between 90 and 250

mm. These values are the minimum and the maximum selected as possible R_{CH} for the four trees. We employed a step-size of 10 mm. For each R_{CH} value, we established the rings before and after this R_{CH} in each of the four trees. We calculated the average of the tree-ring width in these two groups and the quotient between the two averages. Finally, λ was obtained as the mean of the four values obtained for the quotient. On varying R_{CH} the variation of λ

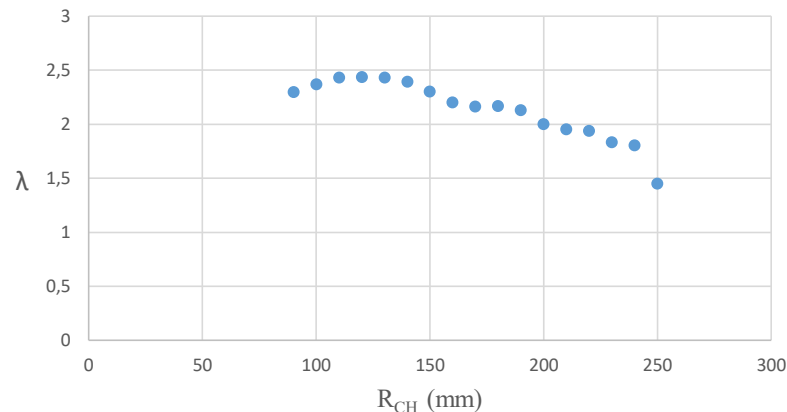


Figure 7. Evolution of λ obtained with the data from 2257, 1318, 477 and 1944 trees, for different values of R_{CH} (mm).
 R_{CH} : Radius at the time of Growth Change. λ : relationship between mean ring width in the juvenile and mature stages.

is determined to be less than 1 for the R_{CH} range previously indicated and less than 0.2 when R_{CH} varies between 90 and 150 mm (a more reasonable range of values for change in growth stage; see Fig. 7). In conclusion, modifications in the estimation of R_{CH} have little effect on the estimation of λ .

However, the influence of R_{CH} in the estimation of MTR can be relevant. We chose two cases presenting different values of R_{CH} but with a similar value for their corresponding λ ($R_{CH}=90$ mm and $\lambda=2.2986$; $R_{CH}=150$ mm and $\lambda=2.3015$).

Both cases provide quite a different estimation of ETR in some trees; however, this is not very relevant on estimating the minimum age (see Table 5).

Application and reliability of the different methods

We employed different methods for estimating *J. thurifera* age, attempting to be as accurate as possible and evaluating the efficiency of each one. All the estimates were based on the geometric radius. Although we are aware that this assumption is inaccurate, this is less relevant than the uncertainties relating to the internal rot or the large perimeter of most of the monumental trees studied (Table 1).

The first two methods, which take into account the constant growth hypothesis, only require information on individual tree-ring series to estimate the age of the trees. They are easy to use and the estimates obtained provide relatively reliable tree ages, although we agree that the TRW-BAI averaged estimator is the most suitable for tree-age estimation (Altman *et al.*, 2016). As additional advantages, method 1 requires little calculation, and method 2 could be adapted to other models, such as the negative exponential model.

The regression method (Method 3) uses the combined information on all the tree-ring series in order to make

estimations. This method requires many samples that are suitable for the conditions in which the tree-age estimation is to be conducted, and these are sometimes difficult or impossible to obtain. This requirement is mostly necessary for monumental trees; and when available samples are relatively scarce, as in our case study, this method proves to be the least reliable of them all.

The fourth method, based on tree growth stages, combines the previous two approaches: estimation of R_{CH} and λ is conducted with the joint information that can be extracted from all the trees sampled, and this is applied to the individual information to estimate the age of each tree. In practice, it is the most difficult method to apply, because it also requires many and suitable samples (although not as many as in the regression method), as well as a greater number of calculations. However, this model is the one that best reflects the radial growth of the trees. The choice of λ as the maximum value of the quotient between mean tree-ring width before and after change in growth stages, makes this method more reliable with regard to estimating the age of the more long-lived or monumental trees. We think that this method would enable particularly reliable estimates to be made if the sampling were conducted in managed populations presenting homogeneous growth.

Many Spanish juniper trees in the “Sabinar de Calatañazor” Nature Reserve are estimated to be several hundred years old; almost half of the trees are over 300 years old and three of them might be older than 400 years. These estimations surpass the ages estimated in Alcalde & Génova (2006), but are more reliable due to the increase of the number of samples and the complexity and quality of the analysis carried out. Such long-lived *J. thurifera* trees have only previously known in three Spanish sites: Sigueruelo (Segovia), where DeSoto *et al.* (2012) indicate a maximum age of 413 years in a tree presenting a perimeter of 85.3 cm; Cabrejas del Pinar (Soria), where Olano *et al.* (2008) establish a maximum age of 350 years; and

Table 5. Estimated tree-age or number of estimated tree rings (ETR) provided by a linear regression model. The table also shows the confidence intervals (CI) for the mean and the prediction (95% confidence level)

ID	ETR	
	($R_{CH}=90$ mm; $\lambda=2.2986$)	($R_{CH}=150$ mm; $\lambda=2.3015$)
2367	127	127
1377	209	206
1623	259	261
844	270	271
1318	281	285
2257	303	306
477	241	248
1944	244	251
2364	278	265
308	202	224
2329	398	339
1370	347	308
854	289	252
1369	373	338
385	317	284
107	288	258
140	316	282
1048	472	418
980	330	296
600	275	249
1070	383	341
1039	275	249
1480	366	329
1246	700	623
590	424	391

ETR: Estimated tree-age or number of estimated tree rings; R_{CH} : Radius at the time of growth change; λ : relationship between mean ring width in the juvenile and mature stages.

Royuela (Teruel), whose chronology ranges from 1681 to 2010 (329 years) according to Esper *et al.*, (2015). However, in the other Spanish sites sampled this species does not surpass 200 years.

Conclusions

We provide dendrochronological data and tree-age estimates to support management and conservation measures of *Juniperus thurifera* in the “Sabinar de Calataña-

zor” Nature Reserve. We have developed a new chronology, with some of the longest-lived trees of this species in the centre of the Iberian Peninsula, ranging from 1738 to 2012.

We employed different methods to estimate tree age, attempting to overcome the main disadvantage arising from the unavailability of data: many trees presented high percentages of missing rings (over 50% of the tree radial growth) as a result of rot or of a large perimeter. Methods based on the mean of the estimations under the classical hypotheses of constant radial increment or constant basal

area, balance the bias of the estimations based on each of the hypotheses separately, thus giving rise to more reliable estimates. We have introduced a new estimation method, based upon the determination of two growth stages (juvenile and mature), an approach more coherent with the biological behaviour of tree growth. Its application in our case study has led to the detection of small differences with respect to the two previous methods. On the other hand, regression model has not been very reliable and it would have been necessary to have much more data to obtain more accurate results.

Our better estimates indicate that the *J. thurifera* trees in this Nature Reserve with radii greater than 30 cm are mostly over 300 years old; we also established the age of some trees as being over 400 years.

Acknowledgments

The present paper is dedicated to the memory of our dear friend and colleague Fernando Gómez Manzaneque, who passed away in December 2018. He took part in the sampling conducted in 2004. Laura Tirado took part in the sampling conducted in 2012 and also measured and analyzed some of the samples. Our thanks to the Castilla y León Regional Government for authorizing the sampling.

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