



Leaf longevity in temperate evergreen species is related to phylogeny and leaf size

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Received: 27 August 2018 / Accepted: 16 August 2019 / Published online: 27 August 2019
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Abstract

Leaf longevity (LL), the amount of time a photosynthetically active leaf remains on a plant, is an important trait of evergreen species, affecting physiological ecology and ecosystem processes. A long LL gives leaves more time to fix carbon but carries higher construction costs, while a short LL allows plants to respond more rapidly to changing environmental conditions. For many evergreen taxa, LL data are not readily available, and it is not known if LL is phylogenetically conserved. To address this gap, we measured LL for 169 temperate and boreal evergreen woody species at the Arnold Arboretum, a botanical garden in Boston, Massachusetts, along with metrics of leaf size and number known to be related to LL. We hypothesized that LL is phylogenetically conserved, and that longer LL is associated with a greater numbers of leaves, smaller leaves, and a colder hardiness zone of the species' native range. We found that average LL ranged from 1.4 years in *Rhododendron tomentosum* to 10.5 years in *Abies cilicia*. LL was phylogenetically conserved, with some genera, such as *Abies* and *Picea*, exhibiting long LL (> 3 years) and others, such as *Ilex* and *Rhododendron*, exhibiting short LL (< 3 years). Leaf length was negatively correlated with LL in conifers, due to differences between *Pinus* and other genera; however, there was no correlation between LL and number of leaves. This study highlights the considerable variation and phylogenetic pattern in LL among temperate evergreen species, which has implications for carbon budgets and ecosystem models.

Keywords Leaf aging · Leaf lifespan · Leaf senescence

Introduction

The physiology and natural history of leaves vary widely across species and ecosystems. This variation can be described with an assortment of leaf traits, which generally combine to maximize carbon gain while minimizing exposure to threats (Diaz et al. 2004; Kikuzawa et al. 2013). One such trait is leaf longevity (LL), the amount of time a photosynthetically active leaf is retained by a plant. Understanding LL is critical to understanding the carbon balance of leaves, and because of its role in carbon and nutrient cycling and ecological interactions, particularly through leaf turnover rate, LL can inform models that describe how climate change is affecting forest dynamics, ecosystem processes, and plant distributions (Gutiérrez and Huth 2012; Reich et al. 2014; Lu et al. 2017). The longer healthy leaves remain on trees and carry out photosynthesis, the longer they sequester carbon and nutrients. This advantage is particularly evident for temperate and boreal evergreen trees that are dominant in cold and resource poor environments (Aerts 1995, 1999). Additionally, the relationships between LL and

Communicated by Gerardo Avalos.

This undergraduate-led study shows extensive variation in leaf longevity (LL) among evergreen species and provides evidence that LL is phylogenetically conserved and subjected to constraint across long time scales. Our study forms the foundation for accurate parameterization of LL in ecosystem process and plant distribution models.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00442-019-04492-z>) contains supplementary material, which is available to authorized users.

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other plant traits (such as leaf size, number of leaves, and tolerance of frost or herbivory) could have important implications for herbivores, epiphytes that grow on leaves, climate change predictions, and models of future plant distributions.

In temperate and boreal regions, LL generally balances the resource cost of producing a new leaf in spring with the cost of retaining a leaf through winter, which requires defenses against both environmental threats (e.g. frost) and herbivory. As such, leaves with high construction costs and low photosynthetic rates, which manifest in adaptations to herbivory, cold temperatures, and low nutrient levels, tend to have longer LL, giving them time to fix more carbon than was used during construction (Mooney and Gulmon 1982; Coley 1988; Chapin et al. 1998; Villar and Merino 2001; Diaz et al. 2004; Wright et al. 2004, 2005; Endara and Coley 2011; Falster et al. 2012; Kikuzawa et al. 2013). The slower resource assimilation of plants with longer LL makes them better suited to water-, nutrient-, and temperature-limited environments than to warm, nutrient-rich, and moist environments, which tend to be populated with shorter-LL plants that can take advantage of seasonal opportunities for high growth rates (Diaz et al. 2004; Endara and Coley 2011; Reich et al. 2014). This resource-oriented view of LL follows from the resource allocation hypothesis, which posits that long-lived plants in resource-poor environments must allocate more resources to defense, whereas short-lived plants with high resource availability can invest instead in rapid growth (Endara and Coley 2011). From this

understanding of LL as a balance between maintenance cost and carbon gain, we might expect leaf senescence to occur when photosynthetic rates can no longer offset the maintenance cost of a particular leaf.

LL has additional connections to the vulnerability of plants to herbivory and environmental damage. Past studies have shown that for broad-leaved plants, species with longer-lived leaves tend to produce leaves that are smaller, more abundant, and more strongly defended (Reich et al. 1999; Kleiman and Aarssen 2007; Yang et al. 2008; Rossatto 2013). Because long-lived leaves are exposed to environmental damage and herbivory for a longer period of time, a plant with long LL must produce leaves that are able to withstand those threats and survive, which often means producing many small leaves (Coley 1988; Wright et al. 2004; Kleiman and Aarssen 2007). This interaction between leaf number, leaf size, and LL is considered part of a suite of trade-offs of long-lived leaves (Kleiman and Aarssen 2007; Rossatto 2013).

While several previous studies have explored various aspects of LL (Table 1), many of them have focused on just a few plant species, sampled from multiple sites with different climates, or used data from studies that varied in collection methods, making comparability uncertain (Wright et al. 2004; Reich et al. 2014). Most notably, a study of four conifer species sampled along a 2160-km gradient in North America found a substantial increase in LL and lower leaf nitrogen content in colder sites (Reich et al. 2014). However,

Table 1 A selection of previous studies that have quantified leaf longevity (LL), the number of species investigated (studies are listed from most species to fewest), and the methods employed to measure LL

| Authors | Number of species | Method of measuring LL |
|--------------------------------|---|--|
| Reich et al. (1999) | 102 total, over 6 sites representing six distinct biomes; max number from one biome is 43 | Broadleaf species—track individual leaves; conifer species—count annual cohorts with at least 50% of their leaves remaining (method we used) |
| Wright et al. (2002) | 75 species over 4 sites with differing rainfall and nutrition | Inverse of leaf mortality (average turnover per year; 2 years recorded) |
| Coley (1988) | 41 | Track individual leaves |
| Dungan et al. (2008) | 34 | Track individual leaves |
| Reich et al. (2004) | 23 | Track individual leaves |
| Mediavilla and Escudero (2003) | 12 | Mean number of leaves per annual growth shoot used to construct static life tables |
| Rossatto (2013) | 12 | Track individual leaves over 2.5 years |
| Falster et al. (2012) | 10 | Space-for-time substitution—i.e., use previously obtained values to calibrate leaf location on branch with leaf age |
| Reich et al. (2009) | 10 | Space-for-time substitution |
| Reich et al. (2014) | 5 | Count annual cohorts with at least 50% of their leaves remaining (method we used); 125 sites |
| Reich et al. (1996) | 2 | Count annual cohorts with at least 50% of their leaves remaining (method we used) |
| Field and Mooney (1983) | 1 | Track individual leaves |
| Laclau et al. (2009) | 1 | Track individual leaves |

there has been no comprehensive survey of LL in a large number of species growing in the same environmental conditions and using a single method. In addition, the trade-off between leaf size, leaf number, and LL found in broad-leaved species has not been investigated in conifers.

In this study, we present what is, to the best of our knowledge, the first large-scale study of LL employing a single method of data collection for species growing at a single site: the Arnold Arboretum in Boston, Massachusetts. With this approach, we are able to investigate inherent differences among species in LL, rather than differences due to the climates in which they are grown, because a wide diversity of species from temperate regions of North America, Europe, and Asia are all growing under the same conditions at the Arnold Arboretum. Using LL data collected from 169 woody species across 36 genera we investigated the effects of phylogeny, climate of origin, leaf size, and leaf number on LL.

We test two main hypotheses: H_1 : LL is phylogenetically conserved; and H_2 : longer LL is associated with a greater number of leaves, with smaller leaves, and with a colder hardiness zone of the species' native range.

Materials and methods

Study site and species

Observations were recorded for woody plants growing outside in the living collections of Harvard University's Arnold Arboretum in Boston, Massachusetts (42°18'N, 71°07'W) at approximately 22 m above sea level. This a temperate site, with cold winters and warm summers, with a mean annual temperature of 10.7 °C and 111 cm of precipitation per year distributed evenly throughout the year. LL and leaf characteristics (see below) were measured for 169 evergreen tree and shrub species, including both angiosperms and gymnosperms, but predominately conifers (Online Resource 1). LL was recorded in June and July 2017, and leaf size and number were measured using herbarium specimens. All plants growing at this temperate 114-ha arboretum experience similar weather and growing conditions, though plants vary in the amount of direct sunlight they receive. Plants at this arboretum are not supplemented with fertilizer or water, except during drought conditions, but they are well-spaced which limits direct competition with other plants; as a consequence, they likely grow under better conditions than plants in the wild.

Leaf longevity

To determine LL, we first counted or estimated (if > 20 leaves on one section) the number of leaves remaining on a

healthy branch for every annual growth section, as delineated by the overwintering buds and resulting bud scale scars, short leaf internodes, and different coloration of twig sections (Online Resource 2). We recorded data on one individual per species; for many species at the Arnold Arboretum only one individual is available on the grounds. We measured two to four healthy, non-reproductive twigs per individual. While the sample size for each species was not large, differences among twigs of the same species were generally small in comparison to differences among species and our goal was to sample as many species as possible (Online Resource 1). Typically, measured twigs were 1–2 m from the ground and were in full or partial sun, and not in deep shade.

For each twig that we measured, we determined the maximum number of leaves grown in any one year and used that value as the estimated potential for annual growth. We used the maximum number instead of the number from the current year because in many cases the section from the 2017 growing season had not yet finished producing leaves. We defined LL as the number of years required for less than half the maximum number of leaves to still be remaining on a twig segment (see Reich et al. 1996, 1999, 2014) (Table 1).

We measured LL for every evergreen species in the Arnold Arboretum for which it was possible to use this method. Certain evergreen species, especially those in the genera *Juniperus* and *Chamaecyparis*, were not measured due to the difficulty of identifying overwintering bud scale scars and distinguishing the age of twig sections.

All of the following analyses were performed using R version 3.4.2 (R Core Team 2017).

TRY database comparison

To compare our data with those previously collected by other researchers, we obtained LL data from the TRY database of plant traits for 25 of our sampled species, collected in 14 different studies using various methods (Kattge et al. 2011; <http://www.try-db.org>). We performed a Wilcoxon signed rank test to determine if LL values were consistently larger or smaller in the TRY data base in comparison with the Arnold Arboretum data. We used a linear regression to determine if species tended to have similar values in the TRY data base and the Arnold Arboretum; LL values were log-transformed prior to analysis to compensate for the right skewness of the data. The log-transformed LL data were normally distributed.

Phylogeny

To determine whether different genera had significantly different LL, we used the R package “nlme” to run a linear mixed effects model (LME) with LL as the response variable, genus as a fixed effect, and species as a random effect

(Pinheiro et al. 2017). In this LME we included only the seven genera with more than five species represented (*Abies*, *Ilex*, *Rhododendron*, *Picea*, *Pinus*, *Taxus*, and *Tsuga*). We then performed a nested analysis of variance (ANOVA) on the LME and used the *glht* function of the “multcomp” package to test general linear hypotheses indicating which genera are different from one another (Hothorn et al. 2008).

To determine whether there were significant differences between species in the same genus, we performed a total of seven ANOVA and Tukey’s HSD tests, one for each genus that had five or more sampled species (listed above).

To examine if LL is phylogenetically conserved, two phylogenetic trees were created by pruning the trees assembled in Panchen et al. (2014) to include only our study species. One tree was created from a composite of trees from previously published literature using the program PHYLOMATIC and is hereafter known as the PHYLOMATIC tree (Panchen et al. 2014; Webb and Donoghue 2005). This method is advantageous because data are available for a wide range of species; however, it does not provide highly accurate branch-length information, and thus the resulting tree is more accurately described as a “pseudo-chronogram,” with lower branch-length variability than more accurately calibrated trees (Molina-Venegas and Rodríguez 2017). The other tree was created in the program PHLAWD using DNA sequence data obtained from GenBank (Smith et al. 2009; Panchen et al. 2014). Because DNA sequence data are not available for as many species, this tree is smaller, but more accurately calibrated than the PHYLOMATIC pseudo-chronogram (Molina-Venegas and Rodríguez 2017). More details of the phylogenetic tree construction can be found in Panchen et al. (2014).

To determine if LL is phylogenetically conserved, we used Pagel’s λ and Blomberg’s K . Both measures provide information about the strength of the phylogenetic signal and allow for comparison between different phylogenetic trees (Münkemüller et al. 2012; Molina-Venegas and Rodríguez 2017). If a trait has a significant phylogenetic signal, and is, therefore, phylogenetically conserved, then closely related species are more likely to have similar values for that trait than would be expected by chance. Both Pagel’s λ and Blomberg’s K are measures of phylogenetic signal for which a value of 0 indicates no phylogenetic conservation and 1 indicates strong conservation (Pagel 1999; Blomberg et al. 2003). Blomberg’s K can also be greater than 1, indicating very strong phylogenetic conservation.

The PHLAWD tree contained 36 of our study species, and the PHYLOMATIC tree contained 121 species; however, the PHYLOMATIC tree was only resolved to the genus level, with 31 genera represented. We calculated Pagel’s λ and Blomberg’s K for the two trees to determine the degree of phylogenetic signal in LL using the *phylosig* function of the “phytools” package version 0.6-20 (Revell 2012). Pagel’s λ

is robust to the polytomies and inaccurate branch lengths in the PHYLOMATIC tree; however, it is less reliable for the small sample size of the PHLAWD tree (Münkemüller et al. 2012; Molina-Venegas and Rodríguez 2017). Unlike Pagel’s λ , Blomberg’s K is slightly more robust with small sample sizes and can detect stronger phylogenetic signals, but has a tendency to over-estimate the signal in pseudo-chronograms leading to type I bias (Blomberg et al. 2003; Münkemüller et al. 2012; Molina-Venegas and Rodríguez 2017). We used both trees and measures to capture the various insights that they provide.

Hardiness zone

We used linear regression to test the relationship between LL and the cold hardiness zone of the native habitat of 149 of our 169 species, using data from Rehder (1940). Cold hardiness zones represent the average annual minimum temperature of a region; we selected the coldest hardiness zone in each species’ native range. Even though the physical location of the hardiness zones and species ranges have shifted somewhat due to climate change (Rubel and Kottke 2010), the zones themselves still describe the approximate temperature profile in which each species typically grows.

Leaf traits

We measured needle length on herbarium specimens at the Arnold Arboretum Herbarium. Herbarium specimens were predominantly collected from plants growing at the Arboretum. Conifer species do not exhibit noticeable changes in shape or size while drying, and, therefore, we expect only minimal differences in size between live and dead needles. We used herbarium specimens for the measurements due to the ability to record many measurements rapidly in lab setting. We measured one needle per twig and three twigs per species whenever possible (Online Resource 1). The variation in needle length within a species was minimal in comparison to differences among species; intraspecific SD ranged from 0.6 to 43.5 mm, with a median of 3.7 mm, whereas needle length among the 78 conifer species ranged from 7.3 to 176.7 mm. We measured annual leaf production for each species on plants growing on the Arboretum grounds and calculated the mean of maximum annual leaf production for all twigs measured of a species.

We examined the relationship between LL and average needle length and annual leaf production for 78 conifer species, using multiple regression with both needle length and annual leaf production as explanatory variables. We performed an ANOVA and Tukey’s HSD test to determine whether needle length differed among genera. We used linear regression to further examine the relationship between LL and needle length, and between needle length and annual

leaf production for all species. Based on the results of the ANOVA, we performed additional, separate linear regressions between needle length and LL for all genera except *Pinus*, and for *Pinus* alone.

Results

Leaf longevity

Across all 169 evergreen species, the average LL was 3.7 ± 1.9 (SD) years and the median LL was 3.0 years. LL ranged from 1.4 ± 0.5 years in *Rhododendron tomentosum* (formerly known as *Ledum palustre*) to 10.5 ± 2.1 years in *Abies cilicica* (Online Resource 1). The genus with the longest average LL was *Abies* (6.2 ± 2 years), while the genus with the shortest average LL was *Rhododendron* (2.2 ± 1.0 years) (Fig. 2, Online Resource 3).

TRY database comparison

Using the Wilcoxon signed rank test, there were no significant differences found between species mean LL values from our study and mean LL values from the TRY database; that is, species did not have a consistently longer or shorter LL in either data set ($p = 0.29$, $n = 25$ species). A linear regression on the log-transformed data showed a significant relationship between our LL values and values from the TRY database (Fig. 1; $p < 0.001$; multiple $R^2 = 0.34$), indicating that species tended to have statistically similar LL values in both data sets. However, for some of the 25 species, such as *Rhododendron maximum*, *Taxus baccata*, *Pinus koraiensis*,

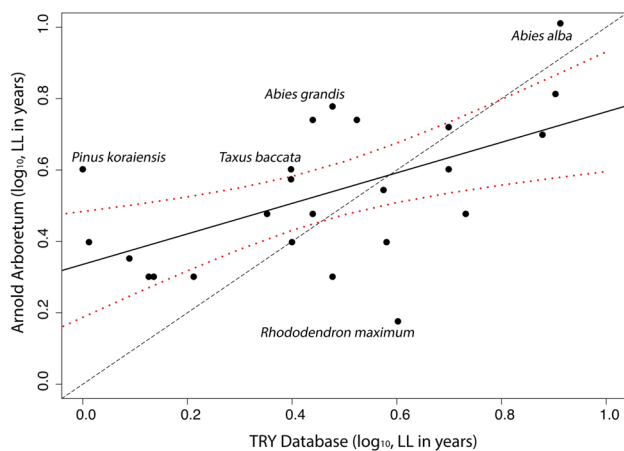


Fig. 1 \log_{10} of our species-level mean leaf longevity (LL) values plotted against \log_{10} of LL values from the TRY database. Each data point represents one species. The dashed line is a one-to-one ratio, while the solid line is a line of best fit ($R^2 = 0.34$; $p < 0.001$; $n = 25$). The dotted red lines represent 95% confidence intervals around the line of best fit (color figure online)

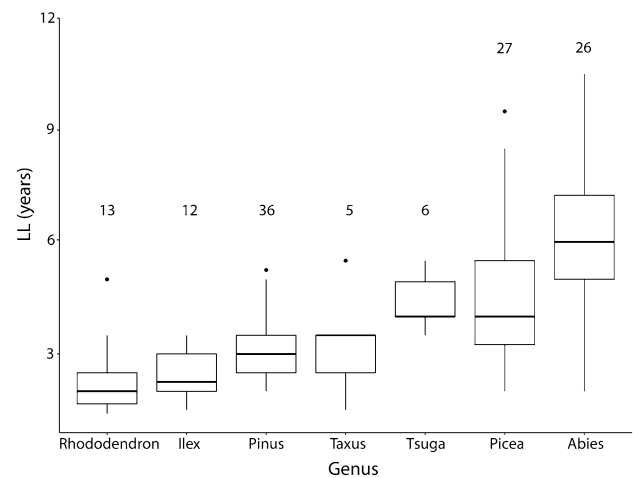


Fig. 2 Boxplot of leaf longevity (LL) for genera with more than five species measured. The number above each genus name is the number of species represented from that genus

and *Abies grandis*, estimates differed substantially between our study and the TRY database (Fig. 1).

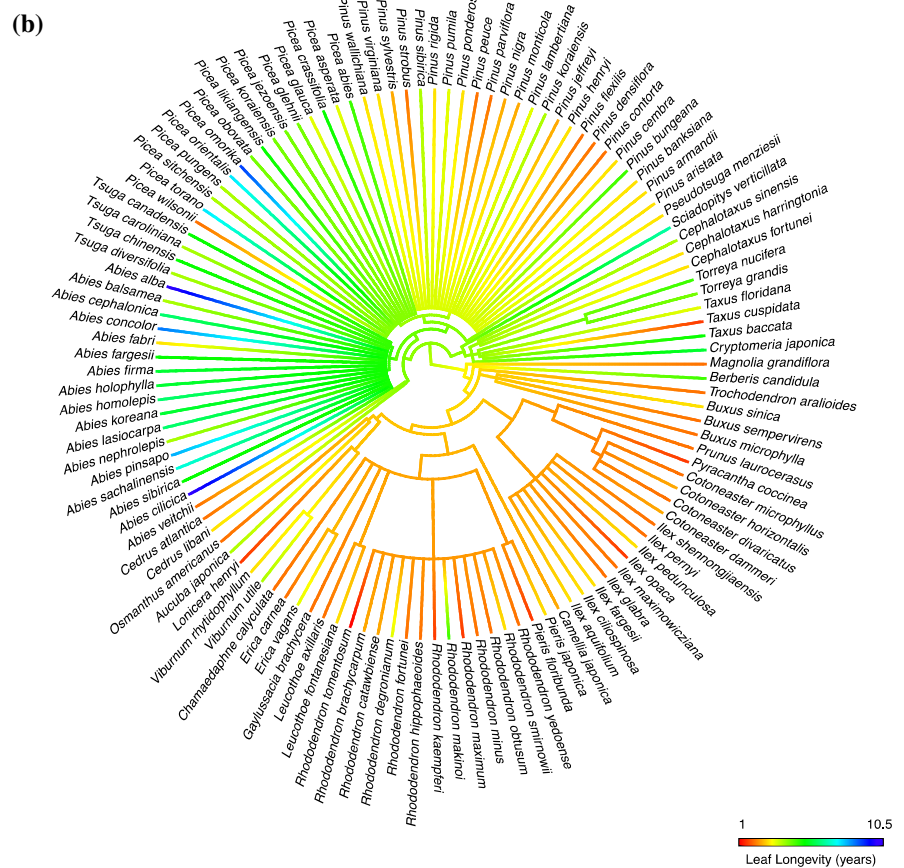
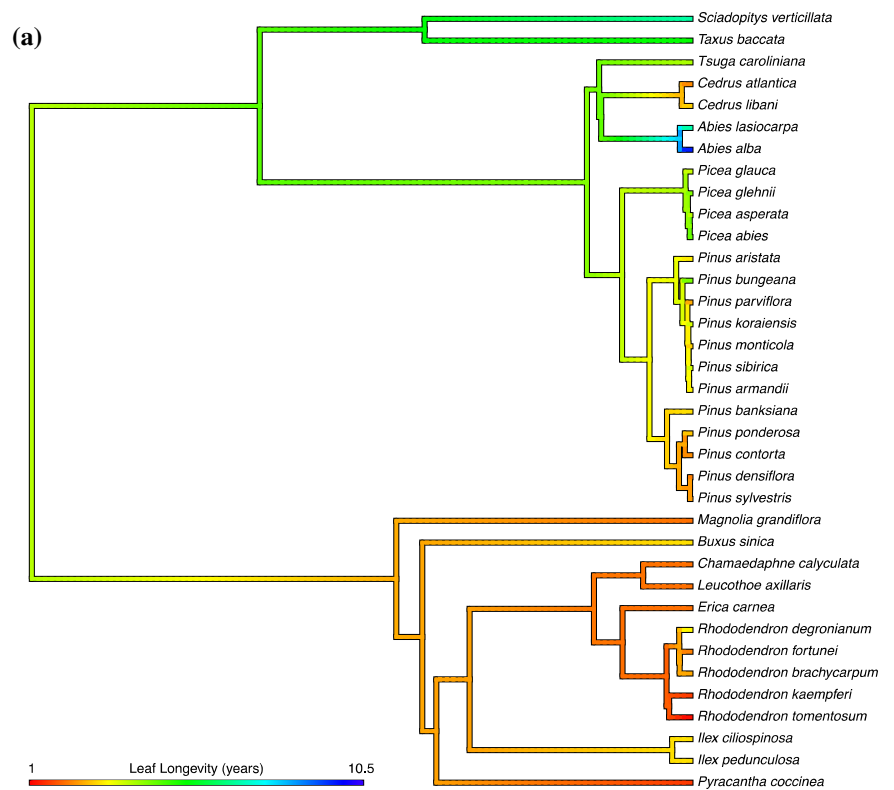
Phylogeny

Both the LME and the nested ANOVA performed on the LME indicated significant differences in LL between genera with five or more species (Fig. 2; Online Resource 3). A Tukey's HSD test on the results of the nested ANOVA showed significant differences in LL between *Abies* and *Taxus*, *Ilex*, *Pinus*, *Picea*, and *Rhododendron*. *Picea* differed significantly in LL from *Ilex*, *Pinus*, and *Rhododendron*. This result confirms that species in the genera *Abies* and *Picea* have longer LL than species of the genera *Ilex*, *Pinus*, and *Rhododendron*, with some other significant differences among particular genera (Fig. 2, Online Resource 3).

In the genera *Abies*, *Picea*, *Pinus*, *Rhododendron* ($p < 0.001$), and *Taxus* ($p < 0.05$), species from within each genus differed significantly from one another in LL.

For the PHLAWD tree, Pagel's λ was 0.90 ($p < 0.01$), and Blomberg's K was 0.05 ($p < 0.05$). For the PHYLOMATIC tree, Pagel's λ was 1.00 ($p < 0.001$) and Blomberg's K was 1.27 ($p < 0.001$). All of these metrics are statistically significant and indicate LL is phylogenetically conserved, meaning closely related species have more similar LL than would be expected by chance (Pagel 1999; Blomberg et al. 2003). The different values between the tests reflect different numbers of species represented in each tree, how branch lengths are calculated, and how each test is carried out (Molina-Venegas and Rodríguez 2017). In particular, Blomberg's K is likely different between the two trees because of its sensitivity to the low branch length variation in the PHYLOMATIC tree. This phylogenetic conservatism is visually illustrated

Fig. 3 Phylogenetic trees illustrating evolutionary conservatism of leaf longevity (LL) for species in this study. Yellow and red branches indicate species with short LL (< 3 years), and green and blue branches indicate species with long LL (> 3 years). **a** The PHLAWD tree, containing 36 species with species-level resolution. **b** The PHYLOMATIC tree, containing 121 species with genus-level resolution. This figure is available in color in the online version



in Fig. 3, in which angiosperms have shorter LL than gymnosperms, and *Pinus* species have shorter LL than *Abies* species, with *Picea* species being intermediate.

Hardiness zone

We found no significant relationship between LL and hardiness zone ($R^2 = 0.005$, $p = 0.19$, $N = 147$), that is, there is no indication that species native to colder regions have longer-lived leaves than species from warmer regions.

Leaf traits

For conifer species, multiple regression with both needle length and annual leaf production as explanatory variables identified leaf length as a significant predictor of LL ($R^2 = 0.14$, $p < 0.005$, $N = 78$) but not annual leaf production ($p > 0.05$) (Fig. 4), that is, longer-lived leaves tend to be shorter in length, though the amount of variation explained is not large. The ANOVA showed significant differences between *Pinus* and *Abies*, *Cedrus*, *Cryptomeria*, *Picea*, and *Tsuga*. In other words, all differences between genera were due to differences between *Pinus* and other genera. When *Pinus* and the other genera were analyzed separately, their respective linear regressions showed no relationship between needle length and LL (Online Resource 4, $p = 0.85$ and $p = 0.25$, respectively). We found no relationship between needle length and annual leaf production in conifers ($R^2 = 0.03$, $p = 0.07$, $N = 78$).

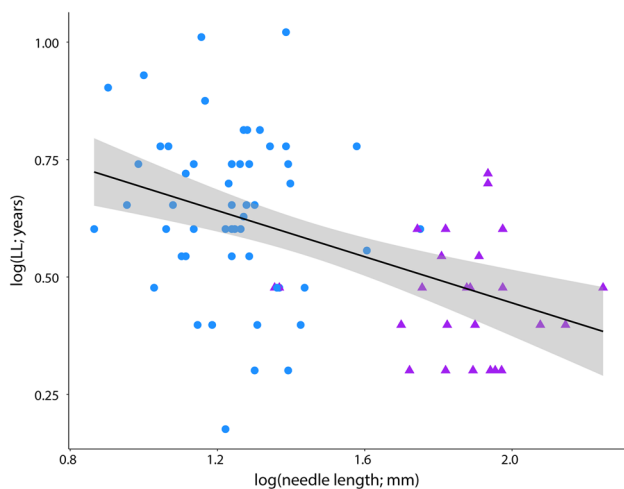


Fig. 4 Linear relationship between \log_{10} needle length and \log_{10} LL of conifer needles. Each data point represents a coniferous species. Blue circles represent species in the genus *Pinus*; purple triangles represent species in the genera *Abies*, *Cedrus*, *Cryptomeria*, *Picea*, and *Tsuga*. The solid line is the line of best fit (adjusted $R^2 = 0.20$; slope = -0.246 ; intercept = 0.936 ; $p < 0.001$, $N = 78$), and the shaded region represents 95% confidence intervals. See Online Resource 4 for separate analyses of *Pinus* and other genera

Discussion

In this study, we measured the LL of 169 evergreen species growing at one location under common climatic conditions. Our phylogenetic analysis indicates that LL is conserved; that is, closely related species are more likely to have similar LL than would be expected by chance. In addition, there are significant differences in LL among genera. The two genera with the longest LL (*Abies* and *Picea*) were significantly different from several of the genera with shorter-lived leaves (e.g. *Ilex*, *Pinus*, and *Rhododendron*). Species of *Abies* and *Picea* are often found growing together in the same forest communities; in light of our results, this suggests that some environmental characteristics of fir and spruce forests—such as cold temperatures, low resource availability, and short growing seasons—are suitable to species with longer-lived leaves (Farjon and Filer 2013).

We expected the climate in the location of a species' origin to at least partially explain LL, because long LL has been shown to be an adaptation to cold, resource-limited climates (Wright et al. 2004; Kikuzawa et al. 2013; Reich et al. 2014). However, in this large sample of evergreen species, there was no significant relationship between LL and the native cold hardiness zone of each species. This lack of relationship could indicate that LL is influenced more by precipitation, soil characteristics, and nutrient levels in the native range than by temperature alone (van Ommen Kloeke et al. 2012; Reich et al. 2014).

We found that longer LL was associated with shorter needles in conifers. This difference, however, is due entirely to the difference in length between *Pinus* needles and needles of other genera, namely that *Pinus* species typically have longer needles and shorter LL than those of other conifer genera. The combination of long needles and short LL for *Pinus*, and short needles and long LL for other genera, could imply a trade-off between longevity and size: because long-lived needles have high construction costs, plants' carbon budgets and nutrient availability could prevent the production of large and long-lived needles (Wright et al. 2005; Kikuzawa et al. 2013). We also found that annual needle production was not related to LL when accounting for needle length. The lack of trade-off between annual needle production and needle length contrasts with the trade-off observed in broadleaf species (Kleiman and Aarssen 2007; Yang et al. 2008) and suggests that in conifers, needle length and LL are driven by different evolutionary pressures than annual needle production.

We found a significant correlation between our measurements of LL for 25 species and independent measures of LL for the same species in the TRY database. This suggests that data from an integrated database could suffice for

an approximate estimate of the LL of a species. However, because variation in the relationship between data sets is high, researchers working with evergreen species should consider taking measurement at their own location, using a standardized method. We note that the largest dissimilarities between our dataset and the TRY dataset are in species with short LL; it may be that the growing conditions at the Arnold Arboretum allow individuals of species with typically very short-lived leaves to plastically extend toward a relatively longer LL. Previous work further supports this recommendation, indicating some of the variation in the LL of evergreen conifer species is due to responses to local temperature and nutrient availability (Reich et al. 2014).

Further work is needed to elucidate the factors which determine LL in evergreen species. Declining photosynthetic efficiency as related to chlorophyll content and leaf mass per area (LMA) could be a driver of leaf senescence and thus LL. A similar pattern has been found for nitrogen use efficiency, where absolute nitrogen content per leaf remains constant through time, but nitrogen per unit mass and hence nitrogen use efficiency decreases through time as LMA increases (Field and Mooney 1983; Mediavilla and Escudero 2003). Leaves may undergo leaf senescence when their costs of maintenance begin to exceed the photosynthetic benefits that they provide to the plant.

Conclusion

In this study of 169 species of evergreen woody species, we found that LL data from the TRY database, which were measured using differing methods, can be combined if necessary to provide useful information on species LL, but that a standardized method is preferable for precise analysis. LL is phylogenetically conserved across evergreen species and is negatively related to needle length in conifers. This suggests separate evolutionary pressures in conifers leading to selection for either smaller, long-lived leaves or larger, short-lived leaves. Further research into the dynamics of LMA as leaves age could test whether photosynthetic efficiency remains the same or decreases as leaves age, potentially providing an explanation for the timing of leaf senescence in evergreen species. Estimates of LL—which are associated with carbon and nutrient cycling and interactions with herbivores and other species—and trade-offs associated with LL described in this study can be further used in modeling forest dynamics and ecosystem processes, predicting distributions of plant functional types and simulating the effects of future climate change. While this study focuses on a broad range of species at one location, further work is needed to determine how LL varies in response to global changes such as

warming temperatures, nutrient inputs, and increased pressure from herbivores.

Acknowledgements We thank the Arnold Arboretum of Harvard University for permission for the fieldwork and sampling, and Irina Kadis of the Arnold Arboretum for help with the leaf length measurements. This study was funded by the BU Undergraduate Research Opportunities Program, and was carried out as part of a BU undergraduate honors thesis (LS) and a BU Academy senior thesis (SP). We acknowledge the use of data provided by the TRY initiative on plant traits (<http://www.try-db.org>). Helpful comments on the paper were provided by Abe Miller-Rushing, Pam Templer, and two anonymous reviewers.

Author contribution statement RBP, LS and SP conceived and designed the study. LS and SP collected data. All authors analyzed the data and wrote the manuscript.

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