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Can the extinction risk of Irish vascular plants be predicted using leaf traits?

Harrison O'Rourke[1,2] · Eimear Nic Lughadha[2] · Karen L. Bacon[1]

Abstract

Globally, 39% of vascular plant species are estimated to be threatened with extinction. Many factors are responsible for this figure; however, in numerous regions the primary drivers of plant extinction remain unknown. In this study, leaf traits were examined to determine whether there is an association between any specific leaf trait and extinction risk for the Irish flora. Ireland has a relatively small flora that is influenced by a temperate, oceanic climate. Fourteen leaf traits were measured for 1,029 angiosperm taxa, primarily from online herbarium images. Extinction risk was based on national Red List assessments for the Irish flora. Multivariate analysis of the data showed no correlation between leaf traits and extinction risk for the Irish flora. One-way ANOVA and Pearson's Chi-squared tests largely supported this result, with some indication that leaf teeth may be associated with extinction risk. The correlation of extinction risk and leaf traits with phylogenetic relatedness was also considered, with the presence of a phylogenetic signal detected for the distribution of extinction risk across the Irish flora and significant phylogenetic signal observed for individual leaf traits. It was concluded that the leaf traits analysed do not significantly correlate with the extinction risk of the Irish flora and that leaf traits are not a good predictor of extinction risk for this flora.

Keywords Extinction risk · Leaf traits · Red List · Irish flora · Plants

✉ Harrison O'Rourke
h.orourke2@nuigalway.ie

1 Botany & Plant Sciences, Ryan Institute, School of Natural Sciences, National University of Ireland, Galway, Ireland

2 Conservation Science Department, Royal Botanic Gardens, Kew, Richmond, UK

Introduction

Drivers of plant extinction

Globally, an estimated two in five plant species are threatened with extinction (Nic Lughadha et al. 2020). Plants are exposed to numerous drivers that may push them towards extinction, many of which act synergistically to increase the pressure on plants (Brook et al. 2008). Currently, habitat loss is reported to pose the greatest threat to plants (Giam et al. 2010; Jantz et al. 2015; Nic Lughadha et al. 2020). Human-dominated land use now accounts for over half of the world's land surface (Watson et al. 2016), with the expansion of intensive agriculture being the primary cause (Foley et al. 2005). Mature vegetation is predicted to decrease by 25-60% in biodiversity hotspots between 2005 and 2100 due to the loss of tropical forests alone (Jantz et al. 2015). Overexploitation of species is another serious threat to plants (Goettsch et al. 2015; Phelps and Webb 2015; Sharrock et al. 2014), with an estimated 21.1% of plants assessed for the global IUCN Red List of Threatened Species (hereafter global Red List) reported to be threatened by overexploitation (Nic Lughadha et al. 2020). Habitat loss can increase the impact of overexploitation by reducing the population size and making the remaining population more vulnerable to overexploitation.

The threat of invasive species, estimated to be an important risk factor for 6.4% of species with a global Red List assessment (IUCN, 2022), can also push local species towards extinction. For example, in Ireland, the invasive *Rhododendron ponticum* has had a significant negative impact on the abundance of native plant species (Stout and Casey 2014). Invasive species are thought to have played a role in 25.5% of recent global plant extinctions (Blackburn et al. 2019). A recent meta-analysis found that when a plant community becomes invaded, plant species richness decreases by 28.4% (Mollot et al. 2017). Habitat loss, overexploitation and invasive species are currently considered to be three of the greatest threats to plant species (Diaz et al., 2019; Nic Lughadha et al. 2020).

In contrast to these three drivers of extinction, climate change thus far appears to have had only a minor impact on plant extinctions (Nic Lughadha et al. 2020). Only 4.1% of plants assessed on the global Red List have climate change as a documented threat to their survival (Nic Lughadha et al. 2020). However, this may partly be due to the longer timescales over which climate change acts compared to these other threats, as well as the difficulty in quantifying the effects of climate change on extinction risk (Trull et al. 2018).

Relationship between leaf traits and extinction risk

Leaf traits, which may refer to morphological, anatomical, physiological, phenological or chemical characteristics of a leaf, are studied for numerous purposes. They are important in identifying and classifying extant plants (Silva et al. 2020); interpreting the fossil record (Soh et al. 2017); and estimating paleoenvironmental and paleoclimatic conditions (Tosal et al. 2021). Leaf traits influence how plants exchange water (Ding et al. 2020), carbon and energy (Reich, 2012) with their environment, thereby impacting important physiological processes such as photosynthetic rates (Shipley et al. 2005). Leaf traits are central to the functioning of terrestrial ecosystems and display a pattern of correlations that are consistent globally (Wright et al. 2004). This pattern of important physiological, structural and chemi-

cal leaf traits is known as the leaf economic spectrum and is largely independent of plant functional type and growth form (Wright et al. 2004). The spectrum is represented at one extreme by long-lived, structurally expensive leaves produced by slow-growing species; and at the other end of the spectrum by short-lived, structurally inexpensive leaves produced by fast-growing species (Wright et al. 2004). Opposite ends of the leaf economic spectrum reflect different ecological strategies that balance resource acquisition versus resource conservation (Wright et al. 2004). Understanding the processes of how plants invest in leaf production may help to predict the response of plant species to the impact of extinction drivers.

Leaf traits are a key indicator of how plants respond to climate change. Leaf trait changes have been associated with climate change in both palaeobotanical studies (Bacon et al. 2013, 2021; Blonder et al. 2014; Soh et al. 2017) and modern studies where leaf traits have responded to a change in climate either in experimental conditions (Bacon et al. 2016) or in transplant experiments (Royer et al. 2009b). Morphological leaf traits have also been used to link change in herbaria specimens over time to climate change (e.g. Guerin et al. 2012). Many leaf traits such as area, shape, length and width can be readily examined in both fossil and herbaria specimen; therefore, insights gained through assessing these traits in palaeobotanical studies can be related to modern taxa, and vice versa.

Palaeobotanical studies often reveal that changes in leaf traits in response to climate were linked to plant survival or local extinction at these times. For example, leaf width and leaf area both showed plasticity across the Triassic–Jurassic boundary in East Greenland. The Triassic–Jurassic mass extinction event saw global temperatures increase by 4°C (McElwain et al. 1999) and atmospheric CO_2 double (Steinthorsdottir et al. 2011; Schaller et al. 2011), as well as increases in aerosol pollutants from massive volcanism (Marzoli et al. 2019; Lindström et al. 2021). Analysis of leaf shape across this boundary in well-preserved fossil floras from East Greenland revealed that species with large entire leaves (e.g. *Ginkgoites obovatus*) were replaced by species with highly dissected leaves (e.g. *Ginkgoites fimbriatus*) (McElwain et al. 1999; Belcher et al. 2010). Modern studies have also indicated that leaf width (Guerin et al.,2012) and leaf area (Ackerly et al. 1992) respond to climate change. The leaf width of *Dodonaea viscosa* subsp. *angustissima* in South Australia decreased by 2mm across a 127 year period that corresponded with a 1.2°C increase in temperature (Guerin et al. 2012). A benefit of narrow leaves at high temperatures is that they are capable of losing heat without substantial rates of evapotranspiration and are also less susceptible to overheating than wider leaves (Yates et al. 2010).

Another leaf trait shown to be responsive to climate change from both palaeobotanical and modern studies is leaf mass per area (LMA). In East Greenland, the Late Triassic forests were dominated by taxa with low LMA, short leaf lifespans and high transpiration rates (Soh et al. 2017). By the Early Jurassic, forests were dominated by taxa with high-LMA and slower metabolic rates (Soh et al. 2017). During the Cretaceous–Paleogene extinction event the LMA of angiosperms in North Dakota decreased, while vein density increased, representing a shift towards fast growth strategies (Blonder et al. 2014). The species that persisted across the boundary had lower carbon investment and higher assimilation rates as a result of their shift in leaf traits (Blonder et al. 2014). The Cretaceous–Paleogene extinction event was important in demonstrating that angiosperms, which dominate almost every ecosystem on the planet today, can shift their leaf traits in response to climate change (Blonder et al. 2014). Other environmental factors that are known to interact with leaf traits

include SO_2 (Bacon et al. 2013, 2021), temperature (Royer et al. 2005, 2009b; Peppe et al. 2011) and light (Evans and Poorter 2001; Aleric and Kirkmann, 2005; Kong et al. 2016).

Climate change is not the only extinction driver that can influence leaf traits. Changes to nutrient supply (Tilman and Lehman 2001), land use change (Cerqueira et al. 2021) and pollution (Dadkhah-Aghdash et al. 2022) have all also been shown to impact leaf traits and elevate the risk of extinction in some plants. For example, changes in the supply of major nutrients can alter the competitive balance of a plant community and push certain species towards extinction (Tilman and Lehman 2001). Nutrient enriched systems can impact on leaf traits by increasing specific leaf area and decreasing rates of leaf turnover, thickness and toughness (La Pierre and Smith 2015). Habitat loss is another driver of plant extinction that can influence leaf traits. In the Brazilian Atlantic Forest, habitat loss was correlated with a lower chlorophyll content, smaller leaf area, higher leaf mass per area, and lower magnesium, nitrogen and phosphorus concentration in the leaves of the endangered palm *Euterpe edulis* (Cerqueira et al. 2021).

Pollution has also been documented as a factor that influences both extinction risk and leaf traits. Air pollution is known to decrease the area, length and width of leaves (Shafiq et al. 2009; Appalasamy et al. 2017; Dadkhah-Aghdash et al. 2022) as smaller leaves experience less contact with air pollution, thereby helping the plant to withstand this stress. Significant increases in the ascorbic acid and polyphenolic content of leaves have also been observed in response to urban air pollution (Mukherjee and Agrawal 2018), potentially as a defence against the production of reactive oxygen species (Conklin and Barth 2004). The impact of climate change, nutrient addition, habitat loss and pollution on leaf traits suggest that a comprehensive trait based approach is needed to understand the role that processes such as ecosystem functioning, ecological interactions and the functional response to shifting environments have on the extinction risk of plant species (Álvarez-Yépiz et al. 2019).

The flora of Ireland

The flora of Ireland is dominated by angiosperms (Parnell et al. 2012) and comprises approximately 2,328 species of native, non-native and invasive vascular plants (National Biodiversity Data Centre, 2017), a relatively low number for a European country (Webb, 1983). The low number of plant species in Ireland is mainly due to the ice sheets that covered Ireland during the last glacial maximum (LGM) until 13,000 BP (Ballantyne et al. 2008). Much of the Irish flora was lost at this time and could only return when the ice sheets had retreated (Molloy and O'Connell, 2014). Most species of the Irish flora can be found in other temperate regions of the northern hemisphere (Webb, 1983); however, some unique species assemblages are seen in Ireland. For example, the cool, wet summers and mild winters of the Burren in Co. Clare allow Mediterranean species (e.g. *Neotinea maculata*) to grow alongside alpine species (e.g. *Dryas octopetala*) (Webb and Scannell 1983; Hanrahan and Skeffington 2015).

The Irish flora is a good model for studying the relationship between leaf traits and extinction risk as it is diverse enough to encompass a range of leaf traits from different functional and phylogenetic groups. The diversity of the Irish flora allows the relationship between a range of leaf traits and extinction risk to be robustly tested. At the same time, the Irish flora is sufficiently limited to allow a full assessment of its extinction risk and leaf traits

to be made, thereby ensuring that taxa from all functional groups within the Irish flora are assessed and that up to date extinction risk assessments are available.

Aim of the study

Several palaeobotanical (McElwain et al. 1999, 2007; Blonder et al. 2014; Soh et al. 2017) and modern flora (Royer et al. 2005, 2009b; Peppe et al. 2011; Bacon et al. 2016) studies have shown that there is a link between leaf traits and the potential to survive environmental upheaval that is driven by climate change. The IUCN Red List (IUCN, 2022) categorises species based on their estimated extinction risk, but does not consider details such as leaf traits (IUCN, 2012a; IUCN, 2012b). The aim of this study was to investigate if there is a relationship between extinction risk and selected morphological leaf traits in a modern flora. We analysed online herbarium images of taxa from the Irish flora that possess a Red List assessment to investigate a potential correlation between leaf traits and extinction risk in a modern context.

Materials and methods

Study location, species selection and leaf traits

The 'Ireland Red List No. 10: Vascular Plants' (Wyse Jackson et al. 2016, hereafter Red List) report was used as the basis for this study. The Red List assessed the threat status of Ireland's plant species and subspecies according to the International Union for the Conservation of Nature (IUCN) categories and criteria (IUCN, 2012b), and guidelines (IUCN 2016). Due to the regional nature of this Red List the IUCN guidelines for the regional level were employed (IUCN, 2012a). The island of Ireland was treated as a single biogeographic unit and assessments encompass the entire island (both Northern Ireland and the Republic of Ireland). Both native and archaeophyte taxa were included, with archaeophytes defined as pre-1500 AD introductions. The angiosperms assessed for the Red List were included in the present study, totalling 1,029 taxa, including 13 Regionally Extinct taxa; 19 Critically Endangered taxa; 25 Endangered taxa; 54 Vulnerable taxa; 90 Near Threatened taxa; and 828 Least Concern taxa. The difference in the number of taxa assessed in the current study (1,029) and the total number of species in the Irish flora (2,328) was due to alien, neophyte and apomictic species being excluded from the Red List. Red List assessments for four species aggregates (*Hieracium, Limonium, Rubus* and *Taraxacum*) were excluded from the present study, as were seven infraspecific taxa (*Callitriche brutia* subsp. *brutia, Cytisus scoparius* subsp. *scoparius, Molinia caerulea* subsp. *caerulea, Nymphaea alba* subsp. *alba, Potentilla erecta* subsp. *erecta, Senecio jacobaea* subsp. *jacobaea, Viola palustris* subsp. *palustris*) either not recognised or judged to be synonyms of other taxa on the Red List by the World Flora Online (WFO, 2021). The nomenclature of this study followed that in the Red List which in turn follows Stace (2010). Fourteen leaf traits were analysed for each taxon (Table1). These leaf traits were chosen as they could be measured from online herbarium images and they are known to display interspecific variation in response to various factors. For example, variation in leaf area has been related to climatic variation (Iio et al.

Table 1 The definition and categories of the 14 leaf traits measured in this study

Leaf Trait	Definition	Categories
Compound	Whether the leaf blade is undivided or divided into several leaflets	Simple; pinnately compound; palmately compound
Lobed	Whether the leaves had distinct lobes or divisions	entire margin; pinnately lobed; palmately lobed
Teeth	Tooth-like projections on the margins of leaves	Teeth absent; teeth present
Regularity of Teeth	Regularly spaced if the length of the basal flanks of two adjacent teeth differs by less than one-third	Teeth absent; regularly spaced teeth; irregularly spaced teeth
Closeness of Teeth	Closely spaced if the basal flanks of the teeth are no longer than three times the apical flanks	Teeth absent; closely spaced teeth; distantly spaced teeth
Apex Form	The shape of the leaf apex	Emarginate apex; round apex; acute apex
Base Form	The shape of the leaf base	Cordate base; round base; acute base; base that merges with the stem
Shape	Overall shape of the entire leaf	Obovate shape; elliptical shape; ovate shape
Area	The area of the leaf blade	Measurement (cm^2)
Perimeter	The perimeter of the leaf blade	Measurement (cm)
Shape Factor	The circularity of the leaf blade, a function of the perimeter and area	Measurement
Length	The distance from the base to the apex of the leaf	Measurement (cm)
Width	The widest distance that can be formed perpendicular to the midrib of the leaf blade	Measurement (cm)
Length-to-Width Ratio	The length of the leaf divided by its width	Measurement

2014), allometric factors (Trueba et al. 2016) and ecological strategy (Pierce et al. 2017); leaf lobation has been related to heat transfer, light environment and hydraulic conductivity (Nicotra et al. 2011); apex shape has been related to precipitation (Malhado et al. 2012); compound leaves related to resource capture ability (Wu et al. 2019); and leaf teeth related to temperature (Royer and Wilf 2006) and water availability (Royer et al. 2009a). Several key leaf traits that have important interactions with environmental variables and therefore may potentially correlate with extinction risk could not be directly measured from online herbaria images and were therefore beyond the scope of this study. These include leaf dry mass, leaf thickness, nitrogen and phosphorous concentration, photosynthetic rate, leaf lifespan and stomatal traits.

Online Herbaria

Several online herbarium databases were used to access herbarium records. The primary database used was the 'herbaria@home' database that catalogues herbarium collections housed in British and Irish universities and museums. Images were preferentially selected for collection localities in Ireland (Table 2). Taxa for which images could not be found on this database were searched for on the 'Kew Herbarium Catalogue', 'Virtual Herbaria JACQ', 'Global Biodiversity Information Facility (GBIF)' and 'Harvard University Herbaria & Libraries' online databases. The herbarium in the National Botanic Gardens of Ireland was

Table 2 The regions from which each herbarium specimen used in this study was collected

Region	Number of taxa
Ireland	424
Great Britain	552
Europe*	52
United States of America	1

* Excluding Ireland and Great Britain

Table 3 The sources that the herbarium images used in this study were accessed from

Online Source of Herbarium Specimens	Number of taxa
herbaria@home	719
Kew Herbarium Catalogue	243
Virtual Herbaria JACQ	35
Global Biodiversity Information Facility	16
Harvard University Herbaria & Libraries	2
The National Botanic Gardens of Ireland*	14

* Not online

visited to score taxa for which images could not be found on any of these online databases (Table 3). Three leaves per taxon from a single herbarium specimen were measured, with undamaged, unfolded leaves preferentially selected. The images were imported into the image processing programme ImageJ (version 1.52) for analysis (Rasband 1997).

Data treatment and statistical analysis

All statistical analysis was conducted using the open-source software R 3.6.2 (R Core Team, 2019). One-way Analysis of Variance (ANOVA) and Pearson's Chi-squared tests were carried out to investigate whether there were any significant differences among the populations in each Red List category for the individual leaf traits. Whenever a significant difference was detected, a multiple comparisons test was carried out. A pair-wise comparison that returns an interval estimate that does not contain zero provides evidence that the pair of population means are significantly different from each other. A post-hoc analysis on the residuals of the Pearson's Chi-squared test (Beasley and Schumacker 1995) was also carried out using the chisq.posthoc.test function in the R package chisq.posthoc.test with a Bonferroni correction applied to control for the family-wise error rate. When a significant difference was detected, an ANOVA test was also carried out between the leaf trait of interest and the year in which each herbarium sample was collected, to determine whether collection year was correlated with leaf physiognomy. Tables of observed and expected values were constructed to indicate which category of the leaf trait and Red List category were associated with any significant result.

The untransformed raw data were analysed by ordination using nonmetric multidimensional scaling (nMDS) (Mather 1976) and applying the Gower distance measure (Gower 1971), which is recommended in cases where there is a mix of continuous and categorical variables (Studer and Ritschard 2016). This ordination was carried out using function metaMDS in the R package vegan (Oksanen et al., 2020). A stressplot was created to explore

the goodness of fit of original distances and projected distances in the nMDS plot. Permutational Multivariate Analyses of Variance (PERMANOVA) tests were carried out to examine whether the populations based on the Red List categories were significantly different from each other. The assumption of homogeneity of multivariate dispersion was checked for this PERMANOVA as the variation in all of the Red List category populations must be equal.

Phylogenetic comparative analysis was carried out to determine if the phylogenetic relationship of the taxa studied was associated with any correlation between extinction risk and leaf traits. The phylogeny of the taxa was built using the function phylo.maker in the R package V.PhyloMaker (Jin and Qian 2019). The eight taxa in the genus *Callitriche* were excluded from this phylogeny as they are not recognised in the V.PhyloMaker package. The function ggtree in the R package ggtree (Yu et al. 2017) was used to construct a phylogenetic tree of the taxa. Pagel's lambda (λ) (Pagel 1999) was calculated for the Red List categories and each leaf trait to quantify their phylogenetic signal. Pagel's λ assesses the phylogenetic dependence of observed trait data and was chosen as a measure of phylogenetic signal as it is efficient at detecting phylogenetic signal while maintaining a low occurrence of false negatives (type II errors) when trait evolution deviates from random (Münkemüller et al. 2012). A value of Pagel's λ=1 indicates that there is a strong phylogenetic signal between species based on a given trait, while λ=0 indicates that the trait has evolved independently of phylogeny and close relatives are not more likely to share the given trait than distantly related species. Pagel's λ was measured using the function phylosig in the R package phytools (Revell 2012).

Fritz and Purvis' D (Fritz and Purvis 2010) statistic was also used to test for a phylogenetic signal of whether the extinction risk of the Irish flora is correlated with phylogeny. The taxa were placed into two extinction risk categories, Threatened (including RE, CR, EN, VU, and NT taxa) and Not Threatened to create a binary trait for extinction risk. The estimated D value can range from less than 0 to greater than 1. A binary trait that is distributed randomly with respect to phylogeny has a D value of 1, while if the binary trait is distributed as expected under a Brownian model of evolution the D value will be 0. Fritz and Purvis' D was measured using the function phylo.d in the R package caper (Orme et al., 2013).

To assess whether there was evidence that taxa with a similar extinction risk were clustered at the tips of the phylogenetic tree or throughout the tree, two community phylogenetic metrics were used; mean nearest taxon distance (MNTD) and mean pairwise distance (MPD), respectively (Tucker et al. 2017). If the standardised versions of these metrics, nearest taxon index (NTI) and net relatedness index (NRI) respectively, exceeded the threshold standard deviation value of 1.96 then this would provide evidence of phylogenetic clustering. A standard deviation value below −1.96 would indicate phylogenetic overdispersion, implying a more even distribution of extinction risk across the phylogenetic tree than would be expected by chance. The standardised versions of these metrics were measured using the functions NTI.p and NRI.p in the R package iCAMP (Ning et al. 2020). These metrics were calculated when the taxa were divided into six Red List categories, and also when the taxa were grouped into two categories, Threatened and Not Threatened. This approach was adopted to test for phylogenetic clustering at both a fine and broader scale of extinction risk.

A phylogenetic PCA was carried out using the function phyl.pca (Revell 2009) in the R package phytools (Revell 2012) to explain the variability between leaf traits and extinction risk while accounting for the association with phylogeny. Continuous variables that did not conform to normality were transformed by log to the base 10, while non-continuous vari-

Table 4 Results of Pearson's Chi-squared and ANOVA tests of leaf traits and Red List categories

Leaf Trait	Statistical Test	*Chi-square*	*F*	*P*
Compound	Pearson's Chi-squared	8.479		0.561
Lobed	Pearson's Chi-squared	13.388		0.197
Teeth	Pearson's Chi-squared	10.244		0.068
Regularity of Teeth	Pearson's Chi-squared	20.07		**0.031***
Closeness of Teeth	Pearson's Chi-squared	13.089		0.202
Apex Form	Pearson's Chi-squared	14.72		0.149
Base Form	Pearson's Chi-squared	12.607		0.604
Shape	Pearson's Chi-squared	18.401		0.062
Log(area)	one-way ANOVA		0.916	0.47
Log(perimeter)	one-way ANOVA		1.001	0.416
Shape Factor	one-way ANOVA		0.988	0.424
Log(length)	one-way ANOVA		1.166	0.324
Log(width)	one-way ANOVA		0.214	0.956
Log(length-to-Width) Ratio	one-way ANOVA		0.431	0.827

*Significant at $P \leq 0.05$

ables were untransformed. Due to the interdependency and interrelationship of the leaf traits studied, a PCA was a suitable choice of multivariate statistic (Abdi and Williams 2010). Phylogenetic principal components provide estimates of the eigen structure that have lower variance relative to nonphylogenetic procedures, reducing type I errors (false positives) relative to a regular PCA (Revell 2009).

Results

Correlation between leaf traits and extinction risk

Stacked bar charts and boxplots of individual leaf traits displayed no obvious patterns between the variables and the Red List categories (Figs. S1-S14 in Supplementary Material 1). When analysing the relationship between individual leaf traits and extinction risk, the ANOVA tests indicated that the none of the leaf traits were correlated with the extinction risk of the Irish flora as all p-values were greater than 0.05 (Table4). Similarly, all of the p-values for the Chi-squared tests were greater than 0.05, except for the trait 'Regularity of Teeth' ($P = 0.031$) (Table4). To further investigate the significant result for 'Regularity of Teeth', a multiple comparisons test was carried out. The 95% confidence level between the Critically Endangered-Vulnerable, Critically Endangered-Near Threatened and Critically Endangered-Least Concern pairs did not contain 0 in their confidence intervals (Table5; Figs S15-S17 in Supplementary Material 1), suggesting that these pairs are significantly different from each other. The sub-categories within the trait 'Regularity of Teeth' that were responsible for the significant result from the Chi-squared test for this trait were investigated through post-hoc analysis on the residuals of the Pearson's Chi-squared test. This analysis, which subsets each possible paired comparison, indicated that the taxa with irregularly spaced teeth in the Critically Endangered category were significantly different ($P = 0.042$) (Table6) from the sub-categories of this trait in the other Red List categories, supporting the results from the multiple comparisons test. Tables of observed and expected values for the

Table 5 Upper and lower bounds of the 95% confidence intervals for the difference in proportions of 'Regularity of Teeth' for each Red List category pair

Red List category pairs	Regularly spaced–Irregularly spaced teeth		Regularly spaced–no teeth		Irregularly spaced–no teeth	
	lower	upper	lower	upper	lower	upper
RE-CR	-0.4709	0.6751	-0.3539	0.1686	-0.5456	0.0045
RE-EN	-0.6411	0.5334	-0.3010	0.1530	-0.3518	0.1428
RE-VU	-0.2196	0.8229	-0.0915	0.2904	-0.2317	0.1689
RE-NT	-0.6988	0.3563	-0.1659	0.1610	-0.1233	0.2410
RE-LC	-0.5850	0.2745	-0.1979	0.0751	-0.1495	0.1774
CR-EN	-0.2406	0.5306	-0.2633	0.2290	-0.4268	0.1027
CR-VU	**0.0576***	**0.7219***	-0.2017	0.2243	**-0.5133***	**-0.0581***
CR-NT	-0.0675	0.5955	-0.2720	0.1077	**-0.5222***	**-0.0892***
CR-LC	**0.0448***	**0.5171***	-0.1863	0.1598	**-0.4582***	**-0.0439***
EN-VU	-0.1107	0.6203	-0.1524	0.2094	-0.3195	0.0627
EN-NT	-0.2362	0.4897	-0.2203	0.0935	-0.3280	0.0252
EN-LC	-0.1252	0.4490	-0.1339	0.1466	-0.2636	0.0637
VU-NT	-0.2000	0.4501	-0.0343	0.2122	-0.0905	0.1427
VU-LC	-0.1739	0.3110	-0.0889	0.1242	-0.1160	0.0759
NT-LC	-0.2825	0.1860	-0.1357	0.0011	-0.1154	0.0255

*95% Confidence intervals that do not contain 0, indicating a significant difference

Table 6 Adjusted p-values from the post-hoc analysis on the residuals of the Pearson's Chi-squared test for the leaf trait 'Regularity of Teeth'

Red List category	Regularly spaced teeth	Irregularly spaced teeth	No teeth
Regionally Extinct	1.000	1.000	1.000
Critically Endangered	1.000	**0.042***	1.000
Endangered	1.000	1.000	1.000
Vulnerable	1.000	1.000	1.000
Near Threatened	0.411	1.000	1.000
Least Concern	0.635	1.000	1.000

*Significant at $P \leq 0.05$

trait 'Regularity of Teeth' further explained the result of this post-hoc analysis by showing that there are more Critically Endangered plants with irregularly spaced teeth than expected if there was no association between leaf teeth regularity and extinction risk (Table S1 in Supplementary Material 1). The relationship between 'Regularity of Teeth' and the year in which each herbarium sample was collected (Table S2 in Supplementary Material 1) was also analysed. Both the ANOVA test ($P = 0.401$) and Eta Squared value ($\eta^2 = 0.00178$) indicate that 'Regularity of Teeth' was not associated with the year in which the herbarium samples were collected.

Another method used to investigate the relationship between leaf traits and extinction risk in the Irish flora was nMDS. A final stress value for the nMDS ordination < 0.20 can be interpreted with confidence (Clarke 1993). The nMDS had a final stress value of 0.146, indicating a good fit of original distances and projected distances in the nMDS plot. This was further supported by the linear increase of data points in the stressplot (Fig. S18 in Supple-
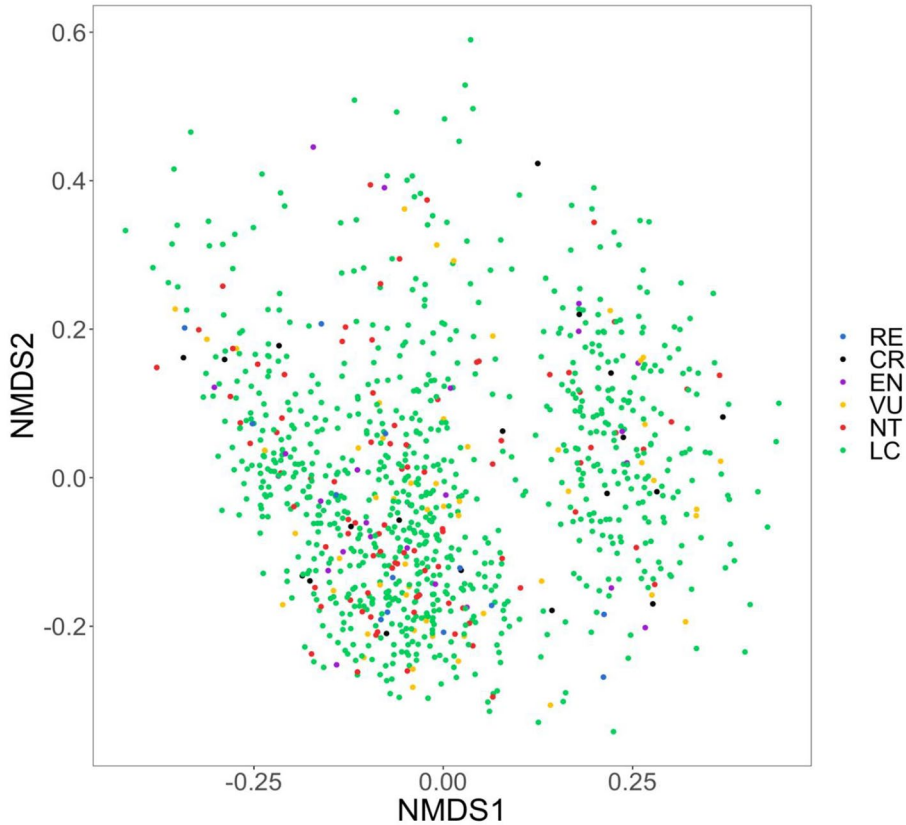
Fig.1 nMDS ordination of the relationship between leaf traits and extinction risk in the Irish flora, with the data clustered into six Red List categories

mentary Material 1). No discernible pattern can be seen in the nMDS plot (Fig.1), suggesting that the leaf traits analysed are not correlated with the extinction risk of the Irish flora. The PERMANOVA test indicated that the six populations based on the Red List categories were not significantly different from each other ($P=0.065$) and the test for homogeneity of multivariate dispersion signified that the variation in all six Red List category populations was equal ($P=0.74$).

Phylogenetic comparative analysis

The phylogenetic tree (Fig.2) and phylogenetic signals indicated a possible relationship between phylogeny and Red List categories. The results of Fritz and Purvis' D (D=0.851, $P=0$ for both Brownian and random phylogenetic structure) indicated that the phylogeny differs significantly from that expected under a Brownian model of evolution, but also differs significantly from that expected under a random phylogenetic structure. The results of Fritz and Purvis' D therefore suggests the presence of a phylogenetic signal within the trait
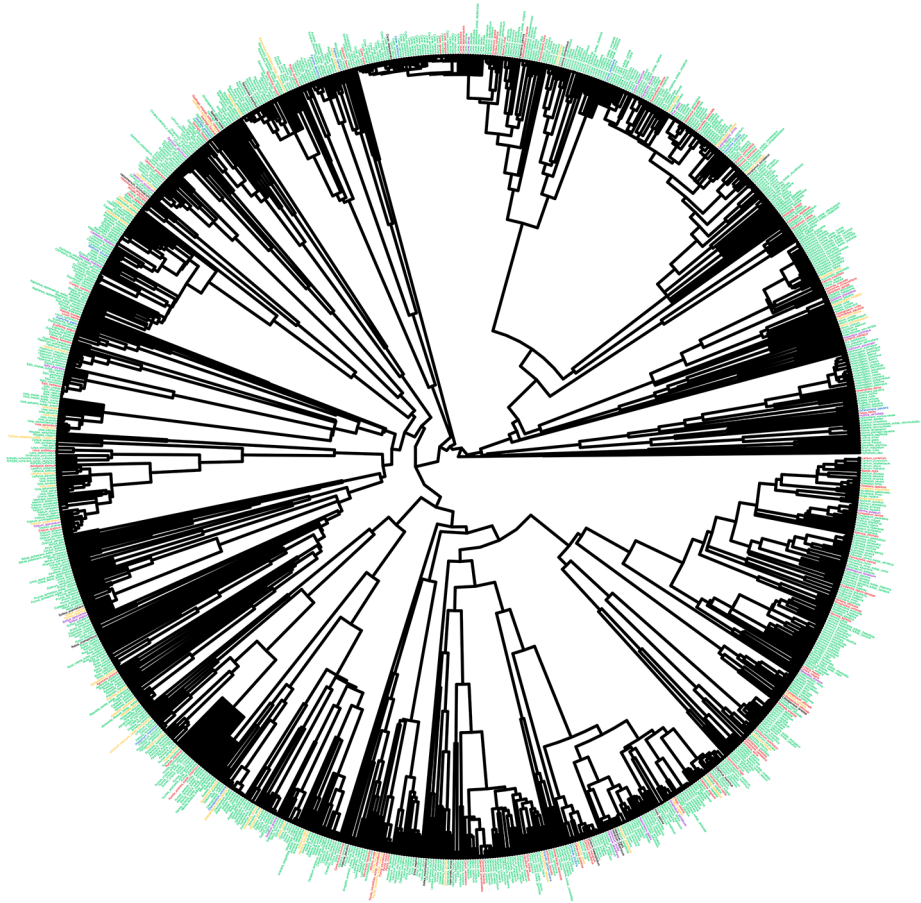
Fig.2 Phylogenetic tree of the taxa of the Irish flora included in this study. The taxa are colour coded based on their Red List category. Blue=Regionally Extinct; Black=Critically Endangered; Purple=Endangered; Yellow=Vulnerable; Near Threatened=Red; and Green=Least Concern

extinction risk, an outcome that is reflected in the slight clustering of Red List categories at the tips of the phylogenetic tree (Fig.2). That the phylogeny did not conform to a Brownian model of evolution is likely due to extinction risk being a derived attribute based on multiple measured or estimated traits. However, no clear phylogenetic signal was detected for extinction risk using Pagel's λ (λ=0.0000661, P=1) (Table7). Pagel's λ indicated that many of the leaf traits analysed are largely explained by their phylogenetic relationships, for example whether a leaf is compound (λ=0.944) and the shape of the base of the leaf (λ=0.871); while other leaf traits such as the leaf shape (λ=0.405) are more independent of phylogenetic relationships (Table7).

Neither the NTI nor the NRI exceeded the 1.96 threshold for significance (0.827 and 0.214 respectively) when the taxa were divided into six extinction risk categories. When the calculation was repeated with the taxa were divided into two categories the NTI rose to 1.234 and the NRI fell to -0.847, again not exceeding the threshold for significance.

Table 7 Results of the phylogenetic signal Pagel's λ for the Red List categories and leaf traits

Variable	λ	Log-likelihood	Log-likelihood (for λ=0)	P-value
Red List category	0.0000661	-1431.64	-0.00802	1
Log(area)	0.855	-1012.93	449.868	<0.001
Log(perimeter)	0.840	-371.464	616.744	<0.001
Log(length)	0.849	-329.112	736.61	<0.001
Log(width)	0.821	-555.754	582.058	<0.001
Log(length-to-width ratio)	0.749	-347.157	1179.85	<0.001
Shape Factor	0.810	301.364	784.009	<0.001
Compound	0.944	-178.518	1137.13	<0.001
Lobed	0.770	-544.993	579.364	<0.001
Apex Form	0.528	-548.608	174.499	<0.001
Base Form	0.871	-1110.69	615.81	<0.001
Teeth	0.816	-331.85	576.393	<0.001
Closeness of Teeth	0.762	-864.366	417.26	<0.001
Regularity of Teeth	0.748	-896.187	416.102	<0.001
Shape	0.405	-740.441	287.327	<0.001
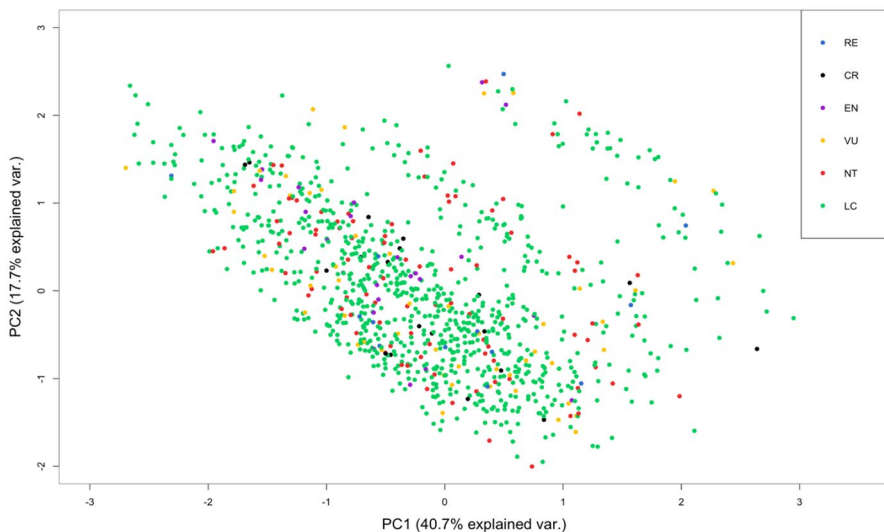
Fig. 3 Phylogenetic PCA plot of 14 continuous and categorical leaf trait variables of the Irish flora, clustered into six Red List categories. This PCA plot explains the variability between leaf traits and extinction risk while accounting for the association with phylogeny

Although these values did not exceed the threshold for significance, they suggest weak clustering within or between closely related taxa, but a lack of clustering across the phylogenetic tree.

Diagnostic tests for normality indicated that all of the continuous variables, except for shape factor, should be transformed for the PCA. The phylogenetic PCA (Fig.3) did not indicate a relationship between phylogeny and extinction risk in the Irish flora as it dis-

played no pattern between the leaf traits and Red List categories. In the phylogenetic PCA axis 1 explained 40.7% of variance and axis 2 17.7% of variance. The variability in axis 1 is largely explained by leaf lobation and area (principal component scores of 0.649 and 0.474 respectively) with taxa possessing lobed leaves and a large leaf area grouped to the right of this axis, while the variability in axis 2 is also largely explained by leaf lobation and area (principal component scores of 0.547 and −0.493 respectively) with taxa possessing lobed leaves grouped at the higher end of this axis and taxa with a large leaf area grouped at the lower end of this axis.

Discussion

Relationship between leaf traits and extinction risk

This study has demonstrated that the leaf traits analysed are not strongly correlated with extinction risk in the Irish flora and that leaf traits are not a good predictor of extinction risk for this flora. The nMDS (Fig.1) plot lacks evidence of a pattern linking the leaf traits of the Irish flora with their extinction risk based on Red List assessments. However, ANOVA and Chi-squared tests detected a level of significance for one leaf trait, 'Regularity of Teeth' (Table4). Post-hoc analysis on the residuals of the Pearson's Chi-squared test indicated that the taxa with irregularly spaced teeth in the Critically Endangered category were significantly different from the sub-categories of this trait in the other Red List categories (Table6). Significant differences were detected for 'Regularity of Teeth' between the Critically Endangered taxa and the taxa in the three categories least at risk of extinction (Vulnerable, Near Threatened and Least Concern) (Table5), suggesting that this leaf trait may be correlated with extinction risk in the Irish flora, although the closely related leaf traits 'Teeth' and 'Closeness of Teeth' had a non-significant relationship with extinction risk. When the Irish flora is grouped into two extinction risk categories (Threatened and Not Threatened) Chi-squared tests did not detect a statistically significant association between 'Regularity of Teeth' and extinction risk ($P=0.3723$), suggesting that more fine-scaled difference is detected by analysing the data at the level of six extinction risk categories.

Impact of drivers of extinction on leaf teeth

The correlation between leaf teeth and extinction risk may be due to the relationship between leaf teeth and temperature. Leaf teeth can increase rates of sap flow early in the growing season when temperatures are limiting, thereby increasing carbon assimilation, a potential adaptation to cold climates (Royer and Wilf 2006). They are able to do this by increasing transpiration and photosynthate production at the beginning of the growing season, thereby ensuring maximum carbon uptake when temperatures are limiting but other necessary resources such as nutrients and water are available. In warmer climates the excess water cost of leaf teeth appears to outweigh the benefits of extending the growing season (Royer and Wilf 2006). Leaf teeth can also prevent freeze-thaw embolisms from occurring by exuding droplets of xylem sap from the leaf teeth (Feild et al. 2005), another benefit of leaf teeth in cold climates. Rapid changes in leaf teeth traits have been recorded in response to changes

in temperature (Royer et al. 2009b; McKee et al. 2019), although only when the magnitude of temperature change was much larger than has been witnessed in Ireland in recent times.

Other ecological controls such as habitat can impact the abundance of leaf teeth through water availability (Royer et al. 2009a). Riparian habitats have been observed to have a higher percentage of species with leaf teeth than non-riparian habitats (Royer et al. 2009a). Therefore, damage to riparian habitats in Ireland could contribute to the relationship found between leaf teeth and extinction risk in the Irish flora. Recent assessment found the conservation status of riparian habitats in Ireland to be unfavourable (NPWS, 2019). Numerous pressures and threats were identified as impacting on these habitats; including modification of the hydrological flow and discharge of waste water (NPWS, 2019).

Association of phylogeny with extinction risk

Phylogenetic analysis was used to address the question of whether shared evolutionary history can explain the prevalence of extinction risk across the Irish flora, and its subsequent implications for relating leaf traits to the extinction risk of the Irish flora. The presence of a statistically significant phylogenetic signal in the extinction risk of the Irish flora was not confirmed by Pagel's λ (Table 7), potentially due to the underlying model of evolution of extinction risk being more complex than can be detected by the Brownian model of evolution that Pagel's λ considers. Modelling extinction risk on Brownian motion has previously been assessed and rejected for the Cape flora of South Africa (Davies et al. 2011). Extinction risk appears to be inconsistent with a simple and heritable model of evolution (Davies et al. 2011), possibly by virtue of extinction risk being a derived attribute based on multiple measured or estimated traits. A global analysis of modern plant extinctions also failed to detect any phylogenetic signal within these extinctions (Humphreys et al. 2019).

A weak but statistically significant phylogenetic signal for extinction risk was detected when the taxa were divided into two categories, Threatened and Not Threatened. Fritz and Purvis's D differed significantly from the values expected for a random shuffle, but also from those expected under Brownian motion, indicating a slight tendency for closely related species to be more similar in their extinction risk than would be expected if extinction risk was independent of phylogeny. The phylogenetic signal detected by Fritz and Purvis's D in the distribution of extinction risk across the phylogenetic tree was primarily a result of clustering at the tips of the phylogeny, as measured by NTI. Although a tendency towards clustering of extinction risk was found at the tips of the tree, this was not statistically significant.

At an individual leaf trait level, strong phylogenetic signals were observed (Table 7). A phylogenetic signal at the leaf trait level has previously been shown for leaf traits such as the presence of leaf teeth (Peppe et al. 2011) and leaf vein pattern (Walls 2011). Numerous other studies show a strong relationship between leaf traits and drivers of extinction such as nutrient addition (La Pierre and Smith 2015; Dangremond et al. 2020), pollution (Shafiq et al. 2009; Appalasamy et al. 2017; Dadkhah-Aghdash et al. 2022) and climate (Royer et al. 2005, 2009b; Nicotra et al. 2011; Peppe et al. 2011; Bacon et al. 2016); therefore, it appears that the variability observed in leaf traits is correlated with both drivers of extinction and phylogeny, and there is a trade-off between these two processes.

Comparison with similar studies

To date, few studies have investigated the relationship between leaf traits and extinction risk. One such study analysed the relationship between maximum leaf lamina length and threat status for 8,542 Fabaceae species (Bradshaw et al. 2008). The species were assigned to two categories; 'threatened' which included Critically Endangered, Endangered, Vulnerable and Near Threatened species, while the remainder of species were classified as 'not threatened'. Bradshaw et al. (2008) found a trend of increasing maximum leaf lamina length with the proportion of threatened species; however, leaf length was only one of thirteen ecological and life-history traits investigated in the study. Within the overall framework of the study, leaf length was not considered a significant predictor of threatened status (Bradshaw et al. 2008). A similar result was found in the current study as the ANOVA test for leaf length ($P=0.324$) (Table 4) indicates no correlation between leaf length and extinction risk.

Other leaf traits such as LMA and leaf water content (LWC) have also been investigated to analyse their correlation with extinction risk. A study in New Zealand analysed 116 species inhabiting a lakeshore ecosystem and classified their threat status based on the New Zealand Threat Classification Framework (Monks and Burrows 2014). Again, all of the species were assigned to two categories; either 'threatened' or 'non-threatened'. Neither LMA nor LWC were significantly associated with threat status (Monks and Burrows 2014). LMA and LWC were not included in the current study as these traits could not be directly measured from online herbaria images. However, leaf area, which is one of the two components of LMA, was measured in the current study and was not found to be a significantly associated with extinction risk ($P=0.47$) (Table 4).

Another study investigated the relationship between specific leaf area (SLA) and threat status at various spatial scales in Germany (Lauterbach et al. 2013). The threat status of 28 perennial species was assessed on a national, regional and local scale. The threat status was calculated using different metrics at each spatial scale: the Ellenberg value 'tendency of change' for the national scale; Red List assessments for the regional scale; and a comparison of historic and recent vegetation records for the local scale. On all three scales, SLA was associated with the degree of threat, with species possessing higher SLA values being less threatened (Lauterbach et al. 2013). SLA was not included in the current study as leaf weight could not be measured. The findings of Lauterbach et al. (2013) were in contrast to those of Monks and Burrows (2014) as SLA is the inverse of LMA. Monks and Burrows (2014) found that threatened species had a lower mean LMA than non-threatened species, although the relationship between threat status and LMA was not statistically significant, while Lauterbach et al. (2013) found that threatened species had higher LMA values than less threatened species. This difference may have been due to the methods used to calculate threat status, which differed between studies. Lauterbach et al. (2013) used a continuous scale from low degree of endangerment to high degree of endangerment; while Monks and Burrows (2014) placed species into two categories, either threatened or non-threatened. Another factor that may have contributed to the contrasting results is the choice of species used in the studies. Monks and Burrows (2014) focused on lakeshore species where all of the threatened species were either semi-aquatic or aquatic, while Lauterbach et al. (2013) used native perennial species from dry grasslands. The small number of species ($N=28$) studied by Lauterbach et al. (2013) may also have contributed to their significant results. To fully understand the association between leaf traits and extinction risk it is necessary to

examine a range of leaf traits in relation to extinction risk across both functional and phylogenetic groups.

Climate change in Ireland

The effects of climate change can already be seen in Ireland. MAT across the period from 1900 to 2012 increased by 0.8°C, with this trend predicted to continue with an increase in MAT of 1.5°C by 2041–2060 compared to the average temperature over the period 1981–2000 (Gleeson et al. 2013). Precipitation levels have also changed in recent years with an increase in mean annual precipitation of 5% for the period 1981–2010 compared to 1961–1990 (Gleeson et al. 2013). However, by 2041–2060 annual precipitation levels are predicted to decrease, with summer months potentially seeing a decrease in precipitation by up to 20% compared to average precipitation over the period 1981–2000 (Gleeson et al. 2013). Such climate change has been observed to influence both the phenology (Menzel et al. 2006) and distribution (Lenoir et al. 2008) of plants on a European scale. It is possible that climate change in Ireland in recent years has not been of a sufficiently large magnitude to correlate with extinction risk. For example, the observed temperature increase of 0.8°C between 1900 and 2012 is considerably lower than the 4°C temperature increase across the Triassic–Jurassic boundary that influenced the extinction of fossil floras in East Greenland (McElwain et al. 1999, 2007; Soh et al. 2017). Palaeobotanical studies also measure changes in climate and extinction over a much longer time period (e.g. 2.2 Myr; Blonder et al. 2014). Therefore, although leaf traits may respond to future climate change in Ireland and become more associated with extinction risk in the Irish flora, currently there is no strong evidence for climate-driven trait change in the Irish flora, or trait change by any other driver of plant extinction, and the leaf traits included in this study are not associated with extinction risk.

There is evidence that anthropogenic drivers such as habitat loss and invasive species exist in Ireland, and these may be associated with plant extinction risk in Ireland. The most recent EU habitats directive assessment for Ireland found that the overall status of 39% of habitats is bad and the overall status of 46% of habitats is inadequate (NPWS, 2019). Multiple pressures were found to be impacting Ireland's habitats, with over 70% of habitats impacted by agricultural practices, and alien and problematic species impacting 42% of habitats (NPWS, 2019). In Ireland, forest cover is at 11% of the total land area, with the non-native plantation species *Picea sitchensis* accounting for 51.1% of this area (NFI, 2017). Current conservation efforts in Ireland focus on tackling invasive species and habitat loss with €1.5m recently being allocated to clear invasive species from National Parks and €14m has been allocated to restore degraded raised bogs in the midlands (Department of Housing, Local Government and Heritage, 2020).

While this study analysed a range of leaf traits, several other leaf traits that have important associations with extinction risk could not be measured via online herbaria images. These include leaf mass (Qaderi et al. 2006) and stomatal traits (Woodward 1987) that have previously been shown to vary with climate. Whether these traits would influence extinction risk remains untested. Similarly to the study by Woodward (1987), the current study demonstrated the importance of well-maintained, digitised herbarium records (Paton et al. 2020). Future research on this topic could look to increase the number of leaf traits examined, control for other factors known to be associated with extinction risk and/or broaden the geo-

graphic scope of the present study. Ireland possesses a relatively depauperate flora; therefore, widening this research to more biodiverse regions (e.g. tropical ecosystems) would allow a greater diversity of plants, and therefore leaf traits, to be analysed. Alternatively, studying a flora where a significant proportion of its species are living at the edge of their climatic envelope (e.g. boreal forests) could produce interesting results.

Conclusion

This study suggests that the extinction risk of the Irish flora is not related to the leaf traits studied and that leaf traits are not a strong predictor of extinction risk in the Irish flora. Phylogenetic analysis indicated that shared evolutionary history was correlated with the prevalence of extinction risk across the Irish flora and demonstrated strong phylogenetic signals for individual leaf traits. These findings are an important first step in understanding how leaf traits may correlate with plant extinction risk in the Irish flora.

Statements and declarations

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