

## LETTER

### Testing Darwin's naturalization hypothesis in the Azores

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#### Abstract

Invasive species are a threat for ecosystems worldwide, especially oceanic islands. Predicting the invasive potential of introduced species remains difficult, and only a few studies have found traits correlated to invasiveness. We produced a molecular phylogenetic dataset and an ecological trait database for the entire Azorean flora and find that the phylogenetic nearest neighbour distance (PNND), a measure of evolutionary relatedness, is significantly correlated with invasiveness. We show that introduced plant species are more likely to become invasive in the absence of closely related species in the native flora of the Azores, verifying Darwin's 'naturalization hypothesis'. In addition, we find that some ecological traits (especially life form and seed size) also have predictive power on invasive success in the Azores. Therefore, we suggest a combination of PNND with ecological trait values as a universal predictor of invasiveness that takes into account characteristics of both introduced species and receiving ecosystem.

#### Keywords

Azorean flora, competition, enemy release, invasive species, molecular phylogeny, phylogenetic nearest neighbour distance, predictors of invasiveness, scale effect.

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#### INTRODUCTION

Invasive species are a major threat to ecosystems worldwide, but particularly on islands where exotic plants frequently form dominant stands, and can alter the three-dimensional structure of native forests or replace them entirely (Asner *et al.* 2008). Even though there is no documented species extinction linked to plant invasion events (Gurevitch & Padilla 2004), invasives have contributed to the decline of endemic species across the globe (Mooney & Cleland 2001). The eradication of invasive species has proven an extremely difficult task (Mooney & Cleland 2001), and therefore, much attention has been paid on predicting the invasive potential of species and banning their import when in doubt. So far, these assessments have been based on a range of ecological and morphological traits with limited predictive power; for example, one of the most reliable criteria is 'invasiveness elsewhere', which has high predictive power in some regions but is not very useful for species that have not been introduced to many places (Pheloung *et al.* 1999; Kolar & Lodge 2001; Pyšek & Richardson 2007). Meta-analyses of studies comparing native vs. invasive plant traits, and non-invasive vs. invasive species traits, found either no significant difference (Hawkes 2007; Hayes & Barry 2008), or differences in performance-related traits like leaf-area allocation, growth rate, size and fitness (Van Kleunen *et al.* 2010). While these results are promising, they are all based on a relatively limited number of comparisons and so far, no study focused on an entire flora.

The invasion process starts when propagules or individuals are transferred to regions outside a taxon's current distribution range (Mooney & Cleland 2001). It can be divided into four stages:

introduction (=transport to a new area), establishment (=germination and/or survival in the new place), naturalization (=self-sustaining population) and finally invasion (=spread into natural habitats). Usually, only 10–15% of all naturalized species become invasive (Kueffer *et al.* 2010), and it is still very difficult to predict individual success because of the influence of factors like introduction frequency (Lockwood *et al.* 2005), residence time (Kueffer *et al.* 2010), characteristics of the invaded ecosystems like resource availability (Blumenthal *et al.* 2009) or new mutualisms acquired in the introduced range (Richardson *et al.* 2000a).

Known as 'Darwin's naturalization hypothesis', one of the earliest theories in the field proposed that introduced plant species are more likely to become invasive in the absence of close relatives in the native flora (Darwin 1859). Darwin's idea was that closely related species are more likely to have similar ecological niches due to common ancestry, and therefore would be competing for the same resources. By contrast, 'unrelated' species would be relatively free from competition, hence they could more easily invade the new environment. To test this hypothesis, adequate phylogenetic information is crucial. Evaluating Darwin's idea using only taxonomic classifications is problematic: genera are often artificial units and several molecular phylogenetic studies have shown that they are rarely monophyletic (natural) groups, even in economically important and well-studied plant families like the grasses (Aliscioni *et al.* 2003; Sungkaew *et al.* 2009), gourds (Schaefer & Renner 2011) and umbellifers (Downie *et al.* 2000). Even if genera are monophyletic, their circumscription often varies considerably between specialists, the 'lumper/splitter' problem (Humphreys & Linder 2009). To quantify evolutionary relatedness based on

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taxonomy alone therefore introduces uncertainties and indeed numerous attempts to test Darwin's hypothesis in this way (starting with a pioneering study using the naturalized European grasses of California; Rejmánek 1996) have provided contradicting and confusing results, so that Diez *et al.* (2008) even renamed this hypothesis 'Darwin's naturalization conundrum'. Lambdon & Hulme (2006) and Diez *et al.* (2009) discuss a number of potential biases and problems in testing Darwin's hypothesis, although surprisingly they did not mention the need for precise relatedness data, which can be obtained using molecular data. So far, the only study that tested Darwin's idea using comprehensive molecular phylogenetic data was in Californian grasses, but only at genus level and following a super-tree approach with uniform branch lengths (Strauss *et al.* 2006). These authors found that introduced grasses are more likely to become invasive if they are more distant to the entire native grass community (not to the single closest native relative), thus confirming in part Darwin's hypothesis within a family. As far as we know, no study has used a species-level phylogeny for an entire flora, which is required to evaluate the general importance of Darwin's hypothesis.

Another problem in recent studies is that they focused on different stages of the process, some of which are less relevant for Darwin's hypothesis. Whereas Darwin was writing about competition and thus had most likely the final stage of invasion in mind, several studies actually tested the earlier 'establishment' and 'naturalization' steps. Indeed, the naming of Darwin's hypothesis is confusing and a more appropriate term may be 'Darwin's invasion hypothesis'. The difference is that 'naturalization' (=establishing self-sustaining populations outside a taxon's range) is likely to depend on abiotic (especially climatic and edaphic) conditions (e.g. Diez *et al.* 2009), whereas 'invasion' (=spread into natural habitats) is likely to depend on the species composition of local ecosystems.

Among the more recent studies, Daehler (2001) rejected Darwin's theory based on a taxonomic analysis of the flora of Hawaii. In his analysis, he distinguishes between 'localized' and 'well-naturalized' species and counts only the latter. This approach has limits because it is likely to mix common naturalized non-invasive species and invasive species that are restricted to localized natural habitats. In another study, Duncan & Williams (2002) rejected Darwin's theory based on a taxonomic analysis of the New Zealand flora. They addressed explicitly only the naturalization step using a database with records for all plant species ever introduced to New Zealand. They found that genera with native congeners are more likely to establish self-sustaining populations, which probably reflects similar climatic preferences (see also Diez *et al.* 2009) but does not address directly the problem discussed by Darwin (1859). Diez *et al.* (2008), in a re-analysis of the New Zealand flora, confirm Duncan & Williams (2002) but find that abundance data either confirm or contradict Darwin's idea of competition between congeners depending on the spatial scale considered. Subsequently, Proches *et al.* (2008) reviewed the subject and emphasized that scale is an important factor, and that competition as suggested by Darwin is likely to have the strongest effect at smaller scales.

We tested Darwin's naturalization hypothesis in the Azores using comprehensive phylogenetic data at various scales. The Azores form an isolated volcanic archipelago on the middle-Atlantic ridge in the Northern Atlantic and comprise nine islands with a total area of 2346 km<sup>2</sup>, a maximum altitude of 2351 m and a maximum age of 5–8 million years (see Schaefer 2003 for maps and extensive literature). The current flora of the Azores consists of *c.* 80% of

introduced species, and of the remaining native species, *c.* 30% are endemic (Schaefer 2003; Silva & Smith 2004; Carine & Schaefer 2010). Examining > 10 000 herbarium specimens and all available literature on the plants of the Azores, we recorded the changes in the introduced Azorean flora since 1494. Based on the geological age of the existing islands and the number of native species, we estimated a natural colonization rate of one angiosperm lineage per 40 000 years since these islands first emerged from the sea (Schaefer 2003) – or even lower rates if there have been islands that are now eroded. In contrast, the naturalization rate of exotic plant species during the past two centuries is more than 100 000 times faster than the natural process and has reached almost three species per year (Schaefer 2003). Because of the lack of strict quarantine regulations and lists of imported species in the Azores (e.g. in contrast to New Zealand or Australia), we cannot assess the number of plants that have been introduced but failed to establish self-sustaining populations in the islands. Therefore, we focus on the final stage of the invasive process: the spread of naturalized species from man-made into natural habitats.

To test Darwin's hypothesis, we reconstructed the phylogeny of the entire Azorean angiosperm flora based on gene sequence data. We could sum branch lengths to measure evolutionary relatedness at various scales, and find that it is correlated to the invasive potential of a species.

## MATERIAL AND METHODS

### Status groups

Classifying status groups is a controversial field (e.g. Pyšek *et al.* 2004). Here, we first distinguish 'native' from 'introduced' species: the former includes all lineages that colonized the Azores before the arrival of the first human settlers in the 15th century. We used all available evidence, including fossil data, sediment core studies, historical accounts by the first inhabitants and visiting botanists, as well as herbarium collections; these data are detailed for each species in Schaefer (2003). Then, we divided the introduced species into 'non-invasive' vs. 'invasive'. This classification is certainly a difficult one (e.g. Richardson *et al.* 2000b). The key question is if 'invasive' status should be based on the 'impact criterion' or rather on a simple 'geographic criterion'. Both approaches are widely used and, depending on the goal of the study, both have their merits. Impact is often difficult to quantify (except in agricultural systems) and can be measured in various ways. However, the impact of agricultural weeds is mainly of interest to farmers, but not so much for ecologists and conservation biologists. Here, we follow Daehler (1998) and call 'invasive' what he classified as 'natural area invaders' in contrast to 'agricultural weeds – undesirable plants that colonize agricultural lands'. This is particularly useful in the Azores, where there is a clear distinction between man-made, frequently disturbed habitats (urban areas, agricultural ground and tree plantations, roadsides, etc.) and natural habitats (e.g. salt marshes, *Festuca* grassland, Laurel and Juniper forests, etc.) with much lower disturbance frequency (e.g. landslides, windbreakage).

The remaining species, called 'non-invasive' hereafter, have no self-sustaining populations in natural areas and are restricted to disturbed grounds. These latter species did not become invasive in historical times (15th century until today) and they would probably not survive in the islands without the constant disturbance in the agricultural areas. In a longer perspective, some of them might become invasive in

response to climate change or the evolution of new genotypes whereas others might go extinct. Our study does not account for these future changes, as they are difficult to model without more detailed ecological datasets.

### Sampling and species inventories

During 2008, we collected leaf samples of all 794 species of flowering plants known to have self-sustaining populations in the archipelago (i.e. the angiosperm flora of the archipelago) plus two outgroup gymnosperms (Appendix S1). Plant species that only exist in cultivation in the Azores or as casuals without self-sustaining populations anywhere in the islands are by definition not part of a region's flora and were not included in our analyses.

We assembled a trait database for all 794 angiosperm species. In total, we measured seven morphological or ecological characters, including two continuous traits (maximum plant height and seed mass) and five categorical, unordered traits: life form, sexual system (monoecious, dioecious, bisexual), seed number, main dispersal mode and main pollination system (Appendix S2).

We performed comprehensive species inventories (1) for each of the nine islands (surveyed 1998–2010), (2) for each  $1 \times 1 \text{ km}^2$  section of three islands with a total area of  $416 \text{ km}^2$  (Flores, Faial, Santa Maria) based on the UTM grid (=520 UTM squares, surveyed 1998–2010) and (3) for 67  $10 \times 10 \text{ m}$  plots of grassland or forest vegetation on three islands: Flores, Faial and Santa Maria (during January 2010). Two of the 520  $1 \times 1 \text{ km}$  squares in the central highland of Flores did not contain non-invasive species and were therefore excluded from the analysis [no comparison of phylogenetic nearest neighbour distance (PNND) values for invasives vs. non-invasives possible]. The  $100 \text{ m}^2$  plots were arranged in *c.* 1 km linear distance along an east–west transect on each of the islands. Of the 67  $100 \text{ m}^2$  plots (45 grassland, 22 forest), 12 did not include non-invasive species and were therefore excluded from the analysis. Complete species lists for all plots are available in Appendix S3.

### DNA sequencing

We used two plastid markers that are conserved enough to be aligned across an entire flora: the maturase K gene (*matK*) and the large subunit of the ribulose 1,5-bisphosphate carboxylase/oxygenase gene (*rbcL*). For *matK*, we designed a pair of new universal primers amplifying the barcoding region of the gene: *matK*-F-uni: 5'-AAT TTA CGA TCH ATT CAT TCM ATW TTT CC-3' and *matK*-R-uni: 5'-AGT TYT ARC ACA AGA AAG TCG AAR TAT ATA-3'. To amplify *rbcL*, we used the primers *rbcL*-1F, *rbcL*-1460R and the internal primers *rbcL*-600F and *rbcL*-724R (Olmstead *et al.* 1992).

### Phylogenetic analyses

Sequences were edited with Sequencher (4.9; Gene Codes, Ann Arbor, MI, USA) and Geneious pro 5.0.4 (Drummond *et al.* 2010), and aligned using MAFFT (Katoh *et al.* 2005).

Maximum likelihood (ML) tree searches were performed using RAxML-HPC2 vs. 7.2.6 (Stamatakis *et al.* 2008). We first analysed each alignment separately. No statistically supported topological conflicts (> 60% likelihood bootstrap support) were detected and we therefore continued the analyses with a combined alignment. Based on the Akaike Information Criterion as implemented in jModeltest

(Posada 2008), we selected the GTR +  $\Gamma$  model (six general time-reversible substitution rates, assuming gamma rate heterogeneity) with model parameters estimated over the duration of specified runs. To detect if the ML analysis had become trapped on a local optimum, we conducted five separate ML searches. The tree with the best likelihood score was then selected as the optimal tree.

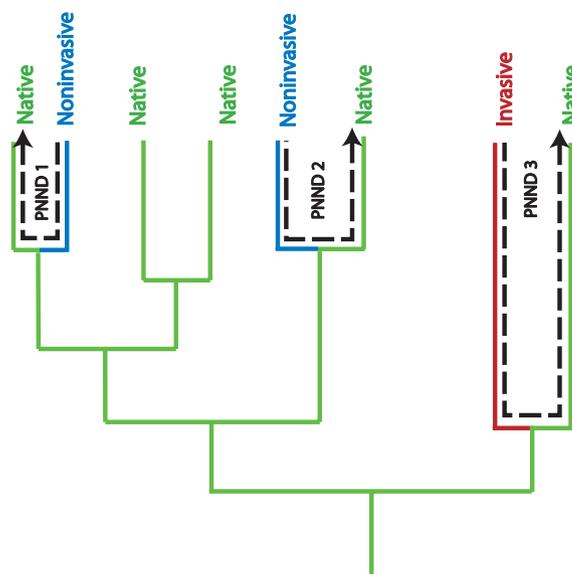
### Molecular dating analyses

To translate genetic distances into absolute times, we used Bayesian time estimation with an uncorrelated-rates model as implemented in BEAST v. 1.5.3 (Drummond & Rambaut 2007) and seven calibration points (Appendix S4). BEAST analyses used the GTR +  $\Gamma$  model with four rate categories. Metropolis coupled Monte Carlo Markov chains were run for 10 million generations, sampling every 1000th generation. Of the 10 001 posterior trees, we left out the first 3000 as burn-in. Convergence was checked using Tracer v. 1.5 (Rambaut & Drummond 2009).

### Relatedness analyses and modelling

We use the ML and BEAST output trees of the Azorean flora to sum branch lengths as a measure of evolutionary relatedness – i.e. the smaller the sum of branch lengths the closer related are the taxa. Specifically, we calculated the 'phylogenetic nearest neighbour distance' between each introduced species (invasive or non-invasive) and the closest relative among the native species (Fig. 1) based on the ML tree (branch length proportional to number of substitutions) or the time-calibrated BEAST consensus tree (branch length proportional to elapsed time). Our metric thus takes into account not only the introduced species but also the receiving environment, which makes it more universal than morphological or ecological traits measured only for the introduced species.

For community-level relatedness comparisons we used Phylocom (Webb *et al.* 2008) and SPACoDi 0.10 (Hardy 2009). In Phylocom, we



**Figure 1** Hypothetical phylogenetic tree showing how to measure phylogenetic nearest neighbour distance (PNND) by summing up the lengths of the branches that connect an introduced species to its closest relative among the native Azorean angiosperms.

computed the mean phylogenetic distance (MPD) between each introduced species (invasive or non-invasive) vs. all natives, or in other words the entire native community (in contrast, above, we only used the distance to the single closest native relative). In SPACoDi, we calculated MPD between species of the three different status groups at different divergence time intervals (i.e. mean divergence time between species having diverged less than a time threshold).

Generalized linear modelling of the probability of invasion success depending on phylogenetic distance and the compiled ecological variables under a binomial error distribution (link = logit) was performed using the R statistical programming language (R Development Core Team 2010). For model averaging, we used the BMA package in R (R Development Core Team 2010). We calculated differences in ecological trait values between an introduced and its closest native relative (Appendix S5) and used these dissimilarities in addition to the absolute ecological trait values (Appendix S2) to test whether evolutionary relatedness alone or in combination with one of these ecological sets can predict invasive success in the Azores. Dissimilarity in ecological traits compared with the closest native relative is needed to test Darwin's competition idea, whereas the absolute character values allow comparison to the entire plant community of the Azores.

## RESULTS

### Classification

In total, 149 species are natives, that is, they arrived in the Azores before the first human settlers via wind, water or bird dispersal. The remaining 645 species have been transported to the islands by settlers and visitors, either accidentally or on purpose, for example, as crops or ornamentals (Schaefer 2003). Out of these 645 species, 56 (from 33 plant families) are natural area invaders following Daehler (1998) ('invasives' hereafter), whereas 589 species are classified as 'non-invasives' following our scheme.

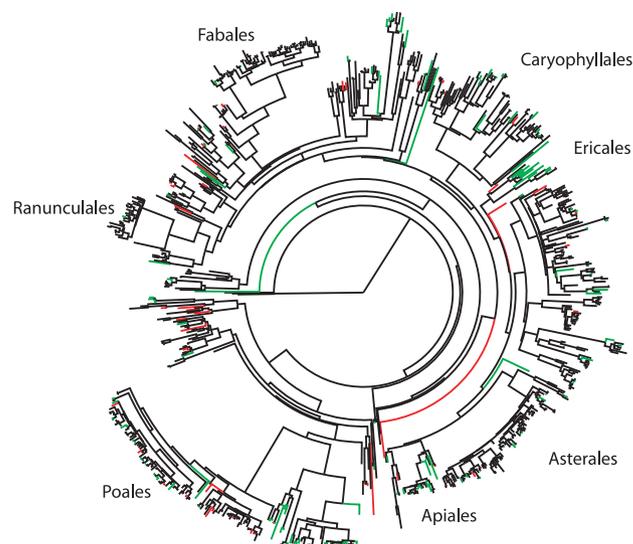
### Phylogenetic and molecular dating analyses

We were able to amplify and sequence *matK* for 93% and *rbcL* for 97% of the 794 species. Sequences generated for this study have been deposited in GenBank under accession numbers FN870382–411, FN908046–75 and HM849734–51178 (Appendix S1).

The combined alignment consists of 2425 aligned nucleotides. Most higher taxa are recovered as monophyletic groups in the tree (Fig. 2; Figure S1) with exceptions only at genus level (e.g. *Radiola* nested in *Linum*, *Lavatera* nested in *Malva*, *Lysimachia* nested in *Anagallis*). Most nodes are statistically supported (likelihood bootstrap  $\geq 60\%$ ) and alternative topologies have similar branch lengths and are thus unlikely to bias our results on PNND. Invasive species are evenly dispersed in the Azorean angiosperm tree (net relatedness index  $-1.84$ ) but show some clustering in relation to the native flora only (Fig. 2).

### Relatedness analyses and modelling of invasiveness

The comparisons of PNND values for invasives, non-invasives and natives were performed with both the ML and the time-calibrated phylogenetic trees. We find that the probability that introduced species become invasive increases significantly with genetic distance to their nearest native relative (as measured by PNND; Fig. 3a).



**Figure 2** Best maximum likelihood phylogram for the 149 native, the 56 invasive and the 589 non-invasive angiosperms of the Azorean flora produced with RAxML-HPC2 vs. 7.2.6 (Stamatakis *et al.* 2008); tip labels not shown (but see Figure S1) and only selected important clades labelled to allow orientation (native species, green; invasive species, red; non-invasive introduced species, black).

A general linear model including PNND alone shows that evolutionary relatedness is significantly correlated with invasiveness (Table 1). As PNND ranges from 1.7 to 270 million years, the probability that introduced species become invasive increases from 0.043 to 0.29 (Fig. 4).

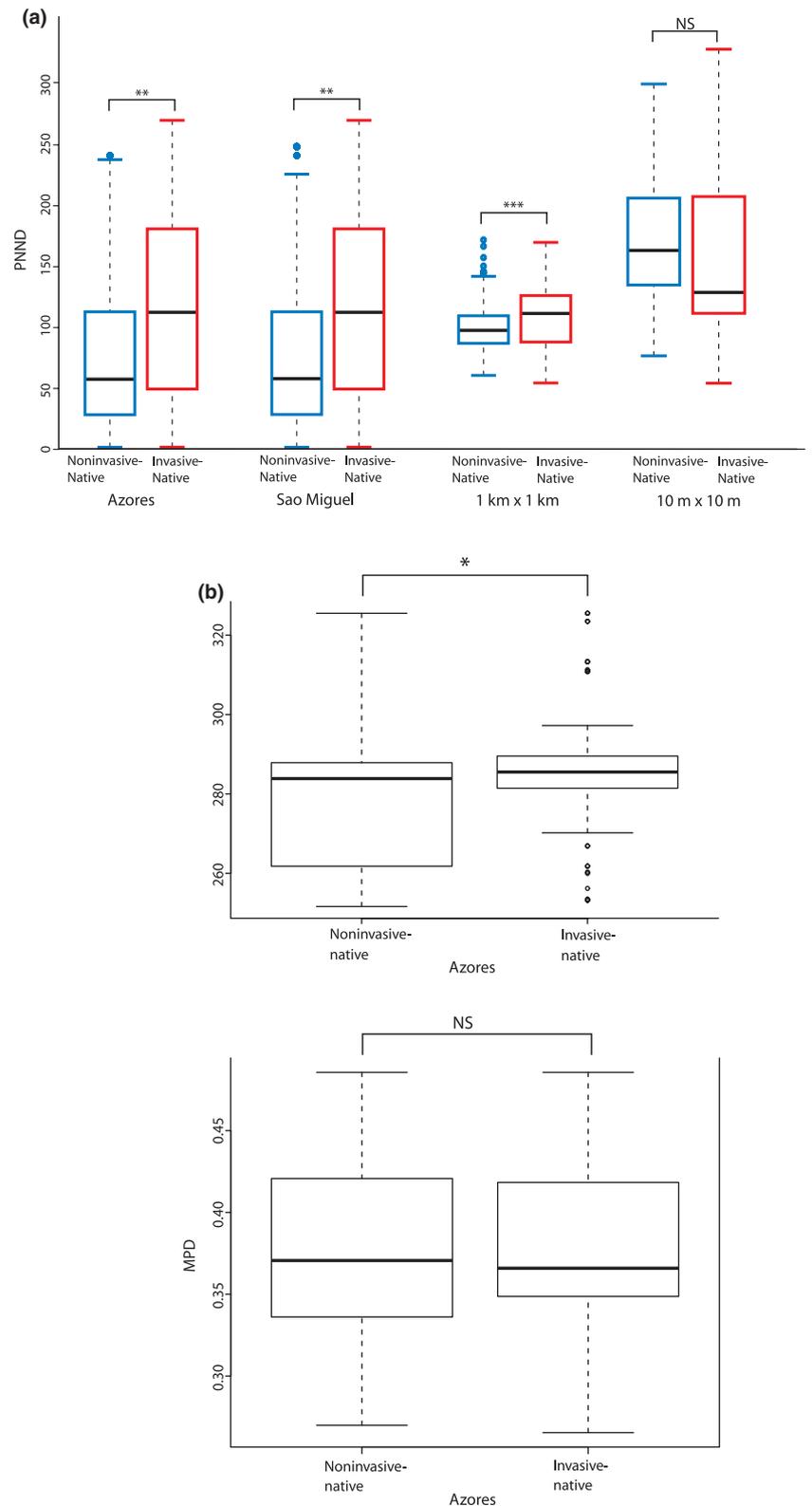
A general linear model including PNND and dissimilarities in ecological traits (between each introduced species and its nearest native relative) shows that only PNND ( $P < 0.0001$ ) and dissimilarity in life form ( $P < 0.01$ ) are significant (Table 2). However, a statistical model including PNND and values of ecological traits shows that values for life form and seed weight both explain more of the variation than PNND (Table 3).

Analyses within islands confirm at the  $\text{km}^2$  scale what we find for the entire archipelago, but the relationship is lost for the  $100 \text{ m}^2$ -plots ( $P = 0.0799$ ; Fig. 3a). Contrary to Strauss *et al.* (2006), we find no difference in PNND at community level (=MPD) (Fig. 3b) and at different divergence time thresholds (Fig. 5).

Regarding the ecological traits, annuals that survive adverse conditions as seeds (therophytes) have a lower probability of becoming invasive than the other common life forms (probability of becoming invasive = 0.018, compared with probabilities  $> 0.1$  for all other life forms with more than 20 representatives). Plants with smaller seeds are more likely to become invasive, but only after taking into account the differences among life forms (Tables 1–3). Other traits like plant height and seed production are uninformative in predicting invasion risks.

## DISCUSSION

Our results confirm Darwin's naturalization hypothesis: introduced plant species are more likely to become invasive in the absence of close relatives in the native flora of the Azores. So, how can we explain this result? Darwin's idea was that closely related species will be competing for the same resources, hence it is less likely that an introduced congeneric will invade the environment already occupied



**Figure 3** (a) Comparison of phylogenetic nearest neighbour distance (PNND) values (in million years, based on the time-calibrated tree) in the Azores archipelago for each non-invasive–native pair ( $n = 589$ ) compared with each invasive–native pair ( $n = 56$ ) ( $P = 0.0008$ ); on a single island, São Miguel, for each non-invasive–native pair ( $n = 411$ ) compared with each invasive–native pair ( $n = 50$ ;  $P = 0.0008$ ); in  $1 \text{ km}^2$  plots on three islands: Flores, Faial and Santa Maria ( $n = 518$ ; comparison of the means for each plot;  $P = 0.0001$ ); and in  $100 \text{ m}^2$  plots ( $n = 55$ ) on three islands, comparison of the means for each plot ( $P = 0.0799$ ). The boxes show the first and third quartiles, the median is indicated by the horizontal line, the range of the data by the vertical dashed line and outliers (more than 1.5 times the interquartile range above/below the first/third quartile) by circles. (b) Mean phylogenetic distance (MPD) between each non-invasive vs. all natives and each invasive vs. all natives based on the ML phylogeny (MPD in substitutions per site) below, and based on the time-calibrated phylogeny (MPD in million years) above. The boxes show the first and third quartiles, the median is indicated by the horizontal line, the range of the data by the vertical dashed line and outliers (more than 1.5 times the interquartile range above/below the first/third quartile) by circles.

by an allied taxon (Darwin 1859). An alternative explanation, the ‘enemy release hypothesis’ proposes that introduced plants lose herbivores and pathogens and thus benefit relative to natives in the same environment (Elton 1958; Keane & Crawley 2002; Mitchell & Power 2003). Exotic plant species will benefit from both the absence

of specialist enemies and the lower relative impact by generalist enemies (Keane & Crawley 2002), except if enemies were co-introduced and/or if enemies of related native plants efficiently regulate the populations of the exotic species. Co-introduction of exotic specialist herbivores is not common in isolated archipelagos like

**Table 1** Generalized linear models with invasiveness as binomial response variable based on time-calibrated PNND values (branch length proportional to elapsed time) for the entire Azorean angiosperm flora (archipelago scale)

Model	Variable	<i>P</i>	<i>R</i> <sup>2</sup>	AIC	Effect size
1	PNND	< 0.0001	0.039	369.75	0.043–0.287
2	Combined		0.056	365.2	0.028–0.32
	PNND	< 0.0001	0.051		
	Difference in life form	0.01	0.017		
3	Combined		0.18	336.5	0.00–0.523
	Life form (categorical)		0.088		
	Log (1000 seed weight)	0.017	0.015		
	PNND	0.021	0.014		
	Log (plant height)	0.054	0.01		

PNND, phylogenetic nearest neighbour distance.

Simplified models were obtained by stepwise deletion using AIC implemented in the step function in the R statistical programming language (R Development Core Team 2010).

**Table 2** Model averaging results for time-calibrated PNND and ecological characteristics of the introduced species

Parameter	Relative importance	Average standard error	CI upper	CI lower	Significance
Life form	0.998	0.418	3.258	1.620	*
Log (seed weight)	0.857	19.831	40.86	-36.872	
PNND	0.768	0.000	0.004	0.004	*
Log (plant height)	0.688	1.544	4.738	-1.316	
Seed number	0.306	5276.095	10341.59	-10340.7	
Dispersal type	0.305	3.661	7.938	-6.414	
Mode of introduction	0.166	5.212	10.64	-9.791	
Pollination system	0.093	6.340	12.67	-12.180	

PNND, phylogenetic nearest neighbour distance.

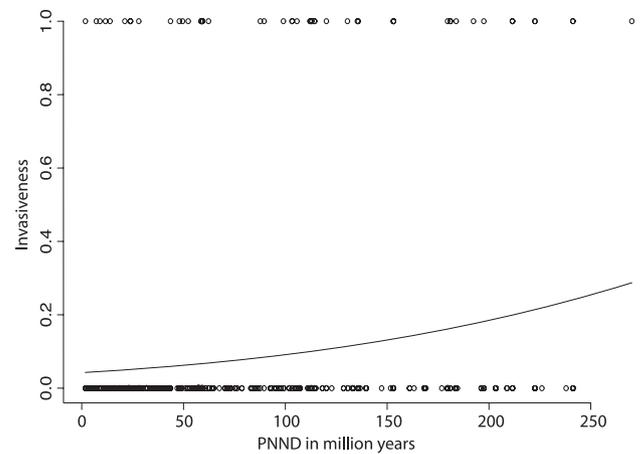
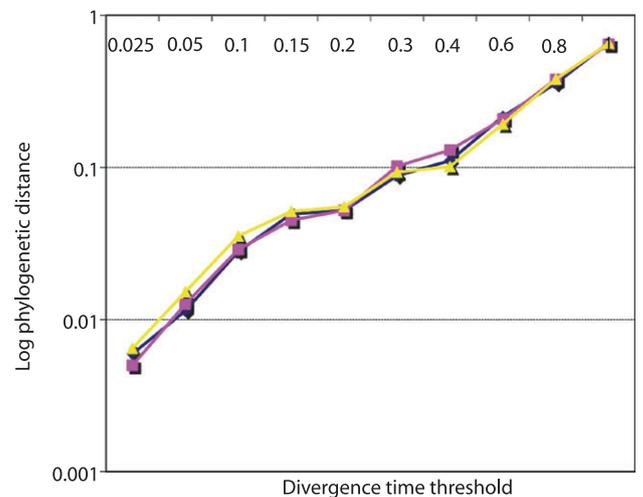
\* A term was considered to be significant if the 95% confidence interval (CI) for its parameter estimate did not include 0.

**Table 3** Model averaging results for time-calibrated PNND and differences in ecological characters between introduced species and nearest native

Parameter	Relative importance	Average standard error	CI upper	CI lower	Significance
PNND	0.999	6.31E-06	0.010	0.010	*
Difference in life form	0.893	1.02E-01	-0.495	-0.894	*
Difference in dispersal type	0.475	1.46E-01	0.084	-0.488	
Difference in log (seed weight)	0.389	6.28E-02	0.037	-0.209	
Difference in log (plant height)	0.288	1.07E-01	0.170	-0.249	
Difference in pollination system	0.273	2.34E-01	0.491	-0.425	
Difference in seed number	0.271	3.76E-02	0.082	-0.065	

PNND, phylogenetic nearest neighbour distance.

\* A term was considered to be significant if the 95% confidence interval (CI) for its parameter estimate did not include 0.

**Figure 4** Relationship invasiveness–phylogenetic nearest neighbour distance (PNND): invasiveness (0 – non-invasive, 1 – invasive) plotted against PNND values (in million years) of all Azorean angiosperms (*n* = 794).**Figure 5** Mean phylogenetic distance over time. Mean phylogenetic distance native vs. non-invasive (purple), native vs. invasive (blue) and invasive vs. non-invasive (yellow), for pairs of species at different divergence time thresholds.

the Azores (but some cases have been reported – see lists for arthropods and other herbivores in Borges *et al.* 2010). Host switching of native specialist predators from indigenous to exotic plants should depend on the degree of relatedness to the native flora. As closely related species share more specialist enemies the ‘enemy-release’ effect should be stronger in the absence of closely related plant species in the new environment. The effect of ‘enemy-release’ is likely to vary strongly between the different predator guilds like vertebrates, insects, fungi, viruses, etc. (Agrawal *et al.* 2005) but seems to be strongest for fast-growing species of nutrient-rich environments (Blumenthal *et al.* 2009). In fact, the most successful invasive plants in the Azores like the South American *Gunnera tinctoria*, the Asian *Hedychium gardnerianum* and the Australian *Pittosporum undulatum* belong to this latter group (Schaefer 2003), and would therefore benefit most from ‘enemy-release’.

Both explanations ‘competition-release’ (Darwin’s original explanation) and ‘enemy-release’, predict the observed dissimilarity between invasive and non-invasive introduced species based on phylogenetic

branch lengths. However, while the competition-release hypothesis also predicts dissimilarity in ecological traits, this is not necessarily the case of the enemy-release hypothesis. Indeed, predator attack is more likely to depend on plant chemicals (nutritional quality, concentrations of defensive compounds) than on morphological traits like seed size or plant height that we measured in this study (Bernays & Chapman 1994; Wardle *et al.* 2002). Here, there are either no significant differences, or when significant, they are contrary to the predictions of the competition hypothesis: Azorean angiosperms are more likely to become invasive if they share a similar life form with their closest native relative. Therefore, the 'enemy-release' explanation is plausible in the Azores. This hypothesis is further supported by our analyses within islands: as competition is expected to be strongest at the level of population or individual, we would predict a strong pattern of higher PNN values for the invasive-native comparisons at small geographic scales but this is not the case in our 100 m<sup>2</sup> plots (Fig. 3a; Appendix S3). In contrast, enemy-release should have similar effects at all scales in a system like the Azores, where distances within an island are too small to prevent fast spread of native or introduced predators or pathogens. A comparative study of predation levels in the native and introduced ranges of the Azorean plant invaders would be needed to test this hypothesis further, but this was beyond the scope of our study.

Another explanation for the scale dependency of our results might be that invaders and native species are found in all kind of habitats, whereas by definition, our non-invasives are found only in disturbed habitats. Therefore, only the plots with some level of disturbance host both invasive and non-invasive species and could be used for the comparison of PNN values. The plots with only natural habitat were excluded because they contain only native and invasive species but not non-invasive species. Several specialist native species cannot survive in even slightly disturbed habitats and are therefore absent from our small-scale plots, which might have an influence on the PNN analyses.

In conclusion, we show that evolutionary metrics like PNN are a key addition to the more traditional ecological assessments of the invasive potentials because they take into account both the traits of the invader and of the invaded ecosystem. With the rapid advance of DNA sequencing technologies, we can expect to recover species-level phylogenies for an increasing number of ecosystems worldwide in the next few years. This will allow testing of our findings in a range of other regions, and hopefully will provide the basis for more efficient approaches to protect the world's insular ecosystems.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Species list with status, distribution in the Azores, voucher information and Genbank numbers.

**Appendix S2** Trait database for the angiosperm flora of the Azores.

**Appendix S3** Species lists for 67 (45 grassland and 22 forest) 100 m<sup>2</sup> plots on Flores, Faial and Santa Maria.

**Appendix S4** Constraints used for molecular clock dating.

**Appendix S5** Trait comparisons for all introduced species and their nearest relative from the native flora.

**Figure S1** Best maximum likelihood cladogram for the Azorean flora produced with *RAxML-HPC2* vs. 7.2.6 (Stamatakis *et al.* 2008).

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