

RESEARCH ARTICLE

Strong phylogenetic signal and models of trait evolution evidence phylogenetic niche conservatism for seagrasses

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Abstract

1. Phylogenetic signal (PS) is the propensity of closely related species to resemble each other. PS has been tested across clades of terrestrial plants; however, insight for seagrasses is still lacking. Signatures of PS and models of niche (trait) evolution can help to detect phylogenetic niche conservatism (PNC), that is, close relatives live in comparable niches.
2. The initial goal of this study was to assess the pattern of PS for the world's seagrasses, by testing the non-independence of phylogenetic relatedness and seagrass species traits. A phylogeny of 49 seagrasses was constructed, together with a matrix of nine traits covering morphological, life-history and reproductive attributes. PS of traits was tested through complementary indices (Pagel's λ , Blomberg's K, Moran's I and Abouheif's C_{mean}). Three models of niche evolution (Brownian Motion, BM; Ornstein-Uhlenbeck, OU; Early Burst, EB) were then fitted to each trait and the multivariate trait matrix.
3. Results supported the existence of strong PS for seagrasses, with a particularly large effect size for seagrass reproductive traits, which followed an EB evolution model. Local Indicators of Phylogenetic Association (local autocorrelation metrics that can help to identify areas of large autocorrelation) supported the presence of PS across seagrass lineages/clades, supported by the dominance of OU as the most parsimonious trait evolution model.
4. The pattern of strong PS seems to be a consequence of long-term PNC of seagrass traits after initial radiation.
5. *Synthesis*: Our study highlights the relevance of evolution from common ancestors and shared history underpinning large seagrass phylogenetic structuring.

KEYWORDS

angiosperm, evolution, functional diversity, multi-trait, phylogeny

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1 | INTRODUCTION

It has long been proposed that closely related species are ecologically similar (Swenson, 2013). Phylogenetic signal (PS) is the propensity of closely related species to phenotypically resemble each other more than less related taxa, which may reflect a shared evolutionary history and the prevalence of common ancestry after many evolutionary processes (Blomberg & Garland, 2002; Losos, 2008). Mathematically, PS is a statistical dependence between traits and a phylogenetic tree under a particular evolution model. Initially, many different evolutionary processes may induce similar PS, suggesting that our capacity to deduce the role of such evolutionary processes (e.g. natural selection) from the measurement of PS is limited (Ackerly, 2009; Losos, 2008; Revell et al., 2008).

To address the limitations of PS, phylogenetic comparative methods help to identify patterns in the evolution of species' niches (e.g. traits), potentially inferring the evolutionary processes that underlie them (e.g. Blomberg & Garland, 2002; Cooper et al., 2010; Harmon et al., 2010; Münkemüller et al., 2015; Revell et al., 2008). The classical Brownian Motion (BM) model of trait evolution, which expects that the correlation structure among trait values is proportional to the amount of common ancestry for a pair of species (Felsenstein, 1973), has been modified in various ways to account for a suite of ecological and evolutionary processes (Pagel, 1999). One of the most commonly used is the Ornstein–Uhlenbeck (OU) model, a modification of the BM model with an additional parameter that measures the strength of return towards a theoretical optimum (Cooper et al., 2016; Hansen, 1997) that is shared across a clade or subset of species. Such OU model may capture the importance of environmental constraints on traits evolution, by placing bounds on BM evolution to model different evolutionary optima (Cooper et al., 2016). When large phenotypic divergence occurs early in evolution (i.e. adaptive radiation), trait evolution is expected to follow an early burst (EB) model (Harmon et al., 2010). Here, rates of evolution slow down through time, being highest at the root of the phylogenetic tree. Additionally, evolution affects a multitude of traits of living organisms simultaneously. Therefore, phylogenetic comparative methods should not only consider individual traits but also multivariate phenotypic evolution, which would provide key insight on the evolution of clades (Clavel et al., 2015). At the end, fitting and comparing models of trait evolution advances our understanding of many macroevolutionary questions.

PS may be expected under the three above-considered evolutionary models. While unconstrained models (like BM) create intense PS, constrained models (like OU) may result, however, in low PS. Testing for PS is of significant interest in macroecology and macroevolution (Münkemüller et al., 2012; Swenson, 2013), shedding light on a wide range of issues in terms of the tempo and mode of phenotypic changes through time. For example, when and why different traits have evolved (Kamilar & Cooper, 2013), particularly when a clear assumption of a specific trait evolution model is assumed (Cooper et al., 2010). Signatures of PS can also help to

determine if vulnerability to climate change or human-mediated disturbances are clustered along phylogenies (Cooper et al., 2010; Rodríguez et al., 2019), or whether niches are conserved along phylogenies (Losos, 2008; Wiens et al., 2010). 'Phylogenetic Niche Conservatism' (PNC), according to which close relatives live in comparable niches, reflecting a tendency of species to retain traits of their niches over time, may be studied combining signatures of PS in conjunction with models of niche (trait) evolution (Münkemüller et al., 2015; Pyron et al., 2015). In particular with reference to several macroevolutionary models that may underlie the patterns of PNC (Cooper et al., 2010).

The phylogenetic dependence of trait distribution, for a particular biological assemblage, may be a priori considered as omnipresent in the natural world (Blomberg et al., 2003). Still, the PS has been only calculated and tested in a few groups, majorly terrestrial plants and animals (e.g. Cano-Barbacid et al., 2022; Harmon et al., 2003, 2010; Swenson, 2013; Swenson et al., 2007). Yet, the level of phylogenetic dependence can considerably vary among phylogenies, and even within clades, sometimes being low when contrasted against the expectations from a random model of BM of trait evolution (Cano-Barbacid et al., 2022; Losos, 2008; Revell et al., 2008). Without a doubt, the advent of molecular data and associated phylogenies, together with robust phylogenetic methods, is catapulting phylogenetic studies to cover a wide spectrum of organisms. Although the phylogenetic position of numerous species is still unknown, deep phylogenetic relationships are reasonably well described for some lineages and, therefore, 'backbone' updated phylogenies are available for different groups of organisms, from terrestrial grasses (Liu et al., 2011) to mammals (Cooper et al., 2010).

Seagrasses are a group of marine angiosperms (i.e. flowering plants), of polyphyletic origin, fully adapted to a submerged life across the world's oceans and distributed from tropical to polar coastal areas of the globe (da Silva et al., 2021; Daru et al., 2017; Den Hartog, 1970; Hemminga & Duarte, 2000; Papenbrock, 2012; Short et al., 2007). These plants evolved from terrestrial ancestors that recolonised the world's seas about 70–100 million years ago (Brasier, 1975; Waycott et al., 2007), at least in three independent times through parallel evolution (Les et al., 1997). Around 72 species of seagrasses create habitats of paramount ecological, socio-cultural and economic relevance, being of considerable conservation interest and facing a range of human-induced stressors (Les et al., 1997; Waycott et al., 2009). Seagrass taxonomy is complex and still unresolved (Papenbrock, 2012; Rock & Daru, 2021), with Den Hartog's (Den Hartog, 1970) study as a seminal contribution to the fundamental phylogeny of seagrass species. Two main clades within Alismatales, petaloid (Hydrocharitaceae) and tepaloid (Cymodoceaceae, Ruppiaceae, Posidoniaceae and Zosteraceae) are typically considered as the backbone of seagrass phylogeny (da Silva et al., 2021; Les et al., 1997; Papenbrock, 2012; Waycott et al., 2014). Seagrass diversity has mostly focused on contemporary, species-level, metrics that ignore the phylogenetic relationships of seagrasses, despite their potential evolution from common ancestors (Rock & Daru, 2021).

The evolution of species' traits can be shaped by ecological factors and/or being constrained by evolutionary history (Ackerly, 2009). Then, variation in morphology (e.g. size) of leaf tissues, life spans and reproduction modes of seagrasses, among key biological attributes, can a priori arise from large-scale variation in the evolutionary histories of species across their ranges of distribution. However, varying environmental scenarios throughout scales of spatial and temporal variability might have also affected the phenotype of seagrasses (Kilminster et al., 2015; Papebrock, 2012). A convergent evolution of seagrasses traits, in terms of shared physiological and morphological characteristics to a marine environment, points to ancient adaptations (da Silva et al., 2021; Lee et al., 2018). To date, however, no study has been carried out to evaluate the prevalence of shared ancestry in seagrass evolution across a range of traits incorporating physiological, morphological and reproductive attributes. The hypothesis of phylogenetic control of functional traits has been recently put forward as a key question in seagrass ecology (Moreira-Saporiti et al., 2023).

The current study aimed to assess the pattern of PS in the world's seagrasses. Mathematically, we tested the statistical non-independence among species' trait values because of their phylogenetic relatedness. To address this question, we compiled information for nine traits covering morphological, life-history and reproductive attributes of 49 species of seagrasses. Then, three models of trait evolution (BM, OU and EB) were fitted to each trait, and the entire multivariate traits data set, and model selection based on information criterion used to assess their parsimony. We expected the strength of PS (i.e. the effect size) to vary among trait types, because early seagrasses had to respond to strong initial selective pressures through varying physiological, morphological and reproductive modifications. By coupling these methods, we aimed to provide insight into the macroevolution of seagrasses. In particular, these approaches finally helped to detect whether PNC, that is, close relatives living in comparable niches, have contributed to shape the macro-evolution of seagrasses.

2 | MATERIALS AND METHODS

2.1 | Phylogenetic structure

We searched for sequences assigned to strictly marine angiosperm species from the NCBI GenBank facility (www.ncbi.nlm.nih.gov/genbank/); those more typical of brackish waters and marshes were not considered (e.g. *Ruppia* spp.). Because several species identifications are synonymised, we initially condensed them under a single current species name, taking advantage of the International Plant Name Index (www.ipni.org) and further corroboration by the World Register of Marine Species (WoRMS, www.marinespecies.org). Information was obtained for a total of 49 species within four families and 12 genera (Table S1). In addition, following Waycott et al. (2007), it was decided to include three freshwater species of the family Lemnaceae (*Lemna gibba*, *Lemna minor* and *Lemna trisulca*),

as outgroups, to root the phylogenetic tree. Most sequences found belonged to three genes: *rbcl*, *matK* and 5.8S (Table S1). The *rbcl* sequence is capable of resolving up to seagrass family and genus level, while the *matK* sequences are reliable in resolving species (Papebrock, 2012). All sequences were downloaded and aligned with the MAFFT 7 package (Kato, 2013) implemented in Geneious; those sequences for which sequence homology was not obtained were discarded. For all remaining sequences, we kept one of each gene for each species, prioritising those that are preserved in official collections (vouchers) and those that have the highest number of unambiguous bases. The sequences of the different genes were trimmed, at both ends, to preserve at least a 50% coverage, thus avoiding areas with very little coverage or inconsistency due to the typical 'edge effect' of Sanger sequencing. To detect incorrect assigned sequences, a maximum likelihood tree was separately carried out for each gene. This allowed us to detect taxa that did not cluster in their respective genera or family, in which case a BLAST was run to identify whether there was a sequence-taxon misassignment in the NCBI databases. Once this refinement was done, a single sequence was created for each species, by concatenating the three available genes, although sequences of the three genes were not available for all of them (Table S1). The final alignment contained the four families and 12 genera that make up the group of seagrasses with eight of the 12 seagrass genera fully represented by all the species (*Amphibolis*, *Enhalus*, *Oceana*, *Phyllospadix*, *Posidonia*, *Syringodium*, *Thalassia* and *Thalassodendron*).

After the phylogenetic database was constructed, we checked which evolutionary model best fitted the sequences, also applying a partitioning scheme with the concatenated genes. The evolutionary models were tested in the ModelTest-NG package, implemented in Cypress, limiting the models to those enabled in RAxML and taking the model that best fits the data according to the Bayesian information criteria (BIC). We obtained that the applicable evolutionary models were the GTR+I+G4 for the complete alignment, the HKY+I+G4 models for the *rbcl* and 5.8S genes and the GTR+I+G4 for the *matK* sequences. We performed both maximum likelihood (ML) and Bayesian Inference (BI) to reconstruct phylogenetic relationships. ML inference was implemented with RAxML 8.2.11 (Stamatakis, 2014) in Geneious 2022.2.1. Statistical reliability of the ML tree was evaluated with rapid bootstrapping (10,000 iterations) to obtain a ML bootstrap consensus tree with a support threshold $\geq 75\%$. A BI analysis was also performed, through the Beast 2 program (Bouckaert et al., 2019), using a separate general time-reversible model with gamma distribution and invariant sites and with 10,000,000 iterations sampled every 1000 trees. The resulting data were checked in the Tracer software, to check the posterior probability results obtained to choose the burning applicable to our data, choosing a final value of 25%. Finally, the tree was collapsed to a value of 0.95 probability. With both approaches, a strict consensus tree was finally implemented, that is, including both the ML and BI models. Although the Bayesian tree has a higher resolution, the posterior probabilities are more susceptible to model violations (Huelsenbeck & Rannala, 2004). The concurrent use of gamma and

invariable-site models has been questioned because of overparameterisation (non-identifiability) of a model (Nascimento et al., 2017). Overparameterisation is not, however, a serious problem for Bayesian analysis (Fabreti & Hohna, 2023). Moreover, the effect of the choice of the evolutionary model has recently been studied both for the topology of the tree, with no major effect on the final result, and for the divergence times, where a good establishment of the priors is more important than the choice of the model.

To reconstruct phylogenetic distances between each pair of seagrass species, an analysis of divergence times (i.e. a time-calibrated tree) was obtained in the BEAST 2.7.3 software. The three above-considered genes, RBCL, MatK and 5.8S, were used, with partitions for each and for the third codon base of these protein-coding genes (RBCL and MatK). The trees and molecular clocks of all partitions were related, but not the substitution models, which were estimated separately using the BEAST Model Test package. The molecular clock model used was the optimised relaxed clock with the estimated mean clock rate (Douglas et al., 2021). The model used for tree construction was the birth death model (Gernhard, 2008), with six fossil calibrations, one calibration for the tree root and one calibration for the common node of all marine phanerogams (Table S2). For the root (Alismatales) and marine phanerogam calibrations, we took the reference data for these groups available on the timetree.org facility (Kumar et al., 2022) and parameterised them via a normal distribution, with the mean at the reference value and a sigma value that fitted the confidence limits of the group origins to 95% of the distribution. For fossil tree calibrations, the oldest fossil found and dated for each group was taken from the literature (Vélez-Juarbe, 2014). Using the minimum (most recent) age in the occurrence of fossils, a uniform distribution was parameterised (as the most conservative calibration approach), with the minimum age of occurrence of the node set to the minimum age of occurrence of the corresponding fossil, and the maximum age of the node set to the maximum (oldest) age of occurrence of the Alismatales group, previously taken from timetree.org. The length of the MCMC (Markov chain Monte Carlo) was parameterised at 100,000,000 samples, of which one in 10,000 was taken. Finally, a 10% burn-in was applied to generate the final maximum clade credibility tree with mean heights as node heights.

2.2 | Multi-trait database and seagrass functional structure

We compiled a total of nine functional traits (Table S3), for each seagrass species, through a literature search (<https://github.com/ftuya/Seagrass-Phylogenetic-Signature>) and available information at the International Union for the Conservation of Nature (IUCN) red list portal (www.iucnredlist.org). Because the IUCN periodically update these databases, this is an accurate and dynamic source of seagrass ecological information (Daru et al., 2017), which here provided values for seagrass generation length times and depth ranges. Traits included morphological, life-history and reproductive attributes of seagrasses. Because trait choice eventually affects the findings

of any multi-trait analysis (Cadotte et al., 2008; Hadj-Hammou et al., 2021), we selected seagrass traits to broadly characterise their functional structure, including a mix of categorical and numerical traits, which are typically included in seagrass studies and, therefore, have a good coverage across seagrass species. As proxies to the light regimes where seagrass species are found, we included the minimum and maximum water depth (m) where each species has been recorded. As morphological traits, we included maximum leaf length (cm) and maximum leaf width (cm). Because seagrass leaf length and width are very plastic within species in response to local conditions (Duffy et al., 2022; Tuya et al., 2019), we searched for the maximum values, as a way to account for inter-species variation. As key life-history traits, we considered the generation length (years) of each species, the sexual reproduction mode (dioecious vs. monoecious), the existence (or lack) of seed banks, the number of seeds per fruit and the maximum seed size (mm). We discarded some seagrass traits that have been previously widely considered. This was the case of the overall leaf morphologies, previously categorised as oval, cylindrical, strap-like leaves on long vertical stems and strap-like on short or no vertical stem (Waycott et al., 2014), because of clear connections with maximum leaf length (Figure S1). Missing trait values, on some occasions, were inferred from conspecific species, that is, within the same seagrass genus or family, and from the same geographic area, based on published literature. The percentage of imputed cases was low and varied between a minimum of 0 (i.e. all traits were obtained for each seagrass species, such as the maximum leaf length and width, or the reproduction mode) to a maximum of 16.3% for generation times (Table S4). Traits such as the dispersion capacity of seagrass species, either via vegetative fragments or seeds, which are very variable among studies as a result of varying techniques (e.g. molecular tools vs. direct measurements), and oceanographic conditions across regions, were not considered.

A 'species-by-species' Gower distance matrix, which is able to accommodate ordinal, nominal and quantitative (numerical) traits, was then obtained. Multi-trait similarities were calculated through the 'gawdis' R package, via the function 'gawdis' (de Bello et al., 2021), which computes dissimilarities between species from species' traits. The distance matrix obtained can be computed to attain a similar contribution of individual traits, or to account for group of associated traits that somehow reflect similar information (e.g. leaf morphology, which includes maximum leaf length and width). We then used the 'weight' argument to vary the contribution of each trait and assess the overall robustness of our functional approach, by considering the correlation of three multi-trait configurations. In addition to a (first) simple average of dissimilarities from individual traits, we carried out a (second) 'weighted average', in which we reduced the weight of categorical traits, such as the binary trait reproduction mode, to reduce their contribution to the multi-trait dissimilarity. In brief, the function searches the best values for the 'weight' argument, to obtain an equal contribution of individual traits. Finally, we used the 'group' argument to define two groups of traits containing overlapping information: leaf morphology (consisting of maximum leaf length and width) and reproduction traits (consisting of

the reproduction mode, seed bank types, the number of seeds per fruit and the maximum seed size), to recalculate a (third) multi-trait dissimilarities matrix. In all cases, correlations between different multi-trait configurations were >0.84 , denoting the robustness of this functional approach. Hence, we finally obtained a triangular matrix of pairwise (Gower) distances between each pair of seagrasses, using the first approach. These dissimilarities were projected into a multidimensional functional space via a principal coordinate analysis (PCoA), using the 'vegan' R package (Oksanen et al., 2008), which visually denoted differences among seagrasses and seagrass families.

2.3 | Testing for PS

In this study, all indices of PS and associated statistical significance tests, as well as graphical outputs to identify PS across the phylogeny, were calculated through the 'phylosignal' R package (Keck et al., 2016). We determined the PS of single quantitative traits through a range of complementary indices, including Pagel's λ (Pagel, 1999), Blomberg's K (Blomberg et al., 2003) and two autocorrelation indices: Moran's I index and Abouheif's C_{mean} , with their associated statistical tests (Münkemüller et al., 2012). Overall, values of λ , K, I and C_{mean} larger than zero denote deep relationships between species' traits and phylogeny (Münkemüller et al., 2012). Under a BM model of trait evolution, Pagel's λ and Blomberg's K are expected to be equal to 1. Hence, trait evolution follows a random walk along the branches of the phylogeny (Münkemüller et al., 2012) and species inherit their traits from ancestors, slowly varying at a constant rate through time (Comte et al., 2014). When values of λ and K are close to 0, there is no PS in the studied trait, that is, there is phylogenetic independence, and the trait has evolved independently of phylogeny and close relatives are not more similar than distant relatives. Values between 0 and 1 suggest some degree of trait lability (Comte et al., 2014). While the upper limit of λ is close to 1, K can take larger values, evidencing stronger trait similarity among related species than expected under BM. Hence, close relatives (i.e. adjacent species in a phylogeny) are phenotypically more similar than expected under BM (Münkemüller et al., 2012). The I and C_{mean} are autocorrelation indices not based on any evolutionary model, with large deviations from 0 representative of strong PS (Münkemüller et al., 2012). The resulting values do not offer any quantitative interpretation when comparing values between different phylogenetic trees. This is because the expected value of the statistic under the assumed model is unknown. We combined these four metrics, because they are complementary and capture different aspects of PS. Their performance depends on the underlying evolutionary model, sample size and the possible existence of errors in the topology of the phylogeny (Münkemüller et al., 2012). Values of these metrics can differ for a particular trait, limiting straightforward interpretation. While approaches under a particular assumption of evolution have the advantage of a direct evolutionary interpretation, autocorrelation approaches have better robustness to imprecise phylogenetic topology under less restrictive expectations (Martins, 1996).

We simulated the efficiency of these metrics to detect PS along a BM influence gradient (Figure S2). The four metrics tended to have varying values when BM reached 100% (Figure S2).

In the case of reproductive traits (reproduction mode, the existence of seed banks, the number of seeds per fruit and the maximum seed size), we initially performed a PCA (Figure S3), after data standardisation (all traits values between 0 and 1), to reduce the dimensionality of this information, as similarly performed for other seagrass traits (Duffy et al., 2022), while avoiding the inclusion of direct categorical traits in the calculation of PS metrics. Axes I and II accumulated a 44.6% and 29.7% of the total variance, respectively, and, therefore, these two component condensed most variation (74.3%) attributed to seagrass reproduction. Hence, the PCA-I and PCA-II loadings, for each species, were considered as two numerical 'artificial' traits in the multi-trait data set. To facilitate visualisation of PS, phylogenetic correlograms (based on Moran's I) were obtained for each trait (Hardy & Pavoine, 2012), which are underpinned by the notion of the spatial correlogram (Sokal & Oden, 1978), to graphically display how the data (here, traits) are autocorrelated at different lags of distance (here, phylogenetic distances). A confidence interval was added through non-parametric bootstrap resampling (Keck et al., 2016). Initially, metrics of autocorrelation (e.g. Moran's I) assume that traits have consistently evolved across the phylogeny. As this is unlikely, and PS can vary among clades, local autocorrelation metrics, that is, Local Indicators of Spatial Association (LISA) such as I_i , the local Moran's I , can help to identify areas of large autocorrelation. When applied to a phylogeny, Local Indicators of Phylogenetic Association (LIPA), through the I_i metric, can help to identify clades, or groups of species, with intense PS (Keck et al., 2016).

To test the null hypothesis of no PS, observed values of each metric were compared with values expected under a random trait distribution, which were numerically simulated by randomly permutating trait values among the tips of the phylogenetic tree (Keck et al., 2016). In these tests, we iteratively randomised the observed trait values across the tips of the tree and computed each metric. By iterating such method a 999 times, a distribution of each metric was obtained under a random trait variation distribution. The only exception was the λ index, whose significance was tested by means of a likelihood ratio test.

2.4 | Models of trait evolution

We fitted multi- and univariate models to test whether seagrass traits followed BM, EB or OU models of trait evolution. Prior to analyses, we resolved the polytomies in the tree using the function 'multi2di' in the 'ape' R package (Paradis & Schliep, 2019), and converted the tree to ultrametric using the function 'force.ultrametric' in the 'phytools' R package (Revell, 2012). Models of trait evolution were run on the whole tree, so we assumed no selective regimes across the phylogeny that might have shifted from one evolutionary process to another. OU models were fitted with one adaptive optimum per trait. Models were compared based on the log likelihood and the

Akaike information criterion corrected for small sample sizes (AICc). Models that were within two units of the lowest AICc model were considered to have similar support (Burnham & Anderson, 2003). The multivariate model was fitted using the 'mvMORPH' R package (Clavel et al., 2015), while univariate models were fitted using the 'geiger' R package (Pennell et al., 2014). For each trait, we mapped the evolution of trait values across the phylogeny using the 'contMap' function in the 'phytools' R package (Revell, 2012).

3 | RESULTS

3.1 | Phylogenetic structure

The trees inferred by both ML and BI (Figure S4) showed no contradictions. The time-calibrated tree (Figure 1) reflects monophyletic relationships across currently accepted genera and families, with large support at the family level, grouping all species

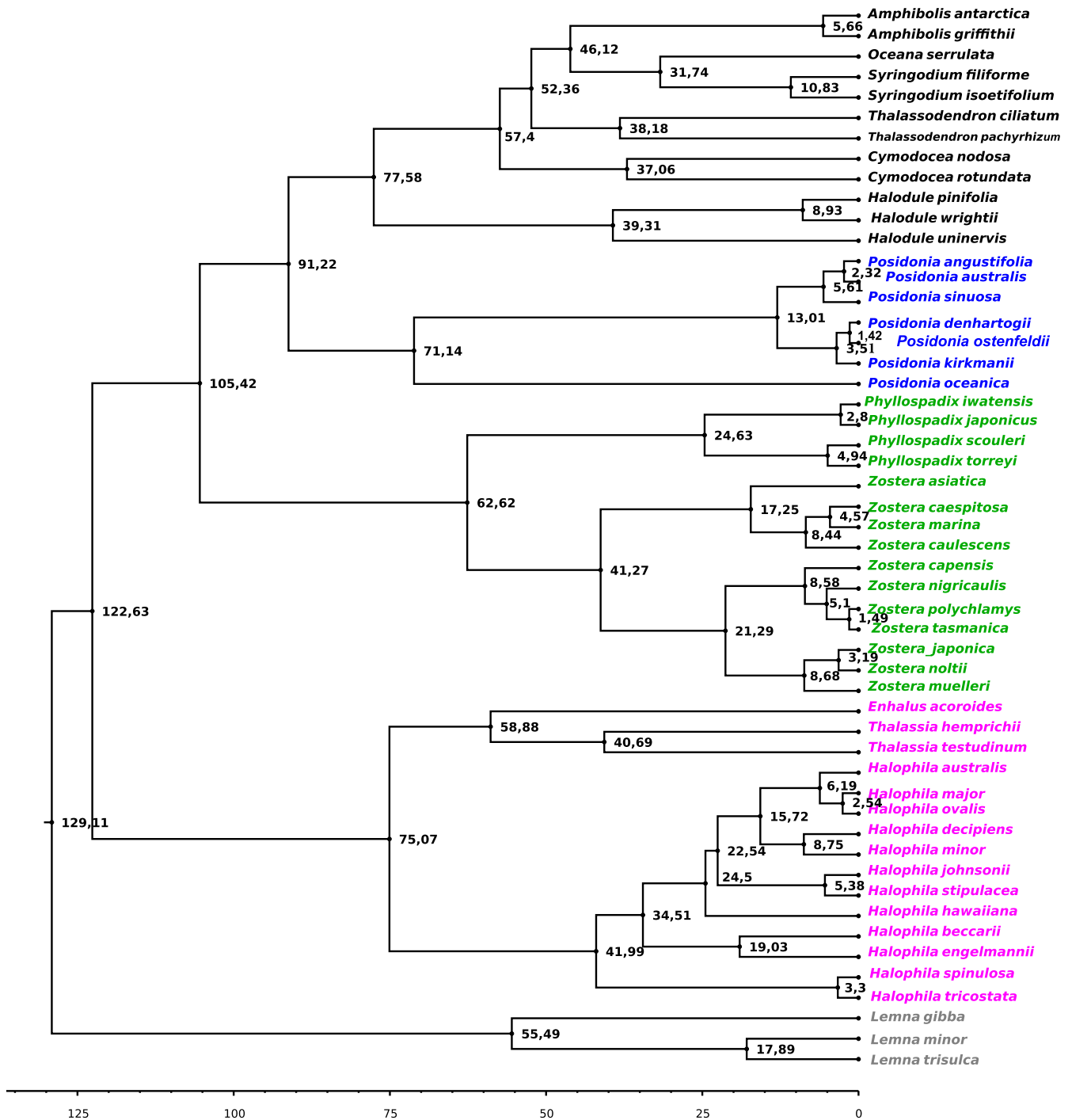


FIGURE 1 Time-calibrated phylogenetic tree of seagrass species. Numbers indicate the estimated age (millions of years) of nodes. Species names are coloured according to seagrass families; black: Cymodoceaceae; magenta: Hydrocharitaceae; blue: Posidoniaceae; green: Zosteraceae; grey: outgroup (*Lemna* spp.).

into monophyletic groups corresponding to Cymodoceaceae, Zosteraceae, Posidoniaceae and Hydrocharitaceae. Despite the varying depth of species-level resolution among the different clades, all currently accepted genera have been grouped with a high level of resolution and support. The tree did not contradict the monophyletic nature of any of the currently accepted genera or families.

3.2 | Multi-trait structure

The PCoA showed that seagrasses varied in their traits, mostly following affinities by families (Figure 2; Table S5 includes pairwise correlations between each of the first two PCoA axes and each trait). The first PCoA axis condensed ca. 23.6% of the overall variation in the multi-trait matrix. Species with positive scores on the first PCoA axis belonged to Hydrocharitaceae and Cymodoceaceae, majorly separating the former family from the other seagrass families (Figure 2). The second PCoA axis accumulated another 10.5% of the total variation, and majorly separated Posidoniaceae from Zosteraceae and, to a lesser extent, Cymodoceaceae (Figure 2).

3.3 | Phylogenetic signal

All traits exhibited significant PS according to the four metrics, including those two metrics (λ and K) denoting no departures from a BM evolution model (Table 1). The only exception was the minimum and maximum depth reached by seagrasses according to K . In general, those two 'artificial' traits condensing seagrass reproductive traits were those that showed a stronger PS (Table 1). For both traits, K was >1 , which reflected a higher degree of trait similarity of related taxa than expected from pure BM. The LIPA analysis (Figure 3, Figure S5) revealed significant local positive autocorrelation in different clades across traits, meaning that seagrass species are positively autocorrelated (e.g. more similar to each other than would be expected by chance). Regular hotspots of autocorrelation across seagrass clades provided concurrent visual evidence of PS. Most phylogenetic correlograms showed large positive, significant, autocorrelation for short and medium lags, indicative of PS (Figure 4). In the case of the seagrass generation time, leaf maximum length and the 'artificial' traits condensing seagrass reproductive traits, significant negative autocorrelation was also observed, which indicated that any two closely related species (i.e. within the same clade) tend to share similar trait values, but two adjacent clades are likely to differ strongly.

FIGURE 2 PCoA ordination showing multi-trait similarities of seagrass species and families (symbols and colours) along the first two PCoA axes. Species names are coloured according to seagrass families; black: Cymodoceaceae; magenta: Hydrocharitaceae; blue: Posidoniaceae, green: Zosteraceae; grey: outgroup (*Lemna* spp.).

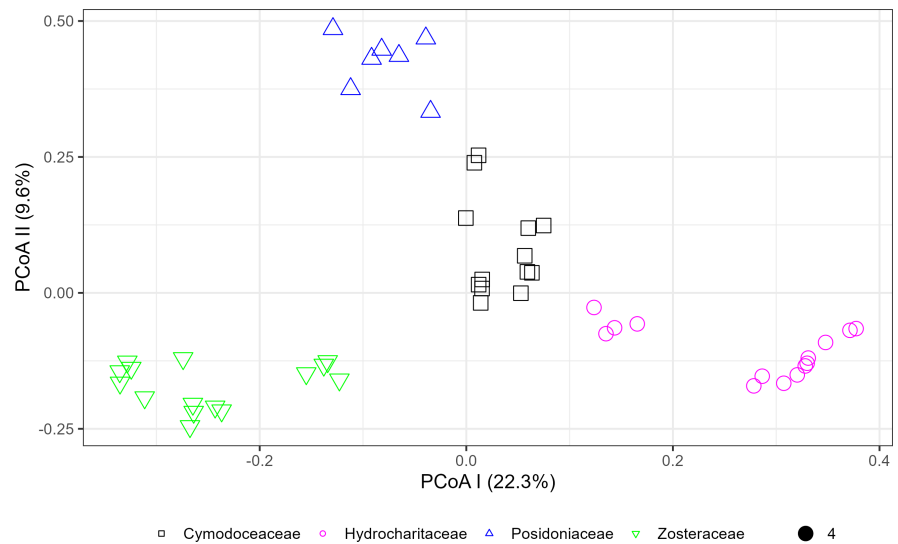


TABLE 1 Measures of PS and associated p -values (<0.05 is highlighted in bold), for each of five seagrass traits and two 'artificial' traits condensing reproductive attributes.

Trait	C_{mean}	p -value	I	p -value	K	p -value	λ	p -value
Minimum depth	0.452	0.001	0.248	0.001	0.098	0.427	0.613	0.001
Maximum depth	0.260	0.007	0.085	0.019	0.123	0.123	0.445	0.040
Leaf max. length	0.536	0.001	0.235	0.001	0.274	0.009	0.784	0.001
Leaf max. width	0.333	0.001	0.164	0.001	0.158	0.013	0.322	0.037
Generation time	0.431	0.002	0.184	0.001	0.488	0.001	0.955	0.001
Reproduction (PC1)	0.709	0.001	0.325	0.001	1.758	0.001	1.011	0.001
Reproduction (PC2)	0.825	0.001	0.445	0.001	2.545	0.001	1.011	0.001



FIGURE 3 Barplot of local Moran's index (I_i) values for each seagrass species and trait, together with the phylogeny used. The value of I_i denotes (black bars) the degree of autocorrelation between closely related species and red bars denote statistically significant (p -value < 0.05) I_i values. Species names are coloured according to seagrass families; black: Cymodoceaceae, magenta: Hydrocharitaceae; blue: Posidoniaceae, green: Zosteraceae; grey: outgroup (*Lemna* spp.).

3.4 | Models of trait evolution

Overall, multi-trait phenotypic evolution was best fitted by an OU model of multivariate trait evolution (Table 2). The minimum and maximum depth of seagrass species followed an OU model of trait evolution (Table 2). Leaf morphology traits also followed OU models (Table 2). Generation time was best fitted by a BM model, while both reproductive traits followed EB models of trait evolution (Table 2). While reproductive traits (which followed an EB model of trait evolution) displayed larger diversification towards the root of tree, the rest of traits (which followed BM and OU models) have a propensity to display larger diversification in trait values towards the tips of the tree (Figure 5).

4 | DISCUSSION

We have demonstrated the presence of strong PS in seagrass traits, consistent with findings of previous studies such as terrestrial plants (Swenson, 2013; Swenson et al., 2007) and grasses (Liu

et al., 2011). Both phylogenetic inertia and adaptation can contribute to trait values. A key question lies in how much of each trait results from adaptation, and how much to common ancestry (Blomberg & Garland, 2002). Seagrasses are aquatic plants adapted to marine environments, which experienced initial adaptations to thrive in a high-salinity domain (Hemminga & Duarte, 2000; Wissler et al., 2011). An initial convergent evolution of seagrass lineages has been considered (Chen et al., 2012; Les et al., 1997), including, for example, species-specific gene loss, as the case of members of the genus *Halophila* and the family Zosteraceae, two lineages that separated ca. 30 million years ago (Chen et al., 2021; Lee et al., 2018). Hence, ancient seagrasses had to face severe initial selective pressures in their habitats through a range of physiological, morphological and reproductive modifications, relative to their terrestrial congeners (Chen et al., 2021; Wissler et al., 2011). Our results provided support for the relevance of evolution from common seagrass ancestors that initially adapted to the marine environment, without, however, major subsequent bottlenecks. In this sense, the two traits condensing reproductive information have followed an EB evolution model, which corresponds to large

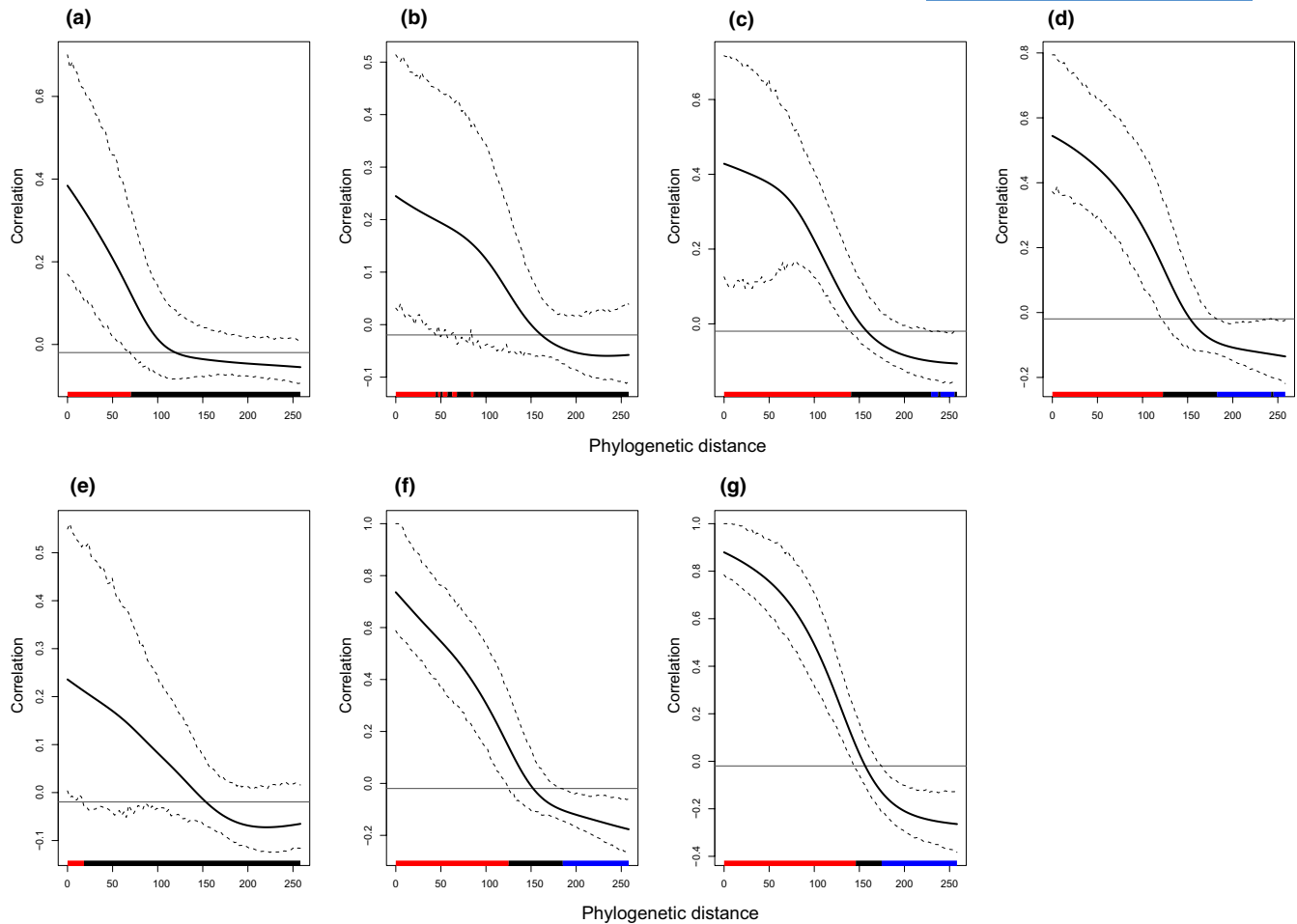


FIGURE 4 Phylogenetic correlograms for seagrass traits, including (a) minimum and (b) maximum water depth, (c) generation length, maximum leaf (d) length and (e) width, (f) PC-I and (g) PC-II of seagrass reproductive traits. The solid line is Moran's I autocorrelation metric and dashed black lines are the lower and upper limits of the confidence envelopes (95% confidence). The horizontal black line denotes expected value of Moran's I under the null hypothesis of no phylogenetic autocorrelation. A red-coloured bar denotes significant positive autocorrelation, while a blue-coloured bar denotes significant negative autocorrelation.

initial phenotypic divergence following adaptive radiation and, therefore, considerable evolution rates at the root of the phylogenetic tree. According to Simpson (1945), adaptive radiations begin when lineages gain access to some previously unexploited area of the niche space, taking advantage of windows of 'ecological opportunity', rapidly diversifying to create new species. Seagrass ancestors colonised the oceans, at least in three independent times; the aquatic habitat then imposed novel selection forces that lead to parallel evolution, such as hydrophilic pollination in their sexual reproduction systems (Wissler et al., 2011). These two traits condensing seagrass reproductive information were, moreover, those that showed a more intense PS ($K > 1$), which reflects a large phylogenetic structure in seagrass reproduction attributes, even larger than that expected based under pure BM. Blomberg's K has been shown to outperform other PS indices in identifying small differences in niche evolution processes that are not related to the strength of BM (Münkemüller et al., 2012), such as fluctuations in the rate of evolution over time (Pagel, 1999), and directional or stabilising selection (Ackerly, 2009; Revell et al., 2008).

When there is shared ancestry, related species are phenotypically similar, and considerable phylogenetic structure reveals common ancestors and shared history (Blomberg & Garland, 2002; Comte et al., 2014). The support of strong PS in seagrass traits, at least for those selected by this study, suggests that most evolution of these traits has been hampered by phylogenetic inertia, which seems a stronger driver of seagrasses traits than recent environmental processes. In other words, phylogenetic inertia is a stronger determinant of trait evolution rather than lineages' recent or independent responses to environmental perturbations, as suggested for other biota (Cooper & Purvis, 2010). Importantly, OU models of trait evolution, which here were the most parsimonious for morphological traits, in addition to the entire multivariate trait data set, capture the importance of constraints on evolution over BM, which tends to move towards adaptive peaks that may concurrently impose limitations on trait diversification (Cooper et al., 2016).

Different evolutionary processes can generate similar results in PS, while different evolutionary rates can produce similar PS (Münkemüller et al., 2012). For example, strong PS may arise not only

	Model	Log-likelihood	AICc	Δ AICc
Multivariate data set	OU	-770.3135	1693.507	0
	BM	-868.9671	1815.617	-122.11
	EB	-868.9671	1818.081	-124.574
Minimum depth	OU	-73.6581	153.8163	0
	BM	-105.2533	214.7516	-60.9353
	EB	-105.2538	217.0076	-63.19131
Maximum depth	OU	-222.6154	451.7309	0
	BM	-246.0816	496.4081	-44.67718
	EB	-246.082	498.6641	-46.93312
Leaf max. length	OU	-275.1353	556.7707	0
	BM	-282.8799	570.0049	-13.23414
	EB	-282.8803	572.2607	-15.48992
Leaf max. width	OU	-32.7579	72.01589	0
	BM	-49.7429	103.7308	-31.71488
	EB	-49.7433	105.9866	-33.97075
Generation time	BM	-150.1586	304.5622	0
	OU	-149.7093	305.9187	-1.356591
	EB	-150.1587	306.8174	-2.255298
Reproduction (PC1)	EB	-10.2250	26.95019	0
	BM	-39.0637	82.37235	-55.42217
	OU	-39.0637	84.62745	-57.67727
Reproduction (PC2)	EB	-13.269	33.03802	0
	BM	-17.274	38.79306	-5.755036
	OU	-17.274	41.04816	-8.010138

TABLE 2 Comparisons of trait evolution models under Brownian Motion (BM), Early Burst (EB) and Ornstein–Uhlenbeck (OU) approaches, for individual traits and the overall multivariate data. Models are ranked according to their AICc and Δ AICc denote differences between each pair of models relative to the most parsimonious model.

from BM but also from a pattern of PNC, which implies the retention of niche-related traits through time (Losos, 2008; Münkemüller et al., 2015; Wiens et al., 2010). PS is a necessary, but insufficient, condition for PNC, which may be otherwise suggested by values of $K > 1$ (Ackerly, 2009; Losos, 2008), as we here have observed, at least for reproductive traits. PNC here refers to the fact that closely related species are more ecologically similar than might be expected solely following a BM trait evolution, so there is a tendency of species to retain ancestral ecological traits (Losos, 2008), slowly diverging during movement to new places. This would follow a model of ‘Niche Retention’ according to the PNC macroevolutionary models of Cooper et al. (2010).

PNC has also been evidenced when the evolution of a trait follows an OU model. In these cases, however, PS indices are usually weaker than predicted under a pure BM model (Cooper et al., 2016; Wiens et al., 2010), as we have here numerically observed (Table 1). In this case, niches of species are constrained, so species do not move far from the optimum niches (Donoghue, 2008), following a model of ‘Phylogenetic Inertia’ according to the PNC macroevolutionary models of Cooper et al. (2010). Subsequently, when corridors for dispersal are available, newly emerging habitats will be filled by species that have been already filtered in from habitats in which key adaptations have already evolved. This seems, for example, to be a plausible scenario for seagrasses after tectonic events in the

Indo-West Pacific, such as the collision of Australia/New Guinea with the Eurasian plate during the late Oligocene and early Miocene (25–20 Myr). This event created considerable shallow water areas between Australia and Indonesia (Wilson & Rosen, 1998) that facilitated expansion of seagrass habitats (Brasier, 1975). Under PNC, novel habitats, as ‘windows of ecological opportunities’, are generally occupied by species that have already adapted, so adaptive evolutionary change is minimised, and similarly related species will maintain comparable ecological traits (Ackerly, 2009). The efficient, long-distance, dispersal of seagrasses, in conjunction with a dual/complementary reproduction (asexual vs. sexual), favours the spread of seagrasses across a wide spectrum of nearshore environments (Kendrick et al., 2012).

The way to demonstrate PNC has been profoundly debated, in addition to whether PNC is a pattern or a process (Münkemüller et al., 2015; Wiens et al., 2010). Still, we believe the strong PS and models of trait evolution have suggested PNC for seagrasses. We here highlight the consistency in results across the four PS metrics, and the range of analysed traits, to support previous considerations. Moreover, the LIPA analysis, via the local Moran’s index for each seagrass species and trait, revealed numerous significant correlations across the phylogeny, to support consistency in the presence of intense PS across varying seagrass lineages/clades. Normally, the degree of PS varies with traits. It has been stated that PS tends to

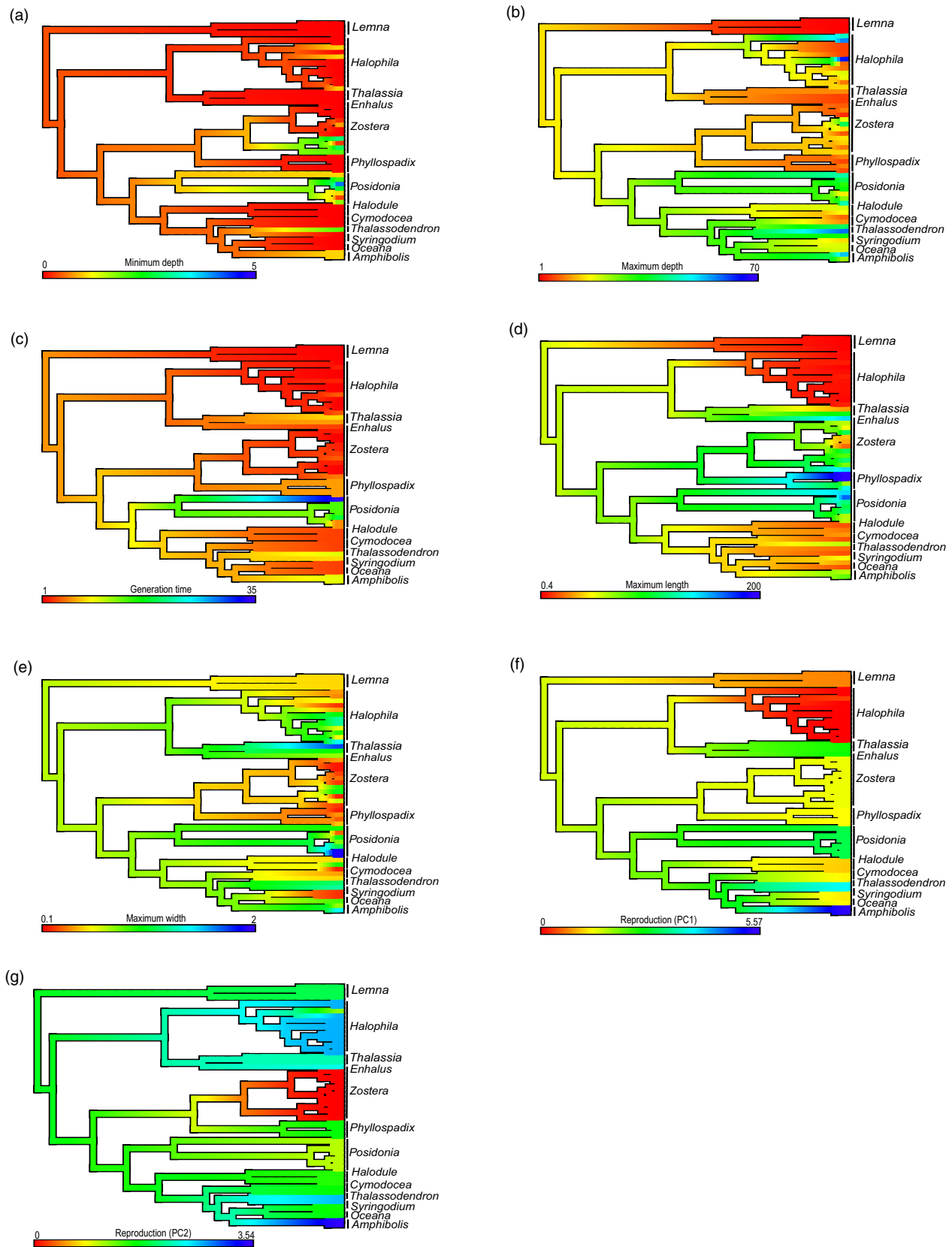


FIGURE 5 Modelled evolution of trait values across the seagrass phylogeny, including (a) minimum and (b) maximum water depth, (c) generation length, (d) maximum leaf length and (e) width, (f) PC-I and (g) PC-II of seagrass reproductive traits.

be strongest in morphological traits, such as body size, intermediate in life-history and physiological traits and low in behavioural traits (Blomberg et al., 2003; Kamil & Cooper, 2013). In our case, all seagrass traits showed clear PS.

The outcomes of our study are, of course, limited by several constraints, and some of them have been already discussed (Rock & Daru, 2021). Firstly, not all seagrass species were covered by this study, as a result of the lack of sequences for a number of taxa. Still, a phylogeny with 49 (out of 72) seagrass species represents a cover of the ca. 70% of the global seagrass species. The lack of a comprehensive seagrass phylogeny, in conjunction with the use of different genes under varying rates of evolution, may considerably limit inferences on seagrass evolutionary patterns (Rock & Daru, 2021). Inaccuracies in phylogenetic topology can generate inconsistencies in estimated PS across metrics (Münkemüller et al., 2012). Despite the presence of terminal polytomies (unresolved evolutionary relationships) in the seagrasses phylogeny, most clades (e.g. families) clearly separated towards the root of the tree. This problem was then minored, and this was reflected in consistency of results across metrics. Second, the number and type of seagrass traits selected, despite covering a range of biological/ecological aspects, could have also influenced results, as widely outlined for other biota (Hadj-Hammou et al., 2021). Seagrasses show a high degree of phenotypic plasticity in a range of structural attributes. It is acknowledged that intraspecific seagrass trait variability and variation in meadow structure can reflect recent evolutionary legacies, from millennia to millions of years (Duffy et al., 2022; Tuya et al., 2019). However, their potential effect is beyond the scope of this study. A lack of a global seagrass multi-trait database limits this approach. Current approaches, for example through the SeagrassTraitDB (<http://seagrasses.cmar.ualg.pt/>), which aims at integrating global empirical data of seagrass traits to promote insight into the functional diversity of seagrasses, are necessary (Moreira-Saporiti et al., 2023). In our functional approach, however, we found consistency when varying weighting traits was undertaken, which somehow reflects the robustness of our selection. In terms of future research, detection of environmental filters on seagrass ecology might benefit from including intraspecific trait variability rather than using mean trait values per seagrass (Moreira-Saporiti et al., 2023).

To date, seagrass diversity has majorly focused on contemporary, species-level, metrics, such as genetic and genotypic diversity descriptors that otherwise ignore phylogenetic relationships when several seagrasses cohabit (Rock & Daru, 2021). Large-scale studies focusing on biodiversity beyond taxonomic metrics need to disentangle functional and phylogenetic relationships of diversity (Bosch et al., 2021, 2022). This has hardly been accounted for in seagrasses (but see Daru et al., 2017), and our results point to a clear connection between functional and phylogenetic metrics of seagrass diversity. For example, recent efforts to map the predicted and observed area where seagrasses can be found (McKenzie et al., 2020) have ignored phylogenetic relationships among coexisting species, while

the global distribution of seagrass diversity has been limited to a mere taxonomic perspective (Short et al., 2007). Within the context of modelling, global and regional projections of contractions and expansions of the distributional ranges of seagrasses, under scenarios of global change, should consider that close seagrass relatives share comparable niches and have a similar functionality, as we have demonstrated.

Within the macroevolutionary puzzle, our study has contributed to our growing knowledge of seagrasses' evolution, by demonstrating strong PS. Despite PS may result from BM, this pattern may be a consequence of long-term PNC after initial radiation. Without a doubt, improvements in seagrass phylogenetic relationships through modern genomic tools, and multi-traits databases, will encourage researchers to revisit connections between functional (ecological) and phylogenetic seagrass similarities in the promising field of seagrass macroevolution.

AUTHOR CONTRIBUTIONS

Fernando Tuya conceived the ideas and designed methodology; Javier Martínez Pérez and Alvaro Fueyo collected all phylogenetic and functional data; Fernando Tuya and Néstor E. Bosch analysed the data; Fernando Tuya led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Our study was a global review and was based on secondary rather than primary data, derived from books and peer-reviewed articles. As such, there was no local data collection. Two coauthors (Javier Martínez Pérez and Alvaro Fueyo) are young (<30 years) PhD students.

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DATA AVAILABILITY STATEMENT

All data are publicly available at Zenodo: <https://doi.org/10.5281/zenodo.10108065> (Tuya et al., 2023). All code used will be permanently archived on GitHub <https://github.com/ftuya/Seagrass-Phylogenetic-Signature>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Differences in leaf maximum length according to the four leaf morphologies.

Figure S2. Behaviour of metrics of PS along a BM influence gradient.

Figure S3. PCA showing similarities in seagrass species according to reproductive traits (reproduction mode, the existence of seed banks, the number of seeds per fruit and the maximum seed size), including vectors for each trait. For each species, the PC1 and PC2 components were used as reproduction traits in detection of phylogenetic signal.

Figure S4. Phylogenetic trees of seagrass species according to Bayesian Inference (BI, right) and Maximum Likelihood (ML, left) to reconstruct phylogenetic relationships.

Figure S5. Distribution of traits mapped across the seagrass phylogeny. Species names are coloured according to seagrass families; black: Cymodoceaceae, magenta: Hydrocharitaceae; blue: Posidoniaceae, green: Zosteraceae; grey: outgroup (*Lemna* spp.).

Table S1. Considered DNA sequences, for each seagrass species, to assess phylogenetic similarities.

Table S2. Occurrence of seagrass species in the fossil record, according to different geological eras and times, to obtain the

time-calibrated seagrass phylogeny. References are included in the bottom of the table.

Table S3. Multi-trait matrix of seagrass attributes. Categorical traits include the reproduction mode (where a dummy variable typifying Dioecious species is denoted by 1), and the existence of seed banks (where a dummy variable of 1 indicates the existence of seed banks). Depths and generation times were rounded (i.e. no decimal).

Table S4. Imputed trait values (in green) from "sibling" species (same genus/family from the same geographical area) for those species that lacked some traits.

Table S5. Pairwise correlations between each of the first PCoA axes and seagrass traits considered by this study.

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