Title

Local adaptation to water-deficit and light limitation suggested from genomic and ecophysiological approaches in a dominant coastal tree

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Local adaptation is often a product of environmental variation in the geographical space and has important implications for species' response to climate change. Ongoing changes in climate are predicted to particularly affect coastal ecosystems, which frequently span broad latitudinal ranges and remarkable environmental variation. We investigated the local adaptation to contrasting environments over the distribution of a widespread coastal tree, based on analyses of key resource-use characteristics. We integrated results from comparative ecophysiology under a common garden experiment, with transcriptome sequencing and genome-wide scans for selection in a commonly found tree in wetlands of the Atlantic coast of South America, *Avicennia schaueriana*. We observed differences related to water and carbon balance between plants from contrasting latitudes, supported by gene expression under field conditions and morphophysiological traits under common garden. Additionally, we found signatures of selection in putative genes associated with photosynthesis, anthocyanin accumulation and the response to osmotic and hypoxia stresses. Our results strongly suggest the existence of a north-south divergence in key resource-use characteristics, likely driven by seasonality in water-deficit and light availability. These findings have implications for conservation and provide the basis for more realistic predictions on coastal plants responses to a rapidly changing climate.

Keywords

Avicennia schaueriana (Black Mangrove); climate change; coastal ecosystems; comparative transcriptomics; genome scans for selection; local adaptation; plant ecophysiology; resource-use.

Introduction

Adaptation is frequently a consequence of spatial variation in selective environmental forces acting on phenotypic variation[1,2]. As selective forces operate, they may reduce the heritable variation within a population, leading to the specialization of individuals[2]. Conversely, in highly stochastic environments, selection can increase the species potential of phenotypic plasticity[3]. Therefore, the investigation of adaptation is fundamental for understanding the ability of a species to respond to environmental changes[4,5].

Alarmingly high rates of environmental changes have been observed in the last decades, particularly affecting coastal ecosystems[6–9]. For instance, bleaching of coral reefs worldwide[10], large-scale dieback of mangrove communities[11] and increased mortality of seagrass beds[12]. Further environmental changes and coastal biodiversity loss threaten critical socio-economical and ecological services provided by coastal ecosystems, including their pivotal role as blue carbon stocks[13]. In this context, studies on the organization of adaptive variation of coastal habitat-forming species are necessary to improve predictions on the impacts of future climate conditions on marine ecosystems[14] and to support efficient conservation practices[15].

Coastal habitat-forming species are generally water-dispersed over long distances[16,17] and often occur across wide latitudinal ranges, thus spanning large environmental variation. The connectivity among populations of these species may be influenced, not only by their dispersion capabilities, but also by means of natural selection, caused by varying establishing success over the broad environmental heterogeneity across latitudes[18]. For instance, distinct coastal species in the North Atlantic show a north-south organisation of diversity with evidences of selection for different thermal regimes[19–21]. Similarly, in the Southwest Atlantic, an overlapping north-south structure of the genetic diversity, with reduced gene flow between populations, has been observed in phylogenetically distant habitat-forming species[22–24]. In this context, one could expect northerly and southerly populations of these species to adapt differently to contrasting environments over their latitudinal ranges, especially due to their large population sizes[2,25]. However, the neutrality of the molecular markers used in previous studies has precluded inferences regarding adaptive variation. The existence of local adaptation remains virtually unknown in species that play a central role in sustaining coastal biodiversity in the South Atlantic. This limited knowledge about the organization of non-neutral variation compromises accurate predictions and suitable conservation efforts for sustainable management.

Here, we tested the hypothesis that latitudinal heterogeneity in selective forces, such as solar irradiance, air temperature and humidity regimens shape the variation of genotypes and phenotypes involved in the optimisation of resource-use in widely distributed coastal species. To reduce the potential for incorrect conclusions about selection[26], we integrated three independent, but complementary approaches. We predicted that: (1) Under a common garden experiment, individuals from contrasting latitudes would show genetically based phenotypic divergence in ecophysiological traits; (2) under contrasting latitudes, transcriptomic changes would be detected in genes involved in the response to the environmental variation; (3) signatures of selection along the species distribution would be detected in genes involved in the response to the latitudinal variation in resource availability. We considered the new-world coastal tree species, *Avicennia schaueriana* Stapf & Leechman ex Moldenke, found in the Lower Lesser Antilles and widespread from Venezuela to the southernmost temperate mangroves of the Atlantic coast of South America (~28 °S)[27] as a model to test this hypothesis. The broad latitudinal range of *A. schaueriana*, spanning wide environmental variation (Fig. 1, Table 1, Supplementary information Fig. S1) and the previously detected north-south structure of neutral variation[22], facilitate ecological predictions and the accumulation of divergence, motivating this choice. We provide

unprecedented insights into the effects of fluctuations in atmospheric water-deficit and light limitation on the phenotypic and genotypic variation and on the regulation of gene expression of a dominant coastal tree. We discuss the implications of our results for the persistence of coastal biodiversity in the context of climate change.

Materials and Methods

Propagule sampling

Mature propagules were collected from 15 *Avicennia schaueriana* Stapf & Leechman ex Moldenke mother trees that were at least 100 m apart from each other, at two genetically contrasting populations[22]: (1) the southernmost range limit of American mangroves, in the subtropical region, and (2) one equatorial site in one of the world's largest macrotidal mangrove forests[28,29], near the northernmost limit of the species range (Fig. 1). We refer to samples collected in the former site as "subtropical" and those in the latter as "equatorial" throughout this work. Sampling in more localities was impracticable due to the absence of mature propagules during fieldwork. A detailed characterisation of each of these two sites can be found in Table 1, in Supplementary Methods and in Supplementary information Figure S1.

Comparative ecophysiology in a common garden experiment

Propagules were germinated as described for *Avicennia germinans*[30]. We grew propagules in trays with local mangrove soil for two months. After this period, 44 similar-sized seedlings from 30 distinct mother trees, 15 from equatorial and 15 from subtropical sites — with an average height of 18 cm, most with three leaf pairs and senesced cotyledons — were transplanted to 6 L pots filled with topsoil and sand (1:1). Seedlings were cultivated for seven months under homogenous conditions in a glasshouse at the University of Campinas, São Paulo, Brazil (22°49' S 47°04' W), where automatic sensors coupled with a data logger (Onset Computer Corp.) measured the atmospheric humidity and temperature every 15 minutes. Seedlings were irrigated daily at 10 a.m. and 5 p.m. with a 3-minute freshwater spray. Twice a week, nutrients were added to the soil using 600 mL of 0.4X Hoagland solution with 15.0 g L^{-1} NaCl per pot. Pots were rotated weekly to reduce the effects of environmental heterogeneity. Because the environmental conditions in the glasshouse differed markedly from those at each sampling site (Supplementary information Fig. S2), none of the individuals benefitted from conditions corresponding to those of their site of origin.

The light reflectance of stems was measured in ten plants from each sampling site using a USB4000 spectrophotometer (OceanOptics, Inc.) coupled to a deuterium-halogen light source (DH-2000; OceanOptics), using a light emission range from 200-900 nm. Photosynthesis, stomatal conductance and transpiration rates were measured every 2.0-2.5 hours in five six-month-old individuals from each sampling site on two different days using a Li-Cor 6400 XT (Li-Cor Corp.).

After harvest, three plants without flowers or flower buds from each sampling site were split into leaves, stems and roots, washed with distilled water, dried for 7 days at 70 °C and weighed. The individual leaf area, total leaf area and leaf lamina angle per plant were measured through photographic analyses using ImageJ[31]. The specific leaf area (SLA, cm^2 leaf area kg^{-1} leaf dry biomass) was also calculated for these samples. Stems were fixed in FAA (Formaldehyde Alcohol Acetic acid), stored in 70% alcohol for wood anatomy analysis and cut into 30 µm thick transverse sections. Sections were stained with a mixture of 1% Astra Blue and 50% alcohol (1:1) followed by 1% Safranin O. Micrographs were taken using an Olympus BX51 microscope coupled to an Olympus DP71 camera (Olympus Corp.). The

following wood traits were quantified using ImageJ and R v.4.0.0: vessel lumen area (A), vessel density in xylem (number of vessels/xylem area), vessel grouping index (mean number of vessels per vessel grouping) and vessel lumen area in sapwood (vessel lumen area/sapwood area). The vessel arithmetic diameter (D) was estimated according to Scholz et al.[32].

Shapiro-Wilk's normality tests and Fisher's F-tests of equality of variances were performed in R 4.0.0 to determine the suitability of group comparison statistical tests. In comparisons between equatorial and subtropical samples, we used unpaired Student's T-tests or, alternatively, unpaired Mann-Whitney-Wilcoxon tests. Multiple-group comparisons were conducted for the analysis of environmental conditions in the field vs. in the glasshouse using one-way analysis of variance (ANOVA) with post hoc Tukey honest significant difference (HSD) tests. A 0.05 significance level was used as alpha for all tests.

Plant material for RNA extraction and RNA-sequencing

Plant material used for RNA-sequencing (RNA-Seq) was collected in the equatorial and subtropical sites described in the "Propagule sampling" section and immediately stored in RNAlater (Ambion, Inc.). Because the precise species identification requires the analysis of both vegetative branches and flower morphology, opportunities of sampling were limited by the reproductive phenology in each visited site. Leaves, stems and flowers from three adult trees at least 100 m apart were collected from July-August of 2014, corresponding to the end of winter at the subtropical site and the beginning of the dry season at the equatorial site. To minimize the detection of differential transcripts expression due to circadian changes, sampling was conducted during the low tide and from 10:30 AM to 4:00 PM. A detailed description of environmental conditions at the time of sampling is available in Supplementary information Table S1.

We extracted RNA according to Oliveira et al.[33] and evaluated its integrity and purity using agarose gel electrophoresis and a NanoVue spectrophotometer (GE Healthcare Life Sciences). lllumina TruSeq RNA Sample Preparation kits (Illumina, Inc.) were used to construct cDNA libraries. cDNA quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies) and concentrations were quantified by qPCR, using the Sequencing Library qPCR Quantification kit (Illumina, Inc.). Sequencing was performed using two 36-cycle TruSeq SBS paired-end kits (Illumina, Inc.) and a Genome Analyzer IIx platform (Illumina, Inc.).

Assembly and characterisation of the transcriptome of A. schaueriana

Adapter sequences were trimmed, and 72 bp paired-end reads were filtered by quality (Phred score ≥ 20 for at least 70% of read length) using the NGS QC Toolkit v.2.3[34]. High-quality reads were used for transcriptome assembly in the CLC Genomics Workbench (https://www.qiagenbioinformatics.com/). We used the default settings, except for the distance between pairs (300-500 bp) and k-mer size (45 bp).

Reads were mapped to transcripts using bowtie1[35] in the single-read mode using default parameters. Transcripts without read-mapping support were removed. Functional annotation was performed using blastx v.2.2.31[36] with an e-value < 1^{-10}. NCBI RefSeq[37], The Arabidopsis Information Resource (TAIR)[38] and NCBI non-redundant (nr) databases were used as reference databases. We excluded transcripts that were exclusively similar to non-plant sequences. Protein family domains were identified using HMMER3[39], which iteratively searched transcripts against the Pfam database. To assign Gene Ontology (GO) terms to transcripts, we used the *Arabidopsis thaliana* gene association file from the GO Consortium[40] and retrieved the information for transcripts showing similar coding sequences in the genome of *A. thaliana*. Redundant transcripts were clustered using CD-HIT-EST v.4.6.1[41] using the

local alignment mode with 95% identity and 70% coverage of the shortest sequence thresholds. Open reading frames (ORF) were identified using Transdecoder (http://transdecoder.sf.net). We reduced the redundancy of transcripts in the final assembly by retaining for each CD-HIT-EST cluster either the sequence with the longest ORF or, in the absence of sequences containing ORF, the longest sequence.

The completeness of the final transcriptome was assessed using BUSCO[42]. Additionally, a reciprocal blastn alignment using an e-value threshold of 10^{-10} and a minimum alignment length of 100 nucleotides with at least 70% identity was used to compare the *A. schaueriana* transcriptome with other publicly available transcriptomes of congeneric species.

Comparative transcriptomics using RNA-sequencing

Tissue-specific count data were obtained from the number of reads uniquely mapped to each transcript of the non-redundant transcriptome using bowtie1[35] and normalised using edgeR[43]. Differentially expressed transcripts (DETs) between tissue-specific samples of equatorial and subtropical trees were detected using the exact test for negative binomial distributions with an adjusted P-value < 0.05. GO term enrichment analyses of the DETs were performed using GOseq[44] with the Wallenius approximation method and P-value < 0.05. Differential expression results were verified using reverse transcription real-time PCR (qRT-PCR) (Supplementary Methods).

Detection of candidate adaptive loci

We sampled leaves from 79 adult plants at ten locations spanning most of the distribution of *A. schaueriana* Stapf & Leechman ex Moldenke (Fig. 1, Supplementary information Table S2). We isolated DNA using the DNeasy Plant Mini Kit (QIAGEN) and NucleoSpin Plant II (Macherey Nagel) following the manufacturers' instructions. DNA quality and quantity were assessed using 1% agarose electrophoresis and the QuantiFluor dsDNA System with the Quantus fluorometer (Promega). Nextera-tagmented reductively-amplified DNA (nextRAD) libraries[45] were prepared and sequenced by SNPsaurus (SNPsaurus) in a HiSeq 2500 (Illumina, Inc.) with 100 bp single-end chemistry. Genomic DNA fragmentation and short adapter ligation were performed with Nextera reagent (Illumina, Inc.) followed by amplification with one of the primers matching the adapter and extending nine arbitrary nucleotides into the genomic DNA. Assembly, mapping and single nucleotide polymorphic loci (SNP) identification were performed using custom scripts (SNPsaurus), which created a reference catalogue of abundant reads across the combined sample set and mapped reads to this reference, allowing two mismatches and retaining biallelic loci present in at least 10% of the samples. We further filtered markers by allowing no more than 65% of missing data, Phred score > 30, 8x minimum coverage, only one SNP per locus and a minor allele frequency ≥ 0.05 using vcftools v.0.1.12b[46]. To reduce paralogy or low-quality genotype calls, we used a maximum read coverage of 56 (the average read depth times 1.5 standard deviation).

After excluding plants morphologically identified as *A. schaueriana* with genomic signs of hybridisation with *A. germinans* (L.) L., we assessed the genetic structure considering all SNPs, using the discriminant analysis of principal components (DAPC)[47] and ADMIXTURE v.1.3.0[48]. For DAPC analyses, we considered the number of groups (K) varying from 1-50 and the Bayesian information criteria for inferring K. Additionally, we used the optim.a.score function to avoid over-fitting during the discrimination steps. For the ADMIXTURE analyses, we performed three separate runs for K varying from 1-15, using the block-relaxation method for point estimation; computing was terminated when estimates increased by < 0.0001, and the most likely K was determined by cross-validation.

We used two programs to minimise false-positive signs of natural selection: LOSITAN[49], assuming an infinite allele model of mutation, using a confidence interval of 0.99, a false-discovery rate (FDR) of 0.1, the neutral mean FST and forcing the mean FST options; and pcadapt 3.0.4[50], which simultaneously identifies the population structure and the loci excessively related to this structure, using FDR < 0.1.

Putative evidence of selection was considered only for SNP loci identified conservatively by pcadapt and five independent runs of LOSITAN, to avoid false-positives[51]. As selection is presumably stronger in coding regions of the genome and there is no reference genome for the species, we used the *de novo* assembled transcriptome, characterized herein, as reference to identify candidate loci within putative coding region. We performed a reciprocal alignment between nextRAD sequences (75 bp) and longer expressed sequences (≈ 300-11600 bp) using blastn v.2.2.31[36], with a threshold of at least 50 aligned nucleotides, a maximum of one mismatch and no gaps.

Data accessibility

Expression data and sequences that support the findings have been deposited in GenBank with the primary accession code GSE116060. A Variant Call Format file and its complementary file, both required for all of the genome-wide SNP diversity analyses are provided as Supplementary Files.

Results

Comparative physiology in a common garden experiment

Seedlings from equatorial and subtropical sites (Fig. 2 and 3) expressed functional divergence in key ecophysiological traits in the common garden experiment. Equatorial plants showed lower stomatal conductance and transpiration rates than did subtropical plants (Fig. 4). Additionally, the inclination angle of the leaf lamina and the average size of individual leaves were smaller in equatorial than in subtropical plants, but total leaf area and specific leaf area did not differ between groups (Fig. 2, Supplementary information Table S3, Supplementary information Fig. S3). Subtropical plants accumulated more biomass in leaves and roots than did equatorial plants. However, the stem dry mass ratio (MR) (stem dry biomass/plant dry biomass) was greater in equatorial plants, whereas the leaf MR (leaf dry biomass/plant dry biomass) was greater in subtropical plants. The dry biomass accumulated in stems and the root MR (root dry biomass/plant dry biomass) were indistinguishable between groups (Supplementary information Table S3). Unexpectedly, 63% of the equatorial plants flowered after six months of growth (Supplementary information Fig. S3g). Since this was not observed in any subtropical plant, flowering plants were not used in biomass allocation analyses.

Mean stem vessel diameter was smaller in equatorial seedlings compared to subtropical seedlings, indicating enhanced hydraulic safety (Fig. 2, Supplementary information Fig. S3h-i). However, vessel density, vessel grouping index, vessel lumen area in sapwood and total hydraulic conductivity of the stems were not significantly different between groups (p-value > 0.05) (Supplementary information Table S3). Plants from contrasting latitudes exhibited different stem epidermis pigmentation, with equatorial seedlings reflecting more red light of long wavelengths (635-700 nm) and less green light of medium wavelengths (520-560 nm) than did subtropical seedlings (Fig. 3).

Characterisation of the first transcriptome of A. schaueriana

In the absence of a reference genome, we obtained the first transcriptome for *A. schaueriana* from leaves, stems and flowers of adult individuals under field conditions (Supplementary information Fig. S4, Supplementary information Table S1). Over 209 million paired-end 72 bp reads showing high quality were *de novo* assembled into a reference, non-redundant transcriptome containing 49,490 sequences, of which 30,227 (61%) were putative protein-coding. Over 91.9% of these reads were mapped to a single transcript, indicating minimum redundancy and wide representation of sequenced data (Supplementary information Table S4). Moreover, 91.8% of universal plant orthologous genes were present in the reference transcriptome (Supplementary information Table S4). Sequences with complete ORF represented approximately 42% (12,796) of all putative protein-coding transcripts (Supplementary information Table S5, Supplementary information Fig. S5). Most of these putative protein-coding sequences (94.33%) showed significant similarity (e-value < 1e-10) to proteins in the Plant RefSeq and TAIR databases (Supplementary information Fig. S5c). More than 80% of protein-coding sequences matched proteins from *Sesamum indicum* or *Erythranthe guttata*, which, as *A. schaueriana*, belong to the order Lamiales (Supplementary information Fig. 6d). We identified 27,658, 18,325 and 13,273 putative orthologs between the *A. schaueriana* reference transcriptome and transcriptomes derived from *A. marina* leaves[52], *A. officinalis* leaves[53] and roots[54], respectively (Supplementary information Table S6).

Comparative transcriptomics between trees from contrasting latitudes

To identify the effects of environmental variation in gene expression and, thus in phenotypes, at contrasting source sites in the field, we compared expression levels in trees under field under conditions as comparable as possible. Sampling was conducted during the low tide, from 10:30 AM to 4:00 PM, at the end of winter at the subtropical site and in the beginning of the dry season at the equatorial site (Supplementary information Table S1). As expected, we observed a consistent source-site pattern in overall transcript expression levels from leaves and stems (Supplementary information Fig. S6a-b). However, flowers transcript expression levels did not show a clear pattern among samples from the same origin (Supplementary information Fig. S6c), leading to the identification of only one DET. Thus, we did not include flowers in the subsequent analyses (Supplementary information Fig. S6f). Conversely, 1,837 and 904 transcripts showed significantly different (FDR < 0.05) relative abundance between equatorial and subtropical samples in leaves and stems, respectively (Supplementary information Fig. S6d-e). Among the total 2,741 DETs, 1,150 (41.91%) were putative protein-coding transcripts.

The assignment of transcripts to GO terms was possible for 25,184 (83.31%) of 30,227 putative protein-coding sequences, allowing GO enrichment analyses. Analyses were conducted separately for leaves and stems and for each of two sets of DETs: one showing higher expression levels in equatorial than in subtropical samples (which we refer to as "DET-Eq") and the other showing higher abundance in subtropical than in equatorial samples (which are referred as "DET-St"). We focused on biological processes associated with key aspects of the response to contrasting climate conditions between equatorial and subtropical sites (Table 1, Supplementary information Fig. S1). The enriched processes among the sets of DET included photosynthesis; cell wall biosynthesis; plant responses to UV, temperature stimulus and water stress (Supplementary information Tables S7-S11, Supplementary information Fig. S6i-l).

Photosynthesis: Among the DET-St, we observed various putative genes participating in the biosynthesis of the photosynthetic apparatus, chlorophyll and photoreceptors; the function of electron transporters and chloroplast movement coordination. Contrastingly, the DET-Eq set showed enrichment in transcripts similar to proteins required for disassembling

the light-harvesting complex of photosystem II in thylakoid membranes[55] (Supplementary information Table S11).

Cell wall biosynthesis: Transcripts similar to 33 distinct proteins and transcription factors that play central roles in the biosynthesis and deposition of cell wall components, such as cellulose, hemicellulose, lignin and pectin, were identified among DET-Eq (Supplementary information Table S10).

Response to UV: Both the DET-St and DET-Eq sets showed enrichment in functions related to the response to UV-light, however, the transcript annotations differed between these sets. The DET-St set included putative UV-B protectants and genes involved in UV-B-induced antioxidants biosynthesis, such as plastid copper/zinc superoxide dismutases, photosystem II repair proteins, and L-ascorbic acid. In contrast, the DET-Eq set showed enrichment of transcripts associated with photo-oxidative damage reduction and the positive regulation of anthocyanin biosynthesis in response to UV. Antioxidants induced by UV irradiation[56], such as putative iron superoxide dismutases and pyridoxine biosynthesis genes, were among the DET-Eq (Supplementary information Table S11).

Response to temperature: In the DET-St set, we observed putative genes presenting critical roles in chloroplast protein translation during cold acclimation and that provide tolerance to chilling temperatures[57,58]. For instance, transcripts similar to the *GLYCINE-RICH RNA-BINDING PROTEIN* (*RZ1A*), which has a chaperone activity during cold acclimation[59], and to the cold-inducible ATP-dependent *DNA HELICASE* ATSGS1, required for DNA damage-repair[60]. Interestingly, DET-St included a putative *AGAMOUS-LIKE 24* (*AGL24*) transcription factor, which is involved in vernalisation-induced floral transition[61]. Contrastingly, various transcripts similar to heat shock-inducible chaperones and to *ADENINE NUCLEOTIDE ALPHA HYDROLASE-LIKE PROTEIN* (*AT3G53990*), involved in chaperone-mediated protein folding[62], were among the DET-Eq set, potentially enhancing tolerance to heat in equatorial plants. Additionally, a transcript similar to the *ETHYLENE-RESPONSIVE ELEMENT BINDING PROTEIN* (*RAP2.3*), which confers resistance to heat and hydrogen peroxide[63], was observed in this group (Supplementary information Table S11).

Response to water stress: Transcripts associated with the response and tolerance to water deficits and with cellular ion homeostasis and osmotic adjustment were enriched among DET-Eq. For instance, a transcript similar to the *ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR* (*RAP2.4*), which regulates the expression of several drought-responsive genes, including aquaporins[64,65]. Accordingly, a putative aquaporin *PLASMA MEMBRANE INTRINSIC PROTEIN (PIP1;4)*[66] was found in this set. We observed in DET-Eq putative genes participating in the synthesis of raffinose, an osmoprotective soluble trisaccharide[67], and also transcripts similar to osmosensitive ion channels belonging to the *EARLY-RESPONSIVE TO DEHYDRATION STRESS FAMILY*. Correspondingly, we observed an ion channel, *SLAC1 HOMOLOGUE 3* (*SLAH3*), required for stomatal closure induced by drought stress[68], and the putative *NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3* (*NCED3*), which increases plant resistance to water deficit through the accumulation of abscisic acid (ABA), leading to stomatal closure. Possibly as a consequence of decreased stomatal conductance, a transcript similar to the photorespiration gene, *ALANINE-GLYOXYLATE AMINOTRANSFERASE 2 HOMOLOG 3* (*AT3G08860*)[69], was also observed among the DET-Eq (Supplementary information Table S11).

We confirmed the results obtained in the computational analyses of RNA-Seq data by qRT-PCR of ten DET in leaf samples (Supplementary information Fig. S7, Supplementary information Table S12, Supplementary Note).

Detection of SNP with signs of selection

RNA-Seq enabled the assembly of a reference transcriptome and the identification of biological processes influenced by the environmental divergence over the South American coastline. To complement these analyses, as one cannot disentangle the effects of plasticity and adaptive selection in the differential expression[70,71], we searched for gene sequence variation among trees sampled along the Atlantic coast of South America (Fig. 1, Supplementary information Table S2). After quality filtering of sequenced data, we selected 77 individuals without evidence of interspecific hybridisation with *A. germinans* for downstream analyses. We identified a set of 6,170 high-quality unlinked biallelic SNP loci with a minor allele frequency ≥ 0.05 and ≥ 8x coverage. The overall genetic structure of genome-wide SNPs corroborated a previous study based on putatively neutral loci[22], dividing the species into two populations: north and south of the northeast extremity of South America (Fig. 5).

We observed 122 loci showing considerable departures from neutral expectations of interpopulational divergence, as conservatively detected[72] by pcadapt and LOSITAN. Fifteen out of these loci aligned to *A. schaueriana* transcripts that were similar to gene models in *A. thaliana* and *S. indicum* (Supplementary information Table S13), enabling screening for their potential functional roles. However, five reference proteins did not have informative functions described for the model plant, hindering functional inferences. Conversely, among the remaining annotated candidates, we found five putative genes involved in processes related to contrasting equatorial and subtropical environmental variables (Fig. 6). One candidate locus was detected in the putative transcription factor *RAP2.4*, which is induced in response to water and salt stress[64] and regulates the expression of aquaporins[65]. Two other candidates showed similarity to the transcription factors *ZINC-FINGER PROTEIN 1* (*ZFN1*), involved in the regulation of the expression of several water stress genes[73], and the *HYPOXIA RESPONSE ATTENUATOR* (*HRA1*), strongly induced in response to low oxygen levels[74]. A putative *UDP GLUCOSYL TRANSFERASE*, an enzyme that catalyses the final step of anthocyanin biosynthesis wherein pigmentation is produced[75], also showed evidences of positive selection. Additionally, one candidate locus was in a transcript similar to a *TETRATRICOPEPTIDE REPEAT PROTEIN* (*AT2G20710, TPR*), which might play a role in the biogenesis of the photosynthetic apparatus[76].

Discussion

Studies that integrate genomic and ecophysiological approaches to investigate local adaptation in dominant tropical trees are virtually absent in the academic literature. We used complementary ecophysiological and molecular approaches to test the hypothesis that latitudinal variation in climate regimens shape genetic and phenotypic variation involved in resource-use strategies in widespread coastal trees, using the mangrove *Avicennia schaueriana* as a model. Overall, our results supported this hypothesis. In a common garden experiment we detected differences between plants from contrasting latitudes in key ecophysiological traits involved in carbon and water balances, indicating an inheritable basis of trait divergence. Accordingly, transcriptomic changes between plants sampled in contrasting latitudes showed enrichment in processes associated with central aspects of the environmental divergence across latitudes, such as responses to temperature, UV light, water deficit, photosynthesis and cell wall biosynthesis (Supplementary information Fig. S6i-l). The relevance of these biological processes in the field was corroborated by the identification of SNP loci with signs of selection present within putative coding transcripts similar to genes involved in response to osmotic stress, accumulation of anthocyanins and photosynthesis, unveiling a genetic basis of environmental adaptation. Figure 6 summarises the integration of

three independent approaches that converges to suggest population adaptation to contrasting environments over the species range.

Evidences of a conservative resource-use strategy in the equatorial population of A. schaueriana

Traits exhibited by equatorial plants compared to subtropical plants in the common garden experiment, such as smaller leaf size and angle, higher levels of red light-reflecting pigments, narrower vessels and lower rates of stomatal conductance, limit carbon acquisition[77] and may have imposed constraints to carbon gain in equatorial plants. Accordingly, these plants also accumulated less biomass than subtropical plants (Fig. 2-4, Supplementary information Fig. S3). Despite causing limitations in growth, such characteristics allow plants to maintain suitable leaf temperature for photosynthesis while reducing the risk of cavitation, UV exposure and water loss through the minimisation of evaporative cooling[77]. Given the relevance of these traits to water and carbon balances, especially under highly saline environments, the prevalence of more conservative resource-use traits among equatorial samples in the common garden experiment suggested a selection favouring drought tolerance, likely being advantageous during hot-dry seasons in equatorial wetlands (August-December). These seasons frequently present a combination of high light and high vapour pressure deficit (VPD), resulting from high temperature (> 30 °C) and air humidity below 60% (Table 1, Supplementary information Fig. S1). Accordingly, equatorial plants also showed lower transpiration rates than subtropical plants in the common garden (Fig. 4). In addition, 63% of the six-month-old equatorial plants flowered from July-August in the common garden experiment (Supplementary information Fig. S3g), whereas subtropical plants did not flower. This period is consistent with phenological observations reported for *A. schaueriana* in both equatorial[78] and southern subtropical forests[79], and could indicate a genetic basis for the observed variation. Early flowering is a phenotype with complex genetic components, rarely studied in non-model organisms, but is renowned as an adaptive mechanism for maximising chances of reproduction under stressful environments[80].

In their native environment, equatorial plants showed increased expression levels of putative heat-shock proteins, drought-induced ion transporters, aquaporins and genes that play central roles in stomatal closure and photorespiration compared to subtropical plants, likely contributing to heat and drought tolerance. These findings provided multiple lines of evidence of heat and water stress responses. Higher expression of aquaporins and genes involved in the accumulation of organic solutes may contribute to lowering the cellular water potential, while improving drought tolerance in equatorial plants compared to subtropical plants[67,81]. Additionally, higher expression of several transcripts associated with secondary cell wall biosynthesis and thickening, may enhance rigidity of cells and reduce the risk of collapse during dehydration-rehydration cycles in equatorial trees[82]. Moreover, equatorial plants showed lower expression of several putative photosynthesis genes compared to subtropical plants. In response to drought, high-light and heat stress plants minimise the photooxidative damage by reducing the photosynthetic activity via repression of photosynthesis genes[83–86]. Remarkably, evidences of selection were detected in two putative transcription factors, *RAP2.4* and *ZFN1*, which play key roles in the regulation of osmotic stress-response[64,73]. These findings support the hypothesis that seasons marked by high VPD, caused by the combination of high temperatures and low air humidity[87] in the equatorial region, play a pivotal selective pressure for coastal trees populations.

Evidences of an acquisitive resource-use strategy in the subtropical population of A. schaueriana

Subtropical plants showed higher stomatal conductance and transpiration rates, higher levels of green light-reflecting pigments, larger leaf area, wider leaf lamina angle and larger xylem vessel diameter than did equatorial plants in the common garden experiment (Fig. 2-4, Supplementary information Fig. S3). These characteristics enhance light energy absorbance and carbon acquisition, at the expense of a higher cavitation risk[88,89]. Conversely, this apparent riskier strategy may compensate for seasonal declines in growth resulting from decreasing temperature, photoperiod and light quality in higher-latitudes[90] (Table 1, Supplementary information Fig. S1). Although low temperatures reduce enzymatic activity[91] and, thus, plant growth, the severity of low-temperature stress in southernmost subtropical mangrove forests of the Americas is likely insufficient to favour the selection of freezing-tolerant adaptations, in contrast to results reported for mangroves at their northernmost edge on the Atlantic coast of the Americas[92]. At the southernmost limit of American mangrove forests, minimum air temperature does not drop below 0 ℃ (Table 1, Supplementary information Fig. S1) and remains within than the expected mangrove physiological thresholds[93]. Additionally, the small population size of *A. schaueriana* at this location[27] and the arrival of maladapted propagules from northerly populations likely reduce the potential strength of selection favouring cold-adaptive traits.

Corroborating the observed differences in morphophysiological traits, we found divergence at the molecular level that may also relate to the increasing amplitude of variation in photoperiod and light quality towards high latitudes. Plants optimise the use of light energy and adjust photosynthetic activity through the regulation of light-harvesting and photosystem-component genes[83]. Thus, the higher expression levels of transcripts associated with photosynthesis in subtropical than in equatorial plants, may have facilitated the absorption of light energy in subtropical plants during winter. Although solar irradiance levels were indistinguishable between sampling sites at the time of sampling (Supplementary information Table S1), transcriptomic changes in putative UV-inducible antioxidant and photodamage repair genes suggest the use of distinct strategies to respond to differential seasonality in photoperiod and light intensity between subtropical and equatorial latitudes. Notably, we observed signatures of selection in two transcripts, one showing similarity to a *UDP-GLUCOSYL TRANSFERASE*, a key enzyme in the anthocyanin biosynthesis pathway, and the other in a transcript similar to a *TPR* protein, required for chlorophyll biosynthesis and for the biogenesis of the photosynthetic apparatus[76]. These results imply that light availability, in addition to VPD, may act as an environmental factor driving selection in *A. schaueriana*.

Although soil oxygen availability affects plant growth in intertidal areas, we did not focus our experiments on the relevance of hypoxia in shaping adaptive divergence in coastal wetlands[94]. However, evidence of selection was detected in a transcription factor highly induced by oxygen deprivation (*HRA1*)[74]. Additionally, the *HRA1* putative homolog also showed a 1.75-fold higher expression in subtropical leaves relative to that in equatorial leaves (Supplementary information Table S1), even though sampling was conducted during the low tide at both sites. As tidal amplitude reduces with increasing latitude along the Atlantic coast of South America[95] (Table 1, Fig. 6), trees are exposed to increasing anoxia conditions southwards. These findings suggest that subtropical populations may have better stress preparedness to hypoxia in comparison to equatorial populations.

Climate change and conservation implications of the results

Our results provide compelling evidences that adaptations to contrasting seasonality in light availability and VPD are involved in the organisation of diversity of the dominant coastal tree, *A. schaueriana*. The functional divergence described herein might differentially affect the sensitivity of northerly and southerly populations to a rapidly changing climate. It

has been suggested that southernmost mangrove forests of the Americas could expand polewards in the near future[96]. We expect that the observed acquisitive resource-use characteristics may indeed favour subtropical trees growth under an increased CO_2 concentration, temperatures and rainfall, as predicted to this region by 2100[97]. However, a great landward relocation of subtropical populations will be necessary to their persistence in the face of rising sea level[98], due to the narrowing of intertidal zones towards mid-latitude areas in the Atlantic coast of South America (Table 1). Even though subtropical populations apparently show a better preparedness to hypoxia in comparison to equatorial populations, this scenario is aggravated by the dense coastal occupation by urban and agricultural areas, which may preclude the landward migration of subtropical mangroves in South America. Contrastingly, equatorial populations frequently have wider plains potentially available for expansion and lower human occupation, but might be threatened by increased VPD during more intense El Niño-Southern Oscillation events[97]. Increased temperature will likely stimulate both respiration[99] and photorespiration[100] and might offset benefits in carbon acquisition caused by increased CO_2 concentration[101]. The critical temperature threshold for photosynthesis may be overcome more frequently, possibly reducing carbon assimilation and productivity[102]. With a conservative resource-use strategy, further limitations in net carbon assimilation could lead to biomass loss or tree mortality triggered by carbon starvation[103]. This scenario could be especially severe in the semi-arid northeast South American coast, particularly in the face of intense human use of adjacent plains and decreasing trends in terrestrial freshwater availability and coastal freshwater input[104,105].

Our results corroborate previous studies of marine species, which associated limited north-south population connectivity with adaptation to latitudinal environmental dissimilarity[19–21]. As widespread coastal trees distributed along the Atlantic coast of South America show an overlapping north-south organisation of genetic variation[22–24], we hypothesise that they may also show divergent adaptation to heterogeneous resource availability over their distribution ranges. Conservation efforts of coastal ecosystems should focus on resilient habitat-forming species, which give shelter and act as climate rescuers for several stress-sensitive species[106]. We recommend that populations of foundation trees occurring north and south from the northeast extremity of South America should warrant attention as distinct conservation management units[107] for the long-term persistence of coastal biodiversity and the ecosystem services they provide.

Conclusions

Studies on local adaptation in tropical trees are frequently limited by difficulties in implementing traditional approaches, as reciprocal transplants, due to their long generation time and the lack of basic biological information, as knowledge of their evolutionary history, reproductive mode or phenological patterns. In developing countries, additional challenges often include budget and logistic issues, as infrastructure limitations and violence risk during fieldwork. Despite limitations, we provided unprecedented ecophysiological and genomic evidences that support the hypothesis that seasonal environmental heterogeneity in light availability and VPD may shape the variation of traits associated with resource-use in *A. schaueriana*, a widespread tree in the Atlantic coastline of South America. The non-neutral trait diversity suggested from the interdisciplinary approach used here provides a perspective into the molecular and phenotypic scales at which environmental selection may shape functional variation of this dominant species, at a continental scale. Thus, we reinforce that the power of our conclusions arise from the combination of independent layers of biological information, rather than from an extensive sampling in each independent approach. Integrating high-throughput sequencing and ecophysiological approaches, has shown to be a promising strategy in the study of adaptation of non-model, long-lived species. To provide

579 more realistic predictions of how dominant coastal trees may respond to current global
580 climate changes we encourage the development of further studies accounting for
581 phenotypically[14] and genetically[108] informed distribution modelling. Since freshwater
582 availability has been decreasing in coastal areas worldwide[104], strongly compromising the
583 productivity of coastal plants[105], such studies should focus on biological variation involved in
584 balance between carbon gain and water loss. This knowledge can improve predictions on the
585 future of ecosystems they form and generate key information for forests conservation and
586 management efforts[14,109]. Without the realization and dissemination of such studies, the
587 success of conservation plans for tropical forests and their potential to mitigate climate
588 change will likely be seriously compromised.

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602 **Author contributions**

603 A.P.S., R.S.O., G.M.M. and M.V.C. designed the study. M.V.C. and G.M.M. conducted
604 fieldwork. M.V.C. and C.S.M. cultivated seedlings and performed analyses of
605 morphophysiological data. M.V.C. prepared samples and performed RNA-sequencing.
606 M.V.C., M.D., D.H.O. and G.M.M. analysed RNA-Seq data. C.C.S. and M.V.C. verified
607 RNA-Seq data through qRT-PCR. G.M.M. prepared samples and performed genotyping of
608 genome-wide SNP. G.M.M. and M.V.C. analysed nextRAD results. A.P.S, M.I.Z., G.M.M.
609 and R.S.O. contributed material/reagents/analysis tools. M.V.C. and G.M.M. wrote the
610 manuscript. All authors discussed the results and contributed to the manuscript.

611 **Competing interests**

612 The authors declare no competing interests.
613

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896

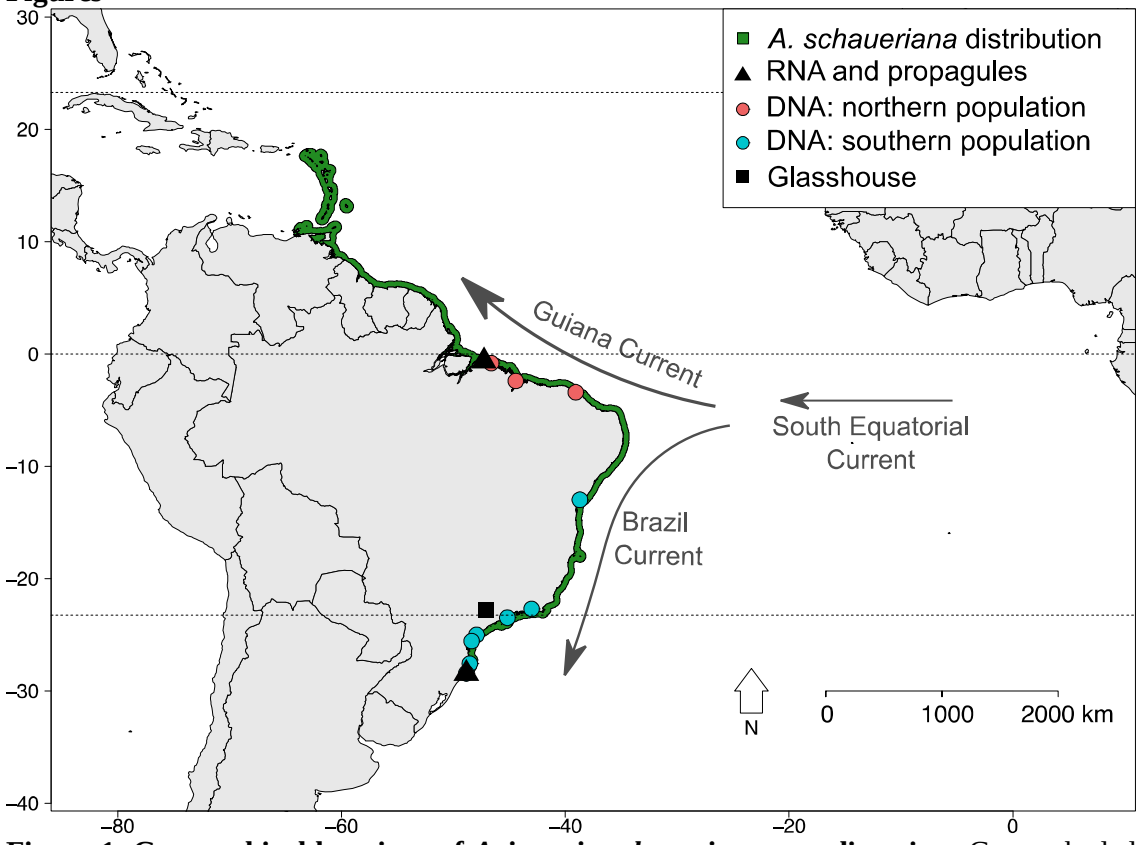
897 **Tables**
898 **Table 1.** Characterisation of subtropical and equatorial sampling sites of propagules used in
899 the common garden experiment and of RNA used for RNA-sequencing. SD: standard
900 deviation of the mean.

	Subtropical	Equatorial
Köppen-Geiger climate characterisation	Temperate oceanic with hot summer, without a dry season (Cfa)	Tropical monsoon (Am)
Latitude (°)	28 S	0
Annual Average tidal amplitude (m)[†]	0.45 (Microtidal)	4.00 (Macrotidal)
Annual mean temperature (°C)[‡]	20.09	26.42
Minimum temperature of the coldest month (°C)[‡]	11.76	22.04
Maximum temperature of the warmest month (°C)[‡]	28.66	31.1
Annual precipitation (mm)[‡]	1,435	2,216
Precipitation in the driest month (mm)[‡]	88	4

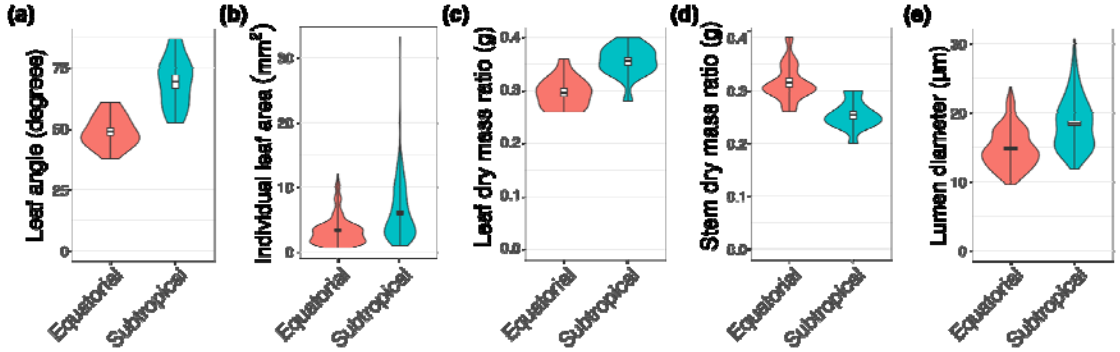
Precipitation in the wettest month (mm)[‡]	162	452
Mean air VPD (KPa)[‡]	1.95	2.82
Maximum air VPD (KPa)[‡]	2.47	2.95
Minimum air VPD (KPa)[‡]	1.48	2.71
Mean irradiance (KJ m^{-2} day^{-1})[‡]	14,270	17,414
Maximum irradiance (KJ m^{-2} day^{-1})[‡]	20,802	21,671
Minimum irradiance (KJ m^{-2} day^{-1})[‡]	8,201	13,874
Mean sea surface salinity (g/kg)[§]	35.50	34.96
Sea surface salinity in the saltiest month (g/kg)[§]	36.24	36.87
Sea surface salinity in the freshest month (g/kg)[§]	33.73	32.54
Average day length (hours (± SD))[¶]	12.103 (±1.251)	12.115 (±0.033)
True mangrove species in the area	*Avicennia schaueriana* *Laguncularia racemosa*	*Avicennia germinans* *Avicennia schaueriana* *Laguncularia racemosa* *Rhizophora mangle* *Rhizophora racemosa* *Rhizophora harriisoni*

VPD: Vapour pressure deficit. [†]Source: Vestbo *et al*. (2018). [‡]Source: BioClim[111]. [§]Source: MARSPEC[112]. [¶]Source: 'daylength' function from R package 'geosphere'[113].

907 **Figures**



908
909 **Figure 1. Geographical locations of *Avicennia schaueriana* sampling sites.** Green-shaded
910 area represents the geographical distribution of the species. Black triangles represent the
911 locations of the equatorial and subtropical sampling sites for propagules and plant tissues
912 used in the common garden experiment and RNA-sequencing, respectively. Coloured dots
913 represent sampling sites of leaves used for the nextRAD genome-wide population diversity
914 analyses (red: population located north of the northeast extremity of South America
915 (NEESA); blue: population located south of the NEESA). Arrows approximately represent
916 the directions of the major sea currents along the Atlantic coast of South America.
917



918
919 **Figure 2. Pair-wise comparison of morphological traits between *Avicennia schaueriana***
920 **seedlings from equatorial and subtropical sites grown under a common garden**
921 **experiment.** Violin plots represent the distribution of observations for plants from equatorial
922 (red) and subtropical (blue) sampling sites. Box plots represent the mean, standard error, and
923 maximum and minimum values. For all variables represented in the figure, the absence of
924 difference between groups was rejected using the unpaired Mann-Whitney-Wilcoxon U-tests

(Wilcox test) or Student's T-test (T-test), at a significance threshold of 0.05. (a) leaf inclination angle (n = 15 leaves per group, 5 plants per group, T-test p-value = 1.02e-06); (b) individual leaf area (n = 250 leaves per group, 3 plants per group, Wilcox test p-value < 2.20e-16); (c) leaf dry mass ratio (leaf dry biomass/plant dry biomass) (n = 15 plants per group, T-test p-value = 7.17e-06); (d) stem dry mass ratio (stem dry biomass/plant dry biomass) (n = 15 plants per group, T-test p-value = 1.38e-05); (e) vessel lumen diameter (n = 700 vessels per group, 3 plants per group, Wilcox test p-value < 2.20e-16).

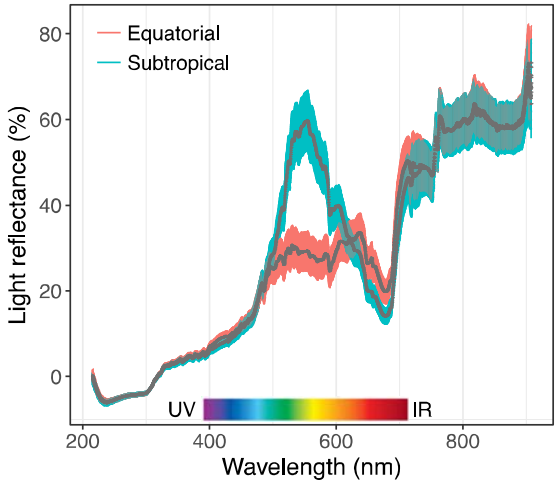


Figure 3. Light reflectance of the stem epidermis of five-month-old *Avicennia schaueriana* seedlings grown in a common garden experiment. Grey lines represent the mean reflectance, and colour-shaded areas represent the standard error for each seedling source site (red: equatorial; blue: subtropical, n = 10 plants per group). The visible light spectrum range is highlighted in the figure.

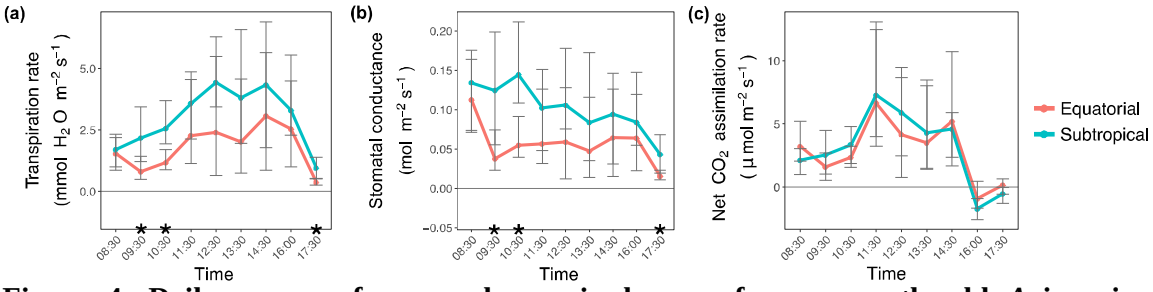


Figure 4. Daily curves of gas exchange in leaves of seven-months-old *Avicennia schaueriana* seedlings grown in a common garden. * represents rejection of the null hypothesis of the absence of a difference based on the unpaired Student's t-test at a significance threshold of 0.05 (n = 10 plants per group). Red line: equatorial origin; blue line: subtropical origin. (a) transpiration rate; (b) stomatal conductance; (c) rate of net CO_2 assimilation.

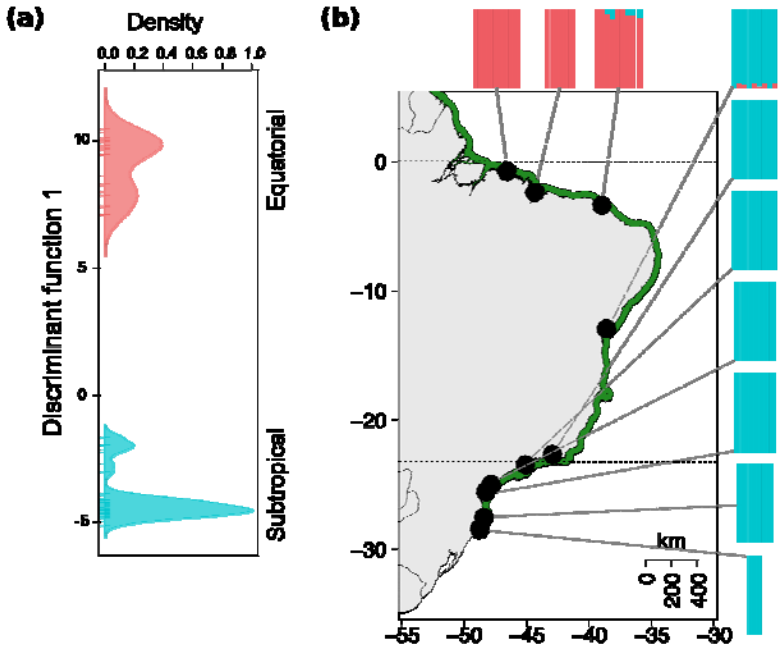


Figure 5. Population structure of *A. schaueriana* inferred from genome-wide genotyping of single nucleotide polymorphic (SNP) loci. Population structure of *Avicennia schaueriana*, inferred from genome-wide genotyping of single nucleotide polymorphic (SNP) loci. (a) Density of individuals on the first retained discriminant function, calculated using a multivariate method, the discriminant analysis of principal components (DAPC); different colours represent inferred populations; (b) map showing the distribution of the species (green shaded area), the geographical location of sampling sites (points) and barplots in which each colour denotes an ancestral cluster and each line represents an individual as inferred by the model-based method implemented in Admixture 1.3.0.

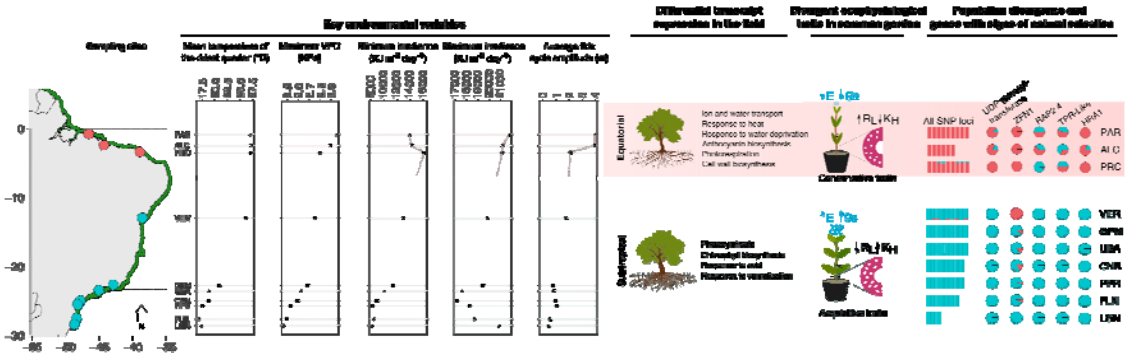


Figure 6. Graphical summary of the integration of ecophysiological and molecular approaches performed in this work. Strong variation in key oceanographic and climatic factors are observed across the broad latitudinal gradient of the Atlantic coast of South America, where dominant trees share a north-south organisation of the neutral genetic diversity. Besides the influence of sea currents in propagules dispersion, the establishing success of propagules over the latitudinal range may play a role in shaping the diversity of genotypes and phenotypes of dominant coastal plants, possibly leading to the evolution of local adaptation. To address this issue, we chose the widespread mangrove, *Avicennia schaueriana*, as a representative species. We combined the assessment of ecophysiological resource-use traits in plants from contrasting latitudes in a common garden experiment,

970 comparative transcriptomics in trees sampled under contrasting latitudes, and natural
971 selection detection tests based on single nucleotide polymorphisms (SNP). Plants from
972 equatorial and subtropical latitudes showed key divergences related to the use of water and to
973 the acquisition of carbohydrates both in the field and in common garden conditions. In
974 addition, north-south genetic divergence was observed in all genotyped SNPs. We also
975 identified signatures of differential selective pressures on specific loci associated to the
976 accumulation of anthocyanins (UDP-GLUCOSYLTRANSFERASE), the response to osmotic
977 stress (RAP2.4 and ZFN1), photosynthesis (TPR) and hypoxia (HRA1). The molecular and
978 ecophysiologic divergences observed through three independent approaches suggest the
979 existence of a north-south divergence in resource-use strategies, likely driven by air water
980 vapour and light availability. These findings highlight the power of using multidisciplinary
981 approaches for the study of adaptation in species for which little basic biological information
982 is available, such as tropical coastal trees. VPD: atmospheric vapour pressure deficit; E:
983 transpiration rate; g_s: stomatal conductance; R_L: xylem vessel lumen resistivity; K_H: xylem
984 vessel conductivity.
985
986