Title

Local adaptation insights from genomics and ecophysiology of a neotropical mangrove

Short title

Local adaptation of a neotropical mangrove

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Abstract

Integrating genomic and ecological data is instrumental for understanding the mechanisms of adaptive processes in natural ecosystems. In non-model species, such studies can be particularly challenging but often yield results with implications for conservation. Here, we integrate molecular and ecophysiological approaches to assess the role of selection in the north-south organisation of genetic variation in the mangrove species *Avicennia schaueriana*, a new-world tree found in tropical to temperate coastal forests along the Atlantic coast of the Americas. We found substantial divergences between populations occurring north and south of the north-eastern extremity of South America, possibly reflecting the roles of contrasting environmental forces in shaping the genetic structure of the species. In a common garden experiment, individuals from equatorial and subtropical forests were found to be divergent in traits involved in water balance and carbon acquisition, suggesting a genetic basis of the observed differences. RNA-sequencing highlighted the molecular effects of different light, temperature and air humidity regimes on individuals under field conditions at contrasting latitudes. Additionally, genome-wide polymorphisms in trees sampled along most of the species' range showed signatures of selection in sequences associated with the biogenesis of the photosynthetic apparatus, anthocyanin biosynthesis and osmotic and hypoxia stress responses. The observed functional divergence might differentially affect sensitivities of populations to our changing climate. We emphasize the necessity of independent conservation management for the long-term persistence of the species' diversity. Moreover, we demonstrate the power of using a multidisciplinary approach in adaptation studies of non-model species.

Keywords

Adaptation genomics, comparative transcriptomics, plant ecophysiology, Avicenniaceae

Introduction

Adaptation to contrasting environments is an ubiquitous consequence of divergent selective forces acting on intraspecific phenotypic diversity (Hereford, 2009; Kawecki & Ebert, 2004). Phenotypic variation can be achieved through plasticity during acclimation to environmental changes or through genetic variation shaped by adaptive processes (Albert, Grassein, Schurr, Vieilledent, & Violle, 2011). Though the occurrence of intraspecific divergence in adaptive traits is well recognised, its molecular basis is not yet fully understood (Savolainen, Lascoux, & Merilä, 2013). The integration of multiple independent approaches to understand the bases of adaptive variation is desirable to minimise the potential for incorrect conclusions (Barrett & Hoekstra, 2011).

In addition to being of fundamental relevance in the field of evolutionary biology, research on the molecular mechanisms underlying adaptive variation may provide valuable information for safeguarding the persistence of populations under environmental challenges. Especially, accelerated rates of contemporary climate change call for studies on functional variation and its consequences for species responses to future climate (Moran, Hartig, & Bell, 2016). Climate change forecasts include a rise in atmospheric CO_2 concentrations up to 550-1000 ppm, leading to a 0.3-4.8 °C increase in mean air temperature, a 0.26-0.82 m sea-level rise and great changes in precipitation regimes by 2100 (Pachauri & Meyer, 2014). These changes are projected to especially affect certain ecosystems, such as mangrove forests (Loarie et al., 2009), since they are distributed in narrow intertidal environments in tropical and subtropical zones and are naturally limited by annual minimum temperatures and average rainfall (Osland et al., 2017). Recent changes have already promoted shifts in the distribution of mangroves and in the density of individuals in populations (Cavanaugh et al., 2014; Duke et al., 2017; Lovelock et al., 2015; Shearman, Bryan, & Walsh, 2013). Negative impacts are

predicted mainly in regions in which aridity is expected to increase and where adjacent areas for expansion are unavailable or do not exist (Alongi, 2015). However, in some regions, mangroves might persist through their ability to adjust soil elevation (Lovelock et al., 2015; McKee, Cahoon, & Feller, 2007) and to rapidly shift their distributions to new suitable areas (Alongi, 2015; López-Medellín et al., 2011; Lovelock et al., 2015).

Yet, these predictions do not account for extant intraspecific variability across the geographical distribution of a given species. For instance, the genetic diversity of all mangrove species studied to date is structured into two populations occurring north and south of the northeast extremity of South America (NEESA) (Francisco, Mori, Alves, Tambarussi, & Souza, 2018; Mori, Zucchi, & Souza, 2015). In the NEESA region, the "South Equatorial Current" bifurcates into "Guiana Current" and "Brazil Current", dispersing mangrove propagules in opposite directions, likely reducing the gene flow between populations (Francisco et al., 2018; Mori et al., 2015; Takayama, Tateishi, Murata, & Kajita, 2008) (Fig. 1). As limited gene flow is a key process determining the magnitude of local adaptation, one could expect these populations to adapt differently to their environments (Kawecki & Ebert, 2004; Savolainen, Pyhäjärvi, & Knürr, 2007). However, the neutral nature of the molecular markers used in previous works precludes inferences regarding environment-driven genetic divergence.

Advances in DNA sequencing now permit the identification of neutral and putatively adaptive genetic variation even in non-model organisms, such as mangrove trees (Andrews, Good, Miller, Luikart, & Hohenlohe, 2016). Here, we used these tools to investigate the non-neutral divergence between previously identified populations of the new-world mangrove tree *Avicennia schaueriana* Stapf & Leechman ex Moldenke. We further explored the potential role of environmental forces underlying such divergence along the Atlantic coast of South

America. *Avicennia schaueriana* is the most widely distributed mangrove species for which genetic diversity information is available over its geographic range. The species is found in the Lesser Antilles (~16 °N) and from Venezuela to the southernmost mangrove forests in the Atlantic (~28 °S) (Soares, Estrada, Fernandez, & Tognella, 2012). To avoid generating incorrect conclusions about the targets of selection (Barrett & Hoekstra, 2011), we integrated three independent but complementary molecular and ecological approaches: (1) comparative physiology of equatorial and subtropical samples grown in a common garden experiment; (2) comparative transcriptomics of trees from equatorial and subtropical localities, sampled in their native environments (Table 1, Supplemental Fig. 1); and (3) genome scans for signatures of selection using high-throughput genome-wide genotyping of individuals, sampled along almost the entire distribution of the species (Fig. 1). We discuss the implications of our results in the context of rapidly changing climate and suggest strategies for mangrove conservation along the Atlantic coast of South America.

Materials and Methods

Propagule sampling

Mature propagules were collected from ten *A. schaueriana* mother trees, at least 100 m apart from each other, from each of two natural populations described in a previous work (Mori et al., 2015): (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 (Kjerfve et al., 2002; Souza-Filho et al., 2006), near the northernmost limit of the species range (Fig. 1). We refer to samples collected in the former site as 'Subtropical' and in the latter as 'Equatorial' throughout this work. A detailed characterisation of each site

can be found in Table 1 and in the Supplemental Information file (Supplemental methods S1, Supplemental Fig. 1).

Comparative ecophysiology in a common garden experiment

Propagules of *A. schaueriana* sampled from the Equatorial and Subtropical sites were germinated as described for *Avicennia germinans* (Reef et al., 2014). After two months, 44 similar-sized seedlings from each sampling site—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 automatically irrigated (daily at 10 a.m. and 5 p.m.) with a 3-minute fresh water 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 (Supplemental Fig. 2), none of the individuals benefitted from conditions that corresponded to those of their origins.

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 (Schneider, Rasband, & Eliceiri, 2012). 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 from slides 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), proportion of solitary vessels (number of solitary vessels/total number of vessels), vessel grouping index (mean number of vessels per vessel grouping), vessel lumen area ratio in xylem (vessel lumen area/xylem area) and vessel lumen area in sapwood (vessel lumen area/sapwood area). The vessel arithmetic diameter (D), vessel hydraulic conductivity (K_H) and lumen resistivity (R_L) were estimated according to Scholz et al. (Scholz, Klepsch, Karimi, & Jansen, 2013).

Statistical comparisons between Equatorial and Subtropical samples were performed in R 4.0.0 using the Mann-Whitney-Wilcoxon unpaired test for non-parametric distributions and unpaired Student's t-test for parametric distributions with 5% significance level. Multiple-group comparisons were conducted using one-way analysis of variance (ANOVA) with post hoc Tukey honest significant difference (HSD) tests.

Plant material for RNA extraction and RNA-sequencing

Plant material used for RNA-sequencing (RNA-Seq) was collected in the sites described in the "Propagule sampling" section. Leaves, stems and flowers from three adult trees at least 100 m apart were collected in each site 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. A detailed description of environmental conditions at the time of sampling is available in Supplemental Table 1. Sampling occurred from 11:00 am to 4:00 pm during the low tide at different altitudes in the intertidal zone. Plant material was washed with sodium hypochlorite solution (0.2%) and immediately stored in RNAlater (Ambion Inc.).

We extracted RNA according to Oliveira et al. (Oliveira, Viana, Reátegui, & Vincentz, 2015) 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 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.) followed by sequencing using two 36-cycle TruSeq SBS Paired End kits (Illumina Inc.) and a Genome Analyzer IIx sequencer (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(Patel & Jain, 2012). High-quality reads were used for subsequent transcriptome assembly in the CLC Genomics Workbench (https://www.qiagenbioinformatics.com/). We used the default settings, except for the distance between read pairs (300-500 bp) and k-mer size (45 bp).

Reads were mapped to the assembled transcripts using bowtie1 (Langmead, Trapnell, Pop, & Salzberg, 2009) in the single-read mode using the default parameters. Transcripts without read-mapping support were removed. Functional annotation was performed using blastx v.2.2.31 (Camacho et al., 2009) with an e-value < 1^{-10}. The NCBI RefSeq (O'Leary et al., 2016), The Arabidopsis Information Resource (TAIR) (Berardini et al., 2015) and the NCBI non-redundant (nr) databases were used as references. To minimize contaminants, we excluded all transcripts that were exclusively similar to non-plant sequences. Protein family domains were identified using HMMER3 (Finn et al., 2014), which iteratively searched all assembled sequences against the Pfam database. To assign Gene Ontology (GO) terms to transcripts, we used the *Arabidopsis thaliana* gene association file from the Gene Ontology Consortium (Blake et al., 2015) and retrieved the information for transcripts with similar coding sequences in the genome of *A. thaliana*. Redundant transcripts were clustered using CD-HIT-EST v.4.6.1 (Li & Godzik, 2006) using the local alignment mode with 95% identity and 70% coverage of the shortest sequence thresholds. Open reading frames (ORF) in putative protein-coding transcripts 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 (Simão, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015). Additionally, a reciprocal blastn alignment (Camacho et al., 2009) 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 (Langmead et al., 2009) and normalised using edgeR (Robinson, McCarthy, & Smyth, 2010). Differentially expressed transcripts (DETs) between tissue-specific samples of trees at the Equatorial and Subtropical sites 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 (Young, Wakefield, Smyth, & Oshlack, 2010) with the Wallenius approximation method and P-value < 0.05. Differential expression results were verified using reverse transcription real-time PCR (qRT-PCR) (Supplemental methods S2).

***Detection of candidate adaptive loci in* A. schaueriana**

We sampled leaves from 79 adult plants at ten locations, spanning most of the geographic range of *A. schaueriana* (Fig. 1, Supplemental Table 2). 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 (Russello, Waterhouse, Etter, & Johnson, 2015) were prepared and sequenced by SNPsaurus (SNPsaurus) in a HiSeq 2500 (Illumina, Inc.) with 100 bp single-end chemistry. Briefly, genomic DNA fragmentation and short adapter ligation were performed with Nextera reagent (Illumina) followed by amplification with one of the primers matching the adapter and extending nine arbitrary nucleotides into the genomic DNA. Thus, the resulting amplicons were fixed at the selective end, and their lengths were dependent on the initial Nextera fragmentation, leading to

consistent genotyping of the amplified loci. Assembly, mapping and single nucleotide polymorphic loci (SNP) identification were performed using proprietary 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 (Danecek et al., 2011). 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* (data not published), we assessed the genetic structure in *A. schaueriana*, considering all SNPs, using discriminant analysis of principal components (DAPC) (Jombart, Devillard, & Balloux, 2010) and ADMIXTURE v.1.3.0 (Alexander, Novembre, & Lange, 2009). For DAPC analyses, we considered the number of groups varying from 1 to 50 and the Bayesian information criteria for inferring the number of groups (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 between 1 and 15, using the block-relaxation method for point estimation; computing was terminated when estimates increased by < 0.0001, and the most likely K-value was determined by cross-validation.

We used methods implemented with two programs to minimise false-positive signs of natural selection: LOSITAN (Antao, Lopes, Lopes, Beja-Pereira, & Luikart, 2008), 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

(Luu, Bazin, & Blum, 2016), which simultaneously identifies the population structure and the loci excessively related to this structure, using FDR < 0.1.

The sequences presenting SNP with putative evidence of selection were identified simultaneously by pcadapt and five independent runs of LOSITAN and were searched within expressed regions of the reference transcriptome. A reciprocal alignment between the short sequences obtained through nextRAD (75 bp) and the longer expressed sequences assembled from RNA-Seq data (≈ 300-11600 bp) was performed. The alignment was conducted using blastn v.2.2.31 (Camacho et al., 2009), with a threshold of at least 50 aligned nucleotides, a maximum of one mismatch and no gaps.

Results

Comparative physiology in a common garden experiment

To identify functional divergence between individuals from Equatorial and Subtropical sites, we conducted a common garden experiment in a glasshouse under a homogeneous, non-climate-controlled environment. We observed differences in key morphophysiological traits between seedlings from contrasting latitudes (Fig. 2 and 3). Subtropical plants showed higher transpiration rates and stomatal conductance than did Equatorial plants (Supplemental Fig. 3). Additionally, the inclination angle of the leaf lamina was smaller and the average size of individual leaves was smaller in Equatorial than in Subtropical plants, but the total leaf area and specific leaf area did not significantly differ between the groups (Fig. 2, Supplemental Table 3, Supplemental Fig. 4). Subtropical plants accumulated more biomass in all vegetative organs (leaves, stems and roots) than did Equatorial plants. However, the stem dry mass ratio (stem dry biomass/plant dry biomass) was greater in Equatorial plants, whereas the leaf dry mass ratio (leaf dry biomass/plant dry

biomass) was greater in Subtropical plants. The root dry mass ratio (root dry biomass/plant dry biomass) was indistinguishable between groups (Supplemental Table 3). Unexpectedly, 63% of the Equatorial plants flowered after six months of growth (Supplemental Fig. 4g). Since this was not observed in any Subtropical plant, flowering plants were not used in the biomass allocation analysis.

Stems from Subtropical samples showed wider vessel diameter than did those from Equatorial samples, but vessel density was not significantly different between the groups (Fig. 2, Supplemental Fig. 4h-i). The sapwood space dedicated to vessel lumen area was greater in Subtropical than in Equatorial plants. Vessels of Subtropical plants showed higher conductivity, at a detriment of hydraulic safety, than did those of Equatorial plants (Fig. 2). The total conductivity of the stems was not significantly different between sample groups. Plants from the contrasting origins 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 used RNA-Seq (Z. Wang, Gerstein, & Snyder, 2009) to obtain a *de novo* assembled transcriptome for *A. schaueriana* from leaves, stems and flowers of six adult individuals from Equatorial and Subtropical sites (Supplemental Fig. 5). Over 209 million paired-end 72 bp reads showing high quality were assembled into a reference, non-redundant transcriptome containing 49,490 sequences, of which 30,227 (61%) were putative protein-coding sequences. Over 91.9% of these reads were mapped to a single transcript, indicating minimum redundancy and a wide representation of sequenced data (Supplemental Table 4). Moreover, searching for universal plant orthologous

genes revealed that 91.8% of the conserved sequences in plants were present in the reference transcriptome (Supplemental Table 4). Sequences with complete ORF represented approximately 42% (12,796) of all putative protein-coding transcripts, with an average length of 1,324 nucleotides (Supplemental Table 5, Supplemental Fig. 6). Most of these putative protein-coding sequences (94.33%) showed significant similarity to proteins in the Plant RefSeq and TAIR databases (Supplemental Fig. 6c). More than 80% of these protein-coding sequences matched proteins from *Sesamum indicum* or *Erythranthe guttata*, which, as does *A. schaueriana*, belong to the order Lamiales (Supplemental 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 (Huang et al., 2014), *A. officinalis* leaves (Lyu, Li, Guo, He, & Shi, 2017) and roots (Krishnamurthy et al., 2017), respectively (Supplemental Table 6).

Comparative transcriptomics between trees from the Equatorial and Subtropical sites

To identify environmental forces associated with variations in gene expression between source sites, we sampled leaves, stems and flowers from trees under uncontrolled field conditions at the end of winter at the Subtropical site and at the beginning of the dry season at the Equatorial site (Supplemental Table 1). We observed a consistency in transcript expression levels from leaves and stems among plants from the same sampling site (Supplemental Fig. 7a and 7b). However, transcript expression levels in flowers were not concordant among samples from the same origin (Supplemental Fig. 7c), leading to the identification of only one DET; thus, we did not include flowers in the subsequent analyses (Supplemental Fig. 7f). Contrastingly, we identified 1,837 and 904 transcripts showing significantly different (FDR < 0.05) relative abundance in leaves and stems, respectively,

between Equatorial and Subtropical samples (Supplemental Fig. 7d and 7e). 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 of the DETs. GO enrichment analyses were focused on biological processes potentially regulating the responses of *A. schaueriana* to the contrasting climatic variables in the Equatorial and Subtropical sites (Table 1, Supplemental Fig. 1). Analyses were conducted separately for leaves and stems and for each of two sets of DETs: one showed higher expression levels in Equatorial than in Subtropical samples (which we refer to these as DET-Eq) and the other showed higher abundance in Subtropical than in Equatorial samples (which are referred as DET-St). The enriched processes among the sets of DET included photosynthesis; plant responses to UV, temperature stimulus and water stress; cell wall biosynthesis and cellular respiration (Supplemental Tables 7-11, Supplemental Fig. 7i-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 and for triggering chlorophyll degradation (Park et al., 2007) (Supplemental Table 11).

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 (Myouga et al., 2008), such as putative iron superoxide dismutases and pyridoxine biosynthesis genes, were among the DET-Eq (Supplemental Table 11).

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 (Goulas et al., 2006; S. Wang et al., 2016). For instance, transcripts similar to the *GLYCINE-RICH RNA-BINDING PROTEIN* (*RZ1A*), which has a chaperone activity during cold acclimation (Kim, Kim, & Kang, 2005), and to the cold-inducible ATP-dependent *DNA HELICASE* ATSGS1, required for DNA damage-repair (Hartung, Suer, & Puchta, 2007). Interestingly, DET-St included a putative *AGAMOUS-LIKE 24* (*AGL24*) transcription factor, which is involved in vernalisation-induced floral transition (Michaels et al., 2003). Contrastingly, various transcripts similar to heat shock-inducible chaperones and to *ADENINE NUCLEOTIDE ALPHA HYDROLASE-LIKE PROTEIN* (*AT3G53990*), involved in chaperone-mediated protein folding (Jung et al., 2015), 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 (Ogawa et al., 2005), was observed in this group (Supplemental Table 11).

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 (Lin, Park, & Wang, 2008; Rae, Lao, & Kavanagh,

2011), was identified in DET-Eq. Accordingly, a putative aquaporin *PLASMA MEMBRANE INTRINSIC PROTEIN (PIP1;4*) (Alexandersson et al., 2005) was also found in this set. We observed in DET-Eq putative genes participating in the synthesis of raffinose, an osmoprotective soluble trisaccharide (Nishizawa, Yabuta, & Shigeoka, 2008), and also transcripts similar to osmosensitive ion channels belonging to the *EARLY-RESPONSIVE TO DEHYDRATION STRESS FAMILY*. Correspondingly, we observed an ion channel protein, *SLAC1 HOMOLOGUE 3* (*SLAH3*), required for stomatal closure induced by drought stress (A. Zhang et al., 2016), 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 *ALANINE-GLYOXYLATE AMINOTRANSFERASE 2 HOMOLOG 3* (*AT3G08860*), which plays a central role in photorespiration (Liepman & Olsen, 2003), showed higher expression in Equatorial than in Subtropical samples (Supplemental Table 11).

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 (Supplemental Table 10).

Cellular respiration: DET-Eq included one putative enzyme from the tricarboxylic acid cycle encoded in the nuclear genome, *ACONITASE 3* (*ACO3*), which converts citrate to isocitrate, and several mitochondrial genes encoding subunits of NADH dehydrogenases, ATP-synthases and cytochrome C oxidases (Supplemental Table 11).

We confirmed the results obtained in the computational analyses of RNA-Seq data by qRT-PCR of ten DET detected across all leaf samples (Supplemental Fig. 8, Supplemental Table 12, Supplemental Results).

Detection of SNP with signs of selection

To complement the analyses of differential gene expression, which could result from plasticity and adaptive selection (Wolf, Lindell, & Backstrom, 2010), we searched for gene sequence variation among trees sampled along the Atlantic coast of South America (Fig. 1, Supplemental Table 2). After quality filtering of the 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 corroborated a previous study based on putatively neutral microsatellite loci (Mori et al., 2015), dividing the samples into two main groups: north and south of the NEESA (Supplemental Fig. 9).

We observed 122 loci showing significant departures from neutral expectations of interpopulational divergence, as conservatively detected (Ahrens et al., 2018) by both pcadapt and LOSITAN. Fifteen of these loci with putative signs of selection were aligned to *A. schaueriana* transcripts that were similar to gene models in *A. thaliana* and *S. indicum* (Supplemental Table 13), enabling screening for their potential functional roles. However, five of the reference proteins did not have informative functions described for the model plant, hindering inferences regarding their function. Conversely, among the remaining annotated candidates, we found five putative genes involved in processes related to the contrasting equatorial and subtropical environments (Fig. 4). One candidate locus was detected in the putative transcription factor *RAP2.4*, which is induced in response to water and

salt stress and regulates developmental processes mediated by light intensity (Lin et al., 2008) and the expression of aquaporins (Rae et al., 2011), which plays a role in plant water homeostasis. Two other candidates showed similarity with the transcription factors *ZINC-FINGER PROTEIN 1* (*ZFN1*), involved in the regulation of the expression of several water stress genes (Sakamoto, Araki, Meshi, & Iwabuchi, 2000), and *HYPOXIA RESPONSE ATTENUATOR* (*HRA1*), strongly induced in response to low oxygen levels (Giuntoli et al., 2014). A putative *UDP GLUCOSYL TRANSFERASE*, an enzyme that catalyses the final step of anthocyanin biosynthesis wherein pigmentation is produced (Tohge et al., 2005), also showed evidence of positive selection. Additionally, one candidate locus was found in a transcript similar to a *TETRATRICOPEPTIDE REPEAT PROTEIN* (*AT2G20710*, *TPR*), which might play a role in the biogenesis of the photosynthetic apparatus (Bohne, Schwenkert, Grimm, & Nickelsen, 2016).

Discussion

We used complementary ecological and molecular approaches to study non-neutral phenotypic and genetic divergences as well as the contribution of contrasting environmental forces between two populations of the mangrove species *Avicennia schaueriana*. The genetic structure previously detected with a few microsatellite loci between forests occurring north and south of the NEESA (Mori et al., 2015) was confirmed using thousands of genome-wide SNP (Supplemental Fig. 9). Equatorial plants showed morphophysiological and transcriptomic signals that appear to minimise the effects of drought, high light and heat, whereas traits observed in Subtropical plants suggest a maximisation of carbon assimilation, which may be beneficial under low temperature and reduced light regime (Fig. 2-3, Supplemental Table 11). Additionally, putative signs of selection were identified in

transcripts associated with contrasting climate variables between equatorial and subtropical latitudes (Fig. 4).

Traits exhibited by Equatorial plants relative to Subtropical plants in the common garden experiment, such as reduced leaf size, smaller leaf angle, higher levels of red light-reflecting pigments, narrower vessels and lower rates of stomatal conductance, limit carbon acquisition (Reef & Lovelock, 2015) and may have imposed constraints to carbon gain in Equatorial plants, which also accumulated less biomass (Fig. 2-3, Supplemental Fig. 3-4). In contrast, such characteristics allow plants to maintain suitable leaf temperature for photosynthesis while reducing both UV exposure and water loss through the minimisation of evaporative cooling (Reef & Lovelock, 2015; Steyn, Wand, Holcroft, & Jacobs, 2002). We argue that the prevalence of these traits among Equatorial samples may be advantageous in their natural environment, especially during the dry season (from August to December), which presents high light intensity, frequently combined with high temperature (> 30 °C) and air humidity below 70% (Table 1, Supplemental Fig. 1). Under high evaporative demand and saline intertidal soils, water acquisition has an elevated energetic cost, representing strong pressure in favour of water-saving adaptations (Reef & Lovelock, 2015). Accordingly, Equatorial plants also showed lower transpiration rates than did Subtropical plants in the common garden (Supplemental Fig. 4). In addition, 63% of the six-month-old Equatorial plants started flowering from July-August (Supplemental Fig. 4g), which is consistent with phenological observations reported for *A. schaueriana* in equatorial sites (Menezes, Berger, & Mehlig, 2008). However, we found no previous records of six-month-old flowering plants in the literature. Although a flowering peak is observed in August in southern subtropical forests (De Alvarenga, Botosso, & Soffiatti, 2017), Subtropical plants did not flower during the glasshouse experiment. Early flowering is a phenotype with complex genetic components and

is rarely studied in non-model organisms; however it is renowned as an adaptive mechanism for maximising the chance of reproduction under stress (Kazan & Lyons, 2016).

Contrastingly, 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. 2-3, Supplemental Fig. 3-4). These characteristics enhance light energy absorbance and carbon acquisition at the expense of greater water loss and higher cavitation risk (Carlson, Holsinger, & Prunier, 2011; Stuart, Choat, Martin, Holbrook, & Ball, 2006). These traits may compensate for declines in net primary production in higher-latitude environments (Saenger & Snedaker, 1993) that result from restrictions in temperature and solar irradiance (Table 1, Supplemental Fig. 1). We argue that the intensity of cold events in southern subtropical populations of *A. schaueriana* is likely insufficient to favour the selection of freezing-tolerant individuals, in contrast to results reported for *A. germinans* at its northernmost distribution limit on the Atlantic coast of North America (Cavanaugh et al., 2014). At the southernmost range edge of *A. schaueriana*, the minimum air temperature does not drop below 0 □ (Table 1, Supplemental Fig. 1), which is higher than the expected mangrove physiological threshold (Osland et al., 2017). Additionally, the small population size of *A. schaueriana* at this location (Soares et al., 2012) and the arrival of maladapted propagules from northerly populations likely reduce the potential strength of selection favouring cold-adapted individuals.

Functional interpopulation divergence at the molecular level was evident under field conditions. Comparative transcriptomics of trees sampled in their native habitats corroborated the suggested effects of environmental variation in light availability, temperature and water stress on the phenotypic divergence observed in the common garden experiment. The

transcriptomic profiles obtained at the beginning of the dry season in the Equatorial site and at the end of winter in the Subtropical site (Supplemental Table 1) showed an enrichment of DETs involved in photosynthesis, cellular respiration, cell wall biosynthesis and plant responses to water stress, temperature and UV light (Supplemental Fig. 7i-l). The adaptive relevance of these biological processes in the field was highlighted through the identification and functional annotation of SNPs putatively under natural selection from populations along the *A. schaueriana* geographic distribution (Supplemental Table 13). In the following subsections, we integrate information derived from three independent approaches explored in this work.

***Water stress as a key selective pressure in equatorial populations of* A. schaueriana**

The increased levels of transcripts similar to heat-shock proteins, to drought-induced ion transporters, and genes that enhance heat tolerance and play central roles in stomatal closure and photorespiration provided multiple lines of evidence of water stress in Equatorial samples. Additionally, Equatorial plants exhibited higher expression of aquaporins and genes involved in the accumulation of organic solutes than did Subtropical plants. These investments improve tolerance to drought (Nishizawa et al., 2008) by lowering cell water potential and actively transporting water through proteins in the cell membrane rather than using passive apoplastic transport (Krishnamurthy et al., 2014; Reef & Lovelock, 2015). Enhanced rigidity of cells reduces risks of damage during dehydration and rehydration, thereby improving resistance to high extra-cellular osmotic pressure (Gall et al., 2015). Thus, we argue that the higher expression of several transcripts associated with cell wall biosynthesis and cell wall thickening in the Equatorial samples may indicate plant responses to water stress. Further evidences of the relevance of water stress in plants at the Equatorial

site were highlighted by the identification of sequence divergence between northerly and southerly populations in two putative osmotic stress-responsive regulatory transcription factors (*RAP2.4* and *ZFN1*). These findings corroborate with the divergence in traits involved in water balance between plants from Equatorial and Subtropical sites, such as vessel diameter, leaf size, leaf angle and transpiration and stomatal conductance rates (Fig. 2, Supplemental Fig. 3-4).

***Latitudinal variation in light quality and intensity may shape functional diversity in* A. schaueriana**

Contrasting seasonal fluctuations in photoperiod and in light quality between low and high latitudes (Pecot, Horsley, Battaglia, & Mitchell, 2005) (Table 1) likely influence the differential expression observed in putative UV-inducible antioxidant and photodamage repair genes. The adaptive relevance of these findings is supported by the sign of natural selection found in a transcript similar to *UDP-GLUCOSYL TRANSFERASE*, a key enzyme to anthocyanin biosynthesis, which confers protection to UV-B. Divergent morphological traits between Equatorial and Subtropical plants grown in the common garden experiment, including leaf inclination angle and stem light reflectance (Fig. 2 and 3) provide additional insights into *A. schaueriana* light-related adaptations.

Low water and low light availability presumably affect photosynthesis and cellular respiration

In response to abiotic stress conditions such as drought, heat and high light, plants optimise the use of light energy and minimise photooxidative damage by reducing the photosynthetic activity via the repression of light-harvesting and photosystem-component

genes (Bray, 2004; Han et al., 2009; Kimura et al., 2003; Moumeni et al., 2011; D. Wang et al., 2011; C. Zhang et al., 2015). We argue that the lower expression of photosynthesis genes in Equatorial than in Subtropical samples likely is further indicative of the role of water stress in shaping divergent phenotypes in the field, but it may also result in the enhanced absorption of light energy in Subtropical plants. Accordingly, we identified increased expression of transcripts associated with chlorophyll biosynthesis among the DET-St set members and with chlorophyll breakdown and leaf senescence among DET-Eq. Chlorophyll degradation followed by leaf senescence allows the remobilisation of nutrients and reduces the water loss through transpiration, contributing to water balance and drought tolerance (Munné-Bosch & Alegre, 2004). Additionally, we detected a putative signature of selection in a transcript similar to a *TPR* protein, required for chlorophyll synthesis and for the biogenesis of the photosynthetic apparatus (Bohne et al., 2016). We suggest that differential seasonality in light and water availability between subtropical and equatorial latitudes may be involved in the divergence of non-neutral variability in the species. Corroborating the results obtained from the genomic approaches, we observed functional trait divergence related to water use and light energy absorbance in plants from contrasting latitudes cultivated under the same conditions (Fig. 2-3, Supplemental Fig. 3-4).

Mitochondrial activity is strongly connected to photosynthesis and chloroplasts function since it generates ATP for carbohydrate synthesis, plays a role in protecting chloroplasts against photoinhibition, participates in dissipating reducing equivalents and exchanges metabolites with chloroplasts during photorespiration (Millar, Whelan, Soole, & Day, 2011; Raghavendra & Padmasree, 2003). Cellular respiration also provides ATP and carbon compounds for secondary metabolism, playing fundamental roles in responses to abiotic stresses, including drought (Atkin & Macherel, 2009; Millar et al., 2011). We suggest

that higher expression levels of cellular respiration genes in Equatorial than in Subtropical samples may be a consequence of the reduced expression of photosynthesis genes and the enhanced energetic demand caused by water stress.

Tidal amplitude variation with latitude as a diverging force along the Atlantic coast of South America

Tidal amplitude is markedly reduced with increasing latitude along the Atlantic coast of South America, ranging from greater than 4 m at low latitudes to less than 1 m at high latitudes (Schaeffer-Novelli, Cintrón-Molero, Adaime, & de Camargo, 1990). The wide variation in tidal amplitude exposes trees to varied durations of hypoxia. The identification of a candidate SNP locus with a putative sign of selection in a transcription factor induced by oxygen deprivation (*HRA1*) may indicate that differential tidal variation acts as a diverging selective force between northerly equatorial and southerly subtropical populations of *A. schaueriana*. The *HRA1* putative homolog also showed 1.75-fold higher expression in Subtropical leaves relative to that in Equatorial leaves under field conditions. However, we did not detect evidences at the phenotypic level in the common garden experiment suggesting the relevance of this environmental variable.

Climate change and conservation implications of the results

The functional divergence described herein might differentially affect the sensitivity of *A. schaueriana* populations to a rapidly changing climate. Although there is no evidence of mangrove expansion at its southernmost range limit in the Americas (Soares et al., 2012), researchers suggest that subtropical populations could expand polewards in the near future as a result from the increased air and ocean temperatures and CO_2 concentrations (Godoy & De

Lacerda, 2015). We expect that the more acquisitive traits, in terms of gene expression in the field and in morphophysiology in the common garden experiment, exhibited by plants from the subtropical range edge may indeed favour growth under increased temperatures and rainfall (Pachauri & Meyer, 2014). However, due to the low tidal variation (< 1 m) observed at the southernmost mangrove distribution edge along the Atlantic coast of South America, a greater relocation of forests will be required for species to keep pace with the rising sea level (Ellison, 2015). In this context, dense human coastal occupations combined with the narrow intertidal zones, characteristic of this region, make mangroves at higher latitudes more vulnerable to habitat loss than in the equatorial region of the Atlantic coast of South America. We expect that in contrast, despite having wider coastal plains potentially available for expansion, we expect that Equatorial populations of *A. schaueriana* might be threatened by the increased mean temperatures and decreased precipitation during the El Niño-Southern Oscillation events (Pachauri & Meyer, 2014). Increased leaf temperature stimulates respiration (Heskel et al., 2016) and photorespiration (Jordan & Ogren, 1984) and might offset the benefits in carbon acquisition caused by increased CO_2 concentration (Drake, Gonzàlez-Meler, & Long, 1997). The critical temperature threshold for photosynthesis is likely to be overcome more frequently in the near future, possibly reducing carbon assimilation and productivity (Saenger & Moverly, 1985) and, in extreme cases, causing biomass loss triggered by cavitation or carbon starvation (Doughty et al., 2015; Rowland et al., 2015).

For the definition of short-term mangrove conservation plans, such as the reforestation or restoration of degraded areas on the Atlantic coast of South America, we recommend that populations occurring north and south of the NEESA should warrant attention as distinct conservation management units(Moritz, 1994).

Conclusions

Based on the combined analysis of our results, we argue that carbon acquisition in plants in equatorial region may be limited by the longer exposure to hypoxia, the higher vapour pressure deficit (VPD) and the higher solar irradiance, especially during the hot and dry season. Furthermore, we argue that in subtropical regions, limitations in carbon gain may result from the lower solar irradiance levels, the lower temperature and the shorter photoperiod during winter (Fig. 4). These environmental differences between equatorial and subtropical latitudes presumably control gene expression in the field, and remarkably, they may have shaped both allele frequencies in genes responding to these variables and morphophysiological traits observed in *A. schaueriana* individuals (Fig. 4). The emergence of this divergence is facilitated by the limited gene flow identified between populations north and south of the NEESA, possibly driven by the movements of the major ocean currents that are active along the Atlantic coast of South America (Fig. 1). Because a similar north-south structure of neutral diversity is also observed for other mangrove and mangrove-associated species on the Atlantic coast of South America (Francisco et al., 2018; Mori et al., 2015; Takayama et al., 2008), it is plausible that the environmental drivers of adaptive divergence in *A. schaueriana* play roles in the divergence of other species.

In addition to revealing that northerly and southerly populations of *A. schaueriana* are genetically and functionally distinct units (Wee et al., 2018), we have provided an in-depth empirical evaluation of the intraspecific variation of a long-living, non-model tree. These results should allow clearer predictions of how *A. schaueriana* and potentially other coastal plants may respond to current global climate changes by accounting for phenotypically

(Moran et al., 2016) and genetically (Ikeda et al., 2017) informed mangrove distribution modelling.

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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 available in the supporting material. Other data are available upon request.

Author contributions

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

Figures and Tables

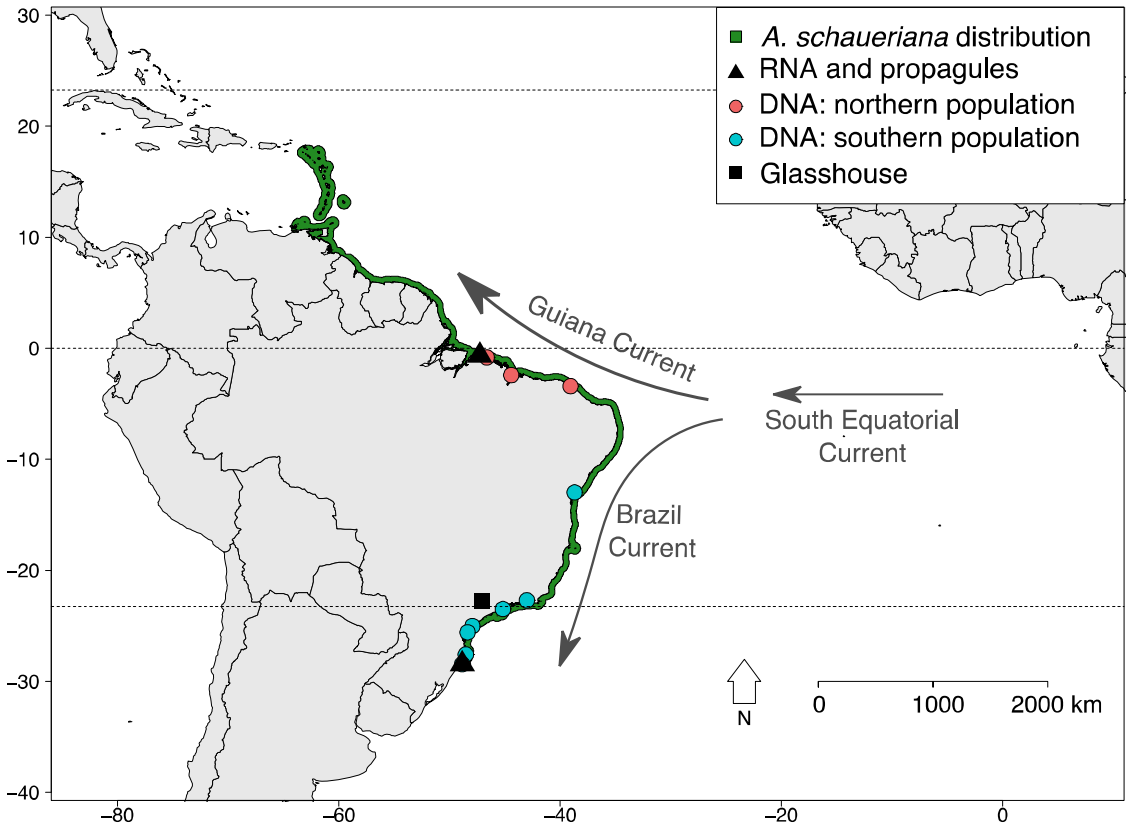

Figure 1. Geographical locations of *Avicennia schaueriana* sampling sites. Green-shaded area represents the geographical distribution of the species. Black triangles represent the locations of the Equatorial and Subtropical sampling sites for propagules and plant tissues used in the common garden experiment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

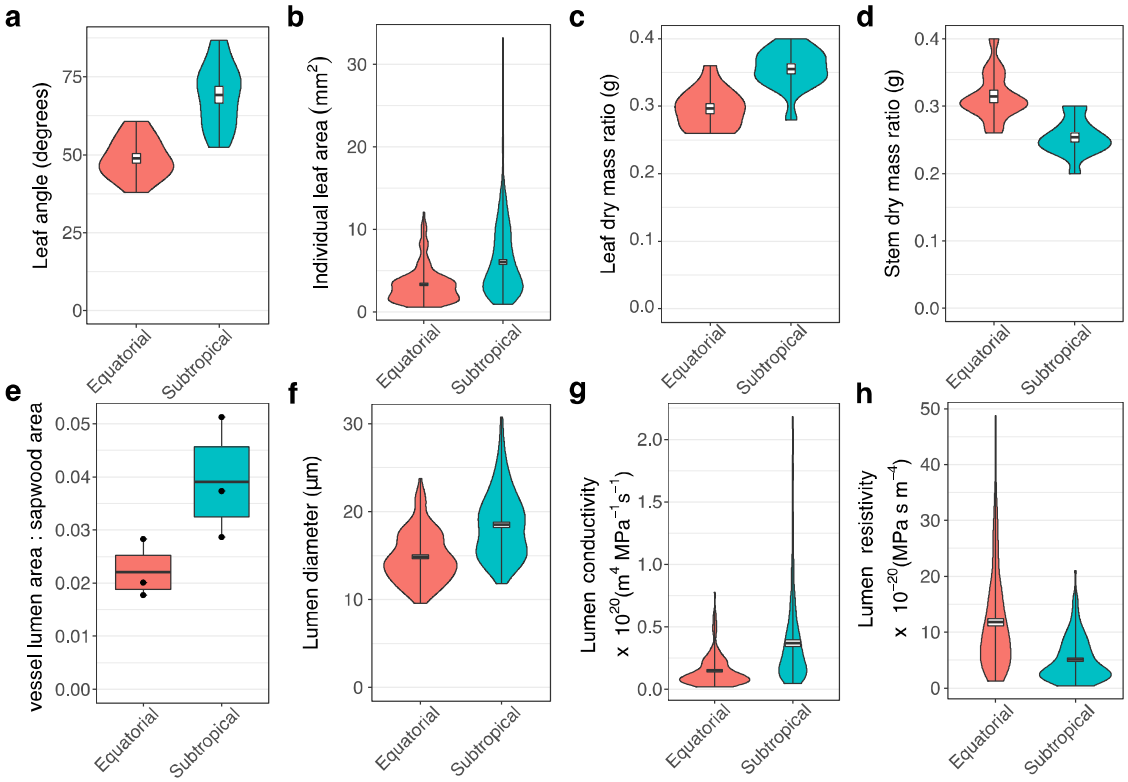

Figure 2. Morphological divergence observed in seedlings of *Avicennia schaueriana* collected from Equatorial and Subtropical sampling sites and grown in a common garden experiment. Violin plots represent the distribution of observations for plants from Equatorial (red) and Subtropical (blue) sampling sites. Box plots represent the mean, standard error, and maximum and minimum values. Two group comparisons were performed using the non-parametric unpaired Mann-Whitney Wilcoxon U-tests. For all variables represented in the figure, the absence of a difference between groups was rejected at a significance threshold of 0.05. (a) leaf inclination angle (n = 15 leaves per group, 5 plants per group); (b) individual leaf area (n = 250 leaves per group, 3 plants per group); (c) leaf dry mass ratio (leaf dry biomass/plant dry biomass) (n = 15 plants per group); (d) stem dry mass ratio (stem dry biomass/plant dry biomass) (n = 15 plants per group); (e) vessel lumen area ratio in sapwood (n = 175 per group, 3 plants per group, observations represented by black points); (f) vessel lumen diameter (n = 700 vessels per group, 3 plants per group); (g) vessel lumen conductivity (n = 700 vessels per group, 3 plants per group); (h) vessel lumen resistivity (n = 700 vessels per group, 3 plants per group).

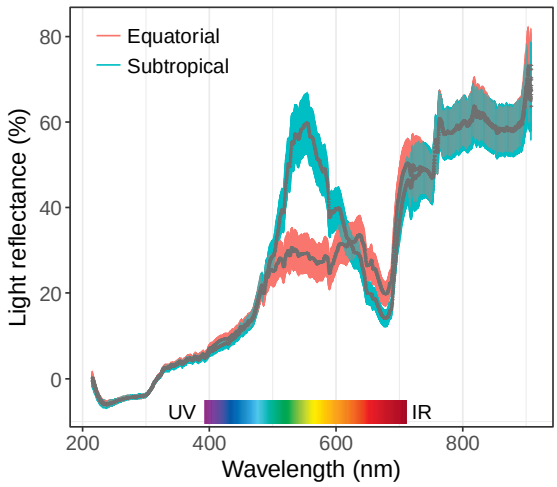

Figure 3. Light reflectance of the stem epidermis of five-month-old *Avicennia schaueriana* seedlings grown in a common garden experiment. Grey lines represent the mean reflectance, and colour-shaded areas represent the standard error for each seedling source site (red: Equatorial; blue: Subtropical,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

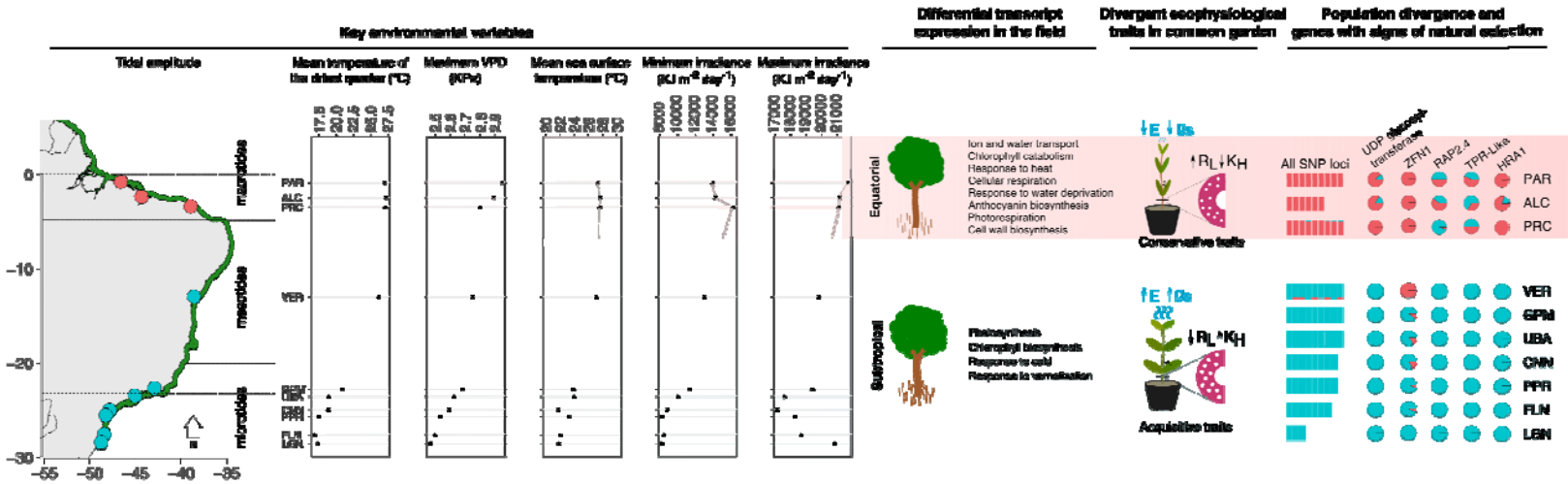

Figure 4. Graphical summary of the integration of ecological and molecular approaches performed in this work. Key oceanographic and climatic factors differ markedly between equatorial and subtropical latitudinal distribution extremes of the distribution of the neotropical mangrove tree, *Avicennia schaueriana*, and possibly shape the diversity of genotypes and phenotypes in the species. To address this issue, we examined the effects of the contrasting environments on overall gene expression, its morphophysiological effects in a common garden experiment and its genomic effects through natural selection detection tests based on single nucleotide polymorphism (SNP). Plants from equatorial and subtropical latitudes showed key divergences related to the use of water and to the acquisition of carbohydrates both in the field and in common garden conditions. In addition, north-south genetic divergence was observed in all genotyped SNPs. We also identified signatures of differential selective pressures on specific loci possibly related to the accumulation of anthocyanin (*UDP-GLUCOSYLTRANSFERASE*), the response to osmotic stress (*RAP2.4* and *ZFN1*), photosynthesis (*TPR*) and hypoxia (*HRA1*). The molecular and ecologic divergences observed through three independent approaches may be related to environmental factors that strongly differ between contrasting latitudes at which the species are found. These findings highlight the power of using multidisciplinary approaches for the study of adaptation in species for which little basic biological information is available, such as tropical trees. VPD: atmospheric vapour pressure deficit; E: transpiration rate; g_s: stomatal conductance; R_L: xylem vessel lumen resistivity; K_H: xylem vessel conductivity.

Table 1. Characterisation of Subtropical and Equatorial sampling sites of propagules used in the common garden experiment and of RNA used for RNA-sequencing. SD: standard 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
Tidal amplitude	Microtidal (< 1 m)	Macrotidal (> 4 m)
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. [†]According to Alvares et al. (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). [‡]Source: BioClim (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). [§]Source: MARSPEC (Sbrocco & Barber, 2013). [¶]Source: 'daylength' function from R package 'geosphere' (Forsythe, Rykiel, Stahl, Wu, & Schoolfield, 1995).