

1 **A high-quality genome assembly and annotation of the gray mangrove, *Avicennia marina***

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3 Running title: Genome assembly of the gray mangrove

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29

30 **Abstract**

31 The gray mangrove [*Avicennia marina* (Forsk.) Vierh.] is the most widely distributed mangrove
32 species, ranging throughout the Indo-West Pacific. It presents remarkable levels of geographic
33 variation both in phenotypic traits and habitat, often occupying extreme environments at the
34 edges of its distribution. However, subspecific evolutionary relationships and adaptive
35 mechanisms remain understudied, especially across populations of the West Indian Ocean. High-
36 quality genomic resources accounting for such variability are sparse. We sequenced and
37 assembled the genome of *A. marina* from the Arabian Gulf, which is the harshest region that the
38 species occupies and at the northern-most limit of its distribution. We used proximity ligation
39 libraries Chicago and Dovetail HiC, and the HiRise assembly pipeline, producing a 456,556,596
40 bp long genome. The largest 32 scaffolds (22.4 Mb to 10.5 Mb) accounted for 98 % of the
41 genome assembly, with the remaining 2% distributed among much shorter 3,777 scaffolds (62.4
42 Kb to 1 Kb). We annotated 23,331 protein-coding genes using tissue-specific RNA-seq data,
43 from which 13,312 were associated to GO terms. Genome assembly and annotated set of genes
44 yield a 96.7% and 92.3% completeness score, respectively, when compared with the eudicots
45 BUSCO dataset. Furthermore, an F_{ST} survey based on resequencing data successfully identified a
46 set of candidate genes potentially involved in local adaptation, and revealed patterns of adaptive
47 variability correlating with a temperature gradient in Arabian mangrove populations. Our *A.*
48 *marina* genomic assembly provides a highly valuable resource for genome evolution analysis, as
49 well as for identifying functional genes involved in adaptive processes and speciation.

50

51 *Key words:* gray mangrove, *Avicennia marina*, genome assembly, HiC, Arabia

52

53 **Significance statement**

54 This study reports the first chromosome-level genome assembly for *Avicennia marina*, the most
55 widely distributed mangrove species of the world. Our new assembly provides an excellent
56 reference for phylogenomics and population genomics studies focused on *A. marina* and related
57 species. An annotation based on tissue-specific mRNA data of unprecedented quality is also
58 provided, enabling functional identification of candidate genes. Overall, the data here reported
59 enables a range of comparative genomics, molecular ecology, and functional divergence tools for
60 the study of an ecological and socioeconomically relevant group of organisms.

61

62 **Introduction**

63 Mangroves are a polyphyletic group of trees and shrubs that inhabit the intertidal zone of the
64 tropic and sub-tropic coasts (Hogarth 2015; Nagelkerken, et al. 2008; Polidoro, et al. 2010;
65 Primavera, et al. 2018). Mangroves share several morphological and physiological adaptations to
66 their harsh intertidal habitat, including traits for tolerance of high salinity, alternating desiccation
67 and submergence of soils across tidal cycles, and exposure of roots to hypoxic, sulfide-rich soils
68 (Tomlinson 2016). Mangroves are of great ecological and economic importance, providing key
69 functions such as high productivity, much of which is exported to surrounding ecosystems, and
70 acting as breeding, nesting, nursery, and shelter areas for a range of biota, as well as serving as a
71 foraging habitat (Carugati, et al. 2018; Lee, et al. 2014; Nagelkerken, et al. 2008; Primavera, et
72 al. 2018). Mangroves also serve as an important carbon sink, supporting climate change
73 mitigation and adaptation potential (Duarte, et al. 2013). The diversity of evolutionary origins
74 and adaptive mechanisms found in mangroves provide compelling systems for studying patterns
75 of trait evolution, lineage divergence and speciation (e.g., Urashi, et al. 2013; Xu, et al. 2017b;
76 Zhou, et al. 2007).

77

78 Of the approximately 70 mangrove species described, the gray mangrove *Avicennia marina* has
79 the broadest latitudinal and longitudinal distribution (Hogarth 2015; Spalding, et al. 2010;
80 Tomlinson 2016). At least three sub-specific, partially allopatric taxa or ‘varieties’ have been
81 described: *A. marina* var. *australasica*, restricted to southeastern Australia and New Zealand; *A.*
82 *marina* var. *eucalyptifolia*, in northern regions of Australasia; and *A. marina* var. *marina*, that
83 ranges from New Caledonia in the Pacific and across the entire Indian Ocean (Duke 1991; Li, et
84 al. 2016; Tomlinson 2016). The broad geographic distribution of *A. marina* is reflected in its

85 presence across diverse environmental gradients (e.g. temperature, freshwater, sediment and
86 nutrient supply, salinity, tidal range) and spatial settings (e.g. open coastlines, coastal lagoons,
87 estuaries, deltas, coral fringes) (Duke 1990; Quisthoudt, et al. 2012). Along with its widespread
88 distribution, several aspects of *A. marina* biology make it a promising model organism among
89 mangroves for the study of evolution based on genomic and molecular approaches. First, the
90 broad environmental gradients present across the *A. marina* range are mirrored by remarkable
91 geographic variation in phenotypic and life-history traits (Duke 1990; Tomlinson 2016), all of
92 which makes them an ideal natural system for studying evolutionary processes related to
93 dispersal, directional selection, and neutral evolution. Previous studies have reported the
94 phylogenetic relationships for the sub-varieties of *A. marina* and congeneric species (Duke, et al.
95 1998; Li, et al. 2016; Nettel, et al. 2008), yet the specific drivers of lineage diversification remain
96 understudied. Furthermore, the extensive gray mangrove populations from the Eastern African
97 and Arabian coasts have rarely been included in reported DNA sequence-based analyses, either
98 for diversity screening or other purposes (e.g., Duke, et al. 1998; Li, et al. 2016). Second, *A.*
99 *marina* can tolerate highly variable and extreme environmental conditions, and often occupies
100 marginal, biologically limiting environments at the edges of its distribution (Morrisey, et al.
101 2010). While several biological structures and mechanisms of the gray mangrove physiology
102 have been described (Naidoo 2016; Nguyen, et al. 2015), the genetic basis and pathways
103 underlying such tolerance are still largely unknown (Jithesh, et al. 2006; Mehta, et al. 2005; Xu,
104 et al. 2017a). Understudied Arabian populations represent a particular gap due to their
105 occurrence at the extremes of air and water temperature, salinity, and aridity (Ben-Hasan and
106 Christensen 2019; Camp, et al. 2018). Third, the *A. marina* genome is moderately small and
107 structurally simple compared with other eukaryotes, and presents a limited amount of

108 transposable elements (Das, et al. 1995; Lyu, et al. 2018; Xu, et al. 2017a), which facilitates the
109 identification of short polymorphisms and structural variants.

110
111 Previous studies on *A. marina* using high-throughput sequencing techniques and genomic
112 approaches have been released. Two multi-species studies, including *A. marina*, explored
113 patterns of convergent evolution at functional genes (Xu, et al. 2017a) and transposable elements
114 loads (Lyu, et al. 2018), based on draft genomes that were recently made available online
115 (<http://evolution.sysu.edu.cn/>). Whole-genome assemblies of *A. marina* and several other
116 mangrove taxa have recently been used for demographic inference (Guo, et al. 2018) and
117 convergent evolution analysis (He, et al. 2020), but the underlying genomic data are not publicly
118 accessible.

119
120 Here, we report a high-quality near-chromosome level assembly obtained using proximity
121 ligation libraries as a resource for genome-based studies on *A. marina* and related mangrove
122 species. An structural and functional annotation based on RNA-seq data from multiple tissues is
123 also provided. We used resequencing data from multiple individuals to evaluate the performance
124 of the assembly as a reference to study patterns of adaptive variability at the genomic level.

125 Resequencing data is also used for assembling the mitochondrial genome.

126

127

128 **Materials and Methods**

129 *Genome sequencing and assembly*

130 The sequenced sample was leaf tissue obtained from an individual located at Ras Ghurab Island

131 in the Arabian Gulf (Fig 1; Abu Dhabi, United Arab Emirates; 24.601°N, 4.566 °E),
132 corresponding to the *A. m. marina* variety. A high-quality genome was produced using proximity
133 ligation libraries and the software pipeline HiRise (Putnam, et al. 2016) at Dovetail Genomics,
134 LLC. Briefly, for Chicago and the Dovetail HiC library preparation, chromatin was fixed with
135 formaldehyde. Fixed chromatin was then digested with DpnII and free blunt ends were ligated.
136 Crosslinks were reversed, and the DNA purified from protein, which was then sheared to ~350
137 bp mean fragment size. Libraries were generated using NEBNext Ultra enzymes and Illumina-
138 compatible adapters, and sequencing was carried out on an Illumina HiSeq X platform. Chicago
139 and Dovetail HiC library reads were then used as input data for genome assembly for HiRise, a
140 software pipeline designed specifically for using proximity ligation data to scaffold genome
141 assemblies (Putnam, et al. 2016). A previously reported draft genome of *Avicennia marina* (Lyu,
142 et al. 2018; Xu, et al. 2017a; available at evolution.sysu.edu.cn/index.html) was used in the
143 assembly pipeline, excluding scaffolds shorter than 1Kb since HiRise does not assemble them.
144 Further details are provided in the Supplementary Information.

145
146 The mitochondrial genome was assembled using NOVOplasty2.7.2 (Dierckxsens, et al. 2017)
147 and resequencing data based on Illumina paired-end 150 bp libraries from a conspecific
148 individual (See below; Supplementary Information). The maturase (*matR*) mitochondrial gene
149 available in NCBI (GenBank accession no. AY289666.1) was used for the input seed sequence.

150

151 *Genome annotation*

152 We performed the annotation of the *A. marina* genome using mRNA data from a set of tissues of
153 conspecific individuals. Samples were collected on the coast of the Eastern Central Red Sea

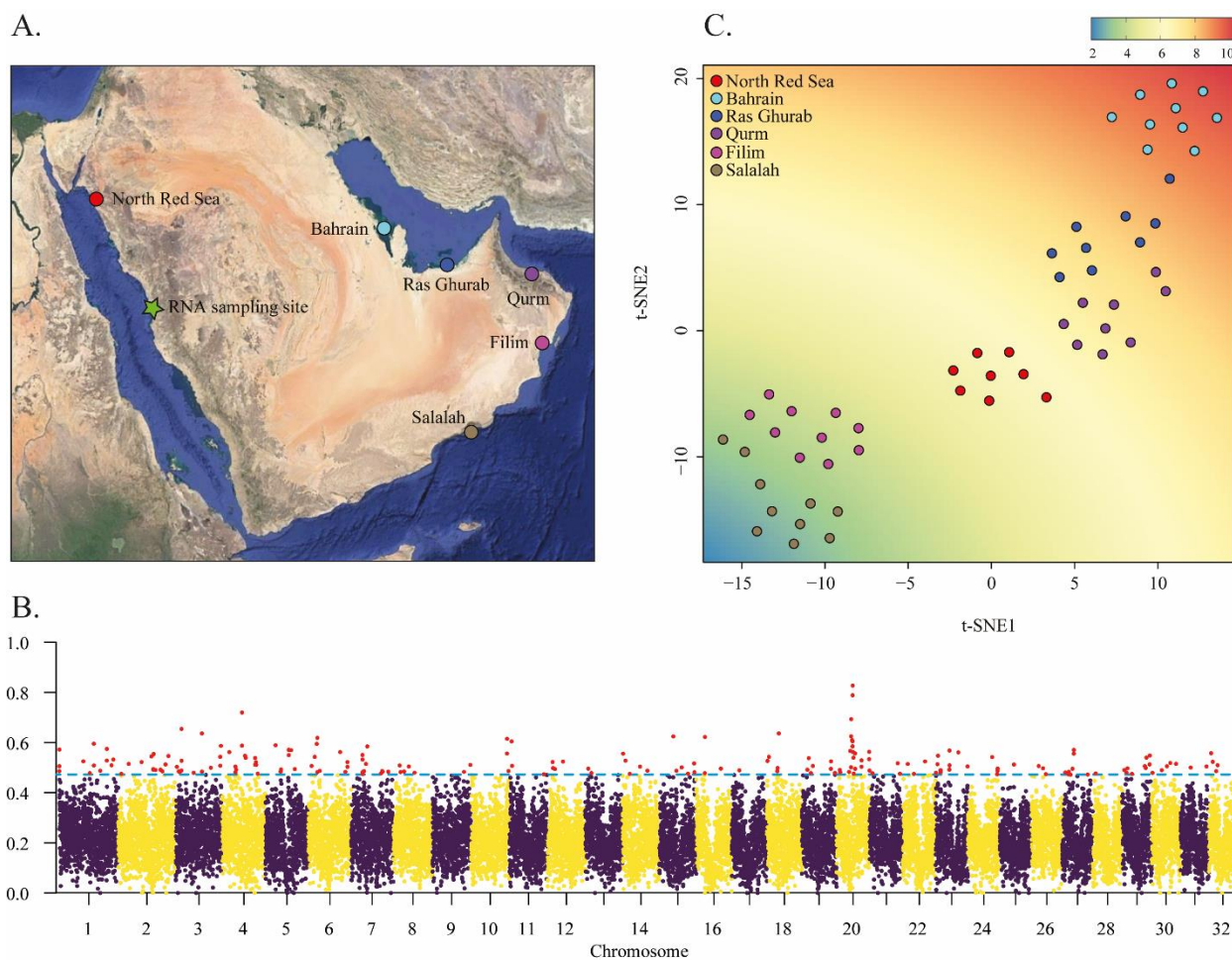
154 north of Jeddah in the Kingdom of Saudi Arabia (22.324 °N, 39.100 °E; Figure 1A). Total RNA
155 was isolated from root, stem, leaf, flower, and seed using TRIzol reagent (Invitrogen, USA).
156 RNA-seq libraries were prepared using TruSeq RNA sample prep kit (Illumina, Inc.), with
157 inserts that range in size from approximately 100-400 bp. Library quality control and
158 quantification were performed with a Bioanalyzer Chip DNA 1000 series II (Agilent), and
159 sequenced in a HiSeq2000 platform (Illumina, Inc.). Messenger RNA reads were mapped with
160 HISAT2 (Kim, et al. 2015), and genome-referenced transcripts for each tissue were produced
161 and merged with StringTie (Pertea, et al. 2015). Prediction of coding regions was performed with
162 TransDecoder (Haas and Papanicolaou 2015). The obtained gene annotation gff3 file was
163 validated and used to generate the reported gene annotation statistics with GenomeTools
164 (Gremme, et al. 2013) and in-house Perl scripts. The Trinotate (Haas 2015) pipeline was then
165 implemented to conduct a homology-based functional annotation by using Swiss-Prot (Bairoch
166 and Apweiler 2000) and pfam (Bateman, et al. 2002) databases, generating a final set of
167 annotated functional genes. Further details on mRNA sequencing and annotation scripts are
168 provided in the Supplementary Information.

169
170 Repetitive regions were first modelled *ab initio* using RepeatModeler v2.0.1 (Flynn, et al. 2019)
171 in all scaffolds longer than 100 Kbp with default options. The resulting repeat library was used to
172 annotate and soft-mask repeats in the genome assembly with RepeatMasker 4.0.9 (Smit, et al.
173 2015).

174

175

176 **Fig. 1.** Geography and adaptive variability in Arabian gray mangroves. (A) Locations of the six
177 stands sampled for whole genome resequencing (colored circles) and for RNA-seq (green star).
178 (B) F_{ST} genome scan based on 22,181 windows of 20 Kb. Boxplot outliers (coefficient=1.5) are
179 marked in red (C) t-SNE based on 200 SNP outliers linked to functional genes. The background
180 shows the correlation between t-SNE1 and t-SNE2 with the annual temperature range registered
181 in each one of the sampling locations. Temperature depicted in the legend is in °C.



185 *Gene completeness assessment*

186 We assessed gene completeness in the genome assembly, and gene annotation, using BUSCO

187 (Benchmarking Universal Single-Copy Orthologs) v4.0.5 (--auto-lineage-euk option;
188 Waterhouse, et al. 2018). BUSCO evaluations were conducted using the 255 and 2,326 single-
189 copy orthologous genes in Eukaryota_odb10 and Eudicots_odb10 datasets, respectively.

190

191 *Adaptive variability analysis and functional assessment of A. marina genome*

192 To test the potential of the assembly and annotation reported here as a resource for genomic-
193 based studies, we checked for regions of high divergence across the genome of *A. marina* using
194 newly generated whole-genome data. We resequenced 60 individuals from six different
195 populations from each of the major seas bordering Arabia (Figure 1A; Table S1, Supplementary
196 Information), including populations in the Red Sea, the Arabian Gulf and the Arabian Sea/Sea of
197 Oman. Arabia's regional seas are characterized by extreme, but divergent, environmental
198 conditions for mangrove growth. The northern Red Sea is characterized by cold winter
199 temperatures and high salinity (Carvalho, et al. 2019), while the southern Persian/Arabian Gulf is
200 the world's hottest sea each summer and is also hypersaline, with both areas considered arid to
201 hyperarid with limited (<200 mm) of rainfall (Vaughan, et al. 2019). In contrast, the Arabian Sea
202 and Sea of Oman have normal oceanic salinity, and summer temperatures that are buffered by
203 cold-water upwelling as a result of the Indian Ocean monsoon, resulting in more benign
204 environmental conditions (Claereboudt 2019). Using vcfTools (Danecek, et al. 2011), we conduct
205 an F_{ST} survey across the six populations based on 20 Kb sliding windows and identify outlier
206 loci associated with functional genes. We then use these loci to explore geographic patterns of
207 adaptive variability by means of t-SNE analysis, testing for correlations between variability in
208 sea surface temperature and t-SNE scores. Details on sequencing, variant calling, and analytical
209 procedures are available in the Supplementary Information.

210

211

212 **Results and Discussion**

213 *Sequencing and genome assembly*

214 We sequenced and assembled a reference genome of a gray mangrove individual from the
215 Arabian Gulf, an extreme environment at the northern limit of the species' distribution. Chicago
216 and Dovetail HiC libraries produced 235 million and 212 million 2x150 bp paired-end reads,
217 respectively, and 134.1 Gb data overall. Genome scaffolding with HiRise yielded an assembly of
218 456.6 Mb for a final sequence coverage of 293X; an L50/N50 equal to 15 scaffolds/13.98 Mb
219 and a relatively large number of ambiguous bases (i.e., N) inserted in the genome (10.6%; Table
220 1). The scaffold length distribution was heavily skewed towards extreme values (Table 1, Figure
221 2). The 32 longest scaffolds, ranging from 22.40 Mb to 10.58 Mb (median = 13.44 Mb)
222 accounted for 98.03% of the whole assembled genome, congruent with a chromosome number
223 $2N = 64$ reported earlier (He, et al. 2020). The remaining 1.97% of the genome was distributed
224 among another 3,777 scaffolds ranging from 62.5 Kb to over 1 Kb (median = 1.8 Kb). The large
225 number of small scaffolds may be due to the high fragmentation of the draft genome used in the
226 assembly pipeline (Dovetails, personal communication). The integrity assessment of the *A.*
227 *marina* genome retrieved a 98.8% and a 96.7% of the tested BUSCO groups for the eukaryote
228 and the eudicots databases, respectively (Table 1). The remarkable discontinuity in length sizes,
229 as well as the integrity and high quality of the scaffolding lends considerable support to the
230 hypothesis of 32 chromosomes; further sequencing efforts involving long-read sequencing are
231 warranted for confirmation. The mitochondrial genome assembly was 22,019 pb long with a
232 46.4% GC content.

233

234

235 **Table 1.** Summary statistics for the genome assembly and annotation of *A. marina*. CDS

236 indicates protein-coding sequences.

Genome assembly	
Total length	456,556,596 bp
Number of scaffolds	3,809
N50/L50	13,979,447 bp/15 scaffolds
N90/L90	11,144,373 bp/29 scaffolds
Chromosome scale	10,583,658 bp/32 scaffolds
Longest scaffolds	22,400,447 bp
Missingness	10.6%
GC content	35.2 %
BUSCO eukaryota database	C:98.8% [S:81.2%, D:17.6%], F:0.8%, M:0.4%, N:255
BUSCO eudicots database	C:96.7% [S:89.2%, D:7.5%], F:0.8%, M:2.5%, N:2,326

Genome annotation	
Number of genes	23,331
Number of annotated genes	21,147
Number of genes with GOs	13,312
Average gene length	5,383.7 bp
Number of CDS	59,445
Average CDS length (bp)	11,46.33 bp
Number of exons	493,357
Average exon length (bp)	307.06 bp
Number of introns	433,912
Average intron length (bp)	625.19 bp
BUSCO eukaryota database	C:97.2% [S:78.8%, D:18.4%], F:1.6%, M:1.2%, N:255
BUSCO eudicots database	C:92.3% [S:85.2%, D:7.1%], F:1.6%, M:6.1%, N:2,326

237

238 BUSCO parameters are C: Complete BUSCO; S: Complete and single-copy BUSCOs; D:

239 Complete and duplicated BUSCOs; F: Fragmented BUSCOs; M: Missing BUSCOs; and N:

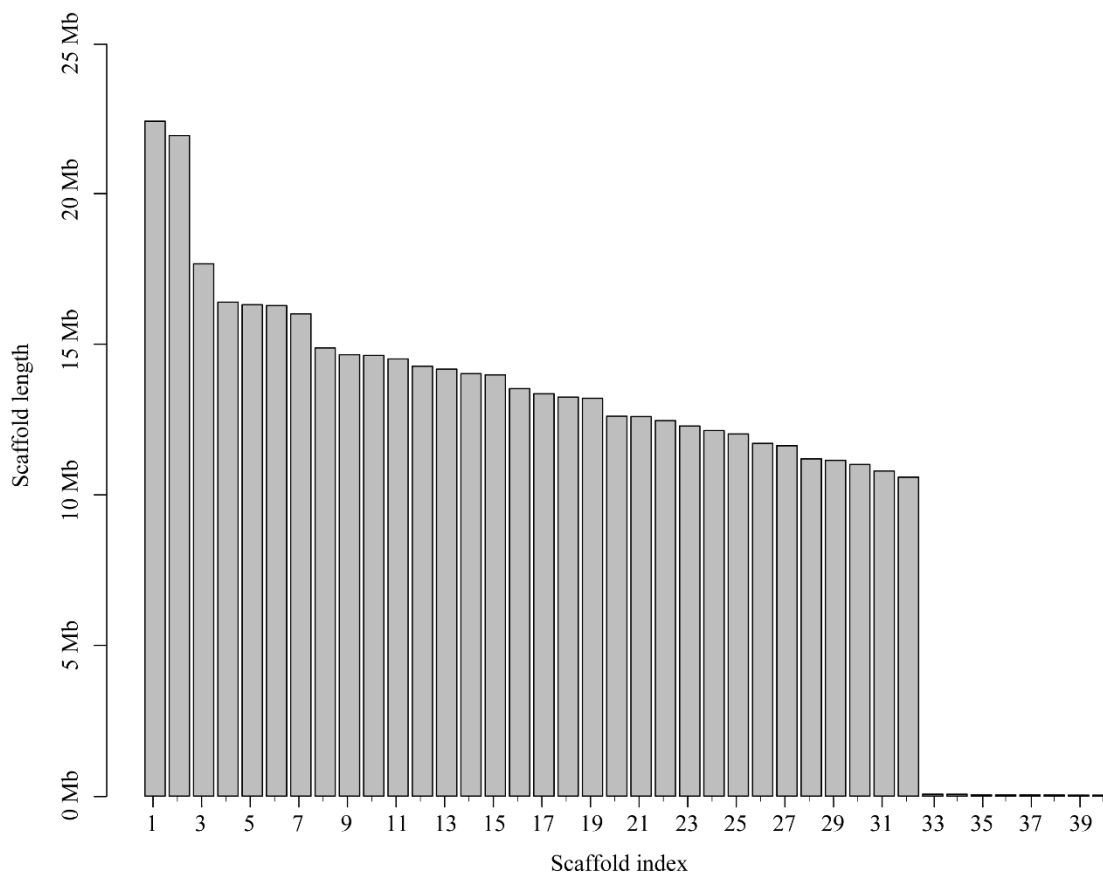
240 Total BUSCO groups searched. CDS indicates protein-coding sequences.

241

242

243 **Fig. 2.** Length bar-plot of the longest 40 scaffolds arranged by decreasing size. The genome was
244 sequenced using proximity ligation libraries Chicago and Dovetails HiC, and the assembly was
245 carried out with HiRise pipeline.

246



247

248

249 *Genome annotation*

250 We identified 23,331 protein-coding genes for which 21,147 orthologs from other species were
251 identified, and 13,312 were associated to GO terms. The average gene length was 5.38 Kb, with
252 a mean of 8.3 exons and 18.6 introns per gene. BUSCO integrity analysis reported a 97.2% of
253 recovered BUSCOs for the eukaryota database, and a 92.3% in the case of the eudicots (Table
254 1). We also found that a total of 40.20% (188.5 Mb) of the *A. marina* assembly consisted of

255 repetitive elements, a value moderately larger than the 30.4% previously reported for the species
256 (Xu, et al. 2017a). The greatest proportions corresponded to long terminal repeats and
257 unclassified elements (20% and 16.7%, respectively; Table S2, Supplementary Information).

258

259 *Adaptive variability analysis and functional assessment of A. marina genome*

260 We resequenced 56 individuals of *A. marina* from six different populations across the
261 environmentally diverse coasts of the Arabian Peninsula (Figure 1A) with a coverage of 85X.
262 After SNP calling and a strict quality filtering, we obtained a dataset of 538,185 SNPs. An F_{ST}
263 scan based on sliding 20 Kb windows revealed a heterogeneous landscape of differentiation and
264 detected a peak of high divergence at the Scaffold 20 (Figure 1B). A total of 200 highly
265 divergent loci were identified, from which 43 overlapped with annotated genes associated to GO
266 terms (Table S2, Figure S1). Several of these genes are involved in the development of shoots,
267 leaves and flowers (BAM2 and BAM7; DeYoung, et al. 2006; Reinhold, et al. 2011), root and
268 seeds (FEI1; Basu, et al. 2016), as well as in protein storage (VTI12; Sanmartín, et al. 2007).
269 Importantly, we also found signals of differentiation in genes involved in plant sensitivity to salt
270 and osmotic stress (WRKY40; Chen, et al. 2010), drought and palatability to detritivorous
271 crustaceans (LOX6; Grebner, et al. 2013), supporting the role of abiotic and biotic factors in the
272 differentiation of Arabian mangroves (Table S3, Supplementary Information). A t-SNE based
273 324 SNPs extracted from the functionally annotated, highly divergent loci showed clear
274 clustering patterns among sampled populations (Figure 1C). Loading scores of retained t-SNE
275 axes revealed a high correlation with the gradient of sea surface temperature (SST; p-values
276 below 8.3×10^{-14} and 2.0×10^{-16} for t-SNE1 and t-SNE2, respectively), also congruent with a

277 pattern of adaptive divergence driven by environmental factors. Further details on analytical
278 procedures are reported in the Supplementary Information.

279

280 In conclusion, we report the first chromosome-scale assembly for the *Avicennia marina* genome
281 along with a comprehensive annotation based on tissue-specific RNA-seq data. The genome is
282 highly contiguous and complete, and we demonstrated that it is valuable resource for variant
283 calling and the identification of functional, candidate genes underlying phenotypic and
284 environmental divergence among mangrove taxa. Improved scaffolding also enables the
285 identification of regions putatively under selection, including structural variants such as
286 chromosome rearrangements or copy number variations, all relevant for investigating questions
287 related to evolutionary biology and molecular ecology in this ecological and socioeconomically
288 important species.

289

290

291 **Data deposition**

292 Genome assembly and annotation, and multi-sample sequence alignment from resequenced
293 individuals have been deposited at DRYAD and can be accessed at DRYAD

294 ([doi:10.5061/dryad.3j9kd51f5](https://doi.org/10.5061/dryad.3j9kd51f5)). The genome assembly has also been deposited at

295 DDBJ/ENA/GenBank under the accession JABGBM000000000. The version described in this

296 paper is version JABGBM010000000. Bioproject (SRA) accession: PRJNA629068; Biosample

297 accession: SAMN14766548.

298

299 Datasets relating to the RNA-seq analysis have been deposited in Mendeley
300 (doi:10.17632/9tsp7fr28r). The RNA-seq reads have been deposited at the National Center for
301 NCBI under the Bioproject (SRA) accession: PRJNA591919, Biosample accession:
302 SAMN13391520.

303

304

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316

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