

1 First description of seagrass distribution and abundance in São Tomé and Príncipe

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10

11 Abstract

12

13 Seagrass meadows in São Tomé and Príncipe, eastern Atlantic Ocean, are described here for  
14 the first time. Specifically, we quantified the biomass and density of seagrasses, characterized  
15 the plant morphology and measure their nutrient content as a proxy of the nutrient  
16 environmental conditions where the meadows develop. The seagrass *Halodule wrightii* was  
17 found in two locations of the northeastern coast of the island of São Tomé: 1) developing  
18 throughout an estimated area of 1500 ha surrounding Cabras islet, at a depth range of 4 - 10  
19 m, on sandy bottom; and 2) at Santana bay with an area of 1500 m<sup>2</sup> at 5-10 m depth, on sandy  
20 bottom. A highly morphologically different population of *Halodule wrightii* was found on the  
21 northeastern coast of the island of Príncipe, off Abade beach, covering an area of 135 m<sup>2</sup> at 4  
22 m depth. Further research is needed to assess if this is a different species. Shoot biomass and  
23 density was 10 and 4-fold higher in São Tomé than in Príncipe, respectively. CN ratios of  
24 above and belowground tissues of plants collected in São Tomé were also significantly higher  
25 than in Príncipe. The carbon content of *Halodule* leaves from São Tomé and Príncipe (41 %)

26 was much higher than that reported for other *Halodule* species, suggesting that meadows may  
27 have an important ecological role for carbon fixation. The presence of *H. wrightii* in São  
28 Tomé and Príncipe raises ecological and evolutionary questions that warrant further research.

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## 30 Highlights

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- 32 • Seagrass meadows in São Tomé and Príncipe, eastern Atlantic Ocean, are described  
33 here for the first time.
- 34 • *Halodule wrightii* was found at two locations in the island of São Tomé.
- 35 • A highly morphologically different *H. wrightii* was found at one location in Príncipe.  
36 Further research is needed to clarify if this is a different species.
- 37 • Results suggest an important ecological role of *H. wrightii* for carbon fixation.

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39 Keywords: distribution, *Halodule wrightii*, description, population, São Tomé and Príncipe,  
40 seagrass

41

## 42 1. Introduction

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44 Seagrasses are marine flowering plants widely distributed along temperate and tropical  
45 coastlines of the world. To facilitate a global assessment of their distribution, seagrass species  
46 have been grouped into six bioregions (Short et al. 2007). The Tropical Atlantic seagrass  
47 Bioregion 2 (Caribbean Sea, Gulf of Mexico, Bermuda, the Bahamas, and both tropical coasts  
48 of the Atlantic) is one of the regions with greatest diversity. Tropical seagrass species of the  
49 genera *Halophila*, *Syringodium*, *Thalassia* and *Halodule* are described for this region, but  
50 only on the western side of the Atlantic. On the tropical coast of Africa only the genus

51 *Halodule* has been reported. Despite the general knowledge of the global distribution of  
52 seagrasses (Green and Short 2003, Short 2007), many regions of the world remain poorly  
53 described, such as the West African seas. The potential for the occurrence of seagrass species  
54 in São Tomé and Príncipe has been reported by those authors but there are no scientific  
55 references of specific locations and no quantitative description of the populations.  
56 São Tomé and Príncipe is a volcanic archipelago of two islands with a total area of 1001 km<sup>2</sup>  
57 (S. Tomé = 852 km<sup>2</sup>; Príncipe = 142 km<sup>2</sup>), located at the Equator in the Gulf of Guinea  
58 (0°25'N; 6°20'E) at 380 km off the continental African coast (Fig. 1a and b). The country lies  
59 within the Tropical Atlantic Bioregion and has a total coastline extension of 240 km. São  
60 Tomé and Príncipe has been designated a marine biodiversity hotspot and a priority for  
61 conservation for its high level of endemic species (Roberts et al. 2002). The region is  
62 particularly relevant for marine turtles as all the five cosmopolitan species nest here  
63 (Schneider 1992, Castroviejo et al. 1994). Seagrass habitats may be locally relevant as feeding  
64 grounds for the green turtle *Chelonia mydas*.

65 Here we describe for the first time the location and extent of seagrass meadows in São Tomé  
66 and Príncipe, quantify their biomass and density, characterize their morphology and measure  
67 their nutrient content as a proxy of the nutrient environmental conditions where the meadows  
68 develop.

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## 70 2. Methods

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72 An underwater survey for seagrasses was conducted in both islands, São Tomé and Príncipe,  
73 in January 2017. On both islands, dominant oceanic currents from southwest render these  
74 coasts too hydrodynamic and unsuitable for seagrasses to develop, restricting to half the total  
75 extension of coastline where seagrasses can potentially occur. Consequently, southwest coasts

76 of both islands were excluded from this survey. Considering a depth distribution of 10 m, we  
77 estimated a total potential area for seagrasses to occur of 1200 km<sup>2</sup>. Satellite images of  
78 Google Earth were used to pre-select potential sites for seagrass development (sheltered bays  
79 and beaches with weak currents). Information from local fishermen and previous knowledge  
80 of turtle feeding grounds were also used to select the most probable sites for the occurrence of  
81 seagrasses. Divers were towed by an outboard motor boat to cover large areas of the  
82 nearshore subtidal zone down to 10 m depth. When spotted, seagrass meadows were sampled  
83 at a depth of ~ 5 cm using a 12 cm diameter core to determine the seagrass biomass and  
84 density. Samples were collected in two different points around Cabras islet, in São Tomé (Fig.  
85 1c, green dots) (total n = 11), and in one point at Abade beach, in Príncipe (Fig. 1e) (n = 6).  
86 Seagrasses were also sighted in Santana, in the island of São Tomé, but no samples were  
87 collected here. After collection, plant samples were kept in cool seawater until analysis. Leaf  
88 length and width, and rhizome diameter were measured, and above and belowground biomass  
89 were determined after drying the samples at 60 °C for 48 h. The carbon (C) and nitrogen (N)  
90 content was determined in leaves and roots in a Vario EL III elemental analyser (Elementar).  
91 Plants were photographed and identified using the identifications keys in Short and Coles  
92 (2001), van Tussenbroek et al. (2002) and Magalhães and Barros (2016). Voucher specimens  
93 were collected, briefly rinsed in fresh water, blotted dry and preserved as herbaria excicatae.  
94 The location of all sites where seagrasses were found was recorded using geographic  
95 positioning systems (GPS) and the seagrass distribution area was estimated by direct  
96 measurements or using Google Earth.

97 Differences in shoot density, above and belowground biomass, leaf width and CN ratio  
98 between populations of the two islands were detected using t-tests. Differences in leaf length  
99 and rhizome diameter between populations were tested using Mann-Whitney nonparametric  
100 test because equality of variances failed even after data transformation. All tests were

101 performed at a level of significance of 0.05 using Sigmaplot for Windows Version 11.0  
102 (Systat Software, Germany).

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### 104 3. Results

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#### 106 3.1. São Tomé

107 Abundant meadows of the seagrass *H. wrightii* were found covering large areas of the  
108 northeastern coast of the island of São Tomé, surrounding Cabras islet (0°24'31''N;  
109 6°42'44''E), at a depth range of 4 - 10 m on sandy bottom (Fig. 1c). The total area where  
110 meadows develop was estimated as 1500 ha. The green seaweed *Caulerpa sertularioides* was  
111 also present, particularly deeper, at 10 m and plus, where it becomes dominant. *Halodule*  
112 *wrightii* was highly covered by an epiphytic alga (*Dasya* sp.), particularly in shallow areas (2  
113 - 5 m) (Fig. 2a). Patches of *H. wrightii* visibly induced the accumulation of sediment, forming  
114 banks with an overall height of 15 - 20 cm. The mean shoot density was  $4349 \pm 1028$  shoots  
115  $\text{m}^{-2}$  (SD,  $n = 11$ ) (Fig. 3a), mean aboveground biomass was  $23.65 \pm 6.36$  g DW  $\text{m}^{-2}$  and  
116 belowground biomass was  $84.38 \pm 39.41$  g DW  $\text{m}^{-2}$  (Fig. 3b and c). Mean leaf length and  
117 width were respectively  $10.5 \pm 3.3$  cm ( $n = 91$ ) and  $0.62 \pm 0.12$  mm ( $n = 34$ ) (Fig. 3d and e),  
118 and mean rhizome diameter was  $0.64 \pm 0.30$  cm ( $n = 14$ ) (Fig. 3f). The C and N content of the  
119 leaves was respectively  $40.6 \pm 0.7$  % and  $2.1 \pm 0.1$  % ( $n = 5$ ) (C:N = 19.3), whereas in  
120 belowground plant parts it was  $33.6 \pm 0.6$  and  $0.9 \pm 0.1$  %, respectively (C:N = 38.8) (Fig. 4).  
121 Plants had bicuspidate leaf tips with a slightly median concave surface (Fig. 5a). No flowers  
122 or fruits were observed.

123 At Santana Bay, three small meadows of *H. wrightii* were also discovered (0°14'45''N;  
124 6°44'47''E) (Fig. 1d), covering a total area of 1500  $\text{m}^2$  at 5-10 m depth, most dense at ca. 7 m.  
125 Plants had bicuspidate leaf tips, similar to those collected in Cabras islet (Fig. 5b). Due to

126 logistical constrains it was not possible to obtain samples from this location for C:N and shoot  
127 density.

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### 129 3.2. Príncipe

130 Only one meadow of a highly morphologically different population of *Halodule wrightii* was  
131 found on the northeastern coast of the island of Príncipe, off Abade beach (1°38'0''N;  
132 7°27'17''E), covering a small area of 135 m<sup>2</sup> at 4 m depth (Fig. 1e). The blade apex had a  
133 rounded median tooth and two shorter lateral teeth (Fig. 5c This leaf tip morphology lies  
134 within the range of shapes described for *H. wrightii* by van Tussenbroek et al. (2010). In  
135 contrast, this leaf tip shape in a short and wide leaf morphotype, as the one found in the  
136 population of Príncipe, is described as *H. beaudettei* by Magalhães et al. (2016). Preliminary  
137 genetic analyses showed equal sequences of nrDNA internal transcribed spacers (ITS) for  
138 both populations. This indicates that, if the morphologically distinct populations from São  
139 Tomé and from Príncipe are two different species, they are still very genetically related, as  
140 expected after a recent split. The meadow was surrounded by smaller seagrass patches of ca. 1  
141 m diameter. The mean shoot density was  $1114 \pm 314$  shoots m<sup>-2</sup> (SD, n = 6), 4-fold lower than  
142 in São Tomé (Fig. 1a,  $p < 0.001$ ). The mean aboveground biomass was  $1.91 \pm 1.11$  g DW m<sup>-2</sup>  
143 and belowground biomass was  $6.07 \pm 2.75$  g DW m<sup>-2</sup>, both about 10-fold lower than São  
144 Tomé (Fig. 1b and c,  $p < 0.006$ ). Mean leaf length and width were respectively  $3.0 \pm 1.1$  cm  
145 (n = 27) (3-fold lower than São Tomé,  $p < 0.001$ ) and  $1.17 \pm 0.14$  mm (n = 11) (2-fold higher  
146 than São Tomé,  $p < 0.001$ ) (Fig. 1d and e), and mean rhizome diameter was  $0.56 \pm 0.16$  mm  
147 (n = 13) (about the same as in São Tomé,  $p = 0.662$ ) (Fig. 1f). The C and N content of the  
148 leaves was respectively  $42.2 \pm 1.6$  % and  $3.3 \pm 0.4$  % (n = 3) (C:N = 12.7), whereas in  
149 belowground plant parts it was  $32.3 \pm 0.7$  and  $1.2 \pm 0.1$  %, respectively (C:N = 26.0) (Fig. 4).  
150 Both CN ratios of above and belowground plant parts were significantly lower than in São

151 Tomé ( $p < 0.005$ ). Specimens were found with rhizomes infected by a parasite, probably  
152 *Plasmodiophora diplanthera*, as described in Braselton and Short (1985) (Fig. 5d).

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#### 154 4. Discussion

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156 We describe for the first time the seagrass populations of São Tomé and Príncipe, in the  
157 eastern Atlantic. *Halodule wrightii* was already reported for the west coast of Africa (Short et  
158 al. 2007). This species is widely distributed in the Tropical Indo-Pacific region and is one of  
159 the three dominant seagrass species in the American Tropical Atlantic coast, along with  
160 *Syringodium filiforme* and *Thalassia testudinum*.

161 The shoot density of *H. wrightii* in São Tomé was similar to that reported by Creed et al.  
162 (2016) in Cape Verde (5998 shoots  $m^{-2}$ , SE = 1247). At the Caribbean Sea, 2.5-fold higher  
163 shoot density was reported (14872 shoots  $m^{-2}$ , SE = 2444) by Gallegos et al. (1994), whereas  
164 at the western Gulf of Mexico, lower densities than São Tomé were reported (2274 shoots  $m^{-2}$ , SE = 439) (Gutierrez et al. 1994). The shoot density of *H. wrightii* throughout the Atlantic  
165 is thus highly variable and might depend on local environmental conditions and putative  
166 genetic variations. The shoot density of *H. wrightii* in Príncipe was similar to that reported by  
167 Magalhães et al. (2016) in Brazil (1955 shoots  $m^{-2}$ , SE = 1475) but plant biomass in Brazil  
168 was much higher, particularly the belowground biomass (28 g DW  $m^{-2}$  compared to 1.91 g  
169 DW  $m^{-2}$  in Príncipe).

171 *Halodule* plants showed great morphological differences between the two islands with respect  
172 to leaf length and width. Plants in São Tomé have long and narrow leaves. In comparison,  
173 plants found at Príncipe have shorter and wider leaves. We hypothesise that the longer and  
174 thinner leaves found in plants from São Tomé are an adaptive plastic response to the higher  
175 shoot density of the meadow compared to the shorter, wider and prostrated leaves in plants

176 from the less dense meadow in Príncipe, as seen for *Halophila ovalis* (Kaewsrihew and  
177 Prathep 2014). However, it should be noted that morphological variation in seagrasses may  
178 occur in response to many other environmental factors such as desiccation, light, temperature  
179 and hydrodynamics (Procaccini et al. 1999, Krupp et al. 2009). Vegetative plasticity with  
180 respect to leaf morphology has been described for *Halodule* species and appears to be related  
181 to habitat type or environmental characteristics, but these species' differences are consistent  
182 for all their geographical records. Previous studies of *H. wrightii* (which in the past included  
183 also the species *H. beaudettei*) report that the narrow leaf type occurs on sandy substrates in  
184 areas with unstable salinity on exposed shores (Den Hartog 1970; McMillan 1983, Bujang et  
185 al. 2008), whereas the wide leaf type has been reported to occur in confined to sheltered and  
186 muddy locations (McMillan 1983), in a variety of substrates, from coralline sand to muddy  
187 and calcareous sandy-mud (Bujang et al. 2008). This suggests that their morphological  
188 differences are related to nutrient availability. Our data on the elemental composition of above  
189 and belowground plant components of *H. wrightii* of Príncipe supports this, as CN ratios are  
190 significantly lower than those of *H. wrightii* from São Tomé. This morphotype develops at a  
191 sheltered bay near the coast in the vicinity of a local community, in contrast with the open  
192 ocean location at São Tomé where the long and narrow leaf morphotype develops. The CN  
193 ratios also suggest that the rhizomes of *H. wrightii* from São Tomé and from Príncipe do not  
194 store relevant quantities of nitrogen as the ratios of belowground parts are about half of the  
195 leaves in both locations, São Tomé and Príncipe. In contrast, the carbon content of *Halodule*  
196 leaves from São Tomé and Príncipe (41 %) was much higher than that reported for other  
197 *Halodule* species (*H. uninervis*, ~ 33 %) (Duarte 1990). This suggests that *H. wrightii*  
198 meadows of São Tomé may be a relevant sink of carbon but further research is needed to  
199 assess their carbon sink capacity.

200 The area around the Cabras islet is locally known as “Sea of Turtles” as it is an important  
201 feeding ground for these animals. It is also an important site of carbon storage. It therefore  
202 represents an ecologically valuable marine habitat that is relevant for conservation.

203

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214 Government of Príncipe for the collaboration and receptivity to the project. We also thank P.  
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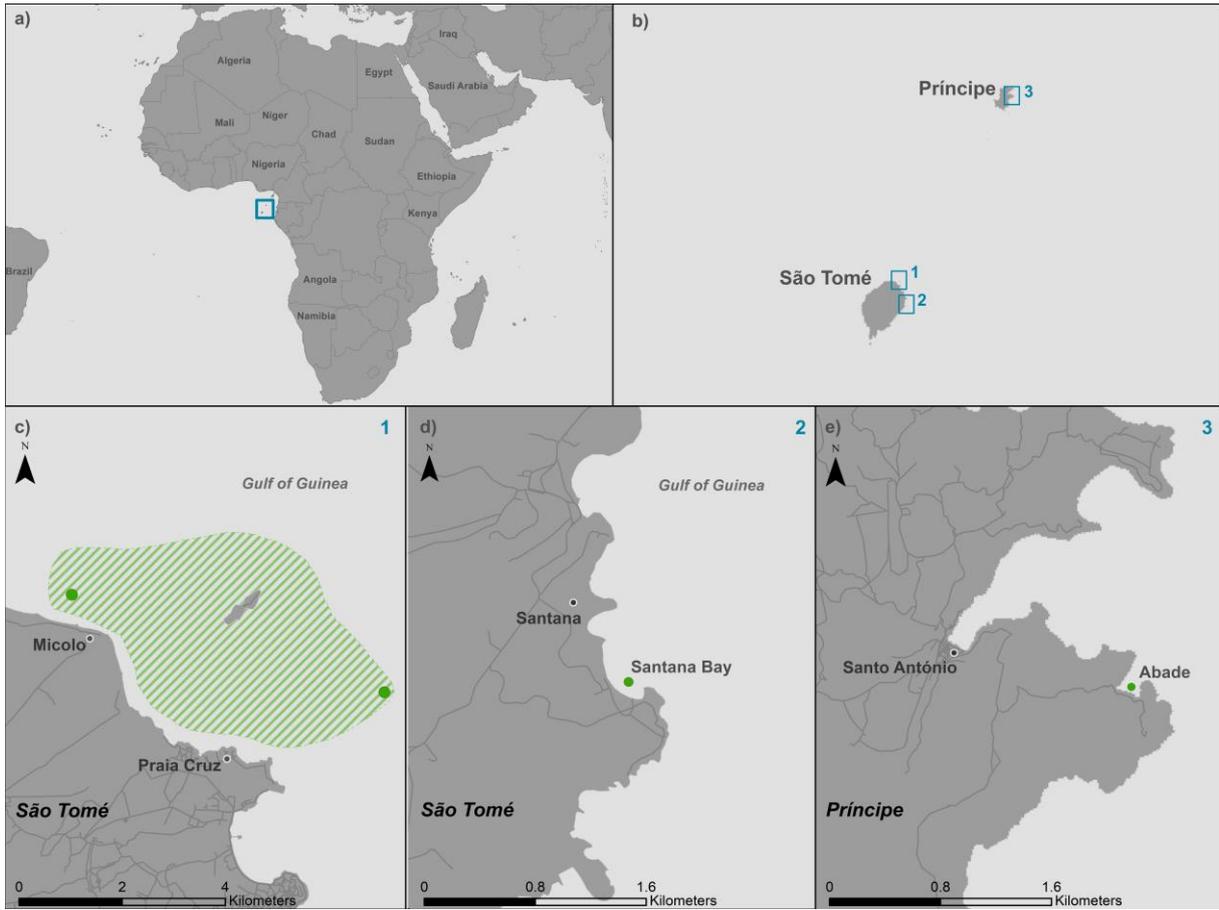
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288 Figures

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290 Figure 1. Maps of the geographical location of São Tomé and Príncipe and the sites where  
291 *Halodule* species were found: (a) location of the archipelago in the Gulf of Guinea, (b) detail  
292 of the islands showing the areas where the seagrass was found, (c) potential distribution area  
293 of *H. wrightii* around Cabras islet in the island of São Tomé and sampling sites (green dots),  
294 (d) location of the meadow at Santana Bay in the island of São Tomé (green dot), (e) location  
295 and sampling site of *H. wrightii* off Abade beach in the island of Príncipe (green dot).

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309 Figure 2. Aspect of the *Halodule* meadows in São Tomé and Príncipe: (a) dense meadow of  
310 *H. wrightii* with epiphytes around Cabras islet, in the island of São Tomé; (b) sparse meadow  
311 of *H. wrightii* off Abade beach, in the island of Príncipe.

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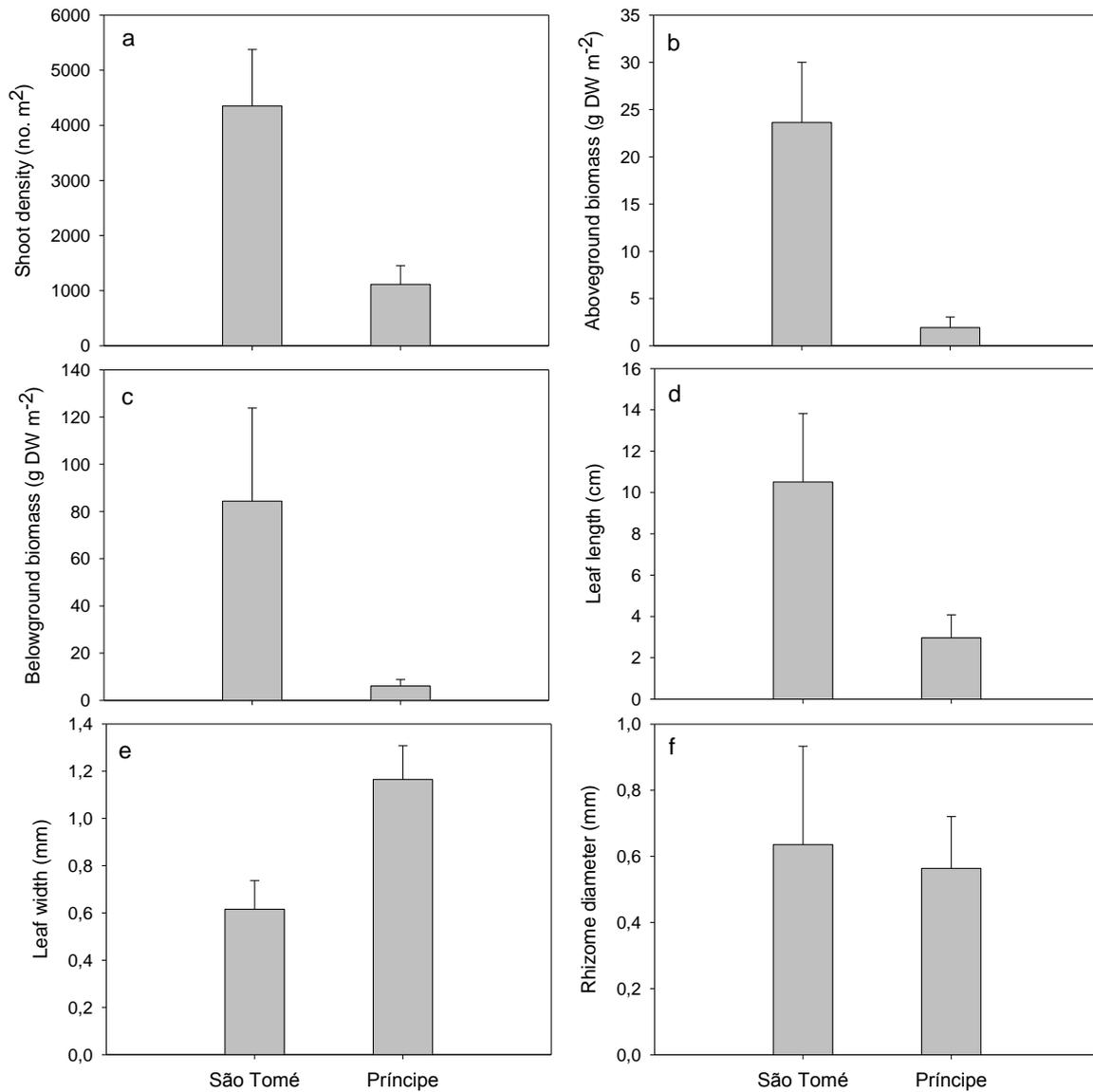
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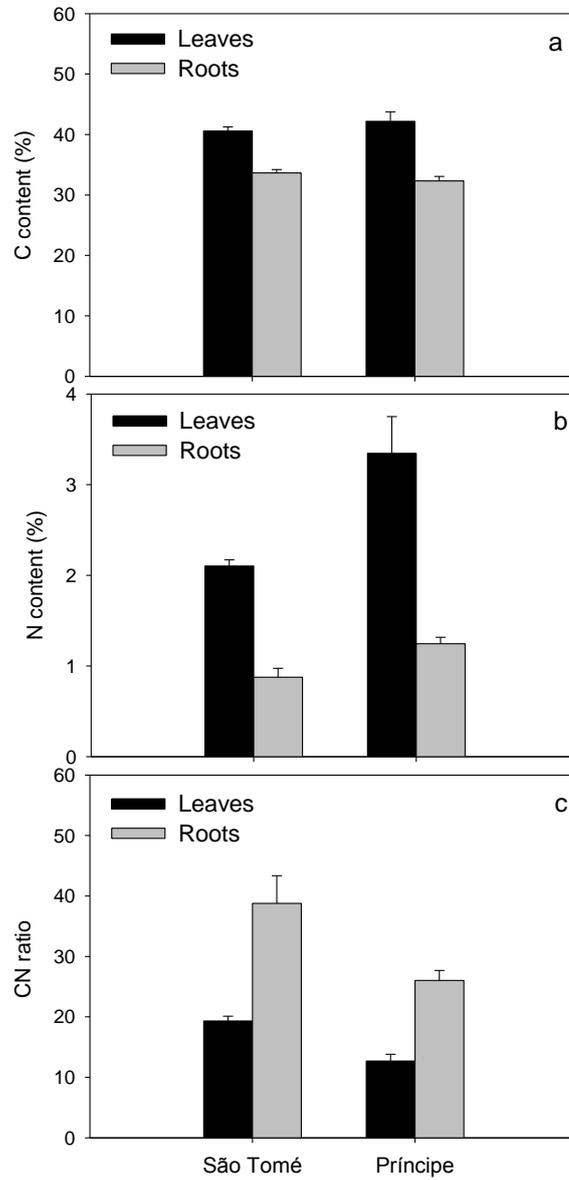
322 Figure 3. Morphometrics of the populations of *H. wrightii* sampled in São Tomé (n = 11) and  
323 in Príncipe (n = 6): (a) shoot density (no. m<sup>-2</sup>), (b) aboveground biomass (g DW m<sup>-2</sup>), (c)  
324 belowground biomass (g DW m<sup>-2</sup>), (d) leaf length (cm), (e) leaf width (mm), and (f) rhizome  
325 diameter (mm).  
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331 Figure 4. Leaf and root content of carbon (a) and nitrogen (b), and CN ratio (c) of *H. wrightii*  
332 from São Tomé and from Príncipe.

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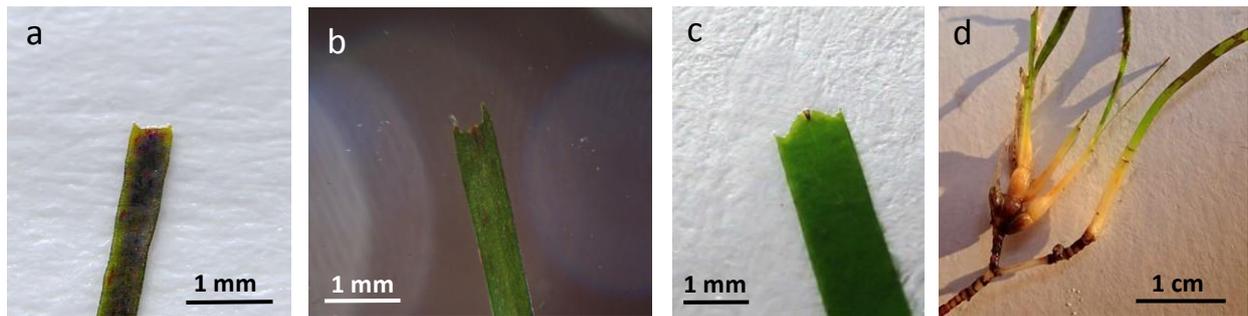
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337 Figure 5. Detail of the leaf tip of (a) *Halodule wrightii* collected around Cabras islet and (b)  
338 collected in Santana (São Tomé) showing bicuspidate blade apex with a slightly median  
339 concave surface, (c) *H. wrightii* collected in Abade beach (Príncipe) showing blade apex with  
340 lateral teeth and convex inner surface, and (d) specimen of *H. wrightii* with internodes  
341 infected by a parasite, probably *Plasmodiophora diplanthera*.

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