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

Ana Alexandre, João Silva, Rogério Ferreira, Diogo Paulo, Ester A. Serrão, Rui Santos

Centre of Marine Sciences (CCMAR), University of Algarve, Gambelas, 8005-139 Faro, Portugal

Corresponding author

Ana Alexandre (aalexandre@ualg.pt)

Abstract

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

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

Highlights

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

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

1. Introduction

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

Halodule has been reported. Despite the general knowledge of the global distribution of seagrasses (Green and Short 2003, Short 2007), many regions of the world remain poorly described, such as the West African seas. The potential for the occurrence of seagrass species in São Tomé and Príncipe has been reported by those authors but there are no scientific references of specific locations and no quantitative description of the populations.

São Tomé and Príncipe is a volcanic archipelago of two islands with a total area of 1001 km^2 (S. Tomé = 852 km^2; Príncipe = 142 km^2), located at the Equator in the Gulf of Guinea (0°25'N; 6°20'E) at 380 km off the continental African coast (Fig. 1a and b). The country lies within the Tropical Atlantic Bioregion and has a total coastline extension of 240 km. São Tomé and Príncipe has been designated a marine biodiversity hotspot and a priority for conservation for its high level of endemic species (Roberts et al. 2002). The region is particularly relevant for marine turtles as all the five cosmopolitan species nest here (Schneider 1992, Castroviejo et al. 1994). Seagrass habitats may be locally relevant as feeding grounds for the green turtle *Chelonia mydas*.

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

2. Methods

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

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

Differences in shoot density, above and belowground biomass, leaf width and CN ratio between populations of the two islands were detected using t-tests. Differences in leaf length and rhizome diameter between populations were tested using Mann-Whitney nonparametric 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).
103
104 3. Results
105
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 (0º24'31''N;
109 6º42'44''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 ± 1028 shoots
115 m^{-2} (SD, n = 11) (Fig. 3a), mean aboveground biomass was 23.65 ± 6.36 g DW m^{-2} and
116 belowground biomass was 84.38 ± 39.41 g DW m^{-2} (Fig. 3b and c). Mean leaf length and
117 width were respectively 10.5 ± 3.3 cm (n = 91) and 0.62 ± 0.12 mm (n = 34) (Fig. 3d and e),
118 and mean rhizome diameter was 0.64 ± 0.30 cm (n = 14) (Fig. 3f). The C and N content of the
119 leaves was respectively 40.6 ± 0.7 % and 2.1 ± 0.1 % (n = 5) (C:N = 19.3), whereas in
120 belowground plant parts it was 33.6 ± 0.6 and 0.9 ± 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 6º44'47''E) (Fig. 1d), covering a total area of 1500 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

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

3.2. Príncipe

Only one meadow of a highly morphologically different population of *Halodule wrightii* was found on the northeastern coast of the island of Príncipe, off Abade beach (1º38'0''N; 7º27'17''E), covering a small area of 135 m^2 at 4 m depth (Fig. 1e). The blade apex had a rounded median tooth and two shorter lateral teeth (Fig. 5c This leaf tip morphology lies within the range of shapes described for *H. wrightii* by van Tussenbroek et al. (2010). In contrast, this leaf tip shape in a short and wide leaf morphotype, as the one found in the population of Príncipe, is described as *H. beaudettei* by Magalhães et al. (2016). Preliminary genetic analyses showed equal sequences of nrDNA internal transcribed spacers (ITS) for both populations. This indicates that, if the morphologically distinct populations from São Tomé and from Príncipe are two different species, they are still very genetically related, as expected after a recent split. The meadow was surrounded by smaller seagrass patches of ca. 1 m diameter. The mean shoot density was 1114 ± 314 shoots m^{-2} (SD, n = 6), 4-fold lower than in São Tomé (Fig. 1a, $p < 0.001$). The mean aboveground biomass was 1.91 ± 1.11 g DW m^{-2} and belowground biomass was 6.07 ± 2.75 g DW m^{-2}, both about 10-fold lower than São Tomé (Fig. 1b and c, $p < 0.006$). Mean leaf length and width were respectively 3.0 ± 1.1 cm (n = 27) (3-fold lower that São Tomé, $p < 0.001$) and 1.17 ± 0.14 mm (n = 11) (2-fold higher than São Tomé, $p < 0.001$) (Fig. 1d and e), and mean rhizome diameter was 0.56 ± 0.16 mm (n = 13) (about the same as in São Tomé, $p = 0.662$) (Fig. 1f). The C and N content of the leaves was respectively 42.2 ± 1.6 % and 3.3 ± 0.4 % (n = 3) (C:N = 12.7), whereas in belowground plant parts it was 32.3 ± 0.7 and 1.2 ± 0.1 %, respectively (C:N = 26.0) (Fig. 4). 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).
153
154 4. Discussion
155
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}
165 , SE = 439) (Gutierrez et al. 1994). The shoot density of *H. wrightii* throughout the Atlantic
166 is thus highly variable and might depend on local environmental conditions and putative
167 genetic variations. The shoot density of *H. wrightii* in Príncipe was similar to that reported by
168 Magalhães et al. (2016) in Brazil (1955 shoots m^{-2}, SE = 1475) but plant biomass in Brazil
169 was much higher, particularly the belowground biomass (28 g DW m^{-2} compared to 1.91 g
170 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 Principe 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* (Kaewsrikhaw 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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Figures

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

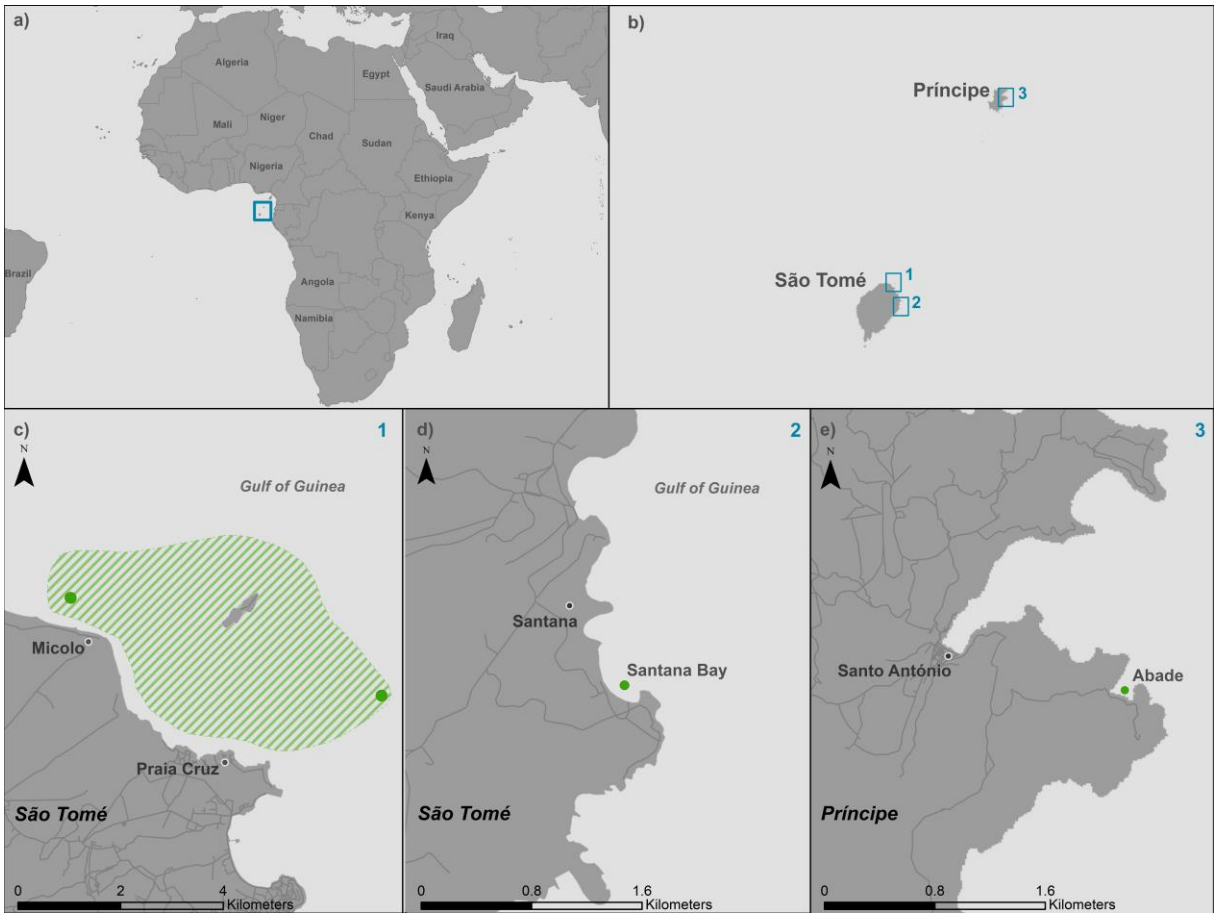
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Figure 2. Aspect of the *Halodule* meadows in São Tomé and Príncipe: (a) dense meadow of *H. wrightii* with epiphytes around Cabras islet, in the island of São Tomé; (b) sparse meadow of *H. wrightii* off Abade beach, in the island of Príncipe.

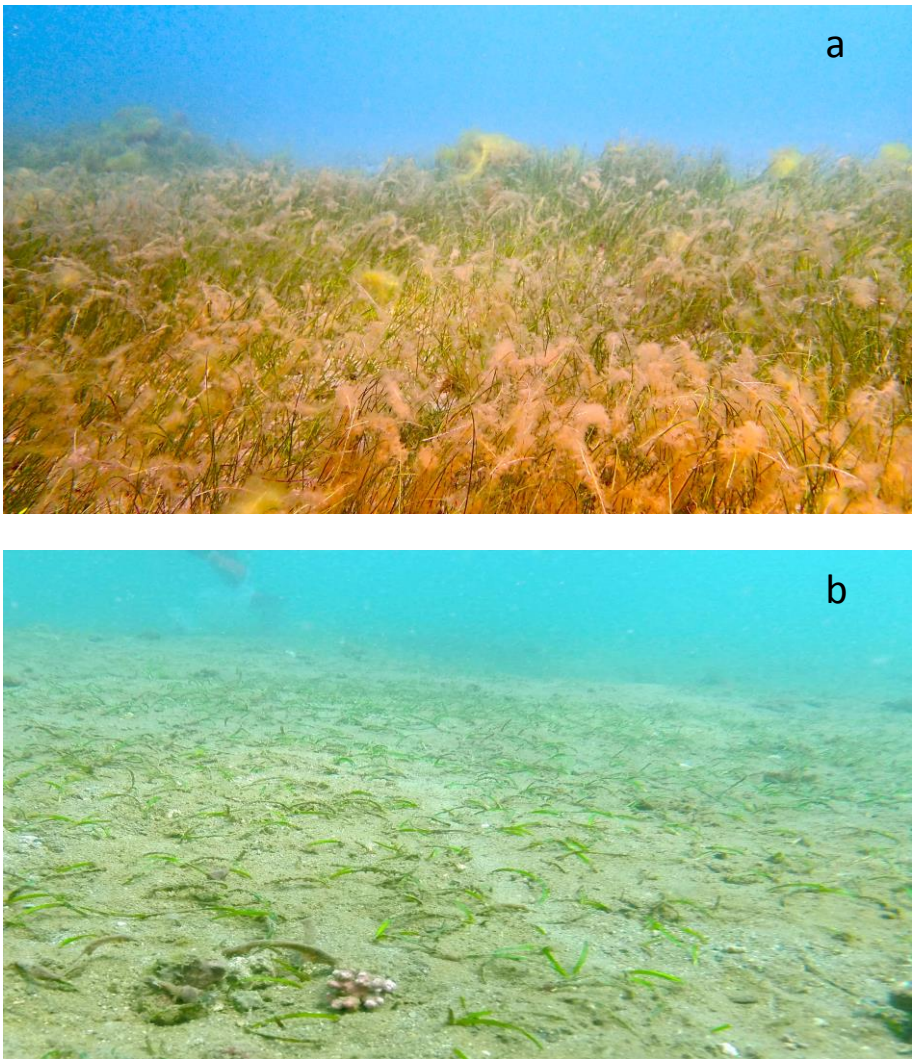

Figure 3. Morphometrics of the populations of *H. wrightii* sampled in São Tomé (n = 11) and in Príncipe (n = 6): (a) shoot density (no. m^{-2}), (b) aboveground biomass (g DW m^{-2}), (c) belowground biomass (g DW m^{-2}), (d) leaf length (cm), (e) leaf width (mm), and (f) rhizome diameter (mm).

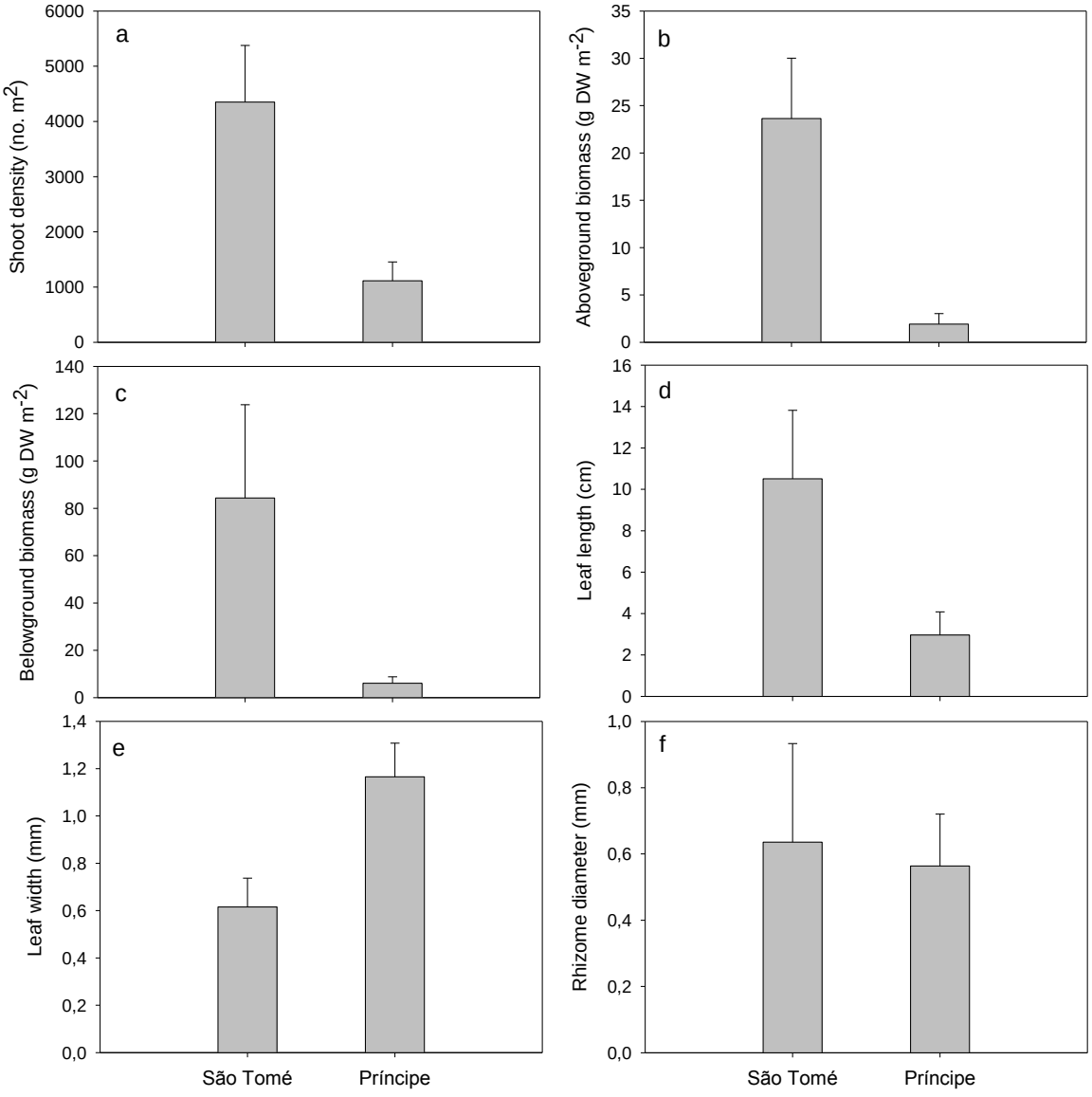

Figure 4. Leaf and root content of carbon (a) and nitrogen (b), and CN ratio (c) of *H. wrightii* from São Tomé and from Príncipe.

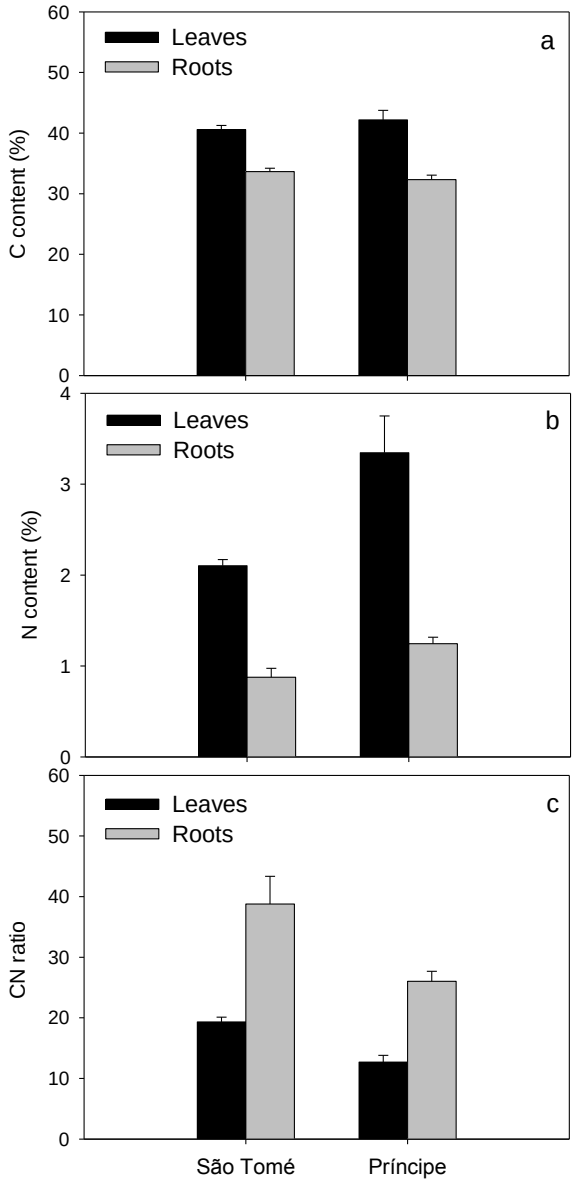

Figure 5. Detail of the leaf tip of (a) *Halodule wrightii* collected around Cabras islet and (b) collected in Santana (São Tomé) showing bicuspidate blade apex with a slightly median concave surface, (c) *H. wrightii* collected in Abade beach (Príncipe) showing blade apex with lateral teeth and convex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