1   **Type:** Research article

2   **Electronic supplements: Supplement_ms_Canepuccia_et_al.docx**

3

4   **Driving forces behind latitudinal variations in plant-herbivore interaction in South West**

5   **Atlantic salt marshes**

6

7   Alejandro D. Canepuccia[1,*], Juan L. Farina[2], Eugenia Fanjul[1], Florencia Botto[1], Jesus

8   Pascual[1], Oscar Iribarne[1]

9

10   [1] - Instituto de Investigaciones Marinas y Costeras (IIMyC), UNMDP, CONICET, CC 1260

11   Correo Central, B7600WAG, Mar del Plata, Argentina

12   [2] - Museo de Ciencias Naturales Lorenzo Scaglia, Area Entomología, Mar del Plata, Av.

13   Libertad 3099, 7600, Argentina

14

15   [*] Correspondence author. E-mail: acanepuc@mdp.edu.ar

16

17   **Running head:** Latitude and plant-herbivore interaction

18   ABSTRACT: Despite long-standing interest in the hypothesis that strength of plant-herbivore

19   interactions decreases at higher latitude, supporting evidence is scarce and the data is

20   conflicting. By field survey and two experiments we examined this hypothesis focusing on the

21   herbivory by the stem-borer moth (*Haimbachia* sp. nov.) on the dominant SW-Atlantic marsh

22   grasses (*Spartina alterniflora* and *S. densiflora*). Field surveys indicate that herbivore

23   abundances and damage, although tend to decrease simultaneously, are unrelated to latitude.

24   Herbivore abundances were related to latitude-dependent variables such as day length and

25   temperature, and also to latitude-independent variables such as precipitation, salinity, and tide

26   amplitude. Abundances were indirectly related to the effects of these variables and sediment

27   characteristics on plant traits like density, height and tissue-composition. After 33-months,

28   herbivore abundances and damage of high-latitude plants transplanted to low-latitude were

29   50-times greater than plants transplanted from low-to high-latitude sites. In a common-garden

30   experiment (38º 56´ S) without herbivore pressure, differences persisted in plant traits from

31   high and low latitude suggesting lack of herbivore induced effects on these plant traits. The

32   persisting conspecific differences in plant-traits translocated along latitude suggest that these

33   variations are under genetic control. Thus, our results provide evidence that although plant-

34   herbivory interactions are more important at lower latitude, many additional and contingent

35   variables unrelated with latitude can divert this geographic pattern.

36

37   KEY WORDS: plant-herbivore interactions · latitude gradients · coastal ecosystem ecology ·

38   trophic interactions · marsh · salinity · *Spartina* · stem-borer moth

INTRODUCTION

Selective pressures on organisms vary throughout latitudinal gradients in abiotic factors (Schemske et al. 2009), and then the nature of their interactions (Więski & Pennings 2014). Furthermore, the evidence has led to hypothesize that species interactions (e.g., herbivory, competition and predation) become stronger toward the tropics (Schemske et al. 2009). In the case of plant-herbivore interactions, it is expected that herbivore pressure would increase at lower latitudes, and correspondingly, plants should evolve more effective defenses (Coley & Barone 1996, Schemske et al. 2009, Więski & Pennings 2014, Hahn and Maron 2016). However, the generality of such hypothesis has been questioned since it is supported only in the Northern Hemisphere (Zhang et al. 2016), and also due to the existence of opposite evidence, or lack of herbivory effects (e.g., Moles et al. 2011, Lim et al. 2015, Zhang et al. 2016). A possible explanation is the existence of many variables affecting plant or herbivores that are not latitudinally controlled (Pennings & Silliman 2005, McCall & Pennings 2012, Anstett et al 2016, Kooyers et al. 2017), which increase the context-dependence of the observed pattern among different world regions. In addition, tests of this hypothesis are relatively scarce (e.g., Long et al. 2011, Więski & Pennings 2014) and deviations from this latitude-herbivory pattern could be due to comparison of different species of plants and/or herbivores, or even changes in the communities along latitude gradients (Anstett et al. 2014). Given that physical factors are expected to change (Adam 2002, IPCC 2014), and that such factors also vary along latitude (e.g., De Frenne et al. 2013), understanding the effect of these geographic variations on the mechanisms driving species interaction is critical to understand ecosystem function and to predict responses to climate change.

Intertidal marshes are some of the most common coastal habitats (Adam 2002). These habitats are dominated by a few plant species, some of which are among the most worldwide

64   distributed plant (e.g., Spartina species, Adam 2002). The herbivore pressure in marshes has

65   been reported as stronger at low than high latitudes (e.g. Pennings & Silliman 2005). Then,

66   these species face very different environmental conditions along latitude (Pennings &

67   Silliman 2005, Canepuccia et al. 2013), varying some of their traits constitutively (genetically

68   based traits), or induced (e.g., Więski & Pennings 2014) in relation to the prevailing local

69   biotic and physical conditions. Southwest Atlantic (SWA) *Spartina* marshes are distributed

70   along a latitudinal gradient (Isacch et al. 2006). These marshes are dominated by two *Spartina*

71   grasses, *S. alterniflora* and *S. densiflora* (Isacch et al. 2006). Within these marshes, there is

72   only one stem boring moth (*Haimbachia* sp. nov.), which is a common *Spartina* specific

73   herbivore (Canepuccia et al. 2011). The moth imagoes emerge in early summer, and as a

74   consequence of larvae damage, the attacked stems die (Canepuccia et al. 2011). A single larva

75   of this moth lives inside of a *Spartina* stem, feeding on a large portion of the basal and middle

76   central stem tissue. This particular herbivore–plant interaction allows to identify easily the

77   herbivore damage as well as the variation of the number of herbivores per area (Canepuccia et

78   al. 2011). Such characteristics, paired with the observed moth density decrease with latitude

79   (Canepuccia et al. 2011), make this plant-herbivore system extremely attractive to examine

80   how changes in physical factors along latitude modify plant-herbivore interaction. The use of

81   this system precludes the existence of artifacts resulting from the comparison of different

82   species of plants or herbivores, and different communities along latitude.

83   By using *Spartina*-moth interaction as a model, our goal was to test the hypotheses that:

84   (i) the strength of plant-herbivore interactions decreases with latitude driven by the variation

85   in physical and environmental conditions and, (ii) variations in plant traits that impact

86   herbivory along latitudes are under genetic control. To test the first hypothesis we describe the

87   gradients in temperature, precipitations, tidal level, and salinity along 8° of latitude in the SW

88   Atlantic coastline (SWA, Fig. 1). Then, we provide an overview of the existent latitudinal

89   variation in these variables, as well as their possible effects on herbivore abundances, plant

90   traits and on plant-herbivore interactions. To test the second hypothesis, we experimentally

91   explored if *Spartina* species transplanted towards higher or lower latitudes from their origin,

92   and therefore growing in different environmental conditions, suffer different herbivory

93   pressure. Here, we were particularly interested in comparing the rates of moth attacks on

94   plants with traits from high and low latitude and to know if these plant traits variations were

95   under genetic control or plastic responses induced by the variation in environmental cues.

96   Thus, we evaluated the hypothesis that herbivory decreases with latitude, using natural

97   gradients and performing field survey and two experiments using the same species of plants

98   and herbivore, within the same habitat.

99

100   **METHODS**

101   **Study area**

102   To evaluate the variation in plant-herbivore interaction we selected eight marshes

103   dominated by *Spartina densiflora* and *S. alterniflora* along the SWA coast (Fig. 1). The sites

104   were: the outlet of Salado River (SAL: 35º 44´ S, 57º 22´ W), San Clemente (SC: 36º 19´ S,

105   56º 46´ W), Bahía Blanca (BB: 38º 51´ S, 62º 07´ W), Bahía Anegada (BA: 40º 31´S, 62º 19´

106   O), the outlet of the river Rio Negro (RN: 41º 01´ S, 62º 48´ W), Bahía San Antonio (BSA:

107   40º 43´ S, 64º 54´ W), Riacho San Jose (RSJ : 42º 25´ S, 64º 37` W), and the outlet of the

108   river Chubut (RCH: 43º 19´ S, 65º 04´ W). These marshes are characterized by two main

109   habitats: (a) the lower marsh, develops at the maritime front and occupy by a monoculture

110   stand of the smooth cordgrass *S. alterniflora,* and (b) the high marsh develops at the upper

111   intertidal height and dominated by the dense-flowered cordgrass *S. densiflora* and the

112   perennial glasswort *Sarcocornia perennis* (Isacch et al. 2006). Along SWA marshes, the

113   larvae of the stem-boring moth *Haimbachia* sp. nov. (hereafter 'moth larvae') is a common

114   herbivore, and its abundance seems to decrease with latitude (Canepuccia et al. 2010a,

115   Canepuccia et al. 2011). This middle latitudinal range of marshes is distributed along of a

116   southward decreasing in precipitations from 900 mm year^{-1} to 200 mm year^{-1} together with an

117   increment of daily thermal amplitudes (Canepuccia et al. 2013). From the ocean side, the

118   sampled marshes are distributed along a gradient of southward increment in tidal amplitudes

119   from microtidal condition (0.75 m above sea level: MASL) to a meso-macrotides condition (3

120   to 6 MASL, Isacch et al. 2006, see Table S1 in the Supplement).

121

122   **Strength and significance of direct and indirect effects on plant-herbivore interactions**

123   To estimate the frequencies of moth larvae, at spring of 2010 (full growing season of

124   the moth larvae), we collected all mature plant stems at 10 randomly selected plots (25 × 25

125   cm) at the center of the spatial distribution of each *Spartina* species (*S. alterniflora* and *S.*

126   *densiflora*) for each study site (Fig. 1). Hereafter sampling plots were, at least, 20 m apart to

127   avoid collecting same plant genotypes (e.g., Richards et al. 2004). All stems were taken to the

128   laboratory and cut longitudinally. We only counted as attacked those stems with larvae inside

129   to prevent overestimate the attack rates by the count of stems attacked in former seasons. We

130   then estimated the frequencies of moth attack as the ratio between the number of attacked

131   stems and the total number of stems in each plots for each *Spartina* species.

132   We estimated plant traits that may be associated with their quality and quantity as food

133   for marsh herbivores (e.g. Siska et al. 2002, Stiling & Moon 2005, Canepuccia et al. 2011).

134   We estimated plant densities, heights of stems, fiber contents and carbon/nitrogen ratios (C:N)

135   for both *Spartina* species for each study site. Densities of plants were measured counting all

136   mature stems in the same plots where moth frequencies were estimated. Plant heights were

137   estimated of a single randomly chosen plant within each plot. Total fiber (following Van

138   Soest et al. (1991) and C:N ratios in plant tissue, were estimated from a pool of 20 stems from

139   five randomly chosen plots for each site and each plant species. All C and N samples were

140   analyzed at the Stable Isotope Facility of the University of California (Davis, USA).

141   To characterize the prevailing environmental conditions at each study site, we

142   estimated major physical variables associated with plant growth, such as precipitation

143   (Canepuccia et al. 2010b, Eiserhardt et al. 2011), air temperature (Speziale et al. 2010),

144   sediment organic matter, salinity and moisture (Bertness et al. 1992, 2008, Dagar 2010) and

145   particularly important for marsh vegetation, anoxic stress, sediment grain size, and tidal

146   amplitude (Bertness et al. 1992, Pennings et al. 2005, Daleo & Iribarne 2009, Canepuccia et

147   al. 2010a). Average of the last 5 years annual precipitation values and the daily maximum and

148   minimum temperatures were calculated from the nearest station from each study site of

149   Argentinean National Weather Service (Servicio Meteorológico Nacional Argentino; see Fig.

150   1) in the latitudinal gradient studied. This 5-years average was performed given that *Spartina*

151   stems do not die in winter but survive almost two years (steams survive in average 18 months,

152   Vera et al. 2009) while plant roots and plant stolons live for several years (Canepuccia per.

153   observation). The mean of the maximum thermal amplitude was estimated for each year as the

154   maximum daily differences between the maximum and minimum temperature for each site.

155   For missing data, as well as for BA site, which did not have a nearby meteorological station,

156   the climate variables were estimated by the Geostatistical Kriging interpolation technique

157   (e.g., Akkala et al. 2010), using latitude and longitude as independent variables. Tidal

158   amplitude, water salinity, sediment grain size distribution, sediment oxygen content (O_2),

159   sediment moisture, and sediment percentage of organic matter (OM) were estimated for each

160   study site. The averages of tidal amplitude were obtained from Isacch et al. (2006). Water

161   salinity values were measured (precision of 1‰) from 10 water samples at a distance of 20 m

162   each along the coastline during high tides from each study site. The grain size distribution was

163   estimated by collecting five samples of sediment (10 cm depth, 10 cm diameter) at each

164   marsh habitat and each site. Sediment was sieved and fractions separated in >1000 μm

165   (cobble and pebble), 1000–125 μm (sand), and 125 μm (silt and clay), dried (60 Cº, 72 h), and

166   weighed. The OM content was estimated in sediment samples (10 cm depth, 10 cm diameter),

167   by loss weight between dry (7 days at 60 °C) and combusted (8 h at 500 °C) sediment.

168   Sediment oxygen content (O_2) was measured in situ by gently pushing a Clark type glass

169   microelectrode (500μm, tip, Unisense A∕S, Denmark) 5 cm into the sediment. Analyses of

170   variance (ANOVAs) or Welch's test (adjusted for inequality of variances Zar 1999), followed

171   by Tukey's HSD (Zar 1999) were used to evaluate the null hypothesis of no differences in

172   frequencies of moth larvae herbivory, plant traits and physical variables among locations. In

173   each case, data were checked for normality with the Kolmogorov-Smirnov test (Lilliefors'

174   correction) and for equal variance with Levene median test.

175   Structural Equation Modeling (SEMs, Grace 2006) were developed to evaluate direct

176   and indirect (mediated by plant traits) effects of latitude and physical variables on the

177   frequencies of moth attack on both *Spartina* species along the SWA coast. The physical

178   variables were included as exogenous variables, and plant traits and moth attack frequencies

179   were included as endogenous variables (see Fig. S1 in the Supplement). Because of the

180   differences in the variables units (e.g., plant density, temperature) standardized coefficients

181   were used to compare the magnitude of the paths. The links exhibiting high collinearity were

182   eliminated to derive in more stable path coefficients. To assess the fit of the models index of

183   the goodness-of-fit chi-square statistic (χ^2), best-fitting possible model the Comparative Fit

184   Index (CFI) and the Tucker Lewis Index (TLI) were used (Bentler & Chou 1987). The root

185   mean square errors of approximation (RMSEA), which is adjusted for degrees of freedom and

186   is considered an index of the parsimonious and good-fitting model were also estimated

187   (Browne & Cudeck 1992).

188   Given the relative high complexity of proposed models for the sample size, a

189   bootstrapping procedure was used to evaluate model stability (Ievers-Landis et al. 2011). For

190   that, a bootstrap procedure (10000 interactions for each regression path in the model) of the

191   initial model was run. After that, if the original sample weights did not differ from the mean

192   of the weights obtained through the bootstrap, the models were considered as unbiased

193   (Ievers-Landis et al. 2011). To compare each regression path in the model, the pairwise

194   differences with the original model values were calculated for each Bootstrap interaction.

195   When zero fell outside the 95% confidence interval of the distribution of resampled

196   differences (Manly 2006), was considered that the estimator did not differ between Bootstrap

197   models and original models. The parameter estimation and testing of model fit were

198   performed using R (R Development Core Team 2012) with the Lavaan libraries (Rosseel

199   2012).

200

201   **Transplant experiments**

202   To analyze if variations in plant traits along latitudes are genetically based or are

203   plastic response to biotic and physical cues in each locality, two experiments of plant

204   translocation were conducted. In the first, both *Spartina* species were propagated clonally. We

205   selected 40 plots (15 cm x 15 cm) of each plant species from high (SC), middle (BB) and low-

206   latitude sites (BSA, see Fig. 1). Attacked stems within each plot were removed. Then, by

207   digging 25 cm deep of sediment with plant rhizome, ten random blocks of each plant from

208   each geographic region were transplanted to the other sites (high, middle and low-latitude

209   sites), while ten plots remained as control. The field-collected clones were within 24 hours

210   transplanted to the different sites, to minimizing the double transplant stress. To eliminate the

211   possible effects of the maternal sediment characteristics, before growing each plant in the

212   transplant locations, we took out the sediment containing the rhizomes in each individual

213 plant. To evaluate the transplants effects on plants, the last ten blocks were transplanted

214 within the same site (auto-transplants), as a treatment control.

215 A second experiment was performed to evaluate if in constant environmental

216 condition of plant growth, and without moth herbivory there are variations in plant traits

217 depending of their latitudinal origin. Ten experimental blocks of *S. densiflora* from each

218 region (high, middle and low latitudes) were transported to the laboratory and grow under the

219 same experimental conditions in an open air-garden environment. Plants were planted (15 cm

220 x 15 cm pot) in standardized substrate of 50:50 mixtures of commercial potting soil and sand

221 (Pennings et al. 2009). To keep plants in intermediate conditions of growth of the latitudinal

222 range studied, the experiment was performed in a coastal and mid-latitude site (Mar del Plata

223 38º 56´ S) free of moths with natural photoperiod and temperature values. We checked to

224 maintain experiment free of herbivores and performed watering twice a day until saturation

225 with fresh water.

226 Both experiments lasted 33 months (three consecutive growing periods) to be sure that

227 all stems in each experimental unity had born and grown under experimental conditions. At

228 the end of this period all stems were cut. The frequencies of moth larvae attack in each block

229 of each treatment of the field experiment, and plant heights (for each block), NDF and C:N

230 (for 5 randomly block) in each block of each treatment of both experiments were estimated as

231 formerly explained. At the end of field experiment, we only found the basal stems section of

232 some plants of the transplant blocks (first 5 to 10 cm), presumably because larvae feeding

233 promoted stem breaking and dragging by tides (Canepuccia et al. 2011). To assure the

234 estimation of herbivory frequency, we discarded these samples, and only used those where the

235 state of the plant allowed us to verify the larvae presence (larvae or signs of larvae: a hole,

236 tunnel or sawdust). Considering the difference in sample numbers and the inequality of

237 variances of data, to test for main effects (Plant or Site) and interaction effects (Plant: Site),

238 we performed a non-parametric statistics (Anderson et al. 2001). Permutation test of the

239 distributions of the F statistics for main effect and interaction effects were used (Manly 2006,

240 Anderson et al. 2001). Permutation testing (10,000 permutations) was applied using R

241 software (3.2.2; https://www.r-project.org/) and the package 'asbio' (Aho 2017). Function

242 'perm.fact.test' was used to conduct permutation testing of the main effect and interaction

243 effect. For pair wise comparisons we performed Monte Carlo analysis (Manly 2006). For

244 these analyses, we used random re-sampling with replacement (10,000 iterations) preserving

245 the sample size. For each iteration we recalculated the pairwise mean differences of variable

246 (plant traits and moth attack frequencies) between treatments. Then, we considered that a

247 variable differed significantly between treatments if zero fell outside the 95% confidence

248 interval of the distribution of resampled differences (following Manly 2006). Analyses of

249 variance (ANOVAs) followed by Tukey's HSD (Zar 1999) were used for evaluate the null

250 hypothesis of no differences among plant traits from different location in the free of moths

251 environment experiment.

252

253

254 **RESULTS**

255 **Strength and significance of direct and indirect effects on plant-herbivore interactions**

256 Environmental variables (Table S1 in the Supplement), plants traits (Table S2 in the

257 Supplement) as well as herbivore densities changed along latitude (Fig. 2). The attack

258 frequencies of moth in both plants varied along the SWA marshes (Welch's test of Box-Cox

259 transformation of attack frequencies in *S. alterniflora*: $F_{6, 27} = 109.45$, $p < 0.01$, theta = 0.7; *S.*

260 *densiflora:* $F_{7, 30} = 21.77$, $p < 0.01$, theta = 0.8), and is higher in lower latitude sites (SAL and

261 SC) decreasing towards higher latitude ones (BB and BSA). However, attack frequencies

262   increased again in the higher latitude sites located at river outlets (RN, for *S. alterniflora,*

263   RCH for *S. densiflora,* Fig. 2).

264   SEM fitted models for plants-herbivore interactions for each marsh habitat (dominated

265   explained variation of: plant densities (53% for *S. alterniflora*, 85% for *S. densiflora*), plant

266   heights (84% for *S. alterniflora*, 91% for *S. densiflora*), plant content in fiber and C:N ratios

267   (100% for both plants) and moth attack frequencies (93% for *S. alterniflora*, 76% for *S.*

268   *densiflora*, Fig. 3; Table S3 in the Supplement). The observed correlation matrix did not differ

269   from the expected matrix (χ^2 = 13.68, df =21; *p*= 0.88 for *S. alterniflora;* χ^2 = 43.39, df = 51;

270   p = 0.73 for *S. densiflora*), and the low RMSEA (< 0.0001 for both plants) indicated a good

271   model fit. The tests comparing the target model with the null model indicated parsimonious

272   and good-fitting models. (CFI = 1.00; TLI = 1.00 for both plants). The non-standardized

273   regression weights for the original models were no different from those of bootstrap samples,

274   suggesting that all of the paths were unbiased for the both plants (Fig. 3; Table S3 in the

275   Supplement), indicating parsimonious and good-fitting models.

276   According to the *S. alterniflora* model, the plant density increased with increasing O_2

277   but decreased with the increase of minimum temperature. Height of this plant increased with

278   OM and decreased with increasing in salinity and tidal amplitudes (Fig. 1A; Table S3 in the

279   Supplement). Fiber content increased with tidal amplitudes; and decreased with maximum

280   and average temperatures, OM, O_2, and water salinity. The C:N ratios increased with

281   increasing in average temperature, O_2 and OM; and decreased with increasing in minimum

282   temperature and water salinity (Fig. 3a, Table S3 in the Supplement). Finally the attack

283   frequencies of the moth on *S. alterniflora* increased with the increased in the average

284   temperatures, and plant traits such as height, densities, C:N ratios and fiber content; while

285   decreased with the increased in tides amplitudes (Fig. 3a, Table S3 in the Supplement).

286   According to the *S. densiflora* model, plant density increased with OM, O_2 and

287   sediment moisture and decreased with the increasing maximum temperature, minimum

288   temperature and salinity. The height of *S. densiflora* increased with increasing precipitations

289   and plant densities, and decreased with increasing salinity, tidal amplitudes, and O_2 (Fig. 3b;

290   Table S3 in the Supplement). The fiber content in plant tissues increased with decreasing in

291   day length and OM, and decreased with increasing in the maximum temperature, minimum

292   temperature, salinity and sediment moisture. The C:N ratios in *S. densiflora* tissue increased

293   with the increasing in fiber, O_2, and salinity, and decreased with the increasing in day length

294   and sediment moisture (Fig. 3b; Table S3 in the Supplement). Finally, the attack frequencies

295   of moth on *S. densiflora* stems increased with plant densities, plant height, content of C:N,

296   tidal amplitudes, and decreased with the increasing fiber and salinity (Fig. 3b; Table S3 in the

297   Supplement).

298

299   **Transplant experiments**

300   At the end of the field experiment plants transplanted to low latitude site (SC) showed

301   higher frequencies of moth attacks than plants transplanted to mid-latitude site (BB), and high

302   latitude site (BSA, Fig. 4). Whereas these attack ratios were different according to the plant

303   origin (interaction affects, Fig. 4 and Table S4 of the Supplement). For example, *S.*

304   *alterniflora* from mid and high latitudes showed all stems attacked by moth larvae (Fig. 4).

305   For both plant species there were no differences in the attack frequencies between control of

306   the transplant treatments (auto-transplants) and control plants in each experimental site.

307   At the end of field transplants experiment, the heights of both plant species tend to be

308   taller at low and middle latitude than at high latitude (Table S4 and S5 of the Supplement).

309   Whereas the heights of plant coming from low latitude tend to be taller than those from mid

310   and high latitude sites (interaction effect, Table S4 of the Supplement). *Spartina alterniflora*

311 at mid-latitude and all high latitude plants transplanted to low latitude site were killed by

312 moths (broken stem with remnant of hollow made from larva feeding, see Canepuccia et al

313 2011). Hence the measurements of plant traits could not be done. At mid and high latitude

314 sites, plants coming from high latitudes showed the lowest heights. While at high latitude site,

315 plants coming from low latitude showed the highest heights (Table S4 and S5 of the

316 Supplement). There were no differences in heights for both plant species between "treatment

317 control" and "control" for each site (Table S5 in the Supplement).

318   Fiber content in *S. alterniflora* tissues showed the highest values in plants transplanted

319 to high latitude sites, while for *S. densiflora* the lowest fiber content were for plants

320 transplanted to mid latitude sites (Site effect, Table S4 of the Supplement). With the exception

321 of *S. densiflora* transplanted to mid latitude, there were no differences in fiber content

322 between control plants of transplant treatments and control plants coming from each site and

323 each species (Table S5 in the Supplement). The C:N ratios in *S. alterniflora* tissues showed

324 the lower values in plants transplanted to mid latitude (Site effect, Table S4 of the

325 Supplement), while for *S. densiflora* the lower C:N ratios were for plants transplanted to mid

326 and low latitude site (Table S5 of the Supplement). There were no differences in C:N ratios of

327 plant coming from different sites within low and high latitude sites (Table S4 and S5 in the

328 Supplement).

329   In the experiment in moth-free environment, we checked continuously and did not see

330 tracks of other herbivores. *Spartina densiflora* from high, middle and low latitude growing in

331 an intermediate latitude values without moths ("common garden" outside the marsh) showed

332 differences in their morphology and tissue composition. Plants from low latitude showed

333 greater height than those from mid and high latitude (ANOVA: $F_{2,27} = 8.84$, p <0.001, Table

334 S6 in the Supplement). The fiber content was lower in plants from low latitude than those

335 from mid and high latitude (ANOVA: $F_{2, 12} = 13.73$, p < 0.001, Table S6 in the Supplement).

336   There were no differences in C:N ratios in plant tissues from low latitude, mid-latitude and

337   high latitude sites (ANOVA: $F_{2, 12} = 0.50$, $p = 0.62$, Table S6 in the Supplement).

338

339   **DISCUSSION**

340   Our results documented that along South Western Atlantic (SWA) marshes, the

341   herbivore abundances and plant damage were related to latitude- and not latitude-dependent

342   variables. Among latitude-dependent variables, the frequency of moth in *S. alterniflora*

343   increased with the increasing average temperature. This association is probably because cold

344   affects herbivore arthropods survival (Deutsch et al. 2008). In addition, herbivore abundances

345   increased with plant densities, which also increased with average temperatures, minimal

346   temperature and low daily thermal amplitude. Along our study range, the minimum

347   temperature consistently decreased to higher latitudes, resulting in a markedly increase of

348   daily thermal amplitude to high latitudes. This decrease in weather stability can have negative

349   effects on plant growth (e.g. Speziale et al. 2010), indirectly enhancing the temperature effects

350   on herbivore abundances and plant damages.

351   But not only plant morphology was affected, C:N ratios in plant tissues also increased

352   with decreasing daylight and minimum temperatures. Consistent with a widespread latitudinal

353   increasing trend of leaf N content (Reich & Oleksyn 2004), plant N contents increased with

354   latitude in salt marshes (Siska et al. 2002). This variation may be because plants can

355   accumulate more N at higher latitudes (He & Silliman 2015) since cold temperatures limit

356   photosynthetic C intake more than root N uptake (Reich & Oleksyn 2004). Moreover, short

357   growing season requires a higher N content to allow rapid growth in high latitude marshes

358   (Siska et al. 2002), which may change the trade-off of food preference with latitude.

359   Surprisingly, moth abundances increased with the increasing in C:N ratios in both

360   plants, despite herbivores often preferring food with higher N content (Gratton & Denno

361 2003). This association could be because larvae may prefer stems with higher proportion of

362 structural tissues like fiber, decreasing the likelihood that debilitated stems (burrowed by the

363 larvae) break down due to tidal action (Canepuccia et al. 2011). Alternatively, this could be as

364 a result of plants facing lower herbivory rates, developing lower chemical defenses and

365 keeping higher N levels (Siska et al. 2002). Whatever the underlying cues, latitudinal proxy

366 variables emerge as important determinants of latitudinal variations in plant-herbivore

367 interactions along SWA marshes.

368   However, there were other factors that varied geographically but are not latitude-

369 dependent, which were also selected for differences in plant-herbivore interactions. For

370 example, the abundance and height of plants increased with increasing OM content, and all

371 tend to increase at lower latitude sites (e.g. Moles et al. 2009). Also, there may be a feedback

372 between OM and the plant community, but most of the detritus produced by marsh plants is

373 exported by tides (Montemayor et al. 2011), which play an important role in the dynamics of

374 these environments. Plants also decreased their heights with increasing in tidal amplitude,

375 which can decrease the risk of stems breaking while tides floods increase. Given the toxicity

376 of Na^+ and Cl^-, plants also reduce their growth and heights as salinity increases (Canepuccia

377 et al. 2010b). Indeed, as rain may dilute salt at high marsh (Canepuccia et al. 2010b),

378 precipitation increment is positively associated with *S. densiflora* height. Since these

379 variations in plant traits imply variation in the amount of food for moth, these variables also

380 have indirect effects on moth abundances through their effects on plant traits.

381   Furthermore, at the low marsh, the C:N in *S. alterniflora* tissues decreased with an

382 increase of salinity, this could be a consequence of the production of osmolytes due to salt

383 stress (e.g., Flowers et al. 1977, Rozema et al. 1985). This variation in tissue composition

384 could have a negative impact on moths. Indeed, at high latitude, in low marshes located in

385 river's outlet, where salinity is lower, the incidence of herbivory increases again. At the same

386   time, fiber content in plant tissues increased with increasing tidal amplitudes. This may

387   happen due to increased need of being resistant to higher tidal power. It is possible that the

388   negative relationship between organic matter and fiber content in the low marsh was

389   indirectly due to the loss of organic matter by outgoing tides (e.g. Tam and Wong 1998) when

390   tidal amplitude increases. So, this higher resistance could be also causing an increased moth

391   frequency in *S. alterniflora*. Indeed, moth abundances also decreased with increasing in tidal

392   amplitude. Then, if moth selects stems with higher C:N and fiber, and with lower risk of

393   being broken by tides, indirectly salinity and tides would be varying the moth selectivity.

394   However, at high marsh, exposed to a higher dryness, higher temperature changes

395   between day and night (Bertness 1999), and salt accumulation (Fanjul et al. 2008), the C:N

396   ratios in *S. densiflora* tissues increased with increasing salinity. These results that contrast

397   with those from the low marsh may be because the highest drying and excessive salinity

398   levels at high marsh increases plant woodiness (e.g., Stiling & Moon 2005). This may also

399   explain why *S. densiflora* height decreased with the increase of sediment O_2 content. A

400   greater degree of soil drainage and aeration in the high marsh may increase the water

401   evaporation, desiccation, and thus salt concentration in the sediment during longer periods

402   between flooding tides. In fact, since high salinity levels negatively impair herbivory (e.g.,

403   Dagar 2010, Canepuccia et al. 2010b), moth abundances decrease directly with increasing

404   salinity at high marsh. In addition, fiber content in *S. densiflora* is about twice as in *S.

405   alterniflora,* which may greatly reduce plant digestibility and palatability (Randolph et al.

406   1991) resulting in an inverse plant fiber-moth abundance relationship. There, tidal effects are

407   milder (weaker force and lower frequency) and thus, the stem rigidity (and plant fiber content)

408   is not so critical for larvae to complete its cycle within stems. Then, salinity and tides

409   indirectly may change the trade-off of food preference between plants species. The

410   geographic differences in plant-herbivore interactions thus, are likely to respond

411   idiosyncratically to the same environmental gradients in different habitat context, even

412   between neighboring habitats.

413   After 33 months, both plants species from high latitude transplanted to low latitude

414   grew smaller, have higher relative fiber content and suffered a higher herbivory than plants

415   located at lower latitude. The opposite result was observed for lower latitude plants

416   transplanted to higher latitude sites, with herbivores strongly preferring high latitude plants,

417   suggesting, as in northern hemispheres marshes (e.g., Salgado & Pennings 2005, Więski &

418   Pennings 2014), latitudinal differences in plant traits and palatability. Plant traits differences

419   still persisted after almost three years of transplant, which suggest that differences among

420   sites in plant traits are under genetic control and thus, that latitudinal variation in herbivory

421   could be selected for the geographical variation in plant traits along latitudes (Salgado &

422   Pennings 2005). However, since the differences were smaller than among plants separated

423   geographically (i.e., controls), also suggest plant plastic response to environmental cues.

424   Many of our observations suggest that the south hemisphere marshes undergo similar

425   processes to those from north hemisphere marshes (e.g., Więski & Pennings 2014). The

426   suggested hemispheric asymmetries in the latitudinal herbivory hypothesis could be due to the

427   fact that studies are biased towards the Northern Hemisphere (see Zhang et al 2016). In

428   addition, in this revision only leaf chewing insects were included, while other herbivores (e.g.

429   sap-sucking, stem borers, Anstett et al. 2014; Canepuccia et al. 2011) were excluded in the

430   analysis. In addition, since some high latitude plants transplanted to low latitudes sites were

431   complete eaten by the end of the experiment, we also found latitudinal differences in plant

432   resistance to herbivory. After the experiment, larvae still preferred plants from high latitudes

433   than those from low latitudes, supporting the idea that the variations in plant anti-herbivore

434   defense among sites are also genetically based traits. Experiment did not allow us to know if

435   there is an induced anti-herbivore defense in these plants. However, plants from different

436 latitudes growing on common garden (without moth herbivory), keep similar pattern of trait

437 than those growing on field transplant experiment (with moth herbivore). These results might

438 suggest that, at least in the assessed traits, the plant defenses are not mediated by the

439 herbivory pressure. Plants can allocate more resources to the constitutive defenses when

440 selective pressure of herbivores is predictably stronger (Optimal defense hypothesis, Stamp

441 2003). To an evolutionary scale, this can help them to cope the higher rates of herbivory in

442 lower latitude, driving in part the geographic differences in plant-herbivore interaction along

443 SWA marshes. The herbivore traits could be also driven by plant traits variation in an

444 evolutionary arms race (Vermeij 1987). Then, moth-plant imbalance of armament may vary

445 the strength of local selection, and physical conditions change the arms-races trajectory (e.g.

446 Toju & Sota 2006) at different rates along SWA coast. Future research is needed to further

447 understand the determining of latitudinal gradients in herbivore traits (e.g. tolerance for plant

448 defenses) along latitudinal gradients of plant traits.

449   Our study shows that plant-herbivore interaction in SWA marshes tend to be more

450 intense at lower latitudes. This variation is given by an increase in abundance of herbivores,

451 and an increase in plant resistance to herbivore damage at low latitudes. However, there are

452 several inconsistencies in the pattern of latitudinal variation of this interaction, which are

453 related to variation in plant or herbivore traits not necessarily related to latitude. Indeed, the

454 plant responses to variables not related to latitude (e.g. salinity, rainfalls, tides amplitudes),

455 indirectly affect traits important to consumers, further increasing the mechanisms responsible

456 for plant-herbivore variations along latitude. Our results, thus, emphasize the value of

457 considering multiple ways in which plants and consumers respond to ecological processes

458 shaped by factors that operate differentially across geography when examining variation in

459 organism interactions along latitude.

460

466

467 LITERATURE CITED

468 Adam P (2002) Saltmarshes in a time of change. Environ Conserv 29:39–61

469 Aho K (2017) asbio: A Collection of Statistical Tools for Biologists. R package version 1.4-2,

470 URL https://CRAN.R-project.org/package=asbio

471 Akkala A, Devabhaktuni V, Kumar A (2010) Interpolation techniques and associated software

472 for environmental data. Environ Prog Sustain Energy 29:134–141

473 Anderson MJ (2001) Permutation tests for univariate or multivariate analysis of variance and

474 regression. Canadian Journal of Fisheries and Aquatic Science 58:626-639

475 Anstett DN, Nunes KA, Baskett C, Kotanen PM (2016) Sources of Controversy Surrounding

476 Latitudinal Patterns in Herbivory and Defense. Trends Ecol Evol 31: 789-802

477 Anstett DN, Naujokaitis-Lewis I, Johnson MTJ (2014) Latitudinal gradients in herbivory on

478 *Oenothera biennis* vary according to herbivore guild and specialization. Ecology

479 95:2915–2923

480 Bentler PM, Chou C-P (1987) Practical issues in structural modeling. Sociol Methods Res

481 16:78–117

482 Bertness MD, Crain C, Holdredge C, Sala N (2008) Eutrophication and consumer control of

483   New England salt marsh primary productivity. Conserv Biol 22:131–139

484 Bertness MD, Gough L, Shumway SW (1992) Salt tolerances and the distribution of fugitive

485   salt marsh plants. Ecology 73:1842–1851

486 Browne MW, Cudeck R (1992) Alternative ways of assessing model fit. Sociol Methods Res

487   21:230–258

488 Canepuccia AD, Alberti J, Daleo P, Pascual J, Farina J, Iribarne O (2010a) Ecosystem

489   engineering by burrowing crabs increases cordgrass mortality caused by stem-boring

490   insects. Mar Ecol Prog Ser 404:151–159

491 Canepuccia AD, Alberti J, Pascual J, Alvarez G, Cebrian J, Iribarne OO (2010b) ENSO

492   episodes modify plant/terrestrial–herbivore interactions in a southwestern Atlantic salt

493   marsh. J Exp Mar Biol Ecol 396:42–47

494 Canepuccia AD, Montemayor D, Pascual J, Farina J, Iribarne OO (2011) A stem-boring moth

495   drives detritus production in SW Atlantic marshes. Mar Ecol Prog Ser 442:1–9

496 Canepuccia AD, Perez C, Farina J, Alemany D, Iribarne OO (2013) Dissimilarity in plant

497   species diversity between salt marsh and neighboring environments decreases as

498   environmental harshness increases. Mar Ecol Prog Ser 494:135–148

499 Coley PD, Barone JA (1996) Herbivory and plant defenses in tropical forests. Annu Rev Ecol

500   Syst 27:305–335

501 Dagar J (2010) Opportunities for sustainable utilization of salt-affected lands and pore quality

502   waters for livelihood security and mitigating climate change through agroforestry

503   systems. In: Thomas RP (ed) Proceedings of the Global Forum on Salinization and

504   Climate Change (GFSCC2010), Thomas R.P. World Soil Resour. Reports., Valencia, p

505   25–29

506 Daleo P, Iribarne OO (2009) Beyond competition: the stress-gradient hypothesis tested in

507   plant–herbivore interactions. Ecology 90:2368–2374

508 De Frenne P, Graae BJ, Rodríguez-Sánchez F, Kolb A, Chabrerie O, Decocq G, De Kort H,

509   De Schrijver A, Diekmann M, Eriksson O, Gruwez R, Hermy M, Lenoir J, Plue J,

510   Coomes DA, Verheyen K (2013) Latitudinal gradients as natural laboratories to infer

511   species' responses to temperature. J Ecol 101:784–795

512 Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR

513   (2008) Impacts of climate warming on terrestrial ectotherms across latitude. Proc Natl

514   Acad Sci 105:6668–6672

515 Eiserhardt WL, Bjorholm S, Svenning J-C, Rangel TF, Balslev H (2011) Testing the water–

516   energy theory on American palms (Arecaceae) using geographically weighted

517   regression. PLOS ONE 6:e27027

518 Fanjul E, Grela MA, Canepuccia A, Iribarne O (2008) The Southwest Atlantic intertidal

519   burrowing crab *Neohelice granulata* modifies nutrient loads of phreatic waters

520   entering coastal area. Estuar Coast Shelf Sci 79:300–306

521 Flowers TJ, Troke PF, Yeo AR (1977) The mechanism of salt tolerance in halophytes. Annu

522   Rev Plant Physiol 28:89–121

523 Grace J (2006) Structural equation modeling and natural systems. Cambridge University

524   Press, Cambridge, UK ; New York

525   Gratton C, Denno RF (2003) Inter-year carryover effects of a nutrient pulse on *Spartina*

526   plants, herbivores, and natural enemies. Ecology 84:2692–2707

527   Hahn PG, Maron JL (2016) A framework for predicting intraspecific variation in plant

528   defense. Trends Ecol Evol 31: 646-656

529   He Q, Silliman BR (2015) Biogeographic consequences of nutrient enrichment for plant–

530   herbivore interactions in coastal wetlands. Ecol Lett 18:462–471

531   Ievers-Landis CE, Burant CJ, Hazen R (2011) The concept of bootstrapping of structural

532   equation models with smaller samples: An illustration using mealtime rituals in

533   diabetes management. J Dev Behav Pediatr 32:619–626

534   IPCC (2014) Climate change 2014: synthesis report (L Mayer and Intergovernmental Panel

535   on Climate Change, Eds.). Intergovernmental Panel on Climate Change, Geneva,

536   Switzerland

537   Isacch JP, Costa CSB, Rodríguez-Gallego L, Conde D, Escapa M, Gagliardini DA, Iribarne

538   OO (2006) Distribution of saltmarsh plant communities associated with environmental

539   factors along a latitudinal gradient on the south-west Atlantic coast. J Biogeogr

540   33:888–900

541   Kooyers NJ, Blackman BK, Holeski LM (2017) Optimal defense theory explains deviations

542   from latitudinal herbivory defense hypothesis. Ecology, 98: 1036–1048

543   Lim JY, Fine PVA, Mittelbach GG (2015) Assessing the latitudinal gradient in herbivory.

544   Global Ecol Biogeogr 24: 1106–1112

545   Long JD, Mitchell JL, Sotka EE (2011) Local consumers induce resistance differentially

546   between *Spartina* populations in the field. Ecology 92: 180–188

547 Manly BFJ (2006) Randomization, bootstrap and Monte Carlo methods in biology, third

548   edition, Chapman and Hall/CRC, Boca Raton, FL

549 McCall BD, Pennings SC (2012) Geographic variation in salt marsh structure and function.

550   Oecologia 170:777–787

551 Moles AT, Bonser SP, Poore AGB, Wallis IR, Foley WJ (2011) Assessing the evidence for

552   latitudinal gradients in plant defence and herbivory. Funct Ecol 25:380–388

553 Moles AT, Warton DI, Warman L, Swenson NG, Laffan SW, Zanne AE, Pitman A,

554   Hemmings FA, Leishman MR (2009) Global patterns in plant height. J Ecol 97:923–

555   932

556 Montemayor DI, Addino M, Fanjul E, Escapa M, Alvarez MF, Botto F, Iribarne OO (2011)

557   Effect of dominant *Spartina* species on salt marsh detritus production in SW Atlantic

558   estuaries. J Sea Res 66: 104–110

559 Pennings SC, Grant M-B, Bertness MD (2005) Plant zonation in low-latitude salt marshes:

560   disentangling the roles of flooding, salinity and competition. J Ecol 93:159–167

561 Pennings SC, Ho C-K, Salgado CS, Więski K, Davé N, Kunza AE, Wason EL (2009)

562   Latitudinal variation in herbivore pressure in Atlantic Coast salt marshes. Ecology

563   90:183–195

564 Pennings SC, Silliman BR (2005) Linking biogeography and community ecology: latitudinal

565   variation in plant–herbivore interaction strength. Ecology 86:2310–2319

566 R Development Core Team (2012) R: a language and environment for statistical computing.

567 Randolph JC, Cameron GN, Wrazen JA (1991) Dietary choice of a generalist grassland

568   herbivore, *Sigmodon hispidus*. J Mammal 72:300–313

569 Reich PB, Oleksyn J (2004) Global patterns of plant leaf N and P in relation to temperature

570   and latitude. Proc Natl Acad Sci U S A 101:11001–11006

571 Richards CL, Hamrick JL, Donovan LA, Mauricio R (2004) Unexpectedly high clonal

572   diversity of two salt marsh perennials across a severe environmental gradient. Ecol

573   Lett 7:1155–1162

574 Rosseel Y (2012) Lavaan: an R package for structural equation modeling. J Stat Softw 4:36

575 Rozema J, Bijwaard P, Prast G, Broekman R (1985) Ecophysiological adaptations of coastal

576   halophytes from foredunes and salt marshes. Vegetatio 62:499–521

577 Salgado CS, Pennings SC (2005) Latitudinal variation in palatability of salt-marsh plants: are

578   differences constitutive? Ecology 86:1571–1579

579 Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K (2009) Is there a latitudinal

580   gradient in the importance of biotic interactions? Annu Rev Ecol Evol Syst 40:245–

581   269

582 Siska EL, Pennings SC, Buck TL, Hanisak MD (2002) Latitudinal variation in palatability of

583   salt-marsh plants: which traits are responsible? Ecology 83:3369–3381

584 Speziale KL, Ruggiero A, Ezcurra C (2010) Plant species richness–environment relationships

585   across the Subantarctic–Patagonian transition zone. J Biogeogr 37:449–464

586 Stamp N (2003) Out of the quagmire of plant defense hypotheses. Q Rev Biol 78:23–55

587 Stiling P, Moon D (2005) Are trophodynamic models worth their salt? Top-down and bottom-
588   up effects along a salinity gradient. Ecology 86:1730–1736

589 Tam NFY, Wong YS (1998) Variations of soil nutrient and organic matter content in a
590   subtropical mangrove ecosystem. Water Air Soil Pollut. 103 245–261

591 Toju H, Sota T (2006) Imbalance of predator and prey armament: Geographic clines in
592   phenotypic interface and natural selection. Amer Nat 167:105-117

593 Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent
594   fiber and non-starch polysaccharide in relation to animal nutrition. J Dairy Sci 74:
595   3583–3597.

596 Vermeij, GJ (1987) Evolution and escalation: an ecological history of life. Princeton
597   University Press, Princeton, New Jersey, USA.

598 Vera F, Gutierrez JL, Ribeiro PD. (2009) Aerial and detritus production of the cordgrass
599   *Spartina densiflora* in a southwestern Atlantic salt marsh. Botany 87:482–491

600 Więski K, Pennings S (2014) Latitudinal variation in resistance and tolerance to herbivory of
601   a salt marsh shrub. Ecography 37:763–769

602 Zar JH (1999) Biostatistical analysis, 4th ed. Upper Saddle River, N.J. : Prentice Hall

603 Zhang S, Zhang Y, Ma K (2016) Latitudinal variation in herbivory: hemispheric asymmetries
604   and the role of climatic drivers. J Ecol 104:1089–1095

605

606

607

608 **Figure captions**

609 Fig. 1. Marshes sampled along the SW Atlantic coast: the outlet of Salado River (SAL), San

610 Clemente (SC), Bahía Blanca (BB), Bahía Anegada (BA), the outlet of Rio Negro (RN),

611 Bahía San Antonio (BSA), Riacho San Jose (RSJ), and the outlet of Rio Chubut (RCH). The

612 coast between the arrows shows the area of development of tidal marshes dominated by

613 *Spartina* spp. grasses. Triangles indicate meteorological stations. The isohyets for the study

614 sites are gray lines with the rainfall values in millimeters.

615

616 Fig. 2. Proportion of stems of (a) *S. alterniflora* and (b) *S. densiflora* attacked by the moth

617 *Haimbachia* sp. nov. along 8 southwestern Atlantic coastal marshes. the outlet of Salado

618 River (SAL), San Clemente (SC), Bahía Blanca (BB), Bahía Anegada (BA), the outlet of Rio

619 Negro (RN), Bahía San Antonio (BSA), Riacho San Jose (RSJ), and the outlet of Rio Chubut

620 (RCH). In this and subsequent figures: limits of the box are 25th and 75th percentiles, vertical

621 lines are the minimum and maximum, black dot inside the box represent the median value.

622 Different letters indicate differences ($p < 0.05$) by Tukey test after Welch ANOVA; *:

623 absence of plants at that location.

624

625 Fig. 3. Path diagrams of the structural equation model estimating the relative importance of

626 direct and indirect effects of physical variables on plant traits and on the moth attack

627 frequencies in *S. alterniflora* (a) and *S. densiflora* (b) across 8 south western Atlantic coastal

628 marshes. Arrows designate the direction of causality; values are standardized coefficient

629 estimates. Arrows widths are proportional to the size of the path coefficients. Solid arrows

630 represent positive relationships and dashed arrows represent negative relationships.

631 Goodness-of-fit statistics are χ^2: chi-square, P = p-values of χ^2, CFI: comparative fit index,

632 TLI: Tucker Lewis Index, and RMSEA: root mean square error of approximation.

633

634   Fig. 4. Interaction plot of frequencies of moth attack (means with standard errors) on low (sc)

635   vs. middle (bb) and high-latitude (bsa) plants (each in separate lines) grew to high (BSA) vs.

636   middle (BB) and low-latitude sites (SC). (a) *Spartina alterniflora* and (b) *S. densiflora* plants.

637   Different letters indicate differences assessed by 10,000 time Monte Carlo simulation of the

638   pairwise differences of the frequencies of moth attack between treatments.
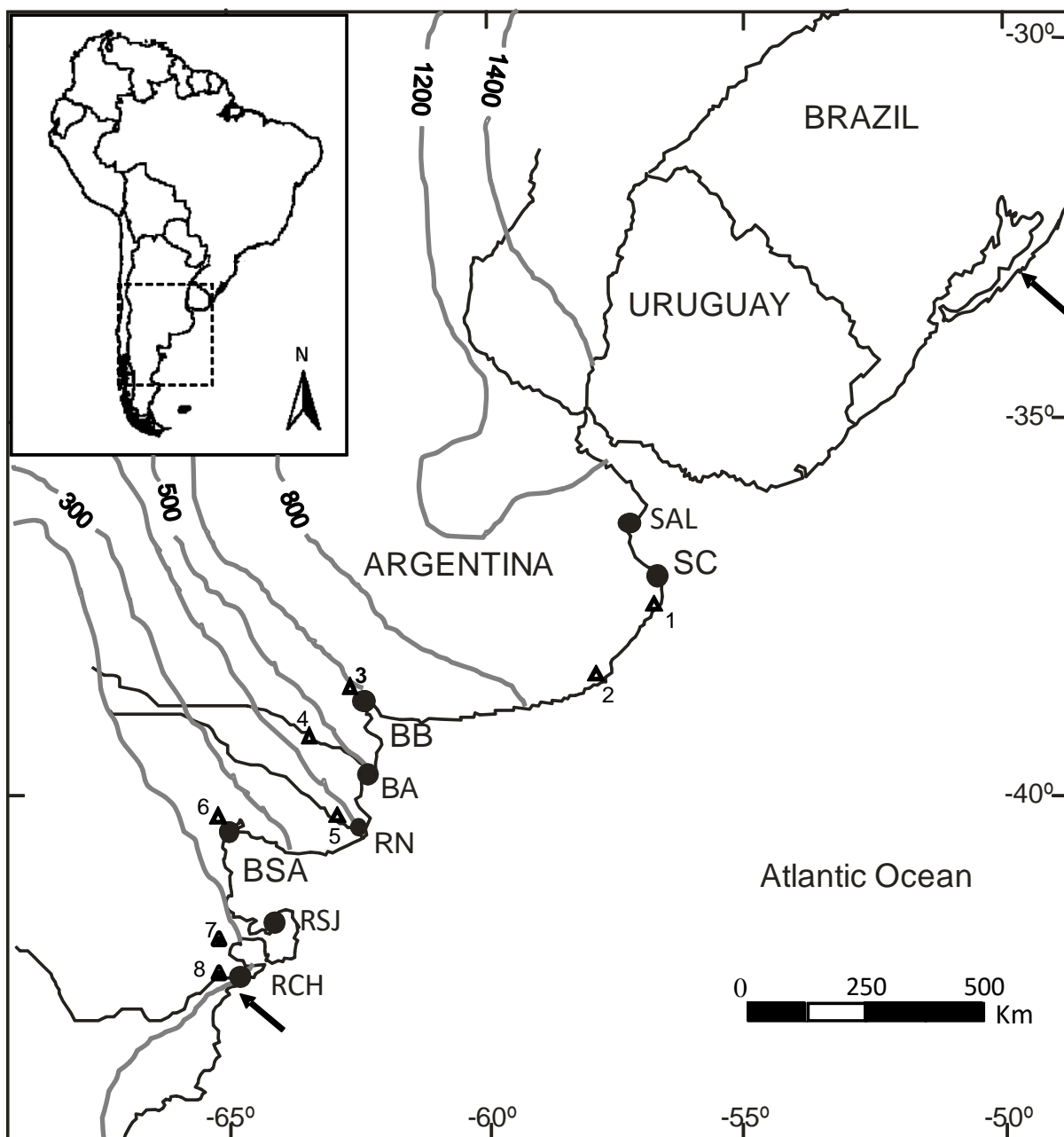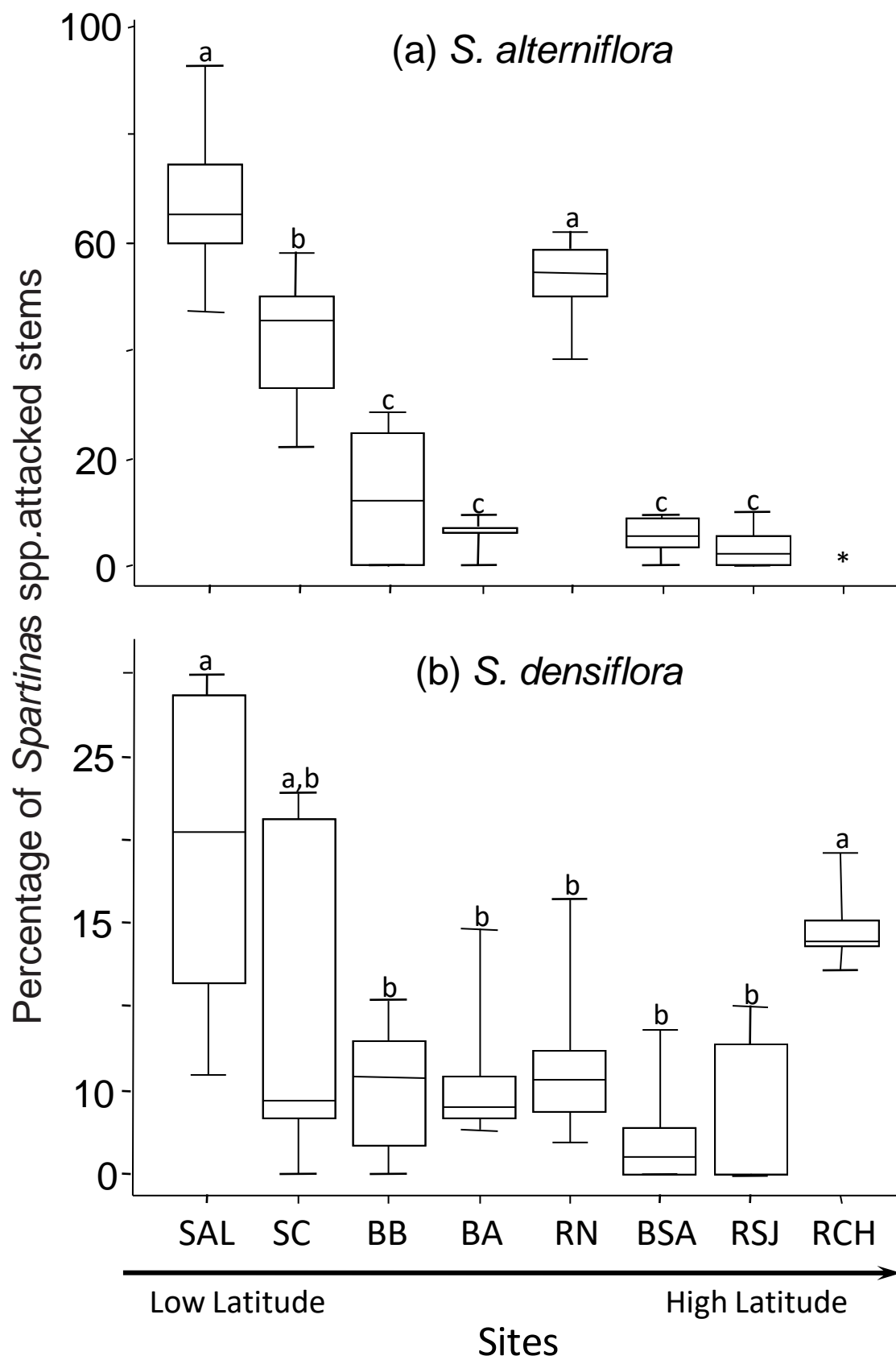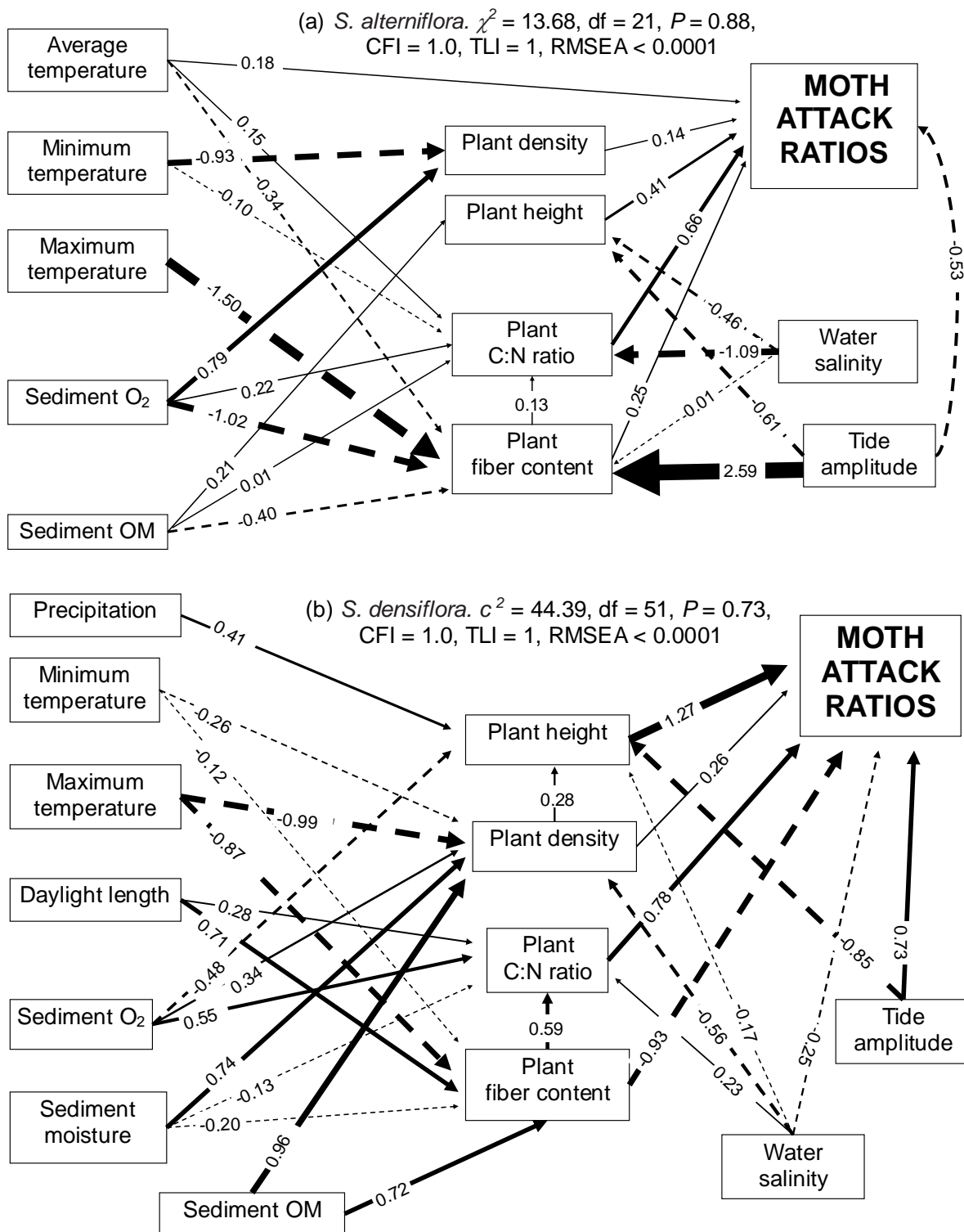
Figure 1

Figure 2

(a) *S. alterniflora*

(b) *S. densiflora*

Figure 3

(a) *S. alterniflora.* χ^2 = 13.68, df = 21, *P* = 0.88, CFI = 1.0, TLI = 1, RMSEA < 0.0001

(b) *S. densiflora.* c^2 = 44.39, df = 51, *P* = 0.73, CFI = 1.0, TLI = 1, RMSEA < 0.0001

Figure 4