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1  **Evolutionary origins of abnormally large shoot sodium accumulation in non-saline**

2  **environments within the Caryophyllales**

3

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29

30  **Heading:** Evolution of extraordinary sodium accumulation in the Caryophyllales.

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32    **Summary**

33

34    •        The prevalence of sodium (Na) "hyperaccumulator" species, which exhibit

35    abnormally large shoot sodium concentrations ($[Na]_{shoot}$) when grown in non-saline

36    environments, was investigated among angiosperms in general and within the

37    Caryophyllales order in particular.

38    •        Shoot Na concentrations were determined in 334 angiosperm species, representing

39    35 orders, grown hydroponically in a non-saline solution.

40    •        Many Caryophyllales species exhibited abnormally large $[Na]_{shoot}$ when grown

41    hydroponically in a non-saline solution. The bimodal distribution of the log-normal $[Na]_{shoot}$

42    of species within the Caryophyllales suggested at least two distinct $[Na]_{shoot}$ phenotypes

43    within this order. Mapping the trait of Na-hyperaccumulation onto the phylogenetic

44    relationships between Caryophyllales families, and between subfamilies within the

45    Amaranthaceae, suggested that the trait evolved several times within this order: in an

46    ancestor of the Aizoaceae, but not the Phytolaccaceae or Nyctaginaceae, in ancestors of

47    several lineages formerly classified as Chenopodiaceae, but not in the Amaranthaceae *sensu*

48    *stricto*, and in ancestors of species within the Cactaceae, Portulacaceae, Plumbaginaceae,

49    Tamaricaceae and Polygonaceae.

50    •        In conclusion, a disproportionate number of Caryophyllales species behave as Na-

51    hyperaccumulators and multiple evolutionary origins of this trait can be identified within

52    this order.

53

54

55    **Key words:** Aizoaceae, Amaranthaceae, Caryophyllales, halophyte, hyperaccumulation,

56    phylogeny, shoot, sodium (Na).

57    **Introduction**

58

59    Sodium (Na) is not considered to be an essential element for plants (White & Brown 2010)

60    although it is required (in micronutrient quantities) for the $C_4$ photosynthetic pathway

61    (Cheeseman, 2015) and some halophytes (euhalophytes) grow better when supplied Na

62    (Greenway & Munns, 1980; Albert, 1982; Flowers & Colmer, 2008; Munns & Tester, 2008;

63    Rozema & Schat, 2013). In addition, in some environments, for example where there is low

64    $K^+$ phytoavailability, plant growth can benefit from a source of Na since $Na^+$ can replace $K^+$ as

65    a cationic osmoticum in the vacuole (White, 2013). The accumulation of excessive Na

66    concentrations in plant tissues is, however, detrimental to plant growth since $Na^+$ interferes

67    with metabolism in the cytoplasm, mitochondria and plastids (Flowers *et al.*, 2015).

68        It is estimated that >6% of the world's land, and 5-15% of the world's agricultural

69    land, is adversely affected by its Na concentration through either salinity or sodicity (Munns

70    & Tester, 2008). Saline soils are generally dominated by NaCl, although there are often

71    significant concentrations of $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$ and $CO_3^{2-}$. They are defined as having <15% of

72    their exchangeable cations as $Na^+$ and soil solutions with electrical conductivity ($EC_e$) >2 dS

73    $m^{-1}$ in a saturated paste extract, which equates to a NaCl concentration of 20 mM, and pH

74    <8.5. Sodic (alkali) soils are generally dominated by $Na_2CO_3$ and are defined as having >15%

75    of their exchangeable cations as $Na^+$ and soil solutions with $EC_e$ >2 dS $m^{-1}$ and pH >8.5 in a

76    saturated paste extract. Saline-sodic soils have >15% of their exchangeable cations as $Na^+$

77    and soil solutions with $EC_e$ >2 dS $m^{-1}$ and pH <8.5 in a saturated paste extract.

78        Halophytes are generally defined as plants that inhabit saline environments or that

79    complete their life cycles in the presence of large concentrations of ions ($\geq$ 200 mM), most

80    commonly NaCl, in the root-zone (Flowers & Colmer, 2008). They can be further classified

81    into miohalophytes, which exhibit maximal growth in non-saline environments, and

82    euhalophytes, which exhibit maximal growth under saline conditions (Greenway & Munns,

83    1980). Halophytes tolerating $EC_e$ >8.0 dS $m^{-1}$ measured in a saturated paste extract

84    (approximately 80 mM NaCl) comprise <0.5% of angiosperm species (1,490/352,000

85    species), but are present in at least 33 orders and 110-120 families of flowering plants (The

86    Plant List, 2013; Flowers *et al.*, 2016). It has been suggested that halophytism is an

87    evolutionarily-labile character that has arisen independently in many angiosperm lineages

88    from pre-adapted genotypes (Flowers *et al.*, 2010; Kadereit *et al.*, 2012; Saslis-Lagoudakis *et*

89    *al.*, 2014; Bromham, 2015; Cheeseman, 2015). Families with a large proportion of

90    halophytes (>10% of species in a family) occur in the Alismatales, Brassicales, Caryophyllales,

91    Ericales, Fabales, Malphigiales, Piperales, Poales, Sapindales and Saxifragales (Saslis-

92    Lagoudakis *et al.*, 2014; Flowers *et al.*, 2016).

93          Halophytes can also be grouped into "ionotypes", which are defined as characteristic

94    ionomic features of plant species that are conserved in diverse environments (Albert &

95    Popp, 1977; Gorham *et al.*, 1980; Albert *et al.*, 2000; Flowers & Colmer, 2008; White *et al.*,

96    2012). Commelinid monocots (e.g. Poaceae, Cyperaceae, Juncaceae) are classed as "Na-

97    excluders" and generally exhibit lower shoot Na concentrations ($[Na]_{shoot}$) than other

98    angiosperms growing in the same environment and Na/K quotients less than unity, whilst

99    many eudicots are characterised by comparatively large $[Na]_{shoot}$ and tissue Na/K quotients

100   greater than unity (Albert & Popp, 1977; Gorham *et al.*, 1980; Albert, 1982; Flowers &

101   Colmer, 2008; Yang *et al.*, 2012). Several families in the Caryophyllales (Amaranthaceae

102   [Chenopodioideae], Caryophyllaceae, Tamaricaceae) exhibit exceptionally large $[Na]_{shoot}$ and

103   tissue Na/K quotients when grown in saline environments (Albert & Popp, 1977; Gorham *et

104   al.*, 1980; Albert, 1982; Flowers & Colmer, 2008; Yang *et al.*, 2012; Zhang *et al.*, 2012). It has

105   also been observed that some Caryophyllales species have exceptionally large $[Na]_{shoot}$ even

106   when grown in non-saline environments (Collander, 1941; Patel *et al.*, 1980; Glenn &

107   O'Leary, 1984; Broadley *et al.*, 2004). For example, in a phylogenetically-balanced study of

108   the ionomes of 117 angiosperm species belonging to 25 orders grown hydroponically in a

109   non-saline solution containing 0.1 mM Na, it was noted that $[Na]_{shoot}$ varied significantly

110   among eudicot orders (P<0.05) and that three of the seven Caryophyllales species studied

111   had conspicuously large $[Na]_{shoot}$ (Broadley *et al.*, 2004). It has been suggested that Na might

112   have a special role in the biology of euhalophyte Caryophyllales, whose maximal growth

113   requires Na accumulation (Flowers & Colmer, 2008), and that the characteristic ionome of

114   the Caryophyllales might reflect their unusual ecology (White *et al.*, 2015). Although

115   Caryphyllales species can inhabit a variety of biomes worldwide, they comprise a significant

116   proportion of the flora of many deserts (Fahn & Cutler, 1992), coastal regions (Kadereit *et

117   al.*, 2012), and soils with unbalanced mineral composition for plant nutrition, such as

118   gypseous (Moore *et al.*, 2014) and ultramafic/serpentine (White & Pongrac, 2016) soils.

119          The Caryophyllales order comprises over 11,000 species currently partitioned into

120   about 700 genera and 38 families (The Plant List, 2013; APGIV, 2016). About 5% of species in

121    the Caryophyllales are halophytes and the order contains 35-40% of all known halophytic

122    angiosperm species (Flowers *et al.*, 2010, 2016; Saslis-Lagoudakis *et al.*, 2014). Of the most

123    populous families in the Caryophyllales (>50 species) the halophytic character is particularly

124    prevalent in the Amaranthaceae (17.3% species), Frankeniaceae (16.7% species) and

125    Tamaricaceae (31.1% species). In contrast to observations on other angiosperm orders, the

126    halophytic character appears to be rarely lost in Caryophyllales lineages, such as the

127    Chenopodioideae and Tamaricaceae, once it has evolved (Bromham, 2015). It has been

128    suggested that the halophytic character might evolve from ancestors with a general

129    complement of stress-tolerance traits that enable lineages to adapt to a wide range of

130    environmental challenges (Kadereit *et al.*, 2012; Saslis-Lagoudakis *et al.*, 2014; Bromham,

131    2015). It is, therefore, noteworthy that the Caryophyllales order contains many succulent

132    species (Kadereit *et al.*, 2012; Rozema & Schat, 2013), many species that possess salt glands,

133    which are specialised multicellular structures that excrete salt onto the leaf surface, or

134    bladder cells, which are modified trichomes that accumulate salt and then burst (Thomson

135    *et al.*, 1988; Fahn & Cutler, 1992; Salama *et al.*, 1999; Flowers *et al.*, 2010; LoPresti, 2014),

136    many species exhibiting $C_4$ and CAM photosynthetic pathways (Silvera *et al.*, 2010; Sage *et*

137    *al.,* 2011; Kadereit *et al.*, 2012), many species that hyperaccumulate potentially toxic

138    elements (White & Pongrac, 2016), and many species adapted to arid (Ehleringer *et al.*,

139    1997) or alkaline (Yang *et al.*, 2012) environments. The $C_4$ photosynthetic pathway has

140    evolved many times within the Caryophyllales (Sage *et al.*, 2011) and Kadereit *et al.* (2012)

141    observed that the rate of gain of the $C_4$ photosynthetic character was greater in salt tolerant

142    Chenopodioideae lineages, which they attributed to shared adaptations between $C_4$

143    photosynthesis and salt tolerance as part of a wider drought tolerance syndrome. A similar

144    dependency of the evolution of $C_4$ photosynthesis with succulence and coastal habitat was

145    also observed (Kadereit *et al.*, 2012). Crassulacean Acid Metabolism has also evolved many

146    times within the Caryophyllales and is associated with succulence and other traits enabling

147    water use efficiency in arid or saline environments (Edwards & Ogburn, 2012).

148        The present study investigated the prevalence of "Na-hyperaccumulator" species,

149    which exhibit abnormally large $[Na]_{shoot}$ (>4 mg Na $g^{-1}$ dry matter) when grown in non-saline

150    conditions (<20 mM $Na^+$ in the rhizosphere solution), among the angiosperms in general and

151    the Caryophyllales in particular. The prevalence of this phenomenon among angiosperms is

152    currently unknown and this study provides an original insight to its occurrence and

153    evolutionary origins within the Caryophyllales order. It is observed that only the

154    Caryophyllales species *Atriplex hortensis* L. and *Beta vulgaris* L. of the ten halophytic species

155    studied, representing eight angiosperm orders, behaved as Na-hyperaccumulators when

156    grown in compost. Similarly, when 334 angiosperm species representing 35 angiosperm

157    orders were grown hydroponically in a non-saline solution containing 0.1 mM Na a

158    disproportionate number of Caryophyllales species exhibited abnormally large $[Na]_{shoot}$. The

159    bimodal distribution of the log-normal $[Na]_{shoot}$ of species within the Caryophyllales

160    suggested at least two distinct $[Na]_{shoot}$ phenotypes within this order. Mapping the trait of

161    Na-hyperaccumulation in non-saline environments onto the phylogenetic relationships

162    between Caryophyllales families (Crawley & Hilu, 2012; Hernández-Ledesma *et al.*, 2015;

163    Yang *et al.*, 2015), and between subfamilies within the Amaranthaceae, suggested that the

164    trait had evolved several times within this order: in an ancestor of the Aizoaceae, but not

165    the Phytolaccaceae or Nyctaginaceae, in ancestors of several lineages formerly classified as

166    Chenopodiaceae, but not in the Amaranthaceae *sensu stricto*, and possibly in ancestors of

167    species within the Cactaceae, Portulacaceae, Plumbaginaceae, Tamaricaceae and

168    Polygonaceae. It is possible that the ability to hyperaccumulate $Na^+$ might benefit plants by

169    providing an alternative osmoticum to $K^+$, especially in environments with low K availability

170    (White, 2013). Thus, Na-hyperaccumulation might have served Caryophyllales during their

171    evolution in overcoming the selection pressures associated with the colonisation of arid or

172    saline environments, which require succulence and water conservation (Fahn & Cutler,

173    1992; Nobel, 2003; Flowers & Colmer, 2008; Kadereit *et al.*, 2012).

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176    **Materials and Methods**

177

178    Responses of halophytic species from different angiosperm orders to salinity

179

180    Responses to salinity were studied in ten halophytic species, from eight angiosperm orders,

181    catalogued in the eHALOPH Halophytes Database (Flowers *et al.*, 2016). These comprised:

182    *Ammi visnaga* (L.) Lam. (Apiaceae, Apiales), *Asparagus officinalis* L. (Asparagaceae,

183    Asparagales), *Atriplex hortensis* L. (Amaranthaceae, Caryophyllales), *Beta vulgaris* L.

184    (Amaranthaceae, Caryophyllales), *Casuarina cunninghamiana* Miq. (Casuarinaceae, Fagales),

185　*Colubrina asiatica* (L.) Brongn. (Rhamnaceae, Rosales), *Hibiscus tilliaceus* L. (Malvaceae,

186　Malvales), *Hordeum jubatum* L. (Poaceae, Poales), *Kosteletzkya virginica* (L.) C. Presl ex A.

187　Gray (Malvaceae, Malvales), *Lobularia maritima* (L.) Desv. (Brassicaceae, Brassicales),

188　*Plantago maritima* L. (Plantaginaceae, Lamiales) and *Scaevola crassifolia* Labill.

189　(Goodeniaceae, Asterales). Species were chosen on the basis of their availability from

190　suppliers and their ability to grow in the glasshouse. Seeds of all species were obtained from

191　Chiltern Seeds (Wallingford, UK) except *C. cunninghamiana*, *H. tilliaceous*, *K. virginica* and *P.*

192　*maritima*, which were obtained from Rareexoticseeds (Montreal, Canada), Kenni Koala's

193　Aussie Seed Store (Australia), Floridawildflowers (Crescent City, Florida, USA) and Scotia

194　Seeds (Brechin, UK), respectively. Seeds were germinated in the dark at between 10 °C and

195　25 °C, according to species requirements, on the surface of filter paper moistened with

196　deionised water. Once a radicle was observed, individual seedlings were transplanted to

197　rockwool plugs (2.5 cm by 2.5 cm by 4 cm; Grodan, Hedehusene, Denmark) held in plastic

198　trays in a glasshouse compartment at The James Hutton Institute, Dundee (UK; latitude

199　56°27'26'' N, longitude 3°4'17'' W) in which the experiment was subsequently performed

200　and irrigated with tap water containing 0.14 mM Na. The glasshouse compartment

201　maintained a maximum of 25 °C by day and a minimum of 15 °C at night using automatic

202　venting and supplementary heating.

203　　　　Established seedlings were transferred to pots containing 1 L Levington Professional

204　compost (ICL, Ipswich, UK) prior to the experiment. Two sets of plants, with up to 12

205　replicate plants per species in each set, were exposed to either non-saline or saline

206　irrigation. Plants were irrigated with 100 mL solution per week. Plants receiving the non-

207　saline treatment were irrigated with tap water containing 0.14 mM Na. The experiment was

208　initiated by increasing the NaCl concentration in the irrigation water of the saline treatment

209　to 50 mM for the first week, then 150 mM NaCl for the second week and finally 300 mM for

210　the third week. Plants were harvested on 12th December 2014, three weeks after the first

211　addition of NaCl to the saline irrigation water. The fresh weight (FW) of whole shoots was

212　determined immediately, then samples were dried in an oven at 70 °C to a constant weight

213　and their dry matter (DM) determined. Dried samples were milled to a powder using a ball

214　mill (C + N Laboratory Mill; Christy and Norris Ltd., Chelmsford, UK), digested using $HNO_3$ in

215　sealed tubes in a microwave oven (MARS Xpress, CEM Corporation, Matthews, NC, USA),

216　cleared using $H_2O_2$, and analysed for sodium (Na) concentration using inductively coupled

217    plasma-mass spectrometry (ICP-MS; ELAN DRCe, PerkinElmer, Waltham, MA, USA) as

218    described by White *et al.* (2012).

219

220    Phylogenetic effects on shoot sodium concentrations in plants grown hydroponically in a

221    non-saline solution

222

223    Phylogenetic effects on shoot Na concentrations in angiosperm species were assessed by

224    combining data from six glasshouse experiments in which plants were grown hydroponically

225    using a Nutrient Film Technique (NFT) essentially as described by Broadley *et al.* (2003). The

226    final dataset comprised 334 species from 35 orders (Table S1). In all experiments, seeds

227    were germinated in the dark on the surface of filter paper moistened with deionised water

228    at temperatures between 4 °C and 25 °C depending on their requirements. Once a radicle

229    was observed, individual seedlings were transplanted to rockwool plugs (2.5 cm by 2.5 cm

230    by 4 cm; Grodan, Hedehusene, Denmark) held in plastic trays and irrigated with tap water.

231    Plastic trays were either placed in a weaning room at 25 °C or in the glasshouse

232    compartment in which experiments were subsequently performed. Once seedlings were

233    established, the rockwool plugs containing plants were transferred to the NFT system.

234    Whenever possible, two rockwool plugs constituted each replicate and up to six replicates

235    were obtained for each plant species. For experiments at both Warwick-HRI, Wellesbourne

236    (UK; latitude 52°12'18'' N, longitude 1°36'00'' W) and The James Hutton Institute, the

237    glasshouse maintained a maximum of 20 °C by day and a minimum of 15 °C at night using

238    automatic venting and supplementary heating. The recirculating nutrient solution contained

239    2 mM $Ca(NO_3)_2$, 2 mM $NH_4NO_3$, 0.75 mM $MgSO_4$, 0.5 mM KOH, 0.25 mM $KH_2PO_4$, 0.1 mM

240    FeNaEDTA, 30 µM $H_3BO_3$, 25 µM $CaCl_2$, 10 µM $MnSO_4$, 3 µM $CuSO_4$, 1 µM $ZnSO_4$ and 0.5 µM

241    $Na_2MoO_4$. This was adjusted daily to pH 6, with $H_2SO_4$, and solutions were replaced

242    completely once or twice each week. Seedlings were harvested during the exponential

243    growth phase, 18-73 days after transfer to the hydroponic system depending upon plant

244    growth rate. Whenever possible, shoots were separated into leaves and stems. The FW of

245    whole shoots or leaves was determined immediately then samples were dried in an oven at

246    70 - 80 °C to a constant weight and their DM determined. Dried samples were milled to a

247    powder using a ball mill, acid digested, and their Na concentrations determined either by

248    inductively coupled plasma emission spectrometry (JY24; Jobin-Yvon, Longjumeau, France)

249　as described by Broadley *et al.* (2003; Experiments 1-4) or by ICP-MS as described by White

250　*et al.* (2012; Experiments 5 and 6).

251　　　Experiment 1, described by Broadley *et al.* (2004), was undertaken in a glasshouse

252　compartment at Warwick-HRI between July and October 2001 to survey calcium (Ca),

253　potassium (K), magnesium (Mg), Na, organic-N and phosphorus (P) concentrations in leaves

254　of a phylogenetically-balanced set of 117 angiosperm species belonging to 25 orders.

255　Experiments 2A, 2B and 2C were undertaken sequentially in a glasshouse compartment at

256　Warwick-HRI between May and November 2003 to survey Ca concentrations in leaves of

257　Magnoliid and monocot orders, with replication at the taxonomic level of the family. Six

258　species representing three Magnoliid orders, 54 species representing eight monocot orders,

259　and nine other angiosperm species were grown in this experiment. Experiment 3, described

260　by White *et al.* (2007), was undertaken in a glasshouse compartment at Warwick-HRI

261　between July and August 2004 to survey selenium (Se) concentrations in leaves of 35

262　angiosperm species chosen to represent the range of ecological strategies for Se

263　accumulation reported in angiosperms. Experiment 4, described by White *et al.* (2015), was

264　undertaken in a glasshouse compartment at Warwick-HRI between June and August 2004 to

265　survey leaf concentrations of Ca and Mg in as many Caryophyllales families as possible, with

266　replication at the taxonomic level of the genus. Forty-six Caryophyllales species were

267　studied, representing eight families and 29 genera, together with 33 other angiosperm

268　species. Experiment 5 was undertaken in a glasshouse compartment at The James Hutton

269　Institute between July and October 2011 to survey leaf Ca and Mg concentrations in a range

270　of serpentine and non-serpentine plant species. These included 28 Caryophyllales species

271　and 35 other angiosperm species. Experiment 6 was undertaken in a glasshouse

272　compartment at The James Hutton Institute between July and November 2015 to survey

273　leaf Ca and Mg concentrations in a range of Arecaceae species, with replication at the

274　taxonomic level of the genus. Twenty three Arecaceae species were studied, representing

275　six genera, together with 11 other angiosperm species. Each Experiment had several species

276　in common with other Experiments allowing cross comparisons (Table S1). In total, 53

277　species, representing 22 families and 15 orders, were grown in more than one Experiment.

278

279　Statistics

280

281  Data are expressed as mean and standard error or standard deviation of the mean of n

282  observations. Statistical differences between treatments were assessed for each species by

283  Student's t-test. Estimates of variation in $[Na]_{shoot}$ were assigned between and within orders

284  ($n$=35), families ($n$=79) and species ($n$=334) using analyses of variance (ANOVA). All

285  statistical analyses were performed using R 3.3.0 (R Core Team, 2016) using a linear model

286  of: $[Na]_{shoot} \sim$ Order+Family+Species.

287

288

289  **Results**

290

291  Ten halophytic angiosperm species were grown in compost in pots that were irrigated with

292  either non-saline or saline solution. The shoot fresh weight (FW) of most of these species

293  did not differ significantly between plants that were irrigated with non-saline and saline

294  solutions (Table 1). However, the shoot FWs of *Asparagus officinalis* ($P$=0.0193) and

295  *Kosteletzkya virginica* ($P$=0.0430) were less in plants irrigated with saline solutions than in

296  those irrigated with non-saline solutions, whereas the shoot FWs of *Atriplex hortensis*

297  ($P$=0.0090) were greater in plants irrigated with a saline solutions than those irrigated with

298  non-saline solutions. Previous studies have also suggested that halophytic *Atriplex* species

299  grow best under slightly saline conditions (Black, 1960; Wallace *et al.*, 1973; Storey & Wyn

300  Jones, 1979; Albert, 1982; Glenn & O'Leary, 1984; Redondo-Gómez *et al.*, 2007; Glenn *et al.*,

301  2012; Norman *et al.*, 2013).

302       The response of shoot Na concentration ($[Na]_{shoot}$) to irrigation with saline solution

303  differed between the species studied, and they could be classified into "Na-excluder", "Na-

304  responder" and "Na-accumulator" species (cf. Baker, 1981). Of the ten angiosperm species

305  studied, four species appeared to exclude Na from their shoot tissues and had small $[Na]_{shoot}$

306  when irrigated with either non-saline and saline solutions (Table 1). These "Na-excluder"

307  species were the two monocot species studied, *Hordeum jubatum* (Poales) and *Asparagus*

308  *officinalis* (Asparagales), *Hibiscus tilliaceus* (Malvales) and *Casuarina cunninghamiana*

309  (Fagales). Five species had relatively small $[Na]_{shoot}$ when irrigated with non-saline solution

310  but when irrigated with saline solution their $[Na]_{shoot}$ increased to more than 10 mg $g^{-1}$ DM.

311  These "Na-responder" species were *Colubrina asiatica* (Rosales), *Kosteletzkya virginica*

312  (Malvales), *Ammi visnaga* (Apiales), *Lobularia maritima* (Brassicales), *Scaevola crassifolia*

313　(Asterales) and *Plantago maritima* (Lamiales). The two Caryophyllales species studied, *Beta*

314　*vulgaris* and *Atriplex hortensis*, both had exceptionally large $[Na]_{shoot}$ when irrigated with

315　non-saline and saline solutions. These species could be designated "Na-accumulator"

316　species.

317　　　　The constitutively large $[Na]_{shoot}$ of "Na-accumulator" species could best be

318　distinguished when plants were irrigated with non-saline solutions (Table 1). The

319　distribution of this trait among angiosperms was, therefore, assessed by growing species

320　hydroponically in a solution containing little Na as described by Broadley *et al.* (2003). Data

321　were combined from six individual glasshouse experiments (Table S1). Since little of the

322　variation in $[Na]_{shoot}$ (3.4%) could be attributed to environment (i.e. experiment), the

323　$[Na]_{shoot}$ for each species was calculated as the arithmetic mean of all experiments in which

324　the species was grown (Table S1). The proportions of the variation in $[Na]_{shoot}$ accounted for

325　at the levels of order, family and species were 13.8%, 54.3% and 28.5%, respectively. This

326　suggests that different plant families show distinct $[Na]_{shoot}$ concentrations. Families with

327　the largest mean $[Na]_{shoot}$ of their constituent species were the Aizoaceae (24.47 ± 5.07 mg

328　$g^{-1}$ DM, *n*=7 species), Cactaceae (17.60, *n*=1 species), Melastomataceae (5.23, *n*=1 species),

329　Portulacaceae (5.20 ± 4.60, *n*=2 species), and Ericaceae (4.51 ± 3.89, *n*=2 species). Three of

330　these families are in the Caryophyllales order.

331　　　　The $[Na]_{shoot}$ differed considerably between angiosperm species grown

332　hydroponically in a non-saline solution (Table S1; Figure 1). Several species had mean

333　$[Na]_{shoot}$ greater than 10 mg $g^{-1}$ DM. These species included nine Caryophyllales species,

334　*Beta vulgaris* (Amaranthaceae; 13.37 ± 2.35 mg $g^{-1}$ DM, *n*=5 experiments),

335　*Echinofossulocactus* sp. (Cactaceae; 17.60 mg $g^{-1}$ DM, *n*=1 experiment), *Carpanthea*

336　*pomeridiana* (Aizoaceae; 19.85 mg $g^{-1}$ DM, *n*=1 experiment), *Hereroa odorata* (Aizoaceae;

337　20.17 mg $g^{-1}$ DM, *n*=1 experiment), *Carpobrotus edulis* (Aizoaceae; 22.76 ± 3.31 mg $g^{-1}$ DM,

338　*n*=2 experiments), *Atriplex hortensis* (Amaranthaceae; 23.75 ± 1.01 mg $g^{-1}$ DM, *n*=2

339　experiments), *Stigmatocarpum criniflorum* (Aizoaceae; 30.81 ± 3.98 mg $g^{-1}$ DM, *n*=3

340　experiments), *Mesembryanthemum cordifolium* (Aizoaceae; 37.42 ± 6.44 mg $g^{-1}$ DM, *n*=2

341　experiments) and *Dorotheanthus bellidiformis* (Aizoaceae; 40.02 mg $g^{-1}$ DM, *n*=1

342　experiment), and two other angiosperm species, C*allistemon rigidus* (Myrtaceae, Myrtales;

343　10.31 mg $g^{-1}$ DM, *n*=1 experiment) and *Gladiolus carneus* (Iridaceae, Asparagales; 10.50 mg

344　$g^{-1}$ DM, *n*=1 experiment).

345    The distribution of $[Na]_{shoot}$ among the angiosperm species studied did not fit a

346    simple normal distribution (Figure 1A) and the log-normal distribution of $[Na]_{shoot}$ appeared

347    to comprise the sum of at least three individual log-normal distributions (Figure 2A). The

348    $[Na]_{shoot}$ of Caryophyllales species differed by several orders of magnitude, from 0.05 mg g$^{-1}$

349    DM in *Lewisia cotyledon* (Montiaceae) to 40.02 mg g$^{-1}$ DM in *Dorotheanthus bellidiformis*

350    (Aizoaceae). The distribution of $[Na]_{shoot}$ among the Caryophyllales appeared to comprise a

351    normal distribution (mean = 0.393, standard deviation = 0.185 mg g$^{-1}$ DM, *n*=42 species) plus

352    up to 19 species with abnormally large $[Na]_{shoot}$ (Figure 1B). The low probabilities of these

353    species being part of the normal distribution suggested that there are at least two distinct

354    $[Na]_{shoot}$ phenotypes among Caryophyllales species. The species with $[Na]_{shoot}$ at the limit for

355    inclusion in the normal distribution were *Plumbago auriculata* (*P*=0.0153, rank #41),

356    *Gomphrena serrata* (*P*=0.0106, rank #42), *Rumex hydrolapathum* (*P*=0.0003, rank #43) and

357    *Limonium sinuatum* (*P*=0.0001, rank #44).

358    The distribution of log-normal $[Na]_{shoot}$ of Caryophyllales species appeared to

359    comprise the sum of two discrete log-normal distributions (Figure 2B). The first log-normal

360    distribution (mean = -0.3717, standard deviation = 0.3299, *n*=49 species) contained 49

361    species and the second log-normal distribution (mean = 1.246, standard deviation 0.2756,

362    *n*=12 species) contained 12 species (Figure 2B). Since these two log-normal distributions

363    differed significantly (P<0.0001), these data suggest that there are at least two distinct

364    $[Na]_{shoot}$ phenotypes among Caryophyllales species. Considering the species with log

365    $[Na]_{shoot}$ at the extremes of these two distributions, the log $[Na]_{shoot}$ of *Psylliostachys*

366    *suworowi* had a greater probability of being in the first rather than the second log-normal

367    distribution (*P*=0.0076 versus *P*=0.0017), whilst the log $[Na]_{shoot}$ of *Spergula arvensis* had a

368    greater probability of being in the second rather than the first log-normal distribution

369    (*P*=0.0210 versus *P*=0.0007). The trait of abnormally large $[Na]_{shoot}$ when plants are grown in

370    non-saline solutions will, henceforth, be termed "Na-hyperaccumulation" and the discrete

371    set of 12 Caryophyllales species with large log $[Na]_{shoot}$ were considered to be "Na-

372    hyperaccumulators".

373    The evolutionary origin of Na-hyperaccumulation was sought by comparing the

374    number of Na-hyperaccumulator species and the mean $[Na]_{shoot}$ in different families of the

375    Caryophyllales (Figure 3). The 12 Caryophyllales species exhibiting Na-hyperaccumulation

376    were distributed across five of the ten Caryophyllales families represented in this study.

377　However, the trait was most prevalent in the Aizoaceae. Six of the seven Aizoaceae species

378　studied exhibited Na-hyperaccumulation. These six species were among the seven

379　Caryophyllales species with the largest $[Na]_{shoot}$ (Table S1). Consequently, the Aizoaceae had

380　the largest mean $[Na]_{shoot}$ (24.47 ± 5.07 mg g$^{-1}$ DM, $n=7$ species) of all the Caryophyllales

381　families. The only Cactaceae species studied, *Echinofossulocactus* sp., also had one of the

382　largest $[Na]_{shoot}$ measured (17.60 mg g$^{-1}$ DM, $n=1$ experiment). In addition, two of the twelve

383　Amaranthaceae species studied (*Atriplex hortensis*, *Beta vulgaris*), two of the twenty

384　Caryophyllaceae species studied (*Silene armeria*, *Spergula arvensis*) and one of the two

385　Portulacaceae species studied (*Portulaca grandiflora*) could also be considered Na-

386　hyperaccumulators (Table S1). However, since (1) there were proportionally fewer Na-

387　hyperaccumulator species in these families and (2) the Na-hyperaccumulator species in

388　these families generally had smaller $[Na]_{shoot}$ than the Aizoaceae Na-hyperaccumulator

389　species, their mean $[Na]_{shoot}$ was less than the mean $[Na]_{shoot}$ of the Aizoaceae (Figure 3). No

390　Na-hyperaccumulator species were observed in the Phytolaccaceae, Nyctaginaceae,

391　Montiaceae, Polygonaceae or Plumbaginaceae. Based on the phylogenetic relationships

392　between Caryophyllales families proposed recently (Crawley & Hilu, 2012; Hernández-

393　Ledesma *et al.*, 2015; Yang *et al.*, 2015) and the data from the experiments reported here

394　(Table S1) it appears that the trait of Na-hyperaccumulation might have evolved several

395　times within the Caryophyllales (Figure 3). It is likely that the trait evolved in an ancestor of

396　the Aizoaceae, but not the Phytolaccaceae or Nyctaginaceae. It is possible that the trait also

397　evolved in ancestors of the Cactaceae and Portulacaceae, which are closely related (APGIV,

398　2016), and in ancestors of the Amaranthaceae and Caryophyllaceae.

399

400

401　**Discussion**

402

403　Angiosperm species can be classified into "Na-excluders", "Na-responders" and "Na-

404　accumulators" based on relationships between their $[Na]_{shoot}$ and the salinity of the

405　irrigation solution (cf. Baker, 1981). This terminology, originally proposed to categorise the

406　responses of plant species to toxic elements ("heavy metals") in the environment, also

407　appears valid for Na accumulation, since the accumulation of excessive Na$^+$ can be toxic to

408　plants and plant species respond to Na$^+$ in their environment either by excluding this cation

409    or accumulating it safely in their tissues. Only two of the ten halophytic species studied in

410    detail in this paper could be classified as Na-accumulators (Table 1). These were the

411    Caryophyllales species *Atriplex hortensis* and *Beta vulgaris*, which both had exceptionally

412    large $[Na]_{shoot}$ when irrigated with either non-saline or saline solutions. A similar response of

413    $[Na]_{shoot}$ to increasing salinity in the root environment has been observed previously for

414    other Caryophyllales species including members of the *Atriplex*, *Salicornia* and *Suaeda*

415    genera (Albert, 1982; Glenn & O'Leary, 1984). However, not all Caryophyllales species

416    exhibit this trait and the response of $[Na]_{shoot}$ to increasing salinity in the root environment

417    of, for example, the miohalophytes *Rumex dentatus* and *Limonium perezii* is typical of a Na-

418    excluders whilst the response of $[Na]_{shoot}$ to increasing salinity in the root environment of,

419    for example, *Sarcobatus vermiculatus* is reminiscent of Na-responders (Glenn & O'Leary,

420    1984).

421         The prevalence of Na-accumulator species, which exhibit abnormally large $[Na]_{shoot}$

422    when grown under non-saline conditions, was assessed by combining data from six

423    glasshouse experiments in which 334 angiosperm species representing 35 angiosperm

424    orders had been grown hydroponically in a non-saline solution containing 0.1 mM Na for 18-

425    73 days (Table S1). It was observed that a relatively large number of Caryophyllales species

426    exhibited abnormally large $[Na]_{shoot}$ (>10 mg g$^{-1}$ DM) when grown in non-saline solutions

427    (Table S1; Figure 1). The distribution of the log-normal $[Na]_{shoot}$ of Caryophyllales species

428    appeared to comprise two discrete log-normal distributions containing 49 and 12 species,

429    respectively (Figure 2), suggesting that there are at least two distinct $[Na]_{shoot}$ phenotypes

430    among Caryophyllales species. The $[Na]_{shoot}$ distinguishing between these two distributions

431    was about 4 mg Na g$^{-1}$ DM.

432         The ability of plants to accumulate Na when growing in non-saline environments is

433    not considered to be an evolutionary advantage (Cheeseman, 2015). Indeed, it has been

434    suggested that grazing by herbivores has selected for glycophyte species that maintain

435    $[Na]_{shoot}$ below about 1-2 mg g$^{-1}$ DM (Cheeseman, 2015). Nevertheless, it is possible that the

436    ability to accomodate large $[Na]_{shoot}$ might be an enabling trait allowing species to adapt to a

437    variety of abiotic environmental challenges. It might confer the ability for osmotic

438    adjustment in environments with low K phytoavailability or contribute to tolerance of arid

439    or saline environments (Flowers & Colmer, 2008; Kadereit *et al.*, 2012; White, 2013).

440    However, it can be observed that the trait of Na-hyperaccumulation within the

441     Caryophyllales is not directly correlated with the expression of either $C_4$ photosynthesis or

442     CAM, tissue succulence, halophytism in general, or the euhalophytic trait in particular (Table

443     S2).

444         The evolutionary origins of the trait of abnormally large shoot Na accumulation

445     when plants are grown in non-saline solutions, termed "Na-hyperaccumulation", can be

446     investigated by mapping this trait on the phylogenetic relationships between Caryophyllales

447     families (Crawley & Hilu, 2012; Hernández-Ledesma *et al.*, 2015; Yang *et al.*, 2015). All

448     Aizoaceae species appear to exhibit Na-hyperaccumulation when grown in non-saline

449     environments (Table S2). Although *Delosperma cooperi* was not classified as a Na-

450     hyperaccumulator in the present study, it has previously been shown to accumulate >4 mg

451     Na $g^{-1}$ DM shoot when grown in a peat substrate (Sunshine Mix #1, SunGro Hort., Bellevue,

452     Washington) and irrigated with tap water with an $EC_e$ of 0.8 dS $m^{-1}$ (Niu & Rodriguez, 2006).

453     In addition to the species studied in the present study, *Galenia pubescens* (Patel *et al.*,

454     1980), *Galenia secunda* (Glenn & O'Leary, 1984), *Sesuvium portulacastrum* (Ramani *et al.*,

455     2006; Slama *et al.*, 2008; Rabhi *et al.*, 2011; Wang *et al.*, 2012), *Sesuvium verrucosum* (Glenn

456     & O'Leary, 1984) and *Tetragonia tetragonioides* (Yousif *et al.*, 2010) have all been reported

457     to accumulate >4 mg Na $g^{-1}$ DM shoot when grown under non-saline conditions (Table S2).

458     In this context, it is noteworthy that many Aizoaceae species possess bladder cells (Thomson

459     *et al.*, 1988; Flowers *et al.*, 2010).

460         The trait of Na-hyperaccumulation in non-saline environments is less ubiquitously

461     exhibited by Amaranthaceae species (Table S2 and references therein). However, it is

462     exhibited by many species formerly classified as Chenopodiaceae. It is exhibited by the

463     Betoideae, *Beta vulgaris* and *Hablitzia tamnoides*, by some Camphorosmoideae (e.g. *Bassia*

464     *hyssopifolia* and *Maireana brevifolia*), by many Chenopoidioideae including most, but not

465     all, *Atriplex* and *Chenopodium* species, by *Corispermum hyssopifolium* and *Corispermum*

466     *pallasii* subsp. *membranaceum*, by all the Salicornioideae studied, including several

467     *Salicornia* and *Tecticornia* species, by many Salsoloideae, and all *Suaeda* species (Table S2).

468     By contrast, the trait is not exhibited by any Amaranthaceae *sensu stricto* (Amaranthoideae,

469     Gomphrenoideae), with the exception of *Ptilotus polystachyus*. Although many

470     Amaranthaceae species possess bladder cells or salt glands (Thomson *et al.*, 1988; Fahn &

471     Cutler, 1992; Flowers *et al.*, 2010; LoPresti, 2014), there does not appear to be a direct

472    correlation between the presence of salt glands and the ability to hyperaccumulate Na in

473    non-saline environments (Table S2).

474    Few Caryophyllaceae species had large $[Na]_{shoot}$ when grown hydroponically in non-

475    saline solutions, with only two of the twenty species examined in the present study (*Silene*

476    *armeria*, *Spergula arvensis*) exhibiting Na-hyperaccumulation (Table S1; Figure 1). This is

477    consistent with previous studies (Sonneveld & Voogt, 1983; Kwon *et al.*, 2005; Heo *et al.*,

478    2007; Jeong *et al.*, 2014). Several species in the Sarcobataceae (*Sarcobatus vermiculatus*),

479    Portulacaceae (*Portulaca grandiflora*; *Portulaca oleracea*) and Cactaceae (*Carnegiea*

480    *gigantea*, *Echinocactus grusonii*, *Echinofossulocactus* sp., *Opuntia ficus-indica*) exhibit large

481    $[Na]_{shoot}$ when grown in non-saline environments (Table S2 and references therein).

482    However, it is clear from the literature that not all Cactaceae exhibit large $[Na]_{shoot}$ when

483    grown in non-saline environments (Table S2; Nobel, 2003; Goodman *et al.*, 2012). No

484    species in the Phytolaccaceae, Nyctaginaceae, Montiaceae, Basellaceae or Simmondsiaceae

485    exhibited the trait (Table S2 and references therein).

486    In the experiments reported here, no Na-hyperaccumulator species were observed

487    in the Plumbaginaceae or Polygonaceae (Table S1; Figure 3). Nevertheless, several species in

488    these families have been reported to accumulate large $[Na]_{shoot}$ when grown in non-saline

489    environments (Table S2 and references therein). In addition, all six species of Tamaricaceae

490    studied to date appear to accumulate large $[Na]_{shoot}$ when grown in non-saline

491    environments (Patel *et al.*, 1980; Ding *et al.*, 2010; Li *et al.*, 2010; Gorai & Neffati, 2011;

492    Sghaier *et al.*, 2015; Sharif & Khan, 2016). It is, perhaps, noteworthy that many species in

493    the Plumbaginaceae and Tamaricaceae possess salt glands, whilst members of the

494    Polygonaceae do not (Thomson *et al.*, 1988; Fahn & Cutler, 1992; Salama *et al.*, 1999;

495    Flowers *et al.*, 2010). Again, there does not appear to be a direct correlation between the

496    occurrence of salt glands and the ability of a species to hyperaccumulate Na in non-saline

497    environments (Table S2).

498    In conclusion, phylogenetic relationships between Caryophyllales families suggest

499    that the trait of Na-hyperaccumulation in non-saline environments has evolved several

500    times within this order (Figure 3). The data presented here suggest that the trait evolved in

501    an ancestor of the Aizoaceae, but not the Phytolaccaceae or Nyctaginaceae. It is also likely

502    that the trait also evolved in an ancestor of species formerly classified as Chenopodiaceae

503    (subfamilies      Betoideae,      Chenopodioideae,      Camphorosmoideae,      Salsoloideae,

504 Salicornioideae, Suaedoideae), but not the Amaranthaceae *sensu stricto* (subfamilies

505 Amaranthoideae, Gomphrenoideae). In addition, it is possible that the trait evolved in

506 ancestors of the Sarcobataceae, Portulacaceae, Cactaceae, Tamaricaceae, Plumbaginaceae,

507 and Polygonaceae, but further studies are required to explore these hypotheses. Future

508 studies should focus on elucidating the evolutionary origin of Na-hyperaccumulation in non-

509 saline environments (1) among species formerly classified as Chenopodiaceae, (2) among

510 species in the Cactaceae and Portulacaceae, which are currently underrepresented in

511 published studies, and (3) among species in the Plumbaginaceae, Tamaricaceae and

512 Polygonaceae, to determine the extent of the trait in these families.

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524

525 **Contributions of Authors**. P.J.W., M.R.B. and H.A.E-S designed the study. H.C.B., A.T., J.A.T.

526 and G.W conducted the experiments. P.J.W., M.R.B. and K.N. compiled and analysed the

527 data. The manuscript was drafted by P.J.W. with contributions from all other authors.

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706

707

708  **Supporting Information**

709

710  Additional supporting information may be found in the online version of this article.

711

712  **Table S1** Shoot sodium concentrations in 334 species from 35 angiosperm orders grown

713  hydroponically in a non-saline solution containing 0.1 mM Na$^+$ in at least one of six

714  glasshouse experiments.

715

716  **Table S2** Occurrence of sodium (Na)-hyperaccumulator species, having shoot Na

717  concentrations >4 mg g$^{-1}$ dry matter when grown in non-saline environments, within the

718  Caryophyllales order, together with their halophytic and photosynthetic characteristics.

719

720    **Table**

721

722    **Table 1** Shoot fresh weight, dry matter (DM) and sodium concentration ([Na]$_{shoot}$) of ten

723    halophytic angiosperm species grown in pots irrigated with either 100 mL non-saline (0.14

724    mM Na) or saline (50-300 mM Na) solution per week. The experiment was initiated by

725    increasing the NaCl concentration in the irrigation water of the saline treatment to 50 mM

726    for the first week, then 150 mM NaCl for the second week, and finally 300 mM for the third

727    week. Plants were harvested three weeks after the first addition of NaCl to the saline

728    irrigation water. Data are expressed as mean ± standard error of the mean of n

729    observations.

730

| Treatment | Species | Family | Order | Fresh Weight (g) | Dry Matter (g) | [Na]$_{shoot}$ (mg g$^{-1}$ DM) |
|---|---|---|---|---|---|---|
| Non-saline | *Hordeum jubatum* L. | Poaceae | Poales | 4.66 ± 2.24 (*n*=3) | 0.43 ± 0.33 (*n*=3) | 0.21 ± 0.02 (*n*=3) |
| Non-saline | *Asparagus officinalis* L. | Asparagaceae | Asparagales | 6.36 ± 0.75 (*n*=10) | 0.74 ± 0.18 (*n*=10) | 0.38 ± 0.05 (*n*=10) |
| Non-saline | *Hibiscus tilliaceus* L. | Malvaceae | Malvales | 2.87 (*n*=1) | 0.19 (*n*=1) | 0.69 (*n*=1) |
| Non-saline | *Colubrina asiatica* (L.) Brogn. | Rhamnaceae | Rosales | 1.47 ± 0.17 (*n*=2) | 0.056 ± 0.003 (*n*=2) | 0.73 ± 0.15 (*n*=2) |
| Non-saline | *Casuarina cunninghamiana* Miq. | Casuarinaceae | Fagales | 0.90 ±0.20 (*n*=2) | 0.045 ± 0.010 (*n*=2) | 1.03 ± 0.24 (*n*=2) |
| Non-saline | *Kosteletzkya virginica* (L.) C. Presl ex A. Gray | Malvaceae | Malvales | 22.69 ± 3.75 (*n*=9) | 2.09 ± 0.63 (*n*=9) | 1.62 ± 0.13 (*n*=9) |
| Non-saline | *Ammi visnaga* (L.) Lam. | Apiaceae | Apiales | 22.56 ± 1.52 (*n*=8) | 2.13 ± 0.18 (*n*=8) | 2.16 ± 0.11 (*n*=8) |
| Non-saline | *Lobularia maritima* (L.) Desv. | Brassicaceae | Brassicales | 20.45 ± 8.68 (*n*=4) | 1.10 ± 0.58 (*n*=4) | 3.40 ± 0.27 (*n*=4) |
| Non-saline | *Scaevola crassifolia* Labill. | Goodeniaceae | Asterales | 49.53 ± 3.92 (*n*=4) | 4.09 ± 0.27 (*n*=4) | 3.78 ± 0.46 (*n*=4) |
| Non-saline | *Plantago maritima* L. | Plantaginaceae | Lamiales | 3.32 ± 0.52 (*n*=12) | 0.053 ± 0.001 (*n*=12) | 4.11 ± 0.33 (*n*=12) |
| Non-saline | *Beta vulgaris* L. | Amaranthaceae | Caryophyllales | 38.95 ± 7.28 (*n*=6) | 2.46 ± 0.63 (*n*=6) | 10.72 ± 1.03 (*n*=6) |
| Non-saline | *Atriplex hortensis* L. | Amaranthaceae | Caryophyllales | 28.18 ± 1.52 (*n*=7) | 4.18 ± 0.47 (*n*=7) | 12.02 ± 0.49 (*n*=7) |
| Saline | *Hordeum jubatum* L. | Poaceae | Poales | 2.52 ± 0.86 (*n*=2) | 0.13 ± 0.07 (*n*=2) | 2.18 ± 0.27 (*n*=2) |
| Saline | *Asparagus officinalis* L. | Asparagaceae | Asparagales | 4.11 ± 0.66 (*n*=9) | 0.36 ± 0.15 (*n*=9) | 2.66 ± 0.97 (*n*=9) |
| Saline | *Hibiscus tilliaceus* L. | Malvaceae | Malvales | 3.36 (*n*=1) | 0.17 (*n*=1) | 4.10 (*n*=1) |
| Saline | *Colubrina asiatica* (L.) Brogn. | Rhamnaceae | Rosales | 1.19 ± 0.36 (*n*=2) | 0.054 ± 0.003 (*n*=2) | 16.73 ± 10.32 (*n*=2) |
| Saline | *Casuarina cunninghamiana* Miq. | Casuarinaceae | Fagales | 0.53 (*n*=1) | 0.060 (*n*=1) | 3.62 (*n*=1) |
| Saline | *Kosteletzkya virginica* (L.) C. Presl ex A. Gray | Malvaceae | Malvales | 15.02 ± 1.58 (*n*=9) | 1.66 ± 0.26 (*n*=9) | 13.60 ± 0.94 (*n*=9) |
| Saline | *Ammi visnaga* (L.) Lam. | Apiaceae | Apiales | 20.38 ± 1.65 (*n*=8) | 1.96 ± 0.22 (*n*=8) | 17.83 ± 1.14 (*n*=8) |
| Saline | *Lobularia maritima* (L.) Desv. | Brassicaceae | Brassicales | 9.54 ± 1.75 (*n*=4) | 0.49 ± 0.18 (*n*=4) | 27.94 ± 2.30 (*n*=4) |
| Saline | *Scaevola crassifolia* Labill. | Goodeniaceae | Asterales | 41.70 ± 9.77 (*n*=4) | 3.41 ± 0.97 (*n*=4) | 19.41 ± 1.97 (*n*=4) |
| Saline | *Plantago maritima* L. | Plantaginaceae | Lamiales | 2.47 ± 0.37 (*n*=12) | 0.052 ± 0.002 (*n*=12) | 27.49 ±1.01 (*n*=12) |
| Saline | *Beta vulgaris* L. | Amaranthaceae | Caryophyllales | 35.72 ± 8.90 (*n*=6) | 2.27 ± 0.60 (*n*=6) | 28.08 ± 3.29 (*n*=5) |
| Saline | *Atriplex hortensis* L. | Amaranthaceae | Caryophyllales | 32.99 ± 0.57 (*n*=6) | 5.04 ± 0.24 (*n*=6) | 35.70 ± 3.45 (*n*=3) |

731

732     **Figure Legends**

733

734     **Figure 1** Frequency distributions of mean shoot sodium (Na) concentrations in **(a)** 334

735     species from 35 angiosperm orders or **(b)** 61 species from ten Caryophyllales families grown

736     hydroponically in a non-saline solution. Shoot Na concentrations >10 mg Na $g^{-1}$ dry matter

737     are designated "more". The solid line indicates the normal (mean = 0.393, standard

738     deviation = 0.185 mg Na $g^{-1}$ dry matter, $n$ = 42 species) distribution fitted to data from the

739     42 Caryophyllales species with the smallest shoot Na concentrations.

740

741     **Figure 2** Frequency distributions of log-normal mean shoot sodium (Na) concentrations in

742     **(a)** 334 species from 35 angiosperm orders or **(b)** 61 species from ten Caryophyllales families

743     grown hydroponically in a non-saline solution. The solid line indicates two log-normal

744     distributions (first: mean = -0.3717, standard deviation = 0.3299, $n$ = 49 species; second:

745     mean = 1.246, standard deviation 0.2756, $n$ = 12 species) fitted to data from the 49

746     Caryophyllales species with the smallest leaf Na concentrations and the 12 Caryophyllales

747     species with the largest leaf Na concentrations, respectively.

748

749     **Figure 3** Phylogenetic relationships between ten families of the Caryophyllales, based on the

750     phylogeny derived by Crawley & Hilu (2012), and their shoot sodium concentrations

751     ([Na]$_{shoot}$). The number of species hyperaccumulating Na (numerator) and the number of

752     species surveyed (denominator) are indicated in parentheses. Families with species

753     expressing the trait of Na-hyperaccumulation are highlighted in yellow and Families without

754     species expressing the trait of Na-hyperaccumulation are highlighted in blue. Data are

755     expressed as mean values with capped lines indicating the standard error of the mean of
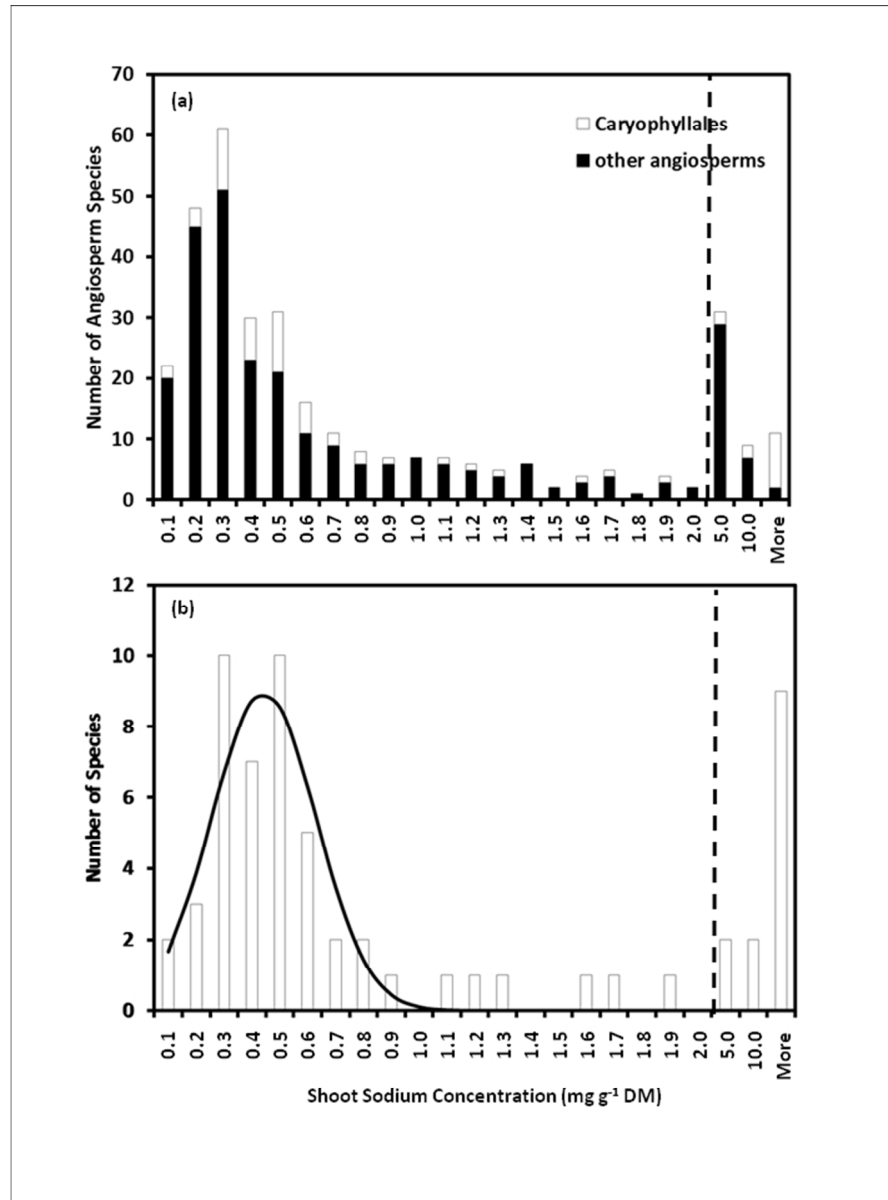
756     species surveyed.

Figure 1 Frequency distributions of mean shoot sodium (Na) concentrations in (a) 334 species from 35 angiosperm orders or (b) 61 species from ten Caryophyllales families grown hydroponically in a non-saline solution. Shoot Na concentrations >10 mg Na g-1 dry matter are designated "more". The solid line indicates the normal (mean = 0.393, standard deviation = 0.185 mg Na g-1 dry matter, n = 42 species) distribution fitted to data from the 42 Caryophyllales species with the smallest shoot Na concentrations.
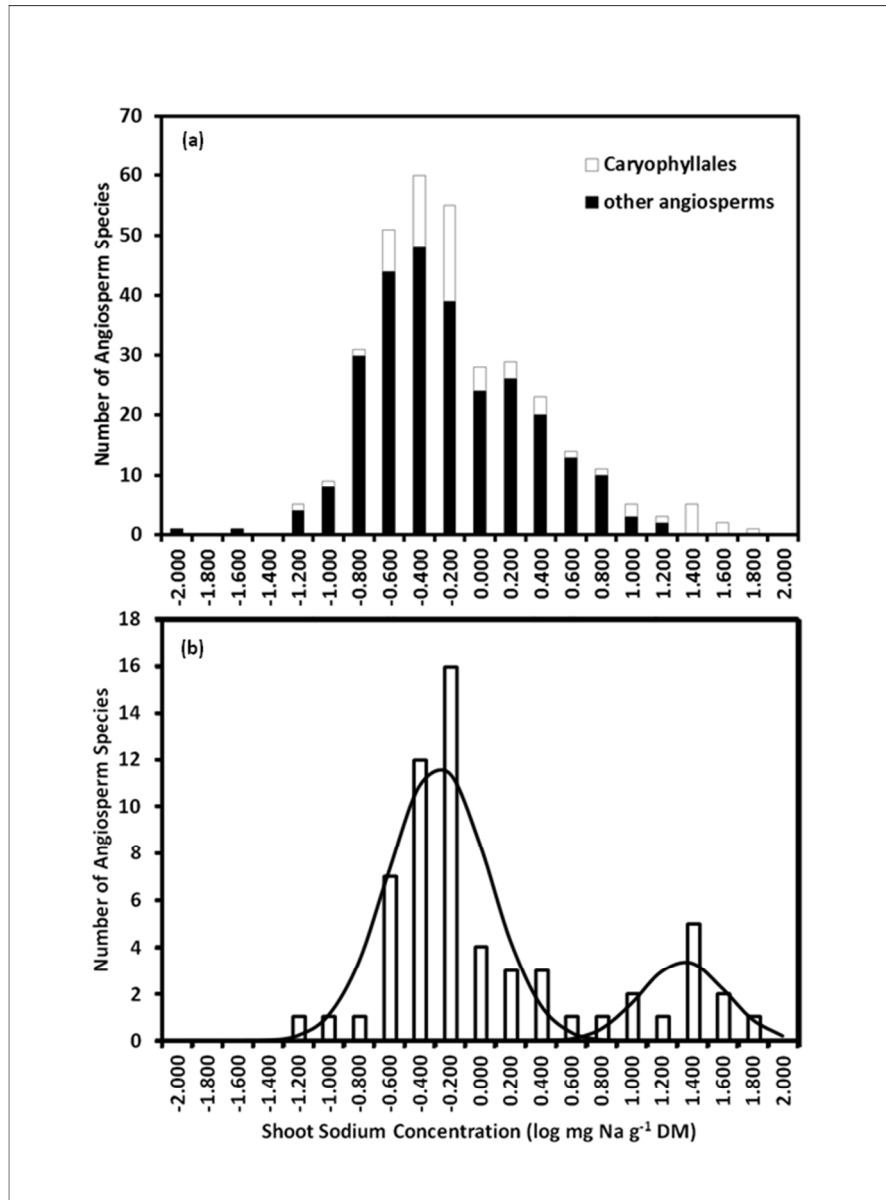
Fig. 1
120x162mm (150 x 150 DPI)

Figure 2 Frequency distributions of log-normal mean shoot sodium (Na) concentrations in (a) 334 species from 35 angiosperm orders or (b) 61 species from ten Caryophyllales families grown hydroponically in a non-saline solution. The solid line indicates two log-normal distributions (first: mean = -0.3717, standard deviation = 0.3299, n = 49 species; second: mean = 1.246, standard deviation 0.2756, n = 12 species) fitted to data from the 49 Caryophyllales species with the smallest leaf Na concentrations and the 12 Caryophyllales species with the largest leaf Na concentrations, respectively.
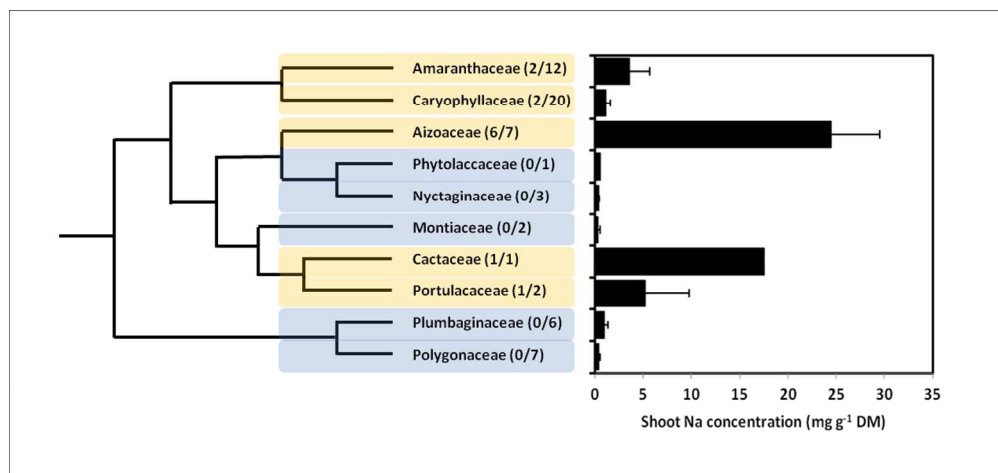
Fig. 2

120x162mm (150 x 150 DPI)

Figure 3 Phylogenetic relationships between ten families of the Caryophyllales based on Crawley & Hilu (2012) and their shoot sodium concentrations ([Na]shoot). The number of species hyperaccumulating Na (numerator) and the number of species surveyed (denominator) are indicated in parentheses. Data are expressed as mean values with capped lines indicating the standard error of the mean of species surveyed.

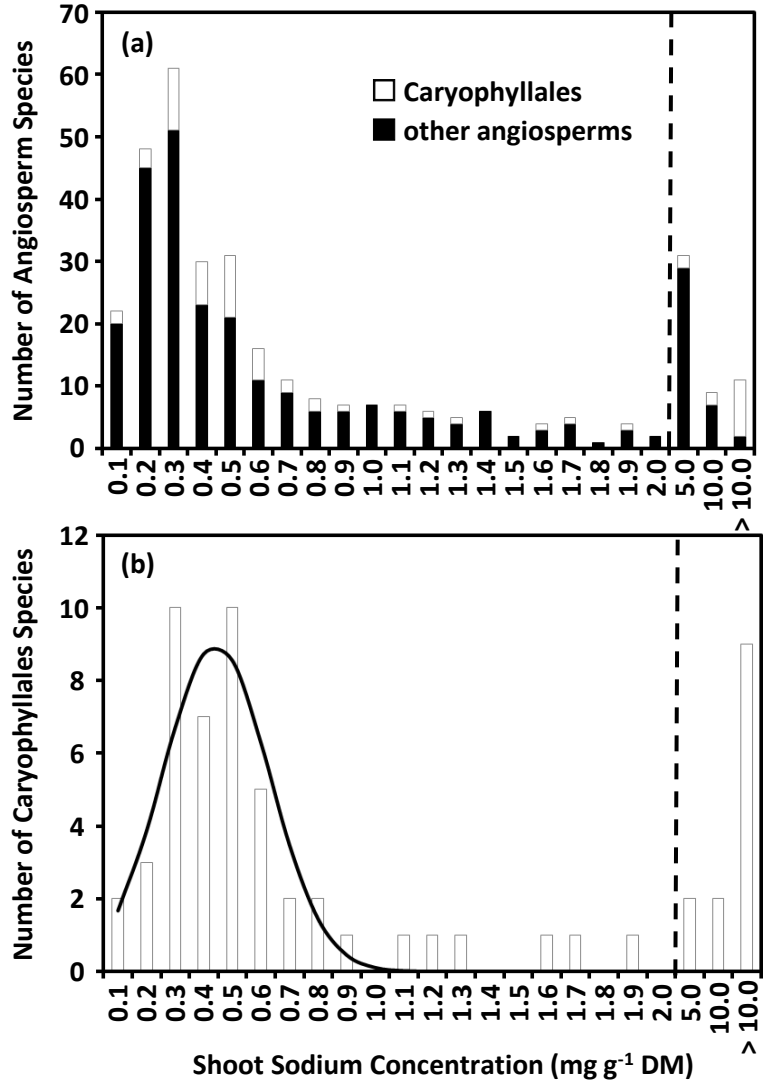Fig. 3
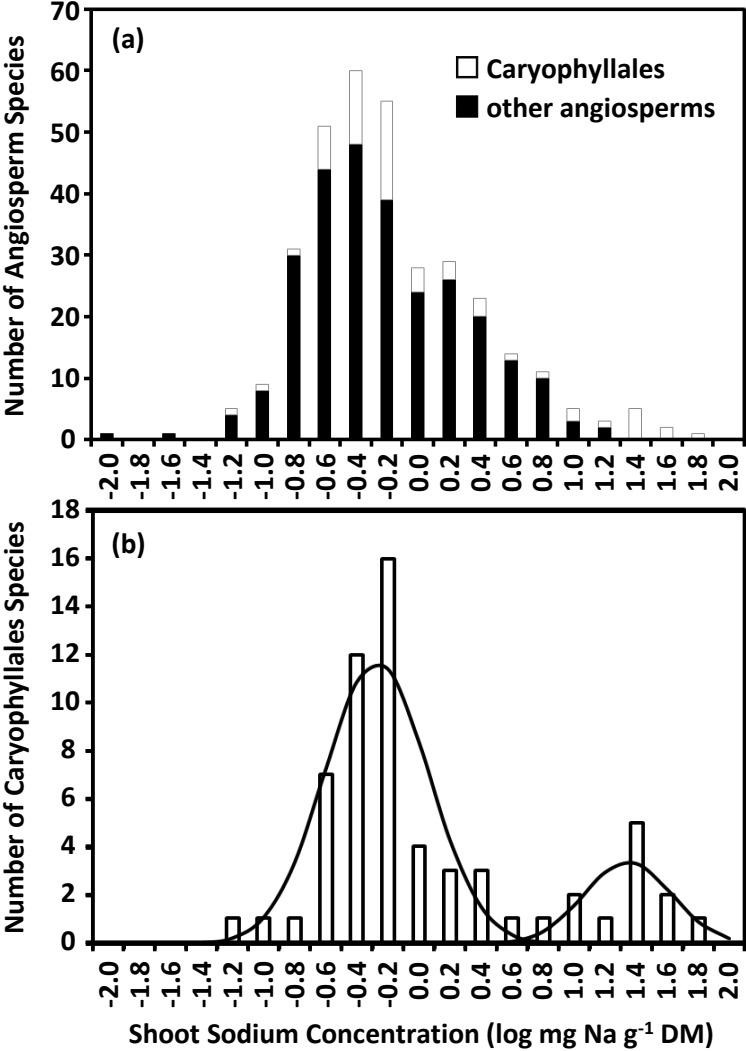215x101mm (150 x 150 DPI)

# Figure 1

# Figure 2

# Figure 3



Amaranthaceae (2/12)
Caryophyllaceae (2/20)
Aizoaceae (6/7)
Phytolaccaceae (0/1)
Nyctaginaceae (0/3)
Montiaceae (0/2)
Cactaceae (1/1)
Portulacaceae (1/2)
Plumbaginaceae (0/6)
Polygonaceae (0/7)

Shoot Sodium Concentration (mg g$^{-1}$ DM)