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Environmental drivers of freshwater macrophyte diversity and community composition in calcareous warm-water rivers of America and Africa

Julissa Tapia Grimaldo[1,2], Matthew T. O'Hare[2], Michael P. Kennedy[3*], Thomas A. Davidson[4], Jaime Bonilla-Barbosa[5], Betzy Santamaría-Araúz[5], Lyn Gettys[6], Sara Varandas Martins[1], Sidinei M. Thomaz[7] and Kevin J. Murphy[1]

[1]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow G12 8QQ, Scotland

[2]Centre for Ecology and Hydrology, Bush Estate, Penicuik, Midlothian EH26 0QB, Scotland

[3]School of Energy, Construction and Environment, Coventry University, Priory Street, Coventry CV1 5FB, England

[4]Department of Bioscience, 25 Vejlsøvej, 8600, Silkeborg, Aarhus University, Denmark

[5]Laboratorio de Hidrobotánica, Departamento de Biología Vegetal, Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, 62209 Cuernavaca, Morelos, México

[6]University of Florida, Fort Lauderdale Research and Education Center, 3205 College Ave, Davie, Florida, FL 33314, USA

[7]Nupélia, Universidade Estadual de Maringá, Maringá, PR, Brasil

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*Correspondence: Michael Kennedy, School of Energy, Construction and Environment, Coventry University, Priory Street, Coventry CV1 5FB, England; ab9280@cov.ac.uk

SUMMARY

1. This study assessed the hypothesis that spatial and environmental drivers of river macrophyte diversity and community composition differ in relative importance in calcareous river systems located in warm regions of America versus Africa.

2. We collected aquatic vegetation and spatio-environmental data, during 2006 – 2011, from >200 hardwater rivers, and associated floodplain waterbodies, located up to 30° North or South of the Equator, in México, Trinidad, Brasil, Argentina, USA (Florida), South Africa, Botswana, and Zambia.

3. Species rarefaction procedures were used to assess the impacts of differing sampling effort in the two continents upon estimation of γ-diversity ("species pool"). We then used a cluster analysis approach (Two-Way Indicator Species Analysis: TWINSPAN) to classify samples into groups based upon species composition. Variation in species richness, community composition and six spatial and environmental variables, among samples making up these groups, were compared using ANOVA and Kruskal-Wallis procedures. Regression trees and redundancy analysis were used to infer the relative importance of spatial and environmental drivers in explaining variation in local species richness and species community composition between the two continents. Sorensen's index (C_s) was calculated to estimate species turnover (β-diversity) between African and American samples.

4. In total 378 macrophyte taxa were recorded, with no significant difference in mean macrophyte α-diversity between African and American sites, but with evidence for high species turnover between the two continents (C_s = 0.17). Rarefaction analysis confirmed the existence of a larger macrophyte species pool in the hardwater rivers sampled in Africa compared to America. TWINSPAN classification identified seven sample end-groups, only one of which contained a mix of sites from both continents. PERMANOVA and nMDS ordination analysis confirmed significant differences in community composition present in

these sample groups. There were substantial differences between the sample-groups for α-diversity, and for spatial and environmental variables.

5. The high species turnover between Africa and America may be accounted for by geographical segregation, along with differences in aquatic habitat characteristics, and varying long-distance dispersal capacities of individual species. The relative importance of spatial and physico-chemical drivers (latitude, pH, altitude, alkalinity and electrical conductivity; but not flow) differed between the continents in influencing variation in both macrophyte diversity and community composition composition. Latitude was a significant, though non-linear and rather complex, spatial driver of macrophyte α-diversity in both American and African hardwater rivers. Water chemistry variables varied in relative importance as drivers of macrophyte α-diversity for African and American sites individually, and for all sites combined, but pH and/or electrical conductivity were more important than alkalinity in each case. In all three cases, altitude was consistently the third most important driver of α-diversity. Spatial and environmental variables played important roles in structuring macrophyte community composition in warm-water calcareous rivers in both America and Africa, with latitude being the strongest individual driver. Thus, this spatial variable, which is a surrogate for numerous enviro-climatic variables, appears to be of importance in determining macrophyte distributions at large spatial scales, for the ecosystem type examined here.

Introduction

Recently, there has been a major effort to improve understanding of the drivers of biogeographic distributions and diversity of freshwater macrophyte species, some (but by no means all) of which have broad planetary distributions (e.g. Bornette *et al*., 1998; Santamaría, 2002; Murphy *et al*., 2003; Makkay *et al.,* 2008; Carvalho *et al*., 2009; Heikkinen *et al*., 2009; Lang & Murphy, 2012; Chappuis *et al*., 2012, 2014; Kennedy *et al*., 2015, 2017; Morandeira & Kandus, 2015; Ranieri *et al*., 2015; Tapia Grimaldo *et al*., 2016; Redekop *et al*., 2016; Alahuhta *et al*., 2017). Most of these studies have examined macrophyte diversity and distributions in cool-temperate river and lake systems, with least attention being paid to warm-water river macrophyte communities. Even fewer studies have directly compared Old and New World freshwater macrophyte ecology: a rare example is Jacobsen & Terneus (2001), on stream vegetation in Ecuador and Denmark. Examples of environmental drivers variously reported to be important, at differing geographical scales, include enviro-climatic factors associated with variation in latitude (e.g. evapotranspiration regime), and environmental heterogeneity associated with a range of physico-chemical factors. Altitude, water and substrate chemistry, flow regime and human-related habitat alteration are often considered relevant in this context.

Freshwater macrophytes are “aquatic photosynthetic organisms large enough to see with the naked eye, that actively grow permanently or periodically submerged below, floating on, or growing up through the water surface” of freshwater systems (Chambers *et al*., 2008). In this study we deal only with vascular freshwater macrophytes, not considering bryophytes or macroalgae.

There is good evidence that the Neotropical biogeographic region, comprising South and Central America, plus a small area of North America, namely part of Texas and most of Florida (Escalante *et al*., 2010), is a global hotspot for vascular freshwater macrophyte biodiversity, with a recorded γ-diversity (species regional pool) of 984 macrophyte species,

97 according to Chambers *et al*. (2008). In contrast the Afrotropical region (Africa and the
98 Arabian Peninsula, south of the Tropic of Cancer) has a lower macrophyte γ-diversity, with
99 614 species, while the Nearctic (Greenland and North America, excluding parts of Texas and
100 Florida) has a macrophyte γ-diversity slightly higher than the value for the Afrotropics, at 644
101 species (Chambers *et al*., 2008).

102 It is not known whether these differences in diversity occur because of natural causes (e.g.,
103 habitat limitations, or for evolutionary reasons), or are due to differences in sampling effort,
104 or both. Afrotropical freshwaters are probably under-recorded for aquatic plant species
105 (examples of, usually quite local, surveys include: Denny, 1973, 1985; Simpson, 1975;
106 Chabwela & Siwale, 1986; Machena, 1988; Sarr *et al.* 2001; Adesina *et al*., 2011; Achieng'
107 *et al*., 2014). A recent survey of 228 sites in Zambian rivers (including both hard- and
108 softwater systems: Kennedy *et al*., 2015) recorded 335 macrophyte taxa, but the cumulative
109 sequential records curve for the dataset showed little sign of reaching an asymptote. It is
110 hence likely that many additional macrophyte species remain to be found in Zambian rivers,
111 and the situation is probably the same for other tropical African countries.

112 In contrast there has been quite a substantial macrophyte survey effort in the Neotropics,
113 particularly in South American freshwater systems (e.g., Bertoli, 1996; Murphy *et al*., 2003;
114 Thomaz *et al*., 2009; Rolon & Matchik, 2006; Amaral *et al*., 2008; Sousa *et al*., 2010, 2011;
115 Varandas Martins *et al*., 2013; Bottino *et al*., 2014; Neiff *et al*., 2014; Bando *et al*., 2015;
116 Schneider *et al*., 2015), though less so in Central America (e.g., Crow, 1993; Philbrick *et al*.,
117 1995; Anonymous, 1999; Bonilla-Barbosa, 2004). Compared to Africa, the macrophyte flora
118 of the Neotropics is probably reasonably well known, although there is evidence that the
119 asymptote of the species-sampling effort curve (for all freshwater habitats combined) has not
120 been reached in this region either (e.g., Ferreira *et al*., 2011).

121 In the Nearctic the survey effort for aquatic macrophyte vegetation has been very
122 substantial, with >2000 publications on the macrophyte ecology (of both the Nearctic and

123 Neotropical parts) of Florida alone held, for example, by the Center for Aquatic and Invasive
124 Plants Aquatic Plant Information Retrieval System (www.plants.ifas.ufl.edu/apirs). It is
125 probable that the freshwater macrophyte γ-diversity of the Nearctic is nearly completely
126 described.

127 In this study we examined variation in river vascular freshwater macrophyte community
128 characteristics, and their potential spatio-environmental drivers, on a broad intercontinental
129 scale, comparing warm regions of the New and Old World. Specifically, we targeted one
130 widespread type of river ecosystem, namely calcareous ("hardwater") rivers and their
131 associated high-connectivity riverine static or slow-flowing waterbodies, occurring in warm-
132 temperate to tropical regions of America and Africa.

133 We define "hardwater systems" as minimally having a mean calcium carbonate
134 concentration ($CaCO_3$) >10 mg L^{-1} (approximately >200 µEq L^{-1}), or bicarbonate
135 concentration (alkalinity: HCO_3^-) >12.2 mg L^{-1} (approximately >200 µEq L^{-1}): following Moyle
136 (1945) and Tapia Grimaldo (2013). Calcareous rivers may have much greater hardness than
137 these minimal values; bicarbonate concentrations >4000 µEq L^{-1} were recorded at several
138 sites in our study. Hardwater rivers arise on a range of catchment geologies, including
139 karstic limestone, softer calcareous rocks such as chalk, gypsum and certain types of
140 sandstone, and calcium-rich alluvial soils (Tapia Grimaldo, 2013). All of these geologies
141 occurred within the set of sites examined here.

142 In this study we tested the hypothesis that significant differences in macrophyte community
143 structure exist between calcareous warm-water rivers (and their associated high-connectivity
144 waterbodies) located in warm-temperate to tropical regions of the New World, versus those
145 in the Old World, taking the Afrotropics as the target Old World region. Specifically, we
146 examined differences in macrophyte diversity and community composition between these
147 regions, in relation to a spatial variable (latitude) and a set of physico-chemical factors
148 (altitude, pH, electrical conductivity, alkalinity and water flow regime) potentially influencing

149 these differences. No previous study has examined this issue, which is of added interest in
150 the context of establishing baseline data to assess potential changes in river floras
151 associated with global climate change and other human stressors. We expected to see
152 differences between these macro-regions primarily because of differences in their physico-
153 chemical characteristics (e.g., Payne, 1986). Historic geographical segregation between the
154 regions, and variation in relevant biotic factors, were also considered likely to influence
155 differences in macrophyte diversity and community composition when comparing African and
156 American warm-water calcareous rivers.

157

158 **Methods**

159 *Study area*

160 A dataset consisting of 292 samples, from Africa (n = 208 samples) and America (n = 84),
161 was collected from sites located on rivers, and associated waterbodies with high connectivity
162 to the river system. Sites were primarily located in flowing river channels. These included
163 main river channels, tributaries, and distributaries (channels which flow into or out of the
164 main river, within its floodplain, depending on main river channel water level: an example
165 within our dataset being the Baia River in the floodplain of the Upper Rio Paraná in Brasil:
166 Varandas Martins *et al.,* 2013). There was a smaller component of sites in static to slow-
167 flowing water channels closely associated with rivers (e.g. backwaters and spring runs); and
168 floodplain riverine lakes, oxbows, and cenotes (sinkholes, produced from the collapse of
169 limestone-bedrock, filled with groundwater derived from underground rivers), which are lentic
170 but closely connected to the river channel.

171 Sites were selected which had hardwater conditions; macrophyte communities present;
172 reflected the range of environmental conditions occurring across each target area; and were
173 reasonably accessible. For safety reasons, some otherwise suitable sites were excluded in

Africa because dangerous animals were present. Within the boundaries of these criteria sampling sites were selected at random along rivers and their associated waterbodies.

In Africa study sites were located in:

(i) Zambia: 176 samples from 130 individual sites throughout the country. Tropical: centred on 13°S, 29°E (latitude range: 8.89090 - 17.8875°S), sampled 2006 – 2011. In Zambia only some sites were repeat-sampled in wet and dry seasons of a single year, or in different years during the study period (for more on this see Kennedy *et al*., 2015, 2016);

(ii) Botswana: tropical: 21 sites in the Okavango Delta, centred on 18.8°S, 22.5°E (latitude range: 18.33908 - 19.57003°S); sampled 2006; and

(iii) South Africa: warm-temperate: 11 sites, in the Highveld area of the Vaal River, centred on 26.5°S, 29.5°E (latitude range: 26.36711 – 26.97082°S); sampled 2009 - 2010.

In America the study areas were in:

(i) USA (northern Florida): subtropical to warm-temperate: 27 sites centred on 29.5°N, 82°W (latitude range: 29.08102 - 30.83998°N); sampled 2011;

(ii) México (Yucatan Peninsula): tropical: 18 sites centred on 19°N, 88.5°W (latitude range: 18.44031 – 21.56547°N); sampled 2011;

(iii) Trinidad: tropical: 17 sites centred on 10.6°N, 61.5°W (latitude range: 10.57670 – 10.71050°N); sampled 2011;

(iv) Argentina, located near the confluence of the Río Paraguay and Middle Río Paraná, in the Provinces of Chaco and Corrientes (warm-temperate, with three sites centred on 27.4°S, 58.7°W (latitude range: 27.245 – 27.460°S); sampled 2010); and

(v) three areas of Brasil (all sampled 2010): (a) Chapada Diamantina in the State of Bahia: tropical, with two sites centred on 12.4°S, 41°W; (b) the Upper Rio Paraná and its floodplain, in the States of Paraná and Mato Grosso do Sul: subtropical, with 17 sites centred on

23.5°S, 54°W; and (c) the Bonito/ Southern Pantanal area of the State of Mato Grosso do Sul: subtropical, with 11 sites centred on 21°S, 56.5°W (total latitude range for Brasil sites: 12.4000 – 25.85909°S).

In the Northern Hemisphere, the total latitudinal range for sample sites was 20.26328° (ranging from a site in Trinidad at 10.57670°N, to a site in northern Florida at 30.83998°N). In the Southern Hemisphere, the site closest to the Equator was located in northern Zambia (8.89088°S), and the furthest-south was a site in Argentina (27.45996°S), giving a latitudinal range of 18.56908°.

Sampling was typically conducted during periods when rivers were experiencing baseflow conditions, during the dry season. This was partly to facilitate access to sites. Dry-season sampling also minimised the possibility of post-flood changes in water chemistry skewing results. Some sites in Zambia were sampled during both wet and dry-seasons and substantial changes to water chemistry were observed following flood events (Kennedy *et al*., 2008; Kennedy *et al*., 2015, 2016). Individual samples from these repeat-sampled sites were, however, treated as discrete units, hence the effects of wet season conditions on analytical results were identifiable.

Biological and environmental data

Data on macrophyte species presence (vascular species only were included in the study) and environmental parameters were collected by field survey, and supporting laboratory analysis of water samples, during 2006-2011, from standard 100 m stretches of each target waterbody. All survey data were personally collected by the authors, to ensure a robust level of standardised quality control for species identification and other field data collection.

Macrophyte surveys broadly followed the international standard EN 14184 (European Committee for Standardization, 2003), to collect qualitative data for macrophyte taxa

223 occurrence (submerged, floating and emergent: Chambers *et al*., 2008) within each survey
224 stretch. A standard macrophyte-sampling grapnel (attached to a 5 m long cord, and thrown
225 from bank or boat as appropriate) was used where necessary as an aid to collection of
226 submerged species. Nomenclature follows The Plant List (www.theplantlist.org). Herbarium
227 voucher specimens were deposited with Coventry University (UK) and the Herbarium of the
228 University of Morelos (HUMO), Universidad Autónoma del Estado de Morelos (México).
229 Plants were identified to species level except where a lack of flowers, or other diagnostic
230 structures, permitted identification only to genus or family level. All macrophyte taxa present
231 at a site were used to calculate α-diversity (S: number of taxa present per sample), but for
232 other data-analysis purposes, only records identified to species level were utilised.
233 Information on the distributional status of each species as endemic, native/ naturalised, or
234 introduced/ invasive, within the Afrotropical and one or both of the Nearctic/ Neotropical
235 biogeographic regions was obtained from various sources. These included e-Monocot
236 (http://e-monocot.org); Flora Zambesiaca: (http://apps.kew.org/efloras); Flora of Zambia:
237 www.zambiaflora.com; Flora of Botswana: www.botswanaflora.com); GBIF (Global
238 Biodiversity Information Facility): http://www.gbif.org/species; Flora acuática vascular del
239 área focal Felipe Carrillo Puerto, Corredor Biológico Sian Ka'an-Calakmul, Quintana Roo,
240 México (Bonilla-Barbosa, 2004): http://www.gbif.org/dataset/7f7f1342-f762-11e1-a439-
241 00145eb45e9a; MEXU/Colección de Plantas Acuáticas: www.gbif.org/dataset/9606752e-
242 f762-11e1-a439-00145eb45e9a; Amaral *et al*. (1998), Scremin-Dias *et al*. (1999), Pott & Pott
243 (2000), Gerber *et al*. (2004), and Cook (2004).

244 Spatial and environmental variables used for this study included latitude: absolute ° (N or S
245 of the Equator); and altitude (m above sea level, a.s.l.), recorded using a hand-held Garmin
246 Etrex (or similar) Global Positioning System (GPS) instrument, and supplemented where
247 necessary by reference to Global Earth or other large scale maps. A subjective assessment
248 of flow (flow categories and approximate corresponding flow velocity intervals follow Lang &
249 Murphy, 2012) was made on a four-point scale: 0 = static: (0 m s^{-1}); 1 = slow flow

(approximately <0.2 m s^{-1}); 2 = moderate flow (approximately 0.2 - 0.4 m s^{-1}); 3 = fast flow: "riffle" or white-water showing (approximately >0.4 m s^{-1}). Electrical conductivity (EC: µS cm^{-1}) and pH were measured on-site, using a Schott 178 Handylab 264 meter, or similar instrument. Water samples were collected at each site (in an undisturbed sediment area) for subsequent laboratory measurement of alkalinity (µEq L^{-1} bicarbonate), using the Gran alkalinity titration method (Neal, 2001).

Statistical methods

Two strategies were used in order to minimise sampling effects and make γ-diversity ("species pool") comparable between continents (Melo *et al*., 2007). The first was construction of rarefaction curves for American and African sites, and the second utilised the incidence-based Chao2 estimator (Chao, 1987; Colwell, 2013). R was used to carry out both analyses.

In order to assess species turnover between sites located in America and those in Africa, β-diversity (Koleff *et al*., 2003) was measured using the Sorensen index (C_s):

$$C_s = 2j/(a + b)$$

where *a* = number of species present in samples surveyed in area *a; b* = number of species present in samples surveyed in area *b*; and *j* = number of species present in common in areas *a* and *b.* Low values for this index imply low commonality between the regional species-sets compared.

To assess variation in macrophyte community composition an ecologically-relevant classification of samples was generated, in terms of species present at each site, using the divisive clustering procedure TWINSPAN (Hill, 1979). A matrix of samples x species for the full dataset was used, including only taxa identified to species level. This produced a set of end-groups of samples (stop-criterion for clustering sample division: division eigenvalue

>0.300), for which spatial, environmental and diversity variables were further compared using inferential statistics.

For inferential statistical testing, to compare mean values of response variables (α-diversity, S; latitude; pH; altitude; alkalinity; electrical conductivity, EC; flow) measured at sites, between TWINSPAN sample-groups, variables were first assessed for normality using Ryan–Joiner testing, and all proved to meet the conditions of normality. Homogeneity of variance was then assessed using Levene's Medians test, and only two variables (pH and α-diversity, S) met the assumption of no significant difference in homogeneity of variance between datasets included in the test. For these two variables, one-factor analysis of variance (ANOVA), with *post-hoc* mean-separation using Tukey's Least Significant Difference test was utilised. The remaining variables were assessed using the non-parametric Kruskal-Wallis procedure. Permutational multivariate ANOVA (PERMANOVA: Anderson, 2001) was used to test for significant differences in community composition composition across the TWINSPAN groups. In order to investigate the relative importance of latitude versus the measured environmental data in influencing community composition a variance partitioning exercise was carried out on the species presence-absence data, using distance-based redundancy analysis (db-RDA) based on Bray-Curtis distance (Anderson & McArdle, 2001). . Variance partitioning is a standard procedure (Borcard *et al*.,1992) used to determine the relative influence of different variables in shaping community composition. A number of db-RDA analyses were carried out: first a full model incorporating latitude and the available environmental data (pH, EC, alkalinity and altitude); second, the model was rerun with latitude as the covariable; then, third, another run with the environmental variables as covariables. By comparing the fractions of variance explained by each model it is possible to calculate the relative influence of latitude versus environment. Permutation tests were applied to assess the significance of the various models.

The above tests are multivariate and investigated the community composition data. In order to investigate the response in the univariate species richness data we used Boosted

Regression Trees (BRTs), which can cope with a combination of categorical and continuous data (De'ath, 2007). BRTs were employed to determine the factors that best predict variation in species richness across the full dataset, and for each continent separately. The approach of Elith *et al*. (2008) was employed to find the optimal number of trees. Tree complexity was set at three with a learning rate of 0.001, and with the bag fraction set at 0.75, meaning each individual tree was constructed using 75% of the data, with its predictive ability tested on the remaining 25% (Elith *et al*., 2008). BRTs are excellent tools for finding patterns in large complex data sets, using thousands of small trees to find variables that (in this case) best predict species richness, but they do not provide a good means to visualise the data. Thus, we used a single univariate regression tree (De'ath, 2002), pruned using a cost-complexity measure, to show how the different explanatory variables relate to patterns in species richness. Indirect gradient analysis ordination, using non-metric multidimensional scaling (nMDS: with Bray-Curtis distance measures), and t-tests were also used in analyses of the dataset. Inferential tests were conducted using Excel (with the Real Statistics add-in package: www.real-statistics.com/free-download/real-statistics-resource-pack), and Minitab version 15.1.0. PERMANOVA, nMDS, and BRTs and regression tree analysis were all carried out in R (R Core Team, 2015). The vegan package was used for PERMANOVA, dbRDA, variance partitioning and nMDS (Oksanen *et al*., 2016); the gbm package (Ridgeway, 2015: www.cran.r-roject.org/web/packages/gbm/gbm.pdf) with additional code from Elith *et al*. (2008) for the BRTs; and rpart (Therneau *et al*., 2015) for the regression trees. Where appropriate, outcomes were considered significant at $p < 0.05$.

Results

In total 378 individual macrophyte taxa were recorded: 154 from America and 242 from Africa (for full macrophyte records see Appendix S1; and for associated geopositional and environmental data recorded for sampling sites see Appendix S2; both attached to the on-

328 line version of this article). Taxonomic resolution varied between individual countries (the
329 strongest being Florida, Argentina and Zambia, with México and Trinidad the weakest,
330 largely reflecting the availability of local literature and expertise available for aquatic
331 macrophyte identification). Overall the total broke down to 291 taxa fully identified to species
332 level, 49 identified to genus, and 38 only to family level.

333 The distinctness of the species composition of the New and Old World floras was compared
334 using both our field data and pre-existing species records from all types of freshwater
335 habitats (see sources for these additional records in Methods). While pre-existing species
336 records from all freshwater habitats indicate that 144 (49.5%) of the 291species that we
337 found, co-occur in both the New and Old Worlds, our results suggest that there is a much
338 greater degree of macrophyte species separation between the continents for calcareous
339 river habitats surveyed in this study. At our sites, just 25 species (8.6%) occur at both
340 American and African sites.

341 From our field data 156 species (53.6%) were found in African samples only, while for all
342 freshwater habitats 80 species (27.5% of the total found in our survey) are recorded from the
343 Afrotropics but not the Neotropical/ Nearctic regions. From our field data 110 species
344 (37.8%) were found only in American samples, while for all freshwater habitats 67 (23.0%)
345 species are recorded from the Neotropical/ Nearctic but not the Afrotropical region. The
346 commonest species, in terms of number of samples in which they were recorded, were
347 mostly those typical of African sites (Fig. 1). Only seven of these 25 common species co-
348 occurred at sites in both continents.

349 Rarefaction plots of cumulative species records collected from the two continents (Fig. 2)
350 approached an asymptote in both cases, and no further increments of the number of species
351 were found, even when doubling the sampling effort by extrapolation. The estimated values
352 and confidence intervals (CI) for total species richness (γ-diversity) produced using the
353 Chao2 estimator were 208.6 (CI 95%: 208.06 – 213.52) and 86.0 (CI 95%: 84.28 – 98.33),

354 respectively for Africa and America. Taken together, these results provide evidence that the
355 sampling effort in both continents was adequate to estimate values for the species pool, and
356 in both cases were close to the real measured values for γ-diversity.

357 Mean α-diversity, directly measured as number of taxa recorded at each sample-site
358 (including taxa not identified fully to species level for each sample), did not significantly differ
359 between America and Africa, with an average of about eight taxa per sample. Endemic
360 species showed fairly similar proportional occurrences in both continents, but there was a
361 higher proportion of introduced/ invasive species at sites in America, compared with Africa
362 (Table 1).

363 Because there was no significant difference in α-diversity between the two regions
364 compared (and also because the data collected were qualitative records), a simple measure
365 of β-diversity was appropriate for use with this dataset (Jost, 2007). The value of the
366 Sorensen coefficient calculated for comparison of macrophyte species turnover between the
367 two sample sets was low at C_s = 0.17, emphasising the dissimilarity between the floras
368 present in warm-water calcareous river systems in Africa and America.

369 TWINSPAN classification of the dataset gave seven end-groups of samples, labelled Groups
370 A – G. These were produced with division eigenvalues in the range 0.347 - 0.780,
371 suggesting reasonable to strong separation of groups based on the macrophyte species
372 composition of their component samples. There were substantial differences in the primary
373 floristic characteristics of the seven sample-groups (Table 2), and also for mean values of
374 the six spatial and environmental variables measured, as well as for α-diversity (Fig. 3).
375 There was strong segregation between groups of samples located in Africa and in America,
376 with only one sample-group (Group D) containing samples from sites located in both
377 continents.

378 Analysis of the species data using PERMANOVA confirmed that the TWINSPAN groups had
379 significantly different community compositions. The results for all-sites combined were F:

16.54, R^2: 0.26, p <0.001; for African sites alone the corresponding outcome was F: 13.22, R^2: 0.16, p <0.001; and for American sites alone: F: 10.08, R^2: 0.27, p <0.001. A clear separation of sample-groups in nMDS ordination space was also apparent (see ordination plots provided as Appendix S3 in Supporting File 3, attached to the on-line version of this article) for all-sites, African sites, and American sites, but particularly so for America, which further emphasises the differences in species-set supported by each group of samples.

The results suggest that both spatial and environmental factors may act as drivers of macrophyte community composition present at the seven sets of survey sites making up the TWINSPAN sample-groups, given the significant and often substantial differences observed between TWINSPAN sample-groups for all six variables measured (Fig. 3a - f). The least variation was, however, seen for flow class, suggesting that this may be weaker than the other variables in driving differences between TWINSPAN sample-groups. Significant variation in α-diversity also occurred between the seven sample-groups (Fig. 3g).

The outcome of the partial db-RDA analysis is in good agreement with these results. For the full data set of environmental variables (pH, conductivity, alkalinity and altitude) and latitude 12.4% of the variation in the community composition data was explained. The variance uniquely attributable to the environmental variables was 6.4%, whereas the variance unique to latitude was only 2.9%, the remainder being shared. The corresponding values for % variance explained by the models for Africa alone were 10.4, 6.6 and 2.7% for full-model, unique to environment, and unique to latitude, respectively. For America the corresponding values were 22.1, 11.6 and 9.8% (alkalinity was not significant within the environmental data for America, but all other outcomes in these analyses were significant at p <0.05). This suggests that both latitude and environmental variables influence the community composition, with environment exerting a greater influence in this case.

The highest mean value for α-diversity was seen in Group B, a small all-African sample-group, dominated by a set of samples from the Okavango Delta in Botswana. Indicators for

406 the group were a diverse set of Afrotropical native and endemic species (Table 2). Fig. 3
407 shows that this set of samples was (within the range of values covered by this study)
408 characterised by low pH and conductivity, intermediate latitude, high altitude, moderate flow,
409 and fairly low alkalinity (similar to that of three other groups, with a mean of c. 1000 µEq L^{-1},
410 indicative of intermediate-hardwater conditions, as defined by Tapia Grimaldo, 2013).

411 In contrast the sample-group with lowest α-diversity, Group A, only contained Neotropical
412 samples, all from Brasil. Indicators for this group (Table 2) consisted of one species native in
413 America, one endemic to the Neotropics, and one invasive in the Neotropics. This group was
414 characterised (Fig. 3) by low conductivity but quite high pH, and had the second highest
415 mean latitude of the seven sample-groups. Sites in this group tended to be located at fairly
416 low altitude. Flow was usually moderate to fast, and the group average for alkalinity was
417 higher than for Group B, at 1500 – 2000 µEq L^{-1}, though this still suggests that most sites
418 were of intermediate-hardwater status (Tapia Grimaldo, 2013).

419 The remaining groups, of intermediate α-diversity, showed quite substantial variability in
420 mean environmental characteristics. For example, Groups F and G were made up of mainly
421 low-lying sites with high mean conductivity (in some cases impacted by marine saline
422 influences, producing very high conductivity values), and rather high pH, located around the
423 Caribbean, together with a few sites further south in South America (Fig. 3). These groups
424 had a quite different macrophyte community from the other five sample-groups, with a mix, in
425 both cases, of samples from three or four Neotropical/ Nearctic countries. The indicator
426 species, however (Table 2), suggest a clear difference in vegetation between the two
427 sample-groups, with Group G (dominated by Florida sites, with sub-tropical to warm-
428 temperate conditions) being indicated by a pair of species native to both the Nearctic and
429 Neotropics. In contrast, lower-latitude tropical American sites made up Group F, mostly from
430 México and Trinidad, and was indicated by four species different from those of Group G: two
431 endemic to the Neotropics/ Nearctic, plus two grass species, one native and the other
432 invasive in both American bioregions.

Single regression tree dendrograms (Fig. 4) for all-sites combined, show average species richness and the number of sites (n) in the “leaves” (end member-groups), with and without latitude included (as a spatial variable, latitude summarises the influence of many other factors, which may have a direct effect on plants, acting across the latitudinal range). When latitude is included it dominates the tree, explaining a high proportion of the variance, and tending to mask the influence of the environmental variables in driving species richness. When the spatial variable is excluded, the principal environmental variables seen to drive α-diversity in this classification are pH, altitude, electrical conductivity and alkalinity. In keeping with the outcome hinted at by the inferential statistical analysis exercise, above, flow was not shown by the regression analysis to be of importance as an environmental driver of macrophyte α-diversity in this dataset.

Partial dependence plots show, in detail, the effect of predictors on the response variable, after taking into account the average effects of all other predictors in the model. So these plots should describe variation unique to the variable in question, though where strong interactions or correlations exist this is less reliable (Elith *et al*. 2008). The outcomes of the BRT analyses (Figs. 5 – 7) provide information to permit determination of the best predictors of macrophyte α-diversity (species richness: S) respectively for the all-sites, American, and African datasets (% deviance explained: 19% for the whole dataset; 26% for the African sites, and 24% for the American sites). The plots show that there are different numbers of influential predictors of species richness for the three datasets, and that their relative importance and the “shape” of their influence (across the gradient-range covered by each individual predictor variable) both vary. The plots also show the proportion of the explained variance that each variable accounts for in the data, and the shape of the relationship - smoothed with the dashed line in the diagrams. The models performed well in terms of observed vs. predicted outcomes (0.70 - 0.80 correlation), with cross validated correlation scores (which compare model predictions with observations left out when building the model) of 0.48 - 0.50 for the three models.

460 The same variables (latitude, EC and altitude) are the most influential for both the African
461 and American data sets, with pH and alkalinity also both influential in the African dataset.
462 What is clear from the plots presented here (Figs. 5 – 7) is that latitude is a powerful
463 predictor of species richness, but there were observable differences in response between
464 the continents. For Africa, species richness increases gradually with distance from the
465 Equator, starting from a relatively low latitude, whereas in America there was no discernible
466 latitude effect until 20º, whereupon there was a rapid increase in richness. There is also a
467 large difference in the response curve for altitude. The largest change in diversity for
468 America occurs below 300 m a.s.l., whilst the lowest-altitude site in the African dataset is
469 around 400m a.s.l. Electrical conductivity is an additional important factor shaping
470 macrophyte species richness in this dataset. The partial dependence plots show that in
471 America it is at the low end of the EC gradient that the influence on richness is greatest, with
472 rising EC corresponding to higher macrophyte species richness. For the African data the
473 pattern is less clear, but an increase is evident in the non-smoothed data.

474

475 **Discussion**

476 Comparisons of diversity and community composition of macrophytes in warm-water
477 calcareous river systems within the two continents provided evidence that Africa and
478 America differ in several ways. For macrophyte diversity, scale of analysis is important.
479 Large scale diversity (γ-diversity) is, on our current evidence (though we think that may
480 change when additional sites, outwith the envelope of site-conditions examined here, are
481 sampled in the future) much higher in these systems in Africa than in America, and this
482 difference cannot be accounted for by sampling effects. However, at local scale (α- diversity)
483 there is similarity between the two regions (Table 1), and this was an unexpected result,
484 given the substantial differences in physical and chemical characteristics of hardwater river
485 habitats sampled in the two continents (e.g., Payne, 1986; also our data presented here).

For example, we observed much greater variation in range of electrical conductivity within the American sites, compared with Africa; while altitude showed generally higher values within African sites, compared with America. While surprising, this result for α-diversity is robust, given the consistency of both sampling strategy and sampling team, across the survey sites in both continents.

There is quite strong evidence for significant variation in α-diversity between the main macrophyte community-types indicated by the TWINSPAN sample-classification (Fig. 3), while the results of PERMANOVA, nMDS sample ordination, and partial db-RDA analysis confirmed the observed species compositional variation across the TWINSPAN groups. The variation in macrophyte community between the two continents was substantial. Only one sample-group (TWINSPAN Group D), contained samples from both Africa and America. Evidence from the species distribution literature, and online distributional databases, for the species found in our survey (see Methods for sources utilised), indicates substantial overlap for their distributions (between all freshwater habitats combined) in the Afrotropics and Neotropics/ Nearctic. However, we found that this was not the case for these plants in warm-water calcareous river habitats in the two continents, with most of the species recorded being found at sites in only one or the other continent, and with only a small proportion of species in common between them. There is, of course, no *a priori* reason why we should expect the same γ-diversity pattern to occur in all individual types of freshwater habitat, and on our evidence warm-water calcareous rivers show substantial differences in species pool (both in diversity and species presence), between America and Africa. This small proportion of species co-occurring in both continents suggests that, at least for the type of ecosystem studied here, it is perhaps not the case that “aquatic vascular plants generally show broad distributional ranges”, as was suggested by Santamaría (2002).

Our thoughts on this are further supported by the level of endemism (at regional level) observed in the dataset, which offers a partial explanation for our results. Approximately one third of the species that we recorded from each continent were endemic either to the

513 Afrotropics or the Neotropics/ Nearctic (Table 1), and so by definition do not occur in both
514 continents.

515 Differences in species niche-breadth may provide a second clue. Niche-breadth values have
516 been calculated (from data collected in Zambia) for 44 of the species found in our survey
517 (Kennedy *et al.,* 2017). Excluding the endemics, it is notable that none of the eight species
518 present in our dataset which were considered by these authors to have narrow niche-
519 breadth (e.g. *Thelypteris confluens* (Thunb.) C.V. Morton, *Tristicha trifaria* (Bory ex Willd.)
520 Spreng.) co-occurred at our survey sites in both Africa and America. In contrast, nine of 31
521 species that were allocated by Kennedy *et al*. (2017) to intermediate/ broad niche-breadth
522 status (e.g. *Ceratophyllum demersum* L., *Cyperus difformis* L.) were found at our survey
523 sites in both continents. It is possible that generalist species, with greater niche-breadth
524 (implying a wide tolerance of habitat conditions, and relatively good dispersal abilities, for
525 example via long-distance endo- and exozoochory, utilising migratory waterfowl: e.g. Agami
526 & Waisel, 1986; Clausen *et al*., 2002; Santamaría, 2002; Coughlan *et al.,* 2017) are likely to
527 have a reasonably high chance of finding suitable conditions for colonization in warm-water
528 calcareous river habitats in both continents. In contrast, narrow-niche species by definition
529 tend to have more specialist survival strategies (Grime, 1979) and narrower ecological
530 tolerances, likely including traits influencing reproductive and dispersal capability, and
531 potentially limiting range size. Recent evidence for this in freshwater organisms, including
532 macrophytes, is provided by Slatyer *et al*. (2013) and Kennedy *et al*. (2017). Such species
533 may, for example, utilise specialised reproductive and dispersal strategies (e.g. underwater
534 pollination; vegetative propagule dispersal mechanisms: Sculthorpe, 1967; Smits *et al.,*
535 1989; Barrat-Segretain, 1996; Donald, 1996; Wingfield & Murphy, 2006; Akasaka &
536 Takamura, 2011; Redekop *et al*., 2016), of possible low efficiency in promoting long-distance
537 dispersal on a broad-scale planetary basis. In turn, this makes it less likely that these
538 species will be present at geographically widely-separated locations. It logically follows that
539 these specialist-strategy species may have more difficulty than generalists in finding

appropriately-similar locations for colonisation in both African and American warm-water calcareous rivers.

Although we do not consider here vicariance factors associated with ancestral phytogeographical influences on current macrophyte distributions (such as those associated with impacts of glaciation events etc.), which are certainly important, but well covered elsewhere in the literature (e.g., Santamaría, 2002; Les *et al.* 2003; Nies & Reusch, 2005; Chen *et al*., 2012a, b; Zhu *et al*., 2015), we do think that more recent actions related to human activities may be relevant. For example, the proportion of invasive/ introduced species in American rivers was substantially higher (at around 9% of total γ-diversity) than in Africa (Table 1), and this is a further likely contribution to explaining the observed community composition differences between the continents. A good example is invasive *Hydrilla verticillata* (L.f.) Royle (thought to be native to the Palearctic/ Oriental bioregions (Zhu *et al*., 2015), though there are also some possibly-native records from Africa: http://www.cabi.org/isc/datasheet/28170). This plant was found at 14 sites in our survey, all in Florida (though it has also recently been recorded as invasive in hardwater river sites in one of the areas of Brasil (the Upper Rio Paraná) that we sampled: e.g., Sousa *et al*., 2010). This species was not present at any of the hardwater river sites sampled in Africa during the study period, though there is a single previous record from a Zambian calcareous river, the Kafue River in 1981: http://www.gbif.org/occurrence/1140612468.

For macrophytes, introductions are frequently related to aquarist activity, remediation, intentional release, and escape from managed environments, such as Botanic Gardens (Brundu, 2015). A recent worldwide survey (Crafton, 2015) also suggested that international trade is a further determinant of invasive success. We suspect that these human-related activities are less intense in Africa than in America, possibly partially explaining differences in invasive species presence between calcareous rivers in the two continents.

565 At many sites, in all the countries of America and Africa examined here, macrophyte α-
566 diversity was low. Our results support previous studies which suggest that, in freshwater
567 systems, local driving factors (chemical, physical or biological) seem to be of overriding
568 importance in determining whether or not macrophyte diversity at an individual site is
569 depressed below the optimal level within a given geographical region (e.g. Baattrup-
570 Pedersen *et al*. 2006; Rolon & Matchik, 2006; Chappuis *et al*., 2012; Lang & Murphy, 2012;
571 Bando *et al*., 2015; Kennedy *et al*., 2015; Morandeira & Kandus, 2015; Schneider *et al.,*
572 2015; Tapia Grimaldo *et al.,* 2016). Physical size of individual rivers, however, seemed to be
573 of little importance in influencing α-diversity. Despite their apparently-large potential area for
574 colonisation, often large rivers are too deep, or their discharge is too great, or they are too
575 turbid, to allow macrophytes to colonise further out into the channel than the marginal zone
576 (e.g. Murphy *et al*., 2003; Sousa *et al*., 2011; Varandas Martins *et al*., 2013).

577 The outcomes of our study emphasised the role of the spatial variable latitude in driving
578 macrophyte diversity and community composition, despite the fairly limited latitudinal range
579 (a band 18 - 20° of latitude wide, commencing about 8 - 10°N or S of the Equator, and
580 running up to about 30°N or S) covered by our study. Latitude integrates a number of enviro-
581 climatic variables, such as maximum and minimum annual temperature, precipitation, and
582 evapotranspiration, which have individually previously been found to be good predictors of
583 large-scale freshwater macrophyte diversity (e.g, Chappuis *et al.*, 2012; Tapia Grimaldo *et*
584 *al.*, 2016).

585 A question remaining to be addressed is whether the variation in environmental
586 heterogeneity seen between sampling sites located in the two continents might be
587 influencing the observed findings of this study. For example, most of the African sites were
588 located at high altitude whilst most sites in American river systems were located at low
589 altitude (although for both altitude and all the other spatio-environmental variables studied
590 there was an overlap in the range of values observed, when comparing sites from the two
591 continents). This apparent sampling bias (at least in the case of altitude) is of course a

592 product of the differences in geography between the areas sampled. Florida and Yucatan,
593 for example, have no high ground at all, whilst Zambia, Botswana and the South African
594 Highveld are all upland regions. The question is whether results obtained from within the
595 envelope of spatial (latitude) and physico-chemical (altitude, pH, conductivity, alkalinity, flow)
596 conditions encompassing our sites apply only within that envelope, or are more widely
597 applicable. Further work is clearly required to address that question, for example by
598 attempting to find and sample low-lying calcareous rivers in Africa, and high-altitude
599 calcareous rivers in America. At present, we conclude that our findings should be considered
600 as being primarily applicable within the environmental envelopes encompassing the river
601 systems studied, pending further research.

602 Our results support the findings of some, but not all, of the relevant previous studies in the
603 literature which have examined large-scale drivers of freshwater macrophyte diversity. For
604 example, Chappuis *et al.* (2012) found that latitude was a major driver of macrophyte
605 diversity (in their case, country γ-diversity) across cool to warm-temperate Palearctic regions
606 of Europe and North Africa. On a broader world scale, evidence is similarly provided by
607 Crow (1993), from Central and North America, and Tapia Grimaldo *et al.* (2016), working
608 with data from Africa and the British Isles, to suggest that both latitude and environmental
609 factors play a role in predicting macrophyte diversity in freshwater systems. However, Viana
610 *et al.* (2014) concluded that environmental and biogeographical factors, rather than latitude
611 *per se*, drive aquatic plant species richness across Europe. Similarly, Alahuhta *et al*. (2017)
612 suggested that, at a global scale, environmental heterogeneity (notably variability in altitude
613 range within a region) plays the main role in driving macrophyte β-diversity, between lakes
614 located in 21 different regions of the world. In our study, altitude was, in every case, third in
615 importance (always behind latitude), in predictive value in this context (Figs. 5 – 7). Other
616 physico-chemistry variables (pH, EC, alkalinity) showed less consistency across the
617 analyses as being useful predictors of macrophyte α-diversity in warm-water calcareous river
618 systems.

The findings of all these studies, including our own, clearly emphasise the need for further work in this field (not just in warm-water calcareous rivers, but in freshwater habitats as a whole, planet-wide) to resolve the relative importance of spatial and environmental drivers in influencing macrophyte diversity. The importance of gaining improved baseline understanding of how such factors may affect freshwater macrophyte distributions and diversity can hardly be over-emphasised in the current context of global climate change.

A criticism of our study is that the snapshot environmental data mainly utilised here are unlikely to represent the longer-term mean values of individual variables at each site. Clearly it would be useful in any follow-up studies to include repeat-sampling to address this issue further. It is also likely that further work may show that other environmental factors, such as nutrient status (e.g., Kennedy *et al*., 2016), as well as biotic interactions, including competition from non-native species (e.g. Michelan *et al*., 2010; Sousa *et al*., 2010), might be of importance in driving both diversity and community composition of warm-water calcareous river plants.

The evidence from the regression tree and boosted regression tree analysis here leads us to conclude that latitude is a significant, though non-linear and rather complex, spatial driver of hardwater river macrophyte α-diversity, within the latitudinal range encompassed by this study. Altitude, pH, conductivity and alkalinity were also of importance in driving diversity, though varying in individual importance between Africa and America. The importance of latitude, even within a narrow range encompassing only low-latitude ecosystems, raises the possibility (see also Tapia Grimaldo *et al*., 2016) that this factor may prove to be a driver of calcareous river macrophyte diversity across larger latitudinal gradients.

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651

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908

909 **Supporting Information**

910 The original datasets collected for this study are available as additional Supporting Files
911 attached to the on-line version of this article.

912

	Afrotropics	America
Total spp. (γ-diversity)	181	135
Native/ naturalised spp.	171	123
% native/ naturalised (of total spp.)	94.5	91.1
Endemic spp. (to Afrotropics or America, respectively)	57	47
% endemic spp. (of total spp.)	31.5	34.8
Introduced/invasive spp. (to Afrotropics or America, respectively)	10	12
% introduced/ invasive spp. (of total spp.)	5.5	8.9
Mean α-diversity (S: mean number of taxa recorded per site) ± standard error	8.8 ± 0.35^{NS}	7.9 ± 0.50^{NS}
Maximum S (recorded number of macrophyte taxa per site)	23	27

Table 1. Total macrophyte γ-, and mean and maximum α-diversity recorded for sites surveyed in African (Afrotropical), and American (Neotropical/ Nearctic) countries, showing data for native/naturalised species (with percentages of endemic species for each region) and introduced/ invasive species. Comparison of mean S by t-test: not significant (NS: $p > 0.05$)

TWINSPAN sample-group							
	A	B	C	D	E	F	G
Samples per group (n)	18	18	32	88	77	26	33
Eigenvalue for group production	0.780	0.519	0.519	0.347	0.347	0.681	0.681
Number of samples per country represented in group	Brasil (18)	Zambia (2), Botswana (15), South Africa (1)	Botswana (5), South Africa (2), Zambia (25)	Zambia (78), Botswana (1), South Africa (1), Trinidad (7)	Zambia (70), South Africa (7)	Trinidad (8), México (17), Brasil (1)	Florida (27), Trinidad (2), México (1), Argentina (3)
Indicator species	IA, IN *Brachiaria arrecta* (= *Urochloa arrecta*) EN *Hymenachne pernambucensis* NaA, NaN *Oxycaryum cubense*	NaA, IN *Cyperus articulatus* EA *Cyperus pectinatus* NaA *Eleocharis dulcis* EA *Miscanthus junceus* NaA, IN *Nymphoides indica* subsp. *occidentalis* NaA, NaN *Oxycaryum cubense* NaA *Schoenoplectus corymbosus* NaA, NaN *Utricularia foliosa*	EA *Panicum subalbidum* NaA *Phragmites mauritianus*	NaA, IN *Nymphaea nouchali* var. *caerulea* NaA, IN *Panicum repens*	NaA, NaN *Commelina diffusa* NaA, IN *Cyperus alopecuroides* NaA, NaN *Cyperus involucratus* EA *Panicum subalbidum* NaA, IN *Pennisetum macrourum* NaA *Persicaria attenuata* subsp. *africana* NaA *Persicaria decipiens* NaA *Schoenoplectus corymbosus*	EN *Eleocharis cellulosa* EN *Fuirena simplex* IA ,NaN *Paspalum notatum* IA, IN *Brachiaria arrecta* (= *Urochloa arrecta*)	NaA, NaN *Lemna aequinoctialis* IA, NN *Pontederia cordata*

Table 2. Characteristics of seven sample end-groups produced by TWINSPAN classification of 292 samples, using only fully-identified species. Indicator species for each group are shown together with information on distributional status of each species in Africa (Afrotropics) and America (Neotropics/

Nearctic combined): $^{IA, IN}$ introduced/ invasive to ^{IA} Afrotropics or ^{IN} Nearctic/ Neotropics; $^{NaA, NaN}$ native/naturalised to ^{NaA} Afrotropics or ^{NaN} Nearctic/ Neotropics; $^{EA, EN}$ endemic to ^{EA} Afrotropics or ^{EN} Nearctic/ Neotropics

Figure Legends

Figure 1. Percentage of (a) African and (b) American samples with records for each of 25 commonest species in the dataset (≥20 records): Bra arr: *Brachiaria arrecta* (Poaceae) = *Urochloa arrecta*; Cer dem: *Ceratophyllum demersum* (Ceratophyllaceae); Com dif: *Commelina diffusa* (Commelinaceae); Cyp alo: *Cyperus alopecuroides* (Cyperaceae); Cyp art: *Cyperus articulatus* (Cyperaceae): Cyp pap: *Cyperus papyrus* (Cyperaceae); Eic cra: *Eichhornia crassipes* (Hydrocharitaceae); Ele dul: *Eleocharis dulcis* (Cyperaceae); Hyd umb: *Hydrocotyle umbellata* (Araliaceae); Lag ili: *Lagarosiphon ilicifolius* (Hydrocharitaceae); Lem aeq: *Lemna aequinoctialis (*Araceae); Lud ads: *Ludwigia adscendens* (Onagraceae); Naj hor: *Najas horrida* (Hydrocharticaeae); Nym noc: *Nymphaea nouchali* var. *caerulea* (Nympheaceae); Pan rep: *Panicum repens* (Poaceae); Pan sub: *Panicum subalbidum* (Poaceae); Per att: *Persicaria attenuata* (Polygonaceae); Per dec: *Persicaria decipiens* (Poygonaceae); Per hyd: *Persicaria hydropiper* (Polygonaceae); Per sen: *Persicaria senegalensis* (Polygonaceae); Phr mau: *Phragmites mauritianus* (Poaceae); Pot sch: *Potamogeton schweinfurthii* (Potamogetonaceae); Sal mol: *Salvinia molesta* (Salviniaceae) = *Salvinia adnata*; Sch cor: *Schoenoplectus corymbosus* (Cyperaceae); Typ dom: *Typha domingensis* (Typhaceae); Val ame: *Vallisneria americana* (Hydrocharitaceae).

Figure 2. Rarefaction plots estimating γ – diversity, using macrophyte taxa records from rivers and associated water bodies for 84 samples collected from 5 countries in America (2010 – 2011), and 208 samples from 3 countries in Africa (2006 – 2011). Black: Africa; grey: America.

Figure 3. Variation in mean values (± standard error) between TWINSPAN sample-groups for spatial and environmental variables measured, and for α-diversity: (a) electrical conductivity (EC: µS cm^{-1}): p <0.001; (b) latitude (absolute ° north or south of Equator): p

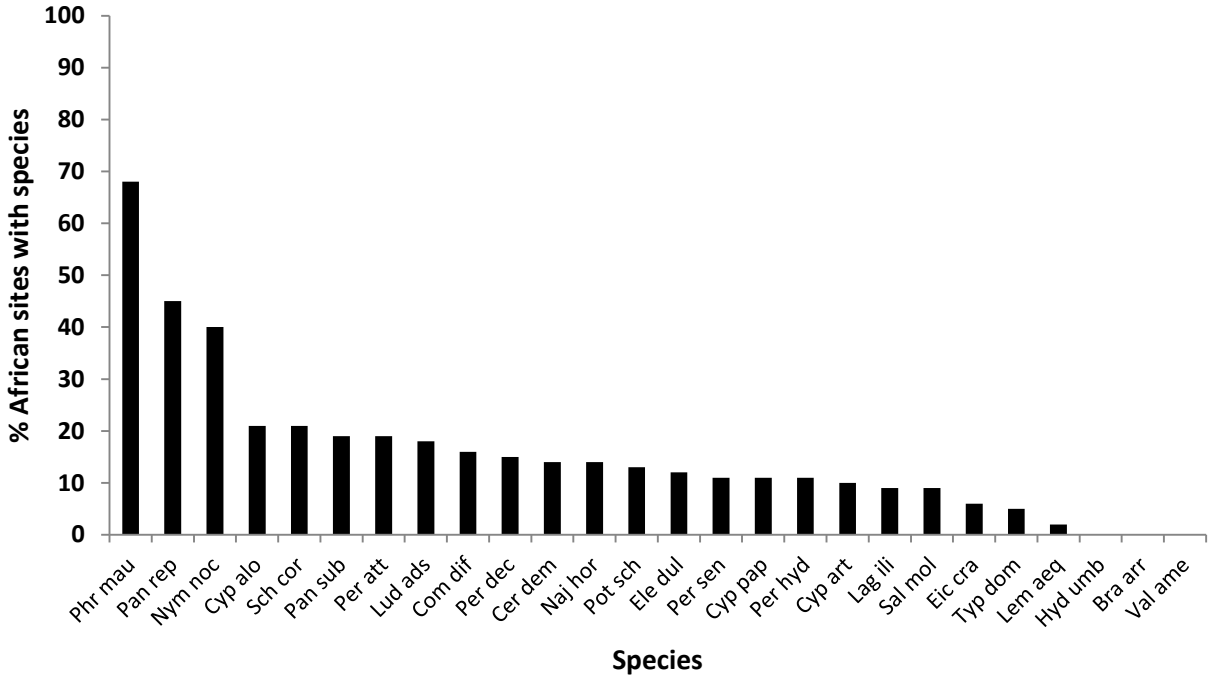
<0.001; (c) altitude (m above mean sea level: a.s.l.): p <0.001; (d) flow class (0: still - 3: fast-flowing): p <0.05; (e) pH: p <0.001, F: 8.513; (f) alkalinity (µEq L^{-1}): p <0.001; (g) S (α-diversity: number of macrophyte taxa recorded per site): p <0.001, F: 15.371. Means for pH and S labelled with a letter in common do not significantly differ (ANOVA outcome with *a-posteriori* Tukey's mean separation test, significant at a minimum of p<0.05). Other variables analysed using Kruskal-Wallis test procedure: overall significance shown for outcome.

Figure 4. Regression tree dendrograms, for all-sites combined dataset, showing average species richness (S) and number of sites (n) in dendrogram end-groups ("leaves"): (a) spatial and environmental variables all included (i.e. latitude included in the analysis); (b) environmental variables only included (i.e. latitude omitted).

Figure 5. Boosted Regression Tree partial dependence plots of fitted function vs. observed values (primary values shown as tick marks on x-axis) for each of 5 spatial/ environmental variables significantly predicting macrophyte α-diversity (species richness: S) for all-sites combined. Continuous line: fitted values; dashed line: smoothed fitted. Abbreviations: Lat: latitude (absolute °); Alt: altitude (m above sea level); Alk: alkalinity (µEq L^{-1}); EC: electrical conductivity (µS cm^{-1}). Values given in brackets are proportion of the explained variance that each variable accounts for in the data.

Figure 6. Boosted Regression Tree partial dependence plots of fitted function vs. observed values for each of 5 spatial/ environmental variables significantly predicting macrophyte α-diversity (species richness: S) for American sites. See caption to Fig. 5 for further details.

Figure 7. Boosted Regression Tree partial dependence plots of fitted function vs. observed values for each of 5 spatial/ environmental variables significantly predicting macrophyte α-diversity (species richness: S) for African sites. See caption to Fig. 5 for further details.


% African sites with species
100
90
80
70
60
50
40
30
20
10
0
Phr mau
Pan rep
Nym noc
Cyp alo
Sch cor
Pan sub
Per att
Lud ads
Com dif
Per dec
Cer dem
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Ele dul
Per sen
Cyp pap
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Lag ili
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Eic cra
Typ dom
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Hyd umb
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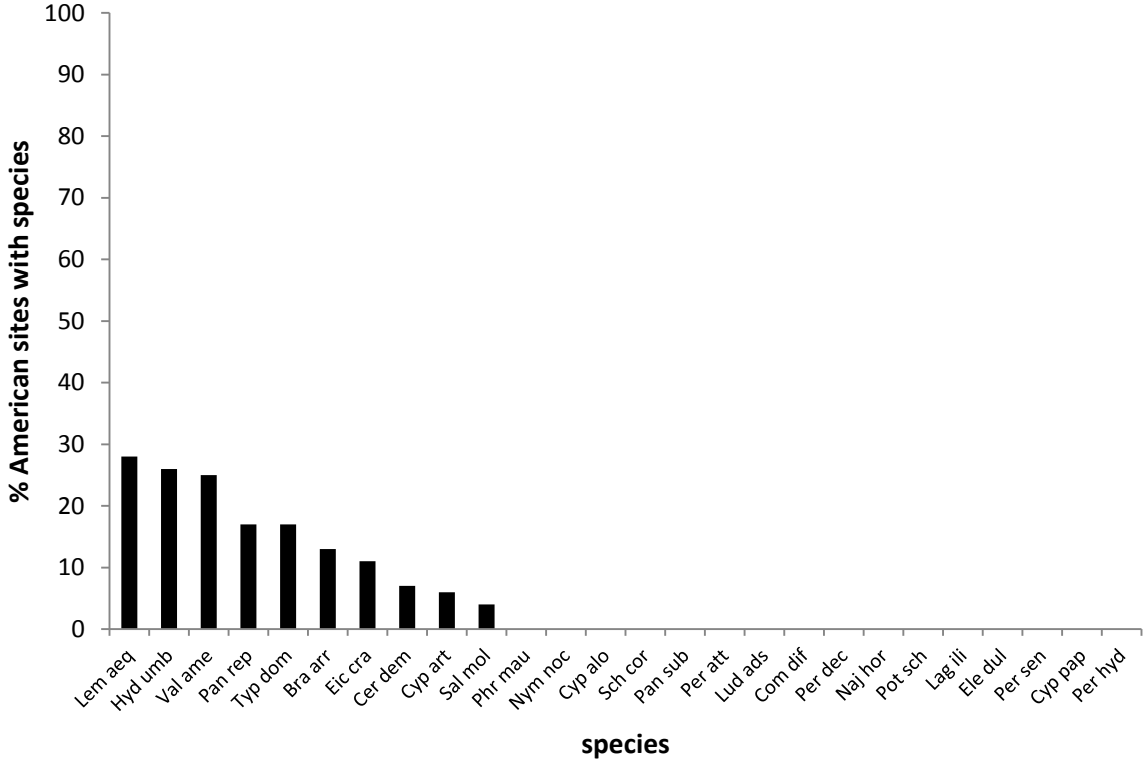
Val ame
Species

(a)


% American sites with species
100
90
80
70
60
50
40
30
20
10
0
Lem aeq
Hyd umb
Val ame
Pan rep
Typ dom
Bra arr
Eic cra
Cer dem
Cyp art
Sal mol
Phr mau
Nym noc
Cyp alo
Sch cor
Pan sub
Per att
Lud ads
Com dif
Per dec
Naj hor
Pot sch
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Per hyd
species

(b)

FIGURE 1

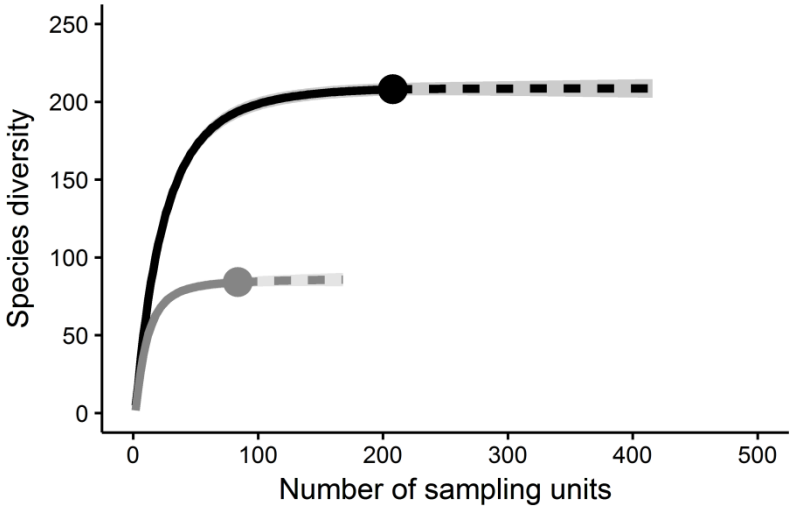
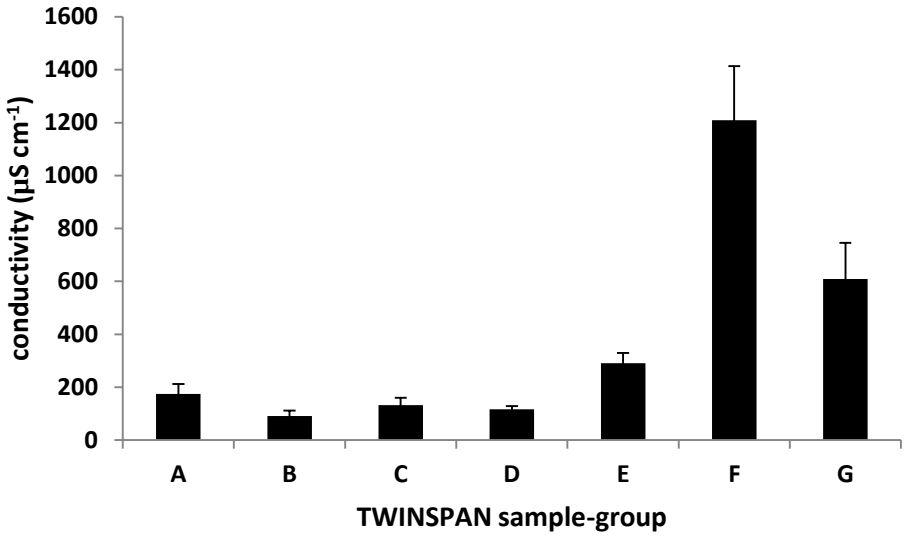
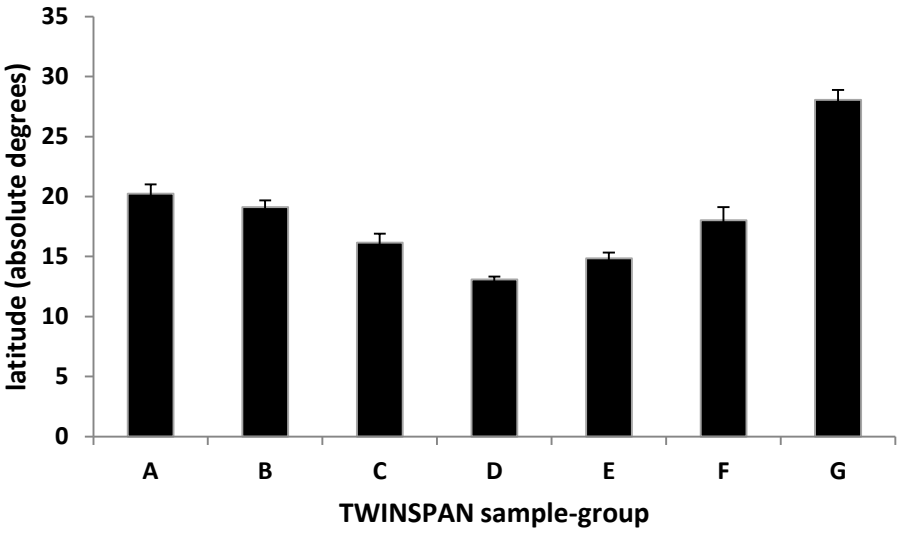

Species diversity
250
200
150
100
50
0
0
100
200
300
400
500
Number of sampling units

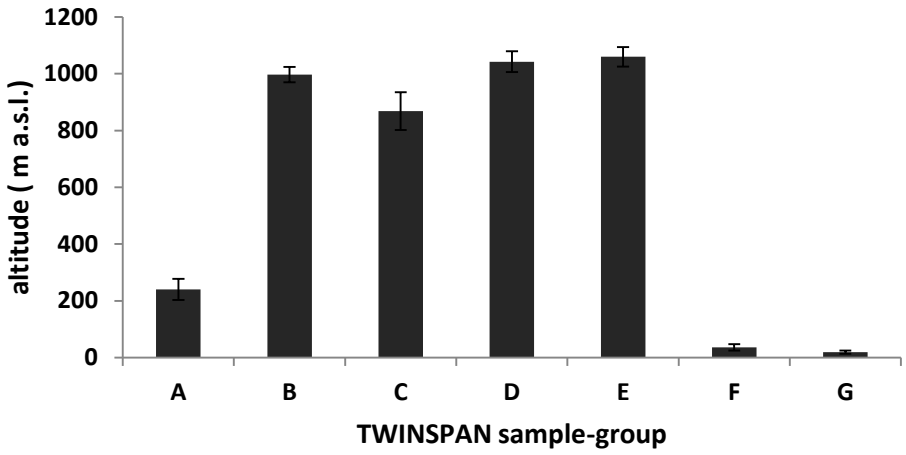
FIGURE 2


1600
1400
1200
1000
800
600
400
200
0
conductivity (µS cm-1)
A
B
C
D
E
F
G
TWINSPAN sample-group

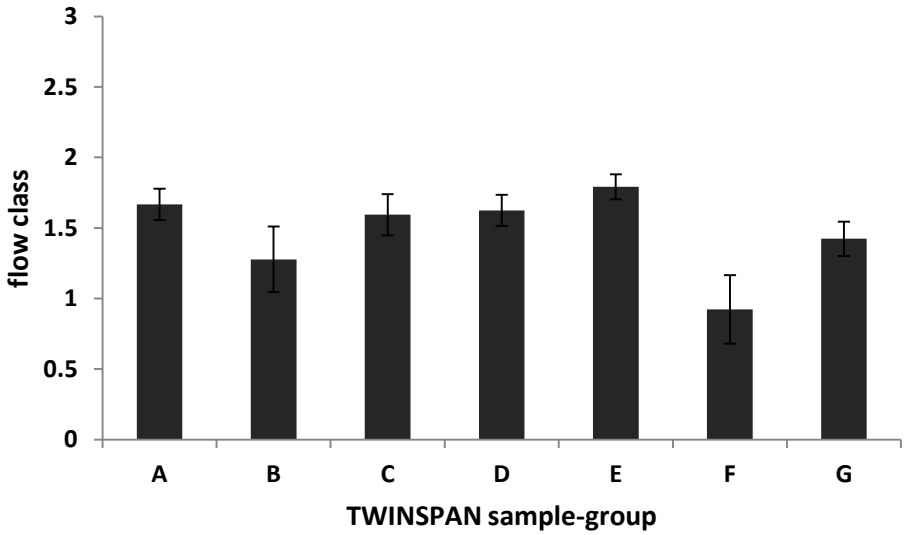
(a)


35
30
25
20
15
10
5
0
latitude (absolute degrees)
A
B
C
D
E
F
G
TWINSPAN sample-group

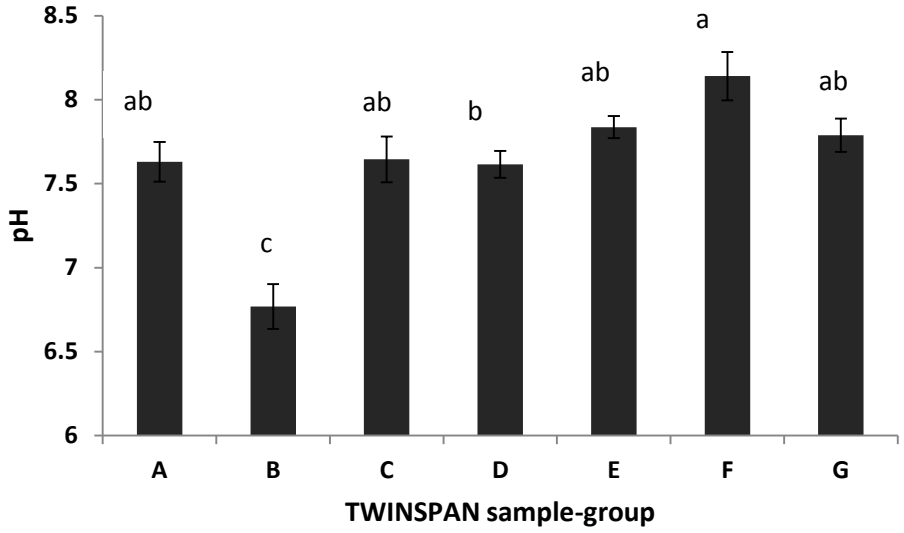
(b)


altitude (m a.s.l.)
1200
1000
800
600
400
200
0
A
B
C
D
E
F
G
TWINSPAN sample-group

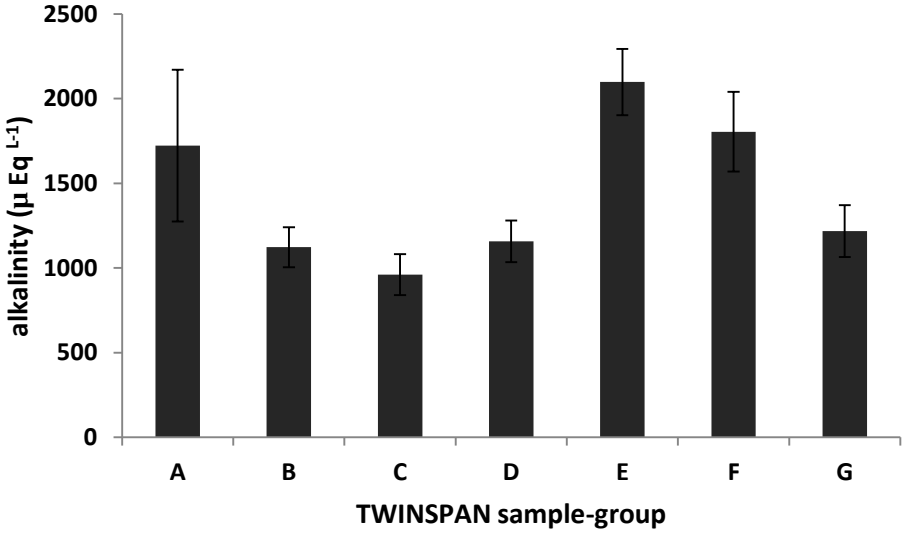
(c)


flow class
3
2.5
2
1.5
1
0.5
0
A
B
C
D
E
F
G
TWINSPAN sample-group

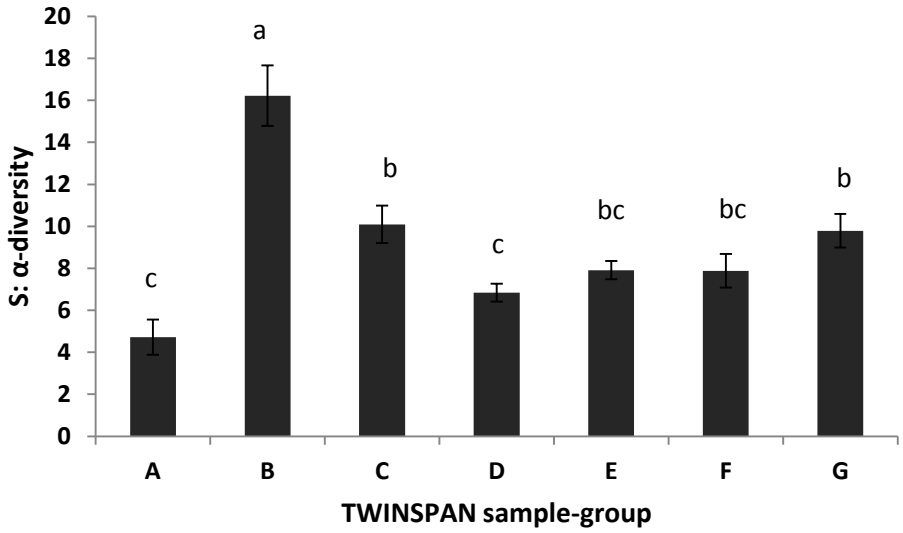
(d)


8.5
8
7.5
7
6.5
6
pH
ab
c
ab
b
ab
a
ab
A
B
C
D
E
F
G
TWINSPAN sample-group

(e)

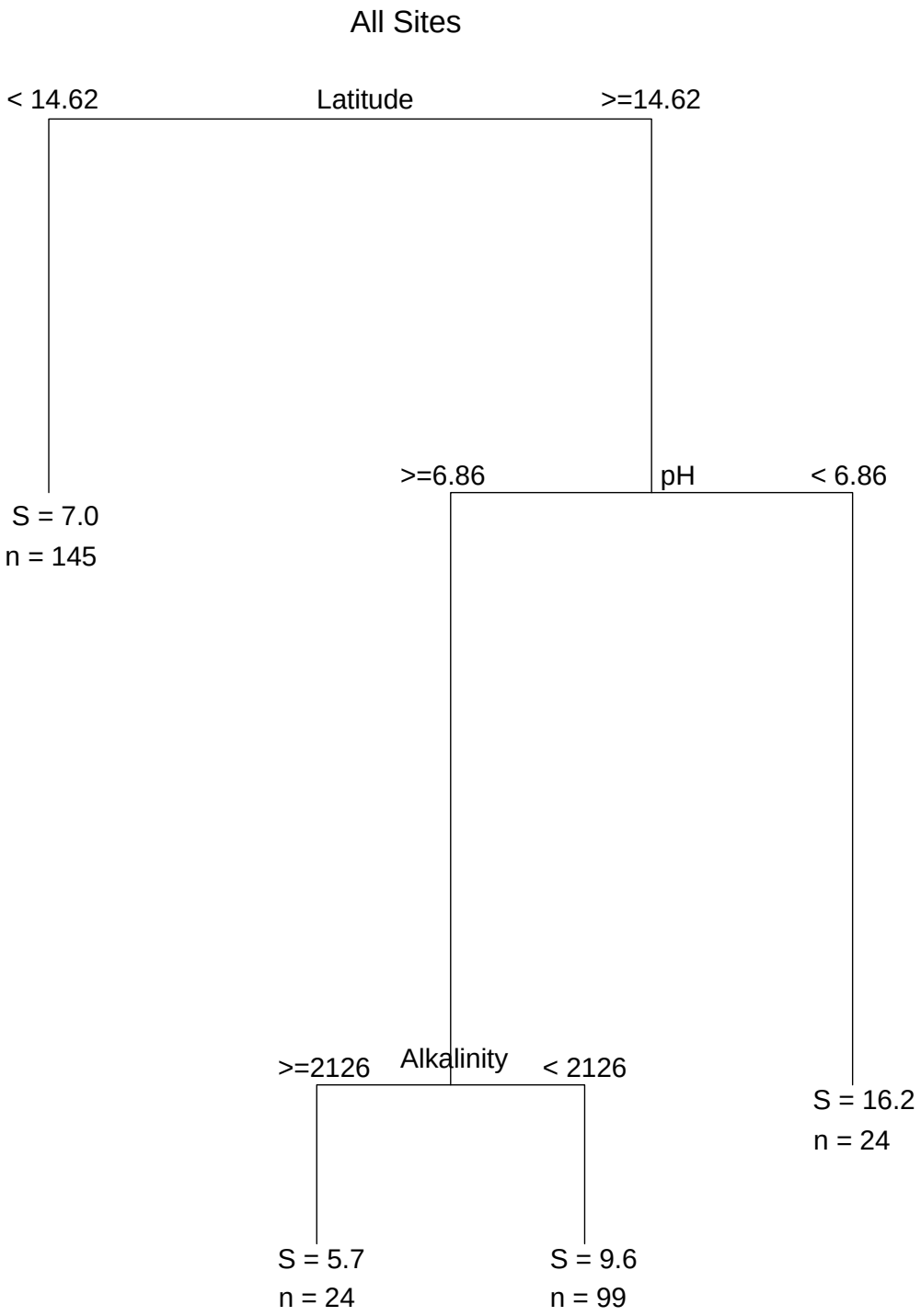

2500
2000
1500
1000
500
0
alkalinity (µ Eq L-1)
A
B
C
D
E
F
G
TWINSPAN sample-group

(f)

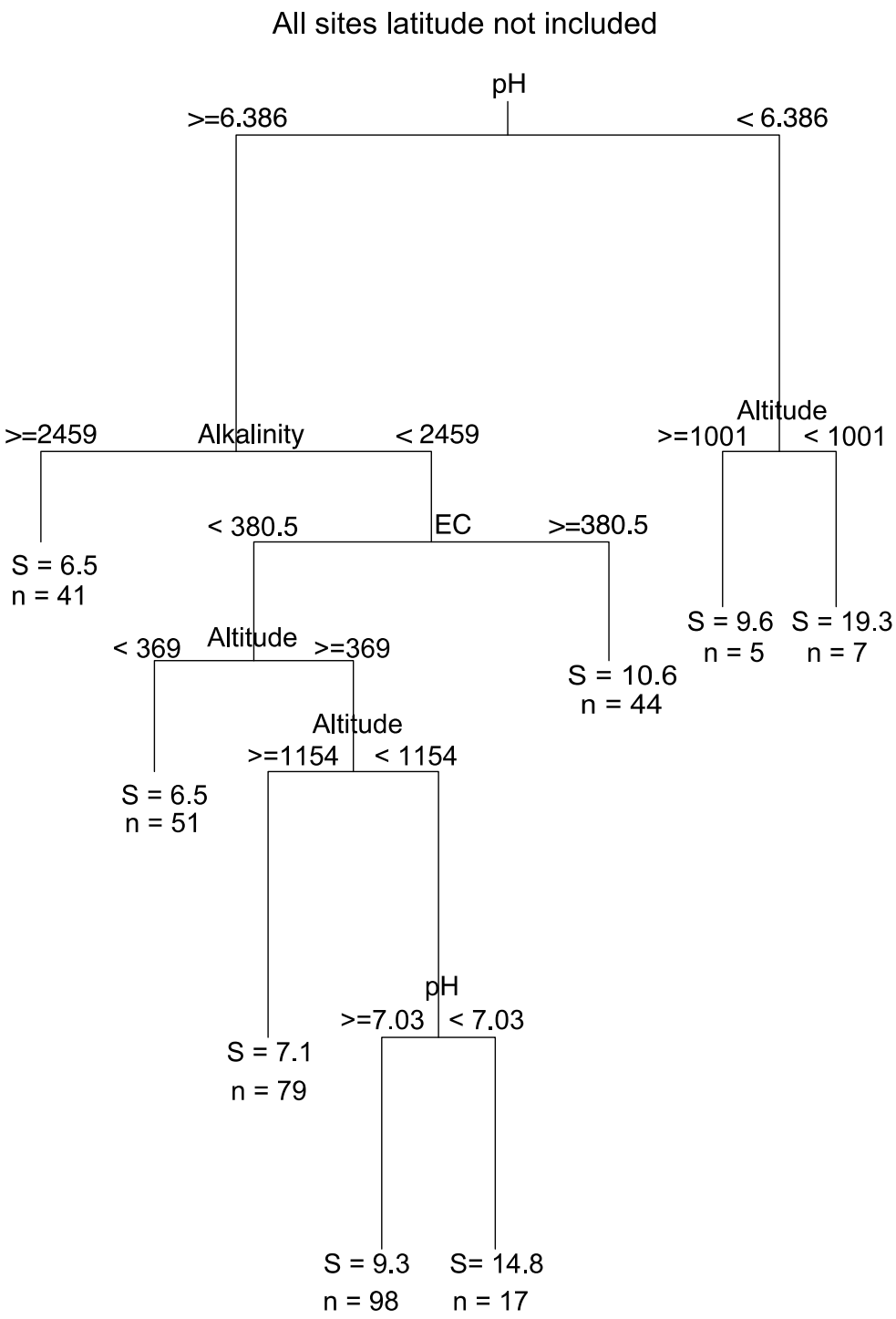

S: α-diversity
20
18
16
14
12
10
8
6
4
2
0
a
b
c
bc
bc
b
c
A
B
C
D
E
F
G
TWINSPAN sample-group

(g)

FIGURE 3


All Sites
< 14.62
Latitude
>=14.62
S = 7.0
n = 145
>=6.86
pH
< 6.86
>=2126
Alkalinity
< 2126
S = 16.2
n = 24
S = 5.7
n = 24
S = 9.6
n = 99

(a)


All sites latitude not included
pH
>=6.386
< 6.386
>=2459
Alkalinity
< 2459
Altitude
>=1001
< 1001
< 380.5
EC
>=380.5
S = 6.5
n = 41
S = 9.6
n = 5
S = 19.3
n = 7
< 369
Altitude
>=369
S = 10.6
n = 44
Altitude
>=1154
< 1154
S = 6.5
n = 51
pH
>=7.03
< 7.03
S = 7.1
n = 79
S = 9.3
n = 98
S= 14.8
n = 17

(b)

FIGURE 4

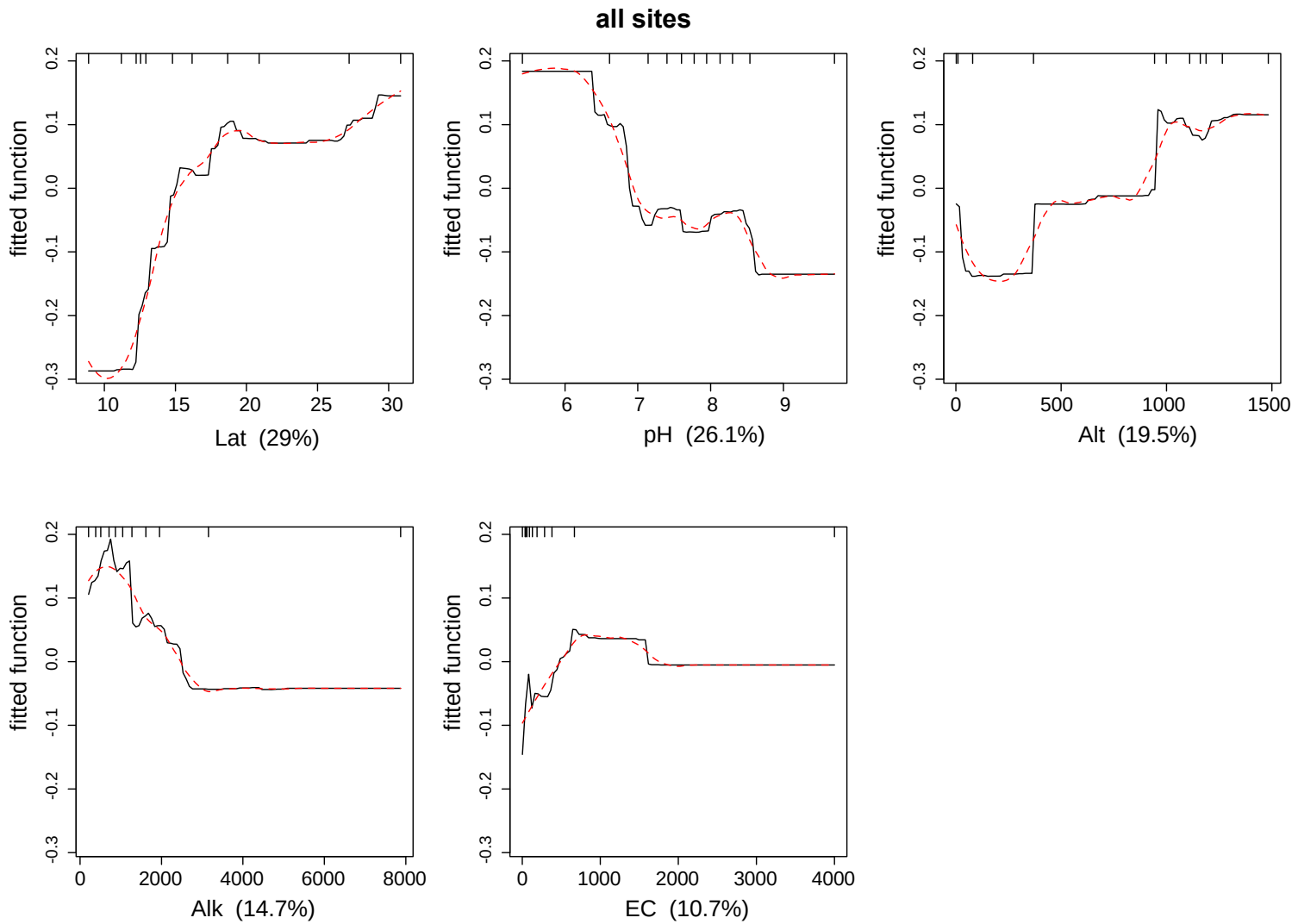


FIGURE 5

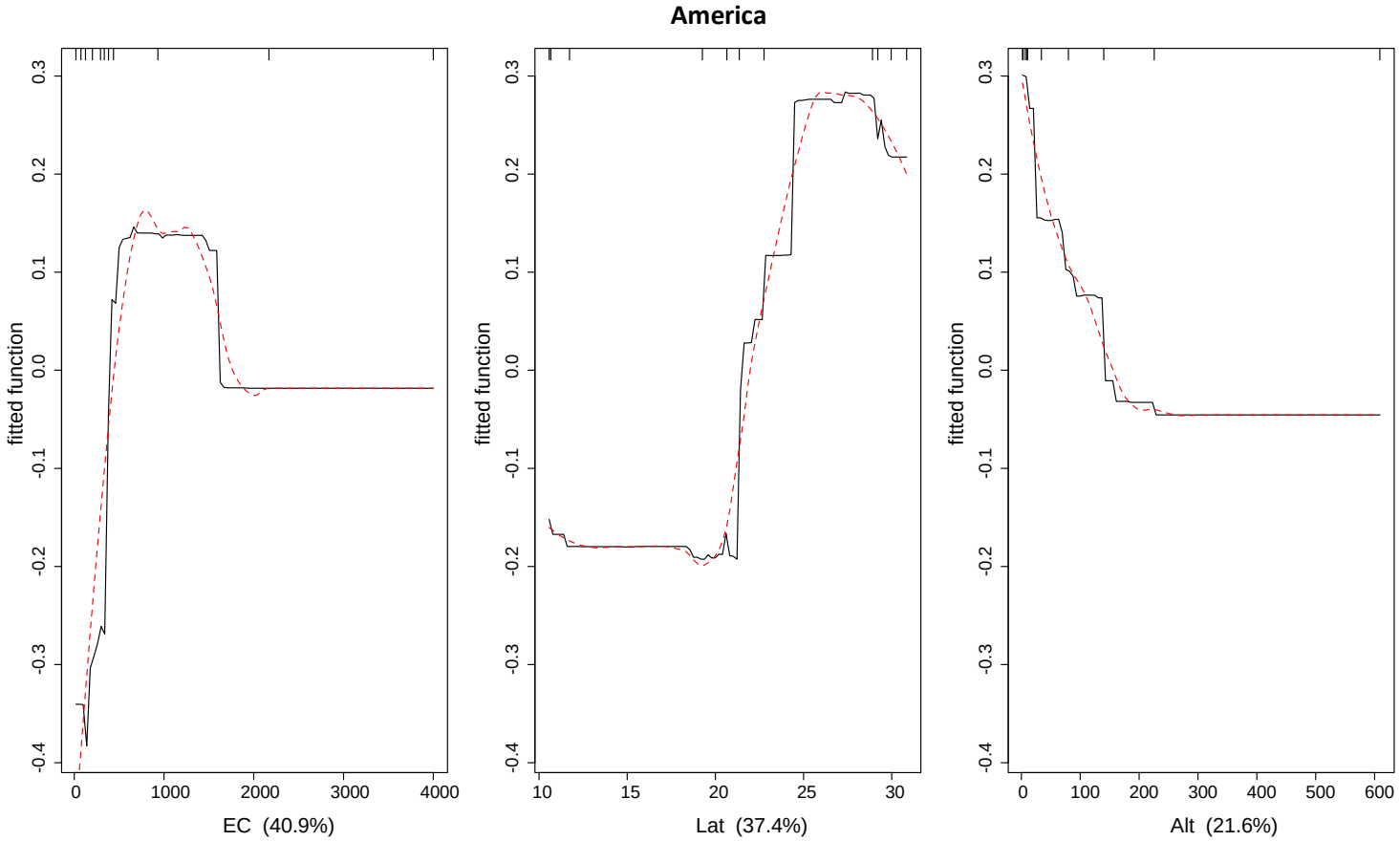


FIGURE 6

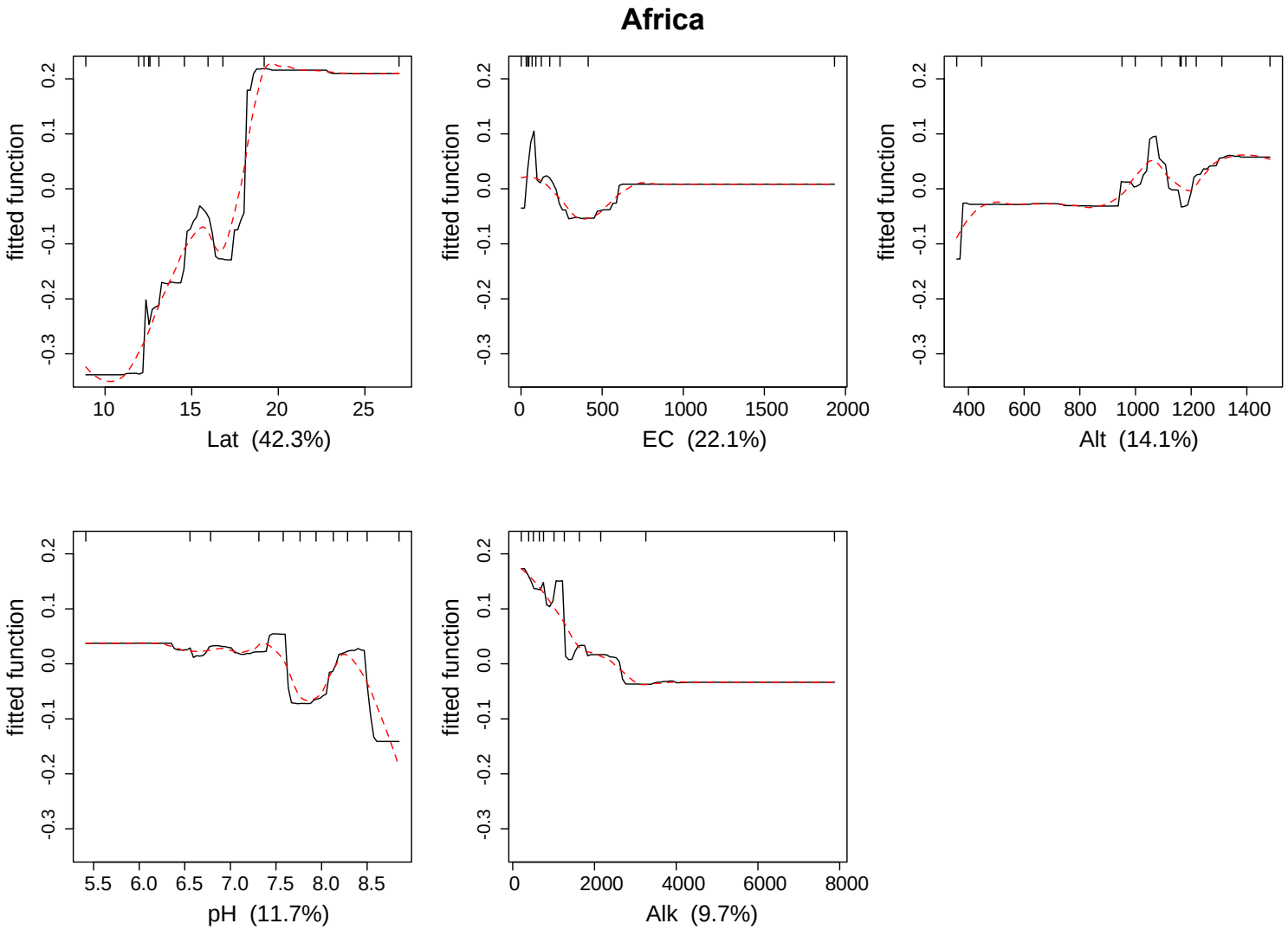


FIGURE 7