Widespread sampling biases in herbaria revealed from large-scale digitization

Barnabas H. Daru[1,*], Daniel S. Park[1,*], Richard B. Primack[2], Charles G. Willis[1], David S. Barrington[3], Timothy J. S. Whitfeld[4], Tristram G. Seidler[5], Patrick W. Sweeney[6], David R. Foster[7], Aaron M. Ellison[7] and Charles C. Davis[1]

[1]Department of Organismic and Evolutionary Biology and Harvard University Herbaria, Harvard University, Cambridge, MA 02138, USA; [2]Biology Department, Boston University, Boston, MA 02215, USA; [3]Pringle Herbarium, Plant Biology Department, University of Vermont, Torrey Hall, 27 Colchester Ave, Burlington, VT 05405, USA; [4]Brown University Herbarium, Department of Ecology and Evolutionary Biology, Brown University, 34 Olive Street, Box G-B225, Providence, Rhode Island 02912 USA; [5]Biology Department, University of Massachusetts, 611 North Pleasant Street, Amherst, MA 01003, USA; [6]Division of Botany, Peabody Museum of Natural History, Yale University, New Haven, CT, USA; [7]Harvard University, Harvard Forest, 324 North Main Street, Petersham, Massachusetts, 01366 USA.

*These authors contributed equally to the study

[1]To whom correspondence should be addressed. Email: barnabas_daru@fas.harvard.edu

Short Title: Sampling bias in herbarium specimens

Manuscript information: 5268 words (Introduction = 723 words, Materials and Methods = 1560 words, Results = 957 words, Discussion = 2028 words | 8 figures (8 color figures) | 3 Tables | 1 supporting information

SUMMARY

1. Non-random collecting practices may bias conclusions drawn from analyses of herbarium records. Recent efforts to fully digitize and mobilize regional floras offer a timely opportunity to assess commonalities and differences in herbarium sampling biases.
2. We determined spatial, temporal, trait, phylogenetic, and collector biases in ~5 million herbarium records, representing three of the most complete digitized floras of the world: Australia (AU), South Africa (SA), and New England (NE).
3. We identified numerous shared and unique biases among these regions. Shared biases included specimens i) collected close to roads and herbaria; ii) collected more frequently during spring; iii) of threatened species collected less frequently; and iv) of close relatives collected in similar numbers. Regional differences included i) over-representation of graminoids in SA and AU and of annuals in AU; and ii) peak collection during the 1910s in NE, 1980s in SA, and 1990s in AU. Finally, in all regions, a disproportionately large percentage of specimens were collected by a few individuals. These mega-collectors, and their associated preferences and idiosyncrasies, may have shaped patterns of collection bias via 'founder effects'.
4. Studies using herbarium collections should account for sampling biases and future collecting efforts should avoid compounding these biases.

Keywords: Herbarium, collector bias, geographic bias, regional flora, sampling bias, temporal bias, trait bias

INTRODUCTION

Herbaria contain a wealth of information about the ecological and evolutionary history of living and extinct species (Funk, 2003). Despite the continuous decline in plant collecting and dwindling support for herbaria (Dalton, 2003; Prather *et al.*, 2004a, b), there has been a recent surge of studies leveraging herbarium collections for diverse research projects not focused on systematics (Pyke & Ehrlich, 2010; Lees *et al.*, 2011; Feeley, 2012; Lavoie, 2013; Hart *et al.*, 2014). These studies include plant demography, current and future species distributions, and temporal changes in phenology and morphology (*e.g.*, Miller-Rushing *et al.*, 2006; Newbold, 2010; Pyke & Ehrlich, 2010; Lavoie, 2013; Staats *et al.*, 2013; Davis *et al.*, 2015; Willis *et al.*, 2017a).

Ideally, herbarium collections used for these studies would include statistically unbiased samples of plant diversity across space and time. However, as the majority of specimens were collected for qualitative taxonomic and/or systematic inquiries, they were usually collected non-randomly and sampling designs were rarely quantified (Wolf *et al.*, 2011; Schmidt-Lebuhn *et al.*, 2013). Because non-random samples may be statistically biased, analyzing them without accounting for biases might lead to spurious results (Syfert *et al.*, 2013).

Sampling biases fall into several broad categories. Taxonomic or phylogenetic bias is the unbalanced sampling of certain taxa or clades over others, typically resulting from the interests of a collector or the attractiveness of plants (Hortal *et al.*, 2007). Geographic bias occurs when specimens are collected more frequently in one place than another often because of differential accessibility (Hijmans *et al.*, 2000). Temporal bias occurs when collection activity is favored in certain years or parts of the year (Cotterill *et al.*, 1994; Funk & Morin, 2000; Norris *et al.*, 2001). Meyer *et al.* (2016) evaluated worldwide terrestrial plant occurrence data using 120 million records from the Global Biodiversity Information Facility (GBIF; Edwards *et al.*, 2000). Their analyses revealed large taxonomic gaps in global plant occurrence data (< 25% of species of land plants were sampled); extensive spatial gaps across regions that harbor high concentrations of plant diversity, especially in Asia, Central Africa, and Amazonia; and strong temporal discontinuities in occurrence records across decades, which can hamper inferences about the effects on plants of recent and future environmental change.

Although Meyer *et al.*'s (2016) study represents the most comprehensive effort to assess biases in plant collections at a global scale to-date, the vast majority of herbarium collections have not been digitized, and of those that have, many of their data are not available, in whole or in part, on GBIF. Thus, Meyer *et al.*'s (2016) assessment of biases may itself be biased, or may reflect inaccurately biases in more complete, regional botanical collections. Furthermore, over two-thirds of the plant records in GBIF are not tied to physical specimens, and cannot be validated by others (Cotterill, 1995). For these reasons we suspect that an analysis of finer-grained collection data, focused on specific regions that have been fully digitized and validated, may reveal clearer patterns of sampling biases between regions than the global trends identified by Meyer *et al.* (2016) (*cf.* Hijmans *et al.*, 2000 for Bolivian potatoes).

Expanding upon Meyer *et al.*'s work, we explored spatial, temporal, and taxonomic/phylogenetic sampling biases in collections from three of the most extensively collected, digitized, and mobilized regional floras in the world: South Africa (SA), Australia (AU), and the New England (NE) region of the United States. The SA flora is a compilation of digitized herbarium specimens from all major herbaria across the country available in a single online portal (South African National Biodiversity Institute [SANBI], 2016; le Roux *et al.*, 2017). The Australian Virtual Herbarium (AVH, 2016) is the main database for AU. It contains digitized herbarium specimens from all the major herbaria in Australia. The Consortium of the Northeast Herbaria database contains digitized specimens from 15 participating herbaria in the NE region of the United States (Schorn *et al.*, 2016). We also examined trait bias – sampling bias due to intrinsic life-history characteristics, including life cycle (annual *vs*. perennial), plant height, and growth form (woody *vs*. herbaceous), and species conservation status. Finally, we examined the contributions of individual collectors to each flora. We identified biases in all five of these categories within each of these regional floras. Our results revealed both commonalities and differences in regional collection biases and identified new sampling foci as collections grow in the future.

MATERIAL AND METHODS

Sources and description of data

We obtained 12,488,200 herbarium specimen records of vascular plants from AU (Australia Virtual Herbarium [AVH], 2016); 2,049,905 herbarium specimen records from SA including Lesotho and Swaziland (South African National Biodiversity Institute [SANBI], 2016); and 879,388 herbarium specimen records from the NE (USA) flora (Consortium of Northeastern Herbaria [CNH], 2016). The records were cleaned in two steps (Fig. S1). First, we standardized the taxonomy of all species using the Taxonomic Name Resolution Service v.4.0 (Boyle *et al.*, 2013). Second, we removed specimens that were duplicates from the same collection locality and date; specimens with clearly erroneous locations (*i.e.*, in oceans); specimens missing exact collection date and/or georeferenced location data; and field observation records not tied to a physical specimen. Following this data cleaning, we retained 32% of the initial specimens for further analysis, including: 24% of the AU records (31,966 taxa; 661,370 records); 49% (20,824 taxa, 4,579,320 records) from SA; and 75% (11,447 taxa, 1,008,206 records) from NE.

Analyses

Spatial biases

First, we evaluated the density of sampling localities across the focal regions using Delaunay triangulation polygons, which measure the land area covered by each sampling locale (Fortune, 1992). Larger triangles indicate sparser collecting effort, whereas smaller triangles indicate more concentrated effort. Second, we examined infrastructure bias by calculating the minimum distance of each collection locality to the nearest major road (GADM, 2015) and herbarium (following Thiers, 2016). Our dataset of roads assumes that the network of major roads in these regions has remained largely unchanged over the past century (Forman *et al.*, 1995). Thus, we focused only on major roads in these three regions and excluded all street roads and dirt tracks in our analysis. We then compared these distances to those generated by a null model (1000 iterations) in which the same number of sample points was randomly (Poisson) distributed across each geographic region. Third, we mapped geographic biases in sampling density, defined as areas of excessive (hotspots) or insufficient (coldspots) collection (Hijmans *et al.*, 2000). Hotspots and coldspots were determined at a spatial grain of 0.25° × 0.25° based on the number of specimens per grid cell, and identified using the 2.5% threshold (Ceballos & Ehrlich,

2006; Orme *et al.*, 2005; Daru *et al.*, 2015), based, respectively, on the 97.5th and 2.5th percentile values in the number of specimens collected per grid cell. Spatial distance calculations were done with the functions *dist2Line* and *spDists* in the R packages sp (Bivand *et al.*, 2013) and geosphere (Hijmans, 2015), respectively. In our final predictive model of sampling density, we also included human population density (CIESIN, 2016), sampling localities, infrastructure (distance to herbaria and roads), number of specimens collected, and elevation or topographic relief.

Temporal bias

For each regional flora, we explored bias at several temporal scales. Collection dates ranged from 20 May 1664 to 9 January 2016 (AU), 15 November 1656 to 6 June 2016 (SA), and 28 July 1687 to 4 May 2016 (NE). We hypothesized that collectors tended to avoid fieldwork during unfavorable conditions (*e.g.*, weekdays, winter, war time). To test for temporal bias, we first re-coded collection dates as days of the week (Sunday = 1, Monday = 2, *etc.*), day of year (DOY; where January 1 = 1 DOY and December 31 = 365 DOY, *etc.*), and decade (*e.g.*, 1901-1910, 1911-1920, *etc.*). We then used a Rayleigh test of directional statistics in the R package circular (Agostinelli & Lund, 2013) to test whether each of these collection dates were randomly distributed against all dates spanning the entire duration of plant collection. If $P < \alpha = 0.05$, we rejected the null hypothesis of temporal uniformity at scales of weeks, days of the year, or decades.

Trait bias

We used customized R scripts to harvest information on growth duration (annual *vs.* perennial), growth form (woody *vs.* herbaceous), and height for each species from online regional databases (all accessed in June 2016), including: New South Wales Flora Online (http://plantnet.rbgsyd.nsw.gov.au); JSTOR Global Plants (https://plants.jstor.org); Atlas of Living Australia (http://bie.ala.org.au); Plants of Southwestern Australia (http://keys.lucidcentral.org); the African Plant Database (http://www.ville-ge.ch); Plants of Southern Africa (http://www.plantzafrica.com); Plant Resources of Tropical Africa (http://www.prota4u.org); Flora of North America (http://www.efloras.org); and the USDA Plants Database (http://plants.usda.gov). We then manually checked these trait

data for inconsistencies among sources, and adjusted the data accordingly. Extinction risk assessments for each species were retrieved from the IUCN Red List database (www.iucnredlist.org, accessed August 2016), which uses the following categories: Data Deficient (DD), Least Concern (LC), Lower Risk/Conservation Dependent (LR/CD), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), and Extinct (EX). We grouped these narrow categories into two broader threat categories, threatened (EX+CR+EN+VU) or not threatened (LR/CD+NT+LC), following Yessoufou *et al*. (2012).

Trait bias was evaluated using a Chi-squared test to contrast the number of observed specimens collected per species with the abundance of a species if specimen collection was equal across all species for each trait category. Because of dramatically unequal sampling effort in some species – *e.g.*, *Senna artemisioides* with 10,167 specimens *vs*. *Eucalyptus cordieri* with only one – and the low coverage of taxa with available trait data, we randomly sampled 50 specimens from each available species with trait data using 1000 randomizations. Species with less than 50 specimens were excluded from this analysis.

Phylogenetic bias

We assessed phylogenetic signal in collection frequency as a measure of phylogenetic bias using two different tests (Wolkovich *et al*., 2013). A strong phylogenetic signal – closely related species sharing similar collection frequency – would suggest phylogenetic bias in collections. We first assembled a phylogeny using Phylomatic (Webb & Donoghue, 2005), enforcing a topology that assumed the APG III backbone (tree R20120829). This phylogeny included all species in our analysis, but provided only an approximate degree of relatedness based on taxonomic hierarchy at family level, thus many relationships, especially within genera, were unresolved. This is problematic because recent theoretical and empirical studies have shown that a lack of resolution in a community phylogeny may mask significant patterns by reducing statistical power (Schaefer *et al.*, 2011) or suggest significant phylogenetic patterns that are not supported by more completely resolved phylogenies (Davies *et al*., 2012).

To alleviate these concerns, we also tested for phylogenetic bias by including only those species sampled in the dated molecular phylogeny inferred from seven genes for 32,223 plant species (Zanne *et al.*, 2014). Although this phylogeny has been criticized (Edwards *et al.*, 2015), it nonetheless represents the single largest phylogeny to date for flowering plants. The taxon sampling for testing phylogenetic bias included 5814 species from AU, 3568 from SA, and 4269 from NE.

We estimated phylogenetic signal using three common metrics: Abouheif's C_{mean} (Abouheif, 1999), Blomberg's K (Blomberg *et al.*, 2003), and Pagel's lambda (λ) (Pagel, 1999). Significance was assessed by comparing observed values to a null distribution created by shuffling the trait values across the tips of the phylogeny 1000 times. Pagel's λ uses a maximum-likelihood method with branch-length transformation to estimate the best-fit of a trait against a Brownian model. Values of Pagel's λ range from 0 (no phylogenetic signal) to 1 (strong phylogenetic signal). Both Blomberg's K (a significant phylogenetic signal is indicated by a K value > 1) and Pagel's λ were calculated using the R package phytools (Revell, 2012). Abouheif's C_{mean} was calculated using adephylo (Jombart & Dray, 2008). We tested the sensitivity of our analysis by exploring phylogenetic signal in collecting effort across nine well-sampled NE clades: Asteraceae, Brassicaceae, Cyperaceae, Ericaceae, Fabaceae, Lamiaceae, Poaceae, Ranunculaceae, and Rosaceae.

In addition to phylogenetic signal, we also used phylogenetic generalized least squares regressions (PGLS) in the R package caper (Orme *et al.*, 2012) to model collecting effort per species in each region as a function of species evolutionary ages, evolutionary distinctiveness (ED), and "evolutionary distinctiveness and global endangerment" (EDGE; Isaac *et al.*, 2007). Species ages were measured as the length of terminal branches (BL) linking species on a phylogenetic tree. ED measures the degree of phylogenetic isolation of a species, whereas the EDGE metric was determined by calculating the ED score of each species (Isaac *et al.*, 2007) and combining it with global endangerment (GE) from IUCN conservation categories: $EDGE = ln(1 + ED) + GE \times ln(2)$, where GE represents expected probability of species extinction over a 100-year period (Redding & Mooers, 2006) categorized as follows: least concern = 0.001, near

Threatened and Conservation Dependent = 0.01, Vulnerable = 0.1, Endangered = 0.67, and Critically Endangered = 0.999.

Last, we examined the phylogenetic structure of collecting efforts across decades to test for patterns of phylogenetic overdispersion and clustering through time. Temporal phylogenetic structure by decade was evaluated using the net relatedness index (NRI) and nearest taxon index (NTI; Webb *et al.*, 2002, 2008). NRI describes a tree-wide pattern of phylogenetic dispersion, whereas NTI evaluates phylogenetic structure towards the tips of the phylogeny. Negative values of NRI or NTI indicate phylogenetic overdispersion whereas positive values indicate phylogenetic clustering.

Collector bias

We determined collector bias by tabulating the number of specimens amassed by each collector in the three floras. We then examined Pearson's product-moment correlation between the numbers of specimens collected per collector with the number of species collected per collector.

Computation and availability of data and code

All statistical analyses were conducted using the Research Computing Clusters of Harvard University (https://rc.fas.harvard.edu/). Data files and custom R scripts will be available from the Harvard Forest Data Archive (http://harvardforest.fas.harvard.edu/data-archive).

RESULTS

Spatial bias

High sampling density was observed in southeast and southwest AU, the Cape region of SA, and two of the six NE states (Connecticut and Massachusetts) relative to other parts of those regions (Fig. 1a-c). When we weighted each sampling locale by the number of specimens, we found a mismatch between hotspots (top 2.5% quantiles) and coldspots (lowest 2.5% quantiles) of sampling intensity (Fig. 1d-f), suggesting hotspots and coldspots are not randomly distributed. Hotspots of collecting tend to cluster around

coasts in AU and SA, whereas coldspots were abundant in interior areas. In NE, hotspots were concentrated in the south and coldspots in the north.

Herbarium specimens tended to be collected closer than expected to roads and herbaria ($p<0.01$; Fig. 2a, b). More than 50% of herbarium specimens were collected within 2 km of roadsides in all three floras ($p<0.01$; Fig. 2a). Moreover, distance to herbaria explained 45% of the variance in collecting effort in AU, 29% in SA and 12.3% in NE (Table 1). Despite substantial gradients in altitudes in each region (-15 – 2022 m a.s.l. in AU; 1 – 3254 m a.s.l in SA; and -3 – 1485 m a.s.l. in NE), most specimens were collected below 500 m a.s.l in AU and NE (81%, 44%, and 93% of specimens in AU, SA, and NE, respectively; Fig. 2c).

Temporal bias

There were historical biases in collection efforts in the three floras: low sampling until 1880 in AU and SA, and a burst of collections in NE in the early 20th century (Fig. 3). Conversely, there was a dramatic increase in botanical collection in SA and AU after World War II, peaking in the 1980s and 1990s, respectively (Fig. 3), 100 years after peak collection activity in NE. Seasonally, specimen collections were biased toward spring and summer for the three floras, with peak collection ranging from September to December in AU and SA (Rayleigh $Z = 0.189$ and $Z = 0.251$ respectively, both $p < 0.001$), and May to September in NE (Rayleigh $Z = 0.718$, $p < 0.001$; Fig. 4a). There was a non-significant trend towards collection on weekends (Saturdays and Sundays) in NE (Rayleigh test $Z = 1.0$, $p < 0.001$) and midweek in SA and AU (Rayleigh test $Z = 0.105$ and $Z = 1.0$, respectively; both $p < 0.001$; Fig. 4a).

Trait bias

Perennials were more frequently collected than annuals in terms of specimens per species in SA and NE, whereas the opposite was true for AU (Fig. 5a). Similarly, graminoid specimens per species were over-represented relative to other habits in AU and SA, whereas herbs and trees were over-represented in NE (Fig. 5b). Relatively short plants were more frequently represented than taller plants in all three floras: 79.3%, 89.3% and

296 84.9% of the plants collected in AU, SA and NE, respectively were less than 5 m in
297 height (Fig. 5c).
298 Threatened species were collected significantly less often than non-threatened
299 plants across all three floras (all $p < 0.001$; Fig. 5d).
300
301 ***Phylogenetic bias***
302 There was a significant, but weak phylogenetic signal in the abundance of specimens per
303 species across all three floras (Table 2). Specifically, closely related species tended to
304 have a more similar number of specimens in a collection than expected (Table 2; Fig. 6).
305 Such phylogenetic bias was strongest in SA (Abuoheif's $C_{mean} = 0.15$ and $\lambda = 0.32$; both
306 $p < 0.01$, but $K = 0.0013$ [NS]). For instance, in SA, collections of the genus *Protea*
307 averaged 115 specimens per species whereas only two specimens were collected per
308 species of *Rytigynia* on average. Most *Amsonia* in NE were represented by < 10
309 specimens per species, whereas many fern species were represented by high specimen
310 numbers (*e.g.*, *Onoclea* with 845 specimens/species). Australian collections showed the
311 weakest phylogenetic bias (Abuoheif's $C_{mean} = 0.12$ and $\lambda = 0.18$, both $p < 0.01$, but $K =$
312 0.00085 [NS]; Fig. 6). Phylogenetic signal varied at the family level as well in NE, with
313 Asteraceae showing the strongest collection bias (Fig. 7), followed by Cyperaceae,
314 Poaceae, and Rosaceae (Table S1).
315 'Evolutionary distinctiveness and global endangerment' (EDGE) was significant
316 predictor of collecting efforts in all three floras ($p < 0.001$), with variance ranging from
317 1.89% (NE) and 3.75% (AU), to 8.89% in SA. In general, EDGE species were generally
318 under-collected in terms of specimens per species (Table 3).
319 Lastly, floristic collecting showed a general trend of phylogenetic clustering
320 within decades for all three floras. The collection of different clades of plants was not
321 evenly distributed across time. NTI was significantly positive in each flora, indicating
322 that clustering occurred near the tips of the phylogeny (Fig. 3). We only observed
323 significant phylogenetic clustering at the deeper nodes of the phylogeny, as indicated by
324 NRI, in SA (Fig. 3); deeper phylogenetic clustering was weak in NE and AU (Fig. 3).
325
326 ***Collector bias***

The number of specimens per collector was highly skewed (Fig. 8). In AU, more than 50% of the examined specimens were amassed by only 2% of the collectors, including A.C. Beauglehole (46,728 specimens), B. Hyland (32,019 specimens), and P.I. Forster (30,280 specimens; Fig. 8a). In SA, more than 50% of the specimens were amassed by 9.5% of collectors, including J.P.H Acocks (19,344 specimens), E.E. Esterhuysen (15,566 specimens), and E.E. Galpin (14,146 specimens; Fig. 8b). In NE, 50% of the specimens were contributed by 3.2% of the collectors, including L.J. Mehrhoff (19,149 specimens), M.L. Fernald (14,368 specimens), and A.S. Pease (12,238 specimens; Fig. 8c). The number of specimens amassed by these collectors was positively correlated with the number of species they collected, suggesting that these collectors were doing general collecting rather than focusing on a particular group of plants ($r = 0.85$ in AU, 0.95 in SA and 0.84 in NE; all $p < 0.01$; Fig. S2).

DISCUSSION

Historically, the primary function of herbaria has been to serve as an institution of taxonomy, allowing users to construct classifications of plants, verify identifications, determine the ranges and morphological characteristics of species, and develop local and regional floras (Greve *et al.*, 2016). Over time, new uses for specimens have arisen, and now more than ever, they are being used in ways that collectors rarely imagined (Pyke & Ehrlich, 2010; Lavoie, 2013; Willis *et al.*, 2017a,b; Nualart *et al.*, 2017; Rudin *et al.*, 2017). Accordingly, attempts to assess and categorize biases inherent in these collections have been made (Rich & Woodruff, 1992; Geri *et al.*, 2013; Schmidt-Lebuhn *et al.*, 2013; Meyer *et al.*, 2016; Stropp *et al.*, 2016). Among these, the most comprehensive investigation is by Meyer *et al.* (2016), who proposed an important conceptual framework for analyzing gaps and biases along taxonomic, geographical, and temporal dimensions. Although Meyer *et al.* (2016) focused more on observational records than herbarium collections, they uncovered numerous biases in 'digitally accessible information' regarding plants and provided an important baseline for evaluating and improving global floristic coverage in collection data. However, vast geographic areas remain where collections data are sparsely available, in part because numerous herbaria have not yet been fully digitized and mobilized. Collection biases in these areas are

difficult to categorize, and may skew global patterns of bias when considered alongside areas whose collection data now are readily available. By focusing on three of the most well-collected and digitized floras in the world, we reduced effects of missing or unavailable data, and most importantly, could evaluate commonalities and differences in patterns of bias among regional collections.

Spatial bias

Our data confirmed the tendencies for botanists to collect along roadsides (*e.g.*, Funk & Richardson, 2002), near herbaria (*e.g.*, Hijmans *et al.*, 2000), in more accessible areas (Rich & Woodruff, 1992), and at lower elevations. Before automobiles became common in the 1920s, people walked or rode domesticated animals (Botkin, 1968; Belasco, 1979). As routes became established, they formed our modern infrastructure, including roads, railroads, and cities that contain herbaria, and spatial biases associated with infrastructure likely increased (Everill *et al.*, 2014). Because roads are known to fragment populations and landscapes (*e.g.*, Forman & Alexander, 1998; Hui *et al.*, 2003; Griffith *et al.*, 2010; Li *et al.*, 2014) and botanists and herbaria predominate in cities, specimens collected in proximity to either are unlikely to represent a random sample across species distributions. Species collected along roadsides are likely to be over-represented by species that thrive with disturbance, and under-represented by forest interior and wetland species.

Collection bias towards lower elevations (< 500m) was most striking in SA, despite extensive collection efforts along the hyper-diverse mountains of the Cape Fold Belt. This is likely because of the presence of the arid and relatively depauperate Great Karoo Plateau, which spreads across over a third of the country, but accounts for only a small proportion of the region's biodiversity. As a result, the low-elevation collection bias in SA may reflect actual species abundance.

Temporal bias

Collections in AU and SA have increased through time until only very recently, but those in NE peaked in the early 1900s. These differences between regional collection activities

387 may parallel broader societal factors influencing plant collection. In NE, the
388 establishment of the New England Botanical Club during the 1890s (NEBC, 1899)
389 preceded a surge and peak in collecting activity associated with prolific botanical
390 expeditions of the region coinciding with the 'Golden Age' of plant collecting in Europe
391 and North America (Whittle, 1970; Musgrave *et al.*, 1999). In SA, collection efforts
392 began much later, peaked during the Apartheid Era from 1948 to 1994, and declined
393 thereafter under the New Democratic Rule, concomitant with the general economic
394 decline of the country and concern for public safety (Ferreira & Harmse, 2000; Lemanski,
395 2004). In AU, the mass immigration of Europeans in 1948 shortly after World War II that
396 included numerous highly skilled professionals (Price, 1998; Leuner, 2007) coincided
397 with a huge increase in botanical collecting. Botanical collecting may have declined more
398 recently because of legislation in AU and SA to regulate collections activities, especially
399 those designed to protect rare and endangered species.
400 Collecting efforts within a season revealed common patterns of bias: specimens in
401 the three regions were collected overwhelmingly in spring and summer. Sampling during
402 these time periods likely reflects efforts to represent the onset and peak of flowering in
403 these temperate regions. However, this seasonal bias likely overlooks key developmental
404 transitions (*e.g.*, Poethig, 2013), including bud formation, bud break, fruit maturation,
405 and leaf senescence (van der Schoot *et al.*, 2014). These temporal patterns also likely
406 reflect favorable conditions for fieldwork. Supporting this argument, these temporal
407 patterns were most pronounced in NE, which experiences the harshest winter climates
408 among the three regions. Collecting was also more likely during holidays and school
409 vacations in NE and AU.
410

411 ***Trait bias***

412 In all three regions, small to medium-height species were over-collected whereas tall
413 species (>5 m) were under-collected. This pattern presumably is related to the relative
414 ease of collecting specimens from shorter species, because reproductive materials are
415 more accessible for shorter specimens, and the dense distribution of numerous short,

frequently herbaceous species. Specimens of trees with woody twigs also are bulkier and more difficult to prepare, which may reduce their collection frequency.

Threatened species were also greatly under-represented in all floras. This is perhaps not surprising given their limited abundance (Palmer *et al.*, 2002) and imposed collecting restrictions (Klemens & Thorbjarnarson, 1995; Pritchard, 1996; Gibbons *et al.*, 2000; Robinson, 2001). Regardless of formal restrictions, botanists now often avoid over-collection of such species by following informal guidelines and collecting plants only in areas with numerous individuals of the species (Iwanycki, 2009). Although careful measures for collecting rare plants is important, under-collection of rare species may lead to incorrect extinction risk assessments (that the species is rarer than it actually is) and greatly limit opportunities to glean historic population and biogeographic data to guide species conservation and restoration.

Annuals were over-represented relative to perennials in AU, while the opposite was observed in SA and NE. Graminoids were over-represented in AU and SA, but they were significantly under-represented in NE. This result may stem from the higher likelihood of common species being collected multiple times by different individuals or expeditions. Along these lines, much of AU is dominated by annual grasses, and the savannas of SA are populated by a variety of native and non-native perennial grasses interspersed with forbs and woody plants (Bond & Parr, 2010). New England, on the other hand, is generally forested, with an abundance of shrubs and perennial herbs below. Lianas and vines simultaneously represent the smallest proportion of growth forms and comprise the least number of specimens per species in all three floras. Such trait-based biases in botanical collections not only influence our perception of species abundance and range, but can also lead to erroneous estimations of functional diversity and ecosystem services, especially for studies relying on specimen databases (Schmidt-Lebuhn *et al.*, 2013).

Phylogenetic bias

Taxonomic biases in collection data have been reported previously (Hijmans *et al.*, 2000; Tobler *et al.*, 2007; Meyer *et al.*, 2016). However, our study is the first, to our knowledge,

446 to demonstrate explicit evidence for phylogenetic bias in herbarium collections.
447 Collection efforts in all three floras were concentrated in particular clades.

448 Previous examination of taxonomic bias has been hampered because they did not
449 evaluate clades lacking formal taxonomic ranks or overlooked other metrics of
450 evolutionary diversity. For instance, our phylogenetic approach not only captured
451 taxonomic bias, but revealed that evolutionarily distinct and globally endangered species
452 are underrepresented in herbarium records relative to other large clades (*e.g.*, Asteraceae,
453 Cyperaceae, Poaceae and Rosaceae in NE). Phylogenetically isolated species that are
454 threatened with extinction represent important targets for future collecting, and for
455 conservation prioritization.

456 Our findings also have strong links for understanding collector behavior. For
457 example, regional collectors with a particular interest in SA *Protea* species or NE *Rubus*
458 species would contribute to phylogenetic collecting bias. Similarly, phylogenetic bias
459 could result from collectors focusing on plants with certain phylogenetically conserved
460 traits, such as showy flowers or the presence of particular secondary metabolites (*e.g.*,
461 medicinal plants). Although our analysis of phylogenetic bias in herbarium collections
462 was far from conclusive and limited by taxa for which phylogenetic data were available,
463 our conclusions are supported by other studies demonstrating widespread taxonomic bias
464 on a global scale (Meyer *et al.*, 2016).
465

466 ***Collector bias***

467 In all regions we identified that a large percentage of specimens were gathered by only a
468 few collectors (Fig. 8). This implies that the habits and preferences of a few individuals
469 likely shaped the establishment and formation of herbarium collections. These “founder
470 effects” propagate across all the dimensions of collection bias examined above. For
471 example, certain collectors may focus on floristic regions and sample all species found
472 therein, whereas others may focus on collecting species of a particular clade across
473 various regions. Professional botanists may tend to collect specimens on weekdays during
474 any time of the year, whereas amateurs and faculty with teaching responsibilities may
475 focus their efforts on weekends and vacation months. Those interested in function and

476 physiology may only collect plants of certain habits or life-histories (*e.g.*, carnivorous or
477 succulent plants). These effects would be compounded when associated with mega-
478 collectors. For instance, the Harvard University Herbaria's collection of Asian plants
479 dates to the early establishment of the institution, and continues to attract scholars of the
480 flora of Asia and their collections. Investigating the historical significance and potential
481 biases created and propagated by these early pioneers is a ripe area for future research.

482

483 **Future collecting**

484 To ensure that herbaria continue to be vital centers for research beyond their importance
485 to taxonomy and systematics, herbarium directors and collectors should consider working
486 to accommodate and possibly reduce biases in plant collections. Biases can be accounted
487 for to a degree using statistical approaches that mitigate their effects (Droissart *et al.*,
488 2012; Feeley, 2012; Grass *et al.*, 2014; Engemann *et al.*, 2015). For instance,
489 comparisons of herbarium species collected near to, and far away from, urban areas and
490 other infrastructure (McCarthy *et al.*, 2012) or using rarefaction methods to predict
491 abundances (Schmidt-Lebuhn *et al.*, 2013) could be useful strategies to improve species
492 distribution models and predict future changes across a flora. Future collecting
493 expeditions should focus on "coldspots" of diversity (Hijmans *et al.*, 2000). Although
494 some of the coldspots we identified may represent inhospitable environments, they often
495 correspond to unique and irreplaceable ecosystems, including the Succulent Karoo of SA
496 and the North Maine Woods in northern NE. Some of these coldspots also may indicate
497 areas where herbarium specimens have yet to be digitized and mobilized, providing
498 additional focus for efforts to make collection data widely available.

499 Phylogenetic and trait biases can be alleviated by targeting collection efforts at
500 specimen gaps along these axes. Temporal bias is more difficult to address, as we cannot
501 add to historic collections. However, we can make efforts to maintain consistent regional
502 botanical records by conducting field surveys at regular intervals.

503 Some of the biases may also be attributed in part to longstanding curation
504 practices. As herbarium collections were amassed for qualitative floristic, taxonomic, and
505 systematic research, duplicate specimens of common species and similar specimens from

the same geographic area have been discarded or sent elsewhere. Indeed, many herbaria refuse to accession new specimens belonging to regions or species that are already represented in their current collections, despite increasing use of herbarium collections for novel applications. This trend is becoming even more pronounced, as herbaria around the world are increasingly constrained by funding, labor, and space. As new uses for biological collections continue to proliferate, curation practices may have to change to accommodate different avenues of research, such as climate change biology and rare plant conservation. Finally, future collecting should strive to overcome "stamp collecting" (*e.g.*, Vul *et al.*, 2014), the tendency to not collect additional specimens of a given species from a specific location and time once another specimen has been collected there and then. Although analyzing the impacts of all of these solutions is beyond the scope of this study, future studies could statistically test these solutions using appropriate null models.

ACKNOWLEDGMENTS

We thank the Harvard University Herbaria for logistic and financial support, and the virtual herbaria in the three regional floras for granting us access to their data: Australian Virtual Herbarium (http://avh.chah.org.au), South African National Biodiversity Institute (http://newposa.sanbi.org/) and Consortium for Northeast Herbaria (http://portal.neherbaria.org/portal/). Digitization of most New England specimens was funded by the ADBC program of the U.S. National Science Foundation (Awards 1208829, 1208835, 1208972, 1208973, 1208975, 1208989, 1209149). Special thanks to T.J. Davies, E.K. Meineke, K.M. Peterson, and K.G. Dexter for valuable discussion during the formation of this manuscript.

Author Contributions: Conceived the project: CCD. Designed the experiment: BHD, DSP. Performed the experiments: BHD. Analyzed the data: BHD with help from DSP. Contributed reagents/materials/analysis tools: BHD, DSP, CGW, AME, CCD. Wrote the paper: BHD with significant comments and editing from all co-authors.

537

538 **References**

539 **Abouheif E. 1999.** A method for testing the assumption of phylogenetic independence in
540 comparative data. *Evolutionary Ecology Research* **1**: 895–909.
541 **Agostinelli C, Lund U. 2013.** *R package 'circular': Circular Statistics (version 0.4-7).*
542 URL https://r-forge.r-project.org/projects/circular/
543 **AVH. 2016.** *Australia's Virtual Herbarium, Council of Heads of Australasian Herbaria*,
544 http://avh.chah.org.au, accessed on 09 June 2016.
545 **Belasco WJ. 1979.** *Americans on the road, from autocamp to motel 1910-1945*.
546 Baltimore: Johns Hopkins University Press.
547 **Bivand RS, Pebesma E, Gomez-Rubio V. 2013.** *Applied spatial data analysis with R,*
548 *Second edition*. Springer, NY. http://www.asdar-book.org/
549 **Blomberg SP, Garland T, Ives AR. 2003.** Testing for phylogenetic signal in
550 comparative data: behavioural traits are more labile. *Evolution* **57**: 717–745.
551 **Bond WJ, Parr CL. 2010.** Beyond the forest edge: ecology, diversity and conservation
552 of the grassy biomes. *Biological Conservation* **143**: 2395–2404.
553 **Botkin BA. 1968.** Automobile humor: from the horseless carriage to the compact car.
554 *The Journal of Popular Culture* **I**: 395–402.
555 **Boyle B, Hopkins N, Lu Z, Garay JAR, Mozzherin D, Rees T, Matasci N, Narro ML,**
556 **Piel WH, Mckay SJ, *et al.* 2013.** The taxonomic name resolution service: an
557 online tool for automated standardization of plant names. *BMC Bioinformatics* **14**:
558 16.
559 **Ceballos G, Ehrlich PR. 2006.** Global mammal distributions, biodiversity hotspots, and
560 conservation. *Proceedings of the National Academy of Sciences USA* **103**: 19374–
561 19379.
562 **CIESIN. 2016.** *Center for International Earth Science Information Network, Columbia*
563 *University.* Gridded Population of the World, Version 4 (GPWv4): Population
564 Density. Palisades, NY: NASA Socioeconomic Data and Applications Center
565 (SEDAC). http://dx.doi.org/10.7927/H4NP22DQ. Accessed 29 August 2016.
566 **CNH. 2016.** Consortium of Northeastern Herbaria. http://portal.neherbaria.org/portal/
567 **Cotterill FPD, Hustler CW, Broadley DG. 1994.** Systematics and biodiversity. *Trends*
568 *in Ecology and Evolution* **9**: 228.

Cotterill FPD. 1995. Systematics, biological knowledge and environmental conservation. *Biodiversity and Conservation* **4**: 183–205.

Dalton R. 2003. Natural history collections in crisis as funding is slashed. *Nature* **423**: 6940.

Daru BH, Van der Bank M, Davies TJ. 2015. Spatial incongruence among hotspots and complementary areas of tree diversity in southern Africa. *Diversity and Distributions* **21**: 769–780.

Davies TJ, Kraft NJB, Salamin N, Wolkovich EM. 2012. Incompletely resolved phylogenetic trees inflate estimates of phylogenetic conservatism. *Ecology* **93**: 242–247.

Davis CC, Willis CG, Connolly B, Kelly C, Ellison AM. 2015. Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms. *American Journal of Botany* **102**: 1599–1609.

Droissart V, Hardy OJ, Sonké B, Dahdouh-Guebas F, Stévart T. 2012. Subsampling herbarium collections to assess geographic diversity gradients: A case study with endemic Orchidaceae and Rubiaceae in Cameroon. *Biotropica* **44**: 44–52.

Edwards EJ, de Vos JM, Donoghue MJ. 2015. Doubtful pathways to cold tolerance in plants. *Nature* **521**: E5–E6.

Edwards JL, Lane MA, Nielsen ES. 2000. Interoperability of biodiversity databases: biodiversity information on every desktop. *Science* **289**: 2312–2314.

Engemann K, Enquist BJ, Sandel B, Boyle B, Jørgensen PM, Morueta-Holme N, Peet RK, Violle C, Svenning J-C. 2015. Limited sampling hampers "big data" estimation of species richness in a tropical biodiversity hotspot. *Ecology and Evolution* **5**: 807–820.

Everill PH, Primack RB, Ellwood EE, Melaas EK. 2014. Determining past leaf-out times of New England's deciduous forests from herbarium specimens. *American Journal of Botany* **101**: 1–8.

Feeley KJ. 2012. Distributional migrations, expansions, and contractions of tropical plant species as revealed in dated herbarium records. *Global Change Biology* **18**: 1335–1341.

Ferreira SLA, Harmse AC. 2000. Crime and tourism in South Africa: international tourists perception and risk. *South African Geographical Journal* **82**: 80–85.

Forman RTT, Alexander LE. 1998. Roads and their major ecological effects. *Annual Review of Ecology and Systematics* **29**: 207–31

Forman RTT, Friedman DS, Fitzhenry D, Martin JD, Chen AS, Alexander LE. 1995. Ecological effects of roads: Toward three summary indices and an overview for North America. In: Canters K, ed. *Habitat fragmentation and infrastructure*. Ministry of Transport, Public Works and Water Management: Maastricht and The Hague, Netherlands, 40–54.

Fortune S. 1992. Voronoi diagrams and Delaunay triangulations. *Computing in Euclidean Geometry* **1**: 193–233.

Funk V. 2003. The importance of herbaria. *Plant Science Bulletin* **49**: 94–95.

Funk VA, Morin N. 2000. A survey of the herbaria of the southeast United States. *Sida, Botanical Miscellany* **18**: 35–52.

Funk VA, Richardson K. 2002. Biological specimen data in biodiversity studies: use it or lose it. *Systematic Biology* **51**: 303–316.

GADM. 2015. *Global Administrative Areas*, version 2.8 (www.gadm.org).

Geri F, Lastrucci L, Viciani D, Foggi B, Ferretti G, Maccherini S, Bonini I, Amici V, Chiarucci A. 2013. Mapping patterns of ferns species richness through the use of herbarium data. *Biodiversity and Conservation* **22**: 1679–1690.

Gibbons JW, Scott DE, Ryan T, Buhlmann K, Tuberville T, Greene J, Mills T, Leiden Y, Poppy S, Winne C *et al*. 2000. The global decline of reptiles, déj·vu amphibians. *BioScience* **50**: 653–666.

Grass A, Tremetsberger K, Hössinger R, Bernhardt K. 2014. Change of species and habitat diversity in the Pannonian region of eastern Lower Austria over 170 years: Using herbarium records as a witness. *Natural Resources* **5**: 583–596.

Greve M, Lykke AM, Fagg CW, Gereau RE, Lewis GP, Marchant R, Marshall AR, Ndayishimiye J, Bogaert J, Svenning JC. 2016. Realising the potential of herbarium records for conservation biology. *South African Journal of Botany* **105**: 317–323.

630 **Griffith EH, Sauer JR, Royle JA. 2010.** Traffic effects on bird counts on North
631 American breeding bird survey routes. *Auk* **127**: 387–393.
632 **Hart R, Salick J, Ranjitkar S, Xu J. 2014.** Herbarium specimens show contrasting
633 phenological responses to Himalayan climate. *Proceedings of the National*
634 *Academy of Sciences USA* **111**: 10615–10619.
635 **Hijmans RJ, Garrett KA, Huaman Z, Zhang DP, Schreuder M, Bonierbale M. 2000.**
636 Assessing the geographic representation of genebank collections: the case of the
637 Bolivian wild potatoes. *Conservation Biology* **14**: 1755–1765.
638 **Hijmans RJ. 2015.** *geosphere: Spherical Trigonometry*. R package version 1.4-3.
639 http://CRAN.R-project.org/package=geosphere
640 **Hortal J, Lobo JM, Jiménez-Valverde A. 2007.** Limitations of biodiversity databases:
641 case study on seed-plant diversity in tenerife, canary islands. *Conservation*
642 *Biology* **21**: 853–863.
643 **Hui C, Shuang-cheng L, Yi-li Z. 2003.** Impact of road construction on vegetation
644 alongside Qinghai-Xizang highway and railway. *Chinese Geographical Science*
645 **13**: 340–346.
646 **Isaac NJ, Turvey ST, Collen B, Waterman C, Baillie JE. 2007.** Mammals on the
647 EDGE: conservation priorities based on threat and phylogeny. *PLoS ONE* **2**: e296.
648 **Iwanycki N. 2009.** *Guidelines for collecting herbarium specimens of vascular plants*.
649 Royal Botanical Gardens Canada.
650 **Jombart T, Dray S. 2008.** adephylo: exploratory analyses for the phylogenetic
651 comparative method. *Bioinformatics* **26**: 1907–1909.
652 **Klemens MW, Thorbjarnarson JB. 1995.** Reptiles as a food resource. *Biodiversity and*
653 *Conservation* **4**: 281–298.
654 **Lavoie C. 2013.** Biological collections in an ever changing world: Herbaria as tools for
655 biogeographical and environmental studies. *Perspectives in Plant Ecology,*
656 *Evolution and Systematics* **15**: 68–76.
657 **le Roux MM, Wilkin P, Balkwill K, Boatwright JS, Bytebier B, Filer D, Klak C,**
658 **Klopper RR, Koekemoer M, Livermore L *et al.* 2017.** Producing a plant
659 diversity portal for South Africa. *Taxon* **66**: 421–431.

Lees DC, Lack HW, Rougerie R, Hernandez-Lopez A, Raus T, Avtzis ND, Augustin S, Lopez-Vaamonde C. 2011. Tracking origins of invasive herbivores through herbaria and archival DNA: the case of the horse-chestnut leaf miner. *Frontiers in Ecology and the Environment* **9**: 322–328.

Lemanski C. 2004. A new apartheid? The spatial implications of fear of crime in Cape Town, South Africa. *Environment & Urbanization* **16**: 101–111.

Leuner B. 2007. *Migration, multiculturalism and language maintenance in Australia*. Peter Lang, Oxford.

Li Y, Yu J, Ning K, Du S, Han G, Qu F, Wang G, Fu Y, Zhan C. 2014. Ecological effects of roads on the plant diversity of coastal wetland in the Yellow River Delta. *The Scientific World Journal* **2014**: 952051.

McCarthy KP, Fletcher JR RJ, Rota CT, Hutto RL. 2012. Predicting species distributions from samples collected along roadsides. *Conservation Biology* **26**: 68–77.

Meyer C, Weigelt P, Kreft H. 2016. Multidimensional biases, gaps and uncertainties in global plant occurrence information. *Ecology Letters* **19**: 992–1006.

Miller-Rushing A, Primack R, Mukunda S. 2006. Photographs and herbarium specimens as tools to document phenological changes in response to global warming. *American Journal of Botany* **93**: 1667–1674.

Musgrave T, Gardner C, Musgrave W. 1999. *The plant hunters. Two hundred years of adventure and discovery.* Seven Dials.

NEBC. 1899. Editorial announcement. *Rhodora* **1**: 1–2

Newbold T. 2010. Applications and limitations of museum data for conservation and ecology, with particular attention to species distribution models. *Progress in Physical Geography* **34**: 3–22.

Norris WR, Lewis DQ, Widrlechner MP, Thompson JD, Pope RO. 2001. Lessons from an inventory of the Ames, Iowa, flora (1859–2000). *Journal of the Iowa Academy of Science* **108**: 34–63.

Nualart N, Ibáñez N, Soriano I, López-Pujol J. 2017. Assessing the relevance of herbarium collections as tools for conservation biology. *Botanical Review* doi:10.1007/s12229-017-9188-z

691 **Orme CD, Davies RG, Burgess M, Eigenbrod F, Pickup N, Olson VA, Webster AJ,**
692 **Ding TS, Rasmussen PC, Ridgely RS, *et al.* 2005.** Global hotspots of species
693 richness are not congruent with endemism or threat. *Nature* **436**: 1016–1019.
694 **Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2012.**
695 *caper: Comparative Analyses of Phylogenetics and Evolution in R.* R package
696 version 0.5.http://CRAN.R-project.org/package = caper.
697 **Pagel M. 1999.** Inferring the historical patterns of biological evolution. *Nature* **401**: 877–
698 884.
699 **Palmer MW, Earls PG, Hoagland BW, White PS, Wohlgemuth T 2002.** Quantitative
700 tools for perfecting species list. *Environmetrics* **13**: 121–137.
701 **Poethig, RS. 2013.** Vegetative phase change and shoot maturation in plants. *Current*
702 *Topics in Developmental Biology* **105**: 125–152.
703 **Prather LA, Alvarez-Fuentes O, Mayfield MH, Ferguson CJ. 2004a.** The decline of
704 plant collecting in the United States: a threat to the infrastructure of biodiversity
705 studies. *Systematic Botany* **29**: 15–28.
706 **Prather LA, Alvarez-Fuentes O, Mayfield MH, Ferguson CJ. 2004b**. Implications of
707 the decline in plant collecting for systematic and floristic research. *Systematic*
708 *Botany* **29**: 216–220.
709 **Price CA. 1998.** Post-war immigration: 1945-1998. *Journal of the Australian Population*
710 *Association* **15**: 17.
711 **Pritchard PCH. 1996.** The Galápagos tortoises: nomenclatural and survival status.
712 Lunenberg (MA): Chelonian Research Foundation. *Chelonian Research*
713 *Monographs* **1**.
714 **Pyke GH, Ehrlich PR. 2010.** Biological collections and ecological/environmental
715 research: a review, some observations and a look to the future. *Biological Reviews*
716 **5**: 247–266.
717 **Redding DW, Mooers AØ. 2006.** Incorporating evolutionary measures into conservation
718 prioritization. *Conservation Biology* **20**: 1670–1678.
719 **Revell LJ. 2012.** phytools: An R package for phylogenetic comparative biology (and
720 other things). *Methods in Ecology and Evolution* **3**: 217–223.

Rich TCG, Woodruff ER. 1992. Recording bias in botanical surveys. *Watsonia* **19**: 73–95.

Robinson JG. 2001. Using 'sustainable use' approaches to conserve exploited populations. In: Reynolds JD, Mace GM, Redford KH, Robinson JG, eds. *Conservation of exploited species*. Cambridge: Cambridge University Press, 485–498.

Rudin SM, Murray DW, Whitfeld TJS. 2017. Retrospective analysis of heavy metal contamination in Rhode Island based on old and new herbarium specimens. *Applications in Plant Sciences* **5**: 1–13.

SANBI. 2016. *South African National Biodiversity Institute.* Botanical Database of Southern Africa (BODATSA), http://newposa.sanbi.org/, accessed on 22 July 2016.

Schaefer H, Hardy OJ, Silva L, Barraclough TG, Savolainen V. 2011. Testing Darwin's naturalization hypothesis in the Azores. *Ecology Letters* **14**: 389–396.

Schmidt-Lebuhn AN, Knerr NJ, Kessler M. 2013. Non-geographic collecting biases in herbarium specimens of Australian daisies (Asteraceae). *Biodiversity and Conservation* **22**: 905–919.

Schorn C, Weber E, Bernardos R, Hopkins C, Davis CC. 2016. The New England Vascular Plants Project: 295,000 specimens and counting. *Rhodora* **118**: 324–325.

Staats M, Erkens RHJ, van de Vossenberg B, Wieringa JJ, Kraaijeveld K, Stielow B, Geml J, Richardson JE, Bakker FT. 2013. Genomic treasure troves: complete genome sequencing of herbarium and insect museum specimens. *PLoS ONE* **8**: e69189.

Stropp J, Ladle RJ, Malhado ACM, Hortal J, Gaffuri J, Temperley, WH, Skøien JO. Mayaux, P. 2016. Mapping ignorance: 300 years of collecting flowering plants in Africa. *Global Ecology and Biogeography* **25**: 1085–1096.

Syfert MM, Smith MJ, Coomes DA. 2013. The effects of sampling bias and model complexity on the predictive performance of MaxEnt species distribution models. *PLoS ONE* **8**: e55158.

Thiers B. 2016. *Index Herbariorum: A global directory of public herbaria and associated staff.* New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/.

Tobler M, Honorio E, Janovec J, Reynel C. 2007. Implications of collection patterns of botanical specimens on their usefulness for conservation planning: an example of two neotropical plant families (Moraceae and Myristicaceae) in Peru. *Biodiversity and Conservation* **16**: 659–677

van der Schoot C, Paul LK, Rinne PLH. 2014. The embryonic shoot: a lifeline through winter. *Journal of Experimental Botany* **65**: 1699–1712.

Vul E, Goodman N, Griffiths TL, Tenenbaum JB. 2014. One and done? Optimal decisions from very few samples. *Cognitive Science* **38**: 599–637.

Webb CO, Ackerly DD, Kembel SW. 2008. PHYLOCOM: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**: 2098–2100.

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* **33**: 475–505.

Webb CO, Donoghue MJ. 2005. Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes* **5**: 181–183.

Whittle T. 1970. *The Plant Hunters*. Heinemann, London.

Willis CG, Ellwood ER, Primack RB, Davis CC, Pearson KD, Gallinato AS, Yost JM, Nelson G, Mazer SJ, Rossington NL *et al.* 2017a. Old plants, new tricks: phenological research using herbarium specimens. *Trends in Ecology & Evolution* **32**: 531–546.

Willis CG, Law E, Williams AC, Franzone BF, Bernardos R, Brun L, Hopkins C, Schorn C, Weber E, Parks DS *et al.* 2017b. CrowdCurio: an online crowdsourcing platform to facilitate climate change studies using herbarium specimens. *New Phytologist* **215**: 479–488.

Wolf A, Anderegg WRL, Ryan SJ, Christensen J. 2011. Robust detection of plant species distribution shifts under biased sampling regimes. *Ecosphere* **2**: 115.

Wolkovich EM, Davies TJ, Schaefer H, Cleland EE, Cook BI, Travers SE, Willis CG, Davis CC. 2013. Temperature-dependent shifts in phenology contribute to

781 the success of exotic species with climate change. *American Journal of Botany*
782 **100**: 1407–1421.
783 **Yessoufou K, Daru BH, Davies TJ. 2012.** Phylogenetic patterns of extinction risk in the
784 Eastern Arc ecosystems, an African biodiversity hotspot. *PLoS ONE* **7**: e47082.
785 **Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG,**
786 **McGlinn DJ, O'Meara BC, Moles AT, Reich PB, *et al.* 2014.** Three keys to the
787 radiation of angiosperms into freezing environments. *Nature* **506**: 89–92.
788
789

LEGENDS TO FIGURES

Fig. 1 Spatial bias in herbarium collections. Geographic distribution of herbarium collecting activity depicting the spatial variation in sampling effort using Delaunay polygon tiles for (a) Australia (857,245 locales), (b) South Africa (n = 61,130 locales), and (c) New England (n = 130, 374 locales). Hotspots (red) and coldspots (blue) of herbarium sampling within quarter degree grids for (d) Australia, (e) South Africa and (f) New England. The hotspots and coldspots are the top and lowest 2.5% quantiles respectively of the number of specimens per locale.

Fig. 2 Comparison of geographic sampling bias of herbarium records in relation to (a) the minimum distance to roads, (b) minimum distance to herbaria (b), and (c) regional altitudes at sampling locales. Black lines in (a) and (b) correspond to sampling locales and red indicates an equal number of random points generated 1000 times. Dark grey shading in (c) corresponds to sampling locales in relation to the regional altitudes *i.e.*, all other altitudes (in red) for all three floras, Australia (left), South Africa (middle) and New England (right). Dotted line in (c) indicates altitude at 500 m above sea level.

Fig. 3 Timeline of herbarium specimen collection density in relation to major historical events in time (indicated in red text) for the three floras: Australia, South Africa and New England. Analysis of phylogenetic structure through time by binning sequences of collection dates into decades and testing for overdispersion *vs*. clustering, are indicated in black font. The red trend line indicates the gross domestic product of each region.

Fig. 4 Temporal biases in herbarium collections. (a) Comparison of density plots of collection dates by seasons of the year of herbarium records (blue line) with the dates spanning the entire duration of collection (red line); blue lines outside the red lines indicate over-collecting at a particular time of year, and (b) Distribution of collection dates by days of the week for the three floras. Australia (n = 4,579,321 collection dates), South Africa (n = 771,991 collection dates), and New England (n = 562,587 collection dates).

821 **Fig. 5** Assessment of bias in plant traits. (a) growth duration, (b) growth form, (c) height,
822 and (d) extinction risk, for the floras of Australia (left pane), South Africa (middle pane)
823 and New England (right pane).
824
825 **Fig. 6** Distribution of phylogenetic bias, the tendency of closely related species to be
826 similarly collected in herbarium records for three floras: (a) Australia, (b) South Africa,
827 and (c) New England. Collecting effort is not phylogenetically random, but tends to be
828 clustered in few selected lineages. The color scales correspond to the log numbers of
829 specimens per species and ranges from red (low number of specimens per species) to blue
830 (high number of specimens per species).
831
832 **Fig. 7** Phylogenetic bias in collection frequency for exemplar families in New England
833 flora. Phylogenetic bias is indicated by significant phylogenetic signal in at least one of
834 three metrics (Abouheif's C_{mean}, Blomberg's K and Pagel's λ. The color bar illustrates
835 values within families: log numbers of specimens per species and ranges from red (low
836 number of specimens per species) to blue (high number of specimens per species).
837
838 **Fig. 8** Collector bias in herbarium collections. The number of herbarium specimens
839 amassed per collector for three regional floras in (a) Australia, (b) South Africa, and (c)
840 New England. The top five collectors in each flora are highlighted in red. Numbers
841 within parentheses correspond to lifespans of the collectors, with collectors that have died
842 highlighted in red and currently living ones in black.
843
844

845 **Table 1.** Model coefficients for multiple regressions of collecting effort in the number of specimens collected per locality.

AUSTRALIA	Predictors (log_{10}-transformed)	Percentage of variance explained (%)	P values	Model adjusted R^2	Model slope	Model intercept
	Distance to roads	0.14	0.001	0.4571	-0.02	11.45
	Distance to herbaria	45.03	0.001		-0.89	
	Human population density	0.50	0.001		0.11	
	Altitude	0.041	0.001		-0.046	
SOUTH AFRICA	Predictors (log_{10}-transformed)	Percentage of variance explained (%)	P values	Model adjusted R^2	Model slope	Model intercept
	Distance to roads	0.00001	0.0003	0.3075	-0.011	11.33
	Distance to herbaria	29.13	0.001		-0.73	
	Human population density	0.0009	0.001		-0.03	

	Altitude	1.62	0.001		-0.15	
NEW ENGLAND	Predictors (log_{10}-transformed)	Percentage of variance explained (%)	P values	Model adjusted R^2	Model slope	Model intercept
	Distance to roads	0.07	0.0009	0.17	0.13	7.03
	Distance to herbaria	12.3	0.001		-0.87	
	Human population density	4.68	0.001		0.30	
	Altitude	0.04	0.001		0.046	

846

847

848

Table 2. Results of the tests of phylogenetic signal in the number of specimens collected per species using three methods (Abouheif's C_{mean}, Blomberg's K and Pagel's λ). Phylogenetic data is derived from Zanne *et al.* (2014). All tests are based on 1000 randomizations. **P < 0.001; *P < 0.01; NS, P > 0.05

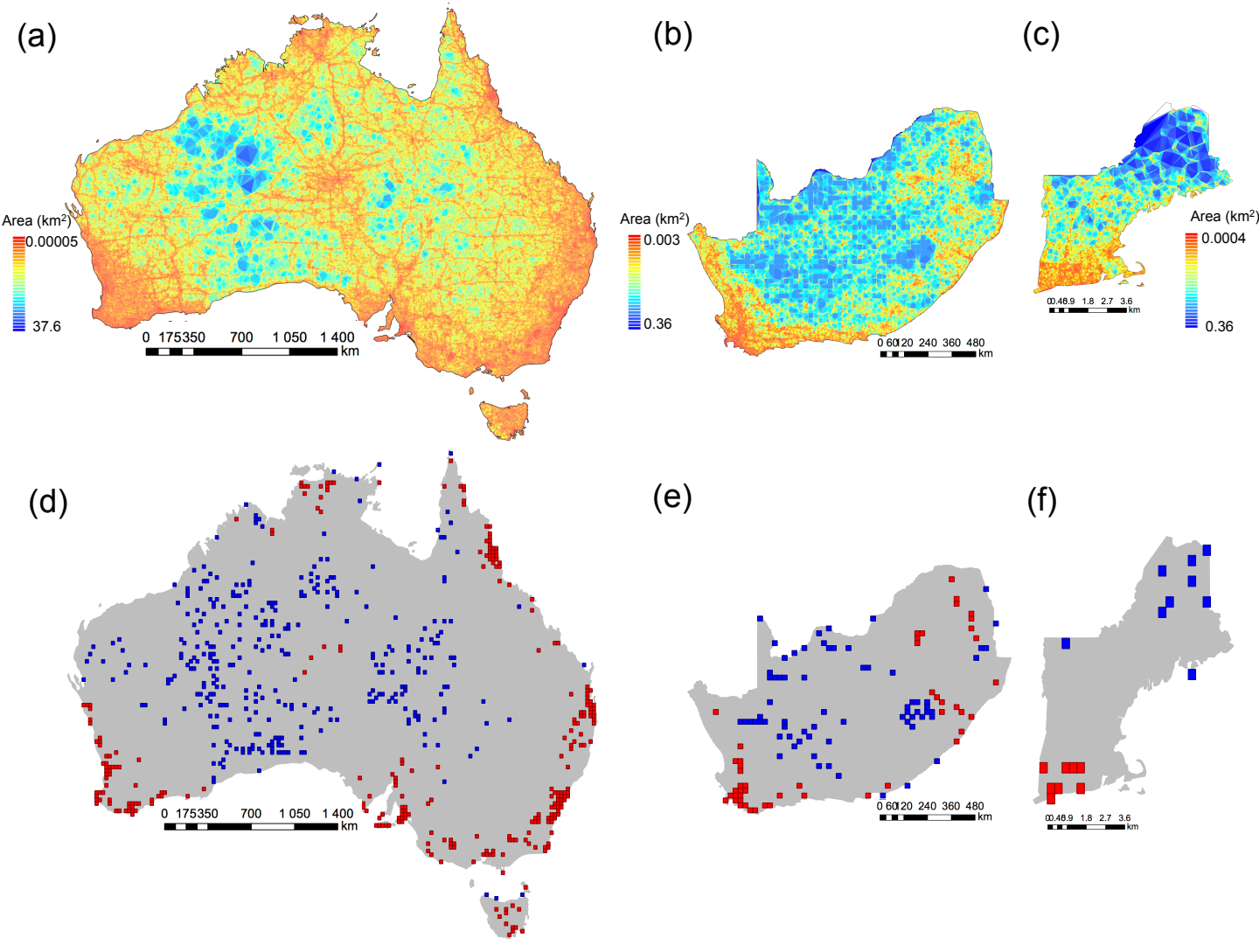
	Australia (n = 5814 species)	South Africa (n = 3568 species)	New England (n = 4269 species)
Abouheif's C_{mean}	0.12**	0.15**	0.12**
Blomberg's K	0.00085^{NS}	0.0013^{NS}	0.0030^{NS}
Pagel's lambda	0.18**	0.32**	0.29**

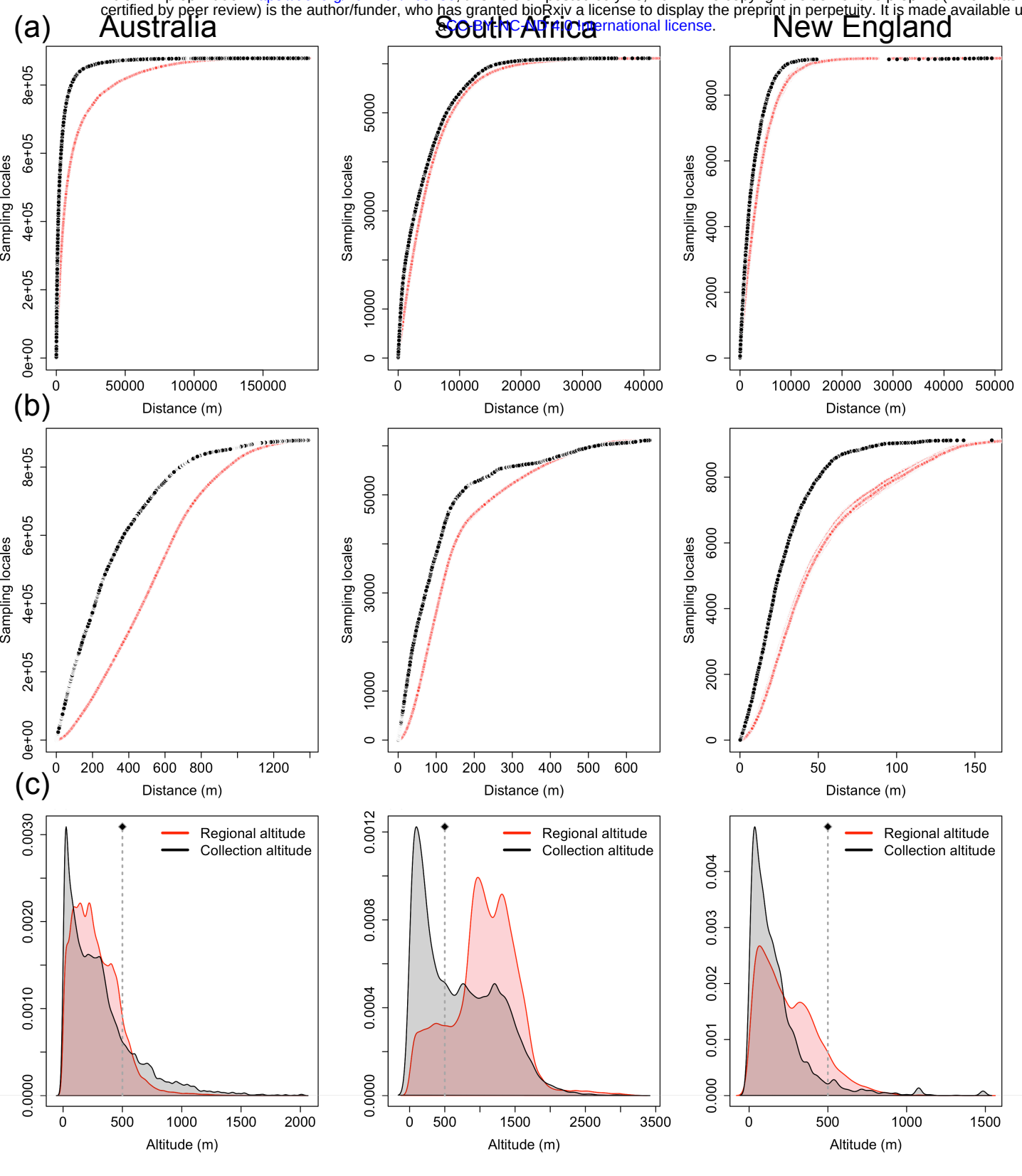
855 **Table 3** Multiple regressions of phylogenetic generalized least squares of collecting effort (frequency) of herbarium specimens with
856 phylogenetic metrics of species uniqueness.

Australia	Predictors (log_{10}-transformed)	Percentage of variance explained (%)	P values	Model adjusted R^2	Model slope	Model intercept
	BL	1.36	0.7	0.049	0.035	4.37
	ED	0.2	0.008		0.44	
	EDGE	3.75	<0.001		-1.23	
South Africa	Predictors (log_{10}-transformed)	Percentage of variance explained (%)	P values	Model adjusted R^2	Model slope	Model intercept
	BL	0.47	0.3	0.09	-0.063	3.63
	ED	0.000015	0.001		0.63	
	EDGE	8.89	<0.001		-1.3	
New England	Predictors (log_{10}-transformed)	Percentage of variance explained (%)	P values	Model adjusted R^2	Model slope	Model intercept

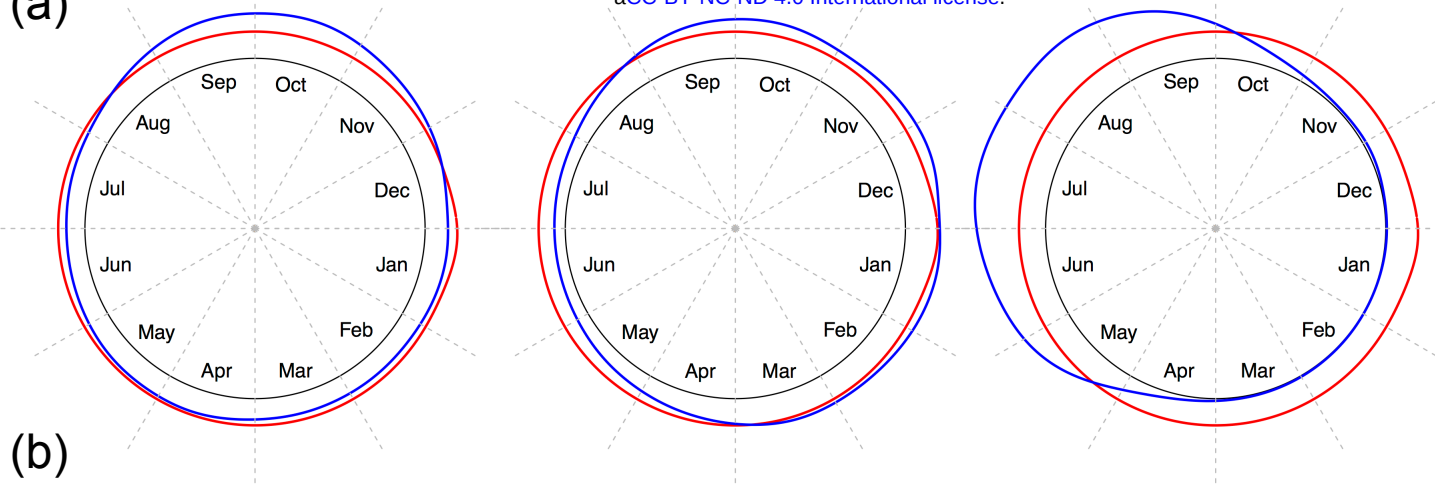
	BL	0.09	0.94	1.70E-02	-0.0052	3.89
	ED	0.054	0.0045		0.79	
	EDGE	1.87	<0.001		-2.28	

857

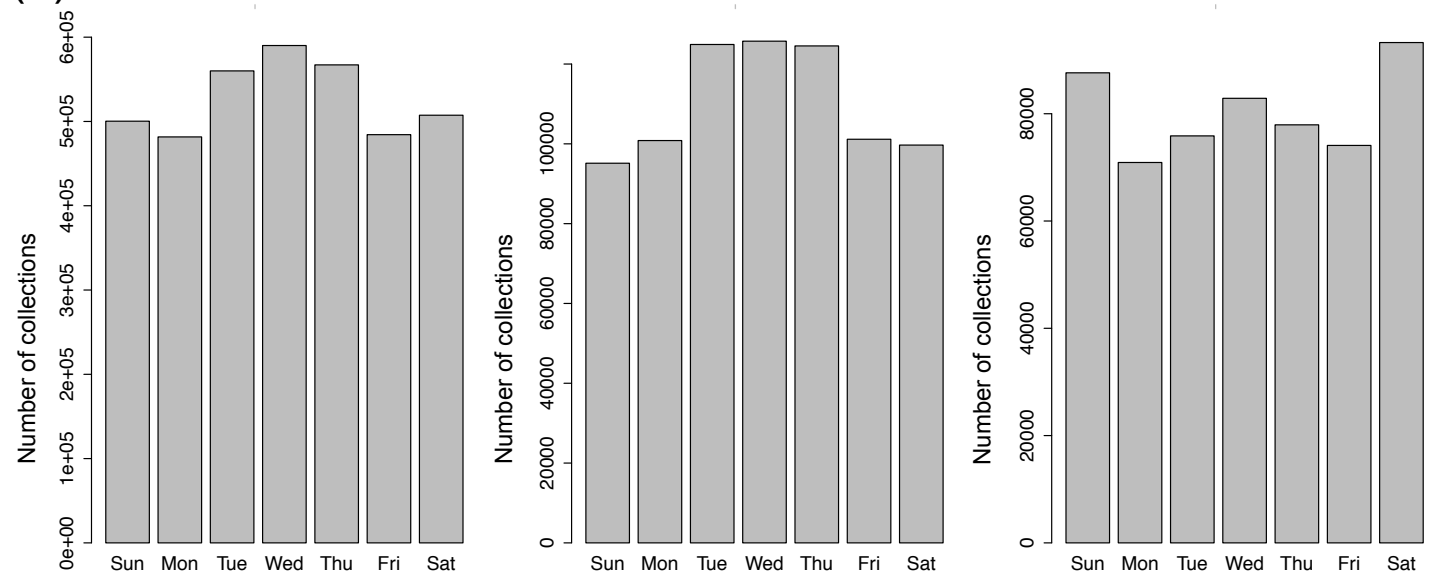




(a)



(b)

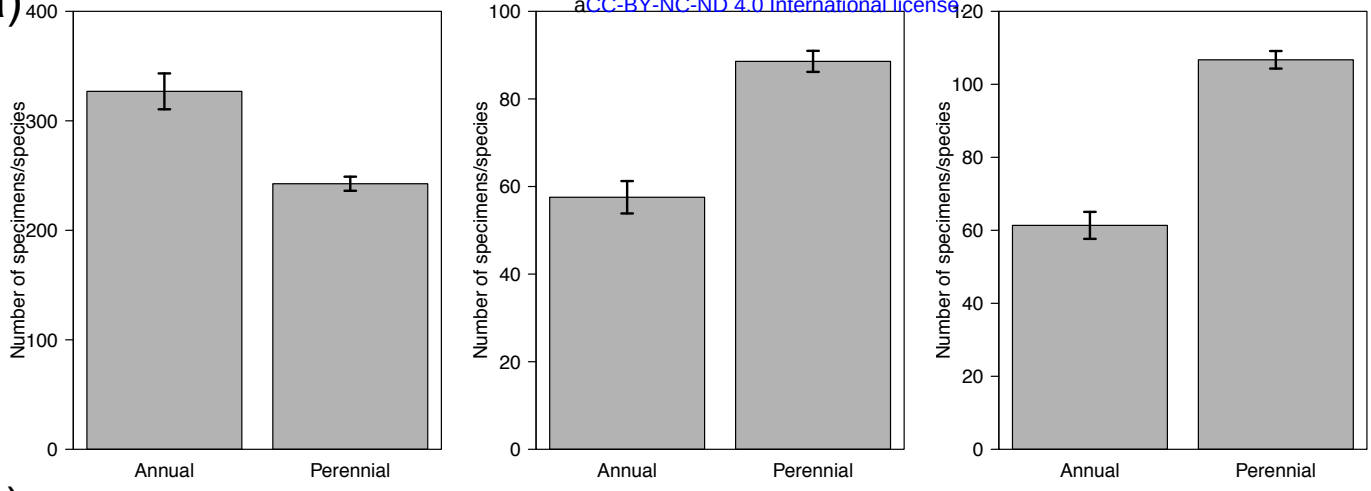


Australia
(1664 to 2016)

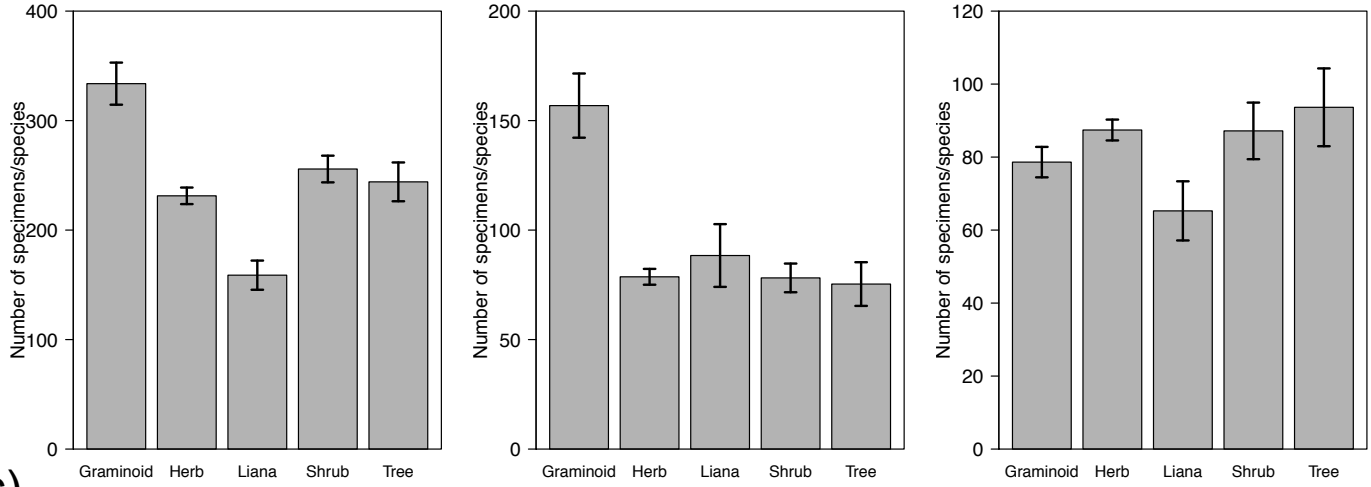
South Africa
(1656 to 2016)

New England
(1687 to 2016)

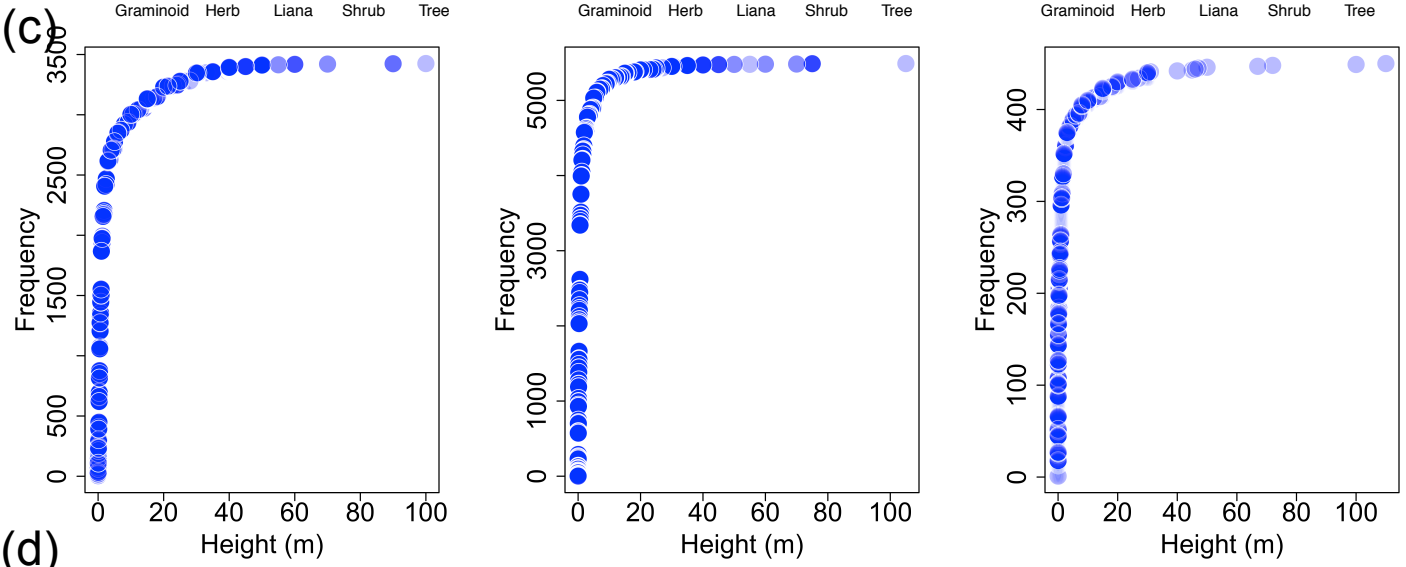
(a)



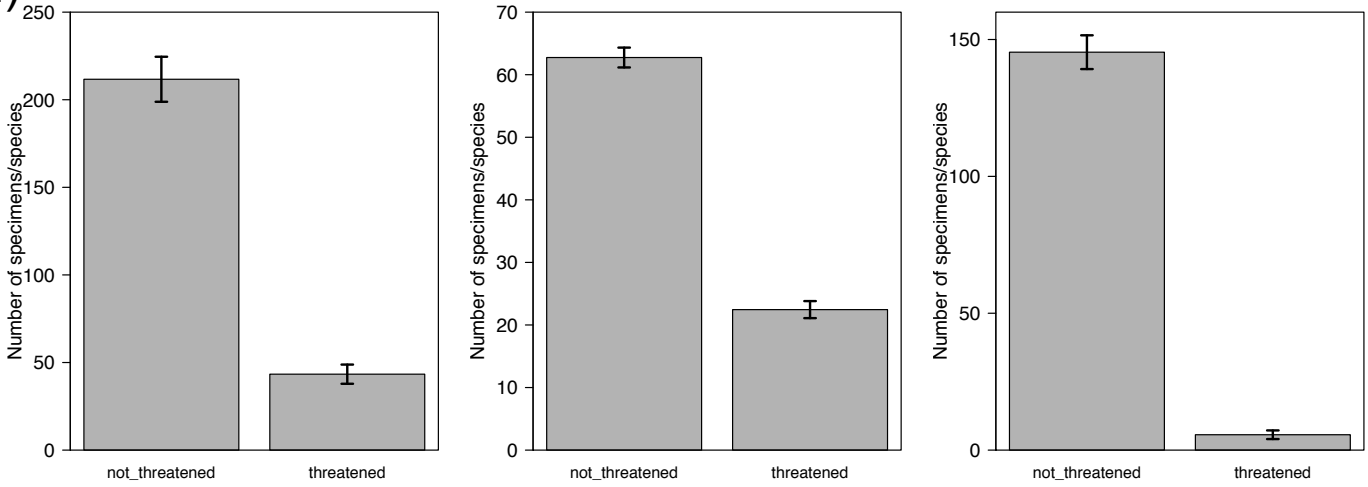
(b)

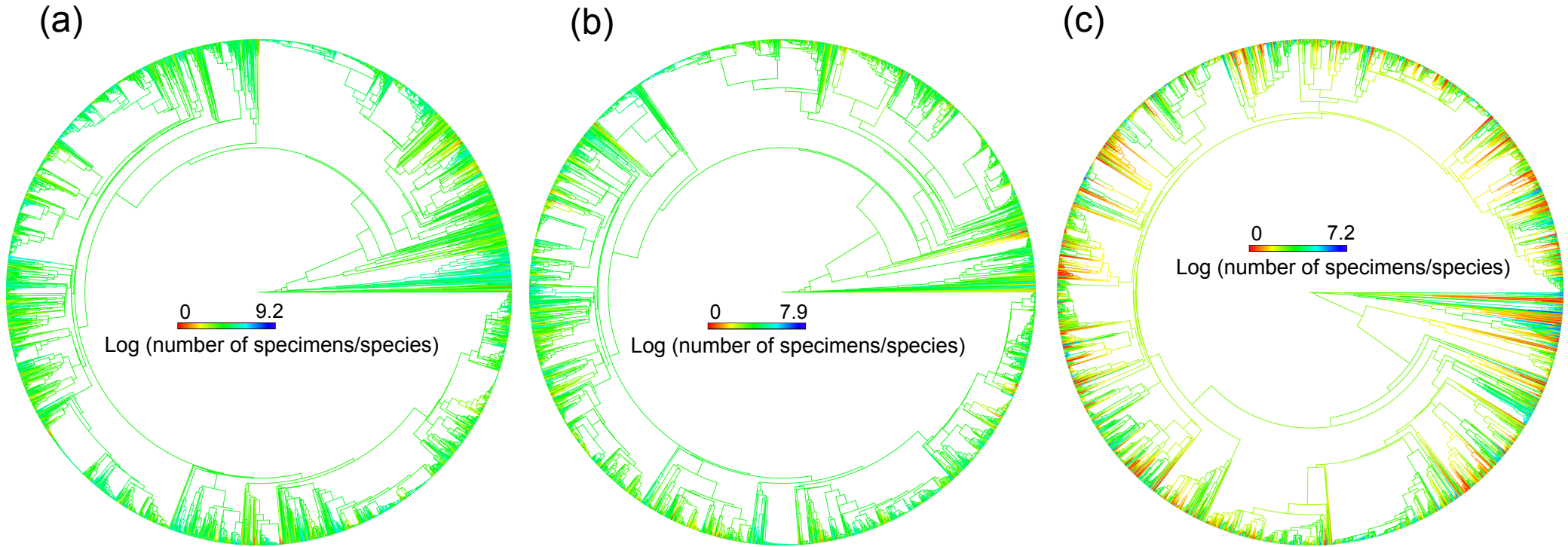


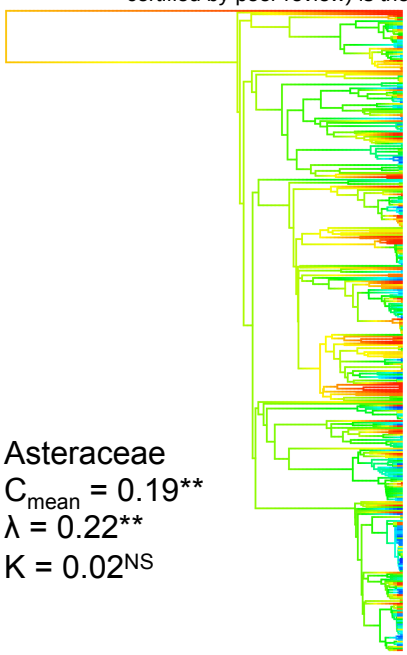
(c)



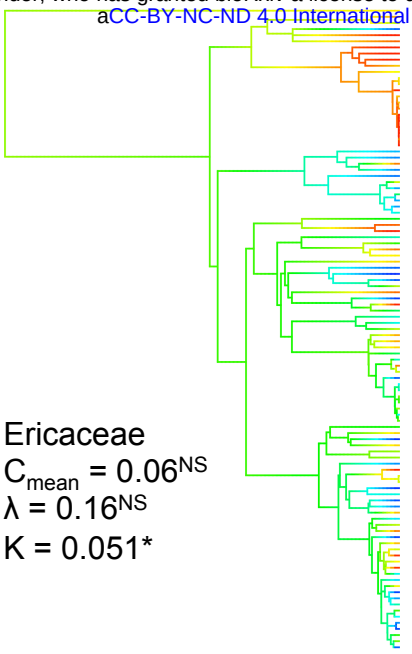
(d)



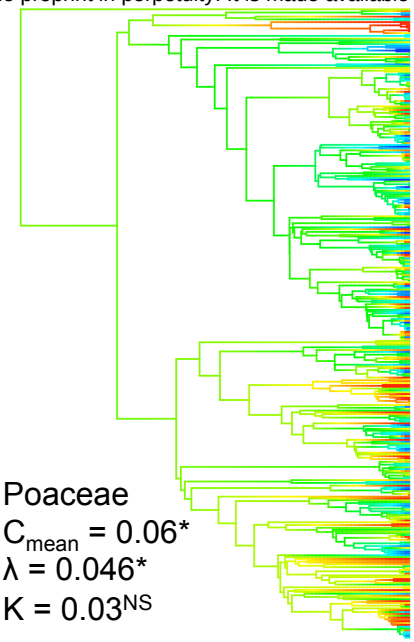

(a)
0
9.2
Log (number of specimens/species)
(b)
0
7.9
Log (number of specimens/species)
(c)
0
7.2
Log (number of specimens/species)



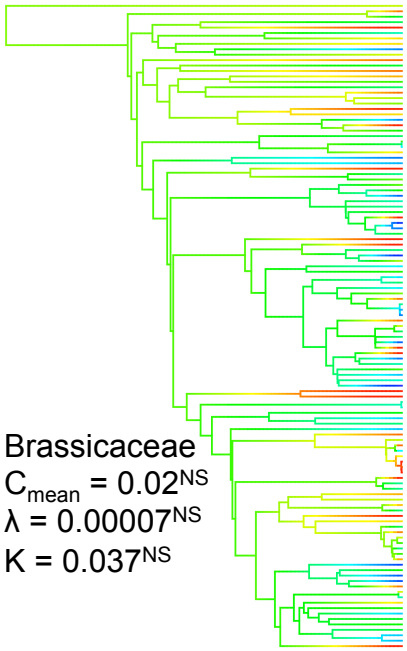
Asteraceae
C_{mean} = 0.19**
λ = 0.22**
K = 0.02^{NS}



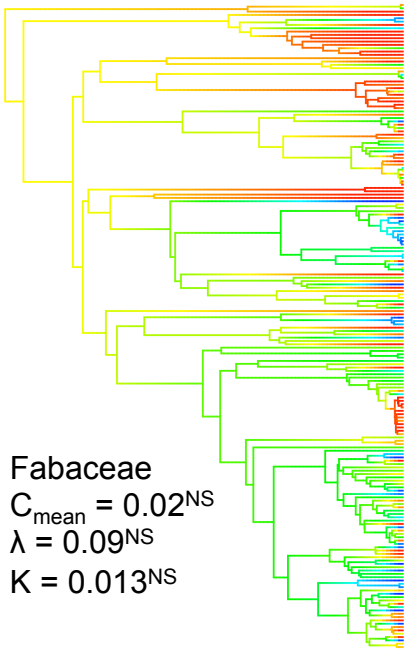
Ericaceae
C_{mean} = 0.06^{NS}
λ = 0.16^{NS}
K = 0.051*



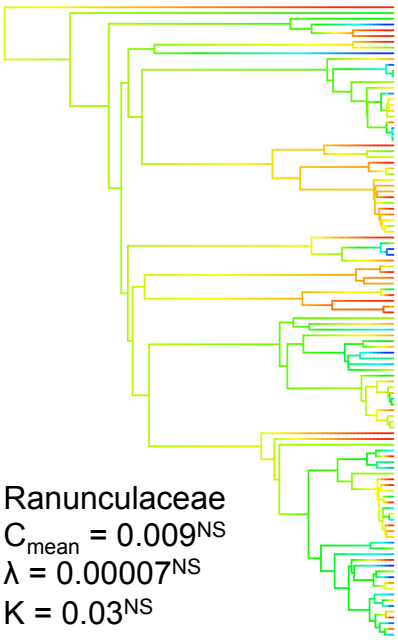
Poaceae
C_{mean} = 0.06*
λ = 0.046*
K = 0.03^{NS}



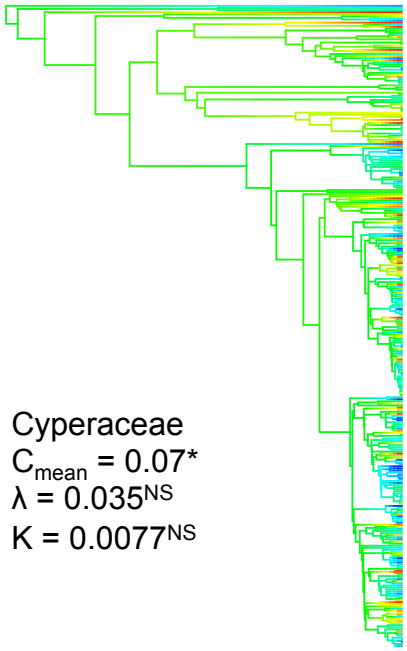
Brassicaceae
C_{mean} = 0.02^{NS}
λ = 0.00007^{NS}
K = 0.037^{NS}



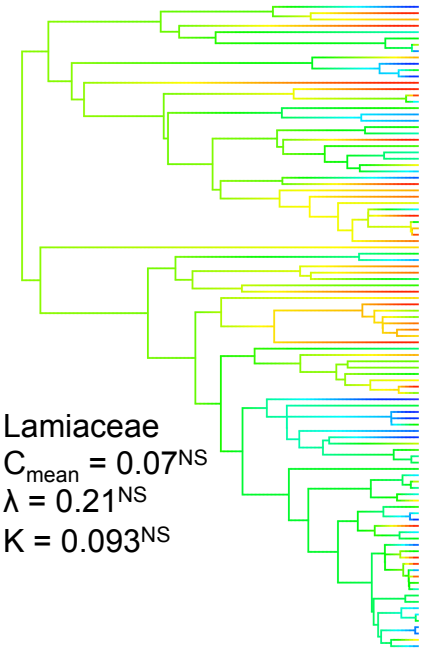
Fabaceae
C_{mean} = 0.02^{NS}
λ = 0.09^{NS}
K = 0.013^{NS}



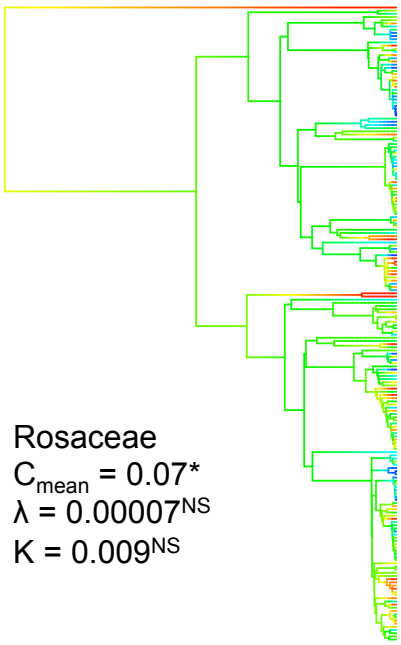
Ranunculaceae
C_{mean} = 0.009^{NS}
λ = 0.00007^{NS}
K = 0.03^{NS}



Cyperaceae
C_{mean} = 0.07*
λ = 0.035^{NS}
K = 0.0077^{NS}

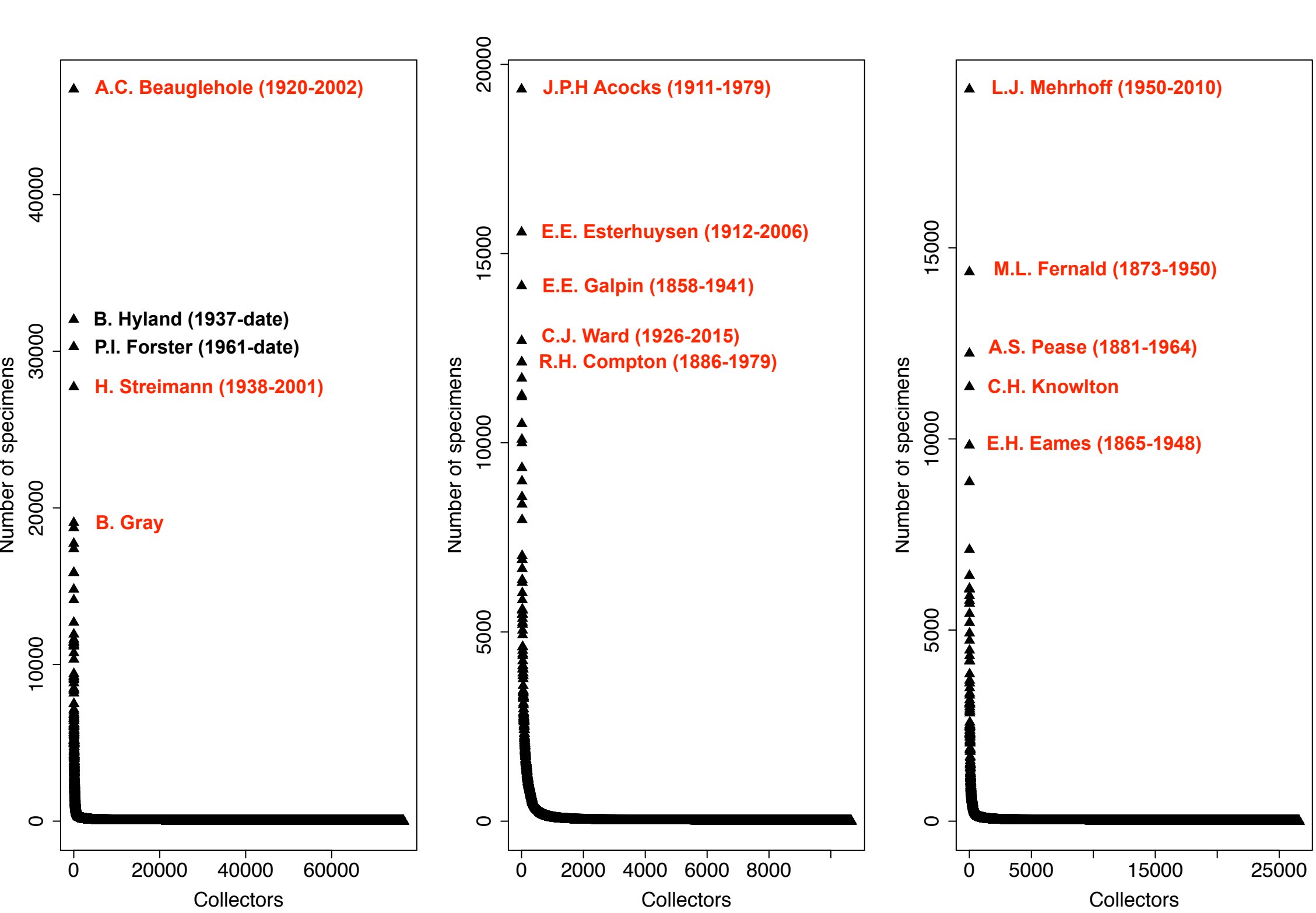


Lamiaceae
C_{mean} = 0.07^{NS}
λ = 0.21^{NS}
K = 0.093^{NS}



Rosaceae
C_{mean} = 0.07*
λ = 0.00007^{NS}
K = 0.009^{NS}

Low High


A.C. Beauglehole (1920-2002)
B. Hyland (1937-date)
P.I. Forster (1961-date)
H. Streimann (1938-2001)
B. Gray
Number of specimens
0
10000
20000
30000
40000
0
20000
40000
60000
Collectors
J.P.H Acocks (1911-1979)
E.E. Esterhuysen (1912-2006)
E.E. Galpin (1858-1941)
C.J. Ward (1926-2015)
R.H. Compton (1886-1979)
Number of specimens
0
5000
10000
15000
20000
0
2000
4000
6000
8000
Collectors
L.J. Mehrhoff (1950-2010)
M.L. Fernald (1873-1950)
A.S. Pease (1881-1964)
C.H. Knowlton
E.H. Eames (1865-1948)
Number of specimens
0
5000
10000
15000
0
5000
15000
25000
Collectors