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SUMMARY

1. Non-random collecting practices may bias conclusions drawn from analyses of herbarium records. Recent efforts to fully digitize and mobilize regional floras online 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, USA (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 biological spring and summer; 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 very few individuals. We hypothesize that these mega-collectors, and along with their associated preferences and idiosyncrasies, 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 to the extent possible.

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 declining 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,b).

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 scientific 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, all of 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 are unavailable, in whole or in part, on GBIF. Thus, Meyer *et al.*'s (2016) assessment of biases may itself be biased, or may inaccurately reflect biases in more complete, regional botanical collections that have been more fully mobilized. Furthermore, over two-thirds of the plant records in GBIF are not tied to physical specimens, and thus cannot be easily 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 predominantly 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 AU. 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, 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). This online tool corrects and standardizes plant names against reference taxonomies, such as Missouri Botanical Garden's Tropicos (http://tropicos.org/) database or the PlantList (http://theplantlist.org/). Second, we removed specimens that were duplicates from the same collection locality and date; specimens with clearly erroneous locations (*i.e.*, in oceans); specimens with zero coordinates and occurrences that fell outside the boundaries of our study; specimens missing exact collection date 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: 24% of the AU records (31,966 taxa; 2,958,195 records); 49% (20,824 taxa, 1,008,206 records) from SA; and 75% (3719 taxa, 661,370 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 derives from the publicly available Digital Chart of the World (http://maproom.psu.edu/dcw/), which was compiled by the US Defense Mapping Agency from 1:1,000,000 scale paper maps (ESRI, 1992). All roads appearing at this scale were included in our analyses. Although this dataset includes only larger roads and has not been updated since 1992, it likely represents the most comprehensive digital record of roads around the world. We

148 then compared these distances to those generated by a null model (1000 iterations) in
149 which the same number of sample points was randomly (Poisson) distributed across each
150 geographic region. Third, we mapped geographic biases in sampling density, defined as
151 areas of excessive (hotspots) or insufficient (coldspots) collection (Hijmans *et al.*, 2000).
152 Hotspots and coldspots were determined at a spatial grain of 0.25° × 0.25° based on the
153 number of specimens per grid cell, and identified using the 2.5% threshold (Ceballos &
154 Ehrlich, 2006; Orme *et al*., 2005; Daru *et al.,* 2015), based, respectively, on the 97.5th and
155 2.5th percentile values in the number of specimens collected per grid cell. Spatial distance
156 calculations were computed with the functions *dist2Line* and *spDists* in the R packages *sp*
157 (Bivand *et al*., 2013) and *geosphere* (Hijmans, 2015), respectively. In our final predictive
158 model of sampling density, we also included human population density (CIESIN, 2016),
159 sampling localities, infrastructure (distance to herbaria and roads), number of specimens
160 collected, and elevation
161
162 ***Temporal bias***
163 For each regional flora, we explored bias at several temporal scales. Collection dates
164 ranged from 20 May 1664 to 9 January 2016 (AU), 15 November 1656 to 6 June 2016
165 (SA), and 28 July 1687 to 4 May 2016 (NE). We hypothesized that collectors tended to
166 avoid fieldwork during unfavorable conditions (*e.g.,* winter, wartime) or certain days of
167 the week (*e.g.*, weekdays for non-professional botanists). To test for temporal bias, we
168 first re-coded collection dates as days of the week (Sunday = 1, Monday = 2, *etc.*), and
169 day of the year (DOY; where January 1 = 1 DOY and December 31 = 365 DOY, *etc.*).
170 We then used a Rayleigh test of directional statistics in the R package *circular*
171 (Agostinelli & Lund, 2013) to test whether each of these collection dates were randomly
172 distributed against all dates spanning the entire duration of plant collection. If $P < \alpha =$
173 0.05, we rejected the null hypothesis of temporal uniformity at scales of weeks, days of
174 the year, or decades.
175
176 ***Trait bias***
177 We used customized R scripts to harvest information on growth duration (annual *vs.*
178 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://ville-ge.ch); Plants of Southern Africa (http://plantzafrica.com); Plant Resources of Tropical Africa (http://www.prota4u.org); Flora of North America (http://efloras.org); and the USDA Plants Database (http://plants.usda.gov). We then manually checked these data for inconsistencies in terminologies for defining certain traits. For example, 'vines' *vs*. 'lianas' for climbers, 'forbs' *vs*. 'herbs' for herbaceous life forms, 'biennial' for perennial growth duration. Extinction risk assessments for each species were obtained 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 following 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 (APG III,

2009) 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; 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; Daru *et al.,* 2017) 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 clades as represented in NE: 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 (*i.e.,* 1901-1910, 1911-1920, *etc.*) 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 all 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 are available from the Harvard Forest Data Archive, dataset HF296 (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 were 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 occurred 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, with a higher density of specimens closer to herbaria (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). We also found a negative correlation between collecting effort and altitude in Australia and South Africa, suggesting a tendency for specimens to be collected at lower elevations. However, the opposite was true for NE, where more specimens tended to be collected at higher elevations than expected by chance (Table 1).

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). This peaking occurred ~100 years after peak collection activity in NE. Seasonally, specimen collections were biased toward spring and summer for all three floras, with peak collections 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 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; the opposite was true for AU where there was a greater tendency for annuals to be collected (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 84.9% of the plants collected in AU, SA and NE, respectively were less than 5 m in height (Fig. 5c).

Threatened species were collected significantly less often than non-threatened plants across all three floras (all $p < 0.001$; Fig. 5d).

Phylogenetic bias

It is possible that closely related species were collected similarly (either under-collected or over-collected) than expected by chance. We tested this assumption phylogenetically and found a significant, but weak phylogenetic signal in the abundance of specimens per species across all three floras (Table 2). Specifically, closely related species tended to have a more similar number of specimens than expected (Table 2; Fig. 6). This phylogenetic bias was strongest in SA (Abuoheif's $C_{mean} = 0.15$ and $\lambda = 0.32$; both $p < 0.01$, but $K = 0.0013$ [NS]). For instance, in SA, collections from the genus *Protea* averaged 115 specimens per species whereas only two specimens were collected for species in the genus *Rytigynia* on average. Most *Agoseris* in NE were represented by < 10 specimens per species, whereas many fern genera were represented by high specimen numbers (*e.g., Onoclea* with 845 specimens/species). Australian collections showed the weakest phylogenetic bias (Abuoheif's $C_{mean} = 0.12$ and $\lambda = 0.18$, both $p < 0.01$, but $K = 0.00085$ [NS]; Fig. 6). Phylogenetic signal varied at the family level as well in NE, with Asteraceae showing the strongest collection bias (Fig. 7), followed by Cyperaceae,

Poaceae, and Rosaceae (Table S1). These families were represented by much higher collection numbers than for other families.

EDGE was a significant predictor of collecting efforts in all three floras ($p < 0.001$), with variance ranging from 1.89% (NE) and 3.75% (AU), to 8.89% in SA. In general, EDGE species (distinctive, endangered species) were generally under-collected in terms of specimens per species (Table 3).

Lastly, floristic collecting showed a general trend of phylogenetic clustering within decades for all three floras. The collection of different clades of plants was not evenly distributed across time. NTI was significantly positive in each flora, indicating that clustering occurred near the tips of the phylogeny (Fig. 3). We only observed significant phylogenetic clustering at the deeper nodes of the phylogeny, as indicated by NRI, in SA (Fig. 3); deeper phylogenetic clustering was weak in NE and AU (Fig. 3).

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 very strongly positively correlated with the number of species they collected, suggesting that these collectors were mainly 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 a resource for botanists carrying out taxonomic and systematic research, allowing users to construct classifications of plants, verify identifications, determine the ranges and morphological

364 characteristics of species, and develop local and regional floras (Greve *et al.,* 2016). Over
365 time, new uses for specimens have arisen, and now more than ever, they are being used in
366 ways that collectors rarely imagined (Pyke & Ehrlich, 2010; Lavoie, 2013; Willis *et al.,*
367 2017a,b; Nualart *et al*., 2017; Rudin *et al.,* 2017). Accordingly, attempts to assess and
368 categorize biases inherent in these collections have been made (Rich & Woodruff, 1992;
369 Geri *et al.*, 2013; Schmidt-Lebuhn *et al.,* 2013; Meyer *et al.,* 2016; Stropp *et al.,* 2016).
370 Among these, the most comprehensive investigation is by Meyer *et al.* (2016), who
371 proposed an important conceptual framework for analyzing gaps and biases along
372 taxonomic, geographical, and temporal dimensions. Although Meyer *et al.* (2016)
373 focused more on observational records than herbarium collections, they uncovered
374 numerous biases in 'digitally accessible information' regarding plants and provided an
375 important baseline for evaluating and improving global floristic coverage in collection
376 data. However, collection biases in large geographic areas are difficult to categorize
377 because the collections data are not yet digitized, and this may skew the global patterns
378 of bias noted by Meyer *et al.* (2016). By focusing on three of the most well-collected and
379 digitized floras in the world, we reduced effects of missing or unavailable data, and most
380 importantly, could evaluate commonalities and differences in patterns of bias among
381 regional collections.

382

383 ***Spatial bias***

384 Our data confirmed the tendencies for botanists to collect along roadsides (*e.g.,* Funk &
385 Richardson, 2002), near herbaria (*e.g*., Hijmans *et al.,* 2000; Moerman & Estabrook,
386 2006; Pautasso & McKinney, 2007), in more accessible areas (Rich & Woodruff, 1992),
387 and at lower elevations. Before automobiles became common in the 1920s, botanists
388 often walked or rode domesticated animals to collection sites (Botkin, 1968; Belasco,
389 1979). As our modern infrastructure developed (*e.g.*, roads, railroads, and cities that
390 contain herbaria) and access to automobiles increased, spatial biases associated with
391 infrastructure likely increased as botanists tended to travel and collect using automobiles
392 (Everill *et al.,* 2014). Because roads are known alter local environmental conditions and
393 facilitate biological invasions (*e.g.,* Forman & Alexander, 1998; Hui *et al.*, 2003; Griffith

394 *et al.*, 2010; Li *et al*., 2014) and botanists and herbaria predominate in cities, specimens
395 collected in proximity to either are unlikely to represent a random sample across species
396 distributions. Specifically, species collected along roadsides are likely to be over-
397 represented by species that thrive with disturbance, and under-represented by forest
398 interior and wetland species that are harmed by disturbance (Gutzwiller & Flather, 2011;
399 Rivers-Moore & Cowden, 2012). As the road network continues to expand and as people
400 become evermore concentrated in cities, this bias toward collecting near roads might
401 become stronger in coming decades.
402 Collection bias towards lower elevations (< 500 m) was striking in SA and AU,
403 despite extensive collection efforts in adjacent hyper-diverse hotspots such as the
404 mountains in the Cape Fold Belt (SA), and Mount Lesueur-Eneabba (Western AU). This
405 is likely due to the presence of the arid and relatively species-poor Great Karoo Plateau
406 (SA), Great Sandy Desert (AU), which each encompass over a third of the respective
407 study sites, but account for only a small proportion of the biodiversity of each region. As
408 a result, the low-elevation collection bias in the floras may reflect actual species
409 abundance. In NE, the trend toward collecting at higher elevation might be due to the
410 strong tendency for botanists to visit the White Mountains and Mount Katadhin to collect
411 alpine species.
412 Although we realize that patterns of species richness may not be randomly
413 distributed across the landscape, accounting for underlying patterns of richness or
414 abundance is difficult because our knowledge of such patterns often derive from (and are
415 thus not independent from) these same (biased) collections. By comparing locations of
416 samples (collections) against a Poisson set of points and specimens per species, and not
417 total collection numbers, we tested only for the non-random distribution of collection
418 locations on a landscape. And indeed, we found that the collection locations were not
419 spatially distributed randomly (Poisson) on the landscape. It is also possible that
420 georeferencing might have introduced additional bias in some specimens. While
421 ascertaining the degree of accuracy of georeferenced records might be challenging
422 because such information is often unavailable, our cumulative curves are likely less
423 affected.
424

425 ***Temporal and seasonal bias***

426 Collections in AU and SA have increased through time until a few decades ago, but those
427 in NE peaked much earlier in the early 1900s. These differences between regional
428 collection activities may parallel broader societal factors influencing plant collection. In
429 NE, for example, the establishment of the New England Botanical Club during the 1890s
430 (NEBC, 1899) preceded a surge and peak in collecting activity associated with prolific
431 botanical expeditions of the region coinciding with the 'Golden Age' of plant collecting
432 in Europe and North America (Whittle, 1970; Musgrave *et al.,* 1999). In SA, collection
433 efforts began much later, peaking during the Apartheid Era (1948–1994), and declined
434 thereafter under the New Democratic Rule, concomitant with the general economic
435 decline of the country and concern for public safety (Ferreira & Harmse, 2000; Lemanski,
436 2004). In AU, the mass immigration of Europeans in 1948 after World War II included
437 numerous highly skilled professionals (Price, 1998; Leuner, 2007) and coincided with an
438 enormous increase in botanical collecting. Botanical collecting may have declined more
439 recently owing to legislation in AU and SA to regulate collections activities, especially
440 those designed to protect rare and endangered species.

441 Collecting efforts within a season revealed common patterns of bias: specimens in
442 all three regions were collected overwhelmingly in biological spring and summer.
443 Sampling during these time periods likely reflects efforts to collect plants in good
444 flowering and fruiting condition. However, this seasonal bias likely overlooks key
445 developmental transitions (*e.g.*, Poethig, 2013), including bud formation, bud break, leaf
446 out, fruit development, and leaf senescence (van der Schoot *et al.,* 2014). Supporting this
447 argument, these temporal patterns were most pronounced in NE, which experiences the
448 harshest winter climates of the three regions. Plants collected during the winter season are
449 almost always in dormant condition, and often lack the leaves and reproductive structures
450 needed for taxonomic research. Collecting was also more likely during holidays and
451 school vacations in NE and AU.

452

453 ***Trait bias***

In all three regions, short to medium-height species were collected more frequently than tall species (>5 m). This pattern is presumably related to the relative ease of collecting specimens from shorter, often herbaceous, species, and because reproductive materials are more accessible and potentially more abundant. Specimens of trees with woody twigs also are typically 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). However, it is also true that collectors sometimes oversample rare or threatened species because of their higher scientific value and avoid the more common ones (Garcillán *et al.,* 2008; Garcillán & Ezcurra, 2011; Minteer *et al.,* 2014). Regardless of past practices and contemporary 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 great care in collecting rare plants is important, under-collection of rare species may lead to incorrect extinction risk assessments (*i.e.,* 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 herbarium collections in AU; the opposite was observed in SA and NE. There was also a high representation of graminoids in herbarium collections in AU and SA. 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 and has an abundance of shade tolerant shrubs and perennial herbs. Graminoids are also considered harder to identify and may be avoided by non-specialists. 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

485 species abundance and range, but can also lead to erroneous estimations of functional
486 diversity and ecosystem services, especially for studies relying on specimen databases
487 (Schmidt-Lebuhn *et al.,* 2013). Whether herbarium records represent true patterns of
488 abundance and diversity remains difficult to untangle from human-mediated collecting
489 biases. However, the large differences among the three floras in the traits of botanical
490 collections almost certainly is reflective of genuine difference species abundance and
491 diversity.

492

493 ***Phylogenetic bias***

494 Taxonomic biases in collection data have been reported previously (Hijmans *et al.,* 2000;
495 Tobler *et al.,* 2007; Meyer *et al*., 2016). However, our study is the first, to our knowledge,
496 to demonstrate explicit evidence for phylogenetic bias in herbarium collections.
497 Collection efforts in all three floras were concentrated in particular clades.
498 Previous examinations of taxonomic bias (*e.g.*, Hijmans *et al.,* 2000; Tobler *et al.,*
499 2007; Meyer *et al.,* 2016) did not use the full complement of modern phylogenetic
500 methods that included patterns of evolutionary relatedness, and so were limited in their
501 ability to detect details of taxonomic bias. In contrast, our phylogenetic approach not only
502 captured taxonomic bias in favor of certain entire families (*e.g.*, Asteraceae, Cyperaceae,
503 Poaceae, and Rosaceae in NE), but revealed that evolutionarily distinct and globally
504 endangered species are underrepresented in herbarium records relative to more common
505 species. Such evolutionarily distinct species, which are threatened with extinction,
506 represent important targets for future documentation or prioritization for conservation
507 (Isaac *et al.,* 2007). However, collecting threatened taxa requires specialized training,
508 compliance with regulation, and awareness of actual collection needs (Minteer *et al.,*
509 2014). Increasingly, DNA barcoding approaches, using small samples from living tissues,
510 combined with GPS-referenced digital photography might be an avenue to document
511 such species.

512

513 ***Collector bias***

In all three regions, a large percentage of specimens was gathered by only a few collectors (Fig. 8). Thus, the habits and preferences of a few individuals likely shaped the establishment and formation of these herbarium collections. These 'founder effects' propagate across all the dimensions of collection bias examined above, and help us to understand past collection behavior. For example, certain collectors may focus on geographically circumscribed floristic zones, often near their place of residence, workplace, or vacation home, and sample all species found therein, whereas others may focus on collecting species of a particular clade across various regions. Professional botanists may tend to collect specimens on weekdays during any time of the year, whereas amateurs and faculty with teaching responsibilities may focus their efforts on weekends and vacation months. Those interested in function and physiology may only collect plants of certain habits or life-histories (*e.g.,* carnivorous, aquatic plants, or succulent plants). These effects would likely be compounded when associated with mega-collectors. For instance, the Harvard University Herbaria's collection of Asian, especially woody plants, was largely built by a few collectors and dates to the early establishment of the institution, and continues to attract scholars of the flora of Asia and their collections. Investigating the historical significance and potential biases created and propagated by these early pioneers is a ripe area for future research.

Future collecting

To ensure that herbaria continue to be vital centers for research beyond their importance to taxonomy and systematics, herbarium directors and collectors should account for and, whenever possible, reduce biases in plant collections. Biases can be accounted for to a degree using statistical approaches (Droissart *et al.*, 2012; Feeley, 2012; Grass *et al.*, 2014; Engemann *et al*., 2015). For instance, inclusion of covariates for distances of collections from herbaria, roads, or other infrastructure (McCarthy *et al.,* 2012), using rarefaction methods to predict abundances (Schmidt-Lebuhn *et al.,* 2013), or including the collector as a variable, would improve species distribution models and associated predictions of future changes across a flora. To remedy such biases, future collecting expeditions should focus on "coldspots" of collection intensity (Hijmans *et al.,* 2000),

that is, places that are under-represented in collections. Although some of the coldspots we identified likely represent more inaccessible environments, they often correspond to unique ecosystems, including the Succulent Karoo of SA and the Great North Woods in northern NE that contain many species of interest. Some of these coldspots also may indicate areas where herbarium specimens have yet to be mobilized, providing additional focus for efforts to make collection data widely available. Equally important is the need to continue modern collecting in well-established "hotspots" so that there are multiple temporal benchmarks against which change can be measured. This is particularly true for non-native invasive species that have rapidly expanding distributions and vulnerable native species that have ranges that are collapsing.

Phylogenetic and trait biases can be alleviated by targeting collection efforts where we know species have been under-collected. Temporal bias is more difficult to address, as we cannot add to historic collections. However, we can make efforts to maintain consistent regional botanical records by conducting field surveys at regular intervals. Also, by linking multiple herbaria into larger digital databases, the temporal biases of individual herbaria can be smoothed out to some extent.

We acknowledge that some of the biases also may be attributed to longstanding curation practices at the herbariums themselves. As herbarium collections were amassed for qualitative floristic, taxonomic, and systematic research, duplicate specimens of common species and non-reproductive material have sometimes been discarded, sent elsewhere, or not accepted in the first place. This trend is becoming even more pronounced as many herbaria around the world are increasingly constrained by funding, labor, and space. As new uses for biological collections continue to proliferate, curation practices should also change to accommodate different avenues of research, such as climate-change biology and rare plant conservation. This will often be most effective through continued collecting of specimens to overcome past biases. And most importantly, researchers analyzing herbarium specimens in a widening array of studies needed to be aware of the biases in these collections, and apply appropriate statistical techniques.

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Supporting Information

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

Fig. S1 Analytical workflow representing the different steps in the development of this study from data compilation, collation, to statistical analysis.

Fig. S2 Relationships of the number of specimens collected per species with number of species collected in each flora for Australia (left), South Africa (middle), and New England of the USA (right).

Table S1 Results of the tests of phylogenetic signal in the number of specimens collected per species.

878 **LEGENDS TO FIGURES**

879 **Fig. 1** Spatial bias in herbarium collections. Geographic distribution of herbarium
880 collecting activity depicting the spatial variation in sampling effort using Delaunay
881 polygon tiles for (a) Australia (857,245 locales), (b) South Africa (n = 61,130 locales),
882 and (c) New England (n = 130, 374 locales). Hotspots (red) and coldspots (blue) of
883 herbarium sampling within quarter degree grids for (d) Australia, (e) South Africa and (f)
884 New England. The hotspots and coldspots are the top and lowest 2.5% quantiles
885 respectively of the number of specimens per locale.
886
887 **Fig. 2** Comparison of geographic sampling bias of herbarium records in relation to (a) the
888 minimum distance to roads; (b) minimum distance to herbaria; and (c) regional altitudes
889 at sampling locales. Black lines in (a) and (b) correspond to sampling locales and red
890 indicates an equal number of random points generated 1000 times. Dark grey shading in
891 (c) corresponds to sampling locales in relation to the regional altitudes, *i.e.,* all other
892 altitudes (in red) for all three floras, Australia (left), South Africa (middle) and New
893 England (right). Dotted line in (c) indicates altitude at 500 m above sea level.
894
895 **Fig. 3** Timeline of herbarium specimen collection density in relation to major historical
896 events in time (indicated in red text) for the three floras: Australia, South Africa and New
897 England. Analysis of phylogenetic structure through time by binning sequences of
898 collection dates into decades and testing for overdispersion *vs*. clustering, are indicated in
899 black font. The red trend line indicates the gross domestic product of each region. NRI,
900 net relatedness index; NTI, nearest taxon index.
901
902 **Fig. 4** Temporal biases in herbarium collections. (a) Comparison of density plots of
903 collection dates by seasons of the year of herbarium records (blue line) with the dates
904 spanning the entire duration of collection (red line); blue lines outside the red lines
905 indicate over-collecting at a particular time of year, and (b) Distribution of collection
906 dates by days of the week for the three floras. Australia (n = 4,579,321 collection dates),
907 South Africa (n = 771,991 collection dates), and New England (n = 562,587 collection
908 dates).

Fig. 5 Assessment of bias in plant traits: (a) growth duration; (b) growth form; (c) height; and (d) extinction risk for the floras of Australia (left pane), South Africa (middle pane) and New England (right pane). Error bars in (a), (b), and (d) represent +/- SE.

Fig. 6 Distribution of phylogenetic bias, the tendency of closely related species to be similarly collected in herbarium records for three floras: (a) Australia; (b) South Africa; and (c) New England. Collecting effort is not phylogenetically random, but tends to be clustered in few selected lineages. The color scales correspond to $\log_{10}$ numbers of specimens per species and ranges from red (low number of specimens per species) to blue (high number of specimens per species).

Fig. 7 Phylogenetic bias in collection frequency for exemplar families in New England flora. Phylogenetic bias is indicated by significant phylogenetic signal in at least one of three metrics (Abouheif's C_{mean}, Blomberg's K and Pagel's λ). The color bar illustrates values within families: $\log_{10}$ numbers of specimens per species and ranges from red (low number of specimens per species) to blue (high number of specimens per species). **P < 0.001; *P < 0.01; NS P > 0.05

Fig. 8 Collector bias in herbarium collections. The number of herbarium specimens amassed per collector for three regional floras in (a) Australia; (b) South Africa; and (c) New England. The top five collectors in each flora are highlighted in red. Numbers within parentheses correspond to lifespans of the collectors, with collectors that have died highlighted in red and currently living ones in black.

935 **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	

936

937

938

939 **Table 2**. Results of the tests of phylogenetic signal in the number of specimens collected per species using three methods (Abouheif's
940 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.
941 **P < 0.001; ^{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 λ	0.18**	0.32**	0.29**

942

943

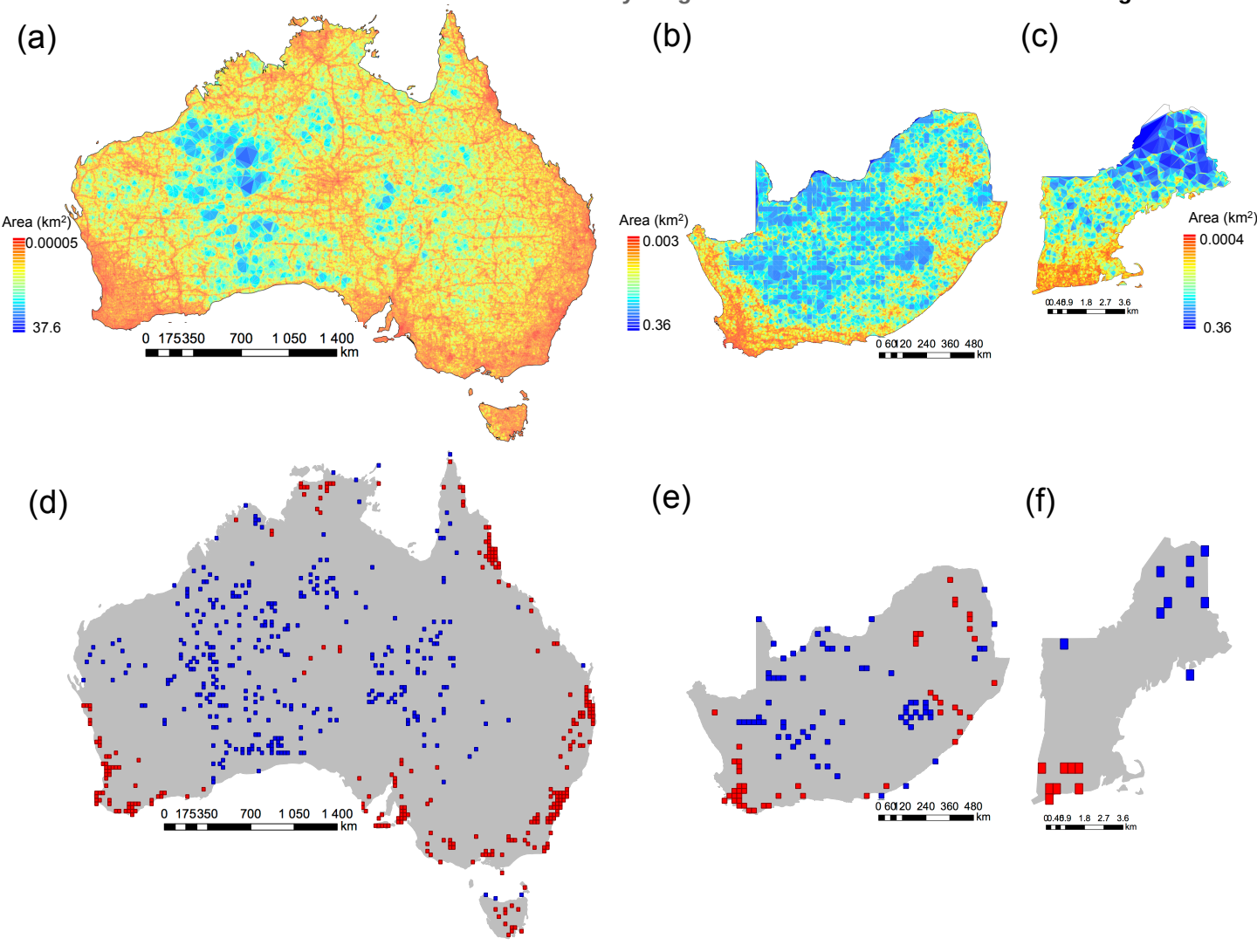
944

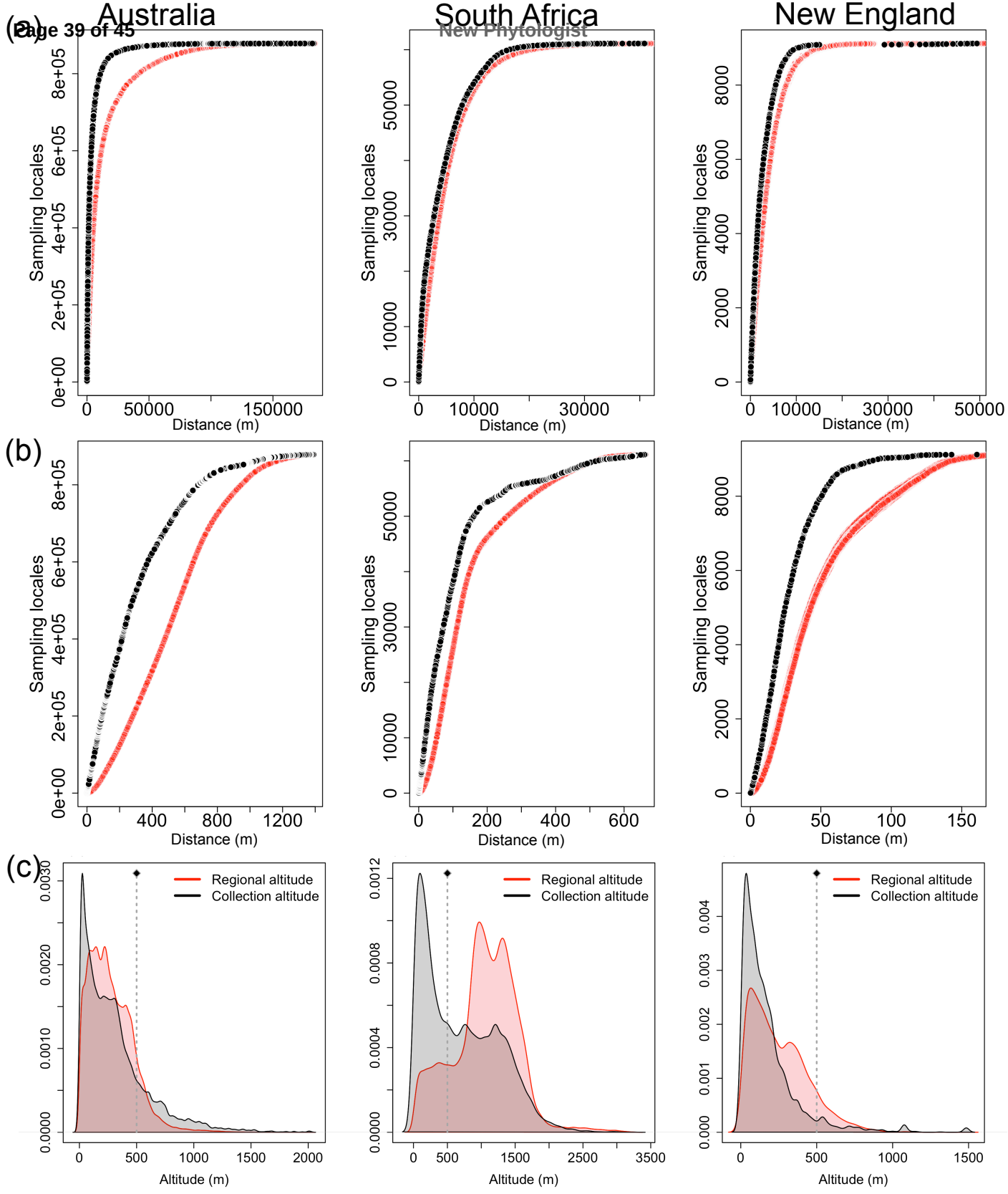
945 **Table 3** Multiple regressions of phylogenetic generalized least squares of collecting effort (frequency) of herbarium specimens with
946 phylogenetic metrics of species uniqueness. BL, terminal branch length; ED, evolutionary distinctiveness; EDGE, evolutionary
947 distinctiveness and global endangerment.

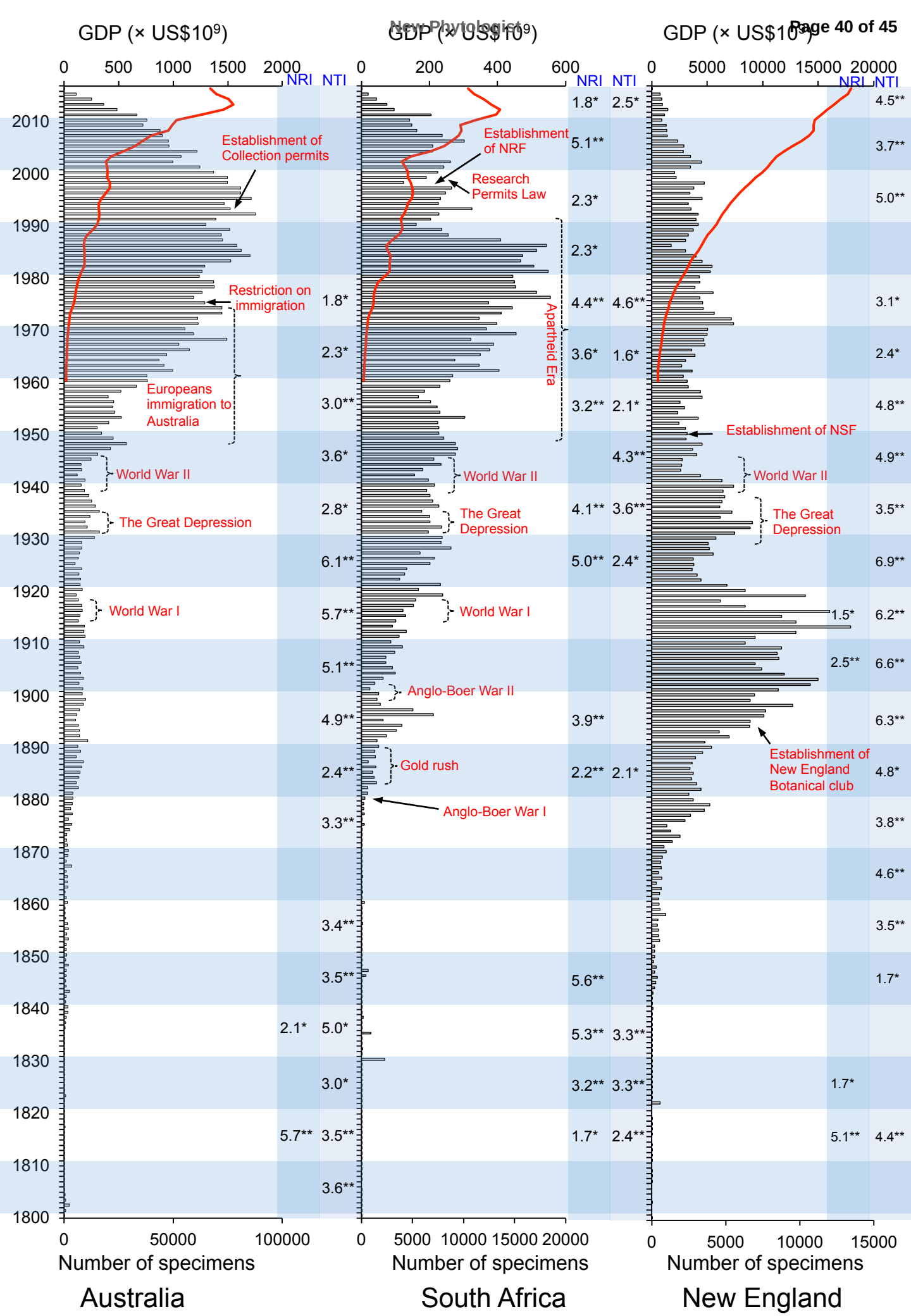
Australia	Predictors (log_{10}-transformed)	Percentage of variance explained (%)	P values	Model adjusted R^2	Model slope	Model intercept
	BL	1.36	0.7	0.049	0.035	4.37
	ED	0.2	0.008		0.44	
	EDGE	3.75	<0.001		-1.23	
South Africa	Predictors (log_{10}-transformed)	Percentage of variance explained (%)	P values	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	P values	Model adjusted R^2	Model slope	Model intercept

		explained (%)				
	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	

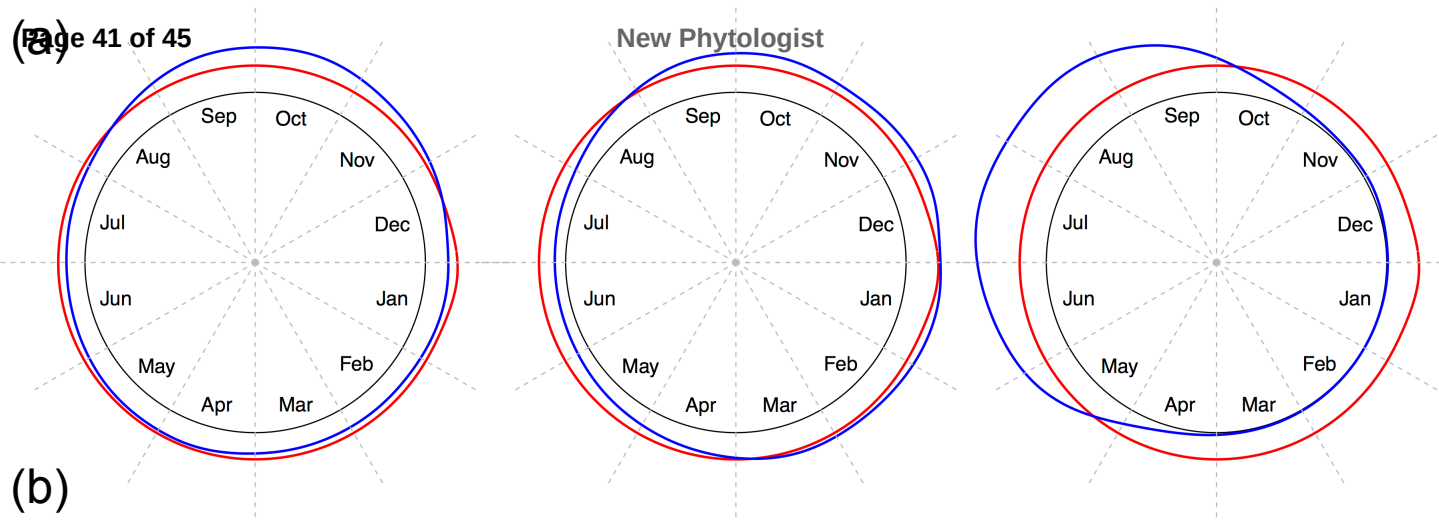
948


(a)
Area (km²)
0.00005
37.6
0 175350 700 1 050 1 400
km
(b)
Area (km²)
0.003
0.36
0 60120 240 360 480
km
(c)
Area (km²)
0.0004
0.36
00.46.9 1.8 2.7 3.6
km
(d)
0 175350 700 1 050 1 400
km
(e)
0 60120 240 360 480
km
(f)
00.46.9 1.8 2.7 3.6
km

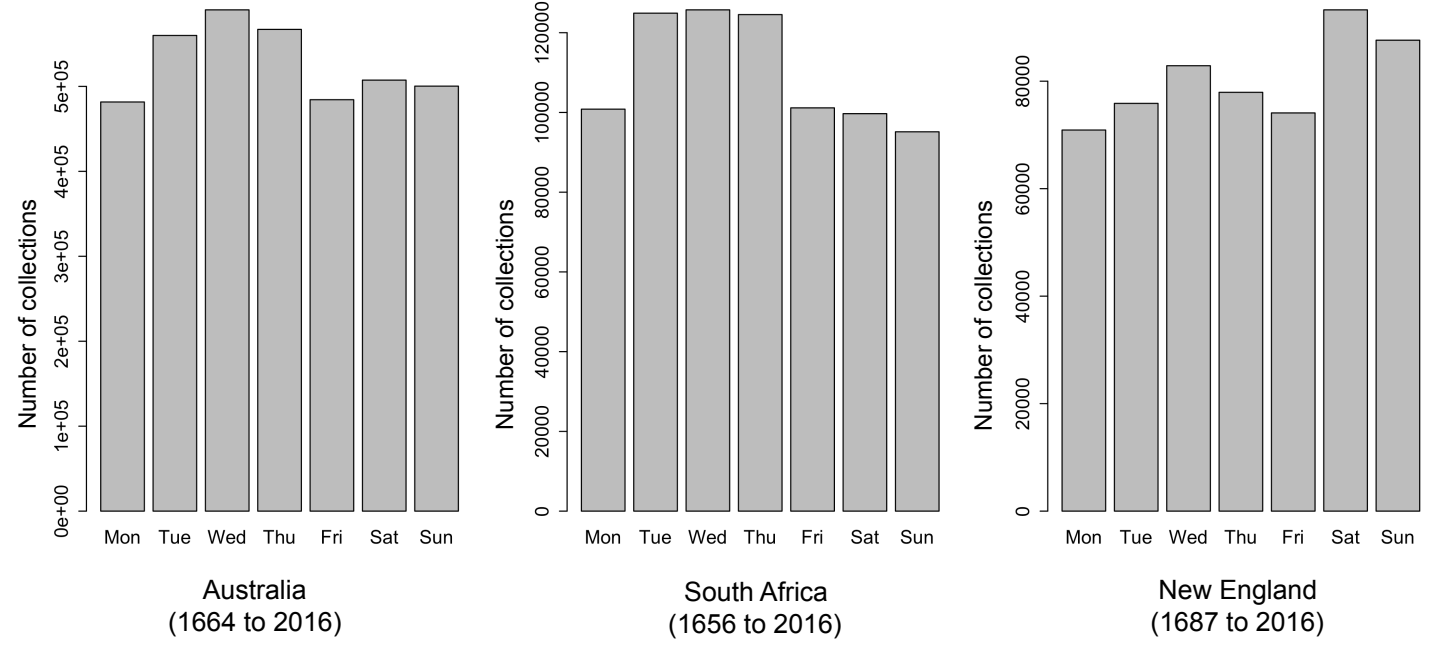

(a)
Australia
South Africa
New England
Sampling locales
Distance (m)
0e+00
2e+05
4e+05
6e+05
8e+05
0
50000
150000
10000
30000
2000
4000
6000
8000
(b)
400
800
1200
200
600
50
100
150
(c)
Regional altitude
Collection altitude
Altitude (m)
0.0000
0.0010
0.0020
0.0030
0.0004
0.0008
0.0012
0.000
0.001
0.002
0.003
0.004
500
1000
1500
2000
2500
3500

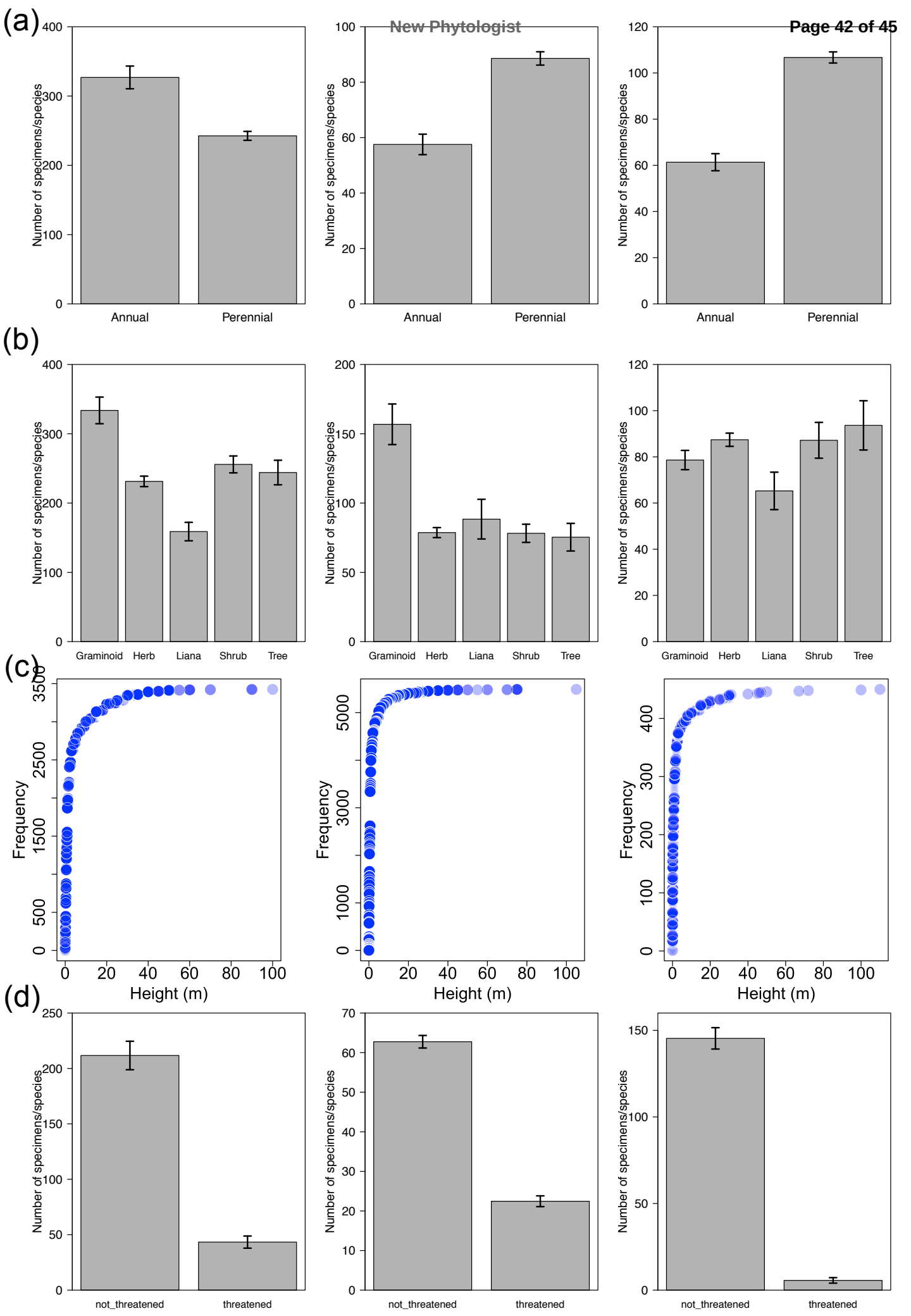

GDP (× US$10^9)
0
500
1000
1500
2000
NRI
NTI
2010
2000
1990
1980
1970
1960
1950
1940
1930
1920
1910
1900
1890
1880
1870
1860
1850
1840
1830
1820
1810
1800
Establishment of Collection permits
Restriction on immigration
Europeans immigration to Australia
World War II
The Great Depression
World War I
1.8*
2.3*
3.0**
3.6*
2.8*
6.1**
5.7**
5.1**
4.9**
2.4**
3.3**
3.4**
3.5**
2.1*
5.0*
3.0*
5.7**
3.5**
3.6**
0
50000
100000
Number of specimens
Australia
GDP (× US$10^9)
0
200
400
600
NRI
NTI
Establishment of NRF
Research Permits Law
Apartheid Era
World War II
The Great Depression
World War I
Anglo-Boer War II
Gold rush
Anglo-Boer War I
1.8*
5.1**
2.3*
2.3*
4.4**
3.6*
3.2**
4.1**
5.0**
3.9**
2.2**
5.6**
5.3**
3.2**
1.7*
2.5*
4.6**
1.6*
2.1*
4.3**
3.6**
2.4*
2.1*
3.3**
3.3**
2.4**
0
5000
10000
15000
20000
Number of specimens
South Africa
GDP (× US$10^9)
0
5000
10000
15000
20000
NRI
NTI
Establishment of NSF
World War II
The Great Depression
Establishment of New England Botanical club
4.5**
3.7**
5.0**
3.1*
2.4*
4.8**
4.9**
3.5**
6.9**
1.5*
6.2**
2.5**
6.6**
6.3**
4.8*
3.8**
4.6**
3.5**
1.7*
1.7*
5.1**
4.4**
0
5000
10000
15000
Number of specimens
New England

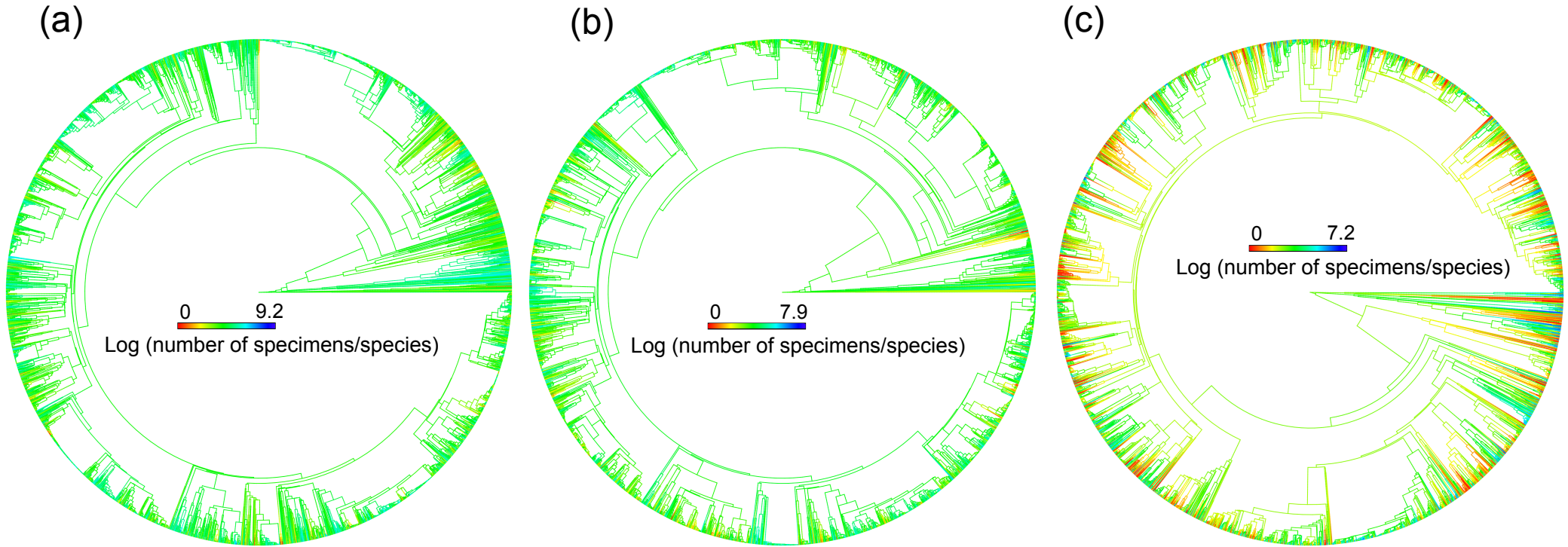
(a)

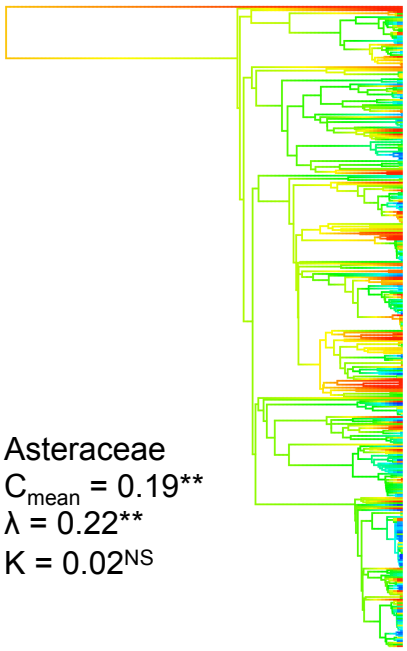

Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug

(b)

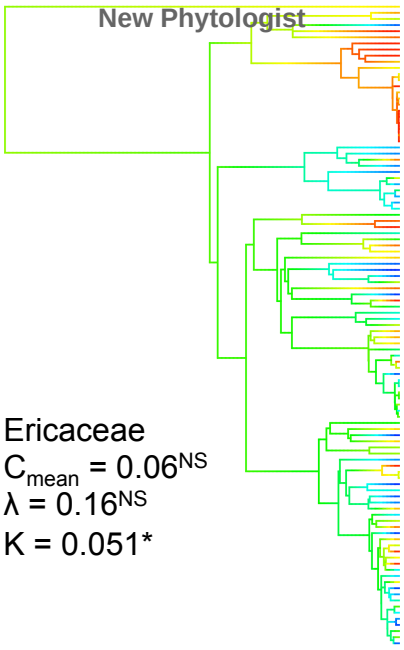

Number of collections
0e+00
1e+05
2e+05
3e+05
4e+05
5e+05
Mon
Tue
Wed
Thu
Fri
Sat
Sun
Australia
(1664 to 2016)
Number of collections
0
20000
40000
60000
80000
100000
120000
Mon
Tue
Wed
Thu
Fri
Sat
Sun
South Africa
(1656 to 2016)
Number of collections
0
20000
40000
60000
80000
Mon
Tue
Wed
Thu
Fri
Sat
Sun
New England
(1687 to 2016)


(a)
Number of specimens/species
Annual
Perennial
(b)
Graminoid
Herb
Liana
Shrub
Tree
(c)
Frequency
Height (m)
(d)
not_threatened
threatened

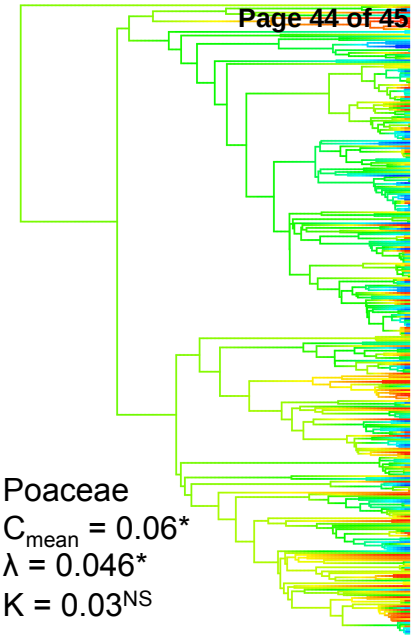

(a)
0
9.2
Log (number of specimens/species)
(b)
0
7.9
Log (number of specimens/species)
(c)
0
7.2
Log (number of specimens/species)



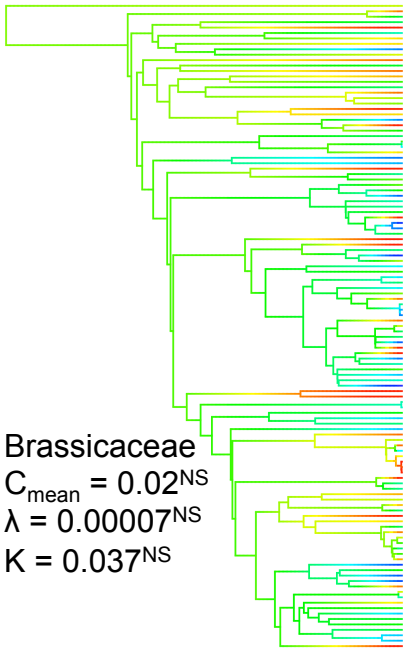
Asteraceae
C_{mean} = 0.19**
λ = 0.22**
K = 0.02^{NS}



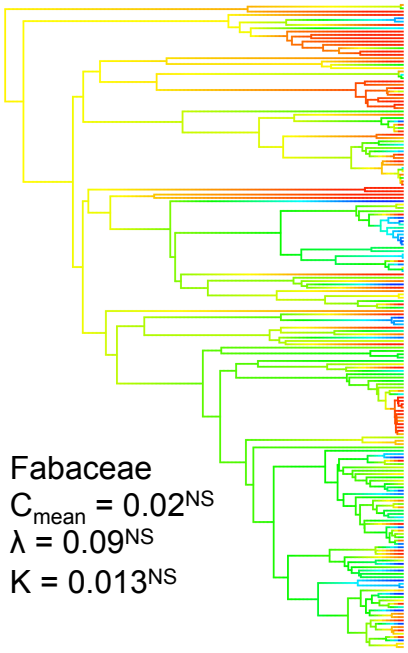
Ericaceae
C_{mean} = 0.06^{NS}
λ = 0.16^{NS}
K = 0.051*



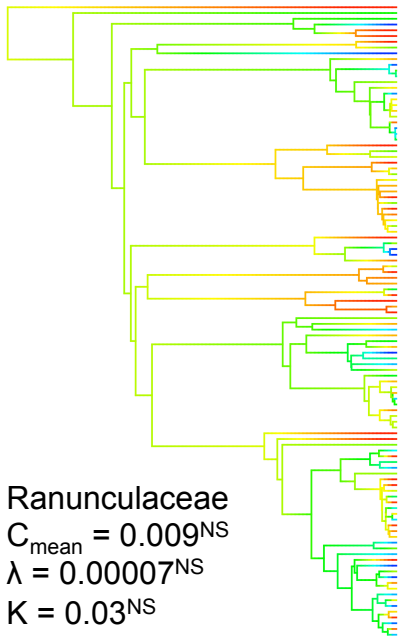
Poaceae
C_{mean} = 0.06*
λ = 0.046*
K = 0.03^{NS}



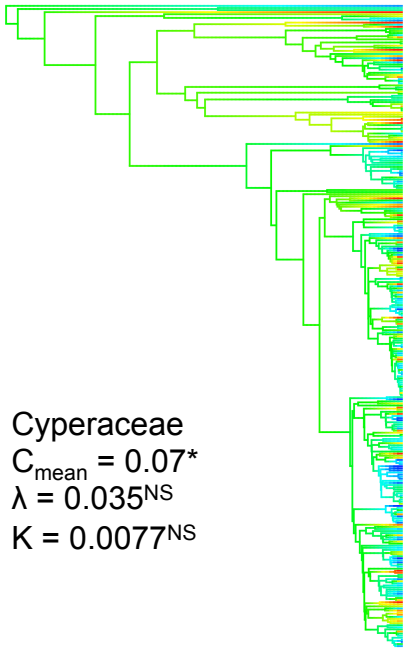
Brassicaceae
C_{mean} = 0.02^{NS}
λ = 0.00007^{NS}
K = 0.037^{NS}



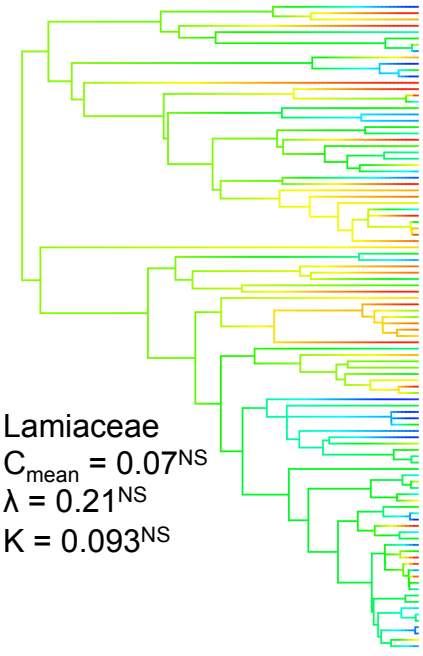
Fabaceae
C_{mean} = 0.02^{NS}
λ = 0.09^{NS}
K = 0.013^{NS}



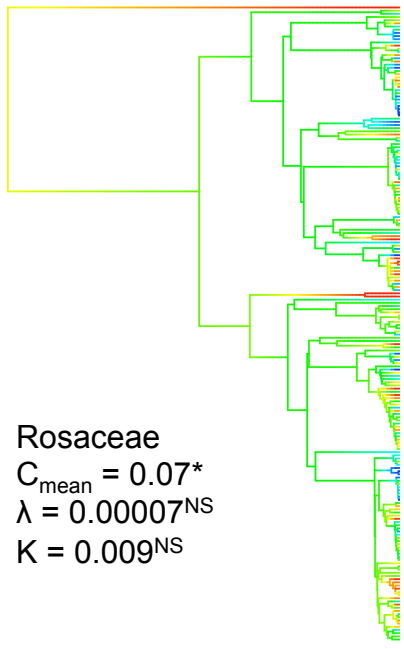
Ranunculaceae
C_{mean} = 0.009^{NS}
λ = 0.00007^{NS}
K = 0.03^{NS}



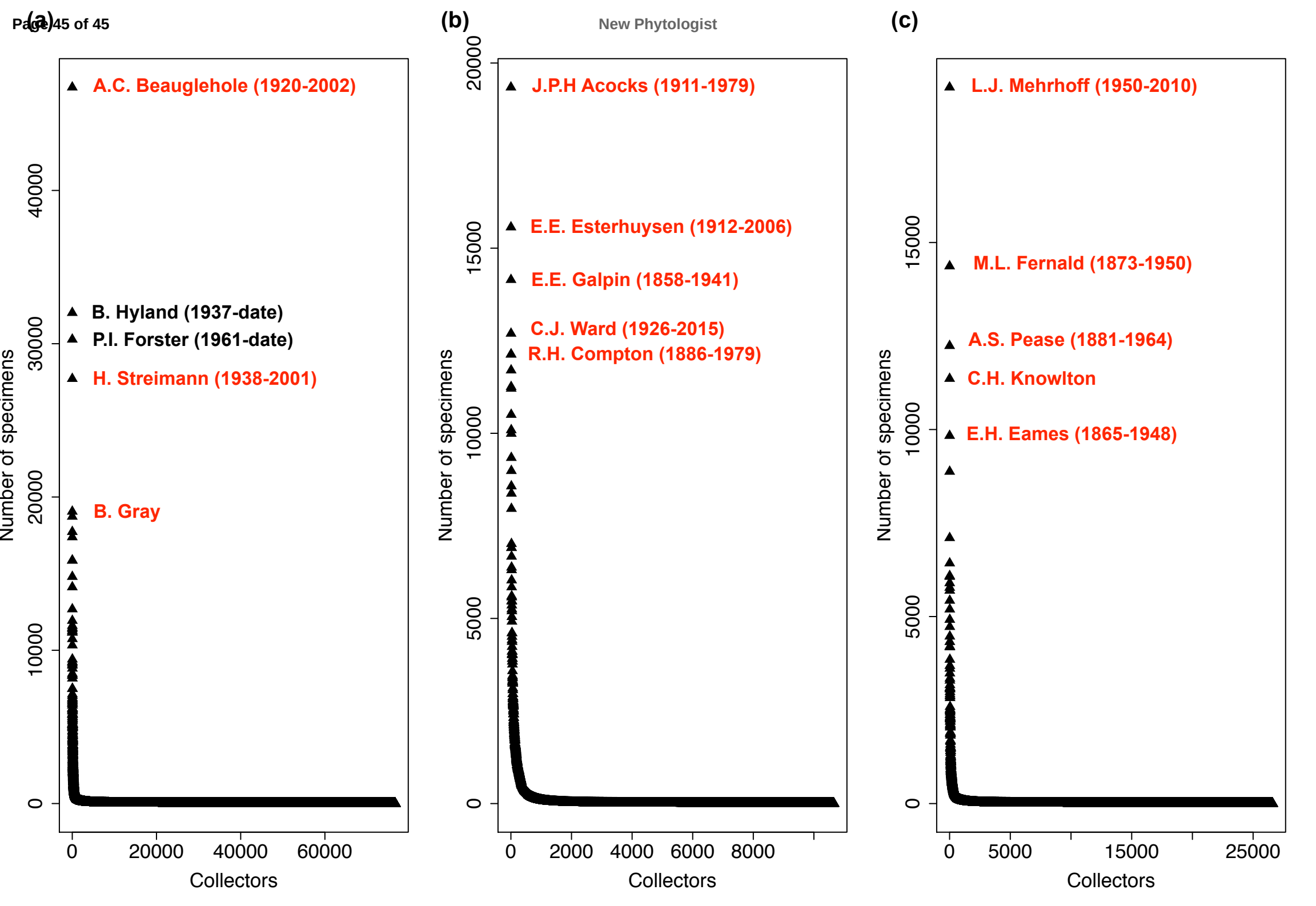
Cyperaceae
C_{mean} = 0.07*
λ = 0.035^{NS}
K = 0.0077^{NS}



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}


(a)
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
(b)
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
(c)
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

New Phytologist Supporting Information Figs S1 & S2 and Table S1

Article title: **Widespread sampling biases in herbaria revealed from large-scale digitization**

Authors: Barnabas H. Daru, Daniel S. Park, Richard B. Primack, Charles G. Willis, David S. Barrington, Timothy J. S. Whitfeld, Tristram G. Seidler, Patrick W. Sweeney, David R. Foster, Aaron M. Ellison and Charles C. Davis

Article acceptance date: 18 September 2017

The following Supporting Information is available for this article:

Fig. S1 Analytical workflow representing the different steps in the development of this study from data compilation, collation, to statistical analysis.

Fig. S2 Relationships of the number of specimens collected per species with number of species collected in each flora for Australia, South Africa, and New England of the USA.

Table S1 Results of the tests of phylogenetic signal in the number of specimens collected per species using three methods for nine exemplar clades in New England.

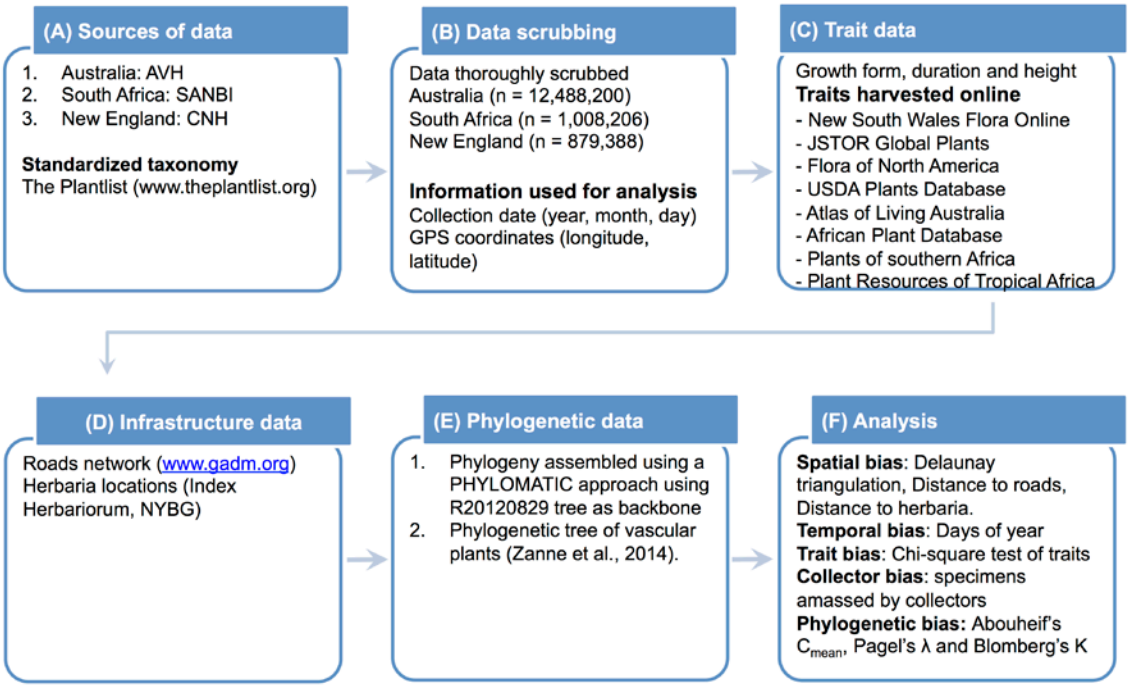


Fig. S1 Analytical workflow representing the different steps in the development of this study from data compilation, collation, to statistical analysis.

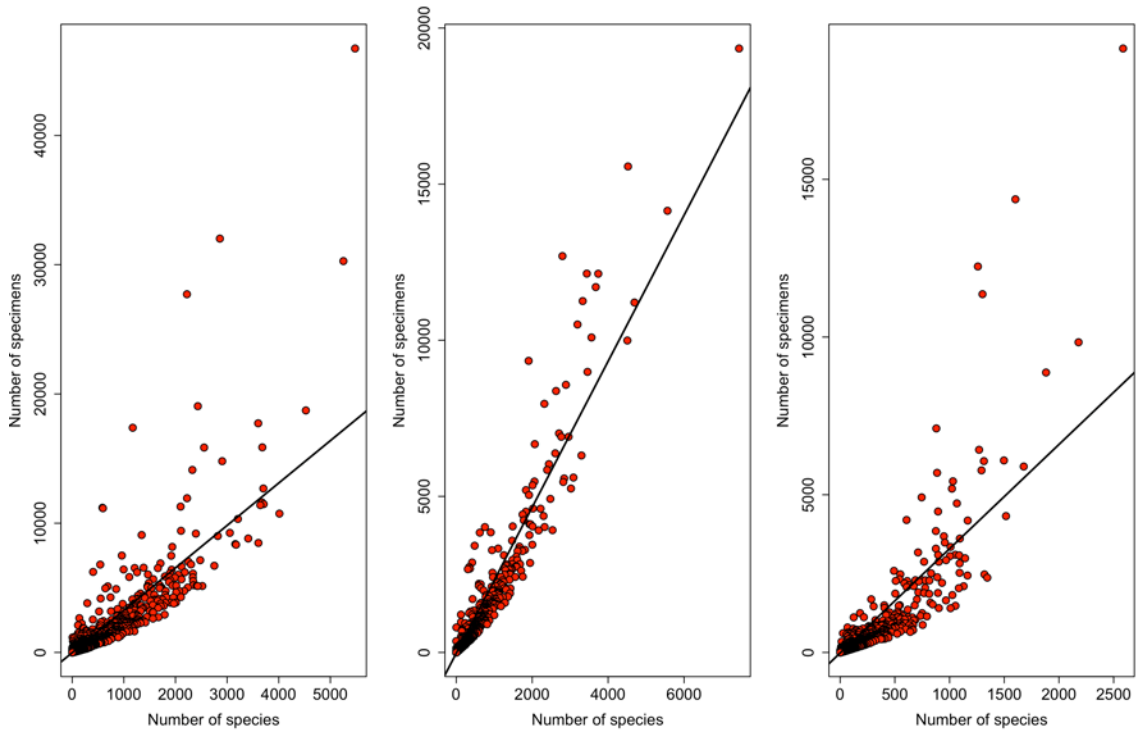


Fig. S2 Relationships of the number of specimens collected per species with number of species collected in each flora for Australia (left), South Africa (middle), and New England of the USA (right).

Table S1 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) for nine exemplar clades in New England: Asteraceae, Brassicaceae, Cyperaceae, Ericaceae, Fabaceae, Lamiaceae, Poaceae, Ranunculaceae, and Rosaceae. Phylogenetic data is derived from Phylomatic (Webb & Donoghue 2005). All tests are based on 1000 randomizations. **P < 0.001; *P < 0.01; NS, P > 0.05

	Asteraceae (n = 593 species)	Brassicaceae (n = 146)	Cyperaceae (n = 518)	Ericaceae (n = 158)	Fabaceae (n = 255 species)	Lamiaceae (n = 146)	Poaceae (n = 565 species)	Ranunculaceae (n = 169)	Rosaceae (n = 346 species)
Abouheif's C_{mean}	0.11**	-0.055^{NS}	0.016^{NS}	0.15*	0.028^{NS}	-0.014^{NS}	0.0026^{NS}	-0.04^{NS}	0.098*
Blomberg's K	0.11^{NS}	0.57^{NS}	0.55^{NS}	0.42^{NS}	0.092^{NS}	0.56^{NS}	0.20*	0.093^{NS}	0.17^{NS}
Pagel's lambda	0.14**	0.00006^{NS}	0.053*	0.27^{NS}	0.23*	0.02^{NS}	0.00008^{NS}	0.00007^{NS}	0.19^{NS}

References to Supporting Information

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

Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG, McGlinn DJ, O'Meara BC, Moles AT, Reich PB, *et al.* 2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* **506**: 89–92.