

Discovery of new Australasian Rare Earth Element hyperaccumulator ferns from screening herbarium specimens

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Abstract

Background and Aims Rare Earth Elements (REE) are essential for the development of clean technologies. Hyperaccumulator plants are metal-loving organisms that can be used to remove metals from contaminated soils. This study aimed to discover new REE hyperaccumulators in the Australasian region among the Blechnaceae and Gleicheniaceae families using specimens stored at the Queensland Herbarium.

Methods A handheld X-ray fluorescence (XRF) instrument was harnessed to scan herbarium specimens, and this data was analysed with Dynamic Analysis in GeoPIXE. Selected specimens were further analysed to validate the XRF results: elemental analysis was conducted with inductively coupled plasma optical emission spectroscopy (ICP-OES), an elemental distribution map through micro-X-ray fluorescence (μ XRF) and scanning electron microscopy (SEM) to rule out airborne contamination of plant samples.

Results From the 3256 specimens analysed with the portable XRF, 73 specimens met the criteria to be considered REE hyperaccumulators (yttrium $>50 \mu\text{g g}^{-1}$ on XRF analysis). Among this group, 11 new hyperaccumulator taxa were discovered, and the elemental analysis reported a total REE concentration around $1000 \mu\text{g g}^{-1}$, i.e. *Diploblechnum neglectum* ($978 \mu\text{g g}^{-1}$), *Sticherus flabellatus* ($1130 \mu\text{g g}^{-1}$), *Sticheropsis milnei* ($1290 \mu\text{g g}^{-1}$). We validated the strong REE hyperaccumulating capacity of the previously reported ferns *Blechnopsis orientalis* ($3850 \mu\text{g g}^{-1}$ total REEs) and *Dicranopteris linearis* ($1280 \mu\text{g g}^{-1}$ total REEs).

Conclusions The use of non-destructive portable XRF to scan herbaria collections is a tool to discover hyperaccumulator plants and this information could also be used as a bioprospecting tool to find REE deposits for potential REE phytomining.

INTRODUCTION

According to the International Union of Pure and Applied Chemistry (IUPAC), rare earth elements (REE hereinafter) are composed of 15 lanthanides: lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu), in addition to scandium (Sc) and Y (yttrium), which behave similarly to the lanthanides (Shan et al., 2003). IUPAC divided the REEs based on their atomic number into light, and heavy REE (Wall, 2014). The light REE are La – Sm and are 200 times more abundant than the heavy REE. The latter group is composed of Eu–Lu, including Sc and Y despite their lower atomic number (McGaughey et al., 2025). Heavy REE have less solubility and alkalinity compared to the light REE (Šmuc et al., 2012). In nature, most REE occur in the +3 valency state, although Ce and Eu are the exceptions. In oxidizing conditions Ce^{+4} forms CeO_2 (weathered and seawater deposits); in reducing conditions Eu forms Eu^{2+} cation (Wall, 2014). REE are widespread in the Earth crust and are as abundant as copper, lead, and bismuth e.g., Ce is the 25th most abundant element (Migaszewski and Gałuszka, 2015).

The unique physicochemical properties of REE as a conductive, electrical, optical, magnetic, and catalytic material significantly increased its use in industry, medicine, and agriculture for the past decade (Alonso et al., 2012). Furthermore, the use of REEs in green renewable technologies for the energy transition reinforced its strategic status as critical elements due to the geopolitical accessibility (Golev et al., 2014). The main REE producer is China (U.S. Geological Survey, 2025), although the supply has been categorized with a high-risk context due to the social, environmental and governance complexity (Lèbre et al., 2020). In this context, secondary resources of REE and innovative technologies are now actively explored (Balaram, 2025). One of the unconventional technologies for REE extraction is phytomining. This phytotechnology uses selected hyperaccumulator plants to extract targeted critical elements from mineralised soils and mine wastes such as tailings and mine impacted water (van der Ent et al., 2015).

Hyperaccumulators are a group of plants that concentrate high levels of specific metals and metalloids in their foliar tissues without any symptoms of stress (van der Ent et al., 2013). Among hyperaccumulators, REE hyperaccumulators have attracted attention because they could contribute to securing REE supply, by extracting REEs from contaminated soil and industry by-products, such as coal ash, red mud, and mine tailings (Gaustad et al., 2021). To be considered an REE hyperaccumulator, a plant must have at least $1000 \mu\text{g g}^{-1}$ REE in its leaves (Baker and Brooks, 1989). The strongest known REE hyperaccumulator is the fern *Dicranopteris linearis*, with 0.7 wt% of REE in dry leaf biomass (Shan et al., 2003) and most studies have focused on this species (Jally et al., 2021; Liu et al., 2020; Liu et al., 2019; Zheng et al., 2023). Despite the growing importance of REE hyperaccumulators, only two were discovered by 2017 (Reeves et al., 2018). The low number of identified REE hyperaccumulators is partly because REEs are present in low concentrations in most soils (Tyler, 2004).

Measuring herbarium specimens is an alternative to locate REE or other hyperaccumulators by saving time and costs to collect samples (van der Ent et al., 2019). For instance, two more REE hyperaccumulators were recently found after scanning 27 000 herbarium specimens (Purwadi et al., 2023). A further study confirmed that these new REE hyperaccumulators occur on REE-bearing rock formations (van der Ent et al., 2023b). An ideal case would be assessing herbarium specimens taken from metal-enriched areas; however, herbarium specimens are not collected considering metalliferous soils. Further, filtering and finding millions of herbarium collections based on their location are more challenging than scanning the entire genera, which is a common strategy in herbarium scanning (Do et al., 2020).

The discovery of new REE hyperaccumulators is essential to develop phytotechnologies for REE extraction and as pathfinders for new REE ore deposits. As such, the objective of this study was to systematically assess and discover incidences of REEs hyperaccumulation in ferns from the Blechnaceae and Gleicheniaceae families curated at the Queensland Herbarium, Australia. Previous studies using handheld – X-ray fluorescence (XRF) scanning of herbarium specimens reported REE hyperaccumulators in *Blechnopsis orientalis* (Blechnaceae) and *D. linearis* (Gleicheniaceae) (Goudard et al., 2024; Purwadi et al., 2024). Therefore, in this study we focus on Blechnaceae and Gleicheniaceae families using handheld XRF scanning of specimens catalogued at the Queensland Herbarium. Handheld XRF was proven to be highly effective for the discovery of REE hyperaccumulators in large herbarium specimen collections (Purwadi et al., 2022). Among the REE, Y is used as a proxy for REEs because the incident energy of the X-ray source (with an Ag-anode) is optimal for

excitation of the Y K α -line (van der Ent et al., 2023b) which is interference-free and has a strong signal (Goudard et al., 2024). The incident energy of the X-ray source is not high enough to excite the K α -lines of the other REEs, while the L α -line of the other REEs, such as Ce and La, interfere with the K α -lines of abundant first-row transition metals making their detection very difficult (Purwadi et al., 2021; Schramm, 2016). The Y-based approach for REE analysis has now been used for several studies that report on REE hyperaccumulation in herbarium specimens (Goudard et al., 2024; Purwadi et al., 2023; Purwadi et al., 2024). Other techniques were used to confirm the hyperaccumulation such as elemental analysis with inductively coupled plasma optical emission spectroscopy (ICP-OES), micro-X-ray fluorescence to understand the spatial distribution of elements in selected specimens and scanning electron microscopy (SEM) to rule out REE airborne contamination in ferns. A phylogenetic tree was also constructed to reconstruct the hyperaccumulation feature in Blechnaceae and Gleicheniaceae fern families.

MATERIALS AND METHODS

Handheld X-ray fluorescence (XRF) scanning of herbarium specimens

The Thermo Fisher Scientific Niton XL3t 950 GOLDD+ analyser (Billerica, Massachusetts, USA) uses a miniaturised X-ray tube (Ag anode, 6–50 kV, 0–200 μ A max) as its excitation source. The X-ray tube irradiates the sample with high-energy X-rays which excite fluorescent (characteristic) X-rays in the sample. These fluorescent X-rays are detected and quantified with a large 20 mm² Silicon Drift Detector (SDD, 185 eV, up to 60 000 counts per second). It can detect a wide range of elements within 15–60 seconds with ideal detection limits of 50–100 μ g g⁻¹ for transition elements such as Ni, Mn, and Zn (but typically >300 μ g g⁻¹ depending on the element in real-life samples). The XRF analysis was undertaken on a sheet of 'herbarium cardboard' on top of pure molybdenum (Mo) and aluminium (Al) plates (~99.995%, 2 mm thick $\times$ 10 $\times$ 10 cm) to provide a uniform background and block transmitted X-rays (see Fig. 1). The XRF analysis used the 'Soils Mode' in the 'Main filter' configuration for 60s duration. Analysis was focused on mature fronds (*i.e.* the most basal leaves on a specimen), and care was taken to fully cover the 6 mm measurement area of the XRF. The selection of specimens to be measured considered previous results of REEs hyperaccumulation reports (Purwadi et al., 2024). Thus, the Blechnaceae and Gleicheniaceae families collected across Australasia were scanned at the Queensland Herbarium, which houses more than 911 000 specimens in plant collection from 150 years of species discovery. Regarding the naming of taxa in Blechnaceae, Queensland Herbarium follows Perrie et al. (2014), although in this work we have referenced Gasper et al. (2017).

Data analysis

The data collected from the herbarium specimens (3256 measurements) using the portable XRF required a further analysis to detect the high Y levels. The REE are not included in the existing calibration dataset, therefore a new calibration was required for quantifying these elements. The derived data was processed using a universal pipeline in GeoPIXE analysis package (CSIRO), with a Dynamic Analysis algorithm developed for nuclear microprobe techniques and synchrotron-based XRF (Ryan et al., 2015). The pipeline for this analysis has been clearly explained by Purwadi et al. (2022). In short, the quantification of elemental concentrations based on XRF spectra using the Dynamic Analysis algorithm is performed by solving a complex physics equation based on information related to the instrument set up and sample properties. The Dynamic Analysis algorithm fits this information into the equation and performs iterations aiming to generate a spectrum as same as the sample. After that, the generated spectrum was decomposed into individual spectrum per detected element, then the individual spectrum is converted to elemental concentrations. To be considered a REE hyperaccumulator plant, Y needs to be > 50 μ g g⁻¹, and the limit of detection of the handheld XRF is between 49–73 μ g g⁻¹ (Goudard et al., 2024).

Scanning electron microscopy with energy-dispersive X-ray spectroscopy

Small leaflet fragments from select specimens in Table 1 were excised from the pinna with a razor blade. The samples were then sealed in a box with silica gel, mounted on stubs, sputter-coated with carbon and analyzed using scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS, Hitachi SU3500), at 100–3000 $\times$ magnification at 15–20 kV, as described previously (Corzo Remigio et al., 2021).

Laboratory-based micro-X-ray fluorescence (μ XRF) elemental mapping

This method reveals the spatial distribution of elements in plant samples and has been used in ecophysiological studies of hyperaccumulator plants (van der Ent et al., 2023a). The University of Queensland (UQ) micro-X-ray fluorescence spectroscopy (μ XRF) facility is a custom-built system assembled by IXRF ATLAS X (TX, USA), which incorporates two 50 kV–1000 μ A sources fitted with polycapillary focussing optics. We used a XOS microfocus Mo-target tube that produces 17.4 keV X-rays (flux of 2.2×10^8 ph s⁻¹) focussing to 25 μ m. To reveal the REEs distribution in fern organs, seven samples with high Y levels were selected from the Queensland Herbarium (see Table 1) and mounted between two sheets of Ultralene thin film (6 μ m) stretched over a Perspex frame magnetically attached to the x-y motion stage at atmospheric temperature (~20°C). The XRF spectra on the UQ μ XRF facility were acquired in mapping mode using the instrument control package, Iridium (IXRF systems).

Elemental analysis of plant tissues

To confirm the REE hyperaccumulation in ferns showing high levels of Y, a pinna from selected ferns from the Queensland Herbarium (see Table 1) were weighed to 100 $\pm$ 5 mg in 6 mL polypropylene tubes for pre-digestion using 2 mL HNO₃ (70%) for 24 hr before being digested in a block heater (Thermo Scientific™ digital dry bath) for a 2-hr programme (1 hr at 70°C followed by 1 hr at 125°C). Ultrapure water (Millipore 18.2 M Ω ·cm at 25°C) was added to the digested samples to make 10 mL volume for elemental analysis using Thermo Scientific iCAP 7400 inductively coupled plasma optical emission spectroscopy (ICP-OES) instrument. Either radial or axial mode was operated considering the approximate element concentration in the analyte. The ICP-OES was chosen to confirm REE hyperaccumulation in plant tissues that were already indicated to be enriched in REEs by prior XRF screening. Previous studies using this setup have demonstrated detection limits as low as 0.01 mg L⁻¹, which are several orders of magnitude lower than typical REE concentrations in hyperaccumulator plants (Purwadi et al., 2024). Matrix-based interferences were compensated using yttrium as in-line internal addition standardization.

Quality controls grouped certified reference solution (Sigma-Aldrich Periodic Table Mix 1 for ICP TraceCERT®, 33 elements, 10 mg L⁻¹ in HNO₃ 70%, v/v), standard reference material (NIST Apple 1515)) treated as samples, and matrix blanks (Supp Table 1).

Ancestral character reconstruction of the hyperaccumulator condition in Blechnaceae and Gleicheniaceae

The phylogenetic relationships among 55 and 19 species within the Blechnaceae and Gleicheniaceae respectively (Supplementary Table 2) were reconstructed using the R package U. phyloMaker (Jin and Qian, 2023) and based on an updated fern tree of life 'ftol_sanger_ml.tre' (Nitta et al., 2022). To understand where and how many times the hyperaccumulation capacity has evolved in these two fern families, the ancestral state for the discrete character "hyperaccumulator" was reconstructed using phylogenetic tree. Then, a Maximum Likelihood Estimation of continuous characters was carried out with the reconstructed tree using the R phytools package (Revell, 2012).

Statistical analysis

The statistical analysis was conducted in R version 4.4.3 with RStudio 2024.12.1. Yttrium concentration gathered with the portable XRF in Blechnaceae and Gleicheniaceae families were compared using the unpaired two-samples Mann-Whitney for non-parametric data. Prior Shapiro-Wilk test was conducted to prove the normality of the Y concentration, and as $p < 0.001$ for both families, the normality test failed. A generalized linear model (GLM) binomial (or Bernoulli GLM) was used to understand the presence or absence of hyperaccumulator plants in Blechnaceae and Gleicheniaceae (Funchs, 2017; Smith and Warren, 2019). The number of scanned specimens was not statistically representative per each species; instead, all the available specimens at the herbarium were scanned. The number of scanned specimens per species were compared against the presence of hyperaccumulators in a binary system. Graphs were created using "ggplot2", "ggpubr", and "plyr" packages.

RESULTS

Discovery of new REE hyperaccumulators

Handheld XRF data acquired consisted of 3256 measurements covering the Blechnaceae and Gleicheniaceae families (Fig. 2a), and 131 species from 31 different countries (Supp. Table 2). The specimens from Australia covered in this study were 2362, making 72.5% from all the data acquired. Seventy-three specimens met the criteria to be considered REEs hyperaccumulator (see Fig. 3 and Supp. Table 3), and in total 13 different species were discovered (Fig. 2b). *Dicranopteris linearis* and *Blechnopsis orientalis* were previously reported as REE hyperaccumulators (Goudard et al., 2024). This data set reports for the first time 11 new discovered REEs hyperaccumulators: *Diploblechnum neglectum*, *Diploblechnum whelanii*, *Doodia aspera*, *Diplopterygium glaucum*, *Diplopterygium longissimum*, *Oceaniopteris cartilaginea*, *Sticheropsis milnei*, *Sticherus cunninghamii*, *Sticherus flabellatus* var. *flabellatus*, *Sticherus hirtus*, and *Sticherus urceolatus*.

The GLM binomial statistical analysis for the Blechnaceae and Gleicheniaceae families (Supp. Table 2) is presented in Supplementary Fig. S1. The GLM binomial test indicated that there is a significantly greater probability of finding a REE hyperaccumulator among Blechnaceae species ($p < 0.001$); in contrast the number of scanned specimens for Gleicheniaceae specimens did not have a significantly probability of identifying a REE hyperaccumulator ($p = 0.0654$). This is also observed in Supp. Fig S1, where more Gleicheniaceae REE hyperaccumulators were identified in a smaller number of scanned specimens (34 %) within the *Dicranopteris*, *Gleichenia*, *Sticheropsis* and *Sticherus* genera, while 91 species in Blechnaceae with 2137 scanned specimens (74 %) among six genera. The REE hyperaccumulators were found in five species within the *Blechnopsis*, *Diploblechnum*, *Doodia* and *Oceaniopteris* genera (Supp. Table 4).

Accumulation of REE in ferns

The elemental chemical analysis of the selected fern species (Table 1) was summarized in Table 2. As the light REE (La, Ce, Pr, Nd, and Sm) are globally more abundant, it was expected that there would be higher concentrations of these elements compared to the heavy REEs (Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, and Sc). Out of the seven fern specimens analysed, five held a total REE concentration higher than 1000 $\mu\text{g g}^{-1}$ (threshold for REE hyperaccumulators); *D. neglectum* accumulated 978 $\mu\text{g g}^{-1}$ REEs, while *O. cartilaginea* only 666 $\mu\text{g g}^{-1}$ REEs. Lanthanum concentration was the highest among the ferns, *Blechnopsis orientalis* (BRI-AQ0145489) accumulated 985 $\mu\text{g La g}^{-1}$, followed by the same species *B. orientalis* (BRI-AQ0568466) with 797 $\mu\text{g La g}^{-1}$. In *S. flabellatus* var. *flabellatus*, *S. milnei* and *D. neglectum*, La concentrations were 480 $\mu\text{g La g}^{-1}$, 360 $\mu\text{g La g}^{-1}$, and 245 $\mu\text{g La g}^{-1}$ respectively. *Oceaniopteris cartilaginea* and *D. linearis* var. *altissima* held the lowest La with 79.8 $\mu\text{g La g}^{-1}$ and 72.9 $\mu\text{g La g}^{-1}$, respectively. Cerium highest concentration was similar to that of La, both *B. orientalis* specimens reported concentrations of 889 and 309 $\mu\text{g Ce g}^{-1}$. The species *D. linearis* var. *altissima*, *S. milnei*, *D. neglectum*, *O. cartilaginea* and *S. flabellatus* var. *flabellatus* followed the decreasing order 146 > 129 > 128 > 120 > 89.4 $\mu\text{g Ce g}^{-1}$. Neodymium concentrations were also higher in both *B. orientalis* with 860 and 661 $\mu\text{g Nd g}^{-1}$. *Sticheropsis milnei*, *S. flabellatus* var. *flabellatus*, *D. linearis* var. *altissima*, *D. neglectum*, and *O. cartilaginea* 300 > 263 > 214 > 211 > 129 $\mu\text{g Nd g}^{-1}$. Yttrium ranged from 119–514 $\mu\text{g g}^{-1}$ for all the ferns. Praseodymium is the fifth highest REE in ferns, and *B. orientalis* specimens accumulated higher levels with 470 and 244 $\mu\text{g Pr g}^{-1}$. *Sticherus milnei*, *S. flabellatus* var. *flabellatus*, *D. neglectum*, *D. linearis* var. *altissima* and *O. cartilaginea* concentrated Pr in the following order: 93.2 > 83.7 > 78.5 > 71 > 57.2 $\mu\text{g Pr g}^{-1}$. For Sm, both *B. orientalis* species accumulated 132 and 115 $\mu\text{g Sm g}^{-1}$, followed by *D. linearis* var. *altissima*, *S. milnei*, *S. flabellatus* var. *flabellatus*, and *D. neglectum*, with the decreasing concentrations of 58.5 > 55 > 42.8 > 37.8 > 26.2 $\mu\text{g Sm g}^{-1}$. The other REE were lower than 100 $\mu\text{g g}^{-1}$ per fern sample and followed this order: Gd > Dy > Er > Yb > Eu > Ho > Tm > Lu > Sc.

The average REE concentration of *B. orientalis* measured in this study was summarized in Table 3 and compared with values reported in two previous studies: the Bangka Island field study (Purwadi et al., 2024), and the Paris Herbarium survey (Goudard et al., 2024). This comparison demonstrates the consistency of REE enrichment in *B. orientalis* across different sample types and locations. Similar to our results, the heavy REE were more abundant

compared to the light REE. Important to note that our study had similar REE concentrations to that of Goudard et al., (2024). This might be explained by the similar methods in both studies (Herbarium specimens), while the REEs values for *B. orientalis* in Purwadi et al. (2024) are lower due to the large number of samples collected in the field where there is more variability in the REE soil concentration. For example, even though Sc is abundant on the earth crust, hyperaccumulator plants concentrate very low quantities of this element ($0.1 \mu\text{g g}^{-1}$) and was below the limit of detection for the reported values in Goudard et al., (2024). Finally, only five species showed a REE hyperaccumulator behaviour in the Blechnaceae family, while with a smaller number of scanned specimens Gleicheniaceae contained nine REE hyperaccumulator species.

REE distribution in ferns revealed by μXRF analysis

The elemental map distribution of selected fern species (see Table 1) is presented in Figure 4 and 5, and the GeoPIXE fitted spectrum is in the Supp Fig 2. To illustrate comparisons between and within species, calcium (Ca) and potassium (K) were mapped along with the highest REE. Potassium distribution is higher compared to that of Ca, and it is depleted from the veins towards the borders of the pinna. Cerium, La and Nd are higher in *B. orientalis* (Fig. 4, c and e, and Fig. 5). These REE, are higher in the midrib and depleted towards the pinna and enriched in the margins, this distribution is similar for *D. neglectum* and *O. cartilaginea* although in less concentration. *D. linearis* var. *altissima*, different to *Blechnopsis* and *Oceaniopteris* sp., the pinna is composed of small pinnules, where Ce is slightly higher in the main vein. *S. flabellatus* var. *flabellatus* showed evenly distributed low La and Nd albeit Ce was even in lower concentrations. *Sticheropsis milnei* had very low and evenly concentrations for Ce, La and Nd.

The SEM images (Fig 6a-d) showed no superficial contamination on the pinna, and the EDS analyses (Fig 6e and 6f) reported REE (Ce, La and Nd) enrichment in specific areas such as the margins and the main vein in two different specimens of *B. orientalis* (areas 2, 3, 4, and 5). Cerium ranged from 0.2 up to 1.8 wt %, La from 0.1 to 0.6 wt %, and Nd from 0.3 up to 0.7 wt %. Areas that were not high in REE were also considered for comparison reasons (1 and 6), where the REE concentration was below the limit of detection (0.1 wt %).

Evolution of the hyperaccumulation capacity

The phylogenetic ancestral reconstruction of the REE hyperaccumulation capacity is shown in Fig. 7-8. In Blechnaceae, this capacity seems to have evolved three times in different clades, whereas in Gleicheniaceae up to five times. In Gleicheniaceae, the REE hyperaccumulator condition is observed in all the clades except for the *Gleichenia-Stromatopteris* clade, which may open the possibility that more species within the Gleicheniaceae can hyperaccumulate. Conversely, this capacity in Blechnaceae might be less recurrent and its evolution may have a stronger phylogenetic component.

DISCUSSION

This study discovered eleven new REE hyperaccumulator taxa from the Blechnaceae (four spp.) and Gleicheniaceae (seven spp.) families by harnessing a portable XRF scanning on the Queensland herbarium collection. Additionally, these results further validated the REE accumulation pattern for previously reported ferns *B. orientale* and *D. linearis* (Goudard et al., 2024). The REE hyperaccumulation for Blechnaceae was found in species from the *Blechnopsis*, *Diploblechnum*, *Doodia* and *Oceaniopteris* genera, while in Gleicheniaceae REE hyperaccumulators were found *Dicranopteris*, *Gleichenia*, *Sticheropsis* and *Sticherus* genera. REE hyperaccumulation is not shared by most species in the Blechnaceae and Gleicheniaceae families. The hyperaccumulation phenomena is rare and only present in less than 800 species, with more than 72% Ni hyperaccumulators (Reeves et al., 2018). The number of REE hyperaccumulators is very low and most physiological studies were conducted on *D. linearis* (Liu et al., 2020) and *Phytolacca americana* (Grosjean et al., 2019). The evolutionary trait for REE hyperaccumulation might be explained by the inadvertent phosphorus uptake as a starting point in evolution for more specialised mechanisms to tolerate and storage high levels of REE (Pollard, 2023).

Scanning herbaria collections using non-destructive handheld XRF is a convenient technique for the discovery of hyperaccumulators, even for elements such as REE that are not considered in the calibration dataset of the instrument; for this purpose the use of Dynamic Analysis in GeoPIXE is an option (Purwadi et al., 2022). The REE are part of critical elements that are being mined and used for modern technologies (Hund et al., 2020); hence, these methods can be transferred to less studied metals for the discovery of hyperaccumulators. As hyperaccumulators are metal-loving plants, they can be used to clean soils impacted by mining activities and store the metals in above ground tissues (Corzo Remigio et al., 2020). This abnormal metal accumulation along with further metallurgical processes can allow purification of metals for an economic return, in a process known as phytomining (Chaney et al., 2021).

The discovery of REE hyperaccumulators from herbaria collections could be a bioprospecting tool to identify REE deposits. Traditionally, handheld XRF analysis of minerals is used for the discovery of valuable ore bodies, and the downsize of this method is the time required depending on the deposit size, and often the in-situ analyses (Vargas Soto, 2022). The chemical composition of the plant can reflect that of the soils if the plant behaves as an indicator, or hyperaccumulator (Baker, 1981). Geophysical remote sensing is a common method used in regional exploration; however, it is rendered useless by the presence of vegetation. The ability to detect hyperaccumulator plants that overlie an enriched regolith could complement geological exploration. The advantages of XRF scanning large collections of plant specimens in herbaria are: (i) the availability of plant species from different parts of the world; (ii) access to the location data of plant specimens; (iii) the non-destructive nature of the XRF analysis; and (iv) the low initial investment, as there is no need to go to the field to get samples.

The multiple applications of REE have been widely addressed; however, the impact of these elements on ecosystems remains poorly understood (Tao et al., 2022). The use of hyperaccumulator plants for remediation is an innovative method, and understanding the underlying mechanisms of REE is essential to minimize the negative impacts and to cover the demand of these elements (McGaughey et al., 2025). Rare earth elements are not considered essential for plants, although they enter inadvertently through calcium, manganese, iron and aluminium channels (Wang et al., 2024), and the response is variable depending on the plant species and the REE concentration in the soil. Some plants exclude REEs in their roots, e.g. wheat (*Triticum aestivum* L.) (Ding et al., 2005), while others are indicators and increase the REE concentration mirroring the concentration in the soil, e.g. *Phytolacca bogotensis* (Grosjean et al.,

2019). There are few explanations for REE uptake and potential proteins involved in the transport, but to date there is no conclusive data. The REE distribution in plants differs considerably (Tyler, 2004), for hyperaccumulators the translocation factor needs to be > 1 , which means that the ratio of REE in shoots / roots has to be > 1 . The subcellular distribution in *Pronephrium simplex*, a REE hyperaccumulator, reported 88.6 % (381.5 $\mu\text{g g}^{-1}$ total REE) in the cell wall, while in molecules *i.e.* crude proteins reported the highest concentration with 2900 $\mu\text{g g}^{-1}$ total REE (Lai et al., 2006).

The elemental analysis of selected ferns in this study reported a higher concentration of light REE, $\text{La} > \text{Ce} > \text{Nd} > \text{Pr} > \text{Sm}$, compared to the heavy ones – common for REE hyperaccumulating Pteridophytes (Grosjean et al., 2024) –, although Y was reported in high concentrations ranging from 119–514 $\mu\text{g g}^{-1}$. As mentioned previously, these elements are not essential and the studies on their effects on plants are contradictory, as it depends on the REE concentration in the soil and the plant species (Kovářiková et al., 2019; Ozturk et al., 2023). For example, La, Ce and Nd were found to improve the photosynthetic capacity in *Arabidopsis thaliana* (Xiaoqing et al., 2009), *Spinacia oleracea* (Hong et al., 2002). The detrimental effects of REE in chloroplasts have been associated to soluble forms of REE such as chlorides, nitrates, sulfates, while insoluble forms such as carbonates, phosphates and hydroxides are less harmful (Kovářiková et al., 2019). The REE uptake by plants is associated with biotic and abiotic factors such as root exudate, microbiota, pH, redox potential, cation exchange capacity (Grosjean et al., 2024; Kastori et al., 2010).

The elemental analysis (ICP-OES method) on limited number of specimens ($n=7$), followed the XRF screening to identify plant tissues likely to contain elevated REE concentrations. As such, these results are not intended to represent population-level averages but to confirm REE hyperaccumulation in selected species, particularly in *B. orientalis* and the newly discovered REE hyperaccumulator taxa such as *Sticherus flabellatus* var. *flabellatus*, *D. neglectum*, *O. cartilaginea* that are geographically distributed in Australia. Formal inferential statistics were not feasible due to the small sample size and lack of raw replicate data from comparable studies. However, a descriptive comparison shows that TREE and individual REE concentrations for *B. orientalis* in this study (TREE: 2490–3850 $\mu\text{g g}^{-1}$) are consistent with, or exceed, previously reported ranges, including from Bangka Island (up to 2900 $\mu\text{g g}^{-1}$ TREE) (Purwadi et al., 2024) and the Paris Herbarium survey (2031–4278 $\mu\text{g g}^{-1}$ TREE) (Goudard et al., 2024). These comparisons support the interpretation that *B. orientalis* has a greater likelihood to be found as hyperaccumulator across different sites and sampling contexts. In our study 223 *B. orientalis* samples were scanned and 34 (16 %) were found with $\text{Y} > 50 \mu\text{g g}^{-1}$. In Goudard *et al.* (2024), 561 *B. orientalis* specimens were scanned and 146 (26%) were reported as REE hyperaccumulators.

CONCLUSIONS

In this study, eleven new taxa of REE hyperaccumulators were discovered from the collection of Blechnaceae and Gleicheniaceae families across the Australasian region and other species could be found if hand-held XRFs were harnessed at other herbarium with well document specimens. This discovery increases the number of REE hyperaccumulators that were reported the last five years, although studies on ecophysiology, tolerance, and phytoextraction still remain scarce. This is not surprising as most of these REE hyperaccumulators are ferns and the germination process varies considerably among species, genera and families, and it is considerably more difficult compared to higher plants. We suggest further studies on the germination protocols and ecophysiology for recently discovered REE hyperaccumulators.

Declarations

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AUTHOR CONTRIBUTIONS

PE and AVDE selected the families to study. PE and NF received funding for this project from the Queensland Government. ACR scanned the plant specimens at the herbarium and undertook the chemical analysis of the samples. IP analysed the raw data in GeoPIXE. PB provided support gathering and validating the data from the herbarium specimens. CM developed the phylogenetic trees. ACR, AVDE, IP, PE, PB, CM and NF wrote the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest relevant to the content of this manuscript.

DATA AVAILABILITY

Not applicable.

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Tables

Table 1. Selected species from the Queensland Herbarium for ICP-OES and SEM analyses to confirm the results from the portable XRF analysis.

ID	Family	Species	Country
BRI-AQ0486332	Blechnaceae	<i>Oceaniopteris cartilaginea</i>	Australia
BRI-AQ0145838	Blechnaceae	<i>Diploblechnum neglectum</i>	Australia
BRI-AQ0145489	Blechnaceae	<i>Blechnopsis orientalis</i>	Australia
BRI-AQ0568466	Blechnaceae	<i>Blechnopsis orientalis</i>	Australia
BRI-AQ0596288	Gleicheniaceae	<i>Dicranopteris linearis</i> var. <i>altissima</i>	Australia
BRI-AQ0437776	Gleicheniaceae	<i>Sticherus flabellatus</i> var. <i>flabellatus</i>	Australia
BRI-AQ0147005	Gleicheniaceae	<i>Sticheropsis milnei</i>	Papua New Guinea

Table 2. Arsenic, and REE concentrations (µg g⁻¹) in plant specimens from the collections of the Queensland Herbarium reported by ICP-OES analysis.

Species	As	Sc	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb
<i>Oceaniopteris cartilaginea</i>	0.292	LOD	190	79.8	120	57.2	129	26.2	5.97	23	0.913	18.5	3.67	6.68	0.664	3.61
<i>Diploblechnum neglectum</i>	8.33	0.125	195	245	128	78.5	211	37.8	3.91	28.5	1.02	26.4	4.92	8.97	1.23	6.35
<i>Blechnopsis orientalis</i> [†]	LOD	LOD	328	985	889	470	860	132	16.3	62	LOD	60.6	9.72	14.8	3.08	19.1
<i>Blechnopsis orientalis</i> [‡]	LOD	0.14	216	797	309	244	661	115	10.6	56.9	1.96	55	7.1	10	1.24	7.74
<i>Dicranopteris linearis</i> var. <i>altissima</i>	LOD	0.514	514	72.9	146	71	214	58.5	15.9	63.3	7.79	53	11.6	28.2	4.15	18.6
<i>Sticherus flabellatus</i> var. <i>flabellatus</i>	4.04	0.336	119	480	89.4	83.7	263	42.8	1.48	22.4	LOD	19.8	2.58	4.06	0.125	1.94
<i>Sticheropsis milnei</i>	3.06	0.371	242	360	129	93.2	300	55	9.34	37	2.94	34.3	5.81	10.8	1.65	10.2

* Total sum of REEs

[†] BRI-AQ0145489

[‡] BRI-AQ0568466

LOD represents the limit of detection, for As is 0.892 µg g⁻¹, for Tb is 0.919 µg g⁻¹, and for Sc is 0.04 µg g⁻¹.

Table 3. Comparison of the average REE concentrations of in *B. orientalis* from this study against previously published values from Bangka Island (Purwadi et al., 2024) and the Paris Herbarium survey (Goudard et al., 2024). For consistency, only old pinna values are shown for the Bangka dataset, which were collected directly from the field in Bangka Island. The Paris Herbarium data were obtained using the same portable XRF instrument model and processing method as this study, but specimens originated from multiple regions worldwide.

Element	This study (n=2)	Goudard et al., (2024) (n=4)	Purwadi et al., (2024) (n=46)
Sc	0.09	< LOD	0.13
Y	272	748	68
La	891	527	70
Ce	599	442	160
Pr	357	118	42
Nd	761	563	49
Sm	124	119	8.8
Eu	13.5	37.8	0.53
Gd	59.5	197	11
Tb	1.41	ND	1.6
Dy	57.8	189	9.6
Ho	8.41	18.5	2.2
Er	12.4	ND	4.4
Tm	2.16	6	0.85
Yb	13.4	44	3.3
Lu	1.4	5.5	0.52

LOD denotes below the limit of detection, and ND non determined.

Figures

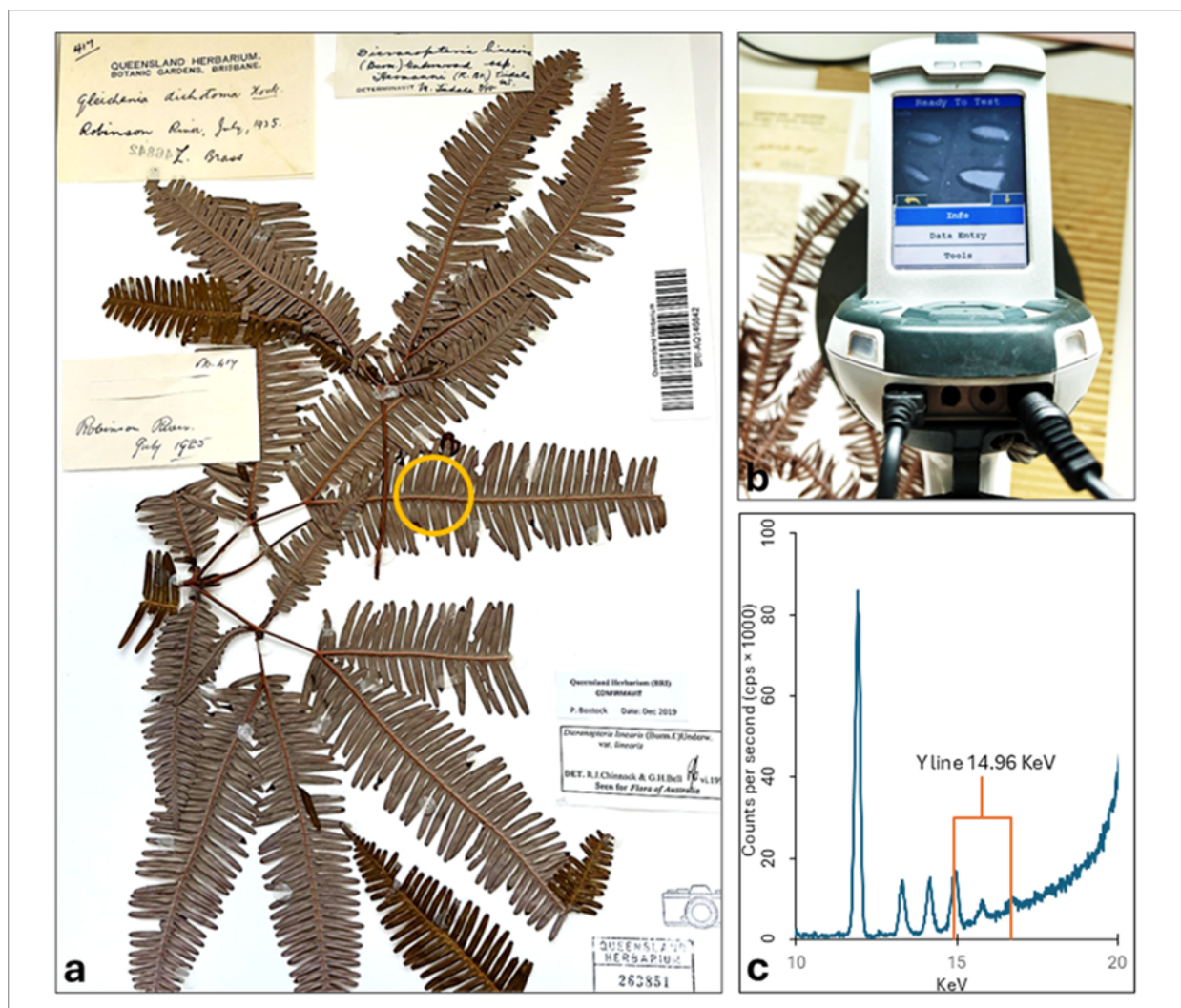


Figure 1

Handheld XRF scanning herbarium fern specimens: a) area aimed for the scan in yellow, which includes the midrib between pinnules; b) view of the scanned area on the screen of the XRF machine; c) Y spectrum obtained in soils mode during 60 s.

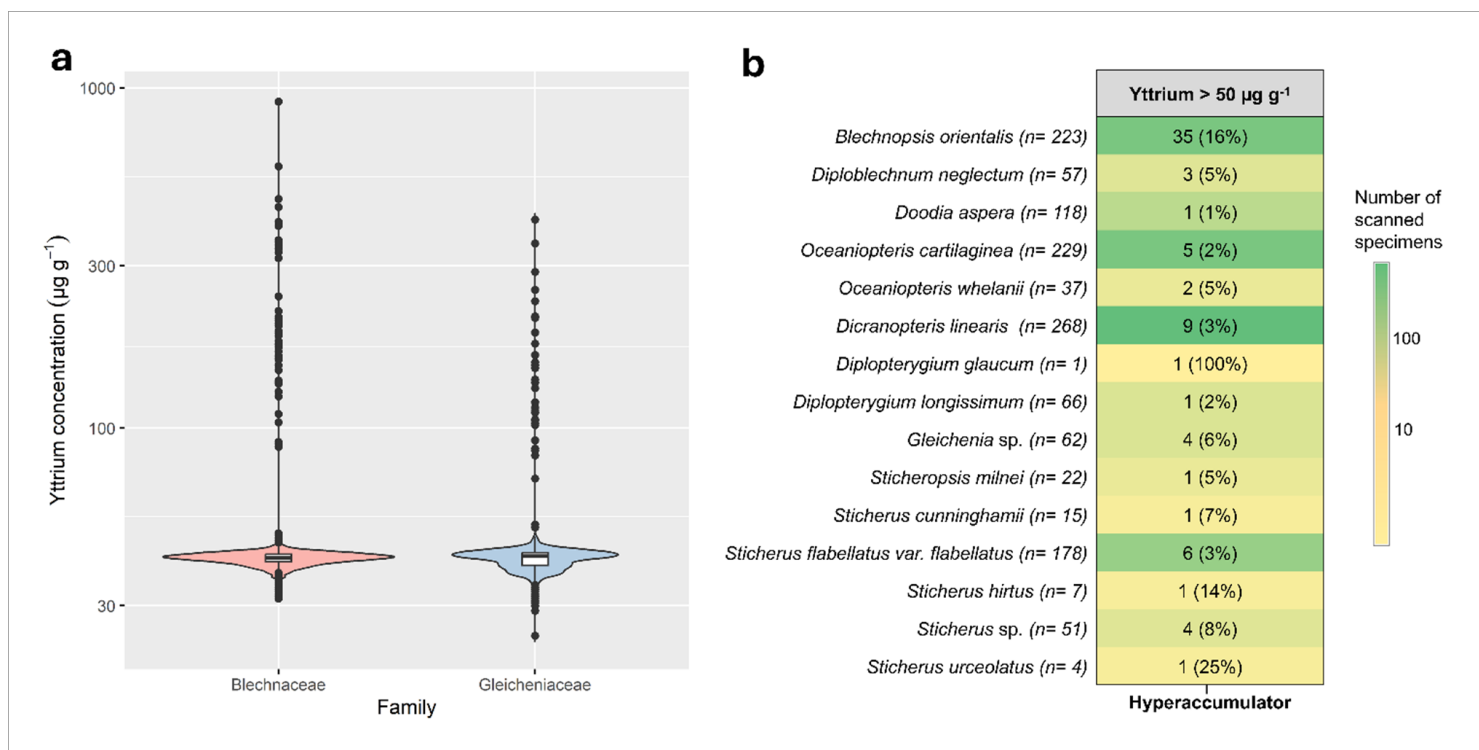


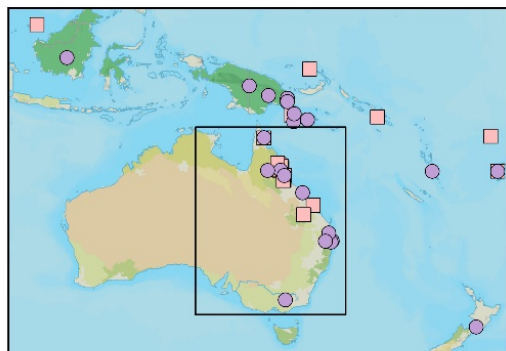
Figure 2

Handheld XRF Y concentration in the Blechnaceae and Gleicheniaceae families: a) violin plots showing the distribution of Y foliar concentration; means and standard error for both families were compared using Mann-Whitney test for non-parametric data ($W=1173287$, $p = 0.3785$), showing no statistical difference between both families; b) Species-level discovery of hyperaccumulators compared with the specimens scanned based on Y concentration, where normal concentrations are set for REE < 10 $\mu\text{g g}^{-1}$, and hyperaccumulator is considered when Y > 50 $\mu\text{g g}^{-1}$.

Map of REE hyperaccumulators

Family

- Blechnaceae
- Gleicheniaceae



Species

- *Blechnopsis orientalis*
- *Diploblechnum neglectum*
- *Doodia aspera*
- *Oceaniopteris whelanii*
- *Dicranopteris linearis* var. *altissima*
- *Dicranopteris linearis* var. *linearis*
- *Sticherus flabellatus* var. *flabellatus*
- *Sticherus urceolatus*

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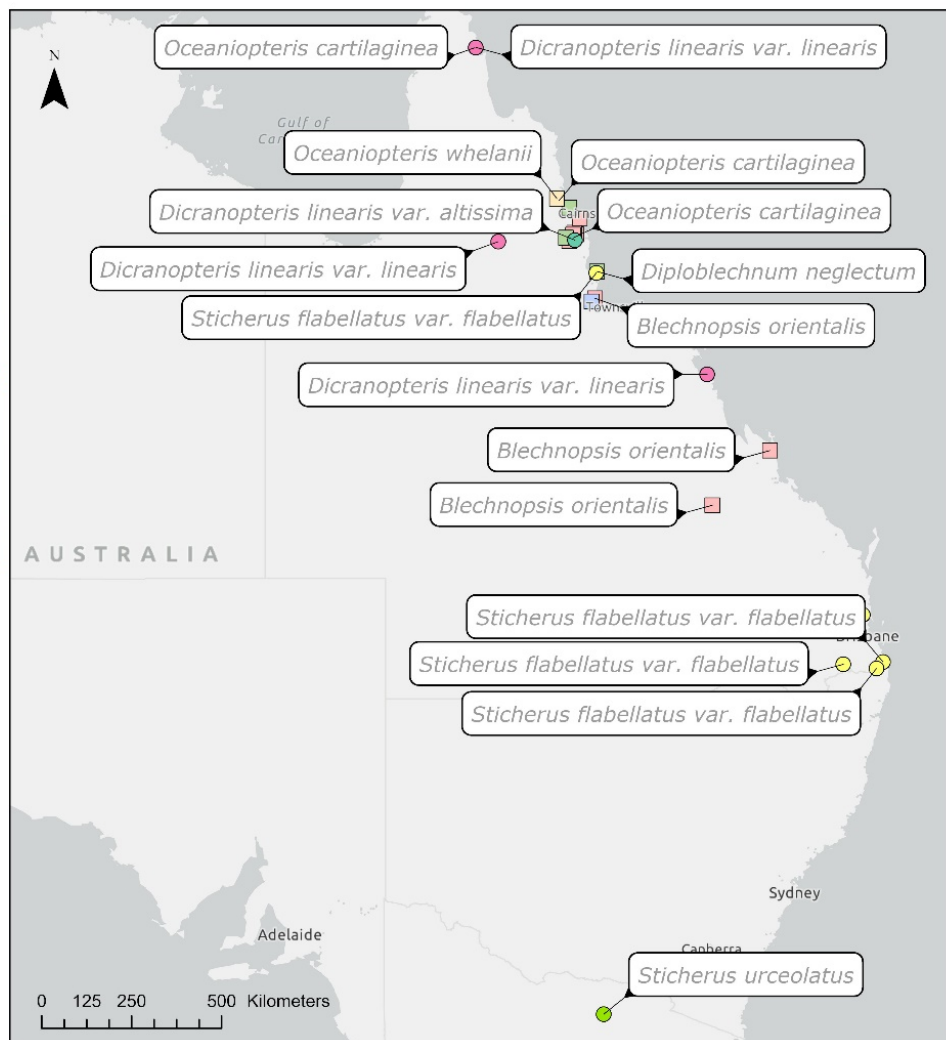


Figure 3

Map of the discovered REE hyperaccumulators from specimens collected across Australia and islands in the Pacific Ocean, and curated at the Queensland Herbarium, Australia.

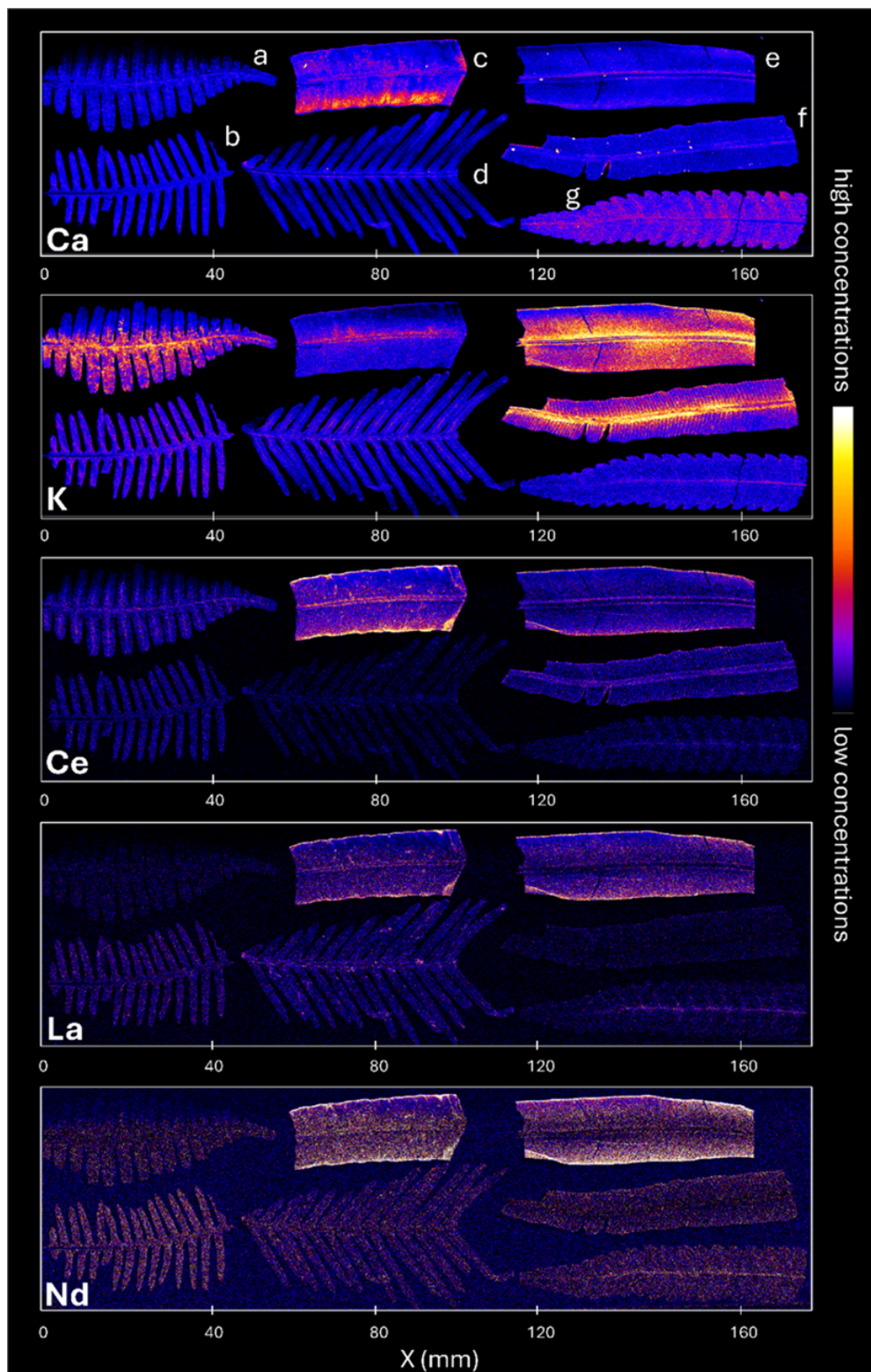


Figure 4

Laboratory based micro-X-ray fluorescence map distribution of calcium, potassium, cerium, lanthanum, and neodymium in selected specimens from the Queensland Herbarium: a) *Dicranopteris linearis* var. *altissima* from Australia, BRI-AQ0596288; b) *Sticheropsis milnei* from Papua New Guinea, BRI-AQ0147005; c) *Blechnopsis orientalis* from Australia, BRI-AQ0145489; d) *Sticherus flabellatus* var. *flabellatus* from Australia, BRI-AQ0437776; e) *Blechnopsis orientalis* from Australia, BRI-AQ0568466; f) *Oceaniopteris cartilaginea* from Australia, BRI-AQ0486332; and g) *Diploblechnum neglectum* from Australia, BRI-AQ0145838.

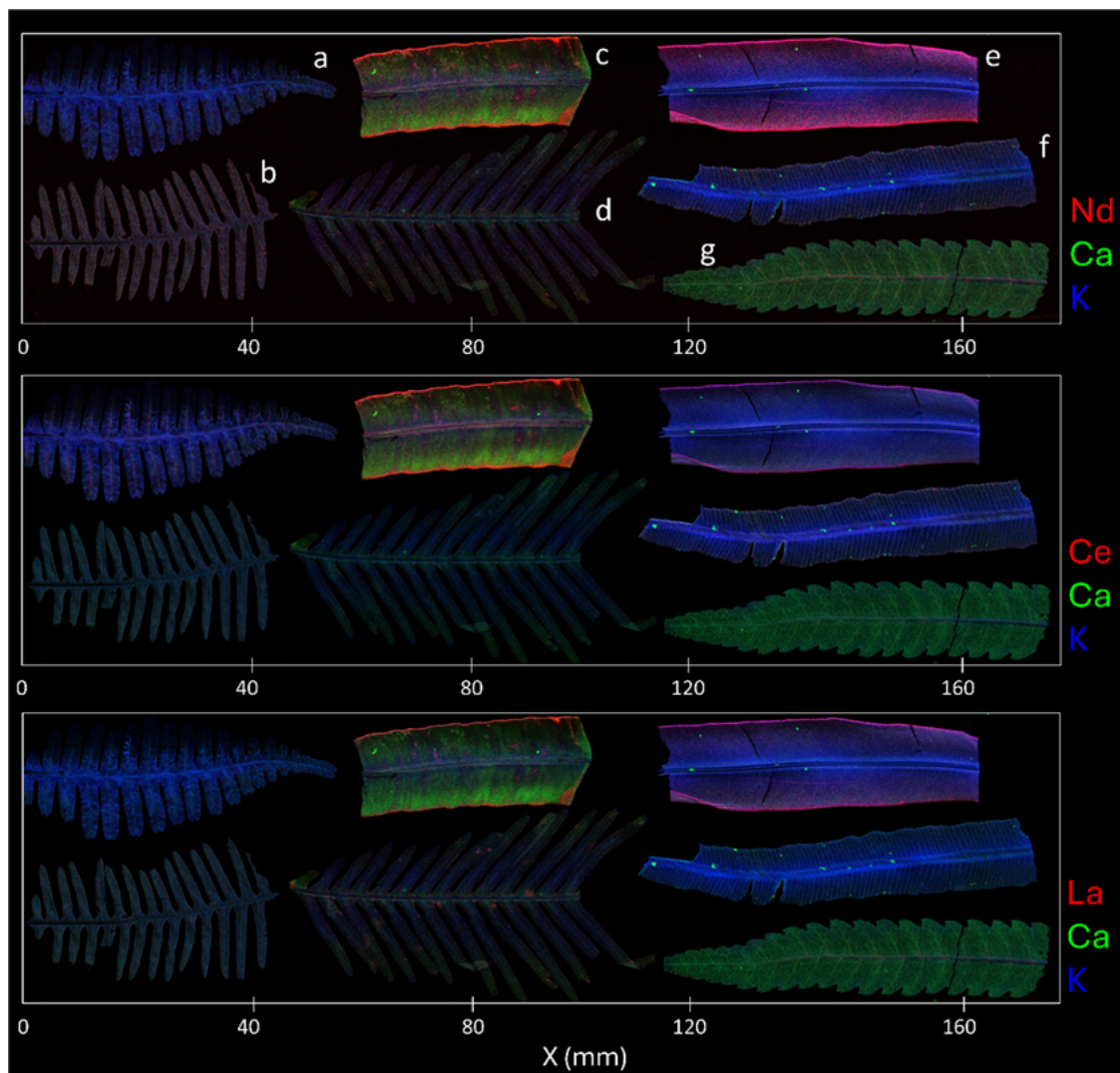


Figure 5

Laboratory based micro-X-ray fluorescence map distribution showing in RGB calcium, potassium in different maps with Nd, Ce and La in selected specimens from the Queensland Herbarium: a) *Dicranopteris linearis* var. *altissima* from Australia, BRI-AQ0596288; b) *Sticheropsis milnei* from Papua New Guinea, BRI-AQ0147005; c) *Blechnopsis orientalis* from Australia, BRI-AQ0145489; d) *Sticherus flabellatus* var. *flabellatus* from Australia, BRI-AQ0437776; e) *Blechnopsis orientalis* from Australia, BRI-AQ0568466; f) *Oceaniopteris cartilaginea* from Australia, BRI-AQ0486332; and g) *Diploblechnum neglectum* from Australia, BRI-AQ0145838.

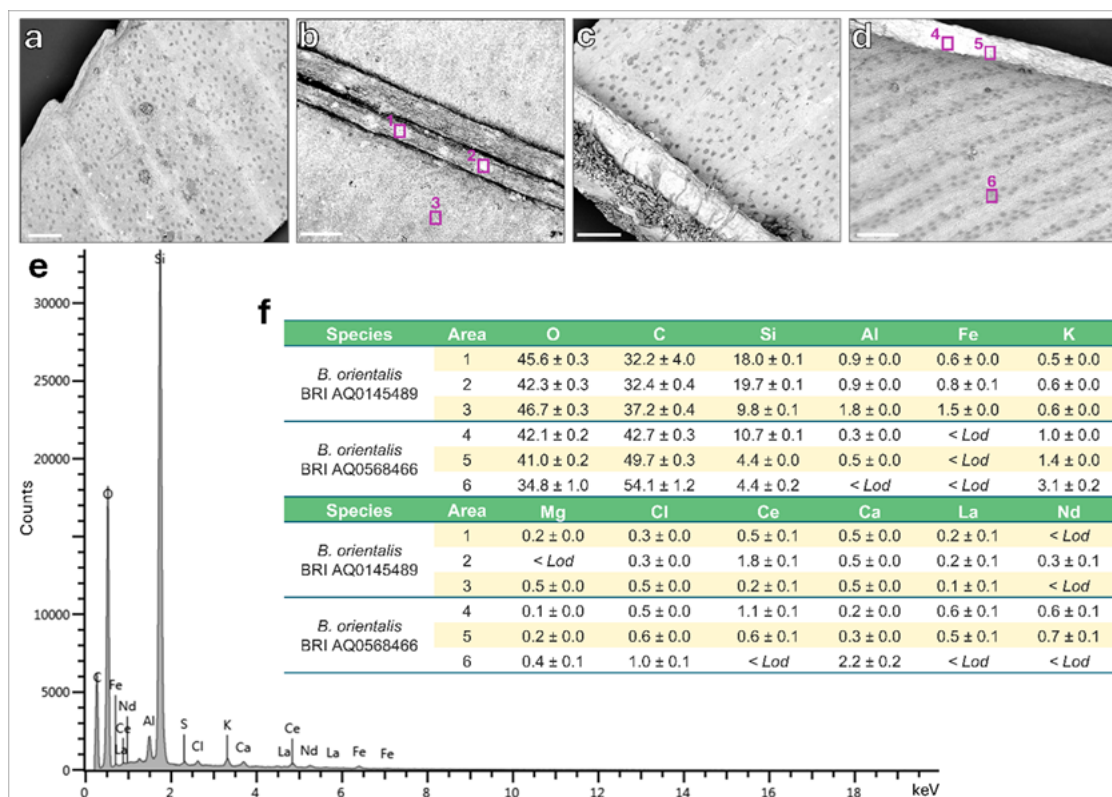


Figure 6

Scanning electron microscopy – backscattered electron (SEM-BSE) coupled with energy dispersive X-ray spectroscopy (EDS) area analysis of Blechnaceae species: a) *O. cartilaginea*, BRI-AQ0486332; b) *B. orientalis*, BRI-AQ0145489; c) *O. cartilaginea*, BRI-AQ0486332; d) *B. orientalis*, BRI-AQ0568466; e) Spectra from the area analysis 5 in Fig 6d; f) Table with EDS results expressed in weight percent (wt %) ± uncertainty (σ). Scale bar denotes 500 μ m.



Figure 7

Ancestral reconstruction of the sampled Blechnaceae species based on the REE hyperaccumulator capacity.

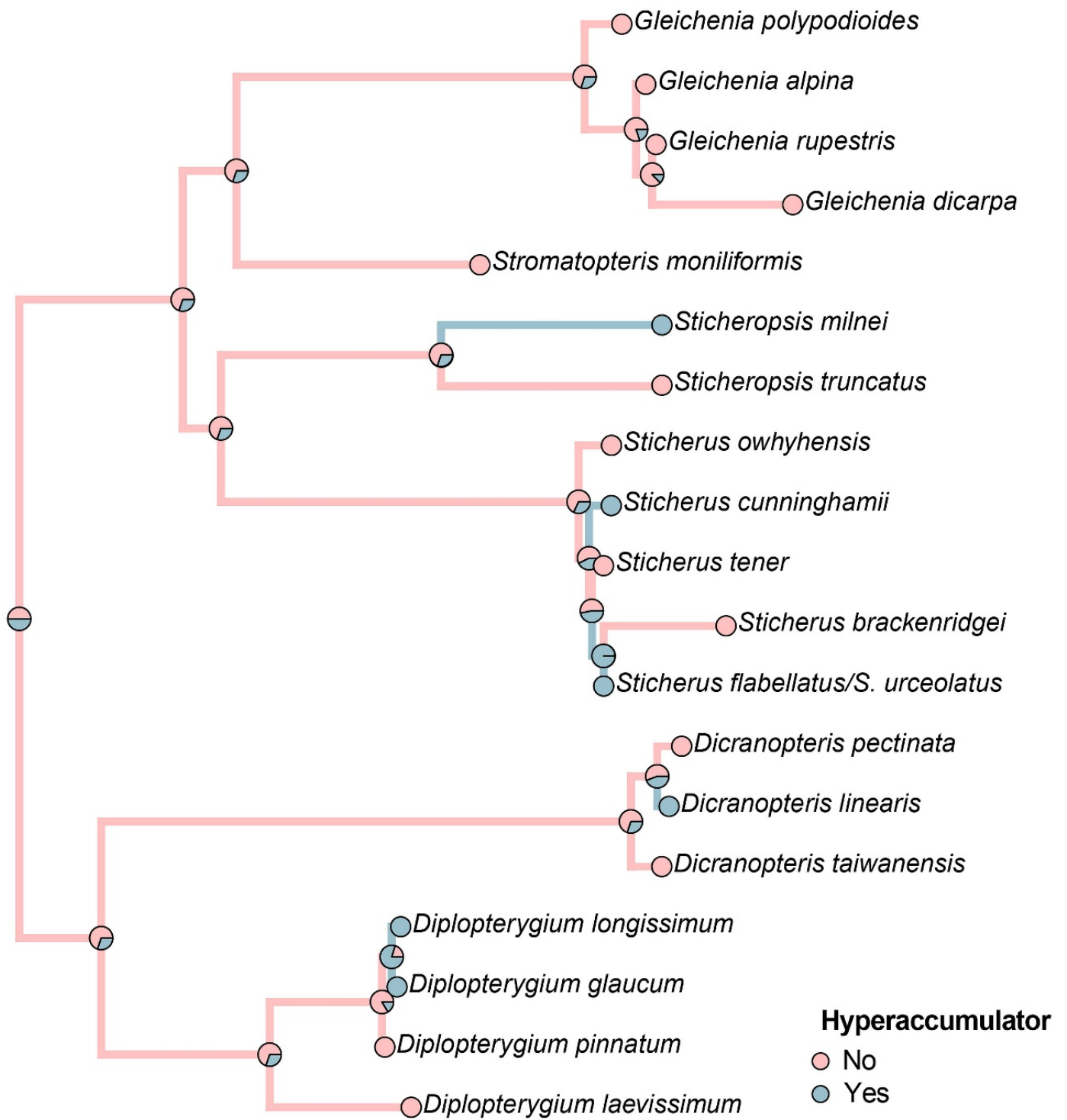


Figure 8

Ancestral reconstruction of the sampled Gleicheniaceae species based on the REE hyperaccumulator capacity. *Sticherus flabellatus* and *S. urceolatus* do not present genetic differences in the phylogenetic tree.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.docx](#)