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SPECIAL FEATURE

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Herbarium and field studies of nickel hyperaccumulator plants from ultramafic soils in Guatemala

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Abstract

Until 2019, there were no published reports of trace element hyperaccumulation in the plants of Central America or Mexico. Recent reports, based mostly on measurement of herbarium specimens using x-ray fluorescence (XRF) spectroscopy, have begun to identify hyperaccumulators of nickel (Ni) in this region. The most extensive deposits of Ni-rich ultramafic rocks and soils in Central America occur along the Polochic-Motagua fault system in eastern Guatemala. This study focused on discovery and characterization of new hyperaccumulators in Guatemala, starting with surveys of herbarium specimens, followed by field studies to understand the ultramafic soils of the region and the ecology of hyperaccumulators that occur there. Initial XRF screening at the Missouri Botanical Garden Herbarium, along with two herbaria in Guatemala, identified four previously unreported Ni hyperaccumulators—*Arachnothryx linguiformis*, *Arachnothryx buddleioides*, *Chionanthus panamensis*, and *Orthion guatemalense*. Field studies in Guatemala characterized the communities in which these species occur, some of which include multiple hyperaccumulator species growing in close proximity. In two taxa that showed phenotypic variation in foliar Ni concentration, there was no statistically significant correlation between Ni concentrations in leaves and either the total or

DTPA-extractable Ni in soils, suggesting that other genetic or environmental variables may influence hyperaccumulation.

KEYWORDS
agromining, ophiolite, phytomining, Polochic-Motagua fault, serpentine

1 | INTRODUCTION

Hyperaccumulators are plants with an unusual ability to store specific chemical elements in their above-ground biomass, achieving elemental concentrations several orders of magnitude higher than in other species (van der Ent et al., 2013), when growing in natural conditions. Although hyperaccumulation has been discussed for a wide range of elements (van der Ent et al., 2021), most research has focused on uptake and storage of metals and metalloids, and about 700 plant species have been reported to hyperaccumulate such elements (Reeves et al., 2021). Of these, the great majority are hyperaccumulators of nickel (Ni), with hyperaccumulation defined as a foliar Ni concentration exceeding 1000 mg kg^{-1} on a dry-weight basis.

The remarkable properties of hyperaccumulators, including uptake, transport, storage, and tolerance of elements at concentrations that are toxic to most organisms, make them inherently interesting for research in plant physiology and ecology. Hyperaccumulators may also have applications in biotechnology for phytoremediation of polluted soils (Chaney & Baklanov, 2017; Mahar et al., 2016; Wójcik et al., 2017) and in agromining for metal recovery (Chaney et al., 2021; Kidd et al., 2018). If optimized, these processes are potentially profitable, yet more environmentally sustainable than conventional alternatives, allowing carbon sequestration as the plants are growing and facilitating habitat maintenance as opposed to soil/subsoil destruction.

The known Ni hyperaccumulators have a global distribution, but until recently none had been reported from Central America (Invernón et al., 2021). This is remarkable considering the well-documented biodiversity of the region and its proximity to Cuba, known to possess one of the world's most diverse Ni-hyperaccumulator floras (Belloeil et al., 2021; Reeves et al., 1996, 1999). Indeed, one of the first indications that hyperaccumulators might occur in Central America was the presence throughout the Neotropics of two species of *Psychotria* (Rubiaceae) that were previously shown to hyperaccumulate Ni in Cuba and the Antilles (Campbell et al., 2013; Reeves, 2003). Those two species (*Psychotria costivenia* and *Psychotria grandis*) were investigated and found to hyperaccumulate Ni at multiple locations across Mexico and Central America, along with two other *Psychotria* species endemic to the region (*Psychotria papantlensis* and *Psychotria lorenciana*), which were newly identified as hyperaccumulators (McCartha et al., 2019).

The initial detection of hyperaccumulation in Central America was based on metal concentration data gathered from herbarium specimens, using a handheld x-ray fluorescence (XRF) spectrometer (McCartha et al., 2019). This technique has proved to be a powerful tool for rapid, non-destructive measurement of the incomparable scientific resource represented by the pressed plant specimens housed in the world's herbaria (Purwadi et al., 2021; van der Ent, Echevarria, et al., 2019). Recent studies using large-scale, XRF-based screening of herbarium specimens have made many additions to the world's known inventory of metal- and metalloid-hyperaccumulator plants (Belloeil et al., 2021; Do et al., 2020; Gei et al., 2020; Jakovljević et al., 2024; van der Ent, Ocenar, et al., 2019). On the other hand, for research on hyperaccumulation to progress beyond mere identification of metal-accumulating species (sometimes criticized as "stamp-collecting"), it will be necessary to combine herbarium identification with detailed studies in the field and/or laboratory (Nkrumah et al., 2017) as a first step to elucidate the significance of metal hyperaccumulation in plant evolution (Jaffré et al., 2013).

Globally, most Ni hyperaccumulators occur on soils derived from Ni-rich ultramafic rocks, which in tropical humid climates weather mainly to deep Ferralsols ("laterites") or sporadic locally serpentinite Cambisols (Echevarria, 2021). Typically, ultramafic rocks are associated with ophiolites, fragments of the oceanic lithosphere usually located near the margins of tectonic plates. Ophiolite-related ultramafic rocks occur at scattered localities throughout Central America (Lewis et al., 2006), so it was not unreasonable to expect hyperaccumulators to be present in the region. However, one of the surprising findings reported by McCartha et al. (2019) was that many specimens of hyperaccumulators originated from localities that are not mapped as ultramafic on geologic maps (French & Schenk, 2004; SGM, 2017), especially in the Mexican states of Chiapas, Tabasco, and Veracruz (i.e. carbonated/clayey sedimentary rocks or basalt). To resolve this paradox, Navarrete Gutiérrez, Pollard, et al. (2021) conducted field studies of hyperaccumulators in Mexico and found that, although the soils were derived from sedimentary parent rock, they contained elevated concentrations of Ni, Fe, and Mn. According to those authors, "*A potential source of Ni in these soils could be lateritic material transported by water from the ultramafic complexes occurring throughout the Polochic-Motagua fault systems in Guatemala*" (Navarrete Gutiérrez, Pollard, et al., 2021). During the field studies in Mexico, other species were newly discovered to be hyperaccumulators, including *Blepharidium guatemalense* (Rubiaceae) (Navarrete Gutiérrez, Nkrumah, et al., 2021; Navarrete Gutiérrez, Pollard, et al., 2021) and several species of the genus *Orthion* (Violaceae), including *Orthion veracruzense* and *Orthion subsessile* (Invernón et al., 2021; Navarrete Gutiérrez et al., 2024; Nkrumah et al., 2021; Reeves et al., 2021).

The most extensive exposures of ultramafic rock in Central America are in the Guatemala Suture Zone (GSZ; Figure 1), including the Polochic-Motagua fault system, which crosses Guatemala from east to west, representing the boundary between the Caribbean and North American plates (Harlow et al., 2011; Lyon-Caen et al., 2006; Martin et al., 2016; Ortega-Gutiérrez et al., 2007). Herbarium specimens of *Psychotria* spp. collected in this region of Guatemala were previously analyzed by McCartha et al. (2019) and also found to have high Ni concentrations, with several labels mentioning collection in the vicinity of a Ni-laterite mine in El Estor, Department of Izabal. (The mine is currently designated CGN-PRONICO, but often referenced on specimen labels by former names including EXMIBAL and FENIX.) The reported geographic range of known hyperaccumulators *B. guatemalense* and *O. subsessile* (Navarrete Gutiérrez et al., 2024; Navarrete Gutiérrez, Pollard, et al., 2021; Reeves et al., 2021) also encompasses the GSZ.

Although recent investigations have begun to identify hyperaccumulators in Central America and Mexico, knowledge is still incomplete and sometimes contradictory. Botanical field studies on ultramafic sites in the region, focused on ultramafic outcrops in Costa Rica (Reeves et al., 2007) and Mexico (Navarrete Gutiérrez et al., 2018), have failed to find any hyperaccumulators. Many of the newly discovered hyperaccumulators grow on non-ultramafic soils (McCartha et al., 2019; Navarrete Gutiérrez et al., 2024; Navarrete Gutiérrez, Pollard, et al., 2021). The ultramafic rocks of the GSZ have been suggested as the regional source of Ni and other elements in such soils. Although some of the recently described hyperaccumulators occur in the general area of the GSZ, there have been no studies, to the knowledge of the present authors, focused specifically on hyperaccumulator plants in Guatemala. The purpose of the research reported here was to determine whether additional hyperaccumulators of Ni occur on the ultramafic soils of Guatemala, based on both herbarium and field research, and to make initial field studies of the distribution and ecology of such plants to confirm their status and initiate physiological and ecological understanding of the hyperaccumulation trait.

2 | MATERIALS AND METHODS

2.1 | XRF screening of herbarium specimens

We conducted a targeted screening of herbarium specimens from the Missouri Botanical Garden Herbarium

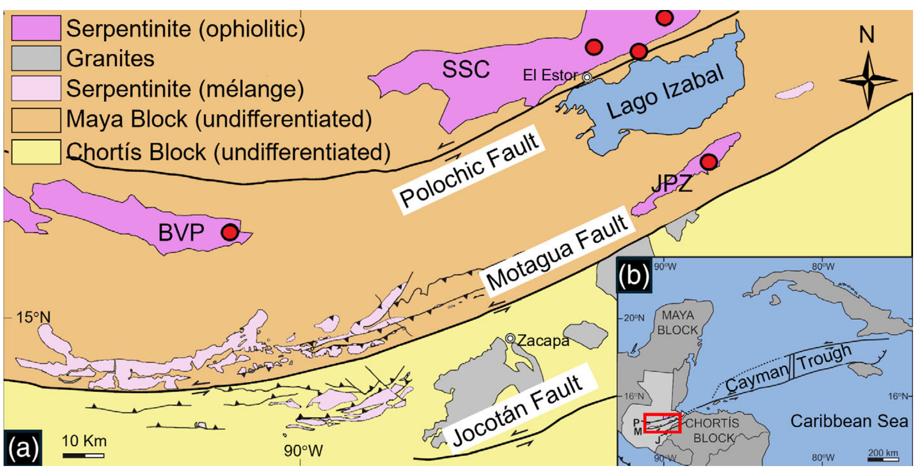

FIGURE 1 (a) Map of the Guatemala Suture Zone, showing the three ophiolitic serpentinite units examined in this study. JPZ = Juan de Paz unit. SSC = Sierra Santa Cruz unit. BVP = Baja Verapaz unit. Approximate location of collection sites is indicated by red dots. (b) Inset map showing the relationship of the Polochic, Motagua, and Jocotán faults in Guatemala to the Cayman Trough in the Caribbean Sea. The red rectangle on the inset map corresponds to the region shown in (a). Maps modified from Harlow et al. (2011).

(MO) using handheld XRF analysis. This herbarium houses extensive collections from Central America (Funk, 2017). Using the "advanced search" functions of the Tropicos database (Tropicos.org, 2019), we obtained a list of all catalogued specimens at MO that were collected in the Municipio de El Estor, a region of approximately 3000 km^2 (Concejo Municipal de El Estor, 2018), which encompasses extensive areas of ophiolitic serpentinite (Giunta et al., 2002; Harlow et al., 2011; Martin et al., 2016), and also includes the CGN-PRONICO Ni-laterite mine. This initial list included 795 taxa and 1465 specimens. The initial list was then cross-referenced with the Global Hyperaccumulator Database (Reeves et al., 2017), to generate a target list of specimens from the El Estor area belonging to genera known to include hyperaccumulators anywhere in the world. A total of 204 specimens representing 98 taxa were thus targeted for XRF-scanning. During the measurement process, if any target specimen had a foliar Ni concentration close to or exceeding the hyperaccumulation threshold of 1000 mg kg^{-1}, we measured all specimens of that particular species, regardless of their provenance, in order to understand the geographic distribution of hyperaccumulation across the range of that species. Additional measurements on species from the target list were made at two herbaria in Guatemala, Universidad de San Carlos de Guatemala (USCG) and Universidad del Valle (UVAL), both located in the capital, Guatemala City. Label information, including locality, was recorded for all measured specimens.

Measurement of herbarium specimens using XRF has been described in several previous publications (McCartha et al., 2019; Navarrete Gutiérrez, Pollard, et al., 2021; van der Ent, Echevarria, et al., 2019; van der Ent, Ocenar, et al., 2019). We used a Thermo Niton XLt3 GOLDD+ handheld analyzer (Thermo Fisher Scientific, Waltham, MA, USA), in "soils" mode, with a 30 s duration of measurement. To provide a uniform background and block transmitted x-rays, a titanium metal sheet (dimensions 100 × 100 mm and 2 mm thick, purity 99.7%; Sigma-Aldrich, St. Louis, MO, USA) was placed beneath the portion of the specimen to be measured. Concentrations of 18 elements were recorded, but this report focuses primarily on Ni.

This study used the same individual instrument described in Navarrete Gutiérrez, Pollard, et al. (2021), and we therefore employed the calibration equation derived empirically in that study to improve the accuracy of Ni measurements on leaf specimens: $y = 1.1216x^{0.953}$, where x = the instrument reading and y = the corrected Ni concentration. The instrument returned Ni concentration readings as low as 40 mg kg^{-1}, with lower concentrations displayed as "<LOD" (Limit of Detection). We have reported the data according to instrumental output, corrected by the calibration equation. Other studies have calculated a somewhat higher LOD based on more theoretical grounds (Gei et al., 2020; van der Ent, Ocenar, et al., 2019). In practical terms the difference is unimportant, as both LOD values are far below the 1000 mg kg^{-1} threshold criterion for hyperaccumulation.

2.2 | Field studies

In July 2019, we conducted field work in Guatemala, concentrating especially on re-visiting localities of hyperaccumulators found in herbarium screening, along with other known ultramafic outcrops. We tested for Ni hyperaccumulation in the field by crushing leaves in filter paper impregnated with dimethylglyoxime (DMG), and also with the handheld XRF analyzer described above. If the tests were positive, samples of corresponding leaves and soils were collected for later analysis using the laboratory procedures described below. At two sites, multiple specimens of a common hyperaccumulator were collected, along with paired soil samples from their root zones, to investigate within-population variation and its relation to soil properties. Voucher specimens were deposited in the herbarium of the Universidad de San Carlos de Guatemala (USCG).

The main study areas were in localities mapped as ophiolitic serpentinite (Giunta et al., 2002; Harlow et al., 2011; Martin et al., 2016), in the Juan de Paz (JPZ), Sierra Santa Cruz (SSC), and Baja Verapaz (BVP) geological units (Figure 1). Sites in the JPZ unit were in low hills south of Lago Izabal (54–64 m above sea level). The SSC unit, north of Lago Izabal, is larger and more diverse, and our study sites included both upland forest at elevations of 250–280 m.a.s.l. and alluvial lowlands (30–60 m. a.s.l.). In the BVP unit, we conducted studies in the Biotopo del Quetzal, a cloud forest reserve (1615–1888 m.a.s.l.). It was not possible to obtain a permit to visit the CGN-PRONICO mine; however, many specimens collected from the vicinity of the mine were measured in our herbarium screening.

2.3 | Analytical protocols for field samples

Leaf samples were digested using the HNO_3–H_2O_2 protocol of Navarrete Gutiérrez, Pollard, et al. (2021), and the Ni concentrations of digests were measured by inductively coupled plasma–atomic emission spectrometry (ICP-AES; Thermo Fisher iCAP 6300 Duo, Waltham, MA, USA). Soil samples were air-dried for 48 h and then measured using the XRF analyzer as an estimate of total trace element concentrations in the soil. Available Ni, Co, and Cr in soil samples were estimated using diethylenetriaminepentaacetic acid (DTPA) as an extractant (adapted from Lindsay & Norvell, 1978), followed by ICP-AES, as described above. Available K, Ca, and Mg were determined by extraction in 1 N ammonium acetate and measurement using flame atomic absorption spectroscopy. Concentrations of total N and available P in soils were measured by micro-Kjeldahl and Olsen methods, respectively (Horta & Torrent, 2007; Nelson & Sommers, 1980). Soil pH was measured on a 1:5 soil: water suspension.

2.4 | Nomenclature and data analysis

Taxonomic nomenclature in this paper follows the *WFO Plant List* (World Flora Online, 2023). Many taxa mentioned here are systematically problematic and currently under revision. Our use of the *WFO Plant List* is not intended as an acceptance or endorsement of a specific classification or nomenclature, but merely an attempt to reference a comprehensive, standardized, and publicly available source. When herbarium sheets or cited literature used a different name, this has been noted.

Statistical analysis was performed with R version 4.0.3 (R Core Team, 2020). Maps were generated using ArcGIS 10.3 (ESRI, 2011).

3 | RESULTS

3.1 | Herbarium studies

A total of 840 herbarium specimens were measured at MO, USCG, and UVAL representing approximately 124 taxa. Of these, 116 species consistently had Ni concentrations far below the 1000 mg kg^{-1} criterion for recognition of Ni hyperaccumulation (van der Ent et al., 2013) and usually below the LOD of the XRF instrument. These non-accumulator species are listed in Table S1 (available as an online supplement). Newly detected accumulators and hyperaccumulators based on herbarium scanning included *Arachnothryx linguiformis*, *Arachnothryx buddleioides*, *Chionanthus panamensis*, and *Orthion guatemalense* (Table 1; Figure 2).

TABLE 1 Nickel accumulator and hyperaccumulator species from Guatemala, detected by x-ray fluorescence spectroscopy of plant specimens from MO, USCG, and UVAL herbaria.

Species name	Family	Number of specimens measured	Number with [Ni] >1000 mg kg^{-1}	Maximum [Ni] (mg kg^{-1})	Minimum [Ni] (mg kg^{-1})
Newly reported hyperaccumulators					
Arachnothryx linguiformis (Hemsl.) Borhidi[a]	Rubiaceae	42	41	16,460	–[b]
Arachnothryx buddleioides (Benth.) Planch.[c]	Rubiaceae	38	0	950	–
Chionanthus panamensis (Standl.) Stearn	Oleaceae	70	13	6040	–
Orthion guatemalense Lundell[d]	Violaceae	6	4	5100	380
Previously known hyperaccumulators					
Psychotria grandis Sw.[e]	Rubiaceae	22	14	11,740	–
Psychotria costivenia Griseb.[e]	Rubiaceae	19	4	12,050	–
Blepharidium guatemalense Standl.[f]	Rubiaceae	28	28	30,240	1163
Orthion subsessile (Standl.) Standl. & Steyerm.[d]	Violaceae	34	30	18,710	450

Note: Foliar Ni concentrations are on a dry weight basis. Minimum Ni concentrations below the instrumental limit of detection are indicated with "–"; means are not reported due to the presence of such values. Individual specimens measured by McCartha et al. (2019) and Navarrete Gutiérrez, Pollard, et al. (2021); Navarrete Gutiérrez, Nkrumah, et al. (2021) are not repeated in these results. Nomenclature follows the *WFO Plant List* (World Flora Online, 2023); specimens labeled with a synonymous name are noted.

[a]Includes specimens labeled as *Rondeletia linguiformis* Hemsl.

[b]Nickel concentration < LOD was in a geographic outlier from Mexico; otherwise, minimum [Ni] was 1190 mg kg^{-1}.

[c]Includes specimens labeled as *Rondeletia buddleioides* Benth.

[d]Described in greater detail in a separate paper in this issue (Navarrete Gutiérrez et al., 2024).

[e]Previously reported as a hyperaccumulator by McCartha et al. (2019).

[f]Previously reported as a hyperaccumulator by Navarrete Gutiérrez, Pollard, et al. (2021).

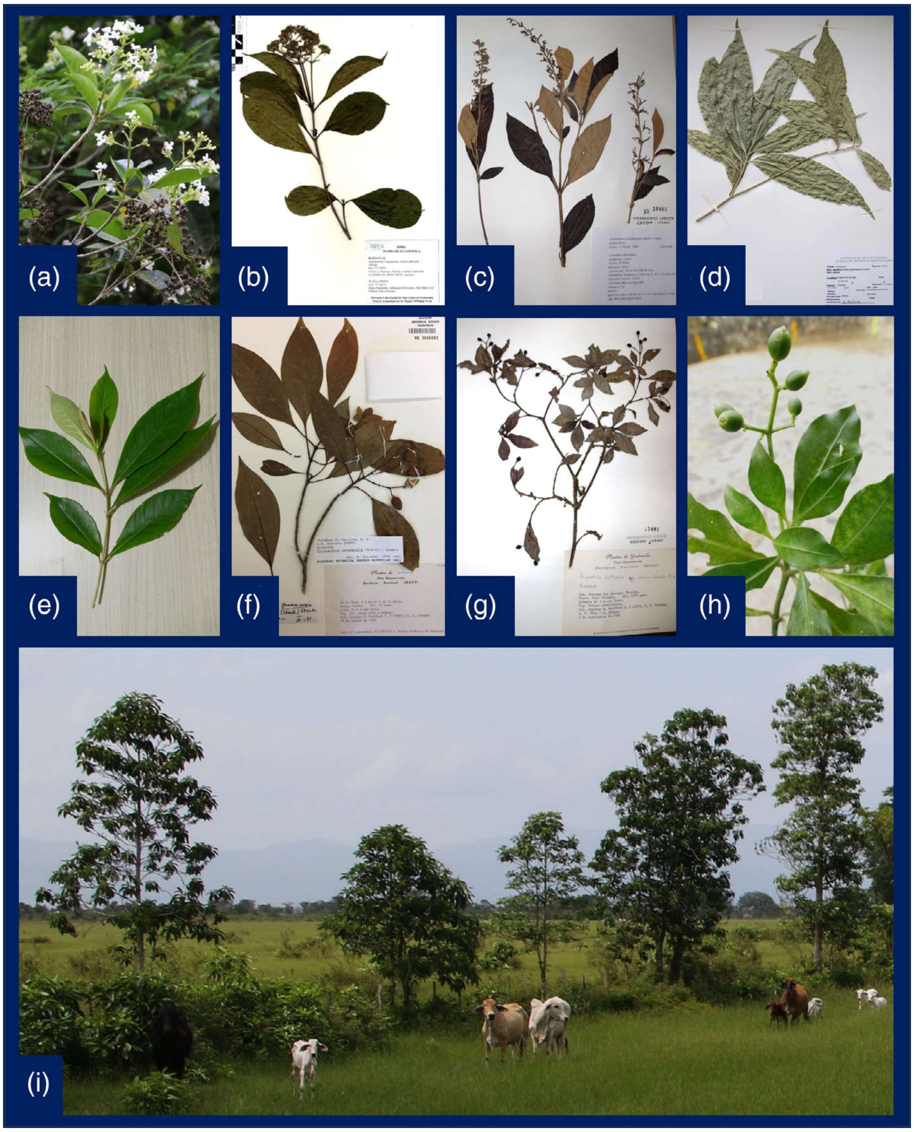

FIGURE 2 Examples of plants and communities mentioned in this paper. See Figure 1 for locations of geological units and collection sites. (a) *Arachnothryx linguiformis*, SSC unit. (b) Voucher specimen of *A. linguiformis* from USCG herbarium, collected in JPZ unit. (c) Specimen of *A. buddleioides*, USCG herbarium. (d) Specimen of *Orthion guatemalense*, UVAL herbarium. (e) Sterile branch of *Chionanthus panamensis*, JPZ unit. (f) Specimen of *C. panamensis*, MO herbarium. (g) Specimen of *Psychotria costivenia* subsp. *altorum*, USCG herbarium. (h) *Psychotria costivenia* subsp. *altorum* with unripe fruit, BVP unit. (i) Agricultural landscape with the hyperaccumulating tree *Blepharidium guatemalense* growing along a fence line, near the northern shore of Lago Izabal.

Arachnothryx linguiformis (Hemsl.) Borhidi is a tall shrub in the Rubiaceae, formerly included in the genus *Rondeletia*. Its distribution, according to published literature, includes Guatemala and the Mexican state of Chiapas (Davidse et al., 2012). However, of the 42 specimens in our herbarium surveys, only one was from Chiapas, whereas the remainder were collected from localities in the Sierra Santa Cruz and Juan de Paz units, near Lago Izabal, Guatemala (Figure 3). All the Guatemalan specimens were hyperaccumulators, with foliar Ni concentrations ranging from 1200 to over 16,000 mg kg^{-1} dry weight (Figure 3). The only specimen below 1000 mg kg^{-1} Ni was the one from Chiapas, with Ni concentration < LOD.

Arachnothryx buddleioides (Benth.) Planch. is a much more common, widespread, and morphologically variable species, ranging from southern Mexico to Panama (Davidse et al., 2012). None of the 38 measured specimens exceeded the hyperaccumulator threshold of 1000 mg kg^{-1}, but 8 of them, all from the department of Izabal in Guatemala, were above 500 mg kg^{-1}, with a maximum concentration of 950 mg kg^{-1}.

Chionanthus panamensis (Standl.) Stearn is a tree in the family Oleaceae. Its reported range extends from southern Mexico to Panama (Davidse et al., 2009) and we measured specimens collected across this range (Figure 4). The frequency distribution of foliar Ni concentrations was distinctly bimodal (Figure 4), with the majority of measurements near or below the LOD, but with a large group in the range from 500 to over 6000 mg kg^{-1}. The hyperaccumulators were all collected from sites in the department of Izabal in Guatemala and the state of Chiapas in Mexico.

Orthion guatemalense Lundell is a tree in the Violaceae, formerly included in the genus *Hybanthus*. It has a highly restricted distribution, endemic to areas surrounding Lago Izabal in Guatemala (Lundell, 1984), and has not previously been recognized as a hyperaccumulator.

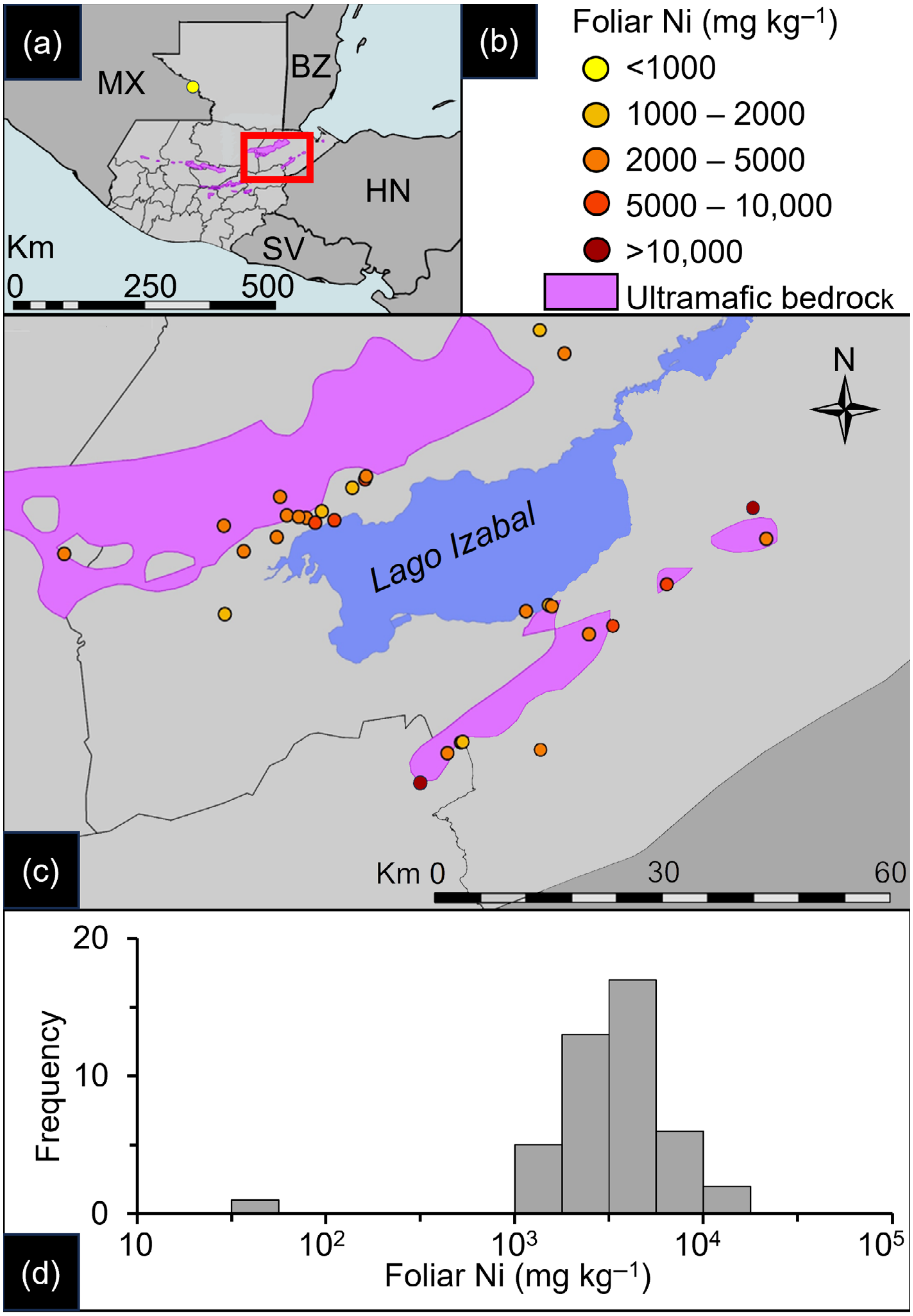

FIGURE 3 Collection localities and foliar Ni concentrations in herbarium specimens of *Arachnothryx linguiformis* (Rubiaceae). (a) Map of Guatemala (lighter gray) and adjacent countries (MX = Mexico; BZ = Belize; HN = Honduras; SV = El Salvador). Red rectangle indicates area of detail map below. (b) Legend for color coding of foliar Ni concentration in specimens. The only specimen with Ni concentration <1000 mg kg^{-1} was a geographic outlier shown in map (a). (c) Detail map of areas surrounding Lago Izabal in eastern Guatemala. All specimens in these areas exceeded the threshold concentration criterion for Ni hyperaccumulation. (d) Frequency histogram of Ni concentration in specimens of *A. linguiformis*, with concentrations plotted on a logarithmic scale.

Hyperaccumulation of Ni in the much more widespread species *O. subsessile* (Standl.) Standl. & Steyerm. has previously been reported (Invernón et al., 2021; Nkrumah et al., 2021; Reeves et al., 2021). A more complete description of hyperaccumulation in this genus is provided in a related paper in this issue (Navarrete Gutiérrez et al., 2024).

3.2 | Field studies of ultramafic communities: General descriptions

Physico-chemical characteristics of soil samples from field sites studied in Guatemala are summarized in Table 2. Sites in the JPZ unit, south of Lago Izabal, had soil properties typical of ultramafic outcrops, including a mean Ca/Mg quotient of 0.44, and elevated concentrations of Ni, Cr, and Co. Samples in the SSC unit, north of the lake, were collected over a larger area and wider range of elevations and substrates, including lacustrine and riparian alluvium at the lower elevations, and their chemistry was correspondingly more varied, with less extreme concentrations of trace elements at some sites and a slightly higher Ca/Mg quotient (mean = 0.61), though still within the typical ultramafic range. The high-elevation site in the BVP unit had more acidic soils, with highly variable concentrations of Ni, Co, and Cr,

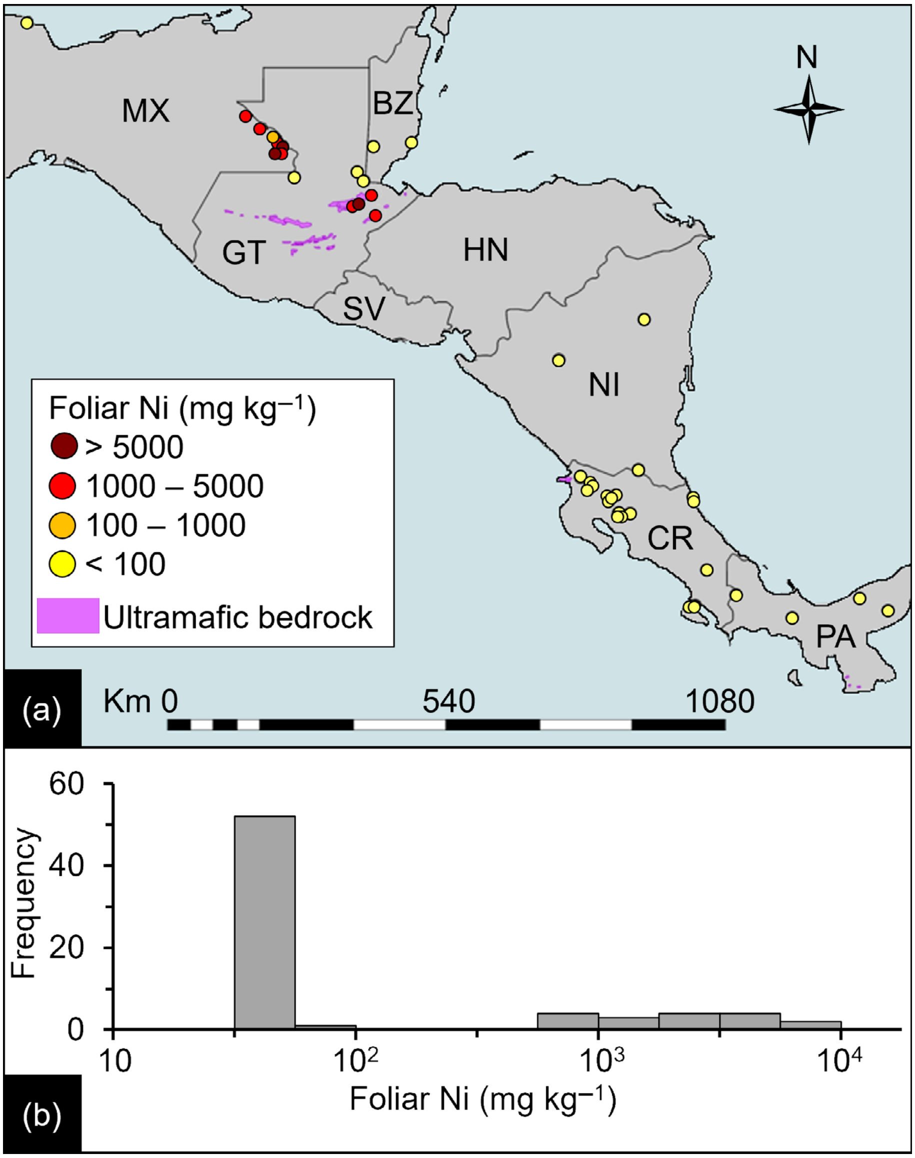

FIGURE 4 (a) Collection localities and foliar Ni concentrations in herbarium specimens of *Chionanthus panamensis* (Oleaceae) across Central America. Country codes: MX = Mexico; GT = Guatemala; BZ = Belize; HN = Honduras; SV = El Salvador; NI = Nicaragua; CR = Costa Rica; PA = Panama. (b) Frequency histogram of Ni concentration in *C. panamensis*, with concentrations plotted on a logarithmic scale.

which were <LOD in some individual samples. Soil profile descriptions made during our exploration of this site revealed the common existence of highly eroded podzols on ultramafic bedrock, which may have led to localized Ni loss from surface horizons. Thus, the geochemical variability of the soils of this region was extremely high.

The plant communities in the JPZ and SSC units appeared broadly similar, although time did not permit a detailed and quantitative community analysis. Much of the accessible area consisted of anthropogenically disturbed landscapes such as roadsides and agricultural land, both active and abandoned (Figure 2). Four hyperaccumulator species were commonly observed in these ruderal communities: *B. guatemalense*, *O. subsessile*, *P. costivenia*, and *A. linguiformis*. In some localities, all four grew immediately adjacent to one another. Only one example of *C. panamensis* was found in our study, growing in the JPZ unit. Nickel concentrations in samples of these five species (Table 3) were somewhat higher than those found in our herbarium surveys, which may simply reflect the fact that collection sites were intentionally located on ultramafic outcrops. We did not observe any living examples of *A. buddleioides*, *O. guatemalense*, or *P. grandis* during the fieldwork reported here.

The BVP unit supported a montane cloud forest community, distinctly different from the other two study areas. Anthropogenic disturbance was much less pronounced, due to the protected reserve of the Biotopo del Quetzal. Only one hyperaccumulator taxon was observed in this area, *P. costivenia* subsp. *altorum* (Standl. & Steyerm.) Borhidi. This entity has been variously recognized at the specific, subspecific, and varietal levels, and its classification remains uncertain (pers. comm., C. M. Taylor, Missouri Botanical Garden). The shrubs we observed were small in stature and had smaller leaves and inflorescences than the more common *P. costivenia* subsp. *costivenia* seen in other areas of Guatemala or Mexico. In their herbarium survey, McCartha et al. (2019) reported that *P. costivenia* subsp. *altorum* is a facultative hyperaccumulator across its range in these two countries. Field tests with DMG paper indicated that some individuals within the Biotopo del Quetzal were Ni hyperaccumulators while others were not, in some cases over a spatial scale of just a few meters. Subsequent analysis of acid digests by ICP-AES confirmed this variability (Table 3).

3.3 | Field studies of ultramafic communities: Plant–soil relationships

In a site in the JPZ unit, leaf samples from seven *A. linguiformis* shrubs were collected, along with corresponding soil samples from the upper 15 cm of soil under the canopy of each. Total soil Ni concentrations of the samples ranged from 1950 to 10,700 mg kg^{-1}, whereas DTPA-extractable Ni concentrations ranged from 290 to 3920 mg kg^{-1}. Total and extractable soil concentrations showed a strong positive correlation with each other (Pearson's $r = 0.98$, $p < 0.001$). Leaf Ni concentrations ranged from 2860 to 12,760 mg kg^{-1}. Leaf concentrations did not show a statistically significant correlation with either measure of soil concentration ($r = 0.63$, $p = 0.14$ for total soil Ni; $r = 0.64$, $p = 0.13$ for extractable soil Ni).

A similar protocol was employed in the cloud forest of the BVP unit to investigate the facultative hyperaccumulator *P. costivenia* subsp. *altorum*. Eight paired leaf and soil samples were collected. Total soil Ni concentrations ranged from 190 to 1710 mg kg^{-1},while DTPA-extractable Ni concentrations ranged from 3 to 180 mg kg^{-1}. Once again, total and extractable soil concentrations showed a significant positive correlation ($r = 0.81$, $p = 0.015$). Leaf Ni concentrations included values both below and above the hyperaccumulation threshold, ranging from 136 to 13,750 mg kg^{-1}. However, leaf concentrations again did not show a statistically significant correlation with either total or DTPA-extractable soil concentrations ($r = 0.34$, $p = 0.40$ for total soil Ni; $r = 0.46$, $p = 0.25$ for extractable soil Ni).

4 | DISCUSSION

Hyperaccumulation of Ni in Guatemalan specimens of *P. grandis*, *P. costivenia*, and *B. guatemalense* has already been reported (McCartha et al., 2019; Navarrete Gutiérrez, Pollard, et al., 2021). The additional specimens examined in this study, from herbaria in Guatemala, confirmed previous findings that *B. guatemalense* is an obligate hyperaccumulator, endemic to ultramafic soils and with high Ni concentrations in all specimens, whereas the two species of *Psychotria* are facultative hyperaccumulators (Pollard et al., 2014), in which some individuals hyperaccumulate Ni while others do not, presumably due to low Ni availability in the soil. Field studies in Guatemala also confirmed the hyperaccumulator status of *P. costivenia* and *B. guatemalense*.

It appears that there are many hyperaccumulator species in *Orthion*, along with its sister genus *Mayanaea* Lundell (syn. *Leonia* Ruiz & Pav.) (Navarrete Gutiérrez et al., 2024). A complete survey of hyperaccumulation in these genera is beyond the scope of this study, because many of the species are endemic to areas outside Guatemala. A more comprehensive analysis of this group is described in a separate paper in this issue (Navarrete Gutiérrez et al., 2024).

Like *B. guatemalense*, the recently discovered hyperaccumulators *A. linguiformis*, *O. subsessile*, and *O. guatemalense* appear to conform closely to the concept of obligate hyperaccumulators. They occur mostly, if not exclusively, on ultramafic soils, and almost always have elevated foliar Ni concentrations. When exceptions occur, they may represent misidentified specimens, which is always a potential risk in herbarium screening. In particular, it is possible that the specimen labeled as *A. linguiformis* that was collected in Chiapas, Mexico, with very low foliar Ni concentration, may have been misidentified. It is important to note in this context that there are many species in the genus *Arachnothryx* which are not hyperaccumulators. In our herbarium surveys, we measured Ni concentrations in 13 species of *Arachnothryx*. In the 11 species other than *A. linguiformis* and *A. buddleoides*, none of the 42 specimens had Ni concentrations above the LOD (Supplementary Table S1).

The maximum Ni concentrations observed in *A. buddleioides* fell just short of the hyperaccumulator threshold, but such criteria should not be regarded as "sacrosanct" or rigid (van der Ent et al., 2013). In any case, it seems likely that further study may find individual plants with higher Ni concentrations. The species also includes individuals with lower Ni concentrations, presumably growing on soils with lower Ni availability; thus, it may be considered a facultative hyperaccumulator.

Chionanthus panamensis is also a facultative hyperaccumulator. It occurs over an extensive geographic range, but hyperaccumulates only when growing on metalliferous soil. Further studies of this species are needed to confirm whether the northern, Ni-accumulating populations in Guatemala and Mexico are conspecific with the more

TABLE 2 Physico-chemical characteristics of soils from field sites in Guatemala.

	Ultramafic unit		
	Juan de Paz (JPZ)	Sierra Santa Cruz (SSC)	Baja Verapaz (BVP)
Number of sites sampled	7	7	8
Elevation (m.a.s.l.)	54–64 (55)	31–280 (139)	1615–1888 (1743)
pH	6.0–6.7 (6.3)	6.2–6.9 (6.6)	3.8–5.4 (4.6)
N	4300–8600 (6700)	4000–9100 (6200)	6200–19,000 (12,600)
P (available)	10.6–16.0 (12.6)	10.0–12.6 (11.4)	9.4–15.7 (12.6)
K (available)	0.23–0.67 (0.44)	0.26–0.33 (0.31)	0.44–0.46 (0.45)
Ca (available)	4.2–8.5 (6.7)	4.5–14.5 (9.9)	1.0–5.7 (3.4)
Mg (available)	11.9–20.6 (17.3)	11.1–26.5 (18.9)	7.0–7.8 (7.4)
Co (total)	840–2079 (1396)	376–816 (604)	92–1225 (521)
Co (available)	92.2–142.9 (108.0)	45.2–155.9 (106.5)	<LOD–22.8 (9.5)
Cr (total)	3530–5460 (4390)	1300–6640 (4760)	140–6650 (2960)
Cr (available)	8.1–12.9 (10.4)	5.0–14.6 (9.3)	<LOD–1.2 (0.6)
Ni (total)	1950–10,700 (4950)	900–2990 (1620)	<LOD–1710 (550)
Ni (available)	293–3920 (1480)	170–945 (424)	3.3–180 (42)

Note: Locations of the three ultramafic units are shown in Figure 1. Data are listed as ranges, with means in parentheses. Elevation is in meters above sea level (m.a.s.l.). Elemental concentrations are in mg kg^{-1}. Protocols for chemical analyses are described in the Materials and Methods. In cases where minimum concentrations were below the instrumental limit of detection (<LOD), the LOD value was used in calculation of means.

TABLE 3 Foliar nickel concentrations in field-collected samples of hyperaccumulators from Guatemala.

Taxon	Location	N	Median [Ni]	Minimum [Ni]	Maximum [Ni]
Blepharidium guatemalense	JPZ, SSC	10	21,370	9070	55,910
Orthion subsessile	JPZ, SSC	5	15,880	10,080	32,440
Psychotria costivenia subsp. *costivenia*	JPZ, SSC	9	10,370	4830	20,140
Arachnothryx linguiformis	JPZ, SSC	14	4690	2450	12,760
Chionanthus panamensis	JPZ	1	6810		
Psychotria costivenia subsp. *altorum*	BVP	8	1100	140	13,750

Note: Collection locations were in the Juan de Paz (JPZ), Sierra Santa Cruz (SSC), Baja Verapaz (BVP) ultramafic units, mapped in Figure 1. Samples were digested in HNO_3 and H_2O_2, and analyzed using ICP-AES (Navarrete Gutiérrez, Pollard, et al., 2021). Ni concentrations are reported in mg kg^{-1} dry weight.

southerly, non-accumulating populations, and whether all plants in the species possess the physiological ability to accumulate Ni. Hyperaccumulation in the related species *Chionanthus domingensis* was previously reported in Cuba (Reeves, 2003; Reeves et al., 1999). During our studies at MO, we also found a specimen of *C. domingensis* with 1070 mg kg^{-1} Ni, collected from the ultramafic soils of Maricao State Forest in Puerto Rico. This represents only the second hyperaccumulator known from Puerto Rico, along with *P. grandis* (Campbell et al., 2013; McAlister et al., 2015). The two hyperaccumulator species now documented in the genus *Chionanthus* D. Royen (syn. *Linociera* Sw. ex Schreb.) are currently the only hyperaccumulators known from the family Oleaceae worldwide (Reeves et al., 2017).

Variation in foliar metal concentration occurs within many hyperaccumulator species (Pollard et al., 2002), and may result from either genetic or environmental differences among plants. We investigated the relationship between soil Ni concentration and foliar Ni concentration in the obligate hyperaccumulator *A. linguiformis*, and in the facultative hyperaccumulator *P. costivenia* subsp. *altorum*. Soil Ni was measured as both total (XRF) and available (DTPA-extractable) concentration. Not surprisingly, total Ni concentrations were higher than available Ni concentrations, but the two soil measurements were strongly correlated, suggesting that the labile pool of Ni in the soil is derived from weathering of less soluble minerals. Although the soil–plant correlation coefficients were positive, none were statistically significant for either species. One hypothesis to explain this lack of correlation is that both species are shrubs with potentially extensive root systems, growing in rocky soil. A single sample of topsoil collected under each plant may not fully represent the elements available throughout that individual's complete root zone (Paul & Chaney, 2023). Hyperaccumulators may also possess active metal uptake mechanisms that are not accurately mimicked by either extraction protocol; however, little is known about such rhizosphere mechanisms, especially in tropical species (van der Ent et al., 2016).

As previously mentioned, the extensive ultramafic areas of Guatemala may be the source of Ni in non-ultramafic soils of southern Mexico, by processes such as transport of lateritic clay particles by water and/or the deposition of volcanic ash (Hernández-Quiroz et al., 2012; Navarrete Gutiérrez, Pollard, et al., 2021). It is also possible that the ultramafic outcrops of the Guatemala Suture Zone may have been a center of biological diversity in the evolution of hyperaccumulators that are now known to occur throughout Central America. Approximately 15 hyperaccumulator species are now documented from Central America and Mexico, all of which have been reported in the last 5 years. However, the biodiversity of Central American hyperaccumulators is much lower at the family level, with all known examples coming from the Rubiaceae, Violaceae, and Oleaceae. Families known to include many hyperaccumulators in the Cuban flora (e.g., Asteraceae, Buxaceae, Euphorbiaceae, Myrtaceae, and Phyllanthaceae) (Belloeil et al., 2021; Reeves et al., 1996, 1999) were also represented in our XRF-based herbarium screening (see Table S1), but no hyperaccumulators were found from these groups on the ultramafic soils of Guatemala.

Both our herbarium screening and our fieldwork were focused on areas of Guatemala mapped as ophiolitic serpentinite. Other ultramafic units of the Guatemala Suture Zone, mapped in Figure 1 as serpentinite mélange (Giunta et al., 2002; Harlow et al., 2011; Martin et al., 2016), have not yet been examined for hyperaccumulation and would be worthy of further study, especially the highly diverse ecosystems of the Sierra de las Minas. On the other hand, it should be remembered that hyperaccumulators are generally uncommon, and many ultramafic soils, such as in the Peninsula of Santa Elena in Costa Rica and the outcrops in central Mexico, are not known to host any hyperaccumulators (Navarrete Gutiérrez et al., 2018; Reeves et al., 2007). Experience to date suggests that hyperaccumulators in Central America and tropical Mexico are typically woody plants growing in humid climates, whereas dry savannas and steep, eroded landscapes are less likely habitats for such species.

The targeted screening method used here, focusing on genera known to include hyperaccumulators in other parts of the world, is efficient; however, it fails to reveal hyperaccumulators in unexpected lineages, and thus may underestimate hyperaccumulator biodiversity. More comprehensive scanning (Belloeil et al., 2021; Gei et al., 2020; Invernón et al., 2021), while time-consuming, will be necessary to avoid bias toward previously known hyperaccumulator groups. An understanding of hyperaccumulator biodiversity is essential to the development of agromining and other biotechnologies. At this early stage, the most promising Central American species for such applications would seem to be *B. guatemalense* and *O. subsessile*, based on their rapid growth and exceptionally high foliar Ni concentrations.

Despite the fact that some plant groups or clades remained understudied, this study and others confirmed the strong tendency of certain plant families to host hyperaccumulators of Ni and other elements. In the Neotropics, this is especially true for the Rubiaceae and Violaceae. Given the highly disjunct geographic distribution of hyperaccumulation (Belloeil et al., 2021; Navarrete Gutiérrez et al., 2024), it would be of great interest to extend research on trace element hyperaccumulation in these particular families throughout the Neotropics.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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