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A SURVEY OF ALUMINIUM ACCUMULATION IN *EUMACHIA* (RUBIACEAE)

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A useful character for spotting specimens of the pantropical Rubiaceae genus *Eumachia* is the leaves drying green. The physiological reasons for this are unresolved. We investigated whether the phenomenon is related to the accumulation of aluminium. Samples of foliage from herbarium specimens of nine species of *Eumachia* (Rubiaceae), including collections from Asia-Pacific, Africa and the Americas, were analysed for elemental concentrations. For comparison, specimens of the closely related genera *Psychotria* and *Palicourea* from similar geographical regions were also analysed. Two species, *Palicourea violacea* (Panama) and *Psychotria pallens* (Brazil) were found to be aluminium hyperaccumulators (≥ 1% Al by dry weight), with a third species, *Eumachia collina* (from New Caledonia), falling just short of the threshold with 0.99% Al. Aluminium accumulation (≥ 0.4% Al by dry weight) occurs but is infrequent among species of *Eumachia* and appears less common than in the close relative *Palicourea*. Aluminium accumulation also appears patchy in *Psychotria* sensu stricto. It seems unlikely that herbarium greenness in *Eumachia* is directly related to aluminium accumulation.

Keywords. Hyperaccumulation, *Palicourea*, Palicoureeae, *Psychotria*, Psychotrieae, shrubs.
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Introduction

The rubiaceous genus *Eumachia*, despite being large (more than 100 species) and almost pantropical (Taylor *et al.*, 2017), is poorly known to tropical botanists. This is readily explained because nearly all the species were formerly included in *Psychotria*, probably the most species-rich genus of woody plants (Davis *et al.*, 2001). Added to this, once plant taxonomists began to separate out this group from *Psychotria* various names, including *Chalaziella* and *Margaritopsis* (Andersson, 2001; Barrabé *et al.*, 2012), were used before *Eumachia* was determined to be the correct one.

Species of *Eumachia* are typically shrubs or small trees with interpetiolar stipules that become hard and straw-coloured and often fragment from the margin, and pyrenes with marginal preformed germination slits (Taylor *et al.*, 2017). Another character that can be useful in determining herbarium material is that in most species of *Eumachia* the leaves

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dry distinctly green. Although there are species of true *Psychotria* that dry green, most dry various shades of red-brown or grey-brown.

The reason for the unusual colour of dry leaves in *Eumachia* species does not seem to be known. One possibility is that it is related to the accumulation of particular elements in the plant tissues. Given the extensive distribution of the genus and the absence of a common association of the species with unusual geological substrates such as ultramafics, aluminium accumulation on the typically acid and aluminium-rich soils of the lowland wet tropics is considered the most likely explanation. Chenery (1948) noted that leaves drying yellowish green were common among aluminium accumulators. There is a report, based on a semiquantitative chemical assay, of *Eumachia acuifolia* (C.Wright) Delprete & J.H.Kirkbr., a species from Cuba, being an aluminium hyperaccumulator (Jansen *et al.*, 2000, as *Margaritopsis nudiflora* K.Schum.). To investigate this further, we measured elemental concentrations in leaf material from herbarium specimens. To provide comparators, we sought to analyse material from species of *Psychotria sensu stricto*.

Materials and methods

The material for investigation all came from specimens in the collection of the Royal Botanic Gardens, Kew (K). Samples were chosen by going through the herbarium cupboards searching for *Eumachia* specimens with extra material of mature leaves loose in packets. These were then sampled for destructive analysis of selected elements including aluminium. Species from across the geographical range of the genus were sought. Specimens obviously from upper montane systems were avoided. To provide a comparator for each *Eumachia* specimen, a specimen of a *Psychotria* species from a similar region was also sought for use in the analysis. It transpired that for two of the American *Psychotria* species selected, the herbarium curation had not caught up with recent transfers to *Palicourea*, a large Neotropical genus. Therefore, the comparator taxon to *Eumachia* included species of both *Psychotria* and *Palicourea*. In total 18 samples were analysed. The specimens sampled are listed in the **Appendix**.

Leaf samples obviously muddy or otherwise exhibiting soil accretion were excluded. It is possible that some of the specimens used may have been treated with mercuric chloride as a prophylactic against insect pests, or contaminated with mercury from treated specimens. This is not likely to influence concentrations of aluminium, however.

The leaf samples (c.100 mg each) were digested in 10 mL of concentrated nitric acid by using a MARSXpress microwave (CEM, Matthews, NC, USA) and made up to 100 mL in ultrapure (18 MΩ) deionised water. Total Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P and Pb concentrations were quantified using an iCAP 6300 Duo inductively coupled plasma optical emission spectrometer (Thermo-Finnegan, San Jose, CA, USA). Reference material (LGC 7162, Strawberry Leaves) was analysed alongside the samples. These showed good recovery for the certified elemental concentrations, but Al recovery was considerably (62%) lower than the indicative (non-certified) concentration of 1000 μg/g. This may mean that our results for Al are lower than the true values, but they are likely to be a good reflection of relative concentrations between species.

In a statistical analysis of a large data set, Metali *et al.* (2011) found that a threshold value of 2300–3900 µg Al/g leaf dry mass was required in tropical species to distinguish aluminium accumulators from non-accumulators. To be conservative, we used a minimum value of 4000 µg/g or 0.4% for aluminium accumulators and 10,000 µg/g or 1% for hyperaccumulators.

To visualise the complete data set, a principal components analysis (PCA) was run using the software R (R Core Team, 2020).

Results

Evidence of aluminium accumulation (> 0.4% by dry weight) was found for four species (**Table**, **Figure 1**). These were *Palicourea conephoroides* (Rusby) C.M.Taylor, *Palicourea*

Table. Aluminium concentration in the leaves of 18 species from three genera in the Rubiaceae[a]

Species	Location	Aluminium (µg/g leaf dry mass)
Eumachia abrupta	Tanzania	1248
Psychotria leucopoda	Tanzania	66
Eumachia boliviana	Peru	140
Palicourea conephoroides	Peru	5515[b]
Eumachia cephalantha	Brazil	667
Psychotria pallens	Brazil	29,628[c]
Eumachia collina	New Caledonia	9910[b]
Psychotria frondosa	New Caledonia	329
Eumachia damasiana	New Guinea	129
Psychotria beaufortiensis	New Guinea	1141
Eumachia leptothyrsa	New Guinea	174
Psychotria lolokiensis	New Guinea	2016
Eumachia microdon	Panama	66
Palicourea violacea	Panama	33,477[c]
Eumachia montana	Sumbawa	197
Psychotria sp.	Sumbawa	296
Eumachia obanensis	Cameroon	1368
Psychotria hypsophila	Cameroon	368

[a] For species authorities, see the Appendix.
[b] Above the 4000 µg/g (0.4%) threshold for aluminium accumulation.
[c] Above the 10,000 µg/g (1.0%) threshold for aluminium hyperaccumulation.

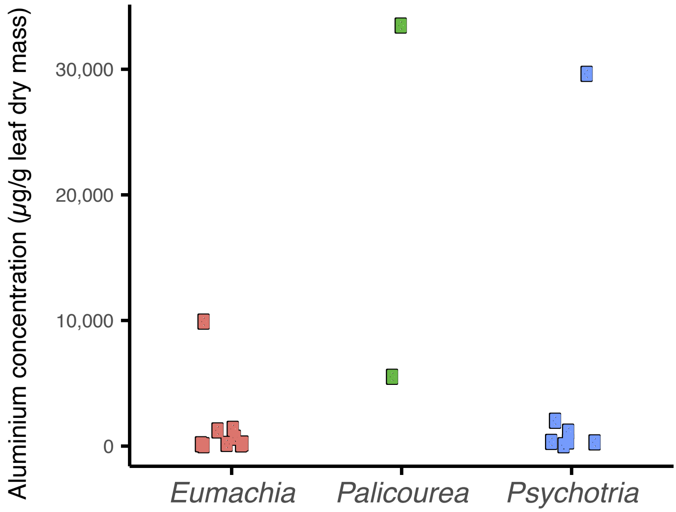

Figure 1. Aluminium concentration in the leaves of 18 species from three Rubiaceae genera.

violacea (Aubl.) A.Rich. and *Psychotria pallens* Gardner from the Neotropics, and *Eumachia collina* (Labill.) Barrabé, C.M.Taylor & Razafim. from New Caledonia. *Palicourea violacea* and *Psychotria pallens* had Al concentrations well above the threshold for hyperaccumulation, with Al concentration in *Eumachia collina* (0.99%) falling just short.

No consistent pattern emerged from the comparison of *Eumachia* species with *Psychotria*/*Palicourea* in terms of foliar Al concentration. For the Asia-Pacific and Neotropical pairs, *Eumachia* species generally had lower Al concentrations than *Psychotria*/*Palicourea*, but for the two African comparators the trend was reversed.

The first two axes of the PCA (Figure 2) accounted for 48% of the variance in the data. The first axis (principal component 1; 32% of variance explained) correlated positively with Al concentration and negatively with the concentration of most of the other elements (see Figure 2B). The second axis (principal component 2; 16% of variance) more effectively discriminated between *Eumachia* and *Psychotria*/*Palicourea*. This axis opposed foliar concentrations of K, P and Mg with those of Mn, Pb and Al (see Figure 2B). *Eumachia* species generally had a higher score on this axis, with *Psychotria* and *Palicourea* species at the lower end, although there was some overlap (see Figure 2A).

Discussion

Our sample size was small and unreplicated within species, but some patterns were evident. Of the nine species of *Eumachia* analysed, only one (*Eumachia collina* from New Caledonia) was found to be an aluminium accumulator, although it very nearly qualified as a hyperaccumulator. Although this adds to the previous record of this phenomenon, from

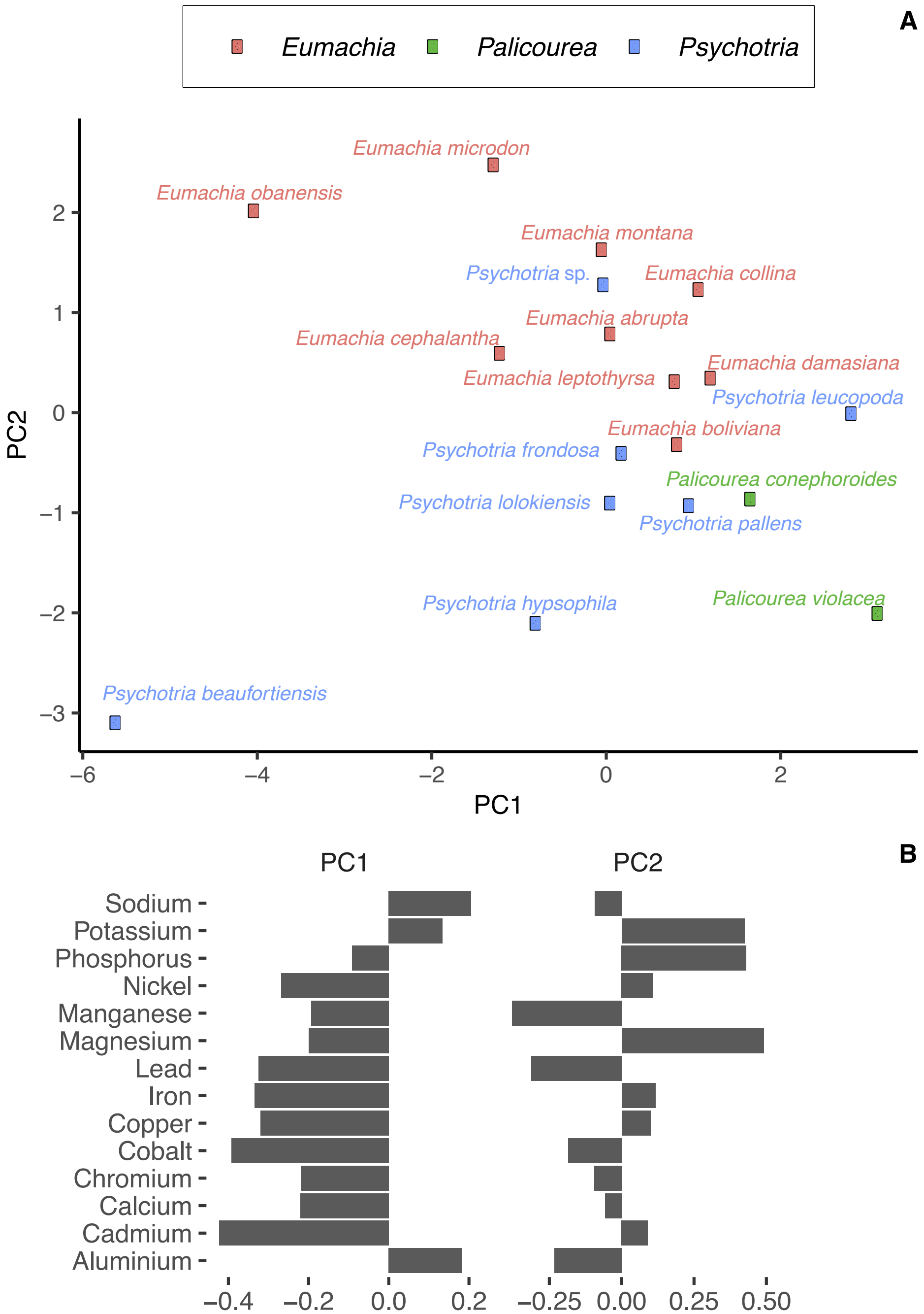

Figure 2. Results of principal components analysis: A, biplot of foliar concentrations of 14 elements; B, correlation between the axis score and elemental concentration for 18 species from three Rubiaceae genera. PC, principal component.

Eumachia acuifolia (Jansen *et al.*, 2000), it is clear that the accumulation of aluminium is not a universal or even frequent phenomenon in *Eumachia*.

The comparison between *Eumachia* and *Psychotria* foliar Al concentration found no consistent differences. One of the seven species of *Psychotria* tested was a clear aluminium hyperaccumulator. Notably, *Psychotria pallens* has foliage that dries a pale green rather than red-brown, which is the general pattern in *Psychotria sensu stricto*. Although only two species of *Palicourea* were analysed, both were found to be aluminium accumulators. One had the highest foliar aluminium concentrations recorded in this study and the other was well over the accumulator threshold. Aluminium hyperaccumulation has been previously reported in species of *Palicourea* (Chenery, 1948; Jansen *et al.*, 2000), but its prevalence in the genus is considerably greater than this, because many of the species listed in these reports have subsequently been transferred to *Palicourea*. So, for instance, of the 24 species of *Psychotria* and *Cephaelis* that Chenery (1948) reported as aluminium hyperaccumulators, only three are now recognised in *Psychotria* and the rest have all been relocated to *Palicourea*.

The taxonomic changes in these Rubiaceae genera have largely been driven by studies of phylogenetic relationships using molecular data, although the morphological overview of Taylor (1996) presaged much of the rearrangement. Phylogenetic analyses have shown that *Psychotria*, as traditionally recognised, contained disparate elements representing various clades (Nepokroeff *et al.*, 1999; Razafimandimbison *et al.*, 2014). The ongoing reorganisation has resulted in considerable change to the systematics of this part of the Rubiaceae. These include the recognition of the tribe Palicoureeae as sister to Psychotrieae (Robbrecht & Manen, 2006), the transfer of much of the heterogeneous *Psychotria* subgenus *Heteropsychotria* to *Palicourea*, and the recognition of *Eumachia* as a large pantropical genus in the Palicoureeae. The currently accepted phylogeny for the genera in this clade of the Rubiaceae is shown in **Figure 3**.

Aluminium accumulation is widely known in the Rubiaceae (Jansen *et al.*, 2000), although apparently commoner in subfamily Rubioideae than in subfamily Cinchonoideae. Our results, and those of others (Chenery, 1948; Haridasan, 1982; Jansen *et al.*, 2000), confirm the presence of aluminium accumulation in the sister tribes Psychotrieae and Palicoureeae. The prevalence of aluminium accumulation seems mixed among the genera of these tribes. *Palicourea* has many species that are aluminium hyperaccumulators, but its cotribalist *Eumachia* is less frequently found to have tissues with abundant aluminium. Interestingly, *Palicourea* species also tend to have leaves that dry with a greenish hue (Berger, 2018), and this is listed as a character of the Palicoureeae in general (Stevens, continuously updated). *Palicourea* species often have blue fruits (Taylor & Steyermark, 2004; Berger, 2018), which is a known correlate of aluminium hyperaccumulation (Chenery, 1948), but in *Eumachia* the fruits generally ripen orange-red (Taylor *et al.*, 2017). It seems likely, therefore, that an inherent tolerance to relatively high internal aluminium concentrations is widespread and

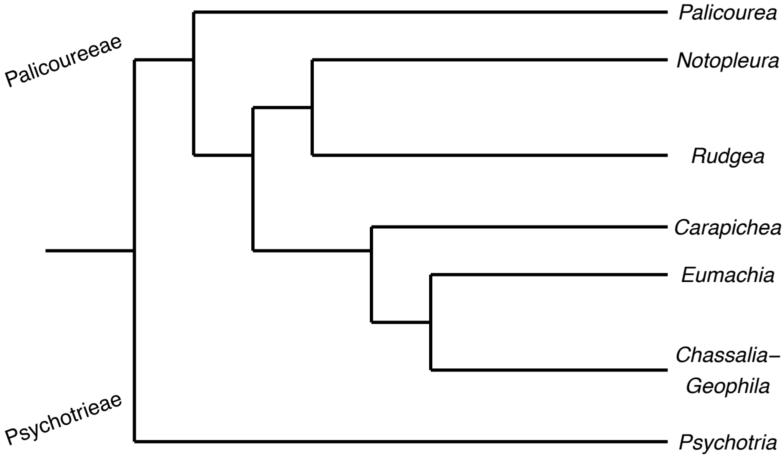

Figure 3. Phylogenetic relationships of the genera in the sister tribes Psychotrieae and Palicoureeae of the Rubiaceae (after Razafimandimbison *et al.*, 2014).

probably long established in this branch of the Rubiaceae – perhaps unsurprising for a set of genera that are very diverse across the lowland tropics, and that often grow on acid and aluminium-rich soils. It may be that in the Palicoureeae the tendency for leaves to dry green is related to the physiology of mineral uptake and deployment in the tissues, but clearly in *Eumachia* it is not always linked to aluminium accumulation.

Interestingly, the overall stoichiometry of foliar elemental concentrations appeared to show some discrimination at the generic level, with species of *Eumachia* exhibiting relatively high concentrations of the physiologically abundant elements Mg, K and P compared with species of *Psychotria* and *Palicourea*. Two of the species of *Psychotria* also had relatively high foliar Mn concentrations, accentuating the discrimatory power of the second principal component in the PCA. Most *Eumachia* specimens sampled had leaves with a very thin, membranous texture. The *Psychotria*/*Palicourea* specimens tended to vary from chartaceous to subcoriaceous in leaf texture rather than the tissue-thin laminas of *Eumachia*. A higher cell wall to cytoplasm ratio in the *Psychotria*/*Palicourea* specimens might be the explanation for the generally lower concentrations of physiologically important elements than in *Eumachia* species.

Clearly, many questions remain unanswered and there is considerable scope for further comparisons within and among widespread tropical genera such as *Eumachia* and *Psychotria*.

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Appendix

LIST OF SPECIMENS ANALYSED FOR THE STUDY

All are from the herbarium collection of the Royal Botanic Gardens, Kew (K).

Eumachia abrupta **(Hiern) J.H.Kirkbr.**

Tanzania. Uzungwa Mountain NP, stream west of Kilombaro village, 12 xi 1997, *P.A. & W.R.Q. Luke* 5151.

Eumachia boliviana **(Standl.) Delprete & J.H.Kirkbr.**

Peru. Coronel Portillo, Chara del Sr Cesar Vela, 25 x 1972, *J. Sibunke V.* 5461.

Eumachia cephalantha **(Müll.Arg.) Delprete & J.H.Kirkbr.**

Brazil. **Sao Paulo**: Piracicaba, estrada para Limeira, 14 vii 1993, *V.C. Souza* et al. 6001.

Eumachia collina **(Labill.) Barrabé, C.M.Taylor & Razafim.**

New Caledonia. Exploitation forestiere Guiraud piste en direction de Mt Ades (800–850 m), sol ferallitique sur peridotites, 25 iii 1988, *Jaffre* 2951.

Eumachia damasiana **(Sohmer) Barrabé, C.M.Taylor & Razafim.**

Papua New Guinea. **New Britain**: north end of Lake Daketana on Willhaumez Peninsula, 30 v 1973, *R.S. Isles* et al. NGF 32277.

Eumachia leptothyrsa **(Miq.) Barrabé, C.M.Taylor & Razafim.**

Papua New Guinea. **Madang Province**: Ramu Subprovince, Walium Station, 20 iv 1979, *Sohmer & Katik* LAE 75127.

Eumachia microdon **(DC.) Delprete & J.H.Kirkbr.**

Panama. OIS, 16 vi 1924, *L.A.M. Riley* SERA 149.

Eumachia montana **(Blume) I.M.Turner**

Indonesia. **Sumbawa**: 1927, *I. Rensch* 593.

Eumachia obanensis **(Wernham) Razafim. & C.M.Taylor**

Cameroon. **Prov. Sud-Oeust**: Dept Fako, Bakingili 50–300 m trail to Mt Cameroon, 25 vii 1984, *S.A. Thompson & J.E. Rawlins* 1662.

Palicourea conephoroides **(Rusby) C.M.Taylor**

Peru. **Pasco**: Oxapampa, Distrito Palcazu, Parque Nacional Yonachagu–Chemillen, Bosque el mirador, 750–820 m, 14 v 2003, *A. Monteagudo* et al. 5228.

Palicourea violacea **(Aubl.) A.Rich.**
PANAMA. El Valle de Anter alt. 1000 m, 16 vi 1946, *P.H. Allen* 3536.

Psychotria beaufortiensis **Valeton ex Sohmer**
INDONESIA. North New Guinea, Cycloop Mountains between Nefor and the coast, vi 1938, *E. Meijer-Drus* 29.

Psychotria frondosa **S.Moore**
NEW CALEDONIA. Col d'Amien, Mont Pembai 800 m, foret humide schistes, 14 iv 1976, *H.S. McKee* 31039.

Psychotria hypsophila **K.Schum. & K.Krause**
CAMEROON. Proposed Ebo NP, near the Njuna River, 7 x 2015, *M. Alvarez* 30.

Psychotria leucopoda **E.M.A.Petit**
TANZANIA. Kwamngumi FR, 350 m, 14 xi 1986, *Ruffo & Mmari* 1980.

Psychotria lolokiensis **S.Moore**
PAPUA NEW GUINEA. Near south-east side of Little Mt Lowes, c.16 miles north of Port Morseby, 25 iv 1967, *R. Pullen* 6813.

Psychotria pallens **Gardner**
BRAZIL. Rio de Janeiro, 24 vii 2012, *J.A. Oliveira* et al. 168.

Psychotria **sp.**
INDONESIA. **Sumbawa**: Mata, 2 i 1910, *J. Elbert* 4106.