Cluster roots are common in *Daviesia* and allies (Mirbelioids; Fabaceae)

FRANCIS J. NGE [1, 2], MARION L. CAMBRIDGE [3, 4], DAVID S. ELLSWORTH [5], HONGTAO ZHONG [3] & HANS LAMBERS [3 *]

[1] *School of Biological Sciences, Faculty of Science, The University of Adelaide, Adelaide, SA, Australia 5005, Australia*

[2] *State Herbarium of South Australia, PO Box 1047, Adelaide, SA 5001, Australia*

[3] *School of Biological Sciences, The University of Western Australia, Crawley, WA 6009, Australia*

[4] *Oceans Institute, The University of Western Australia, Crawley, WA 6009, Australia*

[5] *Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia*

* *Corresponding author:* ✉ *hans.lambers@uwa.edu.au*

Abstract

Cluster roots are best known in the Proteaceae, but also occur in other plant families. Cluster roots are produced by *Viminaria juncea* and some species of *Daviesia*, which belong to the Australian Mirbelioids (Fabaceae). We searched for cluster roots in a number of species in *Daviesia* and its close allies *Gompholobium* and *Sphaerolobium* and found them in all studied species of these three genera. *Daviesia incrassata* subsp. *incrassata*, collected at an unusually waterlogged habitat, had no cluster roots, but they were present in *D. incrassata* subsp. *reversifolia* in a drier habitat. Cluster roots are pervasive in the *Daviesia* group of the Fabaceae, allowing them to persist on low-phosphorus soils.

Keywords: Australia, cluster roots, *Daviesia*, Fabaceae, *Gompholobium*, low-nutrient soils, Proteaceae, *Sphaerolobium*

Manuscript received 3 August 2020; accepted 21 October 2020

INTRODUCTION

Fabaceae are considered a mycorrhizal family (Wang & Qiu 2006), but there are several examples of non-mycorrhizal genera in this family, most prominently *Lupinus* (Vierheilig *et al.* 1994; Treu *et al.* 1995; Trinick 1977; Oba *et al.* 2001). Other species have cluster roots, or both cluster and mycorrhizal roots; for example, some non-mycorrhizal species of *Lupinus* native to the Mediterranean region produce cluster roots (Lambers *et al.* 2013), and the South African fabaceous *Aspalathus linearis* is mycorrhizal and also produces cluster roots (Hawkins *et al.* 2011).

Among Australian Fabaceae, *Viminaria juncea* has long been known to be both mycorrhizal and to produce cluster roots (Lamont 1972; Brundrett & Abbott 1991), whereas *Daviesia cordata* and *D. decurrens* produce cluster roots but are non-mycorrhizal (Brundrett & Abbott 1991; de Campos et al. 2013). *Daviesia physodes* also produces cluster roots, but its mycorrhizal status has not been evaluated (Lambers *et al.* 2019). *Daviesia reclinata* in northern Australia has arbuscular mycorrhizal roots (Brundrett 2017). These taxa belong to the Mirbelioids (Papilionoid tribes Mirbelieae and Bossieeae; Table 1), which contain many species-rich Australian sclerophyllous genera (Crisp & Cook 2003a). *Daviesia* species are scleromorphic shrubs endemic to Australia (Fig. 1a), where they comprise the largest genus of Fabaceae subfamily Papilionoideae, with 131 species (Crisp *et al.* 2017). The genus is distributed across Australia in all major habitats, except wetlands and rainforests. Early suggestions that another Australian pea genus (*Kennedia*) in another tribe (Phaseoleae; Papilionoideae; Table 1) might produce cluster roots (Adams *et al.* 2002) were not substantiated when this was followed up in a detailed survey of carboxylate release from roots of several mycorrhizal and non-mycorrhizal species of *Kennedia* (Ryan *et al*. 2012; Suriyagoda *et al*. 2012.

Viminaria (Fig. 1d) and *Daviesia* belong to the large antipodal group of Mirbelioids, or the *Daviesia* group *sensu* Crisp & Cook (2003b) and a few species have cluster roots. Our aim was first, to discover whether cluster roots are present in additional species of *Daviesia* and, second, whether they are also present in other Mirbelioid genera (Table 1), such as *Gompholobium* (Fig. 1b) and *Sphaerolobium* (Fig. 1c; Crisp & Cook 2003a). Determining whether other species within the group produce cluster roots may give insight into their evolutionary history, and how these species are able to colonise and persist on

Table 1. Formal and informal infrageneric classification of *Daviesia* and allies in Fabaceae mentioned in this study.

Subfamily	Tribe	Group	Genera
Papilionoideae	Mirbelieae and Bossieae (not limited to taxa from '*Daviesia* group')	Mirbelioids or '*Daviesia* group' sensu Crisp & Cook (2003b)	*Daviesia* *Erichsenia* *Gompholobium* *Sphaerolobium* *Viminaria*
	Phaseoleae	n/a	*Kennedia*

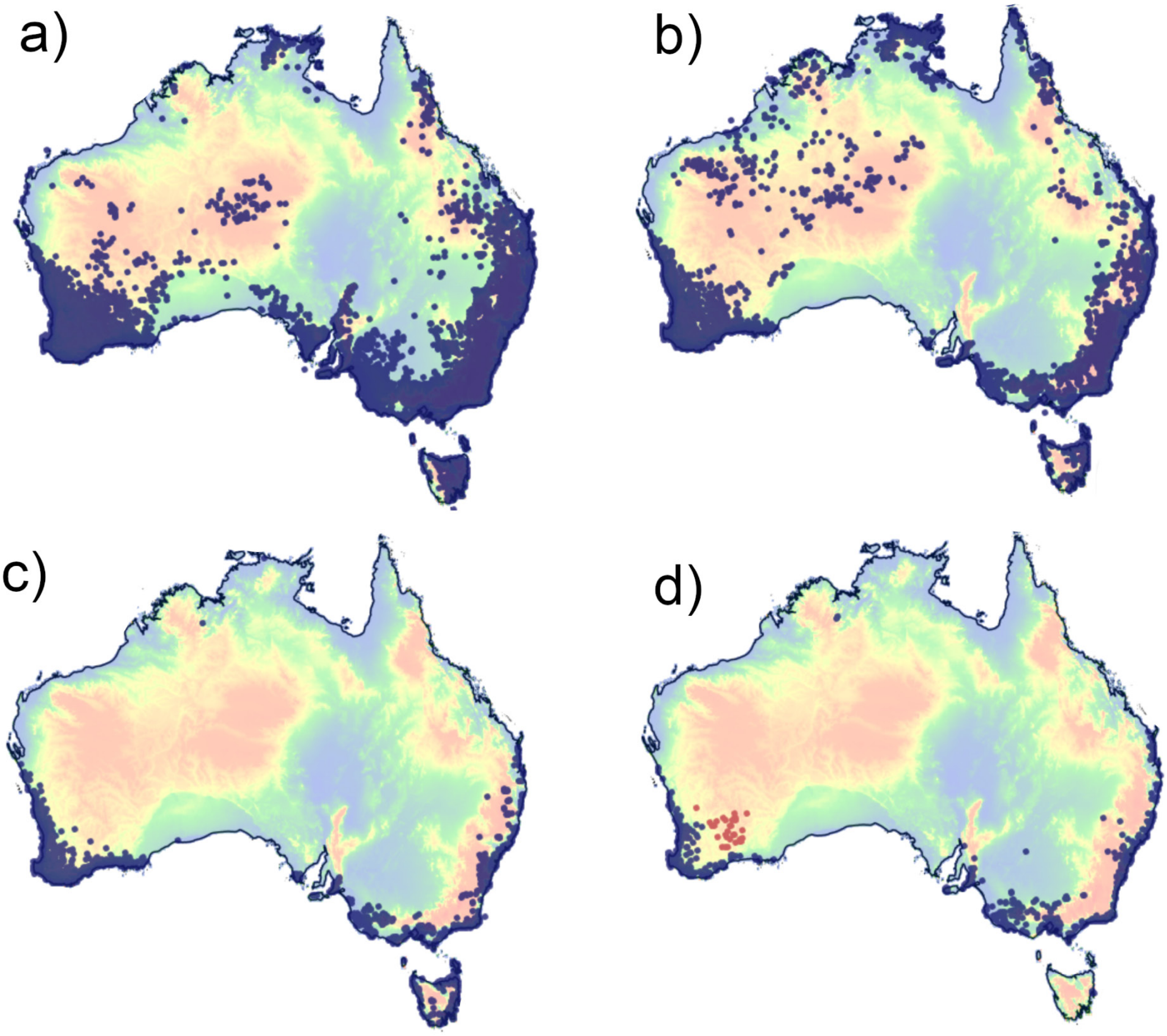

Figure 1. Distributional range of a) *Daviesia*, b) *Gompholobium*, c) *Sphaerolobium*, d) *Viminaria* (blue) and *Erichsenia* (red), sourced from collection records from the Australasian Virtual Herbarium. Map of Australia shaded by topographic contours (orange–high elevation; blue–low elevation) sourced from the spatial portal of Atlas of Living Australia. Details of locations of sampled species are included in Table 2.

low-nutrient, sandy soils, particularly if phosphorus (P) is relatively unavailable. Evidence of how common cluster roots are in this distinct evolutionary lineage within Fabaceae may then give an indication of the importance of this trait.

MATERIALS AND METHODS

We concentrated on sampling sites in southwest Western Australia as most of the *Daviesia* species are found in that region, but we also included sites in South Australia, Victoria and New South Wales (Fig. 2).

Roots of Fabaceae in the *Daviesia* group were collected close to their main stem, making sure to trace them back to the individual stem, to avoid confusion with cluster roots belonging to adjacent species (Abrahão *et al*. 2018). We identified cluster roots on the basis of their distinctive morphology (Shane & Lambers 2005). Purnell (1960) described cluster roots in Proteaceae, as dense clusters of rootlets of limited growth. She referred to each cluster of rootlets as a 'proteoid root' and to that section of the lateral root from which the rootlets arise as the 'axis of the proteoid root'. As these roots have since been found in a range of other families, the term proteoid root has gradually been replaced by 'cluster root' (Shane & Lambers 2005).

RESULTS

We found cluster roots in a wide range of species in the *Daviesia* group at several locations in Western Australia, South Australia, Victoria and New South Wales, and

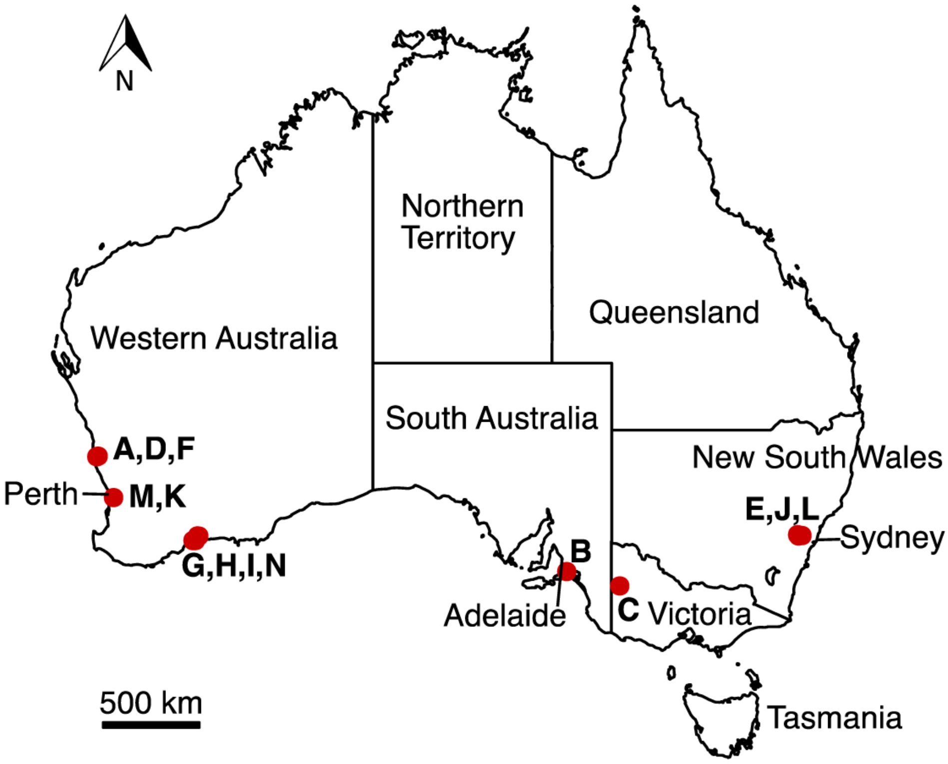

Figure 2. Sampling location of the species sampled in this study – denoted with red dots. The letters on the map refer to the species listed in Table 2.

present evidence for cluster roots in an additional 12 species within the genus (Table 2, Figs 3, 4). Furthermore, *Daviesia* species with cluster roots are scattered across the species level phylogeny from Crisp *et al.* (2017), suggesting that this trait is not clade-specific and most likely common across the genus (Fig. 5). We also document cluster roots in two additional genera within Mirbelioids, – *Gompholobium* and *Sphaerolobium* (Table 2, Fig. 4f).

DISCUSSION

Prior to this study, only two genera in the *Daviesia* group (Mirbelieae), namely *Viminaria* (Lamont 1972; Walker *et al*. 1983) and *Daviesia* (Lambers *et al*. 2019; Brundrett & Abbott 1991), were known to produce cluster roots. We discovered that cluster roots were present in 12 new species of *Daviesia*, and in two additional genera, *Gompholobium* and *Sphaerolobium*. Cluster roots were present in *Daviesia* species across all of our sampling sites except for one waterlogged site. This is significant, because it suggests the trait might be common across all *Daviesia* species, and hence is likely important to the ecology of the genus and its allies. In addition, within *Daviesia* this trait is not clade-specific, with documented species that possess cluster roots scattered across the species-level phylogeny (Fig. 5), again indicating that the trait is likely common across the genus.

Pate *et al.* (1989) noted anomalous secondary thickening in roots of *Daviesia*, which Crisp & Cook (2003b) subsequently referred to as 'cord roots'. No function has been proposed for cord roots (Pate & Dixon 1996). None of these authors mentioned cluster roots for this genus, although they had been firmly established in the *Daviesia* group, first in *Viminaria juncea* (Lamont 1972; Walker *et al.* 1983) and later in two *Daviesia* species (Brundrett & Abbott 1991). We found evidence of cluster roots in all species in the *Daviesia* group we investigated. The lack of cluster roots in *D. incrassata* subsp. *incrassata* was possibly a phenotypic response to an exceptionally wet and clayey habitat, and cluster roots were present in *D. incrassata* subsp. *reversifolia.* It would be of interest to also study the monotypic *Erichsenia* genus that belongs to the *Daviesia* group (Fig. 1d).

The group that is taxonomically closest to the *Daviesia* group, but not within Mirbelieae, is Bossiaeeae (Crisp & Cook 2003a). There are no records of any species producing cluster roots in this group, although several *Bossiaea* species have been studied in great detail (Abrahão *et al.* 2018). We also investigated *Bossiaea rhombifolia* at Davies Park NSW, and did not find cluster roots. Outside Mirbelieae, there are no reports of any Australian Fabaceae producing cluster roots. This might be a reflection of a lack of any investigation, as was the case for many species in the *Daviesia* group. However, Zemunik *et al.* (2015) studied nutrient-acquisition strategies in a range of species in this group, and would have noticed the formation of cluster roots. In a study by Hayes *et al.* (2014), none of the species in Mirbelieae outside the *Daviesia* group showed high leaf manganese concentrations, a proxy for carboxylate release in the rhizosphere (Lambers *et al.* 2015); this suggests that they do not produce cluster roots.

Compared with mycorrhizal roots, cluster roots are a superior P-acquisition strategy on severely P-impoverished soils (Lambers *et al.* 2018). It is likely that

Table 2. Summary of genera and some species in the *Daviesia* group; locations shown in Figure 2.

Genus	Species	Location (letters refer to Fig. 2)	Evidence for cluster roots	Comments
Daviesia	*angulata*	A; Lesueur National Park, WA	Yes	This study
	brevifolia	B; Cox's Scrub Conservation Park, SA	Yes	This study
	brevifolia	C; Broken Bucket Tank Bushland Reserve, Vic	Yes	This study
	chapmanii	D; Lesueur National Park, WA	Yes	This study
	cordata	n/a	Yes	Brundrett & Abbott (1991)
	corymbosa	E; on the Switzerland track in Wentworth Falls, NSW, in a *Banksia ericifolia – Allocasuarina – Hakea – Leptospermum – Kunzea* heath community	Yes	This study
	decurrens	n/a	Yes	Brundrett & Abbott (1991)
	divaricata	F; Lesueur National Park, WA	Yes	This study
	incrassata subsp. *incrassata*	G; Along Horner Road, Kundip Nature Reserve, WA	No	This study; habitat with wet clayey soil
	incrassata subsp. *reversifolia*	H; Along Hopetoun-Ravensthorpe Road, Kundip Nature Reserve, WA	Yes	This study
	physodes	n/a	Yes	Lambers *et al.* (2019)
	teretifolia	I; Fitzgerald River National Park near Hopetoun, WA	Yes	This study
	ulicifolia	J; Minnehaha Reserve near Katoomba, NSW	Yes	This study
Gompholobium	*aristatum*	K; Alison Baird Reserve, WA	Yes	This study
	floribunda	L; Davies Park in Springwood, NSW, in a *Banksia serrata* and *Corymbia gummifera*-dominated woodland community	Yes	This study
	tomentosum	M; Alison Baird Reserve, WA	Yes	This study
Sphaerolobium	*daviesioides*	N; Hamersley Drive, Hopetoun, on the way to the Fitzgerald River National Park, WA	Yes	This study
Viminaria	*juncea*	n/a	Yes	Lamont (1972); Walker *et al.* (1983)

the evolution of this trait in the *Daviesia* group allowed *Daviesia* to rapidly radiate in severely P-impoverished environments (Crisp *et al.* 2017). Cook *et al.* (2014) explored three explanations for biodiversity hotspots: small geographic range, geographic overlap and time for species accumulation. We surmise that the high diversity of *Daviesia* in south-western Australia compared with the rest of Australia also reflects the abundance of severely P-impoverished soils in the region, where cluster roots are of pivotal importance. Cluster roots are pervasive in Proteaceae (Purnell 1960; Shane & Lambers 2005), which also have their greatest diversity in south-western Australia (Pate *et al.* 2001). It is puzzling, however, why *Daviesia* diversified abundantly, whereas two sister genera in the *Daviesia* group (*Viminaria* and *Erichsenia*), of which at least *Viminaria* produces cluster roots, are monotypic.

CONCLUSION

This survey has expanded our knowledge of species and genera that produce cluster roots, and revealed that it is more common in Fabaceae than previously known. *Daviesia* is the most diverse fabaceous genus in Australia (with *ca.* 130 known species), followed by *Pultenaea* and *Gastrolobium*, with ca. 110 species in both genera (Crisp *et al.* 2017). Further work is needed to explore if cluster roots are also found in the other two large Australian Fabaceae genera (*Pultenaea* and *Gastrolobium*).

ACKNOWLEDGEMENTS

HL acknowledges support from the Deputy Vice Chancellor Research at the University of Western Australia towards a field trip during which some of the cluster roots were discovered. FJN was supported through an Australian Government Research Training Program Scholarship. The New Phytologist Trust supported DE's travel to Western Australia, where some of the ideas for this paper were discussed. We acknowledge the Western Australian Department of Biodiversity, Conservation and Attractions (permit no. SW019140 and no. FT61000353), the Victorian Department of Environment, Land, Water and Planning

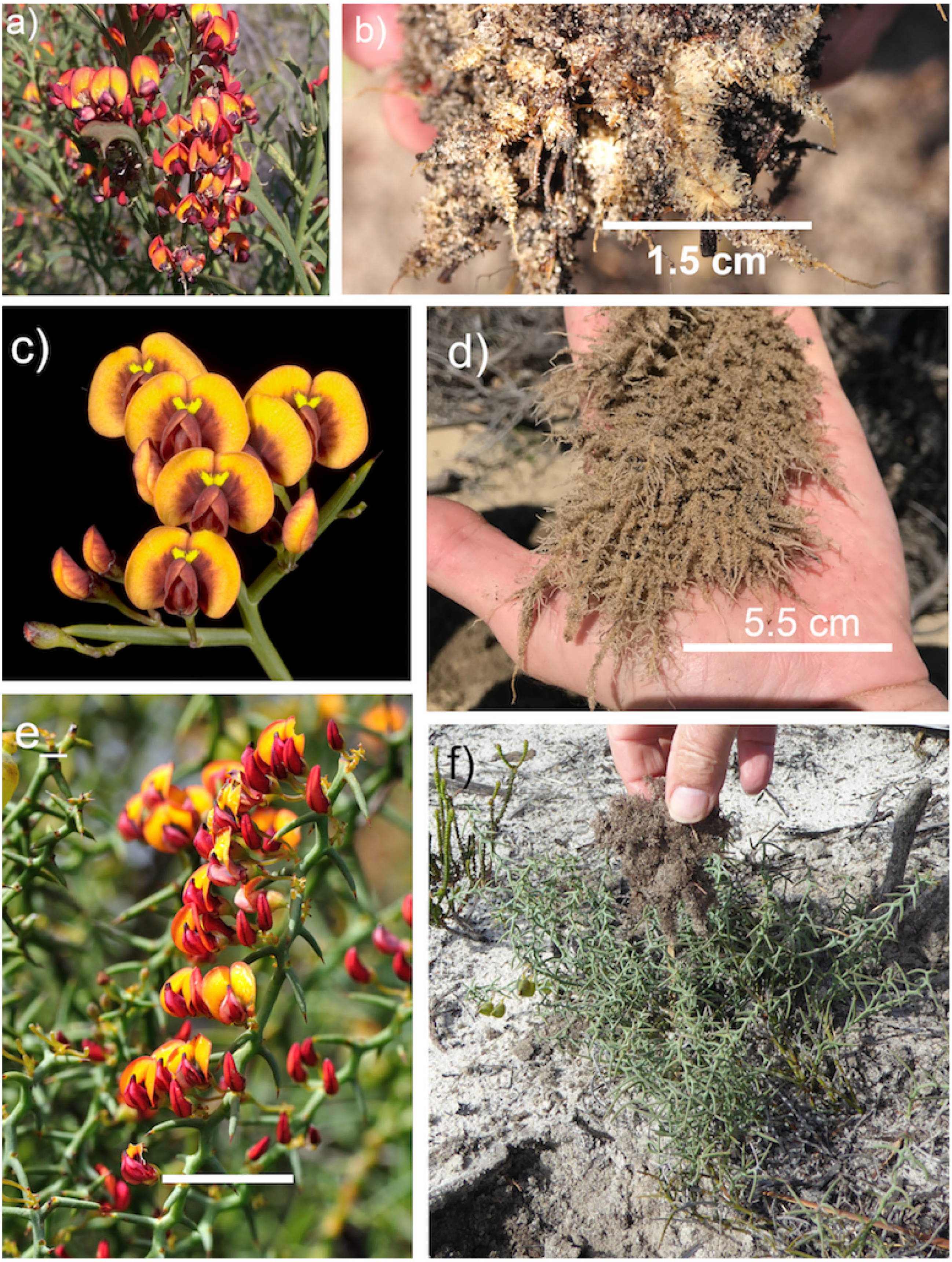

Figure 3. Representative diversity of *Daviesia* with cluster roots: a, b) *Daviesia physodes,* c, d) *Daviesia divaricata,* e, f) *Daviesia incrassata* subsp. *reversifolia.* Photos by authors, except d) by Kevin Thiele. Scale bars are 1 cm unless otherwise stated.

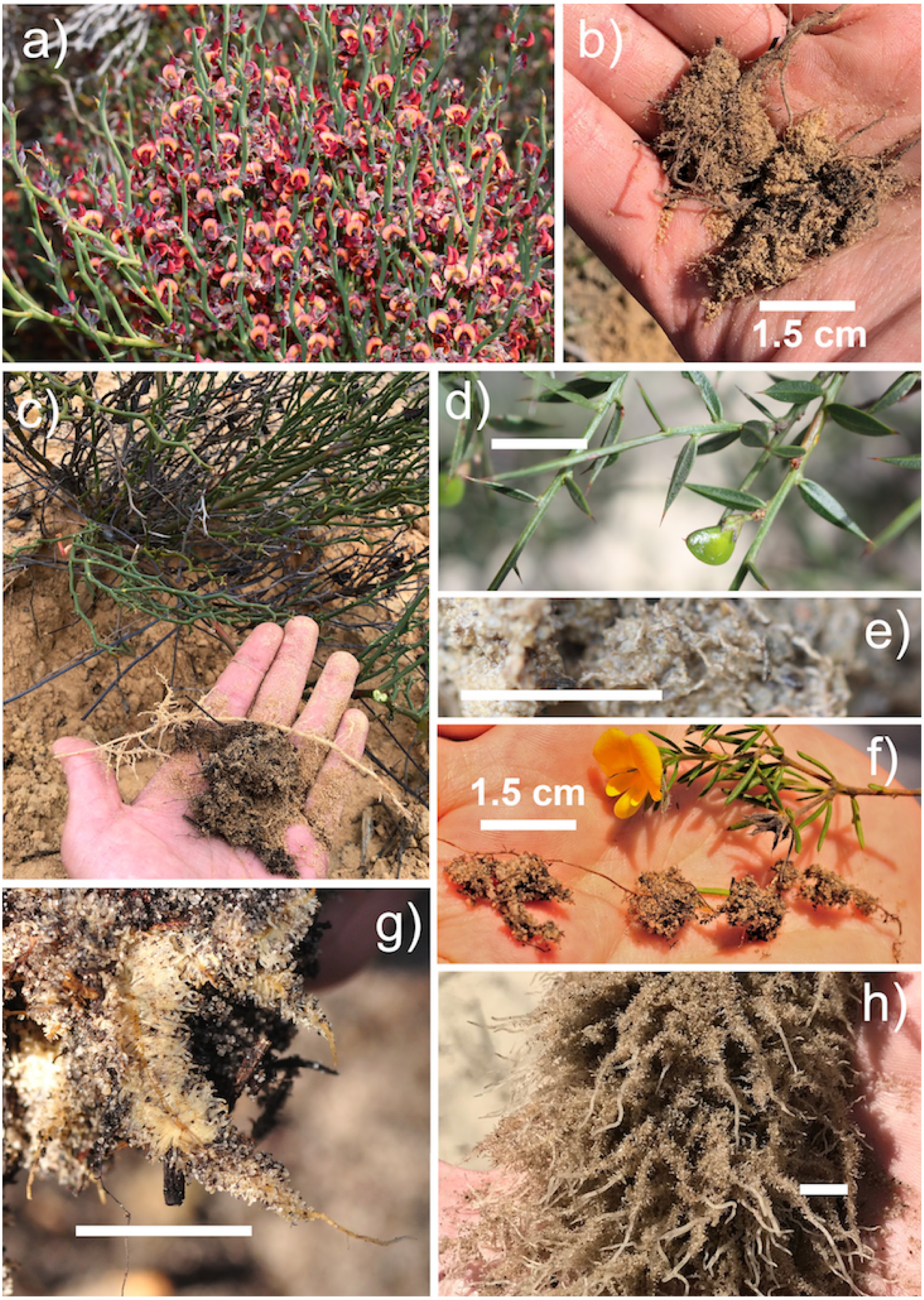

Figure 4. Representative diversity of *Daviesia* and allies with cluster roots: a–c) *Daviesia brevifolia*, d, e) *Daviesia ulicifolia,* f) *Gompholobium tomentosum*, and close up images of cluster roots from g) *Daviesia physodes*, h) *Daviesia divaricata.* Scale bars are 1 cm, unless otherwise stated.

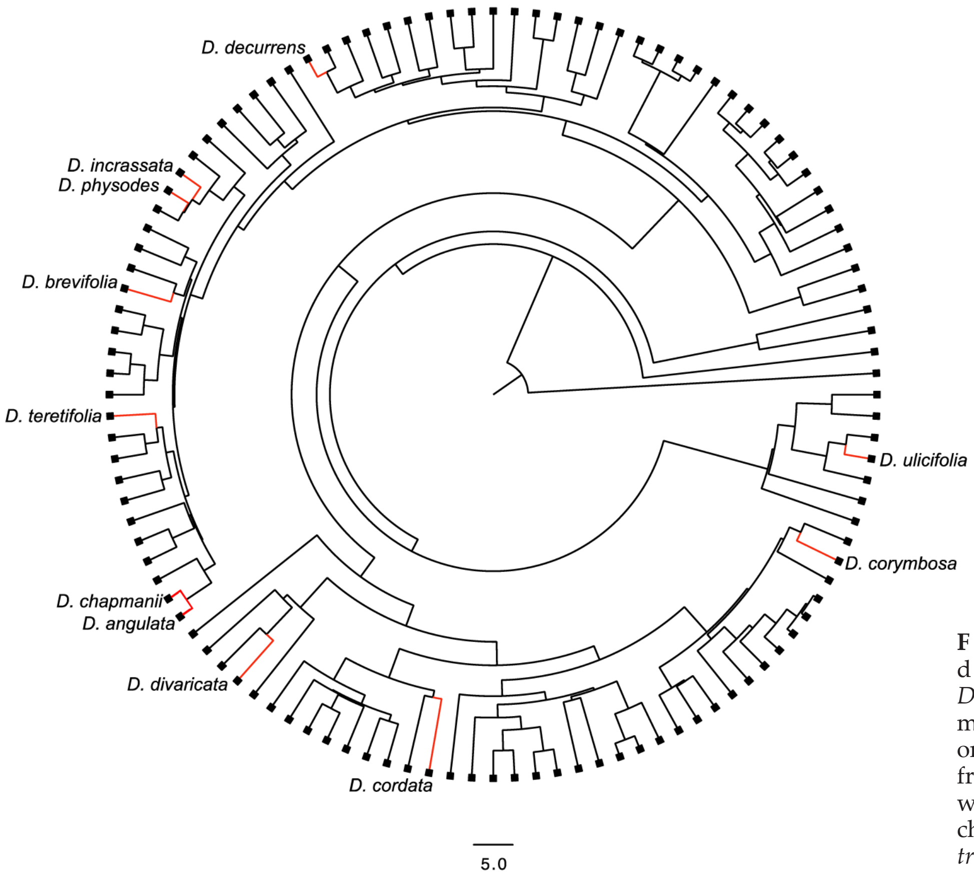

Figure 5. Currently documented species of *Daviesia* exhibiting cluster root morphology highlighted in red on the species-level phylogeny from Crisp *et al.* (2017), which was based on ITS and two chloroplast markers (*ndhF* and *trnL*).

(permit no. 10008523), and South Australian Department of Environment, Water and Natural Resources (permit no. G25787-4) for their permission to examine plants on land under their administration. Material in New South Wales was collected on local council land in accordance with local legislation.

REFERENCES

Abrahão A, Ryan M H, Laliberté E, Oliveira R S & Lambers H 2018. Phosphorus- and nitrogen-acquisition strategies in two *Bossiaea* species (Fabaceae) along retrogressive soil chronosequences in south-western Australia. *Physiologia Plantarum* **163**, 323–343.

Adams M A, Bell T L & Pate J S 2002. Phosphorus sources and availability modify growth and distribution of root clusters and nodules of native Australian legumes. *Plant, Cell and Environment* **25**, 837–850.

Brundrett M C 2017. Distribution and evolution of mycorrhizal types and other specialised roots in Australia. Pages 361–394 *in* Tedersoo L, editor *Biogeography of mycorrhizal symbiosis*. Springer International Publishing, Cham.

Brundrett M C & Abbott LK 1991. Roots of jarrah forest plants. I. Mycorrhizal associations of shrubs and herbaceous plants. *Australian Journal of Botany* **39**, 445–457.

Cook L G, Hardy N B & Crisp M D 2014. Three explanations for biodiversity hotspots: small range size, geographical overlap and time for species accumulation. An Australian case study. *New Phytologist* **207**, 390–400.

Crisp M D & Cook L G 2003a. Phylogeny and embryo sac evolution in the endemic Australasian papilionoid tribes Mirbelieae and Bossiaeeae. Pages 253–268 *in* Klitgaard B B & Bruneau A, editors *Advances in legume systematics, part 10, Higher level systematics* Royal Botanic Gardens, Kew.

Crisp M D & Cook L G 2003b. Phylogeny and evolution of anomalous roots in *Daviesia* (Fabaceae: Mirbelieae). *International Journal of Plant Sciences* **164**, 603–612.

Crisp M D, Cayzer L, Chandler G T & Cook L G 2017. A monograph of *Daviesia* (Mirbelieae, Faboideae, Fabaceae). *Phytotaxa* **300**, 1–308.

De Campos M C R, Pearse S J, Oliveira R S & Lambers H 2013. *Viminaria juncea* does not vary its shoot phosphorus concentration and only marginally decreases its mycorrhizal colonization and cluster-root dry weight under a wide range of phosphorus supplies. *Annals of Botany* **111**, 801–809.

Hawkins H J, Malgas R & Biénabe E 2011. Ecotypes of wild rooibos (*Aspalathus linearis* (Burm. F.) Dahlg., Fabaceae) are ecologically distinct. *South African Journal of Botany* **77**, 360–370.

Hayes P, Turner B L, Lambers H & Laliberté E 2014. Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *Journal of Ecology* **102**, 396–410.

Lambers H, Clements J C & Nelson M N 2013. How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus*, Fabaceae). *American Journal of Botany* **100**, 263–288.

Lambers H, Hayes P E, Laliberté E, Oliveira R S & Turner B L 2015. Leaf manganese accumulation and phosphorus-acquisition efficiency. *Trends in Plant Science* **20**, 83–90.

Lambers H, Albornoz F, Kotula L, Laliberté E, Ranathunge K, Teste F P & Zemunik G 2018. How belowground interactions contribute to the coexistence of mycorrhizal and non-mycorrhizal species in severely phosphorus-impoverished hyperdiverse ecosystems. *Plant and Soil* **424**, 11–34.

Lambers H, Albornoz F E, Arruda A J, Barker T, Finnegan P M, Gille C, Gooding H, Png G K, Ranathunge K & Zhong H 2019. Nutrient-acquisition strategies. Pages 227–248 *in* Lambers H, editor *A Jewel in the Crown of a Global Biodiversity*

Hotspot. Kwongan Foundation and the Western Australian Naturalists' Club Inc., Perth.

Lamont B B 1972. 'Proteoid' roots in the legume *Viminaria juncea*. *Search* **3**, 90–91.

Oba H, Tawaray K & Wagatsuma T 2001. Arbuscular mycorrhizal colonization in *Lupinus* and related genera. *Soil Science and Plant Nutrition* **47**, 685–694.

Pate J S & Dixon K W 1996. Convergence and divergence in the southwestern Australian flora in adaptations of roots to limited availability of water and nutrients, fire and heat stress. Pages 249–258 *in* Hopper S D, Chappill J A, Harvey M S & George A S, editors *Gondwanan heritage.* Beatty & Sons, Chipping Norton, Surrey.

Pate J S, Kuo J, Dixon K W & Crisp M D 1989. Anomalous secondary thickening in roots of *Daviesia* (Fabaceae) and its taxonomic significance. *Botanical Journal of the Linnean Society* **99**, 175–193.

Pate J S, Verboom W H & Galloway P D 2001. Co-occurrence of Proteaceae, laterite and related oligotrophic soils: coincidental associations or causative inter-relationships? *Australian Journal of Botany* **49**, 529–560.

Purnell H M 1960. Studies of the family Proteaceae. I. Anatomy and morphology of the roots of some Victorian species. *Australian Journal of Botany* **8**, 38–50.

Ryan M H, Tibbett M, Edmonds-Tibbett T, Suriyagoda L D B, Lambers H, Cawthray G R & Pang J 2012. Carbon trading for phosphorus gain: the balance between rhizosphere carboxylates and mycorrhizal symbiosis in plant phosphorus acquisition. *Plant, Cell and Environment* **35**, 2061–2220.

Shane M W & Lambers H 2005. Cluster roots: a curiosity in context. *Plant and Soil* **274**, 101–125.

Suriyagoda L D B, Lambers H, Renton M & Ryan M H 2012. Growth, carboxylate exudates and nutrient dynamics in three herbaceous perennial plant species under low, moderate and high phosphorus supply. *Plant and Soil* **358**, 105–117.

Treu R, Laursen G A, Stephenson S L, Landolt J C & Densmore R 1995. Mycorrhizae from Denali National Park and Preserve, Alaska. *Mycorrhiza* **6**, 21–29.

Trinick M J 1977. Vesicular-arbuscular infection and soil phosphorus utilization in *Lupinus* spp. *New Phytologist* **78**, 297–304.

Vierheilig H, Alt M, Mohr U, Boller T & Wiemken A 1994. Ethylene biosynthesis and activities of chitinase and ß-1,3-glucanase in the roots of host and non-host plants of vesicular arbuscular mycorrhizal fungi after inoculation with *Glomus mosseae*. *Journal of Plant Physiology* **143**, 337–343.

Walker B A, Pate J S & Kuo J 1983. Nitrogen fixation by nodulated roots of *Viminaria juncea* (Schrad. & Wendl.) Hoffmans (Fabaceae) when submerged in water. *Functional Plant Biology* **10**, 409–421.

Wang B & Qiu Y-L 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**, 299–363.

Zemunik G, Turner B L, Lambers H & Laliberté E 2015. Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants* **1**, 15050.

The authors declare that they have no conflicts of interest.