CSIRO PUBLISHING
www.publish.csiro.au/journals/asb *Australian Systematic Botany*, **23**, 415–430

Evolution of nickel hyperaccumulation by *Stackhousia tryonii* (Celastraceae), a serpentinite-endemic plant from Queensland, Australia

Dylan O. Burge[A,C] *and W. R. Barker*[B]

[A]Duke University, Department of Biology, Box 90338, Durham, North Carolina, 27708, USA.
[B]State Herbarium of South Australia, Hackney Road, Adelaide, SA 5000, Australia *and* Australian Centre for Evolutionary Biology and Biodiversity, University of Adelaide, SA 5005, Australia.
[C]Corresponding author. Email: dylan.o.burge@gmail.com

Abstract. To elucidate the evolutionary origin of nickel (Ni) hyperaccumulation by the Australian serpentinite-endemic plant *Stackhousia tryonii* Bailey, phylogenetic analyses of chloroplast and nuclear DNA for *Stackhousia* and its close relatives were combined with assays of plant-tissue Ni concentrations. Thirty-five plants from 20 taxa were analysed by sequencing nuclear rDNA (ITS) and the plastid *trn*L–F region. Phylogenetic analysis of sequence data was conducted under maximum parsimony and Bayesian search criteria. In all, 100 plants from 39 taxa, including all 33 *Stackhousia* species, were analysed for Ni concentration by radial inductively coupled plasma atomic-emission spectrometry (ICP–AES). In phylogenetic analyses, *S. tryonii* was monophyletic, nested within a monophyletic *Stackhousia*. Only *S. tryonii* contained concentrations of Ni above the hyperaccumulation threshold (0.1%; 1000 ppm), containing between 0.25% (2500 ppm) and 4.1% (41 000 ppm) Ni by dry weight. Nickel-hyperaccumulation ability appears to have been acquired once during diversification of *Stackhousia*, by *S. tryonii*.

Introduction

Plant adaptation to extreme edaphic conditions has long been of interest to botanists (Mason 1946; Minguzzi and Vergnano 1948; Kruckeberg 1951, 1986; Bradshaw 1952). World-wide, ~368 species of vascular plants, representing 44 families, are known to accumulate Ni in their above-ground tissues, up to concentrations exceeding 0.1% (1000 ppm) by dry weight (Reeves and Baker 2000; Table 1), a phenomenon known as Ni hyperaccumulation (Brooks *et al*. 1977*a*; Reeves 1992). Nickel-hyperaccumulating plants are restricted to Ni-rich soils, particularly those derived from serpentinite rock (Reeves and Baker 2000; Reeves 2003). Serpentinite is formed in the Earth's mantle by metamorphosis of igneous rocks (Brooks 1987). Exposures of serpentinite rock occur world-wide but are most common near tectonic plate boundaries (Brooks 1987). In addition to high concentrations of Mg and Fe, the minerals composing serpentinite also contain high concentrations of heavy metals such as Cr, Co and Ni. When serpentinite is weathered, the resulting soils tend to contain excessive amounts of the elements listed above, especially Ni (Brooks 1987). Hereafter, we refer to these soils as 'serpentinite-derived soils' rather than 'serpentine' (e.g. Brooks 1987), so as to avoid confusion between the rocks themselves and the soils derived from them. It is also important to note that high concentrations of Ni are found in rock types other than serpentinite, some of which may weather to form Ni-rich soils that support Ni hyperaccumulators (Reeves and Baker 2000). Nickel-hyperaccumulating plants are known from Ni-rich soils in Australasia, South-east Asia, Africa, Europe and North America (Reeves and Baker 2000; Table 1), with the greatest diversity occurring in Cuba and New Caledonia (Jaffré 1992; Reeves *et al*. 1996; Reeves 2003). Recently, Ni-hyperaccumulating plants have become the subject of intensive systematic (reviewed in Reeves and Baker 2000), ecological (reviewed in Boyd 2004), physiological (reviewed in Salt and Krämer 2000) and genetic (reviewed in Pollard *et al*. 2002) study. Research is motivated by interest in understanding the Ni-hyperaccumulation trait, as well as potential use of these plants for both detoxification (phytoremediation) of Ni-contaminated soils (reviewed in Baker *et al*. 2000) and phytomining (reviewed in Brooks *et al*. 1998).

Most Ni-hyperaccumulating species are members of families that do not otherwise display a propensity for this trait (Reeves and Baker 2000; Table 1). This observation, combined with the wide phylogenetic spread of the Ni-hyperaccumulating phenotype, encompassing ferns (1 sp.), monocots (4 genera, 4 spp.), and eudicots (97 genera, 363 spp.; Table 1), has led to the idea that Ni hyperaccumulation has evolved independently in multiple plant groups (Reeves and Baker 2000). Recent research points to several potential advantages of Ni hyperaccumulation that may have led to the independent evolution of this trait in multiple plant groups, including resistance to drought, competitive advantage via allelopathy and chemical defence (reviewed in Boyd 2004). Nevertheless, some plant families display a clear propensity for the production

 10.1071/SB10029 1030-1887/10/060415

Table 1. Plant groups containing Ni-hyperaccumulating species

Major group and family are based on Mabberley (2008). The total number of Ni-hyperaccumulating species known for family and the number of genera in a family with Ni-hyperaccumulating species are given; circumscriptions are based on Mabberley (2008). Primary literature or review papers are listed in the References[A]; see also Brooks (1987, 1995, 1998), Baker and Brooks (1989), Reeves (1992, 2006), Baker *et al*. (2000) and Reeves and Baker (2000) for other lists. Species not identified to genus in the primary literature (total of ~4; Reeves *et al*. 2007) are not included in the table. Complete list is available from the corresponding author on request

Major group and family	No. of species	No. of genera	References[A]
Ferns			
Pteridaceae	1	1	13, 46
Monocots			
Commelinaceae	1	1	36
Iridacae	1	1	46
Juncaceae	1	1	16, 52, 54
Poaceae	1	1	16
Eudicots & magnoliids			
Acanthaceae	8	5	9, 13–14, 36, 45–46
Amaranthaceae	1	1	46
Anacardiaceae	1	1	9
Argophyllaceae	2	1	18
Asteraceae	46	12	9, 13–14, 16, 30–31, 37–38, 45–47, 52, 55–56
Boraginaceae	1	1	14, 46
Brassicaceae	87	8	1, 7, 12, 15–16, 27–29, 35, 38–43, 51–54
Buxaceae	17	1	44
Campanulaceae	1	1	16
Caryophyllaceae	6	2	16, 25, 47, 52
Celastraceae	1	1	5, present study
Clusiaceae	4	1	45
Convolvulaceae	4	3	9, 34, 46
Cunoniaceae	8	2	18, 21
Dichapetalaceae	1	1	4, 17
Dipterocarpaceae	1	1	32
Euphorbiaceae	83	12	4, 6, 14, 18, 24, 36, 44, 46, 48
Fabaceae	5	5	9, 16, 34, 46, 52, 56
Lamiaceae	1	1	26
Lythraceae	1	1	46
Malvaceae	7	3	45–46, 57
Meliaceae	1	1	2, 4
Myristicaceae	1	1	57
Myrtaceae	6	2	45
Ochnaceae	3	2	2, 4, 36, 45
Oleaceae	1	1	45
Oncothecaceae	1	1	18
Orobanchaceae	1	1	14, 46
Passifloraceae	6	2	14, 46
Plantaginaceae	1	1	16, 52, 54
Ranunculaceae	1	1	16
Rubiaceae	16	6	3, 14, 19, 36, 45–46
Salicaceae	19	4	22
Sapotaceae	2	2	20, 57
Saxifragaceae	3	1	16, 52
Thymelaeaceae	1	1	36
Velloziaceae	1	1	13, 46
Verbenaceae	5	1	14, 46
Violaceae	8	3	8, 10–11, 18–19, 23, 33–34, 36, 49–50

[A]References: Adigüzel and Reeves (2002) (1); Baker and Proctor (1988) (2); Baker *et al*. (1985) (3); Baker *et al*. (1992) (4); Batianoff *et al*. (1990) (5); Berazaín Iturralde (1981) (6); Brooks and Radford (1978) (7); Brooks and Wither (1977) (8); Brooks and Yang (1984) (9); Brooks *et al*. (1977*a*) (10); Brooks *et al*. (1977*b*) (11); Brooks *et al*. (1979) (12); Brooks *et al*. (1990) (13); Brooks *et al*. (1992) (14); Doksopulo (1961) (15); Gabbrielli *et al*. (1987) (16); Homer *et al*. (1991) (17); Jaffré (1980) (18); Jaffré and Schmid (1974) (19); Jaffré *et al*. (1976) (20); Jaffré *et al*. (1979*a*) (21); Jaffré *et al*. (1979*b*) (22); Kelly *et al*. (1975) (23); Kersten *et al*. (1979) (24); Kruckeberg *et al*. (1993) (25); Lisanti (1952) (26); Menezes de Sequeira (1969) (27); Mengoni *et al*. (2003) (28); Minguzzi and Vergnano (1948) (29); Morrey *et al*. (1989) (30); Morrey *et al*. (1992) (31); Proctor *et al*. (1989) (32); Proctor *et al*. (1994) (33); Rajakaruna and Bohm (2002) (34); Reeves (1988) (35); Reeves (2003) (36); Reeves and Adigüzel (2004) (37); Reeves and Adigüzel (2008) (38); Reeves and Brooks (1983) (39); Reeves *et al*. (1980) (40); Reeves *et al*. (1981) (41); Reeves *et al*. (1983*a*) (42); Reeves *et al*. (1983*b*) (43); Reeves *et al*. (1996) (44); Reeves *et al*. (1999) (45); Reeves *et al*. (2007) (46); Roberts (1992) (47); Schmid (1991) (48); Severne (1974) (49); Severne and Brooks (1972) (50); Vergnano Gambi and Gabbrielli (1979) (51); Vergnano Gambi and Gabbrielli (1981) (52); Vergnano Gambi *et al*. (1979) (53); Vergnano Gambi *et al*. (1982) (54); Wild (1970) (55); Wild (1974) (56); Wither and Brooks (1977) (57).

of Ni-hyperaccumulating species (Table 1), and recent research indicates that differences in tissue Ni concentration among groups of angiosperms may be attributed, at least in part, to ancient evolutionary processes (Broadley *et al*. 2001). Little is known, however, about species-level relationships within angiosperm taxa that contain Ni hyperaccumulators, preventing inference of general patterns in evolution of Ni hyperaccumulation, including the relative contribution of recent versus ancient processes to the evolution of this trait.

The Australasian genus *Stackhousia* contains ~33 species of herbaceous annuals or perennials (Barker in press), 31 of which are endemic to Australia (Barker in press; Fig. 1, Table 2). *Stackhousia* is a member of the Stackhousioideae subfamily of Celastraceae, which also contains the Australia-endemic genera *Tripterococcus* (3 spp.) and *Macgregoria* (1 sp.; Barker 1984; Fig. 1, Table 2). Most species of *Stackhousia* are adapted to seasonally dry habitats, and occupy soils derived from a variety of rock types (Table 2). Although at least two species of

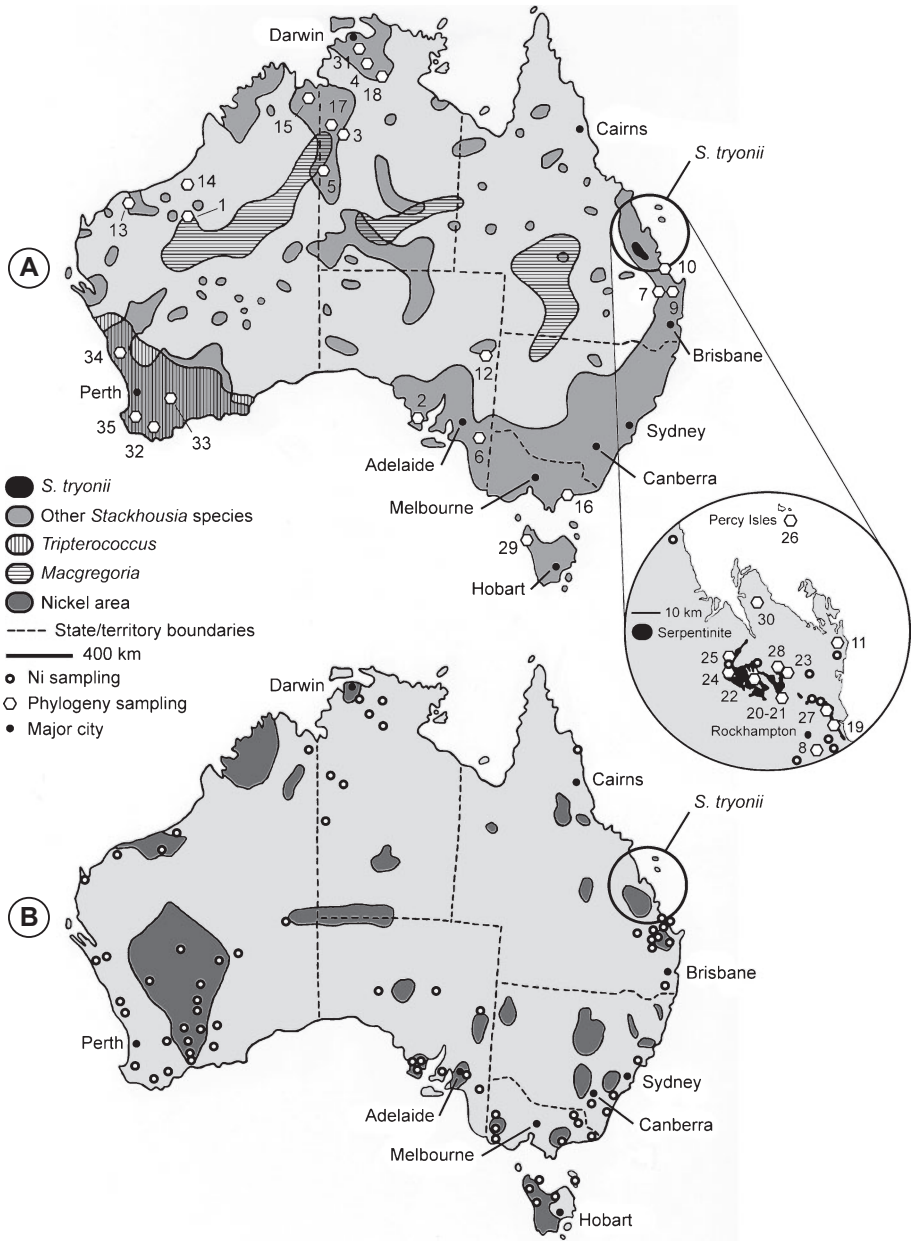

Fig. 1. Distribution of Stackhousioideae in Australia, Ni areas of Australia, and Ni and DNA sampling for the present study. (*A*) Distribution of Stackhousioideae in Australia; samples used for DNA sequencing and phylogenetic analysis are indicated with open, numbered hexagons (Tables 2, 3). (*B*) Ni areas of Australia (Ratajkoski *et al*. 2005); sampling for Ni analysis is indicated with open circles. Inset: extent of serpentinite rock outcrop in Port Curtis region of Queensland (Batianoff *et al*. 1990) and sampling of *Stackhousia* for Ni analysis and DNA sequencing.

Table 2. Taxa comprising subfamily Stackhousioideae (Celastraceae)

Taxon and section are as recognised by Barker (in press; unpubl. data). Ni, the number of populations assayed for nickel concentration in the present study (Appendix 1). DNA, the number of populations for which ITS and *trn*L–F were both sequenced in the present study (Table 3, Appendix 1). Substrate, soil type or geologic parent material on which the taxon is typically found. Taxa with standardised informal names (Barker 2005, in press): *Stackhousia* sp. 1, *S.* sp. *Colliculate cocci (W.R. Barker 6025)* W.R.Barker; *S.* sp. 2, *S.* sp. *McIvor River (J.R. Clarkson 5201)* Queensland Herbarium; *S.* sp. 3, *S.* sp. *Puberulent (N.G. Walsh 4835)* W.R.Barker; *S.* sp. 4, *S.* sp. *Sharply tuberculate cocci (P.K. Latz 8376)* W.R.Barker; *S.* sp. 5, *S.* sp. *West Kimberly (W.R. Barker 6856)* W.R.Barker

Taxon	Section	Ni	DNA	Distribution	Substrate
Macgregoria racemigera	n.a.	2	1	Central Australia	Sand
Stackhousia annua	*Racemosae*	1	0	S Australia	Limestone
S. aspericocca subsp. *aspericocca*[B]	*Racemosae*	2	1	SE Australia	Various
S. aspericocca subsp. *muelleri*[B]	*Racemosae*	3	0	SE Australia	Various
S. cartilaginea[B]	*Stackhousia*	1	0	Central Australia	Sand
S. clementii	*Stackhousia*	2	1	Australia	Limestone
S. dielsii	*Racemosae*	1	0	SW Australia	Sand
S. georgei	*Racemosae*	6	0	SW Australia	Various
S. huegelii	*Racemosae*	1	0	SW Australia	Various
S. intermedia	*Stackhousia*	1	1	Australasia	Various
S. lasiocarpa[B]	*Racemosae*	2	0	W Australia	Granite
S. latzii[B]	*Carinato-alatae*	1	1	Central Australia	Gypseous
S. linariifolia	*Racemosae*	2	1	SE Australia	Various
S. macrantha[B]	*Racemosae*	16	5	E Australia	Various
S. megaloptera	*Carinato-alatae*	3	0	Central Australia	Sand
S. minima	*Sclerococca*	1	0	New Zealand	Various
S. monogyna subsp. *maidenii*[B]	*Racemosae*	1	0	SE Australia	Various
S. monogyna subsp. *monogyna*[A]	*Racemosae*	4	0	SE Australia	Various
S. muricata	*Stackhousia*	1	1	S Australia	Various
S. nematomera[B]	*Stackhousia*	1	0	SW Australia	Various
S. nuda	*Stackhousia*	2	0	SE Australia	Various
S. occidentalis	*Stackhousia*	2	1	W Australia	Various
S. oedipoda[B]	*Stackhousia*	1	1	N Australia	Various
S. pubescens	*Racemosae*	2	0	SW Australia	Various
S. pulvinaris	*Sclerococca*	1	0	SE Australia	Various
S. scoparia	*Stackhousia*	2	0	SW Australia	Various
Stackhousia sp. 1	*Stackhousia*	1	1	W Australia	Sand
Stackhousia sp. 2	*Stackhousia*	1	0	E Australia	Sand
Stackhousia sp. 3	*Racemosae*	1	1	E Australia	Sand
Stackhousia sp. 4	*Stackhousia*	1	1	N Australia	Various
Stackhousia sp. 5	*Stackhousia*	1	1	E Australia	Various
S. spathulata	*Carinato-alatae*	2	0	SE Australia	Various
S. tryonii	*Racemosae*	15	10	E Australia	Serpentinite
S. umbellata	*Stackhousia*	1	0	W Australia	Limestone
S. viminea[A]	*Stackhousia*	8	2	SE Australia	Various
S. virgata	*Stackhousia*	2	1	E Australia	Various
Tripterococcus brachylobus[B]	n.a.	1	1	SW Australia	Various
T. brunonis	n.a.	3	2	SW Australia	Various
T. paniculatus[B]	n.a.	1	1	SW Australia	Various

[A]Taxa known to occur both on and off serpentinite-derived soils. [B]New names being formally published by Barker (in press).

Stackhousia are known to occur on serpentinite-derived soils (Batianoff *et al*. 1990; Bidwell 2000), only one, *Stackhousia tryonii*, is serpentinite-endemic. *S. tryonii* is found only on the serpentinite-derived soils of Queensland's Port Curtis District (Batianoff *et al*. 1990; Fig. 1). Previous research (Batianoff *et al*. 1990) demonstrated that *S. tryonii* is a hyperaccumulator of Ni, sequestering up to 4.1% of this element in its leaves, the third-highest concentration known for a plant (Reeves 2003). *S. tryonii* is the only member of *Stackhousia*, or the Celastraceae, known to hyperaccumulate Ni (Table 1). At least two other species of *Stackhousia* are known to occur on Ni-rich soils; however, they do not hyperaccumulate Ni (Batianoff *et al*. 1990; Bidwell 2000).

The position of *S. tryonii* as the only known Ni-hyperaccumulating member of its genus indicates that this plant represents an independent origin of the Ni-hyperaccumulation trait, making *Stackhousia* a good candidate for study of the evolution of Ni hyperaccumulation. Although past studies have dealt with the physiology of Ni hyperaccumulation in *S. tryonii* (Bhatia *et al*. 2003, 2004, 2005*a*, 2005*b*), the broader evolution of this trait in *Stackhousia* has not been considered in detail. Our study combines phylogenetic analysis of DNA sequence data with information on tissue Ni concentration in a broad sampling of Stackhousioideae to answer the following three related questions concerning the evolution Ni hyperaccumulation in this genus:

(1) do unusual concentrations of Ni accumulation occur in members of Stackhousioideae other than *S. tryonii*; (2) how many times has Ni accumulation evolved in Stackhousioideae; and (3) how does evolution of the Ni hyperaccumulating trait in *Stackhousia* compare with that in other groups of plants?

Materials and methods

Taxonomic and population sampling

DNA and Ni analysis vouchers were selected from existing herbarium collections or from plants collected for this project. Specimens were identified according to Barker (1984, in press).

Several taxon names used in the present paper represent new names for new taxa, or new combinations (Tables 1, 2), that are in the process of being formally described and named in a taxonomic revision of Stackhousioideae (Barker in press). These new names and new combinations are to be treated as manuscript names by the second author if the present work predates publication of the taxonomic paper. Five specimens included in the analysis represent further undescribed species (W. R. Barker, unpubl. data); these are treated using standardised informal names (Barker 2005; Tables 2, 3, Appendix 1). DNA from 35 plants was studied, representing 20 taxa (Tables 2, 3, Appendix 1). An additional 65 specimens, representing all 39 currently recognised Stackhousioideae (Barker in press; W. R. Barker, unpubl. data), were analysed for Ni concentration (Table 2, Appendix 1). To increase the probability of sampling previously unknown Ni hyperaccumulators, an effort was made to select specimens from regions thought to contain Ni-rich soils and/or outcrops of Ni-rich rock. This was accomplished by choosing specimens collected from serpentinite-derived soils (Murray 1969; Batianoff *et al*. 1990; Bidwell 2000) or from areas where Ni-rich rocks are known or likely to occur (Jaireth *et al*. 2005; Ratajkoski *et al*. 2005; Hendrickx 2009; Fig. 1).

Molecular methods

Total genomic DNA was extracted from fresh or herbarium tissue by using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) according to manufacturer instructions. For a majority of herbarium specimens, DNA was extracted using embryos excised from intact seeds. We took this approach following initial herbarium work in which it was discovered that seeds from Stackhousioideae herbarium sheets yield much higher quality total DNA than the vegetative parts of the same specimens, allowing for more rapid and reliable collection of DNA-sequence data. For extractions of seed DNA, up to 10 seeds from a single plant were pooled before preparation. Polymerase chain reactions (PCR) were performed on GeneAmp (Perkin-Elmer Bioscience, Waltham, MA, USA) or Gradient Palm-Cycler (Corbett Research, Mortlake, NSW, Australia) thermocyclers. Excess primer and dNTP were removed using the enzymes exonuclease I (New England Biolabs, Ipswich, MA, USA; 0.2 units μL^{-1} PCR product) and antarctic phosphatase (New England Biolabs, 1 unit μL^{-1} PCR product), incubated for 15 min at 37°C, followed by 15 min at 80°C. PCR reactions were performed using either Qiagen *Taq* DNA polymerase or AmpliTaq Gold DNA polymerase (Perkin-Elmer Bioscience, Waltham, MA, USA). Primers ITS4 (White *et al*. 1990) and ITSA (Blattner 1999) were used to amplify the ITS1–5.8S-ITS2 region of the nuclear rDNA. Primers ‘c’ and ‘f’ (Taberlet *et al*. 1991) were used to amplify the *trn*L–F chloroplast region, comprising the *trnL* intron and the *trn*L–F intergenic spacer. For Amplitaq Gold, amplification was performed using an initial incubation at 95°C for 10 min and 30 cycles of three-step PCR (10 s at 95°C, 30 s at 45°C and 2 min at 72°), followed by final extension at 72°C for 7 min. For Qiagen *Taq* DNA polymerase, both initial incubation and the first step of PCR took place at 94°C, with the former lasting 1 min. DNA sequences were determined bi-directionally on ABI PRISM 3100 Genetic Analyzers (Applied Biosystems, Foster City, CA, USA) at the Duke University Institute for Genome Science and Policy Sequencing and Genetic Analysis Facility.

Outgroup selection

Previous phylogenetic analyses (Simmons *et al*. 2001, 2008; Zhang and Simmons 2006) revealed a close relationship between Stackhousioideae and a group of Austral–Pacific Celastraceae genera. However, none of these studies unequivocally identified the sister-group to Stackhousioideae and we therefore selected outgroups from the Celastraceae genera identified as most closely related to Stackhousioideae by Simmons *et al*. (2008). Previously published sequences (Simmons *et al*. 2008) from each of the following species were used to root individual gene trees and the combined analysis: *Apatophyllum flavovirens*, *Denhamia oleaster*, *Dicarpellum pancheri*, *Hypsophila dielsiana*, *Menepetalum schlechteri* and *Psammomoya choretroides* (Table 3).

Phylogenetic analysis

DNA sequences were assembled and edited using Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Edited sequences were deposited in GenBank (Table 3). As noted by Simmons *et al*. (2008), the internal transcribed spacer regions (ITS1, ITS2) are very highly divergent between the members of the Stackhousioideae and their putative close relatives among the Celastraceae. To avoid problems of ambiguity inherent in aligning such highly divergent DNA sequences, we recoded ITS1 and ITS2 as missing data for the six outgroup taxa (Table 3) but retained these regions for all members of Stackhousioideae. Sequences were aligned using CLUSTAL W (Larkin *et al*. 2007) under default settings. Ambiguously aligned regions of ITS and *trn*L–F were identified and excluded from subsequent analysis (Fig. A1, available as an Accessory Publication on the *Australian Systematic Botany* website).

Maximum parsimony (MP) phylogenetic analyses were conducted using PAUP* 4.0b10 (Swofford 2000), with all characters equally weighted and unordered. Indels were treated as missing data. Heuristic searches were performed using 1000 random sequence addition replicates and tree bisection–reconnection (TBR) branch swapping. Branches were collapsed if their minimum lengths were zero (‘amb-’). Non-parametric bootstrap analyses (Felsenstein 1985) were conducted using 1000 pseudoreplicates and full heuristic tree search settings, with 1000 random sequence addition replicates and TBR branch swapping.

Phylogenetic analyses under the Bayesian criterion were conducted by using the best-fit models of evolution from the

Table 3. Samples examined for genetic analysis

The numbers in the Map column correspond to numbering in Fig. 1; –, indicates an outgroup (not mapped). Taxon or population: for species represented by more than one population, populations are individually numbered for reference to molecular phylogenetic trees; see Appendix 1, for complete specimen data. GenBank accession numbers: both ITS and *trn*L–F accession numbers, respectively, are given, separated by semicolon; for outgroups, two separate *trn*L–F accession numbers are given, separated by a comma (see Materials and methods). Collection [herbarium]; state: NSW, New South Wales; NT, Northern Territory; Qld, Queensland; SA, South Australia; Tas., Tasmania; Vic., Victoria; WA, Western Australia; SN, no collection number given. Substrate, rock or soil type on which the plant was found growing; –, indicates that rock or soil type was not mentioned on the specimen, or that accession represents an outgroup. Ni, the actual or maximum (see Table 2; Appendix 1) tissue Ni concentration for the plant specimen; –, Ni concentration not analysed (outgroups)

Map	Taxon or population	GenBank accession numbers	Collection [herbarium]; state	Substrate	Ni (ppm)
1	*Macgregoria racemigera*	GU169119; GU169154	*RJ Chinnock 9683* [AD]; WA	Sand	<9
2	*Stackhousia aspericocca* subsp. *asp.*[C]	GU169099; GU169134	*D Hopton 235* [AD]; SA	–	<4
3	*S. clementii*	GU169128; GU169163	*WR Barker 2829* [AD]; NT	Limestone	<3
4	*S. intermedia*	GU169120; GU169155	*RM Barker 367* [AD]; NT	–	<6
5	*S. latzii*[C]	GU169115; GU169150	*PK Latz 12870* [AD]; NT	Gypseous	<2
6	*S. linariifolia*	GU169108; GU169143	*ENS Jackson 2317* [AD]; SA	–	<3
7	*S. macrantha* (1)[C]	GU169097; GU169132	*AR Bean 11956* [BRI]; QLD	Ironstone	<5
8	*S. macrantha* (2)[C]	GU169100; GU169135	*D Halford Q8806* [BRI]; QLD	Clay	<7
9	*S. macrantha* (3)[C]	GU169114; GU169149	*PI Forster 31822* [BRI]; QLD	–	<8
10	*S. macrantha* (4)[C]	GU169117; GU169152	*PR Sharpe 5346* [BRI]; QLD	Peat	<9
11	*S. macrantha* (5)[C]	GU169124; GU169159	*T Ritchie SN* [BRI]; QLD	Granite	<20
12	*S. muricata*	GU169123; GU169158	*T Hall 400* [AD]; SA	–	<5
13	*S. occidentalis*	GU169126; GU169161	*WR Barker 2118* [AD]; WA	Sand	<8
14	*S. oedipoda*[C]	GU169125; GU169160	*WR Barker 2042* [AD]; WA	Sand	<2
15	*Stackhousia* sp. 1[A]	GU169129; GU169164	*WR Barker 6825* [AD]; WA	–	<5
16	*Stackhousia* sp. 3[A]	GU169113; GU169148	*NG Walsh 4835* [AD]; VIC	Sand	<7
17	*Stackhousia* sp. 4[A]	GU169116; GU169151	*PK Latz 8376* [AD]; NT	Sand	<5
18	*Stackhousia* sp. 5[A]	GU169130; GU169165	*WR Barker 6856* [AD]; NT	–	<4
19	*S. tryonii* (1)[B]	GU169103; GU169138	*DO Burge 1014b* [AD]; QLD	Serpentinite	41 000
20	*S. tryonii* (2)[B]	GU169102; GU169137	*DO Burge 1013c* [AD]; QLD	Serpentinite	16 400
21	*S. tryonii* (3)[B]	GU169101; GU169136	*DO Burge 1013a* [AD]; QLD	Serpentinite	19 400
22	*S. tryonii* (4)[B]	GU169104; GU169139	*DO Burge 1017a* [AD]; QLD	Serpentinite	30 000
23	*S. tryonii* (5)[B]	GU169105; GU169140	*DO Burge 1018a* [AD]; QLD	Serpentinite	14 100
24	*S. tryonii* (6)[B]	GU169106; GU169141	*DO Burge 1019b* [AD]; QLD	Serpentinite	20 000
25	*S. tryonii* (7)[B]	GU169107; GU169142	*DO Burge 1020a* [AD]; QLD	Serpentinite	15 800
26	*S. tryonii* (8)	GU169109; GU169144	*GN Batianoff 11405* [BRI]; QLD	Serpentinite	20 000
27	*S. tryonii* (9)	GU169110; GU169145	*GN Batianoff 98121* [BRI]; QLD	Serpentinite	10 500
28	*S. tryonii* (10)	GU169112; GU169147	*J Elsol SN* [BRI]; QLD	Serpentinite	8200
29	*S. viminea* (1)	GU169096; GU169131	*GS Hope SN* [CANB]; TAS	Peat	<20
30	*S. viminea* (2)	GU169111; GU169146	*J Brushe 1332* [BRI]; QLD	–	<10
31	*S. virgata*	GU169121; GU169156	*RM Barker 398* [AD]; NT	–	<4
32	*Tripterococcus brachylobus*[C]	GU169098; GU169133	*AS George 15067* [AD]; WA	Sand	<6
33	*T. brunonis* (1)	GU169118; GU169153	*R Bates 4282* [AD]; WA	–	<3
34	*T. brunonis* (2)	GU169127; GU169162	*WR Barker 2256* [AD]; WA	Sand	<2
35	*T. paniculatus*[C]	GU169122; GU169157	*s.d. Hopper 4736* [AD]; WA	Sand	<4
–	*Apatophyllum flavovirens*	EU328714; EU328849, EU328919	See Simmons *et al.* (2008)	–	–
–	*Denhamia oleaster*	EU328724; EU328840, EU328910	See Simmons *et al.* (2008)	–	–
–	*Dicarpellum pancheri*	EU328703; AY935756, AY935756	See Simmons *et al.* (2008)	–	–
–	*Hypsophila dielsiana*	EU328704; EU328846, EU328916	See Simmons *et al.* (2008)	–	–
–	*Menepetalum schlechteri*	EU328710; EU328853, EU328923	See Simmons *et al.* (2008)	–	–
–	*Psammomoya choretroides*	EU328712; EU328852, EU328922	See Simmons *et al.* (2008)	–	–

[A]Taxa with standardised informal names (Barker 2005, unpubl. data; see Table 2, Appendix 1).
[B]Plants for which Ni concentration was determined by using freshly collected silica-dried material rather than tissue obtained from herbarium specimens.
[C]New names being formally published by Barker (in press).

AIC output of the program MrModelltest (Nylander 2004), selecting separate models for the two data partitions. Sampling of trees was performed using the program MrBayes 3 (Ronquist and Huelsenbeck 2003). Three runs of 1 000 000 MCMC generations were performed using one heated and three cold chains, sampling every 1000 generations. Independent chains were then checked for convergence (standard deviation of split frequencies nearing 0.001). Following visual inspection of likelihood-score plots, the initial 100 000 generations of sampling (100 trees) were discarded as burnin and phylogenetic trees were reconstructed from the remaining data. Each of the three independent runs was used for tree building to verify the similarity of the results.

ITS and *trn*L–F provided nearly identical tree topologies under both Bayesian and MP criteria (for ITS, see Figs A2 and A3, and for *trn*L–F, see Figs A4 and A5, all available as

Accessory Publications on the Australian Systematic Botany website). Furthermore, the incongruence length-difference test (Farris *et al*. 1994) did not indicate a significant disagreement between the datasets ($P = 0.18$). Thus, ITS and *trn*L–F data were combined for subsequent analyses. Preliminary analyses of combined data showed that *S. tryonii* and *S. macrantha* were reciprocally monophyletic, with low divergence among sampled populations within each species. To reduce computation time, only two exemplars for each of these species were included in subsequent analyses.

Methods of Ni analysis

Samples (0.03–0.4 g) of leaves, flowers, stems or fruits from herbarium specimens or silica-dried material collected in the field (Tables 2, 3) were assayed for Ni concentration. Preliminary analysis of Ni for freshly collected silica-dried tissue versus herbarium material representing the same population of *S. tryonii* (data not shown) indicated that these two methods of preservation did not strongly influence the Ni concentration. Material for analysis was selected according to availability on each specimen and sometimes consisted of more than one tissue type. This approach was chosen on the basis of previous research (Batianoff *et al*. 1990; Bhatia *et al*. 2003), which showed that all parts of the Ni-hyperaccumulator *S. tryonii* accumulate Ni at comparable concentrations. Analyses were performed by Waite Analytical Services, Adelaide University, Australia (WAS). Samples were oven-dried overnight at 80°C and digested in borosilicate tubes by using a combination of nitric acid and hydrochloric acid, with 5–6 h of heating at up to 140°C (WAS Digestion Code: HA). Concentrations of Ni, as well as 19 other elements known to occur in plant tissues, were determined by radial ICP–AES on a Ciros Vision (SPECTRO Analytical Instruments, Kleve, Germany) spectrometer. For Ni-hyperaccumulating plants, dilutions of digestion products were performed before analysis. For certain low-mass samples, Ni concentration was below the Ni detection threshold of the spectrometer. For these samples, Ni concentration is reported as a maximum based on the predicted detection threshold (reporting limit) for a sample of that mass (Table 3). Some samples contained elevated concentrations of titanium, indicating contamination by soil or processing equipment. Titanium concentration was not significantly correlated with that of any other element (two-tailed *t*-test of Pearson's product–moment correlation, $P > 0.05$), indicating that soil contamination did not contribute significantly to other reported elemental concentrations.

Results

DNA sequences

The nuclear ribosomal ITS region, comprising ITS1, the 5.8S gene and ITS2, but not including bases from the 26S and 18S genes, varied in length from 593 bp (*S.* sp. *Sharply tuberculate cocci (P.K. Latz 8376)* W.R. Barker, Rabbit Flat Roadhouse, Northern Territory; Table 3, *S.* sp. 4) to 625 bp (*S. latzii*, Lake Mackay, Northern Territory; Table 3). The ITS alignment contained 659 characters (Fig. A1), 114 of which were excluded from analysis (see above). Of the included characters, 223 were constant, 85 were variable but not parsimony-informative, and 237 were parsimony-informative. The chloroplast *trn*L–F region varied in length from 949 bp (*S. viminea*, Shoalwater Bay, Queensland; Table 3, *S. viminea* 2) to 989 bp (*S. aspericocca* subsp. *aspericocca*, Eyre Peninsula, South Australia; Table 3). The alignment of *trn*L–F contained 1170 characters (Fig. A1), 421 of which were excluded from analysis. Of the included characters, 555 were constant, 79 were variable but not parsimony-informative, and 115 were parsimony-informative.

Phylogeny

Bayesian and MP analyses of the combined data provided similar topologies (Figs 2, 3). Maximum parsimony analysis of the combined data resulted in four equally parsimonious trees (length = 1138, CI = 0.66, RI = 0.75). In Bayesian and MP consensus trees, *Stackhousia* and *Tripterococcus* were recovered as monophyletic, with the monotypic *Macgregoria* sister to *Stackhousia* (Figs 2, 3). Within *Stackhousia*, *S. tryonii* was recovered as monophyletic, closely related to an accession of *S. viminea* from Sundown Point, Tasmania (Fig. 1: #29; Table 3, Appendix 1) and to the Queensland-endemic *S. macrantha* (Figs 2, 3, Clade B). A second group of *Stackhousia* (Figs 2, 3, Clade A), comprising eight accessions, was also strongly supported. For the single population of *S. tryonii* for which more than one plant was included in the molecular phylogenetic analyses (Canoona Creek, Queensland; Fig. 1, #20, #21; Table 3, Appendix 1), plants grouped strongly with each other in preliminary analyses (97% bootstrap support; results not shown). These plants represent alternate colours in an apparent corolla-colour dimorphism within *S. tryonii*, with some plants blue to purple (Table 3, *S. tryonii* 3) and others white (Table 3, *S. tryonii* 2).

Nickel analyses

For most samples, tissue Ni concentration did not exceed the detection threshold (reporting limit) of the spectrometer (see Materials and methods). Within this group ($n = 76$), Ni concentration did not exceed 30 ppm (Table 3, Fig. 4, Appendix 1). For samples that exceeded the detection threshold of the spectrometer, Ni concentrations for specimens of Stackhousioideae other than *S. tryonii* varied from 0.0001% (1 ppm) to 0.017% (171 ppm) Ni, with an average of 24 ppm ($n = 9$; Table 3, Fig. 4, Appendix 1). Among samples of the known Ni hyperaccumulator *S. tryonii*, Ni concentrations varied from 0.25% (2500 ppm) for a plant from the Limestone Creek watershed near Mount Wheeler, Queensland (Appendix 1, D. O. Burge 1016a) to 4.1% (41 000 ppm) for a plant from Keppel Sands Road, Queensland (Fig. 1: #19, Table 3, Appendix 1). For one population of *S. tryonii* from the Canoona Creek watershed, Queensland (Fig. 1: #20 and #21, Table 3, Appendix 1), two adjacent plants were assayed for their Ni concentration. The first plant contained 16 400 ppm Ni (Table 3, *S. tryonii* 2), whereas the second contained 19 400 ppm (Table 3, *S. tryonii* 3). As described above, these plants represent alternate colours in an apparent corolla-colour dimorphism within *S. tryonii*, blue to purple (Table 3, *S. tryonii* 3) and white (Table 3, *S. tryonii* 2).

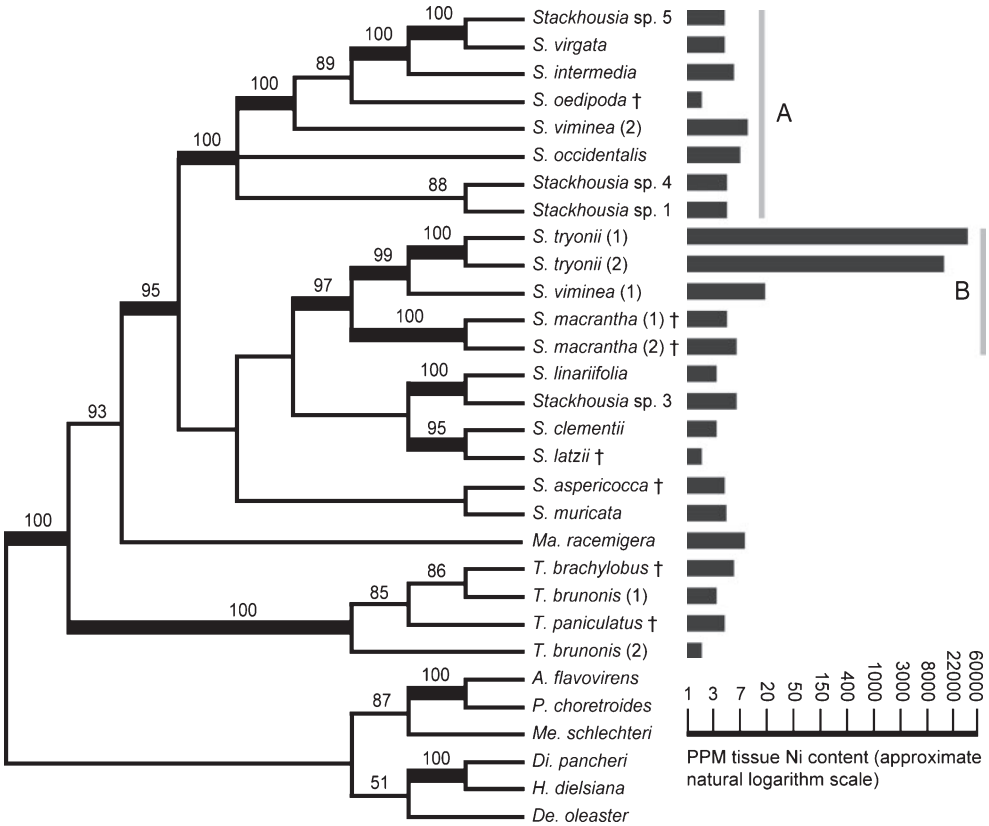

Fig. 2. Maximum parsimony consensus tree from analysis of combined ITS and *trn*L–F data, with Ni concentrations for individual plants mapped using shaded bars. Bootstrap support (BS) values for 1000 replicates of bootstrap resampling, given above branches. Heavy branches indicate BS support values >95%. Ni concentrations are mapped as natural logarithm of ppm Ni (Table 3; Appendix 1), and these are derived from the same specimens as those in DNA sequencing (Table 3). *S. aspericocca* = *S. aspericocca* subsp. *aspericocca* (Table 3). A, B: clades discussed in text. See Table 3 and Appendix 1 for data on individual populations. †, new names being formally published by Barker (in press).

Discussion

Phylogenetic relationships

Our results support recognition of the three genera comprising Stackhousioideae, including the monotypic genus *Macgregoria*, which is the sister taxon to *Stackhousia* (Figs 2, 3). Relationships among *Stackhousia*, *Macgregoria* and *Tripterococcus* recovered in the present study agree with previous work on molecular systematics of Celastraceae (Simmons *et al*. 2008). Although our sampling within *Stackhousia* is not exhaustive (20 of 35 taxa; Table 2), available data have revealed two major well supported clades within *Stackhousia*. The first (Figs 2, 3, Clade A) corresponds to *Stackhousia* section *Stackhousia* (Barker in press; Table 2). The second (Figs 2, 3, Clade B) contains *S. tryonii* and *S. macrantha*, both members of the *Racemosae* section of *Stackhousia* (Barker in press), and one accession of *S. viminea* (section *Stackhousia*) from western Tasmania (Fig. 1: #29, Figs 2, 3, *S. viminea* 1; Table 3). The Queensland-endemic *S. macrantha* is closely related to the widespread *S. monogyna* (Barker in press). *S. tryonii* and *S. monogyna* have long been considered closely related (Batianoff *et al*. 1990), if not synonymous (Barker 1984), which is consistent with the phylogenetic proximity of *S. tryonii* and *S. macrantha* seen in the present study. However, the relationship of a single accession of *S. viminea* (section *Stackhousia*) from Tasmania (Fig. 1: #29) to this group is perplexing, because it violates morphology-based sectional limits (Barker in press) and represents a wide disjunction for a member of a well supported clade otherwise limited to central and southern Queensland. Both ITS and *trn*L–F were sequenced twice using the same DNA extraction obtained from this plant, to test for PCR contamination or sequence-curation mistakes. Although identical sequences were obtained during both attempts, it is possible that the unusual placement of this plant is an artefact of contamination at the level of herbarium sheet or DNA extraction. In contrast, a second accession of *S. viminea*, from the Shoalwater Bay area of Queensland, ~50 km north-east of the northernmost *S. tryonii* population (Fig. 1: #30), is nested within the clade corresponding to section *Stackhousia* (Figs 2, 3, *S. viminea* 2; Table 3), in agreement with morphology-based sectional limits (Barker in press).

The remaining molecular phylogenetic relationships within *Stackhousia* do not tend to agree with the morphology-based sectional limits (Barker in press). However, this conflict is not strongly supported because of an overall lack of phylogenetic resolution at the base of the *Stackhousia* tree (Figs 2, 3). It is

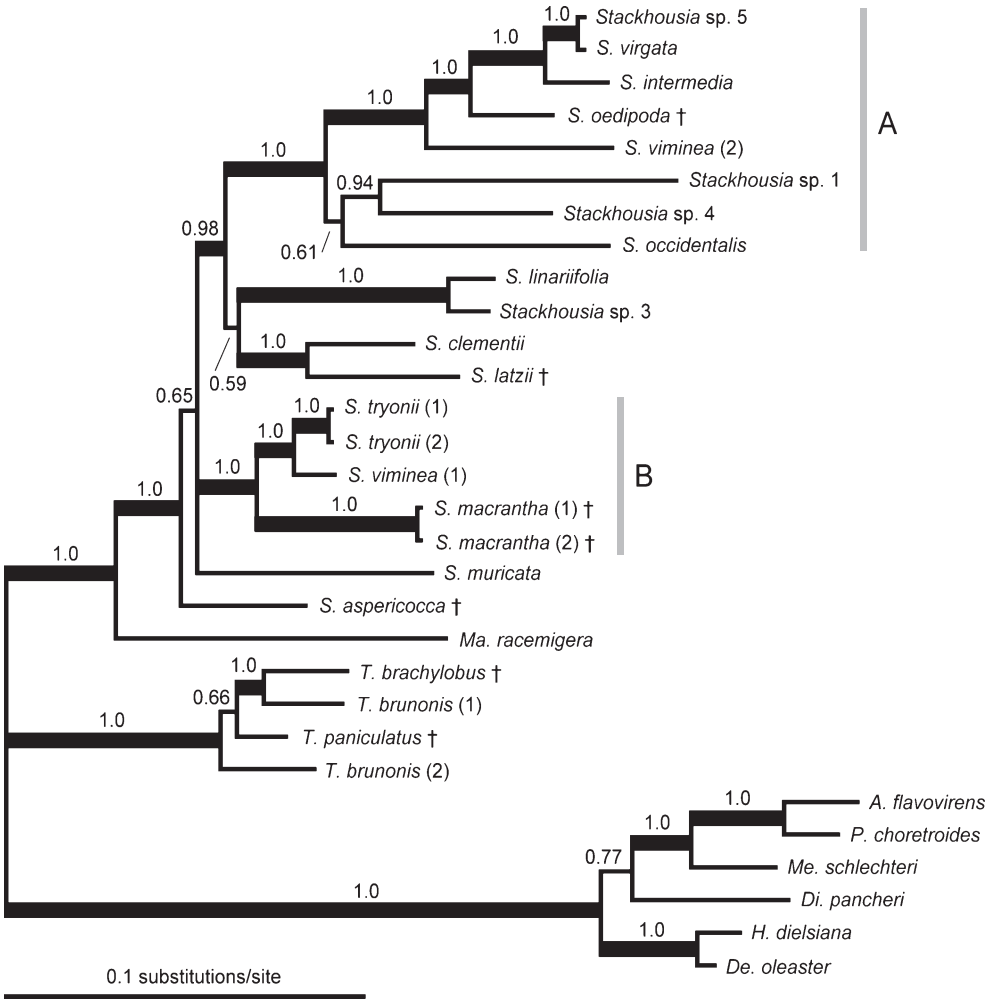

Fig. 3. Bayesian consensus phylogram from analysis of combined ITS and *trn*L–F data. Posterior probabilities (PP) are given above branches. Heavy branches indicate PP of >0.95. *S. aspericocca* = *S. aspericocca* subsp. *aspericocca* (Table 3). Clades A and B are discussed in the text. See Table 3 for data on individual populations. †, new names being formally published by Barker (in press).

interesting to note, however, that *S. clementii* (section *Stackhousia*) and *S. latzii* (section *Carinato-alatae*) are resolved as sister taxa in both MP and Bayesian analyses (Figs 2, 3). The relationship of *S. latzii* to *S. clementii* may indicate a close relationship of the small *Carinato-alatae* section (3 species; Table 2) to the much larger *Stackhousia* section. Overall, however, exhaustive comparison of molecular phylogenetic relationships with relationships implied by morphology (Barker in press) will have to await expanded sampling of DNA sequence data from additional taxa and populations.

Evolution of Ni hyperaccumulation

Our study indicates that the Ni-hyperaccumulation trait was acquired once during diversification of the Stackhousioideae, namely by *S. tryonii*. The relationship of *S. tryonii* to *S. viminea* also bears on the question of how Ni hyperaccumulation evolved in *S. tryonii*. A specimen of *S. viminea* collected from serpentinite-derived soils near the town of Gympie in south-eastern Queensland (Appendix 1, R. L. Specht 810) contained 0.017% (171 ppm) Ni, well in excess of concentrations found in normal (non-Ni-accumulating) plants growing on soils containing ordinary amounts of Ni (Fig. 4; Batianoff *et al.* 1990). Because DNA sequence data are not yet available for this marginally Ni-accumulating plant, it is not possible, at this time, to determine whether it is related to the Ni hyperaccumulator *S. tryonii*. However, as mentioned above, one population of *S. viminea* from Sundown Point, Tasmania (Fig. 1: #29, Table 3, Appendix 1) is closely related to *S. tryonii* (Figs 2, 3, *S. viminea* 1), raising the question of whether the existence of marginal Ni accumulation in *S. viminea* may bear on the evolution of Ni hyperaccumulation by *S. tryonii*. Expanded sampling of *S. viminea* from Tasmania and eastern Australia, particularly in regions of serpentinite or other Ni-rich rock outcrop, might reveal the existence of other Ni-tolerant or Ni-accumulating plants with

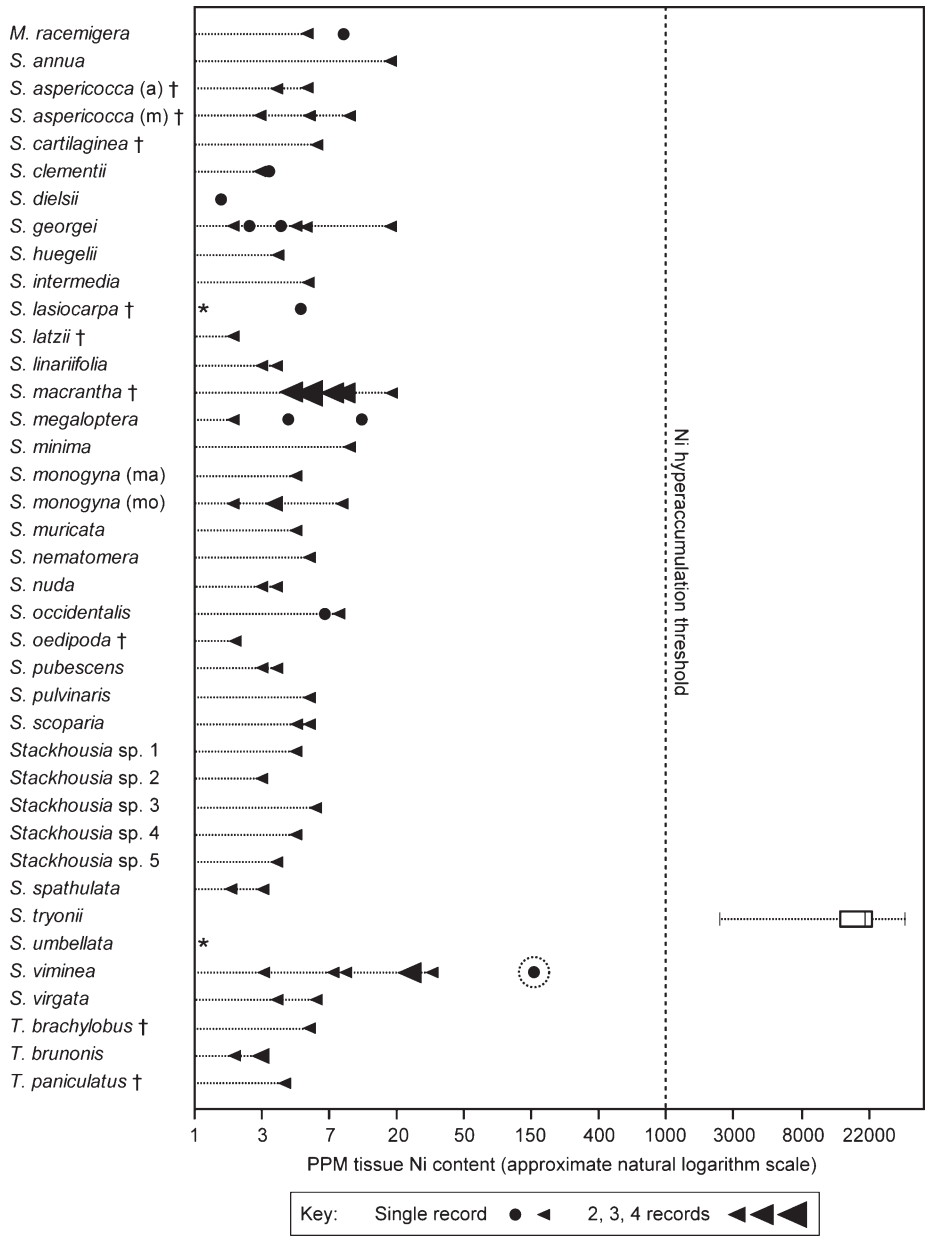

Fig. 4. Results of Ni concentration analysis for all Stackhousioideae taxa. For assays below detection threshold (reporting limit) of ICP instrument (see Materials and methods), maximum values are indicated with solid triangles at the end of dashed lines. Assays within reporting limit are indicated with dark circles or box plot. Asterisk indicates estimated Ni concentration of <1 ppm. *S. aspericocca* (a): *S. aspericocca* subsp. *aspericocca*. *S. aspericocca* (m): *S. aspericocca* subsp. *muelleri*. *S. monogyna* (ma): *S. monogyna* subsp. *madenii*. *S. monogyna* (mo): *S. monogyna* subsp. *monogyna*. †, new names being formally published by Barker (in press). Only the samples of *S. tryonii*, and a single sample of *S. viminea* (R.L. Specht 810, indicated by a dotted circle), are from known serpentinite-derived soils.

close genetic affinities to *S. tryonii*. As noted above, however, the close relationship between *S. tryonii* and *S. viminea* implied by the phylogenetic results may be an artefact of contamination, and should be interpreted with caution.

Our study is the third to report on molecular phylogenetic relationships within a group of plants containing Ni hyperaccumulators. Mengoni *et al*. (2003) reported that Ni-hyperaccumulating members of *Alyssum* section *Odontarrhena* (Brassicaceae) were polyphyletic with respect to non-hyperaccumulating species in ITS-based gene trees, suggesting that Ni hyperaccumulation evolved early during diversification of *Alyssum* section *Odontarrhena*, with subsequent loss in several lineages. Alternatively, it is possible that the trait evolved multiple times in that group. However, the presence

of Ni hyperaccumulators in other Brassicaceae genera (Table 1; Reeves 1988; Reeves and Baker 2000) indicates that the trait may have a more ancient evolutionary origin in the Brassicaceae. In fact, in an expanded molecular phylogenetic study on European members of the Alysseae tribe of Brassicaceae, two independent origins of Ni hyperaccumulation were inferred, one in a group composed of the genera *Leptoplax* (formerly *Peltaria*; Table 1) and *Bornmuellera*, and one in *Alyssum* section *Odontarrhena* (Cecchi *et al*. 2010), in agreement with the findings of Mengoni *et al*. (2003).

Alyssum and *Stackhousia* may represent opposite extremes on a continuum regarding the evolution of Ni hyperaccumulation. In this case, *Stackhousia* would represent an early stage, with a single, isolated, presumably young Ni-hyperaccumulating species. *Alyssum* would represent an advanced condition, comprising a geographically widespread diversification of Ni-hyperaccumulating plants. Alternatively, the difference between *Alyssum* and *Stackhousia* may reflect a difference in ecological opportunity. Specifically, the Ni-hyperaccumulating trait may have developed as early in *Stackhousia* as in *Alyssum*, but resulted in far less diversification of the Ni-hyperaccumulation trait in *Stackhousia* because of the rarity of serpentinite outcrops in Australia (Murray 1969; Jaireth *et al*. 2005; Ratajkoski *et al*. 2005; Hendrickx 2009), compared with the areas of Mediterranean Europe and Turkey where Ni-hyperaccumulating *Alyssum* appear to have evolved (Brooks and Radford 1978; Brooks *et al*. 1979; Reeves *et al*. 1983*a*; Mengoni *et al*. 2003).

Comparison of *Alyssum* and *Stackhousia* with other angiosperm taxa containing Ni hyperaccumulators (Table 1) suggests that the evolutionary dynamics of Ni hyperaccumulation inferred for these two groups may reflect major patterns in nature. Most Ni hyperaccumulators belong to families that contain other Ni hyperaccumulators (Table 1), such as the Brassicaceae (24% of known Ni hyperaccumulators) or Euphorbiaceae (23% of known Ni hyperaccumulators). Although some genera within these families have given rise to a large number of Ni hyperaccumulators, as in *Alyssum*, the overall pattern appears to be one of polyphyly, the Ni-hyperaccumulating trait arising in multiple genera within a family. Apparent multiple origins of Ni hyperaccumulation within families, and within genera that are members of those families (Mengoni *et al*. 2003), suggests the influence of ancient evolutionary events on the present distribution of the trait, as has been suggested by Broadley *et al*. (2001). Specifically, it may be that pre-disposition for Ni hyperaccumulation evolved early during the diversification of these families, or within the diversification of vascular plants as a whole (Broadley *et al*. 2001), and that extant Ni hyperaccumulators have evolved through selection on this underlying genetic architecture, much as has been hypothesised for symbiotic nitrogen fixation in angiosperms (Soltis *et al*. 1995).

In contrast, 23 Ni-hyperaccumulating species belong to families that do not contain additional cases of the trait (Table 1), including *S. tryonii*, the only known Ni-hyperaccumulating member of the entire order Celastrales. Strong phylogenetic isolation of these Ni hyperaccumulators, which represent nearly half of the vascular plant families known to contain Ni-hyperaccumulating species, suggests that the trait may have evolved independently in each of them. The Celastrales, for example, last shared a common ancestor with another lineage containing Ni-hyperaccumulating plants (the Malpighiales) at least 71.6 million years ago (Magallón and Castillo 2009).

The present study relied on tissue from herbarium specimens to determine whether plants were hyperaccumulating Ni. Although most of Ni-hyperaccumulating species are strictly endemic to Ni-rich soils, such as those derived from serpentinite (Brooks 1987), there are examples of hyperaccumulating species that grow both on and off Ni-rich soils (Boyd and Martens 1998). In these species, the trait is usually found to be constitutive, although populations growing off Ni-rich soils do not express the trait because of a lack of available Ni (Boyd and Martens 1998). Because some plants have the ability to hyperaccumulate Ni but not express the trait under low-Ni soil conditions, Ni measurements from field-collected plant tissues should be paired with soil-chemistry data and experimental data, or both, so as to reliably determine the Ni-hyperaccumulating ability of a plant. The results of the present study, as well as other studies based on Ni assays from field-collected tissue, should be interpreted in light of this idea. Overall, future research on evolution of Ni hyperaccumulation among plants will benefit from experiments on hyperaccumulation ecology, improved understanding of Ni physiology, comparative data on the underlying genetic architecture of the trait (Pollard *et al*. 2002), additional phylogenetic studies on specific Ni-hyperaccumulating taxa and phylogenetic reconstruction of plant–Ni relationships at the scale of vascular plant diversification.

Accessory publications (available online)

Accessory publication consists of annotated NEXUS files (in .txt format) for the combined ITS and *trn*L–F DNA alignment (Fig. A1), trees resulting from analyses of ITS (Figs A2, A3) and *trn*L–F alone (Figs A4, A5) and trees resulting from analyses of combined data (Figs A6, A7).

Acknowledgements

We thank the curatorial staff of the State Herbarium of South Australia for their assistance with handling of specimens, as well as volunteer Ronda Atkinson for help in locating study material. Andrew Lowe, Fred Gurgel, Mike Gardner, Hugh Cross, Terry Bertozzi and Dean Cunningham are thanked for assistance in organising laboratory work. We thank Teresa Fowles and Lyndon Palmer of Waite Analytical Services (University of Adelaide, Australia) for assistance with development of tissue-sampling protocols for elemental analyses, and commentary on data interpretation. Fieldwork in Queensland was facilitated by Joy Brushe and Gordon Guymer. We are also grateful to James Beck, Robin Hopkins, Darren Crayn, Amanda Grusz, Carl Rothfels, Gwendolyn Williams, Kathleen Ferris and Kevin Wright for providing constructive criticism of this manuscript and earlier writings. This research was facilitated by an NSF EAPSI fellowship (OISE-0813227) awarded to DOB. The authors dedicate this work to the memory of George N. Batianoff.

References

Adigüzel N, Reeves RD (2002) A new nickel-accumulating species of *Alyssum* (Cruciferae) from western Turkey. *Edinburgh Journal of Botany* **59**, 215–219. doi:10.1017/S0960428602000033

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. *Biorecovery* **1**, 81–126.

Baker AJM, Proctor J (1988) Ecological studies on forest over ultrabasic rocks in the Philippines. *Bulletin of the British Ecological Society* **19**, 29–34.

Baker AJM, Brooks RR, Kersten WJ (1985) Accumulation of nickel by *Psychotria* species from the pacific basin. *Taxon* **34**, 89–95. doi:10.2307/1221569

Baker AJM, Proctor J, van Balgooy MMJ, Reeves RD (1992) Hyperaccumulation of nickel by the flora of the ultramafics of Palawan, Republic of the Philippines. In 'The vegetation of ultramafic (serpentine) soils'. (Eds AJM Baker, J Proctor, RD Reeves) pp. 291–304. (Intercept: Andover, UK)

Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In 'Phytoremediation of contaminated soil and water'. (Eds N Terry, G Bañuelos) pp. 85–107. (Lewis Publishers: Boca Raton, FL)

Barker WR (1984) Stackhousiaceae. In 'Flora of Australia. Vol. 22'. (Ed. AS George) pp. 186–203. (Australian Government Publishing Service: Canberra)

Barker WR (2005) Standardising informal names in Australian publications. *Australian Systematic Botany Society Newsletter* **122**, 11–12.

Barker WR (in press) The taxonomy of subfamily Stackhousioideae (Celastraceae). I. *Journal of the Adelaide Botanic Garden.*

Batianoff GN, Reeves RD, Specht RL (1990) *Stackhousia tryonii* Bailey, a nickel-accumulating serpentinite-endemic species of central Queensland. *Australian Journal of Botany* **38**, 121–130. doi:10.1071/BT9900121

Berazain Iturralde R (1981) Sobre el endemismo de la florula serpentinicola de 'Lomas de Galindo,' Canasi, Habana. *Revista del Jardín Botánico Nacional* **2**, 29–59.

Bhatia NP, Orlic I, Siegele R, Ashwath N, Baker AJM, Walsh KB (2003) Elemental mapping using PIXE shows the main pathway of nickel movement is principally symplastic within the fruit of the hyperaccumulator *Stackhousia tryonii. New Phytologist* **160**, 479–488. doi:10.1046/j.1469-8137.2003.00912.x

Bhatia NP, Walsh KB, Orlic I, Siegele R, Ashwath N, Baker AJM (2004) Studies on spatial distribution of nickel in leaves and stems of the metal hyperaccumulator *Stackhousia tryonii* using nuclear microprobe (micro-PIXE) and EDXS techniques. *Functional Plant Biology* **31**, 1061–1074. doi:10.1071/FP03192

Bhatia NP, Baker AJM, Walsh KB, Midmore DJ (2005*a*) A role for nickel in osmotic adjustment in drought-stressed plants of the nickel hyperaccumulator *Stackhousia tryonii* Bailey. *Planta* **223**, 134–139. doi:10.1007/s00425-005-0133-8

Bhatia NP, Walsh KB, Baker AJM (2005*b*) Detection and quantification of ligands involved in nickel detoxification in a herbaceous Ni hyperaccumulator *Stackhousia tryonii* Bailey. *Journal of Experimental Botany* **56**, 1343–1349. doi:10.1093/jxb/eri135

Bidwell SD (2000) Hyperaccumulation of metal in Australian native plants. PhD Thesis, School of Botany, University of Melbourne, Australia.

Blattner FR (1999) Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *BioTechniques* **27**, 1180–1185.

Boyd RS (2004) Ecology of metal hyperaccumulation. *New Phytologist* **162**, 563–567. doi:10.1111/j.1469-8137.2004.01079.x

Boyd RS, Martens SN (1998) Nickel hyperaccumulation by *Thalaspi montanum* var. *montanum* (Brassicaceae): a constitutive trait. *American Journal of Botany* **85**, 259–265. doi:10.2307/2446314

Bradshaw AD (1952) Populations of *Agrostis tenuis* resistant to lead and zinc poisoning. *Nature* **169**, 1098. doi:10.1038/1691098a0

Broadley MR, Willey NJ, Wilkins JC, Baker AJM, Mead A, White PJ (2001) Phylogenetic variation in heavy metal accumulation in angiosperms. *New Phytologist* **152**, 9–27. doi:10.1046/j.0028-646x.2001.00238.x

Brooks RR (1987) 'Serpentine and its vegetation: a multidisciplinary approach.' (Dioscorides Press: Portland, OR)

Brooks RR (1995) Specific indicator plants. In 'Biological systems in mineral exploration and processing'. (Eds RR Brooks, CE Dunn, GEM Hall) pp. 39–70. (Ellis Horwood: New York)

Brooks RR (1998) Geobotany and hyperaccumulators. In 'Plants that hyperaccumulate heavy metals'. (Ed. RR Brooks) pp. 55–94. (CAB International: Wallingford, UK)

Brooks RR, Radford CC (1978) Nickel accumulation by European species of the genus *Alyssum. Proceedings of the Royal Society of London. Series B. Biological Sciences* **200**, 217–224. doi:10.1098/rspb.1978.0016

Brooks RR, Wither ED (1977) Nickel accumulation by *Rinorea bengalensis* (Wall.) O.K. *Journal of Geochemical Exploration* **7**, 295–300. doi:10.1016/0375-6742(77)90085-1

Brooks RR, Yang X-H (1984) Elemental levels and relationships in the endemic serpentine flora of the Great Dyke, Zimbabwe and their significance as controlling factors for the flora. *Taxon* **33**, 392–399. doi:10.2307/1220976

Brooks RR, Lee J, Reeves RD, Jaffré T (1977*a*) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *Journal of Geochemical Exploration* **7**, 49–57. doi:10.1016/0375-6742(77)90074-7

Brooks RR, Wither ED, Zepernick B (1977*b*) Cobalt and nickel in *Rinorea* species. *Plant and Soil* **47**, 707–712. doi:10.1007/BF00011041

Brooks RR, Morrison RS, Reeves RD, Dudley TR, Akman Y (1979) Hyperaccumulation of nickel by *Alyssum* Linnaeus (Cruciferae). *Proceedings of the Royal Society of London. Series B. Biological Sciences* **203**, 387–403. doi:10.1098/rspb.1979.0005

Brooks RR, Reeves RD, Baker AJM, Rizzo JA, Diaz Ferreira H (1990) The Brazilian serpentine plant expedition (BRASPEX), 1988. *National Geographic Research* **6**, 205–219.

Brooks RR, Reeves RD, Baker AJM (1992) The serpentine vegetation of Goiás State, Brazil. In 'The vegetation of ultramafic (serpentine) soils'. (Eds AJM Baker, J Proctor, RD Reeves) pp. 67–81. (Intercept: Andover, UK)

Brooks RR, Chambers MF, Nicks LJ, Robinson BH (1998) Phytomining. *Trends in Plant Science* **3**, 359–362. doi:10.1016/S1360-1385(98)01283-7

Cecchi L, Gabbrielli R, Arnetoli M, Gonnelli C, Hasko A, Selvi F (2010) Evolutionary lineages of nickel hyperaccumulation and systematics in European Alysseae (Brassicaceae): evidence from nrDNA sequence data. *Annals of Botany* **106**, 751–767. doi:10.1093/aob/mcq162

Doksopulo EP (1961) 'Nickel in rocks, soils, water and plants adjacent to the talc deposits of the Chorchanskaya Group. Tiblisi.' (Izdatel vo Tbiliskovo Universitet: Tbilisi, Georgia)

Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. *Cladistics* **10**, 315–319. doi:10.1111/j.1096-0031.1994.tb00181.x

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791. doi:10.2307/2408678

Gabbrielli R, Pedani F, Vergnano Gambi O (1987) Ulteriori dati sulla composizione minerale della vegetazione degli affioramenti ofiolitici dell'alta Valle di Ayas. *Revue Valdôtaine d'Histoire Naturelle* **41**, 99–110.

Hendrickx M (2009) Naturally occurring asbestos in eastern Australia: a review of geological occurrence, disturbance and mesothelioma risk. *Environmental Geology* **57**, 909–926. doi:10.1007/s00254-008-1370-5

Holmgren PK, Holmgren NH, Barnett LC (1990) 'Index Herbariorum. I. The herbaria of the world.' 8th edn. (The New York Botanical Garden: New York)

Homer FA, Reeves RD, Brooks RR, Baker AJM (1991) Characterization of the nickel-rich extract from the nickel hyperaccumulator *Dichapetalum gelonioides. Phytochemistry* **30**, 2141–2145. doi:10.1016/0031-9422(91)83602-H

Jaffré T (1980) Étude écologique du peuplement végétal des sols dérivés de roches ultrabasiques en Nouvelle Calédonie. *Travaux et Documents de l'ORSTOM* **124**, 1–274.

Jaffré T (1992) Floristic and ecological diversity of the vegetation on ultramafic rocks in New Caledonia. In 'The vegetation of ultramafic (serpentine) soils'. (Eds AJM Baker, J Proctor, RD Reeves) pp. 101–107. (Intercept: Andover, UK)

Jaffré T, Schmid M (1974) Accumulation du nickel par une Rubiacée de Nouvelle Calédonie: *Psychotria douarrei* (G.Beauvisage) Däniker. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences. Série D, Sciences Naturelles* **278**, 1727–1730.

Jaffré T, Brooks RR, Lee J, Reeves RD (1976) *Sebertia acuminata*: a hyperaccumulator of nickel from New Caledonia. *Science* **193**, 579–580. doi:10.1126/science.193.4253.579

Jaffré T, Brooks RR, Trow JM (1979*a*) Hyperaccumulation of nickel by *Geissois* species. *Plant and Soil* **51**, 157–161. doi:10.1007/BF02205937

Jaffré T, Kersten W, Brooks RR, Reeves RD (1979*b*) Nickel uptake by Flacourtiaceae of New Caledonia. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **205**, 385–394. doi:10.1098/rspb.1979.0072

Jaireth S, Hoatson DM, Towner RR, Ratajkoski M (2005) 'Australian nickel resources map.' (Geoscience Australia: Canberra)

Kelly PC, Brooks RR, Dilli S, Jaffré T (1975) Preliminary observations on the ecology and plant chemistry of some nickel-accumulating plants from New Caledonia. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **189**, 69–80. doi:10.1098/rspb.1975.0042

Kersten WJ, Brooks RR, Reeves RD, Jaffré T (1979) Nickel uptake by New Caledonian species of *Phyllanthus. Taxon* **28**, 529–534. doi:10.2307/1219791

Kruckeberg AR (1951) Intraspecific variability in the response of certain native plant species to serpentine soil. *American Journal of Botany* **38**, 408–419. doi:10.2307/2438248

Kruckeberg AR (1986) An essay: the stimulus of unusual geologies for plant speciation. *Systematic Botany* **11**, 455–463. doi:10.2307/2419082

Kruckeberg AR, Peterson PJ, Samiullah Y (1993) Hyperaccumulation of nickel by *Arenaria rubella* (Caryophyllaceace) from Washington State. *Madrono* **42**, 458–469.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics (Oxford, England)* **23**, 2947–2948. doi:10.1093/bioinformatics/btm404

Lisanti EL (1952) Contributo allo studio delle morfosi che si riscontrano sui serpentini. *Nuovo Giornale Botanico Italiano* **14**, 349–360.

Mabberley DJ (2008) 'Mabberley's plant-book, a portable dictionary of plants, their classifications, and uses.' 3rd edn. (Cambridge University Press: Cambridge, UK)

Magallón S, Castillo A (2009) Angiosperm diversification through time. *American Journal of Botany* **96**, 349–365. doi:10.3732/ajb.0800060

Mason HL (1946) The edaphic factor in narrow endemism. I. The nature of environmental influences. *Madrono* **8**, 209–226.

Menezes de Sequeira E (1969) Toxicity and movement of heavy metals in serpentinic soils (north-Eastern Portugal). *Agronomia Lusitana* **30**, 115–154.

Mengoni A, Baker AJM, Bazzicalupo M, Reeves RD, Adigüzel N, Chianni E, Galardi F, Gabbrielli R, Gonnelli C (2003) Evolutionary dynamics of nickel hyperaccumulation in *Alyssum* revealed by ITS nrDNA analysis. *New Phytologist* **159**, 691–699. doi:10.1046/j.1469-8137.2003.00837.x

Minguzzi C, Vergnano O (1948) Il contenuto di nichel nelle ceneri di *Alyssum bertolonii*. *Atti della Società Toscana di Scienze Naturali. Memorie Serie A* **55**, 49–74.

Morrey DR, Balkwill K, Balkwill M-J (1989) Studies on serpentine flora: preliminary analyses of soils and vegetation associated with serpentinite rock formations in the south-eastern Transvaal. *South African Journal of Botany* **55**, 171–177.

Morrey DR, Balkwill K, Balkwill M-J, Williamson S (1992) A review of some studies of the serpentine flora of southern Africa. In 'The vegetation of ultramafic (serpentine) soils'. (Eds AJM Baker, J Proctor, RD Reeves) pp. 147–155. (Intercept: Andover, UK)

Murray CG (1969) The petrology of the ultramafic rocks of the Rockhampton district, Queensland. *Geological Survey of Queensland* **343**, 1–13.

Nylander JAA (2004) 'MrModeltest, v2.' Program distributed by the author. (Evolutionary Biology Centre, Uppsala University: Uppsala, Sweden)

Pollard AJ, Powell KD, Harper FA, Smith JAC (2002) The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences* **21**, 539–566. doi:10.1080/0735-260291044359

Proctor J, Phillipps C, Duff GK, Heaney A, Robertson FM (1989) Ecological studies on Gunung Silam, a small ultrabasic mountain in Sabah, Malaysia. II. Some forest processes. *Journal of Ecology* **77**, 317–331. doi:10.2307/2260752

Proctor AJ, van Balgooy MMJ, Fairweather GM, Nagy L, Reeves RD (1994) A preliminary re-investigation of a plant geographical 'El Dorado'. *Tropical Biodiversity* **2**, 303–316.

Rajakaruna N, Bohm BA (2002) Serpentine and its vegetation: a preliminary study from Sri Lanka. *Journal of Applied Botany* **76**, 20–28.

Ratajkoski M, Huleatt MB, Jaques AL, Jaireth S, Towner R (2005) 'Mineral density map – Nickel deposits, occurrences, and potential.' (Geoscience Australia: Canberra)

Reeves RD (1988) Nickel and zinc accumulation by species of *Thlaspi* L., *Cochlearia* L., and other genera of the Brassicaceae. *Taxon* **37**, 309–318. doi:10.2307/1222140

Reeves RD (1992) The hyperaccumulation of nickel by serpentine plants. In 'The vegetation of ultramafic (serpentine) soils'. (Eds AJM Baker, J Proctor, RD Reeves) pp. 253–277. (Intercept: Andover, UK)

Reeves RD (2003) Tropical hyperaccumulators of metals and their potential for phytoextraction. *Plant and Soil* **249**, 57–65. doi:10.1023/A:1022572517197

Reeves RD (2006) Hyperaccumulation of trace elements by plants. In 'Phytoremediation of metal-contaminated soils, Nato science series (IV): earth and environmental sciences v. 68'. (Eds J-L Morel, G Echevarria, N Goncharova) pp. 25–52. (Springer: Dordrecht, The Netherlands)

Reeves RD, Adigüzel N (2004) Rare plants and nickel accumulators from Turkish serpentine soils, with special reference to *Centaurea* species. *Turkish Journal of Biology* **28**, 147–153.

Reeves RD, Adigüzel N (2008) The nickel hyperaccumulating plants of the serpentines of Turkey and adjacent areas: a review with new data. *Turkish Journal of Biology* **32**, 143–153.

Reeves RD, Baker AJM (2000) Metal-accumulating plants. In 'Phytoremediation of toxic metals: using plants to clean up the environment'. (Eds I Raskin, BD Ensley) pp. 193–229. (John Wiley & Sons: New York)

Reeves RD, Brooks RR (1983) European species of *Thlaspi* L. (Cruciferae) as indicators of nickel and zinc. *Journal of Geochemical Exploration* **18**, 275–283. doi:10.1016/0375-6742(83)90073-0

Reeves RD, Brooks RR, Press JR (1980) Nickel accumulation by species of *Peltaria* Jacq. (Cruciferae). *Taxon* **29**, 629–633. doi:10.2307/1220334

Reeves RD, Brooks RR, MacFarlane RM (1981) Nickel uptake by Californian *Streptanthus* and *Caulanthus* with particular reference to the hyperaccumulator *S. polygaloides* Gray (Brassicaceae). *American Journal of Botany* **68**, 708–712. doi:10.2307/2442798

Reeves RD, Brooks RR, Dudley TR (1983*a*) Uptake of nickel by species of *Alyssum, Bornmuellera*, and other genera of Old World tribus Alysseae. *Taxon* **32**, 184–192. doi:10.2307/1221970

Reeves RR, MacFarlane RM, Brooks RR (1983*b*) Accumulation of nickel and zinc by western North American genera containing serpentine-tolerant species. *American Journal of Botany* **70**, 1297–1303. doi:10.2307/2443420

Reeves RD, Baker AJM, Borhidi A, Berazaín R (1996) Nickel-accumulating plants from the ancient serpentine soils of Cuba. *New Phytologist* **133**, 217–224. doi:10.1111/j.1469-8137.1996.tb01888.x

Reeves RD, Baker AJM, Borhidi A, Berazaín R (1999) Nickel hyperaccumulation in the serpentine flora of Cuba. *Annals of Botany* **83**, 29–38. doi:10.1006/anbo.1998.0786

Reeves RD, Baker AJM, Becquer T, Echevarria G, Miranda ZJG (2007) The flora and biogeochemistry of the ultramafic soils of Goiás state, Brazil. *Plant and Soil* **293**, 107–119. doi:10.1007/s11104-007-9192-x

Roberts BA (1992) Ecology of serpentinized areas, Newfoundland, Canada. In 'The ecology of areas with serpentinized rocks. A world view'. (Eds BA Roberts, J Proctor) pp. 75–113. (Kluwer Academic Publishers: Dordrecht, The Netherlands)

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. doi:10.1093/bioinformatics/btg180

Salt DE, Krämer U (2000) Mechanisms of metal hyperaccumulation in plants. In 'Phytoremediation of toxic metals: using plants to clean up the environment'. (Eds I Raskin, BD Ensley) pp. 231–246. (John Wiley & Sons: New York)

Schmid M (1991) *Phyllanthus.* In 'Flore de la Nouvelle-Calédonie et dépendances, v. 17, Euphorbiacées'. pp. 39–40. (Museum National d'Histoire Naturelle: Paris)

Severne BC (1974) Nickel accumulation by *Hybanthus floribundus. Nature* **248**, 807–808. doi:10.1038/248807a0

Severne BC, Brooks RR (1972) A nickel-accumulating plant from Western Australia. *Planta* **103**, 91–94. doi:10.1007/BF00394610

Simmons MP, Savolainen V, Clevinger CC, Archer RH, Davis JI (2001) Phylogeny of the Celastraceae inferred from 26S nuclear ribosomal DNA, phytochrome B, *rbcl, atpB*, and morphology. *Molecular Phylogenetics and Evolution* **19**, 353–366. doi:10.1006/mpev.2001.0937

Simmons MP, Cappa JJ, Archer RH, Ford AJ, Eichstedt D, Clevinger CC (2008) Phylogeny of the Celastreae (Celastraceae) and the relationships of *Catha edulis* (qat) inferred from morphological characters and nuclear and plastid genes. *Molecular Phylogenetics and Evolution* **48**, 745–757. doi:10.1016/j.ympev.2008.04.039

Soltis DE, Soltis PS, Morgan DR, Swensen SM, Mullin BC, Dowd JM, Martin PG (1995) Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. *Proceedings of the National Academy of Sciences, USA* **92**, 2647–2651. doi:10.1073/pnas.92.7.2647

Swofford DL (2000) 'PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4.' (Sinauer Associates: Sunderland, MA)

Taberlet P, Ludovic G, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**, 1105–1109. doi:10.1007/BF00037152

Vergnano Gambi O, Gabbrielli R (1979) Ecophysiological and geochemical aspects of nickel, chromium and cobalt accumulation in the vegetation of some Italian ophiolitic outcrops. *Ofioliti* **4**, 199–208.

Vergnano Gambi O, Gabbrielli R (1981) La composizione minerale della vegetazione degli affloramenti dell'alta Valle di Ayas. *Revue Valdôtaine d'Historie Naturelle* **35**, 51–61.

Vergnano Gambi O, Brooks RR, Radford CC (1979) L'accumulo di nichel nelle specie italiane del genere *Alyssum. Webbia* **33**, 269–277.

Vergnano Gambi O, Gabbrielli R, Pancaro L (1982) Nickel, chromium and cobalt in plants from Italian serpentine areas. *Acta Oecologica* **3**, 291–306.

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In 'PCR protocols: a guide to methods and applications'. (Eds MA Innis, DH Gelfand, JJ Sninsky, TJ White) pp. 315–322. (Academic Press: San Diego, CA)

Wild H (1970) Geobotanical anomalies in Rhodesia. 3. The vegetation of nickel bearing soils. *Kirkia* **7**(Suppl.), 1–62.

Wild H (1974) Indigenous plants and chromium in Rhodesia. *Kirkia* **9**, 233–241.

Wither ED, Brooks RR (1977) Hyperaccumulation of nickel by some plants of South-east Asia. *Journal of Geochemical Exploration* **8**, 579–583. doi:10.1016/0375-6742(77)90100-5

Zhang L-B, Simmons MP (2006) Phylogeny and delimitation of the Celastrales inferred from nuclear and plastid genes. *Systematic Botany* **31**, 122–137. doi:10.1600/036364406775971778

Manuscript received 9 July 2010, accepted 5 November 2010

Appendix 1. Material examined

List of material used in the study, with taxon, collector, herbarium, locality and Ni assay data. Herbarium acronyms follow Holmgren *et al*. (1990). NSW, New South Wales; NT, Northern Territory; Qld, Queensland; SA, South Australia; Tas., Tasmania; Vic., Victoria; WA, Western Australia. Ni concentration is given as ppm; results below reporting limit are given as a maximum using <symbol. †, new names being formally published by Barker (in press).

Macgregoria *racemigera* F.Muell. – *J.B. Cleland, s.n.* (AD), Giles Tank, WA; Ni < 6. *M. racemigera* – *R.J. Chinnock 9683* (AD), 18.7 km E of Coondiner Pool, WA; Ni < 9; ***Stackhousia*** *annua* W.R.Barker – *T. Hall 181* (AD), Warrenben NP, SA; Ni < 20. *S. aspericocca* Schuch. subsp. *aspericocca* – *D. Hopton 235* (AD), Wanilla Conservation Park, SA; Ni < 4; *S. aspericocca* subsp. *aspericocca* – *W.R. Barker 3652* (AD), W of Dergholm on road to Penola, Vic.; Ni < 6. †*S. aspericocca* Schuch. subsp. *muelleri* (Schuch.) W.R.Barker – *D.E. Albrecht 5140* (AD), Portland–Nelson road, Vic.; Ni < 6. †*S. aspericocca* subsp. *muelleri* – *D.E. Symon 13395* (AD), S of Cherry Gardens, SA; Ni < 10. †*S. aspericocca* subsp. *muelleri* – *W.R. Barker 1418* (AD), SSW of Casterton, Vic.; Ni < 3. †*S. cartilaginea* W.R.Barker – *H.J. Eichler 20268* (AD), near Shire of Esperance, WA; Ni < 7. *S. clementii* Domin – *F.J. Badman 2349* (AD), E of Andamooka Opal Field, SA; Ni < 3. *S. clementii* Domin – *W.R. Barker 2829* (AD), Tanami Desert, NW of The Granites, NT; Ni < 3; *S. dielsii* Pamp. – *W.R. Barker 2181* (AD), NW Coastal Highway, N of Murchison River crossing, WA; Ni 1.29. *S. georgei* Diels – *A.S. George 4489* (AD), E of Laverton, WA; Ni < 20. *S. georgei* – *D.E. Symon 17024* (AD), Fraser Range Station, WA; Ni < 5. *S. georgei* – *Hj. Eichler 21274* (AD), near Norseman, WA; Ni 2.21. *S. georgei* – *P.G. Wilson 3111* (AD), near Londonderry, WA; Ni 4.08. *S. georgei* Diels – *R.V. Smith 66/525* (AD), Queen Victoria Rock, WA; Ni < 6. *S. georgei* – *W.R. Barker 7235* (AD), N of Paynes Find, WA; Ni < 2. *S. huegelii* Endl. – *A.E. Orchard 4254* (AD), road to Nambung NP, WA; Ni < 4. *S. intermedia* Bailey – *R.M. Barker 367* (AD), Daly River Rd off Stuart Hwy, NT; Ni < 6; †*S. lasiocarpa* W.R.Barker – *S. Paust 757* (PERTH), S of Ravensthorpe, WA; Ni 5.48. †*S. lasiocarpa* – *W.R. Barker 2212* (AD), Kalbarri NP, WA; Ni < 1. †*S. latzii* W.R.Barker – *P.K. Latz 12870* (AD), Lake Mackay, NT; Ni < 2; *S. linariifolia* A.Cunn. – *C.R. Alcock 800* (AD), Hundred of Hutchison, SA; Ni < 4. *S. linariifolia* A.Cunn. – *E.N.S. Jackson 2317* (AD), Billiatt NP, SA; Ni < 3; †*S. macrantha* W.R.Barker – *A.R. Bean 10891* (BRI), S of Warwick, Qld; Ni < 5. †*S. macrantha* (*S. macrantha* 1; Table 3, Figs 2–4) – *A.R. Bean 11956* (BRI), State Forest 43, Qld; Ni < 5; †*S. macrantha* – *A.R. Bean 18310* (AD), Burnett Hwy, N of Tansey, Qld; Ni < 5. †*S. macrantha* – *D. Halford Q8067* (BRI), south slope of Mt McCamley, Qld; Ni < 10. †*S. macrantha* (*S. macrantha* 2; Table 3, Figs 2–4) – *D. Halford Q8806* (BRI), N of Rossmoya, Qld; Ni < 7. †*S. macrantha* – *E.R. Anderson 3846* (BRI), W of Ambrose, Qld; Ni < 7. †*S. macrantha* – *I.G. Champion 1246* (BRI), Cape Palmerston NP, Qld; Ni < 7. †*S. macrantha* – *N. Gibson 427* (BRI), Toondoon, Qld; Ni < 10. †*S. macrantha* – *N. Gibson 937* (BRI), State Forest 60, Qld; Ni < 8. †*S. macrantha* – *P. Grimshaw 2569* (BRI), State Forest 210, Qld; Ni < 10. *S. macrantha* – *P.I. Forster 16156* (BRI), Timber Reserve 202, Qld; Ni < 7. †*S. macrantha* (*S. macrantha* 3; Table 3, Figs 2–4) – *P.I. Forster 31822* (BRI), Forest Reserve 1344, Qld; Ni < 8. †*S. macrantha* (*S. macrantha* 4; Table 3, Figs 2–4) – *P.R. Sharpe 5346* (BRI), Deepwater Ck NP, Qld; Ni < 9. †*S. macrantha* – *R.D. Reeves 611* (BRI), E of Jim Crow Mountain, Qld; Ni 9.32. †*S. macrantha* – *T. Eisen 39* (BRI), S of Mt Hope, Qld; Ni < 8. †*S. macrantha* (*S. macrantha* 5; Table 3, Figs 2–4) – *T. Ritchie, s.n.* (BRI), W of Eidsvold, Qld; Ni < 20. *S. megaloptera* F.Muell. – *P. Canty for NPWS 2022* (AD), SE of Immarna Siding, SA; Ni < 2. *S. megaloptera* – *P.G. Wilson 8915* (AD), E of Depot Springs, WA; Ni 13.29. *S. megaloptera* – *R.J. Chinnock 5117* (AD), SW of Yamarna, WA; Ni 4.41. *S. minima* Hook.f. – *W.R. Barker 5796* (AD), Sphinx Ck, New Zealand; Ni < 10. †*S. monogyna* Labill. subsp. *maidenii* (Pamp.) W.R.Barker – *L.A. Craven 1508* (CANB), Burrewarra Point, NSW; Ni < 5. *S. monogyna* Labill. subsp. *monogyna* – *J.H. Ross 3502* (AD), Croajingalong NP, Vic.; Ni < 4. *S. monogyna* subsp. *monogyna* – *W.R. Barker 1639* (AD), Brindibella Range, ACT; Ni < 4. *S. monogyna* subsp. *monogyna* – *W.R. Barker 983* (AD), Holwell Gorge, Tas.; Ni < 9. *S. monogyna* subsp. *monogyna* – *W.R. Barker 988* (AD), Rocky Cape, Tas.; Ni < 2. *S. muricata* Lindl. – *T. Hall 400* (AD), Outalpa Station, SA; Ni < 5. †*S. nematomera* W.R.Barker – *W.R. Barker 2443* (AD), Kamballup, WA; Ni < 6. *S. nuda* Lindl. – *B.J. Lepschi 3981* (AD), Wyee, NSW; Ni < 4. *S. nuda* – *P.R. Sharpe 5515* (BRI), Shoalwater Bay Military Reserve, Qld; Ni < 3. *S. occidentalis* Domin – *A.A. Munir 5236* (AD), S of Goongarrie, WA; Ni 7.30. *S. occidentalis* – *W.R. Barker 2118* (AD), NW Coastal Highway, WA; Ni < 8. †*S. oedipoda* W.R.Barker – *W.R. Barker 2042* (AD), 9 km by road ESE of Gregory Range, WA; Ni < 2. *S. pubescens* A.Rich. – *R.J. Chinnock 4373* (AD), Lake Magenta Rd, WA; Ni < 3. *S. pubescens* – *W.R. Barker 2541* (AD), Mt Short, WA; Ni < 4. *S. pulvinaris* F.Muell. – *H.J. Eichler 14686* (AD), Bogong High Plains, Vic.; Ni < 6. *S. scoparia* Benth. – *A.S. George 7275* (PERTH), Mt Short, WA; Ni < 5. *S. scoparia* – *G.J. Keighery 895* (PERTH), Middle Ironcap, WA; Ni < 6. *S.* sp. *Colliculate cocci (W.R. Barker 6025)* W.R.Barker (*Stackhousia* sp. 1; Tables 2, 3, Figs 2–4) – *W.R. Barker 6825* (AD), Valentine Rockhole, WA; Ni < 5. *S.* sp. *McIvor River (J.R. Clarkson 5201)* Queensland Herbarium (*Stackhousia* sp. 2; Tables 2, 3, Figs 2–4) – *J.R. Clarkson 7833* (AD), McIvor River near Starcke Station, Qld; Ni < 3. *S.* sp. *Puberulent (N.G. Walsh 4835)* W.R.Barker (*Stackhousia* sp. 3; Tables 2, 3, Figs 2–4) – *N.G. Walsh 4835* (AD), Golden Beach, Vic.; Ni < 7. *S.* sp. *Sharply tuberculate cocci (P.K. Latz 8376)* W.R.Barker (*Stackhousia* sp. 4; Tables 2, 3, Figs 2–4) – *P.K. Latz 8376* (AD), Rabbit Flat Road House, NT; Ni < 5. *S.* sp. *West Kimberley (W.R. Barker 6856)* W.R.Barker (*Stackhousia* sp. 5; Tables 2, 3, Figs 2–4) – *W.R. Barker 6856* (AD), 10.8 km by road S of Pine Ck, NT; Ni < 4. *S. spathulata* Sieber ex Sprengel – *I. Crawford 1106a* (AD), Flinders I., Cameron Inlet, Tas.; Ni < 3. *S. spathulata* – *N.N. Donner 11178* (AD), beach at Avoid Bay, SA; Ni < 2. *S. tryonii* Bailey (*S. tryonii* 3; Table 3, Figs 2–4) – *D.O. Burge 1013a* (AD), Canoona Ck watershed, Qld; Ni 19400. *S. tryonii* (*S. tryonii* 2; Table 3, Figs 2–4) – *D.O. Burge 1013c* (AD), Canoona Ck watershed, Qld; Ni 16400. *S. tryonii* (*S. tryonii* 1; Table 3, Figs 2–4) – *D.O. Burge 1014b* (AD), Ross Range, Qld; Ni 41000. *S. tryonii* – *D.O. Burge 1016a* (AD), Limestone Ck watershed, Qld; Ni 2500. *S. tryonii* (*S. tryonii* 4; Table 3, Figs 2–4) – *D.O. Burge 1017a* (AD), Oaky Ck watershed, Qld; Ni 30000. *S. tryonii* (*S. tryonii* 5; Table 3, Figs 2–4) – *D.O. Burge 1018a* (AD), Raspberry Ck Rd, Qld; Ni 14100. *S. tryonii* (*S. tryonii* 6;

Table 3, Figs 2–4) – *D.O. Burge 1019b* (AD), Marlborough Ck watershed, Qld; Ni 20000. *S. tryonii* (*S. tryonii* 7; Table 3, Figs 2–4) – *D.O. Burge 1020a* (AD), Pine Mountain Ck watershed, Qld; Ni 15800. *S. tryonii* – *G. Porter, s.n.* (BRI), South Percy I., Qld; Ni 21000. *S. tryonii* – *G.N. Batianoff 11394* (AD), S Percy I., Qld; Ni 35000. *S. tryonii* (*S. tryonii* 8; Table 3, Figs 2–4) – *G.N. Batianoff 11405* (BRI), near Chase Point, S Percy I., Qld; Ni 20000. *S. tryonii* – *G.N. Batianoff 91086* (BRI), Mount Slopeaway, Qld; Ni 16400. *S. tryonii* (*S. tryonii* 9; Table 3, Figs 2–4) – *G.N. Batianoff 98121* (BRI), Cawarral, Qld; Ni 10500. *S. tryonii* (*S. tryonii* 10; Table 3, Figs 2–4) – *J. Elsol, s.n.* (BRI), Kunwarara Magnesite Mine, Qld; Ni 8200. *S. tryonii* – *R.D. Reeves 645* (BRI), Princhester overpass, Qld; Ni 28000. *S. umbellata* C.Gardner & A.S.George – *R.J. Chinnock 6901* (AD), ridge W of No. 2 Oil Well, WA; Ni < 1. *S. viminea* Sm. – *A.R. Bean 13819* (BRI), Cooloola NP, Qld; Ni < 20. *S. viminea* (*S. viminea* 1; Table 3, Figs 2–4) – *G.S. Hope, s.n.* (CANB), Sundown Point, 10 km S of the Arthur River, Tas.; Ni < 20. *S. viminea* (*S. viminea* 2; Table 3, Figs 2–4) – *J. Brushe 1332* (BRI), Shoalwater Bay, Pine Mountain sector, Qld; Ni < 10. *S. viminea* – *M. Evans 2534* (CANB), Bungonia Lookdown, NSW; Ni < 9. *S. viminea* – *P.I. Forster 9252* (BRI), Biggenden–Maryborough road, Qld; Ni < 30. *S. viminea* – *R.L. Specht 810* (BRI), Upper Kandanga Ck, Qld; Ni 171.5 *S. viminea* – *W.D. Jackson 113* (HO), Corinna, Tas.; Ni < 20. *S. viminea* – *W.R. Barker 1584* (AD), Bogong Township, Vic.; Ni < 3. *S. virgata* Pamp. – *R.M. Barker 398* (AD), road to Middle Arm Jetty, NT; Ni < 4. *S. virgata* – *W.R. Barker 6874* (AD), Kakadu NP, NT; Ni < 7. †***Tripterococcus*** *brachylobus* W.R.Barker – *A.S. George 15067* (AD), N of Crystal Springs, WA; Ni < 6. *T. brunonis* Endl. – *L. Haegi 977* (AD), Lake Johnston, WA; Ni < 3. *T. brunonis* (*T. brunonis* 1; Table 3, Figs 2–4) – *R. Bates 4282* (AD), 20 km N of Hyden, WA; Ni < 3. *T. brunonis* (*T. brunonis* 2; Table 3, Figs 2–4) – *W.R. Barker 2256* (AD), Brand Hwy to Dongara, WA; Ni < 2. †*T. paniculatus* W.R.Barker – *s.d. Hopper 4736* (AD), Ludlow Hithergreen Rd, WA; Ni < 4.