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Mimulus peregrinus (Phrymaceae): A new British allopolyploid species

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Abstract

Polyploidization plays an important role in species formation as chromosome doubling results in strong reproductive isolation between derivative and parental taxa. In this note I describe a new species, *Mimulus peregrinus* (Phrymaceae), which represents the first recorded instance of a new British polyploid species of *Mimulus* (2n = 6x = 92) that has arisen since the introduction of this genus into the United Kingdom in the 1800's. *M. peregrinus* presents floral and vegetative characteristics intermediate between *M. guttatus* and *M. luteus*, but can be distinguished from all naturalized British *Mimulus* species and hybrids based on a combination of reproductive and vegetative traits. *M. peregrinus* displays high pollen and seed fertility as well as traits usually associated with genome doubling such as increased pollen and stomata size. The intermediate characteristics of *M. peregrinus* between *M. guttatus* (2n = 2x = 28) and *M. luteus* (2n = 4x = 60-62), and its close affinity with the highly sterile, triploid (2n = 3x = 44-45) hybrid taxon *M.* × *robertsii* (*M. guttatus* × *M. luteus*), suggests that *M. peregrinus* may constitute an example of recent allopolyploid speciation.

Keywords

Allopolyploidy, *Erythranthe,* hybrid evolution, introduced species, *Mimulus guttatus*, *Mimulus luteus*, rapid evolution, speciation

Introduction

The genus *Mimulus* (Phrymaceae) comprises more than 120 species, the majority (75%) of which occur in western North America, and the remaining having a world-wide distribution including Eastern North America, South America, Australia, the Himalayas, Japan and Madagascar (Grant 1924, Beardsley and Olmstead 2002, Wu et al. 2007).

Species of *Mimulus* have been spread outside their native range due to deliberate and accidental introductions. For example, *M. guttatus*, a native of western North America, is now found in New Zealand and more than 16 European countries (Truscott et al. 2008, van Kleunen and Fischer 2008, Tokarska-Guzik and Dajdok 2010). In some of these areas of introduction, *M. guttatus* has become naturalized and widely distributed, forming a nontrivial component of the local flora (e.g. Wales, Northern England, Scotland, Poland, Germany and New Zealand; Roberts 1964, Stace 2010, Tokarska-Guzik and Dajdok 2010, Vallejo-Marin unpublished). In the United Kingdom (UK), naturalized populations of *Mimulus* are widespread (Preston et al. 2002), and the genus is represented here by three currently extant species (*M. guttatus, M. luteus* and *M. moschatus*), and a complex array of interspecific hybrids, some of which are locally invasive (Stace 2010).

One of the most conspicuous hybridization complexes in the UK involves closely related taxa, of isolated geographic origin: the North American *M. guttatus* DC. (2n = 28, 30, 56, with most North American and British plants 2n = 2x = 28, Mukherjee and Vickery 1962, Vickery 1995, Stace 2010), and the South American taxa *M. luteus* L. (2n = 4x = 60, 61, 62) and *M. cupreus* Dombrain (2n = 4x = 62) (Stace 2010). These taxa belong to *Mimulus* Section *Simiolus* Greene (= *Erythranthe* Section *Simiola* (Green) Nesom & Fraga; Barker et al. 2012). Crosses between *M. guttatus* and *M. luteus/M. cupreus* yield sexually sterile triploids (2n = 3x = 44, 45, 46) that are nevertheless vegetatively vigorous (Roberts 1964, 1968, Stace 2010). In the UK, hybrids between *M. guttatus* and *M. luteus/M. cupreus* have been grown since the 1800's and some of them have become well established throughout the country. For instance, the hybrid between *M. guttatus* and *M. luteus* (= *M. × robertsii;* Silverside 1990) escaped cultivation at least by 1872 (Preston et al. 2002), and currently forms numerous naturalized populations with a scattered distribution in the British Isles (Preston et al. 2002, Stace 2010, BSBI 2011).

Despite being widely distributed and having persisted in the UK for 140 years, the evolutionary fate of *M. guttatus* × *M. luteus/M. cupreus* triploid hybrids has been thwarted by their high pollen- and seed-sterility (Mukherjee and Vickery 1962, Roberts 1964, 1968, McArthur 1974, Parker 1975). Sterility is common in hybrids produced by the merging of genetically differentiated genomes (Mallet 2007), including cases when parents have different chromosome numbers (Stebbins 1950, Stebbins 1958). When hybridization gives rise to viable triploids, these tend to generate high proportions of unbalanced, aneuploid, and usually non-functional gametes (Ramsey and Schemske 1998). However, sterile plant hybrids often recover fertility after genome duplication (Stebbins 1958). Polyploidization in interspecific hybrids — allopolyploidization — has been linked to the restoration of sexual fertility in some natural triploid hybrids (e.g., *Senecio*, Abbott and Lowe 2004).

Polyploidization plays a particularly important role in species formation, as chromosome doubling results in immediate and strong reproductive isolation between the derivative and parental species (Rieseberg and Willis 2007, Köhler et al. 2010). It is therefore not surprising that polyploidization is often thought to be fundamental to angiosperm diversification (Stebbins 1950, Grant 1971, Ramsey and Schemske 2002, Soltis 2005, Wood et al. 2009). In *Mimulus,* speciation by hybridization and polyploidization may have played an important role during the diversification of this group (Vickery 1995, Beardsley et al. 2004). For instance, allopolyploidization between diploid *M. guttatus* and *M. nasutus* has given rise to a widespread North American tetraploid taxon that is strongly reproductively isolated from its progenitors (Sweigart et al. 2008). Despite the importance of hybridization and polyploidization for plants in general, the opportunity to study early events in speciation via this route is limited by the small number of angiosperm species known to have originated via allopolyploidization in the last 150 years (e.g. *Spartina anglica* (Ayres and Strong 2001), *Tragopogon mirus, T. miscellus* (Soltis et al. 2004, 2012), *Senecio cambrensis* and *S. eboracensis* (Abbott and Lowe 2004)). The discovery of a recently formed polyploid hybrid species in the wild therefore would provide a window of opportunity to study the evolution and speciation of polyploid taxa.

In this note, I describe a new, fertile, polyploid (2n = 6x = 92) species of *Mimulus* (Phrymaceae), *M. peregrinus*, which has currently been found in a single locality in the Lowther Hills, Scotland. A comparison of vegetative and reproductive morphology, DNA content, and chromosome number of this new polyploid species against other British *Mimulus*, strongly suggests a hybrid origin for *M. peregrinus* and a close affinity with the sterile triploid hybrid *M.* × *robertsii.* I speculate that *M. peregrinus* may represent the hexaploid derivative of a hybrid between *M. guttatus* and *M. luteus*, although a careful examination of additional populations of both parental and hybrid taxa is required to elucidate the genetic origin, extent and distribution of this new polyploid species. If an allopolyploid origin is demonstrated, *M. peregrinus* has the potential to serve as a study system to understand the evolutionary processes associated with the origin of species through hybridization and polyploidization following the breakdown of geographic barriers caused by human-assisted dispersal.

Methods

Field surveys in August 2011 uncovered the existence of fertile individuals in a large population of *M.* × *robertsii* in South Lanarkshire, Scotland. To further investigate these unusual plants, open-pollinated seeds were collected on 27 August 2011 from multiple seed-bearing fruits in a single patch at Shortcleuch Waters, near Leadhills, South Lanarkshire, Scotland (NS 9029 1578; 55.4237°N, 3.7349°W). Field-collected seeds—accession number 11-LED-seed—were germinated and grown in a controlled environment cabinet (Microclima 1750E; Snijders Scientific, Tilburg, the Netherlands) at the University of Stirling under 16 light-hours at 24°C and 8 dark-hours at 16°C, and 70% constant humidity. Individual plants were grown in 0.37 l round pots, filled with general purpose peat-sand compost (Sinclair, Lincoln, Lincolnshire, UK), and kept on plastic trays with abundant water. Plants were sporadically treated with SB Plant Invigorator (Fargro Ltd, Littlehampton, West Sussex, UK) to control for fungal infections. Seven plants were brought to flowering (F_1 generation; 11-LED-seed-1 to 11-LED-seed-7), and each individual plant was used to generate F_2 offspring via manual self-fertilization of emasculated flowers kept inside the pollinator-free growth cabinet. A representative individual of this F_2 generation (11-LED-seed-2-14) was chosen as the holotype for the type description presented here (deposited at the Herbarium of the Royal Botanic Garden Edinburgh; E).

Pollen measurements were conducted using fresh pollen fixed in 1 ml of 70% ethanol and dyed with 50 µl of lactophenol-aniline blue (Kearns and Inouye 1993). Darkly stained grains were considered viable (Sweigart et al. 2006). Pollen diameter was measured at the widest point in expanded pollen grains using image analysis software (analySIS, Olympus Soft Imaging Solutions, Münster, Germany) at 200× magnification in an Olympus BX50 light microscope.

Stomata size was measured in casts obtained from the adaxiall side of healthy leaves. A negative cast was first obtained with polysiloxane precision impression material (Xantoprene VL Plus, Heraeus Kulzer Gmbh, Hanau, Germany), and a positive cast was then generated with quick-drying nail polish. Measurements of stomata length and width were done using a light microscope at 400×.

Chromosome counts were conducted by John Bailey (University of Leicester) in mitotic cells from root tips of two F_2 individuals (11-LED-seed-3-21 and 11-LED-seed-5-8). Genome size was measured using DAPI-stained nuclei analysed in a CyFlow ML flowcytometer (Partec GmbH, Münster, Germany) in a commercial facility (Plant Cytometry Services, Schijndel, The Netherlands) in six F_1 individuals (11-LED-seed-1 to 11-LED-seed-4, 11-LED-seed-6, 11-LED-seed-7). *Vinca major* was used as internal standard (2n = 92, 2C = 3.80 pg; Obermayer and Greihulber 2006). Because DAPI preferentially binds to AT-rich regions, the flow cytometry results presented here must not be treated as absolute measurements of DNA content.

Data resources

The data underpinning the analysis reported in this paper are deposited at GBIF, the Global Biodiversity Information Facility, http://ipt.pensoft.net/ipt/resource.do?r=mimulus_peregrinus

Taxonomic treatment

***Mimulus peregrinus* Vallejo-Marín, sp. nov.**
urn:lsid:ipni.org:names:77120497-1
http://species-id.net/wiki/Mimulus_peregrinus
Figure 1

Mimulus Section *Simiolus* Green (= *Erythranthe* Section *Simiola* (Green) Nesom & Fraga)

Type. United Kingdom. Scotland: Grown from seed collected in South Lanarkshire near Leadhills, on the banks of Shortcleuch Water. Vice county 77, Ordinance Survey

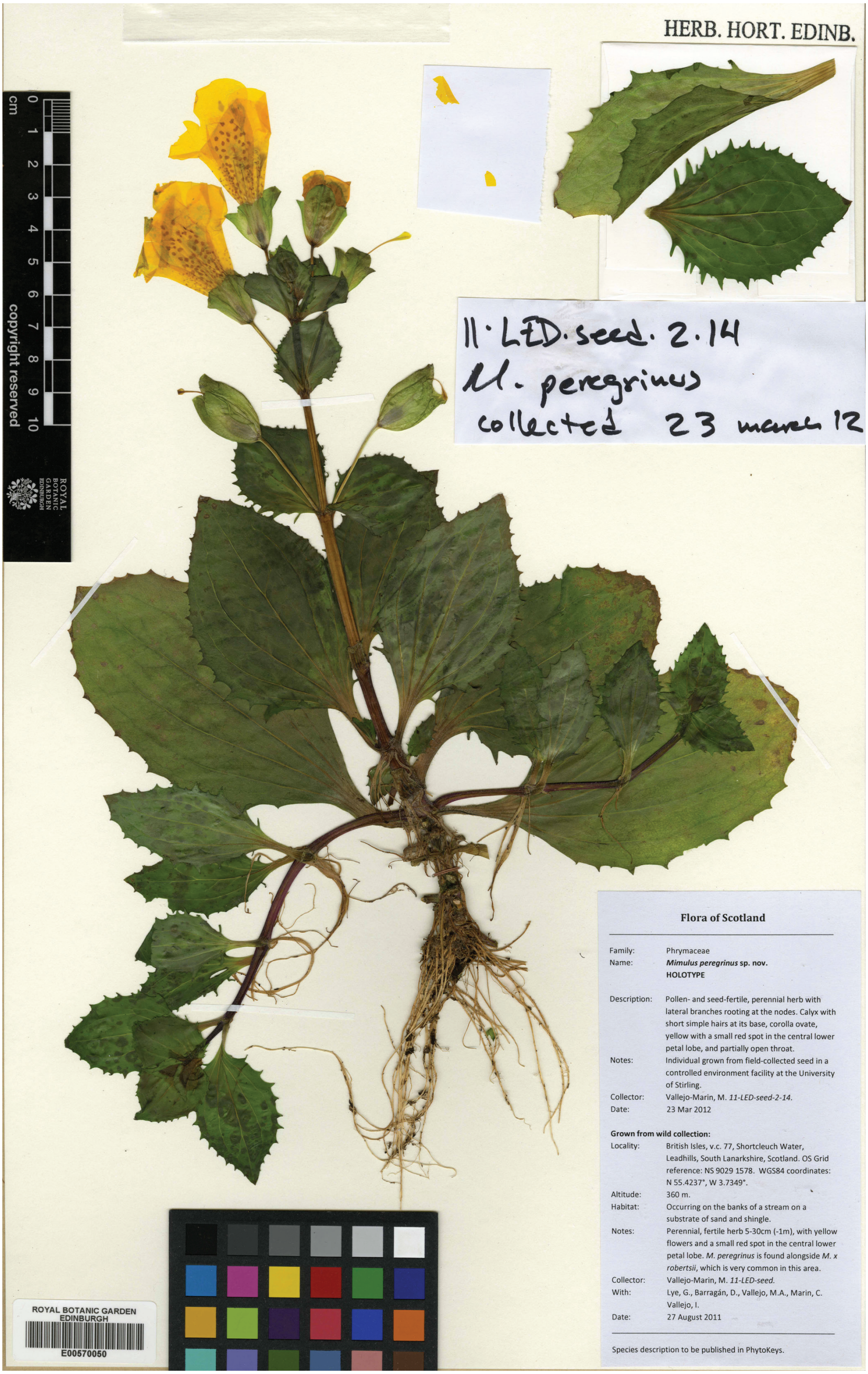

Figure 1. Holotype of *M. peregrinus* Vallejo-Marin [11-LED-seed-2-14; barcode E00570050].

grid reference: NS 9029 1578. WGS84 coordinates: 55.4237°N, 3.7349°W; altitude: 360 m. 27 Aug 2011. M.Vallejo-Marín 11-LED-seed; vouchered as M.Vallejo-Marín 11-LED-seed-2-14 (holotype: E; isotypes: BM, K).

Species nova *Mimulus* × *robertsii* Silverside similis. Herba perennis, pollen et semen fertile, corollae, flavae, lobo centrali cum macula parva rubro. Folia ovata ad oblonga, dentata, regulariter ad irregulariter triangulo-dentata. Calyx interne cum capillis simplicibus instructis.

Description. Perennial herb 5-30 cm (–1 m) high, freely rooting at the nodes. Stem erect or prostrate, glabrous below and glandular pubescent above. Leaves variable, mostly ovate-oblong 3–14 × 1.5–4 cm, with regular to irregularly dentate margins; basal leaves oval to spatulate, with petioles up to three-quarters as long as the blades; upper leaves ovate with much shorter petioles or sessile. Inflorescence racemose, many-flowered; pedicels 2.5–5 cm long, normally equalling or slightly longer than the corolla, but shorter in later flowers. Calyx 1.5–2.5 cm long, with 5 triangular teeth, the upper tooth distinctly longer; pubescent outside covered with glandular hairs throughout, and with short, simple hairs in the base of the calyx extending along the ridges; calyx becoming inflated in fruit, with the lower two calyx-teeth curving upwards and enclosing the fruit. Corolla ovate in frontal view, 4–5 cm wide, 3–5 cm tall, and 4–5 cm long (deep); the lobes almost truncate, particularly the two lateral ones; yellow, with a single faint-red, vertically-elongated 2 × 5 mm spot located approximately half-way on the central lower lobe; throat hairy, spotted with red, more or less open; lobes subequal, the central lower lobe slightly longer (Fig. 2). Style glabrous, ending in a bi-lobed, thigmotropic stigma. Fruit a broadly oblong capsule; seeds striate, very small (<0.02 mg; ~0.1 mm^2). Anthers yielding abundant quantities of viable pollen (percent of viable pollen: 86.39 ± 4.01%, range: 73.24 – 96.40%, N = 6 individuals); pollen diameter from 53.43 ± 1.22 µm (mean ± SE; N = 5 individuals, 100 pollen grains per individual; Hoyer's medium) to 48.78 ± 0.97µm (mean ± SE; N = 6 individuals, 100 pollen grains per individual; 70% ethanol) depending on mounting medium. Sets abundant seed following artificial self-pollination. Germination rates of self-fertilized seed 80% ± 4.2% (N = 6 families, 50 seeds per family). Stomata length 35.44 µm ± 0.99 (N = 7 individuals, 20 stomata per individual). Chromosome number 2n = 92 (J. Bailey).

Distribution. Currently known only from the banks of Shortcleuch Waters, Leadhills, South Lanarkshire, Scotland, UK (v.c. 77).

Ecology. Occurring on the banks of a stream on a substrate of sand and shingle. *M. peregrinus* is found alongside *M.* × *robertsii*, which is locally common. Flowering of *Mimulus* in this region starts in early June. Seeds of *M. peregrinus* were collected in August.

Etymology. The name is taken from the Latin *peregrinus* – foreigner, traveller.

Preliminary conservation status. Currently known only from a single collection outside of a protected area, *M. peregrinus* is provisionally assessed as Critically Endangered (CR D; population size estimated to number less than 50 mature individuals) (IUCN 2011).

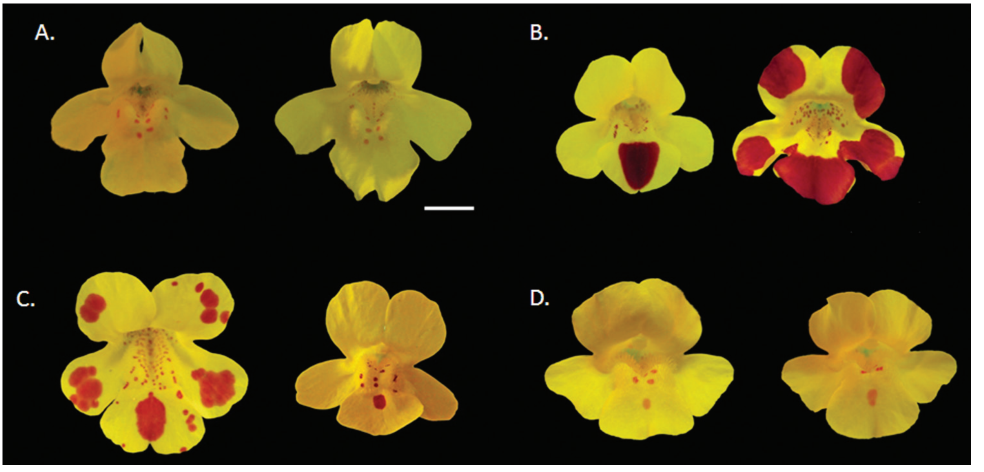

Figure 2. Flowers of *M. peregrinus* and closely related taxa. **A** *M. guttatus* **B** *M.* × *smithii* (*M. luteus luteus* × *M. luteus variegatus*) **C** *M.* × *robertsii* (*M. guttatus* × *M. luteus*), and **D** *M. peregrinus*. Each taxon is represented by flowers from two individuals from a single locality to illustrate within-population variability: *M. guttatus* = Dunblane, Perthshire; *M.* × *smithii* = Coldstream, Scottish Borders; *M.* × *robertsii* = Nenthall, Cumbria; *M. peregrinus* = Leadhills, South Lanarkshire. Scale bar = 1cm.

Specimens examined. United Kingdom. Scotland: Grown from seed collected at South Lanarkshire near Leadhills, on the banks of Shortcleuch Water. 55.4237°N, 3.7349°W; altitude: 360m. 27 Aug 2011. M.Vallejo-Marín, seed voucher: 11-LED-seed. All *M. peregrinus* specimens examined here were derived from open-pollinated seed collected at the type locality and grown in a controlled environment. Some of these first generation seed-grown individuals (11-LED-seed-1 to 11-LED-seed-7) were then used produce a second generation via self-fertilization (e.g. 11-LED-seed-2-14).

Discussion

Mimulus peregrinus can be distinguished from closely related *Mimulus* species and their hybrids in the UK based on a number of morphological and functional characters (Table 1, Fig. 2). Its chromosome number, DNA content, larger stomata and pollen grain size, clearly indicate that *M. peregrinus* is a polyploid species. Although the parentage of this new polyploid has not been firmly established yet, its close affinity with *M.* × *robertsii* suggest that *M. peregrinus* has been derived from hybridization between *M. guttatus* and *M. luteus* and thus it might have arisen through a recent (<140 years) allopolyploidization event. Below I contrast *M. peregrinus* with related *Mimulus* taxa in the UK, and end with a brief discussion on its putative origin.

Table 1. List of main diagnostic characters differentiating *Mimulus peregrinus* from other closely related taxa of *Mimulus* found in the UK. In the cases of the very variable species *M. guttatus* and *M. luteus*, diagnostic characters are taken from those of British populations. For example, although *M. luteus* is polymorphic for corolla-lobe red markings in Chile, the un-marked variety is not naturalized here (Stace 2010). Data presented as mean ± SE (number of individuals analyzed). Data from Stace (2010), Grant (1924) and MVM unpublished results.

Character	*M. peregrinus*	*M. guttatus*	*M. luteus*	*M.* × *smithii*	*M.* × *robertsii*
Corolla lobes with reddish spots or blotches	Yes (one small spot in lower, central lobe)	No	Yes (a single blotch in central lower petal)	Yes (present in 1-5 lobes)	Yes (variable)
Throat of corolla	± open	± closed	± open	± open	± open
Small, simple (non-glandular) hairs on inflorescence and calyx keels	Yes	Yes	No	No	Yes
Seed fertile	Yes	Yes	Yes	Yes	No
Seed size (area in mm^2)	0.167 ± 0.012 (6)	0.126 ± 0.008 (12)	0.103 (1)	0.112 ± 0.006 (8)	---
Seed germination	0.80 ± 0.04 (6)	0.85 ± 0.02 (11)	NA	0.47 ± 0.06 (8)	--
Pollen fertile (proportion viable)	Yes 0.864 ± 0.040 (6)	Yes 0.865 ± 0.053 (6)	Yes (NA)	Yes 0.963 ± 0.006 (2)	No 0.001 ± 0.001 (9)
Mean pollen diameter (μm) [1]	48.77 ± 0.97 (6)	36.72 ± 0.38 (24)	44.08 ± 3.11[2] (2)	45.09 ± 0.39 (25)	37.02 ± 1.70[3] (9)
Stomata size (length, μm)[4]	35.44 ± 0.99 (7)	28.25 ± 0.42 (1)	NA	29.67 ± 0.55 (1)	26.83 ± 0.77 (1)
Chromosomes (ploidy)	2n = 92 (6x)	2n = 28 (2x)	2n = 59,60, 61, 62 (4x)	2n = 60, 61, 62 (4x)	2n = 44, 45 (3x); 2n = 54

[1] = Measured in pollen preserved in 70% ethanol and dyed with lactophenol-aniline blue. [2] = Measured in pollen preserved in Hoyer's medium and dyed with lactophenol-aniline blue. [3] = Inviable (empty) pollen grains are variable in size as they may be fully expanded or partly collapsed. [4] = Measured in 20 stomata per individual.

Comparison with related *Mimulus* in Britain

1. *Mimulus guttatus* DC. (Section *Simiolus* Green) (yellow monkeyflower). *M. peregrinus* has a more open corolla throat, in contrast to the nearly closed corolla throat of *M. guttatus.* The 2–5 mm red spot in the central lower lip of *M. peregrinus*, is absent in most British populations of *M. guttatus*. The margins of the lower leaves of *M. peregrinus* are more triangular and regular than those of most *M. guttatus*, although leaf traits are highly variable in the genus. Field and herbarium specimens could potentially be distinguished by the much larger size of the pollen grains in *M.*

peregrinus. Chromosome number and genome content as measured in flow cytometry are also diagnostic characters to distinguish these two species (Table 1, Fig. 3).

2. *Mimulus luteus* L. (Section *Simiolus* Green) (blood-drop emlets). *M. luteus*, is a group of polymorphic perennial herbs comprising several interfertile varieties that are distinguished based on the presence, size and colour of markings on the corolla lobes. Taxa in this group include *M. luteus* var. *rivularis* Lindl. 1826, with a single large red spot on the middle lower lip; *M. luteus* var. *variegatus* (Lodd.) Hook 1834, with pale yellow corollas tinted with pink at the lobe margins; and *M. luteus* var. *youngana* Hook 1834 (= *M. smithii* Lindl 1835, not Paxton), with deep yellow corollas and lobes with large red spots at the margins (Grant 1924). In the UK, many extant populations of *M. luteus* likely represent crosses between taxa in this interfertile group (e.g. *M. luteus* var. *rivularis* × *M. luteus* var. *variegatus*) (MVM pers. obs.), and present highly variable patterns of spots and blotches in the corolla lobes. *M. peregrinus* can be distinguished from most species and hybrids in the *M. luteus* aggregate by its more robust habit, elliptical leaves with dentate and slightly irregular margins, and the presence of only a small, faint, elongated central spot in the lower lip. Most importantly, *M. peregrinus* possesses simple hairs in the calyx, which are always absent from all varieties of *M. luteus*. Other diagnostic characters of *M. peregrinus* are pollen grain size, stomata size, DNA content as measured in flow cytometry and chromosome number (Table 1, Fig. 3).

3. *Mimulus cupreus* Dombrain (Section *Simiolus* Green) (copper monkeyflower). *M. cupreus* with orange to yellow corollas, and which is closely related to *M. luteus*, has been reported in the UK but most likely in error for the hybrid between *M. guttatus* and *M. cupreus* (*M.* × *burnetii* S. Arn.) (Stace 2010). In contrast with *M. peregrinus,* the copper monkeyflower usually has orange corollas, more open corolla throat, lacks simple hairs in the calyx, and has a smaller chromosome complement (2n = 62).

(3) *M. moschatus* Douglas ex Lindl. (Section *Paradanthus* Grant) (musk). *M. moschatus* is easily distinguished from other British *Mimulus* including *M. peregrinus* by its smaller yellow corollas (1–2.5 cm), glandular-hairy pubescence throughout the plant, and chromosome number (2n = 4x = 32 × = 8, 9, 10, Vickery 1995). *M. moschatus* does not hybridize with other British *Mimulus.*

(4) *M.* × *robertsii* Silverside (*M. guttatus* × *M. luteus*). A highly pollen- and seed-sterile, perennial herb rooting at the nodes, its yellow flowers are marked with orange to red to brown spots of various sizes in the petal lobes (Roberts 1964, Silverside 1990, Silverside 1998,Stace 2010). The corolla is 2.5-4.5cm in length and the throat is partially open (Stace 2010). This is a taxon of variable pubescence, but is usually hairy in the upper parts of the plant (Stace 2010) including the inflorescences which present simple hairs in the base of the calyx (MVM pers. obs.). Of garden origin *M.* × *robertsii* can occasionally arise in the wild; this hybrid is produced by crosses of *M. guttatus* with *M. luteus* var. *rivularis, M. luteus* var. *variegatus* or *M.* × *smithii* Paxton (the latter a hybrid between *M. luteus* var. *rivularis* and *M. luteus* var. *variegatus*, which is phenotypically very similar to *M. luteus* var. *youngana*) (Stace 2010). In the UK it can be found up to 610 m (Ochil Hills, Scotland), and is suggested to be the commonest taxon of high ground (Preston et al. 2002, Stace 2010).

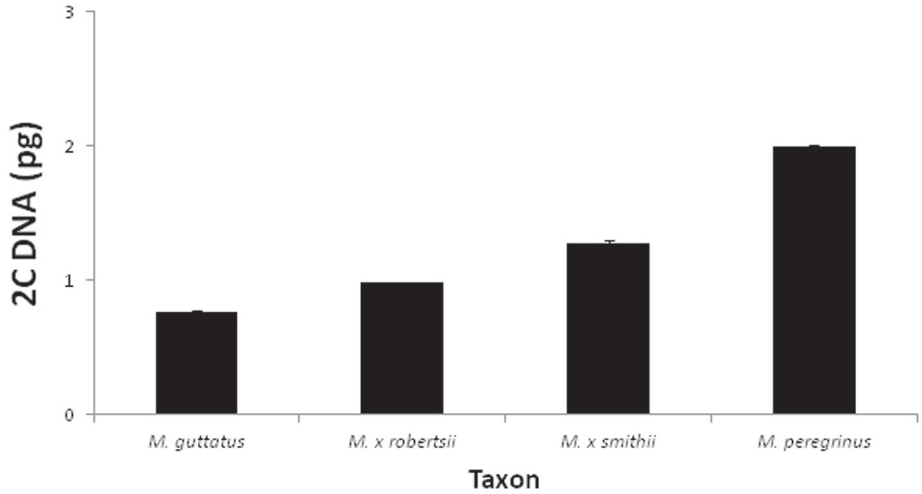

Figure 3. Flow-cytometry estimates of 2C DNA content (DAPI-stained) of British *Mimulus*. Error bars represent standard errors when multiple individuals per taxon were tested. Sample sizes as follows (chromosome numbers for each population are given in parenthesis when available). *M. guttatus*: N = 4 individuals from Dunblane, Perthshire (2n = 28); and 2 individuals from Muckle Roe, Shetland; *M.* × *robertsii* (= *M. guttatus* × *M. luteus*): N = 1 individual from Nenthall, Cumbria (2n = 44, 45); *M.* × *smithii* (= *M. luteus* var. *luteus* × *M. luteus* var. *variegatus*): N = 2 individuals from Coldstream, Scottish Borders (2n = 59, 60, 61, 62); *M. peregrinus:* N = 6 individuals from Leadhills, South Lanarkshire (2n = 92). All chromosome counts kindly provided by J. Bailey.

M. peregrinus resembles *M.* × *robertsii* rather closely in habit, size and general vegetative and floral morphology, suggesting a close affinity between these two taxa (Table 1). *M.* × *robertsii* and *M. peregrinus* can be distinguished by their differences in chromosome number, pollen and seed fertility, pollen grain size, and stomata size (Table 1). *M. peregrinus* presents consistently high levels of pollen fertility (0.86 ± 0.04) and is capable of producing abundant seed set following artificial pollination. In contrast, both natural and artificial specimens of *M.* × *robertsii* present very low levels of pollen viability (proportion of viable pollen = 0.05 ± 0.01, for both naturalized (N = 7) and synthetic hybrids (N = 15)), and do not set seed following artificial pollination (Roberts 1964) (see also Table 1). In addition, the two taxa differ markedly in chromosome number: *M.* × *robertsii* is a triploid (e.g. 2n = 45), while *M. peregrinus* has twice as many chromosomes (2n = 92), and this difference in genome size is clearly seen in flow cytometry analysis of DAPI-stained nuclei (Fig. 3). Finally, associated with the different genome size of the two taxa, *M. peregrinus* has larger pollen grains, larger seeds, and larger stomata than *M. robertsii* (Table 1).

(5) Other hybrids. *M.* × *burnetii* S. Arn. (*M. guttatus* × *M. cupreus*) is a sterile triploid (2n = 45) with copper-coloured corolla, and often presenting a petaloid calyx (Stace 2010). *M.* × *polymaculus* Silverside nom. nud. (*M. guttatus* × (*M. luteus* × *M. cupreus*)) is also a sterile triploid that differs from *M.* × *burnetii* in having dark blotches

in the corolla lobes. Both can be easily distinguished from *M. peregrinus* based on corolla colour, calyx morphology, fertility, and chromosome number. ***M.* × *maculosus* W. Bull ex T. Moore** (*M. cupreus* × *M. luteus*) and ***M.* × *hybridus* Siebert & Voss** (*M. cupreus* × *M.* × *smithii*) are fertile hybrids with variably-coloured corollas, often copper-coloured or with blotches on the petal lobes. They can both be easily distinguished from *M. peregrinus* by their corolla colours, lack of abundant simple hairs in the keels of the calyx, and evenly triangular, flat teeth in the leaf margins. Chromosome numbers for these latter two hybrids are not yet available, but it is to be expected that they are similar to their parental species (2n = 60-62).

Putative origin and distribution of *M. peregrinus*

The intermediate floral and vegetative characteristics of *M. peregrinus* between *M. guttatus* and *M. luteus*, as well as its close morphological similarity to *M.* × *robertsii* clearly suggest a hybrid origin for this new taxon associated with a polyploidization event. The alternative, that *M. peregrinus* is an autopolyploid derivative of a pure *M. guttatus* or *M. luteus* seems highly unlikely based on vegetative and floral characteristics of the different taxa (Table 1). Moreover, both chromosome counts and genome size data are inconsistent with the expectations of an early generation autopolyploid of either *M. guttatus* or *M. luteus* or a backcross between *M.* × *robertsii* and either parent (Fig. 3). The fact that *M. peregrinus* presents approximately twice the number of chromosomes and has double the amount of DAPI-staining DNA than a common cytotype of *M.* × *robertsii* (Fig. 3), immediately suggests that the most parsimonious explanation for the origin of *M. peregrinus* is through hybridization between *M. guttatus* and *M. luteus* linked to a polyploidization event. Given that *M. peregrinus* was indentified amongst a large population of *M.* × *robertsii,* a possible origin of this new taxon is via genome doubling of the triploid hybrid.

The known distribution of *M. peregrinus* is currently restricted to a single locality in Scotland. A preliminary examination of herbarium specimens at the Royal Botanic Gardens in Edinburgh did not uncover any hybrid specimens that were obviously fertile. However, the widespread distribution of *M.* × *robertsii* in the UK suggests, along with anecdotal records of fertility in hybrids (Silverside 1998), may suggest that *M. peregrinus* could be significantly under recorded, and future studies are required to determine its actual distribution.

It is well known that polyploidization can act as a mechanism restoring fertility even in highly sterile triploid hybrids (Dobzhansky 1937, Stebbins 1950, Grant 1971, Ramsey and Schemske 1998, Briggs and Walter 2000), and polyploidization has resulted in the evolution of other non-native allohexaploid species from highly sterile triploids in the UK (e.g. *Senecio cambrensis*, 2n = 6x; Abbott and Lowe 2004). While firmly establishing the origin and distribution of *M. peregrinus* must await further ecological and genetic work, the discovery of this taxon provides an exciting opportunity to study the recent evolution of a new allopolyploid British species.

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RESEARCH ARTICLE

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Aristolochia vallisicola (Aristolochiaceae), a new species from Peninsular Malaysia

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Abstract
A new species in the genus *Aristolochia* (Aristolochiaceae), *A. vallisicola* T.L.Yao, from Peninsular Malaysia is described and illustrated. Among all Peninsular Malaysian *Aristolochia*, it is the only species with a pinnately veined lamina and a disc-liked perianth limb. A distribution map is provided and its conservation status is assessed as Least Concern.

Keywords
Aristolochiaceae, *Aristolochia*, Peninsular Malaysia

Introduction

Aristolochia, the largest genus in the family, consists of about 400 species. It is widely distributed throughout tropics and subtropics, but also in the warm temperate regions. Hou (1984) recognised 28 species in Malesia, 5 of which occur in Peninsular Malaysia while none of them is endemic.

The new species presented here was first collected by a Forest Guard, Kalong (KEP) in 1929 (FMS 24048) from Ulu Kelau, Pahang. The specimen consists of two detached leaves and a detached inflorescence mounted on one sheet. Its vernacular name, *Akar telinga berok* (the pig-tail macaque's ear climber in Malay) indicates that it is a climber. After a lag of 70 years, Kiew collected a flowering specimen (RK 4879) in the Awana waterfall area, Genting Highlands, Pahang. The specimen is complemented by good field notes and was identified as *Aristolochia* sp.

Recently, I was asked to identify a leaf (Kiew s.n., barcode KEP196081) of a butterfly larva food plant collected in the Genting Tea Estate, Pahang. This instigated me to make a visit to the estate, which revealed that the plant is conspecific with the two specimens mentioned above. According to H.S. Barlow and S.K.L. Hok (*pers. comm.*), larvae of the butterfly species, *Parides* (*Atrophaneura*) *sycorax egertoni* (Distant)[1] a member of the family Papilionidae, commonly known as the White Head Batwing (Malay name: *Kepala Putih*) feed on the leaves of this species. Their observations in the Genting Tea Estate revealed that its larvae defoliate young plants and then girdle the stem base just before they metamorphose into pupae. The plant manages to re-sprout later.

Taxonomy

Aristolochia vallisicola **T.L.Yao, sp. nov.**
urn:lsid:ipni.org:names:77120982-1
http://species-id.net/wiki/Aristolochia_vallisicola
Figures 1–3

Note. This species differs from all other Peninsular Malaysian *Aristolochia* L. species in its lamina with pinnate lateral veins, inflorescence with a long peduncle, its disc-shaped perianth limb, annulated hairy perianth mouth and 3-lobed gynostemium. This species is similar to *Aristolochia coadunata* Backer in the lanceolate or oblanceolate lamina with pinnate lateral veins but differs in its larger disc-shaped perianth limb, 58–65 mm diam. versus 15–30 mm diam. in *A. coadunata* and its longer peduncle, 15.5–17 cm long versus up to 2 cm long in *A. coadunata*. This species is also similar to *Aristolochia versicolor* S.M.Huang in the lanceolate or oblanceolate lamina with pinnate lateral veins but differs in its longer petiole, 2.5–7 cm long versus 1–2 cm long, broader leaves, at least 7.5 cm wide versus to 6.5 cm wide, and longer peduncle, 15.5–17 cm long versus 2–3(–10) cm long in *A. versicolor*. The summary and other characters comparison is presented in Table 1.

Type. Peninsular Malaysia. Pahang: Genting Highlands, Awana Waterfall. 26 November 1999 (fl), R.Kiew 4879 (holotype SING!, barcode 78162).

Description. Slender climber. Stem ca 2.5 mm thick, surface shallowly furrowed, sometimes smooth, puberulent, trichomes hooked. **Leaves:** petiole twisted, 2.5–7 cm long, ca 2.5 mm thick, puberulent, indumentum a mix of hooked and straight hairs; lamina lanceolate or narrowly oblanceolate or oblanceolate, 15–24 × 7.5–14 cm; base cordate, auricles rounded, sinus 2–3 mm deep, 8–12 mm wide, margin entire, apex acute; leathery; lamina surface above glabrescent, with scattered black gland dots, lamina surface below puberulent, indumentum a mix of longer straight and shorter hooked hairs; midrib above sunken, below prominent; lateral veins pinnate, above faint, below prominent, basal pair 1, pinnate pairs 5–7; intercostal veins net-like. **Inflorescences**

[1] *Parides sycorax egertoni* is distributed from Southern Myanmar to Peninsular Malaysia and Sumatra (Corbet and Pendlebury 1992).

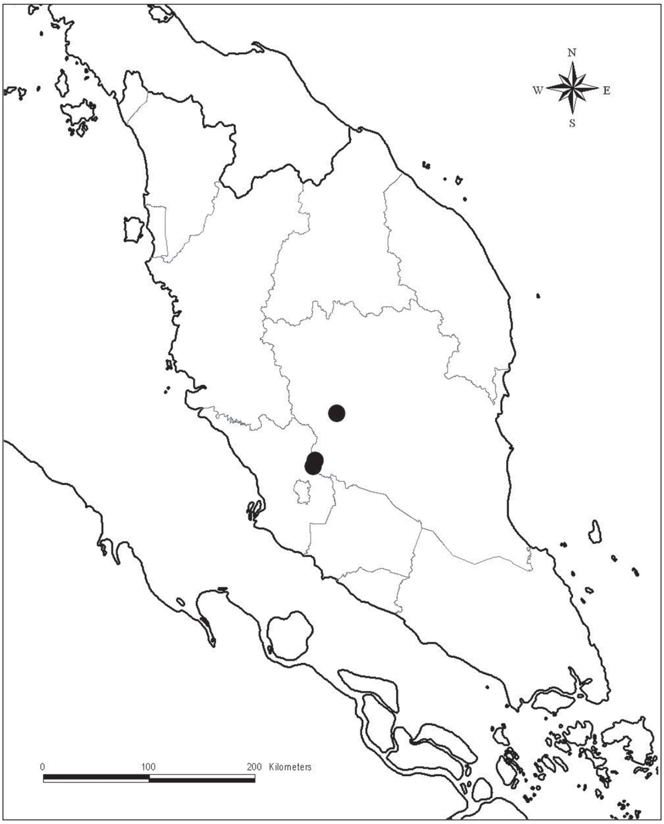

Figure 1. Distribution of *Aristolochia vallisicola* (●).

cauline, solitary; peduncle branched once; 15.5–17 cm long, ca 2 mm thick, puberulent, indumentum mainly of hooked trichomes, scattered with long spreading hairs. Bracts ovate, ca 3 × 1.5 mm, pubescence, base cuneate, apex acute. **Flowers:** pedicel ca 40 mm long, ovary ca 13 × 2 mm, villous; perianth glossy, greyish pale orange with purple tinge, purple beneath, ca 6.5 cm long, outer surface sparsely villose with shorter hooked trichomes, tube geniculately curved, utricle cylindric, ca 30 × 8 mm, inner surface with a glistening white patch of stellate trichomes, perianth tube ca 35 × 8 mm, limb disc-shaped, 5.8–6.5 cm diam., 3-lobed, venation faint, mouth annulate, villous; gynostemium in transverse section faintly trigonal; stamens 6, anthers ca 3 × 0.3 mm; stigmatic lobes 3, conical, ca 0.8 mm long, apex blunt. **Fruit** and **seed** unknown.

Vernacular name. *Akar telinga berok* (Malay).

Distribution. *Aristolochia vallisicola* is endemic in Peninsular Malaysia, Pahang. It has only been found on Titiwangsa Range and its vicinities.

Ecology. This species occurs in highland valleys of lower montane forest about 1000 m altitude and often by rocky streamsides. Specimens with flowers were collected in September and November.

Etymology. The species name *vallisicola* denotes its habitat preference for valleys.

Table 1. Comparison of *Aristolochia vallisicola*, *A. coadunata* and *A. versicolor*.

Characters	***A. vallisicola***	******A. coadunata***	*******A. versicolor*** **(China)**	********A. versicolor*** **(Thailand)**
Petiole length; indumentum	2.5–7 cm long; puberulous	3–9 cm long; pubescent	1–2 cm long; sparsely pilose	1–2 cm long; adpressed hairy
Lamina; length by width (cm)	lanceolate, oblanceolate or broadly oblanceolate; 15–24 × 7.5–14	ovate oblong to lanceolate, rarely ovate; 7.5–33 × 4–12	narrowly elliptic to lanceolate-elliptic; 14–25 × 4–6.5	oblanceolate, oblong-oblanceolate, or elliptic oblong; 11.2–17.5 × 3.4–4.7
Lamina base; sinus depth (mm)	cordate; 2–3	cordate; 5–10	narrowly auriculate; 5–7	narrowly, slightly cordate
Pinnate lateral vein pairs	6–7	4–6	9–10	7–8
Inflorescence	cauline; peduncle 15.5–17 cm long, divided into 4–5 internodes of different lengths	in axils of foliage leaves, rarely cauline; peduncle up to 2 cm long	in axils of foliage leaves, peduncle 2–3 cm long	in axils of foliage leaves, peduncle ca 10 cm long
Bract indumentum	pilose	puberulous	—	pilose
Perianth	tube geniculately curved, utricle cylindric, ca 30 × 8 mm, tube ca 35 × 8 mm; limb disc-shaped, 58–65 mm diam., 3-lobed, mouth annulate	tube geniculately curved, utricle ovoid tubular, 35–30 × 7 mm, tube cylindric, 30–45 × 6 mm, limb disc-shaped, 15–30 mm diam., obscurely 3-lobed, mouth not annulate	tube geniculately curved, basal portion of tube 30–40 × 6–8 mm; limb disc-shaped, 40–60 mm diam., 3-lobed, mouth annulate	tube geniculately curved, utricle ovoid, 8–10 × 8–12 mm, tube ca 13–23 × 5–7 mm; limb disc-shaped, 46–50 mm diam.
Distribution	Peninsular Malaysia	Sumatra, Java, †Peninsular Malaysia (Hou 1984; Igarashi and Fukuda 1999)	China: Guangdong, Guangxi, Yunnan (Huang et al. 2003)	North Eastern Thailand (Phuphathanaphong 1987)

* Images of Backer 26130 (L), Bosscha s.n. (BO-108722) (BO), Schouten s.n. (BO-108723 & BO-108735) (BO), van Steenis 4317, 7326, 12625 (L) seen. Comparison also based on species description and drawings (Backer 1920; Hou 1984, fig. 12; van Steenis 2006, colour plate 4).

** Type specimen could not be located. Comparison based on species description and line drawings (Hwang 1981, fig. 4; Huang et al. 2003, fig. 222, 4–6).

*** Comparison based on images of Beusekom and Phengklai 2985 (L) and its line drawing, and species description (Phuphathanaphong 1987, fig. 13).

† Igarashi and Fukuda (1997) recognised *Aristolochia coadunata* as occurring in Peninsular Malaysia and mentioned that it is one of the food plants of *Parides* (*Atrophaneura*) *sycorax*. I have not seen any *Aristolochia coadunata* specimens from Peninsular Malaysia.

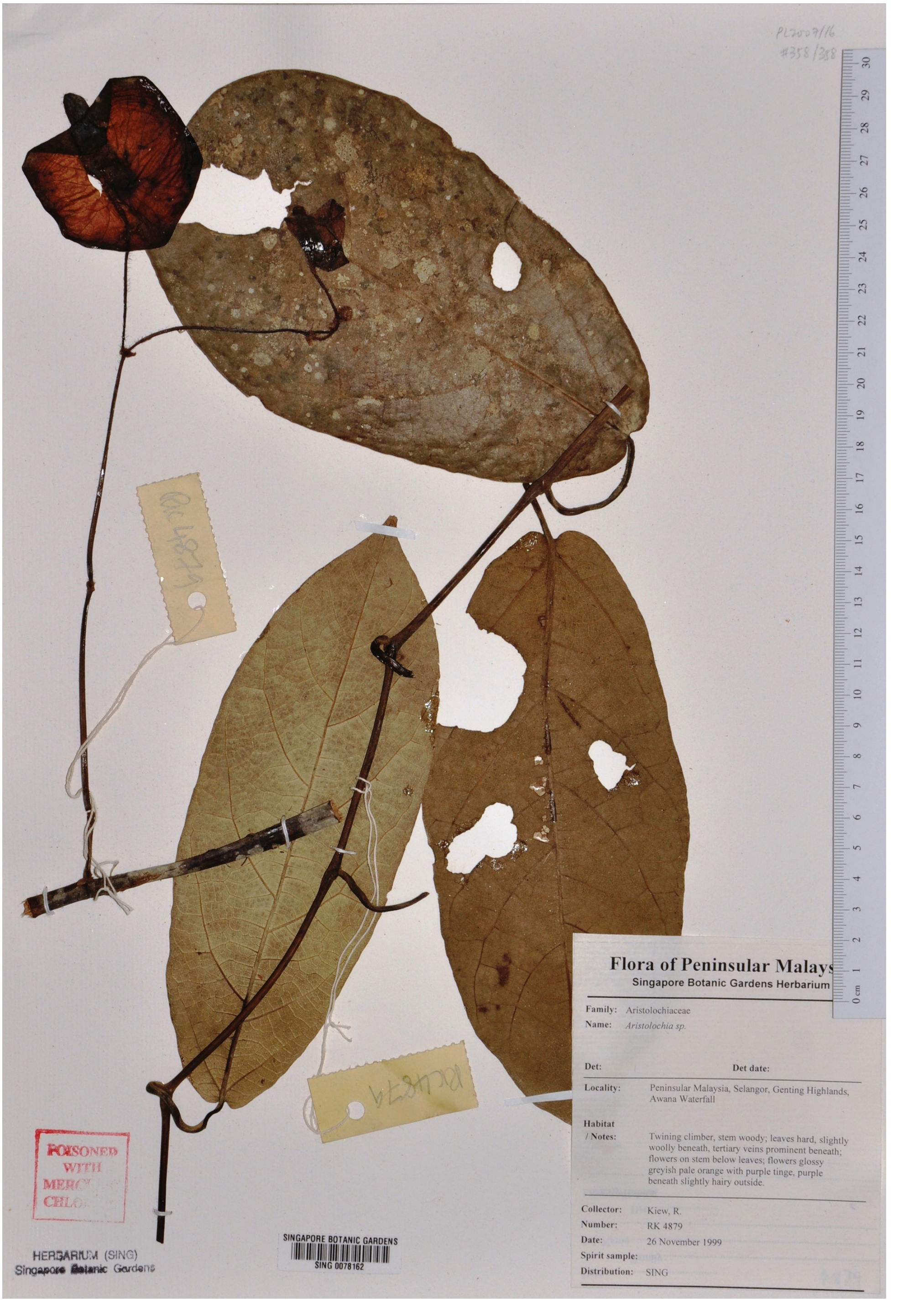

Figure 2. Type specimen of *Aristolochia vallisicola* (Kiew RK 4879, SING, barcode 78162).

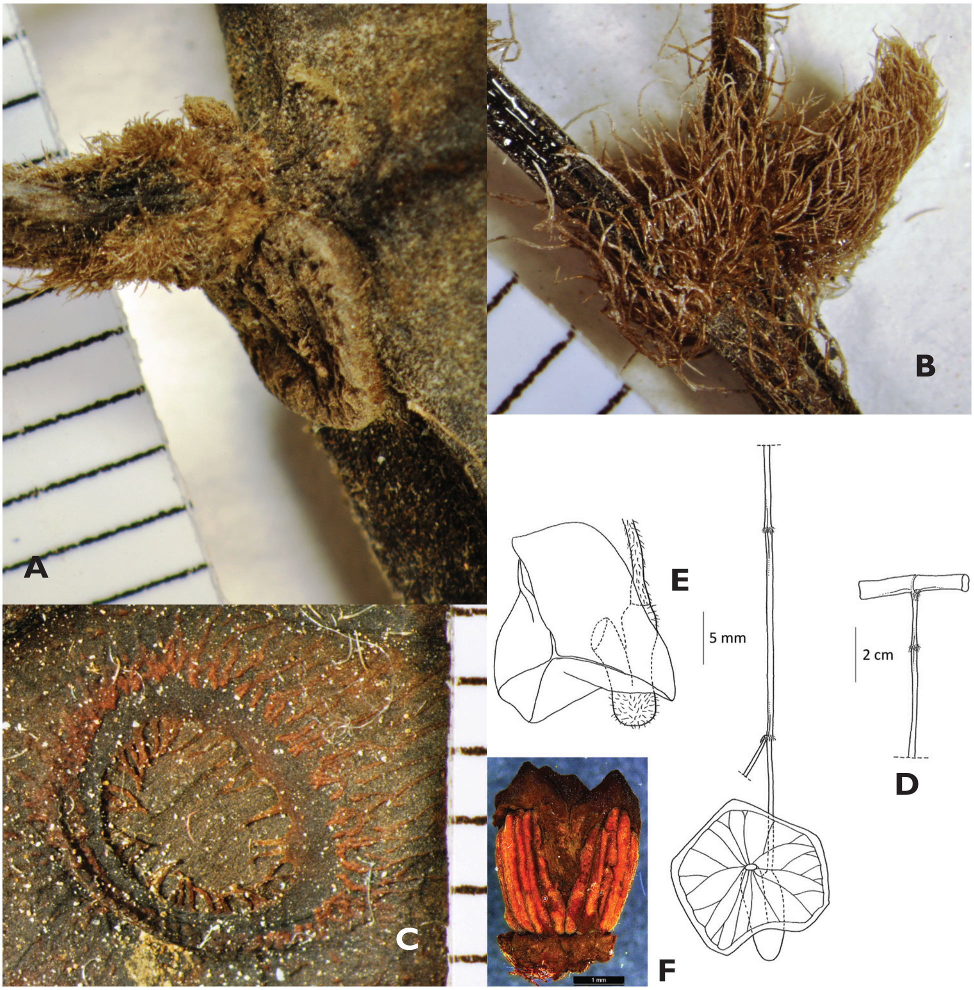

Figure 3. *Aristolochia vallisicola* T.L. Yao, **A** insertion of an inflorescence in axil of petiole scar at thickened stem node **B** villous inflorescence bract **C** annulated perianth mouth **D** an inflorescence with an opened flower **E** flower bud **F** gynostemium. (All from Kiew RK 4879.)

Conservation status. Least Concern. This species occurs above 1000 m altitude, a habitat which is protected by Malaysian legislation.

Specimens examined. Peninsular Malaysia, Pahang: Ulu Kelau, Raub, 24 September 1929 (fl), Kalong FMS 20248 (KEP, barcode 196080); Genting Tea Estate, R. Kiew s.n. (KEP, barcode 196081).

Discussion and conclusion

Aristolochia vallisicola with disk-shaped perianth of 3 lobes which valvate in bud, annulated perianth mouth and gynostemium with 3 segments each consisting 2 stamens belongs to *Isotrema* (Huber 1993). *Isotrema* consists of ca 50 species distributed in temperate and tropical East Asia and in North and Central America. The new species presented here is its first record in Peninsular Malaysia. The position of *Isotrema* clade within *Aristolochia s.l.* is confirmed by phylogenetic studies (Kelly and González 2003; Ohi-Toma et al. 2006).

Old World *Aristolochia* species with a disc-shaped perianth limb are common in northern India (Hooker 1886; Karthikeyan et al. 2010) and southern China (Huang et al. 2003) while 1-lipped or 3-lobed perianth limb are prevalent in Malesian *Aristolochia* species (Hou 1984). *Aristolochia vallisicola* is the only species with a disc-liked perianth limb in Peninsular Malaysia. Apparently, it is a link between the Asian Continental element and Sumatran-Javanese *Aristolochia coadunata*.

Species of *Aristolochia*, a genus of high climber or woody lianas in Malesian forests, are not easy sighted and are very often represented by meagre herbarium specimens. Furthermore, the plants are rarely found in flower. In the past 15 years, 8 new species of *Aristolochia* were described from Thailand (González and Poncy 1999; Hansen and Phuphatanaphong 1999; Phuphathanaphong 2006). This indicates that the species diversity of *Aristolochia* in the Old World, especially in South East Asia is still underestimated. I predict more novelties will be discovered when more specimens from this region are available for taxonomic study.

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RESEARCH ARTICLE

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Cuatrecasanthus (Vernonieae, Compositae): A revision of a north-central Andean genus

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Abstract

Cuatrecasanthus is native to Ecuador and Peru and although several unusual characters define the genus, such as single flowered heads and corolla throat (limb) divided to the base with lobes that are thickened at the margins, the members of the genus were not recognized as especially closely related until relatively recently. All six species are described, including two new to science (*Cuatrecasanthus kingii* H. Rob. & V.A. Funk, **sp. nov.** and *Cuatrecasanthus lanceolatus* H. Rob. & V.A. Funk, **sp. nov.**), and one new combination is recognized (*Cuatrecasanthus giannasii* (Stutts) H. Rob. & V.A. Funk, **comb. nov.**). A key is provided along with images of the types, SEM photographs of the leaf surfaces, a distribution map, and illustrations of the two new species. All species are given a preliminary conservation status of Data Deficient in regard to the IUCN Red List of Threatened Species.

Keywords

Asteraceae, *Critoniopsis,* Ecuador, Neotropics, Peru

Introduction

The Andean genus *Cuatrecasanthus* H. Rob., native to Ecuador and Peru, is one of the most readily distinguished genera in the tribe Vernonieae. The combination of heads with one floret, corollas with the limb divided to the base into five scarcely distorted lobes, lobes with thickened margins, and ten-ribbed achenes is unique in the tribe. Another Andean genus, with similarly deeply cut corolla lobes, *Joseanthus*

H. Rob., differs by its opposite leaves and many florets in each head. Although the distinctions of *Cuatrecasanthus* are clear, it has been subject to problems at the species level that have not been entirely resolved until the present effort to treat the genus for the Flora of Ecuador.

Given the distinctive characters of the genus, it is surprising that the first few species that were described were not recognized as relatives. The first member the group to be described was *Eremanthus jelskii* Hieron. from Peru. When *Vernonia flexipappa* was described by Gleason (1925), the relationship to *E. jelskii* was not recognized. Yet again, *Vernonia giannasii* Stutts (1980) was described without mention of the previously described relatives. It was Robinson and Kahn (1985), at the time of the description of *Vernonia sandemannii* (1985), who first recognized the relationship of the new species to the Hieronymus and Gleason species. At the time of the description of the genus *Cuatrecasanthus* (Robinson 1989) the three species were placed together, with *Vernonia giannasii* being treated as a synonym of *Cuatrecasanthus flexipappus*. The most recent studies have shown some errors in the 1989 treatment, with *V. giannasii* proving to be a distinct species and two additional species needing description. The genus thought to contain three species now proves to contain six with all the additions being based on material from Ecuador.

Although material of *Vernonia flexipappa* was collected by Keeley in 1983, it was not reported in the DNA study of Keeley et al. (2007). Nevertheless, a position for *Cuatrecasanthus* in the subtribe Piptocarphinae near *Critoniopsis* Sch. Bip. is hypothesized on the basis of the woody habit, branching trichomes on the abaxial surface of leaves, and blunt-tipped sweeping hairs on the styles.

Systematics

***Cuatrecasanthus* H. Rob., Revista Acad. Colomb. Ci. Exact. 17(65): 209 (1989).**
http://species-id.net/wiki/Cuatrecasanthus

Type species: *Vernonia sandemanii* H. Rob. & B. Kahn (=*Cuatrecasanthus sandemanii* (H. Rob. & B. Kahn) H. Rob.)

Description. *Erect branching shrubs, scrambling shrubs or trees* (rarely vines) to 3.5 m tall; *stems* terete, striate, minutely pilose (pilosulous) with evanescent simple hairs or thinly tomentose; *pith* solid. *Leaves* alternate, petiolate; *blades* elliptical or lanceolate, base narrowly cuneate to attenuate, subchartaceous, margins entire to remotely subserrulate, narrowly recurved, apex usually sharply acuminate, adaxial surfaces pilosulous with simple non-septate, thick-walled trichomes, with numerous glandular dots, abaxial surfaces covered with thin whitish tomentum of prostrate myceliiform minutely branching trichomes; *secondary veins* 4–9 on each side of midvein, ascending basally at 45–60° angles. *Inflorescence* terminal on leafy stems, rounded corymbiform, branching alternate, with large foliaceous bracts only at lower primary nodes.

Heads clustered and sessile in glomerules at ends of short branchlets (Figs 7C, 9B), individual heads cylindrical; *involucral bracts* ca. 15 in 5–6 gradate series (Figs 7D, 9C), inner bracts easily deciduous, outer bracts persistent; *receptacle* glabrous. *Florets* one per head; *corollas* lavender, outside minutely gland-dotted, distally sometimes pilosulous, basal tube narrow, ca. 2.5–4.0 mm long, throat lacking, lobes 5, linear, separated to base of limb, with somewhat thickened margins, not or scarcely distorted on drying (Figs 7E, 9D); *anther* thecae purple, with short papillose-fimbriate basal appendage, apical appendage ovate-oblong, ca. 0.5 mm long, glabrous; *style* base with stopper-shaped node, with thick-walled cells, sweeping hairs non-septate, obtuse to short-acute. *Achenes* prismatic, 10-costate (Figs 7G, 9F), surface sometimes fleshy, with numerous glandular dots, with few or no eglandular trichomes, with minute short-oblong raphids, base with broad annuliform carpopodium; *pappus* straw-colored, of 45–65 crowded rather persistent capillary bristles, about as long as corolla, barbellate, mostly some somewhat broadened and flattened distally, a few outer shorter bristles rather indistinct. *Pollen* ca. 40–45 μm in diam., spinulose, sublophate, tricolporate, with continuous perforated tectum between colpi.

In addition to the diagnostic generic characteristics are features of special interest such as the marginal teeth of the leaves that are incurved and appressed against the abaxial surface in all but one species (*C. lanceolatus;* Fig. 1A–B) and the finely branching myceliiform hairs on the abaxial surface of the leaves in all the species (Fig. 1C). In addition, there is variation on the leaf surfaces. The surfaces of the leaves have veins that can be exsculpate (above the surface), insculpate (below the surface), or even with the adaxial leaf surface (Figs 2–4). All but one of the species have veins on the adaxial surface that are even with the surface or slightly insculpate; one species has veins that are deeply insculpate (*C. giannasii*) and all six species have veins that are exculpate on the abaxial surface. The style branches are reported on one herbarium label as pale pink almost white; there are no additional data on the color of the styles.

The genus occurs in Ecuador and Peru. The six known species can be distinguished using the following key:

1	Leaf margins with numerous obvious antrorse teeth not strongly incurved against abaxial surface (may vary in prominence); leaf tips narrowly acute, not abruptly short-acuminate	**5. *C. lanceolatus***
–	Leaf margins entire or with obscure inturned teeth; leaf tips usually abruptly short-acuminate	**2**
2	Inflorescence with loose clusters of heads, distinctly exceeding the upper leaves	**3**
–	Inflorescence with dense clusters of heads, not or scarcely exceeding the upper leaves, with interspersed foliiform bracts	**4**
3	Leaf blade broadly elliptical or ovate-elliptical; adaxial surface hispidulous with midvein prominently exculpate and otherwise plane	**4. *C. kingii***

– Leaf blade lanceolate-elliptical; adaxial surface sparsely covered with appressed minute tricihomes with at least the midvein insculpate.. **6. *C. sandemanii***

4 Adaxial surface of leaf with all veins distinctly insculpate; adaxial surface with few short trichomes, veins and trichomes all whitish; distal leaf margins with incurved teeth pressed against abaxial leaf surface; tips of pappus bristles distinctly broadened... **2. *C. giannasii***

– Adaxial surface of leaf with major veins not obviously insculpate, secondary and tertiary veins insculpate; adaxial surface with many prominent stiff trichomes, midvein and trichomes dark brown or yellow; leaf margins with few inturned teeth; tips of pappus bristles not or scarcely broadened................**5**

5 Abaxial surface of midvein of leaf with dense antrorse pubescence mostly on sides; abaxial surface of lamina covered with mostly appressed, stiff, usually brownish trichomes ....**1. *C. flexipappus***

– Abaxial surface of midvein of leaf densely hirsute with spreading hairs; abaxial surface of lamina with erect yellowish trichomes.........................**3. *C. jelskii***

1. *Cuatrecasanthus flexipappus* (Gleason) H. Rob., Revista Colomb. Ci. Exact. 17 (65): 210. 1989.

http://species-id.net/wiki/Cuatrecasanthus_flexipappus

Figs 5A, 10

Type: Based on *Vernonia flexipappa* Gleason

Vernonia flexipappa Gleason, Bull. Torrey Bot. Club 52(5): 186.1925.

Type: Ecuador. Loja: sin. loc., *E. André 2250* (holotype: NY, image US!; isotype: K).

Description. Shrubs or small trees, 1.0–3.0 m tall; *stems* densely pilose with dark brown trichomes, becoming glabrous with age. *Leaves* with petioles 0.5–1.2 cm long; *blades* narrowly to broadly elliptical, mostly 3–9 cm long, 1–3 cm wide, narrowly acuminate at base and apex, margin narrowly but strongly recurved, without evident teeth or with in-turned teeth, adaxial surface dark green, glabrous or with minute appressed pubescence, secondary and tertiary veins insculpate, abaxial surface pale greenish covered with mostly appressed, stiff, brownish trichomes (rarely straw colored) intermixed with less evident whitish prostrate myceliiform branching trichomes, midvein with dense antrorse pubescence mostly on sides; *secondary veins* ca. 5 pairs, spreading from midvein at ca. 45° angles, strongly curved. *Inflorescence* scarcely exceeding vegetative leaves; *branches* densely pilosulous or hirtellous. *Heads* sessile in clusters of 3–7, ca. 10–11 mm tall × 2 mm wide; *involucres* cylindrical to fusiform; bracts mostly deciduous, ca. 15 in ca. 5 series, 1.0–5.5 mm long, ca. 1.2 mm wide, apices short-acute, ovate to narrowly elliptical, yellowish or with reddish median stripe, puberulous to nearly glabrous outside. *Florets* with corollas white to bluish white or lavender, ca. 5.5 mm long, with glandular dots on basal tube and tips of lobes, few small trichomes

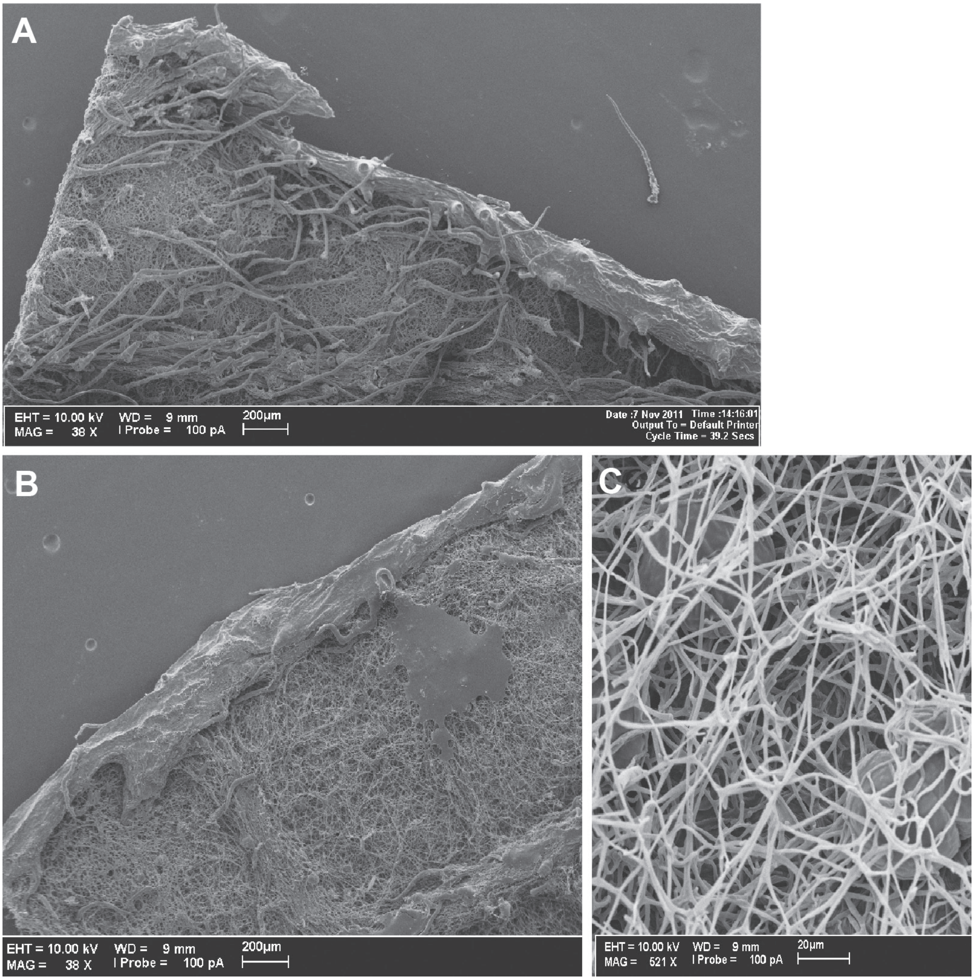

Figure 1. *Cuatrecasanthus* leaves: **A** *C. lanceolatus* showing projecting marginal tooth **B** *C. kingii* showing incurved tooth **C** Myceliform hairs on abaxial surface of leaf of *C. giannasii*.

on lobe tips, tubes ca. 2 mm long, lobes ca. 4 mm long, with some non-glandular trichomes; *anther thecae* ca. 2 mm long. *Achenes* 2.0–2.5 mm long; *pappus* white, of ca. 50 bristles mostly ca. 6 mm long, not or scarcely broadened toward tips. *Pollen* grains 37–47 µm in diam.

Additional specimens examined. Ecuador. **Loja:** along road between Loja and Zamora, ca. km 11 [03°59'0"S, 79°08'16"W, estimated], 2600 m, 2 August 1978, *Zarucchi & Andrade 2304* (US); Carretera Loja–Zamora, km 13, 2500 m [03°59'00"S, 79°07'00"W, estimated], 16 August 1983, *Jaramillo & Winnerskojold 5812* (AAU); Loja–Zamora road, ca. km 15, 03°58'S, 79°08'W, 2400–2700 m, 22–23 April 1984, *Madsen 74081* (AAU, QCA, US); In the páramo of "El Tiro," located at northern terminus of Podocarpus National Park, 500 m from the Loja–Zamora highway, 03°59'S, 79°08'W, 2940–2970 m, 14 April 1992, *Keating 143* (US); In the

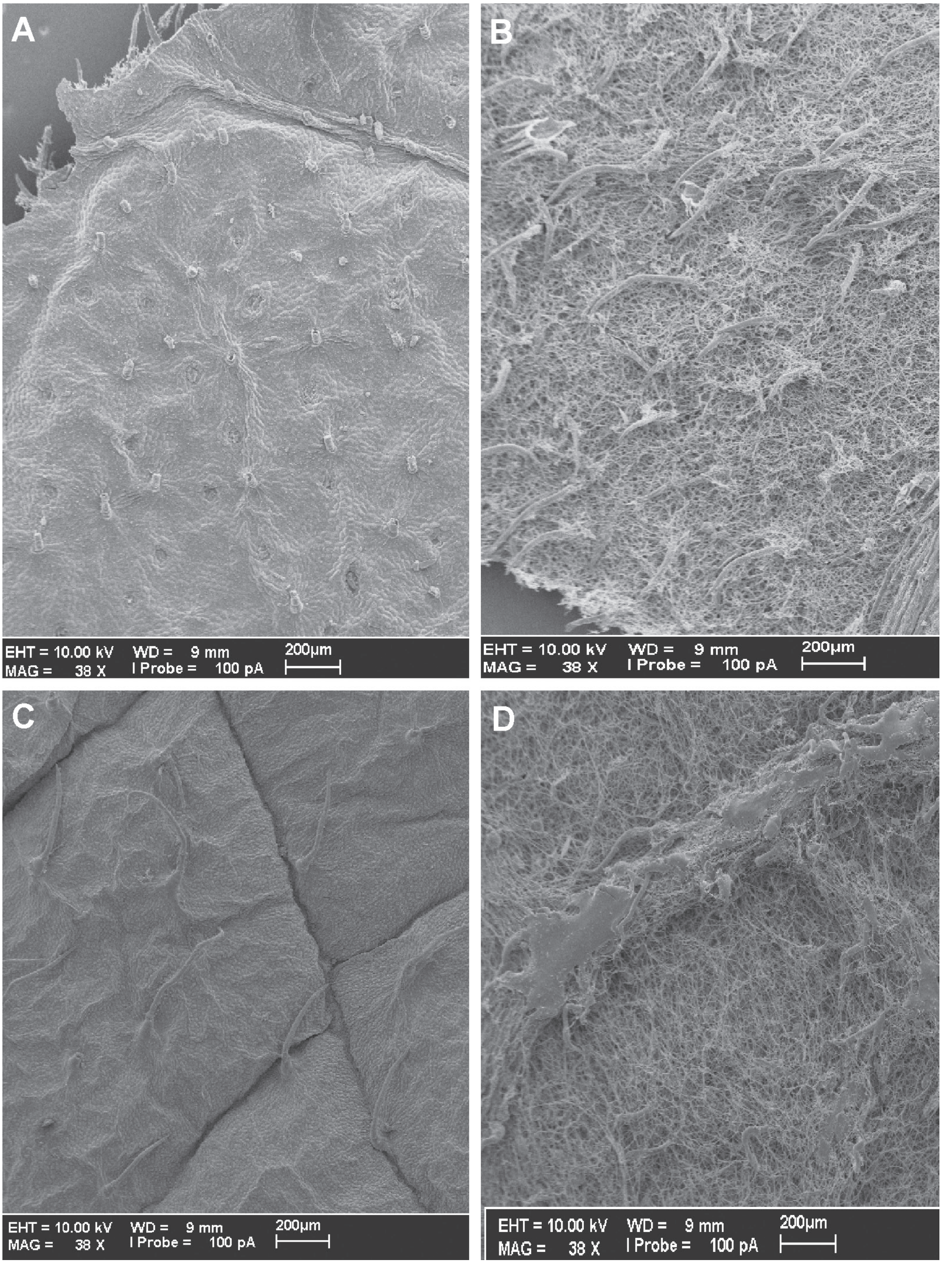

Figure 2. *Cuatrecasanthus* leaf surfaces: **A–B** *C. flexipappus*. **A** Adaxial surface **B** Abaxial surface **C–D** *C. giannasii* **C** Adaxial surface, showing deeply insculpate veins **D** Abaxial surface.

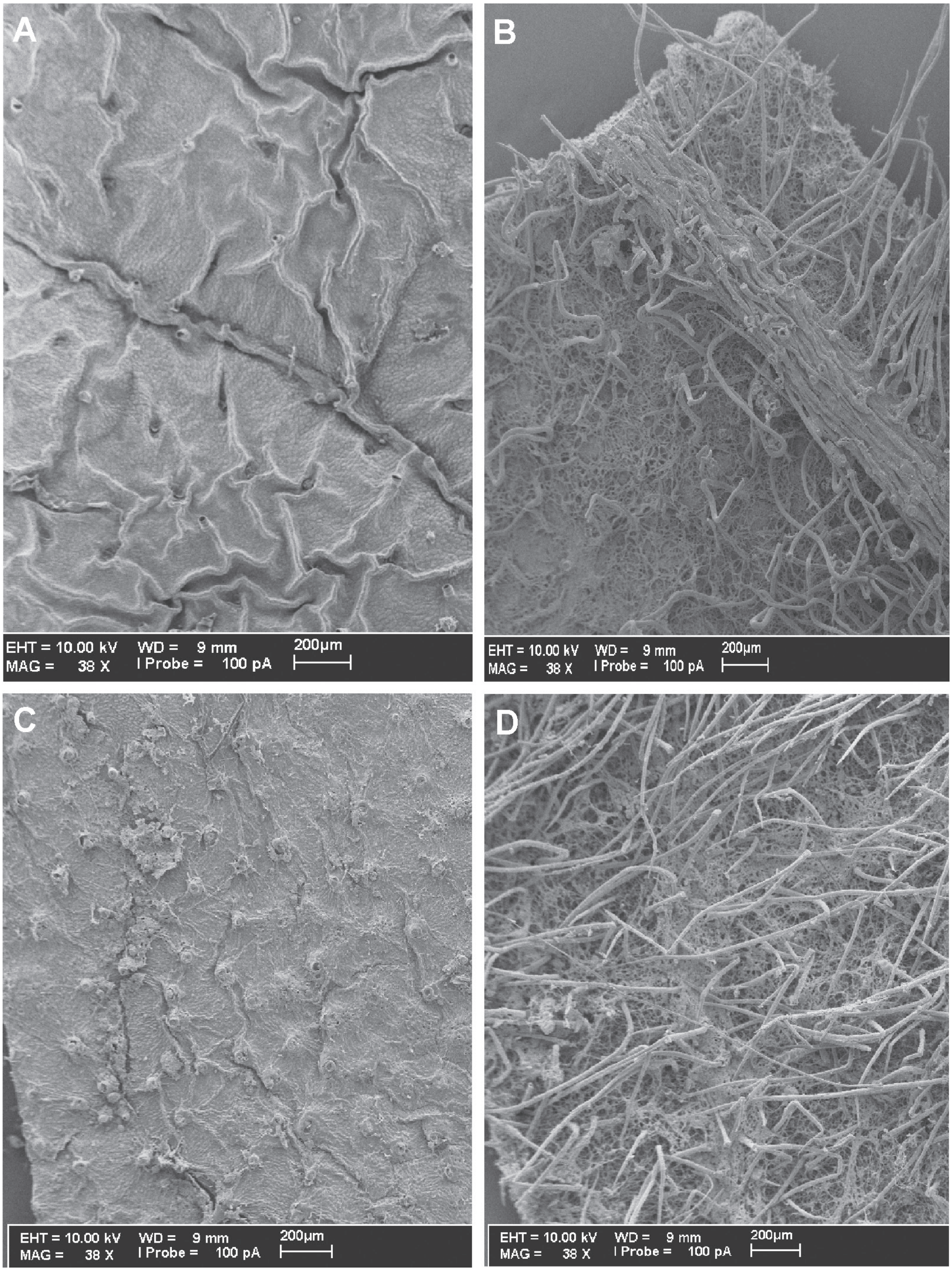

Figure 3. *Cuatrecasanthus* leaf surfaces: **A–B** *C. jelskii*. **A** Adaxial surface **B** Abaxial surface **C–D** *C. kingii*. showing veins even with surface **D** Abaxial surface.

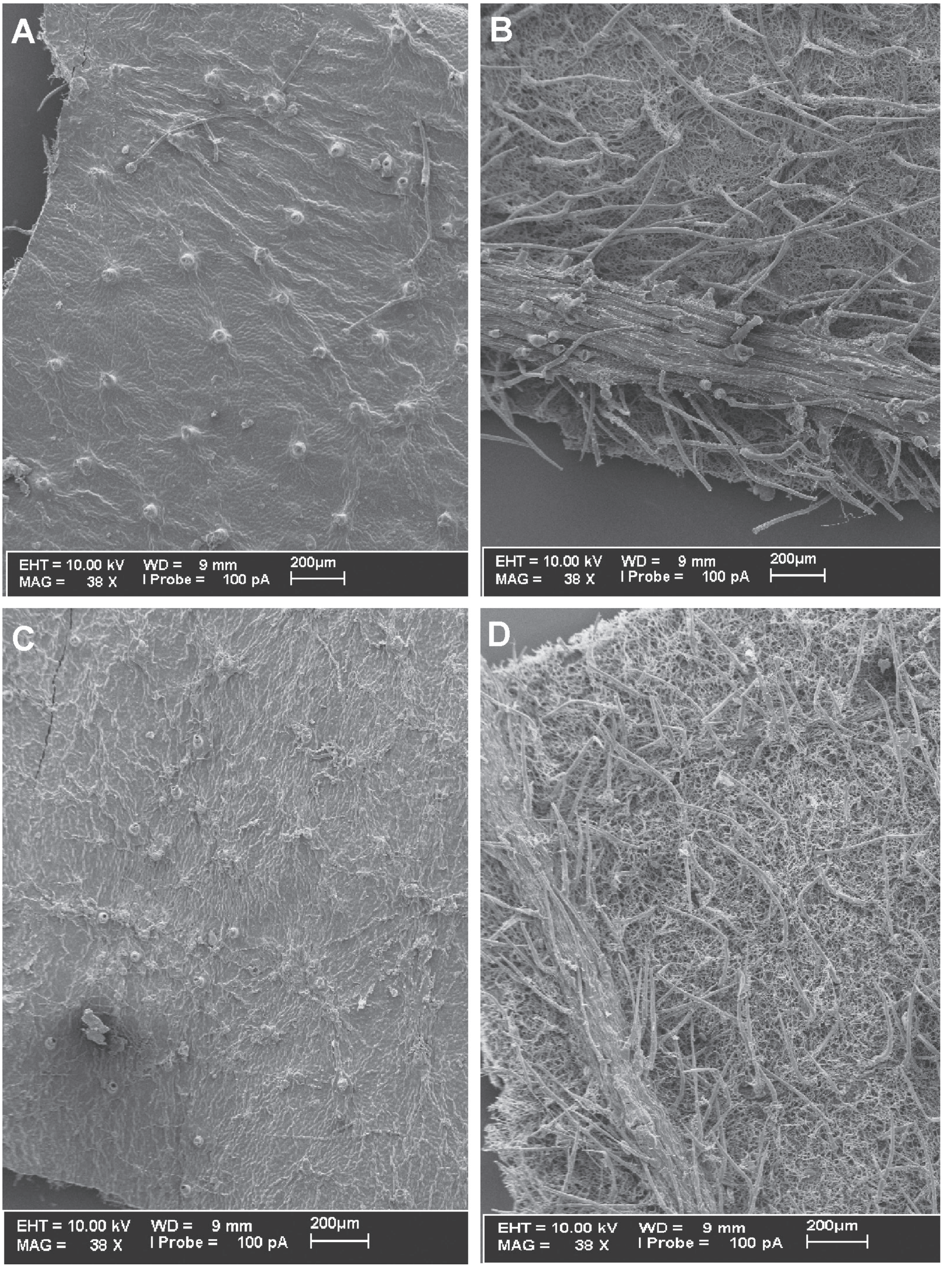

Figure 4. *Cuatrecasanthus* leaf surfaces: **A–B** *C. lanceolatus*. **A** Adaxial surface **B** Abaxial surface **C–D** *C. sandemanii* **C** Adaxial surface **D** Abaxial surface.

páramo and shrub páramo above the Refugio de Cajanuma (Centro de Información), Podocarpus National Park, 04°07'00"S, 79°09'30"W, 2800 m, 31 July 1993, *Keating 409* (US). **Zamora-Chinchipe:** 14.8 km from transit control out of Loja on road

Figure 5. Photographs of *Cuatrecasanthus* types: **A** *C. flexipappus*, holotype (NY) **B** *C. giannasii*, holotype (S).

to Zamora [03°59'10"S, 79°08'02"W, estimated], 2500 m, 8 July 1983, *Keeley & Keeley 4104, 4105, 4106, 4107, 4108, 4109, 4110, 4111, 4115* (K, US); *Keeley & Keeley 4112, 4114* (US); Zamora, carretera Loja–Zamora, Estación Científica San Francisco, sendero hacia las antenas. Colecciones cerca del Francisco 4, en Transecto 2, 03°58'S, 79°04'W, 3000 m, 29 April 2000; *Freire Fierro 3121* (MO, US).

Peru. **Cajamarca:** Prov. Jaen; E slope of Paso de Huascarai, head of Quebrada Granadillas, 15 km SE of Huancabamba, 05°22'S, 79°20'W, 3000 m, 10 June 1947, *Fosberg 27852* (US). **Piura:** Prov. Huancabamba; Los Llanos to Chorro Blanco (Sapalache – Chiguelas), 2650 m, 5°08.2'S, 79°24.6'W, 19 Oct. 2001, *Sagasteguí, Dillon, Leiva, & Zapata 16781* (F, HUT).

Habitat. Roadside, burned over cloud forest on steep south-facing slope; shrub páramo at 2400–3000 m in elevation.

The species is the most commonly collected member of the genus but apparently is sympatric with both *C. kingii* and *C. lanceolatus* in the area near the border between Loja and Zamora/Chinchipe. The species is very closely related to the northern Peruvian *C. jelskii* (Hieron.) H. Rob. The latter differs most obviously by the densely hirsute abaxial surface of the midvein of the leaves and erect rather than appressed trichomes of the abaxial blade surface. The adaxial leaf surface of the latter also has less strongly insculpate veins.

Preliminary conservation status. Data Deficient

2. *Cuatrecasanthus giannasii* (Stutts) H. Rob. & V.A. Funk, comb. nov.
urn:lsid:ipni.org:names:77121072-1
http://species-id.net/wiki/Cuatrecasanthus_giannasii
Figs 5B, 10

Type: Based on *Vernonia giannasii* Stutts
Vernonia giannasii Stutts, Brittonia 32(2): 162 (1980).

Type. Ecuador. Morona-Santiago [formerly Santiago-Zamora]: Camino Cuenca, General Plaza (Limon), 39–41, [02°59'S, 78°41'W, estimated], 2300 m, 19 September 1967, *B. Sparre 18721* (holotype: S!).

Description. *Vines or scrambling shrubs*; stems flexuous, densely pilose with long, mostly single-celled trichomes. *Leaves* with petioles 0.4–0.7 cm long; *blades* subchartaceous, elliptical, mostly 3.5–8.5 cm long, 1.0–2.5 cm wide, acuminate at base, acute to short-acuminate at apex, margins appearing entire, narrowly recurved, with inflexed teeth distally, adaxial surface, dark green, bullulate, sparsely short-scabridulous, secondary and tertiary veins insculpate, abaxial surface pale green with thin white cover of myceliiform branched trichomes, minutely pilosulous with pale trichomes on veins, without dark trichomes, all veins and veinlets exsculpate; *secondary veins* ca. 5 or 6 on each side of midvein, spreading at base at 45–50° angles, curved and more strongly ascending near margins. *Inflorescence* terminal and from axils of uppermost leaves, not or scarcely exceeding the leaves, rounded corymbiform; branches short, puberulous. *Heads* sessile with up to 9 clustered in dense glomerules, 8–10 mm tall, ca. 2 mm wide; *involucre* cylindrical or narrowed distally and fusiform, bracts ca. 16 in ca. 5 series, short-ovate to oblong elliptical, 2.0–5.5 mm long, 1.0–1.5 mm wide, apices short-acute, slightly darkened distally, sometimes with reddish median line, glabrous outside. *Florets* with corollas pale lavender, ca. 6.5 mm long, with glandular dots on basal tube and tips of lobes, tubes ca. 2.5 mm long, lobes ca. 4 mm long; *anther thecae* ca. 2.5 mm long. *Achenes* ca. 2.5 mm long; *pappus* white, of ca. 40 capillary bristles ca. 6.5 mm long, distinctly broadened toward tips. *Pollen* grains ca. 40 µm in diam.

Additional specimens examined. Ecuador. **Loja:** Loja to Zamora, 1876, *André K1152 (*F, NY). **Morona-Santiago** [formerly Santiago-Zamora]: Eastern slope of the cordillera, Valley of the ríos Negro and Chupianza (on trail from Sevilla de Oro to Mendez, Tambo Consuelo to Tambo Cerro Negro, [01°49'S, 78°23'W, estimated], 2400-3000 m, 17 December 1944, *Camp E-1619* (NY, US).

The species is known only from Morona-Santiago and Loja, Ecuador, between 2300 and 3200 m in elevation (Fig. 10).

Camp describes the habit as a vine and this character would easily distinguish the species, but the type specimen has no information on the habit and it appears to be a sturdier plant. Only new collections that document the habit will resolve this issue.

Preliminary conservation status. Data Deficient

3. *Cuatrecasanthus jelski* (Hieron.) H. Rob., Revista Colomb. Ci. Exact. 17 (65): 210 (1989).
http://species-id.net/wiki/Cuatrecasanthus_jelski
Figs 6A, 10

Type: Based on *Eremanthus jelskii* Hieron.
Eremanthus jelskii Hieron., Bot. Jahrb. Syst. 36(5): 462 (1905), non *Vernonia jelskii* Hieron., Bot. Jahrb. Syst. 36(5): 459 (1905).
Type: Peru. Cajamarca: Prope Shanyn (Quebrada Lejia) [probably not far from Tambillo] [05°40'50"S, 79°16'7"W, Cerro Tambillo, estimated], *Jelski 776* (holotype: B, destroyed, photos F, US! [F neg. 14657]; lectotype, designated here: US!).
Vernonia shanynensis MacLeish, Syst. Bot. 9 (2): 135 (1984), nom. nov. for *Eremanthus jelskii.*
Type: Based on *Eremanthus jelskii* Hieron.

Description. *Shrubs or small trees*, 1.0–3.0 m tall; *stems* densely velutinous (short velvety) with dark brown trichomes. *Leaves* with petioles 0.3–0.5 cm long; *blades* narrowly to broadly elliptical, mostly 3–10 cm long, 1–2.5 cm wide, narrowly acuminate at base and apex, margin narrowly but strongly recurved, with few inturned teeth distally, adaxial surface dark green, glabrous or with appressed puberulence, secondary and tertiary veins insculpate, abaxial surface pale green covered with erect, stiff, yellowish trichomes intermixed with less evident whitish prostrate myceliiform branching trichomes, midvein with dense spreading pubescence; *secondary veins* ca. 5–6 pairs, spreading from midvein at 45–55° angles, strongly arched. *Inflorescence* scarcely exceeding vegetative leaves, with intermixed foliiform bracteoles; *branches* densely pilosulous or hirtellous. *Heads* sessile in clusters of 3–7 within larger glomerules, 10–11 mm tall × 1.5–2.0 mm wide; *involucres* cylindrical to fusiform; *bracts* ca. 9–12 in ca. 4 series, 1–5 mm long, ca. 1.2 mm wide, apices short-acute, ovate to narrowly elliptical, yellowish darkened tip, outer bracts puberulous, inner bracts glabrous outside. *Florets* with corollas violet, ca. 6 mm long, with glandular dots on tube and tips of lobes, tubes ca. 2.5 mm long, lobes ca. 3.5 mm long; *anther thecae* deep purple, ca. 3 mm long. *Achenes* 2.0–2.5 mm long; *pappus* white, of 32–ca. 50 bristles mostly ca. 6 mm long, not or scarcely broadened toward tips. *Pollen* grains 37–47 µm in diam.

Additional specimen examined. Peru. **Cajamarca:** Prov. Cutervo; La Pucarilla, entre Sócota y San Andrés [6°16'S, 78°42'W, estimated based on elevation], 2500–2650 m, 24 June 1988, *Sánchez Vega 4868* (CPUN, F, US); 2450 m, 14 November 1986, *Mostacero, Leiva, Mejía, Peláez, & Guevara 1631* (F, HUT); 2 Nov 1991, *Sánchez Vega, Sagastegui & Guevara 5990* (CPUN); Prov. San Ignacio; Cordillera del Condor, Munic. Dist. Huarango, Nuevo Mundo, Caserio Rey del Oriente, arriba, Caseríos Gosén [5°19'S, 78°43'W, estimated for CG but elevation too low] y Pisaguas, 1800 m, 26 July 1997, *Rodríguez & Campos 1816* (MO, US). **Lambayeque:** Prov. Ferrañafe; Paso Upaypecc, Cañaris [6°03'S, 79°16'W, estimated for Cañaris], 3000 m, 25 June 1989, *Llatas Quiroz 2499* (F).

Figure 6. Photographs of *Cuatrecasanthus* types: **A** *C. jelskii*, lectotype (US) **B** *C. kingii*, holotype (US).

Habitat. *Rodríguez & Campos 1816* was described as having been collected in primary forest. The range in elevation that has been reported is 1800–3000 m (Fig. 10).

This species was the first member of the genus to be described. At the time of its description, a comparison was made to Brazilian species of *Eremanthus*, members of the comparatively distantly related subtribe Lychnophorinae. Herbaria that might hold Jelski collections from Peru and therefore might have additional isolectotypes (according to Chaudhri et al. 1972) are F, KRA, NY and W.

Preliminary conservation status. Data Deficient

4. *Cuatrecasanthus kingii* H. Rob. & V.A. Funk, sp. nov.

urn:lsid:ipni.org:names:77121073-1
http://species-id.net/wiki/Cuatrecasanthus_kingii
Figs 6B, 7, 10

Type. Ecuador. Zamora–Chinchipe: 17 km E of Loja on the road to Zamora [03°58'53"S, 79°06'31"W, estimated], 7800 ft [2370 m], 31 January 1979, *King & Almeda 7928* (holotype: US!; isotype: CAS).

Description. *Shrubs* to 1 m tall, bases erect or decumbent to rhizomate; *stems* densely lanulate with tawny mostly single-celled trichomes. *Leaves* with petioles 0.8–

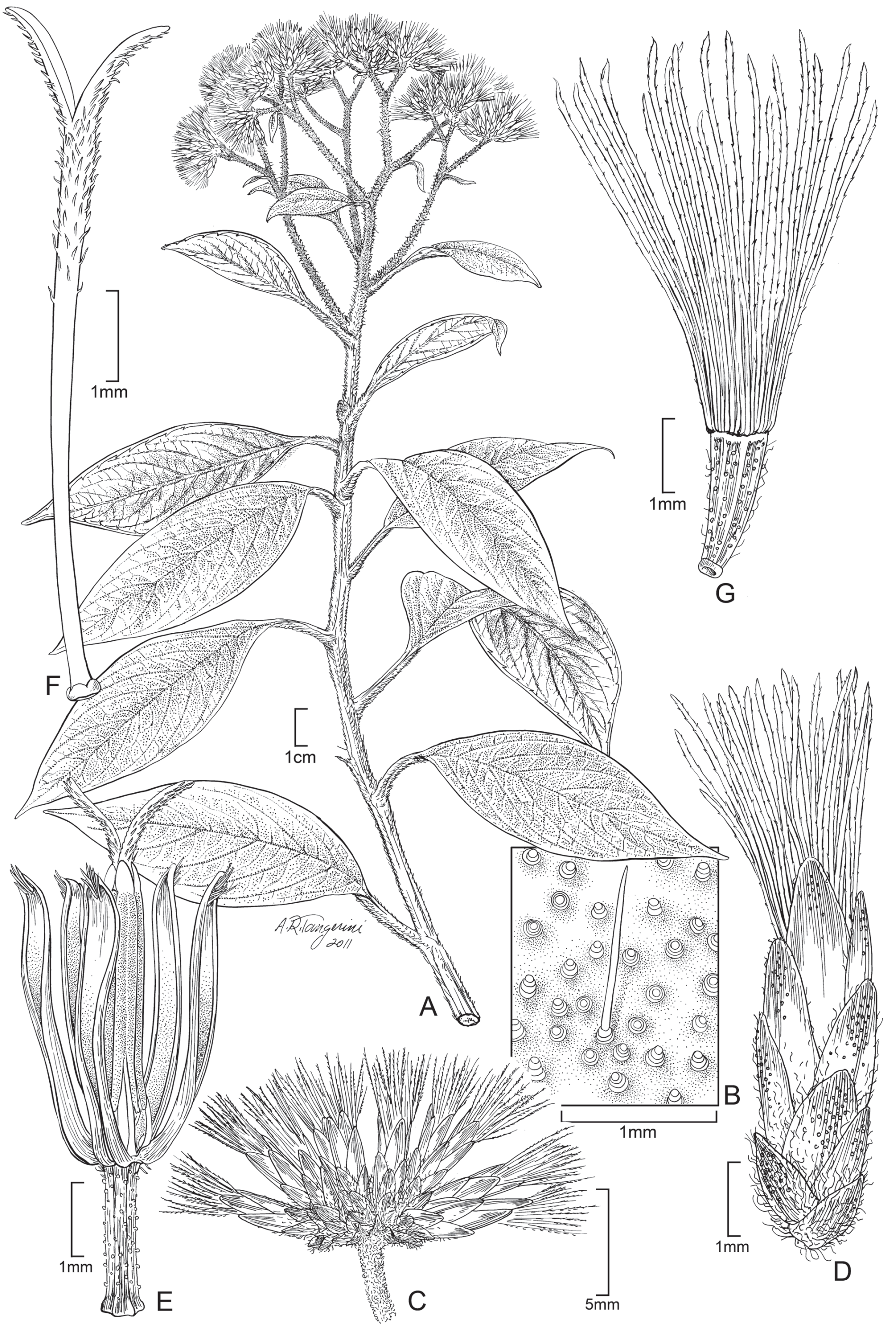

Figure 7. *Cuatrecasanthus kingii*: **A** Habit **B** Detail of adaxial surface of leaf **C** Cluster of heads **D** Single head containing one floret **E** Floret showing corolla lobes divided to base of limb, with thickened margins **F** Style **G** Achene with 8–10 ribs.

2.0 cm long; *blades* ovate to elliptical, mostly 3.5–8.5 cm long, 2–3 cm wide; base acuminate, apex short-acuminate, margins appearing entire, narrowly recurved, with incurved teeth distally, adaxial surface dark, epidermal cells often paler in area along veins, surface plane or with slightly insculpate veins, densely hispidulous with stiff trichome bases, abaxial surface densely lanulate to sericeous with tawny trichomes, at surface with dense white cover of myceliumiiform stellate trichomes; *secondary veins* ca. 5 or 6 on each side of midvein, spreading at base at 45–50° angles, curved and more strongly ascending near margins. *Inflorescence* distinctly exceeding reduced distal leaves, with few long ascending branches; *branches* tomentellous with dark hairs. *Heads* sessile and with up to 12 clustered in dense ultimate glomerules, up to 10 mm tall, ca. 2 mm wide; *involucre* cylindrical or narrowed distally and fusiform, bracts brown, ca. 16 in ca. 5 series, short-ovate to oblong elliptical, 2.0–5.5 mm long, 1.0–1.5 mm wide, apices short-acute, slightly darkened distally, sometimes with reddish median line, scarious, glabrous outside. *Florets* with corollas possibly pale lavender, ca. 6.5 mm long, with numerous glandular dots on basal tube and few on tips of lobes, tips of lobes paucipilosulous, tube ca. 2.5 mm long, lobes ca. 4 mm long; *anther thecae* ca. 2.5 mm long. *Achenes* ca. 2.5 mm long; *pappus* white, of ca. 50 capillary bristles ca. 6.5 mm long, not or scarcely broadened toward tips. *Pollen* grains 35–42 µm in diam.

Additional specimen examined. Ecuador. **Loja:** 10 km E of Loja on road to Zamora [03°59'07"S, 79°08'16"W, estimated], 2500 m, 31 January 1979, *King & Almeda 7920* (CAS, US).

Habitat. Secondary vegetation bordering steep wooded slopes; wet windswept forested ridge interspersed with pastures at elevations of 2370–2500 m (Fig. 10).

The species has the most broadly elliptical leaf blades of any member of the genus. The most distinctive feature, however, is the mostly flat and hispidulous adaxial surface of the leaves. The distribution is restricted to the area near the pass between the Ecuadorian provinces of Loja and Zamora-Chinchipe (Fig. 10).

Preliminary conservation status. Data Deficient

5. ***Cuatrecasanthus lanceolatus*** **H. Rob. & V.A. Funk, sp. nov.**

urn:lsid:ipni.org:names:77121074-1
http://species-id.net/wiki/Cuatrecasanthus_lanceolatus
Figs 8A, 9, 10

Type. Ecuador. Zamora-Chinchipe: Loja–Zamora, km 20.6, 03°57'S, 79°05'W, 2650 m, 9 August 1997, *G.P.Lewis 3424* (holotype: US!; isotypes: AAU!, GB, K, LOJA, MO, QCA, QCNE).

Description. *Shrubs to small trees* up to 2 m high; *stems* flexuous above, hexagonal, densely pilose with brownish trichomes. *Leaves* with petioles mostly 0.5–1.5 cm long; *blades* lanceolate, broadest at basal 1/3, 4.0–9.5 cm long, 1–3 cm wide, apex distally narrowly acute, not acuminate, margins not or scarcely recurved distally, with marginal teeth projecting upward or outward (may vary in intensity), not inward, adaxial surface

Figure 8. Photographs of *Cuatrecasanthus* types: **A** *C. lanceolatus*, holotype (US) **B** *C. sandemanii*, holotype (BM).

dark green, lamina dotted with gland-like persistent or aborted stumps of small scaber, with weakly insculpate veins, abaxial surface gray-green, tawny-pilose, sometimes contorted, denser on veins, with thin grayish prostrate myceliiform branching trichomes; *secondary veins* in 4–5 pairs, strongly ascending. *Inflorescence* distinctly exceeding the reduced distal leaves, main axis and branches mostly deflected at nodes, rounded corymbiform; *branches* tomentellous. *Heads* sessile in clusters of 2–6 congested in larger dense glomerules, 10–11 mm high × 2 mm wide; *involucres* cylindrical or fusiform, ca. 16 in ca. 5 series, 1.0–4.5 mm long, ca. 1.2 mm wide, short-acute, greenish brown, darkened at tips or along midvein distally, glabrous. *Florets* with corollas pink-lilac, ca. 6 mm long, with numerous glandular dots on basal tubes, with a few short hairs at apices of tubes, tubes ca. 2 mm long, lobes ca. 4 mm long; *anther thecae* dark reddish brown, ca. 2.5 mm long. *Achenes* ca. 2 mm long; *pappus* white, of ca. 45 capillary bristles ca. 6.5 mm long, not or scarcely broadened at tips. *Pollen* grains ca. 35 µm in diam.

Additional specimens examined. Ecuador. **Loja:** Road to Zamora from Loja, km 12–14, near top of pass, [03°59'6"S, 79°08'23"W, estimated], 2800 m, 28 September 1961, *Dodson & Thien 781* (US-2!).

Habitat. Local in secondary scrub at 2650–2800 m in elevation (Fig. 10).

Preliminary conservation status. Data Deficient

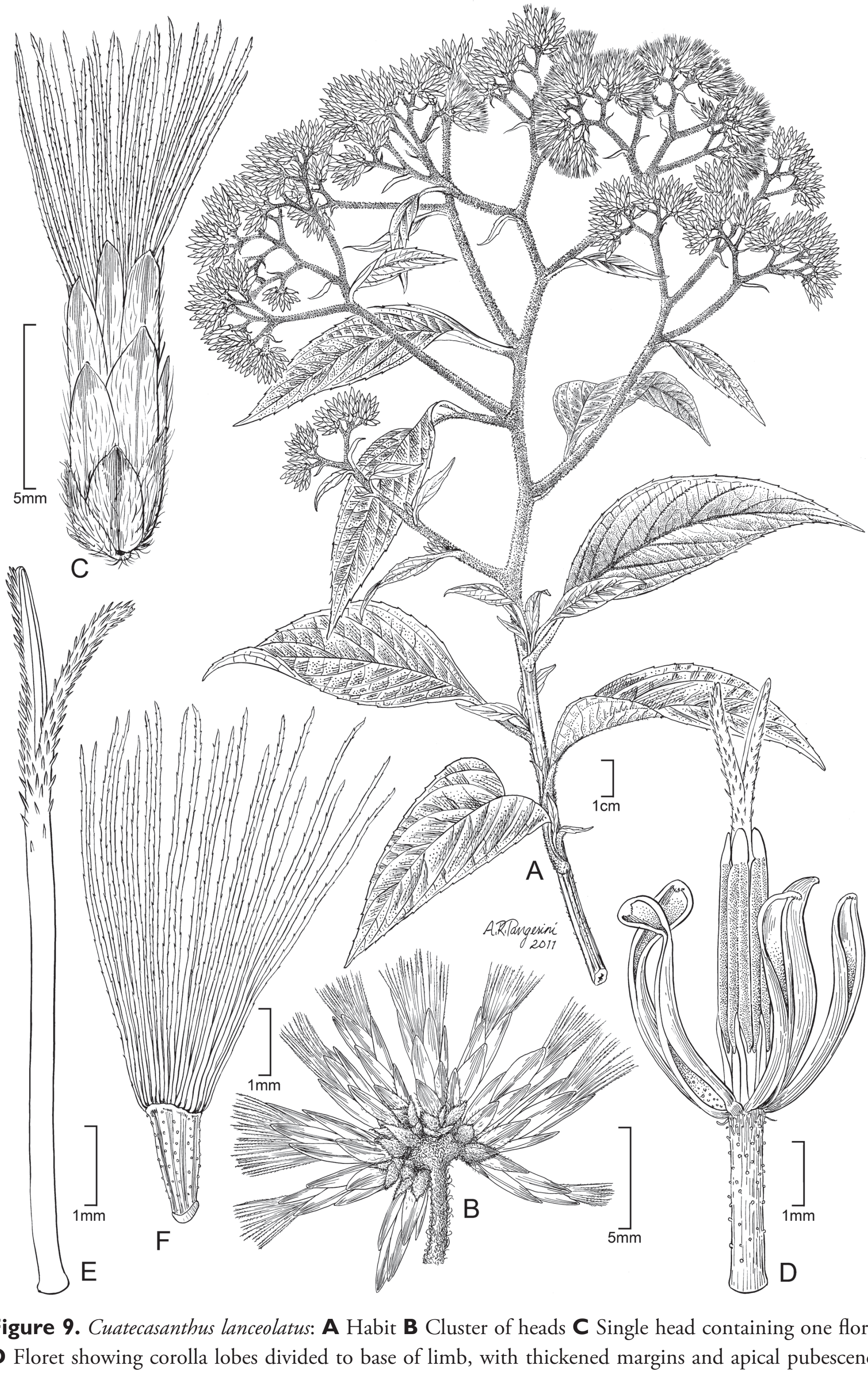

Figure 9. *Cuatecasanthus lanceolatus*: **A** Habit **B** Cluster of heads **C** Single head containing one floret **D** Floret showing corolla lobes divided to base of limb, with thickened margins and apical pubescence **E** Style **F** Achene with 8–10 ribs.

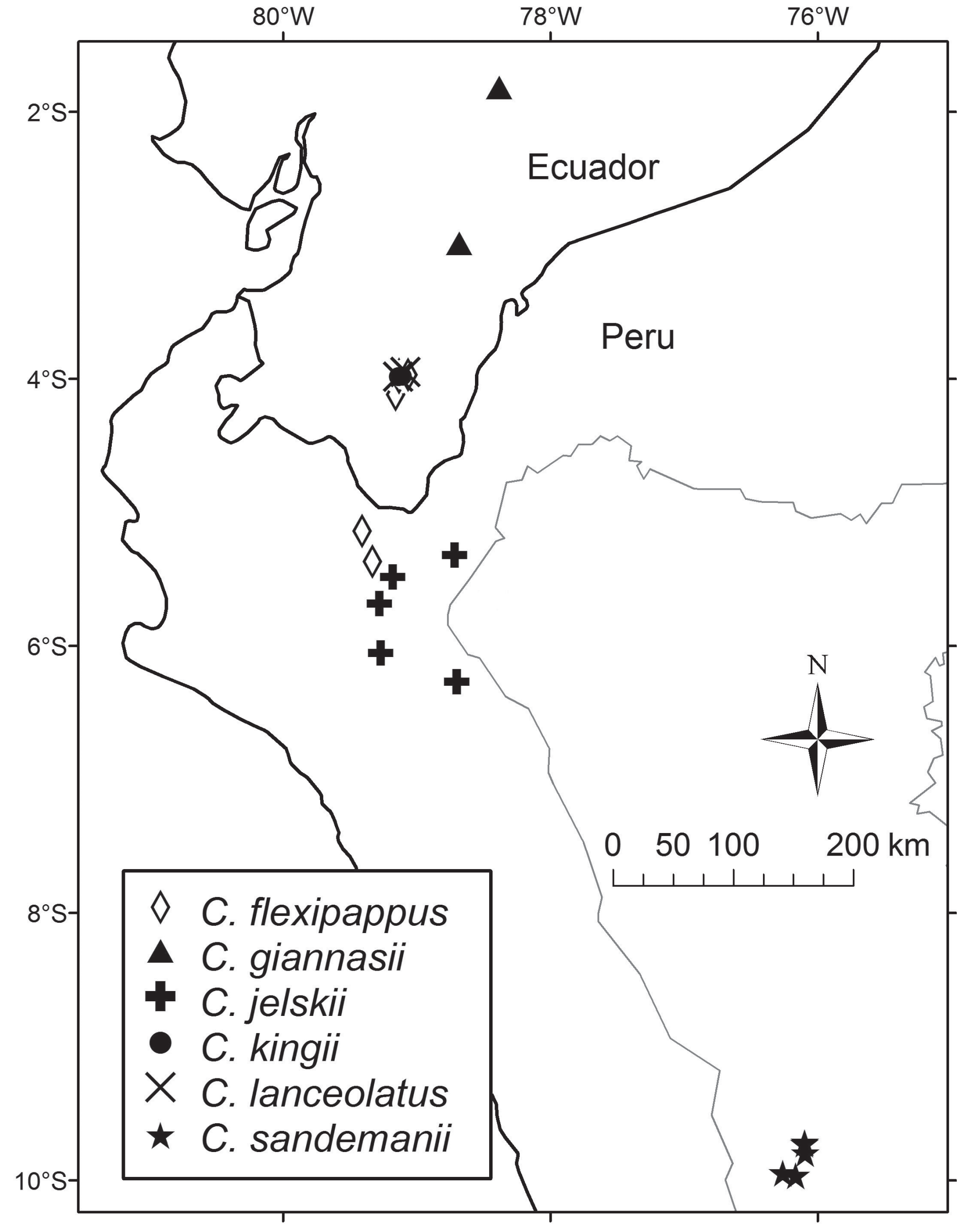

Figure 10. Distribution map of *Cuatrecasanthus* species.

6. *Cuatrecasanthus sandemanii* (H. Rob. & B. Kahn) H. Rob., Revista Colomb. Ci. Exact. 17 (65): 210 (1989).
http://species-id.net/wiki/Cuatrecasanthus_sandemanii
Figs 8B, 10

Type: Based on *Vernonia sandemanii* H. Rob. & B. Kahn
Vernonia sandemanii H. Rob. & B. Kahn, Phytologia 58(4): 253 (1985).

Type: Peru. Huánuco: Carpish (above Huánuco) [09°56'47"S, 76°15'51"W, estimated], 8500 ft [2600 m], June 1938, *C. Sandeman 219* (holotype: BM!; isotype: K).

Description. *Shrubs to small trees*, to 3.3 m high; *stems* brownish, flexuous above, terete, irregularly appressed pilosulous with short pale trichomes. *Leaves* with petioles mostly 1–3 mm long; *blades* thinly papyraceous (the only species), elliptical, broadest near middle, mostly 7–9 cm long, 1.5–2.7 cm wide, base cuneate, apex narrowly acute to short acuminate, margins narrowly recurved, less recurved at apex, margins becoming shortly serrate distally with few inrolled teeth, adaxial surface dark green, rather shiny, sparsely pilosulous, more densely pilosulous on major veins, primary, secondary and tertiary veins insculpate, abaxial surface whitish, pale sericeous on veins, between major veins whitish tomentellous with prostrate myceliiform branching trichomes; *secondary veins* ascending with 5–9 pairs. *Inflorescence* distinctly exceeding the reduced distal leaves, main axis and branches somewhat deflected at nodes, larger foliiform bracts restricted to primary nodes; *branches* densely yellowish sericeous. *Heads* sessile in clusters of 2–6 and clusters congested in numerous larger dense glomerules, 10–12 mm high × 1.5–2.0 mm wide; *involucres* cylindrical or fusiform; involucral bracts greenish brown with exposed parts purplish, ca. 15 in 4–5 series, 1.5–5.0 mm long, ca. 1 mm wide, outer bracts ovate, glabrous to subtomentellous outside, apices rounded to short-obtuse, becoming frayed, linear to narrowly elliptical, mostly glabrous, distally slightly appressed puberulous, short-acute, darkened at tips. *Florets* with corollas violet, ca. 8 mm long, with numerous glandular dots outside, denser on tube and few on tips of lobes, tube 3.5–4.0 mm long, lobes 3.5–4.0 mm long, ca. 0.7 mm wide; *anther thecae* dark reddish brown, ca. 1.3 mm long, bases papillose-fringed; apical appendage oblong, apex rounded. *Achenes* ca. 2 mm long, costae shortly setuliferous, between costae glandular punctate; *pappus* white, of ca. 65 capillary bristles ca. 7 mm long, slightly broadened at tips. *Pollen* grains ca. 45 µm in diam. in fluid.

Additional specimens examined. Peru. **Huánuco:** Prov. Huánuco, alturas de Carpish, entre Huánuco y Tingo María [09°43'11"S, 76°05'56"W, estimated], 2800 m, February 1940, *Ridoutt s.n.* (USM #11579, US); Carpish, entre Huánuco y Tingo María [09°47'56"S, 76°05'47"W, estimated], 2800–2900 m, 22 August 1946, *Ferreyra 1214* (USM, US), 9 August 1947, *Ferreyra 2347* (US); Alrededor del Tunel de Carpish, 09°43'37"S, 76°06'07"W, 2800 m, 2 November 2001, *Salina 230* (US); Prov. Huánuco, Munic. Dist. Amarilis, Sariapampa [9°58'S, 76°10'W, estimated from elevation], 3100 m, 7 May 1946, *Woytkowski 34295* (F, MO).

Habitat: Near the road in cloud forest and rain forest in semi-shade; 2800–3100 m in elevation (Fig. 10).

Preliminary conservation status. Data Deficient

Acknowledgements

Maria Backlund and the staff of S and Mike Dillon and the staff of F are thanked for the loans of material, including the type specimen of *Vernonia giannasii* (from S). In addition, the staff at NY and BM kindly sent images of their types for use in Figure 9. All herbaria abbreviations are from Thiers (continuously updated). We thank Sara Alexander for making the map, Alice Tangerini for the original artwork, and the editors for their suggestions and corrections.

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RESEARCH ARTICLE

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Phylogenetic analyses place the Australian monotypic *Revwattsia* in *Dryopteris* (Dryopteridaceae)

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Abstract

Revwattsia fragilis (Watts) D.L. Jones (Dryopteridaceae), originally described as a *Polystichum* Roth by the pioneer Australian botanist Reverend W.W. Watts in 1914, is a rare epiphytic fern endemic to northeastern Queensland, Australia. Known from only a few populations, it is restricted to tropical rainforests in the Atherton Tablelands. We used the cpDNA markers *psbA-trnH*, *rbcL*, *rbcL-accD*, *rps4-trnS*, *trnG-trnR*, *trnL-trnF*, and *trnP-petG* to infer the relationships of *Revwattsia fragilis* within Dryopteridaceae. Based on our molecular analysis, we were able to reject Watts's 1914 hypothesis of a close relationship to *Polystichum*. Its closest allies are a suite of Asian *Dryopteris* Adans. species including *D. labordei, D. gymnosora, D. erythrosora* and *D. cystolepidota*; maintaining *Revwattsia* renders *Dryopteris* paraphyletic. The epiphytic habit and distinctive long-creeping rhizome of *Revwattsia* appear to be autapomorphies and do not warrant its generic status. In the course of our investigation we confirmed that polyphyly of *Dryopteris* is also sustained by the inclusion of *Acrorumohra* (H.Itô) H.Itô, *Acrophorus* C.Presl, *Arachniodes* Blume, *Diacalpe* Blume, *Dryopsis* Holttum & P.J.Edwards, and *Peranema* D.Don. The epithet *fragilis* is occupied in *Dryopteris*, therefore we provide the name *Dryopteris wattsii* **nom. nov.** to accommodate *R. fragilis* in *Dryopteris.*

Keywords

Biogeography, Australia, Morphology, Polystichum, Rumohra

Introduction

The fern genera *Polystichum* Roth and *Dryopteris* Adans. are now understood to be closely allied members of the Dryopteridaceae. *Polystichum* and its allies *Cyrtomium* C.Presl and *Phanerophlebia* C.Presl are sister to *Arachniodes* Blume and *Dryopteris*

(Schuettpelz and Pryer 2007). The breadth of morphological diversity exhibited by *Polystichum* and *Dryopteris* has, in hindsight, had at least three impacts on the taxonomic history of these genera and their family. First, a large number of segregate genera have been removed from these two genera based on dramatic morphological transformations. Some of these segregates render *Dryopteris* and *Polystichum* paraphyletic; examples include *Sorolepidium* Christ, which belongs in *Polystichum* (Liu et al. 2007a), and *Lithostegia* Ching, which belongs in *Arachniodes* (Liu et al. 2007b). Second, these morphologically innovative lineages are sometimes superficially similar to (i.e. convergent with) remotely related ferns, leading to their circumscription as polyphyletic genera. For example, the morphologically anomalous *Polystichum speciosissimum* (Kunze) R.M.Tryon & A.F.Tryon was originally described in *Cheilanthes* Sw. (Pteridaceae). Third and central here, more remote members of the Dryopteridaceae superficially resemble species of *Polystichum* and *Dryopteris.* For instance, the epiphytic genus *Rumohra* Raddi was long included in *Polystichum*, presumably because of its peltate indusium (Diels 1902). However, Little and Barrington (2003) provided evidence for a close relationship of *Rumohra* to *Megalastrum* Holttum and *Lastreopsis* Ching, a conclusion confirmed in analyses with denser sampling more recently (Schuettpelz and Pryer 2007). This same relationship was implied by Tryon and Tryon (1982) who grouped *Rumohra*, *Megalastrum*, and *Lastreopsis* together in their key to dryopterid genera based on their shared central adaxial costal ridge.

The rare Australian monotypic genus *Revwattsia* D.L.Jones presents a similarly intricate history (Fig. 1A). A high-canopy epiphyte, *Revwattsia fragilis* (Watts) D.L.Jones (Dryopteridaceae) is endemic to northeastern Queensland, where it is known from only a few small populations (Australia's Virtual Herbarium 2012). *Revwattsia fragilis* is confined to mid-elevation rainforest, where it grows inside rotting tree hollows and among other epiphytes (Jones 1998). The Reverend W.W. Watts originally described *R. fragilis* in 1915 ('1914') as a *Polystichum*, presumably because of its perceived similarity to *Rumohra adiantiformis* (G. Forst.) Ching, which was then included in *Polystichum.* In northern Queensland, *Rumohra adiantiformis* is a common species in the humid forests; the two share a few superficial similarities: a long-creeping dorsiventral rhizome and epiphytic habit (Watts 1914)(Fig. 1B). Watts accurately listed characters by which *R. fragilis* differed from *R. adiantiformis*, including its reniform indusia, its less coriaceous texture, and lamina axes lacking a central adaxial costal ridge (Fig. 1D). Andrews (1990) and later Jones (1998) both emphasized what they perceived to be unique characters of *R. fragilis*. Andrews (1990) suggested recognition as a separate genus for the taxon in his treatment of the ferns of Queensland. Jones (1998) followed this lead in establishing the genus *Revwattsia* in his treatment to the Dryopteridaceae of Australia.

Indeed, inclusion of *Revwattsia* in *Polystichum* is untenable morphologically. Long-creeping rhizomes, reniform indusia, and the epiphytic habit are not characteristic of *Polystichum*. The herbaceous dark-brown petiole scales of *Revwattsia* are unknown in *Polystichum*, which has pale scales or dark indurated petiole scales. The extensive glandular indument characteristic of *Revwattsia* (Andrews 1990) is unknown among the mature fronds of large *Polystichum* species. In addition, the symmetrical ultimate

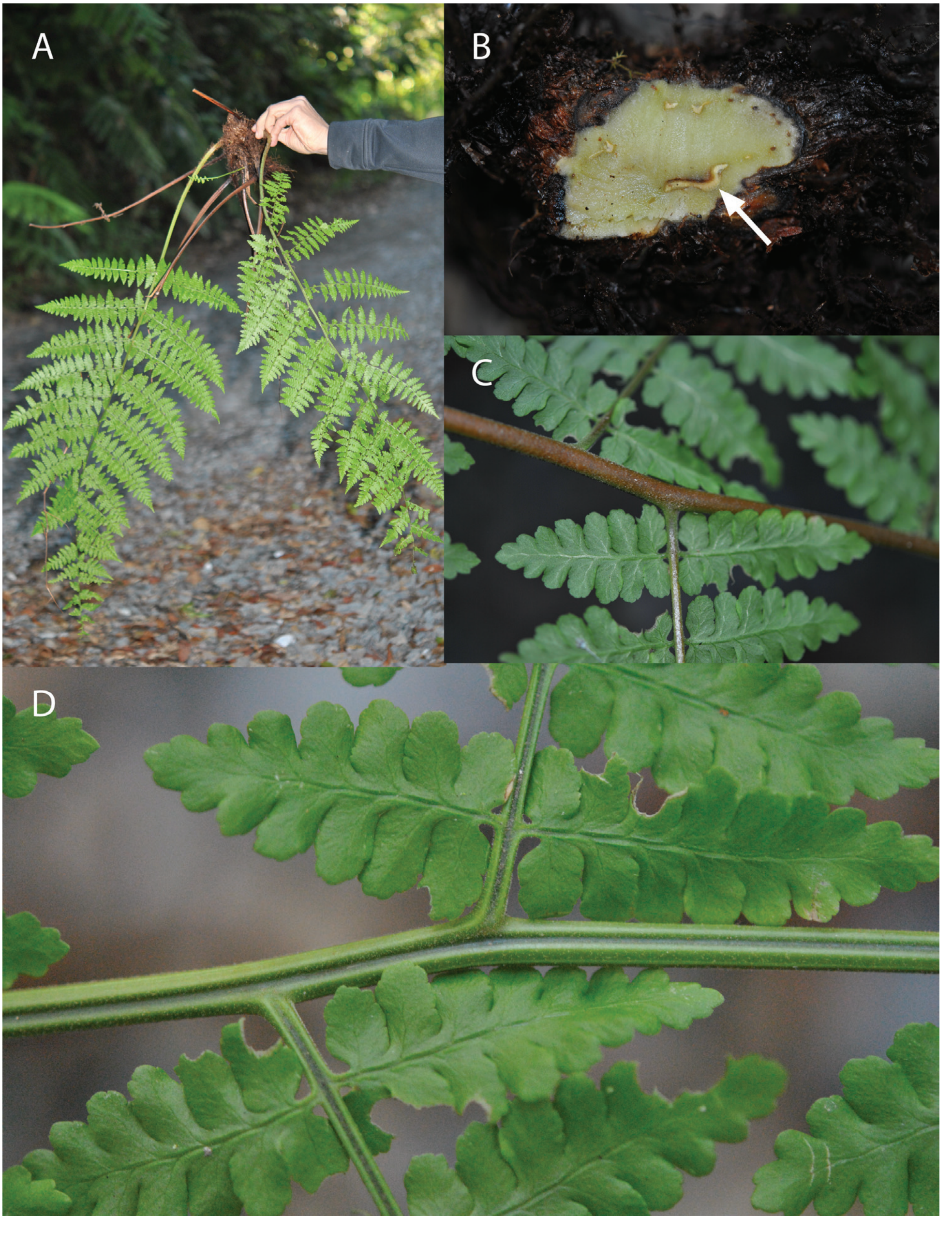

Figure 1. *Revwattsia fragilis*. **A** habit **B** Rhizome in cross section **C** Abaxial rachis and costa **D** Adaxial rachis and costa (M Kessler, M Sundue and M Lehnert 14293).

segments are unknown in *Polystichum* species with large laminae. *Revwattsia* does, however, present morphological features suggestive of a relationship to *Dryopteris*, including the reniform indusium and capitate-glandular indument; characters which are common in *Dryopteris*. On the contrary, the long-creeping rhizome of *Revwattsia* is virtually unknown in *Dryopteris* (present in *D. amurensis* Christ and *D. angustifrons* (T. Moore) Kuntze), as is the epiphytic habit (known in the tropical American species *D. patula* (Sw.) Underw.). Furthermore, the dorsiventral rhizome is absent from the clade that includes *Polystichum* and *Dryopteris*.

Revwattsia presents a taxonomist's classic dilemma; taxonomic placement requires a considered set of decisions about which morphological characters are synapomorphies and which are not. To address this dilemma, we assembled a set of chloroplast DNA nucleotide data from seven markers to infer the phylogenetic relationships of *Revwattsia* and provide insight into its morphological evolution. Included in our inquiry was a test of Jones' 1998 assertion that *Revwattsia fragilis* requires a separate genus within the Dryopteridaceae. In order to understand implications of the taxonomic placement of *R. fragilis*, we also studied its critical morphological characters, namely those of the rhizome, indument, rachis and costa architecture, lamina segment shape, and indusium shape.

Methods

Material

Revwattsia fragilis was collected in the Cook District, Queensland, Australia, along the Mt. Lewis road, ca. 12 km before the shelter at the end of the rd. 16°36'S, 145°17'E, 900 m, M Kessler, M Sundue and M Lehnert 14293 (BRI, VT), 10 Aug 2011. Material for genetic analysis was stored in silica gel until DNA could be extracted. The permit used to collect this material was issued by Dept. of Environment and Resource Management Queensland (Michael Sundue, permit number WISP09438311).

Morphology

Characters for *Revwattsia fragilis* were scored from M Kessler, M Sundue and M Lehnert 14293 at The Pringle Herbarium (VT), and from previously published literature (Watts 1914, Andrews 1990, Jones 1998). We reviewed all salient features, but with particular attention to characters relevant to generic placement i.e. rhizome symmetry and morphology, rachis and costa architecture, lamina dissection, indument, and indusium shape.

Taxon sampling

One-hundred and ninety-eight taxa from 36 genera were used in the phylogenetic analyses including 32 from the Dryopteridaceae. Taxonomic sampling was informed by an initial blast search of the *Revwattsia fragilis rbcL* sequence against the NCBI database (Altschul et al. 1990). The most similar *rbcL* sequences were *Dryopteris erythrosora* (D.C.Eaton) Kuntze, *Dryopteris cystolepidota* (Miq.) C.Chr., and *Dryopteris championii* (Benth.) C.Chr., with 98.6% pairwise identity. Accordingly, our sampling was heaviest in *Dryopteris*, but also included a diverse selection of Dryopteridaceae. We also included more distant outgroups from the Lomariopsidaceae and Pteridaceae. As several generic segregates of *Dryopteris* are suspected to be nested within the genus (Liu et al. 2007b), we included accessions of *Acrorumohra* (H.Itô) H.Itô, *Acrophorus* C.Presl, *Arachniodes* Blume, *Diacalpe* Blume, *Dryopsis* Holttum & P.J.Edwards, *Nothoperanema* (Tagawa) Ching, and *Peranema* D.Don in this study. Some of these taxa have combinations in *Dryopteris*, however recent authors (Liu et al. 2007b, Wu 1999) have treated them under these alternate genera. We use the alternate names to highlight their phylogenetic position. Sequences other than those for *R. fragilis* were downloaded from GenBank; they are primarily from the work reported in Sessa et al. 2012 and Liu et al. 2007a (accession number and herbarium voucher information, Appendix 1).

DNA extraction, amplification and sequencing

Total DNA extraction from silica-dried specimens was accomplished following the CTAB protocol of Doyle and Doyle (1987). Using the Techne TC3000 thermocycler (Techne, Duxford, UK) and the polymerase chain reaction (PCR), two intergenic spacers, *trnG-trnR* and *rps4-trnS*, were amplified for *Revwattsia fragilis*. The primers TRNG1F and TRNR22R (Nagalingum et al. 2007) were used to amplify *trnG-trnR*. Reactions were carried out in 25 mL volumes and included 2.5 mL of 10X PCR buffer, 0.5 mL of 10mM dNTPs, 0.5 mL of 100X BSA, 1.25 mL of the 10 mM forward primer, 1.25 mL of the 10mM reverse primer, 17.85 mL of ddH_2O, 0.15 mL of Ex Taq Polymerase, and 0.5 mL of extracted DNA from *Revwattsia fragilis*. The thermocycler conditions for amplifying *trnG-trnR* comprised an initial denaturation of 2 minutes at 95°C followed by a core sequence of 35 repetitions of 95°C for 30 seconds, 45°C for 30 seconds, and 71°C for 1 minute followed by a final extension of 5 minutes at 71°C. The primers rps4-3er.f (Skog et al. 2004) and trnSr (Souza-Chies et al. 1997) were used to amplify *rps4-trnS*. Reaction conditions for *rps4-trnS* were the same as for *trnG-trnR*. Thermocycler conditions for amplifying *rps4-trnS* comprised an initial denaturation of 3 minutes at 94°C followed by 35 repetitions of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes followed by a final extension of 8 minutes at 72°C. *Revwattsia* rbcL sequences were generated following Schuettpelz and Pryer (2007) using the primers ESRBCL1F and ESRBCL1361R. Resulting PCR products were electrophoresed on a 1% agarose gel in 1x Tris-borate-EDTA (TBE) buffer (pH 8.0) containing ethidium bromide to visualize bands. Automated sequencing took place on an ABI Prism 3130x1 sequencer at the Vermont Cancer Center, Burlington, Vermont, USA. Sequencing primers for *rps4-trnS* were the same primers used for the template amplification. For *trnG-trnR* analysis we used the following sequencing primers: TRNG1F. TRNR22R, TRNG43F, and TRNG63R (Nagalingum et al. 2007). For *rbcL* sequencing we used the amplification primers in addition to ESRBCL628F and ESRBCL654R (Schuettpelz and Pryer 2007).

Sequence alignment and coding

Sequences were edited and aligned using Geneious v5.4.2 (Drummond et al. 2011) and then manually checked for errors. Markers were analyzed separately using Modeltest v3.06 (Posada and Crandall 1998) to determine the model of evolution that each marker most closely fit (Table 1) using the Akaike information criterion (AIC). Indels were coded using the program SeqState 1.4.1 (Müller 2005) and treated in the matrix as standard data.

Phylogenetic analyses

Bayesian inference was conducted on the concatenated data set (*psbA-trnH*, *rbcL*, *rbcL-accD*, *rps4-trnS*, *trnG-trnR*, *trnL-trnF*, and *trnP-petG*) using MrBayes v3.2.0 (Ronquist et al. 2011) using the appropriate evolutionary models determined for each. Sampling of all seven loci was primarily within *Dryopteris*; the remaining taxa, including *Revwattsia fragilis,* had subsets of the seven loci. The Markov chain Monte Carlo permutation of tree parameters was conducted for 2 runs of 5,000,000 generations, sampling every 100th generation. A plot of generations versus log-likelihood was examined using Tracer v1.5 (Rambaut and Drummond 2009) to visually assess stationarity and verify that an appropriate burn-in was achieved. The burn-in was 500,000 generations. The 50% majority-rule tree was examined in FigTree v1.3.1 (Rambaut 2009).

Parsimony analyses using the same data set were conducted using TNT (Willi Hennig Society, Goloboff et al. 2008) implementing the parsimony ratchet (Nixon

Table 1. Characteristics of the cpDNA markers used in the phylogenetic analyses.

Marker	Model (AIC)	Aligned Length of Marker	% Parsimony Informative	Taxa sampled
rbcL	SYM+I+G (26503)	1506	19%	194
trnG-trnR	TIM+I+G (16303)	1290	40%	100
pbsA-trnH	TVM+G (4308)	584	30%	101
rbcL-accD	GTR+I+G (9942)	961	37%	99
trnL-trnF	GTR+G (4594)	297	57%	102
rps4-trnS	TVM+G (7704)	576	51%	101
trnP-petG	TIM+G (9370)	623	50%	99

1999), with the following search parameters: 1000 ratchets with 200 iterations per replicate, 10% weighting, holding 20 trees per ratchet, followed by tree-bisection-reconnection (TBR) branch swapping to completion. Clade support was assessed by implementing a bootstrap analysis of 1000 replicates with 10 ratchets per replicate and holding 20 trees per ratchet. The max RAM was set at 850 MB allowing for storage of 10,000 trees.

Results

Phylogenetic analyses

Of the 5425 total characters, 1717 characters (31.6%) were parsimony informative. In the maximum parsimony analysis (MP) 10,000 most parsimonious trees were retained before maximum storage capacity was reached. The shortest trees had a length of 5531 steps, a consistency index (CI) of 0.40, and retention index (RI) of 0.79. The topology of the Bayesian inference (BI) 50% majority rule tree was largely congruent with the topology of the MP tree but allowed greater resolution of the taxa allied to *Revwattsia fragilis*. Results of the BI and MP analyses place *R. fragilis* in a recently diverged clade within the genus *Dryopteris* (Figures 2 and 3).

In both analyses, there is strong support for placement of *Revwattsia fragilis* within a clade of *Dryopteris* comprising species from southern and eastern Asia. In the Bayesian analysis, *R. fragilis* is sister to the clade comprising *D. cystolepidota*, *D. erythrosora*, *D. gymnosora* (Makino) C.Chr., and *D. labordei* (Christ) C.Chr. (78% posterior probability). This clade in turn is sister to *D. championii* (92% posterior probability), followed by *D. triangularis* Herter (100% posterior probability). These same taxa form a clade in the MP analyses (93% bootstrap support), but relationships between these taxa collapse in the strict consensus of all most parsimonious trees.

Morphological assessment

Revwattsia fragilis exhibits a massive (3 cm diam.) long-creeping rhizome with dorsal leaves and ventral roots (Figure 1A). A rhizome cross-section revealed an elongate ventral meristele (Figure 1B arrow). The rhizome and basal petiole are densely provided with thin, dark brown attenuate scales. The rachis and costa are rounded abaxially (Figure 1C), and are shallowly grooved adaxially (Figure 1D). The grooves are shallowly continuous with the next-order axis (Figure 1D) and they lack a central ridge. These axes are densely provided with short capitate-glandular hairs (Figure 1D). Frond dissection is 2-pinnate-pinnatifid to 2-pinnate-pinnatisect with symmetrical (neither basiscopically nor acroscopically enlarged) pinnae and pinnules (Figure 1A). Fertile fronds have medial sori and light brown reniform indusia.

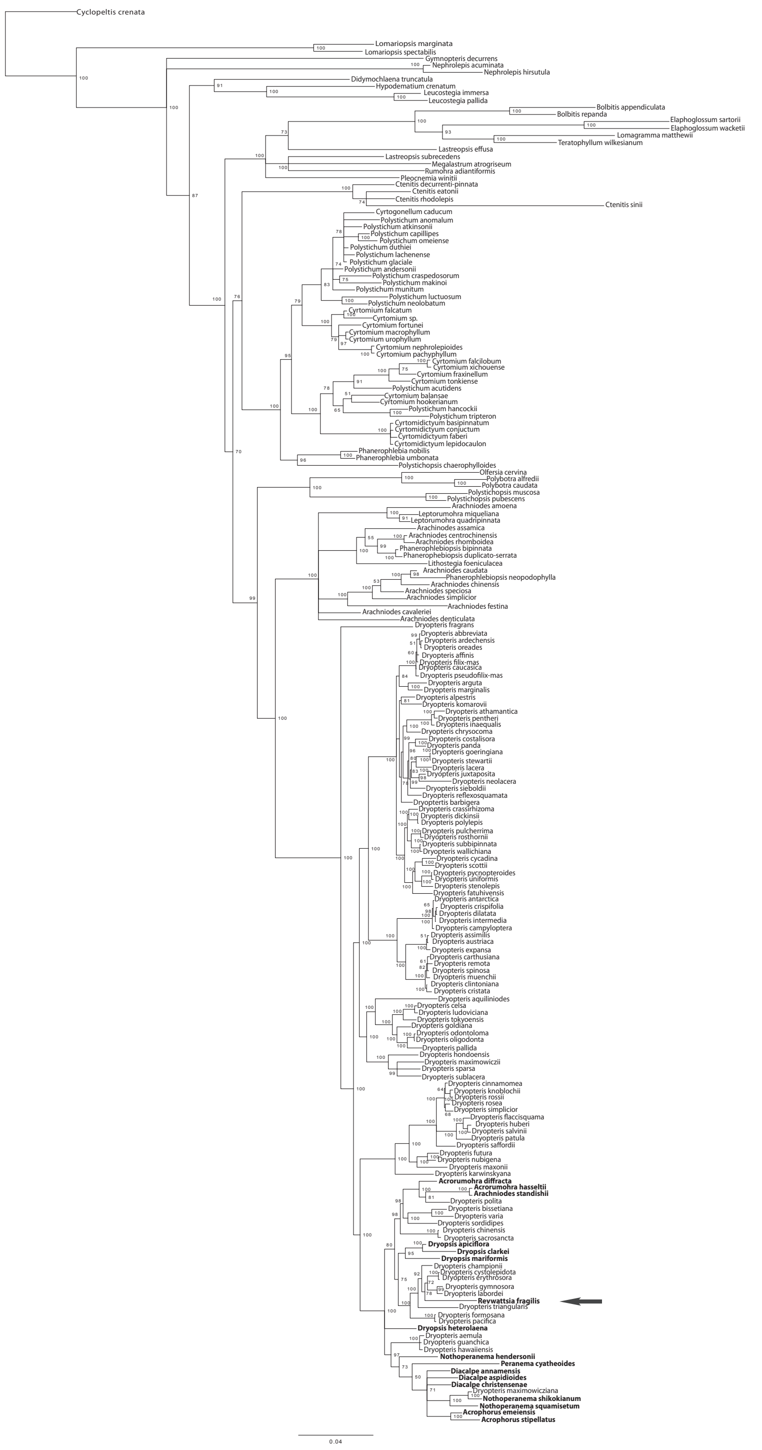

Figure 2. The 50% majority rule tree resulting from Bayesian analysis. Values indicate posterior probabilities, scale bar indicates 0.04 substitutions per site. Arrow indicates position of Revwattsia fragilis.

Figure 3. Strict consensus of 10,000 most parsimonious trees. Values indicate bootstrap support of 1000 pseudoreplicates. Arrow indicates position of Revwattsia fragilis.

Discussion

Monophyly of *Dryopteris*

Results presented here demonstrate that the monotypic genus *Revwattsia* is nested within *Dryopteris* (Figures 2 and 3). Maintaining *Revwattsia* renders *Dryopteris* paraphyletic; we therefore recommend placing the monotypic *Revwattsia* in synonymy under *Dryopteris*.

Paraphyly of *Dryopteris* is further perpetuated by the inclusion of the sampled *Acrophorus* (two species), *Acrorumohra* (two species), *Arachniodes standishii* (T. Moore) Ohwi, *Diacalpe* (three species), *Dryopsis* (three species), *Nothoperanema* (three species), and *Peranema cyatheoides* D. Don. These results do not come as a surprise given the results of other recent phylogenetic studies (Liu et al. 2007b, Geiger and Ranker 2005). The paraphyly of *Dryopteris* presented here corroborates long-standing suspicion about the circumscription of *Dryopteris* segregate genera (Tryon and Tryon 1982) and underscores the need for rich taxon sampling, particularly from Asia, in studies of Dryopteridaceae.

Evolutionary implications

Our assessment of morphological characters largely corroborates those of Watts (1914), Andrews (1990), and Jones (1998). Most of the characters displayed by *Revwattsia fragilis* are known to occur within *Dryopteris*. The dark brown attenuate scales and capitate glandular hairs seen in *R. fragilis* occur frequently in *Dryopteris* (Kramer et al. 1990). The grooved rachis and costae are also typical of *Dryopteris* and many other dryopterid ferns (Holttum 1960). A reniform indusium is characteristic of most Dryopteridaceae and occurs throughout *Dryopteris* as it is currently circumscribed (other indusial shapes, which we take to be autapomorphies, are known from *Acrophorus*, *Diacalpe*, *Notheperanema,* and *Peranema*).

The long-creeping rhizome and elongate ventral meristele of *Revwattsia* (the latter first demonstrated here, Fig. 1B) are distinctive autapomorphies. Although a long-creeping rhizome is known to occur in *Dryopteris amurensis* and *D. angustifrons*, neither is closely allied to *R. fragilis*. These two characters occur in combination sporadically in Eupolypods I (e.g., in *Lomariopsis* Fée (Holttum 1978), the Bolbitidoid clade (Moran et al. 2010), and *Rumohra* (Kato 1974)) and appear to have evolved multiple times. In our experience this combination of characters appears to be correlated with strong dorsiventrality of the rhizome. We take this convergence between our subject species and *Rumohra adiantiformis*, the plant to which Watts presumably thought it most closely related, to be coincidental; Watts never cited these characters in his protolog.

Biogeographic implications

Biogeographic patterns in *Dryopteris* were recently examined by Sessa et al. (2012)—however patterns among Australian taxa were not explicitly addressed. In addition to

Revwattsia fragilis, Australia is home to three species of *Dryopteris*—*D. atrata* (Wall.) Ching, *D. cycadina* (Franch. & Sav.) C.Chr., *D. sparsa* (D.Don) Kuntze (Jones 2012)—and *Acrorumohra hasseltii* (Blume) Ching. All but *D. atrata* are included in our analysis. Unlike *R. fragilis*, these species have relatively broad ranges including India and Sri Lanka, southern China and Japan, and Malesia. In each of these cases, the closest relatives are distributed in southern and eastern Asia, suggesting this region as ancestral for each of the Australian taxa. These species are resolved in clades distinct from each other and from *R. fragilis*, indicating that at least four separate migration events are necessary to explain the current distribution of *Dryopteris* (including *R. fragilis* and *A. hasseltii*) in Australia. The inclusion of the unsampled *D. atrata* in future studies may increase the inferred number of migrations. Our results are comparable to those of Li et al. (2007), who revealed similar migration events from Southern Asia to Australia in the closely related genus *Polystichum*. Although the Sunda and Sahul shelves are currently divided by a deep oceanic trench, these regions were in close proximity 23 mya during the time of the divergence of *Dryopteris* (Sessa et al. 2012, Lohman et al. 2011). It remains unclear whether the migration of *Dryopteris* can be attributed to long distance dispersal or incremental range expansion.

Circumscription of *Dryopteris*

The phylogenetic position of species treated as *Acrophorus*, *Acrorumohra*, *Arachniodes standishii*, *Dryopsis*, *Nothoperanema*, *Peranema,* and *Revwattsia fragilis* demonstrate that the circumscription of *Dryopteris* needs to be expanded. Several of these genera include unique character states that do not occur in *Dryopteris* as currently defined. In addition to the morphological redefinition, expansion of *Dryopteris* to include these segregate genera necessitates numerous nomenclatural innovations. We provide here a name for *Revwattsia fragilis* in *Dryopteris*. The name *Dryopteris fragilis* is previously occupied; therefore a new name is provided.

Taxonomy and nomenclature

Dryopteris wattsii, M. McKeown, Sundue, & Barrington nom. nov. ≡ *Polystichum fragile* Watts, Proc. Linn. Soc. New South Wales 39: 775. 1914 (1915). ≡ *Revwattsia fragilis* (Watts) D.L.Jones [as "*Revwattsia fragile*"], Flora of Australia 48:711. 1998. non *Dryopteris fragilis* C. Chr. TYPE: Australia, Queensland, Majors Homestead, near Ravenshoe, W. W. Watts s.n., Aug 1913 (Syntypes: BRI n.v., MEL n.v., NSW n.v.).

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Appendix

GenBank accession numbers for McKeown et al. 2012 (doi: 10.3897/phytokeys.14.3446.app) File format: Adobe PDF file (PDF).

Explanation note: Genbank Accession numbers are listed in the following order: psbA-trnH, rbcL, rbcL-accD, rps4-trnS, trnG-trnR, trnL-trnF, trnP-petG. The "—" indicates markers that were not available for the taxon.

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RESEARCH ARTICLE

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First instalment in resolution of the *Banksia spinulosa* complex (Proteaceae): *B. neoanglica*, a new species supported by phenetic analysis, ecology and geography

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Abstract

Taxa in the *Banksia spinulosa* Sm. complex (Proteaceae) have populations with sympatric, parapatric and allopatric distributions and unclear or disputed boundaries. Our hypothesis is that under biological, phenetic and diagnosable species concepts that each of the currently named taxa within the *B. spinulosa* complex is a separate species. Based on specimens collected as part of this study, and data recorded from specimens in six Australian herbaria, complemented by phenetic analysis (semi–strong multidimensional scaling and UPGMA clustering) and a detailed morphological study, we investigated both morphological variation and geographic distribution in the *B. spinulosa* complex. All specimens used for this study are held at the N.C.W. Beadle Herbarium or the National Herbarium of New South Wales. In total 23 morphological characters (11 quantitative, five binary, and seven multistate characters) were analysed phenetically for 89 specimens. Ordination and cluster analysis resulted in individuals grouping strongly allowing recognition of distinct groups consistent with their recognition as separate species. Additional morphological analysis was completed on all specimens using leaf, floral, fruit and stem morphology, providing clear cut diagnosable groups and strong support for the recognition of *B. spinulosa* var. *cunninghamii* and *B. spinulosa* var. *neoanglica* as species.

Keywords

Banksia spinulosa, *Banksia cunninghamii*, *Banksia neoanglica,* species limits, phenetics, new species, floral and inflorescence morphology

Introduction

Banksia is a moderately sized genus currently of 212 taxa; viz. 78 species, 9 subspecies and 11 varieties (see Collins et al. 2009), plus 114 species previously included under *Dryandra* (Mast and Thiele 2007). There are 16 named species of *Banksia* in the eastern states of Australia (Collins et al. 2009). Species of *Banksia* are often found in sclerophyllous, heathy shrublands on nutrient poor soils and have spectacular spike-like cylindrical or flattened, head-like conflorescences that are easily recognised (Mast et al. 2005). The growth habit in *Banksia* ranges from small prostrate woody mats to 25 m tall trees. Only one species occurs naturally outside Australia, *Banksia dentata*, which extends to Papua New Guinea, Irian Jaya and the Aru Islands (George 1981; Mast et al. 2002).

According to George's (1999) classification, the *B. spinulosa* complex has the broadest latitudinal, altitudinal and ecological amplitude of any species in the genus (Thiele and Ladiges 1996). The *B. spinulosa* complex consists of four taxa distributed from north-eastern Queensland to eastern Victoria along the coast and highlands. *Banksia spinulosa* var. *spinulosa* occupies both latitudinal extremes but is replaced along the coast between the Sunshine Coast area in south-eastern Queensland and the Hawkesbury River in central eastern New South Wales by *B. spinulosa* var. *collina,* which also has inland outliers west to the Carnarvon National Park area. *Banksia spinulosa* var. *cunninghamii* is mostly a montane taxon distributed mostly between the Hunter River in central eastern New South Wales and eastern Victoria, with a northern disjunction in the McPherson Range along the Queensland–New South Wales border. It is broadly sympatric with, and frequently co-occurs in mixed populations alongside *B. spinulosa* var. *spinulosa* between the northern Blue Mountains and the Moss Vale district. *Banksia spinulosa* var. *neoanglica* is also a montane taxon, distributed from the McPherson Range and along the eastern edge of the New England Tableland, New South Wales south to the Hanging Rock area. *Banksia spinulosa* var. *neoanglica* is parapatric with a montane variant currently attributed to *B. spinulosa* var. *collina* in the Daves Creek area, Lamington National Park; it is allopatric with other taxa in the complex.

Most herbaria follow George (1981, 1988, 1999) in treating this complex as one species with four varieties, viz. *B. spinulosa* var. *spinulosa*, *B. spinulosa* var. *collina, B. spinulosa* var. *cunninghamii*, and *B. spinulosa* var. *neoanglica.* Flora of New South Wales (NSW) Online (1999 onwards) treats the *B. spinulosa* complex as comprising two species, each with two infraspecific taxa: *Banksia spinulosa* var. *collina, B. spinulosa* var. *spinulosa, B. cunninghamii* subsp. *cunninghamii*, and *B. cunninghamii* subsp. A (= *B. spinulosa* var. *neoanglica*), and this paper will use this treatment as a reference point. The primary reason for recognising two species was the broad sympatry of *B. spinulosa* var. *spinulosa* and *B. cunninghamii* subsp. *cunninghamii*. There appears to be no hybridisation between these two taxa, indicating that these two taxa are reproductively isolated from one another and are therefore different biological species (Harden 2002).

These competing taxonomic treatments have created confusion, examples of which can be found in species lists for some National Parks in New South Wales (unpublished visitor brochures), which include *B. spinulosa* var. *neoanglica* and *B. cunninghamii* subsp. A as separate entities. Some herbaria also concurrently use two names for the same entity (see the Atlas of Living Australia). Current circumscriptions of the taxa within the *B. spinulosa* complex are based on intuitive assessment of observed morphological variation, rather than an explicit analysis of the morphological variation. Thiele and Ladiges (1996) conducted a cladistic analysis of the whole of *Banksia* using 92 qualitative characters and 14 morphometric characters in an attempt to clarify interspecific relationships and to provide a phylogenetic classification. As that was a genus-wide study, limited work was conducted on or within individual species.

The aims of this study were (1) to test and set the taxonomic status and circumscription of *B. cunninghamii* subsp. A; and (2) to search for novel diagnostic characters that could be used to distinguish individual taxa within the *B. spinulosa* complex (*sensu* George 1988).

Materials and methods

Study material

Although dried herbarium specimens were available for this study, it was considered necessary to collect fresh material to adequately investigate character homology though a detailed study of different developmental stages. Existing herbarium specimens were deficient in some developmental stages and often were not suitable for destructive sampling. We made collections from locations in New South Wales and Queensland encompassing the full geographic range of *Banksia cunninghamii* subsp. A. Vouchers have been lodged at NE and/or NSW (Table 1). Each site was visited twice; the first time in February to observe the development of the rachis, the second time in May to observe the flowering process. During both visits observations were made and vouchers prepared.

Observations and microscopy

Micromorphology was examined using Leica MZ8 and MZ9 stereomicroscopes fitted with eyepiece graticules. Images were taken using a Wild M400 photomacroscope fitted with a Nikon DS-5M-L1 Digital Sight Camera System. Exploratory scanning electron microscopy of styles was undertaken using air and silca gel-dried samples mounted on double-sided carbon tabs on aluminium stubs. Specimens were coated with gold in a Neocoater sputter coater and examined at 15 kV using a Neoscope JCM-5000 bench-top SEM.

Table 1. Vouchers used in phenetic and morphological analysis of the *B. spinulosa* complex. Numbers in the OTU code are M. L. Stimpson collection numbers. Bcu = *Banksia cunninghamii* subsp. *cunninghamii*; Bco = *B. spinulosa* var. *collina;* Bn = *B. cunninghamii* subsp. A; Bsp = *B. spinulosa* var. *spinulosa*; Bsp? = putative hybrid of *B. spinulosa* var. *collina* × *B. spinulosa* var. *spinulosa*. Abbreviations: NP = National Park; NSW = New South Wales; Qld = Queensland. Voucher codes are herbarium abbreviations following Thiers (continuously updated). All elements of the collections were available at NE and/or NSW during the study, replicates will be distributed.

OTU Code	Location	Voucher
BcuHW30	Hassans Walls, Hartley Vale, NSW	NE, NSW
BcuHW32	Hassans Walls, Hartley Vale, NSW	NE, NSW
BcuHW41	Hassans Walls, Hartley Vale, NSW	NE, NSW
BcuSR43a	Scenic Railway, Katoomba, NSW	NE, NSW
BcuSR43b	Scenic Railway, Katoomba, NSW	NE, NSW
BcuSR43c	Scenic Railway, Katoomba, NSW	NE, NSW
BcuHW114	Hassans Walls, Hartley Vale, NSW	NE, NSW
BcuHW115	Hassans Walls, Hartley Vale, NSW	BRI, NE, NSW
BcuEL117	Evans Lookout, Blue Mountains, NSW	BRI, NE, NSW
BcuCF118	Cataract Falls, Blue Mountains, NSW	NE, NSW
BcuML119	McMahons Lookout, Blue Mountains, NSW	NE, NSW
BcuFF121	Fitzroy Falls, E of Moss Vale, NSW	NE, NSW
BcuFF128	Fitzroy Falls, E of Moss Vale, NSW	NE, NSW
BcuMW122	Medway, W of Moss Vale, NSW	NE, NSW
BcuMW123	Medway, W of Moss Vale, NSW	BRI, NE, NSW
BcuMW126	Medway, W of Moss Vale, NSW	NE, NSW
BcoK25a	Kungala, NSW	NE
BcoK25c	Kungala, NSW	NE
BcoK60a	Kungala, NSW	NE, NSW
BcoK60b	Kungala, NSW	NE
BcoK60c	Kungala, NSW	NE, NSW
BcoKR61	Kremnos, NSW	NE
BcoKR62	Kremnos, NSW	NE, NSW
BcoTG88	Tarragindi, Brisbane, Qld	NE, NSW
BcoDCK102	Daves Creek track, Lamington NP, Qld	NE
BcoDCK85	Daves Creek track, Lamington NP, Qld	NE, NSW
BcoDCK103	Daves Creek track, Lamington NP, Qld	NE
BcoMY93	Mullaway, NSW	NE
BnRN27a	Robinsons Knob Trail, New England NP, NSW	NE, NSW
BnRN27b	Robinsons Knob Trail, New England NP, NSW	NE, NSW
BnRN27c	Robinsons Knob Trail, New England NP, NSW	NE, NSW
BnBP28a	Banksia Point, New England NP, NSW	NE
BnBP28b	Banksia Point, New England NP, NSW	NE
BnBP28c	Banksia Point, New England NP, NSW	NE, NSW
BnNE39a	Point Lookout road, New England NP, NSW	NE
BnNE39b	Point Lookout road, New England NP, NSW	NE, NSW
BnNE39c	Point Lookout road, New England NP, NSW	NE, NSW
BnDCK79	Daves Creek track, Lamington NP, Qld	NE, NSW
BnDCK80	Daves Creek track, Lamington NP, Qld	NE, NSW
BnDCK81	Daves Creek track, Lamington NP, Qld	NE, NSW
BnDCK82	Daves Creek track, Lamington NP, Qld	NE

OTU Code	Location	Voucher
BnBP96	Banksia Point, New England NP, NSW	BRI, NE, NSW
BnTC97	Tom's Cabin, New England NP, NSW	BRI, NE, NSW
BnMM98	Mount Mitchell, NSW	BRI, NE, NSW
BnMM99	Mount Mitchell, NSW	BRI, NE, NSW
BnG100	Girraween NP, Qld	BRI, NE, NSW
BnG101	Girraween NP, Qld	BRI, NE, NSW
BnDCK104	Daves Creek track, Lamington NP, Qld	NE
BnDCK105	Daves Creek track, Lamington, NP, Qld	NE, NSW
BnBB106	Boonoo Boonoo NP, Morgan's Gully, NSW	BRI, NE, NSW
BnBB107	Boonoo Boonoo NP, Morgan's Gully, NSW	NE, NSW
BnBB108	Boonoo Boonoo NP, Morgan's Gully, NSW	NE, NSW
BnBB109	Boonoo Boonoo NP, Cyprus Pine Camp, NSW	NE, NSW
BnGR110	Gibraltar Range NP, Mulligans Hut, NSW	BRI, NE, NSW
BnGR111	Gibraltar Range NP, Mulligans Hut, NSW	NE
BnGR112	Gibraltar Range NP, Mulligans Hut, NSW	BRI, NE, NSW
BnGR113	Gibraltar Range NP, Mulligans Hut, NSW	NE
BspDC42	Darling Causeway, Blue Mountains, NSW	NE, NSW
BspHB44	Hazelbrook, Blue Mountains, NSW	NE, NSW
BspHB45	Hazelbrook, Blue Mountains, NSW	NE, NSW
BspJB46	Jervis Bay, NSW	NE, NSW
BspJB59	Jervis Bay, NSW	NE, NSW
BspML120	McMahons Lookout, Blue Mountains, NSW	NE, NSW
BspFF127	Fitzroy Falls, E of Moss Vale, NSW	NE, NSW
BspMW129	Medway, W of Moss vale, NSW	NE, NSW
BspML130	McMahons Lookout, Blue Mountains, NSW	NE, NSW
BspEL131	Evans Lookout, Blue Mountains, NSW	NE
BspCF132	Cataract Falls, Blue Mountains, NSW	NE, NSW
Bsp?BU36	Bouddi NP, NSW	NE, NSW
Bsp?BU37	Bouddi NP, NSW	NE
Bsp?BU38	Bouddi NP, NSW	NSW
Bsp?CA52a	Calga, NSW	NSW
Bsp?CA52b	Calga, NSW	NE, NSW
Bsp?CA52c	Calga, NSW	NE, NSW
Bsp?CA53a	Calga, NSW	NE, NSW
Bsp?CA53b	Calga, NSW	NE, NSW
Bsp?CA53c	Calga, NSW	NE, NSW
Bsp?M54a	Morisset, NSW	NE, NSW
Bsp?M54b	Morisset, NSW	NE, NSW
Bsp?M54c	Morisset, NSW	BRI, NE, NSW
Bsp?RM66	Morisset, NSW	NE, NSW
Bsp?YM67	Morisset, NSW	NE, NSW
Bsp?JB124	Jervis Bay, NSW	NE
Bsp?MM86	Mount Mee, Qld	NE
Bsp?MM87	Mount Mee, Qld	NE
Bsp?GM89	Glasshouse Mountains, Qld	NE
Bsp?GM90	Glasshouse Mountains, Qld	NE
Bsp?GM91	Glasshouse Mountains, Qld	NE
Bsp?GM92	Glasshouse Mountains, Qld	NE

Phenetic analysis

Selection of characters

The character list was primarily constructed to include leaf, floral, stem and fruit morphology. Assessment of descriptions of the taxa in the *B. spinulosa* complex (George 1981, 1988; Thiele and Ladiges 1996; Harden 2002) led to the selection of characters for the inclusion in the phenetic analysis. Additional characters were considered based on observed differences in the field (Table 2). Wherever possible, quantitative characters were used to reduce subjectivity and to avoid artefacts resulting from the conversion of continuous variables into categorical ones. Qualitative character states were scored as either 1 or 2. Quantitative characters for each OTU were the mean of up to 10 measurements where possible.

Colours, however, were treated as multistate characters to maximise accuracy and repeatability, which allowed for some natural variation, thus avoiding spurious over-

Table 2. Characters used for phenetic analysis for the *Banksia spinulosa* species complex

No.	Character and states
	Quantitative characters
1	Length of complete conflorescence including peduncle ± 1 mm
2	Width of lamina at widest point excluding teeth ± 1 mm
3	Length of lamina including mucro ±1 mm
4	Length from base of lamina to first tooth excluding mucro ± 1 mm
5	Length of seed including wing ± 1 mm
6	Width of wing at widest point ± 1 mm
7	Length of seed excluding wing ± 1 mm
17*	Number of floral pairs around circumference of conflorescence
9	Length of complete infructescence ± 1 mm
10	Circumference of complete infructescence ± 1 mm
12	Lamina interveinal thickness when dry ± 0.05 mm
	Binary characters
11	Lignotuber: 1 = absent 2 = present
20*	Floral bract keel number: 1 = 1 2 = 2
21*	Distal bract margins: 1= plain 2 = recurved
22*	Bract apiculum: 1= absent 2 = present
23*	Bract apiculum: 1 = not incurved 2= incurved
	Multistate characters
8	Lamina apex: 1 = tridentate, 2 = bidentate, 3 = unidentate
13	Colour of lamina adaxial surface when dry~
14	Colour of lamina abaxial surface when dry~
15	Colour of lamina adaxial surface prior to drying~
16	Colour of lamina abaxial surface prior to drying~
18*	Style colour pre anthesis~
19*	Style colour post anthesis~

~ = RHS colours, see Table 3. * = new characters; i.e. not previously used in studies of *Banksia* (cf. Thiele and Ladiges 1996).

precision (see below). Royal Horticultural Society (RHS) colours were used to compare adaxial and abaxial leaf surfaces prior to, and after drying, as well as styles before and after anthesis. Each RHS colour was allocated a number from 1–26 (Table 3).

All leaf measurements were taken from leaves in the middle of a branchlet, selected from the whorl of branchlets subtending a resting terminal bud or conflorescence; leaves were measured after drying. Conflorescence characters such as number of floral pairs were counted live on the plant. Infructescences were measured vertically with a steel ruler and the circumference was measured with a sewing tape measure.

Infructescences were placed on a gas burner for 1–3 min then left on brown paper for two days in a dry place. Seeds were extracted using a pair of forceps and measured under a stereomicroscope using a calibrated eyepiece graticule.

Dataset

A dataset (Appendix 1) was maintained in Microsoft Excel and exported to PATN v. 3 for Windows (Belbin and Collins 2006). The characters were range-standardised and a

Table 3. RHS colour codes used in phenetic analysis

Colours	RHS colours	Coded RHS colours
Green Group	135a–d	1
Green Group	137a–d	2
Yellow Green Group	146a–d	3
Yellow Green Group	147a–d	4
Greyed White Group	156a–	5
Greyed White Group	156b-d	6
Greyed White Group	157a–d	7
Greyed Green group	190a-c	8
Greyed Green Group	190d	9
Greyed Yellow Group	160a	10
Greyed Yellow Group	162a–d	11
Yellow green group	148d	12
Green White group	157b	13
Red Purple Group	59a–d	14
Red Purple Group	61a–d	15
Black Group	202a–d	16
Greyed Yellow group	160b-160d	17
Greyed Green Group	191a–d	18
Greyed Green Group	195a–d	19
Greyed Green Group	196a–d	20
Green Group	139a–d	21
Greyed Green Group	198a–d	22
Yellow Green Group	148a–d	23
Yellow Green Group	145a-d	24
Yellow Green Group	152a-d	25
Greyed Green Group	198a-d	26

distance matrix calculated using the Gower distance metric (Wills et al. 2000). Three-dimensional ordination plots were generated from the distance matrix using semi-strong hybrid multidimensional scaling (SSH MDS) with 100 random starts and 200 iterations to minimise stress. Flexible UPGMA (Beta-value = -0.1) phenograms, 3D ordination scatter plots, and correlation of characters with ordination pattern (PCC) were produced directly within PATN. The criteria for circumscribing distinct taxa were: (1) the OTUs representing the putative taxa formed discrete groups that did not overlap those of any other groups of OTUs in both cluster and ordination analysis and (2) the OTUs within these groups showed an amount of morphological heterogeneity similar to that of the other putative species in the *B. spinulosa* complex included in the analysis (Plunkett et al. 2009). In total 23 characters were used, 11 morphometric, five binary, seven multistate qualitatively coded morphological characters (Table 2).

Diagnostic qualitative morphological characters

Conflorescences

The conflorescences of all taxa in the *B. spinulosa* species complex consist of an elongate woody rachis that has three types of bracts. Below the base of the rachis on the short peduncle are the involucral bracts. The second type of bract is the common bract each of which subtends a flower pair on the conflorescence axis. The third type of bract, a smaller floral bract, subtends each flower in a pair (Johnson and Briggs 1975; George 1981; Thiele and Ladiges 1996). In the early stage of conflorescence development, flower pairs start to develop along the rachis basipetally. The flowers emerge from each side of the floral bracts and above and below each large common bract. Bracts and flower pairs are arranged in vertical columns on the rachis. This pattern is visually enhanced with the development of styles. The vertical striping pattern remains until the perianth and the styles have senesced or fallen from the rachis (George 1981; Thiele and Ladiges 1996; Collins et al. 2009).

Structure of the perianth (floral pairs)

The perianth segments or tepals in *Banksia* each consist of a limb and a claw (Thiele and Ladiges 1996). In *Banksia* and most other Proteaceae the perianth is made up of four tepals (Wrigley and Fagg 1989; Weston 2006).

Structure of the style

The conflorescences in the *B. spinulosa* complex have the appearance of being a particular colour, i.e. black, red, yellow orange, or purple. It is the styles that are most boldly coloured with red, black, green, yellow or purple pigment, not the limb and claw (George

1981; Collins et al. 2009). All styles in the *B. spinulosa* species complex are hooked and extend up to 3 mm past the limb and claw just prior to anthesis. The distal part of the style is modified as a pollen presenter and the stigmatic cavity is located at the apex of the style. The style is released from the limb upon anthesis (Thiele and Ladiges 1996; Weston 2006). All styles in the *B. spinulosa* complex have similar surfaces. Scanning electron microscopy was performed on the style surfaces and no distinguishing features were found.

Leaf morphology

All taxa within the *B. spinulosa* complex have leaves that are scleromorphic in texture, discolourous, and linear in shape. The indumentum on the abaxial leaf surface is felted and the midvein is raised on the abaxial surface of all leaves in all taxa within the complex. Continuous variation was found in the colour of adaxial and abaxial leaf surfaces both within and between populations of all taxa within the *B. spinulosa* complex.

Lignotubers

The term lignotuber refers to a woody swelling which may take the form of an extensive subterranean lignotuber, basal lignotuber, or an above ground lignotuber (Mibus and Sedgley 2000). The development of a lignotuber is considered to have evolved repeatedly in different lineages in response to increased fire frequency (Whelan and York 1998).

Results and discussion

Phenetic analysis

Ordination (Figure 1) and clustering (Figure 2) of the data matrix found five distinct groups of OTUs in the *B. spinulosa* complex: corresponding to *a priori* names *B. spinulosa* var. *collina sens. lat.*, *B. spinulosa* var. *collina* × *B. spinulosa* var. *spinulosa* from near the New South Wales locations of Morisset, Bouddi and Calga, *B. spinulosa* var. *spinulosa*, *B. cunninghamii* subsp. *cunninghamii, B. cunninghamii* subsp. A. The phenogram displays the same five groups of OTUs (Figure 2). Even when we reran the analyses excluding all the binary characters (Characters 11, 20–23; ordination and phenogram not presented), the same five groups of OTUs were obtained, which, along with the very low stress value (Figure 1) indicate that the results are robust. Twelve of the 23 characters, including quantitative, binary and multistate characters had correlated more than 70% with the ordination (Table 4) indicating sound choice of characters, a broad base of evidence for the patterns obtained, and confidence in the results obtained.

The cluster of OTUs of *B. spinulosa* from Morisset, Bouddi and Calga (Table 1) is characterised by red styles, at Morisset and Bouddi and black styles at Calga,

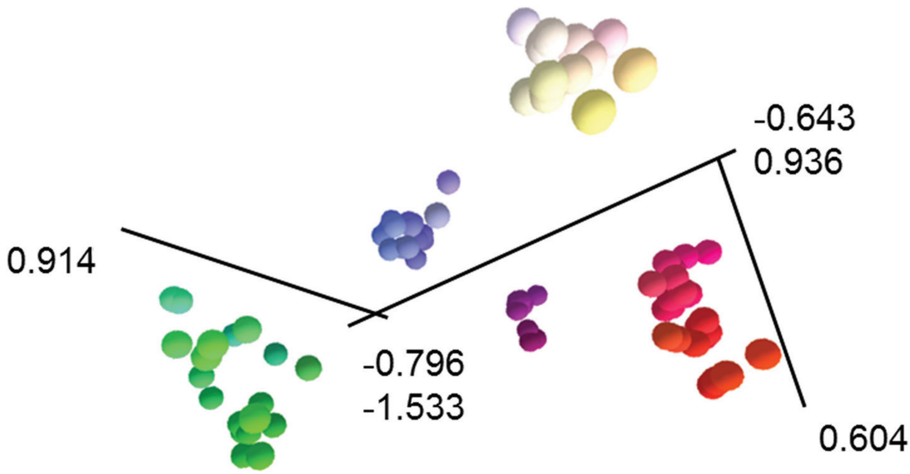

Figure 1. 3D ordination from semi-strong multidimensional scaling of the *Banksia spinulosa* complex. From to left to right**,** *B. spinulosa var. collina sens. lat., B. spinulosa* from Morisset, Bouddi and Calga**,** *B. spinulosa var. spinulosa, B. cunninghamii subsp. cunninghamii, B. cunninghamii subsp. A.* Ordination stress = 0.795. Size and colour of OTUs represents perspective. Ordination orientated to highlight separation of groups of OTUs. See Table 2 for characters and Appendix 1 for data.

Table 4. Principal component correlation (PCC) attributes and ordination vectors for ordination of the *Banksia spinulosa* complex. See Table 2 for Character numbers.

Character	X	Y	Z	Correlation (r^2)
21	-0.115	0.983	0.146	0.978
20	0.35	0.67	0.655	0.953
14	-0.254	0.695	0.672	0.938
23	-0.604	0.473	-0.641	0.938
18	-0.464	0.482	-0.743	0.929
13	0.872	0.195	-0.45	0.917
22	-0.787	-0.327	0.523	0.893
11	-0.534	-0.77	-0.35	0.847
8	0.767	0.269	-0.583	0.84
9	-0.678	0.6	-0.425	0.826
10	0.146	0.72	0.679	0.766
19	-0.104	0.415	-0.904	0.732
3	-0.477	0.868	0.141	0.581
6	0.348	0.868	-0.355	0.537
2	-0.339	0.662	-0.669	0.454
1	-0.47	0.702	-0.535	0.413
16	0.174	-0.25	-0.952	0.391
15	0.45	0.036	-0.892	0.372
12	-0.326	-0.601	0.729	0.308
7	0.433	0.858	-0.277	0.279
5	0.005	0.635	-0.772	0.193
4	0.714	0.694	0.092	0.146
17	-0.614	0.567	-0.549	0.123

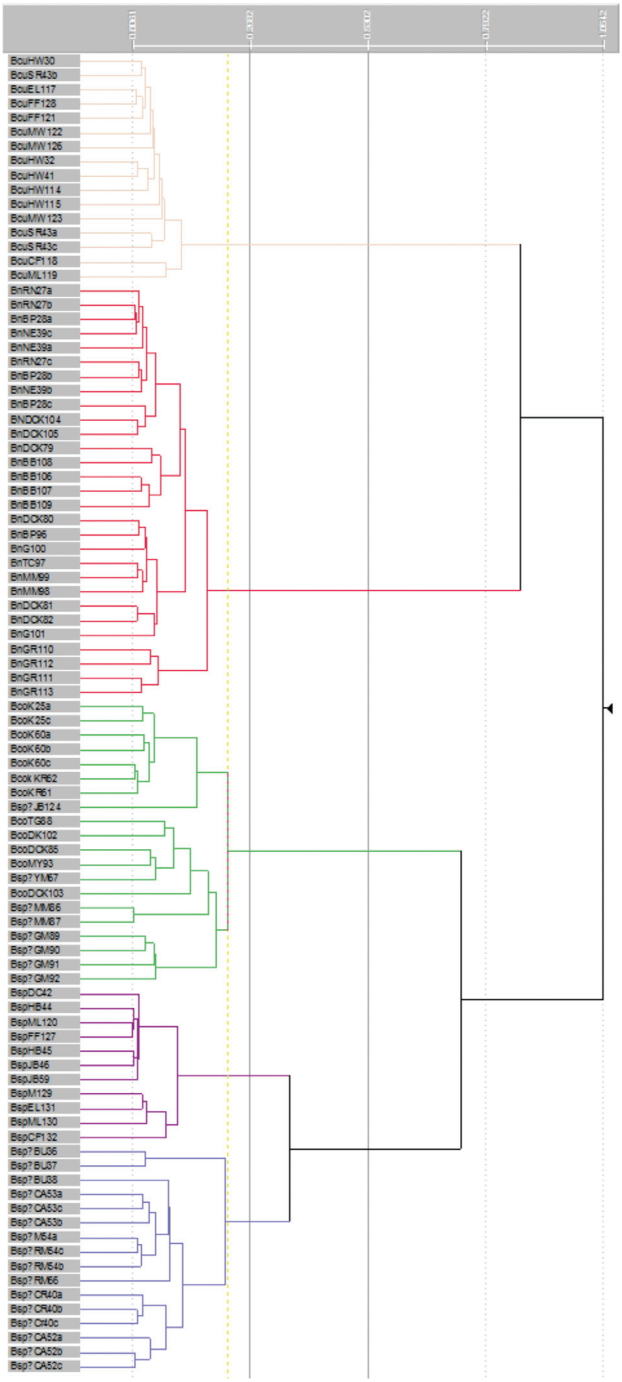

Figure 2. Flexible UPGMA phenogram of OTUs in the *Banksia spinulosa* complex. Major groups from top to bottom: *B. cunninghamii subsp. cunninghamii, B. cunninghamii subsp. A, B. spinulosa var. collina sens. lat., B. spinulosa var. spinulosa, B. spinulosa* from Morisset, Bouddi and Calga. See Table 2 for characters and Appendix 1 for data.

multi-stemmed habit and occurs between the Hawkesbury River and Hunter Valley. Herbarium specimens from these locations have been determined by A.S. George and other botanists as "*B. spinulosa* var. *collina* × *B. spinulosa* var. *spinulosa*". George (1981) considered this group of OTUs to be an intergrade between *B. spinulosa* var. *spinulosa* and *B. spinulosa* var. *collina*. These populations do not fall in a position intermediate between *B. spinulosa* var. *collina* and *B. spinulosa* var. *spinulosa* in the ordination diagram, nor do they segregate into three clusters representing parental species and hybrids. There is thus no clear phenetic evidence of either an intergrade or a mixture of hybrids and parental species between the Hawkesbury River and Hunter Valley. The taxonomic status of these populations and their relationships to others remains unclear. This cluster of OTUs could represent a distinct species, but we will investigate this question and the broader relationship between *B. spinulosa* var. *collina* and *B. spinulosa* var. *spinulosa* further before making any formal taxonomic changes to these taxa.

Slight outliers in the *B. spinulosa* var. *collina* cluster represent some discontinuous morphological variation, which we also plan to investigate.

Taxonomic inference

Given the consistent clear cut groups in the ordination and cluster analysis across a broad geographic and morphological range of OTUs (Table 1), we propose the following taxonomic arrangement, which we use hereafter in this paper: recognising *Banksia cunninghamii* subsp. *cunninghamii* as *B. cunninghamii sensu stricto*; recognising *B. spinulosa* var. *collina* as *B. collina sensu lato*; recognising *B. spinulosa* var. *spinulosa* as *B. spinulosa sensu stricto*; formalising *Banksia cunninghamii* subsp. A at species rank under the name *B. neoanglica* . Although the OTUs of *B. spinulosa* from the Morisset and Bouddi populations could be considered to constitute a distinct species on the evidence we present here, we refrain from recognising these populations as a distinct taxon until we have more thoroughly tested the hypothesis that they are part of an extensive hybrid swarm and searched for any additional populations that might provide evidence for integradation between *B. collina* and *B. spinulosa*.

Morphological analysis

Growth forms within the *Banksia spinulosa* species complex

Banksia cunninghamii sensu stricto is a single-stemmed tree to 7 m tall, and is non-lignotuberous. *Banksia spinulosa sensu stricto* forms a multi-stemmed, rounded shrub to 3 m high. The lignotuber is subterranean. *Banksia collina sensu lato* is a multi-stemmed upright shrub to 3 m tall, with a subterranean lignotuber (Harden 2002; George 1981).

Banksia neoanglica has a variety of growth forms ranging from small rounded multi-stemmed shrubs to single-stemmed trees. The growth forms of *B. neoanglica* appear to be related to the degree of exposure of plants to fire. At sites where there have been no fires for more than 15 years, such as at Binna Burra, Lamington National Park, Queensland and some parts of Gibraltar Range National Park, New South Wales (Pers. Comm. Justin Kreis Ranger Glen Innes National Park), *B. neoanglica* is a single-stemmed tree and exhibits all the traits of an obligate seeder such as a greater infructescence load and spontaneous opening of the follicles. In the tree form, *B. neoanglica* has a slight swelling at the base of the trunk just below the soil or there are epicormic buds which often develop into branches, well above ground level, similar to those of some eucalypts (Burrows 2008). The multi-stemmed form has a substantial subterranean lignotuber and requires fire to open follicles and has a greatly reduced infructescence load.

Individual adult morphological features

Styles: The structure of the conflorescence, including perianth and styles is similar for all taxa in the *B. spinulosa* complex. Size, shape and colour of the individual parts of the conflorescence, however, differ considerably across the species. Style colour in the *B. spinulosa* complex varies depending on the proportions of chlorophyll (green), carotenoid (yellow to orange), anthoxanthin (yellow) and anthocyanin (red to purple) pigments that develop in them (Grotewold 2006). The style colour in *B. neoanglica, B. spinulosa sensu stricto and B. cunninghamii sensu stricto* usually grades from red to maroon to purple during conflorescence development, then the style becomes discolorous at anthesis, with the apex becoming dark purple to black. This is a consistent character within and between populations of three species in the *Banksia spinulosa* complex. The exception is *B. collina sensu lato* which has concolourous green styles both before and after anthesis. We found no black-styled *B. collina sensu lato* within the geographical range of this project.

The style apex *in B. cunninghamii sensu stricto* seems to have substantially more anthocyanin pigment than either *B. spinulosa sensu stricto* or *B. neoanglica*. In *B. cunninghamii sensu stricto* we observed that the style length is usually longer than either *B. spinulosa sensu stricto* or *B. neoanglica* and is a similar length to *B. collina sensu lato*. The black pigmentation of the styles of *B. cunninghamii sensu stricto s*tarts to develop one third of the way along the style above the ovary. In *B. spinulosa sensu stricto* and *B. neoanglica* the dark pigmentation in the style develops one half to two thirds of its length above the ovary. In all populations in the *B. spinulosa* complex with the exception of *B. collina sensu lato* we observed what appeared to be yellow-styled conflorescences. Upon closer inspection they are green styled and appear to have less chlorophyll in both the styles and leaves than is found in *B. collina sensu lato* which is also green-styled. Green styled variants are found in less than 2% of any one population except in *B. collina*. Polymorphism is a common trait in Proteaceae where, for example, 40% of all species of *Protea* exhibit variation in the bract, style and perianth colour (Carlson et al. 2010). It is often unclear whether these variants are transient mutant individuals or this feature is a persistent polymorphism (Carlson et al. 2010). In the case of the *Banksia spinulosa* complex, however, the variants comprise less than 2% of a population and were not found in every population; therefore it is unlikely to be persistent polymorphism.

Perianth colour: The colours of the perianth in the *B. spinulosa* complex vary according to their developmental stage and their exposure to sunlight. The perianth colours can vary within and between populations in all four of the species in the *B. spinulosa* complex. The factor that seems to have the most influence on the perianth colour in the early stages of development is exposure to sun, often mediated by the position of an conflorescence on the outside or inside branches of the plant or by shading from other plants. In *B. spinulosa sensu stricto*, *B. collina* and *B. neoanglica*, the conflorescences that are exposed to full sun tend to have orange or yellow perianths. Those that are exposed to a limited amount of sun tend to be green. The perianth colour of *B. cunninghamii sensu stricto* is diagnostic for the species. At maturity the perianth always has a distinct pink hue and this colouring continues through to anthesis. The pink hue does not vary between and within populations of *B. cunninghamii sensu stricto,* nor does exposure to full sun or full shade effect the colour of the perianth at maturity.

Common bracts: Common bracts have been mentioned in previous studies (Johnson and Briggs 1975; Thiele and Ladiges 1996; George 1981) but the bract surfaces had not been mentioned before this study or used to draw taxonomic conclusions. Close examination, especially at early stages of development, of the abaxial surface of the common (or flower pair) bracts found them to have differences in shape, texture, colour, and surface (Figure 3A–D) which covary in line with the entities recognised here (Figures 1–2) within the *B. spinulosa* complex. We will characterise these differences for use in future expanded phenetic analysis and description of taxa in *Banksia*. Floral bracts were not examined in detail in this study.

Involucral bracts: Involucral bracts appear to be taxonomically informative at the species level in the study group. The involucral bracts of *B. cunninghamii sensu stricto* are caudate with an abaxial 'spine' (Figure 4A). The involucral bracts in *B. spinulosa sensu stricto* (Figure 4B) are longer and more scleromorphic, with little or no hair and no external spine. In *Banksia neoanglica* (Figure 4C) these bracts are more hirsute without an external spine and in *B. collina sensu lato* (Figure 4D) the involucral bracts are shorter, have no external spine and limited hair. There are differences in the distal and proximal portions of the involucral bracts (Figure 4A–D) in each species that warrant further examination.

Taxonomic conclusions

The diversity of species concepts in the biological literature is an asset, not a liability when considering the *Banksia spinulosa* complex and are an integral part of biological theory. We have taken into account the co-varying morphological discontinuities, the phenetic species concept, geographical and ecological isolation and the biological species concept of reproductive isolation. The use of differing concepts has been useful in suggesting multiple lines of evidence for testing taxonomic boundaries in the *Banksia spinulosa* complex (cf. de Queiroz 2007). Clear taxonomic groups were obtained based

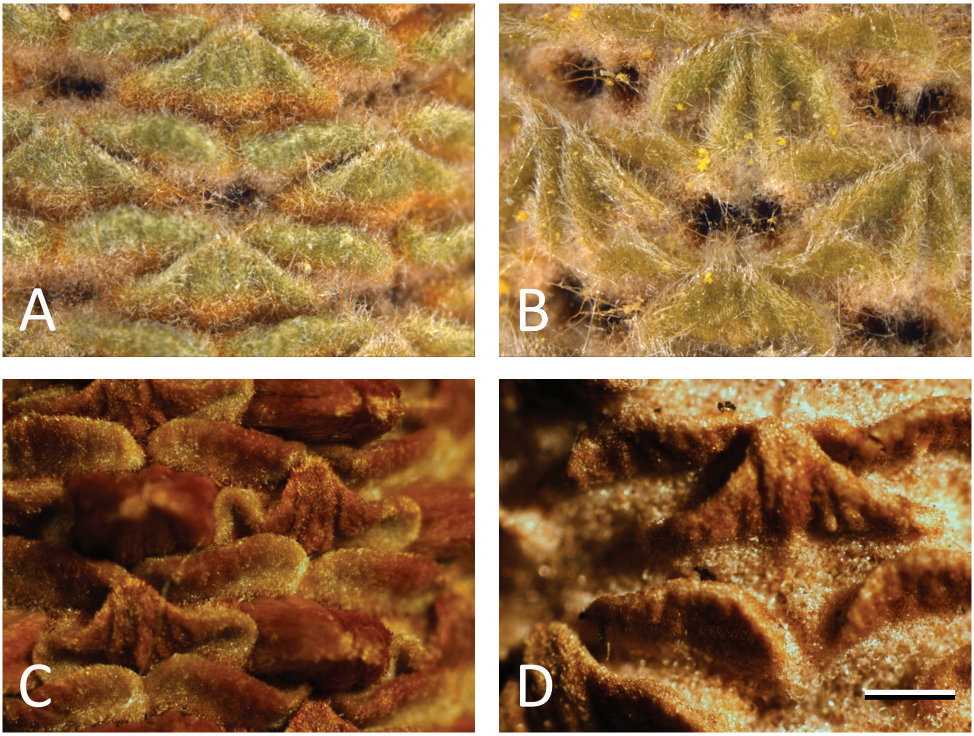

Figure 3. Common bracts on young conflorescences in the *Banksia spinulosa* species complex: **A** *B. neoanglica* (*M.L. Stimpson 98*) **B** *B. cunninghamii sensu stricto* (*M.L. Stimpson 122*) **C** *B. collina sensu lato* (*M.L. Stimpson 25A*) **D** *B. spinulosa sensu stricto.* (*M.L. Stimpson 120*). Scale bar = 1 mm.

on the results of the morphometric analyses and corroborated by new characters (cf. Thiele and Ladiges 1996) such as the abaxial surface of the common bract (Figure 3), the number of floral pairs around the circumference of the conflorescence and obvious differences in the involucral bracts (Figure 4A–D). Additionally, given the ecological isolation, reproductive isolation and morphometric differentiation of at least three of the taxa in the *B. spinulosa* complex, there is a compelling case to recognise *Banksia spinulosa sensu stricto*, *Banksia cunninghamii sensu stricto* and *Banksia neoanglica* as separate species (Table 5). *Banksia collina sensu lato* is considered heterogeneous and in need of further study, but is not readily confused with *B. neoanglica*. Similarly *B. spinulosa* from the Morisset, Bouddi and Calga requires further study but is distinct from *B. neoanglica.*

The geographic distribution of *Banksia neoanglica* falls within the biogeographic region known as the "Macpherson–Macleay Overlap" of Burbidge (1960), which is a biogeographically distinctive and rich area (Crisp et al. 1999) with many species of plants and invertebrates endemic to the area.

Banksia cunninghamii sensu stricto and *B. neoanglica* have often been misidentified because *B. cunninghamii sensu stricto,* on occasions, has a brown indumentum; *B. neoanglica* sometimes also exhibits browning on the abaxial leaf surface. This char-

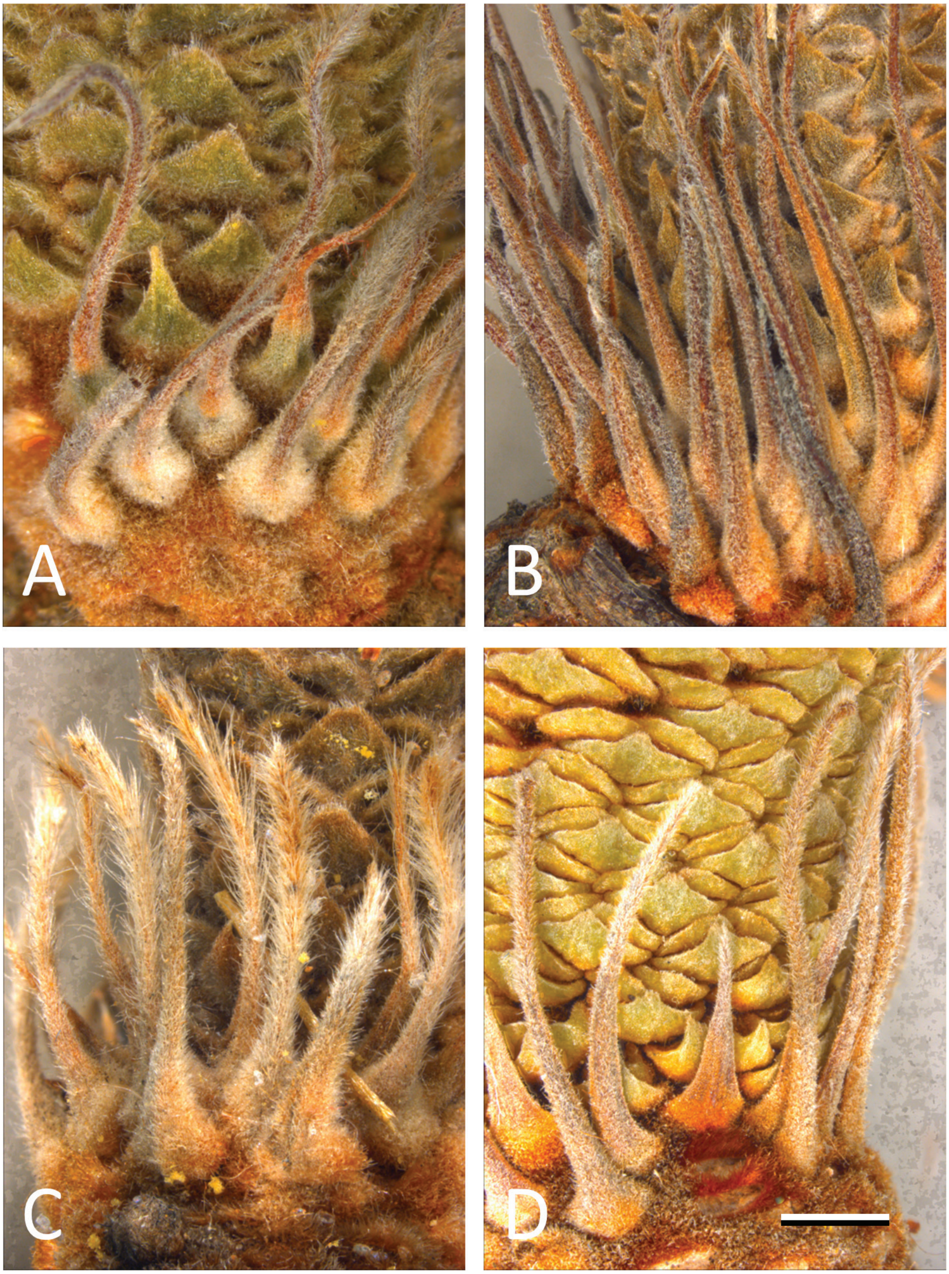

Figure 4. Involucral bracts on young conflorescences in the *Banksia spinulosa* complex. **A** *B. cunninghamii sensu stricto* (*M.L. Stimpson 122*) **B** *B. spinulosa sensu stricto* (*M.L. Stimpson 125*) **C** *B. neoanglica* (*M.L. Stimpson 81*) **D** *B. collina sensu lat*o (*M.L. Stimpson 25A*). Scale bar = 2.5 mm.

acter has been used in the past as an aid to distinguishing *B. cunninghamii sensu stricto* and the two other 'varieties' recognised at that time (George 1981; Harden 2002). Indeed, this attribute occurs in both *B. neoanglica* and *B. cunninghamii sensu*

Table 5. Comparison of some attributes of *Banksia neoanglica, B. spinulosa sensu stricto* and *B. cunninghamii*.

Character	*B. neoanglica*	*B. spinulosa*	*B. cunninghamii*
Lignotuber	present	present	absent
Leaf length	43–75 mm	50–72 mm	53–88 mm
Leaf width	3–4.5 mm	1.5–2.5 mm	3–4.5 mm
Leaf margins	not recurved	tightly recurved	not recurved
Length of inflorescence	84–119 mm	96–144 mm	99–152 mm
Common bract keels	single thickened keel	single keel apex	two thin keels
Common bract apex	apex rounded	apiculate	apex rounded
Number of floral pairs	12–14(–16) pairs	13–16 pairs	12–14 pairs
Perianth colour	orange, or yellow	orange or yellow	pink
Style colour prior to anthesis	red/maroon	red/maroon	red/maroon
Style colour after anthesis	purple/black	purple/black	purple/black
Circumference of infructescence	141–160 mm	153–159 mm	113–125 mm
Length of infructescence	85–120 mm	96–144 mm	113–140 mm

stricto. Drying of the specimens in both of these species can cause browning on the abaxial leaf surface. The browning of the abaxial leaf surface should not be used as taxonomic marker or an identification tool.

Future directions

Disjunct populations in central and northern Queensland currently assigned to *B. spinulosa* var. *spinulosa* warrant inclusion in a more broadly framed analysis, as do the northern and southern populations of *B. collina sensu lato* and Victorian populations of *Banksia cunninghamii sensu stricto*. There are also other populations of *Banksia* that clearly belong with the *B. spinulosa* group but are as yet unstudied. Further work is needed to enable suitable placement of these populations. Analysis using molecular data, together with expanded use of the novel characters presented here, would likely resolve these long-outstanding taxonomic issues.

Taxonomic treatment

***Banksia neoanglica* (A.S.George) Stimpson & J.J.Bruhl, stat. nov.**
http://species-id.net/wiki/Banksia_neoanglica

Banksia spinulosa Sm. var. *neoanglica* A.S.George, *Nuytsia* 6: 315 (1988).

Type. AUSTRALIA: New South Wales: Northern Tablelands, 900 m along Waterfall Way towards Ebor from turn-off to New England National Park, 22 May 2011, *M.L. Stimpson 180, J.J. Bruhl & I.R. Telford*; neotype: NSW; isoneotype: AD, BRI, CANB, CNS, K, MEL, NE, MO, PERTH. Figure 5.

Figure 5. Photograph of the neotype of *Banksia spinulosa* var. *neoanglica* A.S.George (*M.L. Stimpson 180, J.J. Bruhl & I.R. Telford*, NE 98613).

B. spinulosa Sm. var. *cunninghamii* (Sieber ex Rchb.) A.S.George, *Nuytsia* 3: 396 (1981) *pro parte*, excluding type.

B. cunninghamii Sieber ex Rchb. subsp. A: G.J. Harden in G.J. Harden (ed.), *Flora of New South Wales* 1: 71 (1991); G.J Harden, D.W. Harden & D.C. Godden (2000) *Proteaceae of New South Wales*: 170 (2000); G.J. Harden in G.J. Harden (ed.), *Flora of New South Wales* 2, edn 2: 86 (2002).

The protologue of *B. spinulosa* var. *neoanglica* quotes the type:

"1 km N of turnoff to New England National Park, Ebor–Armidale road, N.S.W., 6 April 1986, *S.C. Clemesha*; holo: NSW; iso: CANB, BRI, MEL, PERTH".

No specimens so labelled have been located in NSW, BRI, CANB or MEL herbaria after repeated searches. Alex George (pers. comm. 2010–2011) could find no specimens in PERTH and he believes it likely that specimens were never distributed. Accordingly, we have nominated a neotype, collected from the same population as the type.

Description. Shrubs with 2–8(–10) stems to 2.5 m from a lignotuber or trees to 7 m tall. Juvenile leaves: petiole 2–3.8 mm long; lamina narrowly obovate, 30–66 mm long, 5–11 mm wide, strongly dentate along full leaf margin, apex bidentate. Adult leaves: petiole 1.8–3.5 mm long; lamina linear, 43–75 mm long, 3–4.5 mm wide, occasionally toothed towards the usually unidentate, occasionally bidentate apex; adaxial surface glabrous, with colour after drying RHS greyed green group 195a-d; abaxial surface felted, colour after drying RHS greyed white group 156a–d. Involucral bracts subulate, thickened at base, 3–15mm long, grey-brown pubescent. Conflorescence 84–119 mm long, 70–85 mm diameter at anthesis; floral pairs 12–14(–16) around the circumference of the conflorescence axis. Common bract with a single thickened keel on the abaxial surface that extends from the apex of the bract down to the visible part of the base of the bract, distal margins slightly concave, apex rounded, indumentum villous, lower third of bract uniformly brown and upper two thirds uniformly green (fig. 3A). Perianth 18–23 mm long, pubescent, yellow–orange at maturity but may be green, orange or yellow during developmental stages; limb c. 3.5 mm long; anthers c. 1 mm long. Style 25–38 mm long, apically hooked, colour grading from red to maroon to black just prior to anthesis. Infructescence 85–120 mm long, 35–45mm diam. Seed 15–19 mm long, including wing. Figure 6.

Distribution. *Banksia neoanglica* occurs on the McPherson Range, just north of the Queensland–New South Wales border, Mt Warning and the eastern edge of the New England Tableland southwards to near Hanging Rock, New South Wales. Figure 7.

Ecology. Grows in sandy soil on granite and acid volcanics, rarely on basalt, in *Eucalyptus* open forest (Figure 6), woodland and heath at altitudes of 850–1480 m. The species is sympatric with *Banksia integrifolia* subsp. *monticola* throughout its range, with *B. marginata sensu lato* on the Gibraltar Range and with *B. conferta* in the Daves Creek area.

The growth forms that *B. neoanglica* assume appear to be dependent upon the exposure to fire (Whelan and York 1998). In areas where there have been no fires for more than 15 years, such as Lamington National Park, Queensland, and some parts of Gibraltar Range, New South Wales (pers. comm. Justin Kreis 25 May 2010), a single-stemmed habit is found. Here, the lignotuber is present as a stem thickening just above

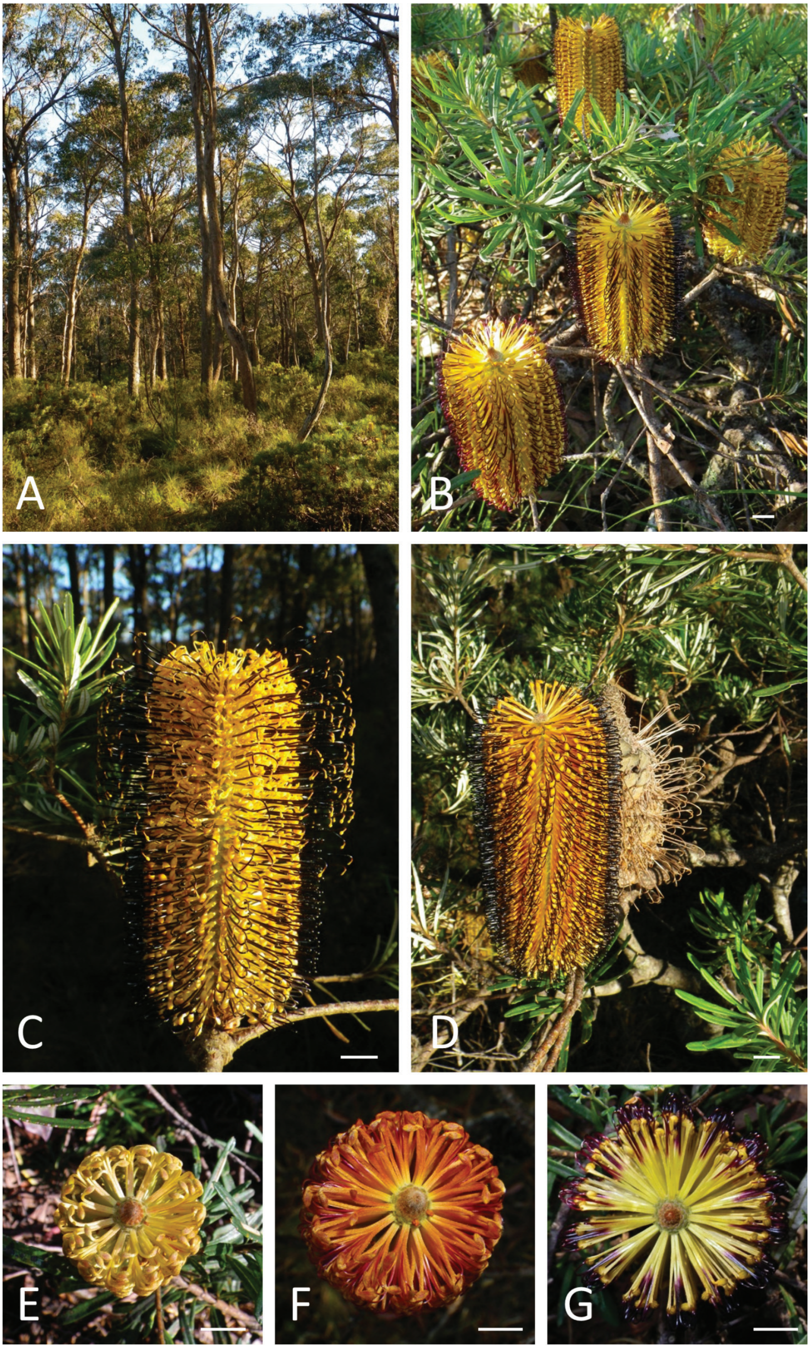

Figure 6. *Banksia neoanglica* at neotype locality. **A** Habitat **B** Conflorescences on shrub **C** Conflorescence from the neotype collection (*M.L. Stimpson 180, J.J. Bruhl & I.R. Telford*) showing basipetal development; upper flowers with pollen on pollen presentors **D** Conflorescence and infructescence with black styles at preanthesis. **E–G** Apex of conflorescences at successive stages of development exhibiting variation in perianth and style colour. Scale bars = 1 cm.

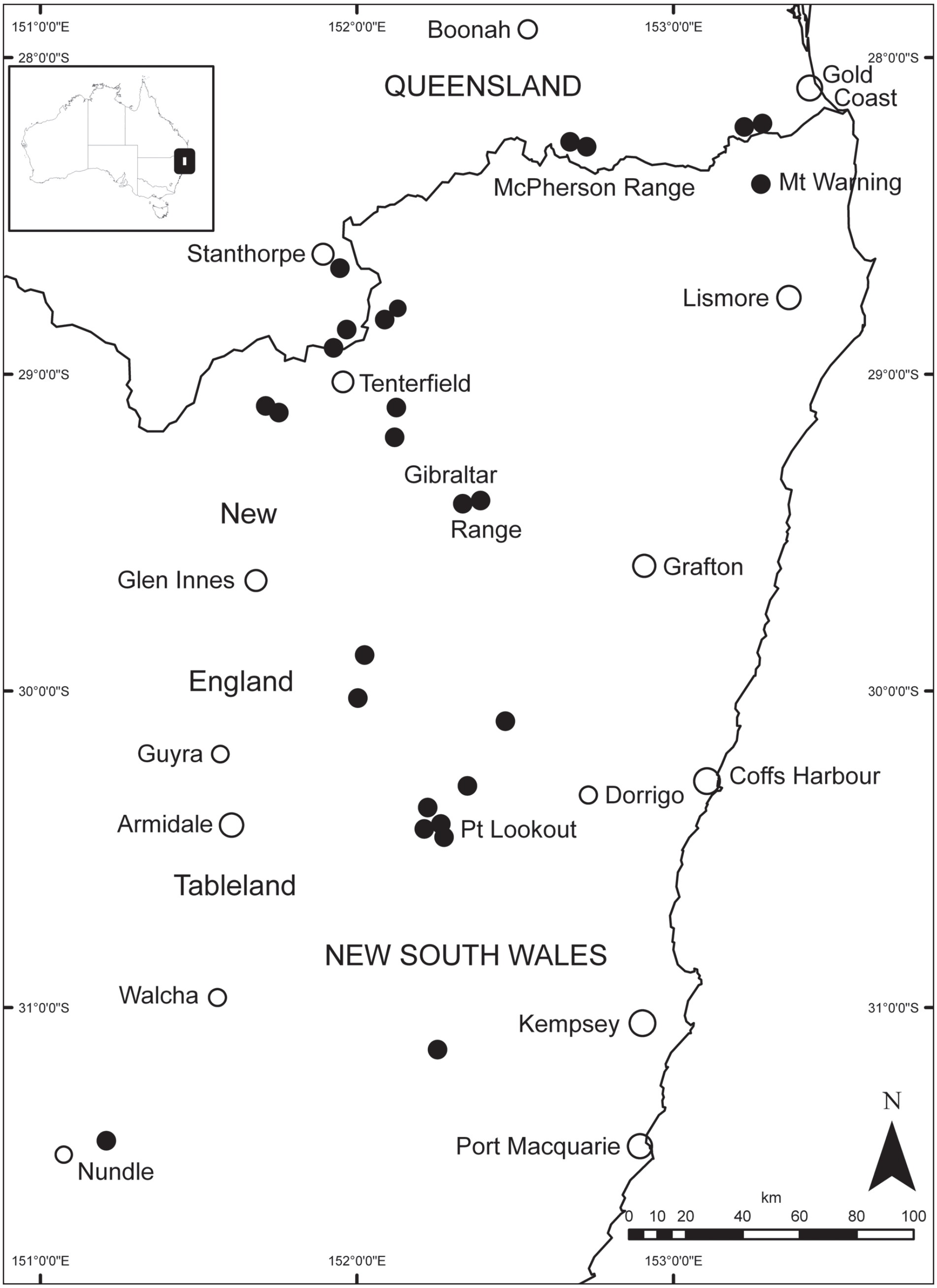

Figure 7. Distribution of *Banksia neoanglica* (solid black circles). Towns and cities indicated by open circles.

or just below the soil surface, and branchlets may sprout from epicormic buds up to 30 cm above the ground. This single-stemmed form of *B. neoanglica* behaves like an obligate seeder with a heavy infructescence load and follicles open spontaneously without fire. More commonly the plants are multi-stemmed, with up to 2–8(–10) stems from a subterranean lignotuber carry a much lower infructescence load, usually 1–3(–5) infructesences per plant. Fire is required to open the follicles.

Conservation status. The species is widespread, often locally common, and is not considered at risk. It is conserved in several reserves: Lamington, Springbrook and Girraween National Parks in Queensland, and Boonoo Boonoo, Gibraltar Range and New England National Parks and Torrington State Conservation Area in New South Wales.

Selected specimens examined. AUSTRALIA. Queensland: Moreton District: McPherson Range, Lamington National Park, Daves Creek track, *M.L. Stimpson 79* (BRI, NE, NSW); Darling Downs District: Girraween National Park, track to Mt Norman, 21 Jan. 2009, *I.R. Telford 13278 & J.J. Bruhl* (NE). New South Wales: North Coast: Mount Warning, 3 Oct. 1939, *F.A. Rodway s.n.* (NSW); Northern Tablelands: 19 km E of Deepwater on Miles Shaw Rd, Butterleaf State Forest, *J.T. Hunter 3750 & P.J. Clarke* (NE);); 0. 4 km N of Torrington, 19 Nov 1972, *J.B. Williams s.n.* (NE); Pheasant Mountain, 32 km NE of Guyra, 24 Apr. 1972, *H.J. Wissmann s.n.* (NE); Mount Chaelundi, E side just below crest, *J.T. Hunter 157 & V.H. Hunter* (NE); New England National Park, Banksia Point, *M.L. Stimpson 28* (BRI, NE, NSW); NE of Bakers Downfall Hill, Nundle State Forest, *J.R. Hosking 1877* (CANB, MEL, NE, NSW).

Phenology. Resting buds start to expand in late January and conflorescences are fully developed by late March with flowering continuing until early July. These times are dependent on climatic conditions.

Breeding system. Extensive experiments conducted between May 1986 and July 1987 found that the New England population of *B. neoanglica* studied was autogamous (Vaughton 1988).

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Appendix 1

Dataset of *Banksia spinulosa* complex used for phenetic analysis. See Table 1 for OTU codes and Table 2 for character list. (doi: 10.3897/phytokeys.14.3415.app) File format: MS Word (DOC).

Explanation note: The dataset (organised in MS Excel) presented here as a MS Word document, includes 23 characters and 92 individuals (operational taxonomic units = OTUs).

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