Volume 16: 127–132
Publication date: 18 August 2014
dx.doi.org/10.7751/telopea20147090

TELOPEA
Journal of Plant Systematics

The Royal Botanic Gardens & Domain Trust

plantnet.rbgsyd.nsw.gov.au/Telopea • escholarship.usyd.edu.au/journals/index.php/TEL • ISSN 0312-9764 (Print) • ISSN 2200-4025 (Online)

Phebalium verrucosum (Rutaceae), new status for a taxon excluded from *P. squamulosum* on morphological and phytochemical evidence

Ian R.H. Telford and Jeremy J. Bruhl

Botany and N.C.W. Beadle Herbarium, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia
Author for correspondence: itelford@une.edu.au

Abstract

Phebalium squamulosum Vent. subsp. *verrucosum* Paul G.Wilson (Rutaceae) is excluded from *P. squamulosum* and raised to the rank of species as *P. verrucosum* (Paul G.Wilson) I.Telford & J.J.Bruhl based on morphological and essential oil data. The distribution of the species is mapped and its conservation status is revised.

Introduction

The genus *Phebalium* Rudge (Rutaceae), widespread through southern and eastern Australia, contains 28 species (Wilson 1970, 2013; Kubitzki et al. 2011; Australian Plant Census 2007–). Several species show considerable morphological variation and several more species may require recognition. In particular, *P. squamulosum* Vent. exhibits considerable polymorphism, with nine subspecies currently recognised by Wilson (2013) in the last major revision of the genus. One of these, *P. squamulosum* subsp. *verrucosum* Paul G.Wilson, then known from few collections, was thought to be narrowly endemic to the Macleay River gorge system (Weston and Harden 2002, 2012) with Wilson (2013) presenting a wider distribution, citing a collection from Gloucester Tops.

Taxonomic utility of essential oils in *Phebalium*

Essential oil data have proved useful as a taxonomic tool in *Phebalium* (Brophy et al. 2006; Pala-Paul et al. 2009) with essential oil profiles characteristic of species. The unusual ketone, dihydrotagetone, first isolated from *Tagetes minuta* L. (Jones and Smith 1925, as *T. glandulifera* Schrank) was shown to be a major component in oils from *P. glandulosum* Hook. subsp. *macrocalyx* R.L.Giles (Lassak and Southwell 1974, as subsp. *glandulosum*). Didydrotagetone appears to be a chemical marker for *P. glandulosum* with essential oil of three additional (of six) subspecies shown to have high concentrations: *P. glandulosum* subsp. *glandulosum* (Brophy et al. 2006), *P. glandulosum* subsp. *nitidum* Paul G. Wilson and *P. glandulosum* subsp. *eglandulosum* Paul G.Wilson (Sadgrove et al. 2013). Besides morphological discontinuities between subspecies, the essential oils of *P. glandulosum* subsp. *angustifolium* contains no dihydrotagetone. Unfortunately, in their recent investigation of *P. glandulosum* subsp. *glandulosum* using morphological and anatomical data, Giles et al. (2008) did not test the other subspecies currently assigned to *P. glandulosum*.

A chemical marker for the *P. squamulosum* group appears to be the sesquiterpene ketone squamulosone, first hydrodistilled from leaves of *P. squamulosum* by Batey et al. (1971). Although no voucher was cited, the locality of collection of the sample, Narrabeen, Sydney, would place it in the type subspecies. Only two other taxa at present referred to *P. squamulosum* have yielded squamulosone as their major essential oil component (Sadgrove et al. 2014): *P. squamulosum* subsp. *lineare* Paul G.Wilson and the Gloucester Tops population assigned by Wilson (2013, p. 474) to *P. squamulosum* subsp. *verrucosum* but which we consider to represent another putative new species (Telford and Bruhl, unpublished data). Analyses from other populations currently assigned to *P. squamulosum* subsp. *squamulosum* in north-eastern New South Wales (Pala-Paul et al. 2009; Sadgrove et al. 2014) and Queensland (Brophy et al. 2006) have identified different chemotypes all lacking squamulosone, most with elemol as the main component of their essential oils, indicating their probable taxonomic misplacement. Essential oil from the several populations of *P. squamulosum* subsp. *verrucosum* sampled (excluding the Gloucester Tops population) lack squamulosone and elemol, but have a high dihydrotagetone content (Sadgrove et al. 2013). Therefore, *P. squamulosum* subsp. *verrucosum* is clearly distinct from the other subspecies currently recognised for this species.

Morphological distinctness of *Phebalium squamulosum* subsp. *verrucosum*

Besides this phytochemical evidence, morphological differences between the named subspecies (Telford and Bruhl, unpublished data) reinforce the need for their taxonomic reassignment. *Phebalium squamuosum* subsp. *verrucosum* differs from most taxa included under *P. squamulosum* in its vegetative and floral morphologies, possessing a dentate calyx and verrucose stems and leaves. Another group of Northern Tableland populations assigned by Wilson (1970, 2013) to *P. squamulosum* subsp. *ozothamnoides* have ovate calyx teeth longer than the tube, not the truncate calyx tube characteristic of *P. squamulosum*; these are currently under investigation (Telford and Bruhl unpublished data) as a probable new species. In regard to oil glands, in all other populations assigned to *P. squamulosum* they are puncate, not verrucose as in *P. glandulosum.* Together, these morphological differences indicate *P. squamulosum* subsp. *verrucosum* has closer affinities with *P. glandulosum,* a possible relationship addressed by Wilson (1970) in the protologue of this taxon. However, discontinuities in foliar morphology, geographic separation and a lack of intergrading populations preclude assigning *P. squamulosum* subsp. *verrucosum* to *P. glandulosum*.

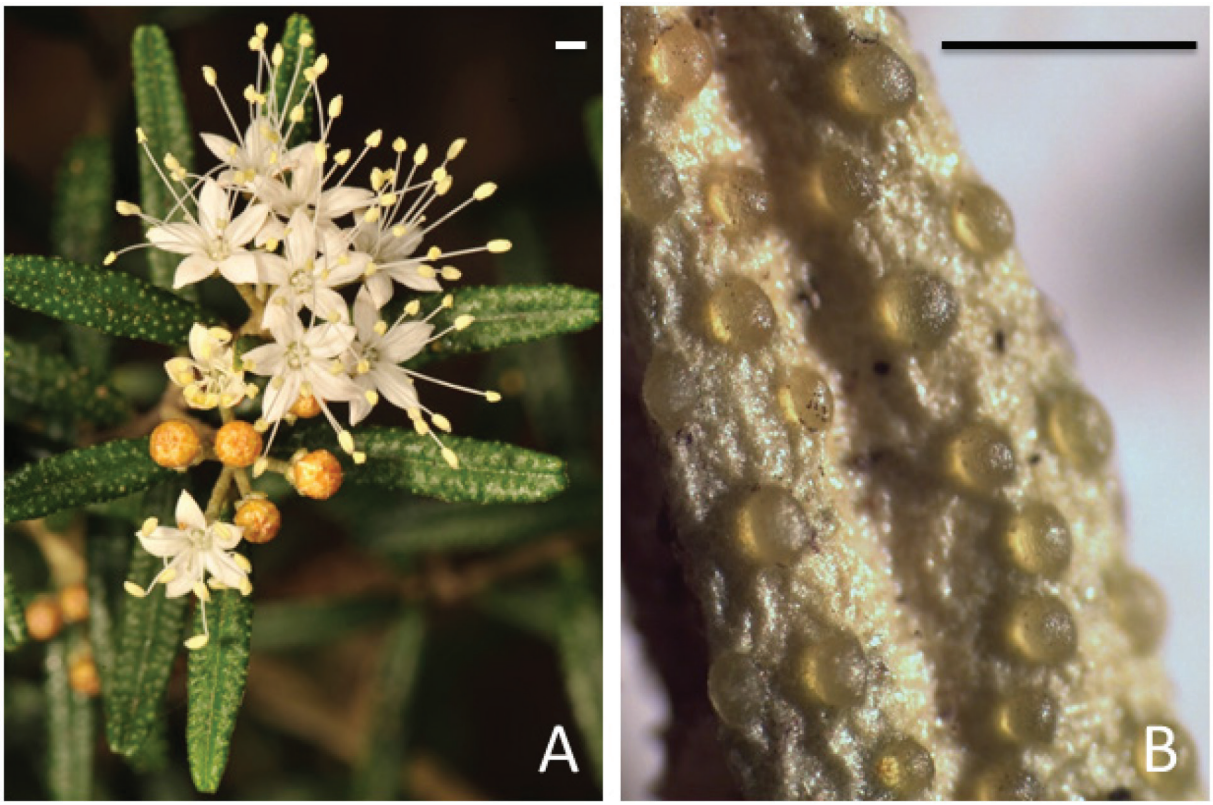

Fig. 1. A. Flowering branchlet of *Phebalium verrucosum* (from plant cultivated at University of New England, Armidale, N.S.W., from cuttings collected at Long Point, Oxley Wild Rivers National Park, N.S.W.); **B**. Photomicrograph of oil glands on adaxial surface of young leaf of *P. verrucosum* (from Copeland 4521, collected Apsley River, N.S.W.) Scale bars = 1 mm.

Phebalium squamulosum subsp. *verrucosum* is removed from *P. squamulosum* and raised to the rank of species below. A comparison of attributes of *P. squamulosum* subsp. *verrucosum, P. squamulosum* subsp. *squamulosum* and *P. glandulosum* subsp. *glandulosum* is presented in Table 1. Here only attributes from the Sydney Basin material matching the type of *P. squamulosum* are presented. Inclusion of attributes of all populations currently assigned to *P. squamulosum* subsp. *squamulosum* would compromise the comparisons as our data suggests the name encompasses a heterogeneous species aggregate as discussed above. Similarly for *P. glandulosum,* with our on-going research indicating that this species is also an unnatural taxonomic assemblage.

Table 1. Comparison of attributes of *P. squamulosum* subsp. *verrucosum, P. squamulosum* subsp. *squamulosum* (from Sydney Basin material matching type) and *P. glandulosum* subsp. *glandulosum.*

Attribute	***P. squamulosum* subsp. *verrucosum***	***P. squamulosum* subsp. *squamulosum***	***P. glandulosum* subsp. *glandulosum***
Stem surface	lepidote & verrucose	lepidote	lepidote & verrucose
Leaf adaxial surface	verrucose	smooth	verrucose
Calyx rim	dentate	truncate	dentate
Adaxial petal colour	cream-white	yellow	cream
Major essential oil component	dihydrotagetone	squamulosone	dihydrotagetone

Status of *Phebalium squamulosum* subsp. *verrucosum*

In the light of the phytochemical data and the polymorphic nature of *P. squamulosum*, its other infraspecific taxa in New South Wales require further critical re-examination. Queensland taxa previously assigned to *P. squamulosum* were studied by Forster (2003), wherein *P. distans* P.I.Forst. was segregated and *P. squamulosum* subsp. *longifolium* (S.T.Blake) Paul G.Wilson reinstated to specific rank. Consistency in the application of rank within a genus is essential. Our future studies in *Phebalium* intend to apply the criteria adopted by Forster (2003) and species concepts consistent with de Querioz (2007).

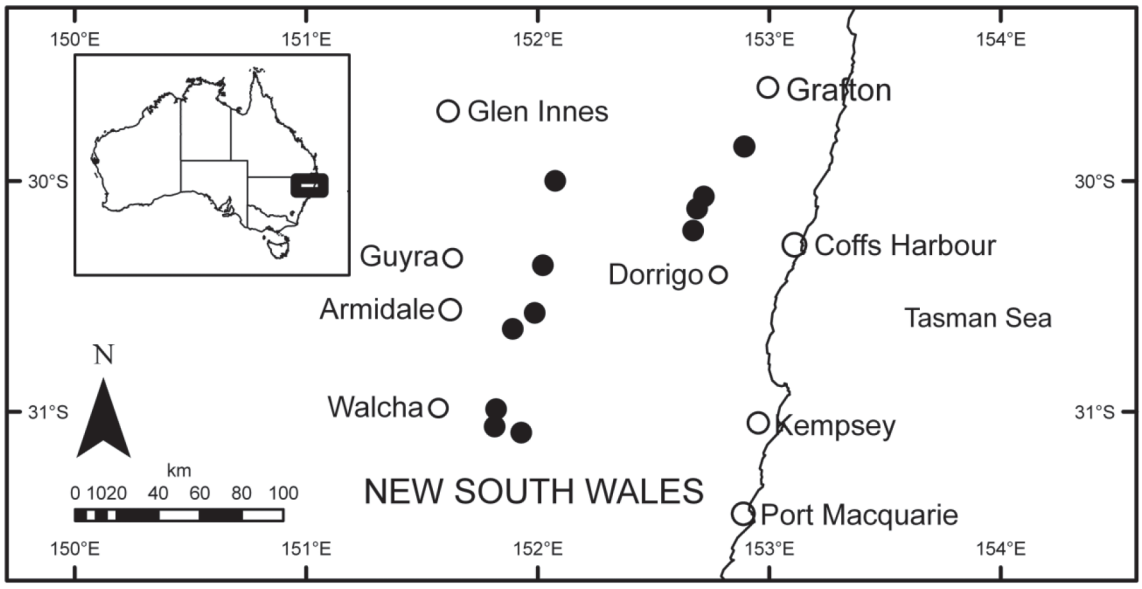

Fig. 2. Distribution of *Phebalium verrucosum* (black dots) in New South Wales, Australia.

Taxonomy

Phebalium verrucosum (Paul G.Wilson) I.Telford & J.J.Bruhl, ***stat. nov.***

P. squamulosum subsp. *verrucosum* Paul G.Wilson, *Nuytsia* 1: 84 (1970); Weston and Porteners (1991); Weston and Harden (2002, 2012); Wilson (2013).

Type: New South Wales: Northern Tablelands: Tia Falls, *W. Forsyth s.n.*, Oct. 1900; holo: NSW69872.

Shrubs or treelets, to 5 m high. Bark grey, becoming corky with prominent pale longitudinal lines of lenticels. Branchlets densely white lepidote. Leaves with petiole 1–2.2 mm long; lamina narrowly elliptic, oblong or linear, 13–38 mm long, 1.8–5.8 mm wide; margin flat or slightly recurved; apex rounded, minutely apiculate, truncate or ± retuse; abaxial surface densely white stellate hairy, rarely with some ferruginous colouring; adaxial surface with midrib impressed, verrucose, initially sparsely stellate hairy, becoming sparsely papillose by loss of hair branches, rarely glabrous. Inflorescence of 1(or 2) terminal umbels; umbels (1–)3–5(–12)-flowered, often with the 2 adjacent upper axils bearing solitary flowers; peduncle 1–3.5(–8) mm long, densely white stellate hairy; pedicels 2.4–6 mm long, densely white stellate hairy. Calyx cup-shaped–broadly obconic, 1.8–2.2 mm diam., verrucose, densely white stellate hairy, 5-lobed; lobes broadly triangular, c. 0.7 mm long. Petals 5, free, ovate, 2.5–3.2 mm long 1.4 mm–1.8 wide, obtusely acuminate, margin slightly incurved, glabrous; abaxial surface silvery or ferruginous lepidote; adaxial surface cream-white coloured. Stamens 10, spreading; filaments 3.5–5.8 mm long; anthers elliptic, c. 0.7 mm long with a minute, globose apiculum. Ovary subglobose, c. 1.2 mm diam.; carpels free, 5, white lepidote; style filiform, c. 4.3 mm long, glabrous; stigma truncate. Fruit of 5 ±spreading cocci; cocci obovoid, 3.5–3.8 mm long, 2.2–3 mm wide, truncate and shortly beaked, white lepidote. Seeds not seen.

Additional specimens examined (selection from 17 seen): New South Wales: Northern Tablelands: Guy Fawkes River National Park, head of Big Scrub Creek, *Floyd 1140*, 10 Nov. 1978 (CFSHB, MEL, NSW); N edge of Wollomombi Gorge, c. 1.5 km ENE of main lookout at Wollomombi Falls, c. 42 km E of Armidale, *Williams s.n. & Floyd*, 20 Sep. 1978 (CANB, MEL, NE, NSW); Oxley Wild Rivers National Park, Long Point rest area, SE of Hillgrove, 200 m downslope on track, *Williams s.n.*, 16 Oct. 1999 (BRI, CANB, NE, NSW); Oxley Wild Rivers National Park, W side of Tia River, 300 m below Tia Falls, *Copeland 4284 & Reckford*, 23 Oct. 2007 (BRI, CANB, MEL, NE, NSW); North Coast: Sara River, Ballards Flat, c. 60 km NE of Guyra, *Williams s.n.*, 25 Oct. 1990 (NE); Nymboida River crossing, 5 km S of Nymboida, *Bean 7668*, 25 Apr. 1994 (BRI, MEL, NSW); Nymboi-Binderay National Park, Nymboida River, Cod Hole, *Telford 12944 & Bruhl*, 9 Sep. 2006 (BRI, CANB, HO, MO, NE, NSW, PERTH).

Distribution: *Phebalium verrucosum* is endemic to the Macleay, Guy Fawkes and Nymboida River drainage basins on the eastern fall from the New England Tableland, on gorge rims of the Northern Tablelands and along the Nymboida River, North Coast, New South Wales. **T**he specimen cited by Wilson (2013, p. 474): 'Gloucester Tops, 1 Jan. 1967, *R. Coveny* (PERTH)' is a misidentification of a species of the *Phebalium squamulosum* core group (*fide* P. H Weston). Plants from this Gloucester Tops population have truncate calyces and the chemical marker squamulosone as the major essential oil component (Sadgrove et al. 2014), not dihydrotagetone as in *P. verrucosum* and *P. glandulosum*

Phenology: flowers recorded April, June, September–November. As in many temperate species of Australian Rutaceae, the first buds open in autumn with flowering continuing sporadically through winter, with the main flush in spring.

Habitat: the species grows on gorge edges at 900–1000 m altitude, and also following suitable rocky stream-side sites down to 180 m altitude. Soils at gorge rim sites are skeletal, derived from metasediments; those of stream banks are on metasedimentary rock outcrops or alluvial gravel. Vegetation communities include *Eucalyptus* shrubby woodland or *Backhousia sciadophora, Olea paniculata* dry rain forest on gorge rims and *Casuarina cunninghamiana* gallery forest on stream banks. Associated understorey species on the gorge rims include *Zieria floydii* at Guy Fawkes River gorge, *Acacia blakei* subsp. *diphylla* at Long Point, *Zieria furfuracea* and *Olearia viscidula* at Wollomombi Falls, *Hakea fraseri* and *Beyeria lasiocarpa* at Tia Falls and *Backhousia myrtifolia*, *Hakea ochroptera* and *Leionema elatius* subsp. *beckleri* along the Nymboida River.

Conservation Status: although previously gazetted as a threatened taxon (see Weston and Harden 2012) under the ROTAP criteria (Briggs and Leigh 1996), recent collections show a considerably larger range than previously known. The species is noted as common at several Oxley Wild Rivers National Park sites (e.g. more than 100 plants at Long Point, pers. obs.) and is now not considered to be at risk. The species is conserved in Guy Fawkes River, Nymboi-Binderay and Oxley Wild Rivers National Parks.

Modification to the key in Flora of New South Wales

The keys to *Phebalium* species in Flora of New South Wales (Weston and Harden 2002) and the New South Wales Flora Online (Weston and Harden 2012) may be modified to accommodate the species as follows:

4 Leaves glandular-warty above, usually with glandular-undulate to crenate margins **5**

Leaves smooth, with entire, straight margins, glands punctate, not prominently raised **6**

5 Leaves cuneate to linear-cuneate ..... ***P. glandulosum***

Leaves oblong to linear or narrow-elliptic ..... ***P. verrucosum***

6 Leaves with midvein not apparent above, 0.8–2 mm wide, linear to narrow-oblong, with margin recurved to tightly revolute..... ***P. stenophyllum***

Leaves either with midvein slightly to strongly impressed above, or >2 mm wide, variously shaped, with margin flat to revolute ..... ***P. squamulosum***

Acknowledgments

Funding was provided by ABRS National Taxonomy Research Grant (Non-salaried Researchers) for part of this project.

References

Australian Plant Census (2007–) Council of Heads of Australasian Herbaria. http://anbg.gov.au/chah/apc/ (accessed 10 June 2013)

Batey LL, Hellyer RO, Pinhey JT (1971) The structure of squamulosone, a new sesquiterpene ketone from *Phebalium squamulosum*. *Australian Journal of Chemistry* 24: 2173–2177. http://dx.doi.org/10.1071/CH9712173

Briggs JD, Leigh JH (1996) Rare or threatened Australian plants, 1995 revised edn. (CSIRO Publishing: Collingwood)

Brophy JJ, Goldsack RJ, Forster PI (2006) Leaf essential oils of the Queensland species of *Phebalium* (Rutaceae: Boronieae). *Journal of Essential Oil Research* 18: 386–391. http://dx.doi.org/10.1080/10412905.2006.9699122

De Queiroz, K (2007) Species concepts and species delimitation. *Systematic Biology* 56(6): 879–886. http://dx.doi.org/10.1080/10635150701701083

Forster PI (2003) *Phebalium distans* P.I.Forst. (Rutaceae), a new and endangered species from south-eastern Queensland, and reinstatement of *P. longifolium* S.T.Blake. *Austrobaileya* 6(3): 437–444.

Giles RL, Drinnan AN, Walsh NG (2008) Variation in *Phebalium glandulosum* subsp. *glandulosum* (Rutaceae): morphometric and anatomical evidence. *Australian Systematic Botany* 21: 271–288. http://dx.doi.org/10.1071/SB07023

Jones TGH, Smith FB (1925) Olefinis terpene ketones from the volatile oil of flowering *Tagetes glandulifera*. Part 1. *Journal of the Chemical Society, Transactions*. 127: 2530–2539. http://dx.doi.org/10.1039/ct9252702530

Kubitzki K, Kallunki JA, Duretto M, Wilson PG (2011) Rutaceae. Pp. 277–356 in Kubitzki K (ed) *The Families and Genera of Flowering Plants,* vol. 10. (Springer: Berlin)

Lassak EV, Southwell IA (1974) Occurrence of some unusual compounds in the leaf oils of *Eriostemon obovalis* and *Phebalium glandulosum* subsp. *glandulosum*. *Australian Journal of Chemistry*. 27: 2703–2705.

Pala-Paul J, Copeland LM, Brophy JJ, Goldsack RJ (2009) Essential oil composition of two new species of *Phebalium* (Rutaceae) from north-eastern New South Wales, Australia. *Natural Product Communications* 4: 983–986.

Sadgrove NJ, Telford IRH, Greatrex BW, Dowell A, Jones GL (2013) Dihydrotagetone, an unusual fruity ketone, is found in enantiopure and enantioenriched forms in additional Australian native taxa of *Phebalium* (Rutaceae: Boronieae). *Natural Product Communications* 8: 737–740. http://dx.doi.org/10.1016/j.phytochem.2013.10.015

Sadgrove NJ, Telford IRH, Greatrex BW, Jones GL (2014) Composition and antimicrobial activity of the essential oils of the *Phebalium squamulosum* species complex (Rutaceae) in New South Wales, Australia. *Phytochemistry* 97: 38–45.

Weston PH, Porteners MF (1991) *Phebalium.* Pp 257–263 in Harden, GJ (ed.) *Flora of New South Wales,* vol. 2 (University of New South Wales Press: Kensington)
Weston PH, Harden GJ (2002) *Phebalium.* Pp 300–304 in Harden, GJ (ed.) *Flora of New South Wales*, vol. 2, revised edn. (University of New South Wales Press: Kensington)
Weston PH, Harden GJ (2012) *Phebalium.* New South Wales Flora Online. http://plantnet.rbgsyd.nsw.gov.au (accessed 10 June 2013)
Wilson, PG (1970) A taxonomic revision of the genera *Crowea*, *Eriostemon* and *Phebalium*. *Nuytsia* 1: 5–155.
Wilson, PG (2013) *Phebalium*. *Flora of Australia* 26: 458–480.

Mansucript received 28 June 2013, manuscript accepted 28 July 2014