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CONTENTS

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Melaleuca uxorum (Myrtaceae), a new species from north-eastern Australia

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Abstract

Melaleuca uxorum Craven, G.Holmes & Sankowsky, a new species in the *M. minutifolia* group, is described from the Herberton Range in north-eastern Australia.

Introduction

Numerous visits by botanists have been made to the northern Herberton Range in tropical Queensland, near the towns of Atherton and Tolga, to explore its woodland vegetation. Despite this attention, populations of a distinctive but undescribed species of *Melaleuca* L. were discovered there recently near Mt Emerald, one of the major peaks in this range. It was collected initially by the second author and J. Holmes in July 2000, and quite independently by the third author in December 2000. Upon investigation, the plant was found to belong to the *Melaleuca minutifolia* F.Muell group of species. It possesses decussate, peltate leaves as do the species of this group but differs *inter alia* in that the floral unit is a triad.

Until now, the *M. minutifolia* group consisted of two species, *M. minutifolia* and *M. monantha* (Barlow) Craven. The former occurs from the Drysdale River district eastwards to south-western Arnhem Land in north-western Australia and the latter from the Palmer River district southwards to Mt Sturgeon in north-eastern Australia. These two species are characterised by the following features: in *M. minutifolia* each floral unit consists of a dyad subtended by a bract, each flower is subtended by three "bracteoles" and the leaf blade apex usually is long acuminate to narrowly acute; in *M. monantha* each floral unit consists of a monad subtended by a bract, each flower is subtended by two bracteoles, and the leaf blade apex usually is shortly to moderately acuminate (Craven & Lepschi 1999).

Further study of the Herberton Range plant has shown that it represents a distinctive new species of the *M. minutifolia* group and it is formally described here.

Taxonomy

Melaleuca uxorum Craven, G.Holmes & Sankowsky, sp. nov.

A *M. minutifolia* F.Muell. et *M. monantha* (Barlow) Craven floribus triadibus, ramulis puberulis minute, foliis latioribus (1.3–2.5 mm) et 11–17-nervibus, et ovulis in quoque loculo numerosioribus (c. 33–37) differt.

Type: Australia: Queensland: Cook District: Herberton Range, 1.2 km NW of Mt Emerald, 4 Dec. 2001, *Craven and Holmes 10422* (holotype BRI; isotypes A, CANB, DNA, L, MEL, NSW, P).

Shrub to 1 m tall (usually 0.5–0.6 m tall). *Bud scales* absent (but prophylls present on lateral shoots). *Branchlets* glabrescent or subglabrous, minutely puberulous, terete,

subcompressed or irregularly angled, very slightly excavated. *Leaves* decussate, imbricate, amplexicaul, peltate, ascending or spreading-ascending, 2–4.5 mm long, 1.3–2.7 mm wide, 1.3–2.3 times as long as wide, sessile; *leaf blade* glabrescent, the abaxial surface glabrous, the margin with stoutish cilia, dull, broadly elliptic, broadly ovate or subcircular, in transverse section lunate, strongly lunate or broadly v-shaped, in lateral view recurved (rarely straight), the base rounded or truncate, the apex acuminate, narrowly acute or obtusely shortly acuminate, with 11–17 longitudinal veins, the oil glands not visible on either surface, sparse. *Inflorescence* a head or short spike of triads, inserted interstitially on the reproductive seasonal growth unit the apex of which continues growth after anthesis, 18–25 mm wide, with 4–12 clustered triads each of which is subtended by a foliage leaf or bract, the subtending bract usually absent at anthesis; *central flower* of each triad ebracteolate, each lateral flower subtended by three "bracteoles" (one narrowly ovate and 1.2–1.7 mm long, the other two narrowly elliptic to linear-elliptic and 1–1.5 mm long). *Hypanthium* glabrous, pinkish-white, not stipitate, cup-shaped, 1.2–2.6 mm long, 1.5–2 mm wide. *Calyx lobes* 5, green, free, overlapping, abaxially glabrous (margin is minutely ciliate), costate or not (distinctly costate when dried), 0.8–1.2 mm long, herbaceous almost to the margin or herbaceous in the proximal-central zone and scarious in a narrow marginal band, the band 0.1–0.2 mm wide, the margin ciliate, broadly ovate, very broadly triangular or broadly elliptic, persistent at least until the immature fruit stage. *Petals* 5, deciduous, glabrous, white flushed pink, not or obscurely clawed, broadly elliptic or subcircular, 1.8–2.2 mm long, the margin ciliate. *Stamens* in 5 bundles, a staminal ring absent, 6–12 per bundle; *filaments* glabrous, pure white, 7.5–11 mm long, the bundle claw 3.7–6 mm long, 0.5–0.6 times as long as the filaments; *anthers* generally uniform in size, broadly obovate or subcircular, 0.3–0.4 mm long, connective not prominently glandular. *Ovary wall* adnate to the hypanthium for the proximal one-quarter only; placentation axile-median; *ovules* c. 33–37 per locule. *Style* glabrous, straight (or more or less so) or hooked, 8–10.25 mm long, the stigma punctiform. *Infructescence* usually as long as wide or longer than wide or sometimes shorter than wide, 7–12 mm in diameter. *Fruiting hypanthium* thick-walled, corky, cup-shaped, 2.5–3.5 mm long, 3–3.5 mm wide, 0.7–0.9 times as long as wide, 1.5–3 mm wide at the orifice; calyx lobes replaced by sepaline teeth or weathering away and not replaced by sepaline teeth; valves inserted. *Seeds* angular-obovoid, 0.6–0.7 mm long, the testa membranous; *embryo* with the cotyledons about one third its length, the cotyledons obvolute.

Etymology: The epithet is derived from the Latin word, *uxor*, wife, spouse, consort, and has been chosen to honour our wives. Kirsty, Jenny and Nada have shared our enthusiasm for plants over the decades. On numerous occasions they have experienced with us both the successes and failures that accompany our bush quests.

Phenology: Flowering period: November to February. Fruits present in all months.

Other specimens examined: **AUSTRALIA: QUEENSLAND**: 1.2 km NW of Mt Emerald, 16 July 2000, *Holmes & Holmes s.n.* (BRI), *ibid.* 26 Mar. 2001, *Holmes & Holmes s.n.* (BRI); near Mt Emerald, 7 km NW of Tolga, 24 Dec. 2000, *Sankowsky 1702* (BRI, CANB), *ibid.* 4 Feb. 2001, *Sankowsky 1703* (BRI, CANB).

Distribution and ecology: *Melaleuca uxorum* has been recorded only from the northern Herberton Range in north-eastern Queensland. The species is known from four sites distributed in an arc, with a linear distance of three kilometres. These sites vary in altitude between 950 and 1050 metres. Soil parent material is acid volcanic, putatively rhyolite. The mainly skeletal soils of the area support a low open woodland dominated by *Eucalyptus lockyeri* Blaxell & K.D.Hill. *Melaleuca uxorum* tends to form low continuous shrubberies on rock pavements where rainfall runoff is concentrated and fire is infrequent. Regular associates include *Acacia aulacocarpa* A.Cunn. ex Benth., *A. calyculata* A.Cunn. ex Benth., *Xanthorrhoea johnsonii* A.T.Lee, *Pseudanthus pimeleoides* Sieber ex

Spreng. and *Borya septentrionalis* F.Muell. Occasional associates also endemic to the district include *Homoranthus porteri* (C.T.White) Craven & S.R.Jones and *Grevillea glossadenia* McGill.

Notes: The species is related closely to *M. minutifolia* and *M. monantha* but differs from them in having three-flowered floral units (two- and one-flowered respectively in *M. minutifolia* and *M. monantha*); glabrescent, minutely puberulous branchlets (glabrous in *M. minutifolia* and *M. monantha*); broader leaves (1.3–2.7 mm wide as against 0.6–1.1 mm and 0.5–1 mm); more veins in the leaf blade (11–17 as against 5–7 in each of the other species); and more ovules per locule (c. 33–37 as against c. 20–30 and c. 20).

In the identification keys in Craven and Lepschi (1999), *M. uxorum* keys out in Key 1 to a group of south-western Australian species that have flowers in triads, and keys out nearest to *M. cucullata* Turcz. with which it has no close relationship. It may be inserted into Key 1 in Craven and Lepschi (1999) by replacing the first lead of couplet 8 with the following:

8. Leaves amplexicaul
   - 8. Leaf blade in transverse section depressed angular-obovate, strongly depressed obtriangular, depressed obovate or shallowly lunate; stamens 3.2–5.5 mm long; ovules 8–10 per locule .....M. cucullata
   - 8A:Leaf blade in transverse section lunate, strongly lunate or broadly v-shaped; stamens 7.5–11 mm long; ovules c. 33–37 per locule.....M. uxorum

Within each triad, the central flower is ebracteolate and each lateral flower is subtended by three "bracteoles". The standard condition in Myrtaceae is for a flower to be subtended by a pair of bracteoles. The "bracteoles" in *M. uxorum* are dissimilar. One is narrowly ovate and 1.2–1.7 mm long, while the other two are narrowly elliptic to linear-elliptic and 1–1.5 mm long. It seems that the floral unit in this plant may be derived from the reduction of an axis bearing several floral units composed of a single bracteate and bracteolate flower to a three-flowered unit with the two lateral flowers maintaining the possession of bract and bracteoles while the central and possibly terminal flower has lost all subtending organs.

Conservation status: By IUCN criteria B and C (IUCN 2001), *Melaleuca uxorum* is Endangered (EN B2ab(iii); C2a). A total population of fewer than 400 plants has been recorded from four sites. These occur within a linear distance of three kilometres and occupy less than one hectare. Recurrent fire is the main threat to survival. Because of the restricted number of known plants, their locations will not be precisely disclosed here.

References

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New species and a new hybrid in the *Viola hederacea* species complex, with notes on *Viola hederacea* Labill.

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Abstract

The *Viola hederacea* Labill. species complex is widespread in south-eastern Australia. It has often been considered problematic, with numerous forms difficult to adequately diagnose and distinguish. This paper results from an assessment of the complex based on extensive field observations, which have shown that the complex is tractable and comprises a number of morphologically and ecologically distinct species. Two new species, *Viola banksii* K.R. Thiele & Prober *sp. nov.*, and *V. eminens* K.R. Thiele & Prober *sp. nov.*, and a new hybrid, *V.* × *zophodes* K.R. Thiele & Prober *nothosp. nov.*, are described and illustrated, and a new circumscription provided for *Viola hederacea sens. str.*

Introduction

The *Viola hederacea* Labill. species complex comprises all taxa in *Viola* Section *Erpetion* (Sweet) Becker, characterised by non-leafy stipules and flowers that are saccate rather than spurred on the anterior petal. Apart from a single doubtful collection from Malaya (Moore 1962), the complex is restricted to Australia, where it has a wide distribution from south-eastern South Australia, through southern Victoria, Tasmania, and eastern New South Wales to Queensland north as far as Atherton (Fig. 1; Adams 1982; Seppelt 1986; James 1990; Entwisle 1996). Many forms are recognisable over this range.

Adams (1982), in the most recent treatment of the complex, described a number of subspecies of *V. hederacea*. Two of these have since been raised to species rank as *V. cleistogamoides* (L.G. Adams) Seppelt and *V. fuscoviolacea* (L.G. Adams) T.A. James. *Viola sieberiana* Spreng., reduced by Adams (1982) and others to a subspecies of *V. hederacea*, has since been reinstated by most authors (e.g. Seppelt 1986; James 1990; Entwisle 1996), while *V. hederacea* subsp. *seppeltiana* L.G. Adams has been rejected as distinct from *V. sieberiana* by Seppelt (1986). The subspecies *V. hederacea* subsp. *perreniformis* L.G. Adams and *V. hederacea* subsp. *curtisiae* L.G. Adams still stand.

Even with these taxa removed, the residual nominal form is still highly variable and problematic. Seppelt (1986), treating the complex in South Australia, noted a 'perplexing array of leaf forms', but considered that 'there is little variation in floral morphology'. James (1990), treating the complex for the *Flora of New South Wales,* described seven 'forms' of *V. hederacea*, based primarily on leaf shapes assessed from herbarium specimens, but regarded that formal recognition of these was premature. Entwisle (1996) remarked that 'there is a need for Australia-wide studies to clarify species concepts and appropriate nomenclature'.

The present paper results from an ongoing study of the *V. hederacea sens. lat.* complex. By contrast with most earlier work, which has been largely herbarium-based, our study has focused on field observation of living material and ecological characteristics from throughout the geographic range of the complex. These have shown that the *Viola hederacea* species complex is tractable, and that it comprises a number of discrete and easily recognisable taxa differing in floral, vegetative and ecological characters. Importantly, almost throughout its range are found areas where two or more distinct taxa grow together or in close proximity, usually with little or no evidence of

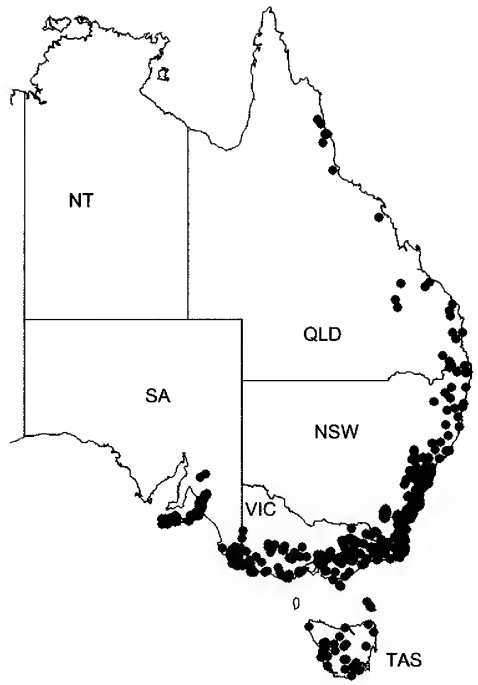

Figure 1. Distribution of the *Viola hederacea* species complex in Australia (based on specimen data from AD, BRI, CANB, MEL, NSW)

hybridisation or introgression and with discrete morphological and ecological differences. Results from the field study have subsequently allowed a more careful appraisal of herbarium material, which has confirmed the distinctness of these taxa over wide geographic ranges.

In this paper we describe and discuss *V. hederacea* Labill. *sens. str.*, *V. banksii* K.R. Thiele & Prober *sp. nov.*, *V. eminens* K.R. Thiele & Prober *sp. nov.* and *V. x. zophodes* K.R. Thiele & Prober *nothosp. nov.* Other taxa will be dealt with in subsequent papers.

Characters in the *Viola hederacea* species complex

All members of the *Viola hederacea* species complex are stoloniferous perennial herbs, often forming large, clonal colonies. Leaves are mostly borne in false whorls on contracted stems at ground level, but in some taxa the stems may become elongated with caulescent, alternate leaves, particularly when the plants are growing amongst shrubs or other dense vegetation. In some taxa (particularly *V. sieberiana*), the contracted stems are woody and densely covered with old leaf bases and stipules, while in others they are fleshier and more or less naked.

Leaves are petiolate, with a flat, irregularly toothed blade. The shape of the blade is variable, even within a single clonal colony. Leaf shape is a useful diagnostic feature for taxa, but with the limitation that exposed leaves are smaller and less distinctive than leaves from lush growth in sheltered sites. Some taxa, such as *V. fuscoviolacea, V. cleistogamoides* and *V. sieberiana* tend to have ovate-rhomboid leaves with a cuneate base, while other taxa tend towards reniform leaves with a broad to narrow basal sinus. Because of the variability of leaves within taxa and individuals, these differences cannot be used consistently to discriminate taxa. Herbarium specimens of *Viola* usually have an inadequate selection of well-developed leaves.

Leaves, petioles and stems may be glabrous or bear sparse, short, unicellular hairs. Degree of pubescence with such hairs is variable, and appears to be of limited taxonomic value.

Flowers are borne singly on short or long, unbranched scapes, each scape bearing a pair of stipule-like bracteoles usually near its middle; the scape is usually slightly geniculate at the bracteoles. Flowers in *V. cleistogamoides* and *V. fuscoviolacea* are characteristically borne on scapes distinctly shorter than the leaves, while in all other taxa the flowers are borne above or well above the level of the leaves.

Flowers are zygomorphic and personate. The calyx comprises five free sepals, and is green or (in some taxa) flushed purple. The corolla comprises five free petals, scarcely exceeding the sepals in *V. cleistogamoides* and *V. fuscoviolacea*, distinctly exceeding the sepals in all other taxa.

In some taxa the flowers are nearly or quite concolorous white, pale blue, pale violet or (in *V. fuscoviolacea*) blackish-violet, while in others they are strongly discolorous violet-and-white or dark violet on a paler violet ground. Among the discolorous taxa some have sharply demarcated boundaries between the violet and white, while others are more graduated, giving a 'washed out' appearance. The brightness and hue of the violet colour differs between taxa. While there is some variation in colour within taxa, the colours and colour patterns are useful diagnostic features, at least on fresh flowers.

The anterior petal (the lowermost with the flower in its natural position) has a slightly saccate base in the position of the spur in other species of *Viola*. The shape, colouration and venation of this petal is an important diagnostic feature.

The lateral petals are twisted in most species (scarcely so in *V. cleistogamoides* and *V. fuscoviolacea*). The degree of twisting of the lateral petals varies between taxa, and is sometimes a useful taxonomic feature. In most species the adaxial surface of the lateral petals is bearded with white or coloured hairs, varying from a few scattered hairs (in e.g. *V. fuscoviolacea*) to a large, dense patch. The hairs are thickened and sometimes slightly club-shaped. Although the presence or absence of bearded lateral petals has been used as a taxonomic feature (e.g. Adams, 1982) it appears to be of limited value, and occasional clones of all taxa are beardless. When present, however, the extent of the beard may be a useful character.

The posterior petals (the uppermost with the flower in its natural position) are generally obovate, reflexed and clawed, but may vary greatly in shape between clones within one taxon. They are of limited taxonomic value.

The androecium comprises five free stamens, connivent around and obscuring the ovary. Each stamen comprises a short, broad filament and a flattened, introrse anther with a terminal, flattened appendage. The connective between the anther cells may be cream or suffused or blotched with purple. The two anthers adjacent to the anterior petal each bear an abaxial nectariferous gland on the connective between the anther cells. The shape, colour and surface texture of this gland is a useful diagnostic feature.

Pollen in some taxa is cream or white, while in others it is yellow to golden. The taxa with yellow pollen also have a yellow colouration on the inner surfaces of the pollen sacs (at least in fresh material).

The gynoecium comprises three fused carpels forming a unilocular ovary with parietal placentas, surmounted by a simple, geniculate style. The ovary varies from greenish-white, sometimes flushed or flecked with violet, to uniformly dark violet. While ovary colour is variable within all taxa, it may have some limited diagnostic value (e.g. the dark-flowered *V. fuscoviolacea* appears to consistently have pale greenish-white ovaries without any purple flushing).

The fruit is a 3-valved capsule opening +/- explosively on maturity to scatter the globose seeds. Freshly exposed seeds may vary in colour, even within a single capsule, from white to dark purplish-black. Colour of the mature, dried seeds is a valuable diagnostic feature, being dull, pale brown or fawn in some taxa and glossy black in others.

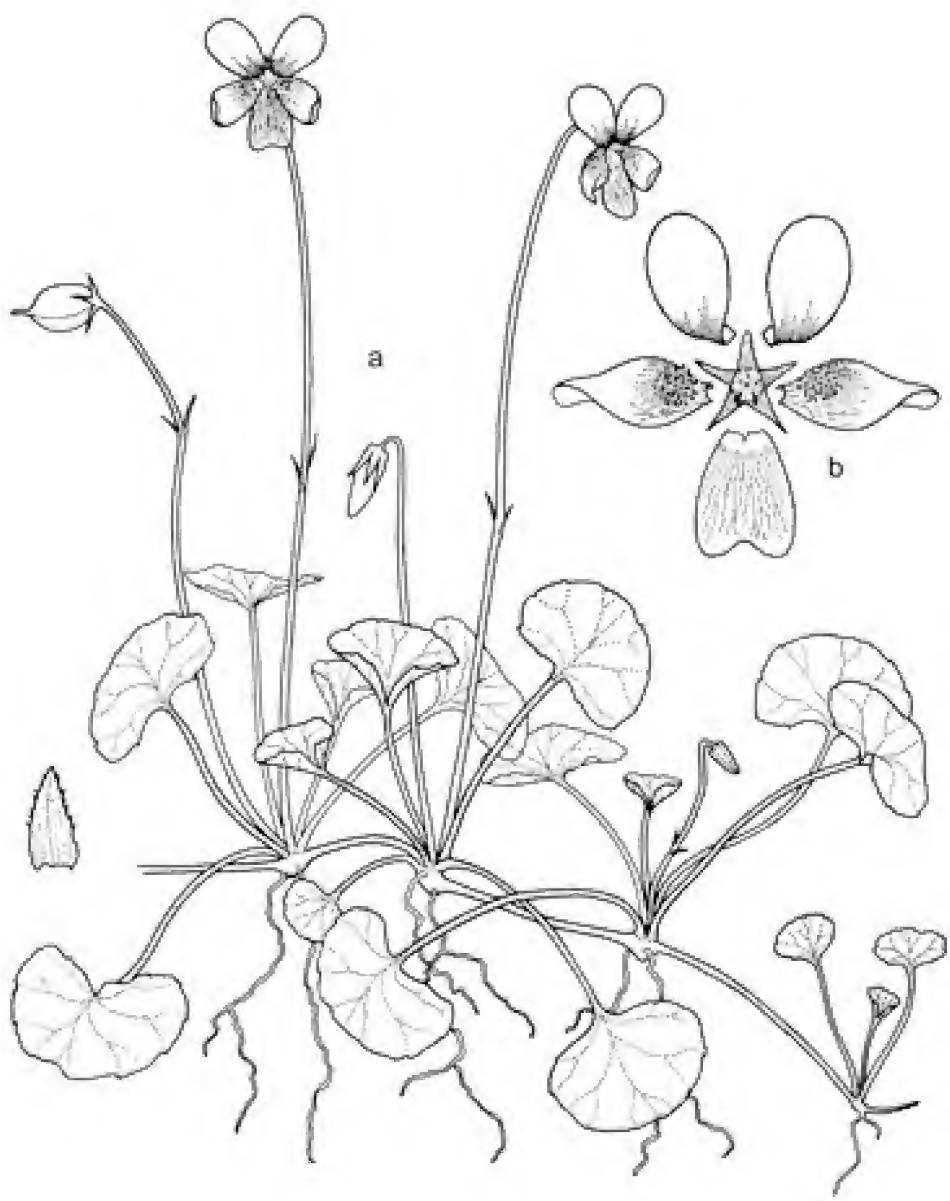

Figure 2. *Viola hederacea.* a Habit x1; b Flower x2 (K.R. Thiele 2542, CANB).

Taxonomy

1. *Viola hederacea* Labill., *Nov. Holl. Pl. Spec.* 1: 66, t. 91 (1805).
Erpetion hederaceum (Labill.) G.Don, *Gen. Hist.* 1: 335 (1831); *Viola hederacea* var. *genuina* Domin, *Biblioth. Bot.* 89: 427 (1928). *Type*: 'In capite Van-Diemen', J.Labillardiere: F, *n.v.*; photo: CANB!

Perennial herb spreading widely by stolons; rootstock sometimes somewhat swollen and bulbous at the stem bases. *Stems* contracted so that the leaves form rosettes, never elongate with caulescent leaves. *Leaves* broad-reniform or semi-circular, the largest (4–)15–20(–30) mm long, (5–)20–30(–50) mm wide, 1–1.5(–2) times wider than long, usually truncate at base or occasionally with a broad sinus or broadly cuneate; lamina with 8–16 obscure teeth, glabrous or with scattered unicellular hairs on the upper surface, dark green above, dull greyish-green beneath; petioles (1–)2–8(–12) cm long; *stipules* narrowly triangular, usually with several small, glandular teeth on each side. *Flowers* on scapes slightly longer to four times as long as the leaves, usually discolorous violet-and-white (occasionally concolorous pale violet or almost white), the colours usually not strongly demarcated; *anterior petal* (4–)8–10(–12) mm long, (3–)5–7(–9) mm wide, narrowly- to broadly obovate or cuneate, broadest in the distal third, usually emarginate, with a small green U-shaped blotch at the base, then usually pale to bright violet for over half its length grading to a white apex, with three to many nerves, the midnerve usually not distinct from the lateral nerves and often anastomosing with them; *lateral petals* spreading, (4–)8–10(–13) mm long, twisted usually to c. 90° (sometimes to 180°), violet at the base grading to white distally; beard covering half or less of the width of the lateral petals, occasionally absent; *dorsal petals* (4–)8–12(–13) mm long, (2–)4–6(–8) mm wide, obovate to broadly obovate (rarely narrowly obovate), erect to strongly reflexed, usually violet at the flexure grading to white for most of their length. *Anthers* 1.3–4.0 mm long, cream, often flushed or flecked with violet, the terminal appendages straw-coloured, with short, irregular hairs on the outer margins of the anther cells; *anther glands* purplish or dull green, shorter than the anther cells, irregularly rugose, broad at the base and each distinctly flattened or depressed towards the other; *pollen* and interior margins of the anther cells white to cream. *Ovary* and *fruit* whitish or pale green, often flecked or flushed purple; *style* distinctly geniculate at its insertion on the ovary. *Seeds* 1.2–2.0 mm long, dull, mottled cream and brown (occasionally uniformly reddish-brown), +/- smooth. *Fig. 2.*

Distribution and habitat. Viola hederacea is common and widespread in south-eastern Australia, from the Mount Lofty Ranges and south-eastern South Australia, throughout Tasmania and southern Victoria, and in eastern New South Wales north to the Northern Tablelands (Fig. 7a). It is typically found on relatively dry soils in forested habitats and on well-drained roadside banks.

In South Australia it is widespread in the South-East region, but is highly localised in the Adelaide Hills, apparently occurring only in the area around Mount Lofty, Belair National Park and near Crafers. Elsewhere on Fleurieu Peninsula and in the Southern Lofty region it is replaced by *V. eminens* (see below). In Victoria it is the most widespread species, occurring commonly in dry to moist forests from the far south-west around Glenelg River and the Grampians Ranges, through the Otway Ranges and thence on both north and south falls of the Great Dividing Range from Melbourne to the New South Wales border. It is replaced on the higher parts of the Great Dividing Range (e.g. summits of Lake Mountain and Mount Baw Baw and on the Errinundra Plateau) by *V. eminens* (see below). *Viola hederacea* is common and widespread throughout much of Tasmania.

In New South Wales *V. hederacea* is the most common species in forests on the eastern part of the Great Dividing Range from the Victorian border to the Blue Mountains and Royal National Park near Sydney. A disjunct but morphologically typical population occurs in the Brindabella Ranges west of Canberra. In northern New South Wales it appears to become patchy and localised, with few collections from widely scattered sites (e.g. Barrington Tops, Glen Elgin). In the Sydney region it is common on shale-derived

soils but is rare on sandstones, where it is replaced by *V. sieberiana* and other as yet undescribed species from the complex. In coastal sites from Ulladulla to the Queensland border it is largely replaced by *V. banksii* (see below).

In the Border Ranges of far northern New South Wales and in Queensland *Viola hederacea* as circumscribed here is replaced by *V. hederacea* subsp. *perreniformis*, a distinct taxon that may be referred to a separate species in a later paper. Note that the description of *V. hederacea* above does not include subsp. *perreniformis*.

Distinguishing features and variation. Viola hederacea sens. str. may be distinguished (Table 1; Fig. 3) from all other taxa in the complex by its obovate to cuneate anterior petals with somewhat irregular venation, usually paler, less sharply discolorous flowers (the violet colouration on the petals grades +/- diffusely into the white rather than being sharply distinct), and reniform or semicircular leaves that are about as broad as long and somewhat discolorous (dark green above, dull greyish-green beneath). Leaf colouration is often a useful though subtle feature on herbarium specimens, dried leaves in *V. hederacea* being characteristically duller and thicker-textured than the other species in the complex. When fresh flowers are available, *V. hederacea* appears to be the only species in the complex in which the anther glands are often (although not invariably) purplish.

Seeds of *V. hederacea* differ from all other taxa currently examined (*V. eminens*, *V. banksii, V. fuscoviolacea*, *V. cleistogamoides*) with the exception of *V. hederacea* subsp. *perreniformis* in being dull brown (often mottled with pale cream) rather than glossy purplish-black. These seed colours only develop on fully matured seed.

Some collections provisionally referred here to *V. hederacea* have very small flowers (petals 3–5 mm long) that are often almost white and with little or no beard on the lateral petals. These usually grow in very shaded, dry or otherwise unfavourable sites adjacent to more typical *V. hederacea*, with intergrading forms, and occur throughout the range of that species. Such collections have sometimes previously been ascribed to *V. hederacea-V. sieberiana* hybrids (e.g. James 1990), but there is no reason to suspect that hybridisation is occurring between those species. These small-flowered forms warrant further investigation.

	V. hederacea	*V. banksii*	*V. eminens*	*V. x zophodes*
Habitat	Moderately dry to moist sites (not swamps), particularly forest habitats	Coastal headlands, lowland swamps and rainforest or moist sclerophyll forest margins	Moist sites; swamps at lower altitudes, wet sclerophyll forest to snow gum woodlands at high altitudes	Moist sites in high altitude swamps
Typical leaf shape	Reniform to semicircular 	Orbicular, with deep sinus 	Broad-reniform 	Broad-reniform 
Flower colour	Weakly to strongly discolorous bright to pale violet with white or pale violet tips, occasionally +/- concolorous	Strongly discolorous, bright violet with prominent white tips	Strongly (rarely weakly) discolorous bright violet with prominent white (occasionally pale violet) tips	Strongly discolorous, dark violet with obscure white tips, occasionally +/- concolorous dark violet
Anterior petal shape	Obovate 	Elliptic to circular 	Ovate 	Ovate 
Anterior petal venation	Obscurely triplinerved, the central nerves anastomosing	Distinctly triplinerved, central nerves scarcely anastomosing	Distinctly triplinerved, central nerves scarcely anastomosing	Distinctly triplinerved, central nerves scarcely anastomosing
Lateral petals	Twisted through c. 90°	Twisted through c. 180°	Twisted through c. 180°	Twisted through c. 180°
Beard on lateral petals	≤1/2 width, sometimes absent	>1/2 width	>1/2 width	≥1/2 width
Anther glands	Short, rugose, flattened, often purple	Long, smooth, narrow and high, scarcely flattened, pale green to whitish	Long, smooth, narrow and high, sometimes slightly flattened, pale green to whitish	Short to long, smooth, flattened, pale green to whitish
Pollen colour	White	Golden	Golden	White to pale yellow (sterile)
Mature seed colour	Dull mottled brown and cream	Glossy purple-black	Glossy purple-black	(no seeds set)

Table 1. Diagnostic features of taxa described and discussed in this paper

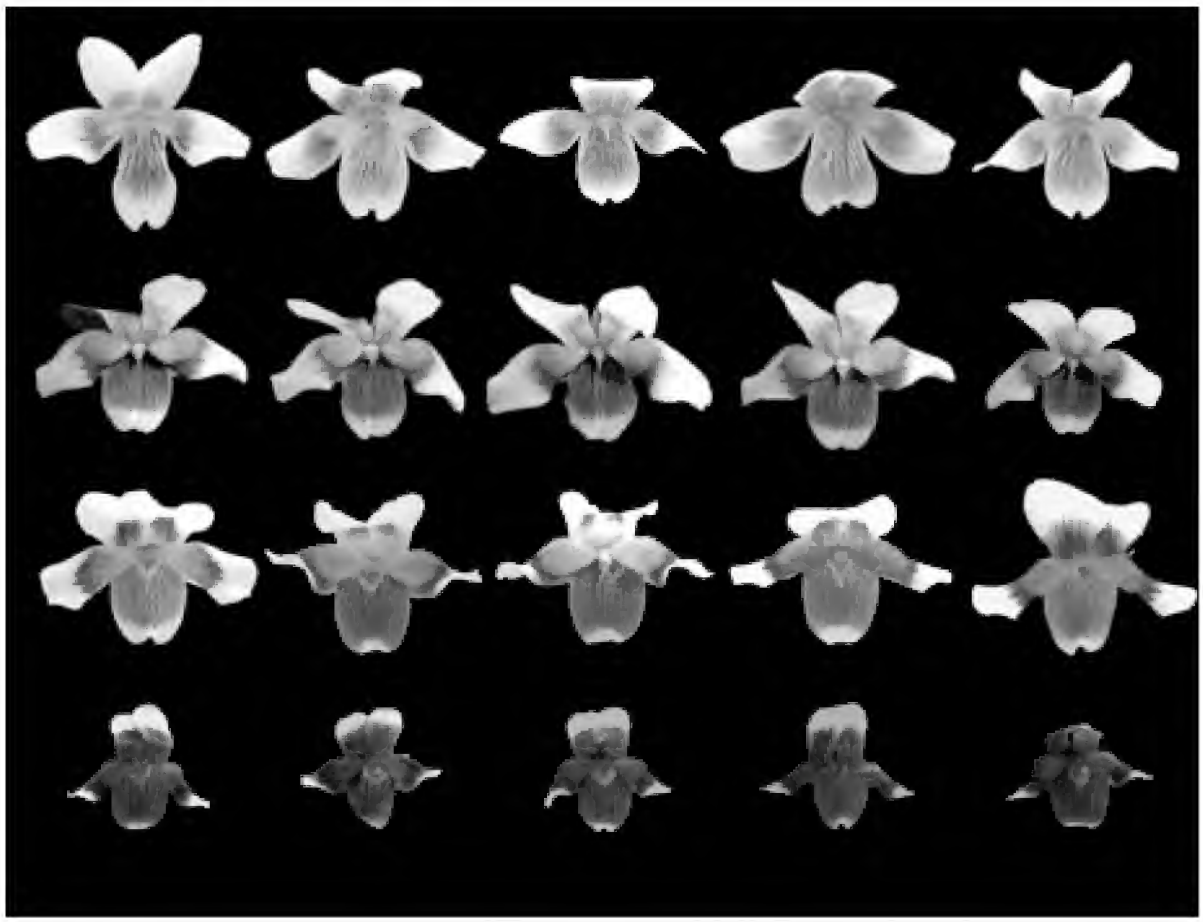

Figure 3. Representative flowers of *V. hederacea* (top row), *V. banksii* (second row), *V. eminens* (third row) and *V. x. zophodes* (bottom row). Approximately natural size.

Notes. The type of *V. hederacea* is from southern Tasmania, collected at Recherche Bay by J.J.H.Labillardiere in 1792. Colour transparencies of the type held at CANB show the typical features for the species, and the illustration accompanying the original description shows well the obovate anterior petals and semicircular leaves that distinguish *V. hederacea* from other taxa in the complex. It is probably the only form occurring in lowland, near-coastal sites in southern Tasmania where Labillardiere would have collected.

Viola hederacea var. *elatines* D.C. was listed as a synonym of *V. hederacea* by Adams (1982). Examination of microfiche photographs of the type material indicate that this variety is a synonym of *V. banksii* (see below).

Selected specimens examined. **South Australia**: Stirling West, 21 Nov 1958, *E.H. Ising s.n.* (AD 95904073); Mount Lofty, Oct. 1917, *E.H. Ising s.n.* (AD 966160089); Mount Lofty Botanic Garden, 12 Jan 1984, *B.R. Moore 67* (AD 98943158); Belair National Park, 2 Nov. 1935, *E.H. Ising s.n.* (AD 98122154); Carpenter Rocks, 5 Nov. 1977, *A.G. Spooner 5490* (AD 97813546); Mount Burr Forest, 12 Sept. 1984, *C. O'Malley 56* (AD 98615266); 2 km N of Donovan's Landing, near Glenelg River, 4 Nov. 1981, *A.A. Munir 5410* (AD 98153296; CANB 350238). **Tasmania**: 3–4 miles from Cradle Mountain on road to Wilmot, 12 Nov. 1965, *M.E. Phillips s.n.* (CBG 14363); 7 miles from Lunawanna, South Bruny Island, towards Cape Bruny, 10 Nov. 1965, *M.E. Phillips s.n.* (CBG 14685); Frenchman's Cap track, at Franklin River, 31 Jan 1969, *E.M. Canning s.n.* (CBG

27186); Russell Falls, Mount Field National Park, 6 Dec. 1977, *L.G. Adams 3364* (CANB 272708); Navarre Plain, 7 km SW of Derwent Bridge on Lyell Highway, 28 Jan 1983, *J.G. West 1983* (CANB 402767); Razorback Mine, Dundas, c. 9 km E of Zeehan, 7 Dec. 1977, *L.G. Adams 3381* (CANB 272716); Lawson Range, 25 Jan 1986, *A. Moscal 11966* (HO 402119; CANB 478725); North Pats River, Flinders Island, 28 Nov. 1976, *J.S. Whinray 2280* (CANB 482308). **Victoria**: Mount Buangor, 18 Dec. 1984, *A.C. Beauglehole 61360* (MEL 2110520); Glenelg River Road, 11 km W of Halls Gap, Grampians Ranges, 4 Oct 1987, *P.C. Jobson 121* (MEL 1561933); About 2.6 miles from Stanley towards Hillsborough, in the Stanley Forest, 19 Oct. 1967, *E.M. Canning s.n.* (CBG 21375); Otway Ranges, about 4 km N of Beech Forest on the Beech Forest to Gellibrand road, 12 Nov 1960, *H.I. Aston s.n.* (MEL 594189); 8 km SE of Lang Lang, 19 Oct 1978, *T.B. Muir 6206* (MEL 577686); 0.3 miles from Granja Gap towards Tallangatta, 28 Oct 1967, *L. Dunn s.n.* (CBG 21377); Lilly Pilly Gully, Wilson's Promontory, 21 Nov. 1961, *M.E. Phillips s.n.* (CBG 2112); Omeo-Corryong road, 2.7 road miles S of Sassafras Gap, 25 Nov 1964, *J. Ackland 188* (MEL 1513775); Quarry Road, Briagolong, 29 Nov 1981, *R.A. Kilgour 136* (MEL 600007); Thompson River Natural Feature Zone, 6 km SE of Walhalla, 22 April 1985, *A.C. Beauglehole 79184* (MEL 677034). **New South Wales and ACT**: Tantawangalo State Forest, 12 km S of Tantawangalo, 24 April 1993, *I.Crawford 2260* (CBG 9317573); "Ngarago", 24.5 km ESE of Nimmitabel, 18 Nov. 1984, *J.G. West 4957* (CANB 454245); 4 km past trig on Merricumbene Fire Trail, Deua National Park, 12 Oct. 1993, *T.R. Lally 155* (CANB 462632); Below Lee's Spring near Blundell's Valley, Brindabella Range, 20 Nov. 1956, *Hj. Eichler 13300* (CANB 389661; AD95814019); Near the start of the walking track from Saltwater Creek to Bittangabee Bay, south of Eden, 15 Sept. 1984, *D.E. Albrecht 698* (CANB 357541); Mt. Dromedary, 25 Jan. 1970, *N.T. Burbidge 7824* (CANB 236770); Claymore Creek aqueduct offshoot, Watson's Crags Spur, Snowy Mountains, 28 Nov. 1970, *J.I. Raine ANU10317* (CANB 247164); 6 miles from Wentworth Falls, near Bedford Creek, 29 Nov. 1971, *J. Pulley 877* (CBG 40518); Glen Elgin, 17 Feb. 1930, *J.W. Haney 56* (CANB 6109); Beside Careys Peak Walking Track, Chichester State Forest, 24 March 1999, *D.J. Mallinson 582* (CBG 99104351); Enmore State Forest, 29 Oct. 1990, *S. McIntyre 1226* (NSW 243735).

2. *Viola banksii* K.R. Thiele & Prober, *sp. nov.*

V. hederacea Labill. affinis foliis grandioribus orbiculatis pluribus, sinu profundiore, floribus grandioribus colore diviore, petalo antico orbiculato plus minusve, glande antherae profunda et polline luteo vel aureo differt.

Typus: Australia, New South Wales, Cook's Rivulet, Kurnell, 12 Nov. 2001, *K.R. Thiele 2671 & S.M. Prober* (Holo: CANB; Iso: MEL, NSW, BRI)

V. hederacea var. elatines DC. *Prodr.* 1: 305 (1824); *Erpetion reniforme* Sweet, *Brit. Fl. Gard.* 2, t. 170 (1826); *Viola reniformis* (Sweet) Endl., *Cat. Hort. Acad. Vindob.* 1 (1842), non Wall. (1824); *Viola hederacea* f. *reniformis* (Sweet) Siebert & Voss, *Vilmorin's Blumengartnerei* 1 (1896). T: Botany Bay, R.Brown: ?BM (not found), *n.v.*

Vigorous *perennial herb* spreading by stolons; rootstock sometimes somewhat swollen and bulbous at the stem bases. *Stems* contracted so that the leaves form rosettes. *Leaves* broad-reniform to orbicular, the largest (12–)18–25(–35) mm long (from the base of the sinus to the apex of the lamina), (20–)30–45(–65) mm wide, 1.0–2.0 times wider than long, with a narrow basal sinus; lamina with (10–)12–18(–20) +/- prominent teeth, glabrous, +/- concolorous bright green; *stipules* narrowly triangular to broadly triangular, usually with several small or elongate, glandular teeth on each side. *Flowers* on scapes to 15 cm long and exceeding the leaves, strongly discolorous violet-and-white; *anterior petal* (7–)8–10(–12) mm long, (5–)6–8(–10) mm wide, distinctly and regularly ovate to broad-elliptic, broadest in the middle third, usually emarginate, with a large green V-shaped blotch at the base then rich violet for over half its length contrasting sharply with a prominent white apex, prominently 3-nerved, the midnerve not or scarcely anastomosing with the lateral nerves which branch +/- regularly towards the margins; *lateral petals* widely spreading, (8–)10–12(–14) mm long, strongly twisted to c. 180°, rich violet at the base grading to white distally; beard covering half or more of the width of the lateral petals; *dorsal petals* (8–)10–12(–15) mm long, (4–)6–8(–9) mm wide, ovate to broadly obovate, erect to strongly reflexed, rich violet at the flexure, white for most of

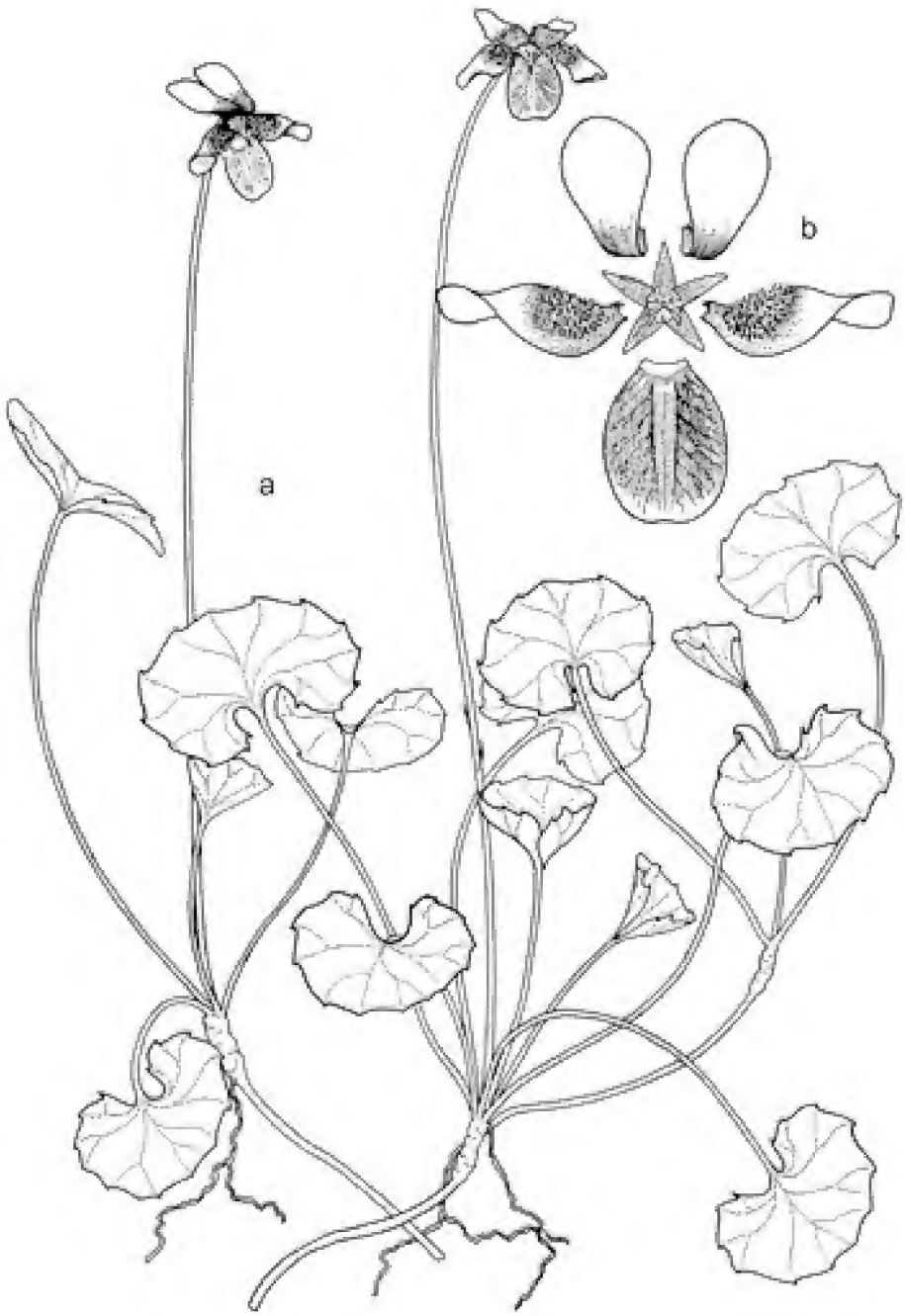

Figure 4. *Viola banksii.* **a** Habit x1; **b** Flower x2 (K.R. Thiele 2671, CANB).

their length. *Anthers* 2.0–5.0 mm long, cream, often flushed or flecked with violet, the terminal appendages straw-coloured, with short, irregular hairs on the outer margins of the anther cells; *anther glands* whitish green (never purplish), almost as long as the anther cells, very prominent, broad and high, +/- smooth; *pollen* and interior margins of the anther cells yellow to golden. *Ovary* and *fruit* whitish or pale green, often flecked or flushed purple; *style* distinctly geniculate at its insertion on the ovary. *Seeds* 1.8–2.5 mm long, glossy purplish-black, +/- smooth to irregularly rugose. *Fig. 4.*

Derivation of name. After Joseph Banks (1743–1820), naturalist on the Cook voyage to Australia and, with Daniel Solander, the first collector of the *Viola hederacea* species complex. The epithet *reniformis*, used for this species by some early authors, is preoccupied by *Viola reniformis* Wall., an Indian species.

Distribution and habitat. Common and widespread in near-coastal sites from near Ulladulla to just north of the Queensland-New South Wales border (Fig. 7b). *Viola banksii* is characteristic of coastal headlands, dune swales and coastal swamp and rainforest fringes, usually in moist areas. At some sites (e.g. at Pebbly Beach south of Ulladulla) *V. banksii* occurs on the margins of *Acmena smithii* rainforest behind the first dune, while *V. hederacea* occurs in drier sites beneath *Eucalyptus maculata* forest immediately adjacent. No intermediates between *V. banksii* and *V. hederacea* have been found, even in ecologically intermediate habitats.

Distinguishing features and variation. V. banksii differs from *V. hederacea* (Table 1; Fig. 3) in having larger, more richly coloured flowers, broad-ovate to broad-elliptic, almost semicircular anterior petals with triplinerved-pinnate rather than irregular venation, more strongly twisted, broader lateral petals with a more extensive beard, very large, prominent anther glands that are tall, broad and smooth, and purple-black seeds. Well-developed leaves of *V. banksii* are generally orbicular with a deep narrow sinus. *Viola banksii* matches most closely *Viola hederacea* 'forma G' of James (1990).

Adams (1982) described *Viola hederacea* subsp. *perreniformis* based on type material from Many Peaks Range, 40 km W of Gladstone, Queensland. This is a distinctive taxon found in inland, mostly mountain localities from the Border Ranges in southern Queensland to the Atherton Tableland. However, Adams included specimens referable to *V. banksii* in his concept of *V. hederacea* subsp. *perreniformis*, including the Banks specimen. *Viola hederacea* subsp. *perreniformis* differs from *V. banksii* in its smaller, less orbicular leaves with broader sinus, smaller flowers that are generally +/- concolorous pale violet, smaller, less prominent anther glands, and brown seeds.

Notes. Viola banksii was collected by Banks and Solander at Botany Bay in 1770, the first member of the *V. hederacea* species complex to be collected. Although a description and drawings were prepared for Banks' *Florilegium*, these were not published until 1900 (Banks & Solander 1900) when the taxon was ascribed to *V. hederacea.*

Banks and Solander collected mostly on the Kurnell Peninsula on the southern side of Botany Bay adjacent to the Endeavour anchorage. *Viola banksii* is still abundant on the banks of the small freshwater stream, Cook's Rivulet, from which the party drew water, and it is possible that this is the same population from which the Banks and Solander specimen was collected. The Cook's Rivulet plants are identical in all respects with the Banks and Solander specimen held at CANB.

Viola hederacea var. *elatines* DC., collected from Botany Bay by Robert Brown, appears to belong to this species. Microfiche photographs held at MEL of material from the De Candolle herbarium at G (not Type material, but presumably seen by De Candolle) show the characteristic orbicular leaves of the species. *Viola banksii* is common around Sydney and Brown would have had ample opportunity to collect material.

The excellent colour plate of *Erpetion reniforme* Sweet (basionym: *V. hederacea* var. *elatines*) in Sweet's British Flower Garden is not typical of the species, having leaves without deep sinuses and flowers with a rather rectangular to ovate anterior petal. It appears superficially closest to *V. eminens* (see below) or to an undescribed species found

on wet rock faces in the Blue Mountains and on Hawkesbury Sandstone as far south as Bundanoon. It is uncertain, however, whether material of these species could have been sent to Europe by 1826.

The plate of *Viola hederacea* in Banks and Solander (1900) comprises two illustrations of clearly different plants. The larger figure is *V. banksii*, clearly drawn from the Banks specimen collected at Botany Bay. The smaller figure is of *V. hederacea sens. str.*, presumably from material incorporated into the Banks herbarium after his return.

Viola banksii is commonly sold in the nursery trade (as *V. hederacea*), and is frequently grown in gardens well outside its natural range. In at least one locality (near Mt. Donna Buang, Victoria) it is adventive, presumably from material derived from a nearby garden or from dumped garden waste. It is likely that *V. banksii* will increase in range.

Selected specimens examined. **New South Wales**: Botany Bay, April 1770, *J. Banks & D. Solander* (CANB 371237); Durras, 24 Feb 1990, *A.M. Lyne 116* (CBG 9013646); St. George's Basin, 24 Jan 1974, *M. McMillan 740111* (CBG 56899); On the W side of Captain Cook Drive, 1.4 km from Cronulla High School, 19 Nov. 1986, *M.M. Richardson 50* (CBG 8603640); Coffs Harbour, 13 May 1967, *C.Burgess s.n.* (CBG 17249); Arrawarra Headland N of Woolgoolga, 7 March 1997, *A.R. Bean 11754* (BRI AQ654667); Iluka, 28 Dec. 1961, *L. Pedley 941* (BRI AQ115419); 1.3 km S of Yamba towards Angourie, 12 Nov. 1994, *A.R. Bean 8025* (BRI AQ633737); Norrie's Headland, Bogangar, 15 km S of Tweed Heads, 25 April 1976, *G.N. Batianoff 12* (BRI AQ169594). **Queensland**: Between Mudjimbah and Mount Coolum, E of Nambour, 14 Aug. 1963, *M.E. Phillips s.n.* (CBG 23396); Eighteen Mile Swamp, North Stradbroke Island, 23 Nov. 1971, *L. Durrington 650* (BRI AQ11278); Point Lookout, Stradbroke Island, 21 April 1935, *D.A. Goy s.n.* (BRI AQ115404); Nerang Creek, 1889, *H. Schneider s.n.* (BRI AQ115406); Southport, 28 Sept. 1952, *A.B. Cribb s.n.* (BRI AQ478596).

3. *Viola eminens* K.R. Thiele & Prober, *sp. nov.*

V. hederacea Labill. affinis foliis latioribus, habitu eminenti, scapis floribus longioribus, petalo antico ovato, colore florum diviore, glande antherae longiore et angustiore et polline luteo vel aureo differt.

Typus: Australia: Victoria: East Gippsland: Swamp on the Delegate River immediately upstream from its crossing with The Gap Road, c. 7.3 km direct line SW of Bendoc (37° 11' 58"S, 148° 49' 44"E), 5 Jan. 1997, *K.R. Thiele 2538 & S.M. Prober* (Holo: MEL; Iso: CANB, NSW, AD).

Perennial herb spreading by stolons; rootstock sometimes somewhat swollen and bulbous at the stem bases. *Stems* contracted so that the leaves form rosettes, or sometimes elongate (to 40 cm) with caulescent, alternate leaves. *Leaves* broad-reniform, the largest (10–)12–15(–25) mm long, (12–)25–35(–45) mm wide, 1.4–3.2 times wider than long, truncate at base or with a broad basal sinus; lamina with (6–)9–20 +/- prominent teeth, glabrous or with scattered unicellular hairs on the upper and/or lower surface, +/- concolorous bright green; petioles 2–12 cm long; *stipules* narrowly triangular to broadly triangular, usually with several small or elongate, glandular teeth on each side. *Flowers* on scapes to 25 cm long and exceeding the leaves, usually strongly discolorous violet-and-white, sometimes sub-discolorous (dark violet and pale violet); *anterior petal* (5–)8–10(–12) mm long, (3–)5–6(–9) mm wide, distinctly and regularly ovate to broad-ovate, broadest in the proximal third (or occasionally to near the middle), usually emarginate, with a large green V-shaped blotch at the base, then rich violet for over half its length contrasting sharply with a small white apex, prominently 3-nerved, the midnerve not or scarcely anastomosing with the lateral nerves which branch +/- regularly towards the margins; *lateral petals* widely spreading, (6–)9–11(–13) mm long, strongly twisted to c. 180°, rich violet at the base grading to white distally; beard covering half or more of the width of the lateral petals; *dorsal petals* (5–)10–12(–13) mm long, (2–)4–6(–7) mm wide, obovate to broadly obovate (rarely narrowly obovate), erect to

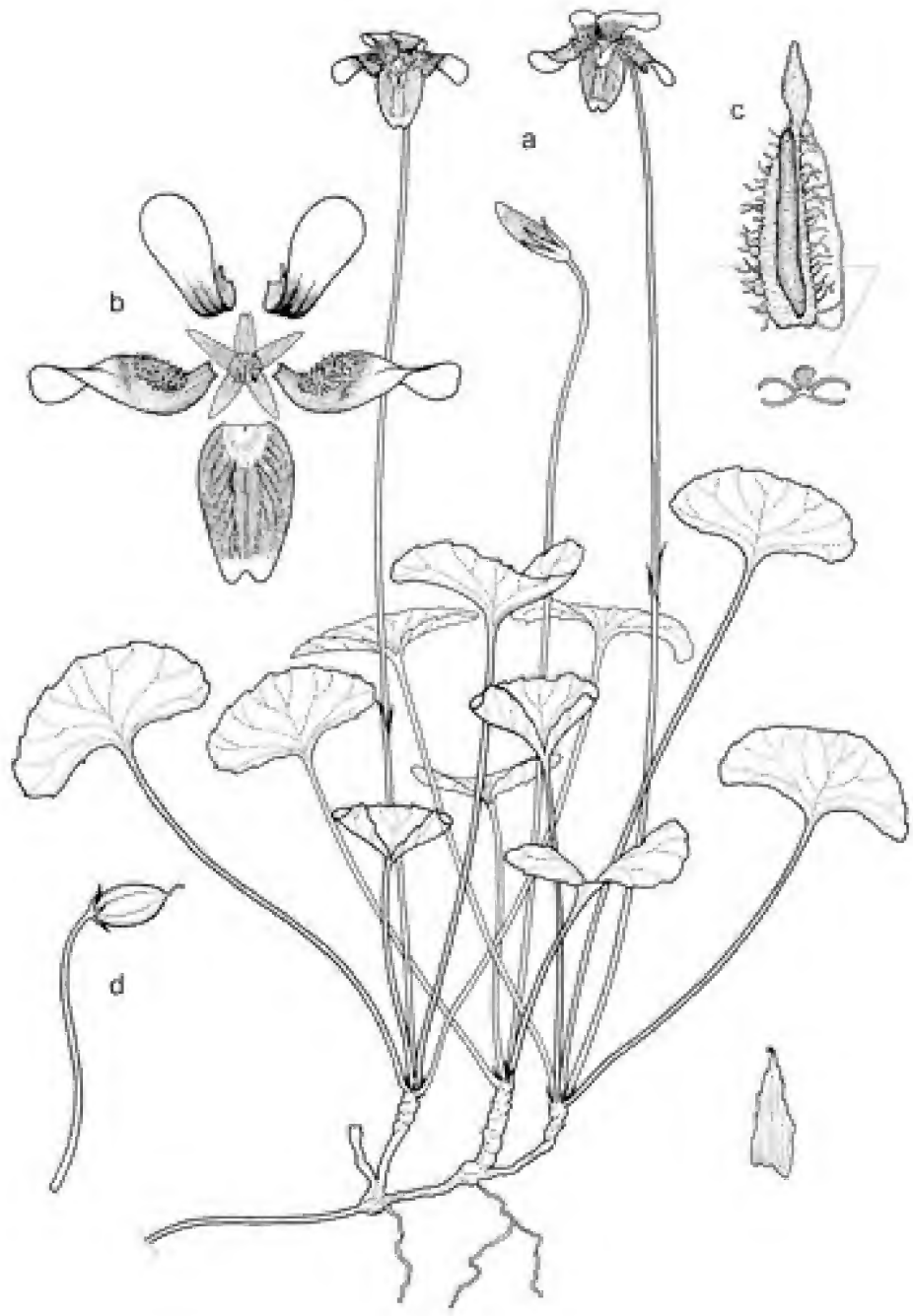

Figure 5. *Viola eminens* **a** Habit x1; **b** Flower x2; **c** ovary and style x? ; **d** Fruit ?1 (K.R. Thiele 2538, CANB).

strongly reflexed, rich violet at the flexure, white for most of their length. *Anthers* 2.0–4.5 mm long, cream, often flushed or flecked with violet, the terminal appendages straw-coloured, with short, irregular hairs on the outer margins of the anther cells; *anther glands* whitish green (never purplish), almost as long as the anther cells, narrow and high, +/- smooth, not or scarcely flattened or depressed; *pollen* and interior margins of the anther cells yellow to golden. *Ovary* and *fruit* whitish or pale green, often flecked or flushed purple; *style* distinctly geniculate at its insertion on the ovary. *Seeds* 1.6–2.6 mm long, glossy purplish-black, +/- smooth to distinctly rugose. *Fig. 5.*

Derivation of name. From the Latin *eminens*, in reference to the distinctively tall, stately flowering scapes that are characteristic of the species.

Distribution and habitat. A common to abundant species in disjunct localities on the Fleurieu Peninsula in South Australia, in the Grampians and Otway Ranges in western Victoria, and from near Melbourne through eastern Victoria to far southern New South Wales (Fig. 7c).

In eastern Victoria *V. eminens* is a characteristic species of moist sites at high altitudes, from Toolangi and Mt Donna Buang to Mt Baw Baw and Mt Wellington, on Mt Buffalo, and in East Gippsland around the Errinundra Plateau. It has not been collected from the Cobberas Range, even though conditions there appear suitable. It almost always occurs in moist sites, either in moist grassland beneath snow gums or on the margins of swamps and in drainage lines. It is infrequent at lower altitudes (e.g. Den of Nargun, Mitchell River National Park near Bairnsdale).

At some sites (e.g. along moist road verges near Goonmirk Rocks, Errinundra Plateau, East Gippsland) *V. eminens* and *V. hederacea* grow in mixed swards, with the flowers of *V. eminens* borne characteristically higher (on longer scapes) than those of *V. hederacea*. No intermediates between them have been found, even in ecologically intermediate habitats.

In the Grampians Range *V. eminens* occurs in moist to very moist sites, e.g. along the banks of the McKenzie River at Zumsteins and on wet, dripping banks at Kalymna Falls. At both these sites *V. hederacea* grows in drier habitats close by the *V. eminens* populations. In South Australia it always occurs in moist sites along stream lines and in swamps, from the southern tip of the Fleurieu Peninsula (e.g. Tunkalilla Creek) north to the Barossa Valley and on Kangaroo Island.

Only two definite collections are known from New South Wales. At Glenbog State Forest, *V. eminens* occupies a small, swampy drainage line with e.g. *Gratiola peruviana*, *Ranunculus pimpinellifolius* and *Hydrocotyle spp.*, while *V. hederacea sens. str.* occurs immediately adjacent on the slopes of the stream gully in drier soil. At Nungatta Mountain it is found in a 'wet site in dry sclerophyll forest'. New South Wales specimens of *V. eminens* probably match 'forma C' of James (1990), although it does not occur on the North Coast as described for that form.

Distinguishing features and variation. Flowers of *V. eminens* are somewhat similar to those of the coastal *V. banksii* (Table 1; Fig. 3). They differ principally in the anterior petal being ovate (broadest toward the base) rather than almost orbicular (broadest about the middle), with a smaller white area at the tip, and in the smaller anther glands. Well-developed leaves of *V. eminens* are broader than long and have a broad sinus, while those of *V. banksii* are usually almost orbicular with a narrow sinus.

Distinguishing features from *V. hederacea sens. str.* are the taller flower-scapes, the richer violet flowers with a more definite demarcation between the violet and white, the distinctively neat, ovate anterior petal with triplinerved-pinnate rather than irregular venation, the more strongly twisted lateral petals with a more extensive beard, the narrower, less rugose, pale greenish-white anther-glands, the purplish-black seeds, and the broader, greener leaves. On herbarium specimens, leaves of *V. hederacea* often dry rather greyish and distinctly discolorous, while those of *V. eminens* remain pale green above and beneath.

Western collections of *V. eminens* (from the Grampians Ranges in Western Victoria and in South Australia) differ from eastern collections (east of Melbourne) in having a strong tendency towards caulescence. In the western populations, plants growing amongst dense vegetation along streamsides or in swamps often become scrambling or climbing, with elongate stems to 40 cm high and widely spaced, alternate leaves. In adjacent more exposed sites plants have contracted stems with leaves in fascicles. Caulescence has never been observed in *V. hederacea*, even in circumstances where plants are growing amongst dense vegetation, and has only rarely been seen in eastern *V. eminens*. It is possible that the western populations of *V. eminens* may warrant subspecific rank, but further work is needed to clarify their status.

A series of collections from near-coastal sites at the western end of Kangaroo Island have distinctively small, angular leaves. These plants are provisionally regarded as a coastal variant of *V. eminens*. Further work, however, may show them to be distinct.

Selected specimens examined. **Victoria**: 14 km S of Bendoc, Errinundra Flora Reserve, Goonmirk Rocks, 29 March 1988, *G.A. Savage 17* (CBG 8800949); Valley below The Castle and near Whale Rock, Mt. Buffalo, 29 Dec 1952, *R. Melville 2622* (MEL 525759); Near junction of Block 10 Road and Thompson Valley Road, 2 km from helipad, 9 Dec 1997, *M.G. Corrick 11567* (MEL 2073182); Blue Range Road, near the crossing at Storm Creek, 2 Jan 1983, *M.G. Corrick 8578* (MEL 657243); Blue Range Road, 1.5 miles N of Mt. Margaret Gap, Marysville district, 21 Dec 1965, *E.J. Carroll s.n.* (CBG 14407); On Ben Cairn Road, where it crosses Walker Creek, 200 m W from road to summit of Mt. Donna Buang, 20 Feb. 1996, *D.B. Foreman 1737* (MEL 2044048); Mt. Baw Baw summit, 1 Feb. 1988, *M. Gray 7153* (CANB 510168); Above Baw Baw Village, 31 Jan 1970, *C.L. Gunn s.n.* (CANB 251717); Echo Flat, Lake Mountain, 22 Dec 1965, *E.J. Carroll s.n.* (CBG 17933); Two miles from Toolangi toward Kinglake, *E.J. Carroll s.n.* (CBG 18007); Against falls at spillway to Lake St. George, Creswick, 30 Dec. 1971, *J.H. Willis s.n.* (MEL 100375); Otway State Forest, rest stop on road from Colac to Gellibrand, 6 km from Gellibrand, 12 Dec 1990, *D. Cunningham 319* (MEL 288838); Top of Mount William and Mount Abrupt, Dec. 1856 – Jan. 1857, *C. Wilhelmi* (MEL 100422). **South Australia**: Section 748, Hundred of Moorooroo, Upper tributary of Tanunda Creek near Schlenke Gully, 25 July 1985, *P.J. Lang 1702* (MEL 1598740; AD 98908074); Warren Conservation Park, 5 Jan 1986, *R. Bates 6756* (CBG 9000713; AD 98607013); Barossa Valley, Upper Tanunda Creek, Schlenkes Creek Gorge, 24 Oct. 1984, *D.N. Kraehenbuehl 4432* (CBG 8908345); AD 98449113); Mount Compass, 6 Oct. 1945, *R.A. Perry* (CANB 19535); Swamp North of Tunkalilla Beach, between Cape Jervis and Victor Harbour, 18 Nov. 1957, *Hj. Eichler 14496* (CANB 389660; AD 95814014); Kangaroo Island, near the permanent pools of Rocky River at Shackle Road, 6 Jan. 1966, *Hj. Eichler 18600* (CANB 318006; AD 96650349). **New South Wales**: N Slope of Nungatta Mountain, c. 48 km SSW of Eden, 16 Feb. 1984, *T. James 511a* (NSW); Glenbog State Forest, Packer's Swamp Road, at creek crossing 2 km SW of Robinson's Road junction, 29 Nov. 1998, *K.R. Thiele 2609* (CANB).

4. *Viola x zophodes* K.R. Thiele & Prober, *nothosp. nov.*

Hybrida e V. eminente et V. fuscoviolacea, a primo floribus minoribus fuscatis, petalis in extremitatibus distalis non-albis vel dealbatis parum; a secundo floribus grandioribus in scapis longioribus fulcratis et foliis latioribus differt.

Typus: Australia: Victoria: East Gippsland: Swamp on the Delegate River immediately upstream from its crossing with The Gap Road, c. 7.3 km direct line SW of Bendoc (37° 11' 58"S, 148° 49' 44"E), 5 Jan. 1997, *K.R. Thiele 2539 & S.M. Prober* (Holo: MEL; Iso: CANB, NSW).

Perennial herb spreading by stolons; rootstock sometimes somewhat swollen and bulbous at the stem bases. *Stems* contracted so that the leaves form rosettes, never elongate with caulescent leaves. *Leaves* broad-reniform, the largest (10–)12–14(–16) mm long, (18–)20–28(–32) mm wide, 1.8–3 times wider than long, usually with a broad basal sinus; lamina with 12–18 +/- prominent teeth, glabrous or with scattered unicellular hairs on the upper surface, +/- concolorous bright green; petioles 2–4 cm long; *stipules*

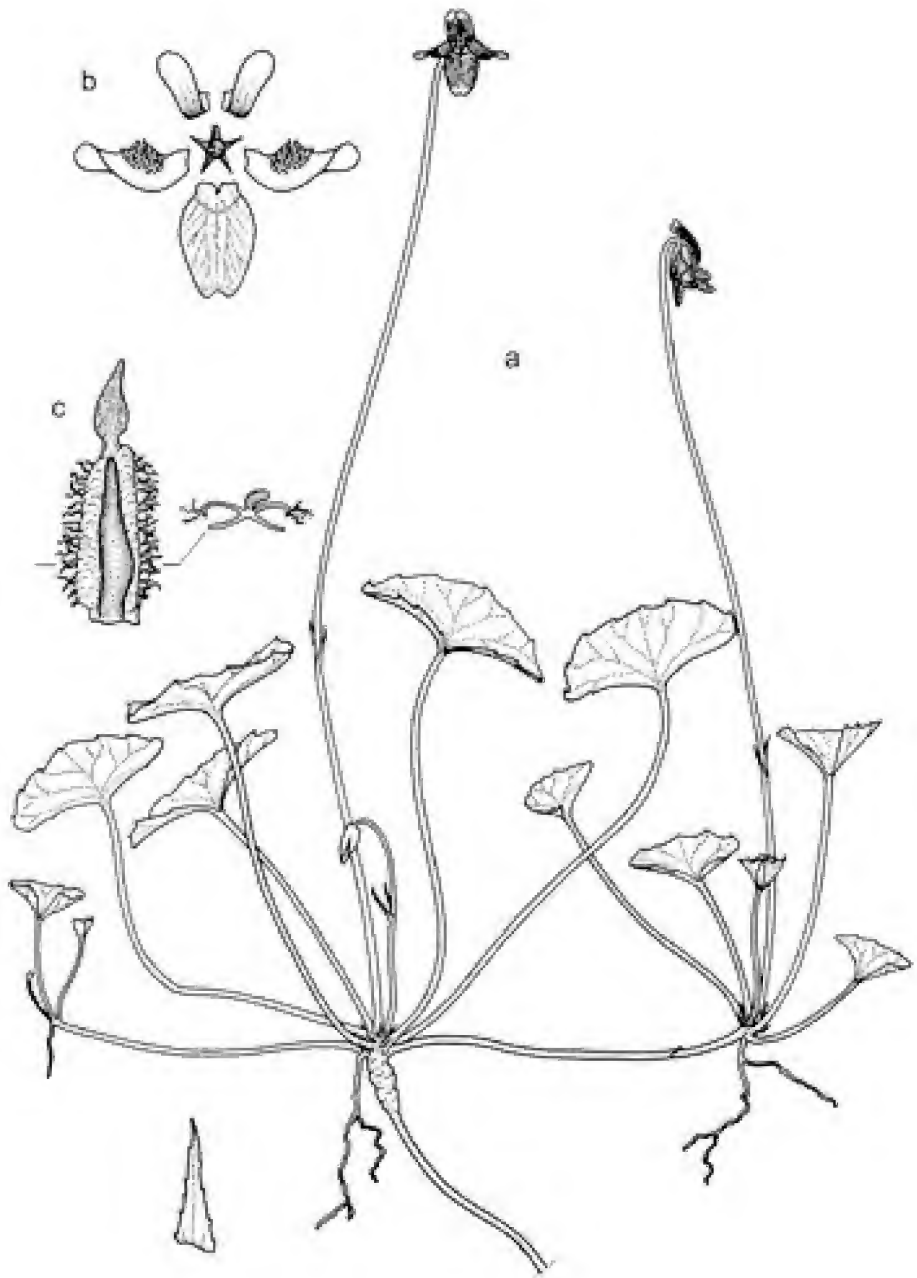

Figure 6. *Viola x zophodes* **a** Habit x1; **b** Flower x2; **c** ovary and style x? (K.R. Thiele 2539, CANB).

narrowly triangular, usually with several small, glandular teeth on each side. *Flowers* on scapes to 12 cm long and exceeding the leaves, blackish-violet with or without small whitish apices to the petals; *anterior petal* (6–)7–8(–8.5) mm long, (4–)5–6(–6.5) mm wide, distinctly and regularly ovate to broad-ovate, broadest in the proximal third, usually broadly emarginate, with a large green V-shaped blotch at the base, then blackish-violet for most of its length sometimes grading to a small whitish apex, prominently 3-nerved, the midnerve not or scarcely anastomosing with the lateral nerves which branch towards the margins; *lateral petals* widely spreading, (7–)7.5–8(–9) mm long, distinctly twisted to c. 180°, blackish-violet sometimes grading to white distally; beard covering about half the width of the lateral petals; *dorsal petals* (7–)8–9(–10) mm long, 3–4 mm wide, narrowly obovate, erect to strongly reflexed, blackish-violet with or without a whitish apex. *Anthers* 3.0–3.5 mm long, cream, often flushed or flecked with violet, the terminal appendages straw-coloured, with short, irregular hairs on the outer margins of the anther cells; *anther glands* green (never purplish), almost as long as the anther cells, +/- smooth, each somewhat flattened or depressed towards the other; *pollen* and interior margins of the anther cells white to cream. *Ovary* and *fruit* whitish or pale green, often flecked or flushed purple; *style* distinctly geniculate at its insertion on the ovary. Mature fruits and *seeds* apparently never produced. *Fig. 6.*

Derivation of name. From the Greek *zophodes*, 'dusky', 'gloomy' (as in twilight), in reference to the very dark, dusky-violet flowers.

Distribution. Scattered in moist sites in the highlands of Victoria e.g. Delegate River near Bendoc (East Gippsland), and near Mt Reynard and Mt. Wellington (Eastern Highlands). *Viola x zophodes* always occurs with *V. eminens* and *V. fuscoviolacea*, and may be expected in other montane to alpine sites where these species co-occur (Fig. 7d).

Distinguishing features and variation. Viola x zophodes is almost certainly an F1 hybrid between *V. eminens* and *V. fuscoviolacea*. It always occurs with these two species, usually occupying ecologically intermediate sites. At all known populations no seed is set, and the pollen grains are empty and ovules slightly discoloured at anthesis. For this reason, introgression with the parents apparently does not occur, and *V. x zophodes* forms a distinctive entity rather than a variable hybrid swarm. Plants are vigorously stoloniferous and often locally abundant.

V. x zophodes differs from *V. eminens* (Table 1; Fig. 3) in its smaller flowers that are blackish-violet compared with the bright violet of *V. eminens*, and in its narrower petals. It differs from *V. fuscoviolacea* in having larger, more openly presented flowers held above the leaves. *V. fuscoviolacea* leaves are usually smaller and more spathulate, but leaves on vigorous plants of *V. fuscoviolacea* are quite reniform and then indistinguishable from small leaves of *V. x zophodes*. The anterior petal of *V. fuscoviolacea* lacks the basal green crescentic mark seen on *V. x zophodes* (and all the other taxa described in this paper).

Different populations of *V. x zophodes* differ somewhat. For instance, on the Delegate River at The Gap Road (East Gippsland), plants are relatively tall and all petals have prominent, though small, white distal patches. Further downstream at the crossing of the Bonang-Bendoc road, and at the Lost Plain, the plants have slightly smaller flowers with very little white at all. Within a population there is little variation, suggesting that hybridisation events giving rise to *V. x zophodes* are rather rare, the plants propagating vegetatively. It is not known which is the male and which the female parent, or whether both crosses are possible.

Selected specimens examined. **Victoria**: Mount Reynard Plateau, c. 1 km NNW of Mt. Reynard, 14 Dec 2000, *N.G. Walsh 5267* (MEL 2089855); Lost Plain, 7 miles NW of Mt. Wellington, 19 Jan 1967, *T.B. Muir 4578* (MEL 100475); Delegate River, on flats at Bidwell Bridge, 1 Dec. 1962, *J.H. Willis s.n.* (MEL 100504).

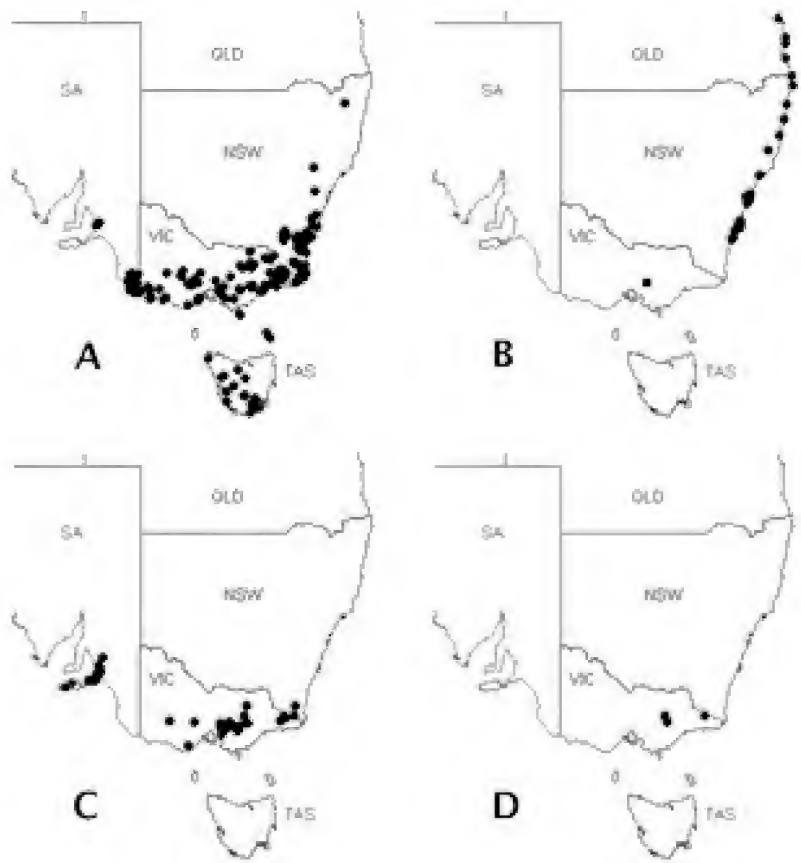

Figure 7. Distribution of *Viola* taxa in south-eastern Australia (based on specimen data from AD, BRI, CANB, MEL, NSW). **A** *V. hederacea sens. str.*; **B** *V. banksii* (● natural populations, ■ adventive population); **C** *V. eminens*; **D** *V. x zophodes*

Key to taxa in the *V. hederacea* species complex

The following provisional key includes all recognised taxa in the *Viola hederacea* species complex (except *V. hederacea* subsp. *curtisiae* and *V. improcera*), as well as a number of undescribed taxa that will be treated in subsequent papers.

V. hederacea subsp. *curtisiae* is described from high elevations at Mount Field in Tasmania; a few collections from the Baw Baw plateau in Victoria have also been allied to this taxon (Entwisle, 1996). *V. improcera* is known only from a few mountain peaks in Victoria (Nunniong Plateau and Mt. Useful) and the Australian Capital Territory (Brindabella Range). Both taxa are rarely collected, poorly understood, and of uncertain status. They could not be included in the key as their diagnostic features are uncertain.

1. Flowers on short scapes hidden among the leaves; petals scarcely exceeding the sepals 2

1a. Flowers on scapes as long as or longer than the leaves; petals clearly longer than the sepals.....3

2. Flowers blackish-violet; usually montane plants.....*V. fuscoviolacea*

2a. Flowers white; lowland plants ..... *V. cleistogamoides*

3. Well developed leaves twice as wide as long or wider; flowers concolorous pale bluish; stems often caulescent; habitat on sandstone in New South Wales .... *V. sp. nov. A* [1]
3a. Leaves not much wider than long or, if about twice as wide as long then flowers distinctly discolorous 4

4. Anterior petal +/- rectangular 5
4a. Anterior petal ovate, almost circular or obovate-obcuneate 6

5. Flowers +/- concolorous pale blue; plants small, usually with cuneate leaf bases .... *V. sieberiana*[2]
5a. Flowers +/- discolorous pale and dark violet (or white and dark violet); plants robust, often trailing; leaves reniform ..... *V. sp. nov. B*[3]

6. Anterior petal broadest in the distal third, its venation usually somewhat irregular, petals with indistinct demarcation between violet and white colouration; seeds dull, cream to brown, usually mottled 7
6a. Anterior petal broadest in the proximal third or the middle, regularly triplinerved, petals with distinct demarcation between violet and white colouration; seeds glossy purple-black 8

7. Leaves usually +/- semi-circular, glabrous to sparsely hairy; flowers usually +/- discolorous; southern plants (S of the New England Tablelands, NSW) .... *V. hederacea sens. str.*
7a. Leaves suborbicular to reniform with a deep sinus, softly hairy; flowers +/- concolorous; northern plants (N from the NSW-Qld Border Ranges) .... *V. sp. nov. C* (=*V. hederacea* subsp. *perreniformis*)[4]

8. Flowers blackish violet, with or without small white tips to the petals .. *V. x zophodes*
8a. Flowers bright violet and white, with prominent white (rarely pale violet) tips to the petals 9

9. Anterior petal broadest in the middle; leaves reniform to orbicular, about as long as wide, often with a deep, narrow sinus; lowland, often coastal plants ..... *V. banksii*
9a. Anterior petal usually broadest in the proximal third; leaves reniform, wider than long, with a broad sinus or truncate; alpine to montane plants in eastern Australia (at lower altitudes in SA) ..... *V. eminens*

[1]A distinctive species from seasonally very dry sandstone sites in New South Wales including Jervis Bay, the Bundanoon area to the Blue Mountains with a disjunct population around Minyon Falls inland from Byron Bay.
[2]Including *V. hederacea* subsp. *seppeltiana*. *V. sieberiana* is restricted to sandstone sites around Sydney in New South Wales. *V. hederacea* subsp. *seppeltiana* is widespread from the Grampians Ranges (western Vic.) to the Mount Lofty Ranges (SA), and may be distinct from *V. sieberiana*. Specimens identified as *V. sieberiana* in intervening areas (e.g. eastern Victoria) are misdetermined, most being *V. fuscoviolacea*, *V. cleistogamoides* or small-flowered *V. hederacea*.
[3]A provisional taxon found on moist sandstone sites particularly on waterfalls and wet soakage areas on sandstone in NSW from Bundanoon to the Blue Mountains.
[4]Preliminary results suggest that this taxon should be raised to species rank, as it is geographically and morphologically distinct from *V. hederacea sens. str.*

Acknowledgements

We would like to thank the directors and staff of AD, BRI, CANB, MEL and NSW for access to their collections; Neville Walsh for the Latin diagnoses; Rod Seppelt (while ABLO at K) and Judy West for help locating material at K and BM; Laurie Adams, Sophie Ducker and Neville Walsh for discussions concerning Viola; and Rob Peace, Denzel Murfet, Rosemary Taplin and Ian McCann for assistance with field work.

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Muelleria 18: 27–32 (2003)

A New Species of *Cardamine* (Brassicaceae) from South-eastern Australia and a Key to *Cardamine* in Australia

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Abstract

A new species *Cardamine tryssa* I.Thomps. from south-eastern Australia is described and illustrated. A key to *Cardamine* species occurring in Australia is also presented.

Introduction

Cardamine L. is a genus of c. 200 species in the Brassicaceae that occurs predominantly in temperate and/or high altitude regions around the world. Twelve indigenous species occur in Australia, mostly in the south-east, and there are four introduced species. During studies of Australian Cardamine (Thompson 1996, Thompson & Ladiges 1996) a few specimens similar to but smaller than typical Cardamine franklinensis I.Thomps. were examined. Subsequent field collections at a Victorian locality and re-examination of herbarium material from interstate herbaria have consolidated the case for recognizing this entity as a new species.

Taxonomy

Cardamine tryssa I.Thomps., *sp. nov.*

A *Cardamine franklinensis* I. Thomps. caulibus gracilioribus, foliis tenuioribus sine pinnis, inflorescentiis paucifloris, floribus minoribus, differt.

Type: Victoria: SW corner of major bridge on Princes Hwy crossing Toorloo Arm of Lake Tyers. Between Lakes Entrance and Nowa Nowa, 17 Dec. 1995, *I.R. Thompson 311* (holotype MEL).

Annual herb to 15 cm high, tap-rooted, glabrous. *Stems* erect, slender, 0.5–0.8 mm diam. *Leaves* mid-green, thin. *Rosette leaves* 5–10, persistent at flowering, simple, 20–80 mm long; petiole 10–60 mm long; lamina elliptic, oblong-elliptic or obovate, 7–20 mm long, 5–10 mm wide; apex obtuse to rounded; base cuneate; margins entire or with 1–3 crenations or lobes per side, the lobes not longer than broad. *Cauline leaves* 0–2, 7–20 mm long; subsessile or petiole to c. 3 mm long; lamina obovate to narrow-elliptic, entire or crenations 1 or 2 per side. *Inflorescences* racemose, indeterminate, of 3–15 flowers; pedicels 2–4 mm long at anthesis. *Flowers* with sepals green or purple, ovate, 1.3–1.8 mm long; petals white internally, usually pink externally, spathulate, 2.5–4 mm long; stamens 6; stigma subsessile at anthesis. *Fruit* with mature pedicels erecto-patent, 5–10 mm long; siliquas erect to suberect, linear, 20–25 mm long, 0.7–1 mm wide; style to 1.5 mm long. *Seeds* elliptic, 0.8–1.0 mm long. (Fig. 1.)

Etymology*: The epithet alludes to its diminutive nature, slender stems, thin leaves and small flowers (Gk: tryssos, delicate).*

Specimens examined*: New South Wales: Cave Creek, 1.5 miles [2.4 km] above junction with Goodradigbee River, 11.iv.1968,* A. Rodd 592a *(NSW). Australian Capital Territory: Near junction of De Salis Creek and Cotter River, Namadgi N.P., 5.xi.1987,* P. Gilmour 6248 *(CBG). Victoria: Forest beside Toorloo Arm (L. Tyers) near Princes Highway Crossing, Sept. 1976,* I.C. Clarke 941 & P.G. Ladd *(MELU); SW corner of major bridge on Princes Hwy crossing Toorloo Arm of Lake Tyers. Between Lakes Entrance and Nowa Nowa, 3.xi.1995,* I.R. Thompson 281, M.F. Duretto & P.G. Neish *(MEL). Tasmania: Pontville, no date, Herb* Spicer *(MEL).*

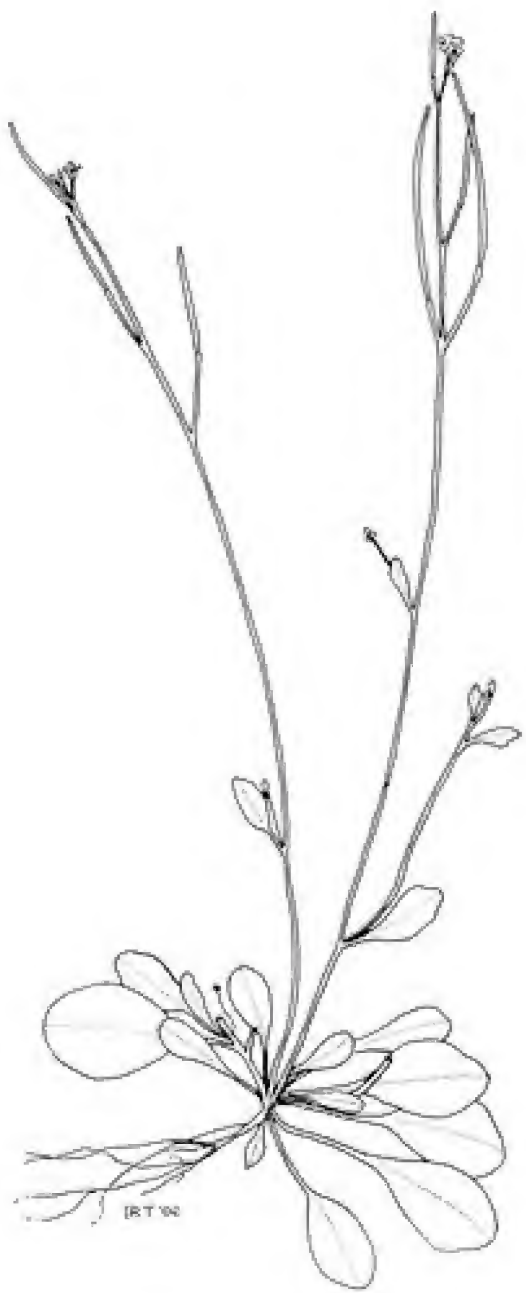

Figure 1. *Cardamine tryssa*, habit. The flowering stems are secondary; and the primary stem has been largely lost. (Holotype: *I.R.Thompson 311*, MEL). Scale bar = 1 cm.

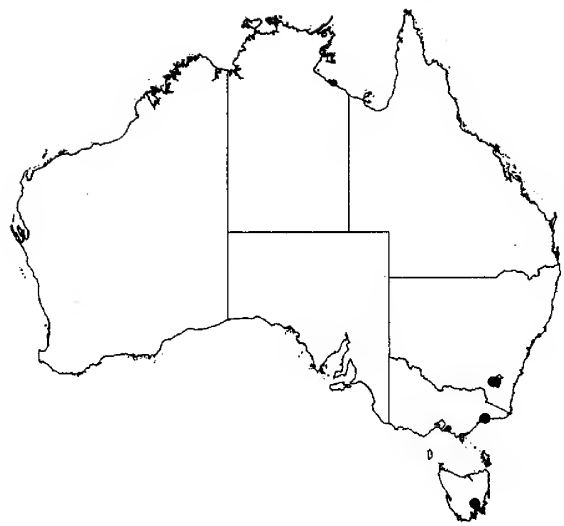

Figure 2. Distribution of *Cardamine tryssa.*

Distribution and Conservation Status. A rare species known from three mainland locations (Fig. 2): near Lake Tyers in East Gippsland, Victoria; from Cave Creek in the Southern Tablelands, N.S.W.; and from De Salis Creek in the A.C.T. There is also an old record from Pontville, in south-east Tasmania. The N.S.W. locality is within Kosciuszko National Park and the A.C.T. locality is within Namadgi National Park.

Habitat. *Recorded from areas of limestone geology in open forest. In the East Gippsland location it occurs in open forest adjacent to a river on a moderately steep slope.*

Notes. *Cardamine tryssa* is similar to *C. franklinensis* in habit and leaf shape. However, *C. tryssa* is not as robust, has thinner leaves, the rosette leaves do not develop pinnatisect segments, inflorescences are fewer-flowered, and flowers are smaller. *Cardamine tryssa* is readily distinguished from other small-flowered (petals < 5 mm long) species of *Cardamine* in Australia by its leaf morphology. Also, petals of *C. tryssa* are pink abaxially in at least some populations, whereas most other small-flowered species have entirely white petals. An exception is a small-flowered form of *C. lilacina* which has been recorded from eastern Victoria. *C. tryssa* appears to behave largely as an annual, but possibly could persist into a second season.

Key to *Cardamine* in Australia

Note: The term pinnate is used for leaves if well-defined petiolule and blade portions are evident; otherwise the term pinnatisect is used.

1 Stems and/or upper surface of leaves at least sparsely haired (close inspection necessary, ideally using low power magnification)
  2 Upper surface of leaves (excluding margins) glabrous
    3 Terminal segment of lower- to mid-cauline leaves with mostly 2–4 lobes per side, sometimes fewer, l:w > 1.2; siliquas erect to sub-erect, forming an angle of < 30° with rachis; natural environments, commonly by streams .... ***C. microthrix***

3: Terminal segment of lower- to mid-cauline leaves with 0–1 lobes per side, or if 2, l:w < 1.2; siliquas strongly divergent, forming angle of > 30° with rachis; urban environments (nurseries, garden beds and gutters)..... ****C.*** aff. ***flexuosa***

2: Upper surface of leaves (excluding margins) bearing scattered hairs
4 Primary stem usually shorter than rosette leaves or lacking and then long-pedicellate flowers arising directly from base; leaves simple or pinnate with 1 or 2 pairs of lateral pinnae; if stem developed, pedicels commonly arising in whorls of 3 or 4; flowers often apetalous or with fewer than 4 petals; siliquas less than 1 mm wide; urban environments .....**C. corymbosa*
4: Primary stem usually longer than rosette leaves; leaves pinnate with 1–6 pairs of lateral pinnae; pedicels ±alternating along rachis; flowers usually with 4 petals; siliquas 1–1.5 mm wide; urban or natural environments
5 Stems glabrous or sparsely hairy; cauline leaves 0–3, rarely more, hairs on leaves not obviously tubercle-based; inflorescence rachis straight, developing fruits usually clearly overtopping open flowers; stamens mostly 4; siliquas forming an angle of < 45° with rachis, valves glabrous or hairy; widespread in urban and a range of natural environments .....**C. hirsuta*
5: Stems sparsely to moderately hairy; cauline leaves mostly > 3, sometimes fewer if plant stunted, hairs on leaves ±distinctly tubercle-based; inflorescence rachis often flexuose, developing fruits not or hardly overtopping open flowers; stamens mostly 6; siliquas forming an angle of > 45° with rachis, valves glabrous; urban and sometimes natural environments in moist, shady habitats .....**C. flexuosa*

1: Stems and leaves glabrous
6 Perennials, horizontal stem growth extensive, sometimes also much branched and forming dense broad clumps; alpine or sub-alpine
7 Stems slender, 1–2 mm in diam., hardly succulent; ascending stems often unbranched, sometimes few-branched, rosettes not developing where stem turns vertical or rosette leaves up to 6; subalpine to alpine areas of N.S.W. and Vic .....*C. astoniae*
7: Stems robust, mostly 2–5 mm diam., succulent, ascending stems often few–several-branched, developing ±loose rosettes of many leaves; endemic to alpine zone of Mt. Kosciuszko region .....*C. robusta*
6: Annuals, or perennials, extensive horizontal growth lacking, but sometimes rootstock of rosetted perennials elongating in small increments each season, and sometimes branching
8 Stems erect or trailing, to c. 60 cm long; cauline leaves 7–many, pinnatisect, segments of higher leaves ± linear; petals > 5 mm long; mature style > 1.5 mm long; lowland swamps .....*C. tenuifolia*
8: Stems erect or ascending, mostly < 30 cm long; cauline leaves mostly 0–6, not divided or division various; petals and mature style various lengths; habitat various
9 Plants developing subtuberous roots; petals white, 5–8 mm long; lowland, westernVic., south-eastern S.A., Tas. (only one collection in past 100 years) .....*C. gunnii*
9: Plants not developing subtuberous roots; petal colour and length various; lowland to alpine; distribution various
10 Annuals; inflorescences indeterminate, primary inflorescence commonly of > 10 flowers; siliquas forming an angle of 30–45° with rachis; urban environments (nurseries, garden beds, gutters) ....**C.* aff. *flexuosa*
10: Annuals or perennials; inflorescences determinate or indeterminate, primary inflorescence of 2–many flowers; siliquas erect or nearly so; natural environments
11 Annuals; rosette usually entirely lost before first fruits mature; all basal leaves with base of terminal blade/segment/pinna attenuate to broad-cuneate; inflorescences from upper cauline leaves commonly

overtopping the primary inflorescence; inflorescences determinate, mostly 2–7-flowered; lowlying areas; inland plains of N.S.W., Vic., and south-eastern S.A.

12 Lower- to mid-cauline leaves undivided and ± narrow-linear or pinnatisect with 1 or occasionally 2 segments per side; segments unlobed, not narrower at base or base >1/2 the width of segment ..... ***C. lineariloba***

12: Lower- to mid-cauline leaves pinnate or pinnatisect with 2 or 3 pinnae/segments per side; segments often lobed, narrower at base, base < 1/2 the width of segment ..... ***C. moirensis***

11: Annuals or perennials; rosette often more persistent than above; at least the earlier basal leaves with base of terminal blade/segment/pinna truncate to cordate, or if cuneate the terminal blade/ segment/pinna > 7 mm wide and/or cauline leaves entire, l:w c. 3; inflorescences from upper cauline leaves not overtopping the primary inflorescence; inflorescences determinate or indeterminate, 2–many-flowered; habitat and region various but not as above

13 Leaves all simple, ± spathulate, or some pinnatisect, the terminal segment longer than broad, narrow to broad cuneate basally, lateral segments sessile, obovate

14 Rosette leaves undivided or usually a proportion with lateral segments; cauline leaves often deeply cleft, sinuses acute; inflorescences of up to 30 or more flowers; sepals 1.8–3 mm long, petals 4-7 mm long ..... ***C. franklinensis***

14: Rosette leaves undivided; cauline leaves not deeply cleft, sinuses not acute; inflorescences of up to 15 flowers; sepals 1.3–1.8 mm long, petals 2.5–4 mm long ..... ***C. tryssa***

13: Commonly at least some basal leaves pinnate, terminal pinna, or blade of simple leaves, c. as long as broad, base truncate to cordate

15 Perennials, rootstock with persistent leaf bases clustered as evidence of previous seasons' rosettes; petals entirely white or pink abaxially, 4–12 mm long; seeds 1.2–2.5 mm long; hills at moderate altitudes to alpine ..... ***C. lilacina***

15: Annuals or biennials, rootstock not developing as above; petals white, 2–4 mm long; seeds 0.7–1.3 mm long; lowland to subalpine

16 Mid-cauline leaves pinnate, lamina of terminal pinna 6–30 mm wide, frequently 2–4 lobes per side, sometimes fewer; margins of pinnae bearing a few to several minute hairs (thorough inspection with magnification may be required) ..... ***C. microthrix***

16: Mid-cauline leaves pinnate or pinnatisect, if pinnate, lamina of terminal pinna < 8 mm wide, entire or with 1 shallow lobe per side; margins of pinnae quite glabrous

17 Cauline leaves 0–3, if 2 or 3 the uppermost cauline leaf mostly < 2.5 cm long; racemes somewhat congested, rachis finally < 4 cm long; pedicels and rachis sometimes minutely papillose; hilly country to subalpine ..... ***C. papillata***

17: Cauline leaves 2–6, uppermost cauline leaf mostly > 2.5 cm long; racemes somewhat lax, rachis of at least primary inflorescence commonly finally > 4 cm long; pedicels and rachis never papillose; lowland regions ..... ***C. paucijuga***

Acknowledgements

I am grateful to staff at MEL for general assistance and for use of facilities and collections, and to the directors/curators of CANB and NSW for the loan of material.

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Muelleria 18: 33–65 (2003)

Seed morphology of Australian species of *Nymphoides* (Menyanthaceae)

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Abstract

Seeds of all known Australian species of *Nymphoides* have been examined externally and measured by light microscope. Representative seeds were selected, and their micromorphology examined by Scanning Electron Microscope (SEM). The results indicate that a combination of seed size, shape, ornamentation and caruncle is distinctive for most species when ornamentation is developed to its fullest extent. However the fullest degree of ornamentation for many species may be reduced or absent even in mature seeds, and sometimes within the same population. Fully ornamented seeds and known variations are described and illustrated. Immature seeds should not be used for comparisons.

Introduction

This work on seeds was undertaken as part of my long-term interest in morphological revisionary studies on the taxonomy of *Nymphoides* within Australia, involving both herbarium and field observations.

The taxonomic value of seed size, shape, ornamentation, and of any caruncular excrescence in Menyanthaceae has been widely recognised. This value is at species level, with well-developed seeds being diagnostic for most species. Botanical drawings in many accounts of Menyanthaceae taxa include seed illustrations from light microscopy (e.g. Aston 1969, 1982, 1984, 1986, 1987, 1997; Raynal 1974; Short 2000) but some SEM studies have been used also (Sivarajan et al. 1989; Chuang & Ornduff 1992).

Seed morphology and its bearing on the systematics of the five genera in Menyanthaceae have been discussed by Chuang and Ornduff (1992). From their work, they concluded that it would not be possible to define any unique set of seed morphological or cellular characters that would distinguish either Menyanthaceae at family level or any of its contained taxa at generic level. However, the high diversity in seed morphology provided ready distinctions between species.

The SEM studies of Chuang and Ornduff (1992) embraced representative seeds of all five genera in the Menyanthaceae, three of which occur in Australia. The studies included the monotypic *Liparophyllum* (sample from New Zealand, but the species is also native in Tasmania), fourteen species of *Villarsia* (1 South African; all 13 Australian species), and six species of *Nymphoides* from different continents (with *N. exigua* from Tasmania). The current study provides descriptions and SEM illustrations of seeds of all 20 species of *Nymphoides* known from Australia, with discussion on the consistency or variation of seed ornamentation in each species. It compliments and expands the work of Chuang and Ornduff in relation to Australian taxa so that data from seed samples of all species of Menyanthaceae known to occur in Australia are now available.

Materials and Methods

Seeds from the several hundred collections housed in all major Australian herbaria, and some from extra-Australian herbaria and localities, were first examined under magnifications of up to 50 times natural size, using a dissecting microscope fitted with a measuring graticule. This allowed seeds to be measured and representative seeds to be

selected for SEM examination. Within each species, seeds were chosen to indicate the kind or kinds of ornamentation, the maximum size and density of tubercles when present, any variation in the kinds of tubercles present or in their size or development, and variation due to seed immaturity. Seeds were also selected to demonstrate geographical variation, including some relevant examples from the extra-Australian range of some species. Collections from which seeds were examined by SEM are detailed in Appendix 1.

SEM examination and photography has been done using the facilities of the Jodrell Laboratory, Royal Botanic Gardens, Kew, England during 1973-1974, the Department of Botany, Monash University, Melbourne in 1989, and the School of Botany, University of Melbourne during 2000-2001.

Results

The number of collections of each species examined by light microscopy, excluding collections without seeds, and the number of those used for SEM samples are given below against each species name (see also Appendix 1). Appendix 2 lists all collections cited in this paper, whether in the main text or in Appendix 1, and indicates the identification of each collection.

In the seed description for each species, seeds with the "peak" or most highly developed ornamentation, or ornamentations, found on mature seeds are described first under the heading of "peak ornamentation". An account of any morphological and/or geographical variations seen is given next under a second heading "variation".

The term "seed body" refers to the whole seed excluding any caruncle, scale, or tubercles. Measurement of the seed body alone allows for consistent comparisons of seed size within species which have seeds which may be either smooth or tuberculate, and between species with differing tuberculate structures.

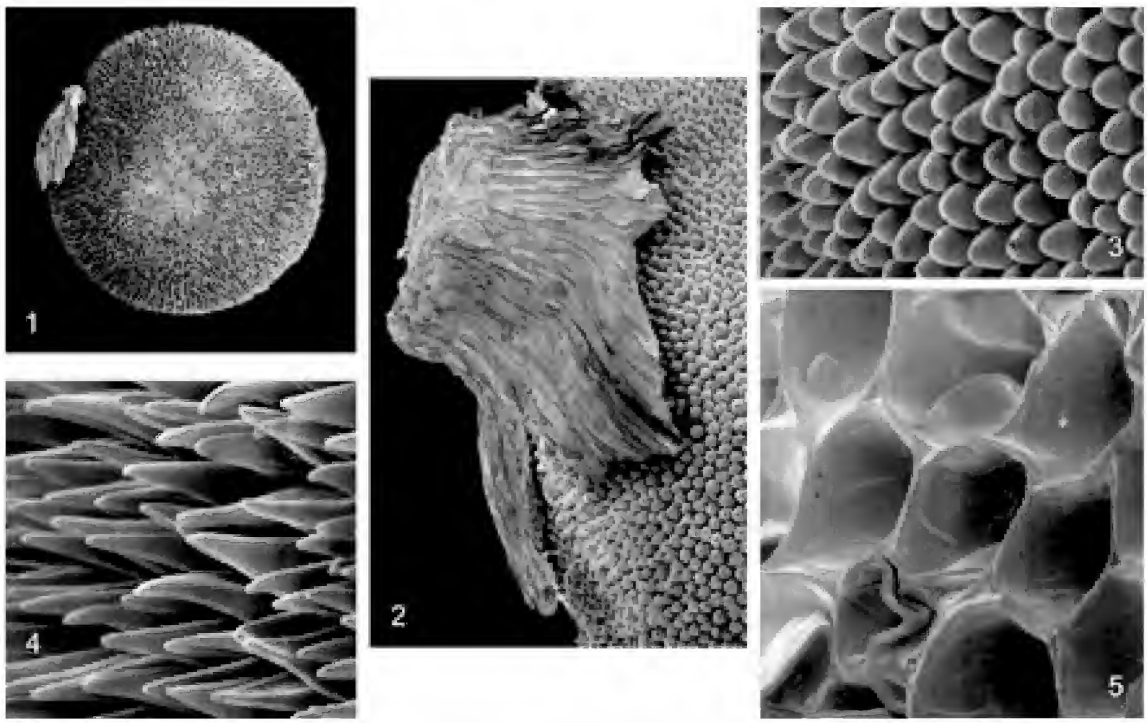
The term "periclinal walls" refers to the visible, i.e. the surface walls of the epidermal cells of the seed. The margins of these walls are most frequently pentagonal and hexagonal (e.g. figs 63, 89), or interdigitate, i.e. with interlocking lobes (e.g. figs 16, 46). Tubercles are actually hollow extensions of the periclinal walls but, for ease and consistency in describing the broad range of tubercular projections involved, they are presented here as independent bodies arising from the walls.

1. *Nymphoides aurantiaca*: (87 collections seen; 8 SEM) Figs 1-5.

Peak ornamentation: Seed body near-globose but very slightly laterally compressed, dark grey-black, 1.45-2.4 mm long, 1.4-2.2 mm wide, 1.125-1.65(-1.9) mm thick. Hilum within a short circular, projection which bears from around its whole perimeter a hard membranous scale appressed to the seed, occasionally the scale absent from a small portion of the perimeter; scale usually oblique, often much longer on one side and reducing to very short or absent on the opposite side, less usually more or less uniformly circular. Periclinal walls with pentagonal or hexagonal margins although the margins are obscured in mature seeds by a dense covering of tubercles. Tubercles are broad-based and basally appressed, each arising singly from the whole wall surface; they are of two kinds, either strongly tapered upwards to a narrowly obtuse apex and to about 85-100(-140) µm long or truncated at about half to two-thirds that length and with broadly obtuse apices. Only tubercles of a single kind have been found on any one seed.

N. aurantiaca is readily identifiable by the seed surface ornamentation, large seed size, and the scale, the scale being unique within the genus.

Variation: The scale varies considerably in size, but there is apparently no linkage of this variation to geographical distribution. For example, both the largest and the smallest scales known occur in northern Western Australia. The largest scale seen on any seed is from the Mitchell Plateau, Kimberleys, Western Australia (*Kenneally 4758*) where the



Figures 1-5. *Nymphoides aurantiaca*; **1** Lateral view of seed with long-tapered tubercles, the scale at left x 20; **2** Lateral view of oblique scale on seed with broad obtuse tubercles x 100; **3** broad obtuse tubercles x 300; **4** long-tapered tubercles x 305; **5** immature seed showing pitted surface, inverted to partly extruded tubercles, and margins of periclinal cell walls x 875 (**1** from *Aston 1979*; **2, 3** from *Lazarides and Adams 309*; **4** from *Brass 19702*; **5** from *Gill/McKean B423*).

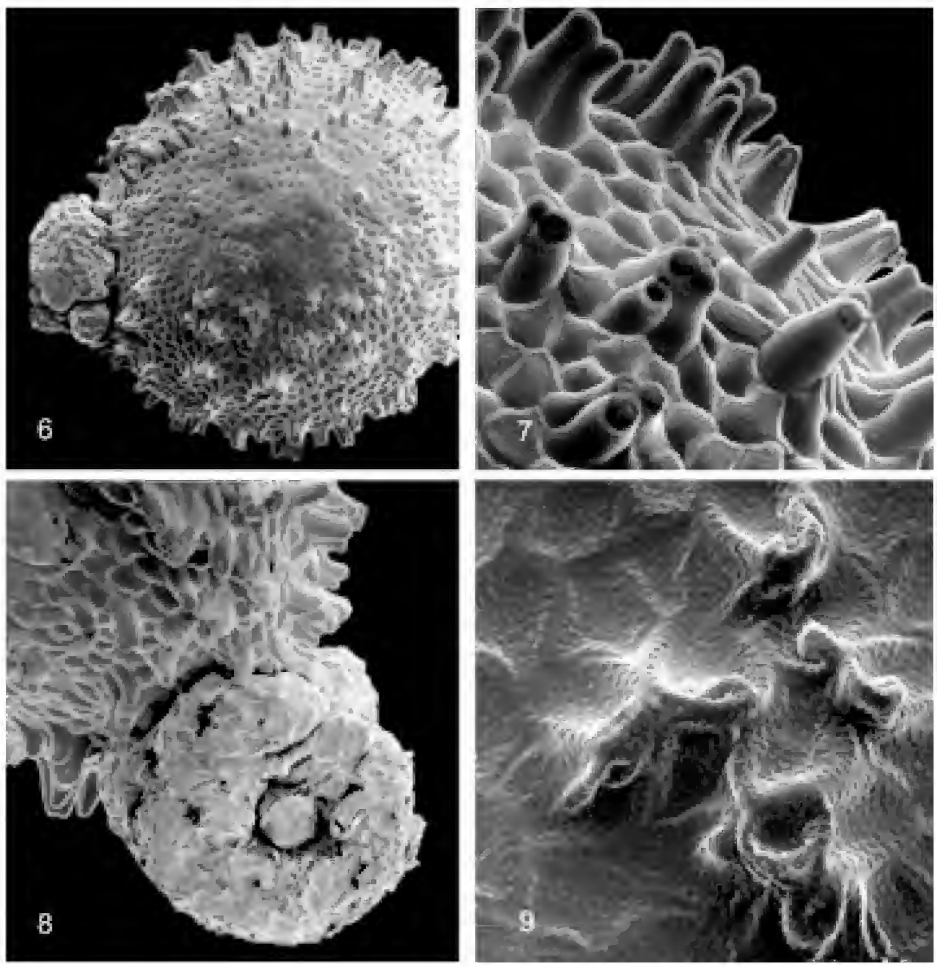
longest side of the scale is about three-quarters of the seed length. Scales at the other extreme of size are reduced to a short, more-or-less inconspicuous membrane only about one-eleventh of the seed length (e.g. *Morton 603*, Weipa, Cape York Peninsula, Queensland; *Beauglehole 52144*, Kimberley district, W.A.). In most populations throughout the Australian range of the species the scales are one-quarter or less of the seed length, but scales to one-third, and sometimes to one-half, seed length are frequent. Ten extra-Australian collections from New Guinea, Sri Lanka, the Moluccas and Thailand which I examined by SEM all had scales which were less than, or rarely one-sixth of, the seed length.

Seeds with the long-tapered tubercles occur in widespread populations throughout northern Australia whereas those with the broadly obtuse tubercles have only been seen from limited areas of the northern Northern Territory. Most collections of the latter seed type come from the area of the South and East Alligator River and their tributary systems, ranging from near Munmarlary, Oenpelli and Nabarlek south through Mudginberri and Jabiru to around Nourlangie (e.g. *Jacobs 1804*; *Lazarides & Adams 309*, figs 2, 3). Other areas from which obtuse-tubercled seeds have been seen are near Daly River (*Craven 4419*) and around the Adelaide River system at Fogg Dam (e.g. *Jacobs 1765*) and Marrakai (*Cousins 178*).

Immature seeds appear deeply pitted over their surface before the tubercles are extruded (fig. 5).

2. *Nymphoides beaglensis*: (7 collections seen; 2 SEM) Figs 6-9.

Peak ornamentation: Seed body near-globose but slightly to moderately laterally compressed, dark grey-brown-black, 0.75-0.95 mm long, 0.70-0.85 mm wide, 0.55-0.70 mm thick. Hilum within a very thick and conspicuous, pale, circular caruncle of many small cells, the circle with an irregular convolution on one side and sometimes somewhat oblique. Epidermal cells of the seed surface all have periclinal walls with pentagonal or hexagonal margins but are otherwise dimorphic. Over much of the seed surface the walls are mildly convex to low-domed, but at intervals of approximately two to four of these convexities the walls form clusters of prominently projecting tubercles. Each cluster consists of one to eight cells, each cell having one simple, thick, tubercle arising from the whole area of the periclinal wall. The tubercles are therefore broad, touching at the base within each cluster, only slightly tapering and a little divergent upwards, broadly obtuse, c. 35-55 μm long and 2.5-3 times as long as wide. Because of the dimorphism of the



Figures 6-9. *Nymphoides beaglensis*; **6** Lateral view of seed, with caruncle at left x 115; **7** portion of seed surface showing cell dimorphism and margins of periclinal cell walls x 405; **8** distal view of caruncle x 215; **9** immature seed showing pitted cells with foveolate surfaces, some with developing tubercles x 720 (**6-8** from *Kenneally* 9451; **9** from *Forrest s.n.*).

epidermal cells, the seed surface appears more or less smooth but with moderately spaced, conspicuous, single- and multi-tuberculate projections.

Variation: The tubercle clusters may be present only on and near the seed edges, but absent from the central areas of the seed faces.

In immature seeds, the convex periclinal walls on mature seeds remain concave so that seeds appear pitted. Tubercles may be only part-formed and semi-immersed within the pits, or non-turgid even when fully extruded. The surfaces of both pits and tubercles are strongly foveolate in *Forrest s.n.*, 1879 (fig. 9).

3. *Nymphoides crenata*: (104 collections seen; 12 SEM) Figs 10-17.

Peak ornamentation: Seed body ellipsoid but strongly laterally compressed, pale straw-coloured to fawn-brown or tan-brown, never black, (0.425-)0.6–1.0(-1.2) mm long, (0.35-) 0.4-0.7(–0.8) mm wide, 0.25-0.55 mm thick. Hilum within a very short, hard, near-basal, ring of the seed body, the ring obliquely circular and forming a minute projection on one side; caruncle absent. Periclinal walls strongly and conspicuously interdigitate, all slightly convex in many populations, giving a more or less smooth seed. In many other populations the walls of some cells each extend into a single central tubercle. Tubercles are thick, broadly obtuse, not touching, from low and domelike to longer and cylindrical, usually 30 μm or less long, rarely to 40 μm; length from less than to 4 times as long as the width, rarely to 6 times the width. Because of the inter-population differences of the shape, placement, and spacing of the tubercles, and their presence or absence, seeds of *N. crenata* are noticeably variable. However, they can be distinguished by the strongly compressed, ellipsoidal, non-carunculate, seed body with hard, near-basal projection.

Variation: Part of the variation in seed ornamentation, shape and size, can be related to geographical distribution, as follows:

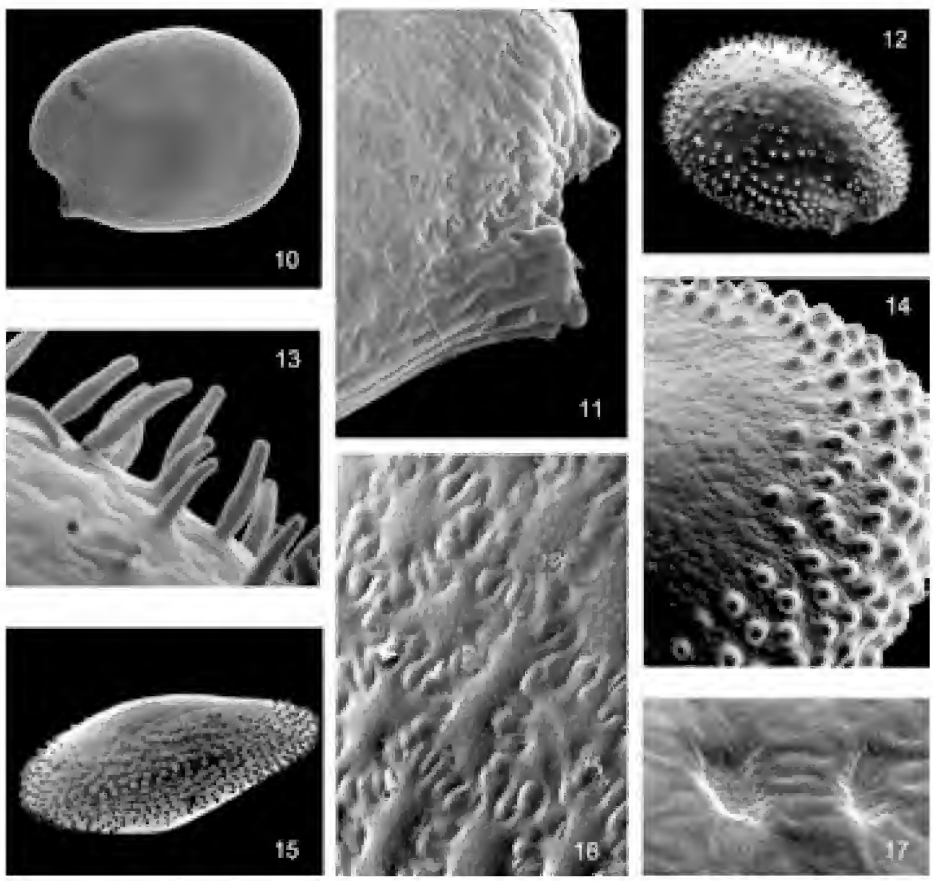
Ornamentation – Mature seeds vary from non-tuberculate and more or less smooth to strongly ornamented with single, non-clustered tubercles. When present, tubercles may occur only on and close to the seed edge, leaving smooth seed faces. They may also extend from the edges over the faces, usually becoming more widely spaced and shorter, or even absent, towards the centre of each face. Edge tubercles occur on all of the edge cells of a seed, whereas face tubercles are usually spaced by one to many non-tuberculate cells. Smooth seeds are prevalent in south-eastern Australia whereas the most highly tuberculate seeds are prevalent throughout tropical areas.

Only smooth seeds have been found on Victorian collections. Most collections from southern Queensland and northern New South Wales between 23° and 32° S latitude and west of 151° E longitude, and also an isolated collection from inland Western Australia, also have smooth seeds (e.g. *Chinnock 808*, Yelma Station, W.A.; *Purdie & Boyland 142*, near Charleville, Qld; *Johnson NSW136695*, near Angledool, N.S.W.). However, a few collections from within this zone have seeds edged with low domes to very short tubercles (e.g. *Wilson & Jacobs 765*, near Pilliga, N.S.W.) and one collection has the seeds shortly tuberculate all over, although very sparsely so on the centre-faces (*Bonney s.n.*, near Jundah, Qld).

Across northern Australia above 18° S. latitude collections show the whole range of seed ornamentation described for the species, without any undoubted correlation of ornamentation with geographical distribution. Although seeds with the longest tubercles all come from five collections from the Northern Territory (e.g. *Byrnes 1817*, fig. 12; *Henshall 3733*, fig. 13) their apparent confinement to the Northern Territory is probably an artefact of the greater number of collections seen from there. Close populations frequently have very different seed ornamentation. For example, *Must 698* (fig. 10) and *N. Byrnes 1817* are from two populations only c. 30 km apart in the vicinity of the Adelaide River, Northern Territory. *Must* has smooth seeds whereas the seeds of the *Byrnes* collection have long tubercles well-distributed over most of their surface. Two collections, *Aston 2275* and *Aston 2279*, from c. 30 km apart in the Normanton area of

Queensland provide another example, seeds of the former having moderately long edge tubercles whereas those of the latter are smooth.

Shape – Four collections from the Cape York and Normanton areas of Queensland (*Aston 2242*, near Laura; *Aston 2269*, Lakefield National Park; *Aston 2280*, near Normanton; *Forster PIF22646*, Bulleringa National Park) and one from the Gulf of Carpentaria region of the Northern Territory (*Thomson 522*, Bing Bong) have more rounded-ellipsoid seeds with mildly to strongly bulged centre-faces. These contrast with the more evenly convex faces of the usual ellipsoid seeds found on other *N. crenata* collections, although some gradations occur. The five collections cited have short to mid-length tubercles on either the seed edges, the edges and side-faces, or over the whole seed surface. Tubercles on seeds of *Forster PIF22646* are short, sometimes only domelike, but are particularly dense, arising from every surface cell.



Figures 10-17. *Nymphoides crenata*; **10** Lateral view of smooth seed × 45; **11** portion of seed showing strongly interdigitate margins of periclinal cell walls and the projection of the seed body which surrounds the hilum × 370; **12** Lateral view of seed with long and widely distributed tubercles × 105; **13** portion of seed with long tubercles × 550; **14** portion of seed with smooth face and low edge tubercles × 275; **15** semi-edge view of seed with smooth centre-face but tuberculate side-faces and edge × 85; **16** surface of mature seed showing strongly interdigitate margins of the convex periclinal cell walls × 1000; **17** immature seed surface showing central foveolate concavity in each periclinal cell wall × 1000; (**10** from *Must 698*; **11** from *Everist 2884*; **12** from *Byrnes 1817*; **13** from *Henshall 3733*; **14** from *Aston 1960*; **15** from *Perry 706*; **16** from *Mckee 8494*; **17** from *Clemens s.n.*, 15 March 1946).

Size – There is an apparent tendency for the smallest seeds to occur in northern tropical Australia and the largest to occur in inland areas of northern New South Wales and southern Queensland.

The smallest seeds measured (< 0.6 mm long) are from six collections from north of 18° S latitude in the Northern Territory and Queensland (e.g. *Byrnes 1817*, Burrell Creek, N.T., fig. 12; *Jacobs 1667*, near Borroloola, N.T.; *Aston 2242*, near Laura, Qld). The largest seeds measured (> 1.0 mm long) are from six collections from southern Queensland and northern New South Wales, between 23° and 32° S latitude and west of 151° E longitude, and from an isolated collection from inland Western Australia (e.g. *Chinnock 808*, Yelma Station, W.A.; *Vogan s.n.*, Upper Mulligan River, Qld; *Boorman NSW136690*, Coolabah, N.S.W.). However, seeds embracing virtually all of the between-extreme lengths of 0.6-1.0 mm occur in all areas throughout the species range. With examination of more collections, the apparent tendency towards a size differential linked to geographical distribution may not be upheld, particularly as the lengths of mature seeds from any one collection frequently vary by up to 0.25 mm (e.g. 0.75-0.95 mm, *Gardner 12220*, Lennard River, W.A.; 0.80-1.05mm, *Johnson & Pedley 61*, Darling Downs, Qld; 0.625-0.825 mm, *Aston 1758*, Yarrawonga, Vic.). Seeds from only one or two capsules from each of 89 collections have been measured.

Immature seeds have concave periclinal walls which appear foveolate (fig. 17).

4. *Nymphoides disperma*: (3 collections seen; 1 SEM) Figs 18-20.

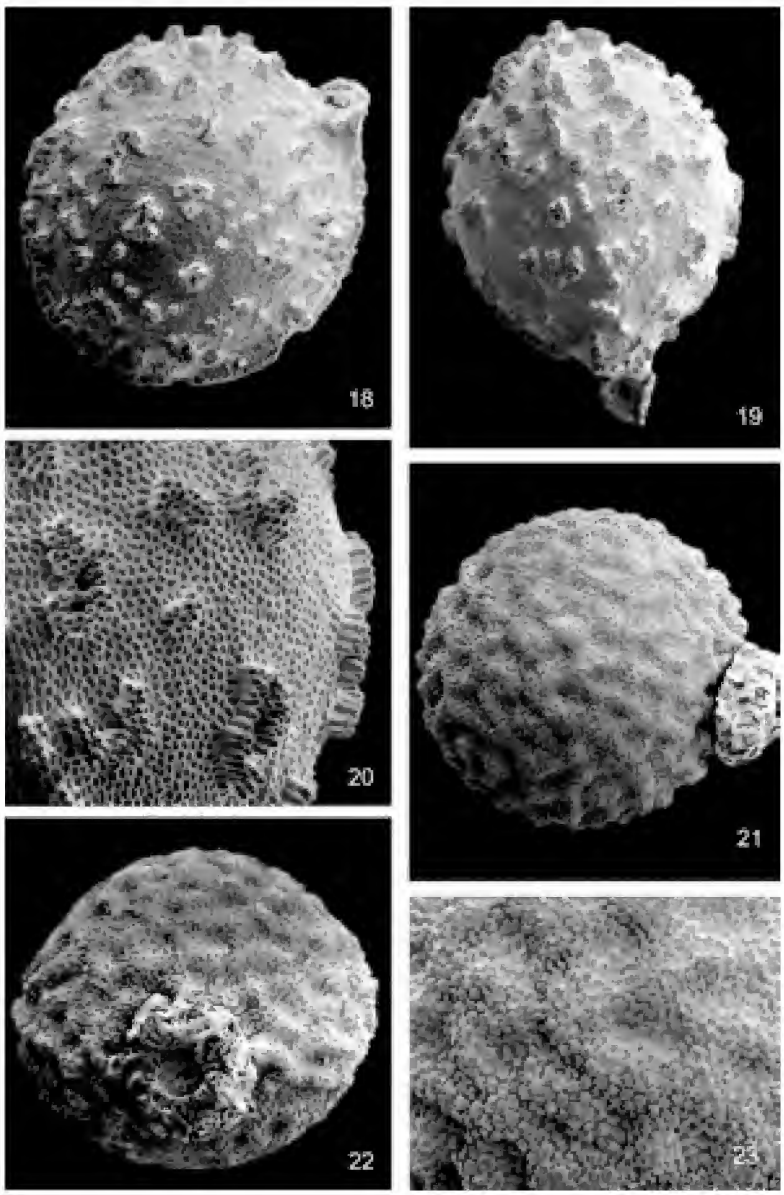
Peak ornamentation: Seed body more or less globose but moderately laterally compressed, straw-coloured (or perhaps finally black), 1.9-2.4 mm long, 1.75-2.3 mm wide, 1.55-1.85 mm thick. Hilum within a very short, thick, oblique projection of the seed body, with most of the perimeter of the projection surrounded by a thin, semi-circular caruncle of many small cells. Periclinal walls with pentagonal or hexagonal margins but otherwise dimorphic. Over much of the seed surface the periclinal walls consist of low convex domes, but at intervals of approximately four to 12 of these domes the walls form clusters of a few to 45 prominently projecting tubercles. Each tubercle arises from the whole area of a periclinal wall. The tubercles are therefore thick, broad and touching at the base, only slightly tapering and a little divergent upwards, broadly obtuse, c. 55-100 μm long and 2.5-5 times as long as wide. Because of the dimorphism of the epidermal cells, the seed surface gives the impression of high, steep-sided plateaus rising abruptly from a uniformly flat plain.

Variation: Seeds of one collection (*George 12508* in part, Prince Regent River Reserve, W.A.) lacked the projecting tubercle clusters but were otherwise comparable with the description given above (see Aston, 1986).

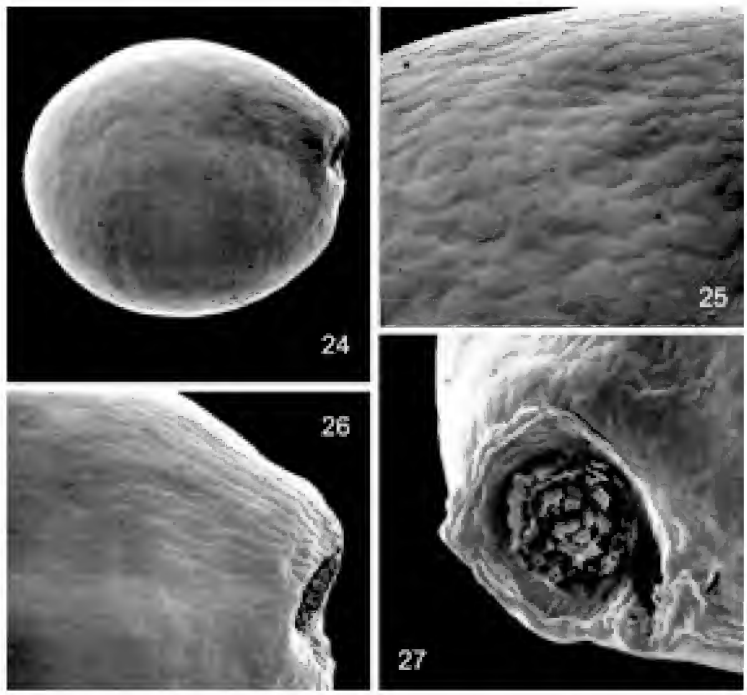
5. *Nymphoides elliptica*: (6 collections seen; 1 SEM) Figs 21-23.

Peak ornamentation: Seed body near-globose but slightly to moderately laterally compressed, dark grey-brown-black, 1.0-1.5 mm long, 1.0-1.3 mm wide, 0.85-1.1 mm thick. Hilum within a pale, circular, moderately thick and prominent, basal caruncle of irregular cells. Periclinal walls with pentagonal or hexagonal margins, each wall bearing a broad-based tubercle arising from virtually all of the wall area. The seed is therefore densely tuberculate with the tubercles often closely appressed. Tubercles are thick, mildly to negligibly tapered upwards, broadly obtuse, often with several minute obtuse micropapillae at their apices, up to 6 μm long and less than twice as long as wide. They vary in length to form a regular, somewhat rugulose, pattern of evenly spaced, more or less circular, depressions separated by reticulate ridging over the whole seed surface. Tubercles of the depressions are shorter than those of the ridging, but the two intergrade gradually. Both the depressions and the ridges are several (c. 6-10) cells wide.

Variation: None observed.



Figures 18-23. *Nymphoides disperma*; **18** Lateral view of seed, the caruncle and hilum at top right x 45; **19** edge view of seed, the caruncle and hilum at bottom x 45; **20** portion of seed surface showing cell dimorphism and margins of periclinal walls x 100 (all from *Forbes 2098*). Nymphoides elliptica; **21** Lateral view of seed, the caruncle at right x 70; **22** edge view of seed, looking on to the caruncle and hilum x 85; **23** portion of seed surface showing depressions, ridging and dense tubercles x 145 (all from *Aston 2260*).



Figures 24-27. *Nymphoides exigua*; **24** Lateral view of whole seed x 35; **25** portion of seed surface showing mildly interdigitate margins of periclinal cell walls x 250; **26** attachment area of seed showing the absence of a caruncle x 80; **27** attachment area with thin caruncular rim x 245 (**24-26** from *Buchanan 2764*; **27** from *Rodway s.n.*, 1896).

6. *Nymphoides exigua*: (4 collections seen; 2 SEM) Figs 24-27.

Peak ornamentation: Seed body rounded-obloid to broad-obloid but strongly laterally compressed, brown-grey, 1.05-1.4 mm long, 0.9-1.1 mm wide, 0.55-0.65 mm thick. Hilum within a small, hard, obliquely circular, near-basal, bulge of the seed body; caruncle absent or represented only by a thin rim or partial rim of cells around the hilum. Periclinal walls with shortly interdigitate margins, each wall slightly convex. Tubercles absent. The seed surface overall appears smooth and shining.

Variation: None observed.

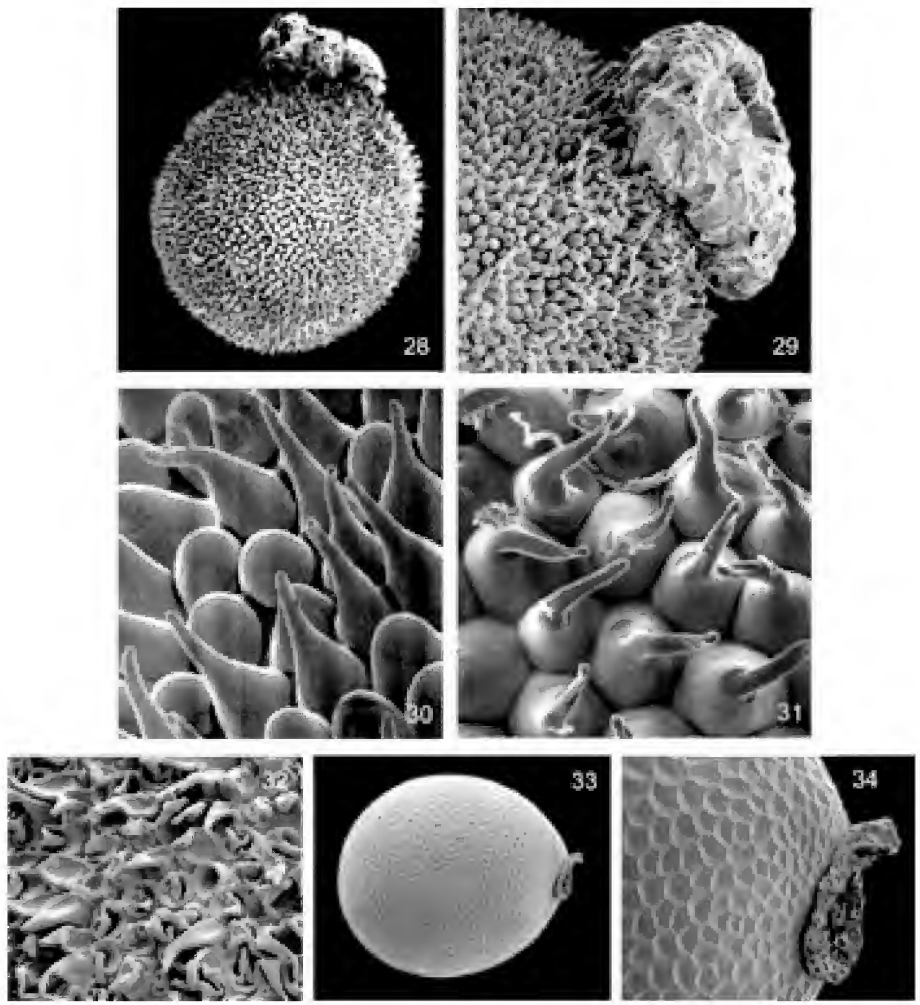
7. *Nymphoides exiliflora*: (41 collections seen; 11 SEM) Figs 28-32.

Peak ornamentation: Seed body ± globular, dark grey-brown to black, (0.4-)0.5-0.8 mm long, (0.4-)0.5-0.75 mm wide, (0.35-)0.45-0.7 mm thick. Hilum within a pale, circular, thick and prominent, basal caruncle of large irregular cells. Periclinal walls with pentagonal or hexagonal margins although the margins are obscured in mature seeds by a dense covering of tubercles. Tubercles broad-based, arising singly from the whole surface of a periclinal wall, and basally appressed; they are of two extremes, the most usual being about 35-50(-70) μm long and bulbous at the base with an abrupt contraction above this into a slender, narrowly obtuse, distal portion. In the less usual extreme, the tubercles are only about half to two-thirds as long, broad throughout, and with a broadly obtuse apex. The dense covering of slender tubercles on most seeds gives a velvety-pubescent appearance to the seed surface.

Variation: The contraction to a slender extremity is less abrupt in the tubercles of some seeds, in which case each tubercle becomes more gently tapered.

All periclinal walls of any seed usually possess the same kind of tubercle, but both distally slender and broad obtuse tubercles have been found intermingled on a few seeds in both Australia and New Guinea (e.g., *Dallachy s.n.*, figs 28, 29; *Ridsdale NGF33531*, fig. 30). Seeds in which all tubercles are tapered occur abundantly throughout the species range, but seeds having only the shorter, broadly obtuse, tubercles have not been found.

Immature seeds appear deeply pitted over their surface before the tubercles are extruded.



Figures 28-34. *Nymphoides exiliflora*; **28** Lateral view of a seed bearing dimorphic tubercles, caruncle at top × 110; **29** portion of seed with dimorphic tubercles, showing latero-distal view of caruncle × 215; **30** surface of seed with both slender-tapered and broad-obtuse tubercles × 1075; **31** bulbous-based, abruptly contracted tubercles × 1150; **32** surface of semi-mature seed showing cell wall margins and developing tubercles x 310 (**28, 29** from Dallachy s.n., [GOET]; **30** from Ridsdale NGF33531; **31** from Dallachy s.n., [MEL]; **32** from Sharpe 1869). *Nymphoides furculifolia*; **33** Lateral view of seed, with caruncle at right x 45; **34** portion of seed showing caruncle and periclinal cell walls × 145 (both from *Lazarides 7645*).

8. *Nymphoides furculifolia*: (33 collections seen; 3 SEM) Figs 33-34.

Peak ornamentation: Seed body near-globose but slightly laterally compressed, dark brown-grey, grey-black or black, 0.55-0.625(-0.75) mm long, 0.50-0.575(-0.65) mm wide, 0.35-0.425(-0.50) mm thick. Hilum within a pale, thin, near-basal, irregularly circular caruncle of small irregular cells. Periclinal walls with pentagonal or hexagonal margins, each wall slightly concave. Tubercles absent. The seed surface overall is glossy and more or less smooth.

Variation: None observed. However, by comparison with the development of seeds of other species it could be expected that the periclinal walls of at least some fully mature seeds may become slightly convex rather than remain concave.

Immature seeds are cream-straw in colour. The most developed show the same size, shape, and surface features as the fully mature black seeds described.

9. *Nymphoides geminata*: (55 collections seen; 19 SEM) Figs 35-43.

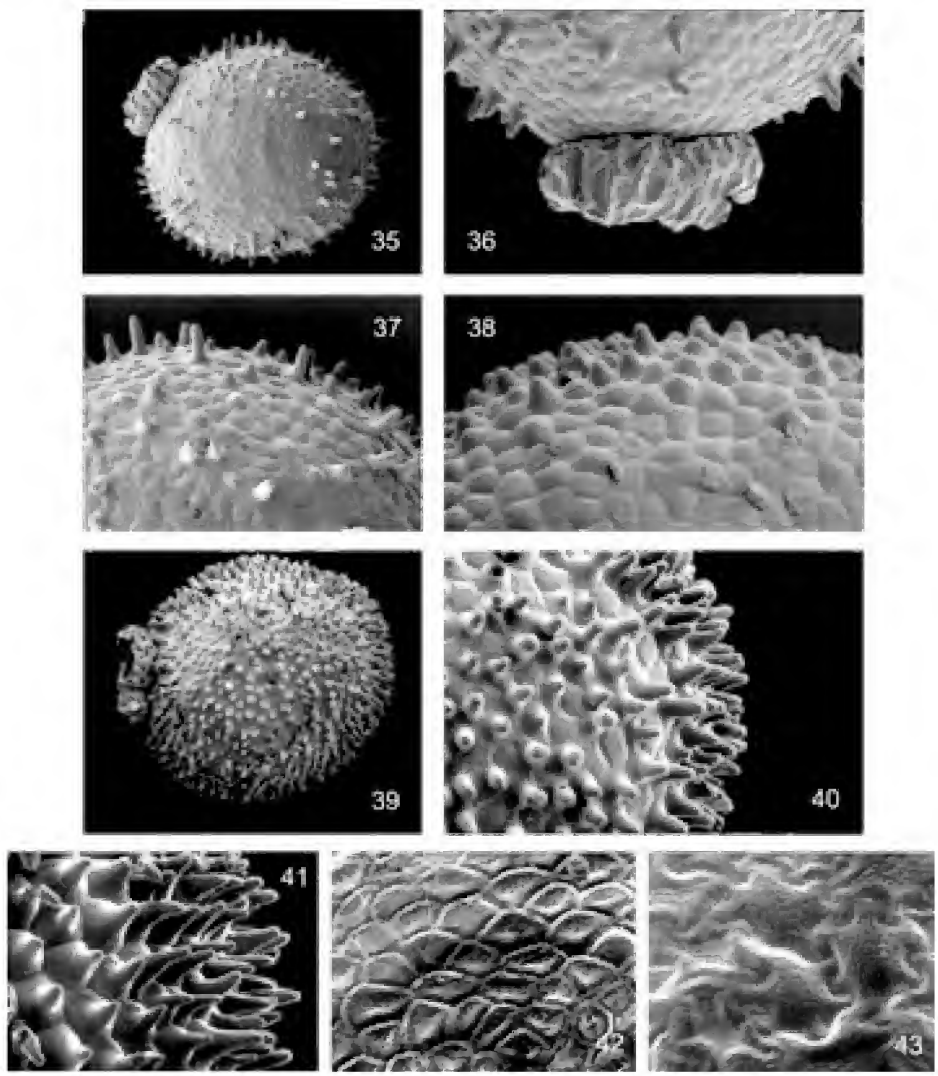
Peak ornamentation: Seed body more or less globular to slightly ellipsoid-globular, slightly to moderately laterally compressed, grey-brown to black, (0.55-)0.6-0.9(-1.2) mm long, (0.5-)0.55-0.75(-0.95) mm wide, (0.4-)0.45-0.65 mm thick. Hilum within a pale, moderately thick and conspicuous, near-basal, circular caruncle of large irregular cells. Periclinal walls with pentagonal or hexagonal margins in most populations, but there are moderately interdigitate margins in some populations (see variation below). Each wall may be slightly convex, or may bear a single tubercle arising centrally from most of the wall area. Tubercles usually non-touching, spaced by one to several cells, often sparse or absent on the seed faces, simple, broadly obtuse, from a low dome or short blunt pyramid up to a moderately broad, slightly tapered tubercle c. 55 μm long and to c. 3 times as long as broad. Seeds may be non-tuberculate and then appear more or less smooth (e.g. *Knoetzsch s.n.*, fig. 42) but they are most commonly tuberculate, with tubercles of all lengths and simple convex periclinal walls included on the same seed (e.g. *Aston 1854*, figs 35-37). More densely tuberculate seeds commonly have tubercles of more or less equal length (e.g. *Aston 1839*, figs 39, 40).

Variation: Seeds from near Tingha, New South Wales (*Aston 1839*, figs 39, 40) and from Glenfield, New South Wales (*McBarron 8556*) are moderately densely tuberculate over their whole surface with even-length, non-appressed tubercles. Many of the tubercles are shortly spaced by non-tuberculate cells. Seeds from two Queensland locations, near Cunnamulla (*Aston s.n.*, Sept. 1969, fig. 41) and near Pentland (*Speck 4602*), are even more densely tuberculate with every cell bearing a broad-based, strongly tapered, prominent tubercle. In the Cunnamulla collection the tubercle bases are somewhat bulbous and appressed so that the seeds are quite similar to those of *N. exiliflora*.

The variation in the length, density and placement of tubercles occurs throughout the geographical range of the species from Queensland to Victoria inclusive. However, I have only seen non-tuberculate seeds (three collections) from Kangaroo Island, South Australia, whereas the only two collections with maximum tubercle density and the most extreme tubercle shape are from the western extremes of the species range in Queensland (see above paragraph).

Fully mature seeds from near Stratford, Victoria (*Aston 1854*, figs 35-37), have moderately interdigitate margins on the periclinal walls of both convex and well-tubercled cells, thereby varying in this character from other Australian collections, including ones from within the same area e.g. Providence Ponds (*Aston 1763A*) and Bengworden (*Aston 1856*). Three collections from New Guinea (*Hoogland & Schodde 7484*, fig. 43; *Walker ANU562*; *Womersley NGF43515*) also have moderately interdigitate margins to the periclinal walls but this may be due to seed immaturity. *Walker ANU562* is very immature, being folded and non-turgid but *Hoogland & Schodde 7484* appears near-mature, with cell walls varying from shallowly concave to low convex.

In immature seeds of Australian collections, the periclinal walls remain inverted so that the seed surface is shallowly to deeply pitted. Wall margins which have not fully lengthened through full cell expansion may have slight curves and appear very mildly interdigitate (e.g., Port Jackson, *F. Bauer s.n.* ?).



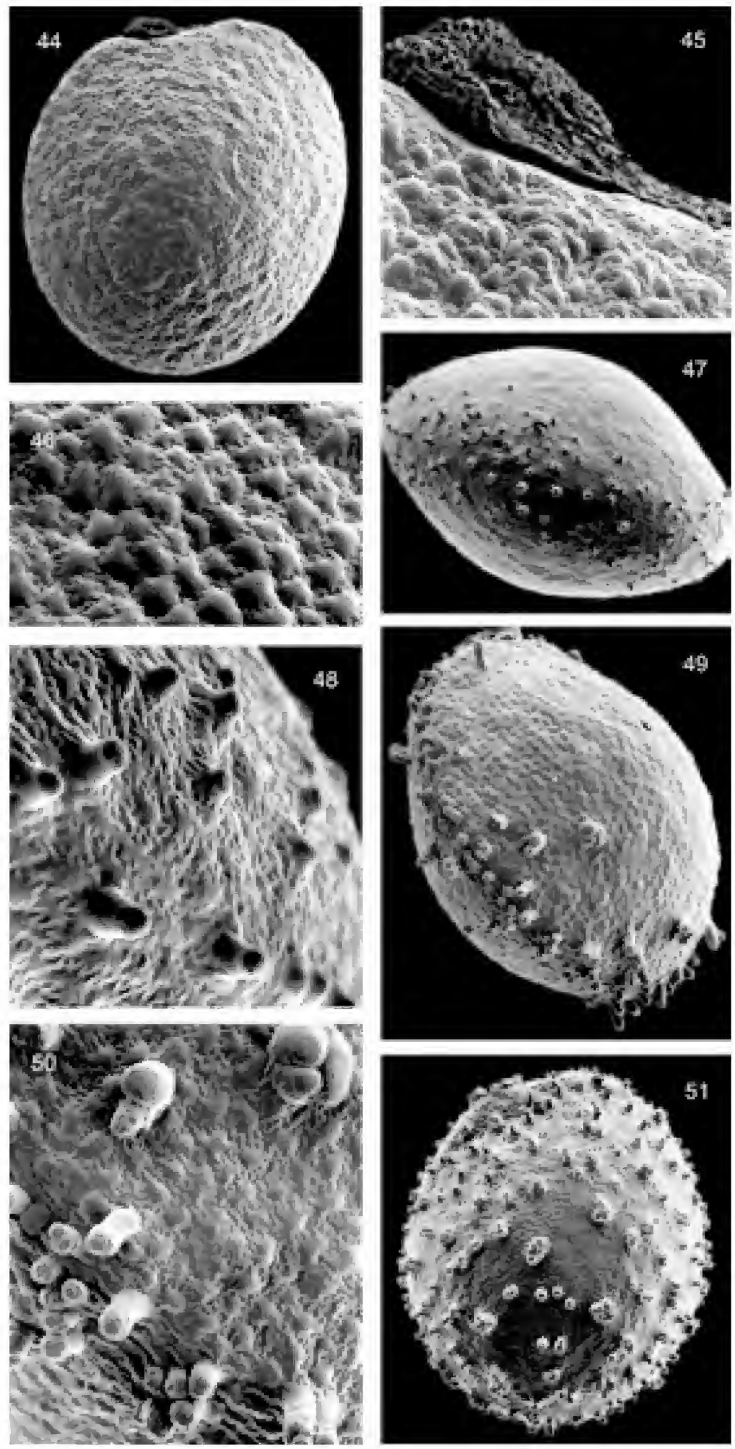
Figures 35-43. *Nymphoides geminata*; **35** Lateral view of seed, with caruncle at left top x 40; **36** portion of seed showing the caruncle x 120; **37** portion of seed showing uneven length tubercles and less usual interdigitate margins of the periclinal cell walls x 100; **38** Portion of seed showing uneven length tubercles and the more usual pentagonal or hexagonal cell margins x 160; **39** Lateral view of seed, with caruncle at left x 40; **40** portion of seed showing even length tubercles and hexagonal or pentagonal cell margins x 120; **41** portion of seed surface showing an even length, unusually bulbous-based, tubercle rising from each periclinal cell wall x 325; **42** seed surface showing pentagonal to hexagonal margins of periclinal cell walls x 320; **43** seed surface showing interdigitate margins x 815 (**35-37** from *Aston 1854*; **38** from *Aston 1840*; **39, 40** from *Aston 1839*; **41** from *Aston*, 26 Sept 1969; **42** from *Knoetzsch*, Jan. 1885; **43** from *Hoogland & Schodde 7484*).

10. Nymphoides indica: (167 collections seen; 14 SEM) Figs 44-58.

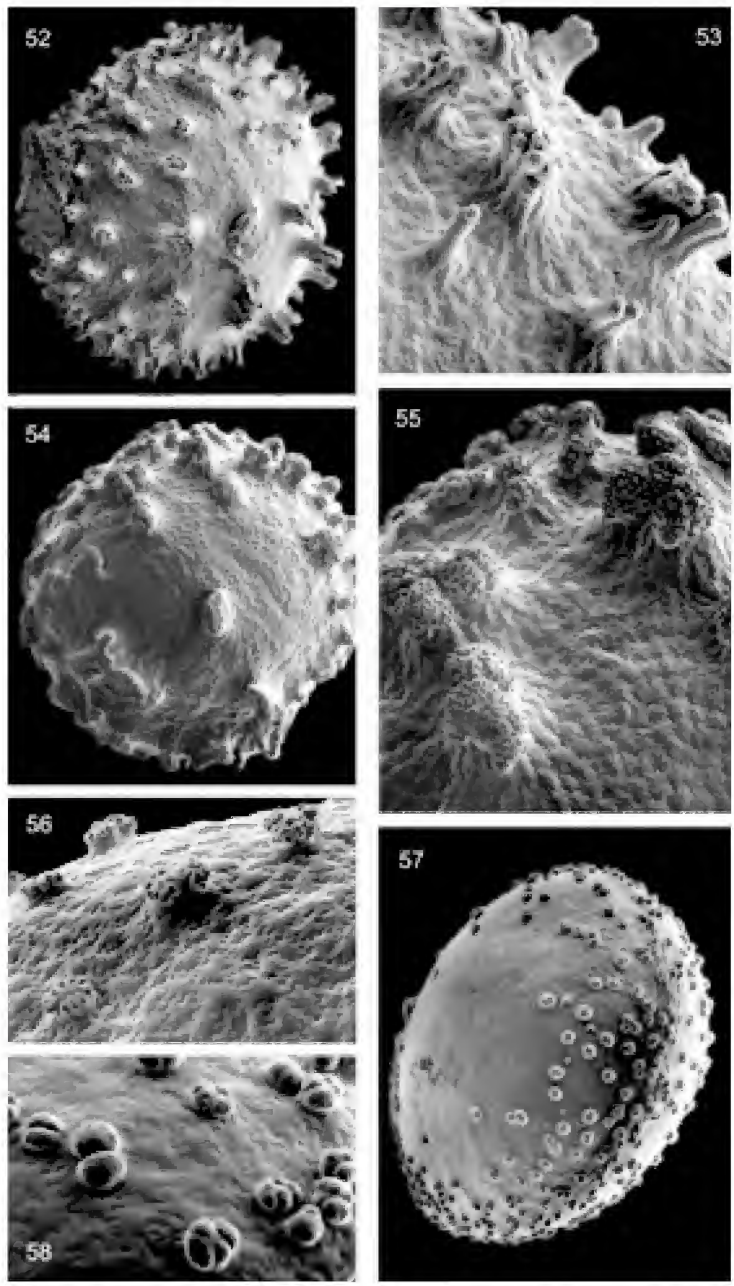
Peak ornamentation: Seed body more or less globose but moderately laterally compressed, cream-straw to fawn-grey to dark grey-brown or black-grey, 0.8-2.1 mm long, (0.725-)0.9-1.7(-1.85) mm wide, (0.5-)0.6-1.2(-1.4) mm thick. Hilum within an evenly circular to oblique, thin and inconspicuous, sometimes apparently absent, basal caruncle, or rarely the caruncle thick and conspicuous. Periclinal walls with strongly interdigitate margins, mostly convex, others variously tuberculate. Tubercles broadly obtuse, varying from solitary to strongly appressed in clusters of 2-11(-20), and from simple to having strongly verrucose extremities. Solitary tubercles are commonly up to c. 96 μm long with the length about four times the width. Tubercle clusters are (20-)50-200(-300) μm long with the length ranging from greater than to lesser than the width. Single tubercles, and/or tubercle clusters, are irregularly spaced over seed surfaces by one to many of the convex-walled cells. Tuberculate seeds therefore appear more or less smooth with spaced tubercles or tuberculate masses. Non-tuberculate seeds have all periclinal walls convex and appear more or less smooth to rugulose throughout.

Variation: *Nymphoides indica* includes a cosmopolitan complex of plants with varied vegetative and reproductive characters which do not lend themselves readily to taxonomic differentiation. The great variability in seed characters illustrates this point. Described below are eight seed variations which encompass the range of seeds which I have seen on Australian material. They indicate morphological progression from non-tubercled to single-tubercled to cluster-tubercled seeds, from simple to distally verrucose tubercles and clusters, and changes in tubercle lengths and surface coverage. Intermediates occur, and some seeds cannot be placed neatly with any one of the forms described. I have not been able to associate the varied seed morphology of Australian populations with any consistent pattern of plant form.

1. Smooth (figs 44-46): seed more or less smooth to rugulose; periclinal walls all convex (*Kenneally 4510*).
2. Single edge tubercles (figs 47, 48): seed as for 1, but with single tubercles 10-30(-60) μm long around edge and side face (*Beauglehole 54910*).
3. Edge clusters (figs 49, 50): seed as for 2, but with some of the tubercles mildly appressed in clusters of 2 to 5 and mildly verrucose at their extremities (*Dixon s.n.*).
4. Overall clusters (fig. 51): seed as for 3, but with the clusters spaced over the whole seed surface, the tubercles mildly adpressed and up to 8 per cluster, 30-50(-80) μm long, each usually slightly broadened toward the summit (*George 15168*).
5. Long-tubercled clusters (figs 52, 53): seed with tubercles (50-)100-210 μm long spaced singly or, most frequently, in clusters of 2-10(-15) over the whole seed surface. Tubercles of each cluster very tightly united along most of their length, their apices verrucose, sometimes divergent. Clusters mostly longer than wide, sometimes appearing spiny when the outer tubercles of the cluster are shorter than the central ones (*Aston 1896*).
6. Long thick clusters (figs 54, 55): seed with all tubercles united into thick clusters 100-250(-300) μm long, with each cluster very strongly verrucose distally. Tubercles fully united and up to 20 (or more) within each cluster. Cluster length mostly more or less equal to the width, but some clusters are narrow and ridgelike (*Aston 2265*).
7. Reduced thick clusters (fig. 56): seed as for 6, but with shorter, fewer-tubercled clusters (30-)40-60(-100) μm long (*Aston 2519*). Most closely related to seed form 4, with intermediates frequent.
8. Very reduced clusters (figs 57, 58): seed as for 7, but with the tubercle clusters even shorter, c. 20-30 μm long, less prominently verrucose, and near-absent from the seed faces (*Edwards s.n.*).



Figures 44-51. *Nymphoides indica*; **44-46** Seed type 1: **44** Lateral view of whole seed × 55; **45** caruncle × 260; **46** seed surface showing interdigitate margins of periclinal cell walls × 290. **47, 48** Seed type 2: **47** Edge view of seed × 95; **48** seed surface at edge showing single tubercles and cell margins × 500. **49, 50** Seed type 3: **49** Semi-edge view of seed × 100; **50** portion of seed surface showing single to few-clustered tubercles with mildly verrucose extremities × 300. **51** Seed type 4: Lateral view of seed × 60.



Figures 52-58. *Nymphoides indica*; **52, 53** Seed type 5: **52** Obliquely lateral view of seed × 60; **53** portion of seed surface showing long, single to clustered, tubercles with more verrucose extremities × 220. **54, 55** Seed type 6: **54** Lateral view of seed × 45; **55** portion of seed surface showing tubercle clusters with strongly verrucose extremities × 145. **56** Seed type 7: portion of seed surface showing shorter, fewer-tubercled clusters × 225. **57, 58** Seed type 8: **57** Semi-edge view of seed × 105; **58** portion of seed surface showing short, almost non-verrucose tubercle clusters × 390. (**44-46** from *Kenneally 4510*; **47, 48** from *Beauglehole 54910*; **49, 50** from *Dixon s.n.*; **51** from *George 15168*; **52, 53** from *Aston 1896*; **54, 55** from *Aston 2265*; **56** from *Aston 2519*; **57, 58** from *Edwards s.n.*

Smooth-seeded collections (89; = 53% of all seeded collections examined) occur through the whole of the species range across northern tropical Australia and down coastal areas of eastern Queensland and north-eastern New South Wales. They were the most prevalent seed form seen in the Northern Territory (37 out of 53 collections; = 69.8%) and Queensland (44 of 76; = 57.9%) and the only form found in the four New South Wales collections. However, smooth-seeded collections from Western Australia were sparse (4 of 34; = 1.2%).

From all the tubercle-seeded collections (78; = 47% of all seeded collections examined), the Kimberley region of Western Australia (30 tubercle-seeded collections seen), the northern Northern Territory (16), and Cape York Peninsula, Queensland (32 from the whole State), each appears to be the centre of development for a different seed form. The most distinctive of these is seed form 6, which has only been seen in ten collections from between 13° and 16° S. latitude on Cape York Peninsula. Seed form 6 and smooth seeds are the only forms which I have seen in collections from the Peninsula. Seed form 5 is most developed in seven collections from the Adelaide River – Kakadu National Park – Katherine area of the Northern Territory, although there are two collections from the Gibb River Road – Durack River area of the Kimberley Region, Western Australia. Seed forms 4 and 7 are connected by intermediates and together form the seed complex which is predominant (15 of 30) in collections from the Kimberley. Forms 2, 3 and 8 also occur in the Kimberley as less frequent gradients of the predominant complex. This whole set of Kimberley gradients also ranges across southern regions of the Gulf of Carpentaria in both the Northern Territory and Queensland, and in the vicinity of Mackay.

Seeds from any one collection have the same seed form but may vary in size of the seed body, e.g. a variation in body length of up to 0.45 mm (1.20-1.65 mm) in *Aston 2536* (Burrells Creek, N.T.). There is no apparent size difference between seeds of different seed forms, with the possible exception of form 6 from Cape York Peninsula. Its seed body lengths of 1.65-2.10 mm average slightly larger than those of other forms but comparatively few seeds have been measured.

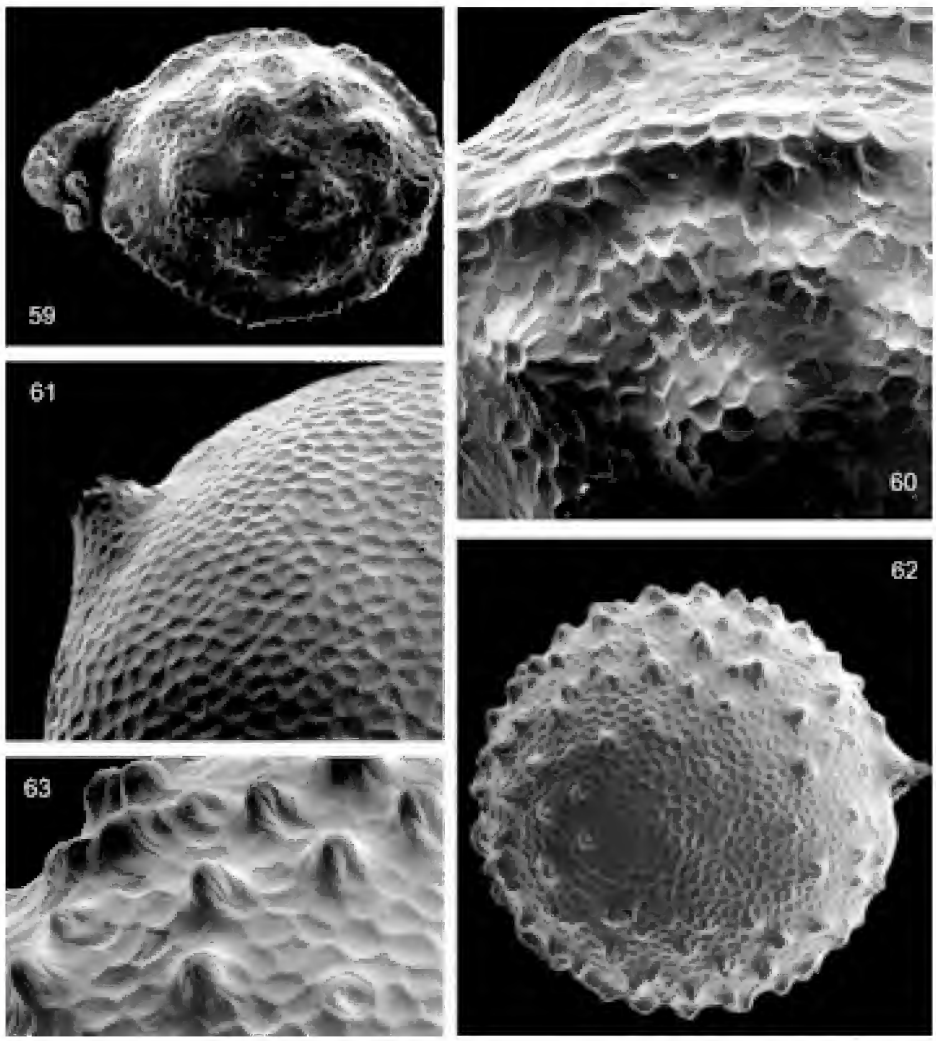
11. *Nymphoides minima*: (68 collections seen; 2 SEM) Figs 59-63.

Peak ornamentation: Seed body near-globose but moderately laterally compressed, cream-straw to fawn-brown or brown-black, 0.5-0.85 mm long, 0.475-0.8 mm wide, (0.275-)0.4-0.6 mm thick. Hilum within a minute, hard, circular, oblique basal projection of the seed body; projection often obscured by a long, looped or coiled, thick-based funicle which may be almost as long as the seed when stretched out; funicle base may be mistaken for a caruncle when the remainder of the funicle breaks off; funicle sometimes short and straight; caruncle absent. Periclinal walls with conspicuous pentagonal or hexagonal margins, each wall concave or occasionally flat to slightly convex. The whole seed surface undulates to form a number of low, broad, multicelled domes on each face and two closely parallel ridges around the seed edge where the domes become denser and more or less united. Most seeds appear shallowly pitted over their whole surface, including the surfaces of the domes and ridges (e.g. *Adams 1767*, figs 59, 60), but some of the darkest, apparently most mature, seeds have flat to mildly convex periclinal walls (e.g. *Aston 2514*, Prince Regent River, W.A.).

Variation: Seeds may lack any development of the domes and ridges and therefore appear uniformly biconvex and more or less smooth, ornamented only by the shallow concavities of the periclinal walls. Various intermediates between these seeds and the well sculptured "peak" form occur. For example, seeds may be smooth and biconvex with only partly-formed edge ridges (*Byrnes 849*, Humpty Doo road, N.T.); they may lack edge ridges but have the broad spaced domes over their whole surfaces (*Byrnes 886*, Jimmy Creek, N.T.; *Rankin 1187*, Berry Springs, N.T.) or over all but the centre-faces

(*Byrnes 1816*, Reynolds Creek, N.T.). Broad domes may be replaced by one- to few-celled projections which simulate narrowed domes of typical seeds (*Aston 1925*, figs 61-63; *Henshall 3661*, Howard Springs, N.T.). Both intermediate and smooth seeds have been found in the same population (*Aston 1925*).

The 68 seeded collections examined comprised 33 from the northern Northern Territory, 23 from the Kimberley region, Western Australia, and 12 (8 N.T.; 4 W.A.) which duplicated localities represented in the other 56 collections, e.g. there are eight different collections from the Edith Falls, N.T. All collections from any one site displayed the same seed form. All Kimberley collections seen possessed the domed and ridged seeds of the well-sculptured "peak" form described, whereas these were fully developed in only three of the 33 Northern



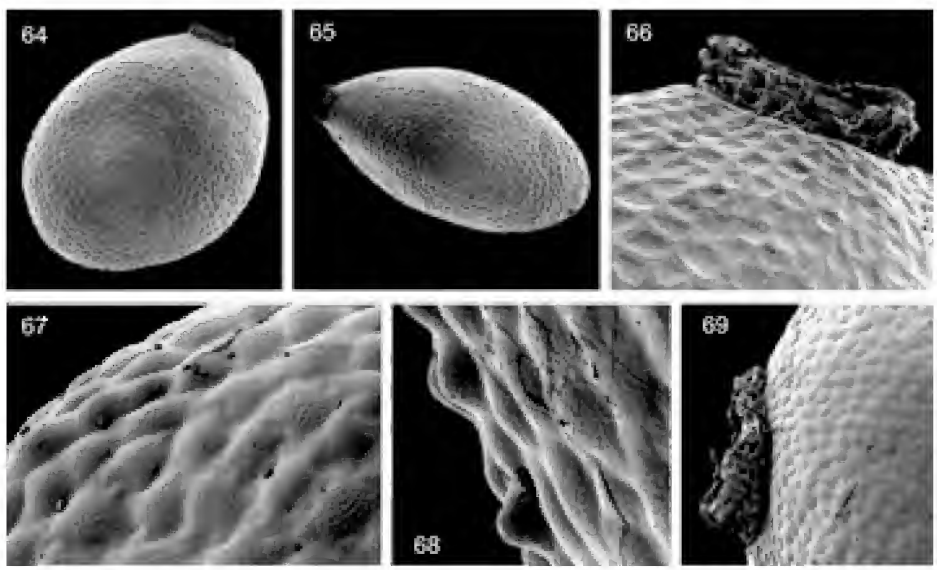
Figures 59-63. *Nymphoides minima*; **59** Lateral view of peak seed form, funicle still attached at left x 105; **60** portion of peak seed showing edge ridges (top) and surface domes x 175; **61** portion of extreme seed form lacking domes or tubercles, with attachment projection at left top x 200; **62** Lateral view of intermediate seed form, with attachment projection at right x 100; **63** portion of intermediate seed showing narrowed domes x 275. (**59, 60** from *Adams 1767*; **61-63** both forms from *Aston 1925*).

Territory locations, namely Allia Creek (*Cowie 4893 & Albrecht*); Edith Falls, (several collections, e.g. *Adams* 1767, figs 59, 60); Upper Fitzmaurice River (*Mueller s.n.*). Of the remaining Northern Territory collections 20 had smooth seeds, or smooth seeds with minute edge sculpturing, and 10 had the various intermediate forms of sculpturing described above. There is therefore a full range of seeds from smooth to the domed/ridged form in the Northern Territory, with apparently a preponderance of smooth or near-smooth seeds. In contrast, only seeds of the domed/ridged form have been found in Western Australian collections.

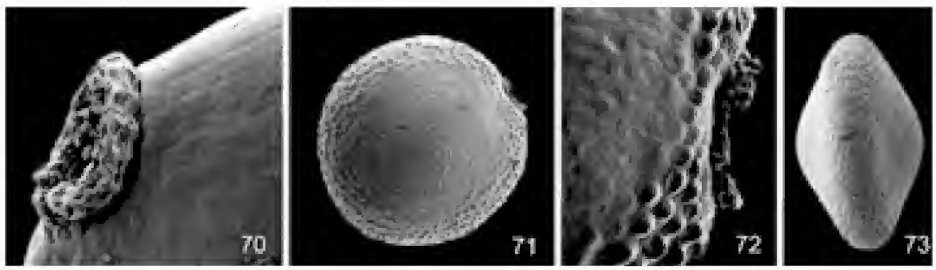
12. *Nymphoides montana*: (31 collections seen; 5 SEM) Figs 64-69.

Peak ornamentation: Seed body ellipsoid but strongly laterally compressed, dark grey-black to black, 1.1-1.55 mm long, 0.825-1.5 mm wide, 0.5-0.725 mm thick. Hilum within a pale, thin, near-basal, circular caruncle of large irregular cells. Periclinal walls with the margins pentagonal or hexagonal, or sometimes tending towards interdigitate, each wall slightly convex. The seed surface overall appears smooth and shining.

Variation: Two collections from the Bentleys Plains, East Gippsland, Victoria (*Beauglehole 36998*, figs 67–69; *Melville 3124*) show a slight tendency towards a tuberculate seed, with the periclinal walls of some spaced cells forming low domes. A few of the walls at the seed edges extend further to form short, tapered, broadly obtuse tubercles about 10-15 µm long, with the width usually about the same as or greater than the length, occasionally only half the length. Two populations near Guyra (*Bell 108*, Billi Bung Lagoon) and Uralla (*Bell s.n.*, Barleyfields Lagoon), both in the Northern Tablelands of New South Wales, have seeds which show the greatest variability known in *N. montana*. These seeds vary from smooth to having the low tubercles moderately densely distributed over the whole seed surface.



Figures 64-69. *Nymphoides montana*; **64** Lateral view of near-mature seed, with caruncle at top × 80; **65** semi-edge view of near-mature seed showing strong lateral compression × 80; **66** portion of near-mature seed showing caruncle and slightly concave periclinal cell walls × 330; **67** portion of mature seed showing convex periclinal cell walls × 320; **68** maximum development of tubercle × 415; **69** portion of mature seed with caruncle at left × 90 (**64-66** from *Adams 1678*; **67-69** from *Beauglehole 36998*).



Figures 70-73. *Nymphoides parvifolia*; **70** Caruncle and seed surface of usual biconvex smooth seed x 210; **71** lateral view of less usual seed with periclinal walls of the edge cells forming low domes x 45; **72** portion of seed showing the caruncle and low surface domes x 175; **73** edge view of seed with the maximum mid-face bulge seen x 45. (**70** from *Aston 2250*; **71-73** from *Clarkson 7794*).

In immature seeds, the convex periclinal walls of mature seeds remain concave so that seeds appear shallowly pitted.

13. *Nymphoides parvifolia*: (34 collections seen; 4 SEM) Figs 70-73.

Peak ornamentation: Seed body near-globose but strongly laterally compressed, from biconvex to broadly and mildly bulged mid-face, brown-black to black, 0.5-0.7 mm long, 0.45-0.675 mm wide, 0.275-0.425 mm thick. Hilum within a basal, circular caruncle of irregular small cells, the caruncle ranging from moderately conspicuous to thin and near-absent. Periclinal walls more or less pentagonal to hexagonal but with mildly interdigitate margins, each wall slightly concave or slightly convex. Tubercles absent. The seed surface appears smooth and shining.

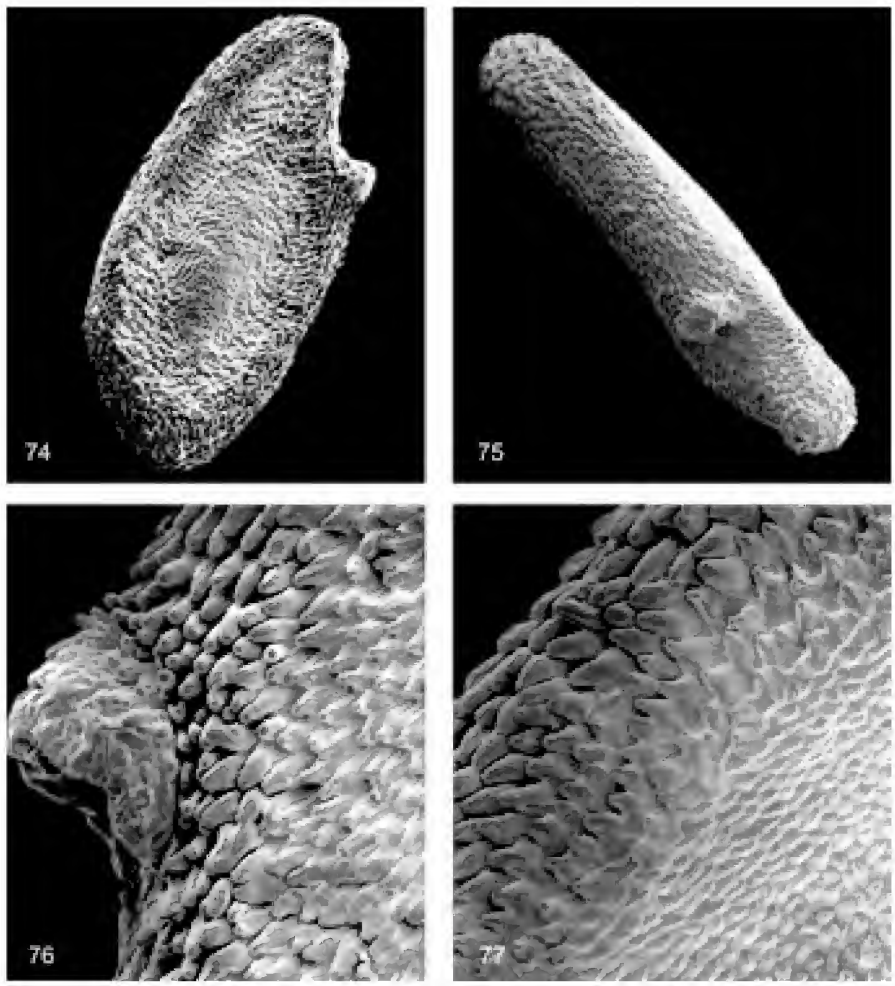
Variation: In a population from Saibai Island [Torres Strait], Queensland (*Clarkson 7794*, figs 71-73) the cells on and close to the seed edge have more pronounced convex periclinal walls, with each wall forming a broad, more or less semicircular dome.

Seeds with unusually large, oblique, conspicuous caruncles which may be 0.14 mm thick at their longest point occur in a collection from the Reynolds River floodplain, Northern Territory (*Cowie 1073*). However, seeds from a second collection from the same vicinity (*Cowie 1080*) possess the usual thinner and non-oblique caruncles.

14. *Nymphoides planosperma*: (5 collections seen; 1 SEM) Figs 74-77.

Peak ornamentation: Seed body narrow-ellipsoid, strongly laterally compressed, black, (1-)1.4-2.25 mm long, (0.5-)0.8-1.05 mm wide, (0.35-)0.45-0.6 mm thick. Hilum within a pale, semicircular, moderately thick caruncle of small irregular cells positioned on the seed edge about one-third of the seed length above the base. Periclinal walls with pentagonal or hexagonal margins. The central area of each seed face is more or less smooth and glossy, somewhat flat to distinctly convex, and has cells with slightly convex to slightly concave periclinal walls. The seed edge is prominently and densely tuberculate, with one broad-based tubercle arising from the whole width of each periclinal wall. Tubercles are regularly tapered upwards, obtuse, directed perpendicularly or slanted unidirectionally toward the seed base, c. 50-65 μm long and more or less twice as long as wide; they reduce in size toward the edge of the non-tuberculate centre-faces. The seed therefore has a distinctive, tuberculate, thickened perimeter strongly contrasting with the comparatively smooth centre-faces. Overall, it appears more or less flat, to flat but centrally bulged, according to the degree of convexity of the centre-faces.

Variation: The tubercles may fail to develop, leaving narrow-ellipsoid, black, glossy, biconvex, more or less smooth seeds, with all cell ornamentation resembling that of the



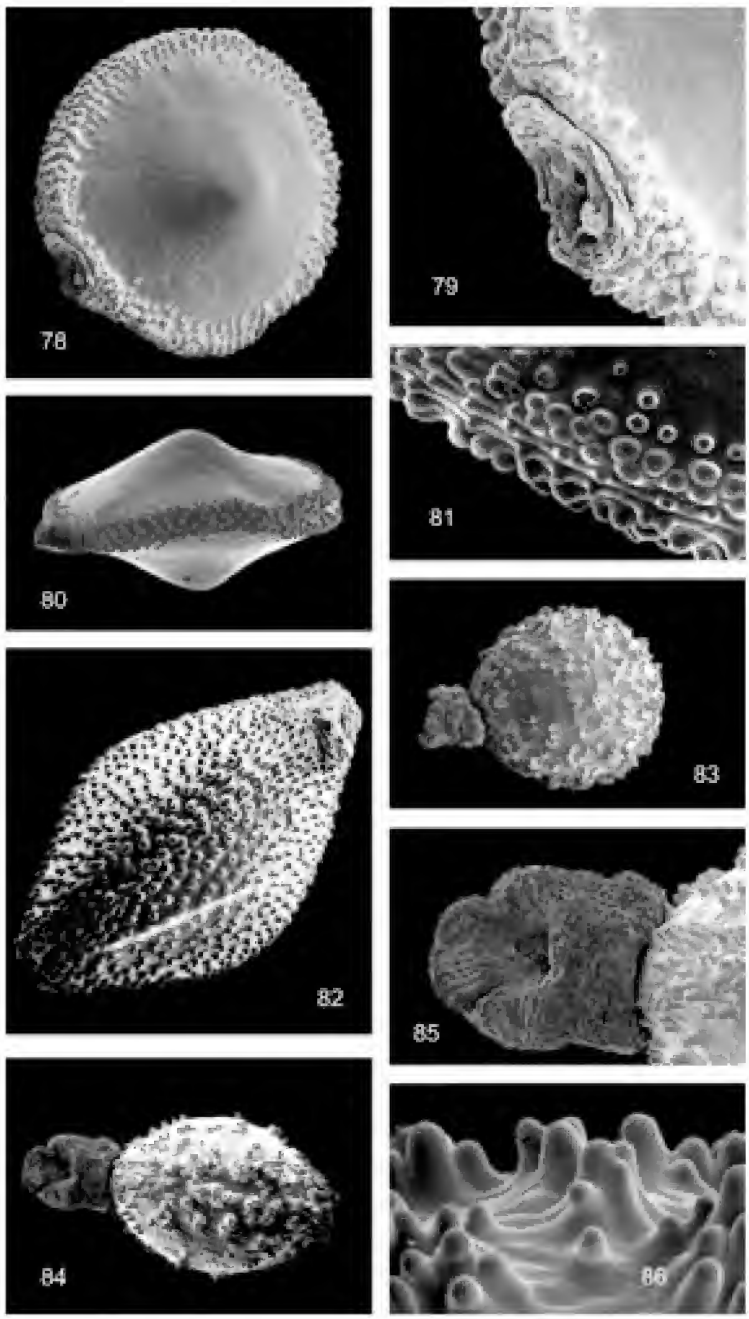
Figures 74-77. *Nymphoides planosperma*; **74** Lateral view of seed x 50; **75** edge view of seed x 50; **76** portion of seed showing the semicircular caruncle x 200; **77** portion of seed showing perimeter tubercles (left) grading to face cells (right) x 200 (all from *Craven 6607*).

seed faces (e.g. *Sanderson & Waterhouse UNSW9634*, Jabiluka Outlier, [between Jabiru and Oenpelli] N.T.). Populations with fully-ornamented seeds (e.g. *Sanderson & Waterhouse UNSW9635*) may exist close to those with smooth seeds. Both these collections were gathered in the same area on the same day but from different pools.

Seed from *Craven 6607* (figs 74-77) is possibly not fully mature. It has fully developed edge tubercles but only moderately developed centre-faces. Seeds from other collections have the centre-faces a little more bulged, with slightly convex rather than concave periclinal walls.

15. *Nymphoides quadriloba*: (48 collections seen: 11 W.A., 30 N.T., 7 Qld; 14 SEM) Figs 78-86.

Two different seed forms of *N. quadriloba*, Typical and Carpentaria, were informally recognised and briefly discussed by Aston (1982). At the same time populations from the



Figures 78-86. *Nymphoides quadriloba*; **78-81** Typical seed: **78** Oblique lateral view of typical seed showing pronounced centre-face bulge and edge tubercles × 100; **79** portion of typical seed showing the caruncle × 280; **80** edge view of typical seed showing the pronounced bulge of centre face × 110; **81** edge of typical seed showing the rectangular cell margins × 335; **82** Carpentaria seed: edge view showing spaced tubercles throughout and the lack of an abrupt central face bulge × 145. **83-86** Kimberley seed: **83** whole seed, face view × 35; **84** whole seed, edge view × 45; **85** caruncle, edge view × 110; **86** portion of seed showing unitubercled to multituberculed projections × 285. (**78, 79** from *Lazarides & Adams 122*; **80, 81** from *Chippendale 7697*; **82** from *Henshall 1938*; **83-86** from *Cowie 4390*).

Kimberley with a third seed form were tentatively placed in the species. I now consider that all three forms are conspecific, and their distinctions and gradations are detailed here.
Peak ornamentations: Typical seeds: [Darwin-Daly River-Katherine-East Alligator River area of the northern Northern Territory and an outlying collection from eastern Arnhem Land (*Cowie 6624 & Bokarra*, Cato River)]: Seed body near-globose but moderately laterally compressed with a pronounced central bulge from each of the faces, cream-straw to brown-black or black, 0.67-1.02 mm long, 0.6-0.95 mm wide, 0.35-0.57 mm thick. Hilum within a thin, circular, basal caruncle of small cells. Periclinal walls mildly interdigitate over the seed faces, the walls becoming more pentagonal or hexagonal near the seed edge and sometimes more or less rectangular over the edge. The walls are dimorphic, being slightly concave to slightly convex over the seed faces, but each produced into a short tubercle at and close to the seed edge. Tubercles smooth, short, thick, only slightly tapered, broad-based and broadly obtuse, c. 15-25 µm long and two or less times as long as wide. The thick, outwardly-directed tubercles occurring densely around the perimeter of the seed form a broad, ± square-cut, tuberculate rim which contrasts strongly with the virtually smooth, prominently bulged, seed faces. Overall, when viewed edge-on, the seed body appears top-shaped or rhomboidal (e.g. *Lazarides & Adams 122*, figs 78, 79; *Chippendale 7697*, figs 80, 81).
Carpentaria seeds: [Corinda-Normanton-Croydon area in the vicinity of the Gulf of Carpentaria, Queensland, and some from the Northern Territory]: Differ from typical seeds by having evenly biconvex instead of centrally-bulged faces, being fully tuberculate, and in having the circular caruncle longer and oblique, i.e. longer overall but even more so on one side (e.g. *Carolin 9104*, Corinda Lagoon, Qld.; *Henshall 1938*, Mary River, N.T., fig. 82). The tubercles are similar in shape to those described above but arise singly from each cell of the whole seed body so that it is covered with moderately spaced, short, thick tubercles. Maximum spacing between tubercles over the central areas of the seed faces is about 1–3 times the width of a tubercle, but decreases towards the edges where many of the tubercles may be touching (e.g. *Henshall 1938*).
Kimberley seeds: [Kimberley district of Western Australia]: Similar to Carpentaria seeds in being evenly biconvex with a long and oblique caruncle. However, the caruncle is usually about twice as long as that of Queensland seeds, with the diameter more or less equal to the greatest length. The largest caruncles seen measure c. 0.1-0.2 mm long at their shortest point and c. 0.175-0.325 mm at their longest point. The most extreme Kimberley seeds (e.g. *Cowie 4390*, Beverley Springs Station, figs 83-86; *Main s.n.*, Woorakin Creek) have this large caruncle and a tubercular surface over which the individual periclinal walls on any one seed may variously lack tubercles, possess a typical single tubercle, or be clustered into multitubercled projections. The two to few tubercles within each projection may be united only at the base or over most of their lengths. They are particularly pronounced in seeds of *Chesterfield 275* (Napier Broome Bay), where single tubercles are sparse and the projections are spaced between areas of low non-tuberculate walls. In contrast, seeds of some collections (e.g. *Kenneally 2193*, Lake Gilbert; *Jacobs 4341*, Mt House, E. of Derby; *Beard 6988*, Mitchell Plateau) are, except for their large caruncle, Carpentaria-like with a uniformly tuberculate surface over which each periclinal wall bears a single tubercle.

Variation: Both Typical and Carpentaria seeds may be modified by absence of the tubercles, giving fully-smooth seeds (e.g. Typical: *Byrnes 1818*, Survey Creek, N.T; Carpentaria: *Aston 2273*, Croydon, Qld). They may also vary in the distribution of the tubercles over the seed surface. In seeds with the Typical bulge-faced shape and thin caruncle, the tubercles may extend onto the side faces, rarely also onto the centre faces (*White s.n.*, June 1955, Mary River, N.T.). In seeds with the Carpentaria biconvex shape and thick caruncle, the tubercles may arise from only some of the cells, may occur over a reduced area of the seed surface, or may be absent, i.e. they may be either spaced by several non-tuberculate cells, present only on the edge and side-faces of the seeds, or missing. The seed variations between populations demonstrate morphological gradients between Typical and Carpentaria seeds in shape (bulged centre-faces to evenly biconvex),

the extent of ornamentation (tubercles absent, to edges only, to edge and side-faces, to whole seed surface), and caruncle (thinly circular to thicker oblique).

Although the Typical and Carpentaria seed shapes are not always as distinctive as described for the extremes, a partial geographical gradient between the two is evident. Typical seeds have not been seen on Queensland collections, and the only Northern Territory fully-tuberculate, biconvex, thicker-caruncled seeds seen are from two populations approaching the Gulf of Carpentaria (*Henshall 1567*, Cox River Station; *Leach 609*, Nathan River Station). The latter, depauperate, collection has been cited by Short (2000) as "*Nymphoides* Nathan River entity".

Kimberley seeds may also be modified by the absence of tubercles to give fully-smooth seeds which retain the large caruncle (e.g. *Kenneally 6589*, Mitchell Plateau; *Kenneally 8243*, Kununurra). The periclinal walls on seeds from Kimberley collections therefore display variations from smooth or mildly convex, to unituberculate and distinct, to clustered with the tubercles united into a multituberculate projection. Typical seeds have not been seen on any Kimberley collection.

In a number of Kimberley collections most of the leaf blades are ovate and comparatively narrower than those from the Northern Territory and Queensland (See Aston, 1982, fig. 3). These include the collections cited above for their most extreme seeds (*Cowie 4390; Main s.n.; Chesterfield 275*), two of the collections cited with uniformly tuberculate seeds (*Kenneally 2193*, *Jacobs 4341*), and smooth-seeded collections. Although the ovate leaves and multituberculate seed ornamentation, best combined in *Cowie 4390*, have not been seen from outside of the Kimberley region, there is apparently no clear correlation of leaf, floral, and seed characters which would allow segregation of a separate taxon.

16. *Nymphoides simulans*: (15 collections seen; 2 SEM) Figs 87-89.

Peak ornamentation: Seeds of this entity have the same characteristics as those of *N. spongiosa*, q.v. There is an apparent tendency for seeds to be smaller on average than those of *N. spongiosa* (0.575-0.85 mm long, 35 seeds measured; cf. 0.65-1.10 mm, 118 seeds measured) but measurements overlap.

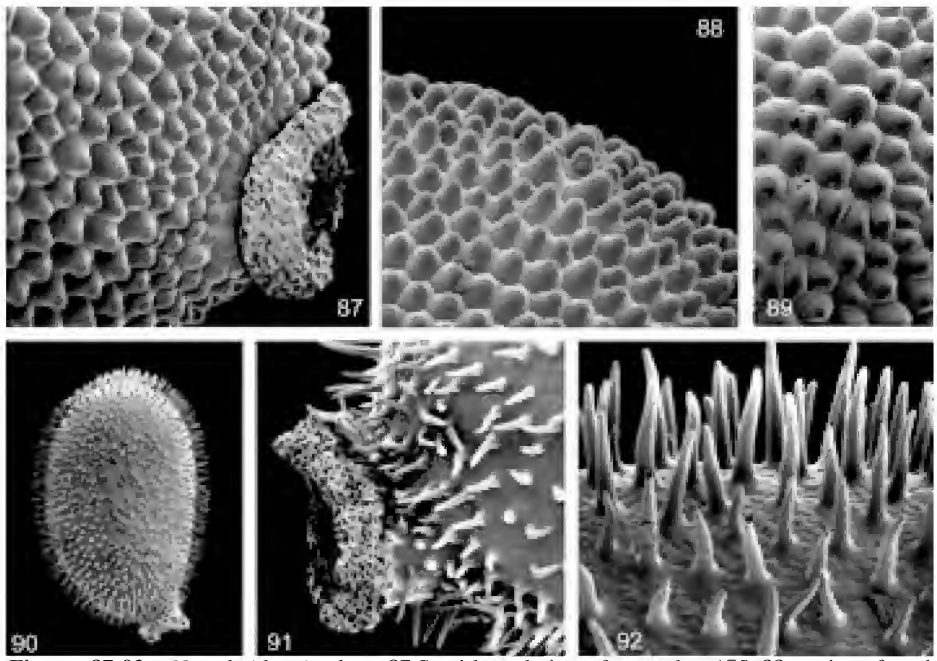
Variation: Variations are the same as those for *N. spongiosa*, e.g. *Craven 3203* (140 km S of Cooktown) has tubercles absent from the centre-faces.

This entity is known chiefly from the Musgrave to Mt Molloy areas of eastern Cape York Peninsula, Queensland, but has also been collected from two locations near the Gulf of Carpentaria at Westmoreland Station, Qld (*Jacobs 1547*, figs 87-89; *Carolin 9203*) and Bing Bong Station, N.T. (*Dunlop 2250*), and from two further locations in the vicinity of the South Alligator River (*Slee et al. 2880*) and East Alligator River, N.T. (*Seddifield s.n.*, DNA 73715). Plants are similar in seeds and leaves to typical *N. spongiosa* of the Northern Territory but differ substantially in several floral characters.

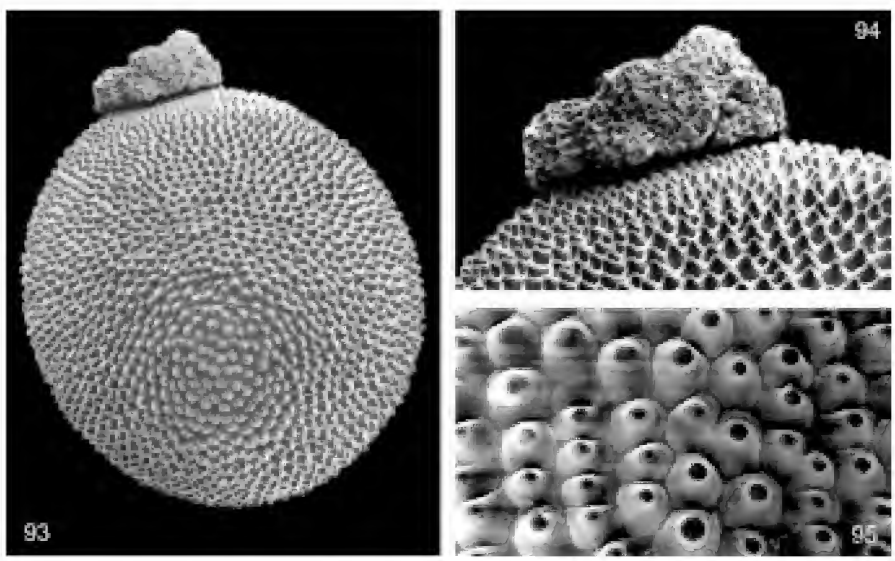
17. *Nymphoides spinulosperma*: (11 collections seen; 1 SEM) Figs 90-92.

Peak ornamentation: Seed body ellipsoid but strongly laterally compressed, black, 1.1-1.5 mm long, 0.8-1.1 mm wide, 0.4-0.5 mm thick. Hilum within a very short, stalk-like, near-basal projection of the cell body, with the end of the projection surrounded by a moderately thick, circular caruncle of numerous small cells. Periclinal walls strongly and conspicuously interdigitate, each bearing a prominent tubercle from its centre, or occasionally tubercles absent from some walls of the seed face. Tubercles smooth, slender, tapered, narrowly obtuse, c. 60-100 μm long and 6 or more times as long as wide. Because of the slender, evenly-spaced tubercles, the whole seed appears moderately densely tuberculate.

Variation: None observed.



Figures 87-92. *Nymphoides simulans*; **87** Semi-lateral view of caruncle x 175; **88** portion of seed surface showing tubercles x 160; **89** vertical view of centre-face of seed surface showing cell periclinal walls x 170 (all from *Jacobs 1547*). *Nymphoides spinulosperma*; **90** Lateral view of seed, with the caruncle at the bottom x 25; **91** portion of seed showing the seed projection with caruncle x 105; **92** portion of seed showing the tubercles and strongly interdigitate margins of the cell walls x 195 (all from *Aston 2870*).



Figures 93-95. *Nymphoides spongiosa*; **93** Lateral view of seed, with caruncle at top x 95; **94** lateral view of caruncle, showing the small caruncular cells x 200; **95** portion of seed surface showing the margins of the cell periclinal walls x 385 (all from *Must 1123*).

18. *Nymphoides spongiosa*: (32 collections seen; 1 SEM) Figs 93-95.

Peak ornamentation: Seed body near-globose but slightly to moderately laterally compressed, cream-straw to light brown-grey or brown-black, 0.65-1.1 mm long, 0.6-0.97 mm wide, 0.35-0.7 mm thick. Hilum within a pale, circular, moderately thick and prominent, (sometimes thin) basal caruncle of small irregular cells. Periclinal walls with pentagonal or hexagonal margins, each wall with a short tubercle arising from the whole wall area. Tubercles broad-based, smooth, strongly tapered upwards, broadly conical but obtuse, c. 15-25 μm long and about as long as wide. The short conical tubercles arising one from each cell give the seed surface a densely granular appearance throughout.

Variation: Tubercles may be more dome-shaped than tapered on some collections (e.g. *Cowie & Cowie 7533*, Howard River Reserve) or may occasionally be confined to the edges and side-faces of seeds, gradually reducing in length from the edges towards the centre-faces which are smooth (e.g. *Lazarides 7639*, vicinity of Nourlangie Creek; *Waterhouse 9649*, near Ja Ja). In immature seeds the periclinal walls are shallowly pitted, each pit consisting of an incipient tubercle not yet extruded (see Aston, 1982).

N. spongiosa is known only from a limited area of the northern Northern Territory, extending east to the East Alligator River and south to about 13° 15' S latitude. See also *N. simulans* above.

19. *Nymphoides subacuta*: (17 collections seen; 2 SEM) Figs 96-97.

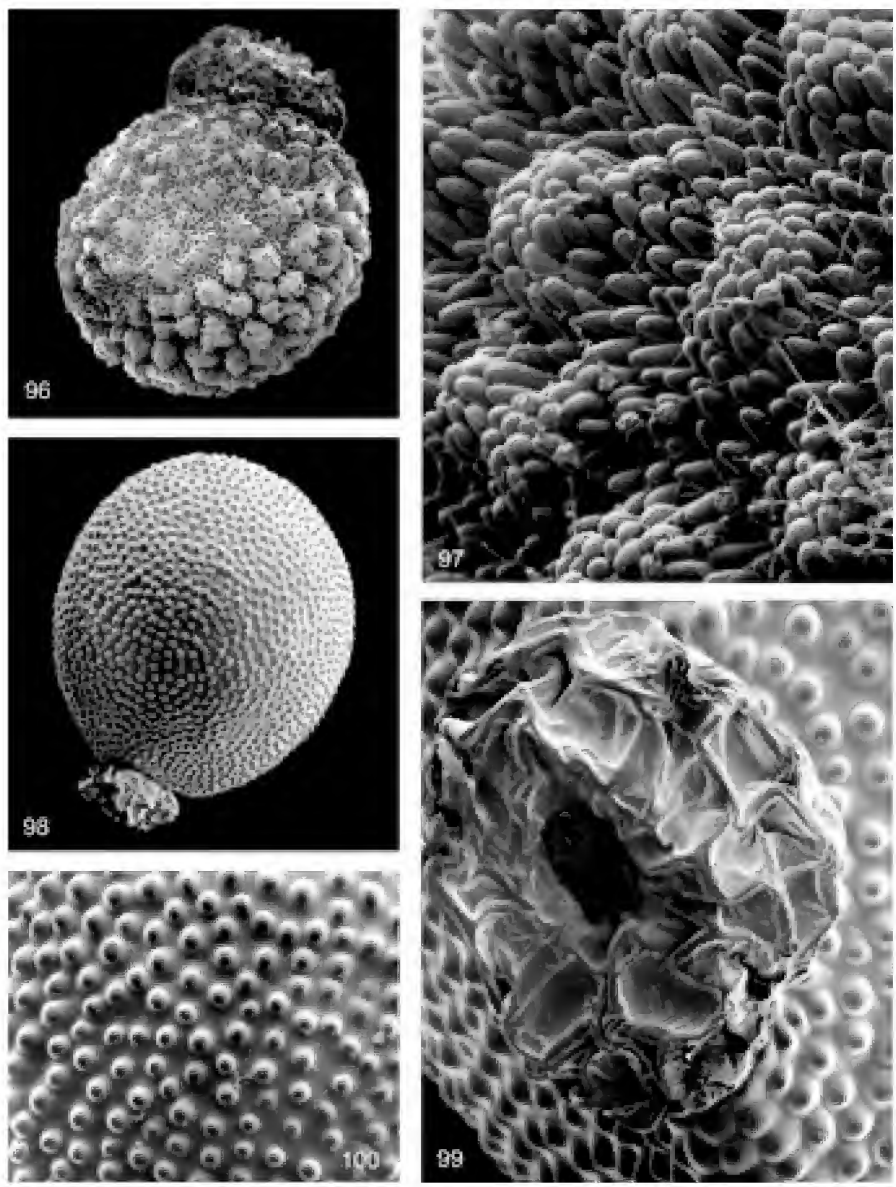
Peak ornamentation: Seed body near-globose but slightly laterally compressed, dark grey-brown-black, 1.4-1.9 mm long, 1.3-1.7 mm wide, 1.1-1.4 mm thick. Hilum within a pale, circular, thick and prominent, basal caruncle of irregular cells. Periclinal wall margins hidden by a dense covering of tubercles. Tubercles one per cell, smooth, broad and tightly appressed at the bases, mildly tapered upwards and broadly obtuse, to c. 70 μm long, the longest ones about three to four times as long as wide. The longest tubercles form dome-like clusters, each cluster consisting of about 20-30 tubercles tightly appressed throughout their length. These clusters are narrowly spaced over the whole seed surface, being separated by and grading into, a reticulate network of lower and narrower inter-dome depressions. This network is covered with shorter tubercles similar to those of the domes but appressed only at their bases. The seed therefore appears to have a regularly domed surface.

Variation: None observed.

20. *Nymphoides triangularis*: (10 collections seen; 1 SEM) Figs 98-100.

Peak ornamentation: Seed body near-globose but slightly to moderately laterally compressed, dark grey-brown-black, 0.45-0.70 mm long, 0.45-0.65 mm wide, 0.25-0.55 mm thick. Hilum within a pale, circular, moderately thick and prominent, basal caruncle of large irregular cells. Periclinal walls with inconspicuous pentagonal or hexagonal margins, each wall with a short, usually dome-like, tubercle arising from most of the wall area. Tubercles broad, smooth, broadly obtuse, c. 6-9 μm long and a little wider than long. Because the tubercles are broad and arise from much of the area of each wall, the tubercles are generally separated from each neighbouring one by less than a tubercle width. The seed surface appears moderately densely granular throughout.

Variation: Tubercles may be present only on and close to the seed edges, gradually diminishing in length from the edge towards the seed face and leaving the face smooth (e.g. *Garnett s.n.,* 5 April 1981, near Edward R. settlement, Cape York, Qld). In *Clarkson 8484 & Neldner*, Wenlock R., Cape York, tubercles are more blunt-conical than dome-like, being fractionally longer than those described, and slightly tapered into a more narrowly obtuse apex.



Figures 96-100. *Nymphoides subacuta*; **96** Semi-lateral view of seed, with caruncle at top x 35; **97** portion of seed surface showing tubercles of the domes and depressions x 260 (both from *Holtze 485*). *Nymphoides triangularis*; **98** Lateral view of seed, the caruncle at bottom x 105; **99** basal portion of seed showing semi-distal view of caruncle surrounding the hilum x 520; **100** portion of seed face showing tubercles x 285 (all from *Aston 2262*).

Discussion

Comparison of mature seeds from Australian collections

The size, shape, ornamentation, caruncle and, to a lesser extent, colour of mature seeds all contribute to the value of seeds in distinguishing Australian species of *Nymphoides*. The following comparisons of these characters should be used in conjunction with the full seed descriptions given under Results and the figures.

Seed size: Seed size is a useful character in distinguishing species, although no species can be identified on seed size alone.

Measurements of the length of the seed body range from 0.4-2.4 mm for all species combined, with a more restricted range for each species. *Nymphoides beaglensis*, *N. exiliflora*, *N. furculifolia*, *N. minima*, *N. parvifolia*, *N. simulans* and *N. triangularis* all have small seeds with the body length consistently less than 1 mm and, except for *N. beaglensis*, 0.85 mm or less. In contrast, the largest seeds occur in *N. aurantiaca*, *N. disperma*, *N. indica*, *N. planosperma* and *N. subacuta*, the only species to have seed bodies which reach 1.6 mm or more long. They may also be shorter, usually no less than 1.4 mm, but as short as 0.8 mm in the exceptionally variable *N. indica*.

The remaining eight species have medium sized seeds with overlapping body lengths in the range of 0.4-1.55 mm. They can be placed in two sub-groups, *N. elliptica*, *N. exigua*, *N. montana* and *N. spinulosperma* (1.0-1.55 mm) contrasting with *N. crenata*, *N. geminata*, *N. quadriloba* and *N. spongiosa* (0.4-1.2 mm). Many seeds of the latter sub-group overlap the range of the consistently small-seeded species.

Seed shape: The length/width ratios of seeds and their degree of lateral compression (thickness) indicate the differences in shape between species. The extreme seed shapes range from narrow-ellipsoid and strongly compressed to near-globular.

Seeds of *N. planosperma* are uniquely shaped in being narrow-ellipsoid, more or less twice as long as wide, and strongly laterally compressed with the width almost twice the thickness. Seeds of *N. crenata*, *N. montana* and *N. spinulosperma* are also strongly laterally compressed, but are broad-ellipsoid with the length only about 1.3 to 1.5 times the width. *Nymphoides exigua* has the same length/width ratio and strong lateral compression as *N. crenata*, *N. montana* and *N. spinulosperma* but is broad-obloid to rounded-obloid rather than ellipsoid.

All other Australian *Nymphoides* species have seeds which are near-globose with the width almost equal to the length. In most of these species the seeds are only slightly to moderately laterally compressed, those of *N. aurantiaca* and *N. exiliflora* being almost totally globular. In contrast, seeds of *N. parvifolia* are the most strongly compressed, with the thickness being only about half to two-thirds of the length and width.

Seeds in some collections of *N. quadriloba* and, to a lesser extent *N. parvifolia*, have centrally bulged faces. These differ from seeds in other collections of the same species, and from those of all other species, which have faces which are evenly curved.

Seed ornamentation: Seeds which are mature and have the most highly developed surface ornamentation, or ornamentations, known for the species concerned, are diagnostic except for distinguishing between *N. spongiosa* and *N. simulans*. Where ornamentations of different species are similar, e.g. as in *N. aurantiaca* and *N. exiliflora*, one or more of the additional characters of seed size, shape and caruncle will allow distinction.

However, ornamentation on even fully mature, turgid, hard, dry, fully-coloured seeds can vary within a species, most notably within *N. indica* and *N. quadriloba*. It may be less prominent because of a reduction in size of the tubercles, a decrease in the number and density of tubercles, or because the tubercles occur over a lesser portion of the seed surface. For example, tubercles commonly develop less in size and density over the central portion of the seed faces compared with their development over the side faces and seed edge (e.g. figs 14, 57). All three reduction features may occur on the one seed, e.g. as in *N. crenata* (fig. 12). In the most extreme reductions, tubercles may be nearly or

entirely absent from mature seeds (e.g. fig. 10). A complex of eight intergrading tubercle variations within *N. indica* (figs 44-58) is described under that species.

The shape of tubercles may also be modified within a species as if tubercle development has ceased before the usual shape has been reached, e.g. as in *N. aurantiaca* (figs 2, 3; cf fig. 4), or in *N. exiliflora* where two tubercle shapes can occur even on the same seed (fig. 30).

The shape of the periclinal walls of mature seeds is generally constant within any one species. In *N. aurantiaca*, *N. beaglensis, N. disperma*, *N. elliptica*, *N. exiliflora*, *N. furculifolia*, *N. minima*, *N. montana*, *N. planosperma*, *N. simulans*, *N. spongiosa* and *N. triangularis* the walls are pentagonal or hexagonal, whereas those of *N. crenata*, *N. indica* and *N. spinulosperma* are strongly interdigitate. *Nymphoides exigua* walls are intermediate between these two extremes, being shortly interdigitate, i.e. more or less shallowly lobed. *Nymphoides quadriloba* walls are usually similar to those of *N. exigua*, although they are sometimes pentagonal and hexagonal at the seed edges. Wall shape in *N. geminata* is generally pentagonal to hexagonal, but rarely shortly interdigitate. It has not been determined for *N. subacuta*.

Periclinal wall shape is best developed over the centre and sides of the seed faces and may also occur over the seed edges. However, quite frequently the edge walls are distorted and differ from those of the centre- and side-faces. Edge distortion is apparently due to a reduced expansion of epidermal cells at seed edges, and becomes more pronounced in immature or less developed seeds.

Caruncle: The hilar region is basal or near-basal in all species of Australian *Nymphoides* except *N. planosperma*, and is accompanied by caruncles or projections of the seed body. These are diagnostic for *N. planosperma* and *N. aurantiaca* and helpful in distinguishing other species.

In *N. aurantiaca* the membranous scale surrounding the hilum is unique, whereas *N. planosperma* is distinguished by the position of the hilum and its semicircular caruncle about one-third of the seed length above the base. Seeds of *N. crenata* and *N. minima* lack caruncles and instead have the hilum within a minute, hard, projection which is continuous with the seed body. In *N. exigua*, the hilum may be within a small circular bulge of the seed body and there may or may not be a thin caruncular rim at the mouth of this bulge. In *N. disperma* there is a short, oblique, projection of the seed body partly edged with a thin caruncle of small cells.

All other species have a circular caruncle composed of cells which are obviously different to the epidermal cells of the seed body. *Nymphoides beaglensis*, *N. exiliflora*, *N. subacuta*, and the Kimberley seeds of *N. quadriloba* have caruncles which are long thick and conspicuous, those of the latter form being exceptionally large (to 0.325 mm long). In contrast, caruncles of *N. furculifolia*, *N. montana* and the Typical seeds of *N. quadriloba* are thin and inconspicuous. Caruncles of *N. indica* (usually thin, rarely thick), *N. parvifolia* (thin to moderately conspicuous), *N. spongiosa* and *N. simulans* (both usually moderately conspicuous, sometimes thin) show variability. In distinguishing species within this group, caruncular size should be used with caution.

Seed colour: Except in *N. crenata*, the darkest colours of fully mature seeds of all Australian *Nymphoides* species range through dark grey-brown to black. Full-sized and expanded seeds which are apparently mature may also be paler grey or straw-coloured in some species.

In *N. crenata* dark seeds have never been seen, all mature seeds being straw-coloured to light tan-brown.

Mature versus immature seeds: It is often difficult to locate capsules with seeds showing the maximum degree of surface ornamentation for the population concerned. Ripening capsules are generally held under water on recurved rotting pedicels which readily break by the time the capsules and seeds are mature. Disturbance usually breaks any tenuous thread still holding a mature capsule, which then sinks, and many collections

therefore lack capsules with fully mature seeds. Before infructescences are disturbed, collectors should search gently for any capsules which are in danger of detachment.

Immature seeds must be viewed with caution, as the degree of development of tubercles and of the periclinal wall shape varies with the degree of seed maturity. Hard, pale, non-turgid seeds approximating the size of mature seeds, will have the same degree of tubercular ornamentation as mature seeds of the same capsule. Even soft (when fresh), half-size, still-developing seeds within the capsule generally give a strong indication of what their mature tubercular ornamentation will be. However, tubercles of immature seeds are often fully or partly inverted within the epidermal cells (fig. 5) before extruding (fig. 32) and inflating at maturity, and immature seeds can therefore give rise to a mistaken interpretation that mature seeds have pitted surfaces.

It is common for the epidermal cells of immature seeds to have sunken, concave, periclinal walls (fig. 9) rather than the raised, convex, walls of associated mature seeds. The shape of periclinal walls is also frequently distorted in immature seeds, particularly at their edges.

Extra-Australian comparisons

There are few published SEM illustrations of seeds of the five non-endemic Australian species from extra-Australian sources, but in a limited study Sivarajan *et al.* (1989) provide figures for Indian collections of *N. aurantiaca, N. indica* and *N. parvifolia*. Their descriptions and figures for *N. aurantiaca* (figs 33-36) agree well with the seed form having a very short scale and short broad obtuse tubercles found in some Australian collections.

For *N. indica*, the illustrations of Sivarajan *et al.* (1989, figs 7-10, 12-14) show similarities to the Australian seed forms numbered 2-4 in the current study whereas the Australian seed form number 1 is shown for a South American collection by Chuang and Ornduff (1992, figs 17 & 18). Sivarajan *et al.* and Chuang and Ornduff examined very few collections. It is possible that enlarged studies would report an increased range of extra-Australian variations for *N. indica* comparable to the large range of variations found in Australian material during the current study. Sivarajan *et al.* noted the need for ".. adequate sampling and more intensive studies on the seed coat patterns of *N. indica* complex" worldwide.

The seed descriptions and figures given by Sivarajan *et al.* (1989, figs 15-17) for *N. parvifolia* in India differ from those reported in the present work. Tubercles on the Indian seeds appear broad, obtuse, and spaced singly or several together over the whole seed surface. In contrast, Australian collections examined had non-tuberculate seeds except in one Torres Strait, Queensland collection. Seeds from this bore short, broad, obtuse, more or less domed tubercles (a little shorter than those on the Indian material) around the edges only. The differences between the Indian and Australian material of *N. parvifolia* fall within the range of seed variations found in other species within Australia (see "Seed ornamentation" under "Discussion" above). They may represent a genuine geographical difference or may simply be due to insufficient sampling.

Conclusions

This study involves the 20 species of *Nymphoides* recognised as occurring in Australia. All of these are native, 15 being endemic and 5 extending outside Australia. The five non-endemic species are *N. aurantiaca*, *N. exiliflora*, *N. geminata*, *N. indica* and *N. parvifolia*.

Morphologically, each species can be placed informally in either a "geminata" or an "indica" group, the two groups differing in flower colour and inflorescence characters (Aston 1982, 1985). The "geminata" group contains 9 species, namely *N. aurantiaca, N. crenata*, *N. disperma*, *N. exigua*, *N. exiliflora, N. geminata, N. montana, N. spinulosperma* and *N. subacuta*. The remaining 11 species are placed in the "indica"

group. The current work demonstrates that neither of these two subgeneric groups can be characterised by any combination of seed characters unique to the group. This is in keeping with Chuang and Ornduff (1992), who found that " ... it would not be possible to characterize each genus of Menyanthaceae by a typical and unique syndrome of morphological and cellular seed structures". Instead, combinations of seed characters, as displayed on fully-ornamented, mature seeds, are diagnostic at species level in Australian species of *Nymphoides*, as they are in the related genus *Villarsia* (Aston, 1969; Chuang and Ornduff, 1992).

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Appendix 1.: *Nymphoides* taxa and Australian collections from which seeds were examined by scanning electron microscopy. Where seed from a collection is illustrated in this paper, the relevant figure number is given in bold at the end of the collection citation. Some New Guinea collections of *N. exiliflora* and *N. geminata* are also included.

1. *N. aurantiaca* (Dalz.) Kuntze: c. 5 km WNW of Cardwell, Qld, Australia. *H.I. Aston 1979* (MEL) (**Fig. 1**); Near Nourlangie Safari Camp, c. 90 miles [145 km] NNE of Pine Creek township, N.T., Australia. *M. Lazarides & L.G. Adams 309* (CANB) (**Figs 2, 3**); Wenlock, Batavia R., Cape York Peninsula, Qld, Australia. *L.J. Brass 19702* (K) (**Fig. 4**); 5 miles [8 km] E. of Humpty Doo, N.T., Australia. *M. Gill (McKean B423)* (L) (**Fig. 5**); Chapman R., Gibb R. to El Questro road, Kimberleys, W.A., Australia. *A.C. Beauglehole 51596* (MEL); Atherton, Qld, Australia. *E.Betche NSW136678* (NSW); 10 km NE of Mudginbarry, N.T., Australia. *S.W.L. Jacobs 1804* (NSW); Mt Molloy, Qld, Australia. *H.S. McKee 9107* (BM).
2. *N. beaglensis* Aston: 8 km E of Beagle Bay, Dampier Peninsula, W.A., Australia. *K. Kenneally 9451* (PERTH, holotype) (**Figs 6-8**); Beagle Bay, W.A., Australia. *A. Forrest s.n.*, 1879 (MEL) (**Fig. 9**).
3. *N. crenata* (F. Muell.) Kuntze: Margaret R. crossing, Deepwater road, N.T., Australia. *J. Must 698* (MEL) (**Fig. 10**); Boatman Station, Maranoa District, Qld., Australia. *S.L. Everist 2884* (K) (**Fig. 11);** Burrell Creek, N.T., Australia. *N. Byrnes 1817* (MEL) (**Fig. 12**); Maryfield Station, Stuart Highway, N.T., Australia. *T.S. Henshall 3733* (DNA) (**Fig. 13**); c. 6 km N of Mareeba, Qld., Australia. *H.I. Aston 1960* (MEL) (**Fig. 14**); James R. crossing on Barkly Highway, N.T., Australia. *R.A. Perry 706* (CANB) (**Fig. 15**); Katherine, N.T., Australia. *H.S. McKee 8494* (K) (**Fig. 16**); Jericho and vicinity, Mitchell District, Qld., Australia. *M.S. Clemens s.n.*, 15 March 1946 (K) (**Fig. 17**); 30 miles [48 km] ENE of El Sherana, N.T., Australia. *L.G.Adams 3070* (CANB); c. 20 miles [32 km] NW of Echuca, Vic., Australia. *H.I. Aston 1701* (MEL); Bing Bong Station, N.T., Australia. *C.R. Dunlop 2248* (DNA); Phillip Creek Station, N.T., Australia. *B.W. Strong 57* (DNA).
4. *N. disperma* Aston: Vansittart Bay, North Kimberley, W.A., Australia. *S.J. Forbes 2098* (PERTH, isotype) (**Figs 18-20**).
5. *N. elliptica* Aston: 10.3 km E of Musgrave, Cape York Peninsula, Qld, Australia. *H.I. Aston 2260* (MEL, holotype) (**Figs 21-23**).
6. *N. exigua* (F. Muell.) Kuntze: Point Hibbs, Tas., Australia. *A.M. Buchanan 2764* (MEL) (**Figs 24-26**); Huonville, Tas., Australia. *L. Rodway s.n.,* Jan. 1896 (MEL) (**Fig. 27**).
7. *N. exiliflora* (F. Muell.) Kuntze: Rockinghams Bay, Qld, Australia. [*J. Dallachy s.n., s. dat.*] (GOET) (**Figs 28, 29**); Weam, Western District, Bensbach Subdistrict, Papua [New Guinea]. *C.E. Ridsdale NGF33531* (L) (**Fig. 30**); Rockingham's Bay, Qld, Australia. *J. Dallachy s.n., s. dat.* (MEL) (**Fig. 31**); 3 km N of Coolum Beach, Qld, Australia. *P. Sharpe 1869* (BRI) (**Fig. 32**); Shoalwater Bay, [Qld, Australia]. *R. Brown s.n., s. dat.* (BM); Hann R., Cape York Peninsula, Qld, Australia. *L.J. Brass 19987* (K); Lawnton, Qld, Australia. *M.S. Clemens 42097* (G); 57 km SSE of Ingham, Qld, Australia. *R. Coveny 6946 & P. Hand* (NSW); Rockingham Bay, Qld., Australia. *J. Dallachy s.n., s. dat.* (K); Kelsey Creek, Qld, Australia. *Rev. N. Michael 945* (BM); Near Bula Village, mouth of Morehead R., Papua [New Guinea]. *R. Pullen 7018* (K).
8. *N. furculifolia* Specht: 13° 12' S., 132° 46' E., N.T., Australia. *M. Lazarides 7645* (CANB) (**Figs 33, 34**); Spencer Range, 26 miles [42 km] E of Oenpelli, N.T., Australia. *L.G. Adams 3000* (CANB); 12° 45' S., 133° 20' E., N.T., Australia. *L.A. Craven 2239* (CANB).
9. *N. geminata* (R.Br.) Kuntze: 4.6 km ENE of Stratford, Vic., Australia. *H.I. Aston 1854* (MEL) (**Figs 35-37**); 3.6 km N of Boonoo Boonoo, N.S.W., Australia. *H.I. Aston 1840* (MEL) (**Fig. 38**); 8.5 km E of Tingha, N.S.W., Australia. *H.I. Aston 1839* (MEL) (**Figs 39, 40**); Between Cunnamulla and Bollon, Qld, Australia. *H.I. Aston s.n.,* 25 Sept. 1969 (MEL) (**Fig. 41**). Boonoo Boonoo via Tenterfield, N.S.W., Australia. *Ch. Knoetzsch s.n.,* Jan. 1885 (MEL) (**Fig. 42**); Yobobos grassland area, Laiagam subdistrict, Western Highlands, New Guinea. *R.D. Hoogland & R. Schodde 7484* (L) (**Fig. 43**); Providence Ponds area, Vic., Australia. *H.I. Aston 1763A* (MEL); 19 km N of Tenterfield, N.S.W., Australia. *H.I. Aston 1841* (MEL); Peecks Road, S. of Bairnsdale, Vic., Australia. *H.I. Aston 1855* (MEL); West of Bengworden, Vic., Australia. *H.I. Aston 1856* (MEL); Port Jackson, N.S.W., Australia. *F. Bauer* [or *R.Brown*] *s.n., s. dat.* (W); Nepean, N.S.W., [Australia]. *R. Brown s.n.*, Dec. 1804. [Type of var. a] (BM); "Nova

Hollandia" [N.S.W.], Australia. *Caley s.n., s. dat.*, (W); Sandy Creek, c. 3 miles [4.8 km] S of Old Bonalbo, N.S.W., Australia. *E.F. Constable NSW66220* (K); Stanthorpe, Qld, Australia. *H.A. Longman s.n.*, 1911 (K); 30 miles [48 km] W of Pentland township, Qld, Australia. *N.H. Speck 4602* (CANB); New England, N.S.W., Australia. [*C. Stuart* ?] (MEL); Sirunki, Western Highlands, New Guinea. *Walker ANU562* (L); Mt Hagen subdistrict, New Guinea. *J. Womersley NGF43515* (L).

10. *N. indica* (L.) Kuntze: Drysdale R. National Park, Kimberley, W.A., Australia. *K.Kenneally 4510* (PERTH) (**Figs 44-46**); Borroloola to Wollogorang road, N.T., Australia. *A.C. Beauglehole 54910* (MEL) (**Figs 47, 48**); Alice R., Qld, Australia. *M. Dixon s.n., s. dat.* (MEL) (**Figs 49, 50**); Traine R., Mt House, Tableland road, W.A., Australia. *A.S. George 15168* (PERTH) (**Fig. 51**); 5–8 km NNE of Katherine, N.T., Australia. *H.I. Aston 1896* (MEL) (**Figs 52, 53**); 5.4 km S of Musgrave, Cape York Peninsula, Qld, Australia. *H.I. Aston 2265* (MEL) (**Figs 54, 55**); Camp Creek, tributary of the Prince Regent R., Kimberley, W.A., Australia. *H.I. Aston 2519* (MEL) (**Fig. 56**); Lennard R., Kimberley, W.A., Australia. *V. Edwards s.n.*, March 1922 (PERTH) (**Figs 57, 58**); 24 km NW of Croydon, Qld, Australia. *H.I. Aston 2272* (MEL); Near Nourlangie Safari Camp, c. 90 miles [145 km] NNE of Pine Creek township, N.T., Australia. *M Lazarides & L.G. Adams 310* (K); Southgate, 8 miles E of Grafton, N.S.W., Australia. *E.C. Macdonald 157* (K); Magella Plain, SW of Cannon Hill, N.T., Australia. *P. Martenz AE259* (CANB); Towards McAdam Range / Arnhem Land [mixed coll.], N.T., Australia. *F.Mueller s.n.*, Oct. 1855 (K); Sideling Creek Dam, Dakabin, near Brisbane, Qld, Australia. *Thorne & Trapnell s.n.*, 10 March 1960 (K).
11. *N. minima* (F. Muell.) Kuntze: Edith Falls, c. 20 miles [32 km] N of Katherine, N.T. Australia. *L.G. Adams 1767* (K) (**Figs 59, 60**); NE of Jimmy's Creek abattoirs, N.T., Australia. *H.I. Aston* 1925 (MEL) (**Figs 61-63**).
12. *N. montana* Aston: Smoker's Gap, Tidbinbilla Range, Paddy's R. district, A.C.T., Australia. *L.G. Adams 1678* (CANB) (**Figs 64-66**); Bentleys Plains, East Gippsland, Vic., Australia. *A.C. Beauglehole 36998* (MEL) (**Figs 67-69**); Camden, County of Cumberland, N.S.W., Australia. *L. Atkinson 11* (MEL); 6 miles [9.6 km] N of Wulgulmerang Post Office, Vic., Australia. *H.I. Aston 1335* (MEL); Dumaresq Dam, c. 12 km NW of Armidale, N.S.W., Australia. *H.I. Aston 1836* (MEL).
13. *N. parvifolia* (Griseb.) Kuntze: Laura to Coen road, Cape York Peninsula, Qld, Australia. *H.I. Aston 2250* (MEL) (**Fig. 70**); Saibai Is., Cook District, Qld, Australia. *J. Clarkson 7794* (MEL) (**Figs 71-73**); 1 mile [1.6 km] S of East Alligator R. crossing, N.T., Australia. *N. Byrnes 841* (MEL); 12° 09' S., 132° 51' E., N.T., Australia. *L.A. Craven 2250* (CANB).
14. *N. planosperma* Aston: c. 22 km NE of Jabiru, N.T., Australia. *L.A. Craven 6607* (MEL, holotype) (**Figs 74-77**).
15. *N. quadriloba* Aston: c. 12 miles [19.3 km] NE of Edith River Siding, N.T., Australia. *M. Lazarides & L.G. Adams 122* (CANB) (**Figs 78, 79**); 2.5 miles [4 km] SW of Fountain Head, N.T., Australia. *G. Chippendale 7697* (K) (**Figs 80, 81**); Mary R., Arnhem Highway, N.T. Australia. *T.S. Henshall 1938* (DNA) (**Fig. 82**); Brolga Swamp, Beverley Springs Station, Kimberley, W.A., Australia. *I.D. Cowie 4390* (DNA) (**Figs 83-86**); c. 2 miles [3.2 km] N of Katherine, N.T., Australia. *L.G. Adams 1747* (K); 5–8 km NNE of Katherine, N.T., Australia. *H.I. Aston 1898* (MEL); c. 41 km NW of Croyden, Qld, Australia. *H.I. Aston 2273* (MEL); Survey Creek, N.T., Australia. *N. Byrnes 1818* (DNA); Napier Broome Bay, Kimberley, W.A., Australia. *E.A. Chesterfield 275* (MEL); 40 km from Normanton on the Croydon road, Qld, Australia. *L.A. Craven 3308A* (MEL); Cox River Station, near Arnold R., N.T., Australia. *T.S. Henshall 1567* (MEL); Lake Gilbert, West Kimberley, W.A., Australia. *K. Kenneally 2193* (PERTH); Near Mitchell Plateau airfield, Kimberley, W.A., Australia. *K. Kenneally 4747* (MEL); Kununurra, Kimberley, W.A., Australia. *K. Kenneally 8243* (PERTH).
16. *N. simulans* Aston [ms, in press, Nov. 2002]: Westmoreland, Qld, Australia. *S.W.L. Jacobs 1547* (NSW) (**Figs 87-89**); E of Musgrave, Cape York Peninsula, Qld, Australia. *H.I. Aston 2261* (MEL).
17. *N. spinulosperma* Aston: 26 km W of St Arnaud, Vic., Australia. *H.I. Aston 2870* (MEL) (**Figs. 90-92**).
18. *N. spongiosa* Aston: Nourlangie Rock area, N.T., Australia. *J. Must 1123* (BRI) (**Figs. 93-95**).
19. *N. subacuta* Aston: Port Darwin, N.T., Australia. *M. Holtze 485* (MEL) (**Figs 96, 97**); 10 miles [16 km] from Darwin, N.T., Australia. *C.E.F. Allen 539* (NSW).
20. *N. triangularis* Aston: 14.8 km E of Musgrave, Cape York Peninsula, Qld, Australia. *H.I. Aston 2262* (MEL, isotype) (**Figs 98-100**).

Appendix 2. Collections cited in this paper, listed in alphabetical order of collector. Identifications are indicated by the species number given in brackets after each collection.

Adams 1678 (12), *Adams 1747* (15), *Adams 1767* (11), *Adams 3000* (8), *Adams 3070* (3), *Allen 539* (19), *Aston 1335* (12), *Aston 1701* (3), *Aston 1758* (3), *Aston 1763A* (9), *Aston 1836* (12), *Aston 1839* (9), *Aston 1840* (9), *Aston 1841* (9), *Aston 1854* (9), *Aston 1855* (9), *Aston 1856* (9), *Aston 1896* (10), *Aston 1898* (15), *Aston 1925* (11), *Aston 1960* (3), *Aston 1979* (1), *Aston 2242* (3), *Aston 2250* (13), *Aston 2260* (5), *Aston 2261* (16), *Aston 2262* (20), *Aston 2265* (10), *Aston 2269* (3), *Aston 2272* (10), *Aston 2273* (15), *Aston 2275* (3), *Aston 2279* (3), *Aston 2280* (3), *Aston 2514* (11), *Aston 2519* (10), *Aston 2536* (10), *Aston 2870* (17), *Aston s.n.* 25.ix.1969 (9), *Atkinson 11* (12), *Bauer* or *Brown s.n. s.dat.* (9), *Beard 6988* (15), *Beauglehole 36998* (12), *Beauglehole 51596* (1), *Beauglehole 52144* (1), *Beauglehole 54910* (10), *Bell 108* (12), *Bell s.n.* 24.ix.1990 (12), *Betche NSW136678* (1), *Bonney s.n.* -.viii.1870 (3), *Boorman NSW136690* (3), *Brass 19702* (1), *Brass 19987* (7), *Brown s.n.* -.xii.1804 (9), *Brown s.n. s.dat.* (7), *Buchanan 2764* (6), *Byrnes 841* (13), *Byrnes 849* (11), *Byrnes 886* (11), *Byrnes 1816* (11), *Byrnes 1817* (3), *Byrnes 1818* (15), *Caley s.n. s.dat.* (9), *Carolin 9104* (15), *Carolin 9203* (16), *Chesterfield 275* (15), *Chinnock 808* (3), *Chippendale 7697* (15), *Clarkson 7794* (13), *Clarkson 8484 & Neldner* (20), *Clemens 42097* (7), *Clemens s.n.* 15.iii.1946 (3), *Constable NSW66220* (9), *Cousins 178* (1), *Coveny 6946 & Hand* (7), *Cowie 1073* (13), *Cowie 1080* (13), *Cowie 4390* (15), *Cowie 4893 & Albrecht* (11), *Cowie 6624 & Bokarra* (15), *Cowie & Cowie 7533* (18), *Craven 2239* (8), *Craven 2250* (13), *Craven 3203* (16), *Craven 3308A* (15), *Craven 4419* (1), *Craven 6607* (14), *Dallachy s.n. s.dat.* (7), *Dixon s.n. s.dat* (10), *Dunlop 2248* (3), *Dunlop 2250* (16), *Edwards s.n.* -.iii.1922 (10), *Everist 2884* (3), *Forbes 2098* (4), *Forster PIF22646* (3), *Forrest s.n.*1879 (2), *Gardner 12220* (3), *Garnett s.n.* 5.iv.1981 (20), *George 12508* in part (4), *George 15168* (10), *Gill (McKean B423)* (1), *Henshall 1567* (15), *Henshall 1938* (15), *Henshall 3661* (11), *Henshall 3733* (3), *Holtze 485* (19), *Hoogland & Schodde 7484* (9), *Jacobs 1547* (16), *Jacobs 1667* (3), *Jacobs 1765* (1), *Jacobs 1804* (1), *Jacobs 4341* (15), *Johnson NSW136695* (3), *Johnson & Pedley 61* (3), *Kenneally 2193* (15), *Kenneally 4510* (10), *Kenneally 4747* (15), *Kenneally 4758* (1), *Kenneally 6589* (15), *Kenneally 8243* (15), *Kenneally 9451* (2), *Knoetzsch s.n.* -.i.1885 (9), *Lazarides 7639* (18), *Lazarides 7645* (8), *Lazarides & Adams 122* (15), *Lazarides & Adams 309* (1), *Lazarides & Adams 310* (10), *Leach 609* (15), *Longman s.n.* 1911 (9), *McBarron 8556* (9), *Macdonald 157* (10), *McKee 8494* (3), *McKee 9107* (1), *Main s.n.* -.viii.1969 (15), *Martenz AE259* (10), *Melville 3124* (12), *Michael 945* (7), *Morton 603* (1), *Mueller s.n.* -.x.1855 (10) (11), *Must 698* (3), *Must 1123* (18), *Perry 706* (3), *Pullen 7018* (7), *Purdie & Boyland 142* (3), *Rankin 1187* (11), *Ridsdale NGF33531* (7), *Rodway s.n.* -.i.1896 (6), *Sanderson & Waterhouse UNSW9634* (14), *Sanderson & Waterhouse UNSW9635* (14), *Seddifield s.n. DNA73715* (16), *Sharpe 1869* (7), *Slee Craven & Brennan 2880* (16), *Speck 4602* (9), *Strong 57* (3), [*C. Stuart* ?] (9), *Thomson 522* (3), *Thorne & Trapnell s.n.* 10.iii.1960 (10), *Vogan s.n.*1889 (3), *Walker ANU562* (9), *Waterhouse 9649* (18), White *s.n.* -.vi.1955 (15), *Wilson & Jacobs 765* (3), *Womersley NGF43515* (9).

Muelleria 18: 67–73 (2003)

Reinstatement of *Epacris Franklinii* Hook. *f.* (Epacridaceae)

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Abstract

Reexamination has been made of the riverine species of *Epacris Cav.* in Tasmania, in order to clarify the identification of plants obtained from the Meander and Mersey Rivers. Some comments are made on the distribution and conservation status of the species. Evidence is presented supporting the reinstatement of *Epacris franklinii* Hook.f. as a species distinct from synonymy with *E. mucronulata* R.Br.

Introduction

In his introductory comments on the genus *Epacris* Cav., Bentham (1868) remarks that "with all its variations in the foliage and shape of the corolla it (*Epacris)* is the most easily recognized (genus) in the Order (Epacridae)... The species, however, (of *Epacris*) are exceedingly difficult to circumscribe by any definite characters, the whole eighteen of the short-flowered ones seeming to pass into each other by small gradations". Unfortunately the passage of time has done nothing to relieve this situation. To the contrary, the discovery of new short-flowered taxa has only added to the problem.

The history of *Epacris* in Tasmanian botany is a good reflection of the problem as the changing status of taxa in the treatments of successive botanists has shown. *Epacris*, a genus of ca. fifty species in eastern Australia, New Zealand and New Caledonia, is represented in Tasmania by some twenty eight taxa (Crowden and Menadue, 2000). Seven species were recognized by Robert Brown (1810) and fourteen, with two varieties, by J.D. Hooker (1860). Ten Tasmanian species are described in Bentham's *Flora Australiensis* (1868), while five others, now or previously recognized as species, were considered by him as varieties, or were given synonymy with other species. Rodway's *The Tasmanian Flora* (1903), considers eleven species with six earlier species considered as varieties (four) or as synonymous (two), while Curtis (1963) describes eighteen species, with no varieties and only one earlier species reduced to synonymy. The most recent treatment of the genus by Crowden and Menadue (2001) considers twenty two species and six unresolved taxa.

In the early literature of Tasmanian botany three riverine species of *Epacris* were described, *E. exserta* R.Br., occurring on the South Esk and Tamar River system of northern Tasmania, *E. franklinii* Hook.*f.* on the large streams flowing to the west coast and *E. mucronulata* R.Br. on the south flowing rivers emptying into Port Esperance. *Epacris franklinii* was later judged by Bentham (1968), to be identical with *E. mucronulata* on the basis of a comparison of some R. Brown specimens of *E. mucronulata*, with a specimen collected by R. C. Gunn from the Gordon R. Bentham's description of *E. mucronulata* carries the footnote "Brown's specimens are in young bud, Gunn's are past flower, but both appear to belong to the same species"; thereafter all *E. franklinii* specimens have been referred to as *E. mucronulata* in Tasmanian literature.

In recent years, 1987 onwards, a number of *Epacris* collections have been made from the north flowing Meander and Mersey Rivers. There had been no collections prior to this time from either river system lodged in HO. The new collections, although apparently all of a single taxon, were lodged under a variety of names, *E. exserta, E.* aff. *exserta, E.* aff. *mucronulata* and *E.* "Union Bridge" (a Mersey R. site), with *E. exserta* becoming a favoured and commonly accepted reference. It became an important matter to determine the correct identity and affinities of these specimens when a proposal to build an

irrigation dam on the Meander R. was opposed, largely on the grounds that it would impact seriously on the habitat of the endangered species *E. exserta*.

Discussion

Floral morphology provides a ready means of distinguishing between *E. exserta* and *E. mucronulata*. In *E. exserta* both the anthers and the stigma are fully exserted above the corolla tube (Fig. 1a). In *E. mucronulata* both anthers and stigma are enclosed (Fig. 1c). It was immediately obvious that none of the specimens from either the Meander or Mersey Rivers, was *E. exserta*, as the anthers and stigmas in all cases were fully enclosed. However, there was an evident affinity of these plants with *E. mucronulata*.

Examination of the HO *E. mucronulata* sheets and comparison with the Meander/ Mersey collections indicated the presence of 2 taxa. Taxon 1 contains all the specimens from the Huon R. system, plus 2 sheets of Gordon R. plants. It matches the type of *E. mucronulata* R.Br. (BM). Taxon 2 contains plants from the King, Gordon, and Pieman River systems and all the plants from the Meander and Mersey Rivers.

The main differences between the two taxa are outlined here. Taxon 1:- young stems sparsely pilose, leaves often distinctly 3-nerved underneath, the apex drawn out to a point, the corolla tube exceeding the calyx, the style bulbous near the base, the stigma below the anthers. Taxon 2:- young stems glabrous or barely pilose, the leaves with only the midrib prominent underneath, the apex acute with a blunt, often upturned mucro, the corolla tube is about equal with the calyx, or slightly longer, the style tapering from the base or with a slight central swelling, the stigma near the top of the anthers.

The distinction in floral morphology, in particular style length, between the two taxa is illustrated in the flower section drawing shown on the cibachrome photo (K) of *E. franklinii* Hook. *f.* (Hook. *f.* nr. 1907, Fig. 2), (later redetermined by Bentham as *E. mucronulata* R. Br.). This drawing matches the flower of Taxon 2, but not that of Taxon 1.

On this evidence it seemed appropriate to reinstate the earlier name *E. franklinii* Hook.f., for all the Taxon 2 specimens, including the Meander/ Mersey plants. *E. mucronulata* R.Br. would then apply only to the Taxon 1 specimens.

The disjunct distribution of *E. franklinii* may well be a consequence of the late quaternary glaciation history in the Central Highlands of Tasmania. It is possible that in a preglacial period of warmer, moister climate, the species was more widespread and at a higher altitude, where the major rivers which are the current habitat of these species all have their source. The onset of cooler and drier climate during the period(s) of glaciation would be expected to force the plants to lower altitudes down the separate river valleys.

Examination of herbarium records, together with field observations of the current distributions of these riverine species, showed *E. exserta*, to be restricted to the South Esk River in northern Tasmania, with its habitat much reduced by over 150 years of farm land development. Indeed some early collection sites are now entirely cleared of native vegetation. Its conservation status is given as endangered. *E. franklinii* and *E. mucronulata*, between them retain most of the distribution on the major river system of the west and south, previously allocated to *E. mucronulata,* and despite some hydroelectric developments and forestry activities, much of their riverine habitat remains intact. Prior to this investigation, the conservation status of *E. mucronulata* was given as vulnerable. This may require reassessment in the light of the significant narrowing of its distribution.

Key to the species

1. Filaments longer than anthers: anthers and stigma exserted.....1. **E. exserta**
1. Filaments shorter than anthers: anthers and stigma enclosed. 2

2. Leaves tri-nerved underneath, apex acute or acuminate: stigma below anthers. .... 2. **E. mucronulata**
2. Leaves with only midrib apparent, apex acute with inturned mucro: stigma amongst anthers. ..... 3. **E. franklinii**

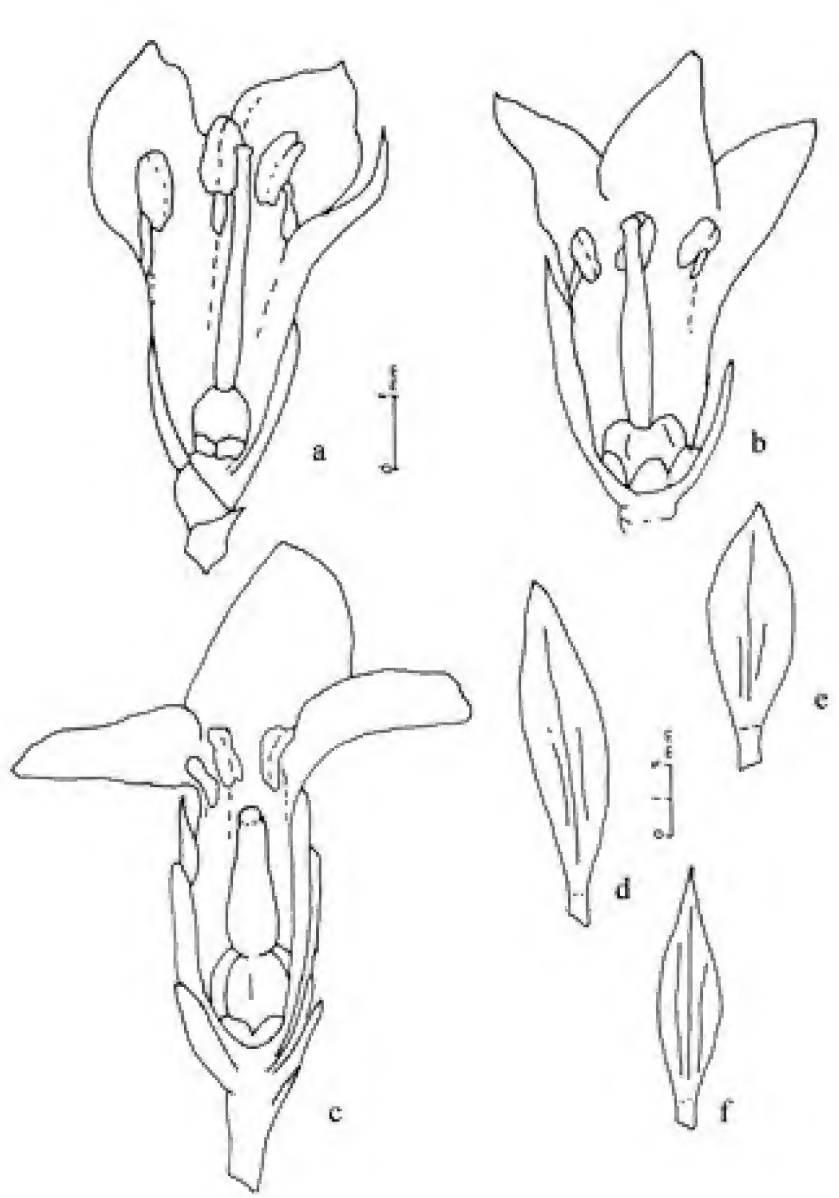

Figure 1. Half flowers of a. *E. exserta* R.Br., *b. E. franklinii* Hook. *f.* and c. *E.mucronulata* R.Br. Leaves of d. *E. exserta* R.Br., e. *E. franklinii* Hook.*f.* and f. *E. mucronulata* R. Br.

Figure 2. Cibachrome photograph from Kew of *E. franklinii* Hook. *f.*, ex Herb. Hookerianum 1867. The notation states "very common shrub growing 6 feet high, overhanging the margin of the River Franklin near Macquarie Harbour. It would be under water much of the year, as the River is subject to heavy flooding and it was commonly so when I was there".

Taxonomy

Description of the Species

1. *E. exserta* R.Br. Prod. 551,1810; DC. Prod. vii. 763, 1839; Hook. *f.*, Fl.Tasm. i, 260, 1831; Bentham, Fl. Australiensis, iv, 238, 1868; Rodway, Tas. Flora, 121, 1903; Curtis, Stud. Fl. Tas. Vol.2. 448, 1963. *Holotype*: Port Dalrymple, Jan. 1804, R. Brown 2485 (BM).

An erect multi-stemmed *shrub* up to 1.5m high. Young *stems* glabrous or nearly so. *Leaves* narrow-lanceolate to elliptical, 7 – 11mm long x 1.1 – 1.3mm wide, the apex acute, blunt, with a short mucro often slightly inturned. *Flowers* clustered in the axils of the top few leaves of new season's branchlets, are on long, bract-clothed pedicels, which bend and project the flowers out from the leaves. *Corolla* tube cylindrical, longer than the calyx and the lobes, the filaments longer than the anthers, projecting the (exserted) anthers clearly above the plane of the corolla lobes. *Style* cylindrical, the stigma at the top of the anthers and usually above them (Fig. 1 a, d).

Distribution: Very rare amongst riverbank boulders in the gorge of the South Esk River at Trevallyn (Launceston), and in riverine vegetation near the picnic grounds. Some early collections were made at Port Dalrymple (near Georgetown) and along the Nile River (Fig. 3.), these populations now appear to be extinct.

Specimens examined: South Esk R., Launceston, Sept. 23, 1842, *R. C. Gunn*, (HO 4248); Launceston, Aug. 21, 1931, *A.M. Olsen* (HO 4253); Cataract Gorge, Launceston, 1911, *F.E. Burbury* (HO 5285); Nile, Oct. 20, 1957, *D.M. Paton*, (HO 4257); Launceston, First Basin, Aug. 27, 1996, *M. Ilowski* (HO 321505); below Trevallyn Dam, Oct. 16, 1987, *R.K. Crowden* (HO 111644).

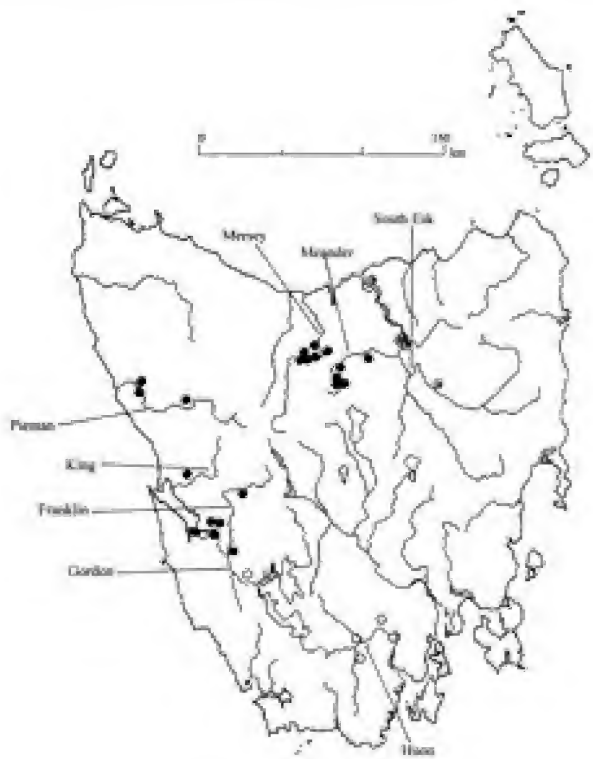

Figure 3. Map showing collection sites in Tasmania of *Epacris exserta* R.Br. (<), *E. franklinii* R.Br. (#), and *E. mucronulata* Hook.*f.* (O).

2. *E. mucronulata* R. Br. Prod. 552, 1810; DC. Prod. vii. 764, 1839; Bentham, Fl. Australiensis, iv, 238, 1868; Rodway, Tas. Flora, 121, 1903; Curtis, Stud. Fl. Tas. Vol.2. 448, 1963. *Holotype*. Port de l'Esperence, banks of the larger river, June 1804, R. Brown 2486 (BM).

An erect *shrub* up to 2m high, many-branched and fastigiated. Young *stems* minutely to moderately pilose. *Leaves* lanceolate 8 – 12mm long, 1.5 – 2mm broad, on longish petioles 1 – 1.2mm, flat to slightly concave, margin slightly thickened and entire, midrib prominent below and usually 2 other veins, apex acute with a short mucro tapering to a blunt tip. *Flowers* relatively few and clustered in the upper axils, borne on long (2.0 – 2.3mm) bract-clothed pedicels. *Bracts* ovate and keeled in upper part, apex acute, the larger bracts becoming mucronate. *Sepals* lanceolate – narrowly ovate, ca. 4mm long x 1. 2mm wide, the midrib indistinct except near the tip, apex acute and mucronate, the margin minutely ciliate in the upper part. *Corolla* tube cylindrical, longer than the sepals and the lobes, the anthers on short filaments and enclosed within the tube. *Style* with a basal swelling, short, the stigma below the base of the anthers (Fig. 1. c, f).

Distribution: Riverbank vegetation just above normal flow levels, but subject to inundation during floods. Huon and Picton Rivers, upstream from Huonville, and the Gordon River (Fig. 3).

Specimens examined: Tahune Bridge, Huon R., Sept. 21, 1979, *S.J. Jarman* (HO 31297); Huon R. at Judbury, Apr. 2. 1988, *A. M. Buchanan* (HO 109634); Huon R. 2km NW of Huonville, Jul. 14, 1991, *P. Collier* (HO 142403; Picton R at bridge, Oct. 30, 1982, *A. Moscal* (HO 66495); Huon R., Tahune Bridge, Sept. 2, 1983, *R.K. Crowden* (HO115366); Gordon R. near Sugarloaf, Nov. 11, 1846, *J. Milligan* (HO 5138); Gordon R., First Splits, Nov. 8, 1978, *S.J. Jarman* (HO 411340).

3. *E. franklinii* Hook.*f.* Fl.Tasm. i. 261. t. 79B, 1831. *Holotype*: Franklin R. Feb 7. 1845. Hook. *f.* nr. 1907, (Herb. Hookerianum 1867), cibachrome from K. *Isotype*: Franklin R. Feb. 7.1845. R. C. Gunn. HO 5944.

An erect spreading *shrub* up to 2m high. Young *stems* mostly glabrous or occasionally sparingly pubescent. *Leaves* lanceolate or elliptic 8 – 11mm long 1.3 –1.4mm broad, on longish petioles 0.9 – 1.1mm, nearly flat or slightly concave near the petiole, margin minutely dentate, midrib only prominent below, apex acute, with a short usually inturned mucro. *Flowers* few, clustered at the branch tips, borne on long, curved, bract-clothed pedicels. *Bracts* ovate and keeled in upper part, apex narrowly ovate. *Sepals* ovate, ca. 4mm long x 1.5mm wide, apex broadly acute, the margin ciliolate. *Corolla* tube cylindrical, as long as or slightly longer than the sepals, but longer than the lobes, the anthers enclosed. *Style* tapering from the base, or with a slight central swelling, the stigma at the top of the anthers (Fig. 1. b, e).

Distribution: Riverbank vegetation along the Meander, Mersey, Pieman, Maxwell, Gordon, Franklin and King Rivers systems. The plants grow above the normal river flow levels, but are subject to inundation during floods (Fig. 3).

Specimens examined: Gordon R., Nov. 1896, *L. Rodway* (HO 5139); King R, Oct. 1903, *L. Rodway* HO 5137); King R. Nov. 24, 1998, *A.M. Buchanan* (HO 329373); Heemskirk Falls on Pieman R., May 27, 1985, *M.J. Brown* (HO 96681); Franklin R. at Irenabyss and downstream to Gordon, Mar. 10, 1980, *A. Moscal* (HO 34118); Pieman R. near Hades Ridge, Nov. 15, 1974, *F. Allen* (HO 106903); Jackeys Marsh, Meander R., Nov. 7, 1986, *K.Williams* (HO 509094); Mersey R. near Weegena, Oct. 1987, *T. Aliano* (HO 322792); Cheshunt Bridge, Meander, Nov. 11,1997, *T. Aliano* (HO 323671); Mersey R. below Alum Cliffs, July 25, 1996, *D. Keith* (HO 320129); Egmont Bridge on Meander R. Jul.25, 1996, *D. Keith* (HO 321356); Long Ridge on Meander R., Jul. 23, 1996, *D. Keith* (HO 321353).

Acknowledgements

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Australasian Sequestrate Fungi 16. *Gastrotylopilus*, a Synonym of *Fistulinella*

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Abstract

Examination of the holotypes of *Gastrotylopilus brunneus* T.H. Li & Watling and *Fistulinella mollis* Watling revealed them to be the same taxon, even though *G. brunneus* was originally described as a gastroid, *i.e.* sequestrate, taxon and *F. mollis* as an epigeous, nonsequestrate taxon. A new genus is accordingly needed to accommodate sequestrate, hypogeous relatives of the epigeous genus *Tylopilus* P. Karst

Introduction

In recent years J.M. Trappe and A.W. Claridge have collected several hypogeous, sequestrate species of fungi related to the epigeous genus *Tylopilus* P. Karst. in south-eastern Australia. This is not surprising, because *Tylopilus* is a relatively diverse genus in Australia (Watling & Li 1999), and its sequestrate derivatives have likely evolved in response to the warm, dry climate characteristic of much of Australia (Trappe *et al.* 2001). We compared our collections to the description of *Gastrotylopilus brunneus* T.H. Li and Watling. None fit that species, and we became aware that the description of *G. brunneus* (Li & Watling 1999) does not fully conform to the usual macromorphology associated with sequestrate boletes.

The sequestrate derivatives of the tubulose Boletaceae are characteristically hypogeous or barely emergent. Their stipes are much reduced or even lacking, and their long, contorted tubes are not vertically oriented (Thiers & Trappe 1969, Thiers, 1989), for example as in the Australian *Gymnogaster boletoides* J.W. Cribb (Fig. 1). Cribb's (1956) illustration of this species graphically portrays the contortion and nonvertical orientation of the tubes usual for sequestrate boletes. *G. brunneus* was described by Watling and Li (1999) as having a stipe 35–45 x 7–14 mm and 'Tubuli ≤ 8–9 mm longi, contorti, sinuosi ad stipen, albi vel leviter fulvo-albi, completi prope poros; pori 1–1.5 per mm, irregulares vel subangulares, rosati, depressionibus' ('Tubules ≤ 8–9 mm long, contorted, sinuous at the stipe, white to light fulvous-white, filled near the pores; pores 1–1.5 per mm, irregular to subangular, pink, with depressions). To better understand *Gastrotylopilus brunneus,* we examined its holotype, Watling 14741, lent by the Royal Botanic Gardens, Edinburgh (E). A second collection (Watling 17785) not included in the original protologue of the type description was also provided by E.

Taxonomy

GASTROTYLOPILUS BRUNNEUS = FISTULINELLA MOLLIS

The holotype of *Gastrotylopilus brunneus* (Figs. 2, 3) is somewhat immature, but both it and Watling 17785 appeared to be normal, dried, epigeous boletes. The slender stipes were tall enough to raise the cap above the soil. The tubes were neither unusually long nor unduly contorted and were generally oriented in the dried specimens such that they

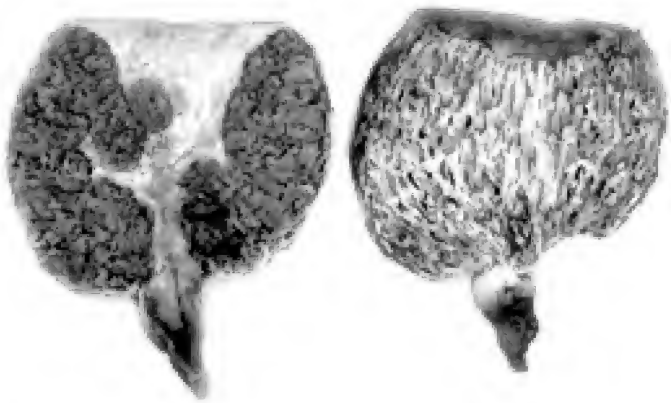
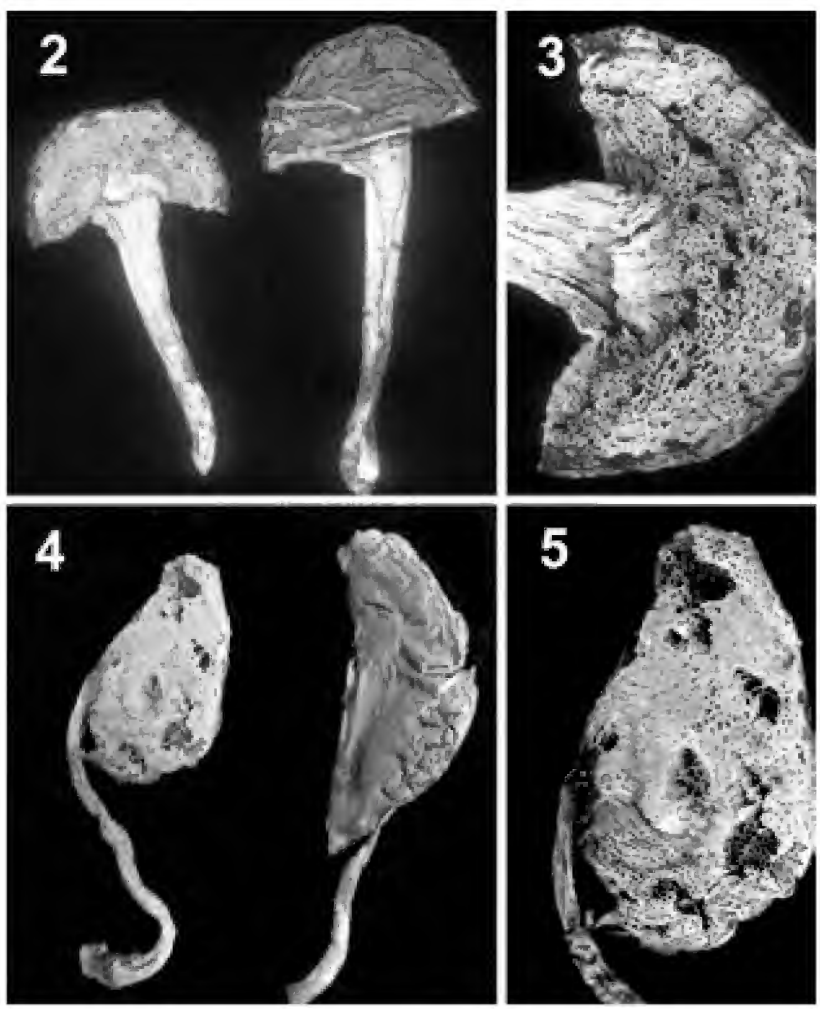


Figure 1. Fresh specimen of *Gastroboletus boletoides*; left, cross section; right, surface view, x 2.5.



Figures 2–5. Dried holotypes of *Gastrotylopilus brunneus* and *Fistulinella mollis*. **2.** *G. brunneus,* x 0.9. **3.** Tube layer of *G. brunneus,* x 2. **4.** *F. mollis,* x 0.9. **5.** Tube layer of *Fistulinella mollis,* x 1.5.

would have been vertical *in situ.* The surface of the tube layer had distinctive depressions of various sizes as noted by Watling and Li (1999). The spores were bilaterally asymmetric and thus most likely ballistospores. We accordingly judged *G. brunneus* to be a nonsequestrate, epigeous bolete.

We then used the key to Australian boletes by Watling and Li (1999) to see if *Gastrotylopilus brunneus* would equate to some other described species. By-passing the key's dichotomy choice that led to *G. brunneus,* 'Tubes contorted; pores irregular, easily collapsed or flattened, with larger or smaller depressions...' we continued through the subsequent choices, arriving finally at the determination of *Fistulinella mollis* Watling as described in Watling and Gregory (1989). Comparison of the original descriptions of both species revealed substantial similarity of macroscopic features, with differences well within the range of variation to be expected between different collections of a species; spore characters matched closely. Comparison of both descriptions with the expanded description and illustration of *F. mollis* by Bougher and Syme (1998) further evidenced conspecificity of the two taxa. Their illustration shows the depressions in the surface of the tube layer also evident on the type collection of *G. brunneus.*

To further test our hypothesis that *Gastrotylopilus brunneus* is a synonym of *Fistulinella mollis*, we examined the holotype of the latter (Watling 10404) from E. The holotype closely matches that of *G. brunneus* in all respects (Figs. 4, 5). The genus name *Gastrotylopilus,* therefore, is a later synonym of *Fistulinella* P. Henn. and thus not available for the sequestrate species related to *Tylopilus.* We shall propose a new generic name for those taxa when we monograph the sequestrate group.

The protologue of *Gastrotylopilus brunneus* contains a minor error that nonetheless is worth correcting to avoid wrong assumptions on habitat of *Fistulinella mollis.* Li and Watling (1999) cite the habitat as 'near the trunk or logs of *Eucalyptus obliqua.*' The holotype collection label in Watling's handwriting, however, reads "track to large *Eucalyptus obliqua."*

Acknowledgements

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Genetic comparison between Victorian and Tasmanian populations of *Prasophyllum correctum* D.L. Jones (Orchidaceae) suggests separate species

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Abstract

Genetic variation within and between Tasmanian and Victorian populations of the Gaping Leek Orchid *Prasophyllum correctum* (Orchidaceae) was investigated using the Random Amplified Polymorphic DNA (RAPD) method. The degree of fixed genetic differences between the two populations was substantial, suggesting that each population constitutes a different species. The Tasmanian population contained very little genetic variation, indicating that asexual reproduction or self-fertilisation may be the predominant reproductive mode, but this population does not appear to be clonal. Individuals from the Victorian population exhibited high levels of genetic variation relative to those from the Tasmanian population. These findings suggest that the Victorian and Tasmanian *P. correctum* populations ought to be managed separately, and cross-pollination or translocation should be avoided, because of the lack of genetic similarity between them.
Keywords: *Prasophyllum*, RAPD, genetic variability, conservation

Introduction

The Gaping Leek Orchid, *Prasophyllum correctum* D.L. Jones, is a small terrestrial orchid from southeastern Australia. *Prasophyllum correctum* (Jones 1994) was believed to be endemic to Victoria until plants collected in 1995 from the Campbell Town golf course in Tasmania were identified as *P. correctum* (Jones 1998). The species is believed to have formerly been widespread throughout lowland Gippsland, but it is currently restricted to two small populations located near Munro and Lindenow South in protected rail reserves (Hoey & Lunt 1995) in *Themeda triandra* Forssk. grasslands or *Eucalyptus tereticornis* Sm. ssp. *mediana* grassy woodlands, which are recognised as endangered ecosystems in Victoria (Coates *et al.* 1999). In Tasmania, the species was known only from the Campbell Town golf course (Hoey & Lunt 1995; Jones 1998) until a single plant was found 7–8 km away in 1999 (Fig. 1). The presence of this outlier is considered to be reasonable confirmation that the species was more widespread before the Tasmanian midlands were opened up for agriculture (Threatened Species Unit 2000).

In Victoria, *Prasophyllum correctum* is listed as threatened under the *Flora and Fauna Guarantee Act* 1988 and has ROTAP code 2E (Briggs & Leigh 1996) with as few as 127 plants estimated to remain (Coates *et al.* 1999). The demise of the species has been attributed to habitat degradation through grazing and clearing for agriculture (Backhouse & Jeanes 1995; Hoey & Lunt 1995). Current threats to the continuing existence of Victorian *P. correctum* include rail reserve maintenance (e.g. rotary hoeing or slashing), grazing and trampling by roaming stock and feral animals, weed invasions, competition from native plant species, and herbicide use (Backhouse & Jeanes 1995; Hoey & Lunt 1995; Coates *et al.* 1999). In contrast, the Tasmanian population is estimated to contain at least 1000 individuals (Coates *et al.* 1999), and appears to be relatively stable in its unusual habitat of the Campbell Town golf course rough. But this population is still sufficiently threatened such that the species is listed as endangered under the Tasmanian *Threatened Species Protection Act* 1995, and under the federal *Environment Protection and Biodiversity Conservation Act* 1999. Although the site is not formally reserved, it is

Figure 1. Known locations of *Prasophyllum correctum* and sites sampled for this study.

subject to a covenant agreement, which does provide some protection (Coates *et al.* 1999). Despite this, the population is still potentially threatened by activities that inadvertently contravene the covenant agreement such as use of fertilisers and recycled effluent (Coates *et al.* 1999).

The primary aim of the present study was to investigate the level of genetic diversity within and between populations of *P. correctum*. The *2000–2002 Recovery Plan* (Coates *et al.* 1999) proposed that such research should be undertaken to inform decisions pertaining to the collection of plant material for *ex situ* conservation programs. The *Recovery Plan* and the *Action Statement* for Victorian plants (Hoey & Lunt 1995) state that translocation of plants is an important management action. For this reason, gaining an understanding of the genetic variation present in both Victorian and Tasmanian populations would play a fundamental role in choosing plants from *ex situ* and *in situ* collections for translocation (Hoey & Lunt 1995; Coates *et al.* 1999). The *Recovery Plan* also noted that the Tasmanian *P. correctum* population contained more morphological variation than the smaller Victorian populations, and may therefore provide a useful reference point for gauging the level of diversity that may once have been present in Victoria.

In light of marked differences in the habitats presently occupied by Victorian and Tasmanian populations of *P. correctum*, and the large degree of geographic isolation between them, an additional aim of this study was to reassess their taxonomic relationship using a molecular approach. The Random Amplified Polymorphic DNA (RAPD) method has previously been used successfully to indicate the level of genetic diversity within populations, between populations and between taxa (e.g. Boehm *et al.* 1993; Sulaiman & Hasnain 1996; Qamaruz-Zaman *et al.* 1998; Tyson *et al.* 1998; Gillies *et al.* 1999; Coleman *et al.* 2000; van der Nest *et al.* 2000; Warburton *et al.* 2000). The RAPD method was chosen because it enables the rapid identification of polymorphic loci that are useful in population-genetic studies and for reflecting taxonomic distinctions, yet requires no prior information on the genetics of the organism. The latter attribute of RAPD is particularly advantageous for work on species for which few genetic data are available, including the present orchid species.

Materials and Methods

POPULATION SAMPLING

In Victoria, *P. correctum* plant material was collected from the largest known population, near Munro. Samples of leaf tissue were collected in August and October 2001 from 15 plants that are the subjects of a long-term monitoring program. Leaf tissue samples were collected from ten individuals at each of the three Campbell Town golf course subpopulations in September 2001. At all sites, leaf material was initially wrapped in moist paper towel and kept in sealed plastic bags on wet ice. Tissue samples were subsequently stored at –86°C until DNA was isolated.

DNA ISOLATION

A section of tissue 2–3 cm long, weighing between 50 and 180 mg was removed from each leaf sample for DNA extraction. All selected leaf sections appeared to be disease free, and were taken from the leaf tip to reduce the risk of sampling regions contaminated by pathogenic or mycorrhizal organisms. Tissue was ground to a fine powder in liquid nitrogen using a ceramic mortar and pestle. Ground material was collected in a pre-chilled 1.5 ml microcentrifuge tube. All samples were assigned a unique alpha-numeric label where the letter refers to the collection locality ('V': Victoria or 'T': Tasmania), and the number refers to an individual plant.

Genomic DNA was extracted using a QIAGEN DNeasy® Plant Mini Kit following the manufacturer's instructions, except 3 µl (rather than 4 µl) of RNase A stock solution was added to each tissue sample in step 2; and DNA was eluted in 75 µl (rather than 100 µl) of elution buffer in steps 12 and 13.

PCR AND ELECTROPHORESIS

DNA was amplified in 20 µl reactions containing 10 µl QIAGEN HotStarTaq™ Master Mix, 9 µl dH_2O, approximately 0.25 µM primer and 1 µl (5-20 ng) of template DNA. Polymerase Chain Reactions (PCR) were performed in an Eppendorf Mastercycler® gradient thermal cycler using the following profile: 95°C for 15 mins, 35°C for 2 mins; 72°C for 90 s (1 cycle), 94°C for 30 s, 38°C for 30 s, 72°C for 30 s (35 cycles) with a final extension step of 72°C for 4 mins 30 s (1 cycle).

A few *P. correctum* individuals were screened for anonymous polymorphic loci using 22 RAPD primers (Operon Technologies, OPA, OPB and OPF series PCR primers). Of the 16 RAPD primers that yielded amplification products, six were randomly selected for population-genetic assessment of *P. correctum*: OPA–03, OPA–04, OPA–13, OPF–04, OPF–09 and OPF–14. Samples of DNA from all individuals were amplified simultaneously for each primer, and a negative control was included in each PCR to facilitate identification of contamination. A small number of samples were rerun either to check that band patterns were reproducible, to clarify poorly amplifying samples, or to compare relative sizes of bands from different individuals. Amplification products were separated via electrophoresis on 1.5% agarose gels stained with ethidium bromide in 1x TBE running buffer at 80 V for 30–120 min (depending on the size of the gel).

SCORING BAND PATTERNS

Gels were viewed with ultraviolet light and photographed either with a Polaroid camera or a Kodak EDAS 120 digital camera. Kodak 1D Version 3.5 imaging software was used to estimate band sizes in digital images and all gels were also scored by eye. Bands were scored as absent (0) or present (1). Duplicate PCR runs were conducted for most sample/primer combinations, and any samples that produced faint or ambiguous bands were rerun. Very faint or ambiguous bands were omitted from the dataset.

ANALYSIS

The dataset was analysed using Genstat for Windows (Version 4.2). A pair-wise similarity matrix between individuals was constructed using Jaccard's coefficient in order to

analyse the relationship between the two populations. The matrix was then used as the basis for ordination by principal coordinate analysis (Gower 1966). The Shannon Diversity Index was calculated for each population and the species as a whole using POPGENE V# (Yeh *et al.* 1997). The index allows a comparison of the degree of variation within each population and is appropriate where Hardy-Weinberg equilibrium cannot be assumed. Partitioning of variation within and among populations was calculated from the index (King & Schaal 1989).

Results

In total, 72 bands were scored for the six primers. Primer OPA–04 produced 4 bands; all others produced between 11 and 15 bands ranging in size from approximately 300 to 2000 bp. Only six bands were common to both Victorian and Tasmanian populations, and five of those were from one primer, OPF–04. The sixth common band (OPF–14) was present in 11 Victorian individuals but only in one Tasmanian plant (T23) (Fig. 2 & 3).

Overall, the Victorian specimens showed a far higher degree of variation than specimens from Tasmania (Fig. 2 & 3). Within the Tasmanian population, 32 bands were present. Fourteen (44%) of those were polymorphic, but 11 of the polymorphic bands were present only in one or two individuals, and often in the same few individuals, leaving a large proportion of the population appearing to be genetically identical. Thirteen individuals showed identical band patterns for all six primers, and another four identical individuals all differed from those 13 by just one band. A further seven individuals were identical to each other and differed from the 13 by two bands and from

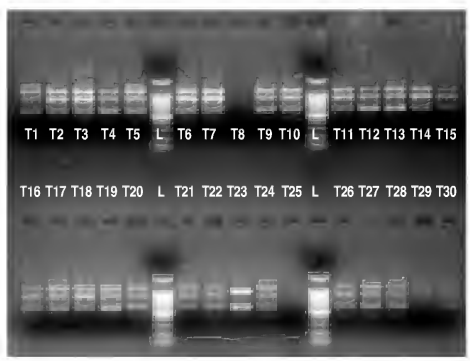

Figure 2. Band patterns of Tasmanian plants for OPF–14. Those that did not amplify well or at all were successfully amplified later. It can be seen from this that band patterns were very uniform, with only one or two polymorphisms (eg T23). The brightest band in the T23 pattern was the only band Tasmanian plants shared with Victorian plants, along with those produced by OPF–04 (not shown).

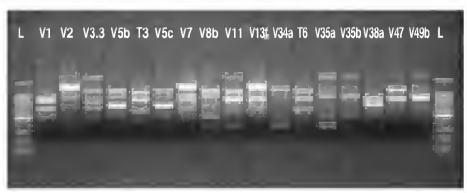

Figure 3. Victorian specimens amplified with OPF–14 and interspersed with Tasmanian specimens to compare band sizes. Note the amount of variation displayed by the Victorian plants compared with the Tasmanian plants in Figure 2.

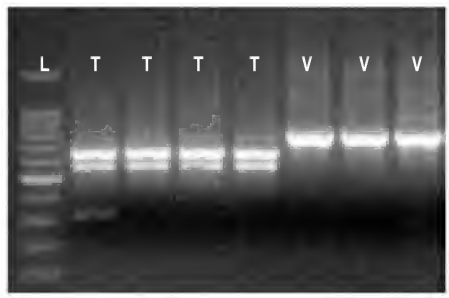

Figure 4. A subset of samples amplified with primer OPA–04. All Victorian samples produced the same pattern as those shown here; all Tasmanian samples showed the top two bright bands shown here, and about half also showed the lower faint band.

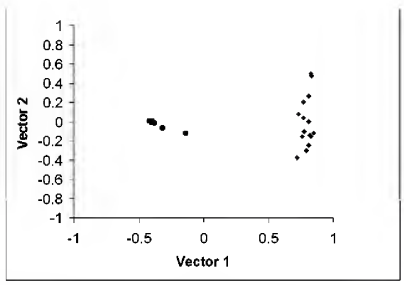

Figure 5. Ordination plot for *P. correctum* showing a strong separation of Tasmanian (●) and Victorian (◆) populations.

the four by one band. Of the remaining six plants, four only differed from the majority by one polymorphism. The other two individuals, T23 and T30, shared four polymorphic states, plus T23 showed six RAPD characters not shared by any other Tasmanian individuals. In other words, most of the variation present was represented by one individual, T23.

In contrast to this striking genetic uniformity among Tasmanian *P. correctum* individuals, the Victorian plants showed great diversity. Of the 46 bands present among Victorians, 38 (83%) were polymorphic. No two individuals showed the same band pattern. This was consistently the case for all primers but one. The exception, OPA–04, produced identical patterns for all Victorian plants (Fig. 4). The minimum difference between two individuals was one band, and the maximum difference was 21 bands.

The analysis of these data using ordination techniques (Fig. 5) showed a very distinct separation between Tasmanian and Victorian plants. Vector 1 contained 64% of the variation present. The Shannon Diversity Index for the Tasmanian population was H_O = 0.076 compared to H_O = 0.236 for the Victorian population indicating a much greater relative genetic diversity in the Victorian population. Partitioning of variation indicates that 65.3% of variation is attributable to among-population variation.

Discussion

GENETIC VARIATION AND TAXONOMY

The distinct genetic separation of the two populations illustrated in the ordination suggests that the Tasmanian and Victorian plants not only share very little genetic variation, but in fact belong to different taxa. The fact that the two populations shared so few RAPD bands suggests that if Tasmanian and Victorian plants share a common

ancestor, they have been genetically separated for a long time, and have had the opportunity to acquire population-wide mutations at many sites in the genome. Alternatively, their origins might involve a complex taxonomic history. The amount of time since the two species can be traced back to a common ancestor cannot be calculated from RAPD data. This may be an area of further study if the taxonomic status of the two populations remains in doubt (see Jones, this issue).

RAPD bands that did appear to be shared by both populations (Fig. 2 & 3) were similar in size but their homology cannot be ascertained absolutely without DNA hybridisation tests (see Rossetto *et al.* 1996). No Victorian plant has a band pattern identical to any of the Tasmanian plants. It seems tenuous at best to maintain that the two populations are one species given this paucity of evidence. Had our starting point been the assumption that the two populations were different species, we would not have considered the six shared bands to be evidence of shared taxonomic status.

In orchids, the RAPD technique consistently detects genetic variation at the specific and interspecific level even. A study of Italian taxa of the *Ophrys bertolonii* aggregrate, separated mainly on subtle differences in morphological characters, found high levels of genetic variability within populations (Gr nanger *et al.* 1998). Distinct species were successfully separated, at a level of 80% similarity using Jaccard's coefficient, but only one of the aggregate species could be distinguished prompting the authors to recognise only a single taxon from the remaining taxa in the aggregrate. In contrast, the two populations of *P. correctum* showed a similarity of 40% based on the simple matching coefficient. The evolution of *Spiranthes hongkongensis* by natural hybridisation and polyploidisation of *S. sinensis* and another species was also supported by RAPD analysis (Chan & Sun 1997).

The low amount of genetic variation present in the Tasmanian population begs discussion. While the population does not appear to be strictly clonal, it seems that either asexual reproduction or inbreeding is common, the population started its existence with only a small amount of genetic variation, or the species has existed in a very stable habitat over a long period of time (James 2000). It has been noted by Bates (1994) that at least one *Prasophyllum* species, *P. goldsackii*, has been observed to produce seed without fertilisation (apomixis) although this has not been verified. Clements (1995) showed that apomixis occurred in the Australian terrestrial orchid *Corunastylis apostasiodes*, a related genus in the subtribe Prasophyllinae. Dixon (1991) observed asexual reproduction by distal daughter tuber formation for some *Prasophyllum* species. It is also possible that the Tasmanian plants are part of a founder colony, begun by very few plants with a reduction in genetic diversity resulting from inbreeding and the accumulation of few mutations since foundation. It is theoretically possible that the population was founded many years ago by a single Victorian plant of *P. correctum*. The Victorian population is variable enough to allow for the possibility that the standard Tasmanian genetic pattern began as a single Victorian plant that has since diverged. However, evidence against this includes the highly conserved regions amplified by OPA–04 that differ strongly between the populations.

Primer OPA–04 (Fig. 4) provides some of the strongest evidence for separating the two populations taxonomically. It showed the least amount of within-population variation, with no differences observed between Victorian individuals. Tasmanian plants showed variation at one band, making this the only primer to show more variation among Tasmanian plants than among Victorian plants. The low amount of within-population variation suggests that sites in the genome that were amplified using this primer were more highly conserved than many of the sites amplified using other primers. If highly conserved sites are not shared, then the two populations are more likely to have evolved as separate species in the distant past, rather than to have diverged more recently.

The high degree of genetic variation present in the Victorian population is clearly the opposite of the expectation expressed in the *Recovery Plan* (Coates *et al.* 1999). Loss of

genetic variation may not be critical yet for the Victorian plants, despite the small size of the populations. Typically, *P. correctum* plants are long-lived, with their tubers being replaced after flowering each year and they can also lie dormant for up to 5 years (Coates *et al.* 1999). There may be more variation present than the genetic analysis revealed because a different combination of plants emerges each year. The genetic diversity revealed in this study may be a reflection of the diversity present when the species was more abundant. But, any detrimental effects of inbreeding may not yet be readily identified.

IMPLICATIONS FOR CONSERVATION MANAGEMENT OF THE SPECIES

The Victorian and Tasmanian populations of *Prasophyllum correctum* must certainly be managed separately and should not be cross-pollinated in *ex situ* collections or translocated. Even if they are not confirmed as different species, they should be considered to be evolutionarily significant units, worthy of separate conservation measures, because of the paucity of overlap in genetic variation.

Jones (1991) noted that *Prasophyllum* species in general routinely present a multitude of taxonomic problems including species complexes. The present-day disjunct distribution of populations that are currently recognised as *P. correctum* may be the result of a vicariance event such as the disappearance of the mainland-Tasmanian land bridge that crossed Bass Straight 13,000 years ago (White 1994) or establishment of populations by for example, long-range seed dispersal. Alternatively, it is possible that although individuals from Victorian and Tasmanian populations are presently morphologically very similar, they actually originated from different evolutionary lineages with their similarity resulting from convergent evolution.

There are risks associated with crossing individuals that are genetically too dissimilar. Long isolated populations may have developed adaptations, specific to particular regions or habitats, that break down by reassortment and recombination if populations hybridise. This can result in outbreeding depression whereby offspring have reduced fitness for example, low vigour and reduced fertility (Barrett & Kohn 1991). Consequently, conservation strategies that involve transplanting individuals from one population to another, encourage gene flow between geographically isolated populations, or store *ex situ* propagules from different geographic locations together, should be approached with caution. Such habitat-specific adaptations have been documented in *Prasophyllum* species (see Jones 1991). In addition, species integrity may be lost by hybrid swamping if different species inadvertently interbreed with closely related taxa (Riesberg 1991). This is particularly pertinent for orchids, which usually evolve ecological or mechanical barriers to hybridisation rather than physiological barriers, making them amenable to interspecific and even intergeneric hybridisation (IUCN 1996). For *Prasophyllum*, however, there is little evidence for species-specific pollen vectors and *P. correctum* has easily accessible pollen with removal possible by a number of vectors (Rouse, pers. comm. 2003). Although ecological barriers to hybridisation such as species-specific pollinator-plant relationships may be highly effective under natural conditions, it is possible that pre-zygotic mechanisms would break down under altered environmental conditions. One such situation might include human-mediated plant introductions for example, translocation of plants between geographically isolated areas such as Victoria and Tasmania. The generally degraded state of the Victorian *P. correctum* habitat could also contribute to reduced integrity of traditional pollination systems.

The Victorian population at Munro appears to have retained a significant amount of the genetic variation presumed to have been present when the species' population sizes were larger. However, the small number of individuals makes the Victorian population likely to be more at risk from habitat destruction and stochastic environmental events than from inbreeding depression or loss of adaptive ability (c.f. the Tasmanian population). It is therefore a priority to establish *ex situ* collections of the species and a translocation

and/or reinforcement program to increase the number of plants growing in Victoria. Coates (2001) has proposed measures to maximise the survival and fecundity of *in situ* plants, such as imposing a regular fire cycle on the sites based on data showing that fire intervals of 2–3 years are beneficial. These measures should be both heeded and implemented, in order to retain as much variation as possible in the wild.

Despite evidence for other species whose limited variation does not appear to be detrimental (James 2000), the minimal genetic variation in the Tasmanian population is cause for concern. Low variation is likely to reduce the ability of the population to respond to changing environmental conditions and selective pressures, thus increasing its risk of extinction. The *Recovery Plan* noted that the Tasmanian plants showed considerable floral variation not evident in Victoria (Coates *et al.* 1999), but this was not reflected in the present genetic data. Further genetic surveys of the Tasmanian plants, combined with tagging and monitoring of individuals, would be useful for maximising the diversity within material collected for *ex situ* propagation. In addition, elucidation of the breeding system for the Tasmanian plants and data on seed production will assist in the development of guidelines for collection of propagules.

In summary, the evidence is strong that the Tasmanian and Victorian *P. correctum* plants should be classified as separate species. Further morphological study (Jones, this issue) has confirmed that reclassification is appropriate. Sampling for *ex situ* material can be based on the population genetic structure found in this study. Information on the reproductive biology of both Victorian and Tasmanian plants can be used to minimise the risk of losing genetic diversity in any seedlings produced from a breeding program based on *ex situ* plants.

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Genetic comparison of populations of the rare Gorae Leek Orchid, *Prasophyllum diversiflorum* Nicholls (Orchidaceae)

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Abstract

The rare Gorae Leek Orchid, *Prasophyllum diversiflorum*, is listed as a threatened species under Victorian legislation. Information on its patterns of genetic variation is urgently required to develop effective conservation strategies for the species. The two remaining populations of *P. diversiflorum* were compared using the Random Amplified Polymorphic DNA (RAPD) method. Despite small population sizes, the level of genetic diversity detected was encouraging for the long-term survival of the species. Both populations showed similar levels of genetic variation. There was little population differentiation with 88% of genetic variation residing within populations.

Key words: conservation; population genetics; *Prasophyllum diversiflorum*; RAPD.

Introduction

The Gorae Leek Orchid, *Prasophyllum diversiflorum* Nicholls, a terrestrial orchid endemic to south-west Victoria, is currently listed as threatened under the Victorian *Flora and Fauna Guarantee Act* 1988 and "endangered 2E" nationally (Briggs & Leigh 1996). The species exhibits considerable variation in floral morphology (Nicholls 1942), and grows along natural watercourses, around swamp margins and on seasonally inundated alluvial flats in open forest or grassy woodland vegetation (Backhouse & Jeanes 1995, Bishop 1996, Rouse 2002). Colonies were once known to have flourished over an area of several hundred acres at Gorae West (Nicholls 1942). But just seven years after the initial discovery of *P. diversiflorum* in 1941, the species was presumed to have become extinct owing to the conversion of its natural habitat to agricultural land. The species was rediscovered 35 years later in the Condah-Hotspur district, 40 km from the type locality (Backhouse & Jeanes 1995, Bishop 1996). Today, *P. diversiflorum* is known from only two small populations, near Dunkeld and Hotspur, which consist of approximately 800 plants and 250 plants, respectively (Rouse 2002).

The taxonomy of the group to which *P. diversiflorum* belongs is complex and there have been differences of opinion as to which populations constitute the species. For example, the *2000-2004 Recovery Plan* (Ingeme & Govanstone 1999) lists six populations, but at this stage, only two remaining *P. diversiflorum* populations can be confirmed. Neither is on reserved land (Rouse 2002). Current threats to the species' survival include grazing by livestock, competitive exclusion by exotic plants, herbicide spraying, and road construction works (Backhouse & Jeanes 1995).

Genetic data is now widely used to guide conservation and recovery programs of endangered plant species (Godt & Hamrick 1999). Yet despite the high percentage of orchid species that are endangered, genetic diversity of orchid taxa has received scant attention (Wong & Sun 1999). Information about a species' genetic diversity can provide useful baseline data for conservation (Geburek 1997) and is often a valuable component of comprehensive conservation management strategies (Hamrick 1983, Falk & Holsinger 1991). Certain aspects of conservation biology, including the loss of genetic diversity in

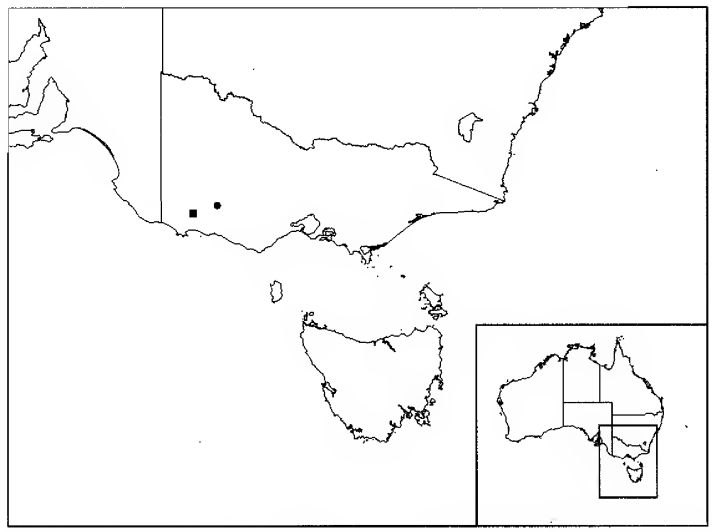

Figure 1. Map of *Prasophyllum diversiflorum* collection localities used in this study (● Dunkeld, ■ Condah-Hotspur).

conservation programs and the restoration of threatened populations, necessarily require detailed population-genetic studies (Hamrick & Godt 1995).

There is virtually no information on the biology and ecology of the Gorae Leek orchid (Ingeme & Govanstone 1999). An objective of the *2000-2004 Draft Recovery Plan* was to investigate factors affecting plant recruitment and population viability including the genetic health and viability of the remnant populations. Information regarding the amount and the partitioning of genetic variation within and between *P. diversiflorum* populations may yield insights into the degree of genetic similarity between the two populations and provide guidance for the species' conservation management.

The apparent ecological specialisation of *P. diversiflorum*, combined with past and present anthropogenic threats, and very small population sizes, make the survival of this species of immediate concern. It is a prime candidate for *ex situ* conservation measures (Backhouse & Jeanes 1995), but prior to the present research, no information has been available to assist decision-making about whether for example, plants from different populations can be safely cross-fertilised with one another.

The aim of this study was to investigate the level of genetic diversity present within and between the two known populations of *P. diversiflorum* and to compare their genetic similarity. The Random Amplified Polymorphic DNA (RAPD) method (Williams *et al.* 1990) was used as it has been successfully employed in the past to indicate levels of genetic diversity within populations, between populations and between taxa in both orchids and other plant species (e.g. Boehm *et al.* 1993, Sulaiman & Hasnain 1996, Qamaruz-Zaman *et al.* 1998, Wong & Sun 1999). The information will assist decision-making regarding the source of germplasm used to establish an *ex situ* collection and breeding program, as well as manipulation of reproduction at field sites.

Materials and Methods

COLLECTION OF PLANT TISSUE

In January 2002, three flowers were collected from inflorescences of 15 individuals at each of the two *P. diversiflorum* populations located near Dunkeld and along the Condah-Hotspur Road, Hotspur, in south-western Victoria (Fig. 1). Each sample was assigned a

unique alpha-numeric label where the letter refers to the collection locality (D: Dunkeld, H: Condah-Hotspur), and the number refers to a particular plant.

ISOLATION AND AMPLIFICATION OF GENOMIC DNA

Plant material was ground in liquid nitrogen using a mortar and pestle. Two flowers per individual were ground up together in order to increase DNA yield following inspection of ovaries to ensure samples had not been fertilised. Genomic DNA was isolated using a QIAGEN DNeasy® Plant Mini Kit following the protocol recommended by the manufacturer with two modifications: 3 µl (not 4 µl) of RNase stock solution was added to each tissue sample (step 2), and 75µl (not 100 µl) of Buffer AE was used to elute DNA (step12 and step 13). Amplification of DNA via Polymerase Chain Reaction (PCR) was performed in 20 µl reactions containing 10 µl Qiagen HotStar Taq® Master Mix, 8.2 µl Millipore dH_2O, 0.8 µl primer and 1 µl template DNA (10-30 ng). All amplifications were performed in an Eppendorf Mastercycle® gradient cycler with the following profile: 15 min at 95°C, 2 min at 35 °C, 90 s at 72 °C (1 cycle), 30 s at 94 °C, 30 s at 38°C, 30 s at 72 °C (35 cycles) with a final extension step of 4 min 30 s at 72 °C (1 cycle). A negative control was included in each PCR run to facilitate identification of contamination.

RAPD ANALYSES

Thirty-eight RAPD primers (Operon Technologies) were assessed. Five primers (OPA-02, OPF-03, OPF-05, OPF-09, OPF-13) were selected from 16 primers that consistently yielded well-resolved polymorphic PCR amplification products. PCR products were separated via electrophoresis on 1.5% agarose gels using a 1µ TBE electrode buffer (90 mM Tris-borate, 2 mM EDTA). Gels were run at 80 V for 30-120 min depending on gel size. PCR products were stained with ethidium bromide then visualised under ultraviolet light at 302 nm. Duplicate PCR runs were conducted to confirm reproducibility of RAPD bands. Matching samples from two independent PCR runs were run together side by side, for all individuals. Gels were photographed with a Polaroid GelCam or Kodak digital camera and DNA fragment sizes were estimated using Kodak Digital Analysis System 120 gel analysis software. All fragment size estimates and RAPD profiles were checked by eye, and any poorly resolved or consistently ambiguous bands were omitted from the data set.

STATISTICAL ANALYSES

The presence or absence of DNA fragments was recorded for each sample in a binary matrix. A similarity matrix using the Simple Matching coefficient was calculated and used as the basis for ordination by principal co-ordinates (Gower 1966) using the program Genstat 5 for Windows (Lawes Agricultural Trust Rothamstead). Shannon's Diversity Index was calculated for each population and for the species using POPGENE software (Yeh & Boyle 1997) and the amount of variation partitioned within and between the populations was derived from them (King & Schaal 1989).

Results

Forty-nine bands were scored for the five RAPD primers used in this study. The number of bands per primer varied from five to 15 and ranged in size from approximately 290 to 2000 base pairs. All but two bands were common to both *P. diversiflorum* populations, so the vast majority of differences between populations resided in the frequency of RAPD fragments rather than the presence of population-specific fragments.

Overall, there was a high degree of genetic variation within the Dunkeld and Condah-Hotspur populations. RAPD profiles from OPF-13 for all individuals are shown in Figure 2. While there is variation within both *P. diversiflorum* populations, it is clear that bands are also shared between populations. The reduced similarity matrix (Table 1) shows the

Table 1. Reduced similarity matrix (%) based on RAPD fragments for the two populations of *P. diversiflorum.*

Population	Dunkeld	Condah-Hotspur
Dunkeld	74.6	
Condah-Hotspur	70.4	74.7

Table 2. Comparison of shared bands (above diagonal) and % similarity (below diagonal) based on RAPD fragments for primer OPF-09 for the two populations of *P. diversiflorum* and the Victorian population of *P. correctum* at Munro.

Population	Dunkeld	Condah-Hotspur	*P. correctum*
Dunkeld	-	8	5
Condah-Hotspur	71.6	-	5
P. correctum	49.9	55.5	-

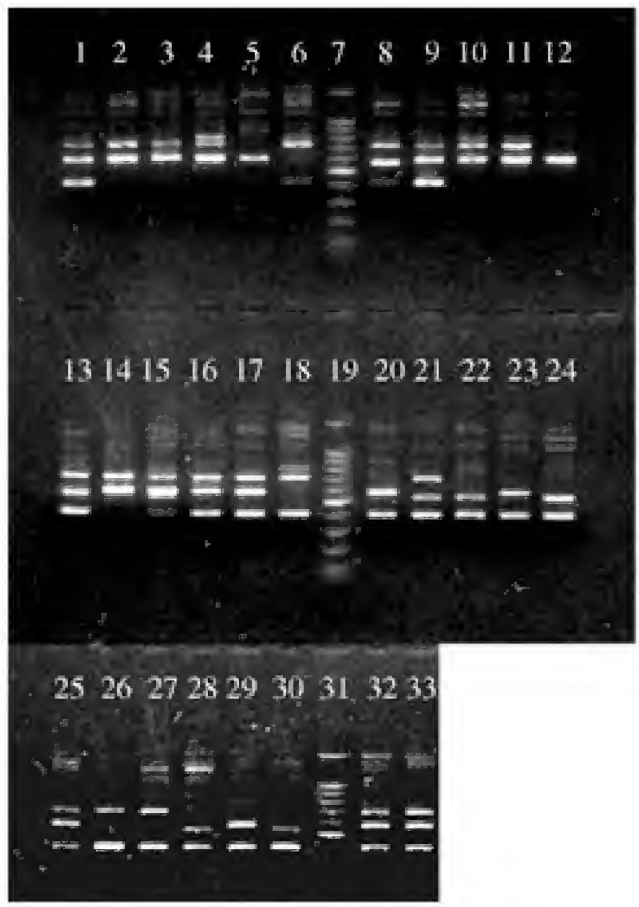

Figure 2. All samples amplified with primer OPF13. Dunkeld: lanes 1-6, 8-16. Condah-Hotspur: 17-18, 20-30, 32-33. Promega 100bp ladder: 7, 19, 31.

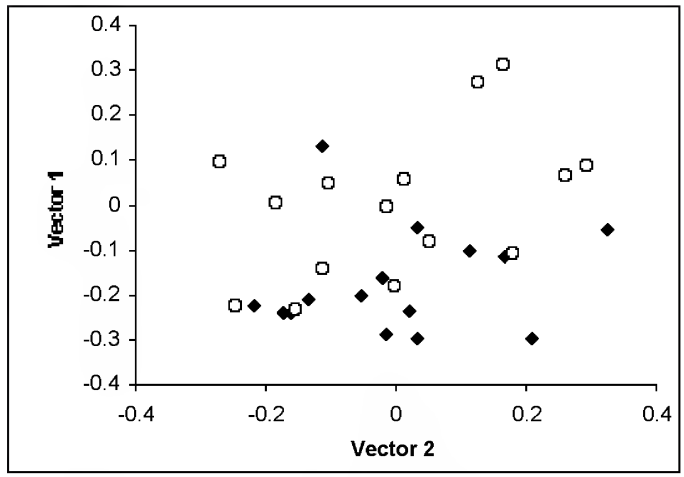

Figure 3. Ordination of RAPD data for *Prasophyllum diversiflorum*. (◆ Dunkeld, O Condah-Hotspur)

age similarity drops only marginally from 75% to 70% when comparing individuals from different populations. In contrast, when RAPD data for OPF-09 are compared, similarity between *P. diversiflorum* and the Victorian population of *P. correctum* drops from 71.6% between the two *P. diversiflorum* populations to 55.2% and 49.9% for Dunkeld and Condah-Hotspur, respectively, (Table 2).

The Shannon Diversity Index, H_O, was very similar for both Dunkeld and Condah-Hotspur populations, 0.357 and 0.364, respectively. Nei's genetic identity was 0.9267 with 88.0% of the total variation attributable to within-populations variation. The gene flow estimate was $N_m = 4.2$.

Ordination of these data shows that while there is some grouping of plants by population, the *P. diversiflorum* individuals form a single group that did not separate out in the third axis. Vectors 1, 2 and 3 combined contained only 34.3% of the variation indicating that there is little differentiation of the populations (Fig. 3).

Discussion

GENETIC VARIATION

The two populations of *P. diversiflorum* share a high degree of genetic variation and the differentiation of populations, shown in the principal coordinate analysis is minor. There is a tendency for plants from the same population to group together, although the differentiation is based largely on the frequency rather than the uniqueness of RAPD fragments occurring in them and only 34.3% of the variation is accounted for by the first three vectors of the principal coordinate analysis. This suggests that the populations share the same origin. The gene flow estimate of $N_m = 4.2$ indicates that there is, or has been recently, significant gene flow between the populations (Brzosko *et al.* 2002). This is further supported from the partitioning of variation where 88.0% is found within populations. This value is indicative of a species that is highly outcrossing (Hamrick & Godt 1995) and provides another parameter for considering the two populations as two components of a single conservation management unit.

A note of caution must be introduced with regard to inferences based on estimates of gene flow between the Dunkeld and Condah-Hotspur *P. diversiflorum* populations. Although it is possible that gene flow is on-going, mediated by long-distance seed dispersal as reported for other orchid species (e.g. Peakall & Beattie 1991), N_m is a measure

of historical gene flow and does not necessarily represent present-day levels (Brzosko *et al.* 2002). The effects on genetic diversity of rapid and relatively recent fragmentation including extinction of the formerly large population at Gorae West may remain undetected for some time. Hence, the longevity of genets (genetic individuals) is an important consideration.

Morphological characters show extensive variability in *P. diversiflorum*. Nicholls (1942, p. 9) noted: "The (new) species is probably one of the most variable, in regard to floral characters, on record". Rouse (2002) commented that Dunkeld plants showed more variation in floral morphology compared to those at Condah-Hotspur but no published data is available. The genetic data provided by the present study agree with the generally recognised diversity of *P. diversiflorum*.

Terrestrial orchids exist as inconspicuous underground tubers for at least some part of their life cycle (Sydes 1994a, Wong & Sun 1999) and the number of plants emerging can fluctuate widely from year to year. In *P. diversiflorum* populations, plant numbers have been observed to fluctuate remarkably depending on climatic conditions with few plants emerging in particularly dry seasons (Ingeme & Govanstone 1999). The genetic variation detected in Gorae Leek Orchid specimens collected during the 2001/2002 flowering season may represent only a small amount of that present. Many members of the population did not emerge (or produce flowers) owing to the unusually dry conditions, and only 15 plants were sampled per population. Sydes (1994b) reported that in years of drought, abortion of flowers because of water stress occurred in *Thelymitra circumsepta* – an orchid that, similar to *P. diversiflorum*, inhabits swampy areas and flowers during summer. Hence, the proportion of the total genetic variation that is represented by reproductive individuals might change considerably from year to year, depending on how many (and which) plants emerge. Temporal fluctuation in the number of emerging individuals has also been observed in populations of the orchid *Cypripedium calceolus* (Brzosko *et al.* 2002). Temporal variation in emergence may have concealed differences in levels of genetic diversity especially if the dry conditions in 2001 promoted the flowering of certain genotypes and suppressed others. The effect of possible temporal variation and sample size limitations must be taken into account when interpreting the present genetic data. Only long-term monitoring of the populations will resolve this issue.

Numerous authors have reported a significant positive correlation between population size and level of genetic diversity in a variety of plant taxa (e.g. McClenaghan & Beauchamp 1986, Peters *et al.* 1990, Billington 1991, van Treuren *et al.* 1991, Raijmann *et al.* 1994, Godt *et al.* 1996, Sun 1996). Given that the Dunkeld *P. diversiflorum* population is larger in size and contains plants that are more variable in floral morphology than the Condah-Hotspur population (Rouse 2002), it might be expected that the former site also harbours the majority of the species' genetic diversity. Yet our results suggest that the level of genetic diversity is very similar across the two populations (Table 1.). Similarly, Wong and Sun (1999) noted that no such correlation was evident in RAPD data obtained from the endangered terrestrial orchid *Goodyera procera*. However, as was highlighted earlier, it may be too soon to recognize the genetic signature of a recent population bottleneck in *P. diversiflorum*.

MANAGING THE SPECIES FOR CONSERVATION

A primary goal of conservation biology is to ensure the maintenance of biodiversity (Stiling 1999), of which genetic diversity is a fundamental component (Moritz & Faith 1998), and effective conservation programs depend on the identification of unambiguous management units (Avise 1994). Based on findings of the present study, the two extant populations of *P. diversiflorum* can be managed as a single unit, yet site-specific management actions may be required. Fluctuating numbers of emergent plants from year to year do not seem to have had a severe effect on the species' genetic diversity at this stage. This may be due to individuals remaining dormant for several seasons if conditions are not suitable, but subsequently re-emerging and contributing reproductively. Similarly,

Wallace (2002) hypothesized that plant dormancy patterns and chaotic fluctuations in population size from year to year may buffer against the stochastic loss of genetic variation, especially in small populations. Therefore, each year some proportion of the total diversity is cryptic in that it cannot be measured, but generally is not lost. Effective survey is a priority for the management and recovery of rare plants (Hogbin & Peakall 2000). Periodic re-sampling of the two populations for genetic assessment would provide more reliable estimates of the diversity present, would gauge more accurately the extent of population differentiation and importantly, the way in which patterns of genetic diversity can vary from year to year.

The development of propagation techniques should be a high priority of the recovery effort (Rubluo *et al.* 1993) so that an *ex situ* collection can be assembled as a safeguard against the loss of diversity (or of an entire population) at one or both of the sites. This would also allow study of the species' breeding system, and facilitate the production of seed for both supplying *ex situ* collections with seedlings and augmenting seed production *in situ* each season. It would also allow the introduction of *P. diversiflorum* to new sites if considered appropriate, without compromising the continued persistence of the two extant populations. Based on data presented here, the crossing of plants from Dunkeld and Condah-Hotspur populations during the recovery process should be considered as a recovery action given that the long-term risk following the historical loss of populations is that the species has lost some adaptive capacity. In particular, seed produced from within- and between-population crosses will be important for ensuring a broad genetic base for *ex situ* material.

Much of the effort involved in the recovery of *P. diversiflorum* will rely on an understanding of its habitat requirements, population dynamics, breeding system, and life history (Hamrick *et al.*1979, 1991, Ackerman 1998, Hogbin & Peakall 2000). Historically, habitat characteristics for *P. diversiflorum* may be broader than those found at the current sites. If reintroduction and augmentation of populations is undertaken as part of the recovery process, a broad base of genetic variation should promote the establishment of populations with an increased ability to thrive under variable conditions. It is probable that there has been some reduction in the genetic diversity within the species as populations have been lost. However, from this study, the amount of genetic diversity revealed is promising for the long-term survival of the species from an evolutionary perspective. At this stage, both populations must be conserved because despite their similarities they do differ and their small sizes and locations make both vulnerable to loss. Increasing plant numbers by conserving both populations and building an *ex situ* collection can contribute to countering the negative effects of genetic processes associated with small population size (reviewed in Burgman & Lindenmayer 1998).

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A revisionary treatment of four species of *Prasophyllum* R.Br. (Orchidaceae) loosely related to *P. correctum* D.L.Jones

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Abstract

The taxonomy of a small group of species loosely related to *Prasophyllum correctum* (Orchidaceae) is resolved. Four species are recognised in the group - *P. correctum* D.L.Jones, *P. bagoensis* D.L.Jones and two species newly described here (*P. crebriflorum* D.L.Jones and *P. incorrectum* D.L.Jones). The taxonomic significance of the latter new species was resolved by an independent group of researchers (Orthia *et al.*) using a molecular technique, the results of this study being reported in an accompanying paper in this issue. Subsequent re-examination of morphological characters supports these findings.

Keywords: *Prasophyllum*, new species, *P. correctum*, *P. bagoensis*, *P. crebriflorum*, *P. incorrectum*, Victoria, Tasmania, Australia.

Introduction

Prasophyllum R.Br. is a complex genus of Australian and New Zealand Orchidaceae which presents difficulties in identification for taxonomists, ecologists and orchid enthusiasts, mainly because of general similarity between many taxa and the difficulty of defining unique characters which can be used as a ready means of identification.

Prasophyllum is the subject of continuing studies which have resulted in the description of new species (Bates 1989b, 1990; Jones 1991, 1994a, 1994b, 1996b, 1997), a review of Tasmanian species (Jones 1998) and the resolution of various complexes (Bates 1989a; Jones 1996a; Jones & Clements 1996). In this paper the taxonomy of four species loosely related to *P. correctum* D.L.Jones is clarified. Two of these taxa are described as new.

Characteristics of the taxa

All four taxa in this group share the following morphological characters: widely gaping flowers with the lateral sepals and petals being well separated from the dorsal sepal; widely spreading, upswept, narrow petals; an erect labellum which is often strongly recurved in the distal half; and, a column, which is fully exposed, or nearly so, when the flower is viewed from the side. *Prasophyllum retroflexum* D.L.Jones and *P. morganii* also share some of these characters and could be considered as part of this group but can be readily distinguished by their densely crowded flowers and retroflexed petals; additionally *P. morganii* has papillae on the labellum and lateral sepals.

Taxonomic history

A species of *Prasophyllum*, originally described as *P. chasmogamum* R.Bates & D.L.Jones (Jones 1991), was later redescribed as *P. correctum* D.L.Jones (Jones 1994a), when it was realised that the wrong specimen had been inadvertently used as the type. In the following year specimens collected from the Campbell Town Golf Course in Tasmania by Hans and Annie Wapstra were identified by the author as *P. correctum* and later included as this species in a review of the genus for Tasmania (Jones 1998). A second species, *P. bagoensis*,

with apparent affinities to *P. correctum*, was described from material collected in the Bago State Forest in south-eastern New South Wales (Jones 2000). In December 1999 a single specimen, provisionally identified by me as having similarities with *P. correctum*, was collected by Mark Wapstra and Brooke Craven from montane tussock grassland in northern Tasmania and further collections were secured by Hans and Annie Wapstra in the following year. Most recently the taxon from Campbell Town identified by me as *P. correctum*, has been shown by a molecular study (Orthia *et al.* 2003) to be distinct at the molecular level; subsequent re-examination of morphological characters have confirmed these distinctions, and the taxon requires formal description.

Key to Species of the *Prasophyllum correctum* Group

1 Dorsal sepal retroflexed; distal petal margins crenulate; labellum straight or in a shallow sigmoid curve (montane areas, south-eastern NSW).....***P. bagoensis***

1. Dorsal sepal deflexed or recurved, not retroflexed; petal margins entire; labellum recurved near the middle **2**

2 Flowers mainly yellowish green; stigmatic plate much longer than the anther and column wings (lowland areas, eastern Vic.) .....***P. correctum***

2. Flowers mainly reddish brown; stigmatic plate of similar length to the anther and column wings.....**3**

3 Flowers densely crowded; apical tail about one-third of labellum length; callus smooth at the base, smooth or rugose distally (montane areas, Tasmania)..... .....***P. crebriflorum***

3. Flowers not crowded; apical tail about one-half of labellum length; callus rugose throughout (lowland areas, Tasmania).....***P. incorrectum***

Taxonomy

1. *Prasophyllum bagoensis* D.L.Jones, *Orchadian* 13(4): 150-151 (2000). Type: New South Wales. Bago State Forest, 6 Jan. 2000, *P. Branwhite 129* (holo CANB!, iso NSW!).

Illustrations: Top plates, page 148, *Orchadian* 13(4) (2000); plate 69, Rouse (2001).

Distribution and ecology: Currently known only from the Bago State Forest in south-eastern New South Wales where it grows in grassy forest in shallow clay loam and in herbfild in peaty soil. Altitude: c. 1000 m.

Phenology: This species flowers in December and January.

Recognition: Within the *P. correctum* group, *P. bagoensis* is recognised by its tawny green flowers which have a strongly retroflexed dorsal sepal; linear-oblong petals with the distal margins crenulate; and, a relatively short, obliquely erect labellum which bends in a shallow sigmoid curve and with a short, thick, smooth callus. In the column the stigmatic plate is of similar length to the column wings and anther. Fig. 1.

Notes: *Prasophyllum bagoensis* is similar to *P. correctum* but that species can be distinguished by its mainly yellowish-green flowers which have a deflexed, not retroflexed, dorsal sepal; linear petals with entire margins; a much larger, obliquely erect labellum which is recurved near the middle; labellum lamina with a long caudate apex which is about half of the total labellum length; the callus being rugose distally; and, the stigmatic plate on the column being much longer than either the anther or the column wings.

Conservation status: Apparently of restricted distribution but poorly known and not conserved. Jones (2000) suggested a conservation status category of 2KV according to the criteria of Briggs and Leigh (1996).

Other specimen examined: **NEW SOUTH WALES.** S end of Bago State Forest, 13 Dec. 2001, *D. Rouse 116* (CANB).

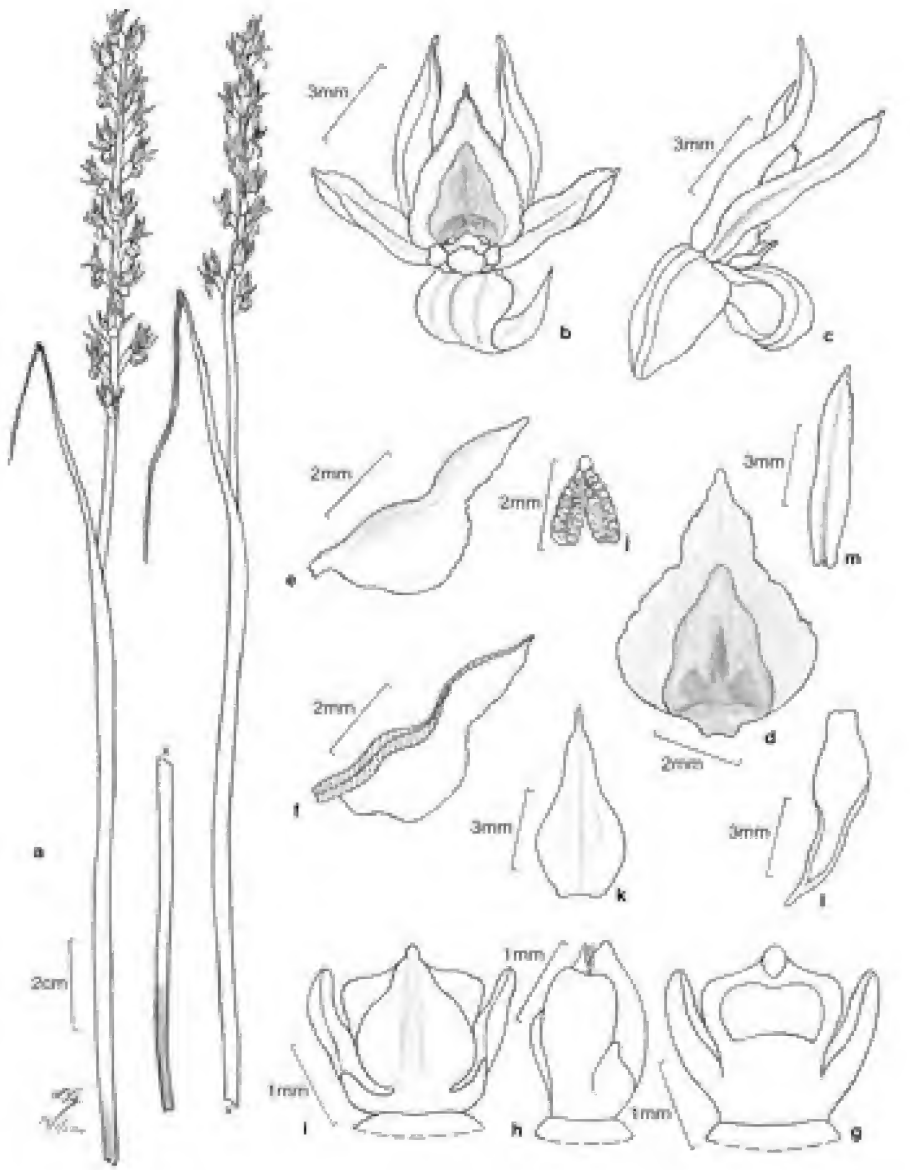

Figure 1. *Prasophyllum bagoensis*, Bago State Forest, NSW, *P. Branwhite 129* (drawn from the type collection): a. flowering plants; b. flower from front; c. flower from side; d. labellum from above, flattened out; e. labellum from side; f. longitudinal section of labellum; g. column from front; h. column from side; i. column from rear; j. pollinarium; k. dorsal sepal; l. lateral sepal; m. petal.

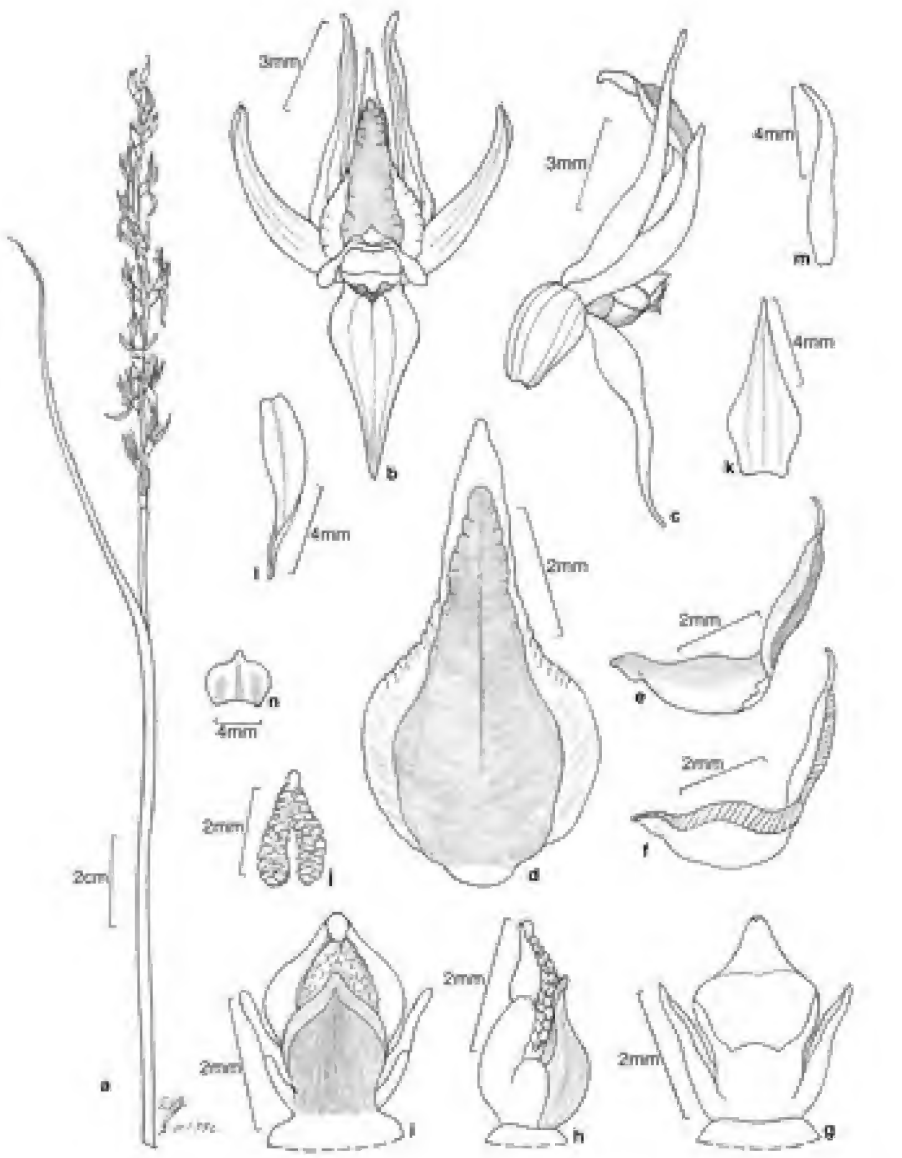

Figure 2. *Prasophyllum correctum*, near Munro, Vic., *D.L.Jones 10689*: a. flowering plant; b. flower from front; c. flower from side; d. labellum from above, flattened out; e. labellum from side; f. longitudinal section of labellum; g. column from front; h. column from side; i. column from rear; j. pollinarium; k. dorsal sepal; l. lateral sepal; m. petal; n. fertile bract.

2. *Prasophyllum correctum* D.L.Jones, *Novon* 4: 106-108 (1994). Type: Victoria, near Munro, 5 Nov. 1992, *J. Jeanes (Jones 10689)* (holo MEL!; iso CANB!).

Illustrations: Page 235, Backhouse and Jeanes (1995); plate 114, Bishop (1996); plate 68, Rouse (2001).

Distribution and ecology: Eastern Victoria, near Munro and Lindenow South. Grows in grassland dominated by *Themeda triandra* and in grassy woodland with *Eucalyptus tereticornis* Sm. as the dominant tree (Coates *et al.* 1999). The soil is a brown clay loam. Altitude: 20-50 m.

Phenology: The species flowers in October and November.

Notes: Within the *P. correctum* group this species can be recognised by its mainly yellowish-green flowers; labellum with a long caudate apex which is about half of the total labellum length; the callus being rugose distally; and, the stigmatic plate on the column being much longer than either the anther or the column wings. It also has more noticeably fragrant flowers than any other member of the group, the largest flowers of the four taxa and very slender spikes. Fig. 2.

The taxon originally described as *P. chasmogamum* (Jones 1991) and treated incorrectly as a synonym of *P. pyriforme* (Jones 1994a), will be included in a revisionary treatment of the *P. rostratum* Lindl. complex, which is in preparation.

Conservation status: This species is listed as threatened under the Victorian Flora and Fauna Guarantee Act 1988. Less than 150 plants remain in two populations (Coates *et al.* 1999). Suggest 2E according to the criteria of Briggs and Leigh (1996).

Specimens examined: **VICTORIA**. None found; all specimens quoted in Jones 1994a are *P. chasmogamum*.

3. *Prasophyllum crebriflorum* D.L.Jones, sp. nov. Affinis *P. correcto* D.L.Jones, sed floribus congestis, rufescentibus; labello parvum insuper medio recurvato, apice caudato tertia parte breviore; et callo laevigato, differt.

Type: Australia. Tasmania: Surrey Hills Freehold (North Forests Burnie), Westwing Plain (precise locality withheld), 670 m, 14 Dec. 2000, *J.E. & A. Wapstra (ORG 3269)* (holo CANB, iso HO, MEL).

Slender tuberous terrestrial *herb* growing singly or in loose groups. *Tubers* not seen. *Leaf* erect, 12-26 cm long, 2-5 mm wide, terete, dark green, base 2-3 mm diam., reddish to purple; free lamina suberect, 6-10 cm long, usually withered at anthesis. *Inflorescence* a moderately dense to dense spike 6-20 cm long. *Floral bracts* transversely ovate, 2-2.3 mm long, c. 3 mm wide, closely embracing the ovary; apex apiculate. *Ovaries* at about 40° to the rachis, obovoid, 5-6 mm long, c. 3 mm wide, bright green, shiny. *Flowers* 6-c.25, 10-12 mm across, reddish brown, opening very widely, sessile. *Dorsal sepal* narrowly ovate-lanceolate, 6.5-8 mm long, 2.5-3 mm wide, sharply deflexed, with 3 indistinct darker veins; apex often recurved, subacute to apiculate. *Lateral sepals* free throughout, linear-lanceolate, 6.5-8 mm long, 1.8-2.2 mm wide, falcate, erect or shallowly recurved, parallel or slightly divergent; base not gibbous; distal margins involute; apex entire or bidentate. *Petals* upswept, widely spreading, linear, 5.5-7 mm long, 0.8-1.2 mm wide; margins entire; apex obtuse to attenuate. *Labellum* very shortly stalked, obliquely erect, usually in a shallow curve but at a sharper angle in old flowers, distal half recurved, the tip erect or recurved; basal claw almost vestigial, c. 0.3 mm long, c. 1.3 mm wide; lamina ovate-lanceolate in outline when flattened, 5-6 mm long, 3-3.5 mm wide, with broad basal margins, constricted in the distal third to half; base not gibbous; proximal margins entire; distal margins slightly irregular. *Callus* extending nearly to the labellum apex, ovate-oblong, 4-5.2 mm long, 2-2.5 mm wide in the proximal third, raised, fleshy, greenish brown, shallowly channelled centrally, constricted sharply in the distal half and extending as a narrow, raised, smooth or rugose caudate section; margins entire or slightly irregular. *Column* porrect from the end of the ovary, c. 2.8 mm

long, c. 3 mm wide, partially exposed by the wide expansion of the tepals; appendages oblong-obovate, c. 2 mm long, c. 0.5 mm wide, straight, pale green, slightly incurved, apex obliquely truncate, shorter than the stigmatic plate. *Anther* ovate, c. 2 mm long, c. 1.8 mm wide, brownish to purplish. *Pollinarium* c. 2.6 mm long; viscidium ovate, c. 0.3 mm long, white; hamulus c. 0.2 mm long; pollinia c. 2 mm long, yellow, sectile. *Stigma* quadrate, c. 2 mm long, c. 2 mm wide, the rostellum higher than the appendages. *Capsules* obovoid, 6-7 mm long, 3-4 mm wide, shiny, pale green. Fig. 3.

Distribution and ecology: Currently known only from Westwing Plain which is south-east of Hellyer Gorge in northern Tasmania. It grows in tussock grassland (*Poa labillardierei* Steud.) with scattered patches of *Hakea microcarpa* R. Br. Some of the orchids were growing in fairly dense patches of *Poa*, but most were in a naturally open area with bare ground and *Herpolirion novae-zelandiae* Hook. f. and *Trachymene humilis* (Hook. f.) Benth. (J.E. Wapstra pers. comm.). The soil is a brown clay loam. Altitude: 660-670 m.

Phenology: This species flowers in late November and December.

Recognition: Within the *P. correctum* group, *P. crebriflorum* is recognised by its crowded, widely opening reddish-brown flowers, the labellum recurved just above the middle, the apical tail-like part of the labellum comprising about one-third of the length of the labellum and the callus being smooth. In appearance it is most similar to *P. incorrectum* but that species, which flowers earlier, has flowers more widely spaced in the spike, the apical tail-like part of the labellum comprising about one-half of the total length of the labellum, and the callus thin and rugose towards the apex. The lowland habitat of *P. correctum* contrasts with the montane habitat of *P. crebriflorum*.

Notes: *Prasophyllum crebriflorum,* which was first collected by Mark Wapstra and Brooke Craven in 1999, is a slender species with crowded, reddish-brown, widely opening flowers; erect, non-gibbous lateral sepals; widely spreading, upswept, linear-oblong petals; an obliquely erect labellum which bends in a shallow curve and with an elongated callus constricted in the distal two-thirds and extended as a narrow, raised, caudate section.

Conservation status: Known from only two almost adjacent grasslands which occur among pine plantations and not located on five other similar grasslands in the area (J.E. Wapstra pers. comm.); not conserved and occurring on private land owned by a timber company; the species remains poorly known. I suggest a conservation status category of 2KE according to the criteria of Briggs and Leigh (1996).

Etymology: From the Latin *creber*, close, crowded and *flos*, flower, in reference to the crowded flowers.

Other Specimens examined: **TASMANIA**. Surrey Hills Freehold (North Forests Burnie), Westwing Plain, 15 Dec. 1999, *M. Wapstra & B. Craven (ORG 2888)* (CANB); Surrey Hills Freehold (North Forests Burnie), Racecourse Plain, 14 Dec. 2000, *J.E. & A. Wapstra (ORG 3268)* (CANB); *ibid*, 4 Jan 2001, *J.E. & A. Wapstra (ORG 3293)* (CANB).

4. *Prasophyllum incorrectum* D.L.Jones, sp. nov. Affinis *P. correcto* D.L.Jones, sed floribus rufescentibus; callo crassiore; lamina stigmatica longitudine anthera et alis columnae similari, differt.

Type: Tasmania. Campbell Town Golf Course, 5 Nov. 1998, *D.L.Jones 16179 & M.A.Garratt* (holo CANB; iso HO, MEL).

Illustration: Page 189, Jones *et al.* (1999) - as *P. correctum*.

Slender tuberous terrestrial *herb* growing singly or in loose groups, occasionally in tufts. *Tubers* ovoid, 0.8-1.2 cm long, 0.6-1 cm wide. *Leaf* erect, 12-30 cm long, 3-5 mm wide, terete, dark green, base 3-5 mm diam., red to purple; free lamina suberect, 8-15 cm long, usually withered at anthesis. *Inflorescence* a sparse to moderately dense spike 5-10 cm long. *Floral bracts* ovate, 3-4 mm long, c. 2 mm wide, closely embracing the ovary, apex

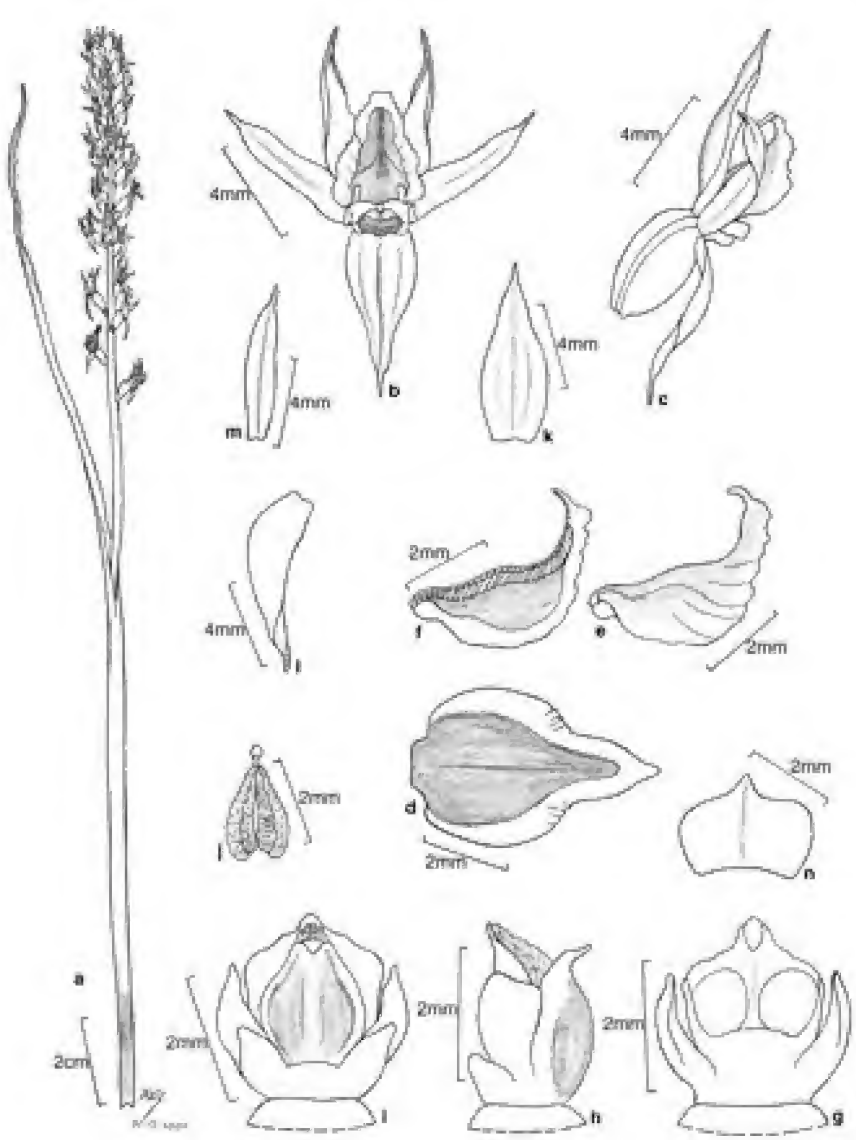

Figure 3. *Prasophyllum crebriflorum*, Surrey Hills Freehold, Tas., *ORG 3268*: a. flowering plant; b. flower from front; c. flower from side; d. labellum from above, flattened out; e. labellum from side; f. longitudinal section of labellum; g. column from front; h. column from side; i. column from rear; j. pollinarium; k. dorsal sepal; l. lateral sepal; m. petal; n. floral bract.

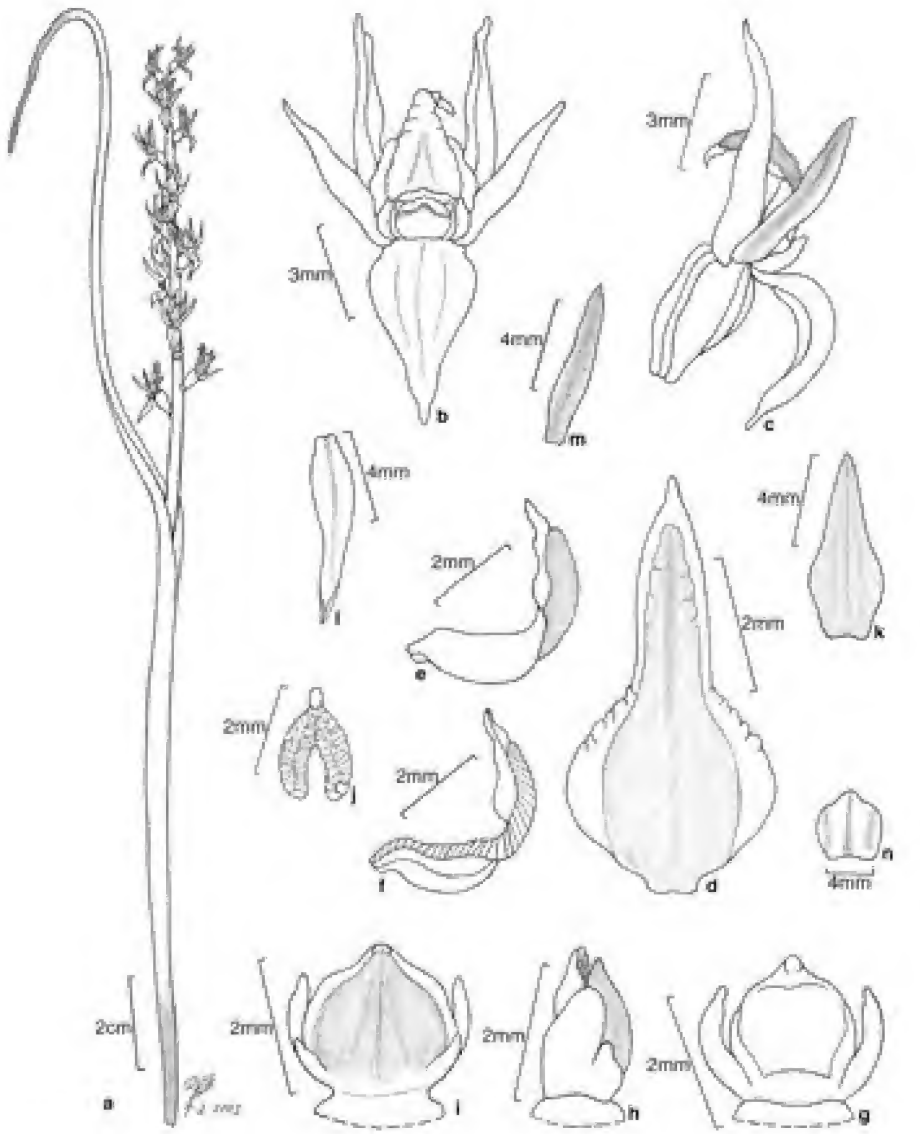

Figure 4. *Prasophyllum incorrectum*, Campbell Town Golf Course, Tas., *H. & A. Wapstra (Jones 14539)*: a. flowering plant; b. flower from front; c. flower from side; d. labellum from above, flattened out; e. labellum from side; f. longitudinal section of labellum; g. column from front; h. column from side; i. column from rear; j. pollinarium; k. dorsal sepal; l. lateral sepal; m. petal; n. floral bract.

apiculate. *Ovaries* at about 30° to the rachis, obovoid, 3-4 mm long, c. 2 mm wide, bright green, shiny, (rarely dark red). *Flowers* 10-20, 7-9 mm across, predominantly yellowish green and light reddish brown, (rarely dark red), opening widely, fragrant, sessile. *Dorsal sepal* linear-ovate, 7-9 mm long, c. 3 mm wide, deflexed, with 3 darker veins, apex subacute to acuminate. *Lateral sepals* connate throughout, partially united or free from the base, linear-lanceolate, 7-9 mm long, 1.5-2 mm wide, erect or recurved, base not gibbous, distal margins involute, apex entire. *Petals* incurved to widely spreading, linear to linear-lanceolate, 7-9 mm long, 1-1.2 mm wide, upswept, green with brown striae, apex subacute. *Labellum* very shortly stalked, obliquely erect, distal half recurved, the tip often projecting through the lateral sepals; basal claw almost vestigial, c. 0.4 mm long, c. 1 mm wide; lamina broadly ovate-lanceolate in outline when flattened, 6-8 mm long, 3.5-4 mm wide, yellowish green (rarely reddish), proximal half almost orbicular, shallowly constricted just above the middle, tapered in the distal half, base not gibbous, proximal margins flat, entire, distal margins entire or slightly crenulate. *Callus* elliptical-lanceolate, 5-6 mm long, 2-2.5 mm wide, raised, fleshy, green (rarely red), channelled centrally, margins entire or crenate, narrowed beyond the bend and extending nearly to the labellum apex. *Column* porrect from the end of the ovary, c. 3 mm long, c. 3 mm wide, fully exposed by the wide expansion of the tepals; appendages linear-oblong, c. 2.3 mm long, c. 0.7 mm wide, pale green (rarely red), divergent, apex truncate or emarginate, about as long as the stigmatic plate. *Anther* ovate, c. 2 mm long, c. 1.6 mm wide, dark red brown. *Pollinarium* c. 2 mm long; viscidium ovate, c. 0.25 mm long, white; hamulus c. 0.2 mm long; pollinia c. 1.6 mm long, yellow, sectile. *Stigma* quadrate, c. 1.5 mm long, c. 1.5 mm wide, the rostellum about as high as the appendages. *Capsules* obovoid, 4-5 mm long, c. 3 mm wide, shiny, green (rarely red). *Fig. 4.*

Distribution and ecology: Endemic to Campbell Town, Tasmania, growing in relatively damp native grassland dominated by *Themeda triandra* and grassy woodland with eucalypts and banksias, in grey sandy loam. Altitude: c. 200 m.

Phenology: This species flowers in October and November.

Recognition: Within the *P. correctum* group, *P. incorrectum* can be recognised by its reddish-brown flowers, the apical tail-like part of the labellum comprising about one-third of the total labellum length and the callus being thick and rugose towards the apex. It is morphologically most similar to *P. correctum* which has mainly yellowish-green, fragrant flowers in a very slender spike, and the stigmatic plate is much longer than either the anther or the column wings. Geographically it occurs closest to *P. crebriflorum* which grows in a montane area, flowers later, has densely crowded flowers and the callus is thinner and smooth throughout.

Notes: Plant habit and floral morphology of the Tasmanian specimens are very similar to collections of *P. correctum* from Victoria (Jones 1998), however the results of a molecular study using RAPD's show conclusively that the Tasmanian and Victorian populations belong to different taxa (Orthia *et al.* 2003).

Morphologically both taxa are very similar and difficult to separate, however after careful re-examination of material from Victoria and Tasmania, the following characters which can be used to separate the two taxa are evident. *Prasophyllum incorrectum* has slightly smaller flowers than *P. correctum* and a thicker labellum callus, however the most distinctive features lie in the column. In *P. correctum* the stigmatic plate is much longer than the anther and the column wings curve outwards, whereas in *P. incorrectum* the anther and stigmatic plate are of similar length and the column wings curve inwards. Flower colour is perhaps the most obvious feature which is easily identifiable. Plants from Victoria have mainly yellowish-green flowers with some brown striations (Backhouse & Jeanes 1995, D.L.Jones pers. obs.), whereas those from Tasmania are mainly reddish brown with some specimens being wholly red.

Conservation status: This species is listed as endangered under the Tasmanian *Threatened Species Protection Act 1995.* The main population of this species, which

occurs on the Campbell Town Golf Course, consists of about 1000 individuals (Coates *et al.* 1999). The site is not part of a formal reserve but the orchids are well known to the owners of the property and are protected by a covenant which includes a management plan. In 1999 a single plant of this orchid was found at a second locality nearby (J.E. Wapstra pers. comm.). Suggest 2E according to the criteria of Briggs and Leigh (1996).

Etymology: The Latin *incorrectum*, incorrect, wrong, in reference to my mistake of including the species in *P. correctum*.

Specimens examined: **TASMANIA**: Campbell Town Golf Course, 5 Nov. 1998, *Jones 16180 & Garratt* (CANB); *ibid*, 20 Oct. 1995, *J.E. & A. Wapstra (Jones 14539)* (CANB); 21 Nov 1995, *J.E. & A. Wapstra (Jones 14681)* (CANB); *ibid*, 9 Nov. 1996, *J.E. & A. Wapstra (ORG 439)* (CANB).

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Muelleria 18: 111–111 (2003)

SHORT COMMUNICATION

A corrected spelling of *Boronia yarrowmerensis* Duretto (Rutaceae)

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In my recent account of *Boronia* (Duretto, *Muelleria* 17: 19-135 (2003)) I inadvertently misspelt the specific epithet of a newly described species as '*yarromerensis*' Duretto (*l.c.*, p. 32). Unfortunately this error was not picked up in the page proofs. The name commemorates 'Yarrowmere Station' [also misspelt in the paper] and the epithet was supposed to be 'yarrowmerensis'. I now take the opportunity to correct the spelling to 'yarrowmerensis'.

Boronia yarrowmerensis Duretto, *Muelleria* 17: 32, Figs 2G-H (2003), as *B. yarromerensis*.

Type: QUEENSLAND: BURKE: About 21 km NNW of Yarrowmere Station homestead on Great Dividing Range, 21°17'S 145°48'E, *R.J. Henderson H2853, G.P. Guymer and H.A. Dillewaard*, 15.x.1983 (holotype BRI *AQ414567*; isotypes CANB, MEL).

Boronia sp. (Yarrowmere R.J.Henderson H2853): E.M. Ross, '*Rutaceae*' In R.J.F. Henderson (ed.) '*Queensland Vascular Plants: names and distribution*, 303 (1994); P.I. Forster, '*Rutaceae*' in R.J.F. Henderson (ed.), *Queensland Plants: names and distribution*, 185 (1997).

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