

Circumscription and phylogenetic relationships of *Prostanthera densa* and *P. marifolia* (Lamiaceae)

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Abstract

Prostanthera densa A.A.Ham. and *P. marifolia* R.Br. (Lamiaceae) are endemic species with restricted distributions within the near-coastal regions of New South Wales (Australia). *Prostanthera marifolia* was previously presumed extinct, but is now known from three small geographically close populations in the Manly-Warringah area of metropolitan Sydney. This species is morphologically very similar to *P. densa*, which is known from five disjunct populations, distributed south from Port Stephens to Jervis Bay. The nucleotide sequence variation of the *psbA-trnH* chloroplast spacer region, and the external transcribed spacer (ETS) region of nuclear ribosomal DNA were analysed to evaluate the phylogenetic relationships and taxonomic integrity of these species. Trees generated from *psbA-trnH* and ETS data provided support for recognising *P. marifolia* as separate from *P. densa*. A multivariate statistical evaluation of the morphological variation of each population of these taxa supported the distinction of these two morphologically similar and closely related species. Based on molecular and morphological data we recommend these two species continue to be recognised. The ETS data highlighted the genetic distinctiveness of four disjunct populations of *P. densa*. Since there is apparently no gene flow between populations of *P. densa*, it was concluded that the conservation of this species requires all populations to be protected.

Introduction

Prostanthera marifolia R.Br. (Lamiaceae) is a small shrub recorded from the Sydney Harbour region of the Central Coast, New South Wales (Brown 1810; Conn 2007). Since first collected in the early 1900s, all subsequent attempts to re-collect this species were unsuccessful. As a result, this species was presumed extinct (Conn 1992). In 2001, a number of plants that broadly agreed with the circumscription of *P. marifolia* were located at three sites in the Manly-Warringah area of Metropolitan Sydney, near Garigal National Park. The conservation status of this species was re-assessed according to the *New South Wales Threatened Species Conservation Act 1995* and is now classified as 'critically endangered' (Conn 2007). *Prostanthera densa* A.A.Ham. is morphologically similar to *P. marifolia* and is confined to a series of disjunct populations throughout coastal and near-coastal New South Wales, occurring at Port Stephens, North Coast Botanical region (Anderson 1961; Jacobs and Pickard 1981); Cronulla, Helensburgh and in the Royal National Park (all three occurring near Sydney, Central Coast Botanical region); and on the Beecroft Peninsula near Jervis Bay (South Coast Botanical region) (Fig. 1). The identity of each species has frequently been confused because *P. marifolia* was poorly known due to local extinctions caused by urban development in central metropolitan Sydney, inadequate circumscription and poor representation in Australian herbaria.

In this study, the morphological variation within and between populations of *P. marifolia* and *P. densa* is assessed empirically. The genetic distinctiveness of individuals attributable to both species is quantified. Together, these data provided insights into the taxonomy, phylogenetic relationships, and conservation status of known populations.

Materials and methods

Molecular phylogenetic analyses

Individuals from five populations of *P. densa*, representing all known extant populations, were included in this study. New collections were made from two of the three extant populations of *P. marifolia* in the Manly-Warringah region. The suitability of several potential outgroup species were assessed using the known molecular phylogeny of *Prostanthera* (Wilson et al. 2012). *Prostanthera granitica* and *P. tallowa* were recovered as suitable outgroup taxa for the ETS data, but only *P. tallowa* formed a sister relationship to the ingroup for the *psbA-trnH* region. Therefore, *P. tallowa* was used as the outgroup species for both molecular regions used in this study. Collection details of all voucher specimens are given in Table 1.

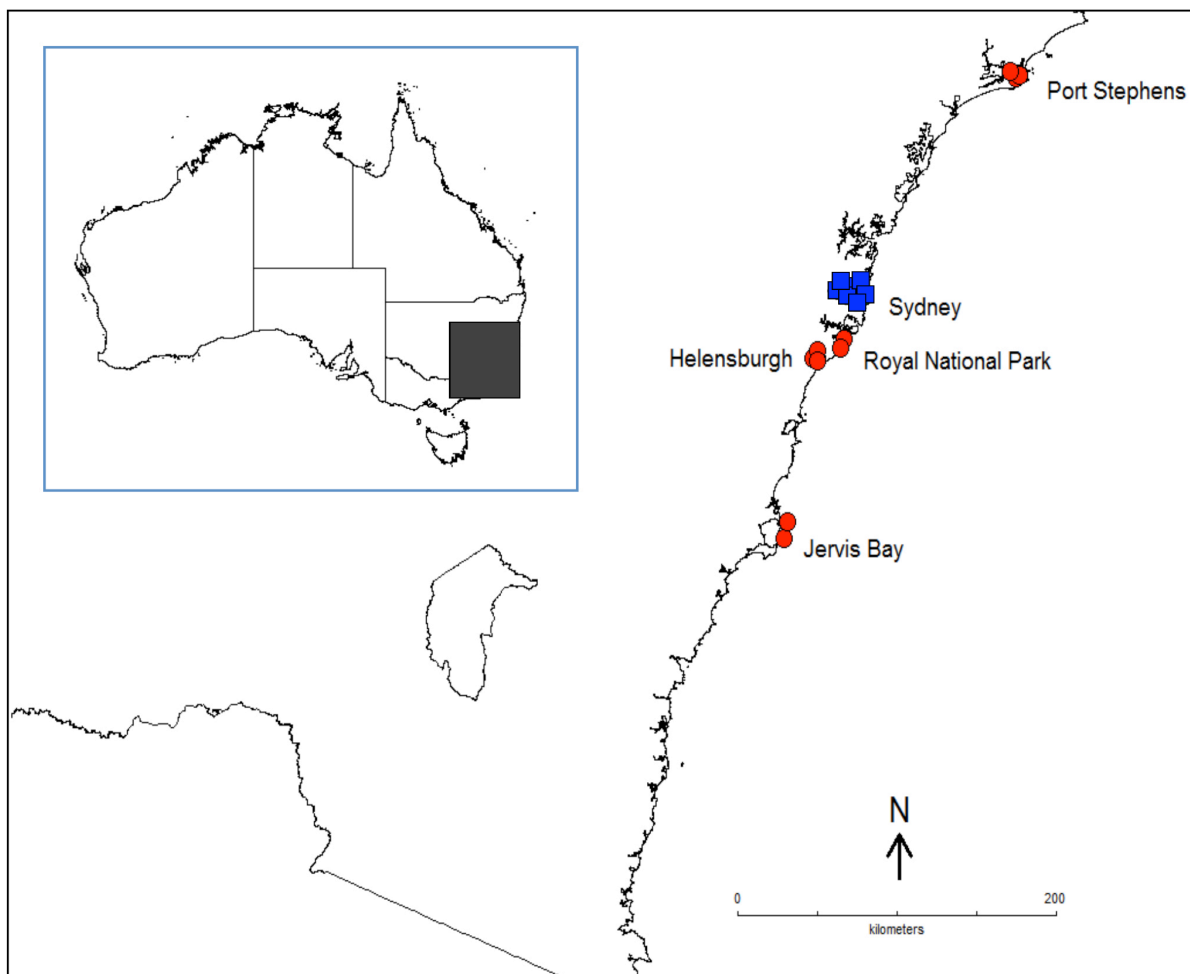


Fig. 1. Distribution of *Prostanthera densa* (red dots) and *P. marifolia* (blue squares) in coastal and near-coastal New South Wales, Australia. Insert map: locality of detailed map in Australia. The geographic distributions are based on collections held at MEL, NSW and SYD.

Table 1. *Prostanthera* specimens of *P. densa* and *P. marifolia* used for DNA sequence and morphological analyses Legend: The collections cited are listed in an approximate north–south order; collection voucher = collector, collection number and herbarium abbreviations (in parentheses); NP = National Park; PW = Parkway; s.n. = sine numero; * = specimen sequenced with only one direction. Genbank numbers are given for each marker.

Taxon	Population	Collection voucher	ETS	<i>psbA-trnH</i>	Morphology	
<i>P. marifolia</i>	Manly-Warringah	Conn 4380A (NSW)	JX047657	KF145108		
		Conn 4444 (NSW)			✓	
		Skelton <i>s.n.</i> (NSW)			✓	
		Conn 5676 (NSW)	KF112054		KF145107	✓
	Longueville	Rupp <i>s.n.</i> (MEL43365)			✓	
<i>P. densa</i>	Port Stephens	Wilson 37 (NSW)	JX047677	KF145100	✓	
		Johnstone 1003 (NSW)	KF112057		✓	
	Cronulla	Conn 2563 (NSW)				✓
		Conn 4381A (NSW)	KF112048		KF145103	✓
	Royal NP	Wilson 173 (NSW)	KF112049*		KF145101	✓
	Helensburgh	Scott 4 (NSW)	KF112053		KF145102	✓
		Wilson 221 (NSW)	KF112050		KF145104	
		Wilson 222 (NSW)	KF112051		KF145105	✓
	Jervis Bay	Wilson 242 (NSW)	KF112052		KF145106	
		Henwood 800 (SYD)	KF112056		KF145110	✓
		Conn 2571 (NSW)				✓
	Miles (NSW496550)	KF112055		KF145109		
<i>P. tallowa</i>	Kangaroo Valley	Wilson 234 (NSW)	JX047664	KF692284		

Selection of molecular markers

Two markers were used in this study: the *psbA-trnH* chloroplast intergenic spacer region and the external transcribed spacer (ETS) region of nuclear ribosomal DNA. The *psbA-trnH* regions have been used at the interspecific level in the Lamiaceae (Albaladejo et al. 2005; Gobert et al. 2006). Similarly, the ETS region of nuclear ribosomal DNA has been found to evolve faster and consequently have greater variability than the internal transcribed spacer (ITS) region, which is more frequently used, making ETS particularly useful for studies below the generic level (Baldwin and Markos 1998; Linder et al. 2000; Stappen et al. 2003; Wilson et al. 2012).

DNA extraction, amplification and sequencing

Total genomic DNA extractions and polymerase chain reaction (PCR) to amplify the selected regions (*psbA-trnH* and ETS) were conducted according to methods used by Wilson et al. (2012). Primers are listed in Table 2.

Table 2. Primers used for amplification of markers

Fragment	Primer Name	Direction	Sequence	Author(s)
<i>psbA-trnH</i>	<i>psbA</i>	5' to 3'	GTTATGCATGAACGTAATGCTC	(Tate and Simpson 2003)
	<i>trnH</i> ^{GUG}	3' to 5'	CGCGCATGCTGGTGGATTACAATCC	(Sang et al. 1997)
ETS	ETS-PROS2	5' to 3'	GCAGCGACGACATCCCAACC	(Baldwin and Markos 1998)
	18S-E	3' to 5'	GCAGGATCAACCAGGTAGCA	(Wilson et al. 2012b)

Sequence alignment and phylogenetic analyses

Multiple sequence alignments were initially performed using the Clustal W alignment tool (Larkin et al. 2007) implemented in the alignment editing program BioEdit 7.0.9.0 (Hall 1999). Alignments were edited manually as necessary in this program. A consensus of the forward and reverse sequences was created for most samples (Table 1).

Maximum parsimony (MP) analysis was conducted using PAUP* 4.0b10 (Swofford 2003) and Bayesian inference (BI) analysis was undertaken using Mr Bayes 3.1.2 (Huelsenbeck and Ronquist 2001). The settings for MP analyses were as follows: nucleotide substitutions and indel events were weighted equally, random taxon addition starting tree with tree-bisection–reconnection (TBR), branch swapping with uninformative characters excluded, and the MULTREES option = yes. Length of the shortest tree (L), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated to test the results of each heuristic search. Branch support was assessed by bootstrap analysis (BS), using 1000 replicates with the above heuristic search settings. Bayesian analysis used datasets without indels with the ‘TVM+G’ model as indicated by Modeltest (Posada and Crandall 1998), and the rate variation across sites was set as gamma-distributed. Tree space was searched using the Metropolis Coupled Markov Chain Monte Carlo analysis, using four chains starting at randomly selected trees. The chains were run for 5,000,000 generations in two simultaneous runs, saving one tree every 100 generations. The ‘burn-in’ value of 30,000 was set for all analyses.

Estimates of the evolutionary divergence between the populations of *P. densa* and *P. marifolia*, using ETS data, was analysed using the Kimura 2-parameter model (Kimura 1980), conducted in MEGA5 (Tamura et al. 2011). The analysis involved 13 nucleotide sequences. All positions containing gaps were treated as missing data. The final matrix contained 327 characters.

Multivariate statistical analyses

Twenty morphological characters were measured (Table 3) from 13 specimens, including most of those sampled for molecular phylogenetic analyses (Table 1). Specimens used in the phenetic analysis represented the morphological range of variation found within and between populations of *P. densa* and *P. marifolia*. The primary aim of the morphometric analyses was to empirically identify any new, or previously recognised, taxonomic units within the sample. To achieve this, a range-standardised distance matrix of the data was produced using the Gower metric association measure (Gower 1971). Cluster analysis was performed using flexible Ungrouped Pair-Group Method using Averages (flexible UPGMA), with β -value = -0.1000 as implemented in PATN Version 3.11 (Belbin and Collins 2006). Association measures were used to ordinate samples in Semi-Strong Hybrid (SSH) multidimensional scaling, with CutOff = 0.900; number of random starts = 100; maximum iterations = 1,000; random seed value = 1,235 (Belbin 1991, 1995). Correlation values for each morphological character in the SSH were calculated using Principal Component Correlation (PCC).

Table 3. Morphological characters used, including character codes, in multivariate analysis of selected specimens of *Prostanthera densa* and *P. marifolia*.

Character code	Definition (units)	Character code	Definition (units)
IntL	Length of 5 th most distal internode (mm)	ProW	Width of prophylls (mm)
IntHD	Density of hairs on distal internodes (mm ²)	KTL	Length of calyx tube (mm)
LfL	Length of leaf lamina (mm)	KABL	Length of abaxial calyx lobe (mm)
LfW	Width of leaf lamina (mm)	KADL	Length of adaxial calyx lobe (mm)
LfW_L	Distance of maximum width of leaf lamina from base/total lamina length ratio	KOHD	Density of hairs on outer surface of calyx (mm ²)
LfHD	Density of hairs on abaxial lamina surface (mm ²)	KIHD	Density of hairs on inner surface of adaxial calyx lobe (mm ²)
LfGD	Density of glands on abaxial lamina surface (mm ²)	CTL	Length of corolla tube (mm)
A1L	Length of a ₁ axis (mm)	CADLL	Length of adaxial corolla lobe (mm)
AntL	Length of anthopodium (mm)	CABLL	Length of abaxial corolla lobe (mm)
ProL	Length of prophylls (mm)	AAL	Length of anther appendage (mm)

Results

Phylogenetic analysis

The total length of the *psbA-trnH* matrix was 403 characters, of which two characters (~0.50%) were variable and one of these was parsimony-informative. The resulting single tree (Fig. 2) placed the *P. marifolia* samples as unresolved members of a polytomy that consisted of a strongly supported clade containing all of the *P. densa* samples (BS=100%; PP=0.97). The relationships between the samples of *P. marifolia* and between accessions within the *P. densa* clade were not resolved.

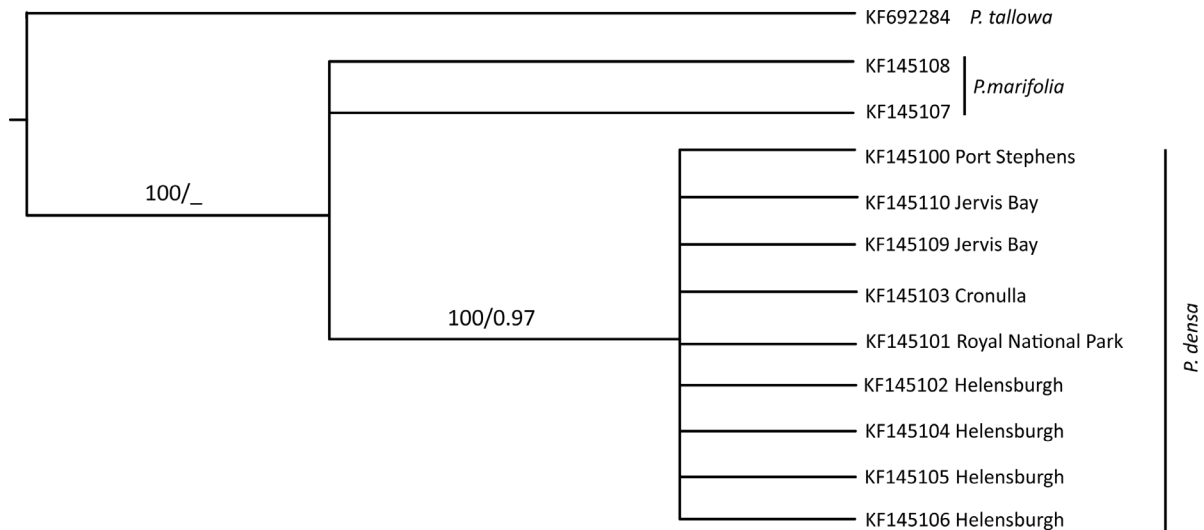


Fig. 2. The single tree from maximum parsimony analysis of the *psbA-trnH* chloroplast sequence data. Bootstrap values (%) from Maximum Parsimony analysis, followed by Bayesian posterior probabilities are reported on branches. Samples used are named according to GenBank identifiers, locality of populations and accepted taxonomy (refer to Table 1 for further details).

The ETS alignment was 351 characters in length (plus two indels) and had 22 variable sites (including one indel), all being parsimony-informative (6.3% of the total sites). A region containing eight base pairs could not be unambiguously aligned and so was removed before analysis. A strict consensus of 18 trees from Maximum Parsimony analysis (not presented), as well as the Bayesian inference tree (Fig. 3), were unable to resolve the relationship between a *P. marifolia* clade (BS=67%; PP=0.99) and accessions of *P. densa*. Four strongly supported clades of *P. densa* formed an unresolved polytomy. Individuals from Cronulla and the Royal National Park were recovered as a clade (BS=100%; PP=1.0), whereas each of the other disjunct populations formed strongly supported clades (Helensburgh: BS=97%; PP=0.93; Jervis Bay: BS=97%; PP=1.0; Port Stephens: BS=97%; PP=0.98).

Our sampled individuals from within Port Stephens and Helensburgh showed no genetic divergence. Accessions of *P. marifolia* from two populations differed from each other (genetic divergence = 0.0031). Two accessions of *P. densa* from the Jervis Bay population were divergent (genetic divergence = 0.0093). However, the single accessions of *P. densa* from the disjunct populations of Cronulla and Royal National Park were genetically identical for ETS.

ETS distance data supported the relative genetic dissimilarity of *P. densa* and *P. marifolia* (genetic divergence = 0.0180). Each of the well-supported populations of *P. densa* were on relatively long branches compared to those of *P. marifolia*, but none of the populations of *P. densa* or *P. marifolia* formed a well-supported relationship with any other population. Within *P. densa*, the Jervis Bay population was genetically the most divergent from the other populations. A comparison of this population to Cronulla–Royal National Park revealed a genetic divergence=0.0362, to that of Port Stephens =0.0331, and to Helensburgh =0.0234. The *P. densa* Helensburgh population is genetically most similar to *P. marifolia* (genetic divergence=0.0139). Thus,

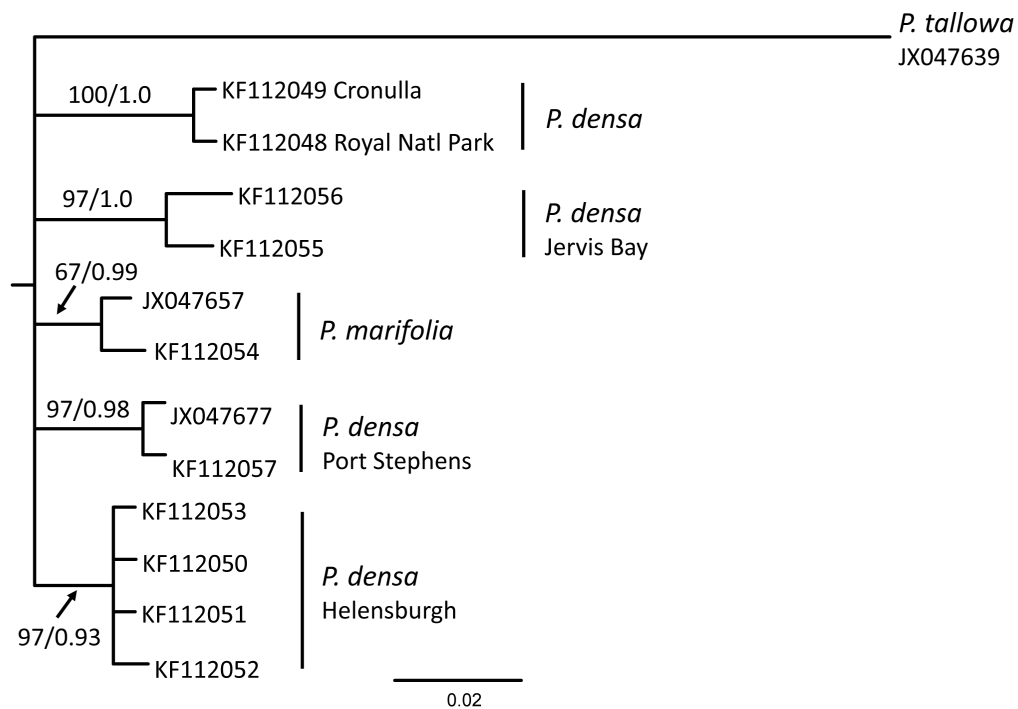


Fig. 3. 50% Majority Rule tree based on Bayesian analysis of the ETS nuclear sequence data. Bootstrap values (%) from Maximum Parsimony analysis, followed by Bayesian posterior probabilities are reported on branches. Samples used are named according to GenBank identifiers, locality of populations, and accepted taxonomy (refer to Table 1 for details). Scale bar = number of nucleotide changes.

the ETS data confirms the contention that populations of *P. densa* and *P. marifolia* are relatively genetically distinct, but cannot provide any insight into relationships between the populations of these two taxa.

Multivariate analysis

Cluster analysis and ordination supported the recognition of *P. densa* as morphologically distinct from *P. marifolia*. However, the Helensburgh accessions of *P. densa* were morphologically more similar to *P. marifolia* than to any other accession of *P. densa* (Figs 4 and 5). Compared to *P. densa*, *P. marifolia* has the following distinguishing characteristics: (1) longer internodes with shorter hairs; (2) leaves that are shorter; (3) leaves narrower and more glandular; (4) prophylls shorter and narrower; (5) shorter anther appendages (Table 4). The disjunct populations within *P. densa* form a morphological continuum. The characters that best separate *P. densa* from *P. marifolia*, based on Kruskal-Wallis and r^2 values, are summarised in Tables 5 and 6.

Discussion

In this study the fast evolving nuclear ETS marker (Baldwin 1992; Baldwin and Markos 1998; Linder et al. 2000), provided about ten times more variability than the *psbA-trnH* marker. The Bayesian inference tree derived from the ETS data (Fig. 3) shows that most of the variation is found between populations of *P. densa*, and reveals a relatively high similarity between the allopatric accessions of *P. densa* from Cronulla and from Royal National Park to the south of Sydney. In contrast, accessions from two geographically close, but functionally allopatric populations of *P. marifolia* received weak bootstrap support for a sister relationship. Analysis of the ETS data did not, however, provide any insights into the phylogenetic relationships between *P. marifolia* and *P. densa*.

The *psbA-trnH* spacer is often useful for distinguishing between species (Kress et al. 2005). In our study it recovered a well-supported clade comprising the *P. densa* populations, but was unable to resolve the relationship between the samples of *P. marifolia*. This result is not inconsistent with the notion that two distinct taxa are present.

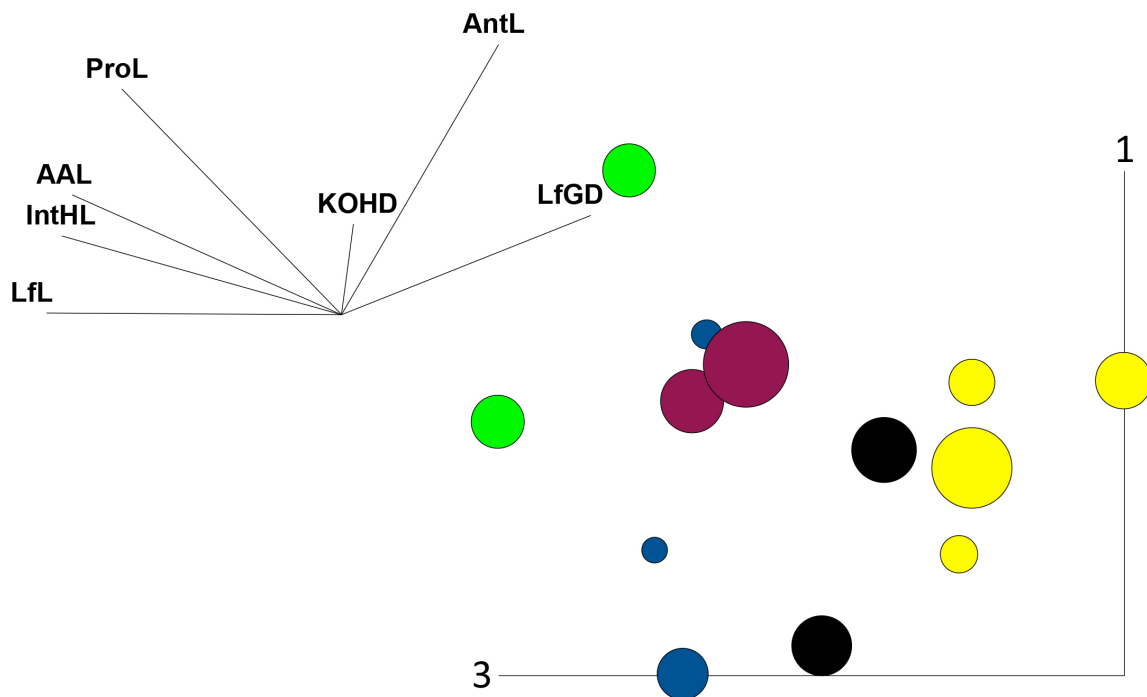


Fig. 4. Three dimensional ordination of the specimens plotted onto axes 1 and 2, with the size of each coloured circle representing its position in the third dimension. A character vector diagram of seven most important characters based on r^2 values is provided (alphabetic character codes as listed in Table 3). Colour codes: yellow = *Prostanthera marifolia*; dark brown = *P. densa* Helensburgh; red-brown = *P. densa* Jervis Bay; blue = *P. densa* Cronulla/Royal National Park; bright green = *P. densa* Port Stephens.

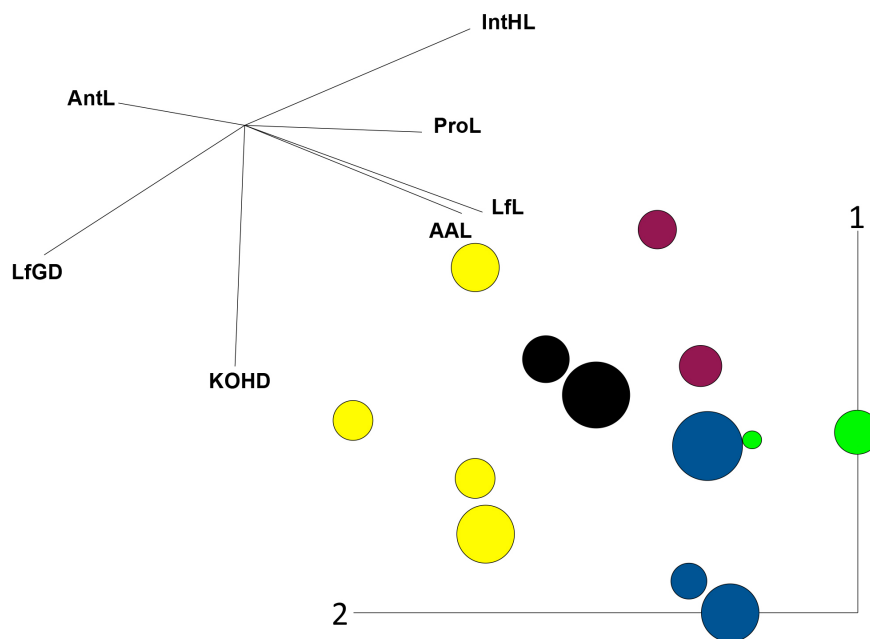


Fig. 5. Three dimensional ordination of the specimens plotted onto axes 1 and 3, with the size of each coloured circle representing its position in the second dimension. A character vector diagram of seven most important characters based on r^2 values is provided (alphabetic character codes as listed in Table 3). Colour codes: yellow = *Prostanthera marifolia*; dark brown = *P. densa* Helensburgh; red-brown = *P. densa* Jervis Bay; blue = *P. densa* Cronulla/Royal National Park; bright green = *P. densa* Port Stephens.

Table 4. Morphological differences between *Prostanthera densa* and *P. marifolia*. Character abbreviations as in Table 3.

Character	<i>P. densa</i>	<i>P. marifolia</i>
Internode length (IntL)	(2–)4–7(–12.5) mm	8.3–9.5(–14.5) mm
Internode hair length (IntHL)	up to 0.96 mm	(0.9–)1–1.4 mm
Leaf length (LfL)	up to 5.5(–8) mm	(6–)8–10(–13) mm
Leaf width (LfW)	2.8–3.5(–5.5) mm	(1.7–)5–7.1 mm
Leaf gland density on abaxial surface (LfGD)	(8.7–)17–22 mm ²	4–8.7(–13) mm ²
Prophyll length (ProL)	up to 2.5 mm	(2–)3.5–4.5(–5.5) mm
Prophyll width (ProW)	up to 0.24 mm	(0.22–)0.3–0.7(–1.7) mm
Anther appendage length (AAL)	up to 0.48 mm	(0.5–)0.8–0.9(–1.2) mm

Table 5. The five most useful characters to discriminate between *Prostanthera densa* and *P. marifolia*, ranked by Kruskal-Wallis values. Character abbreviations as in Table 3.

IntHL 7.7357	LfL 6.1929	IntL 5.4857	LfGD 5.4857	AAL 5.2572
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Table 6. Seven morphological characters with the highest correlation values (as measured by r^2 values) based on Principal Component Correlation (PCC). Character abbreviations as in Table 3.

ProL 0.900	KOHD 0.883	AAL 0.856	LfL 0.846
LfGD 0.835	IntHD 0.799	AntL 0.753	

The multivariate analysis of 20 morphological characters also established the presence of two morphologically discrete taxa, congruent with *P. densa* and *P. marifolia* and supported by the *psbA-trnH* molecular data. The multivariate analysis further supported the conclusion that each taxon comprises a subset of more or less morphologically distinct populations. However, even though the *psbA-trnH* tree and the morphological data support the recognition of *P. densa* as a distinct taxon sister to *P. marifolia*, the morphological circumscription of each taxon is challenging.

The morphological similarity between *P. densa* growing near Helensburgh and *P. marifolia* was first recognised by Hamilton (1920). He characterised *P. densa* as being larger ('9–12 dec') and having an 'upright spreading habit', whereas *P. marifolia* was a 'scrambling undershrub of 3–5 dec'. In general, our field observations support his conclusions. Nevertheless, the density of the surrounding vegetation and degree of exposure to off-shore winds does influence the overall habit of *P. densa*. Although not included in the multivariate analyses, mature leaves of *P. densa* usually have an entire margin, unlike those of *P. marifolia* that appear to always have occasional teeth present. The utility of this character to distinguish these species is unknown. There appears to be no consistent morphological differences between the various populations of *P. densa*. The Port Stephens population tends to have leaves that are longer, with larger prophylls and a podium with a longer a_1 axis. The Helensburgh population has internodes that are longer with shorter hairs than those found in the other populations.

The type collection of *Prostanthera marifolia* (Brown s.n. [Bennett 2371], BM) and the brief description provided in the protologue (Brown 1810) are inadequate to circumscribe precisely this critically endangered species. Furthermore, the morphological similarity between *P. densa* and *P. marifolia* has made it difficult to distinguish between both species. In general, *P. densa* tends to have leaves that are larger, more 'crowded' (with shorter internodes), hairier (with longer hairs) and larger prophylls than *P. marifolia*. Other morphological differences are listed in Table 4. The morphological analyses indicate that most of the studied character states tend to overlap. In particular, populations of *P. densa* occurring in the vicinity of Helensburgh (to the south of metropolitan Sydney) shared many features with *P. marifolia*. Contrary to this, the ETS data provided no support for a relationship between populations of *P. densa* from Helensburgh and *P. marifolia* and (Fig. 3).

In this study it was found that molecular and morphological data assist in the recognition of *P. densa* and *P. marifolia*. We consider the most nomenclaturally stable conclusion is to recognise them at the species level, and to provide an enhanced description of the morphological variation within and between each species.

The conclusions of this study have implications for the conservation of *P. marifolia*, which is listed as 'critically endangered' under the *New South Wales Threatened Species Conservation Act 1995* (Conn 2007), and of

P. densa, which is listed as ‘vulnerable’ (under both the *Commonwealth Environment Protection and Biodiversity Conservation Act 1999* and the *TSC Act*) (Conn 1999). Since the population (consisting of 3 sub-populations) of *P. marifolia* in the Manly-Warringah area is very small (estimated population size: 100 individuals) and currently threatened by anthropogenic disturbance and habitat loss (Hughes 28 February 2011), urgent action is required to protect this species. In addition, the ETS data indicates that the four known populations of *P. densa* (Port Stephens in the north; Cronulla, Royal National Park, and Helensburgh in the centre of its distribution; Jervis Bay in the south) may each be genetically distinct. Although any future studies of the genetic diversity of these populations should use more appropriate population genetic approaches (including increased samplings and investigation of within- and between-population relationships using molecular markers, such as chloroplast and nuclear microsatellites), the conservation of the genetic diversity within *P. densa* requires resource management strategies that conserve all known disjunct populations. Each of the individual populations of *P. densa* appear to be endangered. Although all populations of this species should be prioritised for conservation actions, there is no ‘critically endangered’ category for populations in the *New South Wales Threatened Species Conservation Act 1995* (Anonymous 28 February 2011).

Thirteen priority recovery actions have been identified for *P. densa* by the New South Wales Office of Environment and Heritage (Anonymous without date). Action 9 of these recovery actions, listed as high priority, is to “Review taxonomic classification especially with reference to *Prostanthera marifolia*”. Action 10, listed as a medium priority, is to “Review conservation status endangered/critically endangered (after taxonomic review)”. As this study suggests that all known populations of *P. densa* need to be protected to conserve the genetic diversity of the species, it is strongly recommended that the conservation status of this species be upgraded to endangered or critically endangered. A survey of the current distribution of each population, as recommended in priority recovery action 1, is also likely to be important in this review of conservation status.

Taxonomic descriptions

1. *Prostanthera densa* A.A.Ham., *Proceedings of the Linnean Society of New South Wales* 45 (1920) 263.

Holotype: New South Wales: Central Coast: Cronulla, A.A. Hamilton 9, anno 1911 (NSW134507); iso.: BM, K (refer Notes below).

Erect, more or less compact to openly branched shrub, 0.3–2 m high; faintly aromatic. *Branches* subterete, moderately to densely hairy [(8–)30–90 hairs/mm²]; hairs ± straight to variously curved and/or bent, spreading or slightly retrorse, c. 0.8–1.5 mm long, white; sparsely glandular [4–9(–13) glands/mm²]. *Leaves* green, faintly aromatic (when crushed), usually moderately to densely hairy [(13–)30–90(–160) hairs/mm²]; sparsely glandular [as for branches]; *petiole* (0.4–)0.8–1.5 mm long; *lamina* ovate, often appearing triangular when distal margin strongly recurved, (6–)10–15 mm long, (3.5–)7–12 mm wide [length to width ratio 1.2–1.4(1–1.7), length of maximum width from base to total lamina length ratio 0.8–4.5]; base rounded to ± truncate; margin entire, usually strongly recurved; apex rounded to obtuse; venation not visible, except for midrib raised on abaxial surface. *Inflorescence* a frondose racemiform confluence, uniflorescences monadic; 2–10-flowered [per confluence]. *Pherophylls* absent. *Podium* 0.9–2.5 mm long, moderately hairy and glandular [as for branches]. *Prophylls* inserted near middle or on basal half of podium [a₁ axis to anthopodium ratio 1–3.6], opposite, linear (broadest near middle or on distal half), 2.1–5.5 mm long, 0.2–0.7(–1.7) mm wide [length to width ratio (3.2–)6.5–10.5, length of maximum from base to total lamina length ratio 0.5–0.7], with long spreading hairs [as for branches]; base attenuate; margin entire; apex tapering. *Calyx* green, sometimes with maroon coloration; outer surface sparsely to moderately hairy [13–35(–56) hairs/mm²], hairs spreading, 0.5–0.9 mm long; sparsely to moderately glandular [10–15 glands/mm²]; tube (1.8–)2–4 mm long; *abaxial lobe* very broadly ovate, 2.4–2.8 mm long, 3.5–4 mm wide [length to width ratio c. 0.7], *apex* obtuse to rounded; *adaxial lobe* very broadly ovate, 2–3 mm long, 3–4 mm wide [length to width_ratio 0.5–0.8], *apex* obtuse [adaxial lobe length to abaxial lobe length ratio 0.8–1.1]. *Corolla* 12–15 mm long, pale mauve to mauve, white on inner abaxial surface of tube, with rusty-orange markings in tube; outer surface sparsely hairy [10–25 hairs/mm²], sparsely glandular [10–16 glands/mm²]; inner surface sparsely hairy [6–12 hairs/mm²]; *tube* (5–)6–8 mm long; *abaxial median lobes* very broadly obovate or spatulate, 6.5–10(–12) mm long, 6–7(–9) mm wide [length to width ratio 0.9–1.4], *apex* bilobed (sinus 0.8–2 mm long, 2–4 mm wide distally); *lateral lobes* slightly oblong, 2.5–5 mm long, 2–2.5 mm wide [length to width ratio 1.2–2], *apex* slightly undulate, obtuse to almost truncate; *adaxial median lobe-pair* depressed ovate, 3–4.5 mm long, 4.5–8 mm wide [length to width ratio 0.4–0.6], deeply bilobed (sinus 1.5–2 mm long), *apex* of each lobe rounded. *Stamens* inserted 1.5–2 mm above base of corolla; filaments 2.5–3 mm long, glabrous; anthers 0.8–1 mm long, lobes slightly cristate on basal dorsal surface (trichomes narrowly triangular, c. 0.1 mm long), lobes with basal acumen to 0.1–0.2 mm long, connective extended to form basal appendages 0.5–1.3 mm long, terminating in a few triangular trichomes c. 0.1 mm long. *Disc* ± cylindrical, 0.2–0.3 mm long. *Pistil* 6–8 mm long; *ovary* cylindrical to obovoid,

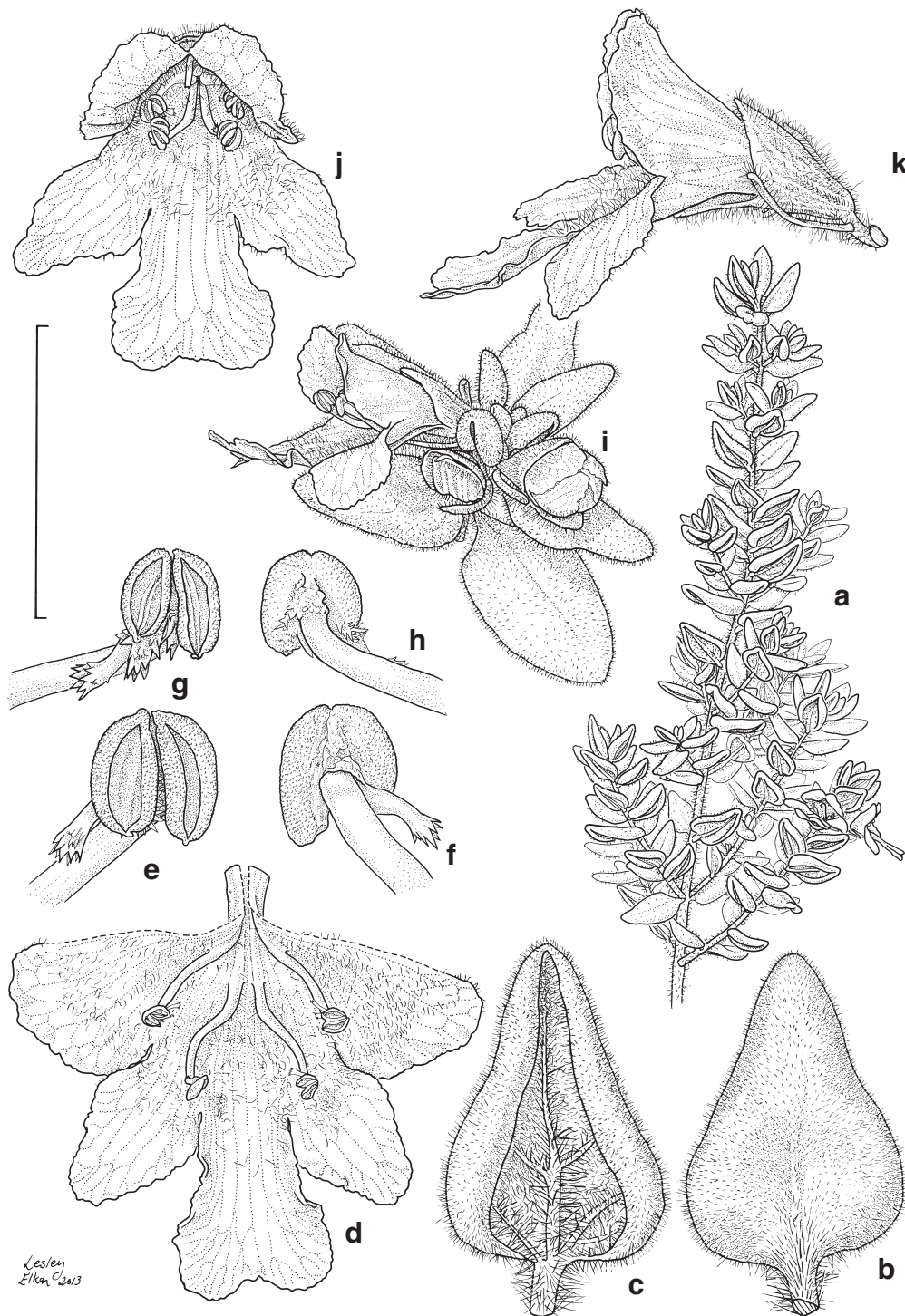


Fig. 6. Illustration of *Prostanthera densa*. **a**, habit with flowers; **b**, detail of adaxial surface of leaf showing indumentum; **c**, detail of abaxial surface of leaf showing recurved margin and indumentum; **d**, open corolla showing corolla lobes, tube and androecium; **e**, abaxial stamen, showing ventral view of dehisced anther locules, anther appendage and filament; **f**, abaxial stamen showing dorsal view of anther locules, connective, anther appendage and filament; **g**, adaxial stamen showing ventral view of dehisced anther locules, triangular trichomes on base of connective, anther appendage and filament; **h**, adaxial stamen, showing dorsal view of anther locules, connective, partial view of trichomes on base of connective, partial view of anther appendage, and filament; **i**, detail of flowering branchlets, with flowers and buds in axils of crowded leaves (internodes reduced), showing podium, prophylls, calyx, corolla and stamens in open flower; **j**, ventral view of open flower, showing open corolla, androecium and partial view of style and stigma; **k**, lateral view of open flower, showing prophylls, calyx and corolla, with abaxial stamens partially visible, and position of style and stigma indicated by stippling (all *B.J. Conn* 2563). Scale bar: **a** = 50 mm; **b**–**d** = 10 mm; **e**–**h** = 2.5 mm; **i** = 15 mm; **j** & **k** = 10 mm. Illustrator: Lesley Elkan

0.4–0.5 mm long, diameter at base 0.3–0.4 mm, lobes 0.1–0.2 mm long, glabrous; style 6–7 mm long; stigma lobes 0.2–0.3 mm long. Fruiting calyx not enlarged. Mericarps 0.8–1 mm long (presumed immature), distally c. 0.5 mm extended beyond base of style, distal diameter c. 1 mm; mature seeds slightly obovoid-cylindrical, 0.8–1.2 mm long, 0.5–0.8 mm diameter (Figs 6, 7a–d).

Habitat: Occurring in sandy soils, amongst sandstone outcrops and on sedimentary conglomerates, in low open forest and coastal shrublands, dominated by *Angophora costata*, *Corymbia gummifera*, *Eucalyptus pilularis*, *E. punctata*, *Syncarpia glomerulifera*, *Allocasuarina distyla*, *A. littoralis*, *Banksia integrifolia*, *B. spinulosa*, *Hakea sericea*, *Acacia terminalis*, *A. myrtifolia* and other myrtaceous and proteaceous shrubs.

Conservation status: This species is listed as ‘Vulnerable’ under the *New South Wales Threatened Species Conservation Act 1995* (Anonymous 07 September 2012). However, since all populations of this species appear to be endangered, these should be prioritised for conservation actions.

Selected specimens examined: NEW SOUTH WALES: North Coast: Port Stephens: Gan Gan Hill, *B.J. Conn 2601* & *B. Timmis*, 28 Sep 1987 (A, L, MEL, NSW, RSA, S); *R.G. Coveny 16459*, *S.F. McCune* & *T.M. Tame*, 10 Mar 1993 (AD, BRI, CANB, CHR, HO, K, L, MEL, MO, NSW, PE, PRE, RSA); *R. Johnstone 1003*, *S. Peel* & *Y. Murakami*, 16 Nov 2001 (NSW); *T.C. Wilson 37* & *A.J. Perkins*, 12 Dec 2006 (NSW).

Central Coast: Cronulla: Bass & Flinders Point, *B.J. Conn 2563*, 4 Jul 1987 (NSW); *4381A* & *H.M. Conn*, 18 Aug 2007 (NSW); Helensburgh: near Ridge Road, *B.J. Conn 2565* & *B. Timmis*, 18 Jul 1987 (NSW); *A.T. Fairley s.n.*, 3 Oct 2001 (NSW497964); Wilson Dam, *J.A. Scott 1–4* & *A.D. Auld*, 6 Jun 2007 (BRI, NSW); Wilson Creek Road, *T.C. Wilson 221*, *222*, *224*, 17 Oct 2009 (NSW); Royal National Park: Audley, *R.G. Coveny 4038* & *R. Bisby*, 21 Mar 1972 (NSW); Marley, *N. Byrnes s.n.*, 2 Jun 1948 (NSW128299); N of Big Marley Beach, *A.T. Fairley s.n.*, 12 Aug 2005 (NSW732721), c. 200 m off Marley Fire Trail, *T.C. Wilson 173*, 16 Apr 2008 (NSW).

South Coast: Jervis Bay: Beecroft Peninsula: Abrahams Bosom Reserve, *B.J. Conn 2566–2571* & *B. Timmis*, 18 Jul 1987 (NSW), *M.J. Henwood 800*, anno 2006 (SYD); ‘Trig Station’, *B.J. Conn 2572–2574* & *B. Timmis*, 18 Jul 1987 (NSW); Cabbage Tree Beach, *L. McDougall 84*, 28 Aug 1988 (NSW); Jervis Bay, *H. Posamentier s.n.*, Nov 1970 (NSW269427); Devils Hole, 2 miles NE of Point Perpendicular, *E.F. Constable s.n.*, 28 Feb 1960 (NSW); Currarong Road at Point Perpendicular Road junction, *T.A. James 2* & *R.G. Coveny*, 29 Sep 1981 (NSW); 30 m N of Currarong Road, *J. Miles s.n.*, 25 Apr 2002 (NSW496550).

Notes: Hamilton (1920) recorded *P. densa* as growing in ‘profusion on the ocean slope of the rocky headland ... at Cronulla ... confined to a limited area between the ocean beach at Cronulla and the northern entrance to Port Hacking.’ The only extant population from the type locality now appears to be restricted to a few plants near Bass and Flinders Point.

2. *Prostanthera marifolia* R.Br. *Prodromus* (1810) 509.

Lectotype (here chosen): *R. Brown s.n. [J.J. Bennett 2371]*, anno 1802–1805 [include data from WT Stearn ‘Introduction’ to Brown (1960)], ‘10 *Prostanthera marifolia* Prodr. 509’ (handwritten label upper left of sheet), ‘New South Wales: North shore of Sydney district (Port Jackson)’ (lower right printed label), ‘North Shore, Port Jackson’ (on reverse side of label/sheet) (BM); probable isolectotypes: ‘*Prostanthera marifolia* Port Jackson’ (upper right handwritten label), ‘New South Wales: North shore of Sydney district (Port Jackson)’ (lower right printed label – as for lectotype) (BM); ‘*Prostanthera marifolia* North shore of Sydney’ (handwritten label near lower centre of sheet attached to following label), ‘R. Brown, Iter Australiense, 1802–5 [Presented by direction of J.J. Bennett 1876] Ex. Herb. Hort. Bot. Reg. Edin.’ (printed label) (LE – lower half of sheet and fragments upper left); ‘*Prostanthera marifolia* Prodr. 509 Port Jackson’ (handwritten label near lower right of sheet, in same hand as lectotype, with ‘Brown’ inserted in another hand), ‘Port Jackson Brown Herb. Swartzii’ (handwritten label lower right of sheet) (S)

Synonym: *Prostanthera* sp ‘Manly Dam’ (Conn 4444). Based on: New South Wales: Central Coast: Manly-Warringah area, *Conn 4444*, *Stevenson* & *Ewings*, 07 Mar 2002 (CANB, BRI, NSW).

Erect, openly branched shrub up to c. 0.3 m high; faintly aromatic. Branches subterete, sparsely to moderately hairy [16–20 hairs/mm²]; hairs ± straight, spreading or slightly retrorse, c. 0.3–0.6 mm long, white; sparsely glandular [up to 6 glands/mm²]. Leaves green, faintly aromatic (when crushed), sparsely to moderately hairy [as for branches]; sparsely glandular [as for branches]; petiole 0.5–1 mm long; lamina ovate to almost elliptic, 8–12(–15) mm long, 4–6(–8) mm wide [length to width ratio 1.7–2.2, length of maximum width from base to total lamina length ratio 4–6]; base shortly attenuate; margin entire or occasionally slightly 1-lobed on each side, rarely with one additional lobe, may appear bluntly 1-toothed on each side because margin slightly recurved; apex rounded; venation not visible, except midrib raised on lower surface. Inflorescence a frondose racemiform conflorescence, uniflorescence monadic; 2–8(–12)-flowered [per conflorescence]. Pherophylls absent. Podium 1–1.5(–2.3) mm long, sparsely to moderately hairy [as for branches], sparsely glandular [as

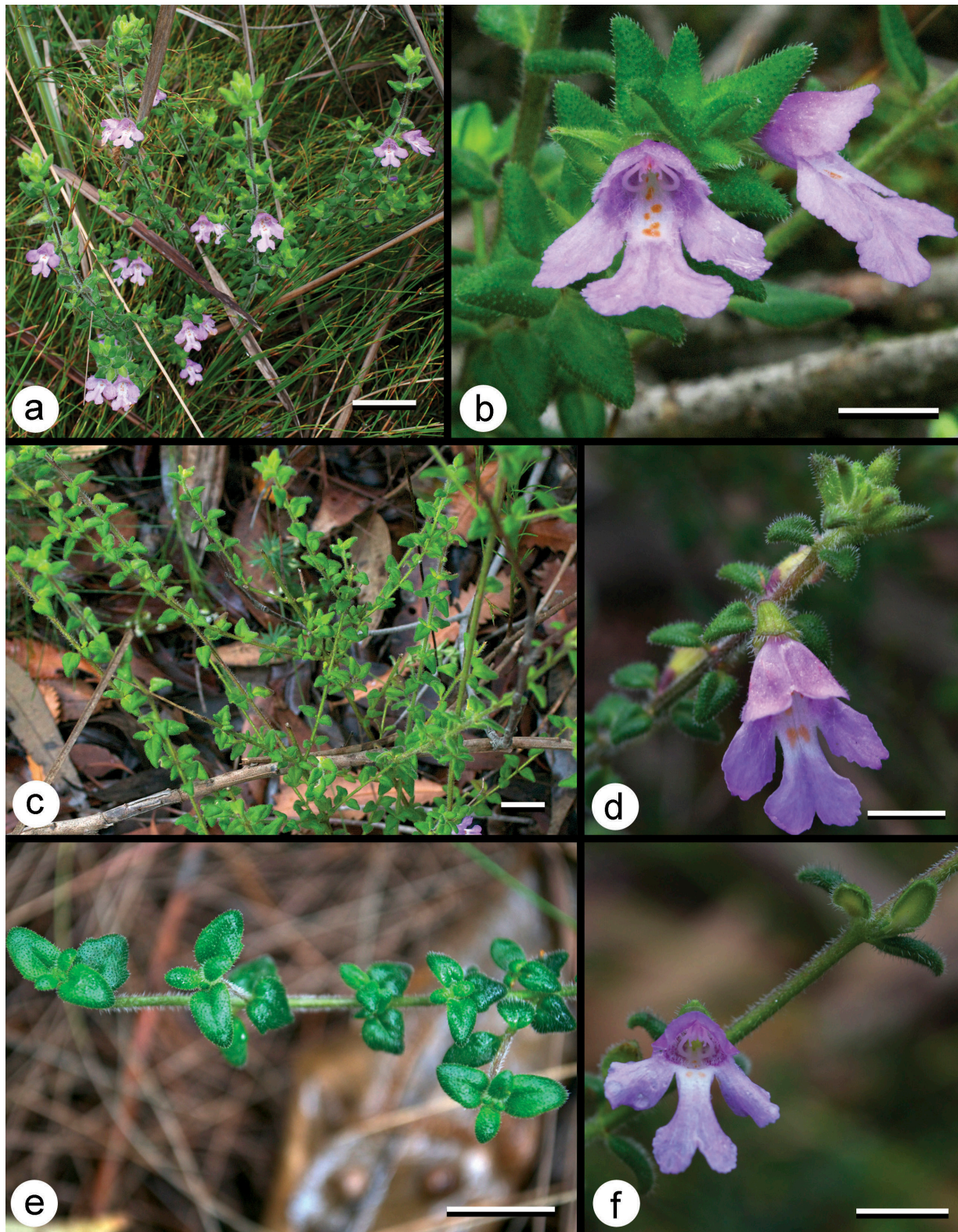


Fig. 7. Images of *Prostanthera densa* and *P. marifolia*. **a**, habit of *P. densa* from Currarong. **b**, flower and leaves of *P. densa* from Currarong. **c**, habit of *P. densa* from near Helensburgh. **d**, flower and leaves of *P. densa* from near Helensburgh. **e**, habit of *P. marifolia*. **f**, flower and leaves of *P. marifolia*. Scale bar: a, c & e = 3 cm; b, d & f = 1 cm. Photographs: T.C. Wilson.

for branches]. *Prophylls* inserted near middle or on distal half of podium [a_1 axis to anthopodium ratio 1.2–1.9], opposite, narrowly elliptic to narrowly obovate, 1–1.5 mm long, c. 0.2 mm wide [length to width ratio 5–8, length of maximum width from base to total lamina length ratio 0.6–0.9], with long spreading hairs [as for branches]; base attenuate or truncate to very slightly broader; margin entire; apex obtuse. *Calyx* light green, with mauve-purple tinge adaxially; outer surface sparsely to moderately hairy [10–18 hairs/mm²], hairs spreading, 0.4–0.8 mm long; sparsely to moderately glandular [10–15 glands/mm²]; tube 1.3–1.8(–2) mm long; *abaxial lobe* very broadly ovate, 2.5–3.8 mm long, 2.2–3.2 mm wide [length to width ratio 1.1–1.2], *apex* obtuse to rounded; *adaxial lobe* very broadly ovate, 2.5–3.6 mm long, 2.5–3 mm wide [length to width ratio 1–1.2], *apex* obtuse [adaxial lobe length to abaxial lobe length ratio 0.9–1]. *Corolla* (9–)10–15 mm long, mauve, white on inner abaxial surface of tube; outer surface sparsely to moderately hairy [10–30 hairs/mm²], sparsely glandular [10–16 glands/mm²]; inner surface sparsely to moderately hairy (similar to outer surface); *tube* (5–)6–8 mm long; *abaxial median lobes* very broadly obovate or spatulate, 8–10(–12) mm long, 6–7(–9) mm wide [length to width ratio 0.9–1.2], *apex* bilobed (sinus 1.8–3 mm long, 2–3 mm wide distally); *lateral lobes* obovate to slightly oblong, 6–8(–9) mm long, 4–6 mm wide [length to width ratio 1–1.5], *apex* slightly undulate, obtuse; *adaxial median lobe-pair* depressed ovate, 3–4(–4.5) mm long, 6.5–8(–9) mm wide [length to width ratio 0.4–0.5], deeply bilobed (sinus c. 3 mm long), *apex* of each lobe rounded. *Stamens* inserted 1.5–2 mm above base of corolla; filaments 3–3.5 mm long, glabrous; anthers 0.8–1 mm long, lobes slightly cristate on basal dorsal surface (trichomes narrowly triangular, c. 0.1 mm long), lobes with basal acumen to 0.1–0.2 mm long, connective extended to form basal appendages 0.6–0.9 mm long, terminating in a few triangular trichomes c. 0.1 mm long. Disc \pm cylindrical, 0.2–0.3 mm long. *Pistil* 6–8 mm long; *ovary* cylindrical to obovoid, 0.4–0.5 mm long, diameter at base 0.3–0.4 mm, lobes 0.1–0.2 mm long, glabrous; *style* 6–7 mm long; *stigma lobes* 0.3–0.4 mm long. *Fruiting calyx* not to very slightly enlarged (abaxial lobe 3.5–3.8 mm long, c. 3.5 mm wide [length to width ratio 1–1.1]; adaxial lobe 3.5–3.7 mm long, c. 3.5 mm wide [length to width ratio 1–1.1]; [adaxial lobe length to abaxial lobe length ratio c. 1]). *Mericarps* 1.5–1.8 mm long, distally c. 0.5 mm extended beyond base of style, distal diameter c. 1 mm; mature seeds slightly obovoid-cylindrical, c. 1 mm long, 0.5–0.7 mm diameter (Figs 7e, 7f and 8).

Habitat: Duffy’s Forest Ecological Community. Woodland dominated by *Eucalyptus sieberi* and *Corymbia gummifera*, growing in deeply weathered clay soil with ironstone nodules.

Conservation status: This species is listed as ‘Critically endangered’ under the *New South Wales Threatened Species Conservation Act 1995* (Anonymous 28 February 2011) and *Commonwealth Environment Protection and Biodiversity Conservation Act 1999* (Anonymous 2013) and as a consequence, the reference to this species being “presumed extinct” (Part 4 of Schedule 1 of the Act) has been omitted. Listing of critically endangered species is provided for by Part 2 of the Act” (Hughes 28 February 2011).

Selected specimens examined: NEW SOUTH WALES: Central Coast: Mosman, R.H. Cabbage 696, 20 Sep 1902 (SYD), Chapman s.n., 13 Jan 1907 (SYD); Manly-Warringah area: A.D. Auld 11, 03 Oct 2002 (NSW 499352); B.J. Conn 4443a, Stevenson & Ewings, 07 Mar 2002 (CANB, MEL, NY, NSW), 4443b (CANB, MEL, NY, NSW), 4444, (CANB, BRI, NSW); 4380A & J.T. Hadiyah, 18 Mar 2007 (NSW); 5676, J.T. Hadiyah & T.C. Wilson, 19 Aug 2011 (NSW); N.J. Skelton s.n., 29 Jan 2002 (NSW495446); D. Stevenson M000–M004, M006, 19 Oct 2001 (NSW); Hunters Hill, A.A. Hamilton s.n., Sep 1906 (NSW134433); ‘Port Jackson’, A. Cunningham s.n., anno 1836 (LE – upper half of sheet); ‘Suspension Bridge’, Johnston s.n., 20 Jan 1903 (SYD); Northbridge, H.S. McKee s.n., 17 Nov 1951 (SYD); Longueville, Lane Cove, H.M.R. Rupp s.n., Apr 1917 (MEL43365).

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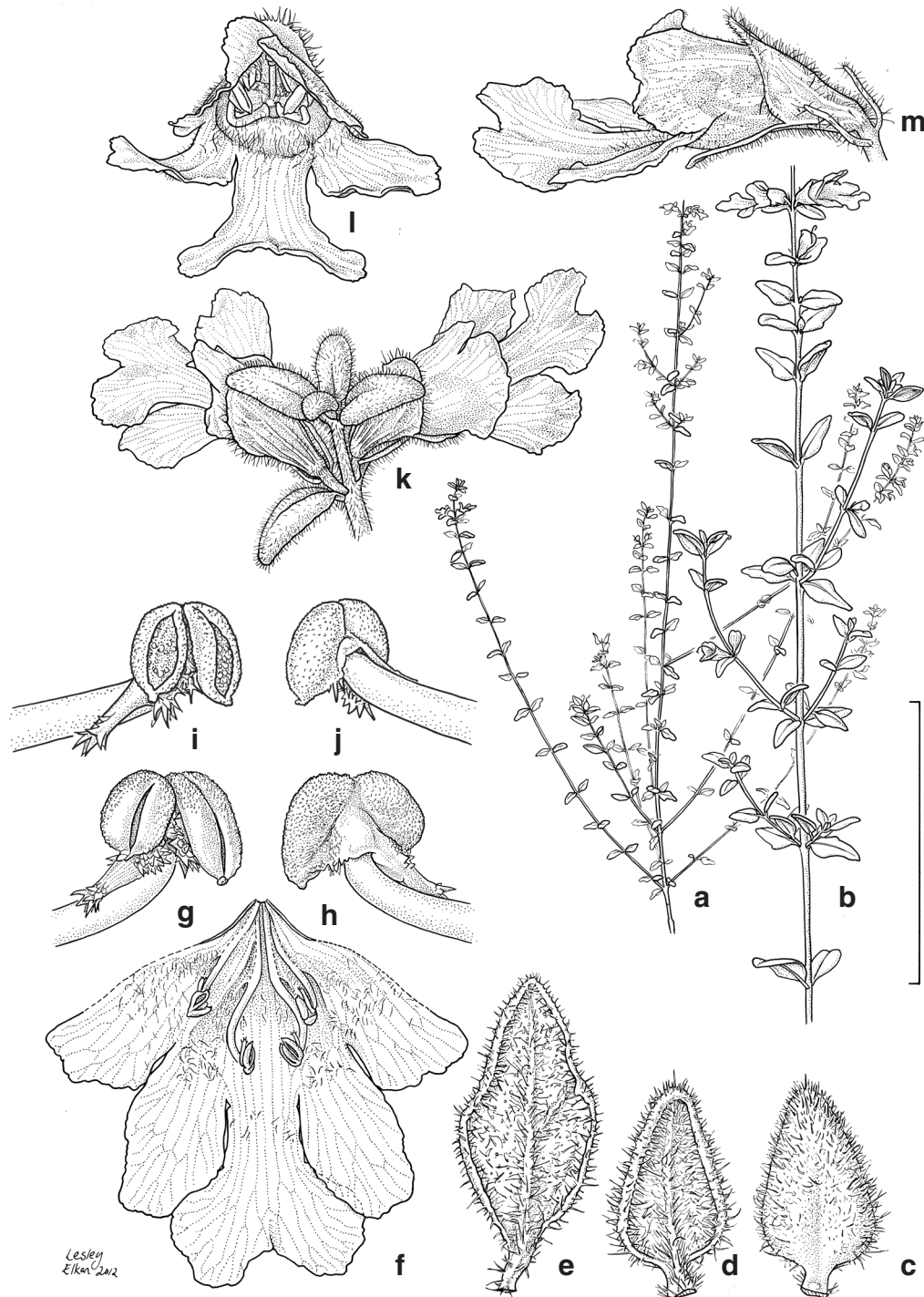


Fig. 8. Illustration of *Prostanthera marifolia*. **a**, branchlet showing open habit; **b**, detail of branchlet with flowers and developing fruits; **c**, detail of abaxial surface of leaf, showing hairs and slightly toothed, recurved margin; **d**, detail of abaxial surface of leaf, with margin lacking teeth; **e**, detail of adaxial surface of leaf; **f**, open corolla showing corolla lobes, tube and androecium; **g**, abaxial stamen, showing ventral view of dehiscent anther locules, anther appendage, triangular trichomes on connective, and filament; **h**, abaxial stamen showing dorsal view of anther locules, connective with triangular trichomes, anther appendage and filament; **i**, adaxial stamen showing ventral view of dehiscent anther locules, triangular trichomes on base of connective, anther appendage and filament; **j**, adaxial stamen, showing dorsal view of anther locules, connective, partial view of trichomes on base of connective, partial view of anther appendage, and filament; **k**, detail of flowering branchlets, with flowers in axils of leaves, internodes extended, showing podium, prophylls, calyx, and corolla of open flower; **l**, ventral view of open flower, showing open corolla, androecium and partial view of style and stigma; **m**, lateral view of open flower, showing prophylls, calyx and corolla, with abaxial stamens partially visible, and position of style and stigma indicated by stippling (a, b & e: B.J. Conn 4443a; c, d, f–m: T.C. Wilson 59). Scale bar: a = 150 mm, b = 50 mm, c–f = 10 mm, g–j = 2.5 mm, k = 15 mm, l & m = 10 mm. Illustrator: Lesley Elkan.

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