Trichome morphology provides phylogenetically informative characters for *Tremandra*, *Platytheca* and *Tetratheca* (former Tremandraceae)

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This paper was published in *Plant Systematics and Evolution* 271: 199-221 (2008)
(published online 21 March 2008).

Abstract. Trichomes of *Tremandra* R.Br. ex DC., *Platytheca* Steetz and *Tetratheca* Sm. (Elaeocarpaceae, former Tremandraceae), together with two outgroup species of *Elaeocarpus* L., are illustrated using Scanning Electron Microscopy, and their distribution on various plant organs is documented. Various trichomes types were identified that relate taxa: simple hairs, stellate hairs, short glandular trichomes, long glandular trichomes, and three forms of tubercules. Both outgroup and ingroup taxa have simple hairs. Stellate hairs are confirmed as unique to *Tremandra*. Prominent and sculptured multi-celled tubercules, some bearing a stout hair, are characteristic of *Platytheca*. Smaller multicelled tubercules occur in both *Platytheca* and *Tetratheca*, except for the Western Australian taxon *Te. filiformis* (possibly plesiomorphic). Unicellular tubercules (papilla) characterise two species of *Tetratheca*. Short glandular trichomes, usually found on the ovary, also occur in both of these genera but not in all species (possibly secondary losses), while long glandular trichomes, usually on stems and leaves, occur only in some groups of *Tetratheca*. Within *Tetratheca*, Western Australian taxa that have five-merous flowers fall into three ‘groups’: seven species (together with one from South Australia) that have short glandular trichomes but no long glandular trichomes; six species that have long glandular trichomes but no short glandular trichomes; and four species that have both trichome types. All other species of *Tetratheca* have four-merous flowers and form two ‘groups’: twelve eastern species (including one from South Australia) that have both short glandular trichomes and long glandular trichomes; four western species and six eastern species that lack short glandular trichomes. Based on these characters, a phylogenetic hypothesis for the three genera is presented.

Keywords: *Tremandra*, *Platytheca*, *Tetratheca*, Elaeocarpaceae, trichomes, scanning electron microscopy, phylogeny

Introduction

Tremandraceae are a small family of three genera endemic to temperate regions of Australia, predominantly in the southern regions of the mainland and Tasmania (Fig. 1). Crayn et al. (2006) concluded that the genera are a dry-adapted clade, related to rainforest taxa within Elaeocarpaceae, that dates back to the Paleocene but radiated during the

Oligocene-Miocene with the onset of greater aridity in Australia. Molecular studies, including exemplar taxa, indicated that Tremandraceae is sister to (Savolainen et al. 2000a, b), or nested within (Soltis et al. 2000; Crayn and Rossetto 2003; Crayn et al. 2006) Elaeocarpaceae. Some recent classifications have formally sunk Tremandraceae into Elaeocarpaceae (APG 2003, Coode 2004), but the group is considered monophyletic.

Fig. 1. Distribution of the tremandroid clade (Elaeocarpaceae, formerly Tremandraceae).

The three genera of the former Tremandraceae (hereafter called the tremandroid clade) are *Tremandra* R.Br. ex DC., *Platytheca* Steetz and *Tetratheca* Sm., including 49 species. Species are small perennial shrubs, ranging from almost prostrate alpine plants to shrubs not much more than one metre in height, and the majority are localised endemics. Stamens open by a terminal pore, situated adjacent to the anther in *Tremandra* or surmounting an anther tube of varied length and colour in *Platytheca* and *Tetratheca*, except in *Te. gunnii* (Thompson 1976).

Tremandra is characterised by the presence of stellate hairs and includes only two species, *Tr. stelligera* R.Br. and *Tr. diffusa* DC., both of which are endemic to south-western Western Australia. *Platytheca* also includes only two species, characterised by verticillate leaves and an inner whorl of anthers that are broader and longer than the outer whorl. Both species are endemic to southwestern Western Australia, with *P. galioides* Steetz widespread (a distribution similar to *Tremandra*) and *P. juniperina* Domin a narrow endemic, known from few, scattered populations.

Tetratheca is the largest of the three genera, with a combination of characters but no clear synapomorphy. Major taxonomic works on *Tetratheca* include those by Steetz

(1845, 1853), Schuchardt (1853), Bentham (1863) and Thompson (1976). Thompson's revision (1976) recognised 39 species of *Tetratheca*, and was the basis of the most recent flora accounts of the family for Australia: Victoria (Jeanes 1999), New South Wales (Gardner and Murray 1992; Carolin and Tindale 1993), Queensland (Stanley and Ross 1983), South Australia (Stove 1986), and Western Australia (Wheeler 1987; Grieve 1998; Wheeler et al. 2002). Since Thompson's revision an additional six species have been described (Alford 1995; Butcher and Sage 2005; Butcher 2007) and there are still several undescribed species in south-western Western Australia (Butcher 2007; Western Australian Herbarium 2007). Also, *Te. procumbens* Hook.f., which Thompson (1976) reduced to synonymy under *Te. pilosa* Labill., was reinstated by Jeanes (1996), and *Te. elliptica* Joy Thoms. is now considered to be a synonym of *Te. setigera* Endl. (Western Australian Herbarium 2007). *Tetratheca* currently contains 45 described species. *Tetratheca* occurs in both eastern and western mainland Australia as well as Tasmania; however, no species is found on both sides of the continent. The group, with its high frequency of regional endemics (Butcher et al. 2007), has the potential to contribute to knowledge of the biogeographic history of regions in south-western and eastern Australia.

Since the time of Steetz (1845), various attempts have been made to divide *Tetratheca* into tribes and sections based on characters such as stamens, seeds, ovules, leaf form and indumentum; none has been satisfactory. Comparative documentation of morphological characters of all taxa is required for phylogenetic analysis, since molecular data may not be fully informative (Crayn and McPherson pers comm.). There have been some anatomical studies, based on only a limited number of species, of wood (Carlquist 1977), flowers (Saunders 1939; Suvatha et al. 1984; Laxmi and Narayana 1987; Rani 1995; Matthews and Endress 2002), pollen (Erdtman 1972; Johri et al. 1972), ovules and seeds (van Tiegham 1906; Berg 1975; Corner 1976; Biddle and Christophel 1978; Boesewinkel 1999). Thompson (1976) and Butcher (2007) noted that the type and/or combination of

trichomes present, of which Thompson (1976) recognised six types, are important characters for identification of species within *Tetradthea*; these trichomes, however, have yet to be documented in detail for potential use in phylogenetic analyses.

Our aim was to define and illustrate the characteristic trichome-types of these taxa using scanning electron microscopy, and document their distribution, combination and abundance on both vegetative and reproductive organs.

Materials and methods

A total of 16 species, covering the three genera, *Platythea*, *Tremandra* and *Tetradthea*, was examined using scanning electron microscopy (SEM). These species were selected to ensure that all trichome types were illustrated and that representative taxa from across the geographic range of the genera were sampled. Two outgroup species of *Elaeocarpus* L., *E. holopetalus* F.Muell. and *E. reticulatus* Sm., were also studied, representing members of the sister clade identified by Crayn et al. (2006). Details of specimens and material examined for the SEM study are given in Table 1. In addition, all 49 species of the tremandroid clade, with the exception of four recently described and rare Western Australian species (Butcher and Sage 2005; Butcher 2007) and *Te. fasciculata* Joy Thoms., for which there was insufficient material available, were surveyed by light microscopy (LM) to document trichome distribution on different plant organs. A total of 775 specimens was examined for the LM analysis. All species observed are given in Tables 2 and 3.

Plant material for SEM was obtained from herbarium collections held at MEL, MELU and from collections made in the field; herbarium abbreviations follow Holmgren et al. (1990). Where possible, all major organs of a specimen were examined under the SEM, including stems, leaves, flowers, fruits and seeds. For some taxa availability of floral material was limited, and fruits and seeds were noticeably under-represented in herbarium collections and could not be scored for a number of species.

Material was preserved either using 70% Ethanol or by air-drying. Specimens preserved in 70% ethanol were dehydrated through a graded ethanol series and then critical point dried. All samples were mounted on aluminum SEM stubs using double-sided carbon tabs and coated with gold using an Edward's S150B Sputter Coater. The samples were viewed and photographed using a Philips XL30 Field Emission Scanning Electron Microscope (FE SEM). One image, of a fruit of *E. holopetalus* (see Fig. 13a), was captured using a Leica DC 300F digital camera. The camera was mounted on a Leica MZ FLIII Dissector Stereo Light Microscope using a 0.63x adaptor.

Results

Trichome types. Trichomes observed by SEM were divided into the following categories: stellate hairs (Fig. 2), simple hairs (Fig. 3), short glandular trichomes (Fig. 4), long glandular trichomes (Fig. 5) and three types of tubercles (Fig. 6). Trichome types are considered to be independent characters since they can occur in combination on a particular plant (Table 2).

Stellate hairs (Fig. 2), with four to eight rays, were only observed in *Tremandra*, in both species. They occur on various organs, including leaves, stem (Fig. 2a), surface of the capsule (Fig. 2b), pedicel (Fig. 2c), ovary and style (Fig. 2d) and sepals (Fig. 2e, f), but vary in size, especially on leaves.

Simple hairs (Fig. 3) are present in all four genera (ingroup and outgroup), with or without other trichome types. Simple hairs are unicellular and unbranched, and may be curled (Fig. 3a *E. holopetalus*) or more or less straight (e.g., Fig. 3b *P. galioides*, Fig. 3c *Te. thymifolia*, Fig. 3d *E. reticulatus*) and erect (Fig. 3e *Te. hirsuta*, Fig. 3f *Te. ciliata*, Fig. 3g *Te. stenocarpa*) to appressed (Fig. 3h-i *Tr. diffusa*). They also vary in length and may be slender (Fig. 3f *Te. ciliata*) or with stout bases (Fig. 3g *Te. stenocarpa*). Simple hairs occur on most plant parts. An unusual form of simple hair was observed on the seeds on both species of *Platythea* (Fig. 3j-k).

Table 1. Taxa, voucher details, method of preservation and material examined for the trichome SEM study. Specimens examined were either preserved in 70% Ethanol (E) or obtained from air-dried herbarium voucher specimens (D). Plant material examined for this study is indicated by +; material not examined for a species is indicated by -. TD = T.L. Downing.

Taxa	Herbarium	Primary Collector	Collector Number	Preservation method	Stem	Leaf	Pedicel	Sepal	Stamen	Ovary	Fruit
<i>Elaeocarpus holopetalus</i>	MELU	I.C. Clarke	1373	D	+	+	-	-	-	-	-
<i>E. reticulatus</i>	MELU 15331	D.H. Ashton		D	+	+	+	+	+	-	+
<i>Tremandra diffusa</i>	HO, MEL	A.C. Rozefelds	1542	E	+	+	-	-	-	-	+
<i>Tr. diffusa</i>	MEL 2244024	P.G. Wilson	6290	D	-	-	-	+	+	+	-
<i>Tr. stelligera</i>	MEL 2243331	A.C. Rozefelds	1554	D	-	-	+	+	+	+	-
<i>Platytheca galioides</i>	MEL	P. Ladd	(TD 25)	D	+	+	+	+	+	+	-
<i>P. galioides</i>	MELU	T.L. Downing	99	E	-	-	-	+	-	+	-
<i>P. juniperina</i>	HO, MEL	A.C. Rozefelds	1559	E	+	+	+	+	+	+	+
<i>Tetratheca affinis</i>	MEL 603471	B.L. Turner	5505	D	+	+	+	+	+	+	-
<i>Te. bauerifolia</i>	MEL	T.L. Downing	38	E	+	+	+	+	+	-	-

<i>Te. ciliata</i>	MEL	T.L. Downing	30	D	+	+	+	+	+	+	+	-
<i>Te. hirsuta</i>	MEL 606108	M.G. Corrick	7826	D	+	+	+	+	+	+	+	-
<i>Te. hispidissima</i>	MEL 293565	T. R. Lally	1522	D	+	+	+	+	+	+	-	-
<i>Te. insularis</i>	MEL	T. L. Downing	66	E	+	+	+	+	+	+	+	-
<i>Te. labillardierei</i>	MEL	M.F. Duretto	1568	E	+	+	+	+	+	+	+	-
<i>Te. nuda</i>	MEL 1008421	E. Rowan		D	+	-	+	+	+	+	+	-
<i>Te. pilosa</i>	MEL	M.F. Duretto	1296	E	+	+	+	+	+	+	+	-
<i>Te. pubescens</i>	MEL	P. Ladd	(TD 40)	E	+	+	+	+	+	+	+	-
<i>Te. stenocarpa</i> (pink)	MEL	T. L. Downing	74	D	+	+	+	+	+	+	+	+
<i>Te. stenocarpa</i> (white)	MEL	T. L. Downing	77	D	+	+	+	+	+	+	+	+
<i>Te. stenocarpa</i>	MEL	T.L. Downing	97	E	+	-	+	+	+	-	-	-
<i>Te. stenocarpa</i>	MEL	T.L. Downing	98	E	+	-	+	+	+	-	-	-
<i>Te. thymifolia</i>	MEL	M.F. Duretto	1285	E	+	+	+	+	+	+	+	-

Table 3. Summary of trichomes across taxa: (1) stellate hairs, (2) simple hairs, (3) short glandular trichomes, (4) long glandular trichomes, tubercles types (5i-iii). Presence +, absence -. St. stamen number. Ov. number of ovules per loculus (following Thompson (1976), Alford (1995), Butcher (2007), Butcher et al. (2007)). * Possibly part of group IV, with loss of glandular trichomes and setae. Geographical distribution of taxa: Western Australia (W) and eastern Australia (E), including New South Wales, Victoria, Tasmania and South Australia (S) which includes Kangaroo Island (KI).

Taxa	Distrib.	St.	Ov.	Characters						
				1	2	3	4	5i	5ii	5iii
<i>Elaeocarpus holopetalus</i>	E	>10	≥2	-	+	-	-	-	-	-
<i>E. reticulatus</i>	E	>10	≥2	-	+	-	-	-	-	-
<i>Tremandra diffusa</i>	W	10	2	+	+	-	-	-	-	-
<i>Tr. stelligera</i>	W	10	2	+	+	-	-	-	-	-
<i>Platytheca galioides</i>	W	10	1	-	+	+	-	+	-	+
<i>P. juniperina</i>	W	10	1	-	+	+	-	-	-	+
Group I										
<i>Tetratea filiformis</i>	W	10	1	-	+	-	-	-	-	-
Group II										
<i>Te. affinis</i>	W	10	4/5	-	+	+	-	-	-	-
<i>Te. aphylla</i>	W	10	1(2)	-	+	+	-	+	-	-
<i>Te. chapmanii</i>	W	10	2	-	-	+	-	+	-	-
<i>Te. efoliata</i>	W	10	2	-	+	+	-	-	-	-
<i>Te. nuda</i>	W	10	1	-	+	+	-	-	-	-
<i>Te. paynterae</i>	W	10	2	-	+	+	-	+	-	-
<i>Te. halmaturina</i>	S (KI)	10	1	-	+	+	-	+	-	-
Group III										
<i>Te. confertifolia</i>	W	10	1	-	+	+	+	+	-	-
<i>Te. hirsuta</i>	W	10	1	-	+	+	+	+	+	-
<i>Te. hispidissima</i>	W	10	1	-	+	+	+	+	+	-
<i>Te. paucifolia</i>	W	8/10	1	-	+	+	+	+	-	-
Group IV										
<i>Te. deltoidea</i>	W	10	1	-	+	-	+	+	-	-
<i>Te. harperi</i>	W	10	1	-	+	-	+	+	-	-
<i>Te. pubescens</i>	W	10	1	-	+	-	+	+	-	-
<i>Te. remota</i>	W	10	1	-	+	-	+	+	-	-
<i>Te. retrorsa</i>	W	10	2	-	+	-	+	+	-	-
<i>Te. setigera</i>	W	10	1	-	+	-	+	+	-	-
Group V										
<i>Te. parvifolia</i>	W	8	1	-	+	-	+	+	-	-
<i>Te. pilifera</i>	W	8	1	-	+	-	+	+	-	-

<i>Te. similis</i>	W	8	1	-	+	-	+	+	-	-
<i>Te. virgata</i>	W	8	1	-	+	-	+	-	-	-
<i>Te. decora</i>	E	8	1	-	+	-	+	+	-	-
<i>Te. juncea*</i>	E	8	2	-	-	-	+	+	-	-
<i>Te. neglecta</i>	E	8	1	-	+	-	+	+	-	-
<i>Te. rubioides</i>	E	8	1	-	+	-	+	+	-	-
<i>Te. rupicola*</i>	E	8	2	-	+	-	+	+	-	-
<i>Te. subaphylla*</i>	E	8	1	-	+	-	-	+	-	-
Group VI										
<i>Te. bauerifolia</i>	E	8	2	-	+	+	+	-	-	-
<i>Te. ciliata</i>	E	8	2	-	+	+	+	+	-	-
<i>Te. ericifolia</i>	E	8	2	-	+	+	+	+	-	-
<i>Te. glandulosa</i>	E	8	1	-	+	+	+	+	-	-
<i>Te. gunnii</i>	E	8	1	-	+	+	+	+	-	-
<i>Te. insularis</i>	S (KI)	8	1	-	+	+	+	+	-	-
<i>Te. labillardierei</i>	E	8	1	-	+	+	+	-	-	-
<i>Te. pilosa ssp latifolia</i>	E	8	1	-	+	+	+	+	-	-
<i>Te. pilosa ssp pilosa</i>	E	8	1	-	+	+	+	+	-	-
<i>Te. procumbens</i>	E	8	1	-	+	+	+	+	-	-
<i>Te. shiressii</i>	E	8	2	-	+	+	+	+	-	-
<i>Te. stenocarpa</i>	E	8	2	-	+	+	+	+	-	-
<i>Te. thymifolia</i>	E	8	2	-	+	+	+	+	-	-

Fig. 2. Stellate trichomes typical of *Tremandra*. *Tr. diffusa*: (a) stem and (b) the surface of the capsule (fruit). *Tr. stelligera*: (c) pedicel (flower stalk), (d) base of style near ovary, (e) and (f) upper sepal surface. Scale bars = 200 μm .

Fig. 3. Simple hairs: (a) lower leaf surface of *E. holopetalus*; (b) style of *P. galioides*; (c) pedicel of *Te. thymifolia*; anther bodies of (d) *E. reticulatus* and (e) *Te. hirsuta*; (f), ovary of *Te. ciliata*; seeds of (g) *Te. stenocarpa*, (h-i) *Tr. diffusa*; (j-k) seed of *P. juniperina*. Scale bars = 100 μ m.

Fig. 4. Short glandular trichomes. *P. galioides* (a, b) edge of sepal, (c) outer sepal surface, also with simple hairs; ovaries of (d) *Te. labillardierei*, (e) *Te. affinis* and (f) *Te. insularis*. Scale bars = 200 μ m.

Fig. 5. Long glandular trichomes (stout, bristle-like trichomes). *Te. stenocarpa* (a) pedicel, showing size variation in long glandular trichomes (simple hairs also present); stems; of (b) *Te. hirsuta* and (c) *Te. pilosa*. *Te. stenocarpa*, showing long and short glandular trichomes together: (d) young stem and (e) pedicel. Scale bars = 500 μm .

Fig. 6. Tubercles. Type i typical of *Tetratheca*: (a) on recurved leaf margin of *Te. thymifolia*, and (b) upper leaf surface of *Te. pilosa*, associated with a seta. Type ii (c-d) on anther tube of *Te. hirsuta*. Type iii typical of *Platytheca*: (e-f) base of anther tube of *P. juniperina*. Unique surface sculpture on stamen surface of *P. galioides* (g-h). Scale bars = 100 μ m.

These hairs are relatively large, flat, narrow-elliptic in outline and have narrowed bases and acuminate tips. They are much larger than the simple hairs which are also present on the seeds. Thompson (1976, her Table 1) referred to them as 'inflated or flat deflated hairs'. Due to the small number of seeds observed in the study this feature (autapomorphic) is not scored in Tables 2 and 3.

Short glandular trichomes (Fig. 4) are stout and topped by a gland of approximately 20-36 cells. They vary in height of the stalk and size of the gland and when observed under the light microscope are often reddish in colour. Short glandular trichomes are sparse but present on leaves and sepals of *Platytheca* (Fig. 4a-b). Approximately half of the *Tetralthea* species examined exhibited short glandular trichomes, usually on the ovary or sepals (e.g. Fig. 4c-d *Te. labillardierei*, Fig. 4e *Te. affinis*, Fig. 4f *Te. insularis*), but sometimes on pedicels and leaves, although there was some variability in distribution among specimens of a single taxon. Short glandular trichomes were not observed in *Elaeocarpus* or *Tremandra* (Tables 2, 3). Butcher (2007, their Fig. 1f) illustrated small glandular trichomes on the ovary of *Te. paucifolia*, which had small simple hairs radiating from their shaft.

Long glandular trichomes are similar to the short glandular trichomes, with which they may occur, but are bristle-like, with the gland at the tip relatively small and sometimes broken off (Fig. 5). They were only observed in *Tetralthea* (Tables 2, 3), where they occur on pedicels, stems and leaves (Fig. 5a, d, e *Te. stenocarpa*, Fig. 5b *Te. hirsuta*, Fig. 5c *Te. pilosa*) and are usually absent from floral parts. *Tetralthea labillardierei*, *Te. parvifolia* and *Te. stenocarpa* were exceptions, with all three taxa having long glandular trichomes on sepals. With LM the stout hairs observed on the leaves and sepals of *P. galioides* may appear similar to long glandular trichomes but are not gland-tipped.

Tubercles (Fig. 6) are wart-like outgrowths categorized as three types. Type (i) tubercles are multicellular and appear to be derived from the base of simple hairs or long glandular trichomes; they were observed on at least one organ in the majority of species of

Tetralthea (Fig. 6 a-b) and in *Platytheca*. Type (ii) unicellular tubercles or papilla are not associated with hairs, and were found on the anther tube of two species: *Te. hirsuta* (Fig. 6c-d) and *Te. hispidissima*. Type (iii) are prominent, sculptured multicellular tubercles characteristic of *Platytheca*, found associated with stout hairs on the stamens of *P. juniperina* (Fig. 6e-f) and *P. galioides*. Tubercles were absent in *Elaeocarpus*, *Tremandra* and a few species of *Tetralthea*, notably *Te. filiformis* and *Te. virgata*. *Platytheca galioides* also had an unusual sculptured epidermis on the stamens (Fig. 6g-h).

Distribution of trichomes. The distribution of trichome types across major plant organs is given in Table 2 for all sampled taxa examined with both LM and SEM.

Stems. The stems of *Tr. diffusa* and *Tr. stelligera* are covered in stellate trichomes, while those of *P. galioides* and *P. juniperina* are glabrous. Simple hairs were found on *Elaeocarpus* and *Tetralthea*. *Tetralthea* exhibits the widest range of variation of stem trichomes, often with more than one type present and with varying degrees of density. For example, stems of *Te. bauerifolia* and *Te. ciliata* only have simple hairs, *Te. hispidissima* (Fig. 7a), *Te. labillardierei* (Fig. 7b) and *Te. pilosa* have more than one type of trichome, while *Te. affinis* and *Te. nuda* are glabrous. In some species, such as *Te. pubescens* (Fig. 7c-d) and *Te. stenocarpa*, the stem becomes glabrous with age.

Leaves. In general, leaves have a denser indumentum on the lower surface than on the upper surface (Fig. 8). Some taxa have few hairs or are glabrous on both surfaces (e.g. *P. galioides*, *P. juniperina*, *Te. bauerifolia* and *Te. stenocarpa*). One or more trichome type may be present on leaves. For example, leaves of *Te. insularis* (Fig. 8g-h) exhibit simple hairs, type (i) tubercles and long glandular trichomes.

Fig. 7. Stems of (a) *Te. hispidissima* and (b) *Te. labillardierei* illustrating combination of trichomes; *Te. pubescens* comparison of (c) young and (d) old stem. Scale bars = 500 μm.

Fig. 8. Comparison of upper and lower leaf surfaces. *E. holopetalus* (a) lower and (b) upper; *Tr. diffusa* (c) lower and (d) upper; *Te. hispidissima* (e) lower and (f) upper; *Te. insularis* (g) lower and (h) upper. Scale bars = 500 µm.

Leaf trichomes were not scored for two species (*T. subaphylla* and *T. affinis*, Table 2) because they are either leafless or have reduced, scale-like leaves.

Sepals. Simple hairs were observed on the inner surfaces of sepals, concentrated on the margin and central vein (e.g., Fig. 9, a,c,e, g,i). Greater trichome variation was observed on the outer surfaces: for example, *Te. labillardierei* (Fig. 7i-j) has both simple and short glandular trichomes on the outer surface.

Stamens. The stamen filament in *Elaeocarpus* is longer than any observed in *Platytheca* or *Tetratheca*, and both the filament and anther body are covered in short simple hairs (Fig. 10a-b). *Tremandra diffusa* (Fig. 10c) is glabrous but *Te. stelligera* has short simple hairs (Fig. 10d). The anthers of *P. galioides* (Fig. 10e) and *P. juniperina* (Fig. 10f) have both simple hairs (long in the latter species) and type (iii) prominent multicelled tubercles, mainly on the anther tube. Most species of *Tetratheca* (Fig. 10g-l) have glabrous stamens, with only a very few scattered and short simple hairs present on the anther body of species such as *Te. affinis* (Fig. 10h) and *Te. nuda*. Type (ii) tubercles (Fig. 4) are evident on the stamens of *Te. hirsuta* (Fig. 10g) and *Te. hispidissima* (Fig. 10k).

Ovary. The ovary of *Tremandra* is covered in stellate hairs (Fig. 11a-b). Both ovary and style of *P. galioides* (Fig. 11c) are densely covered in simple hairs whereas *P. juniperina* (Fig. 11d) is glabrous with simple hairs only on the style. The ovary in *Tetratheca* is more variable (Fig. 11e-l). Some species have a covering of long hairs that may be dense (Fig. 11e *Te. thymifolia*) or less dense (Fig. 11i *Te. pilosa*) or glabrous (Fig. 11g *Te. pubescens*). The ovary of *Te. labillardierei* (Fig. 11f) is densely covered with both short glandular trichomes and simple hairs, while *Te. insularis* (Fig. 11j) has scattered short glandular trichomes among a dense covering of short simple hairs. Only short glandular hairs are present on the ovary of *Te. affinis* (Fig. 11l). In some taxa, including *Te. labillardierei* (Fig. 11f), *Te. stenocarpa* (Fig. 11h), *Te.*

insularis (Fig. 11j) and *Te. ciliata* (Fig. 11k), the distribution of trichomes on the ovary extends onto the lower parts of the style.

Fruits and seeds. Insufficient number of fruits and seeds prevented a comprehensive survey but the pubescence of the capsules (Fig. 12) generally reflects that of the ovary (Fig. 11). In some species as the fruit matures, trichomes are lost. The fruits of *Elaeocarpus holopetalus* and *E. reticulatus* (Fig. 12a) are glabrous drupes. Of seeds observed, all species of *Tetratheca* had simple hairs, but no other trichome. The seeds of *Platytheca* have simple hairs plus large, narrow-elliptic, flat hairs (Fig. 3j-k).

Discussion

Table 3 summarises the types of trichomes as presence/absence characters irrespective of the distribution and density on various plant organs. Table 3 also includes two floral characters and shows the distribution of species in western, southern or eastern Australia. The evolution of trichome and floral characters is hypothesised, using outgroup comparison and summarised as a Hennigian cladogram based on the principle of parsimony (Fig. 13).

Relative to the outgroup *Elaeocarpus*, the presence of simple hairs (character 2) is plesiomorphic within the tremandroid clade. (Note that the sister clade also includes *Aceratium* and *Sericolea*, which have simple hairs and lack complex hairs.) The character simple hairs may be further divided into states to characterise particular species, for example, hairs appressed in *Te. diffusa* (Fig. 3h-i). Stellate hairs (character 1) are unambiguously an apomorphic character of *Tremandra*. Sculptured, multicelled tubercles (character 5iii) are an apomorphic character of *Platytheca*. The large, flat, narrow-elliptic hairs are probably another apomorphy for *Platytheca* but due to the small number of seeds observed in *Tetratheca* this is not certain.

The interesting question is how do the other trichome characters relate *Platytheca* and *Tetratheca*, and species groups within the latter?

Fig. 9. Comparison of inner and outer surfaces of sepals. *Tr. diffusa* (a) inner and (b) outer; *P. galioides* (c) inner and (d) outer; *Te. hispidissima* (e) inner and (f) outer; *Te. insularis* (g) inner and (h) outer; *Te. labillardierei* (i) inner and (j) outer. Scale bars = 500 μ m.

Fig. 10. Stamens. (a-b) *E. reticulatus*: (a) anther and (b) stamen filament. (c-d) *Tremandra*, which lacks an anther tube: (c) *Tr. diffusa* and (d) *Tr. stelligera*. (e-l) *Platytheca* and *Tetratheca*, both of which have an anther tube: (e) *P. galioides*, (f) *P. juniperina*, (g) *Te. hirsuta*, (h) *Te. affinis*, (i) *Te. ciliata*, (j) *Te. bauerifolia*, (k) *Te. hispidissima*, and (l) *Te. insularis*. Scale bars = 1 mm.

Fig. 11. Ovaries of *Tremandra*, *Platytheca* and *Tetratheca*, illustrating some of the observed variation in pubescence. (a) *Tr. diffusa*, (b) *Tr. stelligera*, (c) *P. galioides*, (d) *P. juniperina*, (e) *Te. thymifolia*, (f) *Te. labillardierei*, (g) *Te. pubescens* (h) *Te. stenocarpa*, (i) *Te. pilosa*, (j) *Te. insularis*, (k) *Te. ciliata* and (l) *Te. affinis*. Scale bars = 1 mm.

These two genera are related by the presence of an anther tube. They both also have short glandular trichomes (character 3) and smooth multicelled tubercles (character 5i). However, there are exceptions, notably *Te. filiformis* (Group I), which lacks both these characters. This species may represent the plesiomorphic condition and be basal in the *Platytheca* + *Tetratheca* clade, as hypothesised in the cladogram of Fig. 13 (node 1). Recently, Butcher et al. (2007) published a molecular phylogeny, including a limited number of taxa, that placed *Te. filiformis* as basal within

Tetratheca (although *Platytheca* was used as an outgroup and assumed outside the genus). Further molecular study is required before taxonomic revision, but one possibility is that a fourth genus may be required for *Te. filiformis*.

Other species of *Tetratheca* are listed as five 'groups' (not necessarily monophyletic). Groups II, III and IV are exclusively from Western Australia together with *Te. halmaturina* from Kangaroo Island, South Australia.

Fig. 12. Fruits of: (a) *E. reticulatus* (drupe), (b) *P. juniperina* (capsule lacking trichomes), (c) *Tr. diffusa* (capsule with stellate hairs) and (d) *Te. stenocarpa* (capsule with short glandular trichomes). Scale bars = 1 mm.

They have five-merous flowers (ten stamens), which is plesiomorphic based on outgroup comparison. Seven species in Group II, listed in Table 3 from *Te. affinis* to *Te. halmaturina*, have short glandular trichomes but lack long glandular trichomes (node 2, Fig. 13). The leafless *Te. aphylla* is hypothesised to be related to members of our Group III by Butcher et al. (2007a), and it may then have “lost” characters 2 and 4. Four species in Group III (*Te. confertifolia* to *Te. pauciflora*) have both short glandular trichomes and long glandular trichomes (node 3). Six species in Group IV (node 3) have long glandular trichomes but no short glandular trichomes, interpreted as a secondary loss. Groups V and VI include all species that have four-merous flowers (eight stamens), which it is hypothesised unite them as a clade (node 4). Given that interpretation, Group V, which

includes both western (4) and eastern (6) species, have ‘lost’ short glandular trichomes, in parallel with Group IV. (An alternative hypothesis is that the four-merous condition has evolved twice and that group IV and V are related.) Among the eastern species, *Te. juncea*, *Te. rupicola* and *Te. subaphylla* are possibly related to other eastern species of Group V (*Te. decora*, *Te. neglecta* and *Te. rubioides*). However, the phylogeny of Butcher et al. (2007a), which included some of these species, shows *Te. juncea* to be in a clade with four species from our group VI. Group VI is eastern, including *Te. insularis* from Kangaroo Island (South Australia) and one endemic in Tasmania (Table 3).

freshwater fishes, long-distance dispersal seems an unnecessary *ad hoc* scenario.

Conclusion

Our scanning electron and light microscopy study illustrates the extensive range of variation in the types and combinations of trichomes on various plant organs in the tremandroid clade of Elaeocarpaceae. Trichome types offer informative characters for a phylogenetic analysis of taxa within the family. They confirm that *Tremandra* is distinct, and indicate that *Platytheca* and *Tetratheca* form a clade, with the possibility of the former nested within the latter at a basal node. A combination of morphological and molecular data (see Butcher et al. 2007) may well resolve the relationships of groups and all taxa within *Tetratheca* and contribute to a greater understanding of the biogeographic history of the sclerophyll vegetation of southern Australia.

Acknowledgements

We thank the Directors of herbaria, AD, HO, MEL, MELU, NSW and PERTH for access to material; Dr Simon Crawford (School of Botany, The University of Melbourne) for assistance with the SEM work; Dr Philip Ladd (School of Environmental Science, Murdoch University, Western Australia) for supplying some plant material; and Parks Victoria Rangers (Kinglake National Park, Brisbane Ranges National Park, Kurth Kiln State Park and Bunyip State Park) for access to species in the field. Trisha Downing thanks Greg, Val and Andrew Downing for assistance with fieldwork in 2003 and 2006, and the School of Botany Foundation, for the Megan Klemm Award (2004).

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Title:

Trichome morphology provides phylogenetically informative characters for Tremandra, Platytheca and Tetratheca (former Tremandraceae)

Date:

2008-04-01

Citation:

Downing, T. L., Ladiges, P. Y. & Duretto, M. F. (2008). Trichome morphology provides phylogenetically informative characters for Tremandra, Platytheca and Tetratheca (former Tremandraceae). PLANT SYSTEMATICS AND EVOLUTION, 271 (3-4), pp.199-221. <https://doi.org/10.1007/s00606-007-0622-2>.

Publication Status:

Published

Persistent Link:

<http://hdl.handle.net/11343/34988>