

Polyaperturate pollen types and ratios of heteromorphism in the monocot genus *Conostylis* (Haemodoraceae)

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Abstract. Pollen aperture heteromorphism (variation of aperture number in pollen grains within each flower and in all plants of a taxon) is described in the diverse monocot genus *Conostylis* R.Br. (Haemodoraceae) and that of related genera, all endemic to Western Australia. We report results of pollen observations from 153 specimens. In *Conostylis*, aperture number varies greatly, with biporate, triporate, tetraporate and polyporate pollen present, features considered rare and possibly developmentally significant for monocots. Pollen aperture types and their ratios in this genus are stable and consistent within species and subspecies groupings and phylogenetically informative.

Introduction

The Haemodoraceae R.Br. are a small family of 14 genera and ~100 species of monocots, members of which are native to Australia, southern Africa, northern South America to southern Mexico, some Caribbean islands and eastern to south-eastern North America (Simpson 1998). Family members are characterised as perennial herbs with unifacial leaves, capsular fruits and the consistent occurrence of arylphenalenone chemical compounds (Cooke and Segal 1955; Cooke *et al.* 1958; Cooke and Edwards 1981; Opitz and Schneider 2002; Opitz *et al.* 2003), the latter only sparingly found in some closely related families. Floral morphology is tremendously variable in the family, with members differing, among other features, in perianth symmetry and fusion, stamen number, ovary position, septal nectary anatomy and ovule number (Simpson 1993, 1998).

Simpson (1990), in a phylogenetic analysis of the family Haemodoraceae using morphological data, determined the monophyly of the family and its division into two tribes (now treated as subfamilies), necessitating the transfer of *Phlebocarya* R.Br. from Haemodoroideae to Conostylidoideae, in agreement with McFarlane *et al.* (1987). Hopper *et al.* (1999), in a molecular phylogenetic study of the family, confirmed the monophyly of these subfamilies. All members of the Conostylidoideae – *Anigozanthos* Labill (11 spp.), *Blancoa* Lindl. (1 sp.), *Conostylis* R.Br. (~46 spp.), *Macropidia* J. Drumm. ex Harv. (1 sp.), *Phlebocarya* R.Br. (3 spp.) and *Tribonanthes* Endl. (5 spp.) – are endemic to the south-western region of Western Australia. The Hopper *et al.* (1999) study and a more detailed molecular phylogenetic analysis of subfamily Conostylidoideae (Hopper *et al.* 2006) supported *Tribonanthes* Endl. as basal and sister to the rest of the subfamily, which consists of two major clades: one with *Blancoa* and *Conostylis* as sister taxa and these sister to *Phlebocarya*, and the other with *Macropidia* as sister to *Anigozanthos*. Hopper *et al.* (2006) suggested *Conostylis*

shows a ‘prolonged persistence of relictual taxa, combined with explosive more recent speciation’.

Pollen morphology and ultrastructure of the Haemodoraceae has been studied by Erdtman (1966) and Simpson (1983, 1990). All members of subfamily Haemodoroideae have monosulcate pollen, in contrast to all members of subfamily Conostylidoideae, which have 2–7-porate pollen (Simpson 1983). Additional pollen grain diversity has been noted within the subfamily Conostylidoideae. *Anigozanthos*, *Blancoa*, *Conostylis* and *Macropidia* have mostly cylindrical or fusiform grains (although *Conostylis* spp. may be somewhat polyhedral) with protruding hemispheric aperture walls devoid of exine. *Phlebocarya* has cylindrical grains with flattened apertures with scattered exinous elements. *Tribonanthes* has spherical grains with 5–7 flattened apertures, also with scattered exinous elements (Simpson 1983). The sample size of these previous palynological studies was limited to a small fraction of the taxon diversity in the family. In particular, *Conostylis* was previously known to exhibit heteromorphism in pollen aperture number, having two, three or four apertures; however, only a few species of *Conostylis* were investigated palynologically (Simpson 1983, 1990).

Pollen heteromorphism is often expressed as a variation in aperture number, which has been considered analogous to some animals that produce two types of sperm (Till-Bottraud *et al.* 2005). This variation in the aperture number, ‘the production by a single plant of different fertile pollen types in all of its anthers and thus all flowers, throughout its life cycle,’ is termed *heteromorphism*, in contrast to pollen aperture number variation among individuals within a population, termed *polymorphism* (Till-Bottraud *et al.* 1995). Pollen heteromorphism is common in angiosperms, having been explored mostly in eudicots by, among others, Ressayre *et al.* (2002), Till-Bottraud *et al.* (1995), Dajoz *et al.* (1991, 1993, 1995), Mignot *et al.* (1994), Nadot *et al.* (2000) and Pire and Dematteis (2007).

Monocotyledons have received less attention in studies of pollen aperture heteromorphism owing to reports of their pollen being monoaperturate or monoaperturate-derived (Erdtman 1966; Walker and Doyle 1975; Till-Bottraud *et al.* 1995; Ressayre *et al.* 2005). However, monocots have been explored with success for mechanisms of callose deposition during cytokinesis in the separation of the microspores and formation of the apertures (Ressayre 2001; Ressayre *et al.* 2005).

The present study was undertaken to further explore the pollen aperture type of *Conostylis*, the largest and most diverse genus of the Haemodoraceae, and its relatives in subfamily Conostylidoideae. *Conostylis* is a perennial, rhizomatous herb that has actinomorphic flowers (trichomes characteristically present on adaxial tepal surfaces) with six stamens; however, it has no recognised, morphological autpomorphy (Hopper *et al.* 2006; Simpson 1990). *Conostylis* is endemic to the south-western Kwongan region of Western Australia (Hopper and Gioia 2004). The genus has been extensively treated by Green (1960), Geerinck (1969), Hopper (1977, 1978, 1979, 1980, 1982, 2004) and Hopper *et al.* (1987, 2006). Up to 46 species have been recognised and some additional ones are awaiting description (Hopper *et al.* 2006).

Materials and methods

Pollen samples for the present study came from dried material removed from herbarium sheets at K, PERTH, RSA-POM and SDSU herbaria, obtained during 2006–2007, and in a few cases from alcohol- or formalin–acetic acid–ethanol (FAA)-fixed material. The availability of specimens of different species was extremely variable and sometimes scant, as was the presence of pollen. For the present study, we examined a total of 180 Conostylidoideae specimens, of which we consider here only 131 specimens of *Conostylis* and 22 specimens of other Conostylidoideae. We ascertained the validity of the identifications of these specimens as much as possible by checking annotations that reflect recent taxonomic revisions, and we used more than one specimen of each species, when available. By surveying a large number of specimens, we examined most species of *Conostylis*, although a few were still unobtainable or could not be included (see below). We also incorporated several species we collected at random from around the region at Eneabba, Western Australia, in August–September 2007, specifically for the present study. Pollen was abundant for the most part in these specimens, which were identified for us at the Western Australian Herbarium (PERTH) (see Appendix 1).

We extracted pollen grains from mature anthers of 42 of the ~46 species of *Conostylis* and up to 22 additional *Conostylis* subspecies. Among other members of the Conostylidoideae, we sampled 7 of the 11 species for a total of nine taxa (including subspecies) of *Anigozanthos*, two species of *Phlebocarya*, three of the five species of *Tribonanthes*, and the monotypic *Blancoa canescens* and *Macropidia fuliginosa*. Dried pollen was rehydrated in 70% ethanol and then placed on slides and stained with saturated basic Fuschin solution in 50% glycerol in order to highlight the exine layer to the exclusion of the apertures.

In most cases, we used only one or two anthers from a flower; however, if available, all the anthers of a flower or of several flowers were combined in order to obtain at least 150–200 pollen grains in a slide. No effort was made to limit the samples to one anther or one flower, as we found pollen ratios to be rather stable for each species whether one or more flowers were used. Even if in herbarium specimens there were vast differences in terms of the amount of pollen present (owing to the stage of dehiscence of the anthers, date of collection or state of the specimen itself), on the basis of specimens with a large amount of pollen we found that these factors were not relevant. Pollen heteromorphism ratios were extremely stable across species and subspecies and were constant enough to be evident even in small samples, as long as pollen was present. The emphasis was to sample as much as possible of the genus *Conostylis* in order to ascertain specific trends suggested in preliminary observations. In most cases, we studied more than one sample of a taxon in order to account for potential variability of different populations of the same species or of different plants of the same species. We rejected specimens only when we found none or very little pollen, when the apertures could not be clearly discerned, or if we saw inadequate voucher identification. We personally collected additional species from the field, in order to validate the ratios and pollen types, with good support. (See Appendix 1 for the documentation and raw-data counts for every individual specimen included.) Slides are housed at the herbarium at SDSU.

For each species and subspecies available, we studied pollen samples under light microscopy, and we recorded pollen aperture type and percentages of each type of heteromorphic pollen. When tabulating pollen data, if pollen was very abundant, we used the first-hundred count, followed by two or more counts of one hundred in different and non-overlapping random part of the slides in order to confirm the presence and proportion of the different pollen types. When pollen was less abundant we counted and tabulated every grain in each slide. In the case of highly heteromorphic species it was necessary to employ high magnification and observation at different focal levels, as pollen apertures were not always readily evident. Slides were recounted three times or more until we had at least three counts that were congruent.

We present the results as average percentages, which may represent one, two or as many as five available specimens of any particular species or subspecies. We cannot statistically validate our data as we were not able to comprehensively sample the taxa. Rather, we looked at every specimen available in relevant herbaria that housed *Conostylis* taxa. In some cases, we had only one specimen per taxon; however, on the basis of the stability of pollen type and ratios in those species from which we had many subspecies, or abundant specimens, or where we found abundant pollen, we decided even single specimens were worth including, if only to document pollen type and to approximate heteromorphism ratio, which was the main focus of the present study.

Till-Bottraud *et al.* (1995), in a study of pollen heteromorphism in which ratios were not quantified, designated a plant's pollen as heteromorphic only when the most dominant type represented 90% or less; if other types were present but represented less than 10%, then the pollen was considered 'cryptoheteromorphic'. We found this concept useful and adopted it in our analysis.

Results

The results of the present study showed that the pollen in *Conostylis* may be biporate (Figs 1A, 2A), triporate (Figs 1B, 2B, D), tetraporate (Figs 1C, 2C) or polyporate, defined here as having five or more apertures. (Figs 1D, 2E, F). Pollen aperture number in the rest of subfamily Conostylidoideae is less diverse, with most genera being biporate or showing limited degree of triporate pollen (Table 1, Appendix 1). Pollen of *Anigozanthos* (Fig. 1E), *Blancoa* (Fig. 1F) and *Macropidia* (Fig. 1G) are largely biporate, with the grains being on the whole larger than those of *Conostylis* (Fig. 1A–D). Some species of *Anigozanthos* were found to be cryptoheteromorphically triporate, and some have a fusiform appearance, whereas the triaperturate ratio in *Blancoa canescens* (Fig. 1F) is ~20% (Table 1). *M. fuliginosa* is largely biporate with very limited triporate pollen present, its pollen grains larger than those of *Conostylis*, lengthwise 70 µm on average (Fig. 1G, Table 1). Pollen of *Phlebocarya* is uniformly biporate (Fig. 1H), the grains being somewhat shorter and stouter than the biporate grains of *Conostylis*, whereas pollen of *Tribonanthes* is globose and pantoaperturate (Fig. 1I).

In *Conostylis*, six species are invariant in aperture number, all being 100% biporate. Four of these, *C. angustifolia* Hopper, *C. hiemalis* Hopper, *C. seminuda* Hopper and *C. tomentosa* Hopper, are close relatives in the clade corresponding to section *Appendicula* (Fig. 5, cladogram after Hopper *et al.* 2006; see below); a fifth species, *C. juncea* Endl., is closely related in an adjacent clade, and a sixth, *C. robusta* Hopper, had not been studied phylogenetically at this time. The other 58 species and subspecies we examined are all heteromorphic to varying degrees (Fig. 3). Triaperturate pollen grains are present in the majority of species of *Conostylis*, with proportions varying from a cryptoheteromorphic condition (<10%) in some taxa (10 subsp. of *C. aculeata*, 5 subsp. of *C. candicans*, 2 subsp. of *C. pauciflora*, and four other species) to that of common to very common in other taxa, to almost totally triporate pollen (~90%) in *C. canteriata* Hopper, *C. bealiana* F.Muell. and *C. neocymosa* Hopper (Fig. 3). Tetraporate pollen grains are found for the most part in less abundant proportions than triporate ones, up to 20% in two taxa, *C. androstemma* F.Muell. and *C. drummondii* Benth.; the two subspecies of *C. caricina* Lindl., the two of *C. crassinervia* J.W.Green, and *C. setosa* Lindl. reach or exceed 10%. A very small percentage of pentaporate pollen was found in a few taxa (Fig. 3). *C. phathyantha* Diels was unique in being more than 90% polyporate (Figs 1D, 2E, F).

Pollen aperture type was found to be characteristic of and consistent within the specimens of each species and also within subspecies, as was the ratio of aperture types. For example, nine *Conostylis* species, each of which has at least two or more subspecies, *C. aculeata* R.Br., *C. candicans* Endl., *C. caricina* Lindl., *C. crassinervia* J.W.Green, *C. dielsii* W.Fitzg., *C. pauciflora* Hopper, *C. seorsiflora* F.Muell., *C. setigera* R.Br. and *C. teretifolia* J.W.Green, show little variation within specimens and species in the average percentage of biporate pollen grains among samples examined (Fig. 4). The same is true of examined species with regard to percentage of triporate pollen (not illustrated).

Although many specimens used in the present study had limited pollen, there were only three discrepancies in samples among the 180 examined. One specimen of *C. crassinervia* (PERTH 541343) was highly biporate, whereas all other specimens, including all subspecies of *C. crassinervia*, were highly triporate. The odd specimen was discarded, as its identity had not been annotated subsequent to its initial collection. For *C. serrulata*, the two specimens we examined were very different, one biporate (PERTH 4916867) and the other triporate (K PG Wilson). We elected not to consider either of them, as their validity could not be further ascertained (Table 1, Appendix 1).

Pollen aperture data showed agreement with known phylogenetic relationships derived from molecular data. In their phylogenetic analysis of generic and subgeneric relationships in *Conostylis*, Hopper *et al.* (2006) established two sister clades, A and B, and these two clades are also clearly differentiated by their pollen aperture type (Fig. 5). Clade A is biporate or cryptically triporate (or in one species up to 14% triporate), whereas Clade B shows a great degree of pollen heteromorphism and a high proportion of triporate pollen, with biporate ratios of 2–79% and triporate ratios of 20–90%, and many species showing tetraporate and pentaporate pollen as well. Also consistent with the results of Hopper *et al.* (2006) is that the pollen aperture number of members of section *Appendicula* (Fig. 5) is consistent with the biporate or cryptically triporate aperture type of Clade A and not with the highly heteromorphic subgenus *Pendula* (in Clade B), where section *Appendicula* had been previously placed.

Tetraporate grains are present only in species with a large percentage of triporate pollen, but often with biporate pollen still present (Fig. 3). Very few taxa had tetraporate pollen beyond a mere 5%, and the maximum found was 20%. We also observed that in most species with small percentages of tetraporate pollen, the tetraporate grains are mostly irregular with only one, two or occasionally three pores in the same plane (Fig. 6D, E), whereas in species where tetraporate grains are more abundant, such as *C. canteriata* (See Fig. 6F) or *C. teretifolia* (Fig. 6G), the tetraporate grains are still quite variable in shape, although the pores appear mostly in the same plane.

Even with the stability in pollen aperture ratios across species and species groupings, the average counts presented here are mostly approximate, particularly in highly heteromorphic species (Appendix 1). In many cases, we had only one or two herbarium specimens, which commonly have limited pollen. However, pollen data from the newly collected species consistently matched the taxon-specific pollen aperture types and ratios obtained from herbarium specimens.

Anthers of most species of *Conostylis* measure between 1 and 3 mm in length and the length of most pollen grains is within 30 and 50 µm. Individual refocusing and higher magnification for every grain were necessary in highly heteromorphic species, as many pores were not readily evident (Fig. 6A–D, E). We used Helicon Focus software (<http://www.heliconsoft.com>, verified January 2009) to produce some composite images of pollen grains at different focal lengths (Fig. 6C).

Worth noting is the specimen of *C. caricina* (PERTH 5449723). A rare type of pollen grain was found frequently in this specimen, one somewhat elongated, of a shape not seen

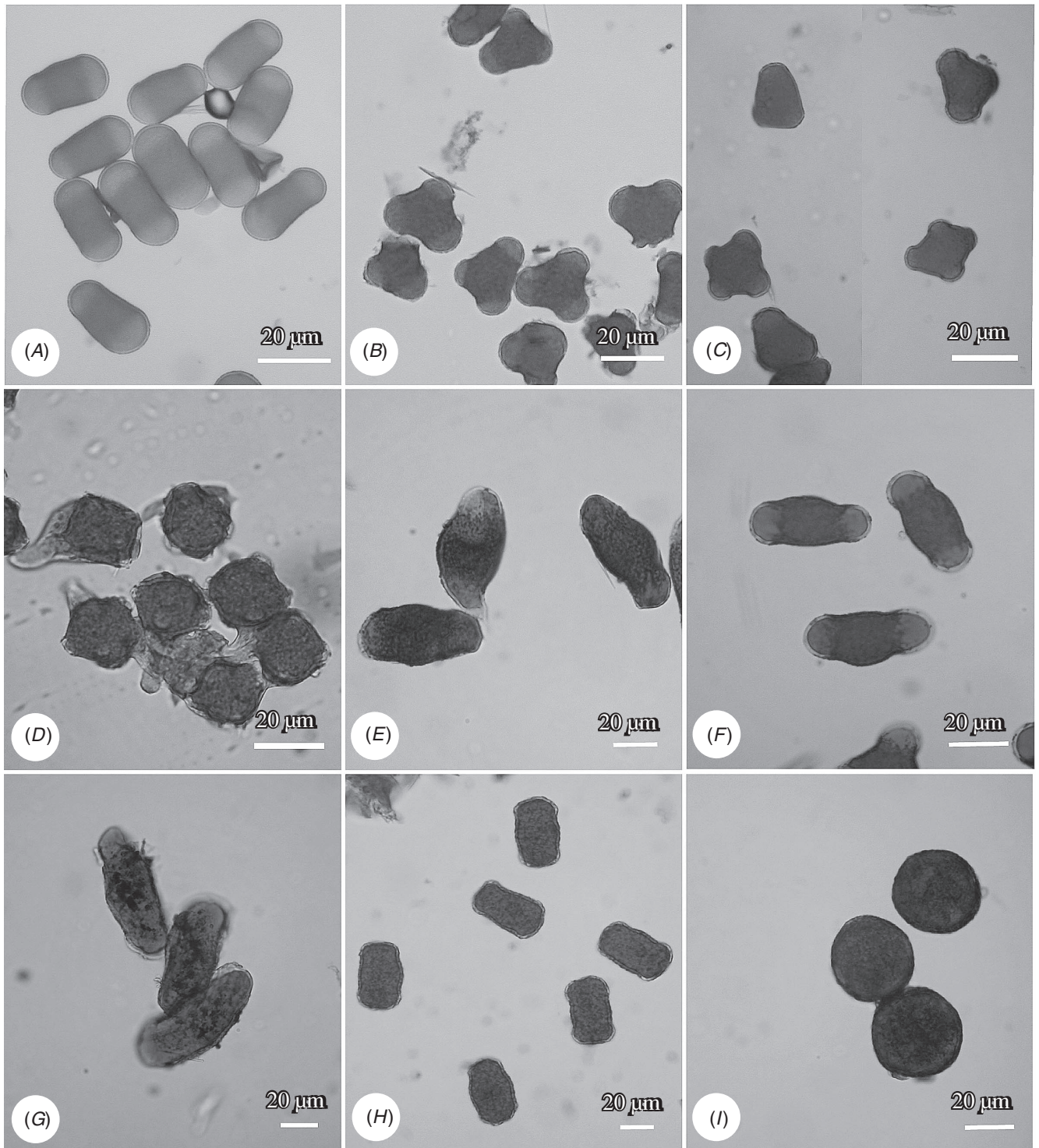


Fig. 1. Pollen grains of subfamily Conostyloideae (by light microscopy). Pollen aperture in *Conostylis*, average lengthwise size of grain 25–35 µm. (A) *C. angustifolia* (SDSU 16999) a 100% biporate species. (B) *C. setigera* ssp. *setigera* (PERTH 228555), a highly triporate species. (C) *C. setigera* (PERTH 5320011), tetraporate grains. (D) *C. phathyrantha* (PERTH 5745748), a polyporate species. (E) *Anigozanthos humilis* (PERTH 5280079), lengthwise pollen grain size range 55–61 µm. (F) *Blancoa canescens* (PERTH 6490875), lengthwise size range 45–50 µm. (G) *Macropidia fuliginosa* (PERTH 2086166), lengthwise size range 60–80 µm. (H) *Phlebocarya ciliata* (SDSU 17022), lengthwise size range 39–42 µm. (I) *Tribonanthes longipetala* (PERTH 7296029), size range in diameter 42–60 µm.

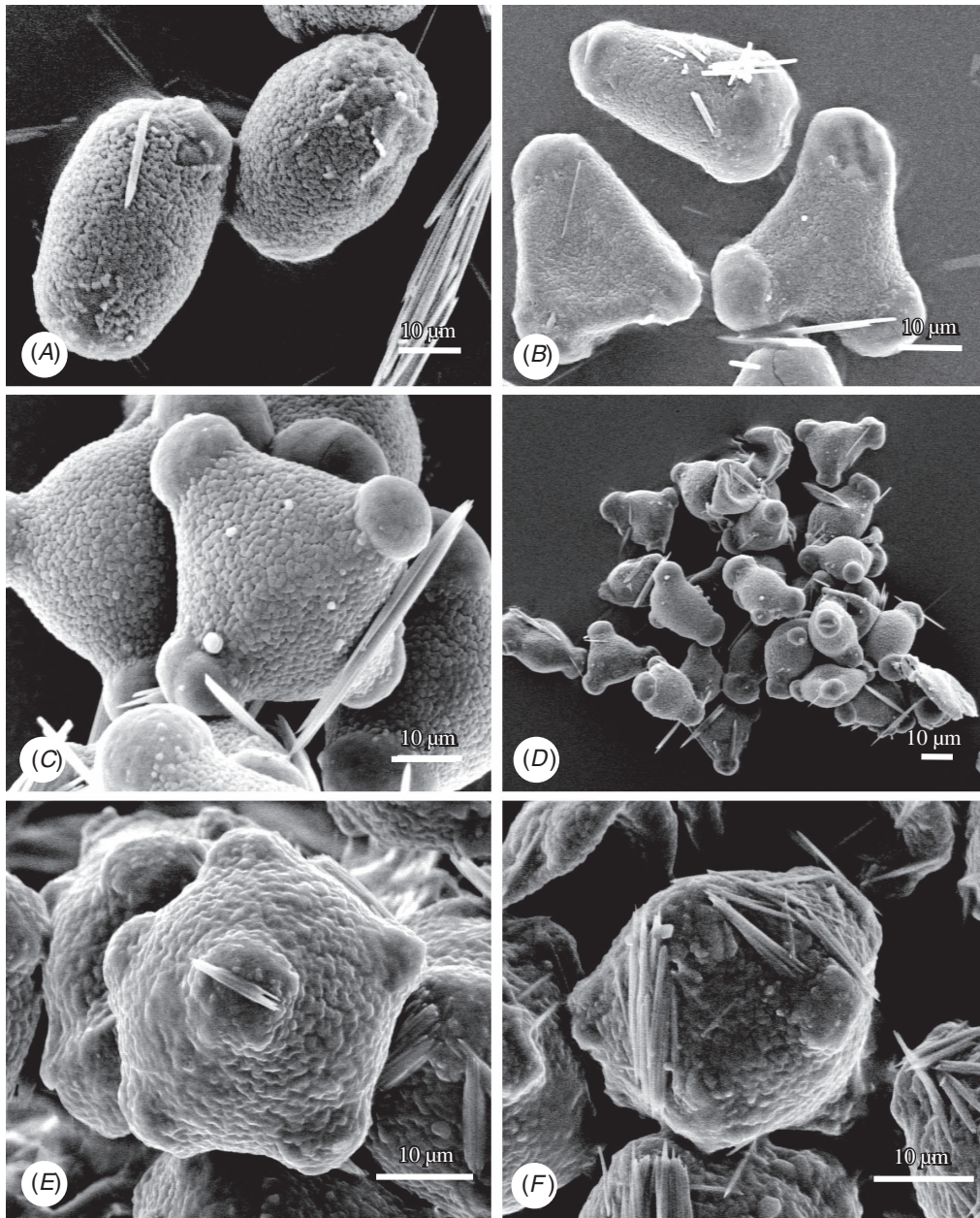


Fig. 2. Pollen grains of *Conostylis* (by scanning electron microscopy). (A) *C. breviscapa* (PERTH 2052385), with biporate grains. (B) *C. vaginata* (PERTH 5645638), with biporate and triporate grains. (C, D) *C. androstemma* (PERTH 2011026), with triporate and tetraporate grains. (E, F) *C. phathyrantha* (PERTH 05745748), showing hexaporate/polyporate grains.

before, with four small apertures in the same plane and an enlargement at one end (Fig. 6H). This type was present at a ratio of 2% of the total count. The enlargement does not appear to represent a pollen tube, as many pollen grains with pollen tubes were also present (Fig. 6I). In addition, the specimen showed a high percentage of tetraporate pollen in many different shapes that were not found in other species or specimens. It is possible that these are transitional to the rarer, elongated type.

Discussion

Pollen aperture number, as a discrete character easy to measure, has been considered often, 'because an evolutionary increase in aperture number in angiosperms since the Mesozoic indicates possible relationship with pollen fitness' (Till-Bottraud *et al.* 1995). Furness and Rudall (2004) considered that the improved chances of contact with the stigmatic surface through an increase in the number of apertures, coupled with the aperture movement

Table 1. Subfamily Conostyloideae

Average aperture-type ratios, arranged within each genus by decreasing biporate percentage. Numbers in parentheses represent number of specimens included

Taxon	Biporate	Triporate	Tetraporate	Polyporate
<i>Anigozanthos humilis</i> Lindl. and its subspp. (2)	100	0	0	0
<i>A. rufus</i> Labill. (2)	100	0	0	0
<i>A. bicolor</i> Endl. ssp. <i>bicolor</i> (1)	99	1	0	0
<i>A. oncys</i> A.S.George (1)	99	1	0	0
<i>A. viridis</i> Endl. and its subspp. (4)	99	1	0	0
<i>A. flavidus</i> D.C. (1)	98	2	0	0
<i>Blancoa canescens</i> Lindl. (3)	76	24	0	0
<i>Conostylis angustifolia</i> Hopper (2)	100	0	0	0
<i>C. hiemalis</i> Hopper (2)	100	0	0	0
<i>C. robusta</i> Diels. (1)	100	0	0	0
<i>C. seminuda</i> Hopper (1)	100	0	0	0
<i>C. tomentosa</i> Hopper (2)	100	0	0	0
<i>C. juncea</i> Endl. (2)	100	0	0	0
<i>C. resinosa</i> Hopper (3)	98	2	0	0
<i>C. prolifera</i> Benth. (2)	98	2	0	0
<i>C. candicans</i> Endl. and its subspp. (10)	97.9	2.1	0	0
<i>C. aurea</i> Lindl. (2)	97	3	0	0
<i>C. laxiflora</i> Benth. (1)	97	3	0	0
<i>C. aculeata</i> R.Br. and its subspp. (23)	96.7	3.3	0	0
<i>C. pauciflora</i> Hopper and its subspp. (3)	96.6	3.3	0	0
<i>C. seorsiflora</i> F.Muell. and its subspp. (4)	91.75	8.25	0	0
<i>C. stylidoides</i> F.Muell. (3)	91.7	8.3	0	0
<i>C. festucacea</i> Endl. and its subspp. (3)	85	14.5	0.5	0
<i>C. lepidospermoides</i> Hopper (1)	79	20	1	0
<i>C. breviscapa</i> R.Br. (2)	76	24	0	0
<i>C. teretiuscula</i> F.Muell. (2)	65	35	0	0
<i>C. pusilla</i> Endl. (2)	62.5	37.5	0	0
<i>C. villosa</i> Benth. (2)	62.5	37.5	0	0
<i>C. wonganensis</i> Hopper (2)	61.5	38.5	0	0
<i>C. dielsii</i> W.Fitzg. and its subspp. (4)	61.3	34.3	1	0
<i>C. vaginata</i> Endl. (4)	60.8	38	0.8	0.4
<i>C. deplexa</i> Green (1)	46	54	0	0
<i>C. latens</i> Hopper (2)	45.5	53	1.5	0
<i>C. argentea</i> (J.W.Green) Hopper (2)	45	54.5	0.5	0
<i>C. albescens</i> Hopper (2)	42	54.5	0.5	0
<i>C. micrantha</i> Hopper (2)	39	61	0	0
<i>C. petrophilodes</i> Benth. (2)	34	65.5	0.5	0
<i>C. rogeri</i> Hopper (2)	28.5	66.5	5	0
<i>C. drummondii</i> Benth. (1)	20	60	20	0
<i>C. caricina</i> Lindl. and its subspp. (4)	16.8	73	10	0
<i>C. androstemma</i> F.Muell. (1)	17	63	20	0
<i>C. setigera</i> R.Br. and its subspp. (8)	17	77.8	5.1	0.1
<i>C. neocymosa</i> Hopper (2)	9.5	90	0.5	0
<i>C. teretifolia</i> J.W.Green and its subspp. (6)	7.7	82.7	9	0.6
<i>C. crassinervia</i> J.W.Green and its subspp. (5)	6.8	81.2	12	0
<i>C. canteriata</i> Hopper (3)	5.3	84	10.7	0
<i>C. setosa</i> Lindl. (2)	4.5	80.5	15	0
<i>C. bealiana</i> F.Muell. (2)	2	93	5	0
<i>C. phathyrantha</i> Diels. (2)	0	0	5	95
<i>Macropidia fuliginosa</i> (Hook.) Druce (2)	100	0	0	0
<i>Phlebocarya</i> R.Br. and its subspp. (2)	100	0	0	0
<i>Tribonanthes</i> Endl. and its subspp. (4)	0	0	0	100

from a polar to an equatorial plane, might have been a key innovation in the diversification of eudicots, which have been defined in terms of the aperture character itself. These changes are known to have happened at the base of the angiosperm clade, with an evolutionary trend towards an increase in the

number of apertures, mostly from one to three (Furness and Rudall 2004). Most taxa have pollen grains with only one or a few apertures, but they 'usually occupy highly stable positions on their surface, with pluriaperturate pollen grains being the derived condition' (Walker and Doyle 1975; and others, cited

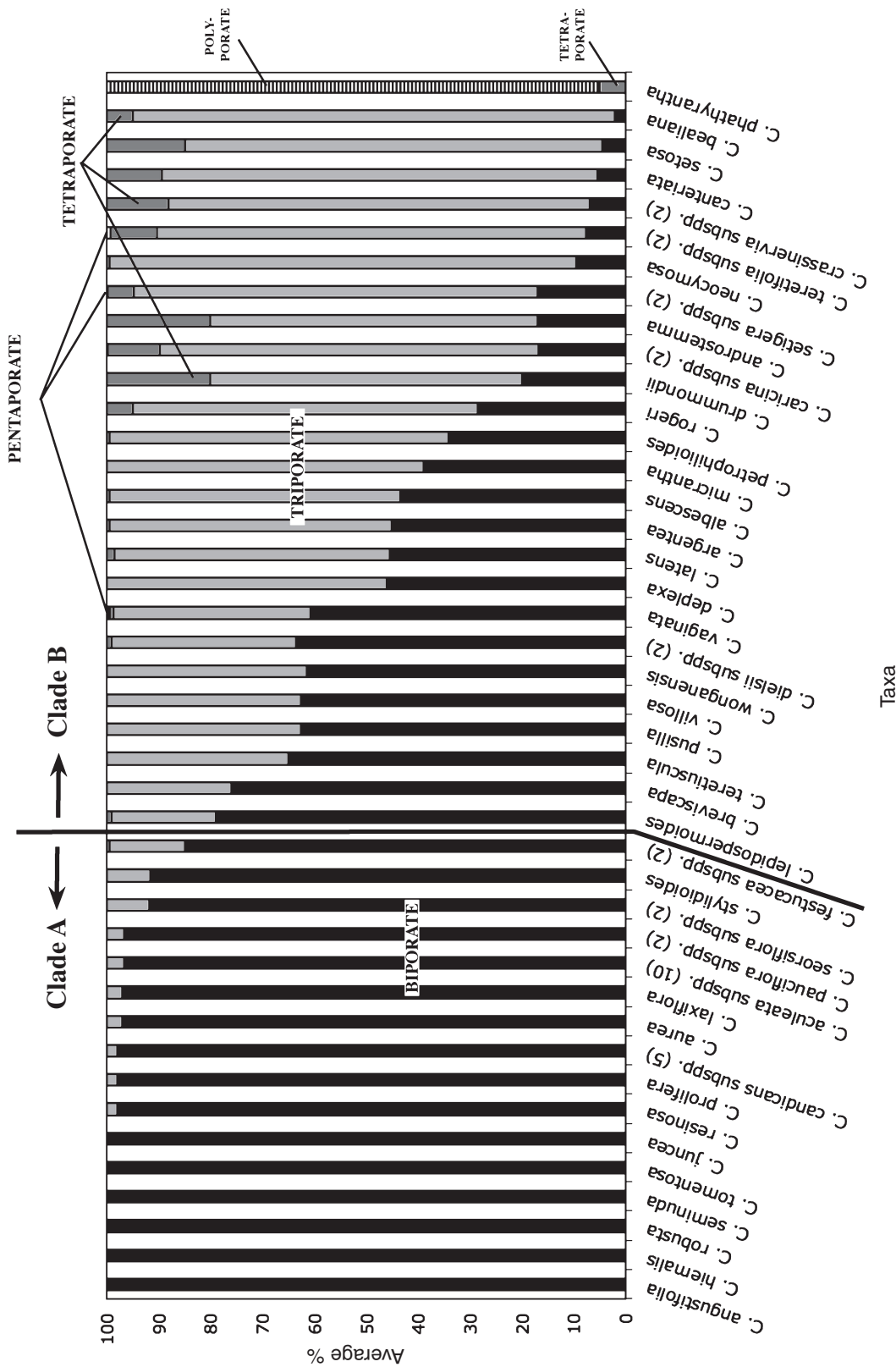


Fig. 3. Bar graph of pollen type ratios in *Conostylis*, illustrating percentages of biporate, triporate, tetraporate and poly-porate pollen grains per species or, in some cases, subspecies groups. Note vertical line, delimiting Clades A and B (after Hopper *et al.* 2006). Number of subspecies in parentheses.

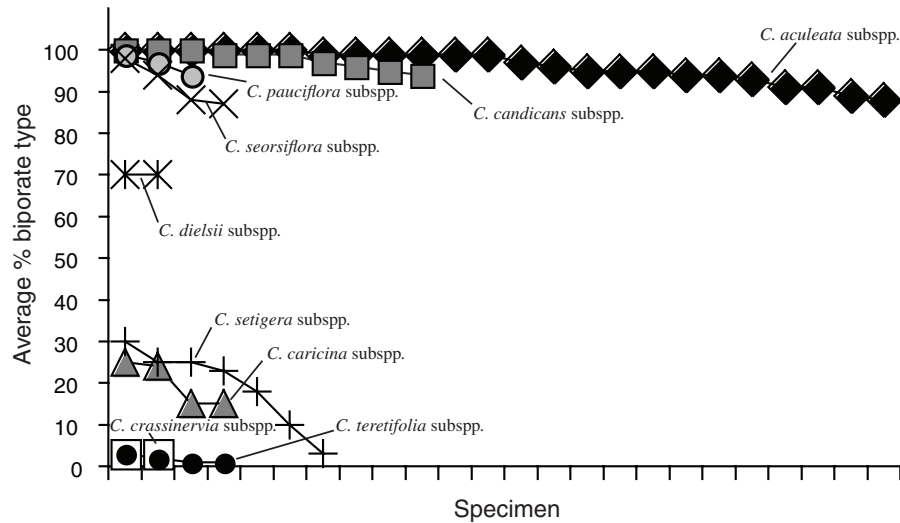


Fig. 4. Average percentages of biporate pollen as a function of the different specimens of *Conostylis*. Considered here are specimens of only those species with one or more subspecies, with number of subspecies indicated below in parentheses. The *x*-axis sometimes represents more than one specimen of the same species or subspecies. Most *Conostylis* species have some proportion of biporate pollen, from 3 to 100%, but these ratios are rather constant within sister subspecies as are the triporate and tetraporate ratios within species/subspecies across the genus (*C. aculeata* subsp. 11; *C. candidans* subsp. 5; *C. caricina* subsp. 2; *C. crassinervia* subsp. 2; *C. dielsii* subsp. 2; *C. pauciflora* subsp. 2; *C. seorsiflora* subsp. 2; *C. setigera* subsp. 2; *C. teretifolia* subsp. 2).

in Dajoz *et al.* 1991). In some lineages of eudicots, aperture number has increased and decreased many times independently (Edlund *et al.* 2004).

Monocots, for their part, have not been widely studied for pollen heteromorphism because their pollen has been widely documented as monoaperturate, and this condition is considered ancestral to eudicots. However, as many as 10 monocot families are known to have triaperturate pollen (e.g. Harley and Dransfield 2003), as documented in an exhaustive review by Harley (2004). Our results with *Conostylis* seem to indicate that an increase in aperture number in pollen grains might be more common than expected in monocots, a view held for all other plant families by Till-Bottraud *et al.* (2005), and that the function of this increase could be the same in all angiosperms. Pollen-aperture variation among closely related species and even within species has been well documented. Ressayre *et al.* (1998) and Till-Bottraud *et al.* (1995) estimated at 30% the number of eudicot species producing several types of pollen grains, differing by aperture number within a plant or showing variation in some populations or species.

In comparisons between four- and three-aperturate pollen grains in *Viola diversifolia* (Dajoz *et al.* 1991, 1993), triaperturate pollen grains were found to live longer, with faster tube growth and increased effectiveness in the long term, whereas four-porate grains were found to mature earlier and have faster germination rates. It has been postulated that a pluriaperturate, more rapidly germinating grain would be more effective at immediate pollination and be favoured at times when pollinators are abundant. The longer lasting pollen grains with fewer apertures could be favoured for their persistence when pollination services are scarcer (Till-Bottraud *et al.* 2001). It is not

known whether different-aperturate pollen functions in the same way in *Conostylis*.

Other authors have found some effect of pollen grain volume on length of the pollen tube and on survival rate (Williams and Rouse 1990; Dajoz *et al.* 1993) or have suggested that different pollen types may be favoured in different ecological conditions. Mignot *et al.* (1994) found pollen heteromorphism was characteristic of the species and could be genetically expressed. In *Conostylis*, the aperture ratio is also species-specific, indicating genetic control, and ratios did not vary noticeably from one population to another.

Many studies have explored genetic or ontogenic influences on aperture number and their relationship with several factors such as polyploidy (Till-Bottraud *et al.* 1995), increase in pollen size or gametophytic ploidy (Dajoz *et al.* 1993), zygomorphy, enantiostyly or pollination strategy (Till-Bottraud *et al.* 2005). Several studies have shown inconclusive results or have attributed heteromorphy of pollen grains to both environmental and genetic factors (Till-Bottraud *et al.* 1995).

A difference in ploidy level of the sporophyte, which is known to influence pollen morphology (many authors, discussed in Till-Bottraud *et al.* 1995), has been associated with increased aperture numbers in pollen grains. However, e.g. Dajoz *et al.* (1995), Mignot *et al.* (1994) evaluated this in studies of the fossil record and proposed that the increase in the aperture number has evolved through selection.

On the basis of the available data, there appears to be no clear correlation between pollen aperture number and chromosome number in *Conostylis*. Two species, *C. setigera* ($2n = 14, 21, 28$) and *C. teretifolia* ($2n = 7, 14$), have a tendency for a polyploid origin and do have greater percentages of higher-aperturate pollen

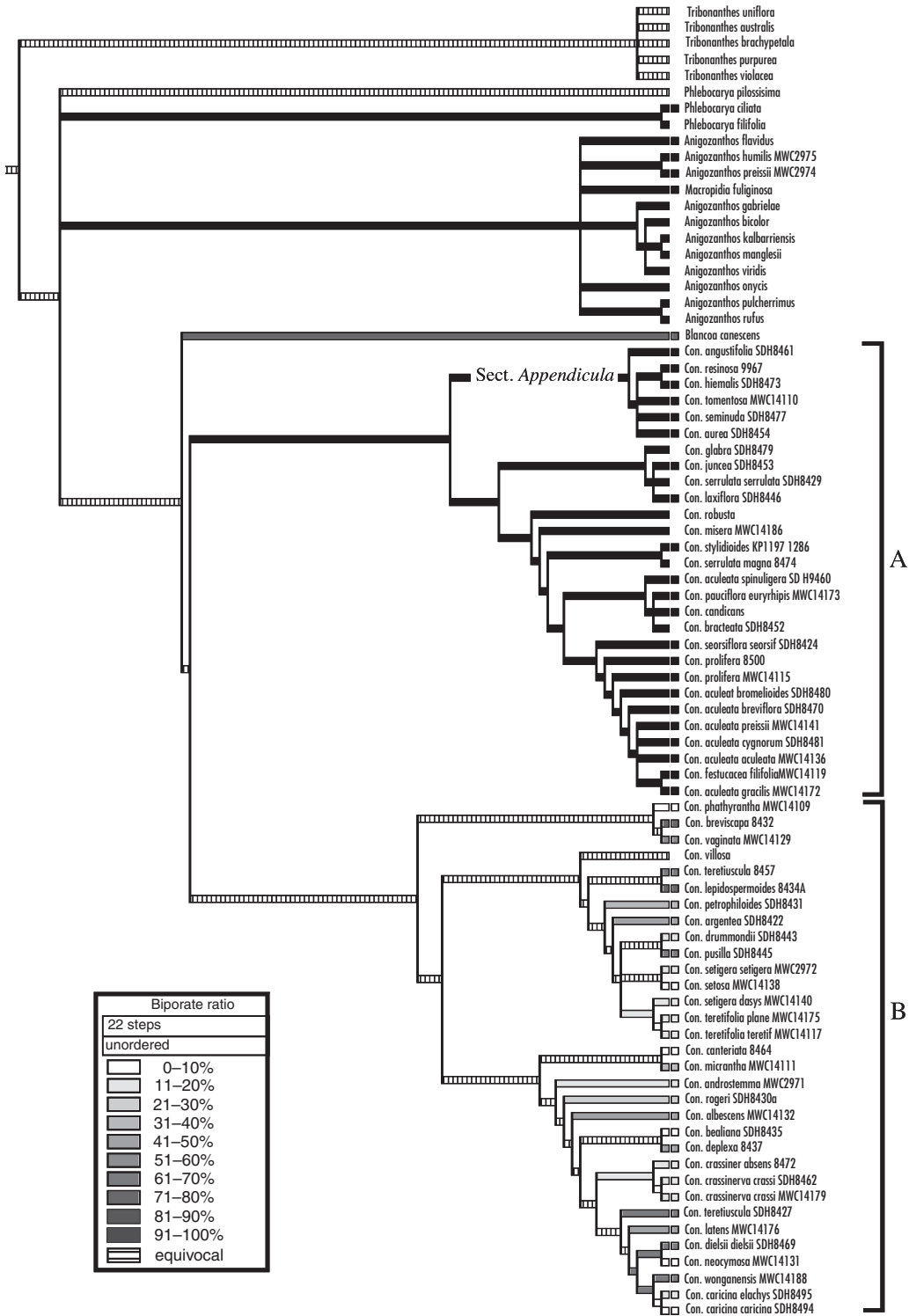


Fig. 5. Cladogram of subfamily *Conostyloideae* (after Hopper *et al.* 2006), with shading reflecting relative percentage of biporate pollen. In *Conostylis*, the presence or absence of pollen heteromorphism and percentage of pollen type are strongly correlated with subgroupings in the genus. Most species in Clade A (from Hopper *et al.* 2006) are biporate to cryptically triporate (90–100% biporate), except for *C. festucacea* Endl., which is 85% biporate (15% triporate). Clade B shows a much greater degree of aperture heteromorphism, with biporate ratios ranging from 2 to 79%, triporate pollen ratios 20–93% and tetraporate ratios up to 20%.

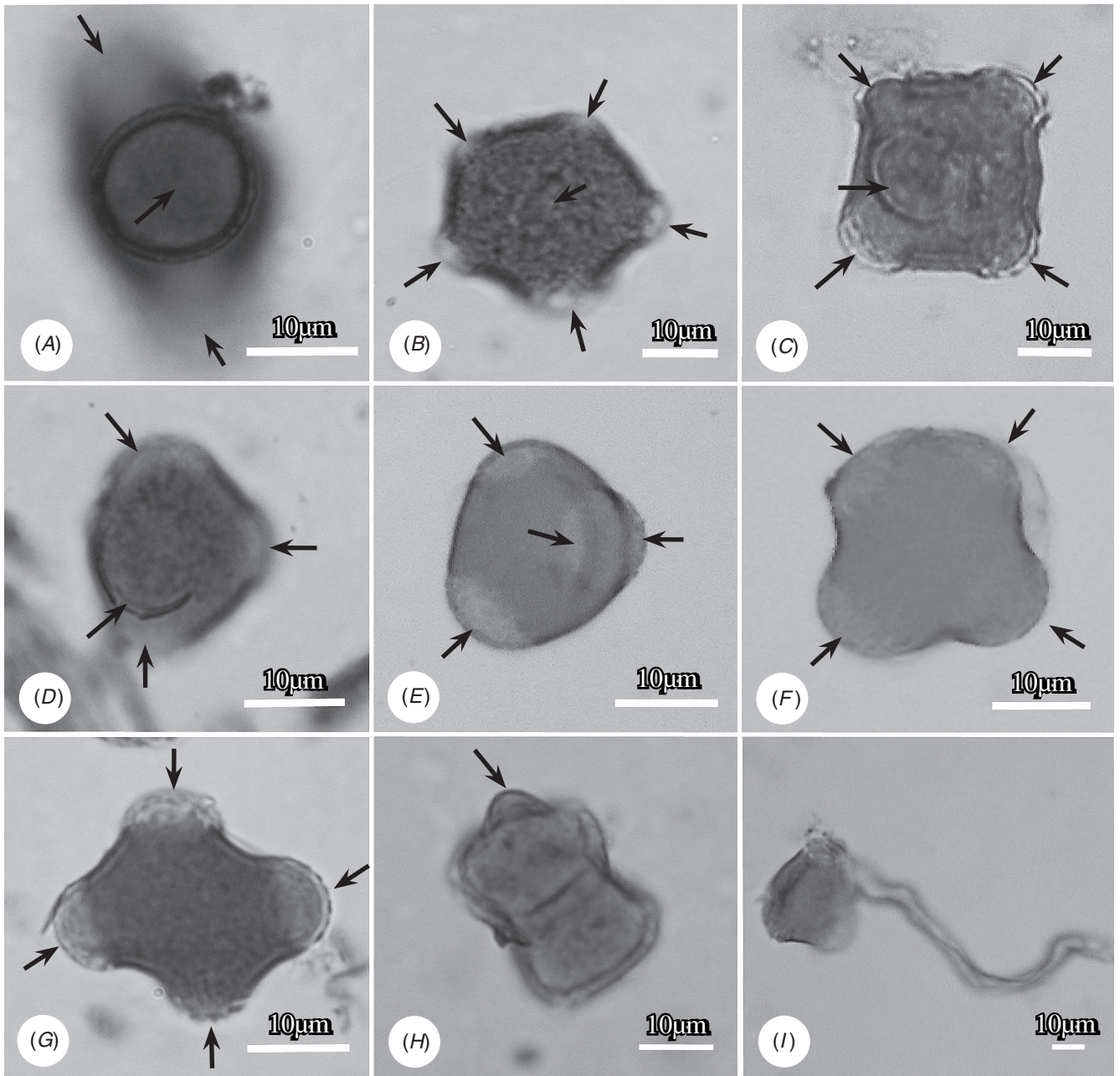


Fig. 6. (A) Triporate grain of *Conostylis androstemma* (PERTH 2011026) that appeared biporate at a lower magnification. (B) Hexaporate grains of *C. phathyantha* (PERTH 5745748), with the central sixth aperture out of focus (centre, arrow). (C) Pentaporate grain in *C. phathyantha* (PERTH 5745748); Helicon Focus composite image of several taken at various focus lengths. (D–G) Examples of tetraporate grains. (D) *C. caricina* (PERTH 449723). (E, F) *C. canteriata* (SDSU 19039). (G) *C. teretifolia* subsp. *teretifolia* (PERTH 05477263). (H) A rare pollen type with four small apertures and an enlargement at one end (where arrow is pointing), present at a ratio of 2% in *C. caricina* (PERTH 5449723). (I) Triporate grain with pollen tube, in the same specimen as (H). Arrows point to apertures, throughout. All images were taken under a light microscope.

types (Appendix 1). However, other species show no such trend. For example, *C. stylioides* ($2n = 16$) has been proposed as being of polyploid origin (Krauss and Hopper 2001) but has a largely biporate condition. Conversely, *C. phathyantha*, which has a high pollen aperture number, is not of polyploid origin, with $2n = 8$ as in most *Conostylis* species.

Apertures in pollen grains have been seen to form relative to the last points of contact of microspores in the formed tetrad before the deposition of callose during cytokinesis, and it is the widespread view that the flexibility in aperture number is related to simultaneous microsporogenesis (Furness and Rudall 2004). Harley (2004) surveyed many studies of monocot genera and

noted the type of pollen, cytokinesis type and other related characters, and found that monocots largely had successive microsporogenesis, in which paired microspores would be already isolated from the other two after Meiosis I and so less likely to have an increased number of apertures. Furness and Rudall (1999) consider that there is little relation between microsporogenesis type and aperture number in monocots. Documenting the large number of apertures in *Conostylis* could shed new light on this issue.

The other genera in the rest of subfamily Conostylidoideae – *Anigozanthos*, *Blancoa*, *Macropidia*, *Phlebocarya* and *Tribonanthes* – are much less speciose than *Conostylis*. We found less pollen diversity and very reduced heteromorphism in pollen grain apertures of these taxa, most being biporate, a few cryptoheteromorphically triporate, and in the case of *Blancoa canescens* (sister to *Conostylis*), 20% triporate. The basal member of the Conostylidoideae, the genus *Tribonanthes*, is unique in having globose, pantoaperturate pollen.

Conclusions

The pollen of the monocot genus *Conostylis* was found to vary greatly in the number of apertures, with biporate, triporate, tetraporate and polyporate (pentaporate and hexaporate) pollen present. Aperturate pollen types and ratios were found to be stable, taxon specific, and consistent within species and subspecies groupings. This heteromorphism in pollen aperture number in *Conostylis*, a condition considered rare in monocots, is correlated well with recently established phylogenetic relationships.

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Appendix 1. Conostyloideae voucher specimen documentation and heteromorphism ratio and origin

Acc. no., Herbarium accession number; Bi, Biporate grain; Coll., collected for the present study; H, Herbarium; Polyp., polyporate grain; Sp.Orig., specimen origin; Te, tetraporate grain; Tri, triporate grain

Taxon	Acc. no.	Collector	% Bi	%Tri	%Te	% Polyp.	Sp.Orig.
<i>Anigozanthos bicolor</i> ssp. <i>bicolor</i>	PERTH 6514170	BJ Keighery 2082 24/08/1994	99	1	0	0	H
<i>A. flavidus</i>	SDSU 17114	MG Simpson 24IX1981J	98	2	0	0	H
<i>A. humilis</i>	PERTH 5280079	JP Pigott 14/09/1992	100	0	0	0	H
<i>A. humilis</i> ssp. <i>humilis</i>	PERTH 7417772	SA Fisher BNC 553 9/10/2005	100	0	0	0	H
<i>A. manglesii</i>	SDSU 17116	K Dixon 16IX1987	98	2	0	0	H
<i>A. oncys</i>	PERTH 2690950	SD Hopper 7862 2/10/1990	99	1	0	0	H
<i>A. rufus</i>	PERTH 2012456	SD Hopper 819 31/10/1974	100	0	0	0	H
<i>A. rufus</i>	SDSU 17111	MG Simpson 27IX1981F	100	0	0	0	H
<i>A. viridis</i>	PERTH 5578531	H Cole 131 24/09/1999	99	1	0	0	H
<i>A. viridis</i> ssp. <i>cataby</i>	PERTH 3911632	SD Hopper 748 28/10/1976	98	2	0	0	H
<i>A. viridis</i> ssp. <i>terraspectans</i>	PERTH 993662	SD Hopper 1784 22/10/1980	98	2	0	0	H
<i>A. viridis</i> ssp. <i>viridis</i>	PERTH 6322581	P Foreman 155 1/10/2002	100	0	0	0	H
<i>Blancoa canescens</i>	PERTH 6490875	TJ Alford 223 23/06/2003	50	50	0	0	H
<i>B. canescens</i>	SDSU 17039	MG Simpson 18IX1981AA	84	16	0	0	H
<i>B. canescens</i>	K	Max Hook 2170 1957	94	6	0	0	H
<i>Conostylis aculeata</i>	SDSU 17035	MG Simpson 239IX1981A	100	0	0	0	H
<i>C. aculeata</i>	PERTH 6799132	B Muir 45 31/10/2001	100	0	0	0	H
<i>C. aculeata</i> ssp. <i>aculeata</i>	RSA 283218	L Haefi 1848 22/8/1917	99	1	0	0	H
<i>C. aculeata</i> ssp. <i>aculeata</i>	PERTH 4968093	R Davis 4325 20/10/1997	94	6	0	0	H
<i>C. aculeata</i> ssp. <i>aculeata</i>	SDSU 17035	MG Simpson 23IX1981A	100	0	0	0	H
<i>C. aculeata</i> ssp. <i>breviflora</i>	SDSU 16995	L Aerne 50 9/26/2005	100	0	0	0	H
<i>C. aculeata</i> ssp. <i>breviflora</i>	SDSU 17034	MG Simpson 17IX1981K	99	1	0	0	H
<i>C. aculeata</i> ssp. <i>breviflora</i>	PERTH 5477646	M Hislop 1546 13/09/1999	95	5	0	0	H
<i>C. aculeata</i> ssp. <i>breviflora</i>	SDSU 19037	NB Pierce 274 2/9/2007	100	1	0	0	Coll.
<i>C. aculeata</i> ssp. <i>bromeliodes</i>	PERTH 5106168	BA Furher 9780 30/09/1997	89	11	0	0	H
<i>C. aculeata</i> ssp. <i>bromeliodes</i>	SDSU 16996	L Aerne 54 9/27/2005	93	7	0	0	H
<i>C. aculeata</i> ssp. <i>cygnorum</i>	PERTH 7028865	K Richardson 221 13/09/2003	95	5	0	0	H
<i>C. aculeata</i> ssp. <i>cygnorum</i>	K	SD Hopper 5193	97	3	0	0	H
<i>C. aculeata</i> ssp. <i>echinissima</i>	PERTH 1143778	SD Hopper 1314 14/09/1979	99	1	0	0	H
<i>C. aculeata</i> ssp. <i>gracilis</i>	PERTH 7527403	D Rooks 197 26/10/2006	95	5	0	0	H
<i>C. aculeata</i> ssp. <i>gracilis</i>	K	SD Hopper 2673	99	1	0	0	H
<i>C. aculeata</i> ssp. <i>preissii</i>	PERTH 7483406	C Danese B11006-51 5/10/2006	94	6	0	0	H
<i>C. aculeata</i> ssp. <i>preissii</i>	RSA 255234	L Deburh 3374	100	0	0	0	H
<i>C. aculeata</i> ssp. <i>riphidion</i>	PERTH 6600069	J Brooker 488 27/09/2002	96	4	0	0	H
<i>C. aculeata</i> ssp. <i>septentrionora</i>	PERTH 5516633	D Bellairs 6047 16/09/1998	91	9	0	0	H
<i>C. aculeata</i> ssp. <i>spinuligera</i>	PERTH 2010429	SD Hopper 1796 23/10/1980	91	9	0	0	H
<i>C. aculeata</i> ssp. <i>spinuligera</i>	SDSU 16997	L Aerne 31 9/25/2005	97	3	0	0	H
<i>C. aculeata</i> ssp. <i>spinuligera</i>	K	HT Mueller 1873	99	1	0	0	H
<i>C. albescens</i>	PERTH 1027212	SD Hopper 1248 25/08/1979	32	68	1	0	H
<i>C. albescens</i>	K 1247/1139	SD Hopper 1247 16/2/1987	52	41	0	0	H
<i>C. androstemma</i>	PERTH 2011026	SD Hopper 188 16/06/1975	17	63	20	4	H
<i>C. angustifolia</i>	SDSU 16999	L Aerne 32 9/25/2005	100	0	0	0	H
<i>C. angustifolia</i>	PERTH 7296037	S Patrick 1560 14/09/1973	100	0	0	0	H
<i>C. argentea</i>	PERTH 6692257	GJ Keighery 48 14/05/1999	42	57	1	0	H
<i>C. argentea</i>	K	CG Kinglsey 7330	48	52	0	0	H
<i>C. aurea</i>	SDSU 17000	L Aerne 26 9/25/2005	99	1	0	0	H
<i>C. aurea</i>	PERTH 5613779	JE Wajon 105 29/09/1999	95	5	0	0	H
<i>C. bealiana</i>	SDSU	MG Simpson 111IX1981D	2	96	2	0	H
<i>C. bealiana</i>	PERTH 6261957	B Archer 1064 28/07/1998	2	90	8	0	H
<i>C. breviscapa</i>	PERTH 2052164	SD Hopper 341 9/9/1976	68	32	0	0	H
<i>C. breviscapa</i>	PERTH 2052385	EM Bennett 3091 15/01/1970	84	16	0	0	H
<i>C. candicans</i>	POM 325144	ES Lathrop 257	99	1	0	0	H
<i>C. candicans</i>	SDSU	L Aerne 62	100	0	0	0	H
<i>C. candicans</i>	PERTH 4305388	M Lewis 64/92 8/8/1992	97	3	0	0	H
<i>C. candicans</i> ssp. <i>candicans</i>	SDSU 19041	NB Pierce 264 2/9/2007	100	0	0	0	Coll.
<i>C. candicans</i> ssp. <i>candicans</i>	PERTH 6392407	MA Langley 6/10/1999	99	1	0	0	H
<i>C. candicans</i> ssp. <i>callicola</i>	PERTH 6417574	J Kelly 32 10/2002	95	5	0	0	H
<i>C. candicans</i> ssp. <i>callicola</i>	SDSU 19040	NB Pierce 260 2/9/2007	99	1	0	0	Coll.

Appendix 1. (continued)

Taxon	Acc. no.	Collector	% Bi	%Tri	%Te	% Polyp.	Sp.Orig.
<i>Conostylis candicans</i> ssp. <i>flavifolia</i>	PERTH 5881900	S Donaldson 1449 5/9/1997	94	6	0	0	H
<i>C. candicans</i> var. <i>leptophylla</i>	PERTH 2035332	SD Hopper 136 28/10/1975	96	4	0	0	H
<i>C. candicans</i> ssp. <i>procumbens</i>	PERTH 6507700	BR Maslin 3020 3/10/1972	100	0	0	0	H
<i>C. canteriata</i>	PERTH 4866355	R Davis 3688 10/7/1997	15	85	0	0	H
<i>C. canteriata</i>	SDSU 19039	NB Pierce 269 2/9/2007	1	77	22	0	Coll.
<i>C. canteriata</i>	SDSU 19038	NB Pierce 267 2/9/2007	0	90	10	0	Coll.
<i>C. caricina</i>	PERTH 5449723	H Bowler 108 8/7/1997	15	76	13	0	H
<i>C. caricina</i> ssp. <i>caricina</i>	PERTH 2054434	SD Hopper 888 18/9/1977	24	66	10	0	H
<i>C. caricina</i> ssp. <i>elachys</i>	SDSU 17004	L Aerne 9/27/2005	15	85	7	0	H
<i>C. caricina</i> ssp. <i>elachys</i>	PERTH 7129300	F Hort 2567 6/8/2005	25	65	20	0	H
<i>C. crassinervia</i>	POM 325146	ES Lathrop 28/8/1976	3	80	17	0	H
<i>C. crassinervia</i>	POM 325147	ES Lathrop 342	10	75	15	0	H
<i>C. crassinervia</i> ssp. <i>absens</i>	PERTH 2078635	SD Hopper 411 8/7/1975	3	75	22	0	H
<i>C. crassinervia</i> ssp. <i>absens</i>	SDSU 17005	L Aerne 42 9/26/2005	14	86	0	0	H
<i>C. crassinervia</i> ssp. <i>crassinervia</i>	PERTH 2054906	SD Hopper 385 17/06/1975	4	90	6	0	H
<i>C. deplexa</i>	K	AS George 7043 30/10/1963	46	54	0	0	H
<i>C. dielsii</i>	K	Mobumbr 14354	70	30	0	0	H
<i>C. dielsii</i> ssp. <i>teres</i>	PERTH 5734215	A Chant 18 19/7/2000	44	43	3		H
<i>C. dielsii</i> ssp. <i>teres</i>	K	SD Hopper 442 5/8/1975	70	30	0	0	H
<i>C. drummondii</i>	PERTH 6238408	F Obbens 6102 3/11/2002	20	60	20	0	H
<i>C. festucacea</i> ssp. <i>festucacea</i>	SDSU 17052	MG Simpson 9IX1981H	100	0	0	0	H
<i>C. festucacea</i> ssp. <i>filifolia</i>	SDSU 17007	L Aerne 52 9/26/2005	87	12	1	0	H
<i>C. festucacea</i> ssp. <i>filifolia</i>	PERTH 2055236	SD Hopper 2609 16/9/1982	83	17	0		H
<i>C. hiemalis</i>	PERTH 2055570	SD Hopper 1661 31/07/1980	100	0	0	0	H
<i>C. hiemalis</i>	SDSU 19127	NB Pierce 273 2/9/2007	100	0	0	0	Coll.
<i>C. juncea</i>	SDSU 17031	MG Simpson 13/9/1981W	100	0	0	0	H
<i>C. juncea</i>	PERTH 6986587	P Foreman 428 13/09/2004	100	0	0	0	H
<i>C. latens</i>	PERTH 1634151	EA Griffin 4944 9/9/1988	43	55	2	0	H
<i>C. latens</i>	SDSU 17009	L Aerne 29 9/26/2005	48	51	1	0	H
<i>C. laxiflora</i>	PERTH 5625300	J Scott 450 3-/09/2001	97	3	0	0	H
<i>C. lepidospermoides</i>	K	SD Hopper 1149 12/2/1967	79	20	1	0	H
<i>C. micrantha</i>	K	SD Hopper 2468	23	77	0	0	H
<i>C. micrantha</i>	PERTH 5414164	RM Evans 11 15/08/1998	55	45	0	0	H
<i>C. neocymosa</i>	K	PJ Randall 50 3/9/1992	10	90	0	0	H
<i>C. neocymosa</i>	PERTH 2056860	EA Griffin 942 2/8/1977	9	90	1		H
<i>C. pauciflora</i>	PERTH 4463684	KJ Keighery 452 8/10/19/92	97	3	0	0	H
<i>C. pauciflora</i> ssp. <i>euryhipis</i>	SDSU 16915	L Aerne 24 9/26/2005	99	1	0	0	H
<i>C. pauciflora</i> ssp. <i>pauciflora</i>	PERTH 1271989	SD Hopper 4878 13/06/1986	94	6	0	0	H
<i>C. petrophilioides</i>	SDSU 17030	MG Simpson 27IX1981B	40	60	0	0	H
<i>C. petrophilioides</i>	RSA 208604	R Lullifer 65141 8/10/1966	28	71	1	0	H
<i>C. phathyrantha</i>	K	CA Gardner 16/9/1934	0	0	0	100	H
<i>C. phathyrantha</i>	PERTH 2079399	MA Burgma 1714 4/8/1983	0	0	10	89	H
<i>C. prolifera</i>	SDSU 16917	L Aerne 51 9/26/2005	100	0	0	0	H
<i>C. prolifera</i>	PERTH 5868238	R Davis WW 0740 25/09/2000	96	4	0	0	H
<i>C. pusilla</i>	PERTH 2102714	EM Camming 68 3777 1/10/1968	76	24	0	0	H
<i>C. pusilla</i>	PERTH 6488927	BR Maslin 767 28/09/1970	49	51	0	0	H
<i>C. resinosa</i>	SDSU 16918	L Aerne 44 9/26/2005	100	0	0	0	H
<i>C. resinosa</i>	PERTH 4975952	SD Hopper 5183 8/8/1986	100	0	0		H
<i>C. resinosa</i>	SDSU 19129	NB Pierce 277 9/4/2006	94	6	0	0	Coll.
<i>C. robusta</i>	SDSU 16985	L Aerne 49 9/26/2005	100	0	0	0	H
<i>C. rogeri</i>	K	SD Hopper 1139 12/2/1987	11	81	8	0	H
<i>C. rogeri</i>	PERTH 6884644	GJ Keighery 5214 23/09/1997	46	52	2	0	H
<i>C. seminuda</i>	SDSU 16986	L Aerne 35 9/25/2005	100	0	0	0	H
<i>C. seorsiflora</i>	RSA 267636	AE Orchard 1640 18/10/1968	98	2	0	0	H
<i>C. seorsiflora</i>	K	EHS Jackson 1242 17/9/1982	87	13	0	0	H
<i>C. seorsiflora</i> ssp. <i>Nyabing</i>	PERTH 5888166	MS Graham 1135 18/10/2000	88	12	0	0	H
<i>C. seorsiflora</i> ssp. <i>seorsiflora</i>	PERTH 6362362	M Bennett 512 28/08/1999	94	6	0	0	H
<i>C. setigera</i>	RSA 302862	HS Stewart 15/8/1959	10	82	8	0	H
<i>C. setigera</i>	RSA 228555	TH Aplin 3193 7/9/1970	18	80	2	0	H
<i>C. setigera</i>	PERTH 5320011	K Kershaw C002 15/09/1998	3	78	18	1	H

Appendix 1. (continued)

Taxon	Acc. no.	Collector	% Bi	%Tri	%Te	% Polyp.	Sp.Orig.
<i>C. setigera</i>	K	A Morrison 20131 6/9/1910	25	67	8	0	H
<i>Conostylis setigera</i> spp. <i>dasys</i>	PERTH 1463667	M Graham 311 19/11/1990	2	96	2	0	H
<i>C. setigera</i> ssp. <i>dasys</i>	SDSU 16987	L Aerne 58 9/27/2005	30	70	0	0	H
<i>C. setigera</i> ssp. <i>setigera</i>	PERTH 2106116	SD Hopper 2624 5/10/1982	23	75	2	0	H
<i>C. setigera</i> ssp. <i>setigera</i>	PERTH 2080060	EM Bennett 2790 2/9/1968	25	74	1	0	H
<i>C. setosa</i>	PERTH 6168272	H Wells 29 11/10/2001	4	69	27	0	H
<i>C. setosa</i>	RSA 189046	TH Aplin 3159	5	92	3	0	H
<i>C. stylidioides</i>	SDSU	MG Simpson 9IX81K	92	8	0	0	H
<i>C. stylidioides</i>	RSA 228567	R Coreny 3032	98	2	0	0	H
<i>C. stylidioides</i>	PERTH 2082438	SD Hopper 552 18/08/1975	85	15	0	0	H
<i>C. teretifolia</i>	PERTH 6392326	MA Langley 2087 22/09/1999	2	71	23	4	H
<i>C. teretifolia</i>	POM 325149	ES Lathrop 26/8/1976	13	85	2	0	H
<i>C. teretifolia</i> ssp. <i>planescens</i>	SDSU 16989	L Aerne 28 9/25/2005	26	73	1	0	H
<i>C. teretifolia</i> ssp. <i>teretifolia</i>	SDSU 16990	L Aerne 36 9/25/2005	1	89	10	0	H
<i>C. teretifolia</i> ssp. <i>teretifolia</i>	PERTH 5477263	M Hislop 1501 13/09/1999	3	90	7	0	H
<i>C. teretifolia</i> ssp. <i>teretifolia</i>	SDSU 19130	NB Pierce 276 2/9/2007	1	88	11	0	Coll.
<i>C. teretiuscula</i>	RSA 281411	S Carlquist 9/23/1974	70	30	0	0	H
<i>C. teretiuscula</i>	PERTH 1633031	EA Griffin 4910 11/8/1988	60	40	0	0	H
<i>C. tomentosa</i>	SDSU 16992	L Aerne 43 9/26/2005	100	0	0	0	H
<i>C. tomentosa</i>	K	SD Hopper 5184 8/8/1986	100	0	0	0	H
<i>C. vaginata</i>	RSA 267643	AE Orchard 1500 12/10/1969	75	25	0	0	H
<i>C. vaginata</i>	PERTH 5645638	JW Horn 2643 28/09/1999	80	20	0	0	H
<i>C. vaginata</i>	K	AH Rodd 5042 17/11/1985	35	65	0	0	H
<i>C. vaginata</i>	SDSU 17036	MG Simpson 27IX1981C	55	42	3	0	H
<i>C. villosa</i>	SDSU 16993	L Aerne 55 9/27/2005	55	45	0	0	H
<i>C. villosa</i>	PERTH 07246331	T Watson 488 28/09/2003	70	30	0	0	H
<i>C. wonganensis</i>	PERTH 1002732	AS George s.n. 31/08/1976	55	45	0	0	H
<i>C. wonganensis</i>	SDSU 16994	L Aerne 53 9/27/2005	68	32	0	0	H
<i>Macropidia fuliginosa</i>	PERTH 2086166	WE Blackall 3632 27/08/1938	100	0	0	0	H
<i>Phlebocarya ciliata</i>	SDSU 17022	MG Simpson 18IX1981A	100	0	0	0	H
<i>P. pilosissima</i> ssp. <i>teretifolia</i>	PERTH 5874084	R Davis 10098 20/09/2001	100	0	0	0	H
<i>Tribonanthes brachypetala</i>	PERTH 2088150	BR Maslin 670A 4/8/1970	0	0	0	100	H
<i>T. brachypetala</i>	PERTH 2088169	R Helms 19/07/1897	0	0	0	100	H
<i>T. longipetala</i>	PERTH 7296029	S Patrick 1509 19/08/1993	0	0	0	100	H
<i>T. purpurea</i>	PERTH 6985181	GJ Keighery 270 1/8/2004	0	0	0	100	H