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A MOLECULAR PHYLOGENETIC STUDY OF GENERIC AND SUBGENERIC RELATIONSHIPS IN THE SOUTHWEST AUSTRALIAN ENDEMICS *CONOSTYLIS* AND *BLANCOA* (HAEMODORACEAE)

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ABSTRACT

We sequenced the plastid gene *mat*K and the nuclear ribosomal spacer ITS for 39 of the 47+ species of *Conostylis* as well as its monotypic sister genus *Blancoa,* which some authors have included within *Conostylis. Conostylis* received 99% bootstrap support as monophyletic, with 100% support that *Blancoa* is its sister. Within *Conostylis,* the study provides strong support for two large sister clades, which we refer to as clades A (100%) and B (99%). Clade A consists of *C.* subgen. *Conostylis* plus the recently discovered *C. glabra* of *C.* subgen. *Pendula* sect. *Divaricata* (100%), and *C.* subgen. *Pendula* sect. *Appendicula* (100%). Clade B consists of species mostly placed within the remainder of *C.* subgen. *Pendula* but also contains members of the other small subgenera. Subgenus *Pendula* can be recircumscribed as monophyletic by excluding sect. *Appendicula, Conostylis phathyrantha,* and *C. glabra* and including subgen. *Androstemma* and subgen. *Greenia.* The status of the other two minor subgenera—*C.* subgen. *Brachycaulon* and *C.* subgen. *Bicolorata*—requires further investigation. *Conostylis* sect. *Divaricata* is polyphyletic. Ancient vicariance events are postulated for *Conostylis* involving separation of major clades in the northern and southern kwongan regions of southwestern Australia. The phylogenetic pattern in *Conostylis* is consistent across several lineages with the prolonged persistence of relictual taxa combined with explosive more recent speciation, the latter pronounced in the northern kwongan. There is evidence of significant divergence in major speciation mechanisms and chromosome number change among the three most species-rich subgenera/sections (dysploidy in *Pendula* and *Appendicula* vs. diploid speciation in *Conostylis*). Further investigation is needed to evaluate these ideas and elucidate the patterns of speciation in this most diverse genus of Haemodoraceae.

Key words: *Blancoa, Conostylis,* Haemodoraceae, kwongan, phylogenetics, southwestern Australia, speciation.

INTRODUCTION

Haemodoraceae, as with many families of monocots, have had a tortuous taxonomic history, which prompted Ornduff (1979) to comment that "the substantial and continuing disagreements concerning the limits of the Haemodoraceae and the relationships of the genera assigned to it indicate that the family merits additional detailed study." Such studies were carried out subsequently. Today, in light of molecular and other data, Haemodoraceae are placed in Commelinales with Commelinaceae, Hanguanaceae, Philydraceae, and Pontederiaceae (Simpson 1990, 1998; Hopper et al. 1999; Chase et al. 2000; Angiosperm Phylogeny Group II [APG II] 2003).

No morphological synapomorphy is known for the family, and the common occurrence of arylphenalenones, once thought to be unique to Haemodoraceae (Cooke and Segal 1955; Cooke and Edwards 1981; Simpson 1990), is now also known from Musaceae, Pontederiaceae, and Strelitziaceae (Greca et al. 1992; Opitz and Schneider 2002; Opitz et al. 2002; Otalvaro et al. 2002). Nevertheless, there is 100% bootstrap support for the monophyly of Haemodoraceae based on *trn*L–F plastid DNA sequences (Hopper et al. 1999). They are a small family with many highly divergent, relictual taxa, as well as a few genera demonstrating a more recent evolutionary radiation.

Two groups have been documented within Haemodoraceae from the earliest descriptions of the family. Whereas Takhtajan (1997) advocated recognition of these as separate families (i.e., Conostylidaceae and Haemodoraceae), most authors have advocated tribal status for them (reviewed by Simpson 1990). We found these to be clades (Hopper et al. 1999) and recognize them as subfamilies, following Dahlgren et al. (1985) and Macfarlane et al. (1987).

Haemodoraceae subfamily Haemodoroideae are widespread, with eight small genera and ca. 30 species in South Africa, the Americas, and Australia-New Guinea. Haemodoroideae have three (rarely one) fertile stamens and mostly glabrous flowers, and their rootstocks usually have red pigments (hence the common name bloodroots). Conostylidoideae are endemic to the southwestern Australian global biodiversity hotspot (Myers et al. 2000; Hopper and Gioia 2004) and have six genera with ca. 77 species. There are six stamens in Conostylidoideae, flowers are usually pubescent, and there is no red pigment in their rootstocks.

Conostylis is the largest genus in Haemodoraceae, with 45 species recognized by Hopper et al. (1987), and a few more are awaiting description based on recent research (Hopper, unpubl. data), all endemic to southwestern Australia (Fig. 1). The genus contains a few relictual, divergent species as well as several species complexes in which patterns of geo-

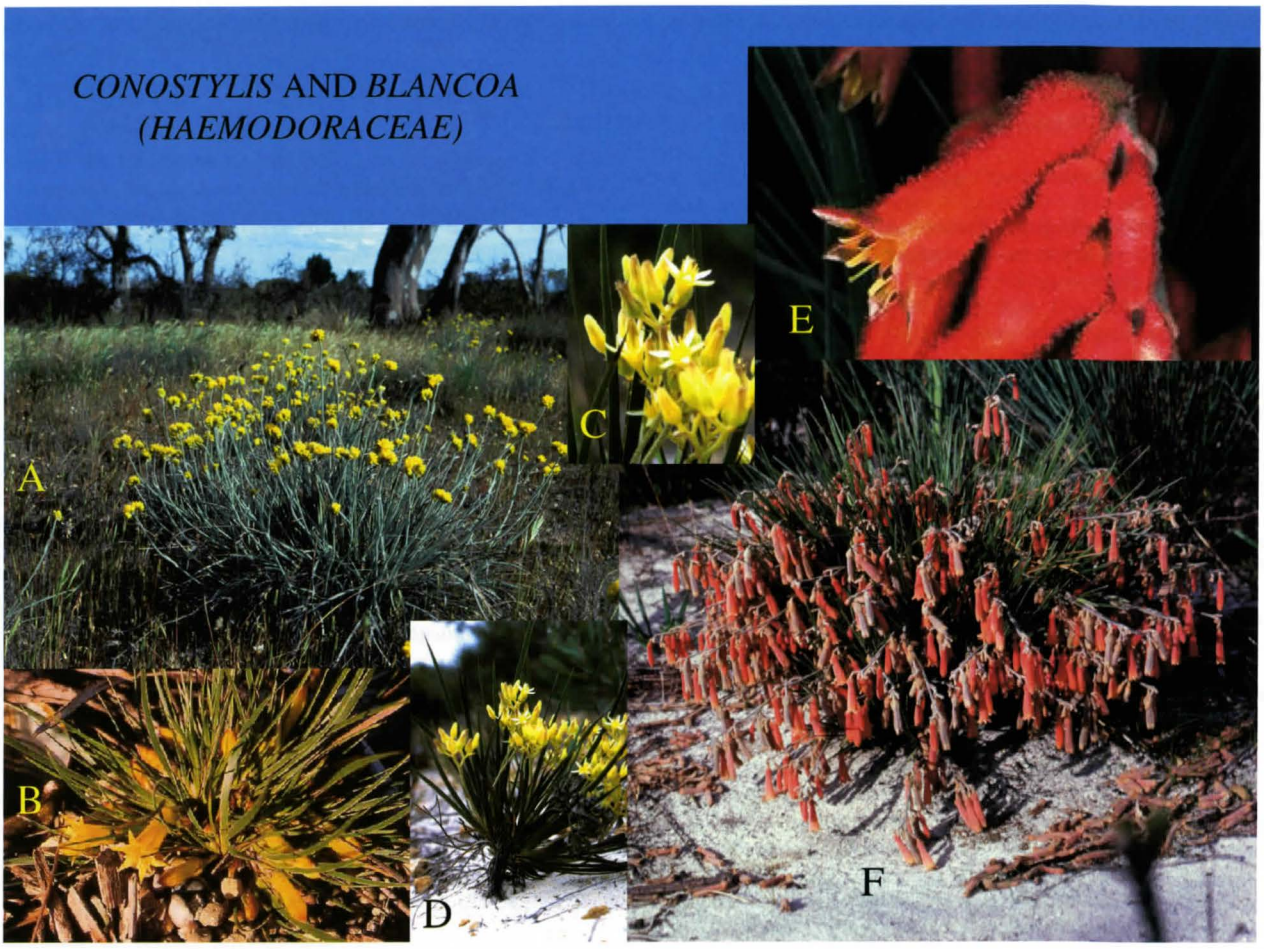


Fig. 1.—Species of *Conostylis* and the monotypic *Blancoa.*—(A). *C. candicans*–C. subgen. *Conostylis.*—(B). *C. bealiana–C.* subgen. *Greenia*—(C, D). *C. neocymosa–C.* sect. *Divaricata*—(E, F). *Blancoa canescens.*

graphical variation, natural hybridization, and dysploidy and/or polyploidy are evident.

European botanists in the nineteenth century faced considerable difficulties in understanding variation in these complexes from specimens collected mainly by James Drummond and Ludwig Preiss. Some 24 species named, for example, by Lindley (1840), Endlicher (1846), and Bentham (1873), are now regarded as synonymous with currently recognized species.

This unusually high level of synonymy was largely resolved by Green (1960), who provided a generic revision based on herbarium and field studies together with anatomical (Green 1959) and cytological observations. Green (1960) recognized only 23 species, including two new endemics from the then recently developed northern kwongan agricultural region centered on Eneabba and Mt. Lesueur.

Subsequently, the genus has been studied in the field and collected extensively, resulting in the description of 15 new species, the reinstatement to species status of six names regarded by Green (1960) as synonyms, and the elevation of one subspecies to full species status (Hopper 1978, 1980, 1982, 1987*a*; Green 1982). A total of 22 species additional to the 23 recognized by Green (1960) were described in the *Flora of Australia* treatment of Hopper et al. (1987). Moreover, 31 subspecies were also recognized in the *Flora of Australia,* most described for the first time there.

Thus, as with many genera endemic to southwestern Australia, more than half the species of *Conostylis* have been described or reinstated in just the past two decades during an exciting phase of modern botanical discovery (Hopper 2003, 2004; Hopper and Gioia 2004). Consequently, much remains to be investigated to develop a sound evolutionary understanding of the genus. Early work on natural hybridization (Hopper 1977; Krauss and Hopper 2001) and seed biology (Tieu et al. 2001*a*, *b*) already indicated the potential for future studies.

The generic relationships of *Conostylis* and subgeneric taxonomy of the genus remain controversial or in need of further elucidation. Whereas most authors recognized the monotypic *Blancoa* as sister to *Conostylis* (e.g., Lindley 1840; Bentham 1873; Keighery 1980; Hopper 1987*b*; Hopper et al. 1999; Fig. 1), others have considered *Blancoa* a synonym of *Conostylis* (Mueller 1873; Geerinck 1969; Macfarlane 1987; Simpson 1990, 1998). In our earlier study of *trn*L–F sequences in Haemodoraceae, Blancoa was sister to three species from divergent subgenera of *Conostylis,* but with only moderate bootstrap support. Further work on this generic relationship was recommended. Moreover, the di-

vision of *Conostylis* into six subgenera, although building on earlier ideas of Lindley (1840), Bentham (1873), Green (1960), and Geerinck (1969), has yet to be evaluated with molecular data.

For these reasons, we embarked upon a study of generic and subgeneric relationships in *Conostylis* and the allied genus *Blancoa.* Given that sequence divergence in the plastid region *trn*L–F found in an earlier study of Haemodoraceae (Hopper et al. 1999) was not high in the three species of *Conostylis* investigated, we used the plastid *mat*K and nuclear ribosomal spacer ITS in this study because they are known in other groups to have more variable sites than *trn*L–F (e.g., Kores et al. 2001). It was anticipated that such a molecular phylogenetic study of *Conostylis* and *Blancoa* would also shed light on the phytogeographic history of southwestern Australia, enabling hypotheses on patterns of speciation to be evaluated (cf. Hopper 1979, 1992; James 1981; James and Hopper 1981; Hopper and Gioia 2004).

MATERIALS AND METHODS

Plant Material

Sources of plant material and locations of vouchers are listed in Table 1. Some 39 of the 47+ species of Conostylis were sampled, together with *Blancoa canescens.* These taxa represented all subgenera and sections within *Conostylis* and most morphologically divergent species known in the genus, as well as several closely related taxa within species complexes. Outgroups were chosen from within subfamily Conostylidoideae based on the earlier *trn*L–F study. They included *Anigozanthos* (3 spp.), *Macropidia* (1 sp.), *Phlebocarya* (1 sp.), and *Tribonanthes* (1 sp.).

DNA Extraction, Gene Amplification and Sequencing

DNA was extracted from 1.0 g fresh or 0.2–0.25 g silica gel-dried leaves (Chase and Hills 1991) using the 2X CTAB method of Doyle and Doyle (1987), except that all samples were purified on cesium chloride/ethidium bromide gradients (1.55 g/mL density).

Amplification of *mat*K and ITS from total DNA was carried out using the primers of H. Sun et al. (2001) and Y. Sun et al. (1994), respectively. Amplified products were cleaned using Magic Miniprep columns (Promega, Annandale, New South Wales, Australia), following protocols provided by the manufacturer.

Modified dideoxy cycle sequencing with dye terminators run on an ABI 377 automated DNA Sequencer (according to the manufacturer's protocols; Applied Biosystems, Inc., Scoresby, Victoria, Australia) was used to sequence the amplification products directly.

Sequence Alignment

Sequences were added to the matrix and aligned manually following the guidelines of Kelchner (2000). The aligned matrix is available from *m.fay@kew.org.*

Data Analysis

We analyzed the combined matrix using heuristic searches with PAUP* vers. 4.0b10 (Swofford 2001) using the following strategy: 1000 replicates of randomized taxon entry with subtree-pruning-regrafting (SPR) swapping and a tree limit of 20 trees per replicate to reduce the time spent swapping on suboptimal islands of trees. We then used bootstrapping (Felsenstein 1985*a*, *b*) to estimate internal support with 500 replicates of simple taxon addition, again with a limit of 20 trees per replicate and SPR swapping.

Two separate analyses were done; both using combined *mat*K and ITS data. Analysis 1 (Fig. 2) includes all 64 taxa indicated in Table 1. Analysis 2 (Fig. 3) includes a subset of 50 of the taxa of Table 1, omitting those for which sequences are missing. For both analyses we show one randomly selected tree to illustrate branch lengths (DELTRAN optimization due to problems with ACCTRAN optimization in PAUP* vers. 4.0b10) and indicate branches not found in the strict consensus with an asterisk. We report all bootstrap percentages (% BS) >50.

RESULTS

We present here the results of analyses 1 and 2 (both using the combined *mat*K and ITS data) (Fig. 2, 3). Both analyses corroborate the hypothesis that *Conostylis* is monophyletic, supported by 99% bootstrap. Moreover, *Blancoa* is corroborated as sister to *Conostylis* with 100% bootstrap support in the two analyses. Within *Conostylis,* two major clades, labeled "A" and "B," were resolved, with strong bootstrap support in both analyses (Fig. 2, 3). In clade A, *Conostylis* sect. *Appendicula* fell as sister to *C.* subgen. *Conostylis* (100% bootstrap in analysis 2). The enigmatic, recently discovered *C. glabra,* which presently keys out as a member of *C.* sect. *Divaricata* within *C.* subgen. *Pendula,* was embedded within *C.* subgen. *Conostylis* as sister to *C. serrulata* subsp. *serrulata* with 95% bootstrap support in analysis 2 (Fig. 3). *Conostylis misera* was also part of a trichotomy (in the strict consensus trees) at the base of the *C.* subgen. *Conostylis* clade. A large clade with 95% bootstrap support (in analysis 2) constituted the majority of this subgenus, but relationships among species were poorly resolved except for a clade comprising *C. bracteata, C. candicans* subsp. *candicans* (Fig. 1A) and *C. pauciflora* subsp. *euryrhipis,* which received 98% support in analysis 2 (Fig. 3).

The second major clade within *Conostylis* (B in Fig. 2, 3) comprised all of *C.* sect. *Catospora* (the largest component of C. subgen. *Pendula*), together with *C.* subgen. *Androstemma, C.* subgen. *Bicolorata, C.* subgen. *Brachycaulon, C.* subgen. *Greenia,* and two of the three species of *C.* sect. *Divaricata* (*C. neocymosa* and *C. phathyrantha*). Excluding the monospecific *C.* subgen. *Bicolorata* and *C.* subgen. *Brachycaulon,* all the above taxa were polyphyletic. *Conostylis phathyrantha* was sister to *C.* subgen. *Bicolorata* and *C.* subgen. *Brachycaulon* in a moderately supported clade (63% in analysis 2) sister to all other taxa in clade B. This remaining moderately supported clade (72% in analysis 2) formed a polytomy with four single species (*C. micrantha, C. androstemma, C. rogeri,* and *C. albescens*) and three strongly supported clades (Fig. 3).

DISCUSSION

Generic Relationships

Our study affirms the earlier work on plastid sequences of a much smaller sample of species (Hopper et al. 1999)

Table 1. Sources of plant material. Classification follows Hopper et al. (1987). Voucher numbers are for S. D. Hopper specimens at Kings Park and Botanic Garden (KPBG) unless otherwise stated.

Taxon	Locality in SW Australia (*Conostylis* only)	Voucher
Conostylis R. Br.		
subgen. *Androstemma* (Lindl.) Hopper		
C. androstemma F. Muell.	Yandan Hill, SE Cataby	*8335*
C. argentea (J. Green) Hopper	Jacup	*8422*
subgen. *Greenia* (Geer.) Hopper		
C. albescens Hopper	Booraan, E Merredin	*8430*
C. bealiana F. Muell.	W Ravensthorpe	*8435*
subgen. *Brachycaulon* (Benth.) Hopper		
C. breviscapa R. Br.	Torradup, E Ravensthorpe	*8432*
subgen. *Bicolorata* Hopper		
C. vaginata Endl.	Jacup	*8423*
subgen. *Conostylis*		
C. aculeata R. Br.		
subsp. *aculeata*	Gracetown	*8447*
subsp. *breviflora* Hopper	W Arrino	*8470*
subsp. *bromelioides* (Endl.) J. Green	Watheroo National Park	*8480*
subsp. *cygnorum* Hopper	Kings Park	*8481*
subsp. *gracilis* Hopper	Gracetown	*8450*
subsp. *preissii* (Endl.) J. Green	Mogumber	*8459*
subsp. *spinuligera* (F. Muell. ex Benth.) Hopper	Cataby	*8460*
C. bracteata Lindl.	Lancelin	*8452*
C. candicans Endl.		
subsp. *candicans*	?	*Chase 185* (NCU)
C. festucacea Endl.		
subsp. *filifolia* (F. Muell.) Hopper	Watheroo National Park	*8478*
C. juncea Endl.	E Lancelin	*8453*
C. laxiflora Benth.	E Karridale	*8446*
C. lepidospermoides Hopper	NE Ravensthorpe	*8434*
C. misera Endl.	?	Ex cult. (KPBG)
C. pauciflora Hopper		
subsp. *euryrhipis* Hopper	Seabird	*8451*
C. prolifera Benth.	W Arrino	*8471*
C. seorsiflora F. Muell.		
subsp. *seorsiflora*	Jacup	*8424*
C. serrulata R. Br.		
subsp. *serulata*	S Kojonup	*8429*
subsp. *magna* Hopper ined.	E Jurien	*8474*
C. stylididoides F. Muell.	Kalbarri	*s.n.*
subgen. *Pendula* Hopper		
sect. *Catospora* Benth.		
C. canteriata Hopper	Eneabba	*8464*
C. caricina Lindl.		
subsp. *caricina*	Avon Valley	*8494*
subsp. *elachys* Hopper	N Dowerin	*8495*
C. crassinerva J. W. Green		
subsp. *crassinerva*	E Jurien	*8462*
subsp. *absens* Hopper	Eneabba	*8472*
C. deplexa J. Green	W Ravensthorpe	*8437*
C. dielsii W. Fitzg.		
subsp. *dielsii*	S Mingenew	*8469*
subsp. *teres* Hopper	N Irwin	*8468*
C. drummondii Benth.	S Kojonup	*8443*
C. latens Hopper	Gillingarra West Rd	*8456*
C. micrantha Hopper	N Irwin	*8467*
C. petrophiloides F. Muell.	E Newdegate	*8431*
C. pusilla Endl.	Albany Hwy	*8445*
C. rogeri Hopper	Hopkins Nature Reserve	*8430a*
C. setigera R. Br.		
subsp. *dasys* Hopper	S Kojonup	*8442*
subsp. *setigera*	Demarz	*11519* (KPBG)

Table 1. Continued.

Taxon	Locality in SW Australia (*Conostylis* only)	Voucher
C. setosa Lindl.	Albany Hwy	*8444*
C. teretifolia J. W. Green		
subsp. *teretifolia*	Eneabba	*8475*
subsp. *planescens* Hopper	E Lancelin	*8455*
C. teretiuscula F. Muell.	Gillingarra West Rd	*8457*
	E Watheroo	*8427*
C. wonganensis Hopper	Wongan Hills	*ex cult.* (KPBG)
sect. *Divaricata* Hopper		
C. glabra Hopper ined.	Watheroo National Park	*8479*
C. neocymosa Hopper	E Watheroo	*8428*
C. phathyrantha Diels	Torradup, E Ravensthorpe	*8436*
sect. *Appendicula* Geer.		
C. angustifolia Hopper	N Cataby, Brand Hwy	*8461*
C. aurea Lindl.	E Lancelin	*8454*
C. hiemalis Hopper	Eneabba	*8473*
C. resinosa Hopper	Arrowsmith River	*8466*
C. seminuda Hopper	Alexander Morrison NP	*8477*
C. tomentosa Hopper	Arrowsmith River	*8465*
Blancoa Lindl.		
B. canescens Lindl.		*Chase 2232* (K)
Phlebocarya R. Br.		
P. ciliata R. Br.		*Chase 2233* (K)
Anigozanthos Labill.		
subgen. *Anigozanthos*		
sect. *Ceratandri* Benth.		
A. flavidus DC.		*Chase 3082* (K)
sect. *Concatenati* Hopper		
A. preissii Endl.		*ex cult.* (KPBG)
subgen. *Haplanthesis* (Benth.) Hopper		
A. humilis Lindl.		
subsp. *humilis*		*Demarz 9866* (KPBG)
Macropidia J. L. Drumm. ex Harv.		
M. fuliginosa (Hook.) Druce		*Dixon s.n.* (KPBG)
Tribonanthes Endl.		
T. australis Endl.		*8441*

that *Conostylis* is monophyletic but with higher bootstrap support (99%). Moreover, we obtained 100% support that *Blancoa* is sister to *Conostylis*. Given our more comprehensive sampling of species, and the far greater number of parsimony informative characters in the *mat*K–ITS sequence data than in Simpson's (1990) morphological study, we can discount his hypothesis that *Blancoa* is embedded within *Conostylis*.

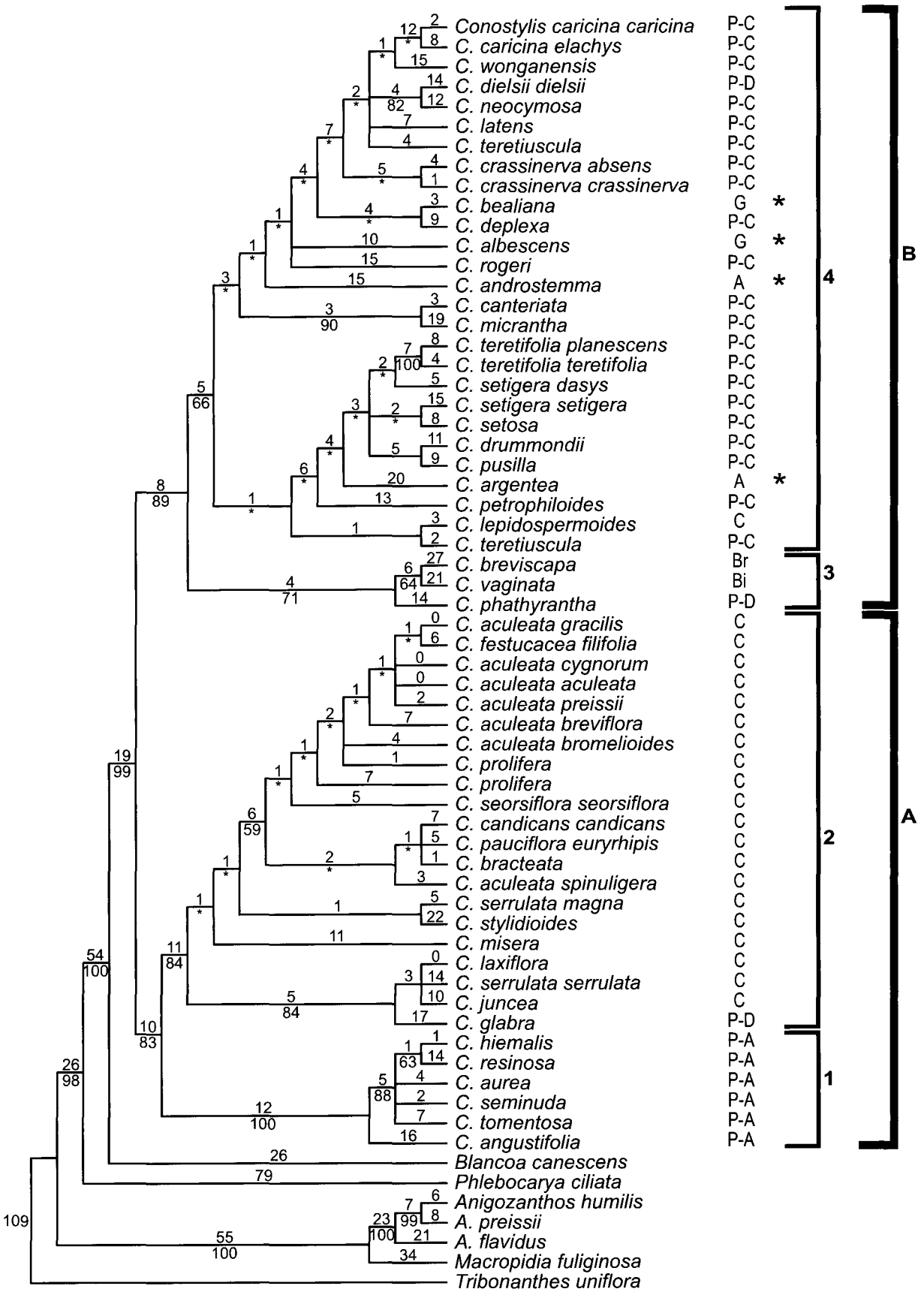
Blancoa differs from *Conostylis* in its large, tubular, pendulous red flowers borne on a unilateral raceme, the ridged adnation of the upper ovary to the perianth at the septa forming three deep nectar pockets, and the ovules several per locule in two rows on a short, vertically elongate, non-dilated placenta (Hopper 1987*b*).

Blancoa canescens is pollinated by birds and honey possums (Keighery 1980; Brown et al. 1997). Its phylogenetic position as sister to *Conostylis*, and its highly modified flowers, indicate that the evolution of adaptations for pollination by vertebrates must have been an old event, predating those that gave rise independently to species of *Conostylis* with tubular flowers such as *C. bealiana* (Fig. 1B), *C. albescens*, *C. androstemma*, and *C. argentea*.

Although *Blancoa* is unequivocally sister to *Conostylis*, sinking *Blancoa* in *Conostylis* is still an option (Backlund and Bremer 1998). However, in this case we prefer to advocate nomenclatural stability. *Blancoa* was first named as a distinct genus by Lindley (1840), with most authors subsequently upholding this view. Consequently, least disruption to nomenclatural use and maximal retrieval of information from the literature will occur if *Blancoa* continues to be recognized at generic rank (Backlund and Bremer 1998).

Subgeneric Relationships within Conostylis

In light of the molecular data, significant modification to the subgeneric treatment of *Conostylis* by Hopper et al. (1987) is needed. Two major clades emerged within the genus—clade A corresponding to *C.* subgen. *Conostylis* + *C.* sect. *Appendicula* (of *C.* subgen. *Pendula*) and the enigmatic *C. glabra* and clade B including all other species in a large


Conostylis caricina caricina
C. caricina elachys
C. wonganensis
C. dielsii dielsii
C. neocymosa
C. latens
C. teretiuscula
C. crassinerva absens
C. crassinerva crassinerva
C. bealiana
C. deplexa
C. albescens
C. rogeri
C. androstemma
C. canteriata
C. micrantha
C. teretifolia planescens
C. teretifolia teretifolia
C. setigera dasys
C. setigera setigera
C. setosa
C. drummondii
C. pusilla
C. argentea
C. petrophiloides
C. lepidospermoides
C. teretiuscula
C. breviscapa
C. vaginata
C. phathyrantha
C. aculeata gracilis
C. festucacea filifolia
C. aculeata cygnorum
C. aculeata aculeata
C. aculeata preissii
C. aculeata breviflora
C. aculeata bromelioides
C. prolifera
C. prolifera
C. seorsiflora seorsiflora
C. candicans candicans
C. pauciflora euryrhipis
C. bracteata
C. aculeata spinuligera
C. serrulata magna
C. stylidioides
C. misera
C. laxiflora
C. serrulata serrulata
C. juncea
C. glabra
C. hiemalis
C. resinosa
C. aurea
C. seminuda
C. tomentosa
C. angustifolia
Blancoa canescens
Phlebocarya ciliata
Anigozanthos humilis
A. preissii
A. flavidus
Macropidia fuliginosa
Tribonanthes uniflora
P-C
P-C
P-C
P-D
P-C
P-C
P-C
P-C
P-C
G
P-C
G
P-C
A
P-C
C
P-C
Br
Bi
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clade centered on *C.* sect. *Catospora* of *C.* subgen. *Pendula* and the remaining four minor subgenera. Each of these two major clades received very strong bootstrap support, but neither has any recognizable morphological synapomorphies.

Clearly, however, all subgenera with two or more species recognized by Hopper et al. (1987) are paraphyletic. Of the two largest subgenera, *C.* subgen. *Conostylis* (14 spp.) was retrieved intact in clade A in our study, requiring only the addition of *C. glabra* to become monophyletic. Although *C. glabra* is not yet formally described, this robust local endemic keys out as a third member of *C.* sect. *Divaricata* within *C.* subgen. *Pendula.* The other two species of this section, *C. phathyrantha* and *C. neocymosa* (Fig. 1C, D), occur at divergent points in clade B. It is clear that the loosely cymose inflorescences with flowers on long pedicels seen in *C. phathyrantha, C. neocymosa,* and *C. glabra* have evolved independently in three divergent lineages of *Conostylis. Conostylis* sect. *Divaricata* as circumscribed by Hopper et al. (1987) should be abandoned as polyphyletic. With the inclusion of *C. glabra,* the monophyletic *C.* subgen. *Conostylis* is diagnosed by numerous ovules per locule borne on a globose placenta and a chromosome number of $n = 8$ (except for *C. stylidioides* which has $n = 16$).

Placement of *C. stylidioides* in clade A (Fig. 2) is interesting, as it has been hypothesized to be an allopolyploid hybrid of *C. aculeata* and *C. candicans* (Hopper 1978, 1995; Krauss and Hopper 2001). The highly divergent *C. stylidioides* fell outside a weakly supported (59% in Analysis 1) clade containing its putative parents, its precise relationships unclear in an unresolved polytomy comprising the *C. aculeata–C. candicans* clade, a novel strongly supported clade (84% in analysis 1) of *C. glabra, C. juncea, C. laxiflora,* and *C. serrulata* subsp. *serrulata,* and two other divergent taxa—*C. misera* and *C. serrulata* subsp. *magna. Conostylis stylidioides* is one of the three most divergent taxa of *Conostylis* revealed in this study, its number of unique base pair changes (22) approximating those for the monotypic *C.* subgen. *Bicolorata* (*C. vaginata*) and *C.* subgen. *Brachycaulon* (*C. breviscapa*). If it is an allopolyploid, it must be of considerable antiquity, or its genome underwent considerable and rapid reorganization following its hybrid genesis (Krauss and Hopper 2001).

On the other hand, *C.* subgen. *Pendula,* with 25 species, has two of its three sections polyphyletic. Only *C.* sect. *Appendicula* was retrieved in our study as a clade. Even this section, however, proved to be sister to *C.* subgen. *Conostylis* in clade A rather than in clade B with other taxa in *C.* subgen. *Pendula* in which *C.* sect. *Appendicula* was originally placed.

The diagnostic characters for *C.* subgen. *Pendula* are the ovules few and pendulous from a peltate placenta or rarely several on the lateral and lower sides of a globose placenta (Hopper et al. 1987). On the basis of our results, ovule number and placentation are subject to convergent evolution, with similar ovule number reductions having arisen independently in several lineages of *Conostylis. Conostylis* subgen. *Pendula* needs to be much more narrowly circumscribed or its diagnostic characters redefined to parallel its relationships in our study.

The arrangement of taxa in clade B calls for a major reassessment of the subgeneric treatment of *Conostylis. Conostylis phathyrantha,* the type species of *C.* sect. *Divaricata,* is sister to the monotypic *C.* subgen. *Bicolorata* (*C. vaginata*) and *C.* subgen. *Brachycaulon* (*C. breviscapa*) in a moderately supported clade (71% in analysis 1, 63% in analysis 2) sister to all other taxa in clade B. Elsewhere in clade B, there is a large unresolved polytomy comprising four single species (*C. albescens, C. androstemma, C. micrantha,* and *C. rogeri*) and three strongly supported clades.

Four vertebrate-pollinated species with large tubular flowers are widely scattered in clade B rather than forming the two monophyletic subgenera: *C.* subgen. *Greenia* (*C. bealiana,* Fig. 1B, *C. albescens*) and *C.* subgen. *Androstemma* (*C. androstemma, C. argentea*). Multiple origins of vertebrate pollination are indicated here, and these subgenera should be abandoned as polyphyletic. However, it is possible that a tubular perianth could represent an apomorphy for *C. albescens, C. androstemma,* and *C. bealiana,* given that their interrelationships are unresolved in strict consensus trees (Fig. 2, 3). A tubular perianth definitely evolved independently in *C. argentea,* according to our analyses.

Further data are needed from additional gene sequences and taxa before the many polytomies in the consensus tree are resolved. Consequently, we refrain here from making formal changes to the infrageneric classification of *Conostylis.* However, we do note that *C.* subgen. *Pendula* could be recircumscribed as monophyletic by excluding *C.* sect. *Appendicula, C. phathyrantha,* and *C. glabra* and including *C.* subgen. *Androstemma* and subgen. *Greenia.*

With a few exceptions, precise sister species relationships are not identifiable in our study. We can say, however, that some subspecies merit elevation to specific rank based on their molecular phylogenetic placement in our consensus tree (e.g., *C. setigera* subsp. *dasys, C. serrulata* subsp. *magna*).

Chromosome Number Change

Knowledge of chromosome numbers in Conostylidoideae has accumulated since the pioneering count of $n = 6$ for *Anigozanthos flavidus* by Stenar (1927). Green (1960) made a major contribution, reporting counts for 24 species of southwestern Australian Conostylidoideae, including confirmation of $n = 6$ for *Anigozanthos,* new records of $n = 4, 5, 7, 8$ and 14 in *Conostylis,* and $n = 8$ for *Blancoa.* James (unpubl. data, 1971) examined hybrids of *Anigozanthos manglesii* D. Don and *A. humilis,* confirming the haploid

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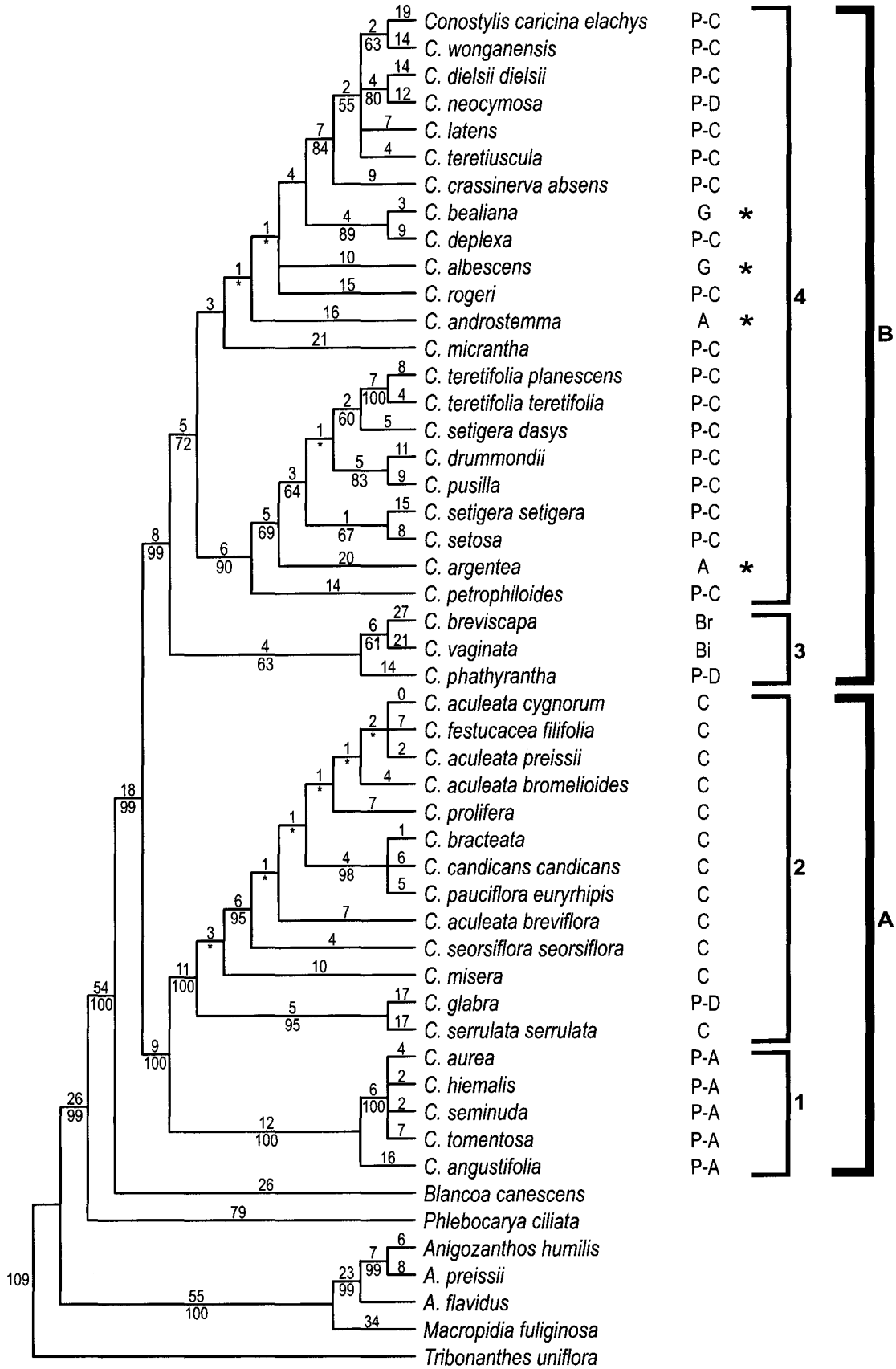
Fig. 2.—Tree from the combined *mat*K and ITS analysis. Number of changes above the line, bootstrap values below. Nodes marked by an asterisk (*) collapsed in the consensus tree. Subgeneric taxa from Hopper et al. (1987) indicated as: P-C = *Conostylis* subgen. *Pendula* sect. *Catospora*; P-D = *C.* subgen. *Pendula* sect. *Divaricata*; P-A = C. subgen. *Pendula* sect. *Appendicula*; G = *C.* subgen. *Greenia*; A = *C.* subgen. *Androstemma*; Br = *C.* subgen. *Brachycaulon*; Bi = *C.* subgen. *Bicolorata*; C = *C.* subgen. *Conostylis.* Large asterisk beside *Conostylis* taxa indicate possession of a tubular perianth. Note monophyletic groups A (containing clades 1 and 2), and B (containing clades 3 and 4).


Conostylis caricina elachys
C. wonganensis
C. dielsii dielsii
C. neocymosa
C. latens
C. teretiuscula
C. crassinerva absens
C. bealiana
C. deplexa
C. albescens
C. rogeri
C. androstemma
C. micrantha
C. teretifolia planescens
C. teretifolia teretifolia
C. setigera dasys
C. drummondii
C. pusilla
C. setigera setigera
C. setosa
C. argentea
C. petrophiloides
C. breviscapa
C. vaginata
C. phathyrantha
C. aculeata cygnorum
C. festucacea filifolia
C. aculeata preissii
C. aculeata bromelioides
C. prolifera
C. bracteata
C. candicans candicans
C. pauciflora euryrhipis
C. aculeata breviflora
C. seorsiflora seorsiflora
C. misera
C. glabra
C. serrulata serrulata
C. aurea
C. hiemalis
C. seminuda
C. tomentosa
C. angustifolia
Blancoa canescens
Phlebocarya ciliata
Anigozanthos humilis
A. preissii
A. flavidus
Macropidia fuliginosa
Tribonanthes uniflora
P-C
P-C
P-C
P-D
P-C
P-C
P-C
G
P-C
G
P-C
A
P-C
P-C
P-C
P-C
P-C
P-C
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P-A
P-A
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4
3
2
1
B
A

number $n = 6$ and noted some meiotic irregularities associated with pollen sterility in the hybrids. Hopper (1978) reported a case of allopolyploidy ($n = 16$) in *C. stylidioides*. More comprehensive data for *Conostylis* and related genera were then reported briefly by Hopper et al. (1987), who listed previously unpublished counts of $n = 5, 6, 7, 8, 14, 16, 21$ and 28 for *Conostylis*, $n = 8$ for *Blancoa*, and $n = 6$ for *Anigozanthos* and *Macropidia*.

Simpson (1990) reported other unpublished counts including $n = 7$ for *Phlebocarya* (vouchers: *Hopper 840* PERTH, *Keighery 691* PERTH), and $n = 7$ for *Tribonanthes* (attributed to G. Keighery pers. comm.). Although $n = 7$ for *Phlebocarya* is consistent with our own data (Hopper unpubl. data), we have obtained $n = 11$ for two species of *Tribonanthes* (Hopper and Stone unpubl. data). Moreover, G. J. Keighery (pers. comm.) has no record of a count of $n = 7$ for *Tribonanthes*, and the report of $n = 7$ for the genus was an error in Simpson 1990 (Simpson pers. comm.).

Now that a well-corroborated molecular analysis of *Conostylis* is in place, the opportunity to explore the evolution of chromosome number change presents itself. Based on outgroup comparison, it is most likely that the ancestor of *Conostylis* had a chromosome number of $n = 8$ (Green 1960; Simpson 1990). *Blancoa* is $n = 8$, *Phlebocarya* $n = 7$, *Macropidia* $n = 6$, and *Anigozanthos* $n = 6$. Only *Tribonanthes*, sister to all the above genera in subfamily Conostylidoideae, has a higher number ($n = 11$). However, members of subfamily Haemodoroideae have higher numbers still, leading to two contrasting models of cytoevolution in Haemodoraceae. Either the common ancestor had $n = 8$, and the higher numbers in subfamily Haemodoroideae and *Tribonanthes* are due to ancient palaeopolyploidy (Simpson 1990), or the common ancestor had $n = 16$, and numbers less than this are due to dysploid decreases.

In *Conostylis*, one species has $n = 16$ (*C. stylidoides*), interpreted as tetraploid on $n = 8$ (Hopper 1978, 1995; Krauss and Hopper 2001). However, an alternative hypothesis is that $n = 16$ is a plesiomorphic number retained in *C. stylidioides* and in no other species. This seems unlikely because all other taxa in *C.* subgen. *Conostylis* are uniformly diploid as $n = 8$. Circumstantial but not yet definitive evidence for an allopolyploid origin of *C. stylidioides* exists (Krauss and Hopper 2001), whereas there is none for the massive extinction of taxa with $n = 15$ to $n = 9$ in *C.* subgen. *Conostylis* required to support the hypothesis of *C. stylidoides* as a plesiomorphic diploid of $n = 16$, and other species in the subgenus derived from dysploid reduction to and stabilization on $n = 8$.

Significant correlations with the chromosomal uniformity on $n = 8$ (excluding *C. stylidioides*) in *C.* subgen. *Conostylis* are the numerous ovules per locule (Hopper et al. 1987) and the frequent occurrence of natural hybridization observed in the *C. aculeata–C. candicans* complex, with only minor levels of pollen sterility in hybrids recorded (e.g., Hopper 1977; Krauss and Hopper 2001). Indeed, the only other species of the genus outside *C.* subgen. *Conostylis* with numerous ovules per locule is *C. androstemma*, deeply embedded in clade B among species with few per locule, indicating that the high ovule number in *C. androstemma* is a parallelism. Moreover, the only case of natural hybridization outside of *C.* subgen. *Conostylis* found among the many sympatric combinations of species is a hybrid of *C. resinosa* × *C. tomentosa* (voucher *Hopper 2473*, PERTH) in *C.* sect. *Appendicula*. This hybrid was highly sterile, with pollen fertility estimated at 3% (Hopper unpubl. data).

There appears to be significant genetic system divergence among the higher clades in *Conostylis*. Indeed, in *C.* sect. *Appendicula* and *C.* subgen. *Pendula*, the two other taxa with species complexes like those in *C.* subgen. *Conostylis*, the low ovule numbers are correlated with dysploid chromosome number reduction series and a virtual absence of natural hybridization when congeners are sympatric. The exceptional speciation in southwestern Australian genera of Haemodoraceae exhibited by *Conostylis* appears to have been based on divergent processes among the three most species-rich subgeneric taxa (sect. *Appendicula*, subgen. *Conostylis*, and subgen. *Pendula*).

This pattern exemplifies endemic speciation in the southwestern region as a whole, which has become known as one of the earth's 25 global biodiversity hot spots (Myers et al. 2000), and has an estimated 8000 species of vascular plants, with 50% endemism (Hopper and Gioia 2004). Hypotheses to account for this exceptional species richness occupy an increasingly diversified literature (references in Hopper and Gioia 2004). Hopper (1979), Lamont et al. (1984), and Cowling et al. (1996) emphasized ecological and geohistorical aspects of speciation, but this approach needs to be combined with a population genetics perspective for an adequate understanding of how populations diverge and ultimately become species. It has become clear that cytoevolution has precipitated or accompanied speciation in a diversity of southwestern Australian groups (e.g., *Stylidium* Sw. ex Willd.: Coates and James 1996; Coates et al. 2003).

Increasing evidence indicates that the majority of southwestern plants have had unparalleled opportunities to persist in small populations of limited dispersal ability for long periods of time, albeit in the face of significant climatic stresses and recurrent fire (Hopper et al. 1996). James (1981, 1992) proposed that the inbreeding and self-fertilization resulting from small population size have placed selection pressures on the evolution of mechanisms that conserve heterozygous genotypes. These mechanisms range from endogenous genetic system responses such as genomic coalescence (e.g., dysploid chromosome number reduction that minimizes the number of randomly assorting elements and increases retention of heterozygous supergenes at meiosis), to exogenous

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Fig. 3.—Tree from the combined *mat*K and ITS analysis, with taxa having significant missing sequences deleted. Number of changes above the line, bootstrap values below. Nodes marked by an asterisk (*) collapsed in the consensus tree. Subgeneric taxa from Hopper et al. (1987) indicated as: P-C = *Conostylis* subgen. *Pendula* sect. *Catospora*; P-D = *C.* subgen. *Pendula* sect. *Divaricata*; P-A = *C.* subgen. *Pendula* sect. *Appendicula*; G = *C.* subgen. *Greenia*; A = *C.* subgen. *Androstemma*; Br = *C.* subgen. *Brachycaulon*; Bi = *C.* subgen. *Bicolorata*; C = *C.* subgen. *Conostylis*. Large asterisk beside *Conostylis* taxa indicate possession of a tubular perianth. Note monophyletic groups A (containing clades 1 and 2) and B (containing clades 3 and 4).

responses like adaptation to mobile vertebrate pollinators that increase the likelihood of outcrossing (an estimated 15% of the southwestern flora is vertebrate pollinated: Keighery1982; Hopper and Burbidge 1986).

An incidental byproduct of those taxa that move down the endogenous response route would be the establishment of barriers to gene exchange among populations—changes in chromosome number or structure selected to maintain heterozygosity within isolated daughter populations would result in meiotic aberrations and sterility in hybrids on secondary contact of daughter and parental populations (Grant 1981). Consequently, fecundity would be low and hybridization would be rare. Rapid and prolific microspeciation would occur. Conversely, taxa that evolve towards vertebrate pollination or other exogenous means of maintaining heterozygosity might be expected to display high levels of hybridization and fecundity associated with chromosomal constancy and much less meiotic aberration. Divergence would require isolation by distance (allopatry), either through ecological divergence, long-distance dispersal, or vicariance imposed by landscape or climatic change.

These predictions hold well in the major clades of *Conostylis*. Thus, *C.* subgen. *Conostylis* has adopted exogenous means of maintaining heterozygosity, primarily through ecological divergence in allopatry of sister taxa (Hopper 1977; Hopper et al. 1987). *Conostylis* sect. *Appendicula* and *C.* subgen. *Pendula,* in contrast, exhibit the features predicted for clades that have followed the endogenous route of conserving heterozygosity through chromosomal and other genetic system change.

The fundamental question, of course, remains as to the actual genetic basis for such divergent evolutionary patterns. What predisposes clades to follow either the endogenous or exogenous route to conserving heterozygosity or adaptive genotypes under intense selection? Excluding artifacts of classification, why are there so many examples of sister taxa, one monotypic and the other highly species-rich (e.g., *Blancoa* and *Conostylis*), in so many independently evolved plant lineages? With the emergence of contemporary techniques enabling DNA sequences to be linked and visualized to positions on chromosomes, we may have the tools to test hypotheses in this most fundamental arena of speciation theory.

Phytogeography of Clades in Conostylis

Isolated species (i.e., those sister to large clades) include in clade A, *Conostylis stylidioides, C. glabra, C. serrulata* subsp. *magna,* and *C. misera* in *C.* subgen. *Conostylis, C. angustifolia* in *C.* sect. *Appendicula,* and, in clade B, *C. breviscapa* (*C.* subgen. *Brachycaulon*), *C. phathyrantha* (*C.* sect. *Divaricata*), and *C. vaginata* (*C.* subgen. *Bicolorata*). Most of these species, as well as *Blancoa canescens,* occur in deep, well-drained, nutrient-poor sands in the northern or southern kwongan of the southwest's Transitional Rainfall and Southeast Coastal Floristic Provinces (Hopper and Gioia 2004), rather than in the laterite, rich loams, or peaty swamps of the forested High Rainfall Province of the deeper southwest. An ancient vicariance event is postulated, with the ancestor of clade A (Fig. 2, 3) confined to the northern kwongan, and that of clade B confined to the southern kwongan.

These two kwongan areas are separated today by the high rainfall forested regions, for which the boundaries extended even further inland during more mesic periods of the Tertiary and Quaternary. There are also complex edaphic barriers in mosaic pattern interspersed through the current wheat belt that separate the large expanses of sands and laterite found in the northern and southern kwongan. The moderating maritime influence on the western and southern coasts since the Jurassic undoubtedly has facilitated the persistence of taxa through arid periods in the northern and southern kwongan, whereas the intervening wheat belt has fewer mesic refuges and may have endured greater extinction rates when rainfall was lower than at present.

There is mounting evidence that many independent clades in southwestern Australian vascular plants exhibit vicariance between the northern and southern kwongan regions, with divergence at varying taxonomic levels from vicarious subspecies to vicarious genera (Hopper 1979; Hopper and Gioia 2004). Consequently, the phytogeographic patterns in major clades of *Conostylis* and between *Conostylis* and *Blancoa* are not unexpected.

Within subgenera of *Conostylis,* as noted above, it is difficult to elaborate on the phytogeography of speciation without better resolution of the molecular pattern. However, the phylogenetic pattern is consistent across several lineages with a hypothesis of the prolonged persistence of relictual taxa combined with explosive more recent speciation, the latter pronounced in the northern kwongan of the Transitional Rainfall Province (Hopper and Gioia 2004). Further investigation is needed to evaluate these ideas and hopefully elucidate speciation in this most diverse genus of the Haemodoraceae.

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