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Chromosome numbers in some Australian Restionaceae (Poales): new counts and an inferred base number for Leptocarpoideae

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Abstract

Chromosome number determinations are reported for 22 species of Australian Restionaceae. The segregation of new species formerly included in *Lepyrodia scariosa* R.Br. sens. lat. has required the redetermination of voucher specimens and some previously reported counts apply to the newly segregated species. A recent DNA-based phylogeny is a basis for discussing these findings together with previously reported counts. In Restionaceae $n = 12$ appears plesiomorphic in Leptocarpoideae, while both $n = 7$ and $n = 9$ are found in Sporadanthoideae.

Introduction

Chromosome numbers in some Australian Restionaceae have previously been reported (Briggs 1963, 1966) but these cover only a minority of the species. Even fewer findings are available for the extra-Australian Restionaceae. Linder et al. (1998) reported that the only published cytological data for African species are three counts by Krupko (1962, 1966), viz *Elegia racemosa* (Poir.) Pers. $2n$ = c. 40; *Staberoha cernua* (L.f.) Dur. & Schinz $n = 16$ and *Hypodiscus aristatus* (Thunb.) Krauss $n = 16$. Dawson (2000) reported numbers for three New Zealand endemic taxa: $2n = 48$ in *Apodasmia similis* (Edgar) B.G.Briggs & L.A.S.Johnson (as *Leptocarpus similis* Edgar), $2n = 18$ in *Sporadanthus ferrugineus* de Lange, Heenan & B.D.Clarkson and in *S. traversii* (F.Muell.) F.Muell ex Kirk. Briggs et al. (2010) present evidence that the clade customarily recognised as family Centrolepidaceae may perhaps be best placed within Restionaceae as subfamily Centrolepidoideae Burnett (see also Briggs & Linder 2009). For that clade, Cooke (1998) notes that chromosome data exist for only a few species of *Centrolepis* although Hamann (1960, 1962) suggested a base number of $n = 10$. Anarthriaceae (sens. lat., including Hopkinsiaceae and Lyginiaceae), the other family of the restiid clade of Poales, show a range of numbers: $n = 11$ or 22 in *Anarthria*, $n = 9$ in *Hopkinsia* and $n = 12$ in *Lyginia* (Briggs 1966; Johnson & Briggs 1981; Bell & Pate 1993). Chromosomes of Restionaceae are small (c. 1–6 µm), those of *Anarthria* are larger (c. 6–13 µm), while *Lyginia* shows intermediate size (5–7 µm).

Of the three currently recognised subfamilies of Restionaceae (Briggs & Linder 2009), Restionoideae is limited to the African region while Sporadanthoideae and Leptocarpoideae are principally Australasian. Sporadanthoideae comprises three genera (species numbers include the new species of *Sporadanthus* and *Lepyrodia* described in this volume by Briggs and Johnson [2012]): *Sporadanthus* (8 spp.), *Lepyrodia* (22 spp.) and *Calorophus* (2 spp.). Subfamily Leptocarpoideae is more diverse, with 28 genera currently recognised and

about 117 species, including some not yet formally named. As noted below, DNA-based phylogenetic studies, however, indicate that the recognition of several of the genera of Leptocarpoideae should be reconsidered. All genera of both subfamilies are represented in Australia. Outside Australia, two species of *Sporadanthus* are New Zealand endemics, *Empodisma minus* (J.D.Hook.) L.A.S.Johnson & D.F.Cutler extends to New Zealand, *Apodasmia* includes one species in New Zealand (*A. similis* (Edgar) B.G.Briggs & L.A.S.Johnson) and one in Chile (*A. chilensis* (Gay) B.G.Briggs & L.A.S.Johnson), while *Dapsilanthus disjunctus* (Mast.) B.G.Briggs & L.A.S.Johnson is in south-east Asia and two *Dapsilanthus* species extend from northern Australia to southern New Guinea.[1]

Methods

As in previous studies (Briggs 1963, 1966), the findings reported below are somatic numbers determined on root-tip material collected in the wild, using c. 130 minutes pre-fixation treatment with saturated aqueous p-dichlorobenzene followed by fixation in 3:1 ethyl alcohol: acetic acid and storage in 70% aqueous ethyl alcohol. Staining and maceration were by ethyl alcohol-hydrochloric acid-carmine (Snow 1963) for approximately 1 hr at 60˚C or for 24 hours or more if refrigerated at c. 4˚C. Stained roots were stored in 70% ethyl alcohol at c. 4˚C. The root cap was largely removed during squash preparation to facilitate spreading of the meristematic cells. Preparations were examined using phase-contrast optics. In one case, *Meeboldina denmarkica*, the finding is also supported by a meiotic haploid count from a developing anther of the same specimen. All voucher specimens (Table 1) have been deposited in the NSW Herbarium. A phylogeny of Leptocarpoideae, inferred by Briggs et al. (2010) from maximum parsimony analysis of *trn*L–F chloroplast DNA data, is used as a basis for presenting new and previously reported findings for this subfamily (Fig. 1). Bootstrap values for this tree are given by Briggs et al. (2010); some nodes are weakly supported but the general structure is consistent with trees based on concatenated data including additional genes (*rbc*L and *trn*K). The *trn*L–F tree is chosen for presenting the data since the taxon sampling of that region in Australian Restionaceae has been more comprehensive than for other DNA regions. The new records include some species of *Leptocarpus* and *Meeboldina* that are identified only by unpublished informal names.

Results

Chromosome numbers are reported for 22 Australian taxa (Table 1). Also chromosome counts (all $2n = 14$) previously reported for *Lepyrodia scariosa* are identified as applying to the newly segregated *L. cryptica* and *L. imitans* (Briggs & Johnson, 2012) as well as to *L. scariosa*.

Discussion

The coverage of taxa is still too limited and uneven to provide a comprehensive picture of chromosomal evolution in Australian Restionaceae and does not indicate a plesiomorphous chromosome number for the family.

In subfamily Sporadanthoideae, *Calorophus* has not been studied but there has been considerable sampling of *Lepyrodia* ($n = 7$) and *Sporadanthus* ($n = 9$). The latter number is shared by the six Australian and the two New Zealand *Sporadanthus* species (Dawson 2000). Indeed the consistent difference in chromosome number was an early indication of these two species groups that are now recognised as genera (Briggs 1963; Johnson & Evans 1963; Briggs & Johnson 1998).

New and previously published records for the largely Australian subfamily Leptocarpoideae are shown in Fig. 1. Reported '*n*' numbers are shown beside the topology of a bootstrap consensus tree based on *trn*L–F data, modified from Briggs et al. (2010). Where a count is available for a species that was not included in the analysis, the name and count are included immediately above other species of the same genus. As noted by Briggs et al.

[1] Note added in proof: in addition there is the recently described *Empodisma robustum* Wagstaff & B.R.Clarkson in northern New Zealand (Wagstaff SJ & Clarkson BR [2012]. Systematics and ecology of the Australasian genus *Empodisma* (Restionaceae) and description of a new species from peatlands in northern New Zealand. *PhytoKeys* 13: 39–79).

Table 1. New chromosome number and voucher records.

Taxon	Chromosome number	Voucher (Herb. NSW)	Sex (dioecious spp.)	Collector & collection number	Locality and comment
Sporadanthoideae					
Lepyrodia cryptica B.G. Briggs & L.A.S.Johnson	*2n* = 14	57107	♀	*L.A.S. Johnson* & B.G.Briggs s.n.	W of Clarence, N.S.W. (count previously published as ***L. scariosa***, Briggs 1963).
L. drummondiana Steud.	*2n* = 14	94265	♂	*B.G. Briggs 512*	Stirling Range, W.A.
L. glauca (Nees) F.Muell.	*2n* = 14	94755	♂	*B.G. Briggs 817*	W of Harvey, W.A.
L. imitans B.G.Briggs & L.A.S.Johnson	*2n* = 14	61061	♂	*E.F. Constable 3030*	Beerwah, Qld., (previously published as ***L. scariosa,*** Briggs 1963)
L. monoica F.Muell.	*2n* = 14	95053	monoecious	*B.G. Briggs 545*	Devils Slide, Porongurup Range, W.A.
Lepyrodia scariosa R.Br.	*2n* = 14	61067	♀	*S.T. Blake 21716*	King John Creek, N of Caboolture, Qld,
	2n = 14	60688	monoecious	*O.D. Evans*	Malabar, N.S.W. (both counts previously published as ***L. scariosa,*** Briggs 1963).
Sporadanthus rivularis B.G.Briggs & L.A.S.Johnson	*2n* = 18	95034	♀	*B.G. Briggs 666*	Fish Creek, Shannon to Walpole road, SE of Shannon River, W.A.
S. strictus (R.Br.) B.G.Briggs L.A.S.Johnson	*2n* = 18	95050	♀	*B.G. Briggs 601*	WNW of Albany, W.A. &
Leptocarpoideae					
Chordifex amblycoleus (F.Muell.) B.G.Briggs & L.A.S.Johnson	*2n* = 14	95093	♂	*B.G. Briggs 700*	Brockman Highway E of Alexandra Bridge, W.A.
Chordifex crispatus (R.Br.) B.G.Briggs & L.A.S.Johnson	*2n* = 24	84806	♀	*B.G. Briggs 415*	W of Esperance on Ravensthorpe road, W.A.
Chordifex microcodon B.G.Briggs & L.A.S.Johnson	*2n* = 14	95102	♀	*B.G. Briggs 850*	S of Regans Ford on Gin Gin road, W.A.
Dapsilanthus elatior (R. Br.) B.G.Briggs & L.A.S.Johnson	*2n* = 24	86989	♀	*C.H. Gittins 1815*	From Wenlock towards Iron Range, Qld.
Empodisma gracillimum (F. Muell.) L.A.S.Johnson & D.F.Cutler	*2n* = 24	95017	vegetative	*B.G. Briggs 737*	SW of Mowen, W.A.
Hypolaena exsulca R.Br.	*2n* = 24	94993	♂	*B.G. Briggs 769*	Near Boallia, S of Busselton, W.A.
	2n = 24	94987	♀	*B.G. Briggs 866a*	Metricup, SW of Bussleton W.A. (counts differ from *2n* = 26 in Briggs 1966)
Lepidobolus quadratus B.G.Briggs & L.A.S.Johnson	*2n* = 24	10248	♀	*C.H. Gittins 1723a*	N of Badgingarra, W.A.
Loxocarya gigas B.G.Briggs & L.A.S.Johnson	*2n* = 24	102490	♀	*C.H. Gittins 1696a*	W of Coorow, W.A.
Meeboldina denmarkica Suess.	*n* = 12 *2n* = 24	90980	♂	*K. Mair*	Manjimup to Albany, W.A.
Meeboldina 'roycei' B.G.Briggs & L.A.S.Johnson unpubl.	*2n* = 24	95139	♂	*B.G. Briggs 814*	Bussell Highway N of Bunbury, W.A.
Meeboldina 'thysanantha' B.G.Briggs & L.A.S.Johnson unpubl.	*2n* = 24	95209	♀	*B.G. Briggs 634*	Kent River, Kenton, W.A.
Melanostachya ustulata (F. Muell.ex Ewart & Sharman) B.G.Briggs & L.A.S.Johnson	*2n* = 14	94874	♂	*B.G. Briggs 786*	S of Busselton, W.A.
	2n = 14	94875	♀	*B.G. Briggs 788*	S of Busselton, W.A.
Stenotalis ramosissima (Gilg) B.G.Briggs & L.A.S.Johnson	*2n* = 24	94333	♀	*B.G. Briggs 523*	S of Chester Pass, W.A.
Taraxis grossa B.G.Briggs & L.A.S.Johnson	*2n* = 24	94933	♂	*B.G. Briggs 653*	Walpole River SW of Walpole, W.A.
	2n = 24	95060	♂	*B.G. Briggs 635*	Peaceful Bay Road S of Bow Bridge, W.A.

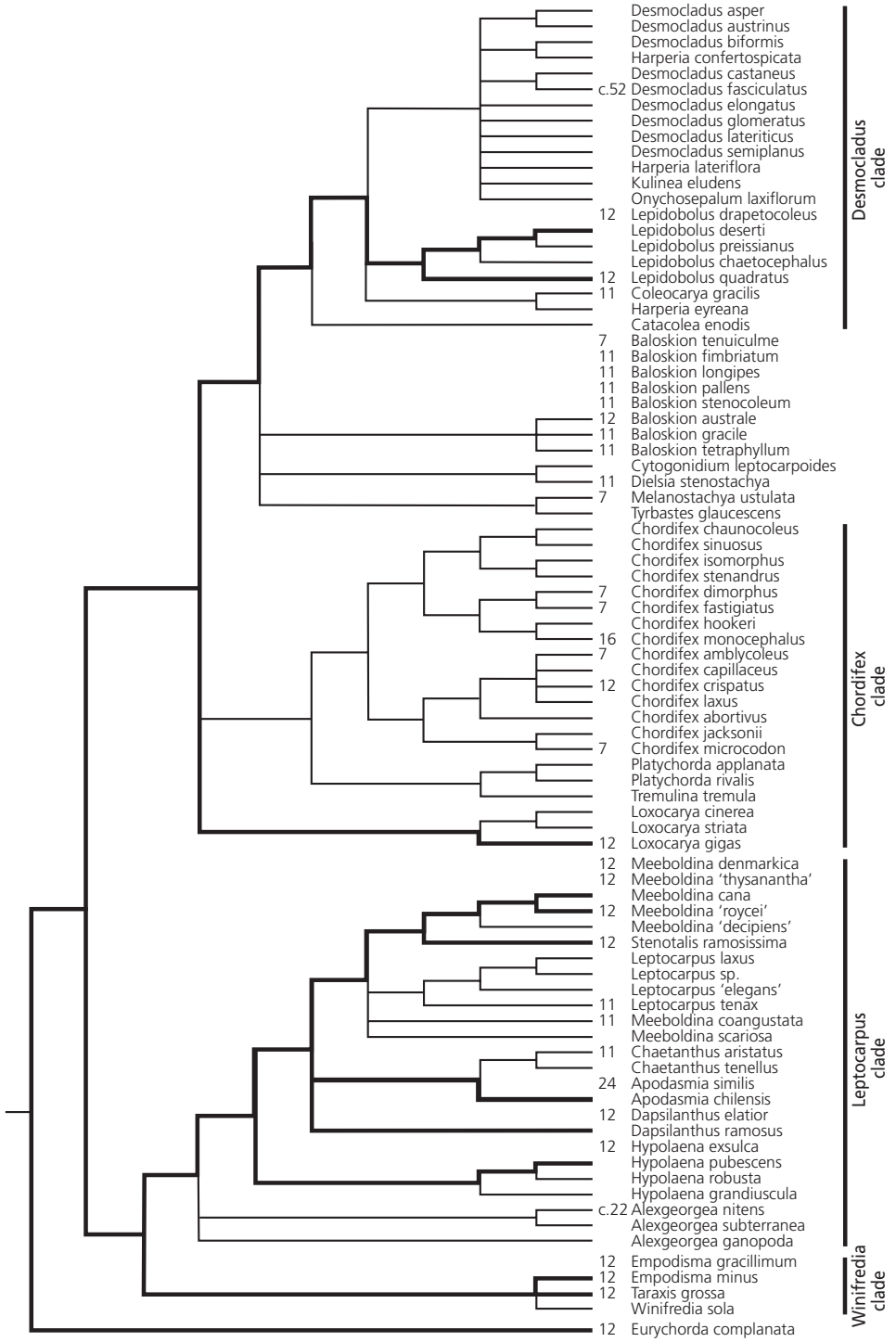

Fig. 1. Chromosome number records (haploid numbers) in Leptocarpoideae, placed beside a phylogenetic tree (bootstrap consensus from *trn*L–F data) modified from Briggs et al. (2010). Bold lines indicate findings of $n = 12$ (or tetraploid $n = 24$), where this may be inferred to retain the plesiomorphic condition for the subfamily. Counted taxa not included in the tree are placed next to (above) congeneric taxa.

(2010) the topology indicates that generic limits around *Desmocladus–Harperia–Kulinia–Onychosepalum* and *Leptocarpus–Stenotalis–Meeboldina* require revision to avoid paraphyly or polyphyly.

From Fig. 1 we note as follows:

There is a considerable diversity of chromosome numbers (n = 7, 11, 12, 16, c. 22, 24, 51–52) although the sampling of taxa is grossly incomplete.

The plesiomorphic chromosome number for Leptocarpoideae is inferred to be n = 12. This number is found in *Eurychorda*, which is sister to the remainder of the subfamily. It also appears in *Empodisma* and *Taraxis*, the counted members of the early-branching Winifredia clade. In a range of other genera (*Hypolaena*, *Dapsilanthus*, several species of *Meeboldina* [including *Stenotalis*], *Loxocarya* and *Lepidobolus*) the topology suggests that the number may also represent a plesiomorphous condition. Other findings of n = 12 are in *Baloskion* and *Chordifex*, but these genera include a variety of numbers and it is less clear whether a plesiomorphous condition has been retained. Bold lines in Fig. 1 indicate a finding of a probably plesiomorphous n = 12 (or n = 24) in a species represented in the tree or in a congeneric counted species.

The finding of n = 24 in the New Zealand *Apodasmia similis* (Briggs in Dawson 2000) identifies this as a tetraploid on the same base number.

If n = 12 is the base number for the subfamily, there has been repeated reduction in number, especially to n = 11 or n = 7, but also number increase in *Alexgeorgea* (n = c. 22) and in *Chordifex monocephalus* (n = 16).

The only notably high number is n = c. 52 in *Desmocladus fasciculatus*, a member of a very poorly sampled clade which does, however, include findings of n = 11 in *Coleocarya* and n = 12 in *Lepidobolus*.

Loxocarya gigas, *Meeboldina* '*roycei*' ms. and '*M. thysananthus*' ms., which have taller and stouter culms than in most congeneric species, do not show higher ploidy levels.

In the genera that show several numbers, these numbers do not appear to be helpful in determining relationships. For example, DNA data show that the eastern Australian *Chordifex* species form a monophyletic group (Briggs et al. 2010) but, among these, *C. dimorphus* and *C. fastigiatus* show n = 7 whereas n = 16 is reported in *C. monocephalus*. By contrast, n = 7 appears elsewhere in *Chordifex* in the Western Australian *C. amblycoleus* and *C. microcodon*, neither closely allied to the eastern Australian species group.

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