

New chromosome reports in the subtribes Diocleinae and Glycininae (Phaseoleae: Papilionoideae: Fabaceae)

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The base chromosome number of $x = 11$ is the most probable in all the subtribes included in tribe Phaseoleae, although some aneuploid reduction is evident in *Collaea* and *Galactia* (Diocleinae) and chromosome duplications are seen in *Amphicarpaea*, *Cologania* and *Glycine* (Glycininae). The aims of this study were to improve the cytological knowledge of some species of *Collaea* and *Galactia* and to examine the anomalous counts reported for *Calopogonium* (Glycininae) and verify its taxonomic position. In addition, a molecular phylogeny was constructed using nuclear ribosomal DNA sequences (internal transcribed spacer region), and the chromosome number was optimized on the topology. In this work, the chromosome counts for *Galactia lindenii*, *Galactia decumbens* and *Collaea cipoensis* (all $2n = 20$), and *Calopogonium sericeum* ($2n = 22$) are reported for the first time. The new reports for *Galactia* and *Collaea* species are in agreement with the chromosome number proposed for subtribe Diocleinae. The study rejects the concept of a cytologically anomalous *Calopogonium* and, based on the phylogenetic analysis, corroborates the position of this genus within subtribe Glycininae. The ancestral basic chromosome number of $x = 11$ proposed for Phaseoleae is in agreement with the evolutionary pathway of chromosome numbers analysed in this work. © 2008 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2008, 158, 336–341.

ADDITIONAL KEYWORDS: *Calopogonium* – chromosome numbers – *Collaea* – *Galactia* – Leguminosae – optimization – phylogenetics.

INTRODUCTION

Diocleinae is one of the eight subtribes currently ascribed to Phaseoleae (Polhill, Raven & Stirton, 1981), which, economically, is the most important tribe of subfamily Papilionoideae (Fabaceae). The circumscription of Diocleinae has been the subject of several studies, especially with regard to the taxonomic position of *Calopogonium* Desv. and *Pachyrhizus* Rich. ex DC. These two genera were assigned to

Diocleinae, together with 11 other genera, although doubts have been raised about their position (Lackey, 1981).

Recently, a number of phylogenetic analyses have been performed involving the different subtribes of Phaseoleae. All agree that *Calopogonium* and *Pachyrhizus* arose outside Diocleinae, being closely related to Glycininae, another subtribe of Phaseoleae (Doyle & Doyle, 1993; Bruneau, Doyle & Doyle, 1995; Doyle *et al.*, 2000; Lee & Hymowitz, 2001; Varela *et al.*, 2004). These studies were based mainly on molecular data. The placement of *Calopogonium* and

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Pachyrhizus in *Glycininae* is also supported by morphological features, as this subtribe is often confused with *Diocleinae* because of a paucity of unique characters (Lackey, 1981; Lee & Hymowitz, 2001).

Chromosome numbers are an important source of taxonomic evidence and, particularly in Fabaceae, this information has proven to be valuable in understanding the evolution of the species (Goldblatt, 1981). Several counts have been made in the 13 genera first ascribed to *Diocleinae*, but the chromosome number in many species still remains unknown. The subtribe has counts mostly of $2n = 22$ ($x = 11$), but *Galactia* P. Browne and *Collaea* DC. consistently have counts of $2n = 20$ ($x = 10$) (Lackey, 1980, Sede *et al.*, 2003, Sede, Fortunato & Poggio, 2006), which makes them cytologically distinct from the remainder of the subtribe.

To date, the counts reported for *Calopogonium* are inconsistent. Some authors have reported a base number of $x = 18$ (Lackey, 1980), whereas others have reported $x = 12$ (Gill & Husaini, 1986). An important point is that nearly all *Glycininae* have somatic counts with base numbers of $x = 11$ or 10 (Lackey, 1980).

To improve the cytological knowledge of some species of *Diocleinae* and to check the taxonomic position of *Calopogonium* we present new chromosome records of one species of the latter genus [*Calopogonium sericeum* (Benth.) Chodat & Hassl.], two of *Galactia* [*Galactia lindenii* Burkart and *Galactia decumbens* (Benth.) Chodat & Hassl.] and one of *Collaea* (*Collaea cipoensis* Fortunato).

In addition, in order to obtain more evidence about the phylogenetic relationships in the subtribes *Diocleinae* and *Glycininae* and to analyse the evolution of chromosome numbers, a molecular phylogeny was constructed using nuclear ribosomal DNA sequences (internal transcribed spacer region, ITS) and the chromosome number was optimized on this topology.

MATERIAL AND METHODS

PLANT MATERIAL AND MITOTIC STUDIES

Accession information for the species analysed is presented in Table 1. The chromosome counts were made from mitotic studies. Root tips were pretreated for 3 h in 0.002 M 8-hydroxyquinoline at 20 °C, fixed in absolute ethanol–acetic acid (3 : 1) and stained in Feulgen after 40 min of hydrolysis in 5 M HCl at 20 °C. Slides were prepared using the squashing technique.

PHYLOGENETIC ANALYSIS AND CHARACTER OPTIMIZATION

Sequences of nuclear ribosomal DNA (ITS1 and ITS2) from subtribes *Diocleinae* and *Glycininae* were

aligned using the DIALIGN program (Morgenstern *et al.*, 1998) with a threshold value of $T = 10$. A matrix of 27 terminals and a total of 995 characters (390 of which were potentially parsimony informative) was constructed. A list of species, GenBank accession numbers and chromosome data are given in Table 1. Five species belonging to allied subtribes of *Phaseoleae* (*Phaseolinae* and *Cajaninae*) were chosen as the outgroup.

The phylogenetic analysis was performed under the parsimony criterion using TNT version 1.1 (Goloboff, Farris & Nixon, 2003). A heuristic tree-searching procedure was used, with 20 random addition sequences plus tree bisection–reconnection (TBR), retaining ten trees per replicate, keeping up to 10 000 trees. Jackknife support values were calculated from 1000 replicates of the original matrix.

Chromosome numbers were optimized on the single tree obtained using WinClada (Nixon, 2002), treating this feature as a non-additive multistate character.

RESULTS AND DISCUSSION

Diocleinae is distinguished by a base chromosome number of $x = 11$, except for *Collaea* and *Galactia*, as several authors have reported a base number of $x = 10$ (Lackey, 1980; Sede *et al.*, 2003, 2006). The chromosome numbers of *G. lindenii*, *G. decumbens* and *Collaea cipoensis* ($2n = 20$) (Table 1, Figs 2–4) are reported here for the first time, and are in agreement with that proposed for the genera by Goldblatt (1981).

The chromosome count for *Calopogonium* is different from those reported by Lackey (1980) and Gill & Husaini (1986) ($x = 18$ and $x = 12$, respectively). We report a chromosome count of $2n = 22$ for *C. sericeum* (Fig. 1), which is more in agreement with the circumscription of *Calopogonium* inside *Glycininae*, as this subtribe has somatic counts of $x = 11$ (Lackey, 1980).

The phylogenetic analysis yielded one most parsimonious tree [length (L) = 1875; consistency index = 0.437; retention index = 0.585]. The tree is shown in Figure 5, in which two main clades can be observed. One comprises species of *Diocleinae*, with strong support (jackknife of 100%). The other group contains the species of *Phaseolinae* species and *Glycininae*, including *Calopogonium*, with a moderately strong jackknife support (84%).

The monophyly of subtribe *Diocleinae* is obtained only by placing *Pachyrhizus* and *Calopogonium* in another subtribe, leaving a monophyletic *Diocleinae* with 100% support. These observations were also found by other authors using a different source of characters (Doyle & Doyle, 1993; Doyle *et al.*, 2000; De Queiroz, Fortunato & Giuliatti, 2003; Varela *et al.*, 2004). The cladogram in Figure 5 shows an unequivocal

Table 1. Species, chromosome numbers, GenBank numbers and accession information for the new reports (indicated with an asterisk)

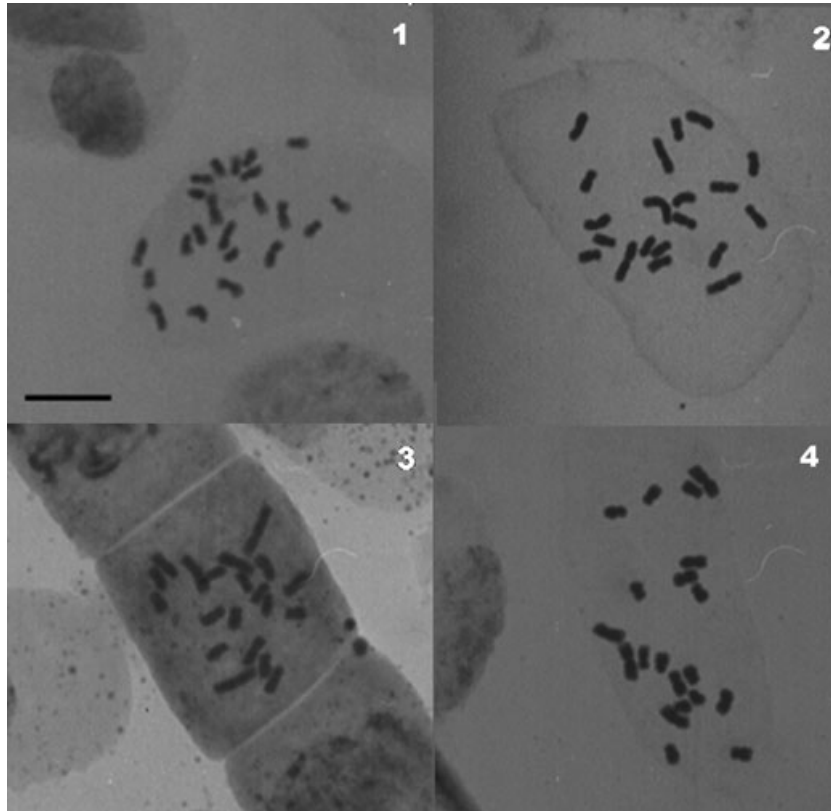
Taxon	$2n$	GenBank number and reference	Voucher number	Country
<i>Rhynchosia hauthalii</i> (O. Kuntze) Grear	22	EU499367 (Espert <i>et al.</i> , this paper)		
<i>Dolichopsis paraguariensis</i> Hassl.	22	AY508744 (Riley-Hulting <i>et al.</i> , 2004)		
<i>Macroptilium psammodes</i> (Lindm.) S.I.Drewes & R.A.Palacios	22	DQ888774-86 (Espert <i>et al.</i> , 2007)		
<i>Phaseolus lunatus</i> L.	22	AF115175 (Delgado Salinas <i>et al.</i> , 1999)		
<i>Strophostyles helvola</i> (L.) Elliot	22	AF115137 (Delgado Salinas <i>et al.</i> , 1999)		
<i>Amphicarpaea bracteata</i> (L.) Fernald	40	DQ006008 (Kress <i>et al.</i> , 2005)		
<i>A. edgeworthii</i> Benth.	40	AF417012 (Parker MA <i>et al.</i> , State University of New York, Binghamton, unpubl. data)		
<i>Calopogonium caeruleum</i> (Benth.) C.Wright ex Sauvalle	22	AY293844 (Varela <i>et al.</i> , 2004)		
<i>C. mucunoides</i> Desv.	22	AY293845 (Varela <i>et al.</i> , 2004)		
<i>C. sericeum</i> (Benth.) Chodat & Hassl.	22*	Sequence not available	Sede 45 & 59 (BAB)	Argentina & Paraguay
<i>Camptosema pedicellatum</i> Benth.	22	AY293841 (Varela <i>et al.</i> , 2004)		
<i>C. rubicundum</i> Hook. & Arn.	22	EU499368 (Espert <i>et al.</i> , this paper)		
<i>Canavalia bonariensis</i> Lindl.	22	AY293839 (Varela <i>et al.</i> , 2004)		
<i>C. grandiflora</i> Benth.	22	AY293840 (Varela <i>et al.</i> , 2004)		
<i>Cleobulia multiflora</i> Mart. ex Benth.	NR	AY881100 (Grangeiro TB <i>et al.</i> , Universidade Federal do Ceara, unpubl. data)		
<i>Collaea cipoensis</i> Fortunato	20*	EU499369 (Espert <i>et al.</i> , this paper)	Fortunato 8411 (BAB)	Brazil
<i>C. stenophylla</i> (Hook. & Arn.) Benth.	20	EU499370 (Espert <i>et al.</i> , this paper)		
<i>Cologania broussonetii</i> (Balb.) DC.	44	AY583501 (Thulin <i>et al.</i> , 2004)		
<i>Cratylia argentea</i> (Desv.) Kuntze	22	AY293842 (Varela <i>et al.</i> , 2004)		
<i>Cymbosema roseum</i> Benth.	NR	AY293836 (Varela <i>et al.</i> , 2004)		
<i>Dioclea megacarpa</i> Rolfe	22	AY293832 (Varela <i>et al.</i> , 2004)		
<i>D. virgata</i> (Rich.) Amshoff	22	AY293835 (Varela <i>et al.</i> , 2004)		
<i>Galactia latisiliqua</i> Desv.	20	AY293843 (Varela <i>et al.</i> , 2004)		
<i>G. lindenii</i> Burkart	20*	EU499371 (Espert <i>et al.</i> , this paper)	Ruiz 2 (HCN)	Colombia
<i>G. decumbens</i> Hassl.	20*	Sequence not available	Fortunato 8414 (BAB)	Brazil
<i>Glycine albicans</i> Tindale & Craven	40	U60541 (Kollipara <i>et al.</i> , 1997)		
<i>G. falcata</i> Benth.	40	U60549 (Kollipara, Singh & Hymowitz, 1997)		
<i>Pachyrhizus erosus</i> (L.) Urban	22	AY293846 (Varela <i>et al.</i> , 2004)		
<i>P. tuberosus</i> (Lam.) Spreng.	22	AY293847 (Varela <i>et al.</i> , 2004)		

NR, no reports.

cal placement of *Collaea* and *Galactia* in subtribe Diocleinae. These two genera comprise a terminal clade and are the only species of Diocleinae to have $2n = 20$. The species of *Collaea* are clustered in a monophyletic group S. Sede, unpubl. data) when including more species and a different source of characters. Their relationship with some *Galactia* species,

observed in the present work, was also found by that author.

In the second major clade visible in Figure 5, it can be seen that *Calopogonium* is grouped within Glyciniinae and forms a clade with *Pachyrhizus*. These two genera should thus be included as members of Glyciniinae.



Figures 1–4. Mitotic metaphases. Fig. 1. *Calopogonium sericeum*, $2n = 22$. Fig. 2. *Collaea cipoensis*, $2n = 20$. Fig. 3. *Galactia decumbens*, $2n = 20$. Fig. 4. *Galactia lindenii*, $2n = 20$. Scale bar, 5 μm .

We optimized the chromosome number onto the tree derived from the analysis (Fig. 5). The ancestral state in the tree is $2n = 22$. Within the Diocleinae clade, the chromosome number evolves to the derived state ($2n = 20$) only in the terminal clade formed by species of *Collaea* and *Galactia*. The clade that comprises the Glycininae species shows an ambiguous reconstruction of the ancestors, as it is equally optimal to consider their character states as $2n = 22$ or 40. *Pachyrhizus* and *Calopogonium* are diploid ($2n = 22$), whereas the other genera studied appear to be polyploids ($2n = 40$ or 44).

A possible explanation for the observation of a derived chromosome state in the Diocleinae clade could be an aneuploid reduction in one of the *Galactia* and *Collaea* ancestors, resulting in a derived chromosome number of $2n = 20$. As for the polyploidy events observed in Glycininae, one hypothesis could be that independent duplication events occurred in each polyploid genus, retaining the ancestral state of $2n = 22$ in *Pachyrhizus* and *Calopogonium*. However, evidence of an ancient duplication exists in the soybean genome, at around 15 Mya (Doyle *et al.*, 2003). Therefore, a more plausible explanation would be that the ancestors of the species of Glycininae might have

undergone chromosome duplications, and *Cologania broussonetti* (Balb.) DC., *Glycine* Willd. and *Amphicarpaea* Elliott ex Nutt. retained these polyploid numbers, whereas *Pachyrhizus* and *Calopogonium* suffered a derived aneuploid reduction, regaining the ancestral number of $2n = 22$. Although more evidence should be collected, we agree with the latter view, with an ancestral polyploidy event probably occurring in subtribe Glycininae, involving *Cologania* Kunth, *Glycine* and *Amphicarpaea*.

CONCLUSIONS

This study rejects the concept of a cytologically anomalous *Calopogonium*. The chromosome count reported here ($2n = 22$) is more in agreement with the number proposed for Phaseoleae. Moreover, based on the phylogenetic analysis, the position of this genus within subtribe Glycininae is verified. The new reports for *Galactia* and *Collaea* species are in agreement with the chromosome number proposed for Diocleinae. The ancestral basic chromosome number of $x = 11$ proposed for Phaseoleae and most of its subtribes, including Glycininae and Diocleinae, is in

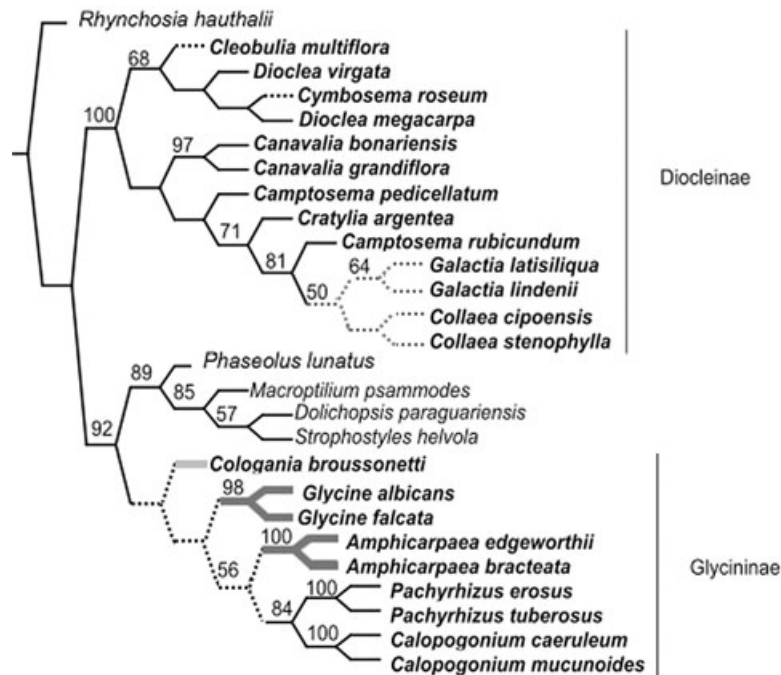


Figure 5. Most parsimonious cladogram obtained with the internal transcribed spacer (ITS) matrix. Jackknife values are shown above the internal nodes. The colours of the branches indicate chromosome number optimization: black line, $2n = 22$; black broken line, ambiguity; dark grey line, $2n = 40$; dark grey broken line, $2n = 20$; light grey line, $2n = 44$.

agreement with the evolutionary pathway of the chromosome number analysed in this work.

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REFERENCES

- Bruneau A, Doyle JJ, Doyle JL. 1995. Phylogenetic relationships in Phaseoleae: evidence from chloroplast DNA restriction site characters. In: Crisp MD, Doyle JJ, eds. *Advances in legume systematics 7: phylogeny*. Kew: Royal Botanic Gardens, 309–330.
- Delgado Salinas A, Turley T, Richman A, Lavin M. 1999. Phylogenetic analysis of the cultivated and wild species of Phaseolus (Fabaceae). *Systematic Botany* **24**: 438–460.
- De Queiroz L, Fortunato R, Giulietti AM. 2003. Phylogeny of the Diocleinae (Papilionoideae: Phaseoleae) based on morphological characters. In: Klitgaard BB, Bruneau A, eds. *Advances in legume systematics 10: higher level systematics*. Kew: Royal Botanic Gardens, 303–324.
- Doyle JJ, Chappill JA, Donovan Bailey C, Kajita T. 2000. Towards a comprehensive phylogeny of legumes: evidence from *rbcL* sequences and non-molecular data. In: Herendeen PS, Bruneau A, eds. *Advances in legume systematics 9*. Kew: Royal Botanic Gardens, 1–20.
- Doyle JJ, Doyle JL. 1993. Chloroplast DNA phylogeny of the papilionoid legume tribe Phaseoleae. *Systematic Botany* **18**: 309–327.
- Doyle JJ, Doyle JL, Rauscher J, Brown AHD. 2003. Diploid and polyploid reticulate evolution throughout the history of the perennial soybeans (*Glycine* subgenus *Glycine*). *New Phytologist* **161**: 121–132.
- Espert SM, Drewes SI, Burghardt AD. 2007. Phylogeny of *Macroptilium* (Leguminosae): morphological, biochemical and molecular evidence. *Cladistics* **23**: 119–129.
- Gill LS, Husaini SWH. 1986. Cytological observations in Leguminosae from Southern Nigeria. *Willdenowia* **15**: 521–527.
- Goldblatt P. 1981. Cytology and the phylogeny of Leguminosae. In: Polhill RM, Raven PH, eds. *Advances in legume systematics*. Kew: Royal Botanic Gardens, 427–463.
- Goloboff PA, Farris J, Nixon KC. 2003. TNT (Tree analysis using New Technology). Program and documentation available from the authors and at <http://www.zmuc.dk/public/phylogeny>
- Kollipara KP, Singh RJ, Hymowitz T. 1997. Phylogenetic and genomic relationships in the genus *Glycine* Willd. based

- on sequences from the ITS region of nuclear rDNA. *Genome* **40**: 57–68.
- Kress W, Wurdack K, Zimmer E, Weigt L, Janzen D. 2005.** Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 8369–8374.
- Lackey JA. 1980.** Chromosome numbers in the Phaseoleae (Fabaceae: Faboideae) and their relation to taxonomy. *American Journal of Botany* **67**: 595–602.
- Lackey JA. 1981.** Phaseoleae. In: Polhill RM, Raven PH, eds. *Advances in legume systematics*. Kew: Royal Botanic Gardens, 301–327.
- Lee J, Hymowitz T. 2001.** A molecular phylogenetic study of the subtribe Glycininae (Leguminosae) derived from chloroplast DNA *rps16* intron sequences. *American Journal of Botany* **88**: 2064–2073.
- Morgenstern B, Frech K, Dress A, Werner T. 1998.** DIALIGN: finding local similarities by multiple sequence alignment. *Bioinformatics* **14**: 290–294.
- Nixon KC. 2002.** *WinClada*, version 1.00.08. Ithaca, NY: Published by the author.
- Polhill RM, Raven PH, Stirton CH. 1981.** Evolution and systematics of the Leguminosae. In: Polhill RM, Raven PH, eds. *Advances in legume systematics, Part 1*. Kew: Royal Botanic Gardens, 1–26.
- Riley-Hulting E, Delgado Salinas A, Lavin M. 2004.** Phylogenetic systematics of *Strophostyles* (Fabaceae): a North American temperate genus within a neotropical diversification. *Systematic Botany* **29**: 627–653.
- Sede S, Fortunato R, Poggio L. 2006.** Chromosome evaluation of southern South American species of *Camptosema* and allied genera (Diocleinae-Phaseoleae-Papilionoideae-Leguminosae). *Botanical Journal of the Linnean Society* **152**: 235–243.
- Sede S, Greizerstein EJ, Dezi R, Fortunato R, Poggio L. 2003.** Chromosome studies in the complex *Galactia-Collaea-Camptosema* (Fabaceae). *Caryologia* **56**: 295–301.
- Thulin M, Lavin M, Pasquet R, Delgado Salinas A. 2004.** Phylogeny and biogeography of *Wajira* (Leguminosae): a monophyletic segregate of *Vigna* centred in the Horn of Africa region. *Systematic Botany* **29**: 903–920.
- Varela ES, Lima JPMS, Galdino AS, Pinto L, Bezerra WM, Nunes EP, Alves MAO, Grangeiro TB. 2004.** Relationships in subtribe Diocleinae (Leguminosae; Papilionoideae) inferred from internal transcribed spacer sequences from nuclear ribosomal DNA. *Phytochemistry* **65**: 59–69.