PhytoKeys 70: 1–10 (2016)
doi: 10.3897/phytokeys.70.6766
http://phytokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
PhytoKeys
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Efficient DNA barcode regions for classifying *Piper* species (Piperaceae)

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Academic editor: *Pavel Stoev* | Received 6 October 2015 | Accepted 2 September 2016 | Published 20 September 2016

Citation: Chaveerach A, Tanee T, Sanubol A, Monkheang P, Sudmoon R (2016) Efficient DNA barcode regions for classifying *Piper* species (Piperaceae). PhytoKeys 70: 1–10. doi: 10.3897/phytokeys.70.6766

Abstract

Piper species are used for spices, in traditional and processed forms of medicines, in cosmetic compounds, in cultural activities and insecticides. Here barcode analysis was performed for identification of plant parts, young plants and modified forms of plants. Thirty-six *Piper* species were collected and the three barcode regions, *matK*, *rbcL* and *psbA-trnH* spacer, were amplified, sequenced and aligned to determine their genetic distances. For intraspecific genetic distances, the most effective values for the species identification ranged from no difference to very low distance values. However, *P. betle* had the highest values at 0.386 for the *matK* region. This finding may be due to *P. betle* being an economic and cultivated species, and thus is supported with growth factors, which may have affected its genetic distance. The interspecific genetic distances that were most effective for identification of different species were from the *matK* region and ranged from a low of 0.002 in 27 paired species to a high of 0.486. Eight species pairs, *P. kraense* and *P. dominantinervium*, *P. magnibaccum* and *P. kraense*, *P. phuwuaense* and *P. dominantinervium*, *P. phuwuaense* and *P. kraense*, *P. pilobracteatum* and *P. dominantinervium*, *P. pilobracteatum* and *P. kraense*, *P. pilobracteatum* and *P. phuwuaense* and *P. sylvestre* and *P. polysyphonum*, that presented a genetic distance of 0.000 and were identified by independently using each of the other two regions. Concisely, these three barcode regions are powerful for further efficient identification of the 36 *Piper* species.

Keywords

DNA barcoding, *matK* gene, *Piper* species, *psbA-trnH* spacer, *rbcL* gene

Introduction

Plants in the genus *Piper* have been used since prehistoric times for a variety of human activities. They are used as spices, in traditional and processed forms of medicines, in cosmetic compounds, in cultural activities and as insecticides (Chaveerach et al. 2006a, Scott et al. 2008, Fan et al. 2011). *Piper betle*, the betel plant, is one of the most important and well-known species of the genus. It contains important chemical substances, such as chavicol, cineol and eugenol, used in essential oils, medicines and insecticides (Yusoff et al. 2005, Misra et al. 2009). Eugenol has been reported as having anti-oxidant and anti-inflammatory properties (Misra et al. 2009). Although the betel plant is of great economic importance, it is challenging to cultivate. The main problem is foot and leaf rot, which is caused by the fungus *Phytophthora parasitica* Dast. In addition, the plant is subject to leaf spot, which is caused by bacteria (Silayoi et al. 1985, Banka and Teo 2000). Investigations of the genus *Piper* in Thailand (Chaveerach et al. 2008, 2009) have found that among the 43 *Piper* species, some produce a betel-like scent. Of these, all are wild species and hardy, producing numerous branches and leaves. They are tolerant and resistant to disease. Some produce a stronger scent than betel. Therefore, these species might be equally or more economically beneficial than the betel plant. The assured advantage is that there would be more choices of plants for use (Sanubol et al. 2014). Medicinal plants have been used in natural and modified forms. The modified forms such as dried sliced plant parts, powder and capsules, are difficult to recognize by physical features. Therefore, reliable identification methods for these plant forms should be developed. DNA barcoding is the most reliable and applicable method for identification. The method was developed in 2003 (Hebert et al. 2003). It principally uses short DNA sequences from appropriate genome regions for the identification of organisms. The *CO1* and 16s rDNA regions have been successfully used for most animals. For example, Hebert et al. (2004) used the mitochondrially encoded cytochrome c oxidase I (*MT-CO1*) to discriminate between bird species. Zhang and Hanner (2012) used sequences of *MT-CO1*, 16s RNA, *MT-CYB* and RNA 18s in 242 species of fish and in 11 *Epinephelus* species.

For plants, however, it is more of a challenge. Currently, several research groups are seeking a suitable genome region, and this effort has led to the identification of appropriate regions for DNA barcoding in some plant groups, such as the *matK* gene (Siripiyasing et al. 2012, Tanee et al. 2012), the *rbcL* gene (Tanee et al. 2012, Kwanda et al. 2013), the *psbA-trnH* spacer region (Chaveerach et al. 2011).

The standard barcodes used for most investigations of plants are the three plastid barcodes, which include *matK* gene, *rbcL* gene and *psbA-trnH* spacer, and one nuclear (ITS) regions identified by the CBOL Plant Working Group (2009), Chaveerach et al. (2011), Hollingsworth et al. (2011) and Monkheang et al. (2011). With the importance of *Piper* species as economically valued plants worldwide and with the plant parts of many species being used, such as the trunk, leaves and fruits, as well as young plants and processed plant materials in the forms of powder and slices, identifying the species used is paramount to verify the authenticity of such goods. Therefore, these products should have a specific marker that identifies a species using barcode for each species.

The aim of this research was to construct barcodes for *Piper* species in Thailand using *matK*, *rbcL* and the *psbA-trnH* spacer regions, as these species are important medicinal plants that have not been fully explored for barcode identification. Here we initiate the development of reference barcodes for plant parts, young plants and plant products.

Materials and methods

Plant materials

Species and sites of *Piper* recently reported in Thailand (Chaveerach et al. 2006a, 2006b, 2007, 2008, Sudmoon et al. 2011) were collected and carefully identified followed the literatures. Leaf samples were kept on ice, transferred to the laboratory, and then stored at -20 °C until further use.

DNA extraction

Whole genomic DNA was extracted using a Plant Genomic DNA Extraction Kit (RBC Bioscience) following the kit protocols.

Amplification of barcode fragments

Polymerase chain reaction (PCR) analyses were performed with primer pairs (5'–3') ATCCATCTGGAAATCTTAGTTC and GTTCTAGCACAAGAAAGTCG (CBOL Plant Working Group 2009) for the *matK* gene, GTCACCACAAACAGAGACTAAAGC and GTAAAATCAAGTCCACCRCG (CBOL Plant Working Group 2009) for the *rbcL* gene, and GTTATGCATGAACGTAATGCTC and CGCGCATGGTGGATTCACAATCC (Hollingsworth et al. 2011) for the *psbA-trnH* spacer region. The reaction mixture (30 µl) consisted of 1× GoTaq Green Master Mix (Promega), 0.5 µM primers, and 30 ng of DNA template. The amplification profile included pre-denaturation at 94 °C for 1 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C (for *matK*) or 55 °C (for *rbcL* and the *psbA-trnH* spacer) for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. The amplified products were subjected to 2% agarose gel electrophoresis.

DNA sequencing and sequences analyses

The specific fragments amplified were sequenced at the DNA Sequencing Unit, Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand. The sequences were then analyzed using Blast tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were aligned for each genome region amplified to determine genetic distance values

by MEGA6 (Tamura et al. 2013) using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. The sequences were submitted to GenBank and corresponding accession numbers were given.

Results

Thirty-six *Piper* species were collected to construct barcodes. Because most of the *Piper* species that we investigated were wild, it was difficult to collect a sufficient amount of samples from all 36 *Piper* species to adequately construct barcodes. Sufficient samples were obtained for four species, *P. nigrum*, *P. betle*, *P. sarmentosum* and *P. retrofractum*, which are all economic plants.

The amplification of barcode bands from the *matK* region was not successful in two species, including *P. montium* and *P. rubroglandulosum* (♀). This may be because the DNAs were fragmented at the primer regions. Table 1 shows the GenBank accession numbers corresponding to 119 sequences from the *matK*, *rbcL* and *psbA-trnH* spacer regions for all 36 species studied.

The intraspecific genetic distances for each region were the following: 1) for the *matK* region, the lowest value of 0.000 was observed in *P. dominantinervium*, *P. hongkongense*, *P. kraense* and *P. longum*, while the highest value of 0.386 was observed for *P. betle*; 2) for the *rbcL* region, the lowest value of 0.000 was observed in *P. dominantinervium*, *P. hongkongense*, *P. longum*, *P. pedicellatum*, *P. pilobracteatum*, *P. polysyphonum*, *P. sarmentosum*, *P. sylvestre* and *P. wallichii*, while the highest value of 0.166 was observed in *P. betle*; 3) for the *psbA-trnH* spacer region, the lowest value of 0.000 was observed in *P. dominantinervium*, *P. khasianum*, *P. kraense*, *P. longum*, *P. montium*, *P. mutabile*, *P. nigrum*, *P. pilobracteatum*, *P. polysyphonum* and *P. sarmentosum* while the highest value of 0.117 was observed in *P. boehmeriifolium.*

The interspecific genetic distances for each region were the following: 1) for the *matK* region the lowest value of 0.000 was observed in the paired species *P. kraense* and *P. dominantinervium*, *P. magnibaccum* and *P. kraense*, *P. phuwuaense* and *P. dominantinervium*, *P. phuwuaense* and *P. kraense*, *P. pilobracteatum* and *P. dominantinervium*, *P. pilobracteatum* and *P. kraense*, *P. pilobracteatum* and *P. phuwuaense* and *P. sylvestre* and *P. polysyphonum*, while the highest value of 0.486 was observed between *P. ribesioides* and *P. pilobracteatum*; 2) for the *rbcL* region, the lowest value of 0.000 was observed between pairs *P. dominantinervium* and *P. caninum*, *P. kraense* and *P. boehmeriifolium*, *P. maculaphyllum* and *P. khasianum*, *P. magnibaccum* and *P. khasianum*, *P. magnibaccum* and *P. caninum*, *P. magnibaccum* and *P. dominantinervium*, *P. montium* and *P. khasianum*, *P. montium* and *P. magnibaccum*, *P. mutabile* and *P. caninum*, *P. mutabile* and *P. dominantinervium*, *P. mutabile* and *P. magnibaccum*, *P. nigrum* and *P. caninum*, *P. nigrum* and *P. dominantinervium*, *P. nigrum* and *P. magnibaccum*, *P. nigrum* and *P. mutabile*, *P. pedicellatum* and *P. khasianum*, *P. pedicellatum* and *P. magnibaccum*, *P. pedicellatum* and *P. montium*, *P. pedicellatum* and *P. pendulispicum*, *P. pendulispicum* and *P.*

Table 1. GenBank accession numbers of DNA barcoding from three regions of *Piper* species.

Scientific name	GenBank accession number[#]		
	matK	***psbA-trnH* spacer**	***rbcL***
Piper argyritis	KM073990	JX442927, KM055176	JX291978, KM055126
P. betle (♀)	GU372747, KM098143	GQ891996, JQ248053	JQ248074
P. betle (♂)	KM098144	JQ248050	JQ248071
P. betloides	KM098135	JQ248051	JQ248072
P. boehmeriifolium	KM073991, KM073992	KM055177, KM055178	KM055127, KM055128
P. caninum	KM073993, KM073994	KM055179, KM055180	KM055129, KM055130
P. colubrinum	GU372751, KM073995	GQ892000	KM055131
P. crocatum	KM098136	JQ248047	JQ248068
P. dominantinervium	KM073996, KM073997	KM055181, KM055182	KM055132, KM055133
P. hongkongense	KM073998, KM073999	KM055183, KM055184	KM055134, KM055135
P. khasianum	KM074000, KM074001	KM055185, KM055186	KM055136, KM055137
P. kraense	KM074002, KM074003	KM055187, KM055188	KM055138, KM055139
P. longum	KM074004, KM074005	KM055189, KM055190	KM055140, KM055141
P. maculaphyllum	KM074006, KM098137	JQ248046, KM055191	JQ248067, KM055142
P. magnibaccum	KM074007, KM074008	KM055192, KM055193	KM055143, KM055144
P. montium	n/a	KM055194, KM055195	KM055145, KM055146
P. mutabile	KM074035	KM055196, KM055197	KM055147, KM055148
P. nigrum	KM074009, KM074010	GQ891994, KM055198, KM055199	KM055149, KM055150
P. pedicellatum var. *eglandulatum*	KM074011, KM074012	KM055200, KM055201	KM055151, KM055152
P. pendulispicum (♀)	KM074013, GU372748	KM055202, GQ891997	KM055153, JX291979
P. phuwuaense	KM074014, KM074015	KM055203, KM055204	KM055154, KM055155
P. pilobracteatum	KM074016, KM074017, KM074018, KM074019	KM055205, KM055206, KM055207, KM055208	KM055156, KM055157, KM055158, KM055159
P. polysyphonum	KM074020, KM074021	KM055209, KM055210	KM055160, KM055161
P. protrusum	KM074032, KM074033	GU980900, KM055223	KM055172, KM055173
P. retrofractum	GU372749, KM074034	GQ891998, KM055224	KM055175
P. ribesioides	GU372750, KM074022	GQ891999, KM055211	KM055162
P. rubroglandulosum (♀)	n/a	JX442926	JX291977
P. rubroglandulosum (♂)	KM098138	JX442925	JX291976
P. sarmentosum	GU372746, KM074023, KM074024	KM055212, KM055213	KM055163, KM055164
P. semiimmersum	KM098139	JQ248045	JQ248066
P. submultinerve	KM098140	JQ248048	JQ248069
P. sylvaticum	KM074025, KM074026	KM055214, KM055215	KM055174
P. sylvestre	KM074027, KM074028	KM055216, KM055217	KM055165, KM055166
P. thomsonii var. *trichostigma*	KM074029	KM055218	KM055167
P. tricolor	KM098141	JQ248049	JQ248070
P. umbellatum	n/a	KM055219, KM055220	KM055168, KM055169
P. wallichii	KM074030, KM074031	KM055221, KM055222	KM055170, KM055171
P. yinkiangense	KM098142	JQ248052	JQ248073

the sequence data deposited at www.ncbi.nlm.nih.gov/Genbank; n/a is "not amplified"

caninum, *P. pendulispicum* and *P. dominantinervium*, *P. pendulispicum* and *P. magnibaccum*, *P. pendulispicum* and *P. mutabile*, *P. pendulispicum* and *P. nigrum*, *P. phuwuaense* and *P. caninum*, *P. phuwuaense* and *P. dominantinervium*, *P. phuwuaense* and *P. magnibaccum, P. phuwuaense* and *P. mutabile*, *P. phuwuaense* and *P. nigrum*, *P. phuwuaense* and *P. pedicellatum, P. pilobracteatum* and *P. caninum*, *P. pilobracteatum* and *P. mutabile*, *P. polysyphonum* and *P. khasianum*, *P. polysyphonum* and *P. magnibaccum*, *P. polysyphonum* and *P. montium, P. sarmentosum* and *P. longum*, *P. sylvestre* and *P. khasianum*, *P. sylvestre* and *P. magnibaccum*, *P. sylvestre* and *P. montium*, *P. thomsonii* and *P. nigrum*, *P. pilobracteatum* and *P. phuwuaense*, *P. polysyphonum* and *P. pendulispicum*, *P. polysyphonum* and *P. pedicellatum*, *P. sylvestre* and *P. pendulispicum*, *P. sylvestre* and *P. pedicellatum*, *P. sylvestre* and *P. polysyphonum*, *P. wallichii* and *P. umbellatum*, *P. protrusum* and *P. phuwuaense*, and *P. protrusum* and *P. pilobracteatum*, while the highest value of 0.213 was observed in the *P. betle* and *P. argyritis* pair; 3) for the *psbA-trnH* spacer region the lowest value of 0.000 was observed in the pairs of *P. montium* and *P. magnibaccum*, *P. pilobracteatum* and *P. caninum*, *P. polysyphonum* and *P. pedicellatum*, *P. ribesioides* and *P. pedicellatum*, *P. sarmentosum* and *P. longum*, *P. sylvestre* and *P. pedicellatum*, *P. wallichii* and *P. khasianum*, *P. wallichii* and *P. pedicellatum*, *P. protrusum* and *P. magnibaccum*, *P. sylvestre* and *P. polysyphonum*, *P. sylvestre* and *P. ribesioides*, *P. wallichii* and *P. polysyphonum*, *P. wallichii* and *ribesioides*, *P. wallichii* and *P. sylvestre*, and *P. yinkiangense* and *P. betle*, while the highest value of 0.228 was observed between *P. semiimmersum* and *P. umbellatum*.

The genetic distance of the *matK* region in Table 2 is a representative example.

Discussion

Most of the 43 species of wild *Piper* in Thailand have many functional uses. Only four species, *P. betle*, *P. retrofractum*, *P. nigrum* and *P. sarmentosum* are economic and cultivated species, and all of these species are also used as ingredients in the products mentioned above in the introduction. *Piper betle* is a well-known species that is important for its chemical substances, including essential oils, chavicol, cineol and eugenol, which can be used for medicinal and insecticidal purposes. Because these plants are widely used, and used in several forms, which include plant parts, powdered preparations, capsule formulations and other preparations, their authenticity should be verified using DNA barcodes to establish the worthiness of these products for medicinal, cosmetics and house-hold use. To overcome the problems associated with identifying species based on morphological characters, DNA barcoding has been employed. For flowering plants in Thailand, the *psbA-trnH* spacer region was suggested as an efficient DNA barcode marker in *Senna* species (Monkheang et al. 2011), as well as *Smilax* and *Cissus* species (Kritpetcharat et al. 2011). In addition, the *rbcL* gene has been suggested as a marker in parasitic plants, including *Scurrula*, *Dendrophthoe*, *Helixanthera*, *Macrosolen* and *Viscum* species (Kwanda et al. 2013) and the *matK* gene marker was identified in some medicinal *Piper* species (Sudmoon et al. 2012). Therefore the authors selected these three regions for barcode promising in the *Piper* species.

Table 2. Genetic distance values of the *matK* region for *Piper* identification, a representative example.

	P. argyritis	*P. betle*	*P. betle*	*P. betle*	*P. betloides*	*P. boehmeriifolium*	*P. boehmeriifolium*	*P. caninum*	*P. caninum*	*P. colubrinum*	*P. colubrinum*	*P. crocatum*	*P. dominantinervium*	*P. dominantinervium*	*P. hongkongense*	*P. hongkongense*	*P. khasianum*	*P. khasianum*	*P. kraense*	*P. kraense*	*P. longum*	*P. longum*	*P. maculaphyllum*	*P. maculaphyllum*	*P. magnibaccum*	*P. magnibaccum*	*P. nigrum*	*P. nigrum*	*P. pedicellatum*	*P. pedicellatum*	*P. pendulispicum*
P. argyritis	.000																														
P. betle	.323	.000																													
P. betle	.076	.362	.000																												
P. betle	.126	.386	.106	.000																											
P. betloides	.249	.423	.262	.260	.000																										
P. boehmeriifolium	.004	.323	.074	.124	.252	.000																									
P. boehmeriifolium	.011	.325	.074	.126	.258	.009	.000																								
P. caninum	.013	.328	.080	.132	.249	.009	.017	.000																							
P. caninum	.013	.328	.080	.132	.249	.009	.017	.000	.000																						
P. colubrinum	.020	.332	.087	.134	.267	.017	.022	.026	.026	.000																					
P. colubrinum	.089	.375	.143	.195	.310	.085	.093	.093	.093	.072	.000																				
P. crocatum	.054	.358	.106	.145	.267	.052	.056	.061	.061	.065	.130	.000																			
P. dominantinervium	.004	.323	.072	.121	.252	.002	.007	.011	.011	.015	.087	.050	.000																		
P. dominantinervium	.004	.323	.072	.121	.252	.002	.007	.011	.011	.015	.087	.050	.000	.000																	
P. hongkongense	.013	.321	.080	.130	.260	.011	.011	.020	.020	.024	.095	.059	.009	.009	.000																
P. hongkongense	.013	.321	.080	.130	.260	.011	.011	.020	.020	.024	.095	.059	.009	.009	.000	.000															
P. khasianum	.007	.325	.076	.126	.249	.002	.011	.011	.011	.020	.087	.054	.004	.004	.013	.013	.000														
P. khasianum	.007	.325	.074	.124	.249	.004	.009	.013	.013	.017	.089	.052	.002	.002	.011	.011	.002	.000													
P. kraense	.004	.323	.072	.121	.252	.002	.007	.011	.011	.015	.087	.050	.000	.000	.009	.009	.004	.002	.000												
P. kraense	.004	.323	.072	.121	.252	.002	.007	.011	.011	.015	.087	.050	.000	.000	.009	.009	.004	.002	.000	.000											
P. longum	.013	.325	.080	.130	.258	.011	.015	.020	.020	.024	.091	.054	.009	.009	.017	.017	.013	.011	.009	.009	.000										
P. longum	.013	.325	.080	.130	.258	.011	.015	.020	.020	.024	.091	.054	.009	.009	.017	.017	.013	.011	.009	.009	.000	.000									
P. maculaphyllum	.065	.369	.130	.171	.282	.065	.072	.074	.074	.080	.141	.111	.065	.065	.074	.074	.063	.063	.065	.065	.072	.072	.000								
P. maculaphyllum	.054	.341	.072	.113	.249	.052	.054	.061	.061	.065	.130	.085	.050	.050	.059	.059	.054	.052	.050	.050	.054	.054	.111	.000							
P. magnibaccum	.007	.325	.074	.124	.249	.004	.009	.013	.013	.017	.089	.052	.002	.002	.011	.011	.002	.000	.002	.002	.011	.011	.063	.052	.000						
P. magnibaccum	.009	.323	.076	.124	.256	.007	.011	.015	.015	.015	.087	.054	.004	.004	.013	.013	.009	.007	.004	.004	.013	.013	.069	.054	.007	.000					
P. nigrum	.015	.334	.087	.130	.262	.017	.022	.026	.026	.030	.102	.061	.015	.015	.024	.024	.020	.017	.015	.015	.022	.022	.072	.063	.017	.020	.000				
P. nigrum	.009	.325	.080	.121	.249	.011	.015	.020	.020	.024	.095	.059	.009	.009	.017	.017	.013	.011	.009	.009	.017	.017	.072	.054	.011	.013	.020	.000			
P. pedicellatum	.011	.328	.080	.126	.252	.007	.015	.015	.015	.024	.091	.054	.009	.009	.017	.017	.009	.011	.009	.009	.013	.013	.067	.046	.011	.013	.022	.013	.000		
P. pedicellatum	.011	.325	.074	.124	.258	.009	.004	.017	.017	.022	.093	.056	.007	.007	.011	.011	.011	.009	.007	.007	.015	.015	.072	.054	.009	.007	.022	.015	.015	.000	
P. pendulispicum	.004	.325	.076	.126	.252	.007	.011	.015	.015	.020	.091	.050	.004	.004	.013	.013	.009	.007	.004	.004	.009	.009	.067	.050	.007	.009	.013	.009	.009	.011	.000

Table 3. Interspecific genetic distance values for identification of the eight pairs *Piper* species by *rbcL* and *psbA-trnH* spacer sequences.

Pairs of species	*matK* region	*rbcL* region	*psbA-trnH* spacer region
P. kraense and *P. dominantinervium*	0.000	0.005-0.008	0.111-0.117
P. magnibaccum and *P. kraense*	0.000	0.008	0.1110-0.123
P. phuwuaense and *P. dominantinervium*	0.000	0.000-0.003	0.021-0.026
P. phuwuaense and *P. kraense*	0.000	0.005-0.008	0.021-0.129
P. pilobracteatum and *P. dominantinervium*	0.000	0.003	0.021
P. pilobracteatum and *P. kraense*	0.000	0.003	0.010-0.123
P. pilobracteatum and *P. phuwuaense*	0.000	0.003	0.016-0.021
P. sylvestre and *P. polysyphonum*	0.000	0.000	0.000-0.010

The results from DNA barcoding 36 *Piper* species using three different marker regions support a previous hypothesis of genetic distance values (Hebert et al. 2003), showing a significant variance in sequences between species and a comparatively small variance within species. Note that the economic and planted species, *P. betle* had the highest intraspecific genetic distance values of 0.386 for the *matK* region, which may have been due to the presence of human growth factors. The interspecific genetic distances for the *matK* region were effective for the identification of different species with 27 pairs of species ranging from a low of 0.002 to a high of 0.486, as shown in Table 2 and eight unidentified species pairs had a genetic distance of 0.000. This result agrees with the study by Hao et al. (2013) who claimed that *matK* had high species identification reliability and suggested that this region should be used for identification of *Piper* species along with the ITS region. Additionally, the *rbcL* and *psbA-trnH* spacer regions are effective for further identification of the other eight species pairs as shown in Table 3. The lowest genetic distance value is 0.010 of the pair *P. sylvestre* and *P. polysyphonum* to the highest value 0.129 for the pair *P. phuwuaense* and *P. kraense* in *psbA-trnH* spacer region. It can be concluded that these three barcode regions are powerful for further efficient identification of the 36 *Piper* species.

The results presented here support those of Newmaster et al. (2007), who proposed to use *matK* and the *psbA-trnH* spacer to identify Myristicaceae plants, Sudmoon et al. (2012) who recommended independent analysis of each barcode region, and CBOL Plant Working Group (2009) who proposed *rbcL* and *matK* as the core DNA barcode regions for land plants.

Acknowledgments

The authors are grateful for the financial support provided by the Thailand Research Fund through the Research Career Development Grant (Grant No. RSA5580054). The authors confirm that there is no conflict of interest.

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PhytoKeys 70: 11–16 (2016)
doi: 10.3897/phytokeys.70.9363
http://phytokeys.pensoft.net

SHORT COMMUNICATION

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Monitoring within non-native ungulate exclosures documents the inherent size of *Crocanthemum greenei* (Cistaceae)

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Academic editor: *Pavel Stoev* | Received 28 May 2016 | Accepted 29 August 2016 | Published 20 September 2016

Citation: Dvorak TM, Catalano AE, Guilliams CM (2016) Monitoring within non-native ungulate exclosures documents the inherent size of *Crocanthemum greenei* (Cistaceae). PhytoKeys 70: 11–16. doi: 10.3897/phytokeys.70.9363

Abstract

Crocanthemum greenei (B.L.Rob.) Sorrie (Cistaceae), a perennial sub-shrub, was measured as part of a demographic monitoring effort on Santa Catalina Island, California, USA (hereafter, Catalina). Introduced ungulate browsers remain present on Catalina. Consequently, many palatable plant taxa on the island are subject to and putatively limited by top-down browsing forces. Historically, introduced ungulates have also been present on each island throughout the range of *C. greenei*. Habitat conservation work, resulting in the construction of ungulate exclosures on Catalina, has now allowed us to measure individuals in their mature, non-browsed form. The published value for *C. greenei* stem (height) is usually 15–30 cm. While the original description hints at a greater potential size, recent descriptions appear to be influenced by observations made during the decades when plants would have been impacted by introduced ungulate herbivores. Here we present stem measurements of 81 adult individuals, with a median of 49 cm and an interquartile range of 42–56 cm. These measurements suggest an expanded stem (height) range of 15–60 cm better describes the taxon and shed light on the historical impacts of invasive ungulates across the islands and those continuing on Catalina.

Keywords

Cistaceae, *Crocanthemum greenei*, *Helianthemum*, Santa Catalina Island, stem measurement

Introduction

Crocanthemum greenei (B.L.Rob.) Sorrie (Cistaceae) is a perennial sub-shrub endemic to the Channel Islands of southern California. It is currently known from Santa Catalina (hereafter, Catalina), Santa Cruz, and Santa Rosa islands. It was also documented on San Miguel Island, but is now considered extirpated from that locale (Thorne 1967, McEachern 2010). *Crocanthemum greenei* is federally listed under the Endangered Species Act as threatened. It has presumably declined due to the browsing impacts of introduced ungulates throughout its range. Eradication efforts have removed all populations of introduced ungulates across the entire Channel Islands archipelago, with the exception of mule deer (*Odocoileus hemionus*) and American bison (*Bison bison*) on Catalina.

A number of ungulate exclosures were constructed from 2008–2011 and have been maintained on Catalina for habitat recovery following wildfires and for focused conservation of select plant taxa. *Crocanthemum greenei* is present within six of these exclosures. We monitored 81 mature individuals within the exclosures as part of an overall island-wide demographic monitoring and study effort that is underway for the species. We measured the main stem of each plant as part of our monitoring protocol. All individuals were measured with a standardized method by the same researcher (A.E.C). A measuring tape was stretched from the base of the main stem to its tallest point, excluding the inflorescence. Photographs were taken of every individual with a standard ruler held or leaning next to the plant for scale (Fig. 1). This documented the physiognomy of each individual measured and permitted later inspection, when necessary.

Results

Crocanthemum greenei stem height measurements within exclosures (n = 81) had a range of 29 cm to 68 cm (Fig. 2). Median and mean were nearly identical at 49.00 cm and 49.02 cm, respectively. The interquartile range of the measurements was 42–56 cm. The most recent taxonomic treatment of *C. greenei* states that stem length is 15–30 cm (Baldwin et al. 2012, Sorrie and Rosatti 2014). These data show that stem (height) of *C. greenei* can be at least as tall/long as 60 cm in the absence of introduced herbivores.

Discussion

Our measurement data show that *C. greenei* may grow substantially taller than previously reported in the absence of browsing by introduced ungulates. Furthermore, during our monitoring efforts we have consistently documented individuals outside of exclosures exhibiting severely browsed growth forms (Fig. 3), which lends evidence toward browsing as the limiting factor to achieving these sizes rather than the possibility of morphological variation due to external factors such as between-year climate (Dvorak

Figure 1. An individual from the current monitoring effort for *Crocanthemum greenei*. This particular individual, growing within an exclosure, had an initial stem measurement of 44 cm and is representative of size for non-browsed individuals.

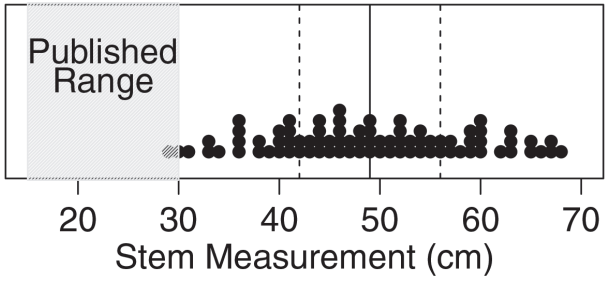

Figure 2. A plot of each exclosure individual in relation to the published stem range of 15–30 cm. The median of the dataset (49 cm) is marked with a solid line and the interquartile range (42–56 cm) lies between the dotted lines.

and Catalano 2016). The measurements summarized here represent well documented, quantitative evidence of the natural growth form of *C. greenei* when not modified by introduced browsers. We feel a particularly significant point is that introduced species

Figure 3. A severely browsed individual from a population not protected by exclosure fencing.

have likely obscured our understanding of some basic aspects of the natural history of this rare, native island-endemic plant.

Crocanthemum greenei was first recognized as distinct from co-occurring *Crocanthemum scoparium* (Nutt.) Millsp. by Edward L. Greene in 1886. Greene named the new species from Santa Cruz Island *Helianthemum occidentale* Greene, but this name was already in use for a European plant (*H. occidentale* Nyman) and was therefore an illegitimate homonym. Regardless, Greene described the new species as suffrutescent, "a foot or more high", and as having an inflorescence densely covered with glandular-viscid hairs; this latter feature distinguished it from *H. scoparium*, which is glabrous or with sparse short-glandular hairs. In his Flora of North America treatment of *Helianthemum*, Robinson (1895) recognized the new plant as *H. greenei* Robinson, providing roughly the same plant height of "6 inches to more than foot in height". Munz (1959) recognized the species in A California Flora, in which he described the plant as having stems 1–2 dm (10–20 cm) high, rarely to 3 dm high (30 cm). In their monograph on *Helianthemum*, Daoud and Wilbur (1965) describe the species as being 14–30 cm tall. Sorrie (2011) later transferred all western North American *Helianthemum* taxa to the genus *Crocanthemum* based on unpublished molecular phylogenetic evidence that *Helianthemum* s.l. is polyphyletic (Arrington 2004). In the most recent treatment of *Crocanthemum* in California (Sorrie and Rosatti 2014), stem height is given

as 15–30 cm. Therefore, the prevailing view since the species was first described was of a plant between approximately 15 and 30 cm tall (stem height 10–20 cm in Munz). We hypothesize that the difference between the prevailing view described above and our observations of the species on Catalina is due to the recent exclusion of introduced herbivores from our study plots.

Since the original description of the species was made on the basis of plants collected on Santa Cruz Island, the primary ungulate impacts relative to those plants would have come from sheep. The first record of sheep introduction on Santa Cruz Island was in the mid-1850s and the first effects on the vegetation due to grazing were reported in 1875 (Hobbs 1980). This timeline places Greene's original collection (1886) during the sheep-grazing period and after the effects of introduced herbivores on the landscape had been noted.

Recent conservation and restoration efforts on the Channel Islands have eradicated ungulates from Santa Rosa and Santa Cruz islands, and resulted in actions on Catalina Island including the creation of exclosure habitats where our measurements of *C. greenei* were made. With browsing pressure removed in some portions of the historical range of *C. greenei*, individuals of the species can now grow to reach their full, inherent size. Beyond initiating a revision of the morphological description of *C. greenei*, we hope that these observations are suggestive of both the capacity for recovery of a rare, island-endemic plant and the continuing need to remove the remaining ungulates from its range, which would bring to completion a critical conservation action for the Channel Islands archipelago.

Acknowledgments

We thank all of those involved with exclosure construction and maintenance on Catalina over the years. Funding was provided by U.S. Fish and Wildlife Service (Grant # F10AC00790), Seaver Institute, and Marisla Foundation.

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PhytoKeys 70: 17–30 (2016)
doi: 10.3897/phytokeys.70.9053
http://phytokeys.pensoft.net

RESEARCH ARTICLE

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Molecular and morphological evidence for recognition of two species within *Harpagonella* (Amsinckiinae, Boraginaceae)

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Academic editor: *James Cohen* | Received 29 April 2016 | Accepted 7 September 2016 | Published 20 September 2016

Citation: Guilliams CM, Jang T, Baldwin BG (2016) Molecular and morphological evidence for recognition of two species within *Harpagonella* (Amsinckiinae, Boraginaceae). PhytoKeys 70: 17–30. doi: 10.3897/phytokeys.70.9053

Abstract

Recent taxonomic treatments of the genus *Harpagonella* have included only one lower taxon, *H. palmeri* A. Gray. However, a larger-fruited variety of *H. palmeri* from Arizona and Sonora was described by I.M. Johnston in 1924. He continued to recognize this taxon – *H. palmeri* var. *arizonica* – in his treatment of the genus in Kearney and Peebles's Arizona Flora in 1960. Here, we provide two lines of molecular evidence and quantitative morphological evidence from calyx characters showing that plants of *Harpagonella* from Arizona, Sonora, and central Baja California, corresponding to Johnston's var. *arizonica*, are distinct from *H. palmeri* of southern California and Baja California. We make the new combination *Harpagonella arizonica* (I.M. Johnston) Guilliams & B.G. Baldwin, **comb. nov.** for the plants from Arizona, Sonora, and central Baja California.

Keywords

Amsinckiinae, Boraginaceae, *Harpagonella*, *Pectocarya*

Introduction

Harpagonella A. Gray is a genus of Boraginaceae, subtribe Amsinckiinae (see Chacón et al. 2016 and Luebert et al. 2016) that occurs disjunctly in western North America, with populations in southern California, USA, and adjacent Baja California, México and other populations in southern Arizona, USA, and adjacent northwestern Sonora, México (Figure 1). The only species recognized in the genus, *H. palmeri* A. Gray, was described in 1876 from an 1875 collection by Edward Palmer on Guadalupe Island, Baja California. In 1924, Ivan M. Johnston recognized two varieties in *H. palmeri*, var. *arizonica* and var. *palmeri*. The former taxon, then known from Arizona and adjacent Sonora, was said to differ from var. *palmeri*, of California and Baja California, in hav-

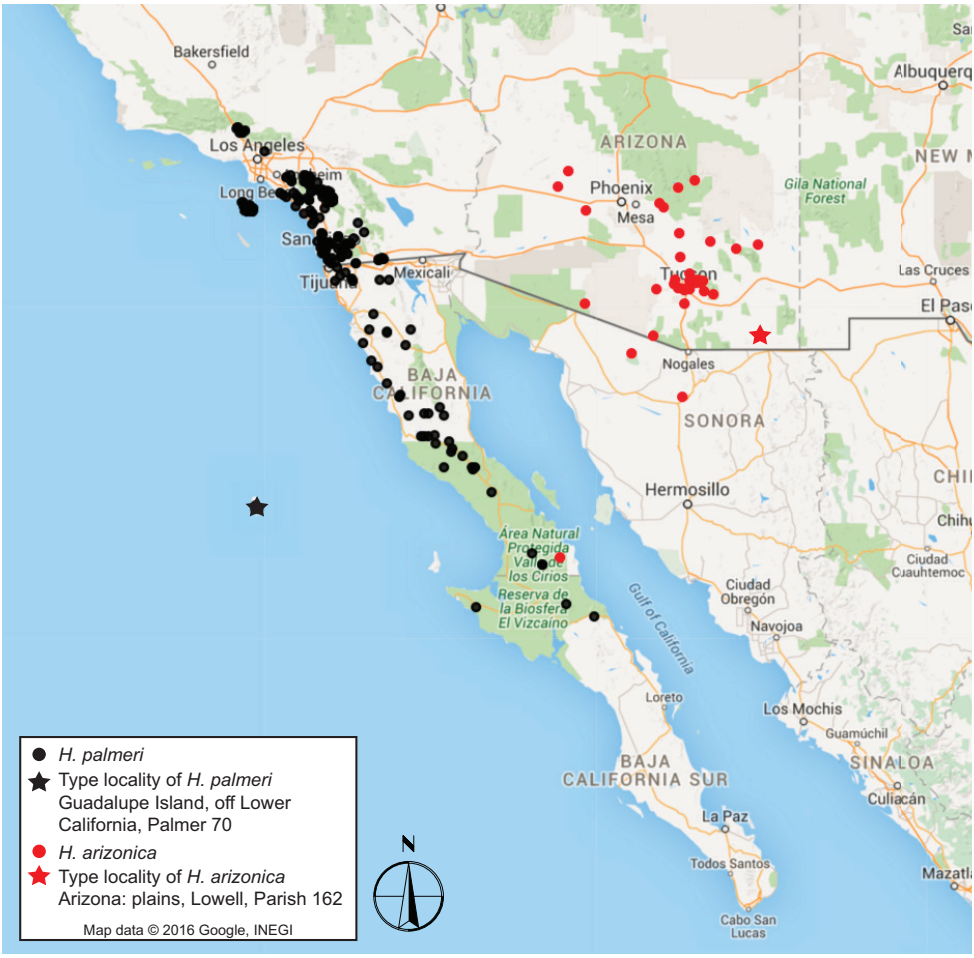


Figure 1. Map of western North America showing *Harpagonella* collections in major herbaria based on available specimen data from GBIF and Bajaflora. Type collection localities are indicated with black star for *H. palmeri* and a red star for *H. arizonica*.

ing longer "cornute processes on the fruiting calyx" and larger nutlets (Johnston 1924). Furthermore, the plants of California and Baja California are often found on clayey soils, while those of Arizona and Sonora often occur in sandy or gravelly soils. In his treatment of the Boraginaceae for the Arizona Flora (Kearney and Peebles 1960), Johnston retained the taxon as a variety, but most other treatments of the genus recognize *H. palmeri* without varieties (e.g., Munz 1973, Veno 1979, Kelley and Messick 2014).

Harpagonella has been regarded as the most morphologically distinctive member of the Amsinckiinae, largely because of ornamentation of the calyx in fruit that is unique to the genus (Johnston 1924, Veno 1979). The genus was placed in its own tribe, Harpagonelleae, for this reason (Gürke 1897). In *Harpagonella*, the calyx is pentamerous, with the two sepals away from the inflorescence axis connate for >80% of their length and the three other sepals free while in flower. The two fused sepals are strongly accrescent, becoming conduplicate, indurate, and often more or less enveloping one nutlet or sometimes both nutlets at fruit maturity (Figure 2). As the fruit matures, five to ten subterete appendages with distal retrorse barbs develop on the pair of fused sepals, giving the fruit the appearance and function of a grappling hook, which is the common name for the genus. The pedicel is also accrescent. It recurves or rarely coils as the fruit matures, placing the lobes of the fused sepals against the inflorescence axis. As Gray (1876) noted, these modifications effectively result in the transfer of dispersal function from the nutlet, as is typical in many Amsinckiinae, to the calyx. The gynoecium in *Harpagonella* is also distinctive. It has been reduced from the typical condition in the Amsinckiinae of four ovules and a fruit of four nutlets to two developing ovules and two nutlets, with the other two ovules early abortive. Unlike the nutlets of many close relatives, e.g. *Pectocarya*, the two nutlets of *Harpagonella* are largely without ornamentation, bearing only short hairs.

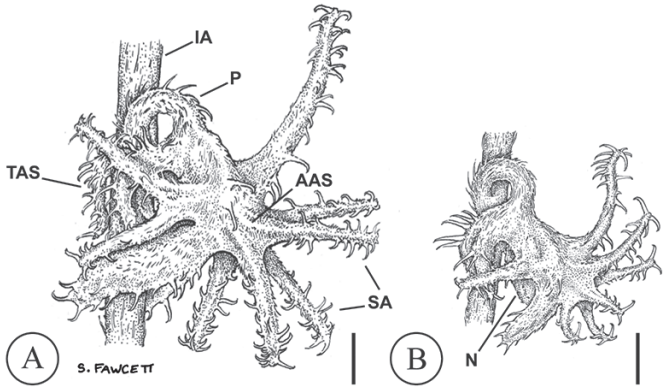


Figure 2. Fruits of *Harpagonella* in lateral view, from A) southern Arizona (*Tedford 1043*, ARIZ403065) and B) southern California (*Bramlet 2301*, ARIZ345225). Although morphologically similar, note overall difference in size. Scale bars are each approximately 1 mm. Labels: (AAS) sepals away from inflorescence axis in flower; (IA) inflorescence axis; (N) nutlet; (P) pedicel; (SA) sepal appendages; (TAS) sepals toward inflorescence axis in flower.

We included *Harpagonella* in broad phylogenetic and taxonomic studies of some members of the Boraginaceae subtribe Amsinckiinae (Guilliams 2015). During the phylogenetic study, we included several samples of *H. palmeri* from throughout its range with the goal of evaluating phylogenetic structure of the included samples, with attention to historical taxonomy. We also examined herbarium sheets representing both previously recognized varieties of *H. palmeri*, taking measurements of the calyx appendages and overall size of the fruit. Although a full phylogenetic study will be published later, we present the results of this study here in reduced form so that the resulting new combination can be available for use in the treatment of *Harpagonella* for the Flora of North America, North of México.

Methods

Phylogenetic analyses

DNA was extracted from 12 samples of *Harpagonella* and 2 samples of *Pectocarya* using a modified CTAB protocol (Doyle and Doyle 1987). Samples included in this analysis are given in Table 1 and were selected on the basis of geographic distribution of the two putative taxa and recency of collection. Six of these samples were from Arizona and were morphologically consistent with *H. palmeri* var. *arizonica* sensu Johnston (1924). The other six samples were from California and adjacent Baja California and were morphologically consistent with *H. palmeri* var. *palmeri*. One sample each of *Pectocarya linearis* DC. var. *ferocula* I.M. Johnst. and *P. recurvata* I.M. Johnst. were included as outgroup taxa.

Polymerase chain reaction (PCR) was used to amplify the internal transcribed spacer (ITS) and the external transcribed spacer (ETS) of nuclear ribosomal DNA, and the *rpl16*, *rps16*, *trnK-rps16*, and *trnL-trnF* regions of the chloroplast genome. All PCR reactions except for those targeting the ETS region were performed using previously published primers and reaction conditions (see Baldwin et al. 1995, Shaw et al. 2005, Shaw et al. 2007). The 5' ETS primer was designed following the protocol of Baldwin and Markos (1998). PCR products were cleaned using USB ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) using the standard protocol. Bidirectional sequencing was performed on an Applied Biosystems 3730xl DNA Analyzer at the Barker DNA Sequencing core facility at UC Berkeley. Contigs were assembled and edited in Geneious R6 (Drummond et al. 2013). Sequences were initially aligned under the default parameters using the Geneious alignment tool in Geneious, then further refined by hand.

For each DNA region, models of sequence evolution were estimated using jModelTest (Posada 2008). Bayesian phylogenetic analyses were performed and summarized using the BEAST suite of programs. Four separate analyses of 10 million generations were performed in BEAST v.1.7.4 (Drummond and Rambaut 2007), with the first 25% of trees discarded as burn-in. Convergence was assessed using Tracer v.1.7.4 (Rambaut and Drummond 2007). Post burn-in runs were combined using Log Com-

Table 1. Specimens of *Harpagonella* and outgroups used in phylogenetic analysis, including collector and collection numbers, herbarium accession numbers, and GenBank accession numbers by DNA region.

Taxon	Collector and Collection Number	Herbarium Accession Number	GenBank Accession Numbers					
			ITS	ETS	rpL16	rps16	trnK-rps16	trnL-trnF
Harpagonella palmeri var. *arizonica*	J.E. Bowers 2395	ARIZ241135	KX151054	–	KX151070	KX151084	KX151098	KX151108
	T.R. Van Devender 88-54	ARIZ278363	KX151052	KX151044	KX151068	KX151082	KX151096	KX151106
	S.P. McLaughlin & J.E. Bowers 4476	ARIZ307288	KX151053	–	KX151069	KX151083	KX151097	KX151107
	A.L. Reina G. & T.R. Van Devender 2003-194	ARIZ364715	KX151056	–	KX151072	KX151086	KX151100	KX151110
	T.R. Van Devender & A.L. Reina G. 2005-842	ARIZ377143	KX151055	–	KX151071	KX151085	KX151099	KX151109
	J. Tedford 599	ARIZ388168	KX151051	KX151043	KX151067	KX151081	KX151095	KX151105
Harpagonella palmeri var. *palmeri*	C.M. Guilliams 1414	n/a	KX151057	KX151045	KX151073	KX151087	KX151101	KX151113
	C.M. Guilliams 1421	n/a	KX151058	KX151046	KX151076	KX151088	KX151102	KX151114
	J.P. Rebman 8348	UC1790083	KX151059	KX151047	KX151075	KX151089	KX151103	KX151111
	S. Boyd & T.S. Ross 7906	UC1871078	KX151062	–	KX151078	KX151092	–	KX151116
	S. Boyd & T.S. Ross 8212	UC1871288	KX151061	–	KX151077	KX151091	–	KX151115
	J.P. Rebman 8031	UC1790065	KX151060	KX151048	KX151074	KX151090	KX151104	KX151112
Pectocarya penicillata	R.B. Kelley 1968	n/a	KX151063	KX151049	KX151065	KX151079	KX151093	KX151117
Pectocarya platycarpa	R.B. Kelley 1983	n/a	KX151064	KX151050	KX151066	KX151080	KX151094	KX151118

biner v.1.7.4. The maximum clade credibility tree (MCCT) was found and clade credibility values calculated using Tree Annotator v.1.7.4.

Separate maximum likelihood analyses for nrDNA and cpDNA were performed using RAxML v1 plug-in in Geneious v8.1.8 (Drummond AJ et al. 2015). Maximum likelihood bootstrap values resulting from these analyses were added to the MCCT.

Morphological analyses

Morphological data were taken from a total of 32 physical specimens of *Harpagonella palmeri* var. *arizonica* and 27 physical specimens of *H. palmeri* var. *palmeri*. Physical specimens measured were those available from the ARIZ, JEPS, and UC herbaria with mature fruits. We also measured high quality digital scans of type material of both taxa. For each specimen, we measured and averaged values from up to five fruits for maximum fruit length along an axis oriented from the pedicel base to the most distant point (including subterete appendages; mm), maximum fruit width along an axis perpendicular to maximum fruit length (including subterete appendages; mm), and maximum length of subterete appendages (mm). Measurements of physical specimens were taken with a digital caliper to the nearest hundredth of a millimeter. Measurement of digital specimens were made in ImageJ (Abramoff MD et al. 2004). Nutlet length has been reported as different between the two varieties, but measuring this feature would have required occasional destructive sampling and was therefore avoided.

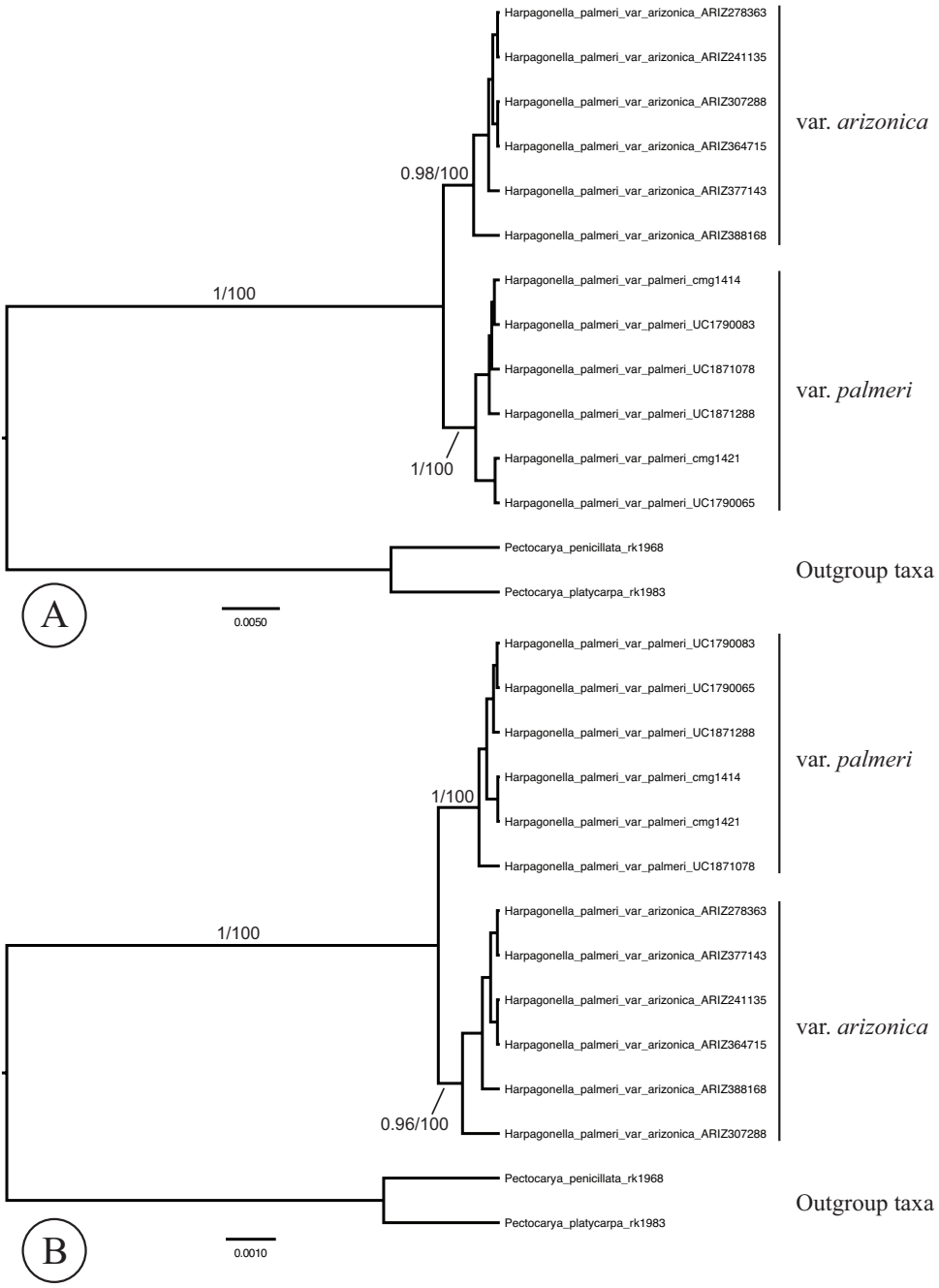
Morphological data were explored using boxplots and basic descriptive statistics. Student's t-tests were performed to evaluate the statistical significance of the differences between the varieties for the features measured. All statistical analyses were performed in R (R Development Core Team 2008).

Results

Phylogenetic patterns in *Harpagonella*

The nuclear dataset comprising ITS and ETS was 1,082 total bases in length. For these loci, jModelTest determined a best-fit model of sequence evolution of GTR+I. In the matrix, 79 positions were variable and phylogenetically informative, 29 were variable and not phylogenetically informative, and 974 were invariant.

The MCCT resulting from the analysis of the concatenated nuclear DNA matrix is given in Figure 3A. Samples of each variety of *Harpagonella* are reciprocally monophyletic and clades by taxon are strongly supported. The clade of samples of var. *arizonica* was supported with a posterior probability of 0.98 and a maximum likelihood bootstrap value of 100. The clade of samples of var. *palmeri* was supported with a



Figures 3. Maximum clade credibility trees from phylogenetic analysis of the: **A** combined, partitioned nuclear DNA regions, and **B** combined, partitioned chloroplast DNA regions. Values on branches are Bayesian posterior probabilities followed by maximum likelihood bootstrap values.

posterior probability of 1 and a maximum likelihood bootstrap value of 100. Support for phylogenetic relationships within each clade was poor.

The chloroplast dataset comprising *rpl16*, *rps16*, *trnK-rps16*, and *trnL-trnF* was 3,442 total bases in length. For these loci, jModelTest determined a best-fit model of sequence evolution of GTR+I. Of these, 51 positions were variable and phylogenetically informative, 30 were variable and not phylogenetically informative, and 3,361 were invariant.

The MCCT resulting from the analysis of the concatenated chloroplast DNA matrix is given in Figure 3B. Samples of each variety of *Harpagonella* are reciprocally monophyletic and clades by taxon are strongly supported. The clade of samples of var. *arizonica* was supported with a posterior probability of 0.96, and a maximum likelihood bootstrap value of 100. The clade of samples of var. *palmeri* was supported with a posterior probability of 1 and a maximum likelihood bootstrap value of 100. Support for phylogenetic relationships within each clade was poor.

The split between *Harpagonella* and outgroup sequences as well as the branches subtending varieties of *Harpagonella palmeri* were all supported by a number of shared nucleotide substitutions as well as insertion/deletions (indels). The *Harpagonella*-outgroup split was supported by 68 substitutions in the nuclear dataset, and 46 substitutions and 31 indels in the chloroplast dataset. The branch subtending the clade of var. *arizonica* samples was supported by 4 nucleotide substitutions in the nuclear dataset, and 1 substitution and 5 separate indels in the chloroplast dataset. The branch subtending the clade of var. *palmeri* samples was supported by 3 nucleotide substitutions in the nuclear dataset and 3 substitutions in the chloroplast dataset.

Morphological patterns in *Harpagonella*

Harpagonella palmeri var. *arizonica* and *H. palmeri* var. *palmeri* differ in all three features measured and the differences are highly significant statistically ($p << 0.001$). Box and whisker plots of the measured morphological features are presented in Figure 4. Values for measurements of type specimens are denoted by an asterisk. Average maximum fruit length ranged from 5.13 to 9.99 mm (average = 7.38 mm; type = 7.58 mm) in *H. palmeri* var. *arizonica* and from 3.04 to 5.87 mm (average = 4.38 mm; type = 5.38 mm) in *H. palmeri* var. *palmeri* ($t = 14.027$, $df = 55.488$, $p < 2.2 \times 10^{-16}$). Average maximum fruit width ranged from 7.33 to 9.33 mm (average = 8.17 mm; type = 8.88 mm) in *H. palmeri* var. *arizonica* and from 3.55 to 6.41 mm (average = 4.84 mm; type = 4.33 mm) in *H. palmeri* var. *palmeri* ($t = 17.912$, $df = 49.56$, $p < 2.2 \times 10^{-16}$). Average maximum subterete appendage length ranged from 3.28 to 5.42 mm (average = 4.08 mm; type = 4.12 mm) in *H. palmeri* var. *arizonica* and from 1.58 to 3.12 mm (average = 2.19 mm; type = 2.10 mm) in *H. palmeri* var. *palmeri* ($t = 16.767$, $df = 55.976$, $p < 2.2 \times 10^{-16}$).

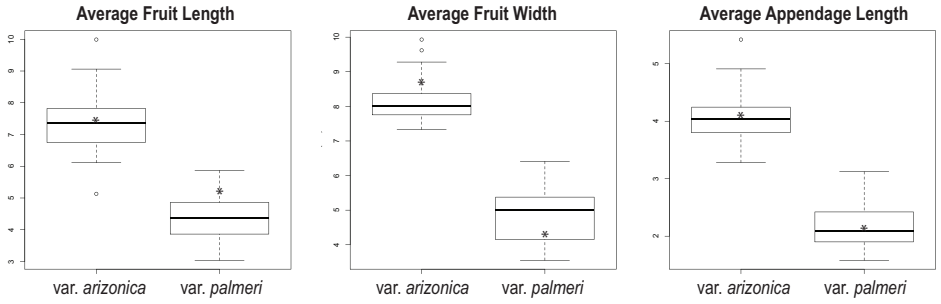


Figure 4. Box and whisker plots by taxon of **A** average maximum fruit length (mm), **B** average maximum fruit width (mm), **C** average maximum subterete appendage length (mm). Asterisks denote the measured values of type specimens. Note significant differentiation in all features measured.

Discussion

The separate phylogenetic analyses of nrDNA and cpDNA presented here each recover two clades within *H. palmeri* corresponding to the two named varieties. Statistical support for these groupings was very high, with posterior probabilities above 0.96 and maximum likelihood bootstrap values of 100 in all cases. The *Harpagonella*-outgroup split as well as clades of samples by variety were each supported by numerous nucleotide substitutions and indels. We take this as strong evidence for two evolutionary lineages in the genus.

Morphologically, these two lineages differ in all measured aspects of fruit size. Plants primarily from Arizona and Sonora are significantly larger in maximum fruit length, maximum fruit width, and appendage length. Box and whisker plots for these features show that the ranges of measurements of these characters between the two lineages are mostly non-overlapping. Although unmeasured here, nutlet size in *Harpagonella* was suggested by Johnston (1924) to be larger in plants from Arizona and Sonora than in plants from California and Baja California. These differences are quantitative, not qualitative, and absent a formal statistical analysis of morphology, Veno (1979) advocated for recognizing no infraspecific taxa in *H. palmeri*, stating that "this feature is variable and somewhat clinal, and does not provide a significant or reliable basis for taxonomic delimitation." The data presented here suggest instead that these quantitative characters appear to be sufficient for reliable delimitation of two taxa corresponding to the evolutionary lineages recovered in the phylogenetic analysis.

Herbarium study of 366 specimens representing 291 gatherings of *Harpagonella* has permitted the evaluation of the geographic range of these morphologically distinct evolutionary lineages, which is especially critical for specimens collected on the Baja California Peninsula, where both named varieties have been reported. Specimens of plants with larger fruits corresponding to Johnston's *H. palmeri* var. *arizonica* are

almost entirely from Arizona and Sonora, with two collections attributable to this taxon made from desert regions of Baja California at mid-peninsula (*Moran 12682*, 28.29007, -113.12146; *Moran 12845*, 28.28333, -113.65). We have observed and confirmed the taxonomic identity of a specimen of the former (DS598325) but not the latter. Specimens of plants with smaller fruits corresponding to Johnston's concept for *H. palmeri* var. *palmeri* are known primarily from southwestern California and the adjacent western coastal areas of the Baja California Peninsula, with collections ranging as far to the south as the Vizcaino Peninsula on the Pacific Coast in Baja California Sur.

The biogeographic pattern displayed by *Harpagonella* – a disjunction between the California Floristic Province sensu Howell (1957) and central, southern Arizona and adjacent Sonora – is somewhat common yet underexplored. Raven and Axelrod (1978) describe this pattern briefly in their important paper on the origin of the California flora, and provide a table of 35 genera, species, or species pairs that have this pattern. To their list of taxa, we add *Harpagonella* based on evidence presented here.

Taxonomic treatment

Based on complete and well-supported reciprocal monophyly in two unlinked genomic partitions, statistically significant morphological differences, and essentially non-overlapping geographic ranges, the two lineages of *Harpagonella* resolved here merit recognition at the species level under the criteria of phylogenetic species concepts (see Mishler and Theriot 2000) as well as longstanding taxonomic practice. To recognize a taxon at species rank for the large-fruited plants found primarily in the deserts of Arizona and Sonora, the following new combination is needed.

Harpagonella arizonica **(I.M. Johnston) Guilliams & B.G. Baldwin, comb. nov.**
urn:lsid:ipni.org:names:77157712-1

BASIONYM. *Harpagonella palmeri* A. Gray var. *arizonica* I.M. Johnston. Contr. Gray Herb. 73: 75. 1924. TYPE: U.S.A. Arizona: "plains, Lowell," *W.F. Parish 162*, May 3, 1884, (holotype: GH! digital image).

SPECIMENS EXAMINED. Specimens listed alphanumerically by collector within a region. (*=specimen measured; è =specimen also used in molecular study; **bold**=type specimen) ***Harpagonella arizonica*****: MÉXICO. Baja California.** *Moran 12682* (DS). **Sonora.** *Keck 3963* (DS, POM), *Reina & Van Devender 2003-194*è (ARIZ, ASU), *Van Devender 2005-842*è (ARIZ). **UNITED STATES. Arizona.** *Abrams 12944* (DS), *Baker 8203* (ASU), *Baker 15963* (ASU), *Barr 67-78* (ASU), *Barr 67-82** (ARIZ, ASU), *Benson 9302* (POM), *Bingham 527** (ARIZ), *Bingham 1402* (ASU), *Bowers 2250** (ARIZ), *Bowers 2280** (ARIZ), *Bowers 2395**è (ARIZ), *Bowers 2461** (ARIZ), *Boyle 8026* (ARIZ), *Brandegee, T.S. s.n. 19 April 1889* (UC), *Butterwick 4349* (ASU), *Butterwick 4550* (ASU), *Butterwick & Hillyard 5793* (ARIZ, ASU), *Butterwick 7419* (ASU), *Carter s.n. 17 March 1936* (ARIZ), *Cave 16* (ARIZ), *Damrel 1618-B8* (ASU), *Daniel*

2581 (ASU), *Daniel & Butterwick 3853* (CAS), *Daniel 3907* (ASU), *Doan 441* (ASU), *Ducote 683* (ASU), *Eastwood 8130* (CAS), *Farruggia 1832* (ASU), *Felger 05-218* (ASU), *Fosberg 10605* (CAS, RSA), *Fosberg 10664* (CAS, POM), *Freeman* (ASU), *Gillespie 5429* (DS), *Griffiths s.n. date unknown** (ARIZ), *Halse 1701* (CAS), *Halverson 379* (ASU), *Harrison & Fulton 6608* (POM), *Harrison & Kearney 6654* (POM), *Higgins 6480* (ASU), *Hitchcock 25598* (DS, RSA), *Imdorf & Rice 427* (ASU, ARIZ), *Imdorf 587* (ASU), *Kearney 6654** (ARIZ), *Keck 2998* (DS), *Keil 1051* (ASU), *Keil 1484* (ASU), *Keil 2864* (ASU), *Keil 4082* (ASU), *Keil 4168* (ASU), *Keil K-11216* (ASU), *Landrum 6656* (ASU), *Landrum 11176* (ASU), *Lane 1035* (ASU), *Lane 1067* (ASU), *Lehto 181* (ASU), *Lehto 307* (ASU), *Lehto 1648* (ASU), *Lehto 1652* (ASU), *Lehto 4594* (ASU), *Lehto 7766* (ASU), *Lehto 10374* (ASU), *Lehto 10389* (ASU), *Lehto 10408* (ASU), *Lehto 10687* (ASU), *Lehto 11733* (ASU), *Lehto 17494* (ASU), *Lehto 17504* (ASU), *Lehto 17541* (ASU), *Lehto 12874-b* (ASU), *Lehto L-19732* (ASU), *Lehto L-19740* (ASU), *Makings 2018* (ASU), *Makings, L. Fertig, & W. Fertig 4346* (ASU, RSA), *Manton 236* (ASU), *Mason 1663** (ARIZ, CAS), *Mauz, Rosen, & Rautenkranz 2005-19* (ARIZ), *McGill LAM1280* (ASU, RSA), *McLaughlin 4476**è (ARIZ), *Orcutt 173* (CAS), *Parfitt 2498* (ASU), ***Parish 162*** **(GH; holotype)**, *Parish s.n. 1909* (DS), *Pase 1599* (ASU), *Peebles 1426** (ARIZ), *Peebles 3693** (ARIZ), *Pierce 296* (ASU), *Pinkava 4672* (ASU), *Pinkava 10122* (ASU), *Pinkava 10261* (ASU), *Pinkava 10893* (ASU), *Pinkava 11655* (ASU), *Price 829* (ASU), *Rand 15* (ASU), *Rand 152* (ASU), *Reeves 6447-a* (ASU), *Reina & Van Devender 97-269* (ARIZ), *Rice 328* (ASU), *Rice 1121* (ASU), *Rice 1586-a* (ASU), *Rice 1598* (ASU), *Jones, S. 1433* (ASU), *Schramm, Bond, & Bond 9* (ASU, RSA), *Shreve 7497* (ARIZ), *Shreve 10113** (ARIZ, DS), *Smith 1577* (ASU), *Swingle s.n. 1914* (ARIZ), *Tedford 582** (ARIZ), *Tedford 599**è (ARIZ), *Tedford 614* (ARIZ), *Tedford & Rose 1034** (ARIZ), *Thornber 2562** (ASU, ARIZ, CAS, RSA), *Thornber 2581** (ARIZ, CAS, RSA), *Thornber 4683* (ARIZ), *Thornber 5488** (ARIZ), *Thornber s.n. 1905** (ARIZ), *Thornber s.n. 1913** (ARIZ), *Toumey 5014** (ARIZ), *Turner 78-41** (ARIZ), *VanDevender 88-54**è (ARIZ), *Van Devender 2003-23** (ASU, ARIZ), *W. Fertig, Makings, & Alcock 29265* (ASU), *Warren 68-25** (ARIZ), *Warren 68-51** (ARIZ), *Wiggins 8420** (ARIZ), *Wiggins 8690* (DS), *Wood* (ASU). ***Harpagonella palmeri*****: MÉXICO. Baja California.** *Bacigalupi 3067* (DS, RSA, UC), *Boyd 5319** (RSA, UC), *Boyd & Ross 5464* (RSA), *Boyd & Ross 5761* (RSA), *Boyd, Gross, O'Brien, & Hamilton 10352* (RSA), *Breedlove 62271* (CAS, RSA), *Carter, Chisaki, & Moran 1056* (UC), *Dressler 668** (ARIZ), *Epling & Stewart s.n. 9 April 1936* (DS), *Haines & Stewart s.n. 7 February 1935* (DS), *Howell 8306* (CAS), *Jones, M.E. s.n. 11 April 1882* (POM), *Moran 6562* (POM), *Moran 6677* (DS), *Moran 6750* (DS, RSA), *Moran 12770* (UC), *Moran 19378* (CAS), *Moran 19992* (POM), *Porter 10551* (RSA), *Rebman & Delgadillo 1638* (ASU), *Rebman & Roberts 4856* (ASU), *Sanders, Rodriguez, West, et al. 5466* (ASU), *Thomas 15730* (DS), *Thorne, Liston, Mistretta 62122* (RSA), *Van Devender 91-348* (ARIZ), *Van Devender, T.R. & R.K. Van Devender 91-239* (ARIZ), *Wiggins & Ernst 12* (UC), *Wiggins & Thomas 67* (CAS), *Wiggins & Ernst 120* (DS), *Wiggins 4265* (DS, POM), *Wiggins 4415* (POM), *Wiggins 4463* (DS, POM), *Wiggins 7600* (DS, UC). **UNITED STATES. California.** *Atwood 17833** (UC), *Bacigalupi 8261** (JEPS), *Banks & Boyd 57* (RSA), *Banks & Boyd 316* (RSA), *Banks & Boyd 398* (RSA), *Banks 1652* (RSA), *Banks 1680*

(RSA), *Bell, Clark, Goss, Green, & Rusiniak 3546* (RSA), *Boyd 1384* (ARIZ, CAS, RSA), *Boyd 1396* (CAS, RSA), *Boyd 1399* (CAS, RSA), *Boyd 1589** (ARIZ, CAS, RSA), *Boyd 1644** (ARIZ, CAS, RSA), *Boyd 1767** (ARIZ, CAS, RSA), *Boyd 1790** (ARIZ, CAS, RSA), *Boyd 1816** (CAS, RSA, UC), *Boyd 3045** (UC), *Boyd, Ross, & Arnseth 3029* (RSA), *Boyd, Ross, & Arnseth 3036* (RSA), *Boyd, Ross, & Arnseth 3045* (RSA), *Boyd, Ross, & Arnseth 3116* (RSA), *Boyd, Ross, & Arnseth 3133* (RSA), *Boyd, Ross, & Arnseth 3196* (RSA), *Boyd, Ross, & Arnseth 3206** (RSA, UC), *Boyd, Ross, & Arnseth 3920* (RSA), *Boyd, Ross, Arnseth, & Bonilla 4008* (RSA), *Boyd, Ross, Arnseth, & Bonilla 4060* (CAS, RSA), *Boyd, Ross, Arnseth, & Bonilla 4110* (RSA), *Boyd, Arnseth, Rasmussen, & Cota 4605* (RSA), *Boyd 6165* (RSA), *Boyd & Mistretta 6311* (RSA), *Boyd 6901* (RSA), *Boyd 6962* (RSA), *Boyd & Ross 7302* (RSA), *Boyd & Ross 7906*è (RSA, UC), *Boyd & Ross 8212*è (RSA, SBBG, UC), *Boyd & Ross 8220* (RSA), *Boyd & Ross 8244* (RSA), *Boyd & Ross 8249** (ARIZ, RSA), *Boyd & Banks 8279* (RSA), *Boyd 10414* (RSA, UC), *Boyd s.n. 28 March 1982* (RSA), *Boyd s.n. 27 April 1982* (RSA), *Bramlet 2301** (ARIZ), *Bramlet 2370* (CAS), *Bramlet 2394* (RSA), *Bramlet 2399* (RSA), *Bramlet & Coleman 2418* (RSA), *Bramlet 2982* (RSA), *Bramlet 2988* (RSA), *Bramlet 3352B* (RSA), *Brandegee T.S. 824** (CAS, POM, UC), *Brandegee s.n. 12 April 1894* (DS), *Brandegee s.n. 15 April 1894 ** (RSA, UC), *Brandegee T.S. s.n. 8 April 1895** (UC), *Gander 1128** (DS, POM, UC), *Gander 3112** (JEPS), *Gander 5072** (JEPS, RSA, UC), *Grant 5218* (DS), *Grant & Wheeler 540* (UC), *Gross, Fraga, Virgen, Thibault 1781* (RSA), *Gross, Fraga, Virgen, Thibault 1845* (RSA), *Hamilton s.n. 17 May 2001* (RSA), *Hirshberg 290* (RSA), *Jones, C. 10* (RSA), *Jones, M.E. 3066* (ARIZ, CAS, DS, POM, UC), *Jones, M.E. s.n. 5 April 1882* (RSA), *Junak, Hoefs, & Crockett SCa-351* (SBBG), *Junak, Hoefs, & Crockett SCa-355* (SBBG), *Junak SCa-361* (SBBG), *Junak, Hoefs, & Crockett SCa-379* (SBBG), *Junak, Hoefs, Takara SCa-399* (SBBG), *Junak, Hoefs, & Stratton SCa-497* (SBBG), *Junak, Hoefs, Takara SCa-514* (SBBG), *Junak & Kirkland SCa-573* (SBBG), *Junak & Kirkland SCa-577* (SBBG), *Junak, Hoefs, Kirkland, & Stratton SCa-631* (SBBG), *Junak, Hoefs, & Kirkland SCa-1439* (SBBG), *Junak SCa-1465* (SBBG), *Junak & Philbrick SCa-1529* (SBBG), *Leatherman 65* (RSA), *Marsh & Marsh s.n. 10 June 1991* (RSA), *Moran & Barber s.n. 8 June 2001* (RSA), *Munz & Johnston 5335a** (CAS, POM, UC), ***Palmer 70* (MO; isotype)** *Parikh 156* (SBBG), *Parikh & Gale 1739* (SBBG), *Parish 12060* (CAS), *Parry s.n. 17 March 1882* (DS), *Peirson 3029* (RSA), *Philbrick & Thorne B67-175* (SBBG), *Pringle 269* (CAS), *Purer 6927** (UC), *Rebman 8031**è (UC), *Rebman 8348**è (UC), *Rebman, Gregory, Mulligan, & Ricks 11673* (RSA), *Rebman, Gregory, Rich, & Principe 12817** (RSA, UC), *Riefner 20-391* (RSA), *Riefner 20-393* (RSA), *Riefner 95-62* (RSA), *Roberts 3870* (RSA), *Roberts & Bontrager 4565* (RSA), *Roberts, Roberts, & Bontrager 4587* (RSA), *Roberts 4855* (RSA), *Roberts & Bomkamp 4981* (RSA), *Roberts & Bramlet 5563* (RSA), *Roberts & Bramlet 5691* (RSA), *Ross 6853** (UC), *Ross 6869* (CAS), *Ross & Takara 6939* (CAS), *Ross, Takara, & Otte 6947* (CAS), *Sanders 26178* (SBBG), *Sanders 32379* (RSA, SBBG), *Sanders, Salvato, Volansky, & Balk 32568* (RSA), *Sanders, Wotipka, Elvin, et al. 26153* (CAS, SBBG), *Thorne 35873* (SBBG), *Thorne 35949** (UC), *True 152* (POM), *Vanderwerff 4235* (RSA), *White 8381* (ASU, RSA), *White & Duchardt 8862* (RSA).

Acknowledgements

We thank the curators at ARIZ, ASU, CAS, JEPS, RSA, SBBG, and UC for allowing use of herbarium specimens for general study, as well as use of a subset of the specimens for the morphological and molecular analyses. We thank the curators of GH and MO for making high quality digital images of type specimens available. We thank Susan Fawcett for preparing illustrations. We also thank the following sources of funding for this work (listed alphabetically): American Society of Plant Taxonomists, California Native Plant Society, JiJi Foundation, the Lawrence R. Heckard Endowment Fund of the Jepson Herbarium, National Science Foundation (DEB-1210833), Northern California Botanists, and the University of California Institute for Mexico and the United States (UC MEXUS). Finally, we thank the reviewers of the manuscript, whose suggestions improved the paper.

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PhytoKeys 70: 31–40 (2016)
doi: 10.3897/phytokeys.70.9093
http://phytokeys.pensoft.net

RESEARCH ARTICLE

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Oenanthe millefolia (Umbelliferae), a new species record for the Turkish and Greek Flora

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Academic editor: *Pavel Stoev* | Received 5 May 2016 | Accepted 30 August 2016 | Published 20 September 2016

Citation: Doğan Güner E, Tekşen M, Bani B (2016) *Oenanthe millefolia* (Umbelliferae), a new species record for the Turkish and Greek Flora. PhytoKeys 70: 31–40. doi: 10.3897/phytokeys.70.9093

Abstract

Oenanthe millefolia (Apiaceae), which is presented as a new recorded species for the Turkish flora, was discovered for the first time in Kırklareli province of Turkey. It is also reported as a new species for the Greek flora based on an unidentified specimen which was collected from the Thrace region of Greece. In this study, an expanded morphological description, the geographical distribution, the habitat properties and the ecological features of the species are exhibited with illustrative figures. Moreover, the micromorphological and anatomical characters of the fruits and the anatomical properties of the stem, petiole, leaves and the palynological features of *Oe. millefolia* are determined and described for the first time.

Keywords

Anatomy, Balkan flora, Bulgaria, Greece, morphology, pollen, *Oenanthe*, taxonomy, Turkey

Introduction

The genus *Oenanthe* L. is represented by nearly 35-40 species in the world and 8 species in Turkey (Hedge and Lamond 1972, Duman 2000). According to Menemen (2012), *Oe. incrassans* Bory & Chaub., which is considered as a synonym of *Oe. pimpinelloides*

L. (Hedge and Lamond 1972), was uncertainly recorded from Turkey. However, the first author of this paper (Doğan Güner 2016) recently confirmed its occurrence in Turkey.

Some unusual specimens were collected from the Kırklareli province of Turkey during a project dealing with the revision of Turkish representatives of the genus *Oenanthe*. These specimens were clearly different from all the taxa of the genus distributed in Turkey (Hedge and Lamond 1972, Duman 2000). After consulting literature dealing with the flora of the adjacent regions and checking the herbarium vouchers, these materials were preliminarily identified as *Oe. millefolia* Janka (Cook 1981). However, these specimens were also similar to *Oe. bulgarica* Velen., which was described by Velenovsky (1898). Detailed investigations of literature and the herbarium vouchers show that *Oe. millefolia* and *Oe. bulgarica* are morphologically very similar taxa (Velenovsky 1898, Hedge and Lamond 1972, Cook 1981, Duman 2000). Moreover, *Oe. bulgarica* has already been synonymised under *Oe. millefolia* by Gandoger (1910) in his "Novus conspectus florae Europae". With this species, the total number of the *Oenanthe* species in Turkey is now 10.

The aim of this study is to describe morphological, anatomical, palynological and fruit micromorphological properties of the species.

Methods

The specimens, collected during the field studies in Igneada-Kirklareli region in 2014 and 2015, were checked with the related literature (Velenovsky 1898, Gandoger 1910, Hedge and Lamond 1972, Cook 1981, Duman 2000).

The specimens of *Oe. millefolia* were compared with the type specimen and the other representative vouchers kept in GOET, E, SOM, W, and WU herbaria (abbreviations following Thiers 2016).

All the samples which were used for the anatomical studies were fixed in 70% ethanol during the field works. They were stained using sartur reagent according to the method described by Çelebioğlu and Baytop (1949). The stained samples were examined under an Olympus E330 microscope and the anatomical properties were clarified. Moreover, related literature was used for the explanation of the anatomical characters (Mauseth 1988, Dickison 2000).

For the palynological studies, pollen slides were prepared according to the Wodehouse method (1935) and were examined under a light microscope (LM). Different 30 pollen grains were measured by using a Leica DM3000 microscope. The pollen samples and mericarps were directly placed on aluminium stubs and coated with gold with Polaron SC 502 Sputter Coater device and observed with the Jeol JSM 6490LV model scanning electron microscope (SEM). For the palynological and micromorphological terminology, Moore et al. (1991), Punt et al. (2007), Hesse et al. (2009) and Doğan Güner et al. (2011) were used.

Results

***Oenanthe millefolia* Janka Oesterr. Bot. Z. 22: 177-178, 1872**
Figures 1–5

= *Oe. bulgarica* Velen. Fl. Bulg. Suppl. 1: 127, 1898.

Specimens examined. BULGARIA. in pratis inter Kalofer et Karlova ad ped. austral. m. Balkan Thraciae, ubi specimina nondum bene efflorata legi d. 2. Junii 1871 (holotype GOET!); In graminosis pagum Susam. July 1910, V. Stribrny s.n. (W!) *(Oe. bulgarica);* Kreis Sliven, Stara Planina (Balkan-Gebirge), Kotlenska Planina (Kotel-10 Str.-km S Kotel Richtung Gradec, Balkan), Rastplatz mit Brunnen, *Quercus*-Mischwald ca. 450 m s.m., 09 August 1978 Kalk., F. Ehrendorfer, F. Sorger, D. Fürnkranz, M.A. Fischer, A. Öztürk s.n. (WU!); Stara Zagora district, around *Quercus robur* forest, in Kilimite area, 26.06.1958, Ivan Ganchev (photo SOM!); South Black Sea coastline, oak forest between Ahtopol and Sinemorets, 09.05.2004, A. Petrova & B. Assyov (photo SOM!); Strandzha mountain, oak forest, by the roadside, Tsarevo-Malko Tarnovo, at fountain, west of Izgrev village, 09.05.2004, A. Petrova & B. Assyov (photo SOM!); Thracian Lowland, pasture, northeast of Malevo village, Haskovo district, 11.06.2004, A. Petrova (photo SOM!); Eastern Rhodopi, above Potocharka village, in forest of *Carpinus orientalis*, *Fraxinus ornus* and *Paliurus spina-christi*, 08.06.2006, D. Dimitrov (photo SOM!); Stara Zagora district, near Sarnevo village, in oak forest, 08.06.1960, Iv. Ganchev, St. Denchev (photo SOM!); St. Iliya hills, 22.07.1964, Iv. Ganchev, St. Denchev (photo SOM!).

GREECE. 3 km from Petrota along road to Pentalofos, open woodland of *Quercus frainetto*, 180 m, 41º 40′ N 26º 09′ E, 12 June 1991, Strid and Kit Tan 31802 (E!).

TURKEY. Kırklareli: Demirköy-Iğneada, to 5–10 km Iğneada, under *Pinus sylvestris* forest, 273 m, 18 July 2014, E. Doğan Güner 2044 & B. Bani (GAZI!); ibid., 03 August 2014, E. Doğan Güner 2075 & B. Bani (GAZI!); Kırklareli: Sarpdere–Armutveren, under *Pinus sylvestris* forest, 33 m, 19 June 2014, E. Doğan Güner 2046 & B. Bani (GAZI!); Tekirdağ: Saray, Kıyıköy district, under *Quercus* forest, 247 m, 16 June 2015, E. Doğan Güner 2101 & B. Bani (GAZI!).

Description. Perennial, 40–70 cm tall, herb, with thickened, fusiform or oblong tubers, tubers generally at stem base, rarely far away. Stem erect, simple or 3 times branched above, hollow, furrowed, minutely scabrid below, glabrous above. Basal leaves lanceolate or oblong in outline, 2-3 pinnate, 17–45 × 5–9 cm, leaves lamina longer than petiole; segments of lamina opposite at rachis, deeply pinnatisect, ultimate segments linear or elliptic up to 6 × 1 mm, excurrent into a setaceaus tip. Upper leaves similar to basal one, but only a few which reduce upwards. Umbel with 12–18 slender rays of sub-equal length, up to 2 cm, becoming slightly thickened in fruit. Umbels 5 cm diam. at flowers and 3 cm diam. at fruit. Bracts 8-9, lanceolate, 8–10 × 1.5–2 mm. Umbellules conical with unequal thickened pedicels in fruit, 20-30 flowered, about

1–1.5 mm diam., pedicels of sterile flowers longer than fertile ones. Bracteoles 9–13, elliptic-linear, 2–3.5 × 0.5–1.5 mm. Petals radiating, white, cordate, to 2.5 mm long. Sepals ovate, 0.3–0.4 mm, acuminate at apex. Filaments at least two times longer than petals. Stylopodium is conical and not exceeding calyx teeth. Styles about as long as the body of the fruit (ca. 3 mm), erect. Fruit ovate to cylindrical, 3 × 2 mm, striate, laterally and base of fruit slightly spongiose margin.

Distribution, habitat and ecology. *Oenanthe millefolia* is distributed in Bulgaria, North-eastern Greece and European Turkey (Fig. 1). No threat factor was observed against the habitat of the species. The populations are represented by many healthy individuals. The flowering time is between June and July, the fruiting time is August. It grows on clearings of *Pinus* and *Quercus* forest between the altitudes of 240–450 m and shares the same habitat with the species of *Oenanthe pimpinelloides* L., *Helianthemum racemosum* (L.) Pau, *Trachystemon orientalis* (L.) G. Don., *Crupina vulgaris* Cass, *Pinus nigra* Arn. subsp. *pallasiana* (Lamb.) Holmboe, *Pulicaria dysenterica* (L.) Bernh., and *Anthemis altissima* L.

Mericarp macromorphology and micromorphology. Mericarps have three dorsal and two lateral primary ribs. The lateral ridges are more prominent and broader than the dorsal ones. The lateral ridges extend towards the base and cover the base of mericarp. Sepals are generally distinctive and persistent in *Oenanthe* species. Stylopodium is conical and not exceeding calyx teeth. Stylopodium ending with style is almost as long as the fruit. The surface ornamentation of the pericarp is longitudinally striate. The pattern is formed by rectangular cells. Stomatal cavities are observed on the pericarp surface and the density increases towards the calyx (Fig. 3). The style surface is ribbed.

Anatomy. *Stem anatomy*: Stems are circular and slightly 7–8 ribbed in cross sections. There is a thin layer of cuticle on the top surface and a single-line epidermis composed of rectangular cells underneath. Collenchyma cells are grouped at the edges. Cortex parenchyma cells are located around disordered collenchyma cells. Collenchyma

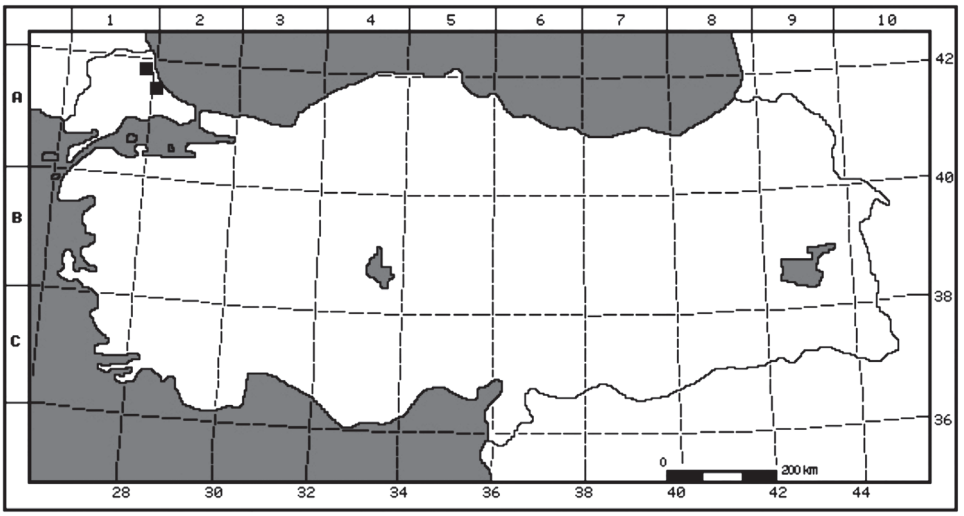

Figure 1. Distribution map of *Oenanthe millefolia* (■) in Turkey.

Figure 2. *Oenanthe millefolia* (E.Doğan Güner 2044) **A** habitat **B** inflorescens **C** root system **D** root system and basal leaves.

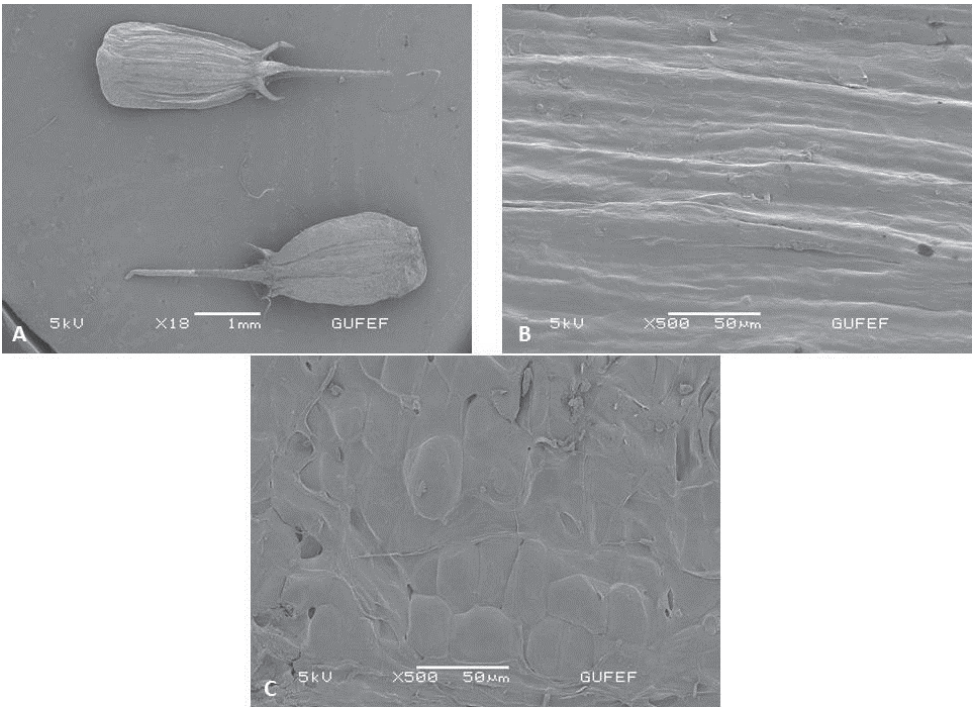

Figure 3. SEM micrographs of mericarp in *Oenanthe millefolia* (E. Doğan Güner 2101) **A** general view **B** dorsal view **C** ventral view.

and parenchyma cells are identified as two layers between edges. Collenchyma has 4–5 rowed, small, and circular cells. Parenchyma has 1–2 rowed, large and circular cells. Secondary slight edges are located between the edges with collenchyma cells. Secretion canals exist in cortex under the collenchyma layer. 5–6 cells in one line surround the canals. Endoderm cell walls are slightly thick one-line oval cells. Vascular bundles are embedded between the cortex and the pith. There are 6–7 rows of sclerenchymatic cell layers between the vascular bundles. Peripheral vascular bundles which are collateral, are large against the edges and small in between the edges. Central vascular bundles are connected with peripherals by sclerenchymatic tissue. Secretion canals are also found under vascular bundles. Slight thickening is seen in pith parenchyma cells. Pith cells have various sizes with large inter-cellular spaces (Fig. 4A–B).

Petiole anatomy: Petiole is almost straight in cross section, ovoid in outline and slightly-canaliculate on the lower surface. Scabrid hairs rarely occur between cubic epidermis cells. Cuticle, epidermis and collenchyma structures are designed in almost the same manner as the stem. Secretion canals between collenchyma and concentric vascular bundles are significant. Xylem elements are dominantly distributed. The pith is composed of large circular cells with a hollow centre (Fig. 4C–D).

Leaf anatomy: The epidermal layer consists of rectangular or circular cells in both adaxial and abaxial directions. Stoma exist on both surfaces. There are large respiratory spaces under the stomata. Mesophyll is composed of two–row palisade and one or two–row sponge parenchyma cells. Large ventilation spaces exist between sponge parenchyma cells. Xylem elements are located through the abaxial side and phloem elements are located through the adaxial side (Fig. 4E–F).

Fruit anatomy: The fruit is schizocarp with two mericarps. The pericarp forms a thin layer around the endocarp and seed. There are single-line and horizontally located epidermal cells on the surface. The mesocarp is formed by 2–3–row small cells. Both epidermis and mesocarp cells have significant thickness. Five ridges are seen on each mericarp. Vascular bundles are located on these ridges. They are reduced. Secretion canals are located on vascular bundles. Pericarp is surrounded with sclerenchymatic tissue which makes a continuous ring up to the carpophore. The sclerenchyma layer is composed of irregular cells with thick walls. There are 4 dorsally and 2 ventrally vittae. Oval-shaped vittae are located in the vallecular region. Endoderm is located as one line under the vittae and seems to be integrated with the testa. The seed is composed of endosperm and testa with a thickened cell wall. Endosperm contains large quantities of lipid and protein. There are many druse crystals in the endosperm. When the section is taken from the middle of the mericarp, the embryo cannot be observed because it is small and close to the tip (Fig. 4G–H).

Pollen morphology. The pollen grains are isopolar symmetric, the aperture is tricolporate type. The pollen shape is prolate with an elliptic equatorial outline, polar axis 29.5–33.5 μm, equatorial axis 15–18 μm. The ornamentation is rugulate. The colpus length is 18–27 μm and width is 0.5–2 μm. The pore length is 4–6 μm and width is 4–6 μm. The exine subtectate is 0.75–1 μm (on equator and polar), the intin is 0.75–1.25 μm (on equator and polar) (Fig. 5).

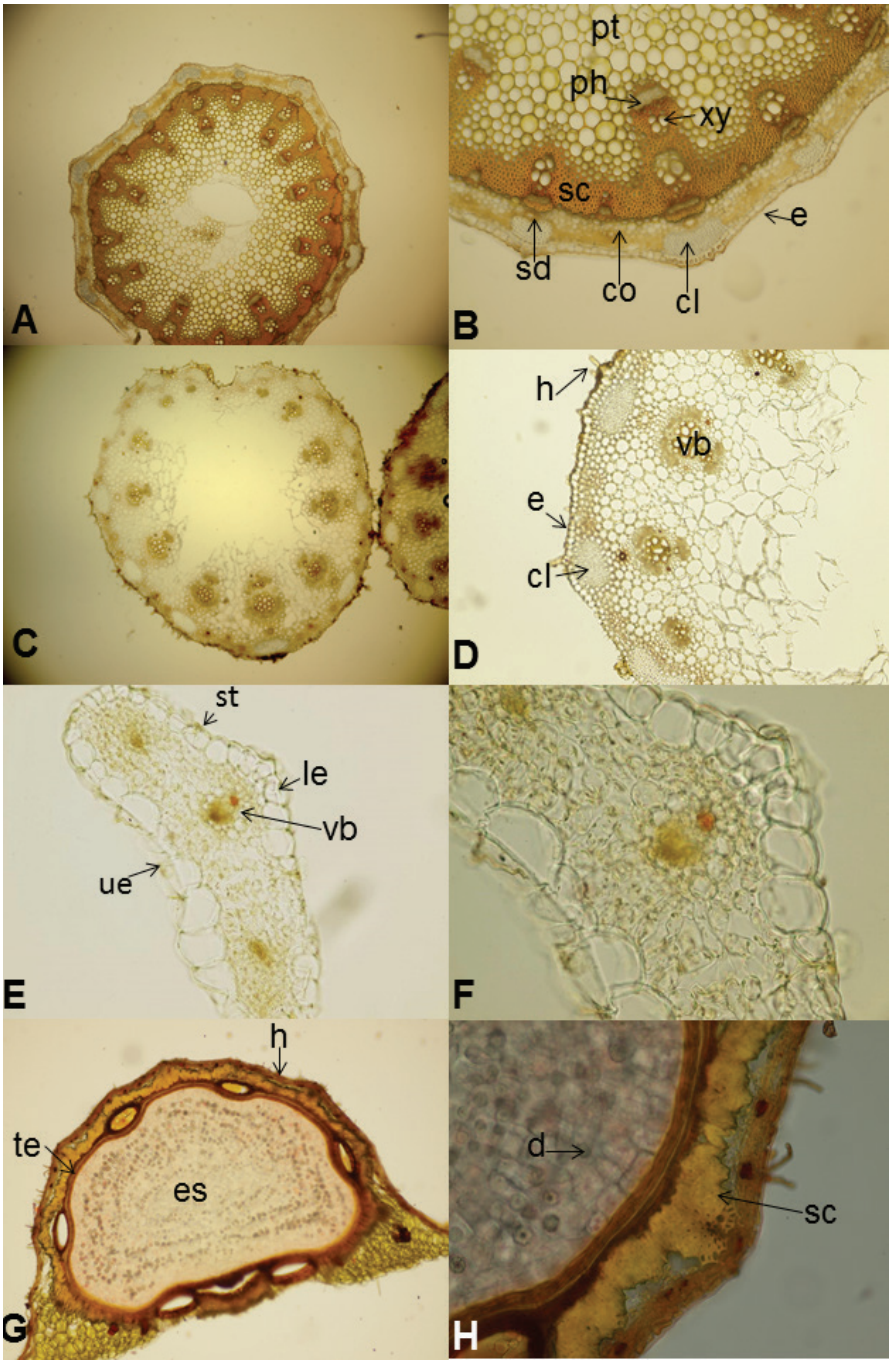

Figure 4. Anatomical structure of *Oenanthe millefolia* (E. Doğan Güner 2044) **A–B** Cross sections of stem (10 × 5), (10 × 20) **C–D** Cross sections of petiole (10 × 5), (10 × 20) **E–F** Cross sections of leaves (10 × 10), (10 × 40) **G–H** Cross sections of mericarp (10 × 10), (10 × 20). (Legend: cl: collenchyma, co: cortex, e: epidermis, es: endosperm, h: hair, le: lower epidermis, ph: phloem, pt: pith, sc: sclerenchyma, sd: secretory duct, st: stoma, t: testa, ue: upper epidermis, xy: xylem, v: vittae)

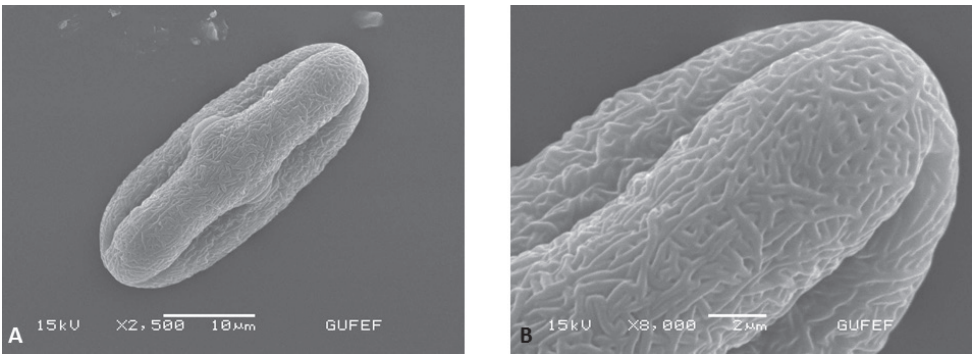

Figure 5. The pollen of *Oenanthe millefolia* (E. Doğan Güner 2044) **A** polar view **B** pollen grain with rugulate.

Discussions

After detailed investigations on the descriptions of *Oe. millefolia* and *Oe. bulgarica*, it was found that these two taxa are conspecific. Gandoger (1910) also previously recognised *Oe. bulgarica* as the synonym of *Oe. millefolia*. However, Cook (1981) did not mention the synonymy of *Oe. bulgarica* under *Oe. millefolia* in Flora Europaea.

The leaf lamina of *Oe. millefolia* is deeply pinnatisect, comprising primer segments with shorter and denser ultimate segments. The primer segment is also remotely and evenly distributed to the apex (Fig. 2D). This leaf morphology clearly resembles the leaf of *Oe. tricholoba* Greuter (Greuter 2012). *Oe. tricholoba* has however ± globular root tubers which are distant from the base of stem (not fusiform or oblong tubers and not very close to the base of the stem).

In this study, the morphological description of the species has been expanded with investigated specimens (especially our collections, the specimens of W and WU herbaria and the photographs of the specimens from GOET, SOM, E herbaria). Some of the morphological characters show high variations. In our collected specimens, root tubers are generally close to the base of the stem but some of them are remote (not only at base of stem), basal leaves 2–3 pinnate (not 2–pinnate) and umbels with 12–18 rayed (not 5–15 or 10–16).

During the studies on the specimens of different herbaria, we realised that one unidentified specimen deposited at E herbarium is identical to *Oe. millefolia*. This specimen was collected from Greece. With this additional record, the distribution of *Oe. millefolia*, so far known as a Bulgarian endemic, has been extended southwards to Turkey and Greece. Therefore, the species should be considered as a Balkan endemic.

The species of *Oenanthe* in Turkey, mostly prefer wetlands and humid areas. It has however been observed that *Oe. pimpinelloides* is distributed in dry areas such as under trees or open woodlands in addition to the aquatic habitats. Moreover, *Oe. millefolia* is observed only in dry areas with *Oe. pimpinelloides*.

This study comprises the discovery of *Oe. millefolia* in the Thracian regions of Turkey and Greece. To date, there has been no comprehensive study dealing with *Oe. millefolia*. This paper provides micromorphological, anatomical and palynological features of the species along with its expanded morphological description. The findings of this study can contribute to further taxonomical investigations on both the genus *Oenanthe* and family Apiaceae.

Acknowledgements

We would like to thank TUBITAK for its financial support (Project number KBAG-114Z005). We are grateful to Dr. Marc Appelhans (Curator of the Herbarium University of Goettingen Albrecht-von-Haller Institute for Plant Sciences, Department of Systematic Botany) and Stoyan Stoyanov (Institute of Biodiversity and Ecosystem research, Bulgarian Academy of Sciences) for their useful contributions.

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PhytoKeys 70: 41–52 (2016)
doi: 10.3897/phytokeys.70.8609
http://phytokeys.pensoft.net

RESEARCH ARTICLE

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Angiosperm flora on the páramos of northwestern Colombia: diversity and affinities

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Academic editor: *P. Stoev* | Received 23 March 2016 | Accepted 19 August 2016 | Published 30 September 2016

Citation: Alzate-Guarín F, Murillo-Serna JS (2016) Angiosperm flora on the páramos of northwestern Colombia: diversity and affinities. PhytoKeys 70: 41–52. doi: 10.3897/phytokeys.70.8609

Abstract

Páramos are high-elevation isolated ecosystems in the Andes characterized by specific flora. This flora includes a number of endemic species and some taxa phylogenetically related to temperate lineages (van der Hammen and Cleef 1986). There are six páramo units or complexes in the Department of Antioquia, located in northwestern Colombia. For five years, we conducted botanic explorations in order to quantify the richness of angiosperm flora in these units. We estimate the richness of angiosperms in these páramos at 693 species, 277 genera, and 86 families, which represent almost 10% of the floral diversity in Antioquia, but contained in only 0.7% of its area. We found that Frontino-Urrao is the most species-rich páramo with 465 species from 225 genera. Our results show that the most diverse angiosperm families of the páramos of Antioquia are Asteraceae, Orchidaceae, Melastomataceae, and Poaceae, which together represent 245 species. Groupings between páramos by Sørensen's similarity index show that the complexes of the Central Andes Cordillera form a cluster of greater affinity than Páramos from other regions. Of the species found, 80 have a CITES or IUCN diagnosis. The expeditions allowed the identification of 21 species not previously registered in Antioquia and a considerable number of endemisms (35 species), further proof of the high plant diversity in these ecosystems.

Keywords

Angiosperms, Andes, Antioquia, páramos, Colombia

Introduction

The páramo ecosystem has been defined in several ways with differing delimitation methods. One of such definitions (Cuatrecasas 1958) considers the páramo as open high-elevation areas characterized by particular vegetation, with the high-Andean forest as the lower limit and the permanent snowcap as the upper limit. Based on altitude and vegetation structure, Cuatrecasas (1968) proposed a subdivision of the páramo into subpáramo, páramo, and superpáramo. Sklenár et al. (2005) define the páramo as intertropical high-mountain ecosystems, located between the continuous band of forest and the upper limit of the permanent snowcap where vegetation can still be found.

Average incident temperatures in páramos range between 3°C and 9°C, with highly marked diurnal fluctuation of up to 20°C. Rainfall ranges between 700 mm and 5000 mm per year, with relative humidity between 80% and 98%. Generally, the lower limit of páramos is defined at about 3000 m.a.s.l. of altitude (Morales-Rivas et al. 2007). Biophysical criteria, such as climate, altitude, soil, biodiversity, and endemisms help establish the limits between forest and páramo (Van der Hammen and Otero 2007).

Colombian páramos are organized under districts and complexes (Sarmiento et al. 2013) as a way to group natural areas that are in many cases divided by geographic accidents or anthropogenic perturbation. In Colombia there are 39 páramo complexes, 20 located on the Eastern Cordillera, 11 on the Central Cordillera, 7 on the Western Cordillera, and 1 on the Sierra Nevada de Santa Marta (Sarmiento et al. 2013). In Colombia, much of the páramo is located in special conservation zones; however, páramo regions are still threatened by the effects of global warming, the expansion of agricultural areas, and, in the particular case of Colombia, the expansion of mining activities (Benavides 2013).

The Department of Antioquia, located in northwestern Colombia, covers an area of 63,612 km^2, comparable to the areas of countries such as Costa Rica (51,100 km^2) or Sri Lanka (65,610 km^2). Antioquia has six páramo complexes, three of them situated in the Western Andes Cordillera (Farallones de Citará, Frontino-Urrao, and Paramillo) and three in the Central Andes Cordillera (Belmira, Valle de Aburrá, and Sonsón). Páramo regions in Antioquia, including new subdivision proposals made by the Alexander von Humboldt Institute for Biological Research (Sarmiento et al. 2013), currently cover an area of about 46,000 ha, representing 0.7% of Antioquia's surface area.

Some efforts have been undertaken to quantify the diversity of flora in Colombian páramos; a recent publication by Marín and Parra (2015) stands out among such efforts. Their work lists a total of 658 plant species for Colombian páramos, a figure that is evidently quite far from the actual diversity found for the páramos in the country. Some detailed inventories of páramo flora have been published for areas such as Chingaza (Madriñán 2012), Chisacá (Pedraza-Peñalosa et al. 2001, 2004), and Sonsón (Alzate et al. 2016), among others. Such inventories have allowed the development of more detailed analyses with regards to the evolution and classification of these areas. Miranda et al. (2002) defined the areas of endemism for Colombian páramos by means of Parsimony Analysis of Endemicity (PAE). Madriñan et al. (2013) inferred

speciation rates of some páramo lineages and found that this ecosystem presents the highest speciation rates known for angiosperms. Londoño et al. (2014) proposed floristic and biogeographic affinities for 30 Colombian páramos by using PAE and Jaccard's similarity index.

In this project we aim to document and evaluate the conservation status of angiosperm species found in the páramos of Antioquia. We also present a floristic affinity analysis based on taxonomic inventories for the six páramos of Antioquia.

Methods

Between 2010 and 2015 we conducted botanical explorations in five of the six páramo complexes in the Department of Antioquia to determine the diversity of Angiosperms in Antioquia's páramo complexes (Table 1). Páramo areas in Antioquia are located in the Central and Western Andes Cordilleras between 2800 and 3969 m of altitude (Arias 2011). We also included a small azonal páramo found at 2600 m that belongs to the Belmira complex (Fig. 1). We collected in different periods and climatic seasons in order to find a higher number of plants in bloom or with fruits. We thoroughly documented Angiosperm specimens collected during the explorations with photographs to illustrate the flora on a web page hosted by the Missouri Botanical Garden. Plant material was processed at the HUA and MO herbariums, where the exsiccata were deposited. We supplemented the species inventory generated from this fieldwork with data from the Flora de Antioquia project, carried out by the Universidad de Antioquia and the Missouri Botanical Garden, available online at http://tropicos.org/Project/CV.

We constructed similarity dendrograms among the six páramo complexes using Sørensen's similarity index (Sørensen 1948), a measure of the number of species shared between two sites compared to the total number of species unique to each site alone. This method was selected because it requires only data on taxa presence/absence rather than abundance indices. We created three presence/absence matrices using taxa (family, genus, and species) as codified characters with 1/0 for each geographic area of the 6 páramo units in Antioquia. We generated a hypothetical zone where all taxa are absent to root the dendrogram. The analysis was carried out with the PAST3.x software package (Hammer et al. 2001) using the UPGMA algorithm and

Table 1. Inventory of Angiosperms for each of the six páramo complexes of Antioquia.

Páramo complex	Extension (ha)*	Families	Genera	Species
Belmira	10.622	62	146	257
Farallones de Citará	11.233	59	112	174
Frontino-Urrao	15.396	79	229	460
Paramillo	1.550	33	68	98
Sonsón	*3.389	61	140	229
Valle de Aburrá	870	59	135	234

*Area within the Department of Antioquia.

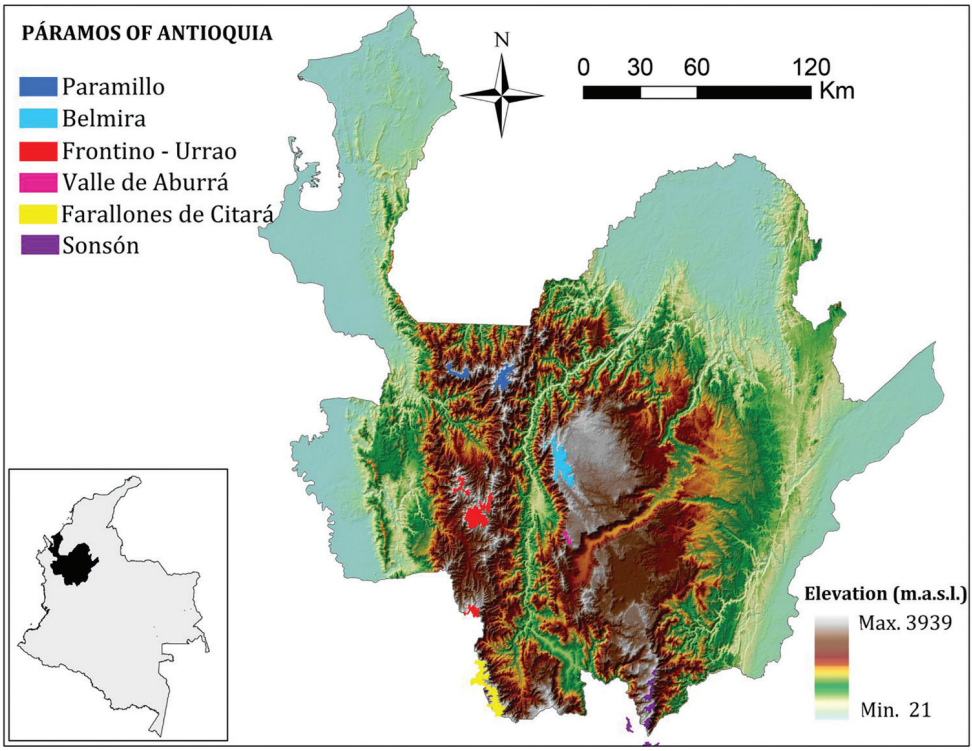

Figure 1. Geographic location of the páramo complexes of Antioquia.

Sørensen's similarity index to evaluate floristic affinities between páramo units. We repeated the analyses excluding the páramos with low sampling in order to observe the possible effects of low-sampled areas. The information generated in this project is being published in parallel on the web portal of the Missouri Botanical Garden at http://tropicos.org/Project/Paramos.

Results

We identified 693 Angiosperm species from 277 genera and 86 families in the six páramo complexes of Antioquia (Table 1). These species represent about 10% of the Angiosperm flora reported for the Department of Antioquia (Idárraga et al. 2011). Our results indicate that Frontino-Urrao is the páramo with the highest richness of angiosperm plant species, genera, and families (460, 229, and 79 respectively). The lowest diversity was found in Paramillo, with only 98 species from 33 families.

Of the 86 families present in the páramos of Antioquia, more than 30 are represented by 6 or more species. Asteraceae and Orchidaceae are the most diverse in species number, each represented by 84 species. (Table 2). With regards to genera, 16 plant families had 5 or more genera in the sampled páramos, with Asteraceae (33 genera)

Table 2. Number of genera and species with their percentages for the 20 most diverse Angiosperm families in the páramos of Antioquia.

Family	Genera	Percentage	Species	Percentage
ASTERACEAE	33	11.91	84	12.12
ORCHIDACEAE	23	8.30	84	12.12
POACEAE	20	7.22	38	5.48
MELASTOMATACEAE	13	4.69	39	5.63
ERICACEAE	11	3.97	30	4.33
RUBIACEAE	8	2.89	20	2.89
CYPERACEAE	8	2.89	18	2.60
ROSACEAE	7	2.53	25	3.61
LAMIACEAE	7	2.53	9	1.30
BROMELIACEAE	6	2.17	29	4.18
SOLANACEAE	6	2.17	13	1.88
APIACEAE	6	2.17	9	1.30
GESNERIACEAE	6	2.17	8	1.15
CARYOPHYLLACEAE	5	1.81	7	1.01
GENTIANACEAE	5	1.81	7	1.01
PLANTAGINACEAE	5	1.81	6	0.87
LORANTHACEAE	5	1.81	5	0.72
CAMPANULACEAE	4	1.44	15	2.16
PRIMULACEAE	4	1.44	9	1.30
BORAGINACEAE	4	1.44	4	0.58

and Orchidaceae (23 genera) being the most diverse families in number of genera. About 20% of the genera and 25% of the species correspond to the Asteraceae and Orchidaceae (Table 2). Of the Angiosperm flora found in the páramos of Antioquia, 35 species are endemic, which represents 5.3% of the total flora registered. Six of the endemic species of the páramos of Antioquia belong to Bromeliaceae, five to Asteraceae, and four to Orchidaceae.

Description of the páramo complexes of Antioquia

Belmira: Located north of the Central Cordillera in the Santa Rosa altiplano. This páramo covers altitudes ranging from 3,000 to 3,340 m.a.s.l. and is one of the largest páramo regions in Antioquia (Sarmiento et al. 2013). We registered 16 species of Bromeliaceae in this páramo, the highest number of species of this family registered in all the páramos studied. In this work, 59 new records of species are described for the Belmira páramo.

Farallones de Citará: Located in the Western Cordillera in the southwestern part of Antioquia. It covers an area of 2,030 ha between 3,350 and 3,940 m.a.s.l., but a proposal has been made to include a 11,233 ha extension (Sarmiento et al. 2013). This

páramo is highly diverse in species of Melastomataceae and Asteraceae. In this study, 118 species are newly reported for Farallones de Citará.

Frontino-Urrao: Located to the southwest of the Department of Antioquia, to the north of the Western Cordillera. It has a large number of wetlands, and its altitudinal range goes from 3,200 to 3,970 m.a.s.l. (Arias 2011). The high diversity of Asteraceae is notable, with 59 species representing 12.8% of the species found in this páramo. Frontino-Urrao is home to vast populations of two *Espeletia* species that are endemic to the region: *E. frontinoensis* Cuatrec. and *E. praefrontina* Cuatrec. Our research revealed 55 new records of Angiosperms for this complex.

Paramillo: Located on the northern extreme of the Western Cordillera inside the Natural National Park Nudo de Paramillo. This páramo ranges from 3,300 to 3,720 m.a.s.l. (Sarmiento et al. 2013). Very few explorations have been carried out in this páramo because of difficulties posed to access it. Because of this, there was limited previous knowledge of Angiosperm diversity in Paramillo. The current inventory shows high diversity of species in Melastomataceae and Orobanchaceae. This is likely the result of low sampling, although these two families are known to be diverse in the páramo, especially Melastomataceae.

Sonsón: Located in the southeast of Antioquia, in the Central Cordillera. Its highest altitude reaches 3,363 m.a.s.l., and despite the fact that its total extension in the Department is 3,389 ha, only a very reduced area has páramo vegetation cover. Sonsón was only acknowledged as a páramo in 2009, because its small extension did not favor its delimitation (Alzate et al. 2016b). Bromeliaceae and Melastomataceae are outstanding for their diversity in this páramo, with 18 and 21 species respectively. In this project, we registered 30 species of Angiosperms not previously reported for the complex.

Valle de Aburrá: Found on the western part of the homonymous valley, it is composed of two steep hills that reach 2,900 to 3,175 m.a.s.l. Human settlements have greatly transformed this páramo and led to ecosystem deterioration and decrease of natural vegetation. Rosaceae (12 spp), Solanaceae (9 spp), and Piperaceae (7 spp) are particularly diverse in this complex. Explorations in this zone registered 33 species not previously reported.

Paramos in the Central and Western Cordilleras form separate clusters based on Sørensen's similarity index when this index is calculated at the species, genus, and family levels (Fig. 2). Of the Western Cordillera, the páramo that is more similar to the ones found in the Central Cordillera is Frontino-Urrao, while the least similar is the Paramillo páramo.

Discussion

This work documents the occurrence of high plant diversity in the páramos of Antioquia, which represents about 10% of the Angiosperm flora known for this Department. In Colombia there are about 238 (Rangel-Churio 2015) of the 425 Angiosperms families recognized worldwide by Reveal and Chase (2011). The páramos of Antioquia have

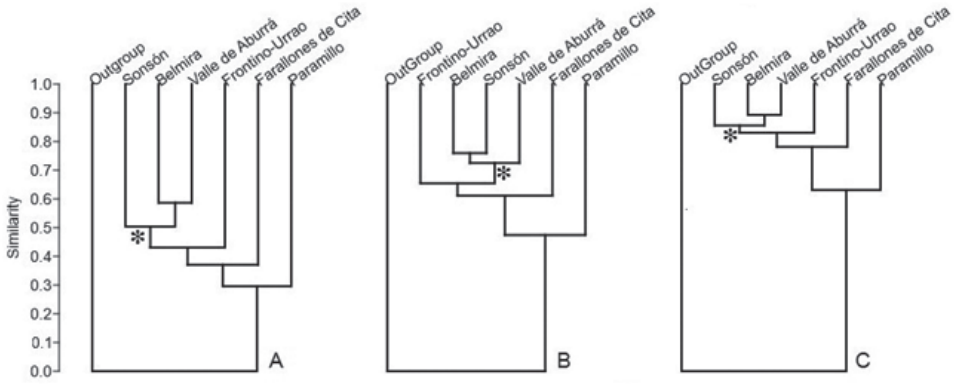

Figure 2. Similarity dendrograms for the six páramo complexes in Antioquia using Angiosperm composition **A** Built using species composition **B** genera composition, and **C** family composition. Asterisk* denotes the node for páramos of Central Cordillera.

representatives of 20% of the total families in the world and around 37% of the ones reported for Colombia. Out of the 3431 vascular species reported for páramos by Luteyn and Churchill (1999), Antioquia has around 20%, but only taking into account Angiosperms; if Monilophythos and Lycophytos diversity is included, this percentage could increase to a much higher value.

Most of the plant diversity in the páramos of the Department is found in the Western Cordillera, especially in the Frontino-Urrao páramo. This high diversity is comparable to the diversity of páramos such as Sumapaz or the Nevados (Londoño et al. 2014). However, Frontino-Urrao is much smaller than these páramos; while the páramo of Frontino-Urrao is only 13,921 ha, Sumapaz and Nevados amount to 180,000 ha and 45,000 ha, respectively. The high diversity registered for Frontino-Urrao may be due to the lack of significant habitat fragmentation by human development or agriculture. Additionally, numerous botanical explorations to the region have allowed for adequate knowledge of its diversity (Idárraga et al. 2011). According to Arias (2011), plant diversity of the Frontino-Urrao páramo is related to its wetlands and is propitiated in its diversity by the landscape heterogeneity represented therein.

The high diversity found in the Sonsón páramo is of great relevance, with 231 species being found in a very small area. This complex has great importance for the connection of the páramo biota of the Central Cordillera because it forms an intermediate point that could permit genetic exchange among the populations located in the Nevado del Ruiz peak and the Belmira páramo. It is worth mentioning that our explorations led to the discovery of a new species of *Espeletia* in the Sonsón páramo, which is in the process of being described and published. This finding will add to the high number of endemic *Espeletia* species in the páramos (Diazgranados 2012).

The similarity among páramo complexes, assessed through species and family composition by using methods of metrical distances, shows Belmira and Valle de Aburrá as the most similar (Fig. 2). These two páramos are located in the Central Cordillera,

quite close to each other, and their current separation is due to anthropogenic influences rather than biogeographical vicariance.

The study of Londoño et al. (2014) presents the Farallones de Citará páramo as quite unlike the remaining páramos of Antioquia. In this analysis, we present a wider sampling of the diversity in Farallones de Citará (174 spp) that allowed us to compare its floral affinities more precisely. The work of Londoño et al. (2014) only considered 62 species for this páramo, probably causing separation of this complex from the remaining Antioquia páramos. We believe that this result is an effect of the low sampling used in the analysis. The páramos with low sampling effort in our study, Paramillo and Farallones de Citará, proved to be more dissimilar to the remaining ones when compared by families, genera, and species. Thus, it is possible that improving the knowledge of its flora might change biological affinities. Affinities remained the same when these analyses were repeated excluding these two areas.

The low diversity reported for the Farallones de Citará and Paramillo complex (Table 1) is likely a consequence of the scarce exploration that has been carried out, rather than being an accurate indicator of species composition (Idárraga et al. 2011). Thus, further studies should be conducted to expand the knowledge of biological diversity and conservation in these páramos. Although the páramo of Belmira is smaller than Farallones de Citará, it has greater diversity, possibly because more botanical expeditions have occurred in Belmira than Farallones de Citará.

The most diverse Angiosperm families in the páramos of Antioquia are the same as reported for other páramos of Colombia's Eastern Cordillera, such as Chingaza (Vargas and Pedraza 2004, Madriñan 2012), Sumapaz (Franco and Betancur 1999), and the Podocarpus National Park in southern Ecuador (Lozano et al. 2009, Keating 1999). Asteraceae and Poaceae are the most diverse families in the Sierra Nevada de Mérida (Ricardi et al. 1997) and in the páramo of Ramal de Guaramacal in Venezuela, according to Cuello et al. (2010). In Colombia, these two families are the most diverse in the páramos of the Serranía del Perijá (Rivera-Díaz 2007) and in Chisacá (Pedraza et al. 2001).

The Asteraceae family has very high diversity in the explored páramos, especially in Frontino-Urrao, where it is represented by 59 species, almost 13% of the species of this páramo. With these values, Frontino-Urrao is a biodiversity hotspot for the Asteraceae family and constitutes an area of great interest for the study and conservation of this group, only comparable to ecosystems such as Chisacá, where 55 species for this family have been reported (Pedraza et al. 2004). An assessment of the floral composition for each of the explored páramos reveals that in Belmira, Farallones de Citará, and Sonsón, Melastomataceae is the family with the second highest diversity of species. Melastomataceae has been reported by Lozano et al. (2009) and Keating (1999) as the most diverse taxon of the Podocarpus Natural Park in the south of Ecuador.

Epidendrum L., *Miconia* Ruiz & Pav., and *Peperomia* Ruiz & Pav. with 21, 21, and 15 species, respectively, were the most diverse genera in the páramos of Antioquia. Both *Epidendrum* and *Miconia* provide a relevant contribution to páramo diversity in páramos such as Sumapaz (Franco and Betancur 1999), but the high diversity found in the páramos of Antioquia for *Peperomia* has not been previously reported for other páramo

regions. Some genera found in the study such as *Aegiphila* Jacq., *Allophylus* L., *Minthostachys* (Benth.) Spach, *Polygala* L., *Ruagea* H. Karst., and *Styrax* L. have not been registered before as páramo flora components (Sklenar et al. 2005). Similarly, through this exploration we added 21 new records of species to the inventory of Antioquia flora.

These explorations allowed us to confirm the presence of species endemic to the páramos of Antioquia, such as *Diplostephium antioquense* Cuatrec., *Pentacalia sonsonensis* (Cuatrec.) Cuatrec., and *P. tomasiana* (Cuatrec.) Cuatrec. In our sampling, some rare species are outstanding too; we collected *Polygala corifolia* Planch. & Triana, a species only known from the type collection carried out in 1837 in the Sabana de Bogotá (Alzate 2016a), in the Belmira complex. Kattan et al. (2004) explained the occurrence of large numbers of endemic groups in páramos through geological history and the isolation of these ecosystems. Endemicity estimated for páramo plants is between 18% (Hofstede 2003) and 60% (Luteyn and Churchill 1999), considered as a whole ecosystem. There is not enough data to estimate endemicity rates of the páramos of Colombia because of missing information and taxonomical inconsistences for many taxa.

Our study confirms páramos as important habitats for threatened species. We found that 80 out of 693 species registered have some degree of vulnerability diagnosis from CITES or IUCN. These species are all members of Orchidaceae and Bromeliaceae, since these are the only families that have been subjects of conservation assesments (Garcia and Galeano 2006; Betancur and García 2006). Of the páramos in this study, Valle de Aburrá has the highest degree of human transformation, mainly due to urban expansion and the almost total destruction of original vegetation cover. Due to cattle ranching and mining very close to the forest-páramo boundary, Belmira has also been significantly altered by human activity. Due to its small and fragmented area, Sonsón is the most endagered páramo. This complex is composed of small areas on mountain peaks with agriculture threatening the remaining habitat. Meanwhile Urrao and Farallones de Citará are the best-preserved páramos of Antioquia, partly due to extreme topography and difficult access.

The inventory presented here is a detailed and wide addition to knowledge of Angiosperm diversity for a considerable extent of the páramos of Colombia and provides support for the high diversity of these ecosystems.

Acknowledgements

The project for the exploration of the páramos of Antioquia has been developed under the support and funding from the Vicerrectoría de Investigación and the Herbario of the Universidad de Antioquia (HUA), and the Missouri Botanical Garden. The authors wish to thank many people that worked for the project, for their support doing fieldwork and identifying vegetal material and in particular to Astrid Álvarez, Santiago Varela, and Oswaldo Díaz. The map was elaborated by Juan Parra of the Universidad de Antioquia. We thank to Dr. Oscar Vargas (University of Texas) and Carrie Tribble (UC Berkeley) for useful comments on the manuscript.

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PhytoKeys 70: 53–96 (2016)
doi: 10.3897/phytokeys.70.9147
http://phytokeys.pensoft.net

RESEARCH ARTICLE

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Diversity and biogeographical patterns of legumes (Leguminosae) indigenous to southern Africa

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Academic editor: *Clifford Morden* | Received 10 May 2016 | Accepted 12 September 2016 | Published 4 October 2016

Citation: Trytsman M, Westfall RH, Breytenbach PJJ, Calitz FJ, Van Wyk AE (2016) Diversity and biogeographical patterns of legumes (Leguminosae) indigenous to southern Africa. PhytoKeys 70: 53–96. doi: 10.3897/phytokeys.70.9147

This paper is dedicated to the memory of Robert Howard (Bobby) Westfall (17 December 1944–21 January 2016), vegetation ecologist and friend whose sudden death during the preparation of this manuscript deprived us of an invaluable collaborator.

Abstract

The principal aim of this study was to establish biogeographical patterns in the legume flora of southern Africa so as to facilitate the selection of species with agricultural potential. Plant collection data from the National Herbarium, South Africa, were analysed to establish the diversity and areas covered by legumes (Leguminosae/Fabaceae) indigenous to South Africa, Lesotho and Swaziland. A total of 27,322 records from 1,619 quarter degree grid cells, representing 1,580 species, 122 genera and 24 tribes were included in the analyses. Agglomerative hierarchical clustering was applied to the presence or absence of legume species in quarter degree grid cells, the resultant natural biogeographical regions (choria) being referred to as leguminochoria. The description of the 16 uniquely formed leguminochoria focuses on defining the associated bioregions and biomes, as well as on the key climate and soil properties. Legume species with a high occurrence in a leguminochorion are listed as key species. The dominant growth form of key species, species richness and range within each leguminochorion is discussed. Floristic links between the leguminochoria are established, by examining and comparing key species common to clusters, using a vegetation classification program. Soil pH and mean annual minimum temperature were found to be the

main drivers for distinguishing among legume assemblages. This is the first time that distribution data for legumes has been used to identify biogeographical areas covered by leguminochoria on the subcontinent. One potential application of the results of this study is to assist in the selection of legumes for pasture breeding and soil conservation programs, especially in arid and semi-arid environments.

Keywords

Agriculture, agronomy, assemblages, biogeography, biomes, bioregions, breeding, diversity, ecology, Fabaceae, flora, floristics, fodder, growth form, legumes, leguminochoria, Leguminosae, pastures, phytochoria, soil conservation, South Africa, southern Africa, species range, species richness, vegetation

Introduction

The legume family (Leguminosae; alternative name Fabaceae) is considered one of the largest, most economically significant plant families (Yahara et al. 2013). It is the third largest angiosperm family with about 19,400 species (Lewis et al. 2005) and its considerable importance in agriculture, its ability to occupy different habitats and diverse life forms are well documented (Yahara et al. 2013). Projects such as the Global Legume Diversity Assessment are a first step in studying the rapid loss of legume species diversity. Asia is proposed to be the first continent to be assessed, resulting in a publication on legume diversity in South East Asia (Raes et al. 2013). As reported by Sprent et al. (2010), the full potential of African indigenous legumes has not yet been realised and South Africa is seen as a valuable source of legumes for possible agricultural use in arid and semi-arid regions. However, Yahara et al. (2013) calculated that currently almost 30% of Leguminosae in South Africa are threatened or are of conservation concern. Greater diversification in the use of legume species for food and forage is also acknowledged as vital in a changing world (Sprent et al. 2011).

Most phytogeographical studies of southern Africa aim to describe plant biogeographical regions (Acocks 1953, Goldblatt 1978, White 1983, Cowling et al. 1998, Linder 2001, Van Wyk and Smith 2001, Bredenkamp et al. 2002, Linder et al. 2005, Steenkamp et al. 2005, Mucina and Rutherford 2006, Linder et al. 2012, Linder 2014). Linder et al. (2012) defined a biogeographical region as a set of grid cells more similar in species composition compared to any other grid cells. It is noteworthy that of all the biogeographical regions in southern Africa, the Cape Floristic Kingdom/Region, confined in its entirety to South Africa, is considered globally the most species-rich temperate flora (Linder 2014) and the only major floristic region matching the range of a single broad vegetation type or biome, in this case the Fynbos biome (Van Wyk and Smith 2001).

For southern Africa, Goldblatt (1978) recognized six floristic units, namely five phytogeographic Regions and one Transition Zone: 1) the Zambezian Region; 2) the Karoo-Namib Region; 3) the Tongaland-Pondoland Region; 4) the Afromontane Region; 5) the Cape Region; and 6) the Kalahari-Highveld Transition Zone. In a recent reassessment of sub-Saharan phytochoria (areas possessing a large number of endemic

taxa), the Cape Floristic Region was clearly delineated from the surrounding Namib-Karoo and Eastern Karoo phytochoria (Linder et al. 2005). Local foci of floristic endemism in southern Africa are described by Van Wyk and Smith (2001) but, for our purpose here, only those regions and centres of endemism corresponding to the classification of grid cells clustering as phytochoria based on the presence/absence of species of Leguminosae, henceforth referred to as leguminochoria, will be compared for their floristic attributes and congruence.

The use of herbarium collection data to generate outcomes such as species richness and biogeographical regions poses several potential limitations (Robertson and Barker 2006, De la Estrella et al. 2012). Sampling efforts may not be consistent, with some quarter degree grid cells (QDGCs) sampled excessively owing to geographical bias (along main roads or in a nature reserve), taxonomic bias (species that are easy to collect or more conspicuous) and temporal bias (collected in one season). QDGCs have historically been used in many African countries for mapping biodiversity data (Larsen et al. 2009). Other weaknesses include: 1) incorrect identification of specimens; 2) outdated taxonomy and 3) incorrect geo-referencing (Soberón and Peterson 2004). The first two comply with the so-called 'Linnean shortfall' as defined by Hortal et al. (2015). The Leguminosae data obtained from the South African National Herbarium (PRE) Computerised Information System (PRECIS) evidently suffered from the deficiencies as stated above. Furthermore, the mean area of 675 km^2 for a QDGC is a fairly large area to categorise in terms of bioregions, biomes, and climatic and soil properties. Some QDGCs lie in ecotonal areas and could therefore not be accurately classified. Hufkens et al. (2009) define an ecotone as a multi-dimensional environmentally stochastic interaction zone between ecological systems with characteristics defined in space and time, and by the strength of the interaction. The history of PRECIS is summarised by Gibbs Russell et al. (1989), and Steenkamp et al. (2005) provide additional information. Despite the shortcomings of herbarium records, they often remain the only available source of major significance with regard to relevant distribution data (Amici et al. 2014).

The principal aim of the present study is to examine the biogeographical patterns displayed by the indigenous Leguminosae in southern Africa and to determine how the resultant broad scale floristic units compare with other such units, i.e. to distinguish ecologically interpretable phytochoria. In the present contribution, hierarchical clustering was applied to distinguish discrete groups that can be named and classified (Kreft and Jetz 2010), the resultant natural regions (choria) being referred to as leguminochoria. In addition to its plant geographical significance, information gathered in this study and the wealth of descriptive and distribution data accumulated by botanists and taxonomists will be of considerable value to plant breeders or rangeland scientists in their search for legume species with pasture and or soil conservation potential, e.g. the need to select increased drought, acidic and salinity tolerant legumes is essential in the light of future predictions of water shortages (Graham and Vance 2003, Niang 2014).

Methods

Distribution data

The Leguminosae records in the South African National Herbarium (PRE) Computerised Information System (PRECIS) were obtained in 2008 and used to map distribution patterns of all species. The recorded presence/absence of species in QDGCs was used for data analysis. The original database contained 33,726 records. Species present outside South Africa, Lesotho and Swaziland were removed, and duplicate records, invalid botanical names, synonyms as well as alien and naturalized legume species were omitted (Trytsman et al. 2011, Trytsman 2013). The edited data resulted in 27,322 records. Where geographical outliers for individual species were noted (–i.e. where a species was recorded outside its main ecological region) it was assumed that the outlier populations was adapted to the given local environmental conditions, and it was therefore not removed from the dataset.

However, the PRECIS database has some inherent weaknesses, especially errors regarding the allocation of taxa to QDGCs. It is estimated that QDGCs for approximately 15% of records may be incorrect (Biodiversity Information Officer, pers. comm). It is noteworthy that an extended QDGC standard has been proposed (Larsen et al. 2009) for mapping biodiversity data across the African continent and as an instrument for sharing biodiversity data where laws, regulations or other formal considerations prevent or prohibit distribution of coordinate-level information. The edited Leguminosae PRECIS data resulted in discarding 19% of the records mainly due to incomplete taxa (only genera, missing subspecies or varieties) and QDGC references resulting in the 27,322 records used. The database does not reflect all herbarium records from southern Africa, but mainly those housed in the National Herbarium in Pretoria and some of its satellite herbaria, notably the KwaZulu-Natal Herbarium (NH) in Durban and the Compton Herbarium (NBG) in Cape Town. Despite its inherent limitations, results of the present analysis have been considered sufficiently meaningful to justify the use of this database, the only one of its kind for the study area.

Names of legume species and intraspecific taxa were verified using the section on the family Leguminosae in the "Plants of Southern Africa, an online checklist" of the South African National Biodiversity Institute (SANBI), at http://posa.sanbi.org/searchspp.php as published in March 2011. Germishuizen and Meyer (2003) was used to describe each species in terms of its growth form, life cycle, height and elevation. These attributes could be useful information in selection and breeding programs. Data on the SANBI website were compared with Germishuizen and Meyer (2003) where discrepancies were found. The reinstatement of *Calobota* Eckl. & Zeyh. and the genus *Wiborgiella* Thunb. were implemented for the division of *Lebeckia* Thunb., whereas the reinstatement of *Euchlora* Eckl. & Zeyh., *Leobordea* Delile and *Listia* E. Mey. and the new genus *Ezoloba* B.-E. van Wyk & Boatwr. were recorded for reclassification of *Lotononis* (DC.) Eckl. & Zeyh. (Boatwright et al. 2009, 2011). For the analyses, 1,580 species representing 122 genera and 24 tribes were considered.

The maps that were used to generate data on climate (mean annual rainfall, mean annual minimum and maximum temperatures) and soil (phosphorus and pH) within each QDGC were supplied by the Agricultural Research Council - Institute for Soil, Climate and Water (ARC-ISCW, 2009). The exchangeable sodium percentage (ESP) assigned to each bioregion was sourced from Nell (2010).

Statistical analysis

A Multivariate Agglomerative Hierarchical Clustering (AHC) was applied to the presence or absence of legume species recorded in the PRECIS database. The input matrix thus contained the 1,580 recorded legume species and the 1,619 QDGCs enclosed within the borders of southern Africa. Some species were recorded only once, but such rare species were not excluded from the data set. The cluster analysis was performed using XLSTAT 2010.6.01 Software (Addinsoft to MS Excel) applying Euclidean distance for dissimilarity and the Ward's linkage method for agglomeration to establish and describe functional legume clusters (leguminochoria). Ward's method is often preferred in broad-scale biogeographical analyses (Kreft and Jetz 2010) and has been applied in several recent biodiversity studies, e.g. Akhani et al. (2013), Divíšek et al. (2014) and Li et al. (2015). The Euclidean distance was used by Biondi et al. (2015) and Abbate et al. (2016) and both Ward's method and Euclidean distance by Boratyński et al. (2013) and Sirisena et al. (2013) in geographical biodiversity studies. The statistical results of the present study are given in the Supplementary material 1 where five main clusters (termed A–E) were noted with a centroid QDGC. Each of the main clusters (A–E) was then examined for meaningful smaller cluster groups with clear geographical boundaries, thus defining ecologically interpretable leguminochoria. Thereafter a discriminant analysis was performed on the leguminochoria (dependent variable) using the same software and mean annual rainfall, mean annual maximum and minimum temperature, soil phosphorus and soil pH (H_2O) (explanatory variables) to identify the possible drivers for discrimination.

The bioregions map of Rutherford et al. (2006) was used as a base layer for plotting the different leguminochoria using the QDGCs (dots on map) assigned to each unique leguminochorion. ArcView GIS 3.2, ESRI Inc. 2002 was used to create the layers. The description of each QDGC was thus based on regional maps where one QDGC average 675 km^2 (± 26 × 26 km). The use of small (megaregional) scale maps as well as assigning abiotic (rainfall, temperature, soil phosphorus, soil pH and ESP) values to an area as large as a QDGC, evidently resulted in a less accurate dataset. This happened especially where two or more bioregions or biomes converged in a QDGC (ecotones), resulting in a considerable loss of descriptive data for many QDGCs. The abiotic data were easier to assign, since QDGCs could be described in transitional terms and classed in a zone closest to those presented in this study. Additional climatology and agrohydrology data (Schulze 2007) were used to describe leguminochoria. These include notes on, for example, extreme maximum temperatures, net primary

production, altitude, days of heavy frost per year, monthly solar radiation and extreme cold spells per year.

Species richness for each leguminochorion was calculated by firstly removing duplicate species present in a leguminochorion. The total number of species was then divided by the total number of QDGCs contained in each leguminochorion. The deletion of duplicate species, however, resulted in a lower total number of QDGCs per leguminochorion, i.e. QDGCs that contained only duplicate species were removed from the dataset.

The percentage occurrence of a species was calculated by dividing the total count of an individual species in a leguminochorion by the number of QDGCs present, i.e. if Species A occurred in 30 of the 50 QDGCs assigned to a leguminochorion, it would have a 60% occurrence in that leguminochorion. The first 20 species with the highest occurrence in a leguminochorion were selected as key species. These species are not indicator species (–i.e. species whose abundance in a given area is believed to indicate certain environmental or ecological conditions or suitable conditions for a group of other species), but rather, from an agricultural viewpoint, a species with potential as a pasture crop being more widely adapted and with a higher occurrence than a rare species with a narrow adaptation. A species is labelled diagnostic when its occurrence is 70% or higher in a given leguminochorion. See Supplementary material 2 for a complete list of species recorded in each leguminochorion. Species present in one cluster only are also noted.

The PHYTOTAB-PC vegetation classification program package of Westfall (1992) was used to form assemblages using the 20 key species recorded in each of the leguminochoria derived from the AHC analyses. The aim of classification is defined as the orderly arrangement of objects according to their differences and similarities (Gabriel and Talbot 1984) and thus, for this study, to ascertain whether floristic links between leguminochoria existed. The method of classification is based on minimum entropy (Westfall et al. 1997) and aims to obtain a cluster sequence where cluster-groups can be formed based on floristic similarities and sequenced according to floristic similarities, delimit cluster-groups and to obtain a species sequence where the cluster-groups and their relations are emphasised (Panagos 1995). This program allows the user to decide on the number of groups classified where the accepted minimum percentage difference between groups is 33%. During the analysis, it was established that six groups were formed by increasing the percentage difference between groups to 38%. A further increase up to 50% resulted in no change in the number of groups (remained at six groups) and therefore the analysis was done at the 38% difference between groups. The resultant classification efficiency for the six groups was 86%, higher than the 60% considered adequate for classification (Westfall 1992).

Results and Discussion

Leguminochoria of southern Africa

Figure 1 shows the dendrogram of the five main clusters (A–E) and the subdivisions within each main cluster formed by the clustering analysis. Cluster A, the second largest

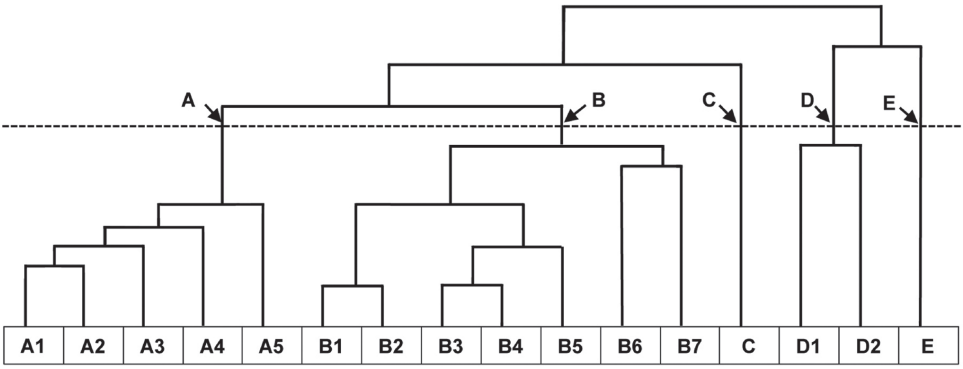

Figure 1. Dendrogram of southern African leguminochoria delimited by Multivariate Agglomerative Hierarchical Clustering. **A1** Southern Afromontane **A2** Albany Centre **A3** Northern Highveld Region **A4** Drakensberg Alpine Centre **A5** Coastal Region **B1** Arid Western Region **B2** Lower-rainfall Cape Floristic Region **B3** Central Arid Region **B4** Generalist Group **B5** Summer Rainfall Region **B6** Northern & Northeastern Savannah Region **B7** Kalahari Bushveld Region **C** Higher-rainfall Cape Floristic Region **D1** Central Bushveld Region **D2** Subtropical Lowveld & Mopane Region **E** Northern Mistbelt.

main cluster, was subdivided into five leguminochoria mainly found in the grassland and savannah regions. Cluster B, the largest main cluster, was subdivided into seven leguminochoria that included one leguminochorion covering a region of South Africa, referred to as the Generalist Group. Cluster C represents the Cape Floristic Region. The two subdivisions of Cluster D represent the savannah regions. Cluster E, the smallest of the five main clusters, represents an Afromontane area. The subdivision of the five main clusters resulted thus in 16 distinct leguminochoria.

The 16 leguminochoria are listed and described in Table 1. The key bioregions (Rutherford et al. 2006) and additional vegetational description (Acocks 1988, Low and Rebelo 1996, Kruger 1999, Van Wyk and Smith 2001, Goldblatt and Manning 2002) delineates the leguminochoria. Leguminochoria B2 and C were formed mainly on the basis of variations in rainfall. Leguminochoria A2 and A4 fall in centres of floristic endemism as described by Van Wyk and Smith (2001). Leguminochorion E is part of the Northern Mistbelt as defined by Mucina and Geldenhuys (2006). Names assigned to the leguminochoria were based on commonly used terms or descriptions contained in the southern Africa vegetation literature.

Sourveld and Mixed Veld Group (medium- to high-rainfall areas) (A)

The Sourveld and Mixed Veld Group lies in the medium- to high-rainfall areas of South Africa, Lesotho and Swaziland. This region receives summer rain with frost occurring in the interior. The region is relatively high in net primary production. The Sourveld and Mixed Veld Group is subdivided into five leguminochoria, namely A1: Southern Afromontane, A2: Albany Centre, A3: Northern Highveld Region, A4: Drakensberg Alpine Centre and A5: Coastal Region.

Table 1. Summary of classification of leguminochoria (A1–E) of southern Africa. Key bioregions from Rutherford et al. (2006) with additional descriptions accessed from published literature.

Cluster	Leguminochorion	Key bioregions[1]	Additional description[2]
A	**Sourveld and Mixed Veld Group (medium- to high-rainfall areas)**		
A1	Southern Afromontane	MHG, SEG, SES	Forest biome (Lo); Moist subtropical (Kr)
A2	Albany Centre	AT, DG, SEG	Albany Centre (Va); Forest biome (Lo); Dry subtropical (Kr)
A3	Northern Highveld Region	CBV, DHG, MHG	Rocky Highveld Grassland (Lo); Moist subtropical (Kr); Bankenveld & N-E Sandy Highveld (Ac)
A4	Drakensberg Alpine Centre	DG, MHG, SEG	Drakensberg Alpine Centre (Va); Forest biome (Lo); Alpine (Kr); *Themeda-Festuca* Alpine Veld (Ac)
A5	Coastal Region	IOCB, LV, SES	Maputaland-Pondoland Region (Va); Coastal Bushveld-Grassland (Lo); Moist & humid subtropical (Kr)
B	**Seasonal Rainfall Group (all-year, winter and summer rainfall)**		
B1	Arid Western Region	NHV, BML	Gariep Centre (Va); Warm desert (Kr); Namaqualand Broken Veld, Succulent Karoo & Strandveld (Ac)
B2	Lower-rainfall Cape Floristic Region	AT, EFR	Maritime (Kr); Coastal Fynbos & Coastal Renosterveld (Ac); Karoo Mountain, Langebaan, Agulhas Plain & Southeastern Centres (Go)
B3	Central Arid Region	EKB, NK	Nama-Karoo and Western Savannah biomes (Ru); Cold & warm desert, Dry subtropical (Kr)
B4	Generalist Group	All regions except: Fynbos, Northern Mistbelt Afromontane, IOCB	Non-specific, Non-Cape group
B5	Summer Rainfall Region	MHG, CBV	
B6	Northern and Northeastern Savannah Region	CBV, LV	Mopane Bushveld, Mixed Lowveld Bushveld, Mixed Bushveld (Lo)
B7	Kalahari Bushveld Region	EKB	Griqualand West Centre (Va); Kimberley Thorn Bushveld & Kalahari Plateau Bushveld (Lo); Kalahari Thornveld (Ac)
C	**Higher-rainfall Cape Floristic Region**	EFR, SWF	Mediterranean (Kr); False Sclerophyllous Bush types & Coastal Renosterveld (Ac); mainly Southwestern and Northwestern Centres (Go)
D	**Savannah Group**		
D1	Central Bushveld Region	CBV	Moist subtropical (Kr); Springbok Flats Turf Thornveld & Sour Bushveld (Ac)
D2	Subtropical Lowveld & Mopane Region	LV, M	Mopane Bushveld & Mixed Lowveld Bushveld (Lo); Dry and moist tropical (Kr)
E	**Northern Mistbelt**	Transitional MHG, LV, CBV	Afromontane Forest (Lo); Inland Moist tropical & moist subtropical (Kr); Tropical Forest Type (Ac)

[1]AT: Albany Thicket; BML: Bushmanland; CBV: Central Bushveld; DG: Drakensberg Grassland; DHG: Dry Highveld Grassland; EFR: Eastern Fynbos-Renosterveld; EKB: Eastern Kalahari Bushveld; IOCB: Indian Ocean Coastal Belt: LV: Lowveld; M: Mopane; MHG: Mesic Highveld Grassland; NHV: Namaqualand Hardeveld; NK: Nama-Karoo; SEG: Sub-Escarpment Grassland; SES: Sub-Escarpment Savannah; SWF: Southwest Fynbos.
[2]Ac: Acocks 1988; Lo: Low and Rebelo 1996; Kr: Kruger 1999, Va: Van Wyk and Smith 2001; Go: Goldblatt and Manning 2002; Ru: Rutherford et al. 2006.

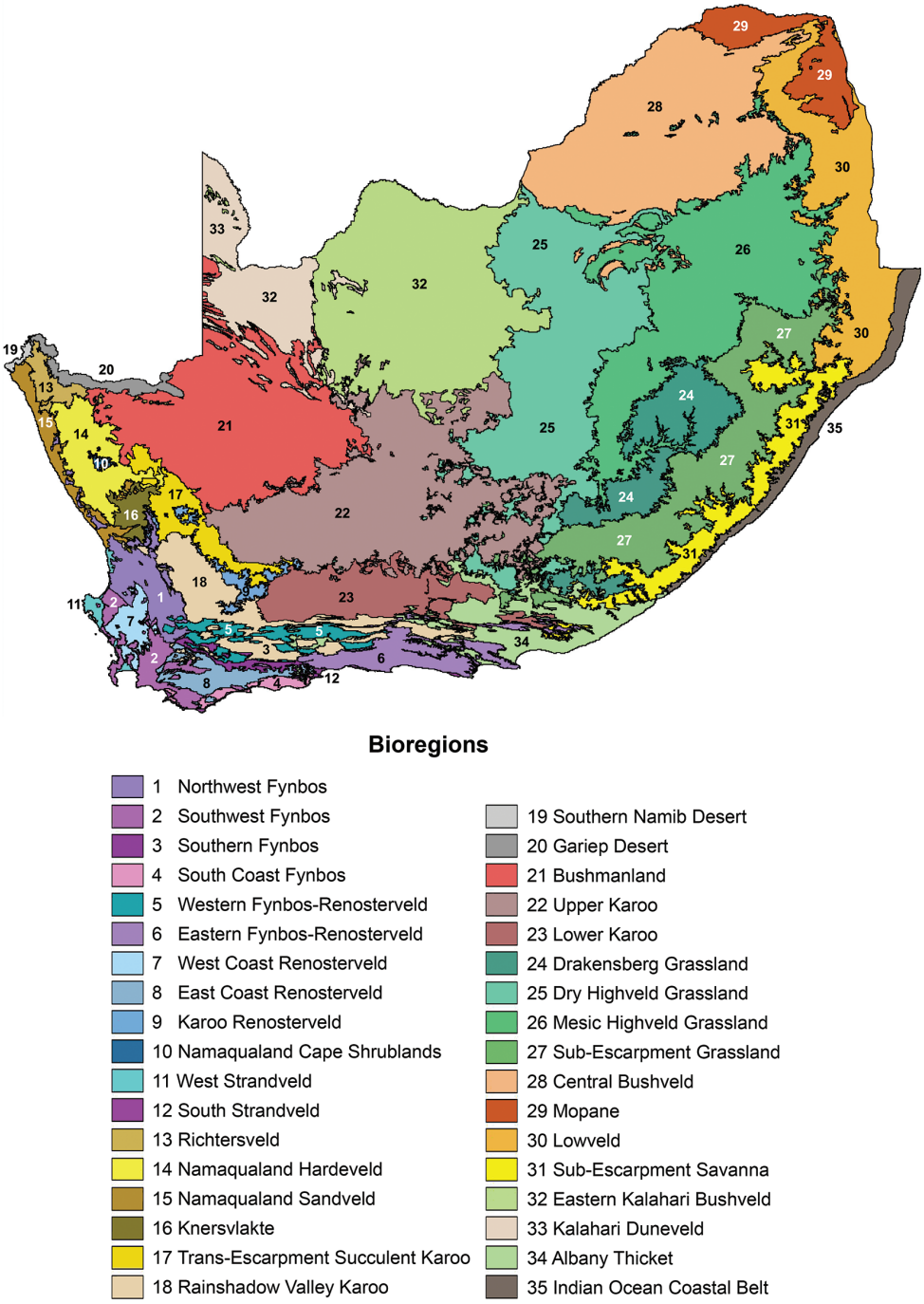

Figure 2. Bioregions of South Africa, Lesotho and Swaziland (Rutherford et al. 2006). The vegetation map shows the 35 bioregions where a bioregion is defined as a composite special terrestrial unit based on similar biotic (vegetation and floristic) and physical features (landscapes and rock types) and processes at the regional scale (Rutherford et al. 2006). The legend should be referred to when comparing the areas covered by leguminochoria.

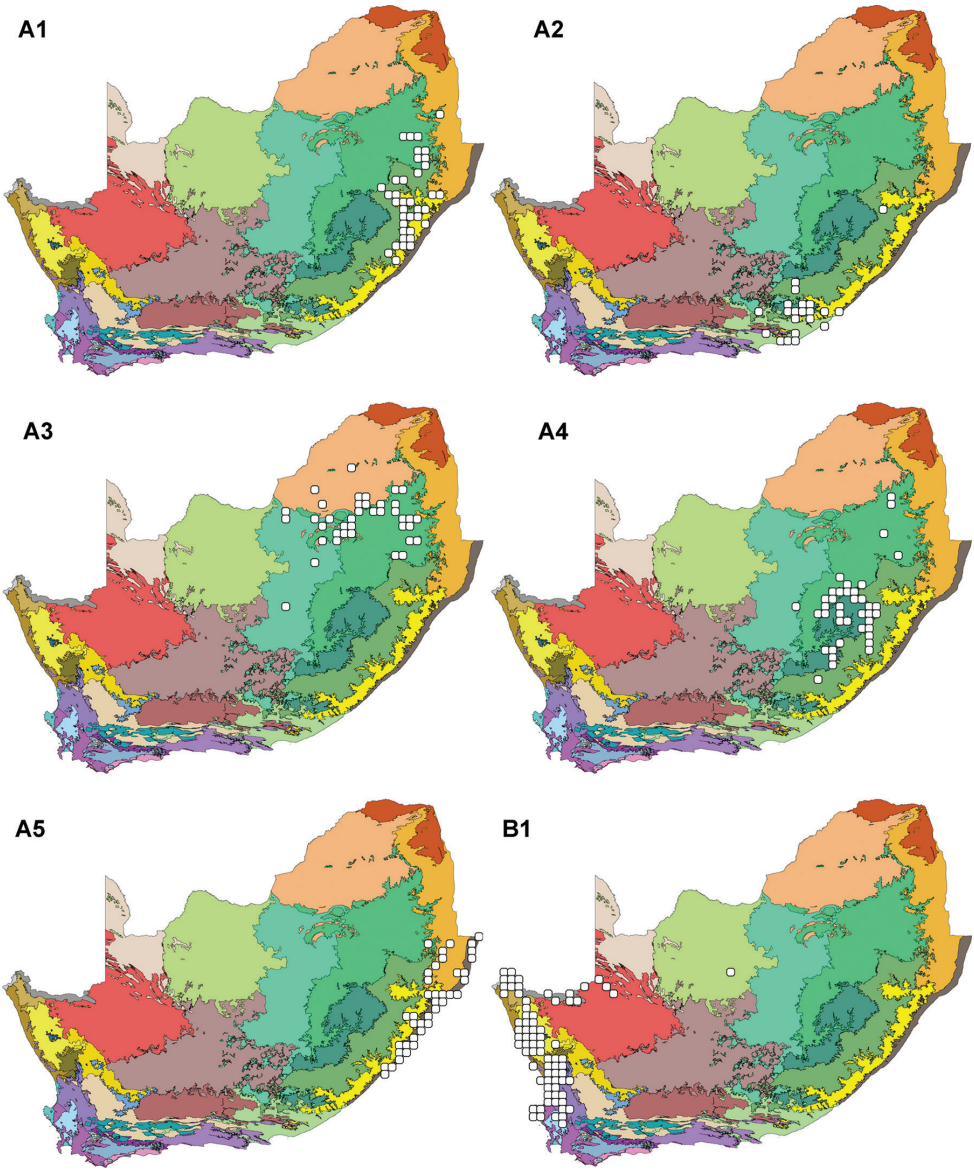

Figure 3. The Leguminochoria **A1–A5** & **B1** superimposed on the Bioregions of southern Africa. Cluster A (Sourveld and Mixed Veld Group) is divided into the Southern Afromontane (**A1**); Albany Centre (**A2**); Northern Highveld Region (**A3**); Drakensberg Alpine Centre (**A4**); and the Coastal Region (**A5**). Cluster B (Seasonal Rainfall Group) is here represented by the Arid Western Region (**B1**); for other subdivisions of cluster B, see Figure 5. The leguminochoria is mapped on bioregions defined by (Rutherford et al. 2006) referring to the legend in Figure 2.

The 35 bioregions of South Africa, Lesotho and Swaziland as defined by Rutherford et al. (2006) is shown in Figure 2. The legend should be referred to when comparing the areas covered by leguminochoria.

Table 2. Representation percentage of key bioregions (Rutherford et al. 2006) within leguminochoria (Cluster A1–E) of southern Africa.

Cluster	A1	A2	A3	A4	A5	B1	B2	B3
AT		**50.0**[a]					**40.0**	
BL						19.1		22.6
CBV			22.2					
DG				35.3				
DHG			16.7					13.0
EFR							**40.0**	
EKB								**26.0**
IOCB					**79.0**			
Low					15.8			
MHG	**50.0**		**61.1**	**41.2**				
NH						**33.2**		
SEG	40.0	**50.0**		23.5				
UK								14.3
Cluster	**B4**	**B5**	**B6**	**B7**	**C**	**D1**	**D2**	**E**
CBV	**18.9**	26.6	**40.8**			**100.0**	21.4	22.2
DHG	13.0							
EFR					**61.5**			
EKB	13.1			**95.0**				
Low			**40.8**				**57.2**	
Mop			18.4				21.4	33.3
MHG		**29.8**						**44.5**
SEG		12.9						
SWF					23.1			

[a]Bold-formatted figures indicate the bioregion with the highest percentage representation in a particular leguminochorion. Only key bioregions with representation values higher than 10% are shown.
AT: Albany Thicket; BL: Bushmanland; CBV: Central Bushveld; DG: Drakensberg Grassland; DHG: Dry Highveld Grassland; EFR: Eastern Fynbos Renosterveld; EKB: Eastern Kalahari Bushveld; IOCB: Indian Ocean Coastal Belt; Low: Lowveld; Mop: Mopane; MHG: Mesic Highveld Grassland; NH: Namaqualand Hardeveld; SEG: Sub-Escarpment Grassland; SWF: Southwest Fynbos; UK: Upper Karoo.
A1: Southern Afromontane; A2: Albany Centre; A3: Northern Highveld Region; A4: Drakensberg Alpine Centre; A5: Coastal Region; B1: Arid Western Region; B2: Lower-rainfall Cape Floristic Region; B3: Central Arid Region; B4: Generalist Group; B5: Summer Rainfall Region; B6: Northern & Northeastern Savannah Region; B7: Kalahari Bushveld Region; C: Higher-rainfall Cape Floristic Region; D1: Central Bushveld Region; D2: Subtropical Lowveld & Mopane Region; E: Northern Mistbelt.

The Southern Afromontane (A1)

The Southern Afromontane includes legume species mainly confined to the Mesic Highveld Grassland, Sub-Escarpment Grassland and Sub-Escarpment Savannah Bioregions evident from Figure 3 and Table 2. The Grassland biome forms the key biome of this leguminochorion (Table 3). Additional information regarding climatology and agrohydrology (Schulze 2007) is shown in Table 4.

Table 3. Representation percentage of key biomes (Rutherford et al. 2006) within leguminochoria (A1–E) of southern Africa.

Leguminochorion	AT	D	FB	GL	IO	NK	SK	SV
A1: Southern Afromontane				**90.9**				9.1
A2: Albany Centre	**50.0**[a]			**50.0**				
A3: Northern Highveld Region				**81.0**				19.0
A4: Drakensberg Alpine Centre				**100.0**				
A5: Coastal Region					**76.5**			23.5
B1: Arid Western Region		4.6	38.6			6.8	**47.7**	2.3
B2: Lower-rainfall Cape Floristic Region	20.0		**75.0**			5.0		
B3: Central Arid Region	0.6	1.1	1.1	14.8		**38.6**	7.4	36.4
B4: Generalist Group	1.1	1.4	1.7	37.0	0.5	14.5	5.6	**38.2**
B5: Summer Rainfall Region	1.4		0.7	**54.6**	5.0			38.3
B6: Northern & Northeastern Savannah Region								**100.0**
B7: Kalahari Bushveld Region				5.3				**94.7**
C: Higher-rainfall Cape Floristic Region			**100.0**					
D1: Central Bushveld Region								**100.0**
D2: Subtropical Lowveld & Mopane Region								**100.0**
E: Northern Mistbelt				9.1				**90.9**

[a]Bold-formatted figures indicate the highest percentage biome in a leguminochorion.
AT: Albany Thicket; D: Desert; FB: Fynbos; GL: Grassland; IO: Indian Ocean Coastal Belt; NK: Nama-Karoo; SK: Succulent Karoo; SV: Savannah.

Table 4. Additional information regarding climatology and agrohydrology (Schulze 2007) of leguminochoria (A1–E) in southern Africa. Not all variables are noted with each leguminochorion.

Leguminochorion	Notes on climatology and agrohydrology
A1: Southern Afromontane	36–42°C extreme maximum temperatures, >6 $tha^{-1}yr^{-1}$ net primary production, early summer to midsummer rain, 600–1200 mm annual rain, 400–1500 m altitude, <20 days heavy frost/year with frost-free areas
A2: Albany Centre	>40°C extreme maximum temperatures, 2–6 $tha^{-1}yr^{-1}$ net primary production, all-year and late and very late summer rain, 200–600 mm annual rain, 0–800 m altitude, <20 days heavy frost/year with frost-free areas
A3: Northern Highveld Region	30–36°C extreme maximum temperatures, 4–8 $tha^{-1}yr^{-1}$ net primary production, early summer to midsummer rain, 400–1000 mm annual rain, 800–2000 m altitude, <60 days heavy frost/year, higher monthly solar radiation compared to A1 and A2
A4: Drakensberg Alpine Centre	Mainly <36°C extreme maximum temperatures, 4–10 $tha^{-1}yr^{-1}$ net primary production, mainly early summer to midsummer rain, 400–1000 mm annual rain, mainly >2000 m altitude, <80 days heavy frost/year, partly high relative relief, >6 extreme cold spells/year lower than -2.5°C on 3 or more consecutive days, high mountains

Leguminochorion	Notes on climatology and agrohydrology
A5: Coastal Region	Mainly >40°C extreme maximum temperatures, >4 $tha^{-1}yr^{-1}$ net primary production, early to mid- to late summer rain, 600–1200 m annual rain, <800 m altitude, frost-free areas, low to medium relief, mainly sourveld, tropically wet with dry winter season
B1: Arid Western Region	Mainly >44°C extreme maximum temperatures, mainly <2 $tha^{-1}yr^{-1}$ net primary production, mainly winter rainfall, <400 mm annual rain, <800 m altitude, mainly frost-free areas and <20 days of heavy frost/year, mainly 25–150 relative relief, high solar radiation during Nov–Feb, sweetveld, arid, hot and dry areas
B2: Lower-rainfall Cape Floristic Region	36–42°C extreme maximum temperatures, 0.5–4.0 $tha^{-1}yr^{-1}$ net primary production, all-year rainfall, mainly 200–600 mm annual rain, mainly 0–200 m altitude, mainly frost-free and <40 days heavy frost/year, mainly >50 relative relief, mainly semi-arid, cool and dry
B3: Central Arid Region	<4 $tha^{-1}yr^{-1}$ net primary production, mainly late to very late summer rain, mainly between 400–1250 m altitude, mainly <50 relative relief, semi-arid to arid, hot, cool and dry, largely sweetveld
B4: Generalist Group	Extremely diverse in terms of given variables
B5: Summer Rainfall Region	>4 $tha^{-1}yr^{-1}$ net primary production, early to mid- to late summer rain, >400 mm annual rain
B6: Northern & Northeastern Savannah Region	Mainly >40°C extreme maximum temperature, midsummer rain, frost-free areas and <20 days of heavy frost, <50 relative relief, sweetveld, semi-arid, hot and dry, the only leguminochorion with 16 occurrences of heat waves >30°C on 3 or more consecutive days/year
B7: Kalahari Bushveld Region	2–6 $tha^{-1}yr^{-1}$ net primary production, mainly late summer rain, 200–600 mm annual rain, 1000–1500 m altitude, mainly 20–60 days heavy frost/year, <50 relative relief, sweetveld, semi-arid and dry, plains and pans
C: Higher-rainfall Cape Floristic Region	Mainly 2–4 $tha^{-1}yr^{-1}$ net primary production, all-year and winter rain, 400–1200 mm annual rain, frost-free areas, mixed veld, mainly long, dry summers hot or cool
D1: Central Bushveld Region	Mainly 36–40°C extreme maximum temperature, 2–6 $tha^{-1}yr^{-1}$ net primary production, early summer to midsummer rain, mainly 400–600 mm annual rain, 600–1500 m altitude, <40 days heavy frost/year, 25–200 relative relief, dry and hot or cool
D2: Subtropical Lowveld & Mopane Region	>40°C extreme maximum temperature, 2–8 $tha^{-1}yr^{-1}$ net primary production, midsummer rain, 200–800 mm annual rain, <800 m altitude, mainly frost-free, <50 relative relief, mainly sweetveld, dry and hot
E: Northern Mistbelt	30–40°C maximum extreme temperature, >4 $tha^{-1}yr^{-1}$ net primary production, mainly early summer rain, >600 mm annual rain, 600–2000 m altitude, mainly frost-free areas, >50 relative relief, sourveld, long winters, low mountains

A summary of the predominant climate and soil characteristics of these regions is given in Figure 4. Data used to construct Figure 4 is available in Supplementary material 3 (rainfall and temperature) and Supplementary material 4 (soil properties). The high rainfall (>600 mm) and moderate minimum (0–8°C) and maximum (25–29°C) temperatures denote this leguminochorion as a relatively highly productive region. Extreme maximum temperatures of 36–42°C are noted for this leguminochorion

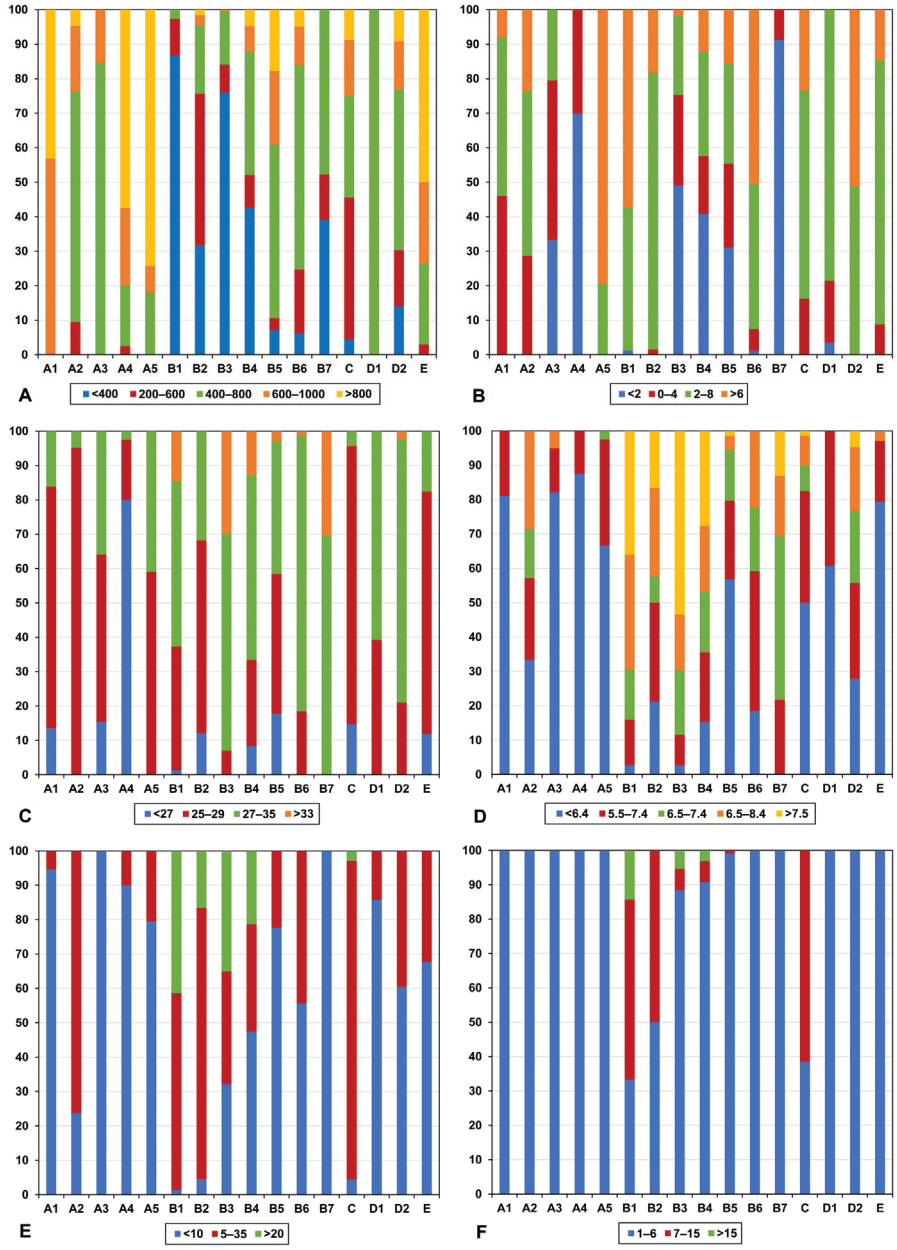

Figure 4. The predominant climate and soil conditions associated with leguminochoria (A1–E) of southern Africa. Climatic conditions shown are mean annual rainfall (**A**) (mm), minimum (**B**) and maximum temperatures (**C**) (°C). The soil properties shown are pH (H_2O) level (**D**), phosphorus content ($mgkg^{-1}$) (**E**) and exchangeable sodium (**F**) (%). The leguminochoria are termed **A1** Southern Afromontane **A2** Albany Centre **A3** Northern Highveld Region **A4** Drakensberg Alpine Centre **A5** Coastal Region **B1** Arid Western Region **B2** Lower-rainfall Cape Floristic Region **B3** Central Arid Region **B4** Generalist Group **B5** Summer Rainfall Region **B6** Northern & Northeastern Savannah Region **B7** Kalahari Bushveld Region **C** Higher-rainfall Cape Floristic Region **D1** Central Bushveld Region **D2** Subtropical Lowveld & Mopane Region **E** Northern Mistbelt.

Table 5. List of key species recorded in leguminochoria of southern Africa, the occurrence percentage within each leguminochorion (% Occ). Key species preceded by a bullet (•) are present in the designated leguminochorion as key species only and bold-formatted diagnostic species has an occurrence of 70% or higher.

Key species	% Occ
A1: Southern Afromontane	
Argyrolobium tomentosum (Andrews) Druce	45
• *Alysicarpus rugosus* (Willd.) DC. subsp. *perennirufus* J.Léonard	28
• *Argyrolobium speciosum* Eckl. & Zeyh.	39
Crotalaria globifera E.Mey.	47
Dalbergia obovata E.Mey.	33
Eriosema cordatum E.Mey.	69
• *Eriosema distinctum* N.E.Br.	42
Eriosema kraussianum Meisn.	58
Eriosema salignum E.Mey.	69
Indigofera hilaris Eckl. & Zeyh. var. *hilaris*	28
• *Leobordea foliosa* (Bolus) B.-E van Wyk & Boatwr.	31
• *Lotus discolor* E.Mey. subsp. *discolor*	31
Otholobium polystictum (Benth. ex Harv.) C.H.Stirt.	33
• *Pomaria sandersonii* (Harv.) B.B.Simpson & G.P.Lewis	31
• *Rhynchosia cooperi* (Harv. ex Baker f.) Burtt Davy	28
• *Rhynchosia sordida* (E.Mey.) Schinz	28
Rhynchosia totta (Thunb.) DC. var. *totta*	33
Tephrosia macropoda (E.Mey.) Harv. var. *macropoda*	33
Trifolium africanum Ser. var. *africanum*	33
Vigna vexillata (L.) A.Rich. var. *vexillata*	56
Zornia capensis Pers. subsp. *capensis*	56
A2: Albany Centre	
Argyrolobium tomentosum (Andrews) Druce	44
• *Aspalathus chortophila* Eckl. & Zeyh.	40
Aspalathus spinosa L. subsp. *spinosa*	55
• *Calpurnia aurea* (Aiton) Benth. subsp. *aurea*	45
• *Crotalaria obscura* DC.	40
• *Eriosema squarrosum* (Thunb.) Walp.	50
Indigofera hedyantha Eckl. & Zeyh.	45
Indigofera sessilifolia DC.	40
Indigofera zeyheri Spreng. ex Eckl. & Zeyh.	65
• *Lessertia brachystachya* DC.	40
Melolobium candicans (E.Mey.) Eckl. & Zeyh.	50
• *Otholobium caffrum* (Eckl. & Zeyh.) C.H.Stirt.	40
Psoralea oligophylla Eckl. & Zeyh.	40
Rhynchosia adenodes Eckl. & Zeyh.	55
Rhynchosia caribaea (Jacq.) DC.	40
• *Rhynchosia ciliata* (Thunb.) Schinz	45
Rhynchosia totta (Thunb.) DC. var. *totta*	50
• *Schotia latifolia* Jacq.	50
Tephrosia capensis (Jacq.) Pers. var. *capensis*	65
Trifolium burchellianum Ser. subsp. *burchellianum*	55

Key species	% Occ
A3: Northern Highveld Region	
Elephantorrhiza elephantina (Burch.) Skeels	42
• *Eriosema burkei* Benth. ex Harv. var. *burkei*	37
Eriosema cordatum E.Mey.	34
Eriosema salignum E.Mey.	34
• *Erythrina zeyheri* Harv.	34
Indigofera hedyantha Eckl. & Zeyh.	34
Indigofera hilaris Eckl. & Zeyh. var. *hilaris*	47
• *Indigofera oxytropis* Benth. ex Harv.	37
• *Leobordea divaricata* Eckl. & Zeyh.	45
Leobordea eriantha (Benth.) B.-E van Wyk & Boatwr.	39
• *Pearsonia cajanifolia* (Harv.) Polhill subsp. *cajanifolia*	34
• *Pearsonia sessilifolia* (Harv.) Dummer subsp. *sessilifolia*	37
Rhynchosia nervosa Benth. ex Harv. var. *nervosa*	37
Rhynchosia totta (Thunb.) DC. var. *totta*	47
• *Tephrosia elongata* E.Mey. var. *elongata*	37
Tephrosia longipes Meisn. subsp. *longipes* var. *longipes*	47
Trifolium africanum Ser. var. *africanum*	37
Vigna vexillata (L.) A.Rich. var. *vexillata*	39
Zornia linearis E.Mey.	39
Zornia milneana Mohlenbr.	37
A4: Drakensberg Alpine Centre	
• *Argyrolobium harveyanum* Oliv.	33
• *Argyrolobium lotoides* Harv.	50
• *Argyrolobium rupestre* (E.Mey.) Walp. subsp. *rupestre*	53
• *Argyrolobium tuberosum* (Andrews) Druce	39
• *Dichilus strictus* E.Mey.	42
• *Dolichos angustifolius* Eckl. & Zeyh.	33
Eriosema salignum E.Mey.	39
Indigofera hedyantha Eckl. & Zeyh.	42
Leobordea eriantha (Benth.) B.-E van Wyk & Boatwr.	33
• ***Lessertia perennans* (Jacq.) DC. var. *perennans***	**72**
• *Lotononis galpinii* Dummer	42
Lotononis laxa Eckl. & Zeyh.	56
• *Lotononis lotononoides* (Scott-Elliot) B.-E.van Wyk	44
• *Lotononis sericophylla* Benth.	58
Melolobium microphyllum (L.f.) Eckl. & Zeyh.	39
• *Melolobium obcordatum* Harv.	42
Otholobium polystictum (Benth. ex Harv.) C.H.Stirt.	47
Rhynchosia totta (Thunb.) DC. var. *totta*	44
Trifolium africanum Ser. var. *africanum*	44
Trifolium burchellianum Ser. subsp. *burchellianum*	58
A5: Coastal Region	
• *Abrus laevigatus* E.Mey.	51
Acacia karroo Hayne	67
• *Aeschynomene micrantha* DC.	54
• *Albizia adianthifolia* (Schumach.) W.Wight var. *adianthifolia*	49
***Chamaecrista mimosoides* (L.) Greene**	**82**

Key species	**% Occ**
• *Crotalaria capensis* Jacq.	62
Crotalaria globifera E.Mey.	64
• *Crotalaria lanceolata* E.Mey. subsp. *lanceolata*	49
• *Dalbergia armata* E.Mey.	51
Dalbergia obovata E.Mey.	67
• *Desmodium dregeanum* Benth.	56
Eriosema cordatum E.Mey.	59
• *Eriosema parviflorum* E.Mey. subsp. *parviflorum*	64
***Eriosema salignum* E.Mey.**	**77**
• *Neonotonia wightii* (Wight. ex Arn.) J.A.Lackey	49
Rhynchosia caribaea (Jacq.) DC.	49
• *Tephrosia grandiflora* (Aiton) Pers.	49
Tephrosia macropoda (E.Mey.) Harv. var. *macropoda*	49
• *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* var. *unguiculata*	51
Vigna vexillata (L.) A.Rich. var. *vexillata*	67
Zornia capensis* Pers. subsp. *capensis	**87**
B1: Arid Western Region	
Aspalathus acuminata Lam. subsp. *acuminata*	15
• *Adenolobus garipensis* (E.Mey.) Torre & Hillc.	15
• *Aspalathus quinquefolia* L. subsp. *virgata* (Thunb.) R.Dahlgren	15
• *Aspalathus spinescens* Thunb. subsp. *lepida* (E.Mey.) R.Dahlgren	22
• *Calobota angustifolia* (E.Mey.) Boatwr. & B.-E.van Wyk	43
• *Calobota sericea* (Thunb.) Boatwr. & B.-E.van Wyk	43
• *Calobota spinescens* (Harv.) Boatwr. & B.-E.van Wyk	19
• *Crotalaria effusa* E.Mey.	20
• *Crotalaria excisa* (Thunb.) Baker f. subsp. *excisa*	18
• *Indigastrum argyroides* (E.Mey.) Schrire	23
• *Indigofera amoena* Aiton	16
• *Indigofera exigua* Eckl. & Zeyh.	15
Indigofera heterophylla Thunb.	19
• *Indigofera pungens* E.Mey.	16
Leobordea platycarpa (Viv.) B.-E van Wyk & Boatwr.	22
• *Lessertia diffusa* R.Br.	28
• *Lessertia excisa* DC.	15
Lotononis falcata (E.Mey.) Benth.	27
• *Lotononis parviflora* (P.J.Bergius) D.Dietr.	19
• *Lotononis rabenaviana* Dinter & Harms	15
• *Melolobium aethiopicum* (L.) Druce	20
• *Melolobium humile* Eckl. & Zeyh.	22
Sutherlandia frutescens (L.) R.Br.	30
• *Wiborgia fusca* Thunb. subsp. *fusca*	15
• *Wiborgia monoptera* E.Mey.	20
• *Wiborgia obcordata* (P.J.Bergius) Thunb.	26
B2: Lower-rainfall Cape Floristic Region	
Acacia karroo Hayne	22
• *Aspalathus collina* Eckl. & Zeyh. subsp. *collina*	31
• *Aspalathus hirta* E.Mey. subsp. *hirta*	17
• *Aspalathus hystrix* L.f.	23

Key species	% Occ
• *Aspalathus kougaensis* (Garab. ex R.Dahlgren) R.Dahlgren	18
Aspalathus nigra L.	25
• *Aspalathus pinguis* Thunb. subsp. *pinguis*	20
• *Aspalathus rubens* Thunb.	32
• *Aspalathus setacea* Eckl. & Zeyh.	26
• *Aspalathus shawii* L.Bolus subsp. *shawii*	18
Aspalathus spinosa L. subsp. *spinosa*	17
• *Aspalathus steudeliana* Brongn.	18
• *Aspalathus subtingens* Eckl. & Zeyh.	31
• *Hypocalyptus sophoroides* (P.J.Bergius) Baill.	17
• *Indigofera denudata* L.f.	17
Indigofera heterophylla Thunb.	23
Lotononis pungens Eckl. & Zeyh.	28
• *Podalyria burchellii* DC.	20
Psoralea affinis Eckl. & Zeyh.	23
Psoralea oligophylla Eckl. & Zeyh.	17
• *Schotia afra* (L.) Thunb. var. *afra*	22
Sutherlandia frutescens (L.) R.Br.	31
Tephrosia capensis (Jacq.) Pers. var. *capensis*	18
B3: Central Arid Region	
Acacia erioloba E.Mey.	6
• *Acacia haematoxylon* Willd.	11
Acacia karroo Hayne	11
Cullen tomentosum (Thunb.) J.W.Grimes	11
Indigastrum argyraeum (Eckl. & Zeyh.) Schrire	8
Indigofera alternans DC. var. *alternans*	29
Indigofera daleoides Benth. ex Harv. var. *daleoides*	7
• *Indigofera meyeriana* Eckl. & Zeyh.	5
Indigofera sessilifolia DC.	10
Leobordea platycarpa (Viv.) B.-E van Wyk & Boatwr.	15
• *Lessertia annularis* Burch.	14
• *Lessertia macrostachya* DC. var. *macrostachya*	5
• *Lessertia pauciflora* Harv. var. *pauciflora*	13
Lotononis pungens Eckl. & Zeyh.	5
Melolobium candicans (E.Mey.) Eckl. & Zeyh.	24
Melolobium canescens Benth.	6
Melolobium microphyllum (L.f.) Eckl. & Zeyh.	6
• *Requienia sphaerosperma* DC.	7
Senna italica Mill. subsp. *arachoides* (Burch.) Lock	12
Sutherlandia frutescens (L.) R.Br.	25
• *Sutherlandia humilis* E.Phillips & R.A.Dyer	6
• *Sutherlandia microphylla* Burch. ex DC.	7
B4: Generalist Group	
Acacia karroo Hayne	8
Crotalaria sphaerocarpa Perr. ex DC. subsp. *sphaerocarpa*	4
Elephantorrhiza elephantina (Burch.) Skeels	3
Indigastrum argyraeum (Eckl. & Zeyh.) Schrire	3
Indigofera alternans DC. var. *alternans*	3

Key species	**% Occ**
Indigofera heterotricha DC.	3
• *Lessertia depressa* Harv.	4
• *Lotononis divaricata* (Eckl. & Zeyh.) Benth.	4
Lotononis falcata (E.Mey.) Benth.	3
Lotononis laxa Eckl. & Zeyh.	4
• *Lotononis pulchella* (E.Mey.) B.-E.van Wyk	3
• *Melolobium calycinum* Benth.	3
Melolobium candicans (E.Mey.) Eckl. & Zeyh.	4
Melolobium canescens Benth.	3
Melolobium microphyllum (L.f.) Eckl. & Zeyh.	6
• *Parkinsonia africana* Sond.	3
Rhynchosia adenodes Eckl. & Zeyh.	3
Rhynchosia caribaea (Jacq.) DC.	3
Senna italica Mill. subsp. *arachoides* (Burch.) Lock	3
Sutherlandia frutescens (L.) R.Br.	4
Tephrosia capensis (Jacq.) Pers. var. *capensis*	4
Trifolium burchellianum Ser. subsp. *burchellianum*	4
B5: Summer Rainfall Region	
Acacia karroo Hayne	11
Chamaecrista mimosoides (L.) Greene	9
Elephantorrhiza elephantina (Burch.) Skeels	8
Eriosema cordatum E.Mey.	9
Eriosema kraussianum Meisn.	9
Eriosema salignum E.Mey.	20
Indigofera hilaris Eckl. & Zeyh. var. *hilaris*	8
Indigofera zeyheri Spreng. ex Eckl. & Zeyh.	7
Listia heterophylla E. Mey	7
Mundulea sericea (Willd.) A.Chev. subsp. *sericea*	16
Rhynchosia adenodes Eckl. & Zeyh.	11
Rhynchosia nervosa Benth. ex Harv. var. *nervosa*	8
Rhynchosia totta (Thunb.) DC. var. *totta*	30
Stylosanthes fruticosa (Retz.) Alston	9
Tephrosia capensis (Jacq.) Pers. var. *capensis*	8
Tephrosia longipes Meisn. subsp. *longipes* var. *longipes*	10
Tephrosia purpurea (L.) Pers. subsp. *leptostachya* (DC.) Brummitt var. *leptostachya*	7
• *Tephrosia semiglabra* Sond.	7
Trifolium africanum Ser. var. *africanum*	20
Vigna vexillata (L.) A.Rich. var. *vexillata*	9
Zornia capensis Pers. subsp. *capensis*	17
B6: Northern and Northeastern Savannah Region	
Acacia burkei Benth.	21
Acacia caffra (Thunb.) Willd.	20
Acacia gerrardii Benth. subsp. *gerrardii* var. *gerrardii*	19
Acacia karroo Hayne	21
Acacia nigrescens Oliv.	20
Acacia nilotica (L.) Willd. ex Delile subsp. *kraussiana* (Benth.) Brenan	19
Acacia tortilis (Forssk.) Hayne subsp. *heteracantha* (Burch.) Brenan	20
• *Colophospermum mopane* (J.Kirk ex Benth.) J.Kirk ex J.Léonard	18

Key species	**% Occ**
• *Crotalaria monteiroi* Taub. ex Baker f. var. *monteiroi*	18
Dichrostachys cinerea (L.) Wight & Arn. subsp. *africana* Brenan & Brummitt var. *africana*	35
• *Faidherbia albida* (Delile) A.Chev.	19
• *Indigastrum costatum* (Guill. & Perr.) Schrire subsp. *macrum* (E.Mey.) Schrire	18
Mundulea sericea (Willd.) A.Chev. subsp. *sericea*	21
Ormocarpum trichocarpum (Taub.) Engl.	26
Peltophorum africanum Sond.	35
Philenoptera violacea (Klotzsch) Schrire	18
• *Pterocarpus rotundifolius* (Sond.) Druce subsp. *rotundifolius*	21
Rhynchosia minima (L.) DC. var. *minima*	18
Schotia brachypetala Sond.	20
Senna italica Mill. subsp. *arachoides* (Burch.) Lock	25
Tephrosia purpurea (L.) Pers. subsp. *leptostachya* (DC.) Brummitt var. *leptostachya*	28
• *Xanthocercis zambesiaca* (Baker) Dumaz-le-Grand	19
B7: Kalahari Bushveld region	
Acacia erioloba E.Mey.	52
• *Acacia hebeclada* DC. subsp. *hebeclada*	57
Acacia karroo Hayne	39
Acacia tortilis (Forssk.) Hayne subsp. *heteracantha* (Burch.) Brenan	30
• *Chamaecrista biensis* (Steyaert) Lock	52
• *Crotalaria griquensis* L.Bolus	35
Crotalaria lotoides Benth.	30
Crotalaria sphaerocarpa Perr. ex DC. subsp. *sphaerocarpa*	48
Cullen tomentosum (Thunb.) J.W.Grimes	39
Elephantorrhiza elephantina (Burch.) Skeels	43
Indigastrum argyraeum (Eckl. & Zeyh.) Schrire	30
Indigofera alternans DC. var. *alternans*	61
• *Indigofera cryptantha* Benth. ex Harv. var. *cryptantha*	30
Indigofera daleoides* Benth. ex Harv. var. *daleoides	**83**
Indigofera filipes Benth. ex Harv.	61
Indigofera heterotricha DC.	43
• *Indigofera rhytidocarpa* Benth. ex Harv. subsp. *rhytidocarpa*	30
Indigofera sessilifolia DC.	57
Listia heterophylla E. Mey	43
• *Rhynchosia confusa* Burtt Davy	61
***Senna italica* Mill. subsp. *arachoides* (Burch.) Lock**	**70**
• ***Tephrosia burchellii* Burtt Davy**	**74**
• *Tephrosia lupinifolia* DC.	30
Zornia milneana Mohlenbr.	35
C: Higher-rainfall Cape Floristic Region	
Aspalathus acuminata Lam. subsp. *acuminata*	41
• *Aspalathus angustifolia* (Lam.) R.Dahlgren subsp. *angustifolia*	44
• *Aspalathus ciliaris* L.	67
• *Aspalathus divaricata* Thunb. subsp. *divaricata*	52
• *Aspalathus hispida* Thunb. subsp. *hispida*	58
• *Aspalathus juniperina* Thunb. subsp. *juniperina*	33
Aspalathus nigra L.	55
• *Aspalathus spicata* Thunb.	45

Key species	**% Occ**
Aspalathus spinosa L. subsp. *spinosa*	50
• *Dipogon lignosus* (L.) Verdc.	41
Indigofera heterophylla Thunb.	42
• *Lessertia herbacea* (L.) Druce	33
• *Otholobium fruticans* (L.) C.H.Stirt.	41
• *Otholobium polyphyllum* (Eckl. & Zeyh.) C.H.Stirt.	38
• *Otholobium virgatum* (Burm.f.) C.H.Stirt.	35
• *Podalyria myrtillifolia* (Retz.) Willd.	55
Psoralea affinis Eckl. & Zeyh.	41
• *Psoralea aphylla* L.	33
• *Rafnia capensis* (L.) Schinz subsp. *capensis*	42
• *Rhynchosia capensis* (Burm.f.) Schinz	39
Sutherlandia frutescens (L.) R.Br.	45
D1: Central Bushveld Region	
Acacia caffra (Thunb.) Willd.	68
***Acacia karroo* Hayne**	**82**
• *Acacia robusta* Burch. subsp. *robusta*	68
• ***Burkea africana* Hook.**	**79**
Chamaecrista mimosoides (L.) Greene	61
Crotalaria lotoides Benth.	61
Crotalaria sphaerocarpa Perr. ex DC. subsp. *sphaerocarpa*	68
Dichrostachys cinerea (L.) Wight & Arn. subsp. *africana* Brenan & Brummitt var. *africana*	61
Eriosema psoraleoides (Lam.) G.Don	68
Indigofera filipes Benth. ex Harv.	64
Indigofera heterotricha DC.	64
• *Indigofera melanadenia* Benth. ex Harv.	64
Listia heterophylla E. Mey	64
Mundulea sericea* (Willd.) A.Chev. subsp. *sericea	**82**
Peltophorum africanum Sond.	61
• *Rhynchosia minima* (L.) DC. var. *prostrata* (Harv.) Meikle	64
Rhynchosia totta* (Thunb.) DC. var. *totta	**75**
• ***Sphenostylis angustifolia* Sond.**	**75**
Stylosanthes fruticosa (Retz.) Alston	61
Tephrosia longipes* Meisn. subsp. *longipes* var. *longipes	**79**
Zornia linearis E.Mey.	64
D2: Subtropical Lowveld & Mopane Region	
Acacia burkei Benth.	41
Acacia gerrardii Benth. subsp. *gerrardii* var. *gerrardii*	49
Acacia nigrescens Oliv.	56
Acacia nilotica (L.) Willd. ex Delile subsp. *kraussiana* (Benth.) Brenan	54
• *Acacia senegal* (L.) Willd. var. *rostrata* Brenan	46
Acacia tortilis (Forssk.) Hayne subsp. *heteracantha* (Burch.) Brenan	41
• *Albizia anthelmintica* (A.Rich.) Brongn.	49
• *Crotalaria laburnifolia* L. subsp. *australis* (Baker f.) Polhill	41
Dichrostachys cinerea (L.) Wight & Arn. subsp. *africana* Brenan & Brummitt var. *africana*	66
Eriosema psoraleoides (Lam.) G.Don	44
Mundulea sericea (Willd.) A.Chev. subsp. *sericea*	59
Ormocarpum trichocarpum (Taub.) Engl.	61

Key species	% Occ
Peltophorum africanum Sond.	61
Philenoptera violacea (Klotzsch) Schrire	54
Rhynchosia minima (L.) DC. var. *minima*	49
Rhynchosia totta (Thunb.) DC. var. *totta*	49
Schotia brachypetala Sond.	56
Senna italica Mill. subsp. *arachoides* (Burch.) Lock	51
Stylosanthes fruticosa (Retz.) Alston	56
Tephrosia longipes Meisn. subsp. *longipes* var. *longipes*	44
Tephrosia purpurea (L.) Pers. subsp. *leptostachya* (DC.) Brummitt var. *leptostachya*	44
E: Northern Mistbelt	
Acacia caffra (Thunb.) Willd.	65
• ***Acacia ataxacantha* DC.**	**88**
• *Acacia davyi* N.E.Br.	65
***Acacia karroo* Hayne**	**71**
• *Aeschynomene rehmannii* Schinz var. *leptobotrya* (Harms ex Baker f.) J.B.Gillett	65
***Argyrolobium tomentosum* (Andrews) Druce**	**74**
• ***Bauhinia galpinii* N.E.Br.**	**79**
• *Desmodium repandum* (Vahl) DC.	68
***Eriosema psoraleoides* (Lam.) G.Don**	**76**
• ***Indigofera sanguinea* N.E.Br.**	**79**
• *Indigofera tristoides* N.E.Br.	65
• ***Pearsonia sessilifolia* (Harv.) Dummer subsp. *marginata* (Schinz) Polhill**	**71**
• ***Pseudarthria hookeri* Wight & Arn. var. *hookeri***	**88**
• *Psoralea arborea* Sims	65
• ***Pterocarpus angolensis* DC.**	**71**
Rhynchosia caribaea (Jacq.) DC.	68
• ***Rhynchosia monophylla* Schltr.**	**76**
Rhynchosia totta (Thunb.) DC. var. *totta*	68
Vigna vexillata* (L.) A.Rich. var. *vexillata	**74**
Zornia capensis* Pers. subsp. *capensis	**82**

(Table 4). Species are adapted to soil with low pH (<6.4), low phosphorus content (<10 $mgkg^{-1}$) and to non-sodic soils.

The Southern Afromontane has some key species in common with the Northern Highveld Region, the Coastal Region, the Summer Rainfall Region and the Northern Mistbelt (e.g. *Rhynchosia totta* var. *totta* and *Vigna vexillata* var. *vexillata*) (Table 5). High occurrences of different species of *Eriosema* is also noted. A numerical study by Linder et al. (2005) could not retrieve the Afromontane, but here it is clearly defined as the Southern Afromontane (A1) and the Northern Mistbelt (E), with various species related to both leguminochoria. Goldblatt (1978) also noted the presence of mutual key species between the Southern Afromontane and the Coastal Region (e.g. *Crotalaria globifera*, *Dalbergia obovata* and *Tephrosia macropoda* var. *macropoda* in this study). This leguminochorion is included in the Maputaland-Pondoland Region (Van Wyk and Smith 2001), Natal (Linder et al. 2005) and Core Afromontane (Steenkamp et al. 2005).

Albany Centre (A2)

In terms of bioregions, the Albany Centre is shared equally in the Albany Thicket and Sub-Escarpment Grassland (Figure 3 and Table 2) and in the Albany Thicket and Grassland biomes (Table 3).

The climate characteristics that prevail in this region (Figure 4) are a medium annual rainfall (400–800 mm), minimum temperatures of mainly 2–8°C and moderate maximum temperatures of 25–29°C. A wide range of soil pH levels is present in this leguminochorion with a phosphorus content of 5–35 $mgkg^{-1}$ and non-sodic soils (Figure 4). The relatively high extreme temperatures (>40°C) noted for this leguminochorion (Table 4) is also noted for the Coastal Region (A5).

The Albany Centre has some key species in common with the Drakensberg Alpine Centre, the Summer Rainfall Region and the Northern Mistbelt (Table 5) (e.g. *Rhynchosia totta* var. *totta*) with high occurrences of *Indigofera zeyheri* and *Tephrosia capensis* var. *capensis*. Van Wyk and Smith (2001) confirm that floristic elements of many other regions converge in the Albany Centre, although it is not strongly evident in the present study. This leguminochorion forms part of the Kalahari-Highveld Transition Zone and Afromontane (Goldblatt 1978), the Albany Centre (Van Wyk and Smith 2001), Natal (Linder et al. 2005) and the Southern Succulent Karoo and Southeastern Fynbos (Steenkamp et al. 2005).

Northern Highveld Region (A3)

The Northern Highveld Region does not fall exclusively in the Afromontane; most QDGCs lie within areas of higher altitude and lower rainfall compared to the Southern Afromontane. The Mesic Highveld Grassland is the key bioregion present in this leguminochorion; while Grassland is the biome that is best represented (Figure 3, Table 2 and 3).

The main difference between the Northern Highveld Region (A3) and Southern Afromontane (A1) is the overall lower rainfall (400–800 mm) noted for the former (Figure 4). The low minimum temperatures (mainly <4°C) and the relatively high number of frost days per year (Table 4) occurring in the Northern Highveld Region are also in contrast to the Southern Afromontane. Low pH (<6.4) and low soil phosphorus values (<10 $mgkg^{-1}$) as well as non-sodic soils are noted for this leguminochorion (Figure 4). Schulze (2007) shows that high altitudes (800–2000 m) are documented for this leguminochorion, higher than for the Southern Afromontane, but lower than for the Drakensberg Alpine Centre (Table 4).

The Northern Highveld Region has some key species in common mostly with the Southern Afromontane, the Drakensberg Alpine Centre and the Summer Rainfall Region (e.g. *Rhynchosia totta* var. *totta* and *Trifolium africanum* var. *africanum*) (Table 5). The Highveld phytochorion, described by Steenkamp et al. (2005), shows similar, but a more confined pattern extending into the Central Bushveld Region. This leguminochorion is included in the Zambezian Region and Kalahari-Highveld Transition Zone (Goldblatt 1978) and in the Highveld (Steenkamp et al. 2005).

Drakensberg Alpine Centre (A4)

The areas covered by the Drakensberg Alpine Centre is shown to be in the Mesic Highveld, Drakensberg Grassland and Sub-Escarpment that forms the key bioregions, with Grassland the only biome part of this leguminochorion (Figure 3, Table 2 and 3).

Figure 4 clearly shows that the Drakensberg Alpine Centre falls in a high-rainfall area (mostly >800 mm) with relatively low minimum (<2°C) and maximum (<27°C) temperatures. Owing to the high rainfall, the soil low pH (<6.4) and phosphorus content of <10 $mgkg^{-1}$ is to be expected (Figure 4). Legume species adapted to low soil phosphorus and pH have an important role to play in subtropical and tropical regions (Oberson et al. 2006). This leguminochorion is further defined by a high number of days of heavy frost per year (a maximum of 80 days) and more than six cold spells per year with temperatures lower than -2.5°C on three or more consecutive days (Table 4). Also noteworthy is that this leguminochorion has the highest elevation range of all the leguminochoria (>2000 m).

The Drakensberg Alpine Centre has some mutual key species with the Southern Afromontane and the Northern Highveld Region (e.g. *Rhynchosia totta* var. *totta* and *Trifolium africanum* var. *africanum*) (Table 5). *Lessertia perennans* var. *perennans* has the highest occurrence (diagnostic species) and is not present as key species in any other leguminochoria. No link with the Cape flora can be established when comparing key species. The Afromontane (Goldblatt 1978), Drakensberg Alpine Centre (Van Wyk and Smith 2001), Natal (Linder et al. 2005) and the Drakensberg Alpine (Steenkamp et al. 2005) are included in this leguminochorion.

Coastal Region (A5)

The Indian Ocean Coastal Belt Bioregion contains most QDGCs found in the Coastal Region, followed by the Lowveld and Sub-Escarpment Savannah Bioregion (Figure 3 and Table 2). In terms of biomes, the Indian Ocean Coastal Belt is highly represented in this leguminochorion, followed by the Savannah biome (Table 3).

High annual rainfall (>800 mm/year), high minimum temperatures (>6°C) and moderate to high maximum temperatures represent the climatic conditions of the Coastal Region (Figure 4). As in the case of most of the "A" leguminochoria, relatively low pH and phosphorus levels as well as non-sodic soils are typical properties of the Coastal Region. The fact that this leguminochorion lies in a frost-free area with extreme maximum temperatures of >40°C (Table 4) could be important when selecting legume species for further evaluation.

The Coastal Region has some key species in common with the Southern Afromontane, the Summer Rainfall and the Northern Mistbelt (e.g. *Zornia capensis* subsp. *capensis*, also a diagnostic species) (Table 5). High occurrences of *Chamaecrista mimosoides* and *Eriosema salignum* is also noted. The Tongaland-Pondoland Region has elements of the Afromontane (Goldblatt 1978) and it is confirmed here. This leguminochorion forms part of the Tongaland-Pondoland Region (Goldblatt 1978), the Maputaland-

Pondoland Region (Van Wyk and Smith 2001), the Natal and Zambezian Central (Linder et al. 2005) and Core Afromontane and Greater Maputaland (Steenkamp et al. 2005).

Seasonal Rainfall Group (all-year, winter and summer rainfall) (B)

Regions in South Africa, Lesotho and Swaziland that receive rain throughout the year or in either winter or summer are essentially grouped in this cluster. Cluster "B" is the largest cluster and includes the Generalist Group containing many QDGC with only one legume species. One manifestation of data deficiency encountered in the present study was that many of the grids containing only one legume species were grouped in this "residue" Generalist Group. The Seasonal Rainfall Group is subdivided into the seven leguminochoria: Arid Western Region (B1), Lower-rainfall Cape Floristic Region (B2), Central Arid Region (B3), Generalist Group (B4), Summer Rainfall Region (B5), Northern & Northeastern Savannah Region (B6), Kalahari Bushveld Region (B7).

Arid Western Region (B1)

The area covered by the Arid Western Region shows that the Namaqualand Hardeveld Bioregion is well represented in this leguminochorion (Figure 3 and Table 2), followed by the Bushmanland Bioregion. The Succulent Karoo and Fynbos are the key biomes present in this leguminochorion (Table 3).

Low annual rainfall (<400 mm) with high minimum and maximum temperatures denotes the Arid Western Region (Figure 4). The high soil pH (>7.5) and medium soil phosphorus content is to be expected in the light of the low rainfall in the region. This is the first leguminochorion noted for its medium (52.4%) and highly sodic soils (14.3%) containing relatively high amounts of sodium (Figure 4). The poor infiltration rate and drainage when the soil is wet and hardness when it is dry are especially problematic for good seed germination and erosion control (Qadir and Oster 2004). The information derived from Schulze (2007) as described in Table 4, shows that the extreme maximum temperatures measured exceed 44°C, with high solar radiation from November to February.

The Arid Western Region has some key species in common with the Lower- and Higher-rainfall Cape Floristic Region (e.g. *Sutherlandia frutescens*), but most key species, mainly belonging to the genus *Aspalathus*, are not common with any other leguminochorion (Table 5). Jürgens (1997) and Goldblatt and Manning (2002) recognised that the Succulent Karoo Region forms part of a greater Cape Flora rather than the Nama-Karoo Region and the present study supports this view. The Succulent Karoo Region, not identified as a phytochorion by Linder et al. (2005), is clearly delineated in this study. The Karoo-Namib Region and Cape Region (Goldblatt 1978), the Namaqualand-Namib Domain and Cape Floristic Region (Cowling et al. 1998), the Gariep Centre, Succulent Ka-

roo and Cape Floristic Region (Van Wyk and Smith 2001), the Namib-Karoo and Cape (Linder et al. 2005) and the Northern Succulent Karoo, Southern Succulent Karoo and the Northwestern Fynbos (Steenkamp et al. 2005) are included in this leguminochorion.

Lower-rainfall Cape Floristic Region (B2)

The Albany Thicket and Eastern Fynbos Renosterveld are well represented in the Lower-rainfall Cape Floristic Region (Figure 5 and Table 2). Fynbos is the predominant biome, followed by the Albany Thicket (Table 3).

The annual rainfall figures in Figure 4 indicate that 200–600 mm annual rain is expected for this leguminochorion, but <400 mm is also a probability. Relatively high minimum (2–8°C) and moderate maximum (25–28°C) temperatures are noted. The soil pH varies greatly, with predominantly acidic soils. Half of the soils in this leguminochorion are medium sodic, similar to those of the Arid Western Region (Figure 4). From Table 4 it is clear that this region is mainly semi-arid, cool and dry, with extreme maximum temperatures 36–42°C. It is mainly a frost-free area, but there is a likelihood of <40 days of heavy frost per year.

The majority of key species of the Lower-rainfall Cape Floristic Region are not present in other leguminochoria, indicating their uniqueness to this leguminochorion (Table 5). Some of the key species are mostly in common with the Higher-rainfall Cape Floristic Region, e.g. *Sutherlandia frutescens* and *Aspalathus nigra*. The floristic link of the Cape Region with the Drakensberg Alpine Centre as acknowledged by Goldblatt (1978) and Steenkamp et al. (2005) could not be confirmed with key legume species. A phytochorion termed Southeastern Fynbos, with a similar pattern except for the inclusion of the eastern part of the Cape Region, has also been defined by Steenkamp et al. (2005). The latter authors further speculate that the orientation of the regional mountains could be responsible for the Southeastern (east-west orientation) and Northwestern Fynbos (north-south orientation) phytochoria as described by them. This hypothesis seems supported for the Lower-rainfall Cape Floristic Region, but not for the Higher-rainfall Cape Floristic Region. Goldblatt and Manning's phytogeographical centres (Goldblatt and Manning 2002) Karoo Mountains, Langeberg, Agulhas Plains and Southeastern Centre closely follow the east-west orientation of the Lower-rainfall Cape Floristic Region. This leguminochorion forms part of the Cape Region (Goldblatt 1978), the Worcester-Robertson Karoo Centre, the Little Karoo Centre and the Cape Floristic Region (Van Wyk and Smith 2001), the Cape (Linder et al. 2005) and the Southeastern Fynbos (Steenkamp et al. 2005).

Central Arid Region (B3)

The area covered by the Central Arid Region clearly shows that this leguminochorion forms mainly in the dry Eastern Kalahari Bushveld, Bushmanland, Dry Highveld Grass-

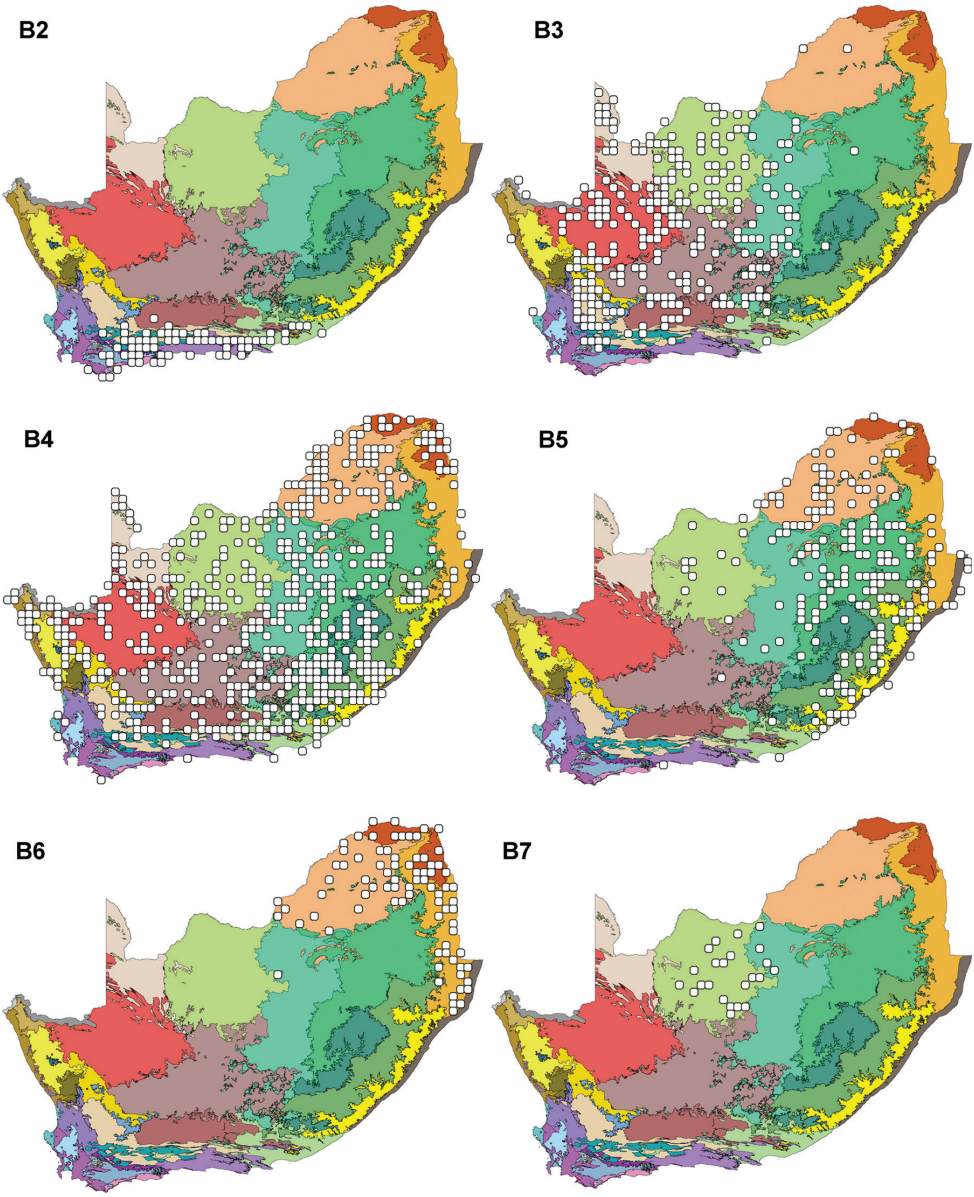

Figure 5. The Leguminochoria **B2–B7** superimposed on the Bioregions of southern Africa. Cluster B (Seasonal Rainfall Group) is divided into the Lower-rainfall Cape Floristic Region (**B2**); the Central Arid Region (**B3**); the Generalist Group (**B4**); the Summer Rainfall Region (**B5**); the Northern & Northeastern Savannah Region (**B6**) and the Kalahari Bushveld Region (**B7**). For the distribution of leguminochorion B1, see Figure 3. The leguminochoria is mapped on bioregions defined by (Rutherford et al. 2006) referring to the legend in Figure 2.

land and Upper Karoo Bioregions (Figure 5 and Table 2). It is noteworthy that the Rainshadow Valley Karoo Bioregion is fairly well represented in this leguminochorion. The Nama-Karoo and Savannah biomes largely represents this leguminochorion (Table 3).

The low annual rainfall of <400 mm noted in Figure 4 is to be expected. The relatively low minimum and high maximum temperatures are also normal for a semi-arid to arid region as Schulze (2007) describes this region in Table 4. The relatively low net primary production as compared to that of the other leguminochoria is noteworthy. The high pH (>7.5) and high soil phosphorus content (>20 $mgkg^{-1}$) defined for the Central Arid Region are expected considering the low annual rainfall (Figure 4). A very small percentage of soils in this leguminochorion are termed medium or highly sodic.

The Central Arid Region lies in the Karoo-Namib Region and the Kalahari-Highveld Transition Zone of Goldblatt (1978). Not surprisingly, most of the key species are also found as key species in the Kalahari Bushveld Region (e.g. *Indigofera alternans* var. *alternans* and *Indigofera daleoides* var. *daleoides*) (Table 5). Other regions that describe this leguminochorion include the Namib-Karoo and Eastern Karoo (Linder et al. 2005) and the Central Karoo and the Southern Succulent Karoo (Steenkamp et al. 2005).

Generalist Group (B4)

Bioregions and biomes not present in the Generalist Group are the Fynbos, eastern parts of the Mesic Highveld Grassland, parts of the Sub-Escarpment Grassland and Savannah, Lowveld and Indian Ocean Coastal Belt. The highest percentage bioregions present are the Central Bushveld, Eastern Kalahari Bushveld and Dry Highveld Grassland Bioregions (Figure 5 and Table 2). Savannah and Grassland biomes are most presented (Table 3).

The wide area covered by the Generalist Group is reflected in the wide-ranging climatic and soil conditions shown in Figure 4. Regions with relatively low annual rainfall (<400 mm), low minimum (<2°C) and high maximum (27–35°C) temperatures form mainly part of this leguminochorion. Soils are generally relatively alkaline (pH >7.5) and low in phosphorus (<10 $mgkg^{-1}$). Owing to the wide area covered, Table 4 gives no additional climatic and agrohydrological information.

Notwithstanding its wide distribution, the Generalist Group has various key species that also occur in the Central Arid Region, the Kalahari Bushveld Region and the Albany Centre (e.g. *Melolobium candicans* and *Indigastrum argyraeum*) (Table 5).

Summer Rainfall Region (B5)

The key bioregions that comprise the Summer Rainfall Region are the Mesic Highveld Grassland and the Central Bushveld, with Grassland and Savannah as key biomes (Figure 5, Table 2 and 3).

The Summer Rainfall Region falls in areas with an annual rainfall of mainly 400–800 mm (Figure 4). Very low minimum temperatures (<4°C) and moderate to high

maximum temperatures are recorded. The phosphorus content of soils grouped in the leguminochorion is mainly below 10 $mgkg^{-1}$, with acidic and non-sodic soils (Figure 4). Owing to the wide area covered, Table 4 gives little additional climatic and agrohydrological information.

The Summer Rainfall Region shares some key species with the Southern Afromontane, the Northern Highveld Region and the Central Bushveld Region (e.g. *Rhynchosia totta* var. *totta* and *Eriosema salignum*) (Table 5). *Tephrosia semiglabra* is the only key species not present as key species in other leguminochoria. Three of Goldblatt's phytogeographical regions fall in this leguminochorion, namely the Zambezian Region, the Kalahari-Highveld Transition Zone and the Tongaland-Pondoland Region (Goldblatt 1978).

Northern & Northeastern Savannah Region (B6)

For the Northern & Northeastern Savannah Region, the Central Bushveld and Lowveld are the two key bioregions, with the Mopane Bioregion listed as a minor component (Figure 5 and Table 2). The Savannah biome represents this leguminochorion in full (Table 3).

Medium annual rainfall (400–800 mm) and relatively high minimum (>6°C) and maximum (27–35°C) temperatures characterise the Northern & Northeastern Savannah Region (Figure 4). Soils are generally acidic, low in phosphorus and non-sodic. This is the only leguminochorion where 16 occurrences of heat waves of >30°C on three or more consecutive days per year are noted in Table 4.

The Northern & Northeastern Savannah Region shares many key species with the Subtropical Lowveld & Mopane Region (e.g. *Dichrostachys cinerea* subsp. *africana* var. *africana* and *Ormocarpum trichocarpum*) (Table 5). Many key species are tree species, e.g. *Pterocarpus rotundifolius* subsp. *rotundifolius* and *Faidherbia albida*. This leguminochorion is included in the Zambezian and the Tongaland-Pondoland Regions (Goldblatt 1978), the Zambezian-central (Linder et al. 2005) and the Greater Maputaland (Steenkamp et al. 2005).

Kalahari Bushveld Region (B7)

It is evident that the Eastern Kalahari Bushveld Bioregion nearly uniquely represents the Kalahari Bushveld Region (Figure 5 and Table 2). In terms of biomes, this leguminochorion lies nearly fully in the Savannah (Table 3).

A relatively medium annual rainfall of 400–800 mm to very low rainfall of <400 mm occurs in the Kalahari Bushveld Region (Figure 4). Low minimum temperatures (<2°C) and high maximum (>27°C) temperatures prevail in this leguminochorion. The slightly acidic (pH = 6.5–7.4), relatively low phosphorus content (<10 $mgkg^{-1}$) and non-sodic soils are described as the main soil properties. Information derived from Schulze (2007) as described in Table 4 indicates that this is a semi-arid, dry area with plains and pans.

The Kalahari Bushveld Region has various key species that are associated with the Central Arid Region and with the Central Bushveld Region (e.g. *Indigofera daleoides*

var. *daleoides* and also a diagnostic species) (Table 5). *Tephrosia burchellii* has a high occurrence and not found as key species in other leguminochoria. Even though the two leguminochoria are from different bioregions, both lie within the Savannah biome and a floristic link is therefore to be expected. The Kalahari-Highveld Transition Zone (Goldblatt 1978), the Griqualand West Centre (Van Wyk and Smith 2001), the Eastern Karoo and the Karoo Transition (Linder et al. 2005) and the Central Karoo (Steenkamp et al. 2005) form part of this leguminochorion.

Higher-rainfall Cape Floristic Region (C)

The key bioregion present in the Higher-rainfall Cape Floristic Region is the Eastern Fynbos Renosterveld with the Southwest Fynbos second highest (Figure 6 and Table 2). This leguminochorion lies entirely in the Fynbos biome (Table 3).

Figure 4 indicates that the annual rainfall is mostly 200–600 mm per year, but that regions of higher rainfall are also included in this leguminochorion. If this is compared with the Lower-rainfall Cape Floristic Region, it is evident that these leguminochoria could be defined individually on the basis of lower and higher annual rainfall. Information derived from Schulze (2007) further confirms the higher rainfall levels in this leguminochorion compared to the Lower-rainfall Cape Floristic Region (Table 4). The minimum temperatures of 2–8°C and maximum temperatures of 25–29°C could be expected in this region. Mostly acidic soils with a wide range of soil phosphorus content is present in this leguminochorion (Figure 4). A high percentage of soils are medium sodic (ESP 6–15%), indicating poor infiltration and drainage, with resultant loss of soil (Qadir and Oster 2004). The leguminochorion forms in a frost-free area (Table 4).

Key species of the Higher-rainfall Cape Floristic Region are found mostly in the Lower-rainfall Cape Floristic Region and only a few in the Arid Western Region (e.g. *Sutherlandia frutescens* and *Indigofera heterophylla*) (Table 5). Most of the key species are not associated with any other leguminochorion, signifying their unique association with this leguminochorion (e.g. *Aspalathus ciliaris* and *Aspalathus hispida* subsp. *hispida*). Key species in this region have no floristic link with the Drakensberg Alpine Centre as acknowledged by Goldblatt (1978) and Steenkamp et al. (2005). Goldblatt and Manning's (2002) phytogeographical centres termed the Northwestern Centre and especially the Southwestern Centre follow the north-south orientation found mainly in this leguminochorion. This leguminochorion forms part of the Cape Region (Goldblatt 1978), the Cape Floristic Region (Van Wyk and Smith 2001), and the Cape (Linder et al. 2005) and the Northwestern and Southeastern Fynbos (Steenkamp et al. 2005).

Savannah Group (D)

The Savannah Group is subdivided into the Central Bushveld Region (D1) and the Subtropical Lowveld & Mopane Region (D2). Relatively high extreme maximum temperatures with early summer to midsummer rain higher than 400 mm rain is described

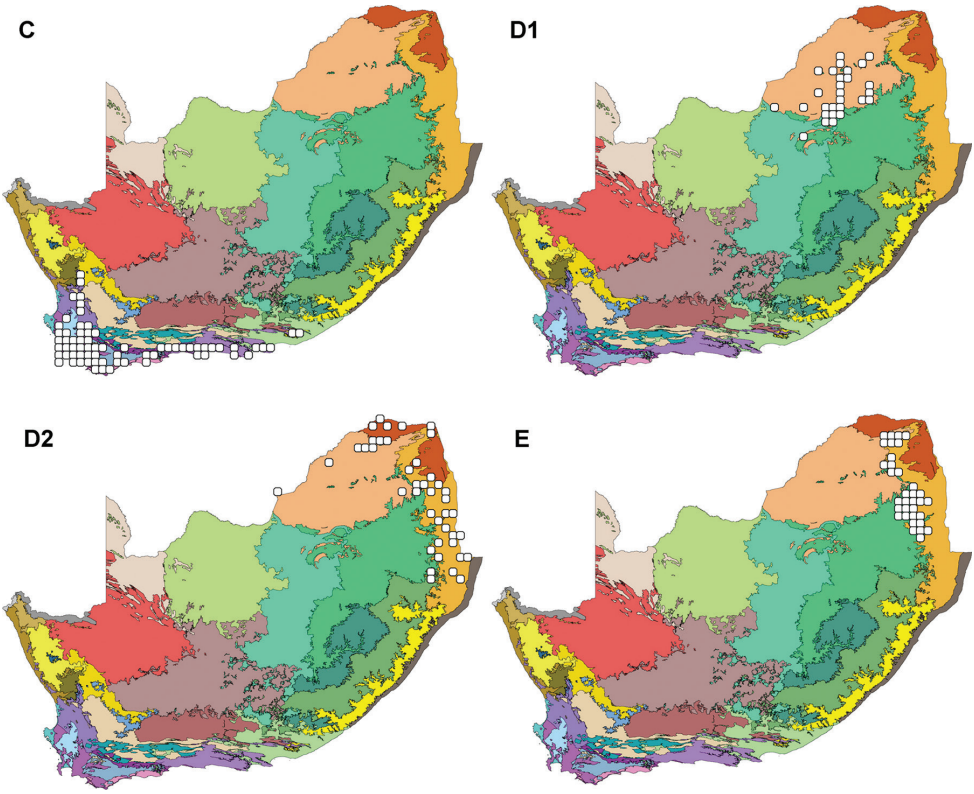

Figure 6. The Leguminochoria **C–E** superimposed on the Bioregions of southern Africa. The Higher-rainfall Cape Floristic Region (Cluster C) and Cluster D (Savannah Group) is divided into the Central Bushveld Region (**D1**) and the Subtropical Lowveld & Mopane Region (**D2**) as well as the Northern Mistbelt (Cluster E). The leguminochoria is mapped on bioregions defined by (Rutherford et al. 2006) referring to the legend in Figure 2.

for this leguminochorion. The region is dry and hot, with a relatively average net primary production (Table 4).

Central Bushveld Region (D1)

Figure 6 shows that the area covered by the Central Bushveld Region is uniquely formed in the Central Bushveld Bioregion and the Savannah biome (Table 2 and 3), but a number of QDGCs lie in the transitional zone between the Central Bushveld and the Mesic Highveld Grassland Bioregion.

The Central Bushveld Region lies in a zone of annual rainfall of 400–800 mm, with relatively high minimum (2–8°C) and maximum (27–35°C) temperatures (Figure 4). Moderately acidic to neutral soils with low phosphorus levels (<10 $mgkg^{-1}$) as well as non-sodic soils occur in this region (Figure 4). Information derived from Schulze (2007) describes this area as dry and hot or cool (Table 4).

Key species of the Central Bushveld Region are found in the Summer Rainfall Region, the Kalahari Bushveld Region and the Subtropical Lowveld & Mopane Region (*Acacia karroo* and *Mundulea sericea* subsp. *sericea*) therefore largely in the Savannah biome (Table 5). *Burkea africana* has a high occurrence and is not noted as key species in other leguminochoria. The Zambezian Region (Goldblatt 1978), the Soutpansberg and Wolkberg Centres (Van Wyk and Smith 2001), the Zambezian-central (Linder et al. 2005) and the Highveld (Steenkamp et al. 2005) form part of this leguminochorion.

Subtropical Lowveld & Mopane Region (D2)

The Subtropical Lowveld & Mopane Region forms part of the Lowveld, followed by the Central Bushveld and Mopane Bioregions (Figure 6 and Table 2). The Savannah is the only biome that represents the leguminochorion (Table 3).

The expected annual rainfall for the leguminochorion is 400–800 mm per year, but lower and higher rainfall figures are also likely (Figure 4). Relatively high minimum (>6°C) and maximum (27–35°C) temperatures predominate this region. The pH range in the Subtropical Lowveld & Mopane Region varies widely, with soils acidic to alkaline, but mostly below 7.4. Most soils are low in phosphorus, but a considerable portion contains more than 10 $mgkg^{-1}$. Only non-sodic soils are found in this leguminochorion. The main differences between the "D" leguminochoria are that wider ranges of rainfall and soil pH are noted for the Subtropical Lowveld & Mopane Region compared to the Central Bushveld Region. Table 4 shows that extreme maximum temperatures of >40°C are expected in this region.

The key species of the Subtropical Lowveld & Mopane Region are linked mostly with the Northern & Northeastern Savannah Region (e.g. *Dichrostachys cinerea* subsp. *africana* var. *africana* and *Ormocarpum trichocarpum*) (Table 5). This leguminochorion is included in the Zambezian Region and Tongaland-Pondoland Region (Goldblatt 1978), the Zambezian-central (Linder et al. 2005) and Greater Maputaland (Steenkamp et al. 2005).

Northern Mistbelt (E)

The Mesic Highveld Grassland, Lowveld and Central Bushveld are the key bioregions found in the Northern Mistbelt whereas Savannah is the main biome prevailing in this leguminochorion (Table 2 and 3). It is clear from Figure 6 that this leguminochorion lies in the transitional zone between the aforementioned bioregions.

A high annual rainfall of >800 mm, noted for most of the region included in this leguminochorion, is to be expected for the Northern Mistbelt (Figure 4). Moderate minimum temperatures of 2–8°C and maximum temperatures of 25–29°C are described for this leguminochorion. Acidic (pH <6.4), low phosphorus (<10 $mgkg^{-1}$) and non-sodic soils are present in this leguminochorion (Figure 4). According to Table 4,

the leguminochorion falls in a frost-free area, with altitudes of 600–2000 m, slightly lower than in the case of the Drakensberg Alpine Centre.

The Northern Mistbelt shares some key species with the Southern Afromontane, the Coastal Region, the Summer Rainfall Region and the Central Bushveld Region (e.g. *Zornia capensis* subsp. *capensis* and *Vigna vexillata* var. *vexillata*) (Table 5). A high occurrence of key species is evident in the presence of a large number of diagnostic species, clearly more than in any other leguminochoria. Goldblatt (1978) speculated that the typical Afromontane taxa may have originated from neighbouring lowland flora termed the Coastal Region in this study. The Afromontane (Goldblatt 1978), the Zambezian-central (Linder et al. 2005) and Core Afromontane (Steenkamp et al. 2005) are incorporated in this leguminochorion.

Species richness, range and growth form

Table 6 gives relevant information on the legume species richness for each leguminochorion as well as the lowest and highest number of legumes collected in the QDGCs within each leguminochorion. The smaller leguminochoria, namely the Higher-rainfall Cape Floristic Region, the Savannah Group and the Northern Mistbelt, have very high species richness, whereas the larger Seasonal Rainfall Group, has a below average species richness. This variation is probably due to the presence of the smaller leguminochoria in the higher-rainfall regions (both temperate and subtropical), while most of the Seasonal Rainfall Group are present in the lower-rainfall (arid) regions. Pausas and Austin (2001) confirm that there is a tendency for species richness to increase with increasing availability of water.

The species range (Table 6) within the Sourveld and Mixed Veld Group, shows that the highest range is recorded in the Coastal Region also noted for recording the highest rainfall. The higher species range of the Lower-rainfall Cape Floristic Region within the Seasonal Rainfall Group is to be expected considering the well-known species richness of the Cape Floristic Region. It is noteworthy that a difference in species richness and species range is recorded between the Lower- and Higher-rainfall Cape Floristic Region. The Lower-rainfall Cape Floristic Region shows average records while the Higher-rainfall Cape Floristic Region shows above average records. Also noteworthy is the relatively high species range of the Savannah Group compared to that of the Northern & Northeastern Savannah Region, the two leguminochoria having similar areas covered in mainly the Savannah Bioregion.

The different growth forms of key species for each phytochorion are shown in Figure 7. As highlighted by Pérez-Harguindeguy (2013), growth form may be associated with ecophysiological adaptation, for example where plant species optimise height and foliage arrangement to avoid or resist grazing by certain herbivores, with prostrate growth forms being correlated with high grazing pressure. The dominant growth form in the Sourveld and Mixed Veld Group (A1–A5) is perennial herbs, with a noteworthy number of climber species. Tree species are the least represented of all

Table 6. Quarter degree grid cell (QDGC) percentage, species richness and range within each leguminochorion of southern Africa. Species richness = #Species/#QDGC in each leguminochorion; Species range = lowest and highest species count/QDGC.

Leguminochorion	% QDGC	Species richness	Species range	Species range mean
A1: Southern Afromontane	2.3	7.7 ±6.0	10–62	26.5 ±11.8
A2: Albany Centre	1.3	11.9 ±13.0	15–65	36.3 ±15.9
A3: Northern Highveld Region	2.4	6.5 ±7.7	10–49	26.8 ±9.5
A4: Drakensberg Alpine Centre	2.5	7.4 ±9.1	8–60	25.4 ±13.6
A5: Coastal Region	2.4	9.1 ±10.7	26–104	51.4 ±20.5
B1: Arid Western Region	4.6	5.3 ±4.4	4–47	17.2 ±9.3
B2: Lower-rainfall Cape Floristic Region	4.1	7.3 ±7.2	9–74	23.4 ±12.3
B3: Central Arid Region	16.7	3.0 ±3.3	1–31	5.3 ±4.8
B4: Generalist Group	34.4	2.0 ±1.7	1–21	3.6 ±3.0
B5: Summer Rainfall Region	12.2	3.2 ±2.6	1–25	9.1 ±5.4
B6: Northern & Northeastern Savannah Region	5.0	4.6 ±4.1	5–36	18.1 ±6.8
B7: Kalahari Bushveld Region	1.4	5.9 ±6.7	11–36	20.6 ±7.5
C: Higher-rainfall Cape Floristic Region	4.2	11.9 ±15.3	34–174	69.6 ±29.1
D1: Central Bushveld Region	1.7	12.6 ±16.6	29–198	67.3 ±34.3
D2: Subtropical Lowveld & Mopane Region	2.7	9.3 ±10.4	4–76	47.6 ±13.8
E: Northern Mistbelt	2.1	13.5 ±19.2	28–213	83.6 ±37.1
Mean	**100.0**	**7.6**	**12–79**	

growth forms. In the Seasonal Rainfall Group (B1–B7), there is a clear increase in the number of shrubs and trees, especially in the Lower-rainfall Cape Floristic Region (i.e. shrubs) and the Northern & Northeastern Savannah Region (i.e. trees). The dominance of dwarf shrubs and shrubs in the Higher-rainfall Cape Floristic Region (C) is similar to the situation in the Lower-rainfall Cape Floristic Region. All growth forms are present in the Savannah Group (D1–D2), with herbs dominating the Central Bushveld Region and trees the Subtropical Lowveld & Mopane Region. Key species of all growth forms in almost equal parts were recorded in the Northern Mistbelt (E). The diagnostic species, i.e. species with occurrences of 70% or higher in a given leguminochoria show dominance in the herb growth form, with nearly equal numbers of the remaining growth forms.

Legume assemblages

The six assemblages computed by PHYTOTAB-PC are listed in Table 7. Group 1 includes the southern and western Cape Region covering the Succulent Karoo and Fynbos biomes. Group 2 includes two relatively low-rainfall leguminochoria and the Generalist Group covering the Nama Karoo and western Savannah. Group 3 represents the Albany Centre, which is noted as a single entity, indicating no floristic links with any

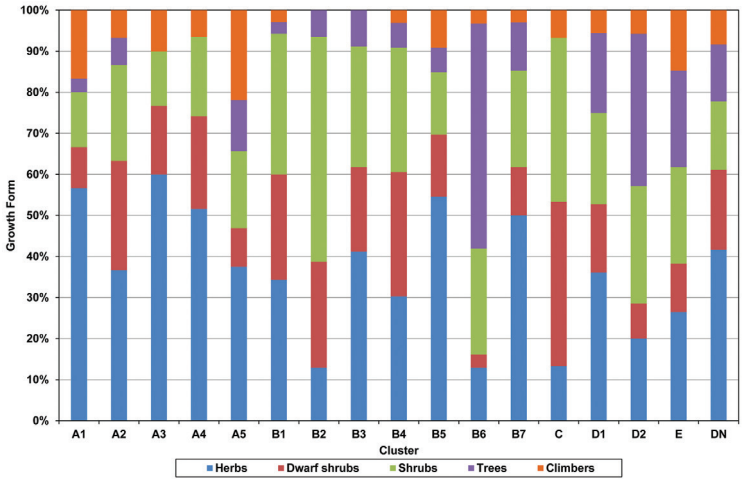

Figure 7. The growth forms of key species recorded in leguminochoria (**A1–E**) of southern Africa. Growth forms are defined as: **1** herb is a small, non-woody seed-bearing plant in which the aerial parts die back at the end of each growing season **2** dwarf shrub is a plant smaller than a shrub which produces wood at its base and has abundant growth branching upward from the base, the upper stems dying back at the end of each growing season **3** shrub is a perennial woody plant less than 10m tall which branches low or near ground level into several main stems although it has no clear trunk **4** tree is a woody plant which grows more than 10m tall, characteristically it has one main stem and **5** climber is a plant with aerial tendrils which it uses to attach itself to a host or surface for support (Germishuizen and Meyer 2003). **DN**: diagnostic species are species with occurrences of 70% or higher. The leguminochoria are termed **A1** Southern Afromontane **A2** Albany Centre **A3** Northern Highveld Region **A4** Drakensberg Alpine Centre **A5** Coastal Region **B1** Arid Western Region **B2** Lower-rainfall Cape Floristic Region **B3** Central Arid Region **B4** Generalist Group **B5** Summer Rainfall Region **B6** Northern & Northeastern Savannah Region **B7** Kalahari Bushveld Region **C** Higher-rainfall Cape Floristic Region **D1** Central Bushveld Region **D2** Subtropical Lowveld & Mopane Region **E** Northern Mistbelt.

of the other leguminochoria. The inclusion of the north-eastern parts of South Africa into Group 4 that covers the Savannah biome is to be expected. The Drakensberg Alpine Centre in Group 5 has no apparent floristic link with the Afromontane regions and forms part of the Grassland biome. Group 6 is a well-defined Afromontane region that includes the coastal areas below the Drakensberg.

The result of the Pearson's correlation matrix for the legume assemblages grouped by PHYTOTAB-PC is shown in Table 8. The Pearson's correlation matrix indicates that for F1, soil pH and mean annual minimum temperature (negative) are the main drivers for distinguishing among legume assemblages, whereas for F2, soil phosphorus level is the main driver. The result for the discriminant analysis is shown in Figure 8 where only the centroids and not all observations are shown due to the large dataset (largely overlying groups). The F1 function (soil pH and mean annual minimum temperature) accounts for 61.43% of the independent variables and the F2 function (soil phosphorus content) accounts for 23.59% of the independent variables (Figure 8).

Table 7. Classification of Leguminochoria of southern Africa in assemblages.

Assemblages	Leguminochoria included within an assemblage
1	Arid Western Region (B1), Lower-rainfall Cape Floristic Region (B2), Higher-rainfall Cape Floristic Region (C)
2	Central Arid Region (B3), Generalist Group (B4), Kalahari Bushveld Region (B7)
3	Albany Centre (A2)
4	Northern & Northeastern Savannah Region (B6), Central Bushveld Region (D1), Subtropical Lowveld & Mopane Region (D2)
5	Northern Highveld Region (A3), Drakensberg Alpine Centre (A4), Summer Rainfall Region (B5)
6	Southern Afromontane (A1), Coastal Region (A5), Northern Mistbelt (E)

Group 6 (Southern Afromontane, Coastal Region and the Northern Mistbelt) positioned to the left on the F1 axis contain species adapted to low soil pH and high minimum temperatures (Figure 8). Group 2 (Central Arid Region, Generalist Group and the Kalahari Bushveld Region) positioned to the right on the F1 axis contain species adapted to high soil pH and low minimum temperatures. Group 1 (Arid Western Region, Lower-rainfall Cape Floristic Region and the Higher-rainfall Cape Floristic Region) positioned at the upper level on the F2 axis contain species adapted to average soil pH and minimum temperatures and high soil phosphorus as opposed to Group 5 (Northern Highveld Region, Drakensberg Alpine Centre and Summer Rainfall Region) that contain species adapted to low soil phosphorus. Group 3 (Albany Centre) and Group 4 (Northern & Northeastern Savannah Region, Central Bushveld Region, Subtropical Lowveld & Mopane Region) are positioned more to the centre and contain species adapted to average soil pH, minimum temperatures and soil phosphorus. It is clear that legume assemblages were grouped mainly based on soil differences, followed by temperature, while rainfall was least important. Other studies, however, showed that the most important abiotic factors that control species distribution are temperature and moisture (Skarpe 1986, Woodward 1987, Ruiz-Vega 1994, Bond et al. 2003). It was corroborated by Greve (2011) that rainfall is the most important variable for the distribution of African vegetation for all vegetation types.

Davis' report (2011) on climate change in southern Africa indicate that small increases in temperature are unlikely to affect plant distribution in a desert (partly enclosed in the extreme northern part of the Arid Western Region), whereas in an arid to semi-arid ecotone (enclosed in the Arid Western Region, Central Arid Region, Kalahari Bushveld Region and Central Bushveld Region), plants could disappear owing to a higher biophysical vulnerability to climate change. In addition to temperature and moisture, Bond et al. (2003) and Midgley et al. (2007) highlight the significant effect of fire on South African vegetation. Fynbos (enclosed in the Lower-rainfall and Higher-rainfall Cape Floristic Region), at least in the more mesic areas, is a fire-dependent ecosystem and could support a forest or thicket. Summer-rainfall areas with an annual rainfall >650 mm (mainly the Southern Afromontane, Northern Highveld Region, Drakensberg Alpine Centre, Summer Rainfall Region and Northern Mistbelt) could become forest with the exclusion of fire, and with <650 mm could show no compositional change in fire-intolerant forest or

Table 8. Pearson's correlation coefficients for Leguminochoria assemblages of southern Africa.

Variables	F1	F2	F3
Mean annual rainfall (mm)	-0.555	-0.550	0.149
Maximum temperature (°C)	0.545	0.145	**0.695**
Minimum temperature (°C)	**-0.646**[a]	0.683	0.332
Soil phosphorus (mgkg^{-1})	0.391	**0.817**	-0.227
Soil pH (H_2O)	**0.798**	0.516	0.195

[a]Values in bold are different from 0 with a significance level alpha = 0.05

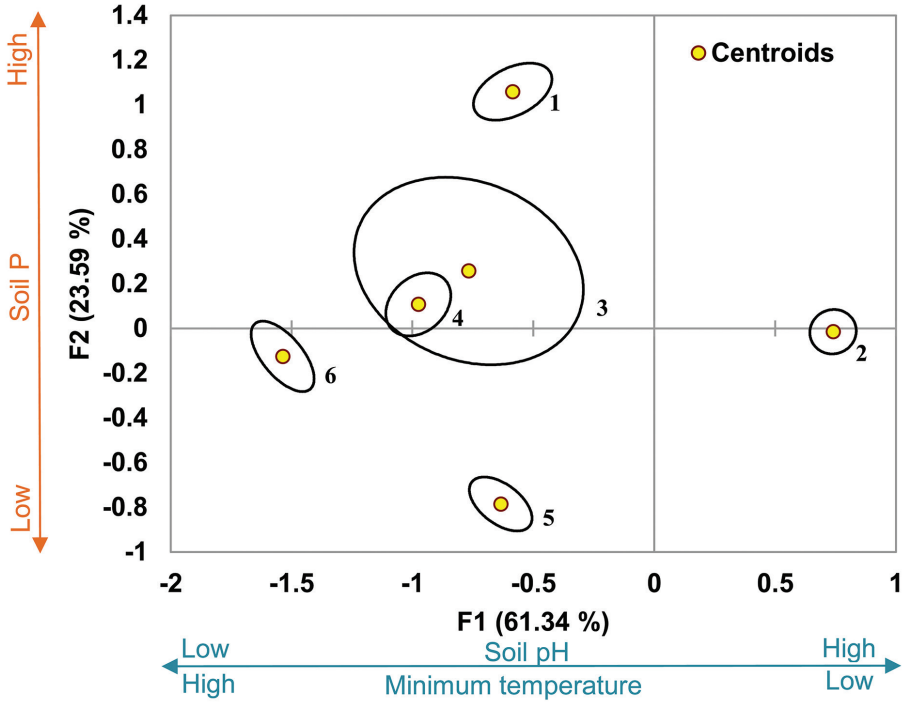

Figure 8. Discriminant analysis for legume assemblages of southern Africa. Only the centroids and not all observations are shown. Confidence ellipses around the centroids and drivers for Factor 1 (soil pH and minimum temperatures) and Factor 2 (soil phosphorus) are shown. The legume assemblages are **1** Arid Western Region, Lower-rainfall Cape Floristic Region, Higher-rainfall Cape Floristic Region **2** Central Arid Region, Generalist Group, Kalahari Bushveld Region **3** Albany Centre **4** Northern & Northeastern Savannah Region, Central Bushveld Region, Subtropical Lowveld & Mopane Region **5** Northern Highveld Region, Drakensberg Alpine Centre, Summer Rainfall Region and **6** Southern Afromontane, Coastal Region, Northern Mistbelt.

thicket species (climate-dependent grassy ecosystems) (mainly the Central Arid Region, Generalist Group, Northern and Northeastern Savannah Region, Kalahari Bushveld Region, Central Bushveld Region and Subtropical Lowveld & Mopane Region).

Conclusions

The Sourveld and Mixed Veld Group represents a group of legume species found mostly in the Grassland and Eastern Coastal Regions and to a lesser extent in the Albany Thicket and Lowveld Regions. The largest leguminochorion, the Seasonal Rainfall Group, includes all regions except the Higher-rainfall Cape Floristic Region and the Northern Mistbelt, being distinctly formed leguminochoria. The Lower-rainfall Cape Floristic Region shares part of the Eastern Fynbos-Renosterveld Bioregion with the Higher-rainfall Cape Floristic Region, although it is also found in the Albany Thicket. The Savannah Group forms part of the Central Bushveld, Lowveld & Mopane Bioregions, similar to the Northern & Northeastern Savannah Region. The smallest leguminochorion, the Northern Mistbelt, is found in the transitional zone between the Mesic Highveld Grassland, the Lowveld and the Central Bushveld Bioregions.

For the Sourveld and Mixed Veld Group, a commonality is the relatively high annual rainfall figures, low pH (< 6.4) and non-sodic soils noted. The minimum and maximum temperatures differ widely within the "A" clusters. It is clear that the Southern Afromontane can be distinguished from the Northern Highveld Region purely based on rainfall figures. The colder conditions that prevail in the Drakensberg Alpine Centre compared to those in the Southern Afromontane are evident from the climatic data, a conclusion also reached by Steenkamp et al. (2005). The Seasonal Rainfall Group shows that the annual rainfall is relatively low and that a relatively high maximum temperature prevails. The soil phosphorus content and pH of this cluster vary widely, but some soils are medium to highly sodic. The difference in climate between the two Cape Floristic Regions is evident where the Lower-rainfall Cape Floristic Region includes areas with annual rainfall figures of <400 mm, while the Higher-rainfall Cape Floristic Region includes areas with annual rainfall figures of >400 mm. The medium annual rainfall and high minimum and maximum temperatures are distinct attributes of the Savannah Group. The climatic and soil conditions for the Northern & Northeastern Savannah Region and the Savannah Group are without doubt comparable owing to similar areas covered. The Northern Mistbelt has a relatively high annual rainfall figure and moderate temperatures, similar to those of the Sourveld and Mixed Veld Group. A low soil phosphorus and pH value are recorded for the Northern Mistbelt.

The six legume assemblages that were identified are geographically sound. The separation of the Albany Centre is unexpected and merits further investigation, especially since some key species were noted as common to other leguminochoria and in the light of Van Wyk and Smith's (2001) observation that floristic elements of many other regions converge in this centre.

It is concluded in this first time study on the African continent that a single plant family, in this case the Leguminosae, do not necessarily follow vegetation units. The vegetation units can be correlated with limiting environmental factors even on a national scale using rainfall, soil pH, soil phosphorus and temperature. In this study, members of the Leguminosae formed clusters based on:

1) Distinctive patterns reflecting either vegetational or geographical regions, for example the Arid Western Region, the Lower- and Higher-rainfall Cape Floristic

Region, the Albany Centre and the Central Bushveld Region; 2) Non-distinctive vegetational patterns, for example the Generalist Group where most vegetational types are present or where residue grids (mainly those with fewer than three species) were grouped; 3) Functional types, for example the Northern Highveld Region with largely herbs and Northern & Northeastern Savannah Region largely trees are the main growth form.

With the exception of a few indigenous legume species (e.g. *Lablab purpureus*, *Lotononis bainesii* and *Vigna unguiculata*) successfully integrated in present-day pasture systems, the vast untapped genetic resources available for pasture screening or soil conservation programs, are evident from this study.

Acknowledgments

We thank the South African National Biodiversity Institute (SANBI) for making available the distribution and descriptive data, Yolande Steenkamp (SANBI) for advice on methods of vegetation analyses, Elsa van Niekerk (ARC-PPRI) for the graphics and two anonymous reviewers for useful comments and suggestions to improve the manuscript. Financial assistance from the University of Pretoria is acknowledged with thanks.

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Supplementary material 1

Statistical results of the clustering analysis using the Agglomerative Hierarchical Clustering method.

Authors: Marike Trytsman, Robert H. Westfall, Philippus J. J. Breytenbach, Frikkie J. Calitz, Abraham E. van Wyk

Data type: phylogenetic data

Supplementary material 2

Species recorded in each leguminochorion (A1–E) of southern Africa.

Authors: Marike Trytsman, Robert H. Westfall, Philippus J. J. Breytenbach, Frikkie J. Calitz, Abraham E. van Wyk

Data type: species data

Supplementary material 3

The predominant mean annual rainfall and minimum and maximum temperatures expressed as a percentage for southern African leguminochoria.

Authors: Marike Trytsman, Robert H. Westfall, Philippus J. J. Breytenbach, Frikkie J. Calitz, Abraham E. van Wyk

Data type: meteorological data

Supplementary material 4

The predominant soil phosphorus content, pH level and exchangeable sodium percentage (ESP) expressed as a percentage for southern African leguminochoria.

Authors: Marike Trytsman, Robert H. Westfall, Philippus J. J. Breytenbach, Frikkie J. Calitz, Abraham E. van Wyk

Data type: meteorological data

PhytoKeys 70: 97–110 (2016)
doi: 10.3897/phytokeys.70.9758
http://phytokeys.pensoft.net

RESEARCH ARTICLE

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Confirming the identity of two enigmatic "spiny solanums" (*Solanum* subgenus *Leptostemonum*, Solanaceae) collected by Jean-Baptiste Leschenault in Java

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Academic editor: *Eric Tepe* | Received 4 July 2016 | Accepted 15 September 2016 | Published 4 October 2016

Citation: Aubriot X, Loup C, Knapp S (2016) Confirming the identity of two enigmatic "spiny solanums" (*Solanum* subgenus *Leptostemonum*, Solanaceae) collected by Jean-Baptiste Leschenault in Java. PhytoKeys 70: 97–110. doi: 10.3897/phytokeys.70.9758

Abstract

Taxonomic revision of the tropical Asian species of *Solanum* revealed two names, *Solanum poka* Dunal and *Solanum graciliflorum* Dunal, whose identities were uncertain and whose application has always been tentative. Material collected in Java at the beginning of the 19th century by Jean-Baptiste Leschenault de la Tour and used to describe these taxa has not been found, despite extensive searches in European herbaria. We here stabilise use of these names by comparing herbarium specimens and drawings of original material made by the artist Toussaint François Node-Véran. Detailed descriptions with synonymy, preliminary conservation assessments and specimen citations are provided for both species. Lectotypes are designated for all names (including synonyms) and epitypes designated for *S. poka* and *S. graciliflorum* to stabilise usage.

Keywords

Exploration, Jean-Baptiste Leschenault de la Tour, Indonesia, Montpellier, Nicolas Baudin, Toussaint François Node-Véran, typification

Introduction

In 1800, shortly after he became First Consul of the Republic of France, Napoléon Bonaparte approved an expedition along the "coasts of New Holland" (Australia). The expedition, led by Nicolas Baudin, has been cited as one of the most ambitious and the most enriching for collections of natural history of the great scientific expeditions of the early 19th century (Cornell 1965, Fornasiero et al. 2010). Naturalists brought back from these distant and previously unexplored lands many new plant species, both as herbarium specimens and as living plants or seeds that were grown out mostly in the plant beds and greenhouses of the Muséum National d'Histoire Naturelle of Paris and in Josephine Bonaparte's gardens at Malmaison (Jangoux 2004, Fornasiero et al. 2010).

The Baudin expedition lasted four years (1800-1804) and its explicit purpose was "observation and research relating to Geography and Natural History". The crew included 24 scientists and artists, among them were three botanists and five gardeners that had been carefully selected by Antoine-Laurent de Jussieu, then director of the Muséum National d'Histoire Naturelle (Proust de la Gironière 2002, Jangoux 2004). By the time the *Géographe* and the *Naturaliste* reached Port Jackson (New South Wales) in June 1802 for a five month stopover, most of the botanical team had either died or left the expedition; only one botanist, Jean-Baptiste Leschenault de la Tour, and one gardener, Antoine Guichenot, remained (Desmet and Jangoux 2010). After collecting in Australia and continuing with the expedition, in 1803 Leschenault was left behind in Timor to recover from illness (Proust de la Gironière 2002, Desmet and Jangoux 2010). After his recovery, he left Timor for Java, but found himself unable to return to France, probably due to instability in Europe at the time. Leschenault was offered the protection of Nicolous Engelhard, the Dutch Governor of the northeastern coast of Java, and given the mandate to collect natural history specimens there (Van Steenis-Kruseman and Van Steenis 1950, Desmet and Jangoux 2010). For two years (1804-1806) Leschenault visited the islands of Java and Madoera where he claimed to have collected ca. 900 plant species (Leschenault 1807), all of which were presumably sent back to the herbarium of the Muséum National d'Histoire Naturelle in Paris (P). Several duplicates of Leschenault's collections in other groups have been found in G, K and L (Van Steenis-Kruseman and Van Steenis 1950), but no catalogue of his collections exists and an accurate estimate of the extant number of collections has yet to be compiled.

In the course of preparing a monographic revision of the spiny solanums from tropical Asia (see Aubriot et al. 2016 for discussion of the Old World clade of subgenus *Leptostemonum* Bitter), we were unable to find the type material for two spiny solanums from Java. *Solanum graciliflorum* Dunal and *S. poka* Dunal were first described by Michel-Félix Dunal in 1814 as part of the supplement of Lamarck's *Encyclopédie Méthodique* edited by Jean Poiret. He cited no herbarium material or collector but cited a drawing ("*Dun. Suppl. Sol. tab.*"; Dunal 1814) from his then unpublished synopsis of *Solanum* (published later as Dunal 1816). In later treatments of these species Dunal (1816, 1852) stated that the collections he had seen were made by Leschenault during his stay in Java (1803-1806). Thorough searches of the herbarium at P where

Leschenault's collections are housed, as well as other herbaria (see Materials and Methods) where duplicates could possibly have been sent, have not revealed any original material upon which the drawings cited in the protologue were based. Toussaint François Node-Véran was the official botanical artist of the Jardin des Plantes in Montpellier in the early part of the 19th century (appointed in 1813 and stayed there until his death in 1852; Denizot et al. 1994) and worked closely with Dunal in preparing the illustrations for the intended major treatment of the taxonomy of *Solanum* (Knapp 2007). Several hundred pen and ink drawings of *Solanum* were made by Node-Véran during the preparation of Dunal's complete treatment of the genus that was never published in its entirety, but only as *Solanorum Synopsis* (Dunal 1816). Political instability in France during the years of the Napoleonic Wars of the early 19th century and Dunal's not being appointed director of the Jardin des Plantes in Montpellier could be contributing factors in his failure to publish the complete illustrated volume (Dulieu 1994, Knapp 2007). Several of the species drawn by Node-Véran were drawn directly from herbarium specimens [e.g., *S. arboreum* Dunal, *Lycopersicon hirsutum* Dunal (=*S. habrochaites* S.Knapp & D.M.Spooner); see Knapp and Spooner 1999, Knapp 2007] that are currently in the herbarium at P. We expect he similarly used herbarium material from P (explicitly cited as herbarium material in Dunal 1816) as the basis for the illustrations of *S. graciliflorum* and *S. poka* cited in the 1814 protologues (Dunal 1814). It is possible that specimens were lost during the turbulent times in Europe in the early 19th century (see Knapp 2007).

Given that no plant specimens corresponding to the protologues have been found, despite extensive searches, we consider the unpublished Node-Véran drawings the most appropriate and only extant possibilities for lectotypifying both *S. graciliflorum* and *S. poka*. These two names have long been treated as confusing, or ignored; they have rarely been used (see below in each species treatment), and few herbarium specimens we have seen have been annotated with either name. Most specimens of the taxa we here recognise as *S. graciliflorum* and *S. poka* have been annotated incorrectly as widespread weedy taxa (e.g., *S. torvum* Sw.) or with names we here consider synonyms (e.g., *S. athroanthum* Dunal); this reflects the limited taxonomic work previously done on tropical Asian *Solanum*, whose taxonomy has not been revised in detail since Dunal's (1852) treatment for Candolle's *Prodromus*. Our purpose here is to secure the application of these names by designating lectotypes for *S. graciliflorum* and *S. poka*, as well as providing complete morphological descriptions for these two species. We also designated interpretative types (epitypes), because details of trichome morphology are extremely important in spiny solanum taxonomy, and these are not visible on the illustrations.

Materials and methods

Searches for type specimens of *S. graciliflorum* and *S. poka* were made using the resources available in Global Plants (http://plants.jstor.org/) and physically in the herbaria where

duplicates could possibly be kept (A, BM, E, G, K, L, LE, MPU and P; abbreviations follow Index Herbariorum; http://sweetgum.nybg.org/science/ih/). Complete details for all specimens examined here are in the data supplement to this article (Suppl. material 1). Morphological descriptions are based on herbarium specimens; we have seen all specimens cited here. Geographical coordinates have been calculated using Google Earth (https://www.google.com/earth/) if not already recorded on specimens.

Taxonomic treatment

Solanum graciliflorum **Dunal, Encycl. [J. Lamarck & al.] Suppl. 3: 763. 1814.**
Fig. 1a, b

Solanum athroanthum Dunal, Prodr. [A. P. de Candolle] 13(1): 208. 1852.
Type. Indonesia. Java: [Prov. Banjinwanyne] "in sylvis prope Sukaradja" [Sukaraja], 1846, *H. Zollinger 2907* (lectotype, designated here: G-DC [G003043306]; isolectotypes: G-DC [G00301684], BM [BM000778325], MPU [MPU012648], P [P00368939, P00368940, P00368941]).

Type. Based on an unpublished illustration of Leschenault collection kept in the Node-Véran collection in Montpellier (lectotype, designated here: Service du Patrimoine Historique de l'Université de Montpellier Node-Véran, Sol. Tab. 47 [MPU028534]); Indonesia. East Java: Blambangan [Sumberwaru, Badjulmati], *T. Horsfield s.n.* (epitype, designated here: BM [BM000886121]).

Description. Scandent shrub to 2 m, armed. Young stems terete, brownish grey, very sparsely stellate-pubescent and prickly, the stellate trichomes porrect, sessile to subsessile, the rays (4-)5–8, 0.1–0.25 mm long, the midpoints to 0.15 mm long, the prickles to 7 mm long, to 8 mm wide at base, curved, deltate, laterally flattened, pale yellow, glabrous; bark of older stems dark brownish grey, glabrescent. Sympodial units difoliate, the leaves geminate, usually similar in size. Leaves simple, the blades (4.5-)7–11 cm long, (1.5-)3–5 cm wide, ca. 2 times longer than wide, elliptic to ovate, chartaceous, slightly discolourous; adaxial and abaxial surfaces sparsely to very sparsely stellate-pubescent and usually with at least some prickles, the stellate trichomes porrect, sessile to subsessile, the rays 6–8, 0.1–0.25 mm long, the midpoint to 0.25 mm long, usually as long as the rays, the prickles 0–10(-12) per leaf side, mostly inserted on the midvein, to 9 mm long, to 2 mm wide at base, straight or slightly curved at the tip, awl-shaped, conical, pale yellow, glabrous; major veins 3–4 pairs drying dark; base attenuate to truncate; margins shallowly to deeply lobed, the lobes 1–3 on each side, 0.5–2.5 cm long, broadly deltate, apically rounded, the sinuses extending up to 2/3 of the distance to the midvein; apex rounded to acute; petiole 0.5–1.8 cm long, 1/10–1/6 of the leaf blade length, sparsely stellate-pubescent with porrect, subsessile trichomes denser at the very base, with 0–2 prickles like those of the blades. Inflorescences leaf-opposed or apparently lateral and borne between leaf pairs, 2–4 cm long, unbranched to up to 6 times branched, with

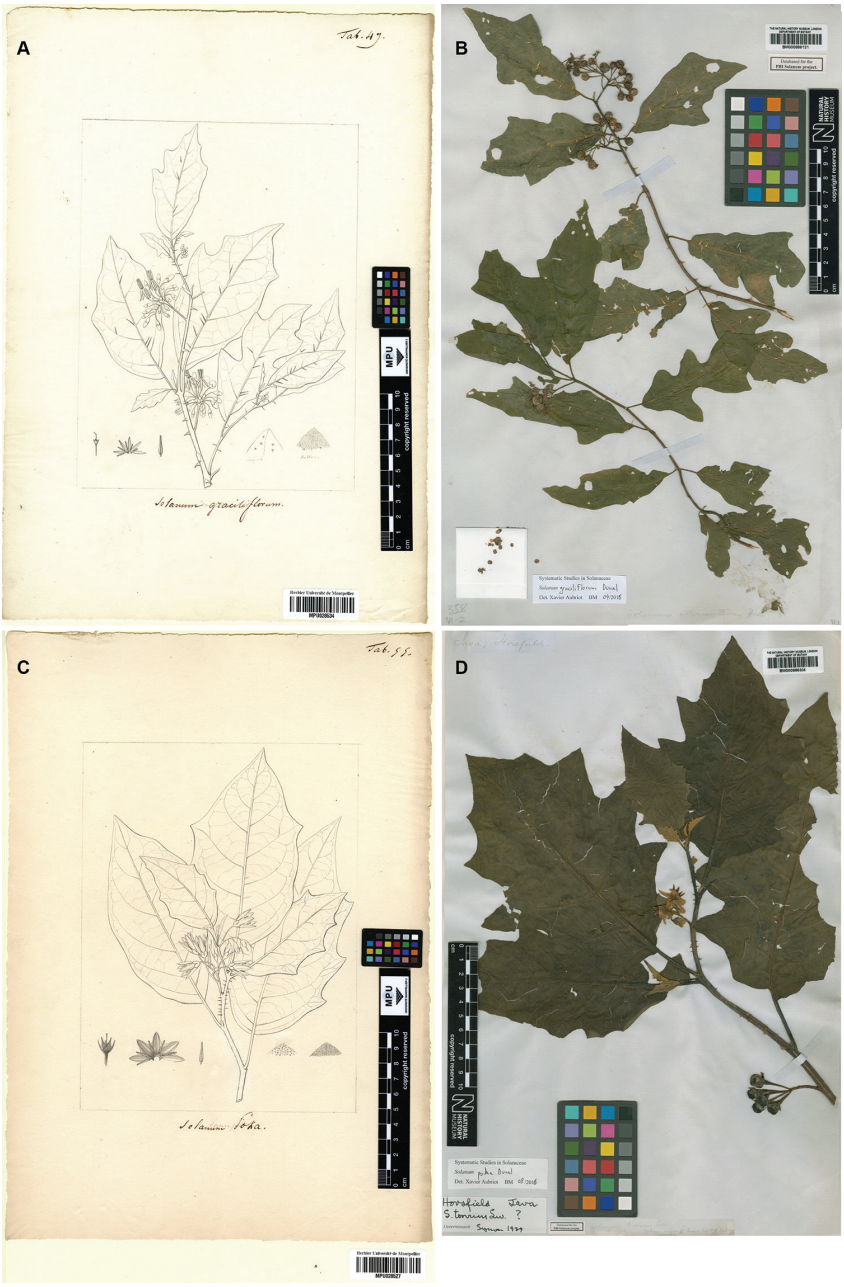

Figure 1. A Lectotype of *Solanum graciliflorum*, illustration *Sol. Tab. 47* [MPU028534] made by T.F. Node-Véran (1773–1852). Reproduced with permission of the Université de Montpellier – Herbier MPU (Service de Patrimoine Historique); copyright Université de Montpellier – Herbier MPU (SPH) **B** Epitype of *Solanum graciliflorum*, *T. Horsfield s.n.* [BM000886121] **C** Lectotype of *Solanum poka*, illustration *Sol. Tab. 55* [MPU028527] made by T.F. Node-Véran (1773-1852). Reproduced with permission of the Université de Montpellier – Herbier MPU (Service de Patrimoine Historique); copyright Université de Montpellier – Herbier MPU (SPH) **D** Epitype of *Solanum poka*, *T. Horsfield s.n.* [BM000886306].

15–50+ flowers; axes sparsely to very sparsely stellate-pubescent, unarmed; peduncle 1–2(-2.5) cm long, with 0–1 prickles like those of the leaves and stems; pedicels 4–7 mm long, erect, articulated at the base, very sparsely stellate-pubescent, unarmed; pedicel scars spaced 1–5 mm apart. Flowers 5-merous, apparently all perfect. Calyx 1.75–2 mm long, campanulate, pubescent with sessile porrect stellate trichomes like those of the stems, unarmed, the lobes 0.25–0.5 mm long, deltate, apically acute. Corolla 0.5–1 cm in diameter, white to pale lilac, stellate, lobed nearly to the base, the lobes 4–5 mm long, ca. 1 mm wide, narrowly deltate to linear, reflexed at anthesis, densely stellate-pubescent abaxially, the trichomes porrect, sessile, the rays 4–6, 0.1–0.2 mm long, the midpoints the same size than the rays or to 0.25 mm long. Stamens slightly unequal; filament tube < 0.5 mm long; free portion of the filaments almost equal, 0.5–1.25 mm long; anthers unequal, three of the five 4.5–5 mm long and two 3–4 mm long, all 0.5–0.75 mm wide, glabrous, connivent, tapering, poricidal at the tips, the pores not lengthening to slits with age. Ovary conical, minutely glandular-puberulent; style ca. 5.5 mm long, slender, curved at the apex, glabrous; stigma capitate, minutely papillate. Fruit a globose berry, 6–50+ per infrutescence, 3–5 mm in diameter, the pericarp shiny, red when mature, glabrous; fruiting pedicels 0.8–1.2 cm long, ca. 0.5 mm in diameter at the base, tapering to a slightly enlarged apex, woody, spreading, unarmed; fruiting calyx lobes slightly expanding to 1.5 mm long, ca. 1/5 the length of the mature fruit, deltate to lanceolate, unarmed. Seeds 6–9 per berry, 3.5–4 mm long, 3–3.5 mm wide, flattened-reniform, orange-brown, the surface minutely pitted, the testal cells pentagonal in outline.

Phenology. The few known collections were flowering and fruiting between May and August.

Distribution and ecology. (Fig. 2) Known from the islands of Java, Bali, Sulawesi and Ambon (Indonesia); growing in forest understory; elevation not recorded on any herbarium material we have seen. The records (as *S. athroanthum*) from the island of Luzon in the Philippines (Merrill 1912, Merrill 1923) are based on misidentifications of specimens of *S. trilobatum* L.

Preliminary conservation status. Data Deficient (DD); known only from seven collections, several of which are of uncertain localities. *Solanum graciliflorum* has not been re-collected since the first half of the 20th century, indicating it is certainly of conservation concern. Recollection of this species and exploration of the type locality are priorities.

Specimens examined. Indonesia. Bali: Perepat Agoeng, 21 Jul 1934, *de Voogd 2177* (A); **Gorontalo:** North Celebes, Jun 1875, *Riedel s.n.* (K); **Java:** sin loc., 1802, *Horsfield 15* (K); West Java, Bogor, *Anonymous s.n.* (K); **Malaku:** "Malay Archipelago, Dawalore [Ambon, Dawa-lour]", Aug 1883, *Riedel s.n.* (K).

Discussion. *Solanum graciliflorum* is a poorly known species represented by very few collections that presents a combination of morphological features that makes it readily recognisable among tropical Asian spiny solanums. It is superficially similar to *S. cyanocarphium* Blume, a sympatric species that is distributed across the Sunda Shelf region, and to *S. retrorsum* Elmer, that occurs mainly in the Philippines. *Solanum gra-*

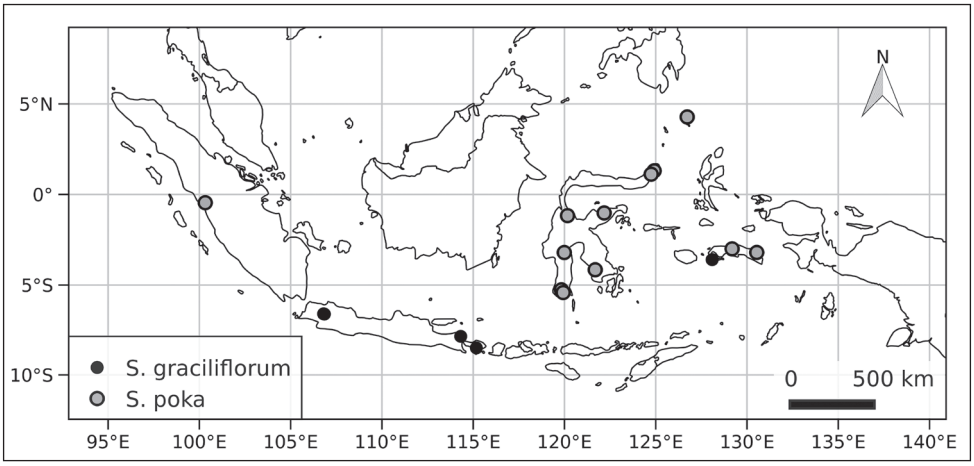

Figure 2. Distribution of *Solanum graciliflorum* and *Solanum poka* in the Malay Archipelago. Geographical information for these collections can be found in the data supplement to this article (Suppl. material 1).

ciliflorum can be distinguished from both of them by its much sparser indumentum, stout, deltate stem prickles (rather than slender and awl-shaped), and tiny, delicate flowers (hence the species epithet) that are clustered in dense, many-flowered inflorescences. Molecular data show that *S. cyanocarphium* and *S. graciliflorum* are not closely related; *S. graciliflorum* is nested within the Sahul-Pacific clade while *S. cyanocarphium* is an unresolved species of uncertain affinities (see Aubriot et al. 2016).

Solanum graciliflorum is the type of section *Graciliflorum* (Dunal) Seithe, a section partly based on the informal grouping made in Dunal's (1852) treatment of *Solanum* in Candolle's *Prodromus*. In Seithe's (1962) circumscription, section *Graciliflorum* included 14 species with stellate trichomes and acicular prickles coming from various region of the world (e.g., *S. bahamense* L. from the Caribbean archipelago, *S. nienkui* Merr. & Chun from Southeast Asia, *S. paniculatum* L. from South America, *S. stelligerum* Sm. from Australia). Symon (1981, 1985) extended the circumscription of the section with the addition of 27 additional species (10 from Australia and 17 from New Guinea), expressing at the same time serious doubts about its coherence. Symon's concerns echoed those expressed in Whalen's systematic treatment of the spiny solanums (Whalen 1984). In this first-ever attempt to include spiny solanums into a morphologically based phylogenetic framework, Whalen did not regard section *Graciliflorum* as a natural group and placed members of the section as defined by Seithe (1962) into several of his informal groups (e.g., *S. bahamense* in the '*Solanum bahamense* group', *S. paniculatum* in the '*Solanum torvum* group', *S. stelligerum* in the '*Solanum ferocissimum* group'). With limited sampling and knowledge of Old World taxa, Whalen did not clarify the identity of *S. graciliflorum*, the type species of the section, and included it in his 'Unusual species group' as a possible synonym of the widespread tropical Asian species *S. violaceum* Ortega. He considered *S. athroanthum* to be

different from *S. graciliflorum*, and placed the former into his '*Solanum dunalianum* group' [= *Solanum* section *Dunaliana* (Bitter) Symon *pro parte*], a group of 20 species distributed across the Malayan archipelago, Australia and the South Pacific that were characterised by lack of broad-based prickles on mature growth, entire leaves with glabrate abaxial surfaces, inflorescences with tightly spaced hermaphroditic flowers, and juicy red berries (Whalen 1984). More recently McClelland (2012) proposed a narrower circumscription of sect. *Dunaliana*, reducing it to six species and excluding *S. graciliflorum* (as *S. athroanthum*) on the basis of its deeply lobed leaves with prickles on the principal veins and its slightly unequal anthers (versus entire to shallowly lobed non-prickly leaves and always equal anthers for all species he recognized as belonging to sect. *Dunaliana*). Instead he suggested a close relationship between *S. graciliflorum* and *S. nienkui*, a Southeast Asian species that also displays anisandry. Recent molecular phylogenetic analysis of tropical Asian spiny solanums incorporating representatives of sections *Dunaliana* and *Graciliflorum* (including *S. dunalianum* Gaudich. and *S. graciliflorum*) showed *S. graciliflorum* to be sister to the Philippine endemic *S. lianoides* Elmer (Aubriot et al. 2016). Both species are prickly vines, but *S. lianoides* differs from *S. graciliflorum* by its denser leaf indumentum, entire leaves and larger flowers. Both species are closely related to *S. dunalianum* (Aubriot et al. 2016), a result consistent with Whalen's (1984) treatment of *S. graciliflorum* (as *S. athroanthum*; see Aubriot et al. 2016 for discussion) but not with McClelland's (2012) hypothesis of relationships.

In the protologue Dunal referred to an illustration made by Node-Véran, '*Dun. Suppl. 7. Sol. Mss. tab. 4.*', an orthographic error for '*Dun. Suppl. Sol. Mss. tab. 47.*' according to the sequence of figure numbers and to the caption on the illustration in Montpellier. We were unable to find any herbarium material matching the illustration in either P or MPU, although Dunal later (Dunal 1816, 1852) cited Leschenault as the collector of the material he had seen. We designate the unpublished illustration of Node-Véran as the lectotype because it is the only extant original material we have identified to date. We have also designated here an epitype specimen that best matches Node-Véran's illustration, and that corresponds to a collection made in the same geographical area as the lost type specimen (i.e. the island of Java in Indonesia) in order to secure the application of the name (Art. 9.8, McNeill et al. 2012).

Dunal (1852) based his description of *S. athroanthum* on *Zollinger 2907* in "hb. DC.". There are two specimens of *Zollinger 2907* in G-DC; we select the more complete of these as the lectotype. The locality data for Zollinger's collections are often not written on all duplicates; for *Zollinger 2907* locality data are only found on P00368940.

Solanum poka **Dunal, Encycl. [J. Lamarck & al.] Suppl. 3: 768. 1814.**
Fig. 1c, d

Solanum torvum Sw. var. *scabrescens* Miq. Fl. Ned. Ind. 2: 648. 1861.

Type. Indonesia. Sumatra: sin. loc., *F.W. Junghuhn s.n.* (holotype: L [L0403917])

Type. Based on an unpublished illustration of Leschenault collection kept in the Node-Véran collection in Montpellier (lectotype, designated here: Service du Patrimoine Historique de l'Université de Montpellier, Node-Véran, Sol. Tab. 55 [MPU028527]); Indonesia. Java: sin. loc., *T. Horsfield s.n.* (epitype, designated here: BM [BM000886306]).

Description. Shrubs to 3 m, armed. Young stems terete, black to dark brownish, moderately stellate-pubescent, usually densely prickly distally, sometimes unarmed, the stellate trichomes porrect, sessile or variously stalked, the stalks to 0.2 mm long, the rays (4-)5–8, 0.1–0.25 mm long, the midpoints reduced to globular glands; prickles to 3.5 mm long, to 2.5 mm wide at base, straight, awl-shaped to deltate, conical, pale yellow, glabrescent; bark of older stems brownish gray, sparsely stellate-pubescent. Sympodial units difoliate, the leaves geminate. Leaves simple, the blades 11–24 cm long, 4–13 cm wide, ca. 1.5–3 times longer than wide, elliptic to broadly ovate, chartaceous, slightly discolorous; adaxial surface moderately stellate-pubescent with porrect, sessile and less often variously stalked trichomes, the stalks to 0.1 mm long, the rays 4–8, 0.1–0.4 mm long, the midpoints to 0.25 mm long; abaxial surface moderately stellate-pubescent with trichomes like those of the adaxial surface, but more often stalked; prickles 0–6 per leaf side, to 6 mm long, to 1.5 mm wide at base, straight or slightly curved at the tip, awl-shaped, conical, pale yellow, glabrous; major veins 6–8 pairs drying yellow; base shortly attenuate to truncate; margins entire or shallowly to deeply lobed, the lobes 1–5 on each side, 0.5–5 cm long, rounded to apically acute, the sinuses extending up to 2/3 of the distance to the midvein, deltate; apex acute; petiole 1.5–4 cm long, 1/10–1/5 of the leaf blade length, densely stellate-pubescent with porrect, sessile trichomes like those of the blades, with 0–5 prickles like those of the stems. Inflorescences apparently lateral or leaf opposed, 2–5 cm long, unbranched to up to 2 times branched, with ca. 5–20 flowers, moderately to densely stellate-pubescent, unarmed; peduncle 0.5–1.5 cm long, with 0–1 prickles; pedicels 0.5–1.2 cm long, erect, articulated at the base, densely stellate-pubescent, unarmed; pedicel scars spaced 2–4 mm apart. Flowers 5-merous, apparently all perfect. Calyx 4–7 mm long, campanulate, moderately stellate-pubescent, densely stellate-pubescent on the midvein, unarmed, the lobes 3–5 mm long, the lower part deltate and abruptly constricting to an elongate acumen, the acumen 3/4 the total lobe length, the abaxial surface more or less strongly keeled along the midvein. Corolla 1–2 cm in diameter, white, lobed for ca. 1/2–2/3 of the way to the base, the lobes 5–8 mm long, 2–3.5 mm wide, deltate, spreading at anthesis, densely stellate-pubescent abaxially on parts exposed in bud. Stamens equal; filament tube < 0.5 mm long; free portion of the filaments 0.75–1.5 mm long; anthers 5–6.5 mm long, ca. 0.75 mm wide, connivent, tapering, poricidal at the tips, the pores not lengthening to slits with age. Ovary conical, minutely glandular-puberulent; style 0.6–1 cm long, slender, curved at the apex, with few scattered hairs at the tip; stigma capitate, minutely papillate, stellate-pubescent. Fruit a globose berry, 8–18 per infrutescence, 0.8–1.5 cm in diameter, the pericarp smooth, bluish green when young turning to dark greyish yellow, glabrous; fruiting pedicels 1.2–2.5 cm long, ca. 1–1.5 mm in diameter at the base, ca. 2–3 mm in diameter at the apex,

woody, erect, unarmed; fruiting calyx lobes not expanding. Seeds 100–200 per berry, ca. 1.75–2 mm long, 1.5–1.75 mm wide, flattened reniform, pale yellowish, the surface minutely pitted, the testal cells sinuate in outline.

Phenology. Flowering and fruiting throughout the year.

Distribution and ecology. (Fig. 2) Widely distributed in the Malay Archipelago, from western Sumatra to the Maluku Islands and across Sulawesi, northwards to the Talaud islands; growing in open woodland, forest edges, degraded vegetation, usually on limestone or volcanic rocks; 0–1600 m elevation.

Preliminary conservation status. Least Concern (LC); EOO > 100,000 km^2 and AOO > 10,000 m^2 (see Moat 2007 for explanation of measurements). Although the EOO and AOO measurement indicate a status of least concern, the few collections coupled with the profound transformation in lowland Indonesian habitats where *S. poka* is found (Margono et al. 2014) suggest that the species is a priority for recollection and reassessment.

Specimens examined. Indonesia. Central Sulawesi: Banggai regency, Luwuk District, Bunta Subdistrict, Sumber Agung, Gunung Hek, Sungai Hek, Cabang Tiga, 980 m, 27 Feb 2004, *Hendrian et al. 964* (E, L); Sigi Regency, near the river S of Tongoa, 650 m, 17 Mar 1981, *Johansson et al. 419* (K, L); **Java:** sin. loc., *Horsfield s.n.* (BM); sin. loc., *Horsfield 786* (BM); **Malaku:** Central Maluku Regency, Wae Mamahala, 1330 m, 11 Nov 1937, *Eyma 2166* (A, L); Central Maluku Regency, Seram Utara District, Manusela National Park, along a trail from Wae Puo to Kali, Ili area, south of Sawai, 830–1230 m, 23 Jan 1985, *Kato et al. C-5431* (A, L); East Seram Regency, Bula District, Luman, 15 km south of Bula, 10–20 m, 26 Feb 1985, *Kato et al. C-7942* (L); **North Sulawesi:** Minahasa Regency, Mt. Soputan, 1080 m, 11 Oct 1973, *de Vogel 2504* (L); Minahasa Regency, Tondano, 1840, *Forsten s.n.* (L); Minahasa Regency, 25 Apr 1895, *Koorders 18035B* (L); Minahasa Regency, 20 m, 28 Apr 1895, *Koorders 18037B* (L); Talaud Islands Regency, Pulau Karakelang, bank of Kuala Bahewa, 30 m, 3 May 1926, *Lam 2772* (K, L); **South Sulawesi:** Gowa Regency, Lombasang, 1000 m, 26 May 1921, *Bunnemeyer 11732* (K, L); Gowa Regency, Lombasang, 1100 m, 31 May 1921, *Bunnemeyer 11813A* (L); Bantaeng Regency, Bonthain [Bantaeng], 1500 m, 12 Jun 1921, *Bunnemeyer 12117* (L); Kolaka Regency, Baula, 150 m, 26 Dec 1909, *Elbert 3224* (L); Enrekang Regency, Enrekang District, Latimojong Mts., in valley 3 km. south west of Bunte Tjejeng and south east of Rantelemo, 1490 m, 14 Nov 1969, *Sands 477* (A, E, K); **Timor:** sin loc., 1882, *Forbes 3806* (BM, L); **West Sumatra:** Agam Regency, Mt. Singgalang, 1600 m, 29 May 1918, *Bunnemeyer 2786* (A, L).

Discussion. *Solanum poka* was long ignored after its first publication (Dunal 1814). It has not been included in classical floristic treatments of Java (Hasskarl 1848, Backer 1965, van Steenis et al. 2006) or Sumatra (Miquel 1862). It was mentioned by Miquel (1856) and Koorders (1912), but both authors merely repeated Dunal's original description, without referring to any specimens. In Koorders's (1918) botanical report on the flora of northeastern Sulawesi he lists several widespread and common

species (e.g., *S. lycopersicum* L., *S. melongena* L., *S. torvum* Sw., *S. tuberosum* L.) as well as two shrubby *Solanum* species for which he did not provide names ("Solanum spec. A" and "Solanum spec. B"). Two previously undetermined Koorders collections of *S. poka* from northeast Sulawesi (Minahasa Regency) in April 1895 (*Koorders 18035B* and *Koorders 18037B*, both L) correspond to *S. poka*. It is possible that these two collections correspond to one (or both) of Koorders' (1918) unnamed species, but since he provided no descriptions or specific localities this is difficult, if not impossible, to ascertain.

Based on morphology, *S. poka* belongs to the *Torva* clade (sensu Stern et al. 2011), with its straight prickles, many flowered inflorescences and corollas with abundant interpetalar tissue (see Fig. 1b). This hypothesis is corroborated by the molecular data (Aubriot et al. 2016). *Solanum poka* is sister to a clade composed of four native Old World species (*Solanum dammerianum* Lauterb. & K.Schum, *S. peikuoense* S.S.Ying, *S. pseudosaponaceum* Blume, *S. torvoideum* Merr. & L.M.Perry) with which it forms a strongly supported group, the 'Old World torvoids' *sensu* Aubriot et al. (2016). Morphologically, *S. poka* most closely resembles *S. pseudosaponaceum*, a widespread species from Taiwan and southern China to Indonesia, but differs in having denser indumentum on the adaxial leaf surface, more numerous straight prickles on the upper stems, fewer, larger flowers with elongate strongly keeled calyx lobes, and much larger fruits. Flowers of *S. pseudosaponaceum* are lilac or purplish-white while those of *S. poka* are always described on labels as white.

In the protologue Dunal referred to an illustration made by Node-Véran, '*Dun. Suppl. 7. Sol. Mss. tab. 55*', but cited no herbarium material. Similarly to the situation of *S. graciliflorum*, we were unable to find any herbarium material matching the illustration in either P or MPU, although Dunal later (Dunal 1816, 1852) cited Leschenault as the collector of the material he had seen. We designate the unpublished illustration of Node-Véran as the lectotype because it is the only extant original material we have identified to date. We designate here an epitype specimen from Java, the cited type locality, (*Horsfield s.n.*, BM000886306) that best matches Node-Véran's illustration, particularly with respect to the diagnostic characters for *S. poka*; leaf shape, prickle shape and calyx lobe morphology.

We have only seen three specimens of *S. poka* from Java, the cited type locality, all collected by Thomas Horsfield, an American physician who collected on Java contemporaneously with Leschenault in the early part of the 19th century (McNair 1942, Van Steenis-Kruseman and Van Steenis 1950). *Solanum poka* is, however, rather broadly distributed across the Malay Archiplago, with the distribution centred on Sulawesi and the surroundings islands (Malaku Islands, Talaud Islands) (Fig. 2). Thorough examination of the extensive holdings in Indonesia (particularly those of the Bogor Botanical Garden Herbarium, BO) and, given the historically extensive natural habitat loss recorded for Java (Margono et al. 2014), additional collecting are both needed to better understand the distribution of *S. poka*.

Dunal (1852) cited the herbarium name '*S. quercifolium* Banks' taken from a specimen in BM collected by Joseph Banks in Java as part of his treatment of *S. poka*. Examination of this sheet (BM000886238) shows it belongs to *S. pseudosaponaceum*.

Acknowledgements

We thank the curators of A, BM, E, G, K, L, LE, MPU and P for permission to access and study the specimens in their care, as well as the Service du Patrimoine Historique of the Université de Montpellier for allowing us to reproduce two of the Node-Véran drawings they hold. We thank Zair Ficinsky for preparing the distribution map. This work was funded in part by the National Science Foundation Planetary Biodiversity Inventory grant DEB-0316614 – 'PBI Solanum: a worldwide treatment' and by the Natural History Museum's Natural Resources and Hazards Initiative. XA's visit to L was funded by the European Community Research Infrastructures program under FP7 SYNTHESYS 'Synthesis of Systematic Resources' grant number NL-TAF-3331.

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Supplementary material 1

List of collections used in the study with full locality and descriptive notes

Authors: Xavier Aubriot, Caroline Loup, Sandra Knapp

Data type: List of collections in xls format

Explanation note: Details of the herbarium material examined for this manuscript (including full locality and descriptive notes).

PhytoKeys 70: 111–137 (2016)
doi: 10.3897/phytokeys.70.9641
http://phytokeys.pensoft.net

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A molecular phylogeny of Caraganeae (Leguminosae, Papilionoideae) reveals insights into new generic and infrageneric delimitations

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Academic editor: *Pavel Stoev* | Received 22 June 2016 | Accepted 25 September 2016 | Published 4 October 2016

Citation: Duan L, Yang X, Liu P, Johnson G, Wen J, Chang Z (2016) A molecular phylogeny of Caraganeae (Leguminosae, Papilionoideae) reveals insights into new generic and infrageneric delimitations. PhytoKeys 70: 111–137. doi: 10.3897/phytokeys.70.9641

Abstract

Based on sequence data of nuclear ITS and plastid *matK*, *trnL-F* and *psbA-trnH* markers, the phylogeny of the subtribes Caraganinae and Chesneyinae in tribe Caraganeae was inferred. The results support the monophyly of each of the subtribes. Within subtribes Caraganinae, *Calophaca* and *Halimodendron* are herein transferred into *Caragana* to ensure its generic monophyly. The subtribe Chesneyinae is composed of four well-supported genera: *Chesneya*, *Chesniella*, *Gueldenstaedtia* and *Tibetia*. Based on phylogenetic, morphological, distributional and habitat type evidence, the genus *Chesneya* was divided into three monophyletic sections: *C.* sect. *Chesneya*, *C.* sect. *Pulvinatae* and *C.* sect. *Spinosae*. *Chesneya macrantha* is herein transferred into *Chesniella*. *Spongiocarpella* is polyphyletic and its generic rank is not maintained. The position of *Chesneya* was incongruent in the nuclear ITS and the plastid trees. A paternal chloroplast capture event via introgression is hypothesized for the origin of *Chesneya*, which is postulated to have involved the common ancestor of *Chesniella* (♂) and that of the *Gueldenstaedtia* – *Tibetia* (GUT) clade (♀) as the parents.

Keywords

Caragana, *Chesneya*, *Chesniella*, chloroplast capture, generic delimitation, phylogeny

Introduction

Caraganeae Ranjbar is a mid-sized tribe in Leguminosae, established by Ranjbar and Karamian (2003) based on five genera: *Calophaca* Fisch. ex DC., *Caragana* Fabr., *Chesneya* Lindl. ex Endl., *Gueldenstaedtia* Fisch. and *Halimodendron* Fisch. ex DC., numbers of genera may be altered when treated by different workers (see below). Caraganeae ranges from eastern Europe, central and western Asia to Mongolia, China and the Himalayas, extending northward to Siberia (Lock 2005; Ranjbar et al. 2014). This tribe is diagnosed by the asymmetrical axillary peduncles or pedicels attached to the slightly gibbous calyx and dehiscent pods (except for *Halimodendron*; Polhill 1981; Ranjbar and Karamian 2003; Ranjbar et al. 2014).

A few recent studies referred to the concept of Caraganeae. Molecular work of Ranjbar et al. (2014) classified Caraganeae into two subtribes: Caraganinae and Chesneyinae Ranjbar, F. Hajmoradi & Waycott. Duan et al. (2015) recognized this tribe based on the genera *Calophaca*, *Caragana* and *Halimodendron*. However, the former was inferred from a limited sampling scheme and few DNA markers, while the latter was subject to the undersampled for Chesneyinae. Hence, the monophyly of this tribe and the division of subtribes need to be further evaluated.

Within the subtribe Caraganinae, the genus *Caragana* has attracted much attention (Komarov 1908; Moore 1968; Gorbunova 1984; Zhao 1993, 2009; Zhou 1996; Zhang 1997; Sanchir 1999; Sanczir 2000; Hou et al. 2008; Zhang et al. 2009). The infrageneric classifications of *Caragana* mainly focused on several morphological characters: leaves paripinnate vs. digitate, with four vs. more leaflets, and petioles and rachises caducous vs. persistent. Recent phylogenetic analyses resolved that *Caragana* was paraphyletic, with *Halimodendron* and *Calophaca* embedded in it (Zhang et al. 2009, 2015a; Zhang and Fritsch 2010; Duan et al. 2015). Thus, proposal of a new generic delimitation for *Caragana* may be possible based on more comprehensive phylogenetic evidence.

The genera *Chesneya* and *Gueldenstaedtia* formed a well-supported clade (Sanderson and Wojciechowski 1996), and were treated as the subtribe Chesneyinae (Ranjbar et al. 2014). Within this subtribe, the generic delimitations were controversial, especially concerning the status of *Chesniella* Boriss. (Borissova 1964), *Spongiocarpella* Yakovl. et Ulzij. (Yakovlev and Sviazeva 1987), and *Tibetia* (Ali) H. P. Tsui (Tsui 1979). The former two genera were separated from *Chesneya*, while *Tibetia* was a segregate of *Gueldenstaedtia* and has been revised in several studies (Cui 1998; Zhu 2004; Zhu 2005a, 2005b; Bao and Brach 2010). Zhang et al. (2015b) supported the monophyly of *Chesneya* and proposed a classification system, but some sections were only weakly supported. Hence, the phylogeny of Chesneyinae and its associated genera needs to be further explored.

We herein employ sequence data from nrDNA ITS and plastid *matK*, *trnL-F* and *psbA-trnH* to a) test the monophyly of Caraganeae and its subtribes; b) estimate the phylogeny of genera in Caraganeae; and c) discuss the taxonomic implications of this phylogeny on the generic and the infrageneric classification of the tribe.

Materials and methods

Taxon sampling

Our sampling was designed largely following the generic demarcations in *Flora Reipublicae Popularis Sinicae* (Liou 1993; Li 1993; Cui 1998). We included 101 accessions, covering 97 species, containing 39 species of Caraganinae (represented by *Calophaca*, *Halimodendron* and all 5 sections of *Caragana* according to Zhang 1997) and 40 accessions (36 species) of Chesneyinae (including *Chesneya*, *Chesniella*, *Gueldenstaedtia* and *Tibetia*, tentatively treating *Spongiocarpella* in *Chesneya*, which were more comprehensively sampled than previous studies [Ranjbar et al. 2014; Duan et al. 2015; Zhang et al. 2015b]). 82 new sequences were generated in this work.

To better resolve the relationships of subtribes Caraganinae and Chesneyinae, 11 Galegeae species (8 genera) and 5 Hedysareae species (4 genera) were also sampled. *Cicer microphyllum* Royle ex Bentham, *Dalbergia hupeana* Hance, *Lathyrus latifolius* L., *Robinia pseudoacacia* L., *Trifolium repens* L. and *Wisteria sinensis* (Sims) Sweet were selected as outgroups based on previous studies (Wojciechowski et al. 2000, 2004; Wojciechowski 2003). Sequences of 40 accessions (representing 40 species) were downloaded from GenBank (see Suppl. material 1 for details). Most accessions we sampled were collected from the field or herbarium specimens. *Onobrychis arenaria* DC. was obtained from seedlings germinated from seeds provided by the Royal Botanic Gardens, Kew.

DNA extraction, amplification and sequencing

Total genomic DNAs were extracted from silica-gel dried leaves or herbarium material using the Plant DNA Extraction Kit - AGP965/960 (AutoGen, Holliston, MA, USA) or the DNeasy Plant Mini Kit (Qiagen, Valencia, USA). Polymerase chain reactions (PCR) were prepared in 25μL containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 mM of each primer, 1 U of *Taq* polymerase (Bioline, Aberdeen, Scotland, UK), and using 10–50 ng (2.5 μL) template DNAs, following Wen et al. (2007). The PCRs for ITS (primer pair: ITS4 and ITS5a) and *psbA-trnH* (primer pair: psbA and trnH) were performed according to Stanford et al. (2000) and Hamilton (1999), respectively. The PCR primer pair for *trnL-F* was "c" and "f" as in Zhu et al. (2013) and Taberlet et al. (1991), and the thermal cycling program followed Soejima and Wen (2006). The barcoding region of the *matK* marker was amplified and sequenced with the primer pair Kim-3F/Kim-1R (CBOL Plant Working Group 2009; China Plant BOL Group 2011), and the amplification conditions were: 95°C (5min) for DNA predenaturation; 94°C (40s), 48°C (40s) and 72°C (100s) for 35 cycles; 72°C (10min) for final extension. PCR products were cleaned using ExoSAP-IT (cat. # 78201, USB Corporation, Cleveland, OH, USA) following the manufacturer's instruction. Purified products were sequenced from both directions with BigDye 3.1 reagents on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analysis

Sequences were assembled with Geneious 7.1 (http://www.geneious.com/), and aligned using MUSCLE 3.8.31 (Edgar 2004), followed by manual adjustments in Geneious 7.1. Because the chloroplast markers putatively evolve as a single molecule, sequences of the three plastid markers (*matK*, *trnL-F* and *psbA-trnH*) were directly concatenated. Topological discordance was investigated by comparing the ITS and the concatenated plastid trees (as in García et al. 2014). To further determine the compatibility between these two datasets, an incongruence length difference (ILD) test and an approximately unbiased (AU) test were conducted with PAUP* (Swofford 2003) and CONSEL (Shimodaira and Hasegawa 2001; using site-wise likelihood values estimated by RA×ML; Stamatakis et al. 2008) programs, respectively. The tests retrieved the p values of 0.01 and 0.0001, respectively, suggesting that the incongruence between these two datasets was significant. The ITS and the concatenated plastid sequences were thus analyzed separately.

Phylogenetic analyses were carried out using Bayesian inference (BI; Rannala and Yang 1996; Mau et al. 1999) with MrBayes 3.2.5 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). Nucleotide substitution model parameters were determined prior to BI using the corrected Akaike information criterion (AIC) in jModeltest 2.1.7. (Posada 2008; Darriba et al. 2012). For the ITS dataset, boundaries of the 5.8S region to the ITS1 and the ITS2 regions were determined by comparison with the published 5.8S sequence of *Vicia faba* L. (Nazar and Wildeman 1981; Yokota et al. 1989), and the sequence substitution models for the ITS1, 5.8S and ITS2 regions were determined separately. Similarly, the models for each of the three plastid markers were estimated for the best-fit models, which were used in the BI analysis for concatenated plastid sequences in a partitioned scheme.

In the BI, the Markov chain Monte Carlo (MCMC) search was run by two replicates for 10,000,000 generations, sampling one tree every 1,000 generations. After the first 2,500,000 generations (2,500 trees) were discarded as burn-in, a 50% majority-rule consensus tree and posterior probabilities were obtained among the remaining trees. Results were checked using the program Tracer 1.5 (Rambaut and Drummond 2007) to ensure that plots of the two runs were converging and the value of the effective sample size for each replicate was above 200. Maximum likelihood (ML) analyses were conducted using RAxML-MPI v8.2 (Stamatakis 2014) with dataset partition scheme the same as in the BI and the following settings: rapid bootstrap analysis with 1,000 replicates and search for best-scoring ML tree in one program run, starting with a random seed, selecting the GTR model. Bootstrap values (LBS), as well as posterior probabilities (PP) were labeled on the corresponding branches of the Bayesian trees.

Results

Sequence characteristics are shown in Table 1. Our ML results are basically congruent in topology with the corresponding BI trees, the support values of the former were thus

Table 1. Sequence characteristics with gaps as missing data: alignment length, the number of the constant, variable and potential parsimony-informative (Pi) sites, and the best-fit nucleotide substitution model determined by AIC.

Dataset	Length	Constant	Variable	Pi	Model
ITS1	266	81	185	148	GTR+I+G
5.8S	164	143	21	14	TrNef+I+G
ITS2	279	113	166	131	GTR+G
matK	807	485	322	189	GTR+G
trnL-F	1412	921	491	279	TVM+I+G
psbA-trnH	793	472	321	175	TIM1+G

labeled on the corresponding branches of the latter (see legend of Figs 1, 2). Thanks to some extra sequences from GenBank (see Suppl. material 1), especially those of *Chesneya* and *Chesniella*, the ITS tree (Fig. 1) was more comprehensively sampled than the plastid tree (Fig. 2), which was of help to increase the general support of the former.

Nuclear data

In the ITS tree (Fig. 1), the Astragalean clade (PP = 1, LBS = 100%; including *Astragalus* L., *Colutea* L., *Eremosparton* Fisch. & C.A.Mey., *Lessertia* R.Br. ex W.T.Aiton, *Oxytropis* DC., and *Swainsona* Salisb.), the Vicioid clade (PP = 1, LBS = 100%; represented by *Trifolium*, *Lathyrus*, *Cicer* and *Galega* L.), tribe Hedysareae (PP = 1, LBS = 98%), subtribes Caraganinae (PP = 1, LBS = 98%) and Chesneyinae (PP = 1, LBS = 100%) were each strongly supported.

Subtribe Caraganinae contained three genera, within which *Calophaca* was monophyletic (PP = 1, LBS = 96%), but *Calophaca* and *Halimodendron* were embedded within the paraphyletic *Caragana*. Within subtribe Chesneyinae, *Gueldenstaedtia* (PP = 1, LBS = 100%) and *Tibetia* (PP = 1, LBS = 100%) were each monophyletic and together they formed a clade (the GUT clade, shown in blue; PP = 1, LBS = 100%). Two accessions of former *Chesneya macrantha* Cheng f. ex H.C.Fu constituted a robustly supported branch nested in a monophyletic *Chesniella* (displayed in green; PP = 0.98, LBS = 89%), while other accessions of *Chesneya* formed another clade (*Chesneya s.s.*; shown in red; PP = 1, LBS = 100%; Fig. 1), which contained three well-supported sections (details see Discussion; PP = 1 & LBS = 100%, PP = 0.98 & LBS = 96% and PP = 1 & LBS = 100%, respectively).

Plastid data

Similar to the ITS results, the plastid tree (Fig. 2) also showed the monophyly of both subtribes Caraganinae (PP = 1, LBS = 100%) and Chesneyinae (PP = 1, LBS = 100%). *Calophaca* and *Halimodendron* were nested in *Caragana* in different places from the ITS tree, but such placement was weakly supported. *Caragana* also showed its paraphyly,

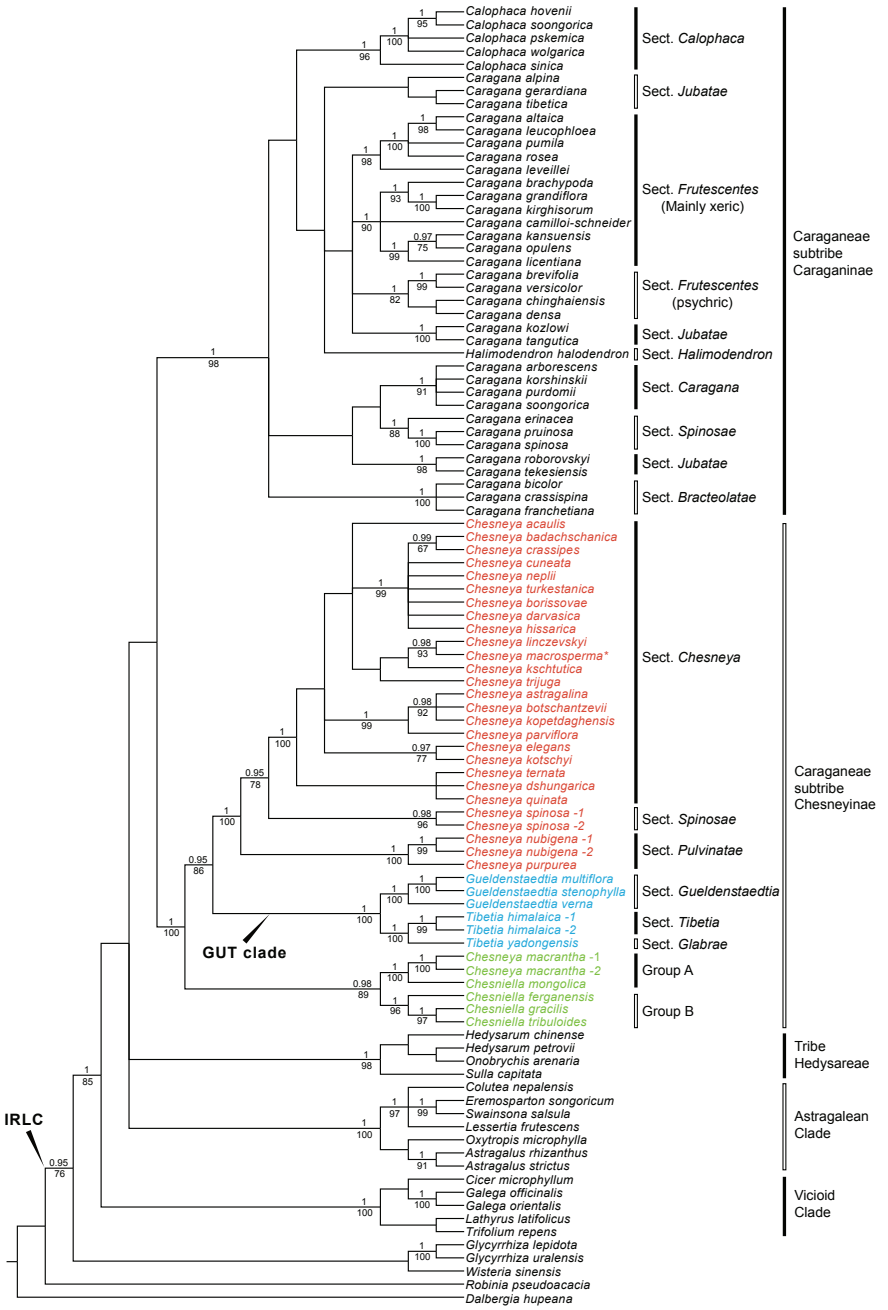

Figure 1. Bayesian tree of the nrDNA ITS data, showing relationships of genera in subtribes Caraganinae, Chesneyinae and their close relatives. The labeled sections of *Gueldenstaedtia* and *Tibetia* followed Tsui (1979) and Zhu (2005a), respectively. Bayesian posterior probabilities (PP ≥ 0.95) and maximum likelihood bootstrap (LBS ≥ 70%) are given above and below branches, respectively. The asterisk indicates the name of *Chesneya macrosperma* has not been published, its voucher was storied in LE (details see Zhang et al. 2015b).

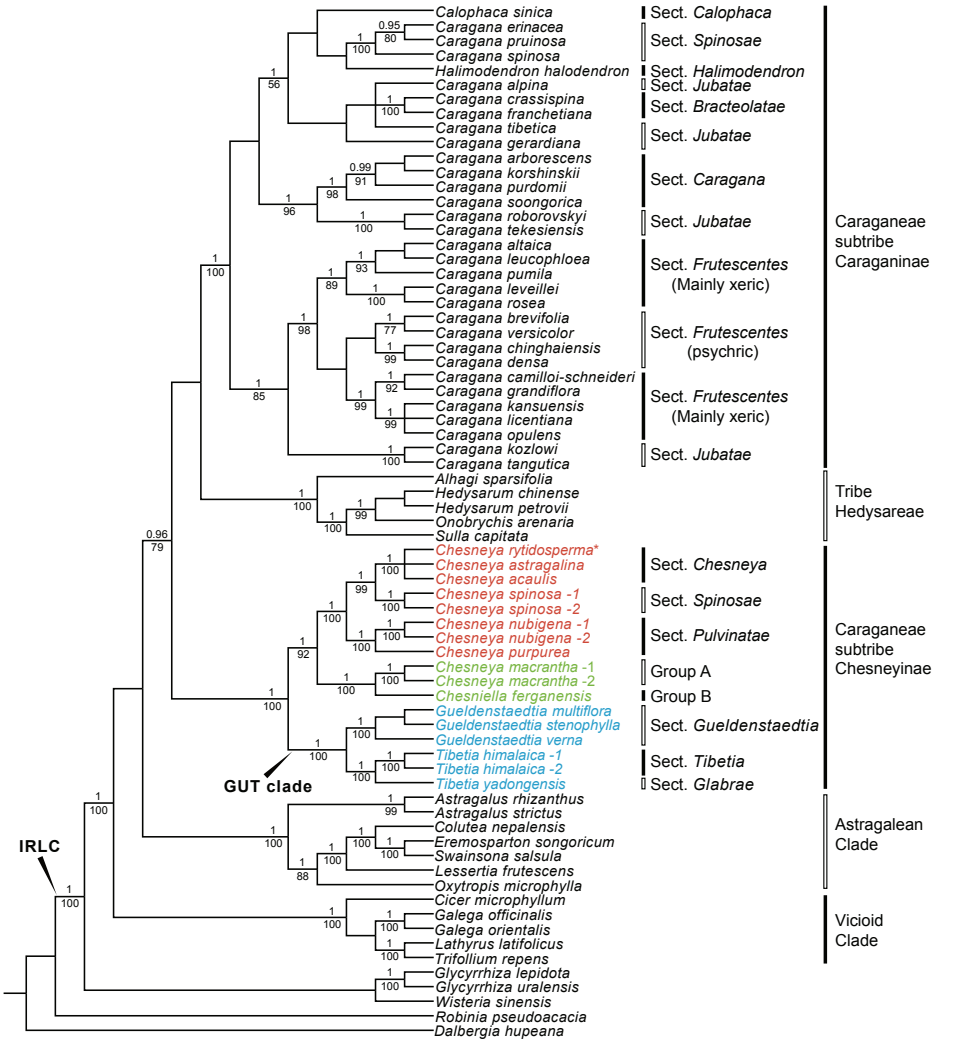

Figure 2. Bayesian tree of the concatenated plastid data of *matK*, *trnL-F* and *psbA-trnH* sequences, showing genera in subtribes Caraganinae, Chesneyinae and their close relatives. The labeled sections of *Gueldenstaedtia* and *Tibetia* followed Tsui (1979) and Zhu (2005a), respectively. Bayesian posterior probabilities (PP ≥ 0.95) and maximum likelihood bootstrap (LBS ≥ 70%) are given above and below branches, respectively. The asterisk indicates the type species of *Chesneya*.

with *C.* sect. *Bracteolatae* (Kom.) M.L.Zhang (PP = 1, LBS = 100%), *C.* sect. *Caragana* Kom. (PP = 1, LBS = 98%), *C.* sect. *Frutescentes* (Kom.) Sanchir (PP = 1, LBS = 98%) and *C.* sect. *Spinosae* (Kom.) Y.Z.Zhao (PP = 1, LBS = 100%) each strongly supported. Unlike in the ITS tree, *Chesneya s.s.* and *Chesniella* were sisters in the plastid tree (PP = 1, LBS = 92%; Fig. 2). As in the ITS tree, the GUT clade (PP = 1, LBS = 100%) contained *Gueldenstaedtia* (PP = 1, LBS = 100%) and *Tibetia* (PP = 1, LBS = 100%), with each genus being monophyletic.

Discussion

Caraganeae comprises ca. 100 species distributed in temperate Asia, extending to eastern Europe (Ranjbar and Karamian 2003; Lock 2005). The two subtribes (Caraganinae and Chesneyinae) recognized by Ranjbar et al. (2014) are each well-supported in our analyses. However, our results did not recover a monophyletic Caraganeae (Figs 1, 2). Similarly, the previously expanded delimitation of Hedysareae *sensu* Lock (2005; also see Cardoso et al. 2013), which included the genera of subtribe Caraganinae and tribe Hedysareae *sensu* Amirahmadi et al. (2014), is not confirmed herein (Figs 1, 2).

Subtribe Caraganinae is composed of *Calophaca*, *Caragana* and *Halimodendron* (Ranjbar et al. 2014). Morphologically, this subtribe differs from Chesneyinae by several characters, including habit (shrubs vs. perennial herbs or subshrubs), leaf type (paripinnate [except for *Calophaca*] vs. imparipinnate) and nerve type on wing petals (pinnate vs. palmate except for *Chesneya*; Lock 2005; Ranjbar et al. 2014; Duan et al. 2015). Caraganinae is also distinct from Hedysareae (as delimited in Amirahmadi et al. 2014 and Duan et al. 2015) based on the following morphological characters: shrubs, rarely small trees; paripinnate, rarely imparipinnate leaves (*Calophaca*); solitary flowers, or a few flowers in fascicles, rarely forming a raceme; pods cylindric, rarely compressed, glabrous or hairy, with dehiscent and twisted valves (except for *Halimodendron*; Polhill 1981; Liu et al. 2010b). Caraganinae is also related to the Astragalean clade; yet due to the morphological diversity of the latter, there are few diagnosable features to differentiate the Astragalean clade from Caraganinae, except for the twisted valves of Caraganinae (*Calophaca* and *Caragana*).

An expanded generic concept of *Caragana*

Within Caraganinae, *Halimodendron* contains only *H. halodendron* (Pall.) Druce with its distribution roughly overlapping with that of *Calophaca* (Lock 2005). This species is morphologically unique in Caraganinae with its inflated pods (Gorshkova 1945; Liu et al. 2010b). Consistent with previous studies (Zhang et al. 2009; Zhang and Fritsch 2010), our results also showed that *Halimodendron* is nested within *Caragana*. The phylogenetic evidence hence supports treating *Halimodendron* as a section within *Caragana*, i.e., *Caragana* sect. *Halimodenron* (Fisch. ex DC.) L.Duan, J.Wen & Zhao Y.Chang. We also resurrect the name *Caragana halodendron* (Pallas) Dumont de Courset based on *Halimodendron halodendron* (Figs 1, 2; see Taxonomic Treatment).

Calophaca morphologically resembles *Caragana*, and it is only distinguished from the latter by its imparipinnate leaves, rachises without thorns, and relatively denser racemes (Borissova 1945; Liu et al. 2010b). *Calophaca* contains 5–8 species mainly distributed in mountainous areas of central Asia, with one species extending to eastern Europe, and one endemic to northern China (Borissova 1945; Tutin et al. 1968; Yakovlev et al. 1996; Lock 2005; Liu et al. 2010b; Zhang et al. 2015a). The embedded position of *Calophaca* within *Caragana* argues that its classification needs to be placed

in the broader phylogenetic framework of *Caragana*, which is supported by our results (Figs 1, 2) and several previous studies (e.g., Zhang et al. 2009, 2010, 2015a, b; Duan et al. 2015). We thus merge *Calophaca* into *Caragana* and recognize it at the sectional level as *Caragana* sect. *Calophaca* (Fisch. ex DC.) L.Duan, J.Wen & Zhao Y.Chang (see Taxonomic Treatment). The species-level nomenclatural changes will be made in a follow-up paper.

The taxonomy of *Caragana* has been investigated by various authors (Komarov 1908; Poyarkova 1945; Moore 1968; Sanczir 1979, 2000; Gorbunova 1984; Zhao 1993; Zhou 1996; Zhang 1997; Sanchir 1999; Chang 2008). However, *Caragana s.s.* as previously circumscribed is clearly paraphylytic (Zhang et al. 2009; Duan et al. 2015). We herein propose the delimitation of *Caragana s.l.* to ensure the generic monophyly (see Taxonomic Treatment). *Caragana* as defined now contains taxa of *Calophaca*, former *Caragana s.s.* and *Halimodendron* (Figs 1, 2), which is classified into seven sections: *Car*. sect. *Bracteolatae* M.L.Zhang, *Car*. sect. *Calophaca*, *Car*. sect. *Caragana*, *Car*. sect. *Frutescentes* (Kom.) Sancz., *Car*. sect. *Halimodenron*, *Car*. sect. *Jabatae* (Kom.) Y.Z.Zhao and *Car*. sect. *Spinosae* (Kom.) Y.Z.Zhao. Although *Caragana s.l.* is morphologically diverse, this genus can be diagnosed by its shrubby habit, saccate, oblique calyx bases, pinnate nerves on the wing petals and twisted, dehiscent pods (except for *Car. holodendron*). The expanded concept of *Caragana* is also supported by cytological evidence (Moore 1968; Chang 1993; Li 1993; Zhou et al. 2002; Chang 2008): most xeric and psychric taxa of *Caragana s.l.* have the same basic chromosome number ($x = 8$).

At the sectional level, our ITS tree (Fig. 1) indicated a strongly supported *Car*. sect. *Calophaca*. On the other hand, former *Caragana s.s.* was divided into five sections mainly based on the combinations of leaf (pinnate or digitate) and petiole/rachis (persistent or caducous) characters (Zhang 1997). Three main sections, *Car.* sect. *Bracteolatae*, *Car.* sect. *Caragana* and *Car.* sect. *Frutescentes*, evolved likely accompanying the rapid uplifts of the Qinghai-Tibet Plateau (QTP) at around 8 Ma (Zhang et al. 2009). These three sections also largely correspond to psychrophytic, mesophytic and xerophytic habitats, respectively (Zhang and Fritsch 2010). Our analyses supported the monophyly of the three sections, with *Car.* sect. *Frutescentes* only being monophyletic in the plastid tree (also see Zhang et al. 2009; Duan et al. 2015; and see below for an exceptional case in *Car.* sect. *Frutescentes*). Our ITS results failed to resolve a monophyletic *Car.* sect. *Frutescentes* (Fig. 1), but this may be due to insufficient informative sites in the ITS data. Furthermore, we only sampled one series for *Car.* sect. *Spinosae* (*Car*. ser. *Spinosae* Kom.), thus cannot assess its monophyly (Figs 1, 2). *Caragana* sect. *Jabatae* was suggested to have experienced a rapid radiation at 3.4–1.8 Ma (Zhang and Fritsch 2010), which may partly explain its poorly resolved relationships in our trees (Figs 1, 2; also see Zhang et al. 2009; Duan et al. 2015).

At the infra-sectional level, *Car.* ser. *Bracteolatae* Kom. and *Car.* ser. *Spinosae* are well-supported by our results (not labeled in the trees). Our results are therefore not completely congruent with Zhang et al. (2009), possibly due to differences in taxon sampling. Interestingly, a strongly supported psychric group is found within the mainly xeric

section *Car.* sect. *Frutescentes* (Zhao 2009). This group is represented by *Car. brevifolia* Kom., *Car. chinghaiensis* Y.X.Liou, *Car. densa* Kom. and *Car. versicolor* Benth. (in Fig. 1; but weakly supported in the plastid tree). Most species of *Car.* sect. *Frutescentes* range from eastern Europe to northern China, Mongolia and Siberia, however, this above-mentioned psychric group is distributed in the southern edge of northern China, extending to Tibet and its neighboring regions. It may represent a vicariant transitional group of *Car.* sect. *Bracteolatae*, *Car.* sect. *Jubatae* pro parte, *Car.* sect. *Spinosae* pro parte (psychrophytic habitat) and *Car.* sect. *Frutescentes*. Other cases of vicariant distributions have been noted in *Caragana*, and vicariance was considered as an important biogeographic pattern for this genus. For example, three closely related species in *Car.* sect. *Caragana*, *Car. microphylla* Lam., *Car. intermedia* Kuang & H.C.Fu and *Car. korshinskii* Kom., show non-overlapping to only slightly overlapping distributions in northeast to northwest China (Shue and Hao 1989; Zhang and Wang 1993; Zhang 1998; Chang 2008).

Phylogeny of Chesneyinae

The subtribe Chesneyinae, as established by Ranjbar et al. (2014), was supported to be monophyletic in our trees (Figs 1, 2). Three main clades can be recognized within this subtribe: the GUT clade, *Chesneya s.s.* and *Chesniella* (Figs 1, 2).

This subtribe contains ca. 50 species and differs from the Astragalean clade by twisted valves (e.g., in *Chesneya*), but a few species of *Astragalus* also have twisted legumes. Taxa of Chesneyinae are distinguished from Hedysareae by their dehiscent pods (Borissova 1945; Yakovlev et al. 1996; Liu et al. 2010a). The genera of Chesneyinae are distributed in central and eastern Asia, Tibet, Mongolia and Siberia, extending to eastern Turkey and Armenia (Fig. 3A; Borissova 1945; Davis 1970; Rechinger 1984; Lock and Schrire 2005; Liu et al. 2010a), which are largely adapted to xerophytic (*Chesneya* and *Chesniella*), mesophytic (*Gueldenstaedtia*) and psychrophytic (*Tibetia*) habitats, respectively, although some species of *Chesneya* (see discussion below) and a few of *Gueldenstaedtia* are psychric taxa. The uplift of the QTP and aridification of the former Tethys region might have driven the origination and divergence of genera in the subtribe Chesneyinae (Wen et al. 2014; Meng et al. 2015; Zhang et al. 2015b).

Topological discordance between ITS and plastid trees in subtribe Chesneyinae

The ITS and plastid topologies are incongruent within Chesneyinae. *Chesneya s.s.* formed a clade with the GUT clade in the ITS tree (Fig. 1), whereas it was sister to *Chesniella* in the plastid tree (Fig. 2). Both relationships were well-supported. Various mechanisms have been proposed to explain discordant topologies between gene trees, such as allopolyploidy, hybridization, horizontal gene transfer, incomplete lineage sorting (ILS), different rate of molecular evolution, and chloroplast capture (Degtjareva et al. 2012; García et al. 2014; Yi et al. 2015).

Allopolyploidy can be ruled out for two reasons. First, taxa within Chesneyinae are diploid (Nie et al. 2002; Yang 2002; Sepet et al. 2014), with no evidence of polyploidy in this subtribe and its allied tribes. Second, deep lineages of Chesneyinae basically display a consistent chromosome number ($x = 8$; Nie et al. 2002; Sepet et al. 2014), with the only exception of *Gueldenstaedtia* ($x = 7$; Yang 2002), which has relatively recently diverged (ca. 15.23 Ma; Zhang et al. 2015b).

ILS and chloroplast capture seem more likely mechanisms for the present case (Tsitrone et al. 2003; Deng et al. 2015; Sun et al. 2015). A time-calibrated phylogeny may facilitate the exploration of the likely mechanism. Incomplete lineage sorting, which rarely occurs in deep lineage (Sun et al. 2015), prevails with bifurcation patterns of the shallow lineages of gene trees (especially at the specific level; Xu et al. 2012), and usually takes place in groups with relatively recent diversification times (García et al. 2014). Zhang et al. (2015b) estimated that the main clades of subtribe Chesneyinae split at ca. 28 Ma, which is beyond the time frame supporting ILS of ancestral polymorphisms (as suggested by Xu et al. 2012). On the other hand, biogeographic patterns can also be taken into consideration (Goodman et al. 1999). Given peripatry and parapatry may have been involved in the evolution of Chesneyinae, if ILS occurred, the main clades would hardly be resolved with well-supported dichotomy as presented herein. Hence, although ILS could not be completely excluded in this case, we regarded chloroplast capture as the most likely cause for the discordant position of *Chesneya s.s*.

Compared to the biparental inheritance of the nuclear genome, plastid DNA of angiosperms is usually uniparentally transmitted, especially maternally (Corriveau and Coleman 1988; McCauley et al. 2007; Wicke et al. 2011). Nevertheless, the plastid DNA of the inverted repeat lacking clade (IRLC; see Figs 1, 2; also as in Lavin et al. 1990; Wojciechowski et al. 2000) in Leguminosae was reported to be inherited paternally or biparentally (Zhang et al. 2003), confirmed by cytoplasmic and phylogenetic studies focusing on *Medicago* L. (paternal transmission; Schumann and Hancock 1989; Masoud et al. 1990; Havananda et al. 2010) and *Wisteria* Nutt. (Hu et al. 2005; Trusty et al. 2007). As *Chesneya s.s.* belongs to IRLC, a paternal inheritance scenario might be the case for the plastid DNA of *Chesneya s.s.*

We herein hypothesize a chloroplast capture event in the origin of *Chesneya s.s.* as follows. The common ancestor of *Chesniella* served as the putative paternal parent of *Chesneya s.s.* (sister to *Chesneya s.s.* in the plastid tree; Fig. 2). The maternal parent most likely was the common ancestor of the GUT clade. Their hybrids, with plastid from the paternal parent, may have continuously backcrossed with the maternal parent, and led to *Chesneya s.s.* inheriting most of the nuclear genome maternally (Fig. 1). Such a chloroplast capture event via introgression likely took place in the Miocene, because the divergence of *Chesneya s.s.* was dated to be 16.56 Ma and that of *Chesniella* was estimated as 19.81 Ma (Zhang et al. 2015b).

Analyses of Zhang et al. (2015b) revealed that the divergence of *Chesneya* and *Chesniella* most likely occurred around the QTP. Our analysis further indicated the psychric group of *Chesneya* diverged first in this genus (*C.* sect. *Pulvinatae*, see Discussion below). It is probable that the common ancestor of *Chesniella* adapted to psychro-

phytic habitats. However, the extant *Chesniella* is rarely distributed on the QTP. As for the GUT clade, *Gueldenstaedtia* possesses a unique chromosome number (x = 7; Yang 2002) within the subtribe. Most species of *Gueldenstaedtia* are adapted to mesophytic habitats of temperate northern and eastern Asia (Fig. 3A), in contrast to the rest of Chesneyinae, which are psychric or xeric taxa. Such a correlation among the variation of chromosome numbers and adaptation to different habitats has also been recorded in other taxa, such as *Hedysarum* (Tang 2005; Duan et al. 2015), *Passiflora* (Hansen et al. 2006) and Amaryllidaceae (García et al. 2014). But the mechanisms of these types of adaptation need to be further explored with robust phylogenetic, ecological and biogeographic analyses in our future efforts.

Phylogeny and treatment of *Chesneya*, *Chesniella* and *Spongiocarpella*

Chesneya is the type genus of Chesneyinae, with ca. 35 species (see Fig. 3B–D). This genus has its distribution from the Himalayan region to northwestern China and Mongolia, through central and western Asia, westward to Turkey and Armenia (Fig. 3A; Borissova 1945; Davis 1970; Yakovlev et al. 1996; Lock and Schrire 2005; Fig. 3A). Our results suggest that the formerly circumscribed *Chesneya*, which contains two well-supported but separated parts: the core *Chesneya s.s.* and the outlier *C. macrantha* (Fig. 3E) (as in Li 1993 & Zhu and Larsen 2010), is not monophyletic (Figs 1, 2). *Chesneya spinosa* P.C.Li (Fig. 3C) of *Chesneya s.s.* is morphologically similar to *C. macrantha* (Li 1981). However, *C. spinosa* is distributed in southern Tibet, while *C. macrantha* is restricted to the dry lands of Mongolia and northwestern China (Li and Ni 1985; Fu 1989). They occupy psychrophytic and xerophytic habitats, respectively, and are clearly not sister to each other (Figs 1, 2).

Chesneya macrantha is nested within a monophyletic *Chesniella* according to our ITS tree (Fig. 1), and in the plastid tree, it is sister to the type of *Chesniella*: *Ch. ferganensis* (Korsh.) Boriss. (Borissova 1964; see Fig. 2, 3F). *Chesneya macrantha* shows some distinct morphologies from the other species in *Chesniella*, including its pulvinate habit and persistent leaf rachis (Li 1993), but this species generally share distribution areas, xerophytic habitats, and some synapomorphies, such as membranous stipules, hairy standard and ovate leaflets with cuneate apices, with *Chesniella* (Li and Ni 1985; Fu 1989; Zhu and Larsen 2010). Therefore, the transfer of *Chesneya macrantha* to *Chesniella* is supported by morphological, geographic and phylogenetic evidence (see Taxonomic Treatment). On the other hand, *Chesneya* was thus re-delimited based on the monophyletic *Chesneya s.s.*

After its establishment by Lindley (1839), *Chesneya* was divided into *C.* sect. *Macrocarpon* Boriss. and *C.* sect. *Microcarpon* Boriss. mainly based on pod morphology (Borissova 1945). The latter was segregated as the genus *Chesniella* by Borissova (1964), and this treatment was followed by Li (1993) and Zhu and Larsen (2010). Zhang et al. (2015b) informally classified *Chesneya* into five sections without detailed taxonomic treatment. Not all their sections were monophylytic, and the diagnostic characters and distributions of several sections were overlapping to some extent.

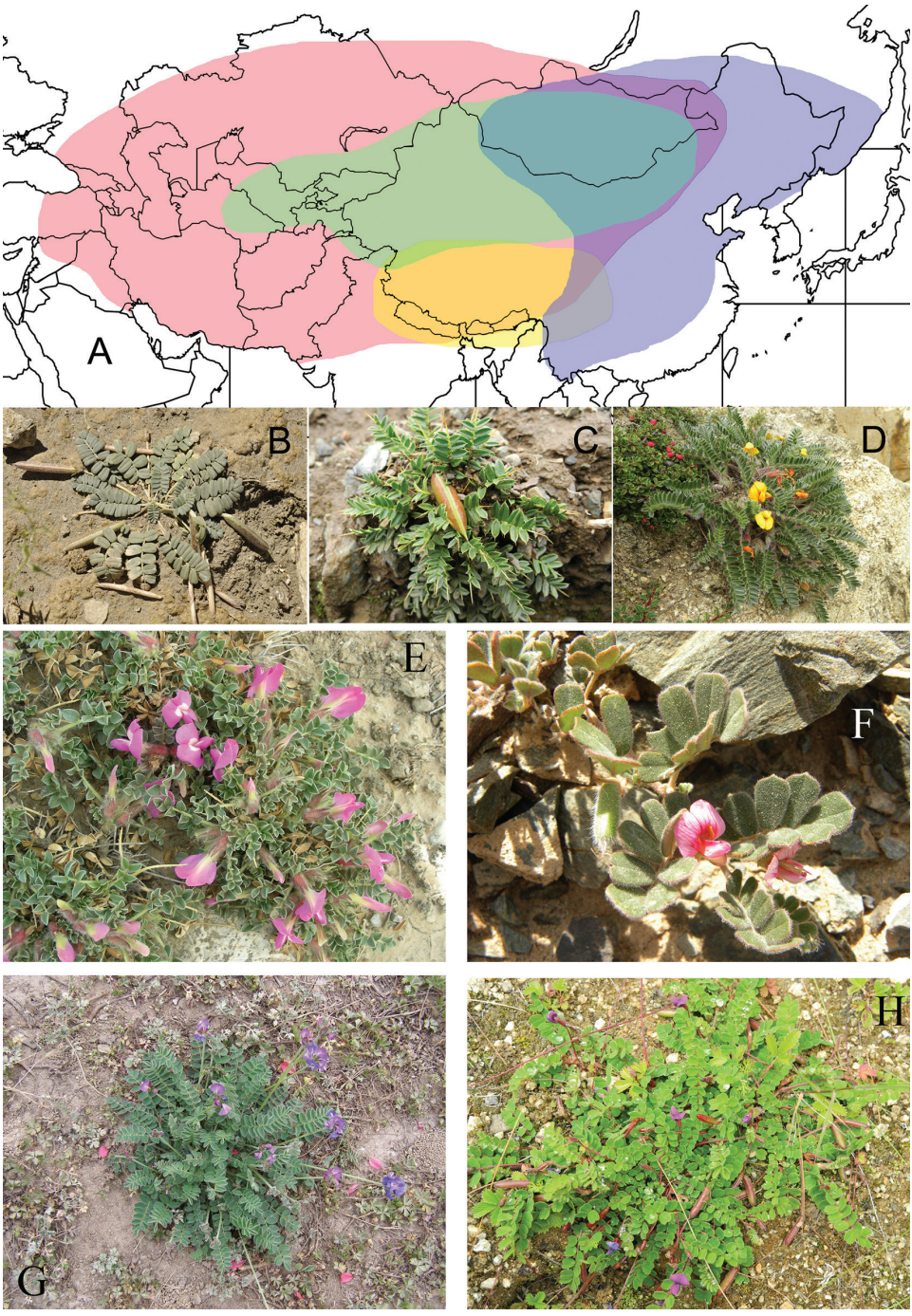

Figure 3. Distribution (**A**) and representative plants (**B–H**) of genera in Chesneyinae. **A** red – *Chesneya*, green – *Chesniella*, blue – *Gueldenstaedtia* and yellow – *Tibetia* **B** *Chesneya acaulis* **C** *Chesneya spinosa* **D** *Chesneya nubigena* **E** *Chesniella macrantha* **F** *Chesniella ferganensis* **G** *Gueldenstaedtia verna* **H** *Tibetia yadongensis*.

The presently demarcated *Chesneya* was assigned into three strongly supported sections herein (as in the key of *Chesneya* proposed by Li 1993; details see Figs 1, 2 and Taxonomic Treatment). *Chesneya* sect. *Macrocarpon* possesses non-pulvinate habit, reduced stems, truncate or emarginate leaflet apices and caducous petiole and rachis (Borissova 1945). This section is composed of most species of *Chesneya*, including the type species: *C. rytidosperma* Jaub. et Spach (see Fig. 2; Borissova 1945; Davis 1970; Rechinger 1984). *Chesneya* sect. *Macrocarpon* was thus treated as *C.* sect. *Chesneya* (Fig. 3B). Unlike this section, petioles and rachises of *C.* sect. *Pulvinatae* M.L.Zhang (Zhang et al., 2015b; see Fig. 3D) are persistent and pubescent. However, most species in *C.* sect. *Pulvinatae* have blackened and curved petioles and rachises, while those of one of its species, *C. spinosa*, are hardened and spiny. Besides, *C. spinosa* formed a clade separated from *C.* sect. *Pulvinatae.* Hence, it is appropriate to segregate this species to form a new monotypic section: *C.* sect. *Spinosae* L.Duan, J.Wen & Zhao Y.Chang (see see Fig. 3C and Taxonomic treatment).

The infra-sectional relationships within *C.* sect. *Chesneya* are basically unresolved in our ITS trees (Fig. 1), and this section is undersampled in the plastid trees (Fig. 2). As for *C.* sect. *Pulvinatae*, two accessions of *C. nubigena* (D.Don) Ali formed a clade, being sister to *C. purpurea* P.C.Li (Figs 1, 2). Based on such well-supported tree topologies and several morphological differences, such as smaller leaflets and purple corollae, the specific status of *C. purpurea* was retained herein (as in Li 1981, 1993).

The xeric *C.* sect. *Chesneya* grows on dry slopes or desert margins of northwestern China, Mongolia and central Asia (see Fig. 3B; Borissova 1945; Rechinger 1984; Lock and Simpson 1991; Yakovlev et al. 1996; Zhu and Larsen 2010). This section is morphologically similar to *Chesniella* (Fig. 3F) and their distributions are more or less overlapping (Borissova 1945; Li, 1993), whereas they are not phylogenetically close to each other (Figs 1, 2). Such a phenomenon may be due to convergent evolution (Degtjareva et al. 2012). *Chesneya* sect. *Spinosae* (Fig. 3C) and *C.* sect. *Pulvinatae* (Fig. 3D) are restricted to Tibet and adjacent regions, adapting to high-altitude psychrophytic habitats (Ali 1977; Zhu and Larsen 2010). The evolutionary history of *Chesneya* appears complex, whereas the elevation of the QTP and the subsequent aridifications may have played an important role (Meng et al. 2015; Zhang et al. 2015b), as in former *Calophaca* (Zhang et al. 2015a), *Caragana* (Zhang and Fritsch 2010) and *Hedysarum* (Shue 1985; Duan et al. 2015).

Most previous workers did not accept the generic status of *Chesniella*, treating it within *Chesneya* (Borissova 1945; Li 1981; Rechinger 1984; Zhu and Cao 1986; Fu 1987, 1989; Yakovlev 1988; Yakovlev et al. 1991). Nevertheless, Li (1993) and Zhu and Larsen (2010) stated that the former is distinguishable from the latter by non-reduced stems, membranous stipules, obviously smaller calyxes, flowers and pods. With the inclusion of *Ch. macrantha* (Fig. 3E), our results justified the monophyly of *Chesniella* (Figs 1, 2), consistent with Zhang et al. (2015b). Within *Chesniella*, two well-supported groups were resolved in our ITS tree (Fig. 1). *Chesniella macrantha* and *Ch. mongolica* (Maxim.) Boriss. constituted group A, the group B included *Ch. ferganensis*, *Ch. gracilis* Boriss. and *Ch. tribuloides* (Nevski.) Boriss. The former confined in Mongolia and Inner Mongolia of China, to the contrast, the latter ranged from northwestern China to central Asia, which implied vicariance caused by Altai Moun-

tain may drive the divergence of these two groups. However, due to undersampling and distinct morphology of *Ch. macrantha* in *Chesniella*, the evolution history and infrageneric taxonomy of this genus needs to be further explored.

Yakovlev and Sviazeva (1987) erected *Spongiocarpella* as a segregate genus from *Chesneya* in the light of the former's spongiose legumes. Such treatment was followed by Yakovlev (1988), Fu (1989) and Yakovlev et al. (1996), but was rejected by Li (1993), Zhu (1996), Qian (1998) and Zhu and Larsen (2010). Based on field and herbarium studies, we concur with Zhu (1996) that the sponge-like pericarp is an unstable character. Additionally, several species formerly assigned to *Spongiocarpella* were represented in our study, including *Chesneya nubigena* (D.Don) Ali, *C. Spinosa* and *Chesniella macrantha*. They did not form a monophyletic group (Figs 1, 2). Thus, our data do not support the generic status of *Spongiocarpella* (as in Zhu 1996; Zhu and Larsen 2010; Ranjbar et al. 2014; Zhang et al. 2015b).

Monophyly of *Gueldenstaedtia* and *Tibetia*

Gueldenstaedtia is a small genus comprised of ca. 10 species and is distinguished from *Chesneya* by its palmately nerved wing petals (vs. pinnately in *Chesneya*) and non-twisted pod valves (vs. twisted) (see Fig. 3G; Liu et al. 2010a). This genus ranges from the Sino-Himalayan region to Mongolia and Siberia (Lock and Schrire 2005; see Fig. 3A). It was established by Fischer (1823) and revised by Fedtschenko (1927), Jacot (1927) and Kitagawa (1936). Ali (1962) divided it into *G.* subg. *Gueldenstaedtia* and *G.* subg. *Tibetia* Ali, but the latter was elevated to the generic rank by Tsui (1979) based on characters of stems, stipules, styles and seeds (see Fig. 3H). The genus *Tibetia* was generally accepted in subsequent revisions (Shue 1992; Yakovlev et al. 1996; Cui 1998; Wu 1999; Zhu 2004, 2005a; Bao and Brach 2010), and it is confined to Tibet and the adjacent regions including southern Gansu, southern Qinghai, western Sichuan and northwestern Yunnan of China, northern India, Nepal and Buhtan (Tsui 1979; Grierson and Long 1987; Lock and Schrire 2005; Zhu 2005a; Bao and Brach 2010).

Gueldenstaedtia and *Tibetia* were each supported to be monophyletic, and the two genera together form the GUT clade (Figs 1, 2). It seems valid to retain the generic status of each genus, which is also supported by karyological studies (Nie et al. 2002; Yang 2002; Zhu 2005b): *Gueldenstaedtia* ($x = 7$) vs. *Tibetia* ($x = 8$). Within *Gueldenstaedtia*, three species were sampled (all belonging to *G.* sect. *Gueldenstaedtia* according to Tsui 1979), but these species were all treated to be *G. verna* (Georgi) Boriss. *s.l.* by some workers (Yakovlev 1988; Zhu 2004; Bao and Brach 2010). Further work is needed to test the delimitation of *G. verna s.l.*

Within *Tibetia*, two accessions of *T. himalaica* (Baker) H.P.Tsui grouped together, which were sister to *T. yadongensis* H.P.Tsui (Figs 1, 2). The tree topology and the morphological characters (e.g., elongate stem and round or retuse leaflets apex) seem to be consistent with treating *T. himalaica* as a distinct species (also see Tsui 1979; Cui 1998; Zhu 2005a; Bao and Brach 2010).

Taxonomic treatment

Caragana **Fabr., Enum. Ed. 2. 421. 1763, emend. nov. L.Duan, J.Wen & Zhao Y.Chang**

Calophaca Fisch. ex DC., Prod. 2: 270. 1825, syn. nov.
Type: *Calophaca wolgarica* Fisch., Prod. 2: 270. 1825.
Halimodendron Fisch. ex DC., Prod. 2: 269. 1825, syn. nov.
Type: *Halimodendron halodendron* (Pall.) Druce, Rep. Bot. Soc. Exch. Club Brit. Isles 4: 626. 1917.

Type. *Caragana arborescens* Lam., Encycl. 1(2): 615. 1785.

Description. Shrubs, subshrubs or rarely small trees. Stipules caducous or persistent. Leaves paripinnate, rarely imparipinnate (*C.* sect. *Calophaca*), 4–27-foliolate; leaflet blades with margin entire. Lax raceme or fascicled flowers axillary, or flowers solitary. Calyx tubular or campanulate, base usually oblique, teeth 5. Corolla yellow, purple, pink or white; standard ovate to suborbicular, clawed or reflexed at margin; wings and keel often auriculate. Stamens diadelphous (9+1). Ovary sessile to stipitate, with ovule 1-many; style filiform. Pod inflated, compressed, cylindric or linear, dehiscent or rarely indehiscent (*C.* sect. *Halimodendron*), with twisted or thickened valve.

Distribution and habitat. This genus contains ca. 100 species, ranging from eastern Europe, Caucasus, western and central Asia, Sino-Himalayan region to Mongolia and Siberia.

Caragana **sect.** ***Calophaca*** **(Fisch. ex DC.) L.Duan, J.Wen & Zhao Y.Chang, stat. & comb. nov.**
urn:lsid:ipni.org:names:77157989-1

Calophaca Fisch. ex DC., Prod. 2: 270. 1825.
Type: *Calophaca wolgarica* Fisch., Prod. 2: 270. 1825.

Distribution and habitat. This section includes 5–8 species, distributed in Caucasus, central Asia, northwestern Xinjaing, Innner Mongolia and Shanxi of China.

Caragana **sect.** ***Halimodendron*** **(Fisch .ex DC.) L.Duan, J.Wen & Zhao Y.Chang, stat. & comb. nov.**
urn:lsid:ipni.org:names:77157990-1

Halimodendron Fisch. ex DC., Prod. 2: 269. 1825.
Type: *Halimodendron halodendron* (Pall.) Druce, Rep. Bot. Soc. Exch. Club Brit. Isles 4: 626. 1917.

Type. *Caragana halodendron* (Pallas) Dumont de Courset, Bot. Cult. 3: 513. 1802.

Distribution and habitat. This section is monotypic and distributes in Caucasus, northeastern Turkey, northern Iran, northern Afghanistan, northern Pakistan, central Asia, western Mongolia, Shanxi and Xinjiang of China.

Key to the sections of *Caragana*

1 Leaves imparipinnate; ovary sessile..... ***Car.* sect. *Calophaca***
– Leaves paripinnate; ovary subsessile or stipitate.....**2**
2 Racemose; pedicel non-articulate; pods inflated, indehiscent, valve thickened; seeds few.....***Car.* sect. *Halimodendron***
– 2–5 flowers in fascicles, or solitary flower; pedicel articulate; pods compressed, cylindric or linear, dehiscent, valve twisted; seeds many.....**3**
3 Petiole and rachis always caducous; leaves pinnate .....***Car.* sect. *Caragana***
– Petiole and rachis persistent, usually spinelike; leaves pinnate or digitate **4**
4 Leave digitate.....***Car.* sect. *Frutescentes***
– Leave pinnate or partly digitate.....**5**
5 Leave digitate or pinnate with 4 leaflets on short branchlets, leave pinnate on long branchlets ..... ***Car.* sect. *Spinosae***
– Leaves pinnate **6**
6 Petiole and rachis persistent ..... ***Car.* sect. *Jubatae***
– Petiole and rachis persistent on long branchlets, caducous on short branchlets..... ***Car.* sect. *Bracteolatae***

***Chesneya* Lindl. ex Endl., Gen.: 1275. 1840.**
Fig. 3B–D

Spongiocarpella Yakovlev & N.Ulziykhutag, Bot. Zhur. 17(2): 249. 1987. syn. nov.
Type: *Spongiocarpella nubigena* (D.Don) Yakovl., Bot. Zhur. 17(2): 249. 1987, based on *Chesneya nubigena* (D.Don) Ali. (see blow)

Type. *Chesneya rytidosperma* Jaub. et Spach, Ill. Pl. Orient. 1(5): 93. 1842.

Chesneya* sect. *Chesneya
Fig. 3B

Chesneya sect. Macrocarpon Boriss., Fl. U.S.S.R. 11: 280. 1945. syn. nov.
Type: *Chesneya rytidosperma* Jaub. et Spach, Ill. Pl. Orient. 1(5): 93. 1842.

Description, distribution and habitat. This section includes the majority of *Chesneya* species. It can be diagnosed by reduced stems and caducous petiole and rachis. It con-

tains ca. 20 xeric species, ranging from desert and dry slope of northwestern China and western Tibet to central and western Asia and Caucasus.

***Chesneya* sect. *Pulvinatae* M.L.Zhang, Biochem. Syst. Ecol. 63: 89. 2015.**
Fig. 3D

Spongiocarpella Yakovlev & N. Ulziykhutag, Bot. Zhur. 17(2): 249. 1987.
Type: *Spongiocarpella nubigena* (D.Don) Yakovl., Bot. Zhur. 17(2): 249. 1987.

Type. *Chesneya nubigena* (D.Don) Ali, Scientist (Karachi) iii: 4. 1959.

Description, distribution and habitat. This psychric section is composed of *C. nubigena*, *C. polystichoides* (Hand.-Mazz.) Ali and *C. purpurea*. It differs from other sections by blackened, curved and non-spiny petiole and rachis, distributed on high-altitude slope in eastern Himalayas and southern and eastern Tibet.

***Chesneya* sect. *Spinosae* L.Duan, J.Wen & Zhao Y.Chang, sect. nov.**
urn:lsid:ipni.org:names:77157991-1
Fig. 3C

Type. *Chesneya spinosa* P.C.Li, Acta Phytotax. Sin. 19(2): 236. 1981.

Description, distribution and habitat. This monotypic section is recognized by its hardened-spiny petiole and rachis. It is restricted in high-altitude psychrophytic rocky slope in southern Tibet.

Key to the sections of *Chesneya*

1 Plant non-pulvinate, petiole and rachis caducous, leaflet apices truncate or emarginate ..... ***C.* sect. *Chesneya***
– Plant pulvinate, petiole and rachis persistent, leaflet apices acute **2**
2 Petiole and rachis hardened and spiny, leaflet apices with short spines ..... ***C.* sect. *Spinosae***
– Petiole and rachis blackened and curved, leaflet apices without short spines ... ***C.* sect. *Pulvinatae***

***Chesniella macrantha* (Cheng f. ex H.C.Fu) L.Duan, J.Wen & Zhao Y.Chang, comb. nov.**
urn:lsid:ipni.org:names:77157988-1

Chesneya macrantha Cheng f. ex H.C.Fu, Fl. Intramongol. 3: 291. 1977.

Note. Information of the type specimen was not included in its protolog, which was recorded in Acta Phytotax. Sin. 19(2): 237. 1981: China. Inner Mongolia: Baganmao, 29 May 1931, *T.N.Liou 2146* (holotype: PE!).

Specimens examined. CHINA. Ningxia: Mt. Helan, 1200m, May 15 1923, *R.C.Ching 108* (US); **Inner Mongolia:** Alasan Left Banner, Xiazi valley, 24 Apr 2009, *Z.Y.Chang et al. 2009054* (WUK); Mt. Yabulai, Agui temple, 1300m, Apr 26 2008, *L.R.Xu 2008008* (WUK); **Xinjiang:** Qomul, 43° 05.330'N, 93° 42.030'E, 1311m, 6 Jun 2004, *Z.Y.Chang et al. 2004516* (WUK).

Distribution and habitat. Dry slopes in Mongolia and Inner Mongolia, Ningxia and Xinjiang of China.

Acknowledgement

We are grateful to the curators of the following herbaria for providing leaf samples, seeds or DNA samples: CS, F, K, TURP, US, WUK and XJBI. We thank Matthew Kweskin, Mingqin Zhou, and Jianqiang Zhang for lab assistance; Natasha Crump for Russian literature translation; Robin Everly for literature search; and Martin F. Wojciechowski, Ying Feng, Shimin Duan, Irina Illarionova and Roman Ufimov for checking voucher information. The Laboratories of Analytical Biology of the National Museum of Natural History (Smithsonian Institution), the Natural Science Foundation of China (Project no. 30270106, 30870155) and the China Scholarship Council are gratefully acknowledged for financial support of the study.

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Supplementary material 1

Voucher information and GenBank accession numbers

Authors: Lei Duan, Xue Yang, Peiliang Liu, Gabriel Johnson, Jun Wen, Zhaoyang Chang

Data type: Multi-records

Explanation note: Data are arranged in the order: taxon name, locality, collector(s), collection number and herbarium, GenBank accession numbers for ITS, *matK*, *trnL-trnF*, *psbA-trnH*. Newly generated sequences are indicated by an asterisk (*); missing sequences are indicated by a dash (–).