

RESEARCH ARTICLE

Characterizing Floral Symmetry in the Core Goodeniaceae with Geometric Morphometrics

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Abstract

Core Goodeniaceae is a clade of ~330 species primarily distributed in Australia. Considerable variation in flower morphology exists within this group and we aim to use geometric morphometrics to characterize this variation across the two major subclades: *Scaevola* sensu lato (s.l.) and *Goodenia* s.l., the latter of which was hypothesized to exhibit greater variability in floral symmetry form. We test the hypothesis that floral morphological variation can be adequately characterized by our morphometric approach, and that discrete groups of floral symmetry morphologies exist, which broadly correlate with subjectively determined groups. From 335 images of 44 species in the Core Goodeniaceae, two principal components were computed that describe >98% of variation in all datasets. Increasing values of PC1 ventralize the dorsal petals (increasing the angle between them), whereas increasing values of PC2 primarily ventralize the lateral petals (decreasing the angle between them). Manipulation of these two morphological “axes” alone was sufficient to recreate any of the general floral symmetry patterns in the Core Goodeniaceae. *Goodenia* s.l. exhibits greater variance than *Scaevola* s.l. in PC1 and PC2, and has a significantly lower mean value for PC1. Clustering clearly separates fan-flowers (with dorsal petals at least 120° separated) from the others, whereas the distinction between pseudo-radial and bilabiate clusters is less clear and may form a continuum rather than two distinct groups. Transitioning from the average fan-flower to the average non-fan-flower is described almost exclusively by PC1, whereas PC2 partially describes the transition between bilabiate and pseudo-radial morphologies. Our geometric morphometric method accurately models Core Goodeniaceae floral symmetry diversity.

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Introduction

Categorization of morphological forms is an important component of comparative biology. Categories can suggest function and/or relatedness, and can be analysed in a comparative framework to assess homology, convergence, and correlation. Some categories are quite obvious, whereas others are less well delineated. Subjective morphological categories are often

sufficient for taxonomic or systematic inquiries; however, new tools in population genetics, evo-devo, and next-generation molecular sequencing are detecting increasingly significant levels of genetic divergence between morphologically cryptic lineages [1, 2]. As such, methods that provide objective, quantitative alternatives to subjective categorization are becoming important, particularly when categories are not immediately obvious or variation appears continuous. Quantifying these traits provides mathematical leverage to studies of variation within populations, and to comparative character evolution and gene expression studies among species to help identify the key molecular targets of adaptive evolution. A textbook example is the adaptive radiation in Darwin's finches. Although their beaks exhibit multiple discrete forms correlating with food sources, the overall shape of the beak is dictated by modulation of two pathways: *Bmp4* for depth, and Calmodulin for length [3, 4], operating along an axis of shape variation discerned by geometric morphometrics [5]. Studies that have shifted from binary or discrete characters to continuous characters have also opened our eyes to previously unseen patterns, as in the example of the punctuated adaptive diversification of the *Aquilegia* nectar spur in response to different pollinators (e.g., [6, 7]).

Floral symmetry provides an interesting morphological character in which to attempt quantitative modelling (e.g., [8]). The basic blueprint of floral development has been well characterized in several model systems and the essential molecular components are well conserved (e.g., [9]). The genes responsible for modifying floral forms are also increasingly being characterized in an evolutionary context in non-model systems through the hybrid field of evo-devo [10]. As adaptive floral morphology is intricately linked with pollinator interactions and environmental cues, quantifying the developmental modules responsible for floral morphology might provide insight into multiple aspects of ecology and evolution. Developing a measurable, quantitative character for floral symmetry is an important factor in clarifying modulations of the basic floral blueprint.

Geometric morphometrics is a methodology that captures quantitative measurements from the shape of complex structures, potentially uncovering patterns of shape evolution or morphological integration [11]. Geometric morphometrics differ from traditional morphometric methods in that they characterize the shape of an object as a whole, rather than in pieces, yielding a unified shape model. Briefly, homologous or semi-homologous morphological landmarks can be applied to a population of comparable images, which provide the raw data for multivariate analyses of shape in developmental and evolutionary contexts [12]. Though these methods have been widely applied and well developed in zoology and related fields, botanists have only begun to adopt them in studies of leaf and floral shape [13]. Floral morphometric variation has been studied in single plant species to address developmental and evolutionary questions [14–19], and across broader sets of species for taxonomic and evolutionary purposes [20–23]. In this study, we use geometric morphometrics to compare and categorize the diverse floral forms in a charismatic clade, Core Goodeniaceae.

The Goodeniaceae is a species-rich family that currently includes more than 420 species across 12 genera [24], with Australia being the centre of diversity. The family is circumscribed into two clades, LAD (*Lechenaultia*, *Anthotium*, and *Dampiera*) and the larger Core Goodeniaceae ([25]: *Brunonia australis*, and sister clades *Scaevola* s.l. (*Scaevola* sensu stricto with embedded monotypic *Diaspasis filifolia*) and *Goodenia* s.l. (*Goodenia*, *Cooperookia*, *Velleia*, *Verreauxia*, *Selliera*, *Scaevola collaris*, and *Pentaptilon*)). Species in Core Goodeniaceae differ in corolla shape and size, petal placement, color, tactile guides, amount of extra-petal “wing” tissue, morphology of the indusium (appendage of the style for secondary pollen presentation unique to the family), and pollinator rewards. A recent molecular phylogenetic study of the family [26] has also provided evidence that within Core Goodeniaceae there have been multiple independent floral symmetry shifts, most notably in *Goodenia* s.l.

With the exception of one species, all Goodeniaceae have pentamerous, bilaterally symmetrical corollas with a dorsal slit opening the tube of fused petal bases between the two dorsal petals. The depth of the slit varies considerably among species, with the most extreme forms having no fusion of the corolla tube above the ovary. The sole taxon that lacks this slit is the monotypic taxon sister to the remainder of Core Goodeniaceae, *Brunonia australis*, which has been considered the only “true” radially symmetrical Goodeniaceae [27]. However, despite the uniform corolla tube, in our observations the distal petal tips rarely have a radially symmetrical arrangement (S1 Fig). Floral symmetry categorization was included in the descriptions of genera in the taxonomic treatment of the family for *Flora of Australia* [24], but not on a consistent basis. The genus *Scaevola* (commonly named fan-flower) and genus *Selliera* are described as “completely split adaxially”, with all five petals towards the ventral side, resembling a fan. *Goodenia* is described as “usually bilabiate”, and *Velleia* is “bilabiate”. *Diaspasis* and *Cooper-nookia* are described as “scarcely bilabiate.” At the subgeneric level, there is no mention of the fan-flower form common to all members of *Goodenia* subgenus *Monochila*, nor is there common or consistent discussion of symmetry types for individual species.

The diverse corolla morphologies and putative convergent evolution of forms make the Core Goodeniaceae an excellent system for the study of floral evolution. Floral shape diversity (and thus potentially developmental or evolutionary lability) appears to be greater in *Goodenia* s.l. than sister clade *Scaevola* s.l. Almost all members of *Scaevola* s.l. have fan-flowers where all five petals are opposite the dorsal slit—a form evocative of the ligulate floret of some Asteraceae. All three major clades of *Goodenia* s.l. contain species with fan-flowers, indicating potential convergent evolution of the fan form. There is also extreme diversity in non-fan-flowered morphs, ranging from clearly bilabiate, with dorsal petals nearly 180 degrees apart from lateral petals, to nearly radial, with similar angles between all five petals. *Goodenia* s.l. also includes more species (226 described versus 103 in *Scaevola* s.l.) and is a more ancient lineage than *Scaevola* s.l. (crown ages of 31.7 Ma versus 13.5 Ma; [28]). We ultimately aim to reconstruct floral symmetry evolution across the Core Goodeniaceae to study the tempo of floral divergence and provide complementary data for detailed taxonomic and evo-devo studies on the group. These efforts are currently limited by uncertainty about the existence or composition of objective categories of floral symmetry in the clade as we rely on the somewhat arbitrary, subjective categories of bilabiate, fan-flowered, and pseudo-radial floral forms, as characterised in the *Flora of Australia*.

Here, we seek to develop and utilize a geometric morphometrics approach that is robust to variation from different photos of individual flowers, captures and describes the major variation in floral symmetry between species, and quantifies and delineates objective floral symmetry categories across the Core Goodeniaceae. Once the robustness of the method to photo variance and potential floral asymmetry and allometric effects is assessed, we apply it across a phylogenetically and morphologically diverse set of Core Goodeniaceae species to test several hypotheses. We hypothesize that the greater variation in floral symmetry form observed in *Goodenia* s.l. rather than *Scaevola* s.l. can be quantified. Additionally, we test whether discrete floral symmetry clusters are recovered through geometric morphometrics, and if these correspond to our subjective pseudo-radial, bilabiate, and fan-flower symmetry categorizations. Finally, we explore how independent patterns of morphological variation among the dorsal and lateral petals support the existence of distinct developmental modules in Goodeniaceae floral morphogenesis.

Materials and Methods

Image collection

Core Goodeniaceae is a diverse clade with many ephemeral, remote species and narrow range endemics [24]. Rather than accurately describing and differentiating individual or closely

related species with extensive intraspecific sampling, the overall goal is to describe floral shape diversity across the breadth of Core Goodeniaceae. To this aim, fewer numbers of floral images are used to represent rarer species than is typical in other geometric morphometrics studies that focus on small numbers of taxa (e.g. [14, 16, 19]). To represent both the phylogenetic diversity and the breadth of floral symmetry morphologies within the group, we captured 335 representative images from 44 species of Core Goodeniaceae from all major clades, as well as one *Dampiera* species from the clade sister to Core Goodeniaceae (Table 1). Images for each species were taken from up to 14 individuals found in multiple populations (7.4 ± 3.3 SD flower images per species). During two collecting trips in southwestern Australia, images of flowers were captured using a digital SLR camera, either in the field or from field-collected living plants maintained in cultivation at Kings Park and Botanical Garden (Perth). For three species not encountered in the field (*Goodenia macmillanii*, *G. disperma*, and *G. stephensonii*), images from the Atlas of Living Australia (<http://bie.ala.org.au/>) were used. We aim to be able to include additional images from various sources in subsequent applications of the method, and so wanted to ensure that such publically available data were useable and comparable to photos taken specifically for morphometrics analysis. Flowers were photographed along the axis of the corolla tube, providing a two-dimensional “head-on” view of the corolla. This imaging method provided a consistent, replicable view of the flowers from both field based collections and those downloaded from an online repository.

Morphometrics

Floral images were landmarked for geometric morphometric analyses using the software tpsDIG2 (available at <http://life.bio.sunysb.edu/morph/> [29]). Initially, three different landmarking schemes, using five, 25, and 45 landmarks per image were employed. The five-point scheme placed one clearly homologous landmark at the apex of each of the five petals. The 25-landmark scheme added four landmarks per petal along the margins of the extra-petal wing tissue at axes oriented 90° from positions halfway and three-quarters of the way down the centerline of the visible petal. The 45-landmark scheme added four more landmarks along the margins of the true petals. When petal margins overlapped, landmark positions were estimated based on the distance from the centerline for the opposite landmark. After analysis, each of the three landmark schemes provided similar models for floral morphology. The five-landmark scheme was continued for further study because of its simplicity and reliance on only clearly homologous landmarks (Fig 1).

The x and y coordinates for these five landmarks from each of 335 images were then subjected to Procrustes transformation to minimize scalar and rotational differences, followed by principle components (PC) analysis on their covariance matrix (see [30]) using MorphoJ [31] to distinguish symmetrical vs. asymmetrical contributions to floral morphology. To examine the role of asymmetry in Core Goodeniaceae floral variation, a Procrustes ANOVA was calculated with MorphoJ to ascertain the magnitude of floral shape variance explained by species determination and by asymmetry. Asymmetrical components were found to be significant in this data set (p-value = 0.0054; S2 Table) however they accounted for less than 2% of the overall variance. We also examined the influence of asymmetry by using centroid sizes (the square root of the sum of squared distances of a set of landmarks from their centroid). If the centroid size of the asymmetric component is small compared to symmetric centroid size, and it is uncorrelated to any of the other factors we are studying, we can focus on the symmetric component for downstream analyses and consider asymmetries to be a part of the overall environmental error in our calculations. The centroid size of the asymmetrical component averaged 0.1010 (± 0.06910 SD), just 4.4% of the symmetrical component (2.305 (± 0.368 SD)). ANOVAs

Table 1. Subjective grouping, mean PC scores, k-means clustering, voucher, and collection information for the plants used in this study.

Taxon	Subjective	# pics	PC1 Mean	PC2 Mean	Cluster Mean	k-means	Voucher photographer	Latitude Longitude (GDA 94)	Collection locality (cultivation collection)	Voucher collector	Voucher collection number
<i>Brunonia australis</i> R.Br.	Fan	12	0.303	0.004	1.00	fan	S.R. Willis	30° 03' S 116° 40' E	Syme Road, east of Wubin, Western Australia	Kelly Shepherd & Spencer Willis	KS 1512
<i>Cooperookia georgei</i> Carolin	Bilabiate	1	-0.332	-0.085	2.00	pseudo-radial	A. Gardner	33° 58' S 119° 48' E	Fitzgerald River National Park, WNW of Quoin head, Western Australia (Kings Park & Botanic Garden living collection KP20070756)	Max Crowhurst	MCRO 44
<i>Cooperookia polygalacea</i> (de Vriese) Carolin	pseudo-radial	5	-0.207	-0.041	2.00	pseudo-radial	S.R. Willis	n/a	Fitzgerald River National Park, Western Australia (Kings Park & Botanic Garden living collection KP19913435)	Luke Sweedman	LSWE 1639
<i>Cooperookia strophiolata</i> (Muell.) Carolin	Bilabiate	8	-0.083	0.101	3.00	bilabiate	S.R. Willis	33° 05' S 119° 42' E	N on Kathleen Road from the Lake King—Norseman Road, Western Australia	Kelly Shepherd & Spencer Willis	KS 1534
<i>Dampiera lindleyi</i> de Vriese	Bilabiate	9	-0.332	-0.129	2.00	pseudo-radial	S.R. Willis	28° 16' S 114° 30' E	W on Rob Road from Chilmony Road, NW of Northampton, Western Australia	Kelly Shepherd & Spencer Willis	KS 1515
<i>Diaspasis filifolia</i> R.Br.	Bilabiate	13	-0.159	-0.074	2.08	pseudo-radial	S.R. Willis	n/a	n/a (Kings Park & Botanic Garden living collection KP1990618)	Luke Sweedman	LSWE 5049
<i>Goodenia berardiana</i> (Gaudich.) Carolin	pseudo-radial	6	-0.088	-0.115	2.00	pseudo-radial	A. Gardner	29° 30' S 117° 00' E	Charles Darwin Reserve, Western Australia	n/a	n/a
<i>Goodenia convexa</i> Carolin	pseudo-radial	6	-0.242	-0.128	2.00	pseudo-radial	S.R. Willis	n/a	Dandaragan-Badgingarra, Western Australia (Kings Park & Botanic Garden living collection KP20130713)	Luke Sweedman	LSWE 8622

(Continued)

Table 1. (Continued)

Taxon	Subjective	# pics	PC1 Mean	PC2 Mean	Cluster Mean	k-means	Voucher photographer	Latitude Longitude (GDA 94)	Collection locality (cultivation collection)	Voucher collector	Voucher collection number
<i>Goodenia berringbinensis</i> Carolin	Bilabiate	14	-0.170	0.082	2.79	bilabiate	S.R. Willis	29° 54' S 120° 31' E	Ularring Wetland, N of Coolgardie, Western Australia	Kelly Shepherd & Spencer Willis	KS 1531
<i>Goodenia decursiva</i> W. Fitzg.	Fan	9	0.190	0.110	1.00	fan	S.R. Willis	33° 54' S 123° 31' E	Mount Pasley, Western Australia (Kings Park & Botanic Garden living collection KP20051416)	Patrick Courtney	PCOU 118
<i>Goodenia disperma</i> F. Muell.	Bilabiate	1	-0.240	-0.102	2.00	pseudo-radial	J. Elliott (0513 07)	n/a	Burra Range, Queensland	n/a	n/a
<i>Goodenia drummondii</i> Carolin	fan	9	0.314	0.005	1.00	fan	S.R. Willis	28° 13' S 114° 29' E	E of Swamp Road, NW of Northampton, Western Australia	Kelly Shepherd & Spencer Willis	KS 1516
<i>Goodenia filiformis</i> R.Br.	pseudo-radial	9	-0.129	-0.038	2.22	pseudo-radial	S.R. Willis	29° 50' S 114° 59' 35' E	Coolimba, Western Australia (Kings Park & Botanic Garden living collection KP19920683)	Luke Sweedman	LSWE 1868
<i>Goodenia hassallii</i> F. Muell.	bilabiate	9	-0.332	-0.006	2.44	pseudo-radial	S.R. Willis	28° 13' S 114° 29' E	E of Swamp Road, NW of Northampton, Western Australia	Kelly Shepherd & Spencer Willis	KS 1517
<i>Goodenia helmsii</i> (E. Pritz.) Carolin	fan	9	0.300	-0.012	1.00	fan	S.R. Willis	30° 2' S 116° 39' E	N on Manuel Road from the Great Northern Highway, NE of Wubin, Western Australia	Kelly Shepherd & Spencer Willis	KS 1511
<i>Goodenia macmillanii</i> F. Muell.	bilabiate	1	-0.366	-0.016	2.00	pseudo-radial	R. Cumming	n/a	n/a (Royal Botanic Garden, Melbourne, Victoria)	n/a	n/a
<i>Goodenia micrantha</i> Hemsl. ex Carolin	bilabiate	9	-0.188	0.008	2.56	bilabiate	S.R. Willis	30° 0.4' S 116° 40' E	W of Richards Road on the Great Northern Highway, NE of Wubin	Kelly Shepherd & Spencer Willis	KS 1510
<i>Goodenia mimuloides</i> S. Moore	pseudo-radial	6*	-0.162	-0.019	2.33	pseudo-radial					

(Continued)

Table 1. (Continued)

Taxon	Subjective	# pics	PC1 Mean	PC2 Mean	Cluster Mean	k-means	Voucher photographer	Latitude Longitude (GDA 94)	Collection locality (cultivation collection)	Voucher collector	Voucher collection number
<i>Goodenia mimuloides</i> S. Moore		3					S.R. Willis	29° 30' S 117° 00' E	N of 7 Mile Well, NE side of Charles Darwin Reserve, Western Australia	Kelly Shepherd & GWG	KS 1550
<i>Goodenia mimuloides</i> S. Moore		3					A. Gardner	n/a	NW on Paynes Find—Thundelarra Road from Great Northern Highway, Western Australia	n/a	n/a
<i>Goodenia occidentalis</i> Carolin	pseudo-radial	4	-0.221	-0.063	2.25	pseudo-radial	S.R. Willis	29° 30' S 116° 55' E	S of Quandong Well, Charles Darwin Reserve, Western Australia	Kelly Shepherd & GWG	KS 1549
<i>Goodenia ovata</i> Sm.	bilabiate	10	-0.278	-0.035	2.10	pseudo-radial	S.R. Willis	n/a	n/a (Zanthorrea Nursery, Maida Vale, Western Australia)	Kelly Shepherd & Spencer Willis	KS 1530
<i>Goodenia phillipsiae</i> Carolin	bilabiate	4	-0.304	0.207	3.00	bilabiate	S.R. Willis	n/a	n/a (Kings Park & Botanic Garden living collection KP20100889)	Anne Cochrane	
<i>Goodenia pinifolia</i> de Vriese	bilabiate	9	-0.145	0.193	2.78	bilabiate	S.R. Willis	31° 50' S 119° 38' E	N of the Hyden—Norseman Road on the Marvel Loch—Forrestainia Road, S of Marvel Loch, Western Australia	Kelly Shepherd & Spencer Willis	KS 1532
<i>Goodenia pusilliflora</i> F. Muell.	bilabiate	11	-0.240	0.002	2.45	pseudo-radial	S.R. Willis	29° 34' S 117° 06' E	Wanarra East Road, W of Great Northern Highway, Western Australia	Kelly Shepherd & GWG	KS 1545

(Continued)

Table 1. (Continued)

Taxon	Subjective	# pics	PC1 Mean	PC2 Mean	Cluster Mean	k-means	Voucher photographer	Latitude Longitude (GDA 94)	Collection locality (cultivation collection)	Voucher collector	Voucher collection number
<i>Goodenia stephensonii</i> F. Muell.	bilabiate	2	-0.211	0.143	3.00	bilabiate	M. Fagg 20137	32° 24' S 150° 11' E	Murrumbo Gap, 49 km W of Denman, New South Wales (Australian National Botanic Garden, Canberra CBG 52947)	H. Streimann	816
<i>Goodenia tripartita</i> Carolin	bilabiate	9	-0.217	-0.069	2.00	pseudo-radial	S.R. Willis	n/a	n/a (Lullfitz Nursery, Wanneroo, Western Australia)	Kelly Shepherd & Spencer Willis	KS 1524
<i>Goodenia varia</i> R.Br.	bilabiate	11	-0.304	0.140	3.00	bilabiate	S.R. Willis	32° 43' S 125° 1' E	Toolinna Cove, Western Australia (Kings Park & Botanic Garden living collection KP19960634)	Luke Sweedman	LSWE 4419
<i>Goodenia viscida</i> R.Br.	fan	7	0.286	0.011	1.00	fan	S.R. Willis	33° 48' S 120° 10' E	N of Hopetoun, Western Australia (Kings Park & Botanic Garden living collection KP20050023)	Luke Sweedman	LSWE 6476
<i>Scaevola anchusifolia</i> Benth.	fan	11	0.319	0.047	1.00	fan	A. Gardner	26° 10' S 113° 11' E	Steep Point, Western Australia (Kings Park & Botanic Garden living collection KP20050795)	Luke Sweedman	LSWE 6585
<i>Scaevola calliptera</i> Benth.	pseudo-radial	6*	-0.001	-0.020	1.67	pseudo-radial					
<i>Scaevola calliptera</i> Benth.		2					A. Gardner	n/a	n/a (Kings Park & Botanic Garden living collection KP19883190)	n/a	n/a
<i>Scaevola calliptera</i> Benth.		2					A. Gardner	31° 39' S 117° 28' E	Tammin, Western Australia (Kings Park & Botanic Garden living collection KP19921313)	Luke Sweedman	LSWE 2368

(Continued)

Table 1. (Continued)

Taxon	Subjective	# pics	PC1 Mean	PC2 Mean	Cluster Mean	k-means	Voucher photographer	Latitude Longitude (GDA 94)	Collection locality (cultivation collection)	Voucher collector	Voucher collection number
<i>Scaevola calliptera</i> Benth.		2					A. Gardner	n/a	n/a (Kings Park & Botanic Garden living collection KP20000404)	n/a	n/a
<i>Scaevola canescens</i> Benth.	fan	5	0.345	-0.040	1.00	fan	A. Gardner	29°47'S 115°15'E	Near Enneaba, Western Australia	n/a	n/a
<i>Scaevola collaris</i> J.M. Black ex E.L. Robertson	fan	9	0.376	-0.066	1.00	fan	S.R. Willis	33° 05' S 119° 35' E	W of Lake King on the Lake King—Newdegate Road, Western Australia	Kelly Shepherd & Spencer Willis	KS 1533
<i>Scaevola crassifolia</i> Labill.	fan	6	0.307	-0.022	1.00	fan	A. Gardner	27°42'S 114° 9'E	Kalbarri beach, Western Australia	n/a	n/a
<i>Scaevola humifusa</i> de Vriese	fan	5	0.341	-0.026	1.00	fan	A. Gardner	28° 13' S 114° 29' E	S on Sandy Gully Road from Rob Road, N of Northampton, Western Australia	Kelly Shepherd & Spencer Willis	KS 1525
<i>Scaevola phlebopetala</i> F. Muell.	fan	10	0.150	0.017	1.00	fan	S.R. Willis	28° 01' S 114° 17' E	Ogilvie West Road, N of Port Gregory, Western Australia	Kelly Shepherd & Spencer Willis	KS 1519
<i>Scaevola platyphylla</i> Lindl.	fan	9	0.302	-0.036	1.00	fan	S.R. Willis	32° 10' S 116° 2' E	Bedfordale, Western Australia (Kings Park & Botanic Garden living collection KP20000831)	Luke Sweedman	LSWE 5356
<i>Scaevola porocarya</i> F. Muell.	fan	9	0.258	0.033	1.00	fan	S.R. Willis	28° 11' S 114° 21' E	E on Yerina Springs Road from Port Gregory, Western Australia	Kelly Shepherd & GWG	KS 1518
<i>Scaevola spinescens</i> R. Br.	fan	11*	0.312	0.025	1.00	fan					
		10					S.R. Willis	30° 03' S 116° 40' E	E on Syme Road from Manuel Road, E of Wubin, Western Australia	Kelly Shepherd & GWG	KS 1543

(Continued)

Table 1. (Continued)

Taxon	Subjective	# pics	PC1 Mean	PC2 Mean	Cluster Mean	k-means	Voucher photographer	Latitude Longitude (GDA 94)	Collection locality (cultivation collection)	Voucher collector	Voucher collection number
<i>Scaevola spinescens</i> R. Br.		1					A. Gardner	29° 30' S 117° 00' E	Charles Darwin Reserve, Western Australia	n/a	n/a
<i>Scaevola thesioides</i> Benth.	fan	5	0.272	0.005	1.00	fan	A. Gardner	n/a	Eneabba-Leeman, Western Australia (Kings Park & Botanic Garden living collection KP19881040)	Herbert Demarz	HDEM 12029
<i>Scaevola tomentosa</i> Gaudich.	fan	10	0.386	-0.030	1.00	fan	S.R. Willis	26° 8' S 113°21' E	Herisson Prong, Shark Bay, Western Australia (Kings Park & Botanic Garden living collection KP19920848)	Luke Sweedman	LSWE 1908
<i>Selliera radicans</i> Cav.	fan	1	0.136	0.066	1.00	fan	S.R. Willis	n/a	n/a (Lullfitz Nursery, Wanneroo, Western Australia)	n/a	n/a
<i>Velleia cycnopotamica</i> F.Muell.	pseudo-radial	4	0.018	-0.114	1.75	pseudo-radial	S.R. Willis	29° 30' S 116° 55' E	S of Quandong Well, Charles Darwin Reserve, Western Australia	Kelly Shepherd & GWG	KS 1548
<i>Velleia discophora</i> F. Muell.	bilabiate	5	-0.272	0.213	3.00	bilabiate	S.R. Willis	29° 56' S 116° 38' E	E of Burgess Road no Dinnie Road, NE of Wubin, Western Australia	Kelly Shepherd & Spencer Willis	KS 1513
<i>Velleia foliosa</i> (Benth.) K. Krause	pseudo-radial	9	-0.265	-0.168	2.00	pseudo-radial	S.R. Willis	n/a	n/a (Kings Park & Botanic Garden living collection KP19990259)	Kelly Shepherd & Spencer Willis	KS 1526
<i>Velleia rosea</i> S. Moore	pseudo-radial	8	-0.084	-0.131	2.00	pseudo-radial	S.R. Willis	30° 0.4' S 116° 40' E	W of Richards Road on the Great Northern Highway, NE of Wubin, Western Australia	Kelly Shepherd & Spencer Willis	KS 1509

(Continued)

Table 1. (Continued)

Taxon	Subjective	# pics	PC1 Mean	PC2 Mean	Cluster Mean	k-means	Voucher photographer	Latitude Longitude (GDA 94)	Collection locality (cultivation collection)	Voucher collector	Voucher collection number
<i>Verreauxia reinwardtii</i> (de Vriese) Beth.	bilabiate	9	-0.288	0.070	3.00	bilabiate	S.R. Willis	28° 11' S 114° 34' E	N on Chilmony Road from North Road, N of Northampton, Western Australia	Kelly Shepherd & Spencer Willis	KS 1514

For species with multiple populations, the species-level information is on a separate row (total of individual images is denoted with *).

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calculated on the asymmetric principle components showed no significance with species (p-value = 0.95) or k-means floral shape cluster (p-value = 0.16) as the grouping variable. Finally, a separate analysis of the same landmark data was performed using the PollyMorphometrics 11.1 package on Mathematica [32] that does not separate asymmetric components.

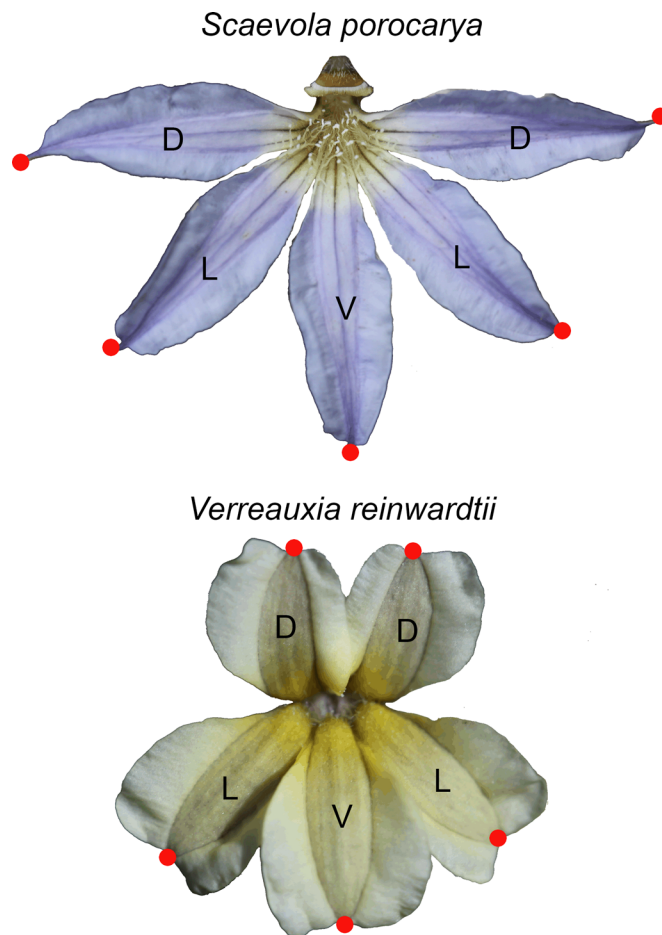


Fig 1. Five point morphometric landmarking scheme for exemplar Core Goodeniaceae taxa. Images of two species (*Scaevola porocarya*—a fan-flower in *Scaevola* s.l., and *Verreauxia reinwardtii*—a bilabiate flower in *Goodenia* s.l.) showing the positions of the 5 landmarks. Dorsal (D), lateral (L), and ventral (V) petals are labelled.

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Models were consistent with those of the MorphoJ symmetric models. All together, these suggest that the flowers are primarily bilaterally symmetrical and that asymmetries in these populations represent a random variance. In addition to asymmetry we explored potential allometric relationships between shape and size using average corolla lengths by species from *Flora of Australia* [24]. Corolla lengths were not found to correlate with the Procrustes transformed centroid size ($p = 0.605$, $r^2 = .006$) or PC scores (PC1: $p = 0.946$; PC2: $p = .731$) by linear regression, and an ANOVA of corolla lengths with k-means floral shape cluster was insignificant (p -value = 0.82; [S3 Table](#)). All of these factors suggest that differences among these flowers arise primarily as a consequence of symmetrical variation in corolla shape, rather than as a consequence of asymmetric or allometric factors. No noticeable performance differences were observed between images obtained during this study compared to those from the publicly available *Atlas of Living Australia* database.

The first two PCs accounted for greater than 98% of the total symmetric variation among the landmarks and were retained for all subsequent analysis. To convert PC scores into five-landmark models of flower shape, we used a script in Mathematica similar to [32]. In short, a table of residual values was constructed for the distance from each individual flower marker to the consensus Procrustes landmark position. The eigenvectors of the residual covariance matrix were then found. By multiplying a vector of PC values times the corresponding vectors and adding this to the consensus position, a morphology (series of x-y coordinate values) specific to the PCs was obtained. This method was used to find shape vectors that mapped between average PC values for clade or categorical floral morphologies.

Goodenia s.l. versus *Scaevola* s.l.

The results of the symmetrical principal components analysis were used to quantify the greater floral variation that *Goodenia* s.l. exhibits compared to *Scaevola* s.l. Data from 214 *Goodenia* s.l. flowers (31 species) and 100 *Scaevola* s.l. flowers (12 species) were analysed by ANOVA using JMP 10 (SAS Institute). To test for heterogeneity of variances we used Bartlett's test [33]. When needed, the non-parametric Wilcoxon rank sum tests of means [34] along with the Levene's tests of heterogeneity of variances [35] were used to accommodate non-normal distributions. *Brunonia australis* and *Dampiera lindleyi* were excluded from these analyses because they fall outside of these two clades.

Morphological clustering of flower data

Each species' flowers were subjectively grouped as fan-flowered, bilabiate, or pseudo-radial. To compare subjective classes with the PCA model, k-means clustering was performed for all 335 individuals with JMP 10, with $k = 3$ to parallel the number of subjectively assigned morphologies. We used the first two symmetrical principal components to quantitatively describe three distinct sub-populations (following the subjective conceptualization) based entirely on landmark position.

Results

Principal components analysis of the landmark data from all individual flowers ($n = 335$) resulted in two components that explained a cumulative 98.5% of the symmetric component of the total variance (PC1: 86.6%, PC2: 11.9%). PC values were then mapped back onto floral morphology using the eigenvectors of the residual covariance matrix (see [Methods](#)). Increasing values of PC1 ventralize the dorsal petals (increasing the angle between them), whereas increasing values of PC2 primarily ventralize the lateral petals (decreasing the angle between them)

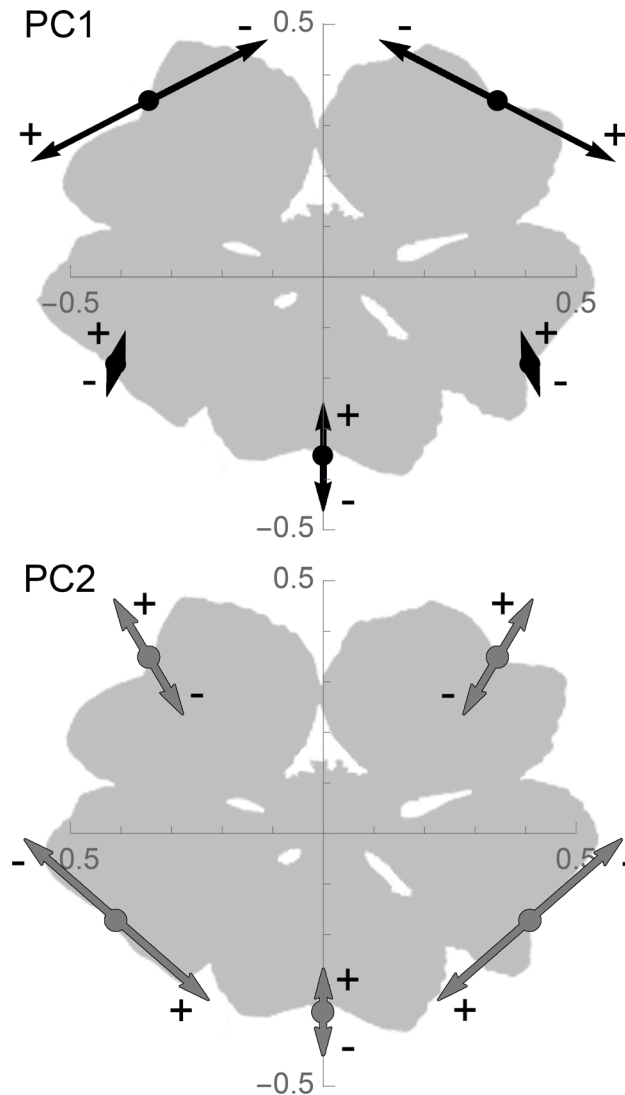


Fig 2. Variation in Core Goodeniaceae floral morphology described by PC1 and PC2. Relative directional influence of the two major principal components of floral landmark variation. The five landmarks are arranged as described by PC scores of zero and zero, with vectors showing shifts associated with PC scores going to ± 0.4 .

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(Fig 2). The manipulation of these two morphological “axes” alone was sufficient to recreate any of the general floral patterns visible in the Core Goodeniaceae (Fig 3).

Goodenia s.l. versus *Scaevola* s.l.

When we partitioned images by clade, the members of *Goodenia* s.l. exhibited lower values for PC1 (greater average dorsalization; -0.105 (0.224 SD)) on average than those of *Scaevola* s.l. (0.219 (0.179 SD)) (Wilcoxon rank sum test p -value < 0.0001 ; Fig 4). The distribution of *Goodenia* is not normal, showing an apparent bimodality on PC1 that includes fan- and non-fan-flowers. Because of this, they have greater variance in PC1 than sister clade *Scaevola* s.l. (Levene’s test p -value = 0.00370). Unlike PC1, PC2 is normally distributed within the floral data. *Goodenia* s.l. has an average PC2 score of 0.00930 (0.112 SD) and *Scaevola* s.l. has and

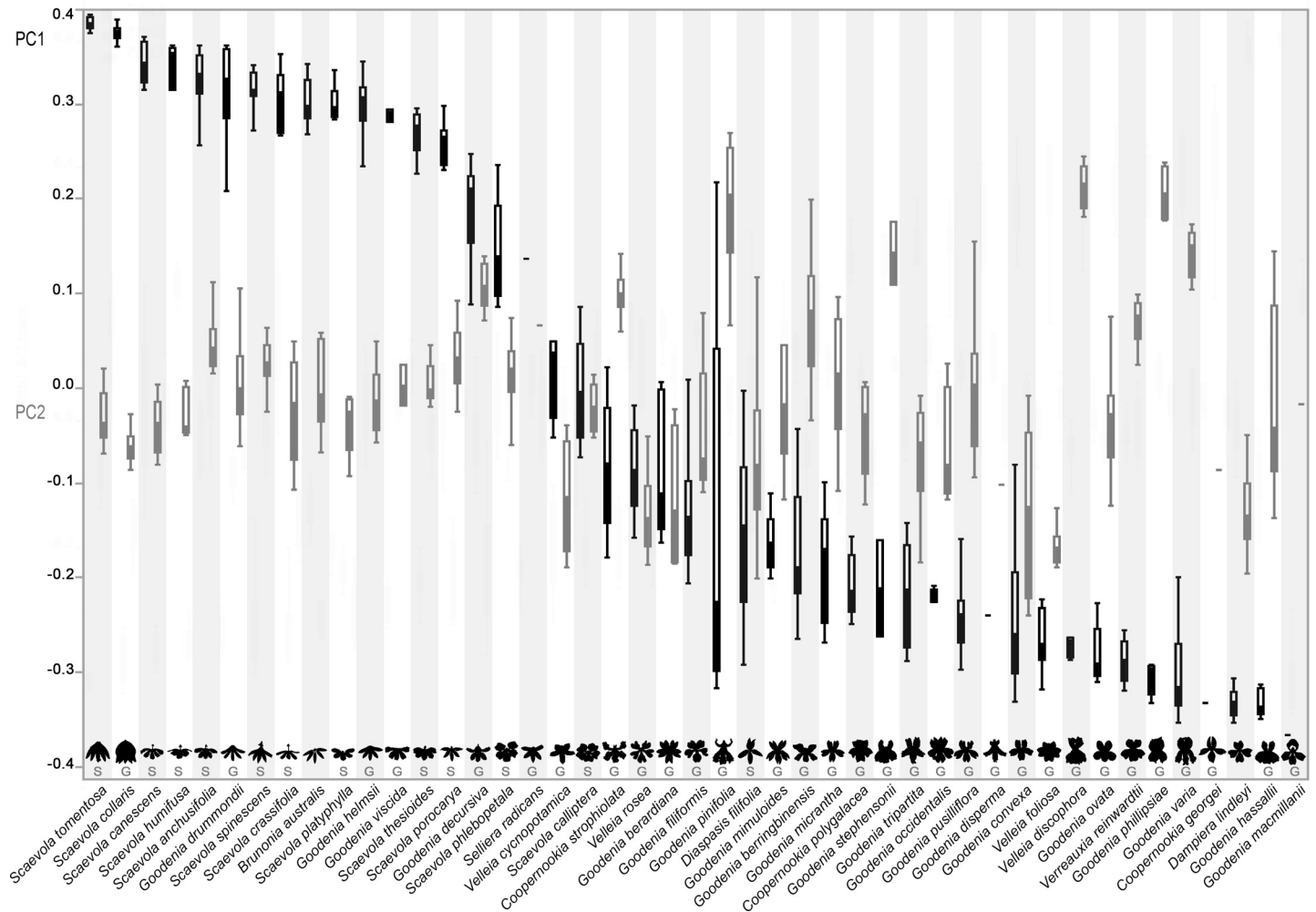


Fig 3. Box plots of variation in 335 PC1 and PC2 scores for 44 Core Goodeniaceae species and *Dampiera lindleyi*. The species are organized by decreasing value of PC1 (black), dorsalizing the dorsal petals (decreasing the angle between them). Increasing values of PC2 (gray) primarily ventralize the lateral petals (decreasing the angle between them). The outline of each flower is shown above clade membership denoted with "G" (*Goodenia* s.l.) or "S" (*Scaevola* s.l.). Boxes denote median values along with quartiles.

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average score of -0.00880 (0.0560 SD). Partitioning the data by clade does not have a significant effect on the differences in mean values (Wilcoxon rank sum test p-value of 0.298), but *Goodenia* s.l. does display an increased variance (Bartlett's p-value of <0.0001).

Morphological clustering of species averages

Geometric morphometrics recovered objective categories of floral symmetry in Core Goodeniaceae (Table 1, Fig 5). All species that had a positive (mean) PC1 value were entirely represented by k-means cluster 1 (fan-flower) with the exception of *Velleia cynopotamica* (PC1 = 0.0183). This species and *Scaevola calliptera* included three individual flowers clustered in k-means = 1; however, these species average k-means scores were 1.75 (*Velleia cynopotamica*, n = 4) and 1.67 (*Scaevola calliptera*, n = 6). All individuals with a PC1 value <0 and positive PC2 correlate to the k-means = 2 (bilabiate) cluster, while species with a negative mean PC1 and PC2 were clustered as k-means = 3 (pseudo-radial) flowers, with the exception of *Goodenia pusilliflora* (PC2 = 0.00220). Ten other species have individuals in two different k-

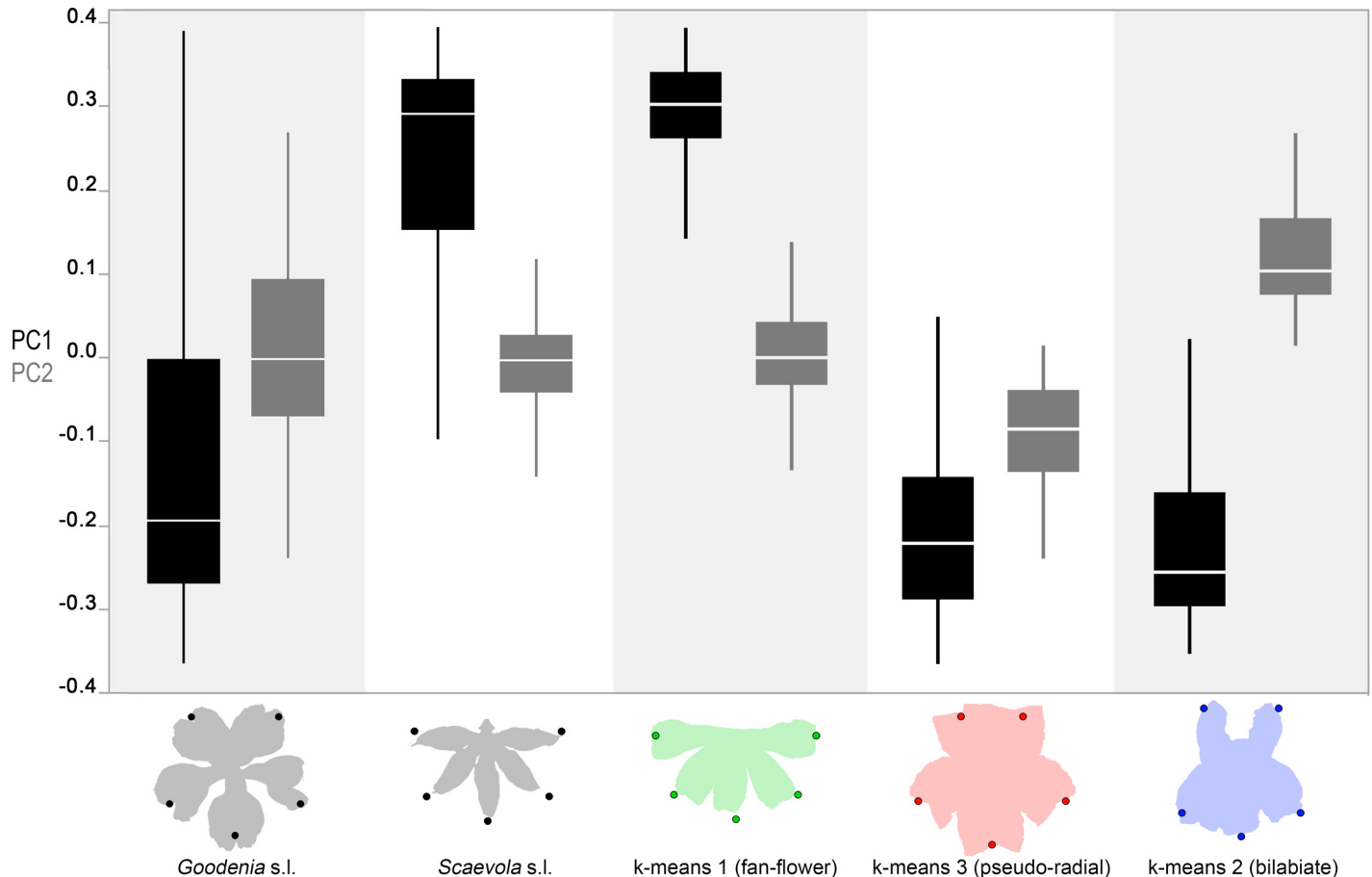


Fig 4. PC1 and PC2 variation in Core Goodeniaceae partitioned by clade and by k-means cluster group. Box plots of PC1 and PC2 scores by clade and by k-means morphological clusters. Boxes denote median values along with quartiles. Below, average landmark positions are superimposed over a flower outline for each group.

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means clusters, grading from nearly completely in cluster 2 (bilabiate), through to individuals evenly divided between clusters 2 and 3 (pseudo-radial), to predominantly among cluster 3.

The three clusters delineated by k-means were very similar to the subjective groups, with total agreement between subjective grouping and k-means cluster species averages about the fan-flowers. However, there was significant overlap between k-means cluster 2 and 3 with bilabiate and pseudo-radial flowers respectively. Of the 28 species with PC1 scores less than 0, subjective classes and k-means clusters did not correlate for nine species with PC2 scores around 0. All were subjectively grouped as bilabiate but fell within the “pseudo-radial” k-means cluster 3 (*Cooperookia georgei*, *Dampiera lindleyi*, *Diaspasis filifolia*, *Goodenia disperma*, *G. hassallii*, *G. macmillanii*, *G. ovata*, *G. pusilliflora* and *G. tripartita*). Both morphometric and subjective methods correlate well with PC1, with adjusted R^2 values of 88%. For PC2, subjective clustering is less well correlated than k-means, at 20% and 62% respectively.

Transitions in floral form

When we examine the relative contribution of the PCs to transitions between floral morphologies (Fig 2), it suggests that the PCs represent independent modules in floral morphogenesis. The clear delineation between fan and non-fan-flowers corresponds almost entirely to PC1,

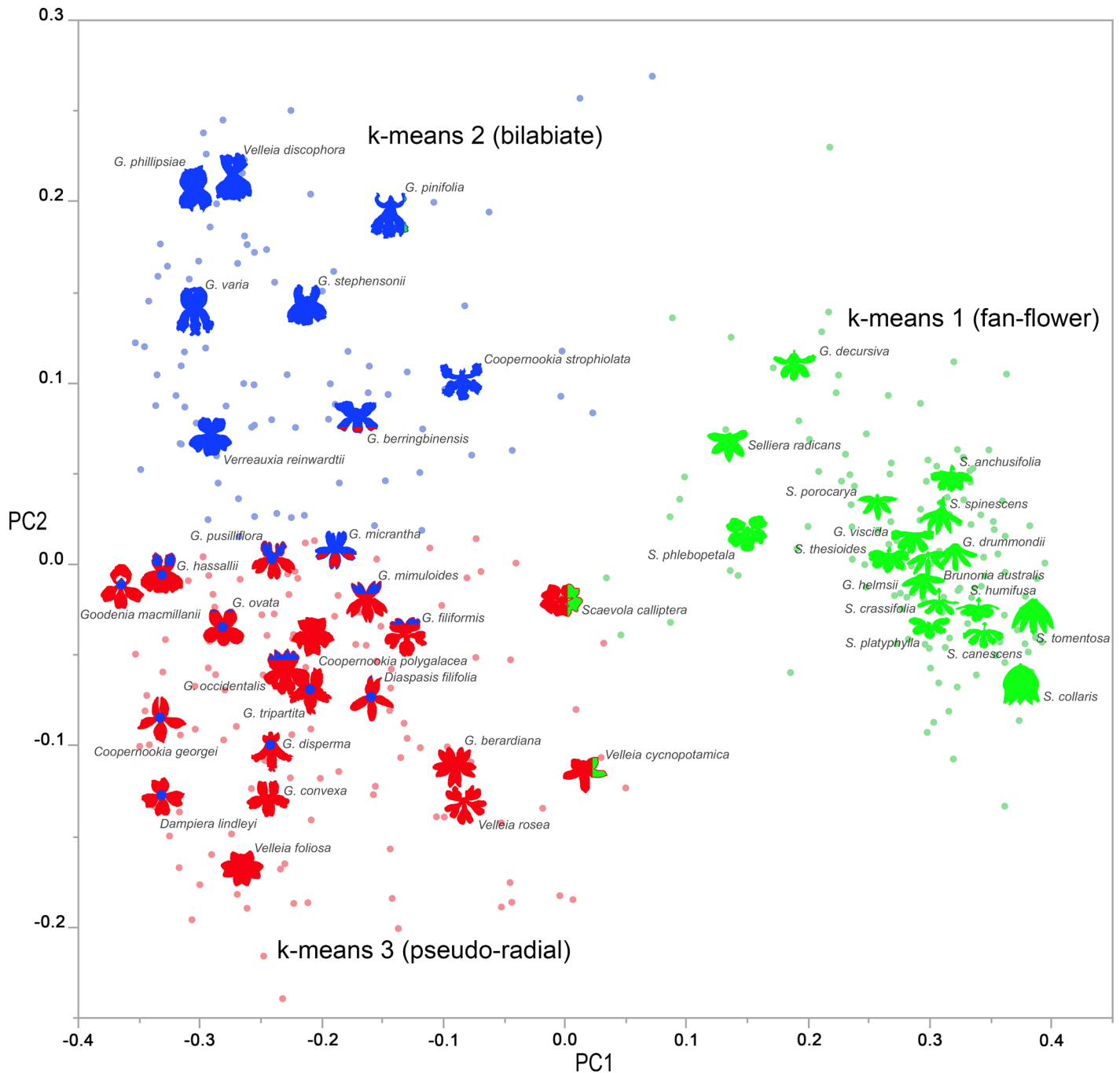


Fig 5. Morphological clustering of Core Goodeniaceae floral diversity. Individuals (dots) and species averages (flower outlines) are depicted in a biplot of their scores for PC1 and PC2 from morphometric analysis. K-means = 1 (fan-flowered) individuals are green, k-means = 2 individuals (bilabiate) are blue, and k-means = 3 (pseudo-radial) individuals are red. Species for which individuals occur in different k-means clusters are illustrated by the proportions of colors depicted in the flower outlines. The flower outline color represents the k-means cluster in the majority, and blue dots indicate when subjective grouping called k-means = 3 (pseudo-radial) species bilabiate.

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with the largest landmark movements in the dorsal petals as they move apart, dorsoventrally. When PC1 is low, pseudo-radial and bilabiate flower shape is described mostly by PC2. As PC2 decreases, bilabiate flowers with lateral petals close to the ventral petal transition to pseudo-

radial flowers with widely spread lateral petals. Ultimately, each of these shifts in morphospace sets up potential hypotheses about gene regulation that may pattern these PC shifts.

Discussion

Floral symmetry diversity in Core Goodeniaceae is highly variable; however, this variation is over simplified by the use of broad subjective terms like fan-flower, bilabiate and pseudo-radial to describe floral form in the group [24]. We aimed to apply a geometric morphometric approach to quantify flower shape in order to better characterize variation in the flowers of Core Goodeniaceae. The five-point landmarking scheme and downstream analyses resulted in two PCs that provide a continuous, 2-dimensional framework to describe aspects of floral morphological variation. The first principle component relates most directly to the dorsal petals and the second component relates most to the lateral petals. When PC1 values (which account for 86.6% of total floral variation) increase, a fan-flower results. Lower values of PC1 decrease the ventralization of the dorsal petals to yield a non-fan-flower, corresponding with a greater range in PC2. Increasing values of PC2 primarily ventralize the lateral petals (decreasing the angle between them), describing bilabiate flowers, whereas decreasing values of PC2 increase the angle between the lateral petals describing pseudo-radial flowers. Together, these capture much of the floral shape diversity of Core Goodeniaceae.

Goodenia s.l. exhibits greater floral variation than *Scaevola* s.l.

If a quantitative measure is to be employed to describe morphology, it must accurately account for observable differences in floral morphologies. *Goodenia* s.l. includes fan-flowered, bilabiate, and pseudo-radially flowered species whereas *Scaevola* s.l. includes representatives that are almost exclusively fan-flowered. We found this observed variation was reflected in our metrics as members of *Goodenia* s.l. exhibit greater variation in both PCs (Fig 4). In PC1, in which the means were significantly different, *Goodenia* s.l. exhibits greater variance and a somewhat bimodal distribution split between six fan-flowered species in addition to the 25 pseudo-radial and bilabiate species. PC2 also exhibits greater variance, which is reasonable considering that the fan-flower morphology (with dorsal petals pushed ventrally toward the lateral petals) inherently limits the variance of PC2.

Floral symmetry clusters are similar to subjective groups

The subjective labelling of floral morphology as fan-flowered, bilabiate or pseudo-radial comes from our attempt at classifying observable patterns in the location of the five petals. We might expect that any mathematical description of floral morphology would also delineate, or at least describe, these classes. We illustrate a strong, shared delineation in both grouping methods between the 17 fan-flowered species and the 28 species exhibiting non-fan morphologies. When the individuals and species are arrayed in a PC1 vs. PC2 biplot, the fan-flowered species occupy a clearly discrete cluster, strongly supporting at least a binary characterization for ancestral floral symmetry reconstruction. In contrast, the bilabiate and pseudo-radial species occupy a semi-continuous and roughly linear vertical axis from low PC2 scores (most pseudo-radial, e.g. *Goodenia berardiana* or *Velleia foliosa*) to high PC2 scores (most bilabiate, e.g. *Goodenia phillipsiae* or *Velleia discophora*).

Given the continuum in form along the PC2 axis, any attempt at grouping is challenging. Subjective grouping and k-means clustering disagree about where the transition from bilabiate to pseudo-radial flowers occurs (Fig 5 and S2 Fig). Subjective grouping discerns 18 bilabiate species and 10 pseudo-radial species, whereas the k-means clustering overlaps these categories, assembling 9 of the subjective bilabiate species into a single cluster, but mixes the remaining 19

subjective bilabiate and pseudo-radial species into a different group. This is of interest, because it is evident that what we may perceive as a bilabiate or pseudo-radial flower, may depend on an interplay between two PCs (reflecting the relative distance between the dorsal and lateral petals), which we may not readily discern. Disagreement might also be further exacerbated by people's apparently diminished ability to distinguish differences in the PC2 (positive and negative resulting in bilabiate and pseudo-radial forms respectively), based on the low R^2 value between its scores and subjective clustering, especially compared to PC1. Additionally, our more restricted perception of pseudo-radial symmetry may have caused us to assign fewer species to that group. Again, these are the types of problems inherent in subjective classification that are not encountered in the mathematical description of clusters, though natural variation among species presents challenges to clustering. Notably, there were 12 species with individuals along the margins of the pseudo-radial cluster, and three species whose individuals split almost evenly between the pseudo-radial and bilabiate clusters (*Goodenia hassallii*, k-means avg = 2.44, n = 9; *G. pusilliflora*, k-means avg = 2.45, n = 11; and *G. micrantha*, k-means avg = 2.56, n = 9). This suggests that any given species may exhibit variation around some typical mean for PC1 and PC2 from a developmental perspective, without strictly adhering to a preconceived classification of morphology.

The lack of a clear delineation between pseudo-radial and bilabiate morphologies makes it difficult to argue for three morphological character states with the current data. Instead, we may consider that the change in morphology characterized by PC2 may be of a continuous nature. It is possible that the addition of more species to analyses such as these may yield a more bimodal or even trimodal distribution along the PC2 axis, but it may also reinforce the apparent continuum we find among these 28 species. Because of this, phylogenetic reconstruction of the evolution of Core Goodeniaceae flowers would require this character (PC2) to be scored as a continuous trait. Our inability to distinguish additional clusters may also be limited by our morphometric method, especially by the limitations of the "head-on" floral images, as many of the species in the bilabiate cluster have characteristically three-dimensional corollas and recurved dorsal petals. Future work should consider the use of multiple floral views or another method to build 3D geometric morphometric models of these flowers [20], which may reveal additional axes of shape variation.

Another interesting result of these analyses is the fact that much of the potential morphospace is currently unoccupied in the Core Goodeniaceae. This could be due to mechanical, genetic, or selective constraints on Goodeniaceae floral morphological evolution. For example, when PC1 scores are above 1.0 (and the dorsal petals are farther separated), this limits the capacity for movement on the PC2 axis (less space between the dorsal and lateral petals) without overlapping the petals substantially, though we do see some petal overlap in many species, particularly with conspicuous extra-petal wings. Alternatively, there could be an underlying genetic cause, similar to epistasis that restricts the expression of a "PC2" developmental module when a "fan-flower" pathway is active. In either case, if PC2 is restricted in a large portion of the study group, its contribution to phenotypic variance is possibly underestimated, as would normally be the case with an epistatic effect.

Modularity and potential candidate genes

The independent patterns of morphological variation among the dorsal and lateral petals support the existence of distinct developmental modules in Goodeniaceae floral morphogenesis. Several transcription factor candidate genes studied in other groups may play a role in the transition of floral forms described by both PCs. The most studied across angiosperms are members of the *CYC2* clade of *CYCLOIDEA*-like (*CYC*-like) genes [10, 36–38]. Restriction of

expression of these genes to the dorsal region of the corolla has been correlated in many angiosperm groups with a transition to bilateral symmetry. Specifically, in core eudicots, *CYC2* genes independently shift from equivalent dorsoventral expression across the corolla (either ubiquitously expressed or not expressed) in radially symmetric flowers to dorsally restricted expression in bilaterally symmetrical flowers. Data from Dipsacales suggests that the extent of dorsal restriction is correlated with petal location [10, 39], allowing us to hypothesize that a similar pathway could potentially play a role in morphological shifts described by changes in PC1. Closely related to Goodeniaceae, the Asteraceae have undergone multiple duplications of *CYC2* members, with one paralog exclusively expressed in ray florets [40–44]. Changes in expression patterns of *CYC2* clade members have been shown to have an effect on petal growth in both Asteraceae and Brassicaceae [45, 46], suggesting the possibility that they could regulate the PC2 morphological changes. Candidate genes involved in floral symmetry and corolla fusion, could be mapped across a clade of species with quantified morphometric shape in order to more clearly hypothesize how gene changes effect subtler shifts in morphology.

Conclusions and broader picture

This study describes a relatively simple application of geometric morphometrics for characterizing flower symmetry in the Core Goodeniaceae, which could be applicable to other plant groups. We found that the majority of floral shape variation among the Core Goodeniaceae individuals we sampled is symmetrical, and that variation in the ventralization of the dorsal petals dominates floral shape diversity. It confirms the strong distinction between the fan-flower morphology and all others within the clade. The simplicity, both in construction and interpretation, of the five-landmark model used in this research could argue for its application in other pentamerous angiosperm groups.

Further research is necessary to characterize the genetic factors that modulate these morphological shifts and to what extent the multiple transitions to fan-flowers in this clade [26] have been in parallel. The repeated transitions to fan-flowers in Goodeniaceae may be driven by pollinator selection, however much additional research is necessary to identify the pollinators that may influence them and to characterize Goodeniaceae floral evolutionary dynamics.

Supporting Information

S1 Fig. Core Goodeniaceae floral diversity. Species images are arranged by their scores for the first principal component (PC) of floral variation, which is depicted in the upper boxes. The lower boxes correspond to the second PC scores. Additionally, the results of subjective grouping and k-means clustering are depicted with icons for bilabiate, pseudo-radial, and fan-flowers. When there are two icons, they correspond to subjective grouping (left) and k-means clustering (right). When the methods agreed, the consensus is indicated with a single icon. Following [25 and 26], species depicted in red are included in *Scaevola* s.l., in blue are in *Goodenia* s.l. (note *Scaevola collaris* is placed within this clade), while *Brunonia* and *Dampiera* (depicted in grey) are not in these clades.

(TIF)

S1 Table. Raw x,y landmark coordinates for all individuals.

(XLSX)

S2 Table. Procrustes ANOVA calculated for shape. Data from all images were analyzed with MorphoJ to ascertain the overall variance in shape explained by species and by asymmetries (side) in floral morphology.

(XLSX)

S3 Table. Analysis of corolla size variation. Total variance of the average corolla lengths of the 44 species used in this study (source) were analyzed with k-means floral shape cluster as a factor.

(XLSX)

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Author Contributions

Conceived and designed the experiments: AG JF JM KS RJ. Performed the experiments: AG JF KS. Analyzed the data: AG JF. Wrote the paper: AG JF JM KS DH RJ.

References

1. Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. PNAS. 2004; 101:14812–17. PMID: [15465915](#)
2. Heinrichs J, Hentschel J, Bombosch A, Fiebig A, Reise J, Edelmann M, et al. One species or at least eight? Delimitation and distribution of *Frullania tamarisci* (L.) Dumort. s. l. (Jungermanniopsida, Porellales) inferred from nuclear and chloroplast 50 DNA markers. Mol Phylogenet Evol. 2010; 56:1105–14. doi: [10.1016/j.ympev.2010.05.004](#) PMID: [20460161](#)
3. Abzhanov A, Protas M, Grant BR, Grant PR, Tabin CJ. Bmp4 and morphological variation of beaks in Darwin's finches. Science. 2004; 305:1462–5. PMID: [15353802](#)
4. Abzhanov A, Kuo WP, Hartmann C, Grant BR, Grant PR, Tabin CJ. The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. Nature. 2006; 442:563–7. PMID: [16885984](#)
5. Foster DJ, Podos J, Hendry AP. A geometric morphometric appraisal of beak shape in Darwin's finches. J Evol Biol. 2008; 21:263–75. PMID: [18021202](#)
6. Hodges SA, Arnold ML. Spurring plant diversification: are floral nectar spurs a key innovation? Proc R Soc Lond B Biol Sci. 1995; 262:343–8.
7. Whittall JB, Hodges SA. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. Nature. 2007; 447:706–9. PMID: [17554306](#)
8. Kitazawa MS, Fujimoto K. A dynamical phyllotaxis model to determine floral organ number. PLoS Comput Biol. 2015; 11:e1004145. doi: [10.1371/journal.pcbi.1004145](#) PMID: [25950739](#)
9. Sablowski R. Control of patterning, growth, and differentiation by floral organ identity genes. J Exp Bot. 2015; 66:1065–73. doi: [10.1093/jxb/eru514](#) PMID: [25609826](#)
10. Specht CD, Howarth DG. Adaptation in flower form: a comparative evodevo approach. New Phytol. 2015; 206:74–90. doi: [10.1111/nph.13198](#) PMID: [25470511](#)
11. Klingenberg CP. Evolution and development of shape: integrating quantitative approaches. Nat Rev Genet. 2010; 11:623–35. doi: [10.1038/nrg2829](#) PMID: [20697423](#)
12. Slice DE. Geometric morphometrics. Annu Rev Anthropol. 2007; 36:261–81.
13. Viscosi V, Cardini A. Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. PLoS One. 2011; 6:e25630. doi: [10.1371/journal.pone.0025630](#) PMID: [21991324](#)
14. Gómez JM, Perfectti F, Camacho JPM. Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. Am Nat. 2006; 168:531–45. PMID: [17004224](#)
15. Gómez JM, Bosch J, Perfectti F, Fernández JD, Abdelaziz M, Camacho JPM. Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. Proc R Soc Lond B Biol Sci. 2008; 275:2241–9.
16. Medel R, Botto-Mahan C, Kalin-Arroyo M. Pollinator-mediated selection on the nectar guide phenotype in the Andean monkey flower, *Mimulus luteus*. Ecology. 2003; 84:1721–32.
17. Benitez-Vieyra S, Medina AM, Cocucci AA. Variable selection patterns on the labellum shape of *Geoblasta pennicillata*, a sexually deceptive orchid. J Evol Biol. 2009; 22:2354–62. PMID: [20069726](#)

18. Gómez JM, Perfectti F. Evolution of complex traits: the case of *Erysimum* corolla shape. *Int J Plant Sci*. 2010; 171:987–98.
19. Savriama Y, Gómez JM, Perfectti F, Klingenberg CP. Geometric morphometrics of corolla shape: dissecting components of symmetric and asymmetric variation in *Erysimum mediohispanicum* (Brassicaceae). *New Phytol*. 2012; 196:945–54. doi: [10.1111/j.1469-8137.2012.04312.x](https://doi.org/10.1111/j.1469-8137.2012.04312.x) PMID: [22988918](https://pubmed.ncbi.nlm.nih.gov/22988918/)
20. van der Niet T, Zollikofer CP, León MS, Johnson SD, Linder HP. Three-dimensional geometric morphometrics for studying floral shape variation. *Trends Plant Sci*. 2010; 15:423–6. doi: [10.1016/j.tplants.2010.05.005](https://doi.org/10.1016/j.tplants.2010.05.005) PMID: [20541450](https://pubmed.ncbi.nlm.nih.gov/20541450/)
21. Shipunov AB, Bateman RM. 2005. Geometric morphometrics as a tool for understanding *Dactylorhiza* (Orchidaceae) diversity in European Russia. *Biol J Linn Soc Lond*. 2005; 85:1–12.
22. Kaczorowski RL, Seliger AR, Gaskett AC, Wigsten SK, Raguso RA. Corolla shape vs. size in flower choice by a nocturnal hawkmoth pollinator. *Funct Ecol*. 2012; 26:577–87.
23. Fernández-Mazuecos M, Blanco-Pastor JL, Gómez JM, Vargas P. Corolla morphology influences diversification rates in bifid toadflaxes (*Linaria* sect. *Versicolores*). *Ann Bot*. 2013; 112:1705–22. doi: [10.1093/aob/mct214](https://doi.org/10.1093/aob/mct214) PMID: [24142920](https://pubmed.ncbi.nlm.nih.gov/24142920/)
24. Carolin RC, Rajput MTM, Morrison P. Goodeniaceae. In: George AS, editor. *Flora of Australia Volume 35*. Canberra: Australian Government Publishing Service; 1992. pp. 4–300.
25. Jabaily RS, Shepherd KA, Gustafsson MHG, Sage LW, Krauss SL, Howarth DG et al. Systematics of the Austral-Pacific family Goodeniaceae: Establishing a taxonomic and evolutionary framework. *Taxon*. 2012; 61:419–36.
26. Gardner AG, Sessa EB, Michener P, Johnson E, Shepherd KA, Howarth DG, et al. Utilizing next-generation sequencing to resolve the backbone of the Core Goodeniaceae and inform future taxonomic and floral form studies. *Mol Phylogenet Evol*. 2016; 94:605–17. doi: [10.1016/j.ympev.2015.10.003](https://doi.org/10.1016/j.ympev.2015.10.003) PMID: [26463342](https://pubmed.ncbi.nlm.nih.gov/26463342/)
27. Dumortier BCJ. *Analyse des familles des plantes, avec l'indication des principaux genres qui s'y rattachent*. Tournay: Imprimerie de J. Casterman; 1929.
28. Jabaily RS, Shepherd KA, Gardner AG, Gustafsson MHG, Howarth DG, Motley TJ. Historical Biogeography of the predominantly Australian family Goodeniaceae. *J Biogeogr*. 2014; 41:2057–67.
29. Rohlf FJ. tpsDIG2. Stony Brook, NY: Department of Ecology and Evolution, State University of New York. 2003.
30. Zelditch ML, Swiderski DL, Sheets HD. *Ordination Methods*. In: Zelditch ML, Swiderski DL, Sheets HD, editors. *Geometric Morphometrics for Biologists (Second Edition)*. San Diego: Academic Press; 2012. pp. 135–167.
31. Klingenberg CP. MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Resour*. 2011; 11:353–7. doi: [10.1111/j.1755-0998.2010.02924.x](https://doi.org/10.1111/j.1755-0998.2010.02924.x) PMID: [21429143](https://pubmed.ncbi.nlm.nih.gov/21429143/)
32. Polly PD. *Geometric morphometrics for Mathematica*. Version 11.0. Department of Geological Sciences, Indiana University: Bloomington, Indiana. 2014.
33. Bartlett MS, Kendall DG. The statistical analysis of variances—Heterogeneity and the logarithmic transformation. *J R Stat Soc Series B Stat Methodol*. 1946; 8:128–138.
34. Welch BL. On the comparison of several mean values: an alternative approach. *Biometrika*. 1951; 38:330–6.
35. Levene H. Robust tests for equality of variances. In: Ingram O, Hotelling H, et al., editors. *Contributions to probability and statistics: Essays in honor of Harold Hotelling*. Stanford University Press; 1960. pp. 278–292.
36. Luo D, Carpenter R, Vincent C, Copsey L, Coen E. Origin of floral asymmetry in *Antirrhinum*. *Nature*. 1996; 383:794–9. PMID: [8893002](https://pubmed.ncbi.nlm.nih.gov/8893002/)
37. Howarth DG, Donoghue MJ. Phylogenetic analysis of the “ECE” (CYC TB1) clade reveals duplications predating the core eudicots. *Proc Natl Acad Sci USA*. 2006; 103:9101–6. PMID: [16754863](https://pubmed.ncbi.nlm.nih.gov/16754863/)
38. Preston JC, Hileman LC. Developmental genetics of floral symmetry evolution. *Trends Plant Sci*. 2009; 14:147–54. doi: [10.1016/j.tplants.2008.12.005](https://doi.org/10.1016/j.tplants.2008.12.005) PMID: [19231272](https://pubmed.ncbi.nlm.nih.gov/19231272/)
39. Howarth DG, Martins T, Chimney E, Donoghue MJ. Diversification of *CYCLOIDEA* expression in the evolution of bilateral flower symmetry in Caprifoliaceae and *Lonicera* (Dipsacales). *Ann Bot*. 2011; 107:1521–32. doi: [10.1093/aob/mcr049](https://doi.org/10.1093/aob/mcr049) PMID: [21478175](https://pubmed.ncbi.nlm.nih.gov/21478175/)
40. Broholm SK, Tähtiharju S, Laitinen RAE, Albert VA, Teeri TH, Elmaa P. A TCP domain transcription factor controls flower type specification along the radial axis of the *Gerbera* (Asteraceae) inflorescence. *Proc Natl Acad Sci USA*. 2008; 105:9117–22. doi: [10.1073/pnas.0801359105](https://doi.org/10.1073/pnas.0801359105) PMID: [18574149](https://pubmed.ncbi.nlm.nih.gov/18574149/)

41. Chapman MA, Leebens-Mack JH, Burke JM. Positive selection and expression divergence following gene duplication in the sunflower *CYCLOIDEA* gene family. *Mol Biol Evol* 2008; 25:1260–73. doi: [10.1093/molbev/msn001](https://doi.org/10.1093/molbev/msn001) PMID: [18390478](https://pubmed.ncbi.nlm.nih.gov/18390478/)
42. Kim M, Cui ML, Cubas P, Gillies A, Lee K, Chapman MA et al. Regulatory genes control a key morphological and ecological trait transferred between species. *Science*. 2008; 322:1116–9. doi: [10.1126/science.1164371](https://doi.org/10.1126/science.1164371) PMID: [19008450](https://pubmed.ncbi.nlm.nih.gov/19008450/)
43. Fambrini M, Salvini M, Pugliesi C. A transposon-mediate inactivation of a *CYCLOIDEA*-like gene originates polysymmetric and androgynous ray flowers in *Helianthus annuus*. *Genetica*. 2012; 139:1521–9.
44. Tähtiharju S, Rijpkema AS, Vetterli A, Albert VA, Teeri TH, Elomaa P. Evolution and diversification of the *CYC/TB1* gene family in Asteraceae—a comparative study in *Gerbera* (Mutisieae) and sunflower (Heliantheae). *Mol Biol Evol*. 2012; 29:1155–66. doi: [10.1093/molbev/msr283](https://doi.org/10.1093/molbev/msr283) PMID: [22101417](https://pubmed.ncbi.nlm.nih.gov/22101417/)
45. Broholm SK, Tähtiharju S, Laitinen RAE, Albert VA, Teeri TH, Elmaa P. A TCP domain transcription factor controls flower type specification along the radial axis of the *Gerbera* (Asteraceae) inflorescence. *Proc Natl Acad Sci USA*. 2008; 105:9117–22. doi: [10.1073/pnas.0801359105](https://doi.org/10.1073/pnas.0801359105) PMID: [18574149](https://pubmed.ncbi.nlm.nih.gov/18574149/)
46. Busch A, Zachgo S. Control of corolla monosymmetry in the Brassicaceae *Iberis amara*. *Proc Natl Acad Sci USA*. 2007; 104:16714–9. PMID: [17940055](https://pubmed.ncbi.nlm.nih.gov/17940055/)



Species	id	x1	y1	x6	y6	x11	y11	x16	y16	x21	y21
Brunonia australis	0	114	643	294	290	612	112	1120	268	1215	504
Brunonia australis	1	155	488	455	95	911	53	1267	260	1274	582
Brunonia australis	2	276	595	509	246	976	111	1495	416	1678	899
Brunonia australis	3	153	602	543	269	934	84	1391	259	1779	818
Brunonia australis	4	115	645	297	241	793	60	1297	227	1379	588
Brunonia australis	5	101	913	388	261	940	138	1302	227	1602	530
Brunonia australis	6	123	525	353	229	682	142	1078	308	1236	523
Brunonia australis	7	130	535	409	185	657	115	1190	378	1364	811
Brunonia australis	8	112	630	257	304	682	120	1022	256	1413	725
Brunonia australis	9	163	324	326	214	804	131	1104	265	1390	692
Brunonia australis	10	91	705	217	333	816	108	1509	268	1769	629
Brunonia australis	11	111	835	358	348	884	55	1284	217	1620	814
Cooperhooikia georgei	0	661	1298	201	561	721	263	1246	628	752	1300
Cooperhooikia polygalacea	0	1251	2695	739	897	2299	245	3484	1439	2604	2596
Cooperhooikia polygalacea	1	1174	2685	586	1024	1749	302	2868	1048	2099	2735
Cooperhooikia polygalacea	2	1446	2188	1257	925	2272	314	3417	1174	2313	2637
Cooperhooikia polygalacea	3	583	2649	376	1340	1409	291	2513	769	1592	2785
Cooperhooikia polygalacea	4	1635	2722	345	1091	1732	242	2696	776	2861	2497
Cooperhooikia strophiolata	0	590	2502	734	448	2085	175	3328	656	3161	2533
Cooperhooikia strophiolata	1	613	2604	422	803	1691	155	2781	764	2610	2711
Cooperhooikia strophiolata	2	520	2485	474	483	1566	185	2662	718	2499	2568
Cooperhooikia strophiolata	3	983	2594	1075	985	2216	315	3277	783	3289	2808
Cooperhooikia strophiolata	4	850	2757	479	520	1842	172	2989	413	2700	2622
Cooperhooikia strophiolata	5	873	2721	725	530	1767	156	2913	731	2453	2884
Cooperhooikia strophiolata	6	562	2462	458	551	1602	158	2639	366	2575	2648
Cooperhooikia strophiolata	7	851	2657	491	550	1858	178	2827	635	2546	2678
Dampiera lindleyi	0	2545	2555	1264	1301	2483	418	3589	1123	2527	2588
Dampiera lindleyi	1	2070	2614	1149	1243	2249	490	3484	1422	2107	2606
Dampiera lindleyi	2	2214	2670	1229	1364	2486	595	3601	1727	2251	2631
Dampiera lindleyi	3	2221	2520	1003	839	2410	274	3603	1178	2245	2550
Dampiera lindleyi	4	2158	2544	1020	1033	2337	448	3608	1359	2169	2487
Dampiera lindleyi	5	2089	2485	1220	887	2451	487	3452	1447	2125	2433
Dampiera lindleyi	6	2191	2437	1139	1069	2226	402	3288	973	2219	2385
Dampiera lindleyi	7	2485	2542	1380	947	2328	440	3649	1216	2369	2567
Dampiera lindleyi	8	2275	2526	1289	946	2305	428	3379	967	2313	2532

Diaspasis filifolia	0	571	2643	393	545	1393	72	2814	1476	1670	2847
Diaspasis filifolia	1	746	2554	314	1000	1632	88	3139	1523	2596	2627
Diaspasis filifolia	2	1283	2803	220	1233	1798	85	3176	1005	2581	2727
Diaspasis filifolia	3	1272	2582	145	1355	1505	153	2581	1288	1984	2258
Diaspasis filifolia	4	1316	2452	202	952	1152	74	2391	1233	1837	2540
Diaspasis filifolia	5	223	2105	289	551	1464	151	1961	698	2153	2202
Diaspasis filifolia	6	408	2130	383	1049	1805	109	2937	1165	1674	2936
Diaspasis filifolia	7	692	2784	391	699	1432	134	2634	1074	2395	2037
Diaspasis filifolia	8	1590	2780	873	1723	2484	138	3829	1495	2929	2897
Diaspasis filifolia	9	792	2403	182	1256	1772	201	2959	709	2209	2783
Diaspasis filifolia	10	1037	2027	1116	914	2522	178	3557	920	1708	2880
Diaspasis filifolia	11	1243	2783	221	978	1515	161	2767	1190	2564	1915
Diaspasis filifolia	12	1592	2845	352	822	1667	121	2996	926	2260	2703
Goodenia berardiana	0	753	357	869	656	601	857	328	651	435	349
Goodenia berardiana	1	1800	1797	1570	1201	2000	930	2517	1150	2434	1782
Goodenia berardiana	2	398	667	309	423	617	218	907	474	799	687
Goodenia berardiana	3	607	815	385	777	391	430	697	329	821	537
Goodenia berardiana	4	1003	770	675	950	467	680	574	408	953	475
Goodenia berardiana	5	1065	1127	593	1205	481	723	910	430	1200	767
Goodenia berringbinensis	0	113	436	138	73	293	51	459	93	462	441
Goodenia berringbinensis	1	119	423	70	201	243	47	423	135	314	427
Goodenia berringbinensis	2	156	479	181	59	316	64	508	174	405	522
Goodenia berringbinensis	3	216	459	110	135	272	86	466	171	407	496
Goodenia berringbinensis	4	165	488	103	168	300	73	526	202	405	438
Goodenia berringbinensis	5	148	462	81	96	269	55	454	154	387	501
Goodenia berringbinensis	6	180	481	84	130	313	57	509	149	500	501
Goodenia berringbinensis	7	207	579	136	227	346	142	585	267	429	601
Goodenia berringbinensis	8	153	463	138	94	302	54	460	86	480	475
Goodenia berringbinensis	9	196	478	147	134	331	88	472	177	387	489
Goodenia berringbinensis	10	166	515	105	124	262	115	460	190	322	510
Goodenia berringbinensis	11	99	451	70	163	281	70	449	165	440	466
Goodenia berringbinensis	12	247	426	99	150	291	76	495	126	437	465
Goodenia berringbinensis	13	197	442	165	170	288	108	451	182	386	440
Goodenia convexa	1	1977	1823	1547	1286	2217	832	2801	1431	2201	1849
Goodenia convexa	2	1905	1905	1486	1022	2219	695	2827	1139	2267	1940
Goodenia convexa	3	2073	1882	1608	1148	2171	917	2646	1156	2173	1888

Goodenia convexa	4	2179	942	2905	1458	2296	2130	1518	1639	1939	1003
Goodenia convexa	5	1736	1122	2435	856	2901	1323	2714	2000	1849	1987
Goodenia convexa	6	2136	1882	1358	1048	2129	631	2947	1106	2308	1906
Goodenia decursiva	0	592	2145	1319	538	2331	262	3403	623	3958	2272
Goodenia decursiva	1	682	2353	1077	644	2333	283	3401	608	3910	2357
Goodenia decursiva	2	357	2034	782	440	1951	160	3024	617	3418	2034
Goodenia decursiva	3	213	1876	809	459	1898	155	2907	569	3409	2054
Goodenia decursiva	4	797	2608	1203	456	2430	171	3644	654	3930	2687
Goodenia decursiva	5	399	2150	778	376	1928	107	3063	366	3420	2213
Goodenia decursiva	6	468	2140	1212	492	2423	169	3627	627	4238	2421
Goodenia decursiva	7	230	2034	1051	329	1978	86	3231	415	3876	2226
Goodenia decursiva	8	156	2162	1123	685	2417	256	3544	826	3953	2362
Goodenia disperma	0	398	904	167	467	566	202	939	452	658	939
Goodenia drummondii	0	400	807	889	516	1306	286	1940	499	2306	954
Goodenia drummondii	1	237	769	615	387	1288	136	1951	462	2169	795
Goodenia drummondii	2	146	719	430	313	1086	83	1723	288	2027	791
Goodenia drummondii	3	80	619	232	418	722	107	1515	151	1867	986
Goodenia drummondii	4	50	645	474	202	1061	192	1253	117	2002	552
Goodenia drummondii	5	88	1212	345	499	992	78	1714	272	1997	1252
Goodenia drummondii	6	273	952	423	415	1113	166	1810	258	2306	976
Goodenia drummondii	7	206	1185	557	488	1343	220	2117	476	2450	1135
Goodenia drummondii	8	214	580	692	427	1155	174	1826	364	2109	810
Goodenia filiformis	0	1030	2454	657	1115	1736	516	2726	1326	2157	2513
Goodenia filiformis	1	1106	2372	680	1298	1720	543	2701	1255	2212	2413
Goodenia filiformis	2	901	2233	678	972	1696	651	2529	1043	1958	2377
Goodenia filiformis	3	834	1906	521	710	1454	500	1922	737	1659	2048
Goodenia filiformis	4	1079	2389	683	730	1655	511	2609	1090	2053	2387
Goodenia filiformis	5	845	2062	545	919	1328	379	2320	1042	1847	1943
Goodenia filiformis	6	636	2161	474	1027	1340	430	2139	811	1831	2153
Goodenia filiformis	7	730	2013	493	1089	1256	539	2163	1223	1800	2049
Goodenia filiformis	8	964	2124	627	956	1760	466	2707	1065	2532	2035
Goodenia hassallii	0	1309	2352	323	636	1712	208	2841	825	1555	2264
Goodenia hassallii	1	2353	2465	989	771	2464	164	3852	811	2429	2285
Goodenia hassallii	2	1901	2541	671	574	2042	118	3401	883	1898	2602
Goodenia hassallii	3	1109	2636	363	186	1288	116	2067	254	1328	2592
Goodenia hassallii	4	2344	2808	1046	466	2250	210	3435	567	2527	2953

Goodenia hassallii	5	1262	2364	259	517	1514	64	2771	946	1275	2621
Goodenia hassallii	6	2218	2776	1089	846	2339	270	3695	889	2550	2824
Goodenia hassallii	7	2061	2815	1136	553	2304	276	3383	526	2668	2867
Goodenia hassallii	8	1260	2251	363	361	1312	117	2195	253	1559	2304
Goodenia helmsii	0	152	447	336	227	638	124	951	225	1155	555
Goodenia helmsii	1	133	407	332	193	519	130	886	199	1017	373
Goodenia helmsii	2	114	298	252	153	523	47	802	204	926	488
Goodenia helmsii	3	168	807	352	254	724	157	1102	315	1222	636
Goodenia helmsii	4	111	599	238	290	691	161	1031	384	1124	704
Goodenia helmsii	5	54	620	278	260	681	116	1107	266	1298	635
Goodenia helmsii	6	69	544	159	334	501	112	921	206	1082	510
Goodenia helmsii	7	68	462	186	235	558	93	945	231	1096	520
Goodenia helmsii	8	95	508	233	241	545	84	972	193	1127	546
Goodenia macmillanii	0	226	360	37	228	115	123	250	124	222	362
Goodenia micrantha	0	950	276	1365	896	840	1251	257	936	608	253
Goodenia micrantha	1	1068	275	1352	1125	803	1263	299	831	701	248
Goodenia micrantha	2	767	180	919	712	631	815	290	766	349	189
Goodenia micrantha	3	950	240	1094	831	663	933	313	813	421	209
Goodenia micrantha	4	988	284	1290	987	844	1252	329	1090	399	364
Goodenia micrantha	5	1034	192	1279	855	789	1068	250	885	506	181
Goodenia micrantha	6	869	209	900	802	548	852	216	741	336	181
Goodenia micrantha	7	850	311	1068	952	596	1094	244	845	470	260
Goodenia micrantha	8	732	176	1037	852	648	967	195	845	451	194
Goodenia mimuloides	0	1238	1025	703	1381	371	844	672	389	1238	633
Goodenia mimuloides	1	2507	1706	1850	1866	1630	1320	2003	965	2561	1206
Goodenia mimuloides	2	2521	1665	2034	1823	1746	1429	1998	1011	2551	1203
Goodenia mimuloides	3	1717	1214	2303	824	2717	1093	2646	1623	1924	1805
Goodenia mimuloides	4	1857	2046	1595	1030	2278	765	2873	1056	2611	2120
Goodenia mimuloides	5	2342	1124	2453	1545	2178	1732	1849	1588	1915	1145
Goodenia occidentalis	0	486	779	290	446	548	309	855	431	699	761
Goodenia occidentalis	1	462	739	323	425	630	233	898	454	663	791
Goodenia occidentalis	2	336	435	765	396	908	634	732	919	312	704
Goodenia occidentalis	3	618	750	500	348	799	358	971	537	790	760
Goodenia ovata	0	1027	2851	433	525	1727	138	3005	434	2131	2837
Goodenia ovata	1	1432	2768	263	578	1466	145	3030	662	2136	2737
Goodenia ovata	2	1134	2742	431	766	1618	213	2687	1194	2194	2625

Goodenia ovata	3	1295	2757	311	801	1731	118	3125	946	1840	2819
Goodenia ovata	4	1367	2781	342	891	1677	124	3046	965	2037	2864
Goodenia ovata	5	1556	2769	235	1001	1680	102	3157	828	2053	2798
Goodenia ovata	6	1715	2792	258	587	1860	183	3164	495	2224	2736
Goodenia ovata	7	931	2488	359	600	1375	117	2953	859	1556	2865
Goodenia ovata	8	961	2849	467	882	1861	101	3520	833	2044	3002
Goodenia ovata	9	1328	2996	335	1212	1855	161	3342	1201	2151	2868
Goodenia phillipsiae	0	723	2398	628	340	1162	577	1673	375	1062	2563
Goodenia phillipsiae	1	928	2742	463	271	1283	211	2090	421	1663	2742
Goodenia phillipsiae	2	751	2685	539	308	1110	208	1653	428	1337	2764
Goodenia phillipsiae	3	857	2489	413	399	1005	286	1659	371	1100	2414
Goodenia pinifolia	0	130	1094	330	193	726	154	1000	178	1181	1113
Goodenia pinifolia	1	147	743	497	197	780	110	964	154	1420	856
Goodenia pinifolia	2	133	771	341	96	573	55	758	102	983	697
Goodenia pinifolia	3	462	839	258	187	628	85	919	204	632	851
Goodenia pinifolia	4	474	825	279	223	577	82	841	154	698	849
Goodenia pinifolia	5	348	987	339	163	539	135	716	257	726	963
Goodenia pinifolia	6	238	964	185	197	507	103	764	208	591	957
Goodenia pinifolia	7	323	872	218	161	456	111	696	206	523	848
Goodenia pinifolia	8	228	879	149	139	418	63	680	145	658	868
Goodenia pusilliflora	0	520	884	392	661	578	542	762	603	697	872
Goodenia pusilliflora	1	1816	1342	2554	968	2785	1392	2589	1881	1841	1600
Goodenia pusilliflora	2	2009	1698	1668	1162	2060	969	2517	1095	2195	1695
Goodenia pusilliflora	3	1958	1685	1883	1137	2116	1013	2331	1129	2261	1698
Goodenia pusilliflora	4	1656	1230	2547	1025	2724	1467	2525	1950	1680	1704
Goodenia pusilliflora	5	1883	1681	1671	1000	2119	837	2514	1029	2268	1716
Goodenia pusilliflora	6	509	633	390	335	645	237	918	360	753	666
Goodenia pusilliflora	7	616	909	483	596	705	501	919	592	784	893
Goodenia pusilliflora	8	424	808	254	542	495	429	723	515	581	799
Goodenia pusilliflora	9	386	698	301	374	511	284	680	392	551	702
Goodenia pusilliflora	10	407	910	244	446	535	352	794	464	616	934
Goodenia stephensonii	0	101	246	94	76	181	55	268	88	225	241
Goodenia stephensonii	1	127	238	97	77	161	56	213	76	196	229
Goodenia tripartita	0	896	2616	279	1110	1558	264	2582	960	1939	2686
Goodenia tripartita	1	1559	2653	919	1029	2346	465	3391	1262	2940	2712
Goodenia tripartita	2	1916	2734	1163	988	2364	390	3572	1221	2574	2702

Goodenia tripartita	3	1724	2788	1023	1420	2264	261	3609	1314	2715	2721
Goodenia tripartita	4	1758	2665	1058	1106	2498	214	3652	1072	2875	2793
Goodenia tripartita	5	1671	2654	687	1221	2298	350	3683	1533	2939	2758
Goodenia tripartita	6	1876	2733	1138	937	2391	334	3493	922	2724	2745
Goodenia tripartita	7	1953	2844	899	667	2343	277	3712	957	3220	2502
Goodenia tripartita	8	2145	2665	978	750	2048	261	3643	770	2823	2777
Goodenia varia	0	964	2245	428	393	1178	171	1821	401	1075	2325
Goodenia varia	1	745	2312	369	287	988	149	1704	361	1095	2360
Goodenia varia	2	846	2369	371	238	1013	131	1782	287	1121	2348
Goodenia varia	3	730	2030	415	297	1082	196	1594	450	1168	1988
Goodenia varia	4	780	1695	316	302	989	336	1558	390	1077	1759
Goodenia varia	5	852	1836	413	344	968	224	1575	365	1000	1825
Goodenia varia	6	583	2214	551	511	1233	256	2056	591	1680	2252
Goodenia varia	7	658	2234	474	384	1158	266	1954	409	1404	2268
Goodenia varia	8	1031	2227	566	481	1265	219	2055	426	1462	2088
Goodenia varia	9	1055	2282	530	350	1328	246	2114	586	1353	2369
Goodenia varia	10	456	1948	508	358	1146	261	1763	578	1227	2219
Goodenia viscida	0	306	1141	764	433	1280	278	2010	357	2447	1193
Goodenia viscida	1	168	1157	339	499	1104	179	1858	417	2092	1274
Goodenia viscida	2	200	1055	495	343	1308	161	1944	373	2315	1066
Goodenia viscida	3	139	890	448	403	1102	154	1858	307	2196	887
Goodenia viscida	4	193	766	561	313	1188	159	1861	441	1941	1022
Goodenia viscida	5	55	871	425	243	1048	142	1610	437	1793	924
Goodenia viscida	6	137	1090	544	307	1271	121	2120	568	2291	1115
Scaevola anchusifolia	0	179	484	463	140	810	154	1210	250	1467	637
Scaevola anchusifolia	1	636	1231	268	1077	146	653	280	285	716	165
Scaevola anchusifolia	2	182	871	579	186	1051	179	1514	239	2030	768
Scaevola anchusifolia	3	2217	2205	1469	1769	1543	1168	1897	554	2695	615
Scaevola anchusifolia	4	196	376	779	145	1325	280	1666	674	1633	1284
Scaevola anchusifolia	5	291	535	452	273	719	156	1000	176	1282	451
Scaevola anchusifolia	6	269	406	430	222	668	157	918	210	1130	417
Scaevola anchusifolia	7	227	454	427	283	659	249	852	308	1026	487
Scaevola anchusifolia	8	201	477	366	241	619	130	891	204	1111	461
Scaevola anchusifolia	9	342	435	505	245	741	186	1006	215	1222	387
Scaevola anchusifolia	10	217	484	436	207	702	153	935	183	1176	400
Scaevola calliptera	0	1705	1682	1669	1076	2217	910	2708	1133	2595	1771

Scaevola calliptera	1	1602	1902	1452	912	2208	622	2873	929	2653	1897
Scaevola calliptera	2	1761	1849	1616	1145	2189	906	2780	1202	2572	1834
Scaevola calliptera	3	1753	1807	1620	1134	2179	914	2659	1151	2560	1836
Scaevola calliptera	4	1279	2010	1287	928	2255	448	3118	1037	3069	1971
Scaevola calliptera	5	1462	1741	1452	970	2247	556	3002	955	2762	1891
Scaevola canescens	0	262	404	388	226	667	188	848	248	1001	416
Scaevola canescens	1	1739	1408	1891	1191	2142	1138	2426	1229	2507	1361
Scaevola canescens	2	552	238	611	323	635	438	597	570	540	641
Scaevola canescens	3	507	176	560	233	648	414	571	614	420	669
Scaevola canescens	4	255	314	374	202	599	135	792	227	909	307
Scaevola collaris	0	146	157	242	93	509	81	711	120	861	195
Scaevola collaris	1	87	103	189	60	398	61	624	69	750	142
Scaevola collaris	2	88	126	185	66	381	107	605	115	733	136
Scaevola collaris	3	164	123	216	89	414	75	620	91	776	166
Scaevola collaris	4	209	120	310	76	505	83	690	113	756	134
Scaevola collaris	5	189	130	272	124	462	73	643	100	671	104
Scaevola collaris	6	193	136	335	98	487	98	660	111	770	176
Scaevola collaris	7	170	163	293	113	489	132	667	196	781	241
Scaevola collaris	8	193	273	352	169	782	141	1116	204	1446	361
Scaevola crassifolia	0	205	502	299	297	670	158	1012	341	1100	456
Scaevola crassifolia	1	190	451	240	254	537	69	795	113	943	401
Scaevola crassifolia	2	217	281	349	169	522	161	720	155	816	322
Scaevola crassifolia	3	213	417	267	241	466	185	624	186	719	401
Scaevola crassifolia	4	264	529	396	366	757	178	1132	327	1237	624
Scaevola crassifolia	5	235	588	399	412	746	221	1093	227	1292	575
Scaevola humifusa	0	194	525	359	280	597	217	899	296	999	510
Scaevola humifusa	1	182	401	320	286	513	186	741	249	849	357
Scaevola humifusa	2	254	468	407	363	536	285	784	256	913	400
Scaevola humifusa	3	298	409	359	275	522	222	688	301	759	393
Scaevola humifusa	4	257	541	472	367	822	188	1224	335	1429	548
Scaevola phlebopetala	0	483	1884	890	669	2030	169	3026	701	3148	2495
Scaevola phlebopetala	1	302	2134	568	724	2252	218	3118	685	3470	2110
Scaevola phlebopetala	2	228	2141	467	698	2054	157	3081	755	3383	2164
Scaevola phlebopetala	3	238	1804	677	777	1692	206	2737	693	3095	1889
Scaevola phlebopetala	4	86	1859	473	597	1851	101	2944	1189	2916	2157
Scaevola phlebopetala	5	421	2161	632	576	1755	107	3010	730	2987	2231

Scaevola phlebopetala	6	180	1907	559	586	1842	176	3028	1005	2843	2444
Scaevola phlebopetala	7	1102	2255	1264	781	2465	241	3688	1052	3821	2294
Scaevola phlebopetala	8	340	2084	615	516	1700	92	2856	738	2862	2184
Scaevola phlebopetala	9	355	2214	498	647	1655	154	2804	953	2860	2385
Scaevola platyphylla	0	152	1250	700	457	1981	103	3694	843	3985	2196
Scaevola platyphylla	1	311	1782	618	888	2491	118	3823	957	4310	1887
Scaevola platyphylla	2	108	1982	621	619	2230	125	3757	685	4304	1971
Scaevola platyphylla	3	441	2292	1159	1022	2447	529	3840	1287	4260	2360
Scaevola platyphylla	4	178	1577	782	602	2171	114	3256	841	4264	1647
Scaevola platyphylla	5	170	1726	645	833	2121	113	3648	929	4007	1758
Scaevola platyphylla	6	103	2034	736	893	2270	124	3506	797	4293	1700
Scaevola platyphylla	7	117	2057	594	1088	2158	137	3360	471	4123	1911
Scaevola platyphylla	8	67	1885	701	672	2251	103	3566	744	4176	1689
Scaevola porocarya	0	250	2234	797	649	2482	160	3353	810	4067	2418
Scaevola porocarya	1	567	1924	1247	678	2459	393	3439	794	4193	1961
Scaevola porocarya	2	106	1722	300	694	1135	40	2518	189	3272	1613
Scaevola porocarya	3	366	2348	782	898	1875	117	3742	683	4342	2138
Scaevola porocarya	4	373	2095	949	770	2317	163	3659	715	4305	2177
Scaevola porocarya	5	176	1998	591	794	1904	102	3138	616	3818	1925
Scaevola porocarya	6	516	2354	1240	809	2248	501	3239	1031	4024	2172
Scaevola porocarya	7	144	1679	610	737	1860	163	3049	820	3470	1939
Scaevola porocarya	8	116	2358	540	795	1676	100	3539	412	4354	2270
Scaevola spinescens	0	1324	1532	1712	970	2208	839	2685	992	3018	1398
Scaevola spinescens	1	1168	1107	1572	616	2038	447	2516	696	2769	1089
Scaevola spinescens	2	1332	1115	1762	590	2248	418	2730	666	2967	1086
Scaevola spinescens	3	1827	1327	2004	1007	2693	856	3002	1028	3261	1440
Scaevola spinescens	4	1623	1441	1924	947	2532	656	3067	745	3554	1237
Scaevola spinescens	5	1297	1400	1692	862	2225	685	2920	812	3474	1441
Scaevola spinescens	6	1356	1262	1648	908	1937	733	2466	803	2822	1152
Scaevola spinescens	7	1482	1432	1754	1009	2129	859	2562	926	2941	1366
Scaevola spinescens	8	1456	1696	1746	1153	2044	920	2781	949	3200	1424
Scaevola spinescens	9	236	253	334	133	483	117	633	242	616	396
Scaevola spinescens	10	224	406	361	201	626	152	841	275	891	535
Scaevola thesioides	0	333	699	494	306	914	125	1293	290	1454	640
Scaevola thesioides	1	260	713	472	294	970	185	1455	395	1581	869
Scaevola thesioides	2	1482	1406	1661	966	2089	780	2529	952	2711	1297

Scaevola thesioides	3	1761	1367	1854	1012	2115	923	2444	1007	2547	1334
Scaevola thesioides	4	326	496	405	293	662	183	951	240	1063	479
Scaevola tomentosa	0	185	715	934	536	1596	222	2061	528	2985	724
Scaevola tomentosa	1	108	511	525	337	1530	118	2004	474	2791	408
Scaevola tomentosa	2	54	438	554	274	1156	271	1823	269	2392	372
Scaevola tomentosa	3	150	641	959	106	2005	198	2818	411	3854	1066
Scaevola tomentosa	4	58	913	879	532	1980	193	2631	479	3669	856
Scaevola tomentosa	5	179	529	767	190	2110	141	3199	346	3857	781
Scaevola tomentosa	6	103	449	928	434	1720	165	2651	408	3334	763
Scaevola tomentosa	7	157	381	989	338	1845	190	2928	433	3524	947
Scaevola tomentosa	8	99	585	699	265	1660	88	2582	208	3155	535
Scaevola tomentosa	9	186	671	763	412	1603	125	2545	481	3745	883
Selliera radicans	0	209	1377	394	536	1194	159	1832	415	1985	1540
Velleia cynopotamica	0	212	444	173	188	399	91	575	239	577	437
Velleia cynopotamica	1	206	567	121	290	413	185	697	329	620	588
Velleia cynopotamica	2	243	716	184	470	451	239	704	435	675	650
Velleia cynopotamica	3	312	419	251	294	409	186	556	312	488	433
Velleia discophora	0	761	1930	650	282	1190	213	1583	364	1390	1950
Velleia discophora	1	636	1735	552	268	1020	475	1277	300	952	1688
Velleia discophora	2	731	1975	475	295	1021	215	1655	412	1402	1930
Velleia discophora	3	1512	2201	1264	265	1726	175	2422	380	2109	2162
Velleia discophora	4	1365	1863	1239	350	1781	258	2184	348	1960	1911
Velleia foliosa	0	1211	2595	508	1328	1969	342	3114	1586	1955	2728
Velleia foliosa	1	1857	2639	1047	1311	2390	441	3543	1440	2558	2594
Velleia foliosa	2	1947	2609	1067	1390	2473	415	3653	1310	2685	2671
Velleia foliosa	3	1975	2607	868	1229	2321	348	3655	1530	2449	2785
Velleia foliosa	4	2130	2527	1060	1311	2343	337	3649	1213	2554	2502
Velleia foliosa	5	1799	2625	684	1036	2130	304	3524	1183	2310	2609
Velleia foliosa	6	2171	2607	979	1251	2380	309	3787	1225	2351	2641
Velleia foliosa	7	1386	2473	559	815	1646	284	2955	1310	1558	2456
Velleia foliosa	8	1439	2424	432	1106	1773	310	3080	1059	1853	2403
Velleia rosea	0	474	1224	425	601	906	333	1398	726	1170	1278
Velleia rosea	1	779	1686	405	899	1355	233	2139	936	1836	1595
Velleia rosea	2	511	1217	248	595	874	200	1495	636	1198	1230
Velleia rosea	3	371	1259	203	633	955	201	1501	768	1091	1408
Velleia rosea	4	402	1107	181	570	676	192	1168	514	962	1094

Velleia rosea	5	641	1432	298	606	1007	244	1769	740	1308	1453
Velleia rosea	6	510	1095	302	609	929	247	1407	565	1243	1088
Velleia rosea	7	422	1010	247	575	813	178	1351	596	1069	1043
Verreauxia reinwardtii	0	1038	2043	548	671	1301	280	1935	564	1644	2028
Verreauxia reinwardtii	1	1204	1760	674	472	1415	192	2067	532	1702	1744
Verreauxia reinwardtii	2	881	1903	427	519	1177	242	1785	493	1552	1842
Verreauxia reinwardtii	3	2162	2701	1562	1036	2425	680	3291	997	2632	2755
Verreauxia reinwardtii	4	2139	2502	1606	912	2271	618	3100	870	2549	2540
Verreauxia reinwardtii	5	1980	2042	1510	735	2262	550	2966	856	2465	2261
Verreauxia reinwardtii	6	2051	2481	1557	1174	2335	747	3158	1015	2541	2456
Verreauxia reinwardtii	7	1972	2506	1494	980	2333	624	3148	916	2749	2506
Verreauxia reinwardtii	8	2003	2375	1510	1006	2151	609	2904	870	2484	2374

S2 Table. Procrustes ANOVA calculated for shape

Data from all images were analyzed with MorphoJ to ascertain the overall variance in shape explained by species and by asymmetries (side) in floral morphology.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Species	108	23.7	0.219	103.9	<.0001
Individual	840	1.77	0.00211	2.04	<.0001
Side	3	0.0131	0.00436	4.23	0.0054
Ind*Side	948	0.978	0.00103		

S3 Table. Analysis of corolla size variation

Total variance of the average corolla lengths of the 44 species used in this study (source) were analyzed with k-means floral shape cluster as a factor.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Floral Morphology	2	13.7482	6.8741	0.2022	0.8177
Error	42	1427.8018	33.9953		
Total	44	1441.55			