

A phylogenomic investigation of *Scutellaria* reveals distinct patterns in medicinal flavonoid diversity

Bryce Askey¹, Yeong Hun Song¹, Yoonkyung Lee², Ru Dai¹, Sangtae Kim², and Jeongim Kim^{1,3}

¹Horticultural Sciences Department, University of Florida, Gainesville, FL, USA

²Department of Biology, Sungshin Women's University, Seoul, Republic of Korea

³Plant Molecular and Cellular Biology Graduate Program, University of Florida, Gainesville, FL, USA



Figure 1. (A) Maximum likelihood phylogenetic tree inferred from 3 chloroplast genomic regions for 51 species of *Scutellaria* and 1 outgroup. To facilitate downstream analysis, the tree was subdivided into 5 color-coded clades.

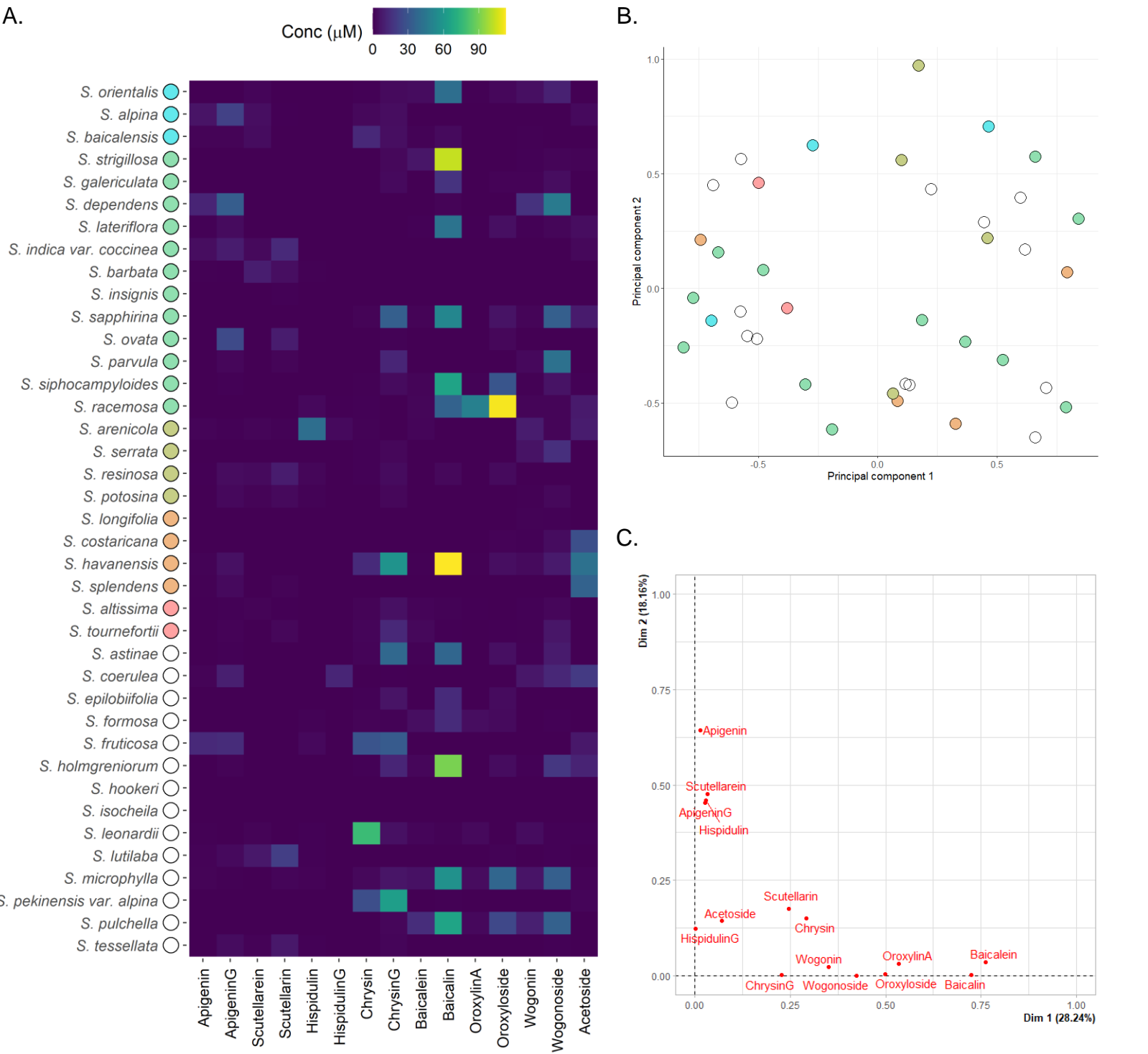


Figure 2. Aerial flavonoid concentrations measured with High Performance Liquid Chromatography (HPLC) for # species of *Scutellaria*. (A) Heatmap of collected data. Colored circles next to species names indicate phylogenetic clade, as shown in Figure 1. An empty circle indicates that the species was not included in the tree from Figure 1. Data for fresh tissue samples is shown at 5000 ppm (5 mg sample / 1 mL solvent). Data for herbarium tissue samples is shown at 500 ppm (0.5 mg sample / 1 mL solvent). (B) Multiple Correspondence Analysis (MCA) results generated from binary version of flavonoid data. (C) Variable representations from MCA.

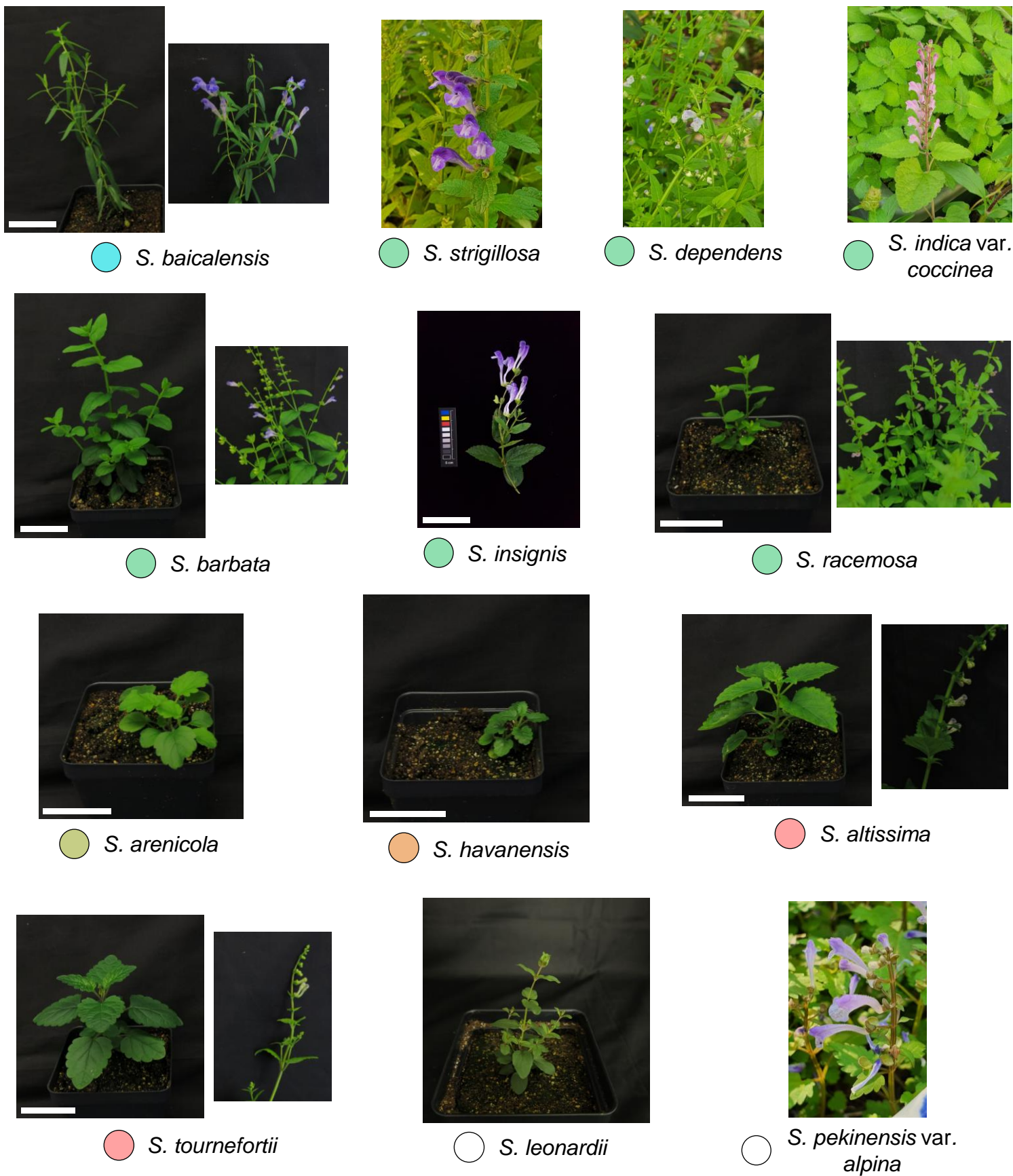


Figure 3. Representative images of 13 species of *Scutellaria* selected for additional flavonoid and genome size profiling. Scale bar in bottom left of images represents a length of 5 cm. Images without a scale bar didn't have a ruler included in the image. Colored circles next to species names indicate the clade which the species belongs to.

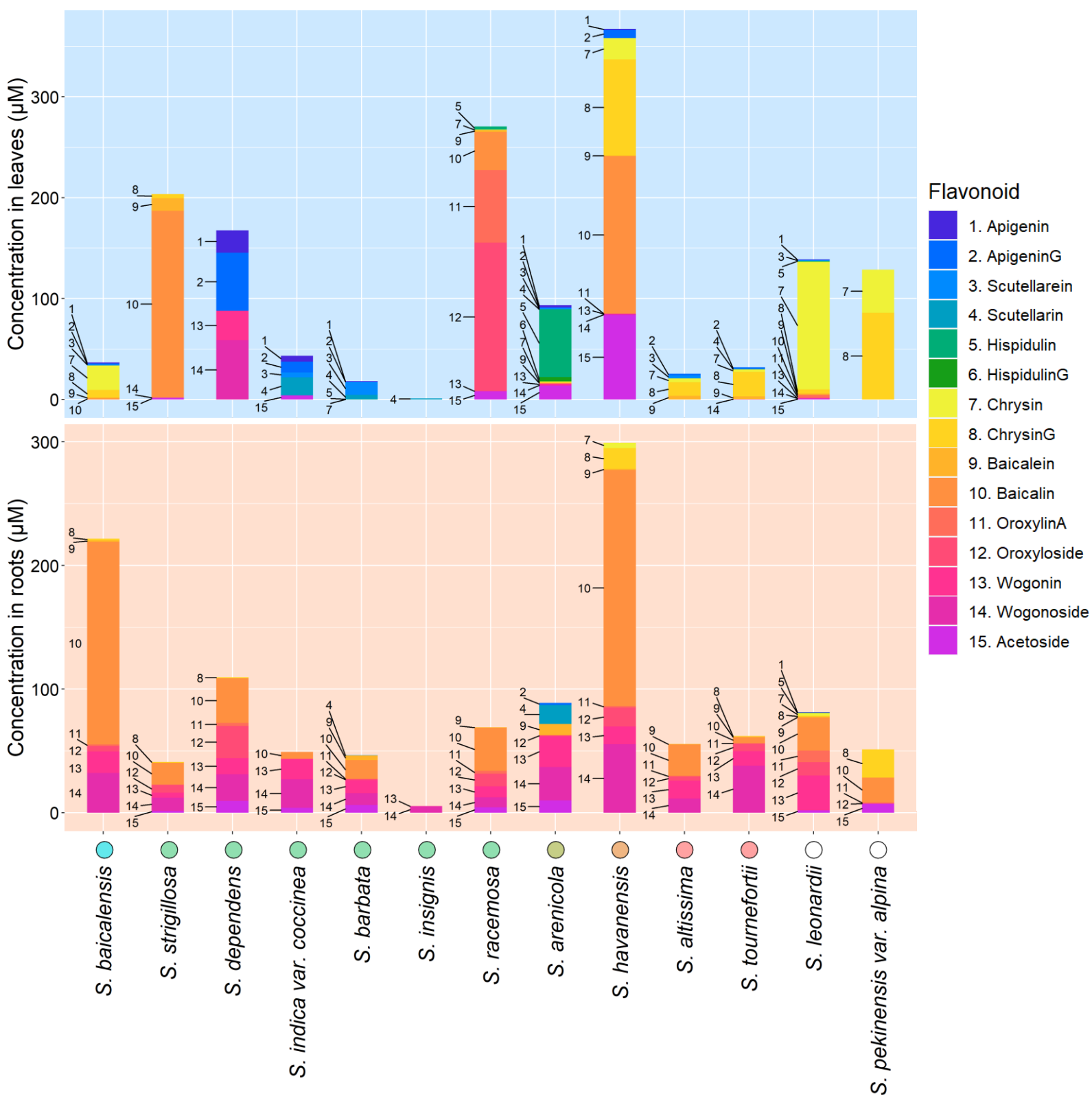


Figure 3. Organ-specific flavonoid data collected from 13 *Scutellaria* species via High Performance Liquid Chromatography (HPLC). Species are ordered based on phylogenetic relationship determine from chloroplast genome data, shown in Figure 1. Colored circles next to species names indicate phylogenetic clade, as shown in Figure 1. An empty circle indicates that the species was not included in the tree from Figure 1. Flavonoids are ordered based on proposed flavonoid pathway for *S. baicalensis*.

Table 1. Genome sizes as estimated by flow cytometry. Cell colors indicate phylogenetic clade, as shown in Figure 1.

Species	Estimated genome size (Gbp)		Chromosome number
	Measured in this study	Published previously	Published previously
<i>S. baicalensis</i>	0.54	0.38 (Xu et al., 2020)	2n = 18 (Xu et al., 2020)
		0.41 (Zhao et al., 2019)	2n = 18 (Zhao et al., 2019)
		0.41 (Cole et al., 2008)	
<i>S. strigillosa</i>		0.38 (Lee & Kim, 2017)	
<i>S. dependens</i>	0.44		
<i>S. indica</i> var. <i>coccinea</i>		0.38 (Lee & Kim, 2017)	
<i>S. barbata</i>	0.52	0.35 (Xu et al., 2020)	2n = 26 (Xu et al., 2020)
<i>S. insignis</i>		0.46 (Lee & Kim, 2017)	
<i>S. racemosa</i>	0.44	0.37 (Cole et al., 2008)	2n = 18 (Cole et al., 2008)
<i>S. arenicola</i>	0.85		
<i>S. havanensis</i>	0.37		
<i>S. altissima</i>	0.39		
<i>S. tournefortii</i>	0.39		
<i>S. leonardii</i>	0.50		
<i>S. pekenensis</i> var. <i>alpina</i>	0.38		

Table S1. Source of *Scutellaria* tissue samples

	Species	Condition	Organ	Herbarium	Voucher label
1	<i>S. alpina</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	T. H. Everett s. n. (NY)
2	<i>S. altissima</i>	Dried	Leaf	Steere Herbarium, NY, USA	M. Churadze 1007 (NY)
3		Fresh	Leaf, stem, root	To be submitted	Herbarium name not recognized
4	<i>S. angulosa</i>	Dried		Steere Herbarium, NY, USA	K. M. Naid s. n. (NY)
5	<i>S. angustifolia</i> ssp. <i>angustifolia</i>	Dried		Steere Herbarium, NY, USA	R. Olmstead 558 (NY)
6	<i>S. angustifolia</i> ssp. <i>micrathum</i>	Dried		Steere Herbarium, NY, USA	A. Tiehm 16758 (NY)
7	<i>S. antirrhinoides</i>	Dried		Steere Herbarium, NY, USA	N. D. Atwood 28625 (NY)
8	<i>S. arenicola</i>	Dried		University of Florida Herbarium, FL, USA	(FLAS)
9		Fresh	Leaf, stem, root	To be submitted	Herbarium name not recognized
10	<i>S. astinae</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	J. T. Howell 38532 (NY)
11	<i>S. baicalensis</i>	Fresh	Leaf, stem, root	To be submitted	Herbarium name not recognized
12		Fresh	Leaf, stem, root	To be submitted	Herbarium name not recognized
13	<i>S. barbata</i>	Fresh	Leaf, stem, root	Sungshin Herbarium, Seoul, Republic of Korea	S. Kim 2019-056 (SWU)
14	<i>S. blepharophylla</i>	Dried		Steere Herbarium, NY, USA	G. B. Hinton 1086 (NY)
15	<i>S. brittonii</i>	Dried		Steere Herbarium, NY, USA	S. P. Churchill 7376 (NY)
16	<i>S. californica</i>	Dried		Steere Herbarium, NY, USA	R. Olmstead 434 (NY)
17	<i>S. coccinea</i>	Dried		Steere Herbarium, NY, USA	D. Cardenas et al. 42257 (NY)
18	<i>S. coerulea</i>	Dried	Leaf	Steere Herbarium, NY, USA	T. S. Quedensley 10037 (NY)
19	<i>S. costaricana</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	A. Cronquist 8834 (NY)
20	<i>S. dependens</i>	Fresh	Leaf, stem, root	Sungshin Herbarium, Seoul, Republic of Korea	S. Kim 2019-065 (SWU)
21	<i>S. discolor</i>	Dried		Steere Herbarium, NY, USA	A. A. Bullock 740 (NY)
22	<i>S. drumondii</i>	Dried		Steere Herbarium, NY, USA	L. C. Higgins 17229 (NY)
23	<i>S. elliptica</i>	Dried		University of Florida Herbarium, FL, USA	(FLAS)
24	<i>S. epilobiifolia</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	C. E. Garton 22860 (NY)
25	<i>S. formosa</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	H. et al. 11073 (NY)
26	<i>S. fruticosa</i>	Dried	Leaf	Steere Herbarium, NY, USA	H. Field & Y. Lazar s. n. (NY)
27	<i>S. galericulata</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	D. Erskine & A. J. Smith 2118 (NY)
28	<i>S. glabriuscula</i>	Dried		Steere Herbarium, NY, USA	R. Kral s. n. (NY)
29	<i>S. guatemalensis</i>	Dried		Steere Herbarium, NY, USA	D. Alvarez 11028 (NY)
30		Dried		Steere Herbarium, NY, USA	J. K. Small s. n. (NY)
31	<i>S. havanensis</i>	Fresh	Leaf, stem, root	To be submitted	Herbarium name not recognized
32	<i>S. heterophylla</i>	Dried		Steere Herbarium, NY, USA	A. Bertschinger s. n. (NY)
33	<i>S. heydei</i>	Dried		Steere Herbarium, NY, USA	W. Koelz 6242 (NY)
34	<i>S. hispidula</i>	Dried		Steere Herbarium, NY, USA	R. D. Worthington 12501 (NY)
35	<i>S. holmgreniorum</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	G. Schoolcraft 1222 (NY)
36	<i>S. hookeri</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	L. O. Williams et al. 28585 (NY)
37	<i>S. incanca</i>	Dried		Steere Herbarium, NY, USA	R. D. Thomas 150388 (NY)
38	<i>S. indica</i> var. <i>coccinea</i>	Fresh	Leaf, stem, root	Sungshin Herbarium, Seoul, Republic of Korea	S. Kim & S. T. Lee 2015-0298 (SWU)
39	<i>S. insignis</i>	Fresh	Leaf, stem, root	Sungshin Herbarium, Seoul, Republic of Korea	S. Kim 2015-0110 (SWU)
40	<i>S. integrifolia</i>	Dried		University of Florida Herbarium, FL, USA	(FLAS)
41	<i>S. isochelia</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	A. M. Brenes s. n. (NY)
42	<i>S. javalambrensis</i>	Dried		Steere Herbarium, NY, USA	A. Charpin et al. s. n. (NY)
43	<i>S. javanica</i>	Dried		Steere Herbarium, NY, USA	S. K. Lau 1915 (NY)
44		Dried	Leaf	Steere Herbarium, NY, USA	G. F. Buddell II 2352 (NY)
45	<i>S. lateriflora</i>	Fresh	Leaf, stem, root	To be submitted	Herbarium name not recognized
46		Dried		Steere Herbarium, NY, USA	J. C. Arthwz s. n. (NY)
47	<i>S. leonardii</i>	Fresh	Leaf, stem, root	To be submitted	Herbarium name not recognized
48	<i>S. leptosiplonsipkon</i>	Dried		Steere Herbarium, NY, USA	L. Maplcoba s. n. (NY)
49	<i>S. longifolia</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	J. J. Castillo & J. M. Vargas 2722 (NY)
50	<i>S. lutea</i>	Dried		Steere Herbarium, NY, USA	Jorge A. Molina 30005 (NY)
51	<i>S. lutilaba</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	Hinton et al. 21760 (NY)
52	<i>S. microphylla</i>	Dried	Leaf, stem, root	Steere Herbarium, NY, USA	H. D. Ripley 14966 (NY)
53	<i>S. multicularis</i>	Dried		Steere Herbarium, NY, USA	Assadi, Edmondson & Miller 2135 (NY)
54	<i>S. multiglandulosa</i>	Dried		Steere Herbarium, NY, USA	D. S. Correll & H. B. Correll 51703 (NY)
55	<i>S. multiglandulosa</i>	Dried		University of Florida Herbarium, FL, USA	(FLAS)
56	<i>S. muriculata</i>	Dried		Steere Herbarium, NY, USA	C. L. Lundell 14978 (NY)
57	<i>S. oblonga</i>	Dried		Steere Herbarium, NY, USA	L. H. Cramer 4871 (NY)
58	<i>S. orientalis</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	E. Serowa & E. Ryschowa s. n. (NY)
59	<i>S. ovata</i>	Dried	Leaf	Steere Herbarium, NY, USA	R. D. Thomas et al. 170488 (NY)
60	<i>S. parvula</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	R. D. Thomas & C. Slaughter 103883 (NY)
61	<i>S. paucifolia</i>	Dried		Steere Herbarium, NY, USA	P. Jaeger 8796 (NY)
62	<i>S. pekinensis</i> var. <i>alpina</i>	Fresh	Leaf, stem, root	Sungshin Herbarium, Seoul, Republic of Korea	S. Kim 2015-0268 (SWU)
63	<i>S. peregrina</i>	Dried		Steere Herbarium, NY, USA	H. Field & Y. Lazar s. n. (NY)
64	<i>S. pinnatifida</i>	Dried		Steere Herbarium, NY, USA	K. H. Rechinger 686 (NY)
65	<i>S. potosina</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	D. S. Correll & H. B. Correll 30562 (NY)
66	<i>S. prostrata</i>	Dried		Steere Herbarium, NY, USA	W. Koelz 21180 (NY)
67	<i>S. przewalskii</i>	Dried		Steere Herbarium, NY, USA	L. Janczenko s. n. (NY)
68	<i>S. pseudoserrata</i>	Dried		Steere Herbarium, NY, USA	R. Kral s. n. (NY)
69	<i>S. pulchella</i>	Dried	Root	Steere Herbarium, NY, USA	R. Schischkin et al. s. n. (NY)
70	<i>S. purpurascens</i>	Dried		Steere Herbarium, NY, USA	A. M. Brenes s. n. (NY)
71		Dried		University of Florida Herbarium, FL, USA	(FLAS)
72	<i>S. racemosa</i>	Fresh	Leaf, stem, root	To be submitted	Herbarium name not recognized
73	<i>S. relenorskyi</i>	Dried		Steere Herbarium, NY, USA	A. Donmez 3627 (NY)
74	<i>S. resinosa</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	G. C. Freeman & R. E. Brooks 3682 (NY)
75	<i>S. sapphirina</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	S. L. Welsh 20585 (NY)
76	<i>S. scordiifolia</i>	Dried		Steere Herbarium, NY, USA	H. H. Iltis et al. 307 (NY)
77	<i>S. seleriana</i>	Dried		Steere Herbarium, NY, USA	C. G. Pringle s. n. (NY)
78	<i>S. serrata</i>	Dried	Leaf	Steere Herbarium, NY, USA	J. J. Carter s. n. (NY)
79	<i>S. siphocampuloides</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	S. D. White 4380 (NY)
80	<i>S. splendens</i>	Dried	Leaf	Steere Herbarium, NY, USA	A. Villegas H. 00063 (NY)
81	<i>S. strigilloso</i>	Fresh	Leaf, stem, root	Sungshin Herbarium, Seoul, Republic of Korea	S. Kim 20140705 (SWU)
82	<i>S. suffrutscens</i>	Dried		Steere Herbarium, NY, USA	J. L. Reveal 3390 (NY)
83	<i>S. sumatrana</i>	Dried		Steere Herbarium, NY, USA	C. Hamel & R. S. Toroes 767 (NY)
84	<i>S. tessellata</i>	Dried	Leaf, stem	Steere Herbarium, NY, USA	R. D. Worthington 13323 (NY)
85	<i>S. tournefortii</i>	Fresh	Leaf, stem, root	To be submitted	Herbarium name not recognized