

Crataegus ×ninae-celottiae and C. ×cogswellii (Rosaceae, Maleae), two spontaneously formed intersectional nothospecies

Knud Ib Christensen¹, Mehdi Zarrei², Maria Kuzmina³, Nadia Talent⁴, Charlotte Lin^{4,5}, Timothy A. Dickinson^{4,6}

Assoc. Prof. M.Sc. Ph.D. Knud Ib Christensen (born 13 October 1955, deceased 16 January 2012), formerly at the Botanical Garden, Natural History Museum of Denmark, University of Copenhagen 2 The Centre for Applied Genomics (TCAG), The Hospital for Sick Children, Peter Gilgan Centre for Research and Learning, Rm. 139715, 686 Bay St., Toronto, Ontario, M5G 0A4 3 Department of Botany, MRC-166 National Museum of Natural History Smithsonian Institution Rm W106 Washington, DC 20013-7012 USA 4 Green Plant Herbarium (TRT), Department of Natural History, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario Canada M5S 2C6 5 School of Education, University of Stirling, Scotland UK FK9 4LA 6 Department of Ecology & Evolutionary Biology, University of Toronto, 25 Willcocks St., Toronto, Ontario, Canada M5S 3B2

Corresponding author: Timothy A. Dickinson (tim.dickinson@utoronto.ca)

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Abstract

Crataegus monogyna Jacq. is naturalized in North America, where it has hybridized with native diploid hawthorns at least twice. We provide names for the two nothospecies (as well as for the corresponding nothosections and nothoseries), referring to existing documentation in the literature for **nothosp. nov**. *Crataegus ×ninae-celottiae* K.I. Chr. & T.A. Dickinson (*C. monogyna × C. punctata* Jacq.). New data are provided to further document **nothosp. nov**. *Crataegus ×cogswellii* K.I. Chr. & T.A. Dickinson (*C. monogyna × C. punctata* Jacq.). New data are provided to further document **nothosp. nov**. *Crataegus ×cogswellii* K.I. Chr. & T.A. Dickinson (*C. monogyna × C. suksdorfii* (Sarg.) Kruschke). In both cases, the striking differences in leaf shape between most New World hawthorns and Old World section *Crataegus*, and the intermediacy of the hybrids, account for the relative ease with which these hybrids can be recognized. Finally, new sequence data from ITS2 and chloroplast DNA barcoding loci confirm the genetic relationships between the two nothospecies and their respective parents.

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Keywords

North America, hawthorn, hybridization, diploid, leaf shape, ITS2, DNA barcodes

Introduction

Crataegus monogyna Jacq. is a widespread species of Crataegus sect. Crataegus that occurs in much of Europe, northern Africa and western Asia. Within the area of its natural distribution it hybridizes with several other species of sect. Crataegus, e.g., C. laevigata (Poir.) DC., C. rhipidophylla Gand., C. meyeri Pojark., C. pentagyna Waldst. & Kit. ex Willd., C. orientalis M. Bieb., and C. azarolus L., as well as C. nigra Waldst. & Kit. of sect. Sanguineae (Albarouki and Peterson 2007; Byatt 1975; Christensen 1983; Christensen 1992a, b, 1994; Christensen and Zielinski 2008; Dönmez 2004). In fact, Christensen (1992) applied the term "compilospecies" to C. monogyna. This term, coined by Harlan and DeWet (1963), describes species that aggressively acquire genes from other species by introgressive hybridization, potentially explaining the "...great variability of C. monogyna and also its wide distribution" in the Old World (Christensen 1992a). Crataegus monogyna was introduced to the U.S.A. and Canada by the early European settlers (Billings 1862; Douglas 1914; Kirk 1819; Provancher 1863). It has often escaped from cultivation and, e.g., in abandoned fields and woodlands with extensive hawthorn colonization, it may hybridize with native diploid species of *Crataegus* such as C. punctata Jacq. (sect. Coccineae Loudon; Phipps pers. comm.; Wells and Phipps 1989) and C. suksdorfii (Sarg.) Kruschke (sect. Douglasia Loudon; Dickinson et al. 2008; Love and Feigen 1978; Talent and Dickinson 2005). Because of the striking contrast in leaf shape between members of C. sect. Crataegus and most North American Crataegus species, these hybrids are currently the best-known examples of diploid-diploid hybridization in the North American Crataegus flora. We provide names for these two nothospecies (as well as for the corresponding nothosections and nothoseries), referring to existing documentation in the literature for *Crataegus × ninae-celottiae* K.I. Chr. & T.A. Dickinson (C. monogyna × C. punctata Jacq.; Wells and Phipps 1989). We also document variation in leaf shape for the second hybrid, Crataegus ×cogswellii K.I. Chr. & T.A. Dickinson (Crataegus suksdorfii × C. monogyna), and provide new sequence data from ITS2 and chloroplast DNA barcoding loci that confirm the genetic relationships between the two nothospecies and their respective parents.

Methods

Sampling. Because the occurrence of *C. monogyna* and its hybrids is sporadic, most of our samples are non-random, and merely attempt to document the co-occurrence of the parental species and (or) their hybrids (Table 1). Only in the case of the hybrid swarm found at the Cogswell-Foster Preserve in Linn Co., Oregon (site OR1), have we used either the throw of a pair of dice or ignorant person sampling (Ward 1974) in or-

Table 1. Sites in Canada and the United States at which collections of native and naturalized diploid (unless indicated otherwise) *Crataegus* were made as vouchers for morphological, chemical, and molecular (boldface) observations (Fig. 1–3; Tables 2–4). Sampled individuals are listed by their collector and collection number; principal collector is T. A. Dickinson (D) unless indicated otherwise, as follows: JC, J. Coughlan; CAR, Rebecca Dotterer; EH, E. Harris; EL, E. Y. Y. Lo; RML, R. M. Love; MP, M. A. Purich; Z, P. Zika.

State	/Province			
	Site	Location	Taxon	Individuals
Britis	h Columbia			
	BC16	Central Kootenay R.D., Robson, Broadwa- ter Road, Broadwater Road S side	C. monogyna	2008-26
	ВС	Central Kootenay R.D., Winlaw, next to Winlaw general store (10 miles S of Slocan) on bank of small creek (tributary of Slocan River).	<i>C. suksdorfii</i> Probably polyploid	RML9313
Califo	ornia		·	·
	CA11	Humboldt Co., Hwy 36, 6.8 air km W of Bridgeville	C. monogyna	JC001
	CAR4	Trinity Co., T37N R7W S17	<i>C. suksdorfii</i> Polyploid?	CAR042
	CAR5	Siskiyou Co., flood plain of the Scott R., N side of Fay Lane, between jct. Hwy 3 and bridge	C. suksdorfii	2006-16, 2006-18, 2006-19, 2006-22 , CAR044
	CAR7	Siskiyou Co., T26N R11W S17	<i>C. suksdorfii</i> Polyploid?	CAR048
	CRRR01	Sonoma Co., Ragle Ranch, W of Sebas- topol	C. monogyna	JC003
Idaho)			
	ID10	D10 Benewah Co., T44N R1W S8, Soldier Creek, W side of Hwy 3 just N of RR crossing and St. Mary's R. C. suksdorfii Probably polyploid		D1608
Mont	ana			
	MT1	Powell Co., Dry Creek, N side, edge of meadow and gallery forest	4× C. suksdorfii	D1614, D1619
Onta	rio		7	1
	NTON23	City of Toronto, Centennial Park, Etabicake	C. punctata	MP71
	11101125	City of foloito, Centennia Faix, Etobleoke	C. ×ninae-celottiae	MP24, MP73
	ON21	Bruce Co., Eastnor Twp., Barrow Bay, E side Hwy 9 at S.R. 15	C. punctata	Dickinson & Nguyen BB4
	ON31	Middlesex Co., Ilderton, SE corner Denfield Side Road and Ilderton Road (Hwy 16)	C. punctata	EH52, MP56, MP61, 2003-79
	ON40	City of Toronto, Ashbridges Bay Park	C. punctata	MP35
	ON45		C. monogyna	MP82, MP83, MP98
		5 Durham R.M., Bowmanville, floodplain of Bowmanville Creek	C. ×ninae-celottiae	2002-13, MP84, MP85, MP86
			C. punctata	MP81
	ON46	Perth Co., E side Thames R. North Branch 2 km S of Motherwell	C. punctata	2008-72A

State	Province			
	Site	Location	Taxon	Individuals
Orego	on			
			<i>C. monogyna</i> (diploid)	EL74, EL78, EL80, EL83, OR1-5, OR1-8, OR1-9, OR1-10, OR1- 11, OR1-12, OR1-16
			Triploid <i>C. monogyna</i>	RML C-2003-25
	OR1	Linn Co., Willamette Valley, Cogswell- Foster Preserve	C. ×cogswellii	99FW7-1, 99FW7-2, 99FW7-3, 99FW7-6, 99FW7-7, 99FW7-8, 99FW7-9, 2009-36, EL68, EL71 , EL73, EL76, EL77, EL79 , EL81, EL82, EL84, EL85 , OR1-2, OR1-3, OR1-4, OR1-6, OR1- 7, OR1-13, OR1-14, OR1-15, OR1-17, OR1- 18, OR1-19, OR1-20, RML8718
			C. suksdorfii	EL68 , EL69, EL72, EL75, OR1-1, RML8709
	OR	Lane Co, City of Eugene	C. ×cogswellii	RML C-2003-12, RML C-2003-13, RML9304
	OR4	Douglas Co., Upper Elk Meadow, 28 miles SSE Cottage Grove	<i>C. suksdorfii</i> Probably polyploid	RML8758, RML8767, RML8768
		Columbia Co., Sauvie Island, Willow Park	C. monogyna	EL108
	OP11	Island, Willow Bar Islands beach, just N	C. ×cogswellii	Z18482
	OKII	of Columbia-Multnomah county line, on bank of Columbia River	C. suksdorfii	JC117, JC118, JC119
	OR18	Jackson Co., Rogue River, Old Stage Rd. 80 m NE of Rogue River Hwy/99	C. suksdorfii	JC039
	OR22	Linn Co., Corvallis, KOA Campground, 440 m from hwy 34 on Oakville Rd. SW. specimen 150 m SE of camp entrance	C. suksdorfii	JC060
	OR35	Skamania Co., Cascade Locks, 110 m N of Cascade Locks Rd., on N side of Forest Ln.	C. suksdorfii	JC092
	OR37	Multnomah Co., Columbia River Gorge National Scenic Area, 1.5 km NE of Trout- dale	C. suksdorfii	JC098, JC102
	OR38	Columbia Co., Diblee Pt., Site 350 m N of Dike Rd., 1.8 km WNW of Lewis and Clark Bridge	C. suksdorfii	JC136
Washington				
	WA	Clark Co. S of mouth of Lewis River, ca. 1.5 air miles NNW of Ridgefield	C. suksdorfii	Z18485
	WA8	Skamania Co., Gifford Pinchot National Forest, Zig Zag Lake, 9 mi NW of Wind R.	<i>C. suksdorfii</i> Probably polyploid	Brooks s.n.
	WA10	Skamania Co., Gifford Pinchot National Forest, Upper Goose Creek Meadow	<i>C. suksdorfii</i> Probably polyploid	RML8909

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der to draw more unbiased samples, with the inevitable consequence that these samples reflect the greater frequency of the introduced species and its hybrids. To mitigate this, we have included individuals of mostly diploid *C. suksdorfii* from other sites in order to reflect the variation found in this taxon.

Note that we distinguish the taxon referred to here as *C. suksdorfii* from the other western North American black-fruited hawthorn with 20 stamens per flower, *C. gay-lussacia* A. Heller. This is because these two taxa are allopatric (Coughlan 2012 and unpubl. data), and differ in morphology and cytotype. *Crataegus gaylussacia* has shorter petioles and thorns that are thicker at their base than is the case with diploid *C. suks-dorfii* (Dickinson unpubl. data). Molecular data are consistent with *C. gaylussacia* being an autotriploid derivative of diploid *C. suksdorfii* (Zarrei et al. http://2012.botany-conference.org/engine/search/index.php?func=detail&aid=536 and unpubl. data; see also Lo et al. 2009). In contrast, the *C. suksdorfii* complex has been shown to comprise, in addition to diploids, allotriploids and allotetraploids (Zarrei et al. http://2012.botany-conference.org/engine/search/index.php?func=detail&aid=536 and unpubl. data).

In order to increase our sample for molecular studies we have supplemented field collections of leaf tissue and herbarium vouchers with tissue removed from existing specimens in the ROM Green Plant Herbarium. Historical records of the distribution of *C. monogyna* were collected from five herbaria across Canada (TRT, MTMG, MT, QFA and UBC). Online databases of Canadian and U.S. herbaria used included ACAD, the Invader Database System of the University of Montana (which contains information for five northwestern states: Idaho, Montana, Oregon, Washington, Wyoming), OSC, and WTU. Distribution maps were prepared from specimen locality data using SimpleMappr (Shorthouse 2010). Names of *Crataegus* sections and series used here follow those published by VASCAN (Brouillet et al. 2010), and are accepted names sensu FNA Ed. Comm. in prep.

Morphology. For this study we concentrated on capturing and analyzing leaf shape data, as described elsewhere (Dickinson et al. 2008). Many previous studies of hybridization involving C. monogyna (Bradshaw 1953; Byatt 1975; Love and Feigen 1978), and of leaf shape variation in Crataegus generally (e.g. El-Gazzar 1980; Phipps and O'Kennon 2007), have attempted to quantify leaf lobing by means of a ratio of two measurements, x and y, where x is the distance from the tip of a lobe (usually the most basal one) to the deepest point of the sinus between that lobe and the adjacent one above it, and y is a measure of leaf size, usually the parallel distance from the tip of the lobe to the midrib. This approach can be effective when comparisons involve only leaves that have some degree of lobing (e.g. studies of hybridization between C. monogyna and C. laevigata in Europe, or of the lobed leaves of many species belonging to North American C. sect. Coccineae, such as C. punctata). However, when lobing is absent altogether the necessary landmarks (lobe tip, deepest point of the sinus) are absent, and the distance x is undefined or is set to zero (Love and Feigen 1978). In this case, a better approach is to carry out multivariate analyses of additional measurements of leaves and other organs (Wells and Phipps 1989), or to quantify variation in the leaf outline as a whole. Elliptic Fourier coefficients obtained from digitized leaf outlines

captured using MorphoSys (Meacham and Duncan 1991), or the Fourier amplitudes derived from them, provide a useful method for doing just this (Dickinson et al. 2008; McLellan and Endler 1998; Rohlf and Archie 1984).

Leaf outline data were collected from two overlapping samples: (1) short shoot leaf spectra (Dickinson and Phipps 1984) collected from a random sample of individuals at the Cogswell-Foster Preserve (comprising one *C. suksdorfii*, seven *C. monogyna*, and 12 putative hybrids), and (2) leaves on herbarium specimens from the Cogswell-Foster Preserve and other locations in the Pacific Northwest. In the latter the attempt was made to sample the leaf shape variation seen in *C. suksdorfii* as widely as possible. In both cases, variation in the shape of the leaf blade (i.e. excluding the petiole) was summarized by means of 39 Fourier amplitudes, and displayed by means of principal components analysis.

For each leaf outline we also obtained the area (A) and perimeter (P), so as to calculate the inverse of the dissection index described by Kincaid and Schneider (1978), i.e. $inv(D.I.) = 2(A\pi)^{\frac{1}{2}}/P$, a parameter that has an upper bound of one for a perfect circle regardless of size, and approaches zero as the length of the perimeter increases with increased lobing of the outline (Dickinson 2003; Dorken and Barrett 2004). In addition to outline data we made linear measurements with which to index overall leaf shape: X, leaf blade length above the widest point; Y, leaf width; and Z, leaf blade length below the widest point (Marshall 1978). On some of the flowering specimens in our sample we collected additional data on stamen number, style length and style number (in fruiting specimens, equivalently, pyrene number), and stigma width, in order to compare these with data collected by others from the introduced species and C. punctata. After transformation to a common [0,1] range these data were also summarized using principal components analysis. Analyses of variance were carried out on selected measurements. All data analyses described above were carried out using the R environment for statistical computing (R Core Team 2013). Significance of individual principal component axes was evaluated using the broken-stick criterion (Frontier 1976) with the help of R function *evplot* (Borcard et al. 2011).

Molecular methods. Four DNA barcodes (*rbcL, matK, trnH-psbA*, and ITS2; CBOL Plant Working Group 2009; Chase et al. 2007; Hollingsworth et al. 2011) were generated directly from genomic DNA for a worldwide sample of *Crataegus* (Dickinson et al. http://2011.botanyconference.org/engine/search/720.html; Zarrei et al. unpubl. data). The plastid origin of the markers was used to establish the maternal parentage of the hybrids. DNA was extracted and amplified from leaf tissue of individuals representing the two hybrids and their parent species (Table 2) using Canadian Centre for DNA Barcoding (CCDB) protocols (Ivanova et al. 2011; Kuzmina and Ivanova 2011a, b). This sample overlapped partially with the cloned ITS2 one (below), and provided an additional two *C. suksdorfii*, 10 *C. monogyna*, and five *C. punctata* individuals, as well as one more of each of the two hybrids (Table 2).

We also analyzed data from another project (Zarrei et al. http://2012.botanyconference.org/engine/search/index.php?func=detail&aid=536 and unpubl. data) in which ITS2 was cloned for a sample of individuals that included 14 *C. suksdorfii*, four *C. monogyna*, three *C. punctata* and two each of the two hybrids (Table 3). Meth-

Table 2. Results of Neighbor-joining clustering of sequence data for chloroplast DNA barcode loci. GenBank accession numbers indicate cluster affiliation (Cluster 1 or 2) for *Crataegus* species and their putative hybrids. Details of the BOLD data can be found at dx.doi.org/10.5883/DS-CRATMONO. See Table 1 for sites and collectors; eight-digit ROM Green Plant Herbarium (TRT) accession numbers identify vouchers.

	Cluster 1 – sections Coccineae		Cluster 2 continue Containing	
	and <i>Douglasia</i>		Cluster 2 – section Crataegus	
Taxon /site /BOLD /tree / TRT	rbcL-a	trnH-psbA	rbcL-a	trnH-psbA
Crataegus punctata				
NTON23 TRT103 MP71 TRT00002237	KC251377	KC251652		
ON31 TRT096 MP61 TRT00002228	KC251375	KC251650		
ON31 TRT105 MP56 TRT00002223	KC251372	KC251647		
ON40 TRT101 MP35 TRT00002203	KC251374	KC251649		
ON45 TRT104 MP81 TRT000047	KC251378	KC251653		
ON46 TRT210 2008-72A TRT00000908	KC251373	KC251648		
Crataegus ×ninae-celottiae				
NTON23 TRT106 MP24 TRT00002199			KC251376	KC251651
NTON23 TRT203 MP73 TRT00002239	KC251350	KC251624		
ON45 TRT201 MP85 TRT00002250			KC251348	KC251622
ON45 TRT202 MP86 TRT00002251			KC251351	KC251625
ON45 TRT204 MP84 TRT00002249			KC251349	KC251623
Crataegus monogyna				
BC16 TRT209 2008-26 TRT00002452			KC251343	KC251617
CA11 TRT274 JC001 TRT00020101			KC251338	KC251612
CRRR01 TRT275 JC003 TRT00020102			KC251341	KC251615
ON31 TRT109 2003-79 TRT00000395			KC251340	KC251614
ON45 TRT108 MP82			KC251342	KC251616
ON45 TRT190 MP83 TRT00002248			KC251339	KC251613
ON45 TRT211 MP98 TRT00029476			KC251336	KC251610
OR1 TRT005 EL80 TRT00000413			KC251347	KC251621
OR1 TRT006 EL83 TRT00000415			KC251346	KC251620
OR1 TRT007 EL74 TRT00000416			KC251344	KC251618
OR TRT030 RML C-2003-25			KC251227	VC251611
TRT00000420			KC2)1337	KC2)1011
OR11 TRT143 EL108 TRT00000417			KC251345	KC251619
Crataegus ×cogswellii			1	
OR1 TRT206 EL71 TRT00002650		KC251627		
OR1 TRT207 EL85 TRT00002654		KC251626		
OR1 TRT208 EL79 TRT00002657	KC251352			
Crataegus suksdorfii				
CAR5 TRT129 2006-19 TRT00001569	KC251419	KC251692		
CAR5 TRT133 2006-22 TRT00001563	KC251418	KC251691		
CAR5 TRT140 2006-16 TRT00001567	KC251417	KC251690		
CAR5 TRT141 2006-18 TRT00001568	KC251416	KC251689		
OR1 TRT205 EL68 TRT00001724	KC251424	KC251699		
WA TRT146 Z18485 TRT00001805	KC251415	KC251688		

Table 3. Voucher specimens for cloned ITS2 data, listing site number (Table 1), collection number, ROM Green Plant Herbarium (TRT) accession numbers, and the GenBank accession numbers for individual clones.

Taxa	Voucher	GenBank accession number
	OR18 Coughlan, Zarrei, and Shaw JC039	KC173887, KC173888, KC173889, KC173890,
	(TRT00020137)	KC173891, KC173892, KC173893
	OR22 Coughlan, Zarrei, and Shaw JC60	KC173587, KC173588, KC173589, KC173590,
	(TRT00020146)	KC173591, KC173592
	OR35 Coughlan, Zarrei, and Shaw JC092	KC173957, KC173958, KC173959, KC173960,
	(TRT00020153)	KC173961, KC173962, KC173963, KC173964
		KC173595, KC173596, KC173597, KC173598,
	OR3/ Coughlan, Zarrei, and Shaw JC98	KC173599, KC173600, KC173601, KC173602,
	(1R100020159)	KC173603, KC173604
	OR37 Coughlan, Zarrei, and Shaw JC102	KC174113, KC174114, KC174115, KC174116,
	(TRT00020163)	KC174117
	OR11 Coughlan, Zarrei, and Shaw JC117 (TRT00020172)	KC174118, KC174119
	OR11 Coughlan, Zarrei, and Shaw JC118	KC174178, KC174179, KC174180, KC174181,
$C \rightarrow L + C$	(TRT00020232)	KC174182, KC174183
C. suksaorfu	OR11 Coughlan, Zarrei, and Shaw JC119	KC174144, KC174145, KC174146, KC174147,
	(TRT00020234)	KC174148, KC174149, KC174150
	OR38 Coughlan, Zarrei, and Shaw JC136	KC173605, KC173606, KC173607, KC173608,
	(TRT00020242)	KC173609
	CAR5 Dickinson and Lo 2006-16	KC173531, KC173532, KC173533, KC173534,
	(TRT00001567)	KC173535, KC173536, KC173537, KC173538
	CARS I. and Diskinger 2006 22	KC173522, KC173523, KC173524, KC173525,
	(TPT000015(2)	KC173526, KC173527, KC173528, KC173529,
	(1K100001909)	KC173530
	OP1 Lo Dishinson and Namura EL 68	KC173577, KC173578, KC173579, KC173580,
	(TRT00001724)	KC173581, KC173582, KC173583, KC173584,
	(11(100001/24)	KC173585, KC173586
	WA Zika 18485 (=18430, 18417;	KC173513, KC173514, KC173515, KC173516,
		KC173517, KC173518, KC173519, KC173520,
		KC173521
	OR1 Lo, Dickinson, and Nguyen EL-71	KC173663, KC173664, KC173665, KC173666,
	(TRT00002650)	KC173667, KC173668
	OR1 Lo, Dickinson, and Nguyen EL-79	KC173682, KC173683, KC173684, KC173685,
C × cooswellii	(TRT00002657)	KC173686, KC173687
0. 1003.000		KC173669, KC173670, KC173671, KC173672,
	OR1 Lo, Dickinson, and Nguyen EL-85	KC173673, KC173674, KC173675, KC173676,
	(TRT00002654)	KC173677, KC173678, KC173679, KC173680,
		KC173681
	OR1 Lo, Dickinson, and Nguyen EL-74	KC173650, KC173651, KC173652, KC173653,
	(TRT00000416)	KC173654
C monogyna	BC16 Dickinson, Lee, and Talent 2008-26	KC173655, KC173656, KC173657, KC173658,
C. monogynu	(TRT00002452)	KC173659, KC173660, KC173661, KC173662
	ON45 Purich MP98 (TRT00029476)	KC173643, KC173644, KC173645, KC173646,
		KC173647, KC173648, KC173649
C ×ninae-celottiae	ON45 Purich and Talent MP84	KC174184, KC174185, KC174186, KC174187,
	(TRT00002249)	KC174188, KC174189

Taxa	Voucher	GenBank accession number
	ON45 Purich and Talent MP85	KC174190, KC174191, KC174192, KC174193,
	(TRT00002250)	KC174194, KC174195
	ON45 Purich and Talent MP86	KC173688, KC173689, KC173690, KC173691,
	(TRT00002251)	KC173692, KC173693
	ON21 Dickinson and Nguyen BB4 (TRT)	KC174266, KC174267, KC174268, KC174269,
		KC174270, KC174271
C. punctata	ON31 Purich s.n (TRT)	KC174272, KC174273, KC174274, KC174275
	NTON23 Purich, Talent, Nguyen, and Lo	KC173694, KC173695, KC173696, KC173697,
	MP73 (TRT00002239)	KC173698, KC173699, KC173700, KC173701

ods for extracting total genomic DNA, marker amplification, cloning, DNA sequencing, and collapsing original sequences to unique sequences (ribotypes) are described elsewhere (Zarrei et al. http://2012.botanyconference.org/engine/search/index. php?func=detail&aid=536 and unpubl. data). Here we report on analyses of a total of 160 ribotypes (Table 3). A recombination test was performed using RDP4 Beta 4.14 (Martin et al. 2010). The Neighbor-Net analysis (Bryant and Moulton 2004) was undertaken using SplitsTree v.4.12.3 (Huson and Bryant 2006) to visualize incompatible splits in the network from uncorrected p-distances calculated with MEGA5 (Tamura et al. 2011). Bootstrap support (BS) was estimated using 1,000 bootstrap pseudoreplicates (Felsenstein 1985) implemented in SplitsTree.

Flow cytometry. Flow-cytometric methods for quantifying nuclear DNA in embryo and endosperm followed Talent and Dickinson (2007a). Embryo DNA amounts of 1.48–1.70 pg were taken to indicate diploids, and an endosperm to embryo ratio of approximately 1.5 was taken to indicate sexual reproduction with meiosis.

Results and discussion

Morphology. Despite differences in sample size, the Pacific Northwest hybrid, *Crataegus ×cogswellii*, appears more variable than either of its putative parents, *C. monogyna* or *C. suksdorfii* (Fig. 1). The hybrid is clearly intermediate with respect to both leaf lobing (the inverse Dissection Index; Fig. 1) and style number (STYLE; Fig. 1). Principal components analyses of leaf outlines from Pacific Northwest *C. monogyna*, *C. suksdorfii*, and their putative hybrid, demonstrate variation in leaf shape both within and between these three entities (Fig. 2A, B). The first principal component reflects the contrast between the unlobed leaves of *C. suksdorfii* and the markedly lobed ones of *C. monogyna*, as well as the intermediacy of the hybrid (Fig. 2A, B), much as illustrated earlier by Love and Feigen (1978; their Fig. 3), and by Wells and Phipps (1989) for the Ontario hybrid and its parents (their Fig. 4). The second principal component reflects variation in the relative overall lengths and widths of the leaf outlines (Fig. 2A).

DNA barcode loci. Analyses of both the directly sequenced and the cloned ITS2 ribotypes demonstrate the parentage of both putative hybrids (Fig. 3; Table



Figure 1. Principal components analysis biplot for five morphometric descriptors averaged for each of 41 *Crataegus* herbarium specimens from the Cogswell-Foster Preserve and other locations in the Pacific Northwest (*C. suksdorfii* (s), *C. monogyna* (m), and the putative hybrid, *C. ×cogswellii* (h)): *relX*, leaf length above the widest point, scaled by the width; *relZ*, leaf length below the widest point, scaled by the width; *relZ*, leaf length below the widest point, scaled by the width; *invDI*, inverse dissection index = $2(A\pi)^{1/2}/P$, where *A* is the leaf area and *P* is the leaf perimeter; *STAM*, number of stamens per flower; *STYL*, number of styles per flower. Both axes shown account for significant portions of the total variance according to the broken-stick criterion (Frontier 1976).

3); no signs of recombination were detected in the cloned ITS2 dataset. ITS2 sequences from the hybrids resemble either *C. monogyna* or one of the native North American species. The way in which both parental ribotypes are maintained in each of the hybrids examined here is probably due to how recently the hybrids have been formed: less than 200 years ago in the case of the Ontario hybrids (Douglas 1914; Kirk 1819; Provancher 1863), and less than 100 years ago in the case of the Oregon ones (the earliest specimen of *C. monogyna* was collected in 1914 in Douglas Co. Oregon; Phipps 1998). These time periods are evidently too short for genome homogenization (concerted evolution) to have taken place, even in diploids reproducing sexually. Our small sample of seed from the hybrids (Table 4) parallels earlier



Figure 2. A Principal components analysis of 39 Fourier amplitudes for 86 subterminal short shoot leaves from 20 *Crataegus* individuals at the Cogswell-Foster Preserve in Linn Co., Oregon (one *C. suks-dorfii* (s), seven *C. monogyna* (m), and 12 putative hybrids (h), *C. ×cogswellii*). Leaf outlines illustrate the shape contrasts responsible for the ordination: in grey, six subterminal leaves from short shoots of a single individual (OR1–8) **B** Principal components analysis of 39 Fourier amplitudes averaged for leaves sampled regardless of position on short shoots of 64 herbarium specimens from the Cogswell-Foster Preserve and (circled points) other locations in the Pacific Northwest (Table 1). In both A and B the two PCA axes shown are significant according to the broken-stick criterion (Frontier 1976). In **B** arrowed point 1 represents the single individual of *C. suksdorfii* for which individual leaves are represented in **A**, while arrowed point 2 represents the averaged data for the six leaves of *C. monogyna* shown in grey in **A**.

results (Talent and Dickinson 2007a) showing diploidy and sexual reproduction in both parental taxa.

Only two of the three chloroplast genome barcode loci showed sufficient variation for individuals from *Crataegus* section *Crataegus* to be distinguished from ones belonging to either *C*. section *Coccineae* or *C*. section *Douglasia* (Table 2). Sequence data from both *rbcL-a* and the *trnH-psbA* spacer region showed the same two clusters, *C*. sections *Coccineae* and *Douglasia* (Cluster 1), and *C*. sect. *Crataegus* (Cluster 2; Table 2). The way in which the hybrids fell into one of these clusters or the other demonstrates that, with one exception, *C. monogyna* is the female parent of the Ontario hybrids with *C. punctata* studied here, while *C. suksdorfii* is the female parent of the Pacific Northwest hybrids.

These results corroborate earlier observations based on seed-set in artificial crosses between the parent species (Love and Feigen 1978; Wells and Phipps 1989). In reciprocal pollinations seed set was greatest (32–34%) when *C. monogyna* stigmas received pollen from *C. punctata* (Wells and Phipps 1989). Fruit set was most successful when *C. monogyna* pollen was applied to the stigmas of *C. suksdorfii* flowers (mean 42%, range 25–73%, compared to a 29% mean fruit set by *C. suksdorfii* with open pollination; Love and Feigen 1978). However, all reciprocal crosses between *C. monogyna*, *C. suksdorfii*, and their hybrid yielded seeds (R. M. Love, personal communication).



Figure 3. A Neighbor-joining tree calculated by BOLD for ITS2 DNA barcode sequences amplified directly from genomic DNA (labels include corresponding collector and GenBank number; see dx.doi. org/10.5883/DS-CRATMONO and Table 1 for details). Dashed lines indicate the sectional affinity of the sequences **B** The corresponding Neighbor-Net network for the cloned ITS2 sequences has three branches representing: (a) ribotypes from individuals of *C. monogyna*, and from its hybrids with both *C. suksdorfii* and *C. punctata*; (b) ribotypes from individuals of *C. suksdorfii* and *C. xcogswellii*; and (c) ribotypes from individuals of *C. suksdorfii* and *C. punctata* and *C × ninae-celottiae* (Table 3). The numbers shown are the % bootstrap support for each of the three branches.



Figure 4. Geographic distribution of *Crataegus ×ninae-celottiae* K.I. Chr. & T.A. Dickinson nothosp. nov. and *C. monogyna* in Ontario. Filled square, holotype of *Crataegus ×ninae-celottiae*; Crosses, TRT specimens of *C. ×ninae-celottiae*; asterisks, *C. ×ninae-celottiae* specimens cited by Wells and Phipps (1989); stars, specimens of *C. monogyna* in MT, MTMG, QFA, TRT, and UBC. *Crataegus punctata* occurs throughout the region depicted (Phipps and Muniyamma 1980; this paper also maps additional records for *C. monogyna*).

Table 4. Flow-cytometric results from seeds of the two described *Crataegus* nothospecies. The ratios shown for endosperm and embryo nuclear DNA contents are well within the ranges observed for sexually reproducing *C. monogyna* (Talent unpubl. data) and diploid *C. suksdorfii* (Lo et al. 2013).

Taxon/TRT accession/site/collection	Total number seeds	Mean embryo DNA	Mean endosperm:embryo ratio (number of seeds)
Crataegus ×ninae-celottiae			
ON45 2002-13 (TRT00000406)	2	1.58 pg	1.56 (2)
ON31 EH52 (TRT00002256)	1	1.67 pg	1.53 (1)
Crataegus ×cogswellii			
OR1 EL-79 (TRT00002657)	3	2.08 pg	1.58 (1)
OR1 2009-36 (TRT00002568)	1	1.87 pg	1.60 (1)

Our use of data from DNA barcoding is not a test of the value of DNA barcoding in *Crataegus*, as this is discussed elsewhere (Dickinson et al. http://2011.botanyconference.org/engine/search/720.html; Zarrei et al. unpubl. data). Rather, we have taken advantage of our barcode sequence data from individuals unequivocally identifiable as *C. monogyna*, *C. punctata*, *C. suksdorfii* and their hybrids in order to use sequence similarity to inform us about the hybridization process.

Hybridization. Since its introduction to North America during the late 18th and the 19th centuries (Kirk 1819; Provancher 1863; Douglas 1914), first on the east coast and



Figure 5. Geographic distribution of *Crataegus ×cogswellii* K.I. Chr. & T.A. Dickinson nothosp. nov. and its parental species in the Pacific Northwest. Filled square, holotype of *Crataegus ×cogswellii*; crosses, TRT specimens of *C. ×cogswellii*; circles, diploid *C. suksdorfii*; stars, *C. monogyna* (specimens in OSC, TRT, UBC, and WTU).

then on the west, *C. monogyna* has become widely naturalized in the U.S.A. (EDDMapS 2013) and Canada (Phipps and Muniyamma 1980; Phipps 1998; Lin 2009). Nevertheless, except for isolated occurrences in northern Delaware and adjacent Pennsylvania, as well as in Kentucky, Utah, and the San Francisco Bay area in California, *C. monogyna* in North America is not found south of 40°N latitude. In Ontario, *C. punctata* appears to be the only native diploid with a similarly late flowering period that is also frequently sympatric with *C. monogyna* (Fig. 1 in Campbell et al. 1991; Fig. 4). *Crataegus suksdorfii* is the only native hawthorn in the Pacific Northwest known to include diploid individuals, and these are restricted to Oregon and adjacent California and Washington, west of the Cascades (Fig. 5; Lo et al. 2013). Where they co-occur, diploid *C. suksdorfii* and *C. monogyna* flower at the same time, the latter species much more abundantly than the former (Love and Feigen 1978).

Crataegus monogyna may never have been commonly planted in boundary hedges in Canada as it was in Europe. Fences and hedges appear to have been only rarely constructed in 17th Century Canada by European settlers to confine ruminant animals (Greer 2012); the animals were instead fed indoors, but allowed to roam the arable land for a short season after harvest, confined by the wall of surrounding forest. To this day, the hawthorn commonly growing along Ontario fence lines consists of native species, perhaps naturally occurring there. In Ontario forests we often encounter remnants of zig-zag post-and-rail fences, and these had the advantage over a hedge that they could be rapidly constructed as needed to mark property boundaries or to keep animals out of particular areas. In the United States hedging had its advocates in the early nineteenth century, but one of these described the superiority of native species like *C. crus-galli* ("cockspur" or "Newcastle thorn") and *C. marshallii* ("parsley-leaved" or "Virginia thorn") over introduced *C. monogyna* (Kirk 1819; "to sow or plant without fencing, would (in this country) be a useless labour").

Flow cytometry of seeds from both hybrids was consistent with diploid embryos and triploid endosperm, except that the embryos from *C. ×cogswellii* show slightly higher than diploid measurements, higher than the 1.39–1.66 pg measurements previously obtained from leaf data (Table 4; Talent and Dickinson 2005). Whether the seeds involved would have germinated is unknown, but in contrast to the large healthy looking seeds from *C. ×ninae-celottiae*, those from *C. ×cogswellii* had smaller embryos and were variously misshapen. We noted that some individual trees of *C. ×cogswellii* have a high degree of parthenocarpy—completely seedless fruit and the seeds we collected may therefore have been supernumerary to any strongly viable seeds. We can only state that *C. ×cogswellii* apparently carries out both meiosis and fertilization, as expected of other diploid *Crataegus* (Table 4; Talent and Dickinson 2007b).

In her examination of hybridization between *C. punctata* and *C. monogyna* in Ontario, Purich (2005) found that the styles of *C. punctata* are significantly longer than those of *C. monogyna* (mean_{mono} = 4.1 mm; mean_{punc} = 7.3 mm; sample sizes 5/52 and 7/116, individuals/styles). Differences between the two species in pollen grain diameter, hence volume, are not significant (Purich 2005). No such difference in style length is present when comparing *C. monogyna* and *C. suksdorfii*. These results suggest that in Ontario, at least, the longer styles of *C. punctata* could act as a barrier to the successful penetration of *C. punctata* ovules by pollen tubes from *C. monogyna* and *C. suksdorfii* similar (Dickinson unpublished data), it may be that the more abundant flower production of *C. monogyna* (Love and Feigen 1978) contributes to its role as the predominant pollen parent of *C. xcogswellii*. The exception to the summary above (TRT203 in Table 2; *C. punctata* as the maternal parent) reflects the way in which differences in style length likely act to influence the direction of hybridization in a probabilistic rather than an absolute way.

Taxonomy

Crataegus nothosect. Coccitaegus K.I. Chr. & T.A. Dickinson nothosect. nov. (Crataegus sect. Coccineae × sect. Crataegus)

Crataegus nothoser. *Punctaegus* K.I. Chr. & T.A. Dickinson **nothoser. nov.** (*Crataegus* ser. *Crataegus* × ser. *Punctatae*)

Crataegus ×ninae-celottiae K.I. Chr. & T.A. Dickinson **nothosp. nov.** (Fig. 6). – Type: CANADA, Ontario, Peel R M, Don Gould Park and E side of Erin Mills Parkway (ON22), 43°31.960'N, 79°39.591'W, woodlot and fields with extensive hawthorn colonization, 2 Jun 1989, Dickinson D1492 (holotype TRT00002197!; isotype S!) (♀*Crataegus monogyna* × ♂*C. punctata*)

Ramunculi pubescenti vel glabri. Folia distalia ramorum fertilium non profunde quinqueundecim-partita, 30–55 mm longa, 16–38 mm lata, nervi supra profunde impressi; stipulae caducae, 3–4 mm longae, plusminusve denticulatae. Inflorescentiae 5–17-florae, laxae, pubescentae; bracteae caducae, plusminusve denticulatae. Sepala integra, rarius sparsim glandulosa, post anthesin reflexa. Fructus 9–12 mm longus, 12–14 mm latus, ruber vel aurantiacus; pulpa lutea, mitis et succida; pyrenae 2–3, ventraliter sulcatae vel foveatae.

Remarks. Shrub or tree up to ca. 6 m tall. Twigs of the current year densely to sparsely hairy or glabrous, hairs appressed to patent, straight or slightly curly; twigs of the previous year pale grey or ash-grey; aphyllous thorns 0.5-2 cm long, stout, straight; spinetipped, leaf- and dwarf-shoot-bearing branchlets lacking. Leaf blades ovate, obovate or elliptical, acute at apex, attenuate, cuneate or rounded at base, shallowly or deeply and regularly lobed, lobes with an acute apex, basal pair of veins convergent, straight or slightly divergent, intercalary veins running to the sinuses partly present, upper surface with ± deeply impressed veins at maturity, dull or lustrous bright or dark green, sparsely hairy and often becoming glabrous except along the veins, hairs appressed or semi-patent; lower surface dull, pale green, sparsely hairy throughout or only along the major veins and in the vein axils, hairs appressed or semi-patent; margin regularly crenate-serrate or serrate, teeth minutely glandular, glands less than 0.1 mm; petiole eglandular, narrowly winged in upper part. Subterminal leaf blade of flowering shoots 30–55 mm long, 16–38 mm wide, shallowly and regularly lobed, lobes 2–5 pairs, basal pair extending 0.2-0.4 times the width of lamina to midrib, each lobe with 6-11teeth, basal pair of sinuses in apical 1/4 to basal 1/3 of lamina; petiole 6–20 mm long; stipules caducous, membranous or herbaceous, 4-8 mm long, irregularly or regularly glandular-denticulate, with 20-30 teeth. Leaf blades of elongate shoots 35-45 mm long, 25-35 mm wide, shallowly or deeply and regularly lobed, lobes 3-5 pairs, basal pair extending 0.2–0.6 times the width of lamina to midrib, each lobe with 4–11 teeth, basal pair of sinuses in basal 1/2-1/3 of lamina; petiole 8-12 mm; stipules caducous, herbaceous, ca. 6 mm long, regularly glandular denticulate-serrate, with ca. 15 teeth.



Figure 6. Holotype of *Crataegus ×ninae-celottiae* K.I. Chr. & T.A. Dickinson nothosp. nov. (\bigcirc *Crataegus monogyna* × \bigcirc *C. punctata*): TRT00002197, CANADA, Ontario, Peel R M, loc. ON22, Don Gould Park and E side of Erin Mills Parkway, 43°35'N, 79°40'W, abandoned fields with extensive hawthorn colonization, 2 Jun 1989, Dickinson D1492.

Inflorescence 3–4 cm long, lax, corymbose, 5–17-flowered, densely to sparsely hairy, hairs appressed, semi-patent or patent, straight or slightly curly; pedicels 3–18 mm, densely to sparsely hairy, hairs appressed, semi-patent or patent, straight or slightly curly; bracts caducous, membranous or herbaceous, 3–4 mm long, 0.2–0.4 mm wide, linear-lanceolate, 10–15 times as long as wide, irregularly glandular-denticulate, with 5–7 teeth. Hypanthium 3–4 mm long, densely to sparsely hairy, hairs appressed, semi-patent or patent, straight or slightly curly; sepals 2–4 mm long, 1.5–2 mm wide, triangular-lanceolate or triangular, 1–2.7 times as long as wide, entire or rarely irregularly and minutely glandular-serrate, teeth 0–2, apex acute or obtuse; petals 6–7 mm long and wide; stamens 18–20, anthers 1–1.2 mm long, pink or purple; styles 2–3; hypostyle pilose. Fruit 9–12 mm long, 8–12 mm in diameter, 1.0–1.1 times as long as wide, globose, broadly ellipsoidal or obovoid, ± lustrous, red or orange, punctate with small, pale brown lenticels, up to ca. 0.2 mm in diameter, sparsely hairy, crowned by the persistent, reflexed sepals; calyx tube indistinct, ca. 0.5 mm long, 3–4 mm wide; flesh yellowish, hard and mealy; pyrenes 2–3, ventro-laterally smooth; hypostyle pilose.

Phenology. Flowering in May–June. Fruiting in August–September.

Reproductive biology. Sexual. 2n = 2x (2n = 34? Muniyamma and Phipps 1979; Talent and Dickinson 2005); diploid embryos and triploid endosperm.

Distribution. Eastern Canada. Ontario (Fig. 4).

Etymology. *Crataegus* ×*ninae-celottiae* honors Nina Celotti (1971–1995), who studied the pollination pathway of the two parent species, *C. punctata* and *C. monogyna*.

Similar taxa. *Crataegus* ×*ninae-celottiae* differs from *C. monogyna* in: spine-tipped, leaf- and dwarf-shoot-bearing branchlets lacking; leaf blades with \pm deeply impressed veins above; subterminal leaf blade of flowering shoots shallowly lobed, lobes 2–5 pairs (not \pm deeply lobed and lobes 1–3 pairs); stipules caducous, often membranous, irregularly or regularly glandular-denticulate, with 20–30 teeth (not \pm persistent, herbaceous and \pm entire); styles and pyrenes 2–3 (not 1–(2)); fruit often orange, punctate with pale brown lenticels up to ca. 0.2 mm in diameter.

Crataegus ×*ninae-celottiae* differs from *C. punctata* in: aphyllous thorns shorter, 0.5–2 cm long (not 2–5 cm long); leaf blades regularly lobed almost to the base (not unlobed or shallowly lobed towards apex), intercalary veins running to the sinuses sometimes present; subterminal leaf blade of flowering shoots usually smaller, up to ca. 55 mm long, and veins 2–5 pairs (not up to ca. 85 mm and veins 6–10 pairs); stipules often herbaceous and irregularly glandular-denticulate; sepals shorter, 2–4 mm long, and wider, 1–2.7 times as long as wide (not 3–7 mm long and 2–4.7 times as long as wide); styles and pyrenes 2–3 (not 3–5); fruit usually smaller, up to ca. 12 mm long and in diameter (not up to ca. 15 mm long and in diameter) and less distinctly punctate with smaller lenticels up to ca. 0.2 mm in diameter (not up to ca. 0.4 mm in diameter).

Crataegus \times *ninae-celottiae* was studied by Phipps and Muniyamma (1980) and by Wells (Wells and Phipps 1989), who documented the intermediacy of the hybrid relative to its parents in leaf, thorn, flower, and fruit characteristics. In addition, paper chromatography was used to compare phenolic profiles of the three entities, which also demonstrated intermediacy. These results have been corroborated using thin layer chromatography (Harris 2001). Both parents and the hybrid are diploids (x = 17, as in other Maleae; Muniyamma and Phipps 1979; Talent and Dickinson 2005), and both parents are highly pollen fertile (stainability > 80%). Pollen stainability in the hybrid was found to be variable (27–97%, mostly in the range 60–80%; Purich 2005).

Specimens examined, paratypes (in bold, specimens in Tables 2–4). CANADA, Ontario: Peel Co., City of Mississauga, Don Gould Park and E side of Erin Mills Parkway (ON22), 1989-06-02, Dickinson D1480 (TRT00000408!); 1989-06-02, Dickinson D1482 (TRT00000407!); 1989-05-31, Dickinson D1485 (TRT00000409!); 2000-05-19, Talent NT-03 (TRT00002306!); 2011-05-28, Christensen & Dickinson s.n. (TRT00024869!). Middlesex Co., Denfield Twp., SE corner Denfield Side Road and Ilderton Road (ON31), 2001-05-17, Harris & Dickinson EH-52 (TRT00002256!); 2001-05-17, Harris & Dickinson EH-54 (TRT00002257!); 2002-07-30, Talent & Dickinson EH52 (TRT00000405!). Durham R.M., Bowmanville, floodplain of Bowmanville Creek (ON45), 2002-09-30, Dickinson & Nguyen 2002-13 (TRT0000406!), 2004-06-03, Purich 85 (TRT00002250!), 2004-06-03, Purich 86 (TRT00002251!).

Crataegus nothosect. Crataeglasia K.I. Chr. & T.A. Dickinson nothosect. nov. (Crataegus sect. Crataegus × sect. Douglasia)

Crataegus nothoser. Crataeglasianae K.I. Chr. & T.A. Dickinson **nothoser. nov.** (Crataegus ser. Crataegus × ser. Douglasianae)

Crataegus ×cogswellii K.I. Chr. & T.A. Dickinson nothosp. nov. (Fig. 7.). – Type: U.S.A., Oregon, Linn Co., Cogswell-Foster Preserve, 44°19.985'N, 123°7.353'W, 3 Sep 2009, Dickinson & Dickinson 2009-40 (holotype TRT00002574!; isotype TRT). (♀ Crataegus suksdorfii × ♂ C. monogyna)

Ramunculi glabri vel rarius sparsim villoso-lanati. Folia distalia ramorum fertilium quinque-novem-partita, rarius integra, 25–70 mm longa, 15–50 mm lata; stipulae caducae, 4–8 mm longae, plusminusve denticulatae. Inflorescentiae 4–25-florae, laxae, glabrae vel rarius villoso-lanatae; bracteae caducae, plusminusve denticulatae. Sepala integra vel rarius sparsim glandulosa, post anthesin reflexa. Fructus 9–12 mm longus, 12–14 mm latus, lampro-atro-purpureus vel anthracinus; pulpa lutea, mitis et succida; pyrenae 2–5, ventraliter sulcatae vel foveatae.

Remarks. Shrub or tree up to ca. 12 m tall. Twigs of the current year glabrous, rarely sparsely villous-lanate; twigs of the previous year dark reddish-brown or pale- or dark-grey; aphyllous thorns 0.5–2 cm long, stout, straight or slightly recurved; spine-tipped, leaf- and dwarf-shoot-bearing branchlets lacking, rarely present. Leaf blades broadly or narrowly obovate, ovate, rhombic-ovate or elliptical, acute at apex, attenuate, cuneate or rounded at base, deeply or shallowly and regularly lobed, rarely some leaves unlobed, lobes with an acute or obtuse apex, basal pair of veins divergent or straight, intercalary

veins running to the sinuses usually present; upper surface dull, dark green, sparsely hairy especially along the veins, hairs appressed or semi-patent; lower surface dull, pale green, villous in the vein axils and occasionally along the major veins; margin regularly and \pm coarsely or finely crenate-serrate or serrate, teeth eglandular or minutely glandular, glands less than 0.1 mm; petiole eglandular or rarely sparsely glandular, narrowly winged in upper part. Subterminal leaf blade of flowering shoots 25-70 mm long, 15-50 mm wide, deeply or shallowly and regularly lobed, rarely unlobed, lobes (0-)2-4 pairs, basal pair extending 0.2-0.8 times the width of lamina to midrib, each lobe with 5-18 teeth, basal pair of sinuses in apical 1/3 to basal 1/3 of lamina; petiole 5-15 mm long; stipules persistent or caducous, herbaceous, 5-12 mm long, irregularly or regularly glandular denticulate-serrate or serrate, with 4-30 teeth. Leaf blades of elongate shoots 40-90 mm long, 30-50 mm wide, deeply or shallowly and regularly lobed, lobes 1-4 pairs, basal pair extending 0.4-0.9 times the width of lamina to midrib, each lobe with 7-20 teeth, basal pair of sinuses in basal 1/2–1/5 of lamina; petiole 10–20 mm; stipules persistent or caducous, herbaceous, 6-14 mm long, regularly glandular denticulate-serrate or serrate, with 15-30 teeth. Inflorescence 2.5-5 cm long, lax, corymbose, 4-25-flowered, glabrous, rarely sparsely villous-lanate; pedicels 4-11 mm, glabrous, rarely sparsely villous-lanate; bracts caducous or very rarely persistent, membranous or herbaceous, 3-10 mm long, 0.2-2.5 mm wide, linear-lanceolate, 4–10 times as long as wide, regularly glandular-serrate or ± irregularly glandular-denticulate, with 4–22 teeth. Hypanthium 2–3 mm long, glabrous or rarely sparsely villous-lanate; sepals 1–2.5 mm long, 1.5–2 mm wide, triangular, 0.5–1.7 times as long as wide, entire or very rarely irregularly and minutely glandular-serrate, teeth 0-2, apex acute or obtuse; petals 4-6 mm long and wide; stamens 18-20, occasionally vestigial, anthers 0.6–1 mm long, purple; styles 2–5; hypostyle pilose. Fruit 6–9 mm long, 6-8 mm in diameter, 1-1.2 times as long as wide, globose-subglobose or broadly ellipsoidal, epruinose, ± lustrous, blackish purple or black, glabrous-subglabrous, crowned by the persistent, reflexed sepals; calyx tube indistinct, 0.4-1 mm long, 3.5-4.5 mm wide; flesh yellowish, soft and juicy; pyrenes 2–5, irregularly ventro-laterally pitted; hypostyle pilose.

Phenology. Flowering in April–May. Fruiting in September. Some individuals strongly parthenocarpic.

Reproductive biology. Sexual. $2n = 2x [\approx 34]$ (Talent & Dickinson 2005); diploid embryos and triploid endosperm. Chromosome number: 2n = 2x = 34, estimated from flow cytometry data (Table 4); chromosome counts have not been made.

Distribution. Northwestern U.S.A.; western Oregon (Figure 5); potentially present in adjacent northwestern California and southwestern Washington where the parent species are sympatric.

Etymology. Crataegus ×cogswellii honours the Cogswell family, and Mr. and Mrs. Lee Foster, of Halsey, Oregon. In 1872 John Cogswell, Mrs. Foster's grandfather, purchased the land that the Fosters gave to the Oregon Nature Conservancy as the Cogswell-Foster Preserve (Lopez 1971), and at which *C. ×cogswellii* has been most intensively studied (Love and Feigen 1978).

Similar taxa. Crataegus ×cogswellii differs from C. monogyna in: leaf- and dwarfshoot-bearing branchlets usually lacking; stipules of leaves of flowering shoots irregu-



Figure 7. Holotype of *Crataegus ×cogswellii* K.I. Chr. & T.A. Dickinson nothosp. nov. (*♀Crataegus suksdorfii × ♂C. monogyna*): TRT00002574, U.S.A., Oregon, Linn Co., Cogswell-Foster Preserve, 44.333082°N 123.122547°W, 3 Sep 2009, Dickinson & Dickinson 2009-40.

larly or regularly glandular denticulate-serrate or serrate (not \pm entire); styles and pyrenes 2–5 (not 1–(2)); fruit blackish purple or black (not bright or dark red).

Crataegus ×*cogswellii* differs from *C. suksdorfii* in: twigs of the current year occasionally sparsely villous-lanate; leaf- and dwarf-shoot-bearing branchlets occasionally present; leaf blades usually deeply or shallowly and regularly lobed, intercalary veins running to the sinuses usually present; inflorescence, pedicels and hypanthia occasionally sparsely villous-lanate; hypostyle pilose (not glabrous or sparsely pilose).

Specimens examined, paratypes (in bold, specimens in Tables 2-4). U.S.A., Oregon: Columbia Co., Sauvie Island (OR11), 2003-06-14, Zika 18482 (TRT00002651!); 2005-08-31, Lo & Dickinson 103.2 (TRT00001918!), Lo 105.2 (TRT00001917!); Lane Co. Eugene, 1993-05-07, Love 9304 (TRT00002644!), 2003-05-13, Love C2003-12 (TRT00002646!), C2003-13 (TRT00002647!); 2003-06-01, Zika 19571 (TRT00001890!); Linn Co., Cogswell-Foster Preserve (OR1), 1987-04-7, 1987-04-27, 1987-09-20, Love 8707 (TRT00001895!, TRT00001907!, TRT00001912!), 8714 (TRT00001897!, TRT00001899!, TRT00002643!), 8715 (TRT00001901!, TRT00001902!, TRT00001910!), 8716 (TRT00001894!, TRT00001913!), 8717 (TRT00001900!, TRT00001909!), 8718 (TRT00002645!), 8719 (TRT00001893!, TRT00001905!, TRT00001906!), 8720 (TRT00001904!), 1993-05-18, Barbour, Evans & Love 93064 (TRT00001896!), 1997-07-27, Love 9726 (TRT00002196!); 2004-06-10, Lo, Dickinson & Nguyen 71 (TRT00002650!), 73 (TRT00002660!), 76 (TRT00002658!), 77 (TRT00002659!), 79 (TRT00002657!), 81 (TRT00002655!), 82 (TRT00002656!), 84 (TRT00002653!), 85 (TRT00002654!); 2009-09-03, Dickinson & Dickinson 2009-22 (TRT00002555!), 2009-23 (TRT00002556!), 2009-24 (TRT00002557!), 2009-28 (TRT00002560!), 2009-33 (TRT00002565!), 2009-34 (TRT00002566!), 2009-36 (TRT00002568!), 2009-38 (TRT00002570!), 2009-39 (TRT00002571!), 2009-41 (TRT00002573!), 2009-42 (TRT00002572!), 2009-43 (TRT00002575!); Marion Co., Salem, 2003-05-01, Zika 18296 (TRT00001889!). Washington: Clark Co., 2003-06-01, Zika 18431 (TRT00001891!).

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RESEARCH ARTICLE



Taxonomy of Atlantic Central African orchids 2. A second species of the rare genus Distylodon (Orchidaceae, Angraecinae) collected in Cameroon

Vincent Droissart^{1,2,3,4}, Phillip J. Cribb⁵, Murielle Simo-Droissart³, Tariq Stévart^{2,4,6}

I Institut de Recherche pour le Développement (IRD), Unité Mixte de Recherche AMAP (Botanique et Bioinformatique de l'Architecture des Plantes), Boulevard de la Lironde, TA A-51/PS2, F-34398 Montpellier Cedex 5, France 2 Missouri Botanical Garden, Africa & Madagascar Department, P. O. Box 299, St. Louis, Missouri 63166-0299, U.S.A. 3 Plant Systematic and Ecology Laboratory, Higher Teacher's Training College, University of Yaoundé I, Yaoundé, Cameroon 4 Herbarium et Bibliothèque de Botanique africaine, CP 265, Université Libre de Bruxelles, Boulevard du Triomphe, B-1050, Brussels, Belgium 5 Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK 6 Botanic Garden, Meise, Domein van Bouchout, Nieuwelaan 38, B-1860 Meise, Belgium

Corresponding author: Vincent Droissart (vincent.droissart@ird.fr)

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Abstract

While conducting field inventories in South Cameroon, we collected two specimens of a new species that we considered to belong to the genus *Angraecopsis*. Afterwards, a careful examination of specimens housed at main herbaria, along with the nomenclatural types, allows us to place it in *Distylodon*, a monotypic genus previously known from East Africa. *Distylodon sonkeanum* Droissart, Stévart & P.J.Cribb, **sp. nov**. was collected in the lowland coastal forest of Atlantic Central Africa. It is known from a single locality in the surroundings of the Campo-Ma'an National Park. The species differs from *D. comptum*, by its several-flowered inflorescences, longer leaves and spur, and shorter pedicel and ovary. The species appears to be rare and is assessed as Critically Endangered [CR B2ab(iii)] according to IUCN Red List Categories and Criteria. New field investigations are required to attempt to find it in the low-elevation parts of the Campo-Ma'an National Park in Cameroon.

Keywords

Angraecoid, Cameroon, Campo-Ma'an National Park, IUCN Red List Categories and Criteria, new species

Introduction

During fieldwork conducted in April 2007 by the first and the third authors (Droissart 2009) in the surroundings of the Campo Ma'an National Park, near the village of Bifa (South Region, Cameroon), two living specimens of an unknown orchid were collected without flowers and were thus cultivated in an orchid shadehouse at Yaoundé. These specimens flowered in July 2007 and were initially placed in *Angraecopsis* through the trilobate shape of the lip. Then, a detailed examination and comparison with material at the Orchid Herbarium of the Royal Botanic Gardens, Kew, and with literature (e.g. Summerhayes 1933, 1951, Szlachetko and Olszewski 2001), allowed us to identify these specimens as belonging to a new species of the monotypic genus *Distylodon* Summerh.

Complementary surveys in the same area and examination of closely related material preserved at BR, BRLU, K, P, WAG and YA (herbaria acronyms according to Thiers continuously updated) did not reveal any additional specimens of this novelty. Seven years after our discovery in Cameroon, we describe the new species collected from a single locality, resulting in the present paper. This paper represents the second in a series of publications based on recent intensive field work (Stévart 2003, Droissart 2009) and focusing on collections-based taxonomic revisions of Orchidaceae in Atlantic Central Africa.

Materials and methods

Collections of BR, BRLU, K, P, WAG and YA were examined. Measurements, colors and other details given in the description are based on living material, alcohol-preserved specimens, and data derived from field notes. Morphological investigation used a Nikon SMZ645 stereomicroscope. The conservation status of the species was assessed by applying the IUCN Red List Categories and Criteria (IUCN 2001, 2008).

Taxonomic treatment

Distylodon sonkeanum Droissart, Stévart & P.J.Cribb, sp. nov.

urn:lsid:ipni.org:names:77137691-1 http://species-id.net/wiki/Distylodon_sonkeanum Figs 1, 2

Diagnosis. *Distylodon sonkeanum* Droissart, Stévart & P.J.Cribb, is close to *Distylodon comptum* Summerh. but differs from it in having several-flowered inflorescences, longer leaves and spur, and shorter pedicel and ovary.

Type. Cameroon. Bifa (piste sur la route Kribi-Ebolowa), à 5 km au SE de Zingui, le long d'une piste de chasseurs entre les rivières Nyété et Nyamefoo, 02°41.308'N,



Figure 1. Illustration of *Distylodon sonkeanum (Droissart, Stévart & Simo M. 585*): A *habitus* B flower, diagonal view C flower, side view D dorsal sepal E lateral sepal F petal G lip H column with pollinium, sideview I column with anthercap, frontal J pollinium with stipe K stipe.



Figure 2. Photographs of living specimen of *Distylodon sonkeanum*: **A** front view of the flower **B** side view of the flower **C** inflorescence.

010°16.406'E, 5 July 2007, Droissart, Stévart & Simo M. 585 (holotype: BRLU!; iso-type: YA!).

Description. Dwarf epiphytic herb. Stem short, up to 8 mm long, 2-2.5 mm in diameter, leafy, unbranched. Roots more than 120 mm long, 1-1.8 mm in diameter, numerous, distributed at the base of the plant, unbranched. Leaves few (up to 5), 25- $37 \times 6-11$ mm, obliquely narrowly elliptic, slightly coriaceous, with entire margins, with an indistinct and irregular reticulate venation; leaf apex obliquely subacute, unequally bilobed, with distance between the two lobes less than 1 mm. Inflorescences up to 41 mm long, 2- to 5-flowered, almost horizontal to pendant, unbranched, emerging at the base of the stem, with peduncle 13 mm long; floral internode about 6-7 mm, one flower per node; rachis terete. Bracts up to 1.2 mm long, tubular. Flowers 17-25 \times 6–8 mm, not opening widely, green, the larger one situated at the apex of the inflorescence. Ovary and pedicel not twisted, 5-7 mm long. Dorsal sepal 4-7 × 1.6-1.9 mm, linear lanceolate, acute, slightly to markedly reflexed, with entire margins. Lateral sepals 5.5-9 × 1.8-2.0 mm, obliquely linear-lanceolate, acute, slightly to markedly reflexed, with entire margins. Petals $3.0-4.8 \times 1.1-1.3$ mm, linear lanceolate, acuminate, slightly curved forward, with entire margins. Lip $4.5-6.0 \times 2.0-3.5$ mm, slightly to markedly trilobed in the basal half; side lobe obliquely triangular, subacute to acute, 0.8–1.8 mm long; mid lobe much longer, 3–4 mm long, linear-subulate, acuminate, fleshy, somewhat curved forward; spur 13.5–18.0 mm long, 0.8–1.1 mm in diameter,



Figure 3. Distribution of *Distylodon sonkeanum* and *D. comptum* in tropical Africa.

cylindrical, straight, slightly inflated in the apical part in larger flowers, apex rounded. Column $1-1.2 \times 1.0$ mm, almost cylindrical with truncate apex. Rostellum 0.3 mm long, consists of two erect, subulate teeth or fangs. Anthercap 1.0×1.0 mm, deltoid. Pollinia two, spherical. Viscidia two, with two stipites 0.7-0.8 mm long, independent of each other, flattened, bifurcate.

Distribution and habitat. *Distylodon sonkeanum* is endemic to the coastal part of the Lower Guinea Domain (White 1979) of the Guineo-Congolian Region. It is known from a single locality in South Region of Cameroon (Fig. 3). The only population known so far was found in the lowland evergreen forest at 100 m elevation, growing epiphytically on a fallen branch.

Conservation. IUCN red list category: Critically Endangered, [CR B2ab(iii)]. *Distylodon sonkeanum* is only known from one very restricted subpopulation in Cameroon which represents one 'location'. The main threats known to the species in the southern Cameroon are deforestation for logging and oil palm (*Elaeis guineensis* Jacq.) and rubber (*Hevea brasiliensis* (Willd. Ex A. Juss.) Müll.Arg.) plantations, resulting from a strong international demand. The ongoing loss of the forest leads us to expect a continuous decline of its habitat in the only known locality, and therefore of its extent of occurrence and area of occupancy. Moreover, this location is accessible to local residents and through their practices of shifting agriculture and small-scale timber exploitation; they are gradually transforming this area into secondary forest.

Distylodon sonkeanum appears to be rare but, due to its inconspicuous habit, further fieldwork is required to ascertain its conservation status more objectively. Considering

the general habitat of *D. sonkeanum*, it is to be hoped that more specimens and additional sites will be found in the low-elevation parts of the adjacent Campo Ma'an National Park. Based on our current knowledge of the ecology and the distribution of the species (one location and AOO less than 10 km²), and using the Criteria B of the IUCN Red List Categories and Criteria, the species was assessed as CR B2ab(iii).

Etymology. The species epithet 'sonkeanum' refers to the Professor Bonaventure Sonké of the University of Yaoundé I, who is an internationally recognized taxonomist, specialized in the Rubiaceae's family. He has collected extensively in the Bipindi-Akom II area, and adjacent Ngovayang massif and Campo-Ma'an National Park. He was one of the first to highlight the high biodiversity of this area and he greatly facilitated our work in Cameroon.

Key to the species of Distylodon

1	Inflorescence several-flowered (2- to 5-flowered	ed), leaves 25–37 mm long, spur
	13.5–18.0 mm long, pedicel and ovary 5–7 mr	m long <i>Distylodon sonkeanum</i>
2	Inflorescences one-flowered, leaves 7-17 n	mm long, spur 6.5 mm long,
	pedicel and ovary 9 mm long	Distylodon comptum

Discussion

The genus *Distylodon* was established about 50 years ago by Summerhayes (1966). Before our discovery, it was represented only by *Distylodon comptum* Summerh., known from one specimen collected in Uganda in 1944 (*Eggeling 5381*, holotype K!). As suggested by its etymology, this genus is mainly characterized by the shape of the rostellum which is bilobated, the two lobes standing up parallel to one another forming two narrow almost subulate acute teeth or fangs (Fig. 1 H, I).

Distylodon sonkeanum is easily distinguished from the only taxon described in the genus so far, *D. comptum*. The new species produces pluriflorous inflorescences, longer leaves and spur, and shorter pedicel and ovary. As pointed out by Summerhayes (1966), the genus appears to be closely allied to *Angraecopsis* Kraenzl. Both genera present short stemmed plants with distichous, conduplicate leaves with unequally lobed tips; their inflorescences are short bearing small, spurred and pale yellow to green colored flowers. Nevertheless, the genus *Distylodon* can be distinguished by its sepals with similar shape and size, and the characteristic shape of its rostellum. Further molecular evidences are required to test the monophyly of *Distylodon*. Unfortunately, no material suitable for DNA studies has been collected so far and consequently the phylogenetic placement of the species remains to determine.

The distance between the localities of *Distylodon sonkeanum* and *D. comptum* is more than 2,000 km (Fig. 3). The Albertine Rift is well known for the concentration of many narrow endemics, being a hotspot of plant diversity in East Africa (Brooks et

al. 2004). The territories surrounding the Campo-Ma'an National Park also harbor many orchids that are endemic to Atlantic Central Africa (Droissart 2009), and our discovery stresses the need of further explorations and plant protection in this area. The large gap between the two taxa, covering the Congolian sub-centre of endemism (White 1979), remains largely unsampled and future botanical explorations may reveal that the geographic disjunction between the two species is not as large as we may believe today.

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RESEARCH ARTICLE



Dysaster cajamarcensis, a new shrubby genus and species of Astereae (Asteraceae) from Peru

Harold Robinson¹, Vicki Funk¹

I Department of Botany, MRC 166, NMNH, P.O. Box 37012, Smithsonian, Washington, DC. 20013-7012

Corresponding author: Harold Robinson (robinsoh@si.edu)

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Abstract

Dysaster cajamarcensis is a spreading broad-leaved shrub named as a new genus and species of the tribe Astereae subtribe Hinterhuberinae collected in northern Peru. It has bisexual disc florets, disc style branches with strong stigmatic lines and hairy appendages, compressed achenes in both ray and disc florets, and papyraceous involucral bracts.

Keywords

Asteraceae, Astereae, Diplostephium, Hinterhuberinae, Peru

Introduction

There is something very unsatisfying about a plant, sent for identification, that has no strikingly distinctive feature, but has a combination of characteristics that excludes it from any already known genus. It is particularly unsatisfying when the plant involved is a member of a tribe such as the Astereae in which phyletic studies using DNA (Brouillet et al. 2009) are not yet adequately correlated with morphological and anatomical studies. Nevertheless, such a plant has been collected in northern Peru. The specimen of the broad-leaved shrubby plant arrived with a field identification of *Diplostephium*, the latter a genus of shrubby Astereae that is common in the Central and Northern Andes. The plant is not a *Diplostephium* Kunth, and has characteristics that do not agree with any other genus in the tribe.

Attempts to identify the plant have involved the use of keys in Hoffmann (1890– 1894), Cuatrecasas (1969), Nesom and Robinson (2007), Strother and Brouillet (2006), and lists of genera and species in Braco and Zarucchi (1993), and the genera sequenced in the treatment by Brouillet et al. (2009). Results were as follows.

The treatment by Brouillet et al. (2009) includes no unaccounted for elements among the listed South American Astereae. More importantly, all of the Astereae listed in the Catalogue of the Flowering Plants and Gymnosperms of Peru (Brako and Zarucchi 1993) and in the treatment of the tribe in Colombia (Cuatrecasas 1969) can be excluded. The keys to genera in various treatments are not much more helpful. In Hoffmann (1890–1894) the plant keys into the relationship of *Sommerfeltia* Less., but the latter is a distinctive element from southeastern South America with deeply dissected leaves. In Cuatrecasas (1969) the new entity keys to *Aster* L., a concept that in that work was based on two introduced species now known to be *Symphyotrichum* Nees. The Peruvian plant also keys to the *Symphyotrichum* relationship in Nesom and Robinson (2007), but the involucral bracts are totally non-herbaceous. When keyed among North American genera in Strother and Brouillet (2006), the Peruvian plant comes to *Ampelaster* G.L. Nesom, another member of the *Symphyotrichum* relationship.

One further possibility exists. The involucral bracts have a median dark stripe that might be indicative of the resin duct characteristic of the subtribe Conyzinae. Among the genera of that subtribe, the new entity would key in Nesom and Robinson (2007) to *Darwinothamnus* Harling. The latter is endemic to the Galapagos Islands, and it is a linear-leaved rather ericoid-looking plant with inflorescences not or scarcely exserted. It has chaffier, more recurved involucral bracts and small narrow limbs on the ray florets. The achenes in *Darwinothamnus* are sparsely setuliferous on the faces rather than densely spiculiferous, and the marginal ribs contain enlarged resin ducts. The pappus lacks a well-defined outer series, and bristles have tenuous rather than broadened tips.

A comparison on a broad scale using preliminary DNA sequencing (ITS1 & 2) places the new entity among previously sequenced Astereae that are almost all members of the subtribe Hinterhuberinae. The genera that show closest correlation are *Hinterhubera* Sch. Bip. ex Wedd., *Parastrephia* Nutt., *Guynesomia* Bonifacino & Sancho, the diminutive epappose *Laestadia* Kunth ex Less., and *Diplostephium*. Of these, *Hinterhubera* is an ericoid, mostly narrow-leaved genus of Colombia and Venezuela that has narrow corolla lobes and functionally male disc florets. *Parastrephia* is a genus of cupressiform resinous shrubs with bisexual disc florets and nearly terete achenes from mostly southern Peru, Bolivia and Chile. *Guynesomia* is a plant with sparse linear leaves, bisexual florets and scarcely compressed achenes that is endemic to Chile. There remains *Diplostephium* which is the only genus in the group that has species that are remotely similar in habit to the unknown entity from northern Peru. None of these show DNA correlation closer than 97%.

In spite of all the results from various keys and DNA results, it is the genus *Diplostephium* in which the Peruvian plant was placed by the collectors, and it is that genus with which it is most likely to be confused on brief observation. The new genus and *Diplostephium* differ in five significant characteristics.

- The achenes of the new entity are compressed with only two ribs in both ray and disc florets;
- (2) The disc florets are fully bisexual with style branches having well-developed stigmatic lines;
- (3) Involucral bracts are narrowly lanceolate and sharply pointed with a dark median stripe outside;
- (4) the outer pappus is a strongly differentiated series of squamae; and
- (5) the inflorescence is exserted well beyond the foliate parts of the branches and has few heads on long peduncles.

Diplostephium has more triangular and prismatic achenes, functionally male disc florets lacking stigmatic lines on their style branches, involucral bracts that are more ovate, less pointed, and without an external median stripe, a less strongly differentiated outer pappus series that has shortened bristles of variable lengths, and an inflorescence that is usually dense and mostly sessile, rarely subumbellate.

The Peruvian entity is named here as new at both the generic and species level.

Taxonomic treatment

Dysaster cajamarcensis H. Rob. & V.A. Funk, gen et sp. nov. urn:lsid:ipni.org:names:77138096-1 http://species-id.net/wiki/Dysaster_cajamarcensis

Type. Peru. Dept. Cajamarca: Prov. Contumazá. 14 km S of Contumazá on gravel road, rocky slopes, Western Cordilleran evergreen forest, 2520 m, 17 Jul 1992, *T.F. Stuessy, D.W. Crawford & A. Sagástegui 12686* (holotype US; isotypes OH, HUT).

Shrubs with spreading branches, with scattered upright branchlets ca. 2 dm long; stem surfaces densely white tomentose, internodes mostly 5-10 mm long. Leaves alternate, sometimes with small axillary fascicles. Petioles ca. 5 mm long; blades elliptical, 1.0–2.5 cm long, 0.4–0.8 cm wide, bases cuneate, margins with 5–8 small teeth, apices obtuse to subacute, upper surface dark green with some arachnoid tomentum, bullate with veins insulcate, lower surface densely whitish tomentose with strongly exsulcate veins; venation pinnate, with 5-6 veins on each side, spreading at ca. 45° angles. Inflorescences strongly excerted on tips of foliose branchlets, branching with usually 3 capitula; peduncles slender, 4–7 cm long, thinly whitish tomentose, with few scattered minute bracteoles above base. Capitula radiate, heterogamous, with rays to 3-4 cm wide; involucres campanulate, ca. 0.8 cm high, ca. 1.5 cm wide, bracts ca. 70 in 3-4 series, 2–8 mm long, ca. 0.8 mm wide, linear-lanceolate with slender tips, narrowly scarious and often reddish at margins and tips, mostly papyraceous, pale greenish outside with dark longitudinal median stripe; receptacle epaleaceous. Ray florets 30-35, fertile, female; corollas pink-purple, basal tubes ca. 2.5 mm long, limbs ca. 12 mm long, 2 mm wide, scarcely trilobed at tip, without evident glands or trichomes except



Figure 1. Holotype of *Dysaster cajamarcensis* H. Rob. & V.A. Funk (*Stuessy, Crawford & Sagastequi* 12686, US).



Figure 2. Floral details of *Dysaster cajamarcensis* H. Rob. & V.A. Funk **A** Disc achene **B** Style appendage **C** style branches showing stigmatic lines and appendages.

abaxially at base of limb, style branches with stigmatic lines continuous along margins and apex; disc florets ca. 75; corollas yellow, narrowly funnelform, ca. 7 mm long, basal tube ca. 2 mm long, glabrous, throat ca. 4.7 mm long, with some short, pointed, septate hairs near base, lobes ca. 0.7 mm long, oblong-ovate, with few short, septate hairs at tip; anther thecae ca. 2 mm long, slightly pointed at base with few sterile cells at base; anther appendage ca. 0.2 mm long; style base slightly broadened. Ray and disc achenes alike, ca. 2 mm long, lenticular, compressed with 2 costae along margins, costae not containing enlarged resin ducts, lateral surfaces densely covered with short spicules, setulae numerous near base, few setulae distally; pappus of ca. 17 slender bristles ca. 5 mm long, slightly broadened distally. Outer series of numerous scale-like squamae ca. 0.3 mm long. Pollen in fluid ca. 30 µm in diam.

Distribution. Known only from the type from Cajamarca, Peru.

Ecology. Rocky slopes, Western Cordilleran evergreen forest, elevation 2620 m. **Etymology.** *Dys-* – bad, + *aster* – for the genus.

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RESEARCH ARTICLE



The systematics of the worldwide endoparasite family Apodanthaceae (Cucurbitales), with a key, a map, and color photos of most species

Sidonie Bellot¹, Susanne S. Renner¹

I Systematic Botany and Mycology, University of Munich (LMU), Menzinger Strasse 67, 80638 Munich, Germany

Corresponding author: Sidonie Bellot (sido.bellot@gmail.com)

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Abstract

Using morphological, nuclear, and mitochondrial data, we here revise the taxonomy of Apodanthaceae and allocate the 36 names published in the family to ten biological species in two genera, *Apodanthes* and *Pilostyles*. All species are endo-parasites that live permanently inside trees or shrubs of the families Salicaceae or Fabaceae and that only emerge to flower. Because of this life history, Apodanthaceae are among the least known families of flowering plants. Nevertheless, the World's herbaria as of 2013 hold at least 785 collections that, in combination with DNA phylogenies, permit well-founded species circumscriptions and geographic range maps. We also provide a key to all species, discuss the newly accepted or synonymized names, and make available color photos of six of the ten species.

Keywords

Apodanthaceae, genus circumscriptions, mitochondrial DNA sequences, nuclear DNA sequences, parasitic plants, species circumscriptions

Introduction

Apodanthaceae Tiegh. ex Takht. (Cucurbitales) is a family of endoparasites that live entirely in their host's stems and only become visible once the strictly unisexual flowers have burst through the bark. This life style, added to the small size of the flowers and patchy occurrence of the apparently mostly dioecious populations, has made it difficult to collect good and complete herbarium material (including both sexes and flowering and fruiting specimens). While populations once identified may be recollected at the same time year after year, usually only local botanists will have the opportunity to carry out such recollections. Apodanthaceae are disjunctly distributed in North and South America, mainland Africa, Iran, and Australia. They occur in arid as well as humid tropical environments. Two genera have been validly described, the worldwide *Pilostyles*, and *Apodanthes* from Central and South America.

The taxonomy of the genera and species of Apodanthaceae has not been studied since the work of Ida de Vattimo-Gil (Vattimo-Gil 1950, 1955, 1971, 1973). Modern molecular-phylogenetic work based on representatives of most of the so-far named species (Bellot and Renner in review), together with study of collections deposited in the World's herbaria since the end of the 19th century, has revealed the need to synonymize many superfluous names, a task that we carry out here. We also up-date the circumscription of the family and its two genera, and clarify that they have specialized on different hosts, namely Salicaceae (mainly *Casearia*) and Fabaceae.

To achieve a better understanding of species boundaries and relationships, and to clarify the species' geographic and host ranges, we compared loans from numerous herbaria, dissected flowers, and isolated DNA from multiple collections. Molecular markers useful for these obligatory holoparasites are the nuclear 18S ribosomal RNA region and mitochondrial *matR* (Barkman et al. 2004; Bellot and Renner in review), and we show here that these markers can be used to place incomplete collections (for example, those of only one sex or only with fruits) in the correct species. Lastly, we provide an annotated key to all species that we recognize, and brief descriptions of their diagnostic traits along with color images and comments on their geographic and host ranges.

Methods

Plant material, DNA extraction and sequencing, phylogenetic analyses

We enlarged the DNA data matrix of Bellot and Renner (in review) by extracting DNA from additional specimens representing either unusual individuals or potential new species. No DNA sequences could be obtained from *P. stawiarskii*, known only from two collections in R, and *P. holtzii*, the only collection of which was destroyed in World War II. Suppl. material 1 shows species names and their authors, herbarium vouchers, and GenBank accession numbers. In total, 10 sequences (3 of 18S and 7 of *matR*) were newly generated for this study.

Total genomic DNA was extracted from herbarium specimens using the commercial plant DNA extraction Invisorb[®] Spin Plant Mini Kit (Stratec molecular, Berlin, Germany). The mitochondrial *matR* and the nuclear 18S genes were amplified using the primers listed in Bellot and Renner (in review). PCR products were purified with the ExoSAP or FastAP clean-up kits (Fermentas Life Sciences, St. Leon-Rot, Germany), and sequencing relied on the Big Dye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3130-4 automated capillary sequencer. Chromatograms were checked and sequences were edited using Geneious R7 (Biomatters, available from http://www.geneious.com), and contigs were then blasted against GenBank to rule out contamination. Alignments of the clean sequences were performed using the program MAFFT v. 7 (Katoh 2013) resulting in matrices of 1626 and 1727 aligned nucleotides for *matR* and 18S, respectively. We failed to amplify the gene *matR* from the African *Pilostyles aethiopica* and from the Iranian *P. haussknechtii*. Phylogenetic reconstructions relied on maximum likelihood (ML) as implemented in RAxML-7.2.8-ALPHA (Stamatakis 2006), using the GTR + G model of nucleotide substitution with 100 bootstrap replicates under the same model. Trees were rooted on *Corynocarpus laevigatus* (Corynocarpaceae; Cucurbitales), based on Filipowicz and Renner (2010).

Morphological data and assessment of the host ranges of Apodanthaceae

We geo-referenced locality data from 785 herbarium collections on loan from the herbaria B, G, C, GH, K, M, MO, MSB, W, NA, PMA, and SI and added data from the Global Biodiversity Information Facility (GBIF Backbone Taxonomy, 2013-07-01, http://www.gbif.org/species/7279680). We also recorded host names, up-dating their taxonomy as relevant. All label information was compiled in a database using the Botanical Research and Herbarium Management System (BRAHMS, http://herbaria. plants.ox.ac.uk/), and maps were produced using DIVA-GIS 7.5 (http://www.diva-gis. org). Collections were sorted by geography, flowering specimens were sexed to evaluate sexual dimorphism, and a representative number of flowers were then dissected under a stereoscope. For each dissected flower, the first author recorded the number, arrangement and size of the tepals, shape and ornament of the pistil/central column, number of pollen sacs, presence of hairs and presence of a nectary at the base of the flower. Pictures of representative organs were taken using a Dino-Lite USB microscope model AM413ZT (Dino-Lite Europe) and the DinoCapture Imaging software version 2.0 of the same company.

Results and discussion

Genus and species boundaries in Apodanthaceae

The dissections showed that species have characteristic flower sizes, number of tepals, tepal cilia, and number of anthers rings. For the American species, we use these differences in the key (below). Suppl. material 2 shows measurements and counts from the 123 dissected flowers. Six collections could not reliably be assigned to these groups because their flowers were slightly unusual: *R. Callejas et al. 8062*, a male plant from Colombia identified as *Apodanthes caseariae* by A. Idarraga in 2002; *Y. Mexia 4540*, a female plant from Brazil that is the type of the name *A. minarum*; *H. S. Irwin et al. 20350*, a female plant from Brazil identified as *Pilostyles ulei* by Ida de Vattimo

in 1975; *H.S. Irwin 31560*, a male plant identified as *P. blanchetii* by the first author but parasitizing an uncommon host (*Dioclea*, Fabaceae); *J. Rzedowski 11303*, a female plant from Mexico identified by the collector as *P. thurberi*; and *F. Chiang 9034*, a female plant from Mexico identified as *P. thurberi* by J. Henrickson in 1972.

The 18S and *matR* molecular trees show the *Pilostyles* collections that we wanted to identify (in red on Fig. 1) grouped with P. thurberi or P. blanchetii. The collections R. Callejas et al. 8062 and Y. Mexia 4540 grouped with two undoubted representatives of A. caseariae. R. Callejas et al. 8062 is a male plant and comes from the border with Panama, a country where A. caseariae has been repeatedly collected. The host of R. Callejas et al. 8062 was originally identified as Trema (Cannabaceae), but a partial matR sequence of this host BLASTed to Casearia nitida, making it likely that the host was in fact a Casearia. If that is the case, this would suggest that the collection represents an Apodanthes. The few male flowers of Apodanthes caseariae that have so far been dissected (Suppl. material 2) do not allow assessing the full morphological variability of the male flowers of this species. Therefore we had to rely on DNA for identification. In terms of its matR (Fig. 1A) R. Callejas et al. 8062 was embedded among other sequences of A. caseariae, while in terms of its 18S (Fig. 1B), it was sister to them. We identified the specimen as A. caseariae. Other matR and 18S sequences in the A. caseariae clade are from the type of the name A. minarum (Mexia 4540) from Brazil. Its host was a *Casearia* and its (female) flowers match those of *A. caseariae* (Suppl. material 2). We therefore synonymize *A. minarum* under *A. caseariae* (an action carried out below).

In combination, the present morphological and molecular results show that Apodanthaceae comprise at least ten biological species that can be allocated to two mutually monophyletic genera. In the Americas, these are *Pilostyles thurberi* in the southern United States of America and Mexico, P. mexicana in Mexico, Guatemala and Honduras, the widely distributed P. blanchetii from Panama to Jamaica to Brazil and Uruguay, and P. berteroi in Chile and Argentina. The Americas also harbor A. caseariae from Guatemala to Brazil (Fig. 2). Australia has three species, P. coccoidea, P. collina, and P. hamiltonii; Iran has P. haussknechtii, and Africa has P. aethiopica. The second African species, P. holtzii has not been recollected since 1907, when its type collection was made. Another species, the southern Brazilian P. stawiarskii, is only known from two specimens (one of them the type) collected at the same locality in Jan./Feb. 1948 and Dec. 1949; morphologically it resembles P. blanchetii (Vattimo, 1950). The host ranges of our accepted genera and species do not overlap. Apodanthes parasitizes only Salicaceae, whereas Pilostyles parasitizes only Fabaceae. As seen on Figure 3, there is a correspondence, although not perfect, between the phylogenies of host genera and parasitic species, and host specialization may have played a role in speciation of Apodanthaceae. At the species level, Table 1 shows that species of Apodanthaceae can grow on one or up to thirteen host species. As seen in Figures 2 and 3, our species concepts are corroborated by geographic and host ranges, except in the case of Apodanthes caseariae and Pilostyles blanchetii, both widespread in Brazil. These two species have different sized flowers (see below), and parasitize phylogenetically distantly related hosts (Fig. 3).





Figure 1. Phylogenetic relationships in Apodanthaceae obtained under maximum likelihood from the mitochondrial gene *matR* (\mathbf{A}) and the nuclear ribosomal RNA gene 18S (\mathbf{B}). Shown in red are the specimens we wanted to identify to species. Numbers indicate bootstrap support >70%.



Figure 2. Geographic distribution of Apodanthaceae based on label information from 785 herbarium collections.



Figure 3. Phylogenetic relationships among the hosts of Apodanthaceae (legume relationships from Wojciechowski et al. 2006) and among the species of Apodanthaceae (from Bellot and Renner, in review). Identical colors link parasite species and their host(s) and are also used in Figure 2. Dashed lines represent associations with rarely reported hosts; hosts in bold are the most common ones.

Table 1. Hosts of Apodanthaceae based on label information from 785 herbarium collection	ns. Upper
case numbers refer to the references below the table.	

Parasite	Host genera	Host species		
	Berlinia ¹ , Brachystegia, Jul-	Brachystegia boehmii Taub., Brachystegia glaucescens x spiciformis,		
P. aethiopica	bernardia, Pseudoberlinia ¹ ,	Brachystegia spiciformis Benth., Brachystegia taxifolia Harms., Julber-		
	Westia ¹	nardia globiflora (Benth.) Troupin		
		Adesmia arborea Bert. ex Savi, Adesmia aff. spinosissima Meyen,		
		Adesmia obovata Clos, Adesmia bedwellii Skottsb., Adesmia miraflo-		
P. berteroi	Adesmia	rensis Remy, Adesmia uspallatensis Gill ex H. & A., Adesmia gracilis		
		Meyen ex Vogel, Adesmia microphylla H. & A., Adesmia monosperma		
		Clos, Adesmia pinifolia Gillies, Adesmia trijuga Gillies		
		Bauhinia candicans Benth., Bauhinia divaricata L., Mimosa claussenii		
		Benth., Mimosa cyclophylla Taub., Mimosa aff. setosa Benth., Mimosa		
P. blanchetii	Bauminia, Cassia, Dioclea,	maguirei Barneby, Mimosa scabrella Benth., Mimosa setosissima Taub.,		
	Galactia ² , Mimosa, Schnella	Mimosa uraguensis H. & A., Mimosa cf. xanthocentra Martius, Sch-		
		nella cumanensis Britton & Rose		
		Casearia aculeate Jacq., Casearia arborea Urb., Casearia decandra Jacq.,		
A. caseariae	Casearia, Xylosma	Casearia grandiflora Cambessèdes, Casearia guianensis Urb., Casearia		
		hirsute Swartz, <i>Casearia nitida</i> Jacq.		
P. coccoidea	Jacksonia			
P. collina	Gastrolobium, Oxylobium	Gastrolobium euryphyllum Chandler & Crisp		
D L!!	Durint	Daviesia angulata Benth., Daviesia decurrens Meissner, Daviesia pecti-		
1: namiitonii	Daviesia	<i>nata</i> Meissner, <i>Daviesia preissii</i> Lindley		
		Astragalus brachycalyx Fisch., Astragalus brachystachys DC., Astragalus		
		cephalanthus DC., Astragalus chalaranthus Boiss. & Hausskn., Astra-		
ת ביין ביין ב	Astragalus, Halimodendron,	galus compactus Reiche, Astragalus floccosus Boiss., Astragalus gossypinus		
1. naussknechti	Onobrychis	Fisch., Astragalus microcephalus Willd., Astragalus rhodosemius Boiss.		
		& Hausskn., Astragalus spinosus Muschler, Astragalus susianus Boiss.,		
		Astragalus verus Olivier, Halimodendron halodendron (Pall.) Druce		
P. mexicana	Calliandra	Calliandra houstoniana (Miller) Standley		
Delaurhani	Dalea, Psorothamnus,	Dalea bicolor Humb. & Bompl. in Willd., Dalea formosa Torrey,		
1: thurbert	Parosela ³	Dalea frutescens Gray, Psorothamnus emoryi (Gray) Rydberg		

¹Verdcourt, B., 1998. Flora of tropical East Africa - Rafflesiaceae. Flora of tropical East Africa 175, 1–2. CRC Press.

²Ule, E., 1915. Rafflesiaceae. Notizblatt des Königl. botanischen Gartens und Museums zu Berlin-Dahlem 6, 292–293.

³Rose, J. N., 1909. Studies of Mexican and Central American Plants n°6. Contributions from the United States National Herbarium 7, 26–265.

Description of the family

Stem-endoparasites, non-photosynthetic. No leaves, stem or roots, instead an endophytic system of cells inside the stem parenchyma of the host, flowers bursting through the host bark. Flowers unisexual, plants dioecious or monoecious, a point still insufficiently known; flowers of both sexes on the same host or not. Pollination by flies and bees, possibly also wasps (Bellot and Renner 2013; Sipes et al. 2014), based on the fruit color and size, dispersal is probably by birds. Flowers white or yellow (*Apodanthes*), or white, pink, orange, red, purple or brown (*Pilostyles*),



Figure 4. Tepals of Apodanthaceae. **A** Tepal of the outer whorl of *Apodanthes caseariae* **B** Tepal of the middle whorl of *A. caseariae* **C** Tepal of the inner whorl of *A. caseariae* **D** Tepal margin of *A. caseariae*. The scale bar corresponds to 0.2 mm.



Figure 5. Sexual organs of Apodanthaceae from rehydrated herbarium material. **A** Androecium of *Pilostyles haussknechtii*, note the two rings of pollen sacs topped by a ring of hairs **B** Style and stigma of *P. haussknechtii* **C** Ovary locule and ovules of *P. haussknechtii* **D** Androecium of *Apodanthes caseariae* after bursting of the pollen sacs, note the hairs covering the column apex **E** Gynoecium of *A. caseariae*. The scale bar corresponds to 0.2 mm.

aggregated on the host stems, minute (1.5 to 15 mm long when dried), usually with radial symmetry. Perianth composed of 2 or 3, rarely 4, whorls of tepals (Fig. 4A–C), the latter sometimes with hairs along their margins (Fig. 4D), or a hair cushion at their basis (Blarer et al. 2004). In male flowers, the staminal filaments completely fused and forming a tube around a central column that is usually fused to the column (Fig. 5A, D), the up to 72 pollen sacs arranged in 1–4 rings around the column apex (Fig. 5A), the column apex dome-shaped and circled or covered by single-celled hairs (Fig. 5A, D), a basal nectar cushion in both sexes (Fig. 5E). Female flowers without staminodes and with a single thick style topped by the

dome-shaped stigma (Fig. 5B, C, E). Ovary semi-inferior, placentation parietal with 50–300 ovules (Fig. 5C, E). Seeds ca. 0.5 mm long (Bouman and Meijer 1994). Fruit a fleshy berry.

Key to the genera and species of Apodanthaceae

1a	From the Neotropics, parasitizing Salicaceae, tepals always in 3 whorls with
	(from the outside) 2, 4, and 4 tepals, the inner whorl easily deciduous, female
	flowers >5 mm long 1. Apodanthes caseariae
1b	From the Neotropics, Africa, Iran, or Australia, parasitizing Fabaceae, num-
	ber of outer tepals usually >2, female flowers <5 mm long2
2a	Occurring in Australia
2b	Not in Australia5
3a	Tepals in 3 whorls
3b	Tepals in 2 whorls
4a	Flower diameter >3 mm
4b	Flower diameter <3 mm
5a	Occurring in Africa
5b	Not in Africa
6a	Occurring in Iran6. P. haussknechtii
6b	Occurring in the America7
7a	Tepals in 3 whorls, each with 2 to 7 tepals, anthers in 4 whorls (spiral), num-
	ber of anther lobes >70, on <i>Adesmia</i> 7. <i>P. berteroi</i>
7b	Tepals in 3 whorls, each with 3 or 4 (rarely more) tepals, anthers in 2 or 3
	whorls, number of anther lobes <70, not on Adesmia
8a	Middle tepals ovoid, on Calliandra, Dalea, Parosela or Psorothamnus, anthers
	in 3 whorls (at least if on <i>Dalea, Parosela</i> or <i>Psorothamnus</i>)9
8b	Middle tepals mostly diamond-shaped, apparently never on Calliandra,
	Dalea, Parosela or Psorothamnus, anthers in 2 whorls (females of the three
	species cannot be securely distinguished)
9a	On Calliandra, tepals in 3 whorls, each with 4 tepals9. P. mexicana
9b	On Dalea, Parosela, or Psorothamnus, tepals in 3 whorls, each with 3 or 4
	tepals

Allocation of all species names so far described in Apodanthaceae

1. *Apodanthes caseariae* Poiteau, Ann. Sci. Nat. (Paris) 3: 422, t. 26. 1824. http://species-id.net/wiki/Apodanthes_caseariae

Apodanthes flacourtiae Karsten, Linnaea 28: 413. 1857. Type: Venezuela, Aragua, Choroni, parasitic on "Flacourtieae" [most like a species that today would be placed in Salicaceae], *H. Karsten s.n.* (W, destroyed in WWII), **syn. nov.**

- Apodanthes tribracteata Rusby, Descr. S. Amer. Pl. 15. 1920. Type: Bolivia, near Inglis-Inglis, 8 Aug. 1902, R. S. Williams 1580 (NY), syn. nov.
- Nom. inval. Apodanthes matogrossensis Vattimo, Vattimo-Gil, Rodriguésia 26(38): 45. 1971, without Latin descr. Type: Brazil, Mato Grosso, parasitic on *Casearia, J. G. Kuhlmann 53076* (R, not seen).
- Apodanthes panamensis Vattimo-Gil, Rodriguésia 26(38): 45. 1971, without Latin descr., Latin diagnosis in Rev. Brasil. Biol., 33(1): 140. 1973. Type: Panama, Canal Zone, Aug. 1984, *R. E. Woodson Jr. and R. W. Schery 965* (NY, MO). Already synonymized by A. Gentry (1973).
- *Apodanthes surinamensis* Pulle, Recueil Trav. Bot. Néerl. 6: 259. 1909. Type: Suriname, along the Marowijne River, July-Dec. 1903, parasitic on Flacourtiaceae [most like a species that today would be placed in Salicaceae], *G. M. Versteeg s.n.* (U0007645), **syn. nov.**
- Apodanthes roraimae Ida de Vattimo, Rodriguésia 29(44): 48-49. 1978. Type: Brazil, Roraima, 24 Jul. 1974, parasitic on Flacourtiaceae [most likely a species that today would be placed in Salicaceae], G. T. Prance et al. 21353 (NY), syn. nov. Comment: George Yatskievych, a curator at the Missouri Botanical Garden, also studied the NY isotype in 2004 and annotated it as A. caseariae.
- Apodanthes minarum Vattimo-Gil, Rodriguésia 26 (38): 45. 1971, without Latin descr.; Latin diagnosis in Rev. Brasil. Biol., 33(1): 140. 1973. Type: Brazil, Minas Gerais, Viçosa, 31 Mar. 1930, Y. Mexia 4540 (L, MO), syn. nov.

Type. French Guiana, Karouany, c. 1802, parasitic on *Casearia* spec., *P. A. Poiteau s.n.* (P: P00686413).

Note. Tepals white to yellow, female flowers >5 mm long, tepals in 3 whorls, the outer with 2 tepals, the inner one easily deciduous (Figs 4A–D; 6C, D). Growing in trunk and branches of *Casearia* and occasionally *Xylosma* (Salicaceae, Fig. 3) in Guatemala, Honduras, Costa-Rica, Panama, Colombia, Venezuela, Suriname, French Guiana, Brazil, Peru and Bolivia (Fig. 2).

2. Pilostyles collina Dell, Nuytsia 4: 293–294. 1983.

http://species-id.net/wiki/Pilostyles_collina

Type. Australia, Western Australia, Peak Charles, 10 Jan. 1982, parasitic on *Oxylobium*, *B. D. Dell 8216* (G, MO).

Note: Tepals orange to red, in 3 whorls. Growing in young stems of *Gastrolobium* and *Oxylobium* in Western Australia (Figs 2, 3, see Thiele et al. 2008 for pictures of flowers).

3. *Pilostyles hamiltonii* Gardner, J. Roy. Soc. Western Australia 32: 77. 1948. http://species-id.net/wiki/Pilostyles_hamiltonii **Type.** Australia, Western Australia, Darling District, Helena Rover, Mundaring Weir, Mar. 1946, parasitic on *Daviesia pectinata* Lindl., *C. D. Hamilton s.n.* (PERTH, not seen).

Note: Tepals dark burgundy, in 2 whorls, flowers >3 mm in diameter. Growing in young stems of *Daviesia* in Western Australia (Figs 2, 3, see Thiele et al. 2008 for pictures of flowers).

4. Pilostyles coccoidea K.R. Thiele, Nuytsia 18: 273-284. 2008.

http://species-id.net/wiki/Pilostyles_coccoidea

Type. Australia, Western Australia, Waddi Road, 30°33'26"S, 115°28'10"E, 7 Mar. 2008, parasitic on *Jacksonia, K.R. Thiele 3495* (PERTH 07692447).

Note. Tepals pale orange to brown, in 2 whorls, flowers <3 mm in diameter. Growing in stems of *Jacksonia* in Western Australia (Figs 2 and 3, see Thiele et al. 2008 for pictures of flowers).

5. *Pilostyles aethiopica* Welwitsch, Trans. Linn. Soc. London 27: 66–70. 1871 = *Berlinianche aethiopica* (Welw.) Vattimo-Gil, nom. inval.

http://species-id.net/wiki/Pilostyles_aethiopica

Pilostyles holtzii Engler, Bot. Jahrb. Syst. 46: 293. 1912 = Berlinianche holtzii (Engl.) Vattimo-Gil, not validly published. Type: Tanzania, Kilimatinde, July 1907, parasitic on Berlinia eminii Taub., W. Holtz 1422 (B, destroyed during World War II), syn. nov. (based on the protologue).

Syntypes. Angola, Huila, 12 May 1860, parasitic on *Berlinia paniculata* Benth. = *Pseudoberlinia paniculata* (Benth.) P.A.Duvign., *F. M. J. Welwitsch 529, 529b* (C, G).

Note. Tepals pink to brown, in 3 to 4 whorls each with 3-6 tepals. Male flowers with 1 or 2 ring(s) of ca. 15 pollen sacs, stamen filaments free from the central column (Fig. 6F), hair cushion at the basis of the inner tepals (Blarer et al. 2004). Growing in branches of *Julbernardia* and *Brachystegia*, maybe also on *Berlinia*, *Westia* and *Pseudoberlinia*, in Zimbabwe, Zambia, Tanzania, Angola and Malawi (Figs 2, 3).

6. *Pilostyles haussknechtii* Boissier, Arch. Sci. Phys. Nat. 25: 255–261. 1866. http://species-id.net/wiki/Pilostyles_haussknechtii

Type. Middle East, parasitic on Astragalus, J. E. Haussknecht s.n (G-BOISS, not seen).

Note: Tepals pink to brown in 2 whorls, each with 6 to 10 tepals (Fig. 6G). Found at the basis of young branches of *Astragalus* and occasionally *Onobrychis* and *Halimo- dendron* in Iran (Figs 2, 3).

7. *Pilostyles berteroi* Guillemin, Ann. Sci. Nat., Bot., sér. 2, 2: 21. 1834 = *Apodanthes berteroi* (Guill.) Gardner, Hooker's Icon. Pl. 7: t. 655. 1844. syn. nov. http://species-id.net/wiki/Pilostyles_berteroi

Syntypes. Chile, Quillota, parasitic on *Adesmia*, [in Chile 1828-1831] *C. L. G. Bertero s.n.* (P, not seen); Chile [from the collection number this was in "various localities in the Andes", during the period from 27 Oct.-26 Nov. 1841], *T. Bridges 1273* (BM, not seen, K, not seen).

Note: Tepals purple to brown with clearer margins (Fig. 6A), 9–18 in number, stamens in 4 whorls (spirals), with > 70 pollen sacs. Growing in older stems of *Adesmia* shrubs in Chile, Argentina, Peru, and Bolivia (Figs 2, 3). Our morphological (Suppl. material 2) and molecular data (Fig. 1) show that the species is nested among other species of *Pilostyles*, indicating that Gardner's transfer was erroneous.

8. *Pilostyles blanchetii* (Gardner) R.Br., Trans. Linn. Soc. London 19(3): 247. [6 Nov 1844] = *Apodanthes blanchetii* Gardner, Icon. Pl. 7: t. 655 b. 1844 [Jul 1844] = *Frostia blanchetii* (Gardner) H.Karst., Nov. Actorum Acad. Caes. Leop.-Carol. Nat. Cur. 26: 922. 1858.

http://species-id.net/wiki/Pilostyles_blanchetii

- Pilostyles calliandrae (Gardner) R.Br., Trans. Linn. Soc. London 19(3): 247. [6 Nov 1844] = Apodanthes calliandrae Gardner, Icon. Pl. 7: t. 644. 1844 [Jan 1844] = Frostia calliandrae (Gardner) H. Karst., Nov. Actorum Acad. Caes. Leop.-Carol. Nat. Cur. 26: 921. 1858. Type: Brazil, Amazonas, near Maynas [Manaus], Feb. 1840, G. Gardner 3639 (K000601222), syn. nov.
- Pilostyles caulotreti (Karsten) Hook.f., Prodr. (DC.) 17: 116. 1873 = Sarna caulotreti Karsten, Linnaea 28: 415. Jun 1857 [1856]. Type: Venezuela, H. Karsten s.n. (W, destroyed in WWII). Comment: Gentry (1973) considered this name as synonym of P. blanchetii, and we agree with this assessment.
- Pilostyles ingae (Karsten) Hooker f., Prodr. (DC.) 17: 116. 1873 = Sarna ingae H.Karst., Linnaea 28: 415. Jun 1857 [1856]. Type: Colombia, Cauca, Popayán, parasitic on Inga, H. Karsten s.n. (W, destroyed in WWII), syn. nov. (based on the protologue).
- *Pilostyles galactiae* Ule, Notizbl. Königl. Bot. Gart. Berlin 6: 292. 1915. Type: Brazil, Amazonia, Surumu River, tributary of the Rio Branco, Oct. 1909 and Mar. 1910, parasitic on *Galactia jussiaeana* Kunth., *E. Ule 7895* (B, holotype destroyed in WWII; isotype NY), **syn. nov.**
- Pilostyles goyazensis Ule, Ber. Deutsch. Bot. Ges. 33: 475. 1915. Syntypes (all parasitic on *Mimosa*): Brazil, Goias, region near city of Corumba, Sobradinho, Aug. 1892, *E. Ule 3097*; Serra dos Pyreneos, Mun. Corumba, Dec. 1892, *E. Ule 3098*; same location, Dec. 1892, *E. Ule 3099*; in the Corumba region, Aug. 1892, not flowering, *E. Ule s.n.*; Serra dos Pyreneos, Aug. 1892, not reproductive, *E. Ule s.n.* (all in B, material destroyed in WWII), syn. nov. (based on the protologue).



Figure 6. Flowers of Apodanthaceae species A *Pilostyles berteroi* on *Adesmia* in Chile (picture by C. Heibl) B Male flowers of *P. thurberi* on *Psorothamnus emoryi* in California (picture by L. Hendrickson)
C Male and female flowers of *Apodanthes caseariae* on *Casearia* (?) in Panama (picture by G. Gerlach)
D Close-up of a male flower of *A. caseariae* (picture by G. Gerlach) E *Pilostyles blanchetii* on *Bauhinia* in Panama; insert: close-up of a flower (pictures by C. Galdames) F *Pilostyles aethiopica* on *Julbernardia globiflora* in Zimbabwe (picture by S. Bellot); insert: close-up of male flowers (picture by S. Bellot); no Astragalus in Iran (picture by S. Bellot). The white scale bars correspond to 1 mm and the black ones to 5 mm.

 Pilostyles globosa (S.Watson ex Robinson) Hemsl., J. Linn. Soc., Bot. 31: 311. 1896 = Apodanthes globosa S.Watson ex Robinson., Bot. Gaz. 16: 83, tab. 9, 1891. Type: Mexico, Northern part, Sierra Madre, parasitic on *Bauhinia lunarioides* A. Gray, C. G. Pringle 1950 (G), syn. nov.

- Pilostyles stawiarskii Vattimo-Gil, Revista Brasil. Biol. 10: 196. 1950. Type: Brazil, Paraná, Mun. de Palmas, parasitic on *Mimosa scabrella* Benth. [incl. its synonym *Mimosa bracaatinga* Hoehne], Jan. 1948 and Feb. 1948, *V. Stawiarski* R50.591 and 50.592 (R, photos). There is also a topotypical collection from Dec. 1949, syn. nov. (based on the protologue).
- Pilostyles ulei Solms-Laub., in Goebel, Organogr. Pfl. 2,1: 434. Figure 292 (1900), descr. in Endriss, Flora, Ergänz.-Bd. 91: 209. 1902. Type: Brazil, Goias, parasitic on Fabaceae, *E. Ule s.n.* (B, destroyed in WWII; R has E. Ule 34, E. Ule 36, E. Ule 38, E. Ule 148, E. Ule 367, E. Ule 482, and E. Ule 483 labeled as this species, not seen). Comment: already Solms-Laubach (1901) and Endriss (1902) considered *P. ulei* as a synonym of *P. ingae*.

Type. Brazil: Bahia, 1839, J. S. Blanchet 2861 (NY).

Note. Tepals purple to brown sometimes with clearer margins (Fig. 6E), in 3 whorls with usually 4 (rarely 3-6) tepals, the middle tepal diamond-shaped. Stamens in 2 whorls. Found in branches of *Mimosa* and *Bauhinia*, but also *Cassia*, *Dioclea*, *Galactia* and *Schnella*, in Jamaica, Cayman Islands, Costa-Rica, Panama, Colombia, Venezuela, Guyana, Brazil, Argentina and Uruguay (Figs 2, 3).

9. *Pilostyles mexicana* (Brandegee) Rose, Contr. U.S. Natl. Herb. 12(7): 264. 1909 = *Apodanthes mexicana* Brandegee, Zoe 5(11): 245. 1908. http://species-id.net/wiki/Pilostyles_mexicana

Type. Mexico, near Zacuapan, Tenampa, parasite on *Calliandra grandiflora* Benth., Oct. 1906, *C.A. Purpus 2207* (NY).

Note. Tepals red to brown, in 3 whorls, each with 4 tepals. Growing in branches of *Calliandra* in Guatemala, Honduras and Mexico (possibly further south; Figs 2, 3).

10. Pilostyles thurberi Gray, Pl. Nov. Thurb. 326-327. 1854.

http://species-id.net/wiki/Pilostyles_thurberi

- *Pilostyles covillei* Rose, Contr. U.S. Natl. Herb. 12: 263. 1909. Type: USA, Texas, Dickens county, Matador ranch, 14 June 1894, parasitic on *Parosela formosa* (Torr.) Vail, *F. V. Coville 1860* (US, not seen).
- Pilostyles glomerata Rose, Contr. U.S. Natl. Herb. 12: 263. 1909. Type: Mexico, Puebla, near Tehuacán, 31 Aug. 1905, parasitic on Parosela, J. N. Rose and J. H. Painter 9942 (NY, G). The protologue gives the collection number as 8942.
- Pilostyles palmeri Rose, Contr. U.S. Natl. Herb. 12: 263. 1909. Type: Mexico, San Luis Potosí, near Alvarez, May 1887, parasitic on *Parosela*, *E. Palmer 584* (US-570088).
- Pilostyles pringlei (Watson) Hemsl., J. Linn. Soc., Bot. 31: 311. 1896 = Apodanthes pringlei Watson ex B.L.Rob., Bot. Gaz. 16: 83, tab. 9. 1891, no Latin descr.; Pilo-

styles pringlei (Watson) Rose, Contr. U.S. Natl. Herb. 12: 264. 1909, superfluous transfer. Type: Mexico, Sierra Madre, near Monterey, 27 June 1888, parasitic on *Dalea frutescens* A. Gray, *C. G. Pringle 1949* (NY, M, G).

Pilostyles sessilis Rose, Contr. U.S. Natl. Herb. 12: 263. 1909. Type: male flowers: Mexico, Hidalgo, Ixmiquilpan, 1905, parasitic on Parosela, J. N. Rose 9041 (NY); female flowers: Mexico, Querétaro, hacienda Ciervo, 20 Aug. 1905, parasitic on Parosela tuberculata (Lag.) Rose, J. N. Rose and J. H. Painter 9636 (NY, US).

Type. USA, probably Arizona, near Gila river, June 1850, parasitic on *Psorothamnus emoryi* (A. Gray) Rydb., *G. Thurber 682* (NY).

Note. Tepals white, red to brown, in 3 whorls, each with 3 or 4 tepals, rarely more (Fig. 6B). Growing in branches of *Dalea, Parosela* and *Psorothamnus* in the southern United States of America and Mexico (Figs 2, 3). New York (NY) has a specimen from Mexico of this species annotated as "*Pilostyles mortoni*", a nomen nudum, by Ida de Vattimo in 1952.

Note on an invalid genus name

Harms (1935) tried to place the two African names, *Pilostyles aethiopica* Welw. and *P. holtzii* Engl., in a separate section, *Pilostyles* section *Berlinianche*, named for their legume host species in the genus *Berlinia*, but failed to include a Latin diagnosis for the new section. Later, Vattimo-Gil (1955, 1971) decided to rank this section as a separate genus because of the hair cushions on the inner perianth whorl and strictly tri- and hexamerous flowers compared to the tetramerous flowers of the American species of *Pilostyles*. This assessment, however, could only have been based on specimens of *P. aethiopica*, since the only collection of *P. holtzii* burnt in World War II. Unfortunately, Vattimo-Gil also neglected to provide a Latin diagnosis, and the genus name is therefore not valid. Based on our results (Fig. 1), *P. aethiopica* does not deserve generic status because it is embedded among the other species of *Pilostyles*.

Note on a possible new species of Pilostyles

Flavio González and Natalia Pabón-Mora, at the university of Antioquia in Colombia, are studying the ecology and morphology of Apodanthaceae in Colombia (González and Pabón-Mora accepted a) and are describing a new species of *Pilostyles* (González and Pabón-Mora accepted b). This species is the first *Pilostyles* parasitizing the legume genus *Dalea* in South America and occurs in dry valleys of the Colombian Eastern Cordillera at altitudes above 2000 m. Morphologically, the new species is most similar to *P. berteroi*, which grows in the Chilean and Peruvian Andes at up to 3000 m of altitude (Fig. 2) and parasitizes *Adesmia* (closely related to *Dalea*, see Fig. 3).

Conclusion

By combining morphological and molecular information, we show that Apodanthaceae comprise 10 species and that morphological distinctions fit well with geographical disjunctions and specializations on different hosts (Salicaceae vs. Fabaceae). DNA sequences of mitochondrial *matR* and nuclear 18S rDNA, along with morphology and geography permit identifying any collection of Apodanthaceae. A wider sampling of the morphological variation, especially of male *Apodanthes caseariae* and female *Pilostyles blanchetii*, *P. mexicana* and *P. thurberi*, however, is needed to determine whether some unusual specimens might deserve to be ranked as subspecies.

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Supplementary material I

List of accessions used in this study with author names for each species, herbarium vouchers, and GenBank accession numbers

Authors: Sidonie Bellot, Susanne S. Renner

Data type: species list

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Link: doi: 10.3897/phytokeys.36.7385.app1

Supplementary material 2

Results of the dissections of 123 flowers from 82 specimens of Apodanthaceae

Authors: Sidonie Bellot, Susanne S. Renner

Data type: measurements

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RESEARCH ARTICLE



Gymnanthemum koekemoerae (Compositae, Vernonieae), a new species from South Africa

Harold Robinson¹, Vicki A. Funk¹

1 US National Herbarium, Department of Botany, NMNH, Smithsonian Institution, Washington, D.C. 20560 USA

Corresponding author: Vicki A. Funk (funkv@si.edu)

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Abstract

A new species of *Gymnanthemum* (Compositae, Vernonieae) from South Africa is described. It can be distinguished from other species in the genus by the five-flowered capitula and widely obtuse leaf blades.

Keywords

Asteraceae, Compositae, Gymnanthemum, South Africa, Vernonieae

Introduction

In the course of preparing a monograph covering all of the Vernonieae of southern Africa (Botswana, Namibia, South Africa) a specimen sent from PRE was determined to be a previously undescribed species of *Gymnanthemum*. Here we describe this new taxon, provide a key to the endemic species from South Africa, and an original illustration.

Taxonomic treatment

Gymnanthemum Cass.

http://species-id.net/wiki/Gymnanthemum

Bull. Soc. Philom. Paris 1817: 10. 1817. Type: Gymnanthemum cupulare Cass. = G. coloratum (Willd.) H. Rob. & B. Kahn

Bracheilema R. Br. ex Salt, Abyss. Append.: 65. 1814, nom. nud.

Decaneurum DC., Arch. Bot. (Paris) 2: 516. 1833, nom. superfl., type same as Gymnanthemum.

Plectreca Raf., Fl. Tellur. 4: 119. 1838. Type: Staehelina corymbosa Thunb.

Keringa Raf., Sylva Tellur.: 144. 1838. Type: Vernonia amygdalina Del.

Cheliusia Sch. Bip. in Hochstetter, Flora 24(1, Intelligenzbl.): 26. 1841, nom. nud.

Vernonia subsect. *Urceolata* S.B. Jones, Rhodora 83: 67. 1981. Type: *Vernonia sphaerocalyx* O. Hoffm.

Remarks. Shrubs or small trees moderately to densely branching; stems often felted, hairs rarely asymmetrically T-shaped. Leaves alternate; petioles short, winged or elongate; blades membranaceous to rather coriaceous, margins entire to serrate or dentate, upper surfaces essentially glabrous and somewhat glossy to arachnoid tomentose. Inflorescences terminal, densely corymbiform, with small bracteoles; peduncles short; involucral bracts coriaceous, 25–35, 4–5-seriate, inner bracts often deciduous. Florets 3–50; corolla white to violet, anther base broadly tailed, tails often long, apical appendage glabrous; style base without or with scarcely distinct node; style branches with stout, pointed sweeping hairs. Achenes 5–10-costate, raphids short, elongate or not evident; pappus of many rather persistent capillary bristles, often with broadened tips, with outer series of short squamellae. Pollen sublophate. Chromosome number n = 10, 20. More than 43 species are found in sub-Saharan Africa, Madagascar, Southern Asia, and also introduced into Brazil.

The genus *Gymnanthemum* was described by Cassini (1817), included in *Vernonia* by Candolle (1836) and Bentham (1873) and resurrected by Robinson and Kahn (1986) and Robinson (1999). The generic limits have changed and are now more narrow than in 1999. Currently the genus has nine species in southern Africa (Robinson et al. in prep.), five of which are endemic to South Africa; a key to those is provided here. The four more widespread species are *G. theophrastifolium* (Schweinf. ex Oliv. & Hiern) H. Rob., *G. coloratum* (Willd.) H. Rob. & B. Kahn, *G. amygdalinum* (Del.) Sch. Bip. ex Walp. and *G. myrianthum* (Hook. f.) H. Rob. The still unfinished monograph will cover all species of Vernonieae from Southern Africa with descriptions, keys and pollen images (Robinson et al. in prep.).

Gymnanthemum koekemoerae H. Rob. & V.A. Funk, sp. nov.

urn:lsid:ipni.org:names:77138105-1 http://species-id.net/wiki/Gymnanthemum_koekemoerae

Type. South Africa. Limpopo Province. Thohoyandou District. Thathe-Vonde Nature Reserve. Grassland at rocky outcrop near entrance, 1233 m, 22°55'10"S, 30°19'36"E [2230CD], 23 March 2002, *Koekemoer 2273* (holotype PRE!, isotype US!). Figs 1–3.

Description. Sparsely branched shrubs 1.3–1.5 m tall; stems brown, terete and striate, hispid to hirtellous and gland-dotted, hairs unicellular, with short branches and spurs. Leaves alternate; petioles 3-4 mm long; leaf blades chartaceous, suborbicular, 4.5-6.5 cm long and broad, bases rounded to broadly obtuse, abruptly terminating at petiole, margins with c. 5 broad dentations above basal 1/4, apex with broad obtusely triangular tip; adaxial surface dark green when dry, essentially glabrous, primary and secondary veins priminulous in shallow grooves, tertiary veins flush with surface; abaxial leaf surface somewhat paler, sparsely pilosulous on larger prominulous veins, surface with numerous yellow glandular dots; secondary veins widely spreading at 50–80° angles, usually 4 on each side, quaternary veins minimally prominulous. Inflorescence broadly corymbiform, terminal on stems and distal branches; peduncles 3-8 mm long, capitula 13-15 mm high; involucres 4-5 mm wide, to 7-8 mm wide when in fruit; involucral bracts subimbricate in c. 5 gradate series, round to oblong, 2-7 mm long, 2.5-3.6 mm wide, inner bracts somewhat ranked, apices broadly rounded to subtruncate, with broad rounded surface outside, greenish or brownish with darker and gland-dotted distal 1/4; florets 5 in a capitulum; corollas pale lavender, c. 9.5 mm long, essentially without hairs, sparsely gland-dotted outside, basal tube c. 5 mm long, funnelform distally, throat c. 0.5 mm long, lobes evenly tapered, c. 4 mm long; anther thecae c. 4.5 mm long, apical appendage triangular, c. 0.6 mm long, 0.25 mm wide; achenes c. 5 mm long, 10-ribbed, with numerous short, spreading setulae mostly on ribs, with glandular dots between ribs; pappus mostly c. 9 mm long, becoming tawny, of c. 90 crowded capillary bristles, bristles scarcely broadened distally.

Related taxa. *Gymnanthemum koekemoerae* is closest to *G. mespilifolium* in its leaf pubescence, but it has an abrupt base on the leaf blade, totally unlike the narrow acumination in *G. mespilifolium* that gives the leaves of the latter a long-petiolate appearance. The blades of the new species are also more chartaceous, and the dentations of the leaf are more numerous and are as broad as long. The dentations in *G. mespilifolium* are long and narrowly acute, and are restricted to the distal 1/3 of the leaf blade.

Notes. The holotype (PRE) has both flowering and fruiting material while the isotype (US) material is mostly fruiting. The specimen of the new species was distributed as *Vernonia triflora* Bremek. (now *Gymnanthemum triflorum* (Bremek.) H. Rob.) which has only 3 florets in its capitula, has stiffly and densely hispid stems, and has ovate to oblong leaf blades with hispidulous abaxial surfaces.

Etymology. The species is named for Dr. Marinda Koekemoer (PRE) who collected the type material and who has done so much to further our knowledge of the Compositae of southern Africa.

Distribution. This species is known only from the type locality.



Figure 1. Type specimens. **A** Photograph of the isotype (US) **B** Photograph of the inflorescence of the holotype (PRE).



Figure 2. Original Illustration of *Gymnanthemum koekemoerae* H. Rob. & V.A. Funk: **A** Habit **B** Abaxial surface of leaf **C** Head **D** Outer involucral bract **E** Inner involucral bract **F** Floret **G** Longitudinal section of floret showing anthers **H** Style **I** Achene with pappus. [Illustration by Alice Tangerini (US)]



Figure 3. Illustration of heads from the holotype: **A** Head when in flower **B** Head with mature achenes. Note: there is an increase in dark coloration on the more mature head, possibly caused by fungi. [Illustration by Alice Tangerini (US)]

Key to the endemic species of Gymnanthemum from South Africa

1	Abaxial surface of leaves sparsely puberulous to essentially glabrous2
_	Abaxial surface of leaves hispid to tomentose
2	Leaf blades chartaceous with broadly obtuse bases; stems puberulous often
	with dark hairs (fungus), especially in fruiting specimens
	Gymnanthemum koekemoerae H. Rob. & V.A. Funk
_	Leaf blades rather membranaceous with long-acuminate bases; stems essen-
	tially glabrousGymnanthemum mespilifolium (Less.) H. Rob.
3	Leaf blades oblong to ovate with obtuse bases; stems hirsute; capitula with 3
	florets Gymnanthemum triflorum (Bremek.) H. Rob.
_	Leaf blades obovate to oblanceolate with cuneate bases; stems tomentose;
	capitula usually with 4–5 florets
4	Stems and abaxial surfaces of leaves completely covered with appressed
	tomentum; inflorescence narrowly corymbose
_	Stems with tomentum of cottony hairs, abaxial surfaces of leaves with mixed
	erect and arachnoid hairs that do not totally obscure green surface; inflorescence
	broadly corymbose, much broader than high

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