

## Spores of *Serpocaulon* (Polypodiaceae): morphometric and phylogenetic analyses

VALENTINA RAMÍREZ-VALENCIA<sup>1,2</sup> & DAVID SANÍN <sup>3</sup>

<sup>1</sup>Smithsonian Tropical Research Institute, Center of Tropical Paleocology and Archeology, Grupo de Investigación en Agroecosistemas y Conservación de Bosques Amazonicos-GAIA, Ancón Panamá, Republic of Panama, <sup>2</sup>Laboratorio de Palinología y Paleoecología Tropical, Departamento de Ciencias Biológicas, Universidad de los Andes, Bogotá, Colombia, <sup>3</sup>Facultad de Ciencias Básicas, Universidad de la Amazonia, Florencia Caquetá, Colombia

### Abstract

The morphometry and sculpture pattern of *Serpocaulon* spores was studied in a phylogenetic context. The species studied were those used in a published phylogenetic analysis based on chloroplast DNA regions. Four additional Polypodiaceae species were examined for comparative purposes. We used scanning electron microscopy to image 580 specimens of spores from 29 species of the 48 recognised taxa. Four discrete and ten continuous characters were scored for each species and optimised on to the previously published molecular tree. Canonical correspondence analysis (CCA) showed that verrucae width/verrucae length and verrucae width/spore length index and outline were the most important morphological characters. The first two axes explain, respectively, 56.3% and 20.5% of the total variance. Regular depressed and irregular prominent verrucae were present in derived species. However, the morphology does not support any molecular clades. According to our analyses, the evolutionary pathway of the ornamentation of the spores is represented by depressed irregularly verrucae to folded perispore to depressed regular verrucae to irregularly prominent verrucae.

**Keywords:** *character evolution, ferns, eupolypods I, canonical correspondence analysis*

*Serpocaulon* is a fern genus restricted to the tropics and subtropics of the Americas that contain 48 named taxa (Smith et al. 2006; Labiak & Prado 2008; Rojas-Alvarado & Chaves-Fallas 2013; Schwartsburd & Smith 2013; Sanín 2014, 2015, personal observation, October 2015; Sanín & Torrez 2014; Chaves-Fallas et al. 2015) and its monophyly is strongly supported (Schneider et al. 2004b). However, despite the strong support at the clade level, interspecific relationships of the genus for some clades are still not completely resolved using only molecular information (Kreier et al. 2008). In this context, the incorporation of new macro- and micro-morphological characters can provide evidence for species delimitation in the genus and allow for the reconstruction of phylogenetic character evolution.

Spore morphology in *Serpocaulon* has not been studied in this context, although spores have been

useful in phylogenetic analyses of several other groups of ferns (Wagner 1974; Pryer et al. 1995; Moran et al. 2007, 2010; Schneider et al. 2009). Here, we use spore morphology as a phylogenetic tool to (a) explore the evolution of different spore traits within different species of *Serpocaulon*, (b) qualify the sculptural elements and the complexity of the surface ornamentation or patterns that they form, and (c) provide a potential tool to classify monolete verrucate spores and increase the taxonomic resolution of spore records.

### Material and methods

Spores were obtained from herbaria specimens that represent 25 of the species of *Serpocaulon*. This sample means 47% of the 48 described taxa (Table I). Those exemplars are deposited in the following herbaria:

COL, FAUC, HUA, PSO and UPCB; abbreviations follow Thiers (2016); see ‘Specimens examined’. Generally, one specimen per species and 20–50 spores per stub were examined. We studied all species included in the phylogeny of Kreier et al. (2008), with the exception of *S. giganteum*, *S. gilliesii*, *S. latissimum*, *S. loriciforme* and *S. silvulae*. *Serpocaulon giganteum* and *S. loriciforme* were excluded because we do not accept their specific entity. The other three species were excluded because we were not able to obtain any of them. We used four genera as outgroup taxa, including *Campyloneurum*, *Pleopletis*, *Polypodium* and *Microgramma* (see ‘Specimens examined’) (Schneider et al. 2004b; Smith et al. 2006; Kreier et al. 2008)

Spores were processed without treatment from herbarium specimens to aluminium scanning electron microscope (SEM) stubs with double-sided bonding tapes, covered with gold/palladium in a sputter-coater for five minutes, and then, imaged digitally using a Zeiss mod EVO 40 vp SEM at 15 kV (acceleration voltage) at magnifications of 5000× and 15000× in the SEM laboratory at the Smithsonian Tropical Research Institute (STRI), Panama City. Spore images, detailed morphological description and measurements of all species examined for this study can be found at STRI Pollen Database <http://biogeodb.stri.si.edu/jaramillo/palyno/morph/>.

Four discrete morphological spore characters (perispore, prominent verrucae, depressed regular verrucae and depressed irregularly verrucae) and one vegetative character (blade dissection) described for the tree principal morphologies (pinatisect, pinnate and simple (Lellinger 2002); Figure 1A–D, Table I) were observed, studied and optimised using Mesquite 2.75 (Maddison & Maddison 2011) onto the cpDNA strict consensus tree of Kreier et al. (2008, Figure 2); using the principle of parsimony. We have used binary character states for all qualitative characters, except the blade dissection that was multistate. The final trees and figures were made in Mesquite and R (R Development Core Team 2014).

Ten continuous characters, including four indexes (verrucae width/verrucae length [VW/VL], verrucae length/spore length [VL/SL], verrucae width/spore length [VW/SL], verrucae width/spore width [VW/SW], perimeter verruca [PV], area verruca [AV], diameter verruca [DIV], density verruca [DEV], degree of sphericity [RV] and Outline [O]; obtained from the average of three measurements from a central oval) were measured (Figure 1E–G) following the suggestions of Raghavan et al. (2005), Mander et al. (2013) and Han et al. (2014). They were analysed using Image J

(Rasband 1997) (Figure 1E–G, Table II) and optimised on the same topology (Figure 3) using R.

Morphological descriptions follow Tryon and Lugardon (1991) and Punt et al. (2007). Verrucae patterns were quantified from four drawings based on pictures of a 5000× SEM image of each specimen, using CorelDraw, Photoshop and Illustrator software packages for the creation of patterns (Figure 1).

In order to characterise the relationships between species morphology on a quantitative basis, a canonical correspondence analysis (CCA) was performed (Figure 4). A Hellinger transformation of species measurements was made in order to increase the statistical weight. The statistical analyses were performed using R.

## Results

### *Spore morphology and ornamentation*

SEM images revealed that the surface ornamentation of the 25 *Serpocaulon* species are considerably diverse despite them being grouped in the same ornamentation category. All species are characterised by verrucate ornamentation consisting of an element more than 1 µm wide that is wider than long and not constricted at the base. However, the spores of *Serpocaulon* species show different patterns of morphology: irregularly depressed verrucae, depressed regular verrucae, irregularly prominent verrucae and with the presence of folded perine (Figure 1A–D).

All studied species of *Serpocaulon* and four outgroup species are assigned to five classification patterns of ornamentation (Figure 2). Group one includes 16 species that show spores with irregularly depressed verrucae, including three members of the outgroup (*Campyloneuron brevifolium*, *Microgramma lycopodioides*, *Polypodium vulgare*, *S. attenuatum*, *S. crystalloneuron*, *S. falcaria*, *S. fraxinifolium*, *S. intricatum*, *S. lasiopus*, *S. levigatum*, *S. loriceum*, *S. menisciifolium*, *S. polystichum*, *S. ptilorhizon*, *S. subandium* and *S. triseriale*). Group two contains five species whose spores exhibited folded perine (*S. catharinae*, *S. dissimile*, *S. latipes*, *S. sessilifolium* and *S. wagneri*). Group three contains two species with spores with depressed regular verrucae (*S. eleutherophlebium* and *S. patentissimum*), and group four contains six species with spores with prominent verrucae (*S. appressum*, *S. caceresii*, *S. dasypleuron*, *S. maritimum*, *S. polystichum* and *S. richardii*) (Table I). This last group represents the principal polytomy in the phylogenetic analysis. Additionally,

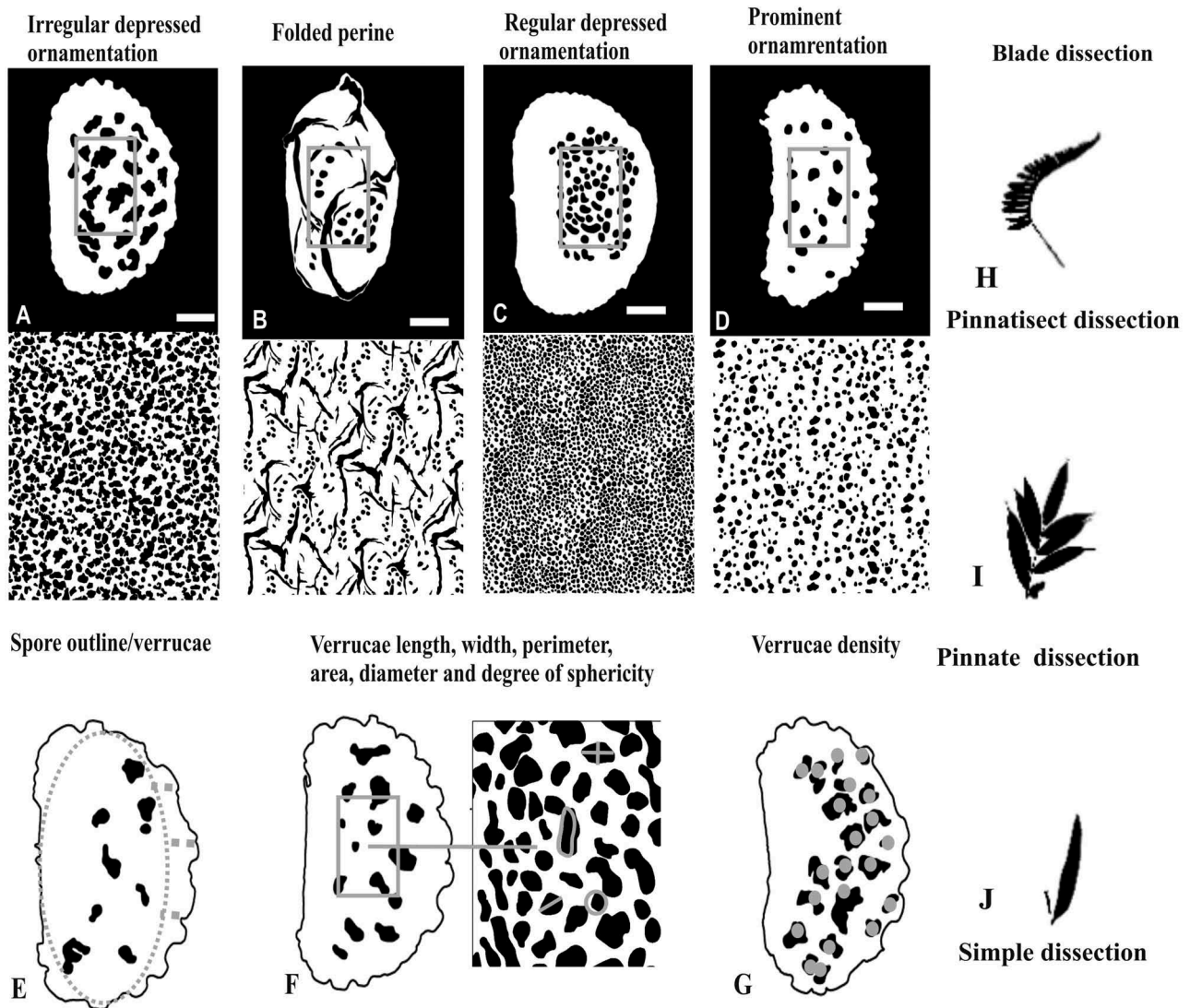


Figure 1. Ornamentation type verrucae: **A.** Spores with irregularly depressed verrucae – (*Serpocaulon triseriale*). **B.** Spores with perine (*Serpocaulon sessilifolium*). **C.** Spores with depressed regular verrucae (*Serpocaulon patentissimum*). **D.** Spores with prominent verrucae (*Serpocaulon caseresii*). **E.** Spore outline/verrucae index measure. **F.** Graphic representation of some measures. **G.** Graphic representation of the verrucae density measure. **H.** Pinnatisect dissection; incised all the way down to the axis, the segments not contracted at their base. **I.** Pinnate dissection; divided into entire to lobed pinnae that are contracted at the base. **J.** Simple dissection; not divided. Definitions for (**H–I**) follow Lellinger (2002). Scale bars – 10  $\mu$ m.

*Pleopeltis bombycina* was the only species exhibiting spores with different ornamentation (Figure 2, Table I, II).

#### Phylogenetic mapping of the qualitative and quantitative characters

The presence of irregularly depressed verrucae was optimised in basal taxa such as *Serpocaulon attenuatum*, *S. menisciifolium* and *S. triseriale*. It is generally common throughout the genus and in some species of the outgroup (Figure 2). The presence of folded perispore was observed as homologous for

clades III, V and VII (Figure 2) and also is a useful character to recognise *S. catharinae* (Figure 5H, I), *S. dissimile* (Figure 5L), *S. latipes* (Figure 6F, G), *S. sessilifolium* (Figure 6C, D) and *S. wagneri* (Figure 5J, K) that occur in the clade IX. Spores with prominent verrucae were presented in derived species and represent a synapomorphy of clade IX, especially exhibited by species in subclade a, and with a reversal to spores without prominent verrucae in *S. polystichum* and *S. fraxinifolium* (Figure 2). Regular depressed verrucae represent a synapomorphy of *S. eleutherophlebium* and *S. patentissimum* (Figures 2, 7).

Table I. Characters and state scored.

	Species	P	DRV	DIV	PV
1	<i>Serpocaulon appressum</i>	1	1	1	0
2	<i>Serpocaulon attenuatum</i>	1	1	0	1
3	<i>Serpocaulon caceresii</i>	1	1	1	0
4	<i>Serpocaulon catharinae</i>	0	1	1	1
5	<i>Serpocaulon crystalloneuron</i>	1	1	0	1
6	<i>Serpocaulon dasypleuron</i>	1	1	1	0
7	<i>Serpocaulon dissimile</i>	0	1	1	1
8	<i>Serpocaulon eleutherophlebium</i>	1	0	1	1
9	<i>Serpocaulon falcaria</i>	1	1	0	1
10	<i>Serpocaulon fraxinifolium</i>	1	1	0	1
11	<i>Serpocaulon intricatum</i>	1	1	0	1
12	<i>Serpocaulon lasiopus</i>	1	1	0	1
13	<i>Serpocaulon latipes</i>	0	1	0	1
14	<i>Serpocaulon levigatum</i>	1	1	0	1
15	<i>Serpocaulon loriceum</i>	1	1	0	0
16	<i>Serpocaulon maritimum</i>	1	1	1	0
17	<i>Serpocaulon menisciifolium</i>	1	1	0	1
18	<i>Serpocaulon patentissimum</i>	1	0	1	1
19	<i>Serpocaulon polystichum</i>	1	1	1	0
20	<i>Serpocaulon ptilorhizon</i>	1	1	0	1
21	<i>Serpocaulon richardii</i>	1	1	1	0
22	<i>Serpocaulon sessilifolium</i>	0	1	1	1
23	<i>Serpocaulon subandinum</i>	1	1	0	1
24	<i>Serpocaulon triseriale</i>	1	1	0	1
25	<i>Serpocaulon wagneri</i>	0	1	1	1
Outgroup					
26	<i>Polypodium vulgare</i>	1	1	0	1
27	<i>Campyloneurum brevifolium</i>	1	1	0	1
28	<i>Microgramma lycopodioides</i>	1	1	0	1
29	<i>Pleopeltis bombycina</i>	1	1	1	1

Spore characters: P, perispore; DRV, depressed regular verrucae; PV, perimeter verruca; DIV, diameter verrucae. Character states: 0, present; 1, absent.

*Serpocaulon patentissimum* presented the highest value for verrucae density versus *S. crystalloneuron*, *S. ptilorhizon*, *S. subandinum* and *S. levigatum*, which presented the lowest values (Figure 3A). The highest verrucae diameter and perimeter were found in *S. dasypleuron* and the lowest values in *S. lasiopus* and *S. sessilifolium* (Figure 3B, C). The degree of sphericity in verrucae was very high in *S. dasypleuron* presenting much higher values compared with the rest of the species, and followed by *S. ptilorhizon*, *S. intricatum* and *S. latipes* (Figure 3D). Likewise, *S. dasypleuron* presented the largest value of verrucae coverage, which is logical, because it presents the highest values of diameter and perimeter, followed by *S. ptilorhizon* and *S. latipes* (Figure 3E). The character of spore outline was well discriminated in *S. dasypleuron*, *S. ptilorhizon*, *S. subandinum*, *S. wagneri*, *S. dissimile* and *S. patentissimum* spores (Figure 3F). Those morphological indexes allowed us to distinguish the following species: *S. ptilorhizon*, *S. levigatum*,

*S. eleutherophlebium*, *S. wagneri* and *S. appressum* (Figure 3G–J).

The performed CCA represents a useful technique to establish groups more objectively than descriptive methods, reducing the subjectivity in morphological characterisation of spore ornamentation using quantitative information. This is a good tool to explore and assign taxa of *Serpocaulon* based in the spore morphology. The best morphological indices for discriminating and grouping species according to the CCA were: VW/VL, VW/SL and VL/SL (Figure 4, Tables III, IV). DEV, AV and PV are also good quantitative discriminants (Figures 3, 4, Tables III, IV) allowing for the separation of species.

The first two functions of the discriminant function analysis (CC1 and CC2) explained 76.8% of the species variation in morphometric variables. CC1 alone explained 56.3% of variation. Eight of 29 species were very well discriminated by DEV (Figures 3, 4, Table IV), whereas VW/VL could discriminate seven species (Figures 3, 4, Table IV) and WW/SL separate along the short to long axis (long verruca). However, eight species were separated by width of the verruca axis and three species by the length and width spore characters (Figure 4, Table IV). VW/SL separated two species, DIV one species, PV three species, and O eight species (Figure 4, Table IV), according to the short to long axis. No distinct assemblages were defined from the ordination diagram (Figure 4), but four groups were assigned from qualitative analysis (Figure 2).

## Discussion

This study is the largest done on spore morphology in *Serpocaulon* (Coelho & Esteves 2011; Ramírez-Valencia et al. 2013). A comparison of our results to similar studies published in other groups suggests that spore morphology is generally a useful tool to distinguish several taxa and explore its phylogeny (Wagner 1974; Korall & Taylor 2006; Moran et al. 2007, 2010; Sundue et al. 2011; Wei & Dong 2012; Wang et al. 2015). However, each specific group could be defined according to its spore morphology, for example, in Aspleniaceae, Holttum (1974) used differences in spore morphology to distinguish two subspecies of *Asplenium phyllitidis* D. Don, where the perispore is uniformly winged in subsp. *phyllitidis* but echinate in subsp. *malesicum*. Wei and Dong (2012) observed that four taxa included in the *A. nidus* L. complex could be distinguished by its

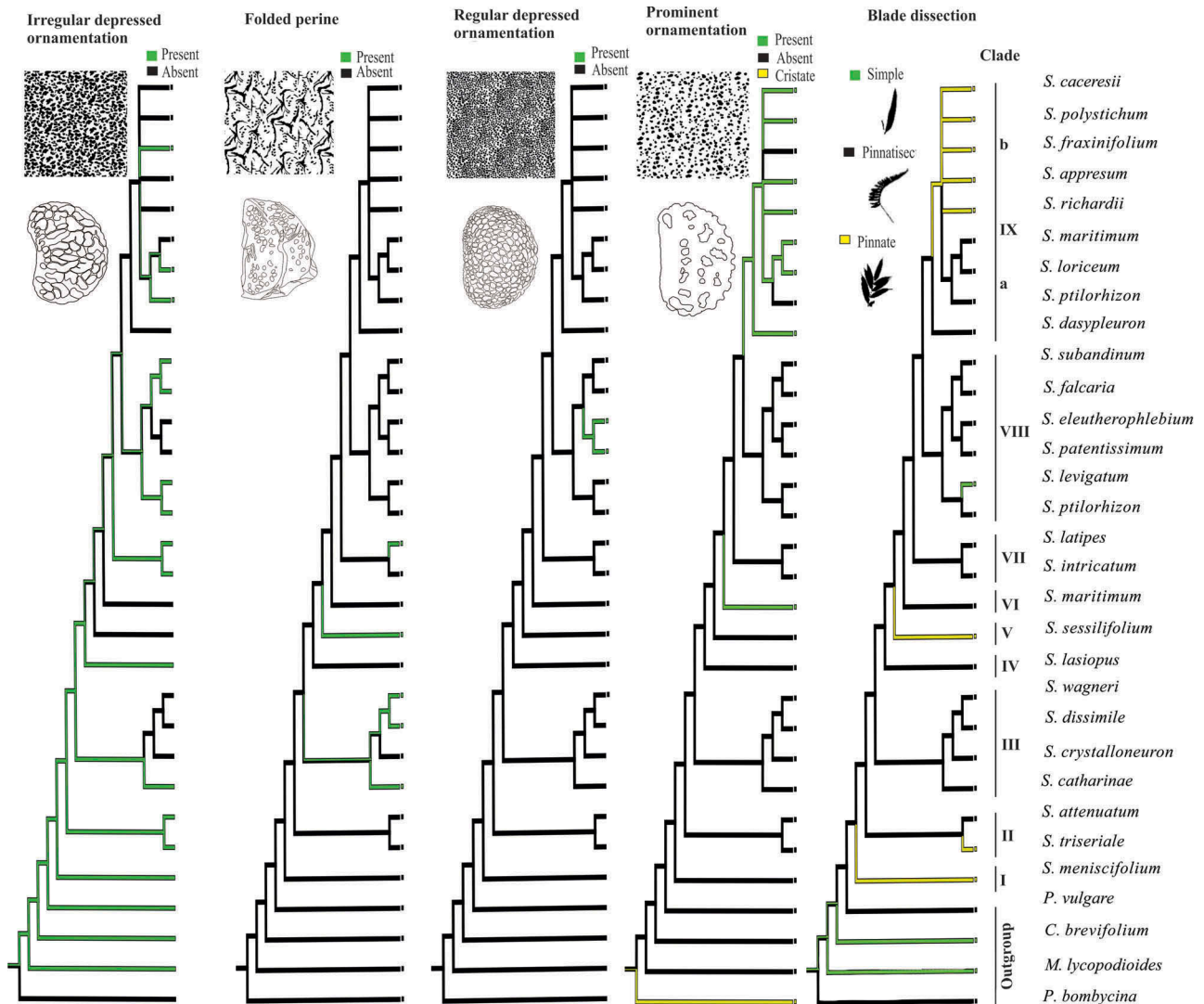


Figure 2. Optimisation of discrete characters of spore and blade dissection on to the pruned strict consensus tree of *Serpocaulon* from Kreier et al. (2008).

unique perispores. Moran et al. (2010) found that the perispore characters are very relevant to define clades in bolbitidoid ferns (Dryopteridaceae), and Korall and Taylor (2006) found that the spore patterns closely correspond to DNA sequence phylogeny in Selaginellaceae.

Contrastingly, in the section *Squamipedia* of *Elaphoglossum* (Dryopteridaceae), the molecular data support its monophyly, but when the spore morphology of the species in this section is compared, the Madagascan species show non-echinulate spores versus the Neotropical ones that are echinulate (Vasco et al. 2015). A similar situation occurred in the new described fern family Desmophlebiaceae and the genus *Desmophlebium*, which exhibits strong molecular and macro-morphological support, whereas the spores of *Desmoph-*

*lebium* have cristate or broadly folded perines and do not strongly distinguish the genus and the family from Aspleniaceae or Hemedictyaceae (Mynssen et al. 2016).

In *Serpocaulon*, and despite that its monophyly is strongly supported (Schneider et al. 2004b) and several molecular information exist (Smith et al. 2006; Kreier et al. 2008), the morphometric and sculptural surface patterns do not exactly correspond to phylogeny, where their characters are homoplastic, and do not support any proposed clade (Figure 2).

#### Spore morphology and ornamentation

*Serpocaulon* spores fell within lengths ranging from 35 to 110  $\mu\text{m}$  and widths between 35 and 95  $\mu\text{m}$ ,



Figure 3. Optimisation of continuous characters of spore and verruca on to the pruned strict consensus tree of *Serpocaulon* from Kreier et al. (2008). **A.** Density verrucae. **B.** Diameter verrucae. **C.** Verrucae perimeter. **D.** Verrucae degree of sphericity. **E.** Area verrucae. **F.** Spore outline. **G.** Verrucae length/spore length. **H.** Verrucae width/spore length. **I.** Verrucae width/spore width. **J.** Verrucae width/verrucae length.

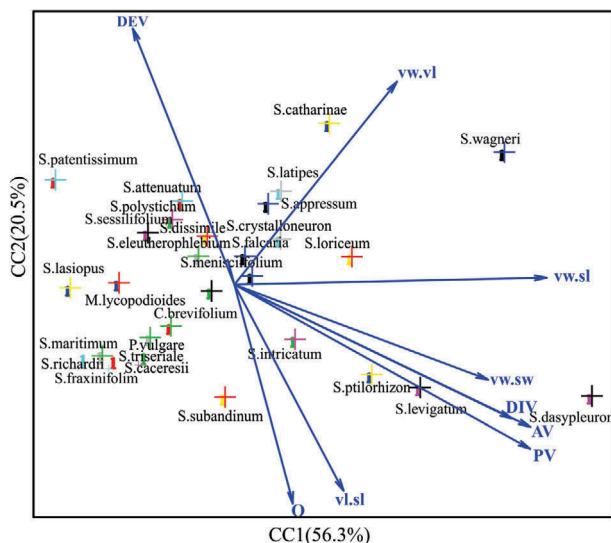


Figure 4. Scatterplot of the first two discriminant functions from a morphometric analysis in 25 species of *Serpocaulon*. CC1 explains 56.3% of the variation and CC2 explains 20.5%.

which is similar to that reported for other Polypodiaceae (Tryon & Lugardon 1991). *Serpocaulon lasiopus* had the largest spore sizes (63–110  $\mu\text{m}$ ), followed by *S. subandinum* (55–104  $\mu\text{m}$ ) and *S. eleutherophlebium* (65–80  $\mu\text{m}$ ) (Table II). A comparison of our spore images to those published elsewhere (Murillo & Bless 1978; Hensen 1990; Tryon & Lugardon 1991; Solé De Porta & Murillo 2005; Contreras-Duarte et al. 2006; Coelho & Esteves 2011; Ramírez-Valencia et al. 2013) suggests that sculpture pattern and perispore morphology is generally consistent within a given species.

There are some remarkable resemblances in spore ornamentation, especially among the types with shallowly tuberculate verrucae or papillate sculpturing, which involves the type of spore common in Polypodiaceae and Davalliaceae (Wagner 1974; Wang et al. 2015). These include such genera as *Campyloneurum*, *Goniophlebium*, *Microgramma*, *Pecluma*, *Phlebodium*, *Pleopeltis*, *Polypodium*, *Pyrrrosia*, *Selliguea*, *Serpocaulon* and *Synammia* in Polypodiaceae (Wagner 1974; Hovenkamp 1986; Tryon & Lugardon 1991; Tejero-Díez et al. 2009; Coelho & Esteves 2011; Ramírez-Valencia et al. 2013; Smith & Tejero-Díez 2014) and *Araioستيgiella*, *Davallia*, *Davallodes*, *Humata* and *Nephrolepis* in Davalliaceae (*sensu* Wagner 1974; Smith et al. 2006; Wang et al. 2015).

Similar spore morphology can be found in the fossil record, including: *Polypodiisporites* aff. *Polypodiisporites speciosus* Sah, *Polypodiisporites* sp. 3 and *Polypodiisporites pachyexinatus* Jaramillo et Dilcher

(Jaramillo & Dilcher 2001). All of these taxa share the verrucae shape of several genera of Polypodiaceae, especially *Phlebodium* (Tejero-Díez et al. 2009) and *Serpocaulon* (Coelho & Esteves 2011; Ramírez-Valencia et al. 2013). This presents a viable possibility to trace the fossil record of these taxa in Cenozoic deposits, especially between the Oligocene and Miocene (Schneider et al. 2004a; Schuettpelz & Pryer 2009).

In a taxonomic context, the perispore in Polypodiaceae has been suggested as a feature that could be useful in delineating some groups (Lloyd 1981). For example, the psilate perispore without folds in most species studied here (*Serpocaulon catharinae*, *S. dissimile*, *S. sessilifolium* and *S. wagneri*) is also reported for other Polypodiaceae genera, such as *Goniophlebium* (Röd-Linder 1990) and *Pyrrrosia* (Hovenkamp 1986), possibly indicating parallel evolution of this spore ornamentation (Coelho & Esteves 2011). However, until now the perispore are absent in the fossil record (Selling 1946).

In addition, our results supports the data of Ramírez-Valencia et al. (2013) that suggest how in *Serpocaulon ptilorhizon*, a sister species and usually confused with *S. funckii* (Moran 1995; Sanín personal observation, October 2015), the size, shape and distance of the verrucae are important characters to distinguish these two taxa, where the first species shows the higher values. In the same line, the clade IXb that represents the principal polytomy (Figure 2) is composed mainly by pinnate blade dissection species such as *S. appressum*, *S. caceresii*, *S. fraxinifolium*, *S. polystichum* and *S. richardii* that could be distinguished morphologically by the correlation of two or more characters like its spore ornamentation (Figure 8A–H), rhizome scale shape, and its blade indumenta (dense trichomes, appressed or patent scales) (Sanín personal observation, October 2010). In this sense, the spore morphology represents a valuable tool for the taxonomy of the genus.

#### Phylogenetic mapping of morphological characters

Clade I includes *Serpocaulon menisciifolium*, which is sister to all other *Serpocaulon* species (Kreier et al. 2008). *Serpocaulon menisciifolium* is characterised by ancestral features, such as pinnate blades (De la Sota 1973; Jarret 1980; Vasco et al. 2014), and spores with irregularly depressed verrucae (Figure 2). The most widespread exospore sculpturing in ferns seems to be either very finely ornamented or essentially smooth (Wagner 1974), which was also found in the earliest-diverging species (clade I and II), in 12 of 25 sampled

species of *Serpocaulon*, and in some species of the outgroup. A remarkable feature in clade II was that those species share the irregularly depressed verruca and incidentally, these species also occur at lowland elevation (Kreier et al. 2008; Sanín personal observation, October 2010).

We found that all the species analysed in the clades III and IV to VII share pinnatisect lamina, which represents a synapomorphy for that clade (Figure 2). The pinnate blade exhibits a relation with prominent verrucae (except in *Serpocaulon dasypleuron*, which presents a pinnatisect blade, and *S. polystichum* that shows irregularly depressed verrucae). This character state changes from pinnate to pinnatisect and reverses back to pinnate two times on the tree. However, the pinnatisect blade is present in 18 of the 25 species evaluated. *Serpocaulon lasiopus* and *S. sessilifolium*, in clades IV and V, respectively, share the presence of perispore with *S. dissimile* and *S. wagneri* (subclade c from clade III), and also share the presence of short creeping rhizomes, thick (5–15 mm; for *S. dissimile* and *S. sessilifolium*; Moran 1995; Sanín personal observation, October 2010) or relatively thick (2.7–6.4 mm; for *S. lasiopus* and *S. wagneri*;

Sanín personal observation, October 2010), and patent scales, which are imbricate, numerous, dense, and cover the rhizome (Moran 1995; Sanín personal observation, October 2010). Interestingly, the wide but short creeping rhizomes, with dense, patent, imbricate scales are features shared by *S. attenuatum*, *S. dissimile*, *S. menisciifolium*, *S. triseriale* and *S. wagneri*, which tend to grow at low or seldom at middle elevations (Smith et al. 2006; Sanín personal observation, October 2010). Along those lines, McHenry and Barrington (2014) suggested that different fern lineages moved to higher elevation, with concomitant morphological changes, including increase in spore size. That was confirming with the species of *Serpocaulon* that inhabits the high Andean forest and Páramo, since those taxa exhibit the higher sizes of the spores in our sample.

The species in the clades VI (*Serpocaulon maritimum*), VII (*S. latipes* and *S. intricatum*) and VIII (*S. eleutherophlebium*, *S. falcaria*, *S. levigatum*, *S. patentissimum*, *S. pilorhizon* and *S. subandinum*) share the long-creeping and thinnest rhizomes (Smith et al. 2006), as well as the pinnatisect

Table II. Quantitative spore characters evaluated in the *Serpocaulon* species.

No.	SL	SW	VL	VW	VW/VL	VL/SL	VW/SL	VW/SW	PV	AV	DIV	DEV	RV	O
1	36	20.3	1.5	3.6	2	0.031	0.075	0.112	9	7	3	23.8	81	2.67
2	43	28	2	3	0.982	0.0405	0.045	0.069	6	3	2	39.4	16	1.44
3	44	23	2	3.5	0.669	0.091	0.060	0.117	9	6	3	14.4	63	3.64
4	49	30	2.5	2.3	1.232	0.007	0.076	0.093	10	7	3	36.6	90	1.44
5	50	39	2.5	5	1.179	0.0475	0.055	0.071	9	7	3	10.4	76	3.00
6	52	40	5	6	0.658	0.154	0.100	0.178	18	26	6	11.4	1076	4.67
7	68	36	3.6	3	0.588	0.064	0.037	0.062	8	5	2	45.9	33	1.20
8	68	35	2.7	3	0.673	0.060	0.040	0.079	9	7	3	39.2	78	1.62
9	44	25	2.3	4.5	1.254	0.053	0.067	0.105	9	7	3	18.6	69	2.90
10	53	29	2.6	4	0.68	0.095	0.044	0.076	7	4	2	15.6	24	3.41
11	40	27	5	2.7	0.617	0.116	0.071	0.112	10	7	3	12.2	91	2.52
12	83	44	2	2.4	0.329	0.047	0.015	0.025	4	1	1	21.4	3	1.86
13	43	28	2	2.4	1.544	0.046	0.071	0.112	10	7	3	44	91	1.54
14	56	35	2.5	7	0.473	0.159	0.075	0.110	13	14	4	12.8	311	3.38
15	50	31	3	6.5	1.261	0.059	0.075	0.111	12	11	4	13.5	205	2.59
16	53	45	2.3	4.4	0.473	0.085	0.040	0.064	7	4	2	11.8	24	3.86
17	39	23	2.6	5	1.1525	0.066	0.076	0.119	9	7	3	15.7	81	3.10
18	56	30	2.5	3.3	0.551	0.055	0.030	0.049	5	2	2	85.6	7	1.19
19	44	31	3	2.7	0.618	0.069	0.042	0.062	6	3	2	51	12	1.63
20	56	49	4	8	0.496	0.121	0.060	0.084	14	15	4	6.7	359	4.41
21	43	28	3	4	0.633	0.086	0.054	0.107	8	5	3	17.5	47	3.83
22	62	39	2	2.5	0.587	0.036	0.021	0.035	5	2	1	30	5	1.29
23	75	50	3.5	6	0.594	0.091	0.054	0.106	13	14	4	5	296	5.18
24	57	34	2	5.3	0.441	0.087	0.038	0.064	8	5	2	21.4	36	2.78
25	56	36	1.5	3.3	1.175	0.082	0.094	0.096	10	8	3	34	96	1.19
26	50	30	7.5	3.2	0.493	0.058	0.028	0.043	11	10	4	13.6	162	2.96
27	63	32	3.6	1.5	0.563	0.124	0.071	0.110	5	2	2	40.8	5	1.65
28	50	27	2.3	1	0.405	0.063	0.026	0.045	3	1	1	25	1	1.13
29	62	34	NA	NA	0.4109	0.045	0.019	0.038	0	0	NA	NA	0	NA

Quantitative spore characters (mean). 1–29: Species evaluated; see Table I; SL, spore length; SW, spore width; VL, verrucae length; VW, verrucae width; VW/VL, verrucae width/verrucae length; VL/SL, verrucae length/spore length; VW/SL, verrucae width/spore length; VW/SW, verrucae width/spore width; PV, perimeter verruca; AV, area verruca; DIV, diameter verruca; DEV, density verruca; RV, rounded verruca; O, Outline.



Table III. Scores of morphological characters according to the two first axes of the canonical correspondence analysis (CCA).

Character	CCA1	CCA2
VW/VL	182.535.032	<b>10.228.095</b>
VL/SL	198.941.250	-18.152.680
VW/SL	<b>511.708.510</b>	<b>27.800.745</b>
VW/SW	-297.289.482	-18.096.609
DEV	-0,73491253	0,9589851
O	-232.562.442	-0,7488698
DIV	-278.048.235	-0,6521758
AV	<b>371.984.882</b>	0,6261982
PV	<b>-0,01567034</b>	-0,3688515

Note: Bold numbers correspond to parameters, which are best correlated to the axes. Abbreviations: see caption of Table II.

blades, except for *S. levigatum*, which is the only species in the genus that exhibits a simple lamina (Smith et al. 2006; Sanín 2014, 2015; Sanín & Torrez 2014), this feature is recognised as an auto-apomorphy for the species and has been found in other derived eupolypod I genera like *Elaphoglossum* (Dryopteridaceae; Wagner 1974; Moran et al. 2010), *Campyloneurum*, *Grammitis*, *Microgramma* and *Niphidium* (Polypodiaceae; Schuettpelz & Pryer 2007; Vasco et al. 2015). The entire blade represents a derived character from ancestors with more divided leaves (De la Sota 1973; Vasco et al. 2015). In this sense, the hypothesis of Smith et al. (2006), supported and complemented by Kreier et al. (2008), that suggest how the current taxa of *Serpocaulon* arose from species growing primarily in lowlands to mid-elevations rainforest, but that species exhibiting more derived characters occur predominantly in the Andean rainforest at higher elevations. This hypothesis could also be tested by the morphologic variation of the ornamentation of the character transition from basal to derived species, represented by the following transitions: irregularly depressed verrucae to conspicuous perispore to irregularly prominent verrucae to depressed regular verrucae (Figures 1, 2).

The subclade IXa is characterised by species that have irregularly depressed verrucae and correlated with pinnatisect lamina, long creeping rhizomes (Smith et al. 2006) and rounded, appressed scales. Subclade IXb also shares the feature of long creeping rhizomes. Additionally, with the exception of *Serpocaulon dasyleurum*, *S. fraxinifolium*, *S. maritimum* and *S. polystichum*, the other species of subclade IXa share pinnate lamina and prominent verrucae. Interestingly, all the species in this clade (except the widely distributed *S. fraxinifolium*) tend to inhabit lowlands, and some of them are widely distributed in the Amazon basin (*S. caceresii* and *S. richardii*) (Sanín personal observation, October 2010) In addition, some of the

pinnate species present a wide range of growth habits, from epiphytic, epipetric to terrestrial, which partially fits with the suggestions of Kluge and Kessler (2007), who mention that the species with highly dissected lamina tend to present terrestrial habits or a combination of growth habits.

The pinnatisect species and *Serpocaulon polystichum* (pinnate lamina) are the only exception to support the relationship between the features of prominent verruca and pinnate lamina in clade IX. This could represent a reversion of characters for these species. However, it is a possible misidentification of several taxa into this clade, because, as Schwartzburd and Smith (2013) suggest, several taxonomic uncertainties exist in the genus. This was the case of *S. giganteum* (= *S. articulatum*) (Schwartzburd & Smith 2013). In addition, several taxonomic problems are still unresolved in the species complexes of *S. fraxinifolium* and *S. loriceum* (Moran 1995; Smith et al. 2006; Kreier et al. 2008; Sanín personal observation, October 2010).

## Conclusion

The spores of 25 *Serpocaulon* species and three outgroup taxa used in this study exhibit four types of ornamentation patterns that can be represented by the following evolutionary pathway, according to our analyses: depressed irregularly verrucae to folded perispore to depressed regular verrucae to irregularly prominent verrucae. The micro-morphological characters studied here of the verrucae are important to differentiate the taxa of *Serpocaulon* and to contribute to the classification of monolete verrucate spores to increase taxonomic resolution of spore records in different periods of time, their phylogenetic relationships and morphological evolution. However, these kinds of characters (especially quantitative characters) are little used or often omitted in almost all previous systematic studies on *Serpocaulon* and in general in monolete, verrucae spores. The irregularly depressed verruca was present in ancestral species. The presence of folded perispore not closely appressed was mapped as a synapomorphy shared by the clades III, V and VII, and could be linked with species with pinnatisect blades. Our results support previous hypotheses that suggest how *Serpocaulon* arose from species growing primarily in lowland to colonising the mid- to high-elevation Andean mountain forests of South and Central America.

## Acknowledgements

The authors especially thank Carlos Jaramillo (Smithsonian Tropical Research Institute [STRI],

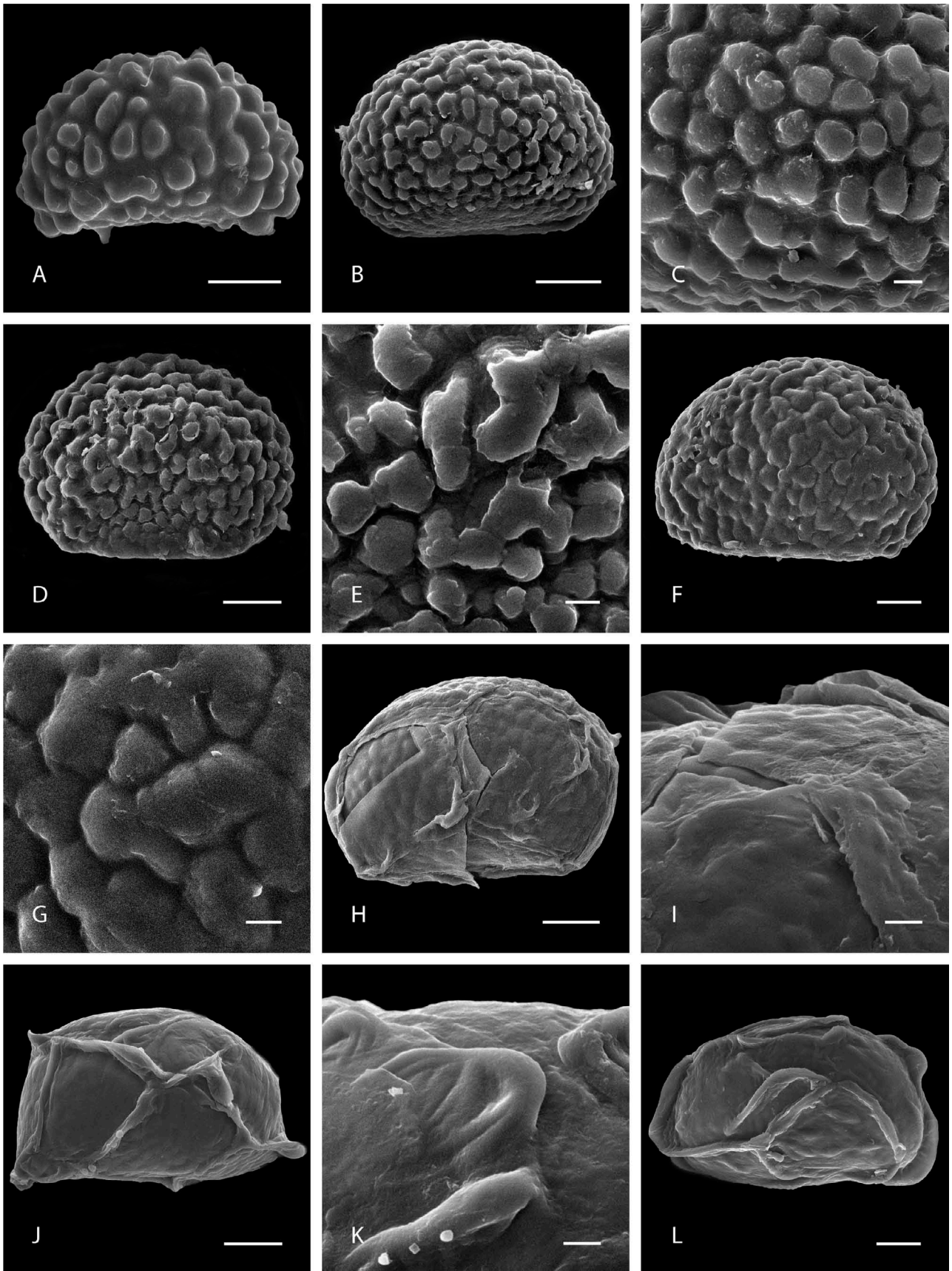


Figure 5. Spores of *Serpocaulon*. **A.** *Serpocaulon menisciifolium*. **B, C.** *Serpocaulon attenuatum*. **D, E.** *Serpocaulon triseriale*. **F, G.** *Serpocaulon crystalloneurum*. **H, I.** *Serpocaulon catharinae*. **J, K.** *Serpocaulon wagneri*. **L.** *Serpocaulon dissimile*. Scale bars – 10  $\mu\text{m}$  (A, B, D, F, H, J, L), 5  $\mu\text{m}$  (C, E, G, I, K).

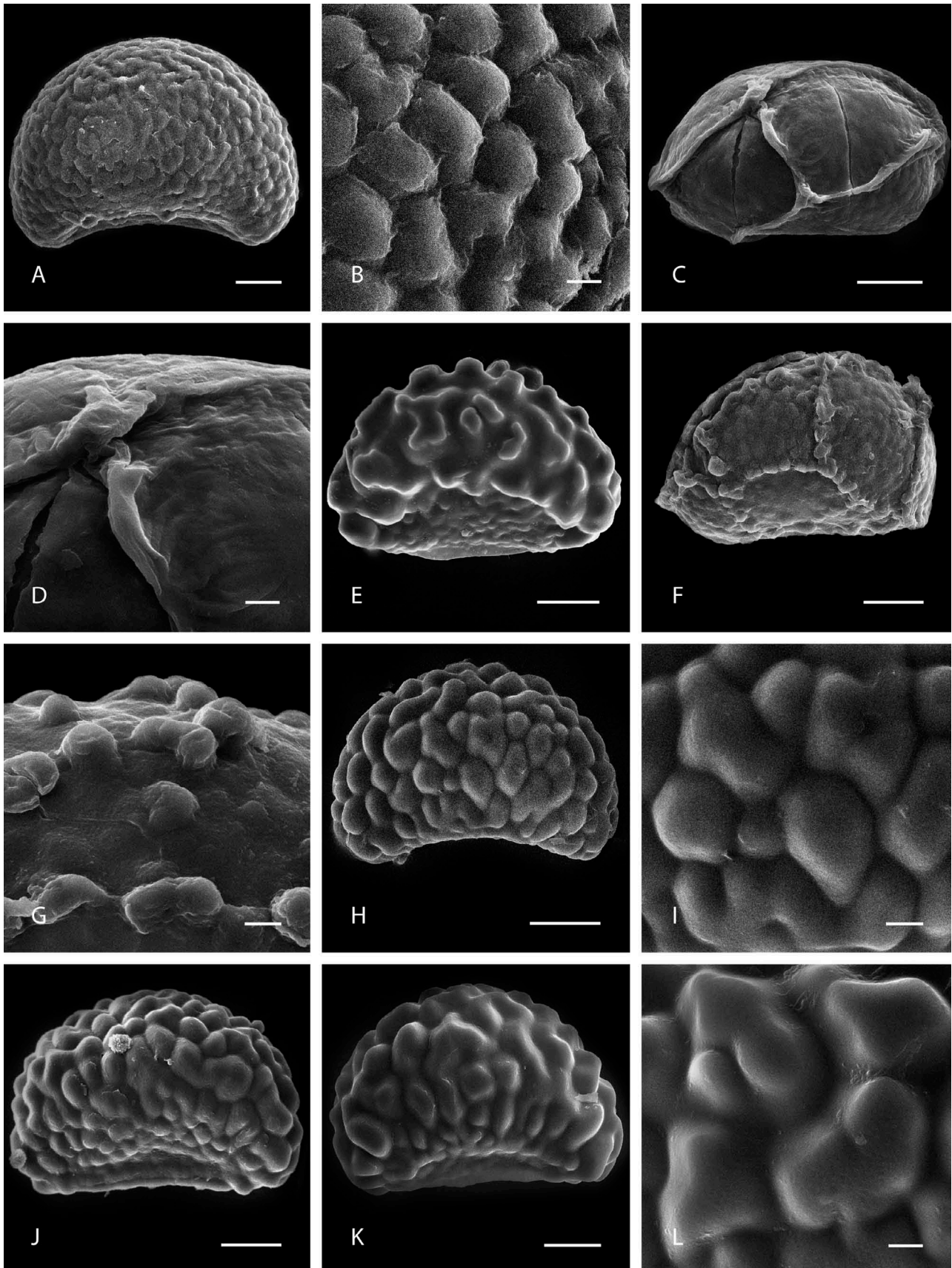


Figure 6. Spores of *Serpocaulon*. **A, B.** *Serpocaulon lasiopus*. **C, D.** *Serpocaulon sessilifolium*. **E.** *Serpocaulon maritimum*. **F, G.** *Serpocaulon latipes*. **H, I.** *Serpocaulon intricatum*. **J.** *Serpocaulon levigatum*. **K, L.** *Serpocaulon ptilorhizon*. Scale bars – 10  $\mu\text{m}$  (A, C, E, F, H, J, K), 5  $\mu\text{m}$  (B, D, G, I, L).

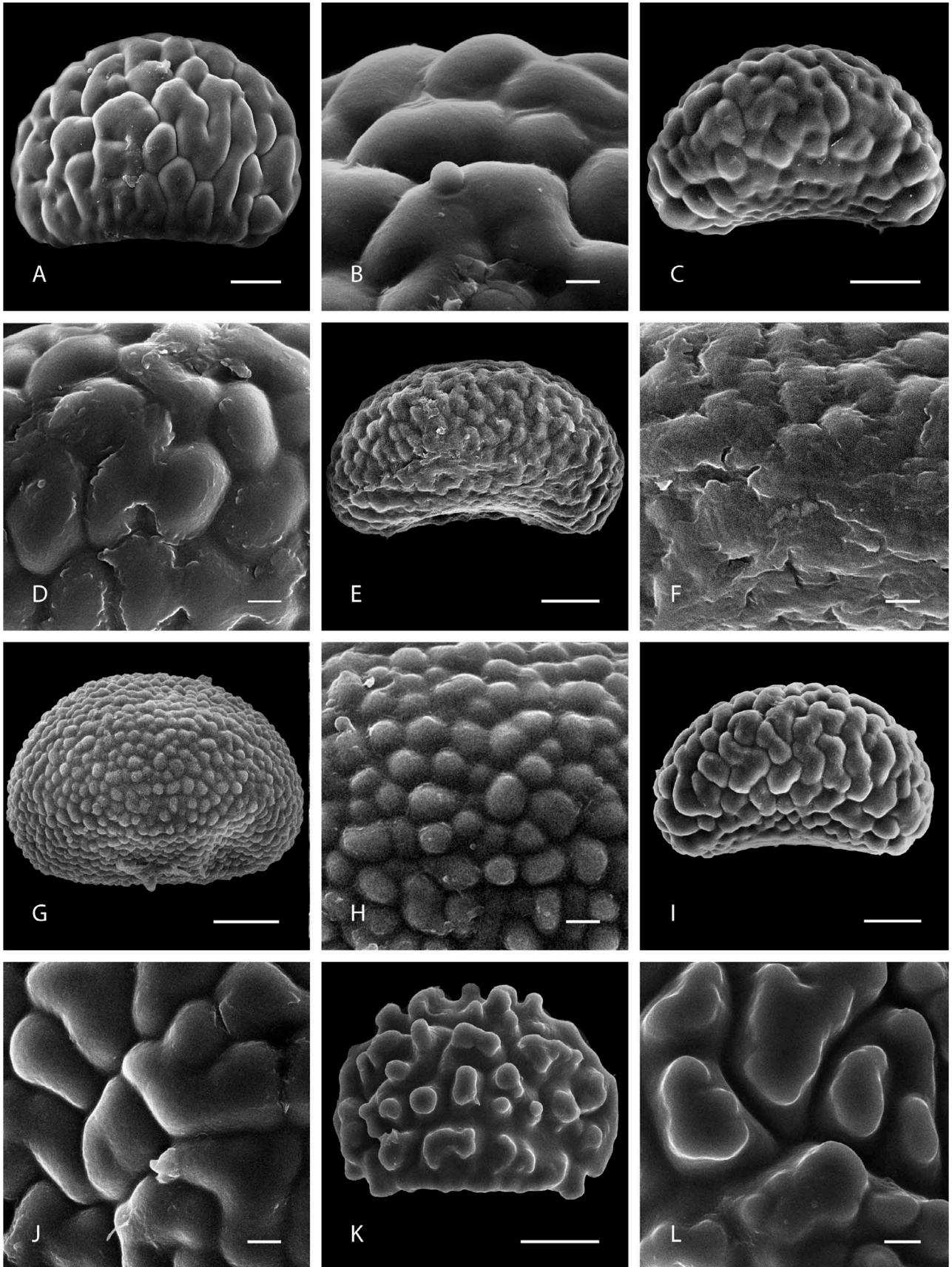


Figure 7. Spores of *Serpocaulon*. A, B. *Serpocaulon subandium*. C, D. *Serpocaulon falcaria*. E, F. *Serpocaulon eleutherophlebium*. G, H. *Serpocaulon patentissimum*. I, J. *Serpocaulon loriceum*. K, L. *Serpocaulon dasypleuron*. Scale bars – 10  $\mu\text{m}$  (A, C, E, G, I, K), 5  $\mu\text{m}$  (B, D, F, H, J, L).

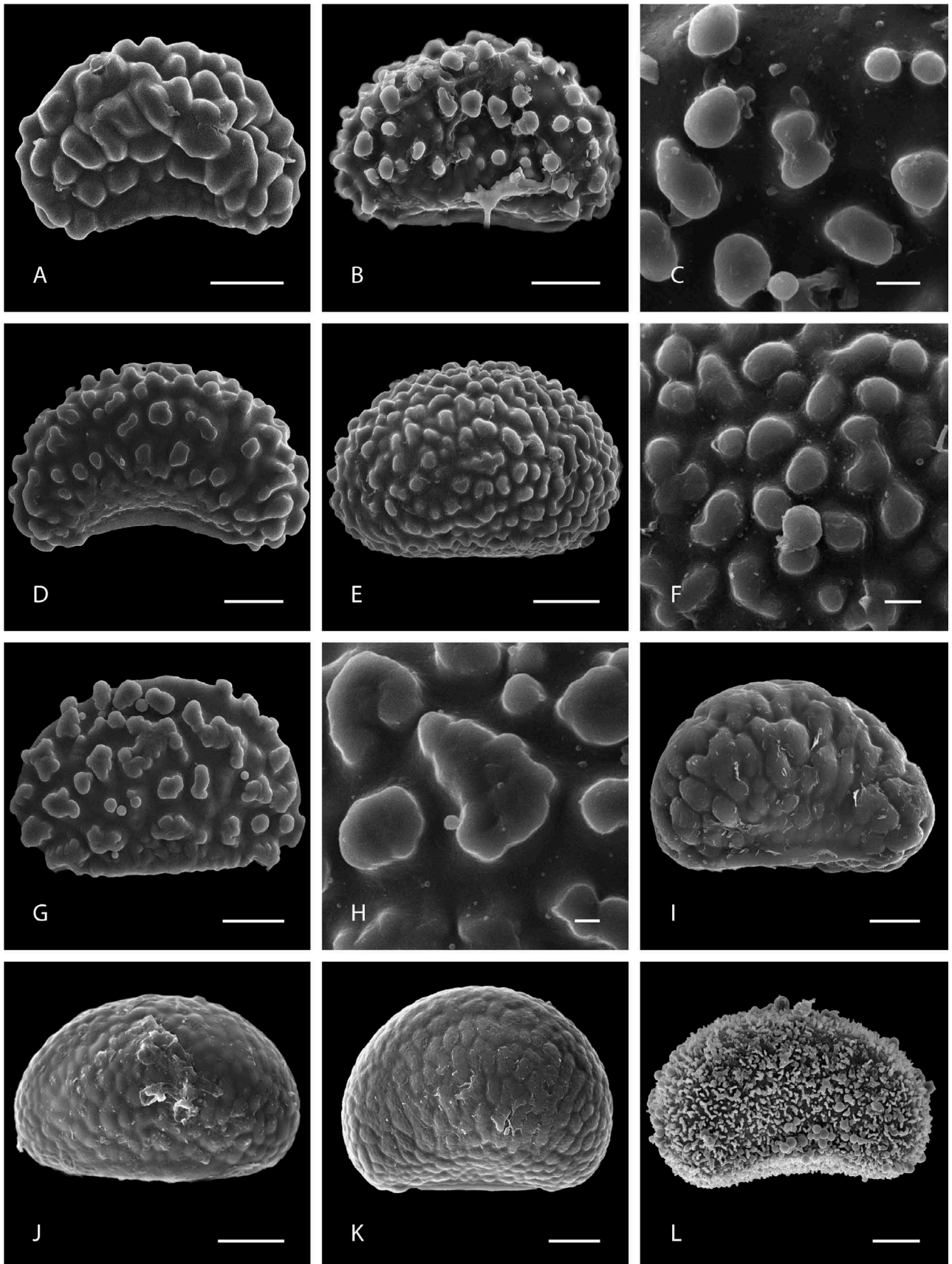


Figure 8. Spores of *Serpocaulon*. **A.** *Serpocaulon fraxinifolium*. **B, C.** *Serpocaulon richardii*. **D.** *Serpocaulon appressum*. **E, F.** *Serpocaulon polystichum*. **G, H.** *Serpocaulon caceresii*. **I.** *Polypodium vulgare*. **J.** *Campyloneuron brevifolium*. **K.** *Microgramma lycopodioides*. **L.** *Pleopeltis bombycina*. Scale bars – 10  $\mu\text{m}$  (A, B, D, E, G, I, J, K, L), 5  $\mu\text{m}$  (C, F, H).

Table IV. Scores of species variability according to the two first axes of the canonical correspondence analysis (CCA).

Species	VW/VL	VL/SL	VW/SL	VW/SW	DEV	O	DIV	AV	PV
<i>Microgramma lycopodioides</i>	-8.00E+03	-6.08E+01	-4.53E+01	-6.46E+01	-1.58E+04	-5.20E+02	-1.00E+03	-1.00E+03	-2.00E+03
<i>Polypodium vulgare</i>	-3.00E+03	-6.59E+01	-4.33E+01	-6.71E+01	-2.72E+04	1.31E+03	2.00E+03	8.00E+03	6.00E+03
<i>Serpocaulon appressum</i>	1.80E+04	-9.33E+01	3.67E+00	2.31E+00	-1.70E+04	1.02E+03	1.00E+03	5.00E+03	4.00E+03
<i>S. attenuatum</i>	1.00E+04	-8.39E+01	-2.58E+01	-4.04E+01	-1.40E+03	-2.10E+02	-8.96E-15	1.00E+03	1.00E+03
<i>S. caceresi</i>	8.00E+03	-3.30E+01	-1.06E+01	7.21E+00	-2.64E+04	1.99E+03	1.00E+03	4.00E+03	4.00E+03
<i>S. catharinae</i>	1.70E+04	-1.17E+02	4.76E+00	-1.66E+01	-4.20E+03	-2.10E+02	1.00E+03	5.00E+03	5.00E+03
<i>S. crystalloneuron</i>	1.60E+04	-7.69E+01	-1.55E+01	-3.84E+01	-3.04E+04	1.35E+03	1.00E+03	5.00E+03	4.00E+03
<i>S. dasyleuron</i>	7.00E+03	2.99E+01	2.96E+01	6.79E+01	-2.94E+04	3.02E+03	4.00E+03	2.40E+04	1.30E+04
<i>S. dissimile</i>	2.00E+03	-6.04E+01	3.35E+01	-4.78E+01	5.10E+03	-4.50E+02	-5.84E-15	3.00E+03	3.00E+03
<i>S. elutherophlebium</i>	9.00E+03	-6.39E+01	-3.06E+01	-3.06E+01	-1.60E+03	-3.00E+01	1.00E+03	5.00E+03	4.00E+03
<i>S. falcaria</i>	1.30E+04	-7.06E+01	-3.91E+00	-4.67E+00	-2.22E+04	1.25E+03	1.00E+03	5.00E+03	4.00E+03
<i>S. fraxinifolium</i>	-6.00E+03	-2.92E+01	-2.69E+01	-3.41E+01	-2.52E+04	1.76E+03	2.29E-14	2.00E+03	2.00E+03
<i>S. intricatum</i>	4.00E+03	-7.97E+00	5.23E-01	2.16E+00	-2.86E+04	8.70E+02	1.00E+03	5.00E+03	5.00E+03
<i>S. lasiopus</i>	-9.00E+03	-7.70E+01	-5.56E+01	-8.49E+01	-1.94E+04	2.10E+02	-1.00E+03	-1.00E+03	-1.00E+03
<i>S. latipes</i>	1.50E+04	-7.79E+01	5.23E-01	2.16E+00	3.20E+03	-1.10E+02	1.00E+03	5.00E+03	5.00E+03
<i>S. levigatum</i>	-4.00E+03	3.46E+01	3.72E+00	3.80E-01	-2.80E+04	1.73E+03	2.00E+03	1.20E+04	8.00E+03
<i>S. loriceum</i>	1.40E+04	-6.47E+01	3.82E+00	1.51E+00	-2.73E+04	9.40E+02	2.00E+03	9.00E+03	7.00E+03
<i>S. maritimum</i>	-5.00E+03	-3.92E+01	-3.13E+01	-4.59E+01	-2.90E+04	2.21E+03	5.52E-14	2.00E+03	2.00E+03
<i>S. meniscifolium</i>	1.10E+04	-5.83E+01	4.80E+00	9.10E+00	-2.51E+04	1.45E+03	1.00E+03	5.00E+03	4.00E+03
<i>S. patenissimum</i>	-1.00E+03	-6.93E+01	-4.11E+01	-6.07E+01	4.48E+04	-4.60E+02	1.96E-14	-1.42E-13	6.24E-13
<i>S. polystrichum</i>	5.00E+03	-5.52E+01	-2.85E+01	-4.81E+01	1.02E+04	-2.00E+01	8.09E-14	1.00E+03	1.00E+03
<i>S. pilorhizon</i>	-2.00E+03	-2.56E+00	-1.12E+01	-2.53E+01	-3.41E+04	2.76E+03	2.00E+03	1.30E+04	9.00E+03
<i>S. richardii</i>	6.00E+03	-3.85E+01	-1.71E+01	-2.54E+00	-2.33E+04	2.18E+03	1.00E+03	3.00E+03	3.00E+03
<i>S. sessilifolium</i>	1.00E+03	-8.79E+01	-4.98E+01	-7.48E+01	-1.08E+04	-3.60E+02	-1.00E+03	7.58E-13	6.28E-13
<i>S. subandinum</i>	3.00E+03	-3.32E+01	-1.73E+01	-3.63E+00	-3.58E+04	3.53E+03	2.00E+03	1.20E+04	8.00E+03
<i>S. triseriale</i>	-7.00E+03	-3.68E+01	-3.27E+01	-4.54E+01	-1.94E+04	1.13E+03	-1.11E-13	3.00E+03	3.00E+03

Note: Bold numbers correspond to species, which are best correlated to axes. Abbreviations: see caption of Table II.

Panamá) and Andres Pardo Trujillo (IIES, Colombia) for their kind support. The authors would like to thank colleagues at the COL, CAUP, HUA, FAUC, PSO and UPCB herbaria for allowing to study the collections, and to Jorge Ceballos (STRI) for his assistance in the SEM laboratory, María José Leño (UNIANDES) for his graphic assistance and Simon Quintero (UNIANDES) for special support. Sincere thanks to Michael Sundue (UVM), Lucas Majure (DBG), Robbin Moran (NYBG) and the anonymous reviewers for providing constructive comments. This work was supported by the Centre of Paleoecology and Archaeology (CTPA) at the STRI and Caldas University (IIES Institute and FAUC Herbarium).

### Disclosure statement

No potential conflict of interest was reported by the authors.

### Specimens examined

- Serpocaulon appressum* (Copel.) A. R. Sm., Bolivia, Provincia de Nor Yungas: Municipio de Coroico, Parque Nacional Cota Pata, Camino antiguo de Sillutinkara, 2444 m, 16° 15' S, 67° 53' W, 15 January 2012, Sanín et al. 5535 (HUA).
- Serpocaulon attenuatum* (C. Presl) A. R. Sm., Colombia, Meta: Reserva Natural la Macarena, margen izquierdo del río Guaya-bero, 1er raudal, 7 June 1970, Echeverry 2037 (COL).
- Serpocaulon caceresii* (Sodirol) A. R. Sm., Colombia, Antioquia, Cocorná, vereda La Piñuela, carretera a San Francisco, 1000–1500 m, 13 abr 1991, Giraldo-Cañas 71 (HUA).
- Serpocaulon catharinae* (Langsd. et Fisch.) A. R. Sm., Brasil, Paraná: Curitiba, campus Politécnico Universidad Federal de Paraná, frente a la Facultad de Ciencias Biológicas, 23 June 2010, Sanín 4199 (HUA).
- Serpocaulon crystalloneuron* (Rosenst.) A. R. Sm., Bolivia, Provincia de Nor Yungas: Municipio de Coroico, Parque Nacional Cota Pata, Camino antiguo de Sillutinkara. 14 January 2012, Sanín et al. 5530 (HUA).
- Serpocaulon dasypleuron* (Kunze) A. R. Sm., Colombia, Amazonas: río Miriparaná, Cerro de la Gente Chiquita in head waters of Quebrada Guacayá, sin dat., 18 May 1952, Schultes 16501 (COL).
- Serpocaulon dissimile* (L.) A. R. Sm., Costa Rica, San Vito de Coto Brus: Jardín Botánico Robert y Catherine Wilson, Estación Biológica Las Cruces, O.E.T., sendero vía al Río Java, 1211 m, N 08°47'0.3', W 82°57'34', 14 June 2011, Sanín & Santiago 5019 (HUA).
- Serpocaulon eleutherophlebium* (Fée) A. R. Sm., Colombia, Cauca, Popayán, Parque Nacional Natural Puracé, sector anexo a las bases militares, 3887 m, 02° 22' N, 76° 26' W, 26 July 2009, Sanín & Sanín 3293 (FAUC).
- Serpocaulon falcaria* (Kunze) A. R. Sm., Costa Rica; Cordillera de Talamanca; Cerro de la Muerte, al margen de la carretera vía Savegre, 2484 m, 09° 32' N, 83° 48' W, 20 June 2011, Sanín & Torrez 5031 (HUA).
- Serpocaulon fraxinifolium* (Jacq.) A. R. Sm., Bolivia, Provincia de Nor Yungas: Municipio de Coroico, Parque Nacional Cota Pata, Camino antiguo de Sillutinkara, 2444 m, 16° 15' N, 67° 53' W, 15 January 2012, Sanín et al. 5537 (HUA).

- Serpocaulon intricatum* (M. Kessler et A. R. Sm.) A. R. Sm., Bolivia, Provincia de Nor Yungas: Municipio de Coroico, Parque Nacional Cota Pata, Camino antiguo de Sillutinkara, 2178 m, 16° 14' N, 67° 53' W, 15 January 2012, Sanín et al. 5526 (HUA).
- Serpocaulon lasiopus* (Klotzsch) A. R. Sm., Colombia, Cundinamarca: Laguna de Ubaque, 2000 m, 11 July 1974, Acosta-Arteaga 595 (COL).
- Serpocaulon latipes* (Langsd. et L. Fisch.) A. R. Sm., Brazil, Paraná: Curitiba, Paranaguá, río Guaraguaçu, Entrada para Sambaqui, 11 m, 25° 39' S, 48° 30' W, 1 July 2010, Sanín & Labiak 4200 (HUA).
- Serpocaulon levigatum* (Cav.) A. R. Sm., Colombia, Caldas: Manizales-Villamaria, vereda Montaña, finca San Antonio, relicto al nor-oriente de la casa, 2226 m, 4° 59' N, 75° 27' W, 7 Ago 2009, Sanín et al. 3315 (FAUC).
- Serpocaulon loriceum* (L.) A. R. Sm., Costa Rica, San Vito de Coto Brus: Jardín Botánico Robert y Catherine Wilson, Estación Biológica Las Cruces, O.E.T., sendero via al Río Java, Sanín & Santiago 5014 (HUA).
- Serpocaulon maritimum* (Hieron.) A. R. Sm., Colombia, Nariño: Junín, en dirección a Barbacoas, 01° 20' N, 78° 02' W, 1000 m, 20 Dic 1972, Haegemann & Leist 1750 (COL).
- Serpocaulon menisciifolium* (Langsd. et Fisch.) A. R. Sm., Brasil, Paraná: Antonina, Reserva Natural Rio Cachoeira (SPVS), estrada Sede-Gervásio, Matos 544 (UPCB).
- Serpocaulon patentissimum* (Mett. ex Kuhn) A. R. Sm., Colombia, Nariño: Reserva Natural la Planada, sendero el Rondón cerca 2 km del Centro de Investigación, 1900 m, 01° 09' N, 77° 58' W, 19 January 1997, Herrera 9140 (PSO).
- Serpocaulon polystichum* (Link) A. R. Sm., Colombia, Antioquia: Valdivia, vereda San Fermín, 1 km de la vía Ventanas (Mun. Yarumal) Briceño, 1700 m, 07° 08' N, 75° 30' W, 22 March 1988, Arbeláez et al. 313 (HUA).
- Serpocaulon ptilorhizon* (Christ) A. R. Sm., Colombia, Antioquia: Guatapé, vereda Santa Rita, finca Montepinar, bosque pluvial, 1850 m, 06° 16' N, 75° 10' W, 6 February 1990, Echeverry 195 (HUA).
- Serpocaulon richardii* (Klotzsch) A. R. Sm., Colombia, Boyacá: carretera a Pauna-Otro Mundo, 1350 m, 16 October 1967, Jaramillo-Mejía et al. 3650 (COL).
- Serpocaulon sessilifolium* (Desv.) A. R. Sm., Bolivia, Provincia de Nor Yungas: Coroico, Parque Nacional Cota Pata, Camino antiguo de Sillutinkara, 1962 m, 16° 13' S, 67° 52' W, 15 January 2012, Sanín et al. 5534 (HUA).
- Serpocaulon subandinum* (Sodirol) A. R. Sm., Bolivia, Provincia de Nor Yungas Municipio de Coroico, Parque Nacional Cota Pata, Camino Precolombino de Sillutinkara, 3069 m, 16° 16' S, 67° 53' W, 14 January 2012, Sanín et al. 5521 (HUA).
- Serpocaulon triseriale* (Sw.) A. R. Sm., Colombia, Caldas: Neira, vía Aránzazu, 4 km del casco urbano, 1350 m, 05° 12' N, 75° 35' W, 3 March 2008, Sanín 2602 (FAUC).
- Serpocaulon wagneri* (Mett.) A. R. Sm., Colombia, Cundinamarca: Ubalá, Vereda San Roque, camino a Campo Hermoso, 1150 m, 30 June 1998, Fernández-Alonso 16217 (COL).
- Campyloneurum brevifolium* (Lodd. ex Link) Link. Colombia, Antioquia, Anorí, Región Providencia, sector Aljibes-La Tirana, 500 m, 18 January 2004, Rodríguez 4484 (COL).
- Microgramma lycopodioides* (L.) Copel. Colombia, Norte de Santander, San Cayetano, Vereda Ayacucho, Finca Bellavista, 1355 m, 11 May 2009, Infante-Betancour 623 (COL).
- Pleopeltis bombycina* (Maxon) A.R. Sm. Colombia, Antioquia, Municipio de San Rafael, Morro Pan de Azúcar, 1100–1354 m, 14 October 1981, Orozco, et al. 754 (COL).
- Polypodium vulgare* L. Alemania, Poiana, Uzului, 500 m, 22 August 1970, Barabas & Mititelu 1085 (COL).

## ORCID

David Sanín  <http://orcid.org/0000-0003-0680-5531>

## References

- Chaves-Fallas JM, Moran RC, Oviedo-Brenes F. 2015. *Serpocaulon rojasianum* (Polypodiaceae): A new fern hybrid from Costa Rica. *Brittonia* 67: 185–190. doi:10.1007/s12228.015.9368.2.
- Coelho C, Esteves L. 2011. Spore morphology of *Serpocaulon* A. R. Sm. and related taxa from Brazil (Polypodiaceae). *Grana* 50: 165–181. doi:10.1080/00173134.2011.614727.
- Contreras-Duarte AR, Bogotá-Ángel J, Jiménez-Bullla LC. 2006. Atlas de las esporas de Pteridofitos de Chipaque (Cundinamarca, Colombia). *Caldasia* 28: 327–357.
- De la Sota ER. 1973. On the classification and phylogeny of the Polypodiaceae. In: Jermy AC, Crabbe JA, Thomas BA, eds. The phylogeny and classification of ferns, 229–244. London: Academic Press.
- Han JH, Cao H, Barb A, Punyasena SW, Jaramillo CA, Shyu C. 2014. A Neotropical Miocene pollen database employing image-based search and semantic modeling. *Applications in Plant Sciences* 2: 1–17. doi:10.3732/apps.1400030.
- Hensen RV. 1990. Revision of the *Polypodium loricum*-complex. *Nova Hedwigia* 50: 279–236.
- Holtum RE. 1974. *Asplenium* L. sect. *Thamnopteris* Presl. *Garden Bulletin of Singapur* 27: 143–154.
- Hovenkamp PH. 1986. A monograph of the fern genus *Pyrrhosia*. *Leiden Botanical Series* 9: 1–280.
- Jaramillo CA, Dilcher D. 2001. Middle Paleogene palynology of central Colombia, South America: A study of pollen and spores from tropical latitudes. *Palaeontographica Abt. B* 258: 87–123.
- Jarret FM. 1980. Studies in the classification of the leptosporangiate ferns: I. The affinities of the Polypodiaceae *sensu stricto* and the Grammitidaceae. *Kew Bulletin* 34: 825–833. doi:10.2307/4119077.
- Kluge K, Kessler M. 2007. Morphological characteristics of fern assemblages along an elevational gradient: Patterns and causes. *Ecotropica* 13: 27–43.
- Korall P, Taylor W. 2006. Megaspore morphology in the Selaginellaceae in a phylogenetic context: A study of the megaspore surface and wall structure using scanning electron microscopy. *Grana* 45: 22–60. doi:10.1080/00173130500520453.
- Kreier HP, Rex M, Weising K, Kessler M, Smith AR, Schneider H. 2008. Inferring the diversification of the epiphytic fern genus *Serpocaulon* (Polypodiaceae) in South America using chloroplast sequences and amplified fragment length polymorphisms. *Plant Systematics and Evolution* 274: 1–16. doi:10.1007/s00606-008-0021-3.
- Labiak P, Prado J. 2008. New combinations in *Serpocaulon* and a provisional key for the Atlantic rain forest species. *American Fern Journal* 98: 139–159. doi:10.1640/0002-8444(2008)98139:NCISAAJ2.0.CO;2.
- Lellinger DB. 2002. A modern multilingual glossary for taxonomic pteridology. *Pteridologia* 3: 1–263.
- Lloyd RM. 1981. The perispore in *Polypodium* and related genera (Polypodiaceae). *Canadian Journal of Botany* 59: 175–189. doi:10.1139/b81-028.
- Maddison WP, Maddison DR. 2011. Mesquite: A modular system for evolutionary analysis. Version 2.75. <http://mesquiteproject.org>.
- Mander L, Li M, Mio W, Fowlkes CC, Punyasena SW. 2013. Classification of grass pollen through the quantitative analysis of surface ornamentation and texture. *Proceedings of the Royal Society* 280: 1–7. doi:10.1098/rspb.2013.1905.
- McHenry MA, Barrington D. 2014. Phylogeny and biogeography of exindusiate Andean *Polystichum* (Dryopteridaceae). *American Journal of Botany* 101: 365–375. doi:10.3732/ajb.1300191.
- Moran RC. 1995. Polypodiaceae. In: Davidse G, Sousa M, Knapp S, eds. *Flora Mesoamericana*, Psilotaceae a Salviniaceae, 333–366. México: Universidad Nacional Autónoma de México.
- Moran RC, Hanks JG, Labiak P, Sundue MA. 2010. Perispore morphology of bolbitidoid ferns (Dryopteridaceae) in relation to phylogeny. *International Journal of Plant Sciences* 171: 872–881. doi:10.1086/655856.
- Moran RC, Hanks JG, Rouhan G. 2007. Spore morphology in relation to phylogeny in the fern genus *Elaphoglossum* (Dryopteridaceae). *International Journal of Plant Sciences* 168: 905–929. doi:10.1086/518269.
- Murillo MT, Bless MJ. 1978. Spores of recent Colombian Pteridophyta II: Monolet spores. In: Van der Hammen T, ed. *The quaternary of Colombia*, 319–365. Amsterdam: Elsevier.
- Mynssen C, Vasco A, Moran RC, Sylvestre LS, Rouhan G. 2016. *Desmophlebiaceae* and *Desmophlebium*: A new family and genus of Eupolypod II ferns. *Taxon* 65: 19–34. doi:10.12705/651.2.
- Pryer KM, Smith AR, Skog JE. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. *American Fern Journal* 85: 205–282. doi:10.2307/1547810.
- Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A. 2007. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* 143: 1–81. doi:10.1016/j.revpalbo.2006.06.008.
- R Development Core Team. 2014. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. <http://www.R-project.org>; accessed 24 December 2014.
- Raghavan ML, Ma B, Harbaugh RE. 2005. Quantified aneurysm shape and rupture risk. *Journal of Neurosurgery* 102: 355–362. doi:10.3171/jns.2005.102.2.0355.
- Ramírez-Valencia V, Sanín D, Pardo-Trujillo A. 2013. Análisis morfológico de las esporas de *Serpocaulon* (Polypodiaceae) de la Cordillera Central de Colombia. *Caldasia* 35: 177–197.
- Rasband WS. 1997. Image J: Image processing and analysis in Java. Bethesda, MD: U.S. National Institutes of Health. <http://rsb.info.nih.gov/ij/>; accessed 24 December 2014.
- Röd-Linder G. 1990. Monograph of the genus *Goniophlebium*. *Blumea* 34: 277–423.
- Rojas-Alvarado AF, Chaves-Fallas JM. 2013. A new hybrid of *Serpocaulon* (Polypodiaceae) from Costa Rica. *American Fern Journal* 103: 175–181. doi:10.1640/0002-8444-103.3.175.
- Sanín D. 2014. *Serpocaulon obscurinervium* (Polypodiaceae), a new fern species from Colombia and Ecuador. *Plant Ecology and Evolution* 147: 127–133. doi:10.5091/plecevo.
- Sanín D. 2015. *Serpocaulon tayronae* (Polypodiaceae), a new fern species from the Sierra Nevada de Santa Marta. *Phytotaxa* 213: 243–252. doi:10.11646/phytotaxa.213.3.
- Sanín D, Torrez V. 2014. *Serpocaulon ×manizalense*: A new hybrid between simple- and pinnate-leaved species of *Serpocaulon* (Polypodiaceae) from Colombia. *Blumea* 59: 123–130. doi:10.3767/000651914X685375.
- Schneider H, Schuettpelz E, Pryer KM, Cranfill R, Magallón S, Lupia R. 2004a. Fern diversification in the shadow of angiosperms. *Nature* 428: 553–557. doi:10.1038/nature02361.
- Schneider H, Smith AR, Cranfill R, Hildebrand T, Hauffler CH, Ranker TA. 2004b. Unraveling the phylogeny of polygrammoid ferns (Polypodiaceae and Grammitidaceae): Exploring aspects of the diversification of epiphytic plants. *Molecular*



- Phylogenetics and Evolution 31: 1041–1063. doi:10.1016/j.ympev.2003.09.018.
- Schneider H, Smith AR, Pryer KM. 2009. Is morphology really at odds with molecules in estimating fern phylogeny? Systematic Botany 34: 455–475. doi:10.1600/036364409789271209.
- Schuettpelz E, Pryer KM. 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. Taxon 56: 1037–1050. doi:10.2307/25065903.
- Schuettpelz E, Pryer KM. 2009. Evidence for a Cenozoic radiation of ferns in a angiosperm-dominated canopy. Proceedings of the National Academy of Sciences of the United States of America 106: 11200–11205. doi:10.1073/pnas.0811136106.
- Schwartzburd P, Smith AR. 2013. Novelties in *Serpocaulon* (Polypodiaceae). Journal of the Botanical Research Institute of Texas 7: 85–93.
- Selling OF. 1946. Studies in Hawaiian pollen statistics, Part I. Bishop Museum Special Publications, Volume 37. Honolulu, HI: Bishop Museum.
- Smith AR, Kreier HP, Haufler CH, Ranker TA, Schneider H. 2006. *Serpocaulon*, a new genus segregated from *Polypodium*. Taxon 55: 919–930. doi:10.2307/25065686.
- Smith AR, Tejero-Diez JD. 2014. *Pleopeltis* (Polypodiaceae), a redefinition of the genus and nomenclatural novelties. Botanical Sciences 92: 1–16. doi:10.17129/botsci.29.
- Solé De Porta N, Murillo MT. 2005. Estudio palino-botánico de algunas especies de Pteridofita de Colombia. Revista Academia Colombiana de Ciencias Exactas, Físicas y Naturales 29: 183–218.
- Sundue M, Vasco A, Moran RC. 2011. Cryptochlorophyllous spores in ferns: Non-green spores that contain chlorophyll. International Journal of Plant Sciences 172: 1110–1119. doi:10.1086/662071.
- Tejero-Diez JD, Mickel JT, Smith AR. 2009. A hybrid *Phlebodium* (Polypodiaceae, Polypodiophyta) and its influence on the circumscription of the genus. American Fern Journal 99: 109–116. doi:10.1640/0002-8444-99.2.109.
- Thiers B. 2016. Index herbariorum: A global directory of public herbaria and associated staff. New York: New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/>; accessed 13 My 2015.
- Tryon AF, Lugardon B. 1991. Spores of the Pteridophyta. Surface, wall structure, and diversity based on electron microscope studies. New York: Springer.
- Vasco A, Loring J, Rouhan G, Ambrose BA, Moran RC. 2015. Divided leaves in the genus *Elaphoglossum* (Dryopteridaceae): A phylogeny of *Elaphoglossum* section *Squamipedia*. Systematic Botany 40: 46–55. doi:10.1600/036364415X686323.
- Vasco A, Moran RC, Ambrose BA. 2014. The evolution, morphology, and development of fern leaves. Frontiers in Plant Science 4: 1–16.
- Wagner WH. 1974. Structure of spores in relation to fern phylogeny. Annals of the Missouri Botanical Garden 61: 332–353. doi:10.2307/2395061.
- Wang F, Liu H, He C, Yang D, Xing F. 2015. Taxonomic and evolutionary implications of spore ornamentation in Davalliaceae. Journal of Systematics and Evolution 53: 72–81. doi:10.1111/jse.12115.
- Wei LL, Dong SY. 2012. Taxonomic studies on *Asplenium* sect. *Thamnopteris* (Aspleniaceae) II: Spore morphology. Nordic Journal of Botany 30: 90–103. doi:10.1111/j.1756-1051.2011.01224.x.

# Corregimiento El Caraño, Florencia, Caquetá – Colombia

## SERPOCAULON (Polypodiaceae) de EL CARAÑO

David Sanín<sup>1,2</sup> & Jorge Luis Peña-Núñez<sup>2</sup>

<sup>1</sup> Grupo de Investigación en Agroecosistemas y Conservación de Bosques Amazónicos (GAIA), Universidad de la Amazonía  
<sup>2</sup> Semillero de Investigación en Biogeografía Evolutiva (SIBE), Programa de Biología, Facultad de Ciencias Básicas, Universidad de la Amazonía

Fotos de: Jorge L. Peña y David Sanín. Producido por David Sanín y Jorge L. Peña-Núñez con el apoyo de Keller Science Action Center del Field Museum.  
 © David Sanín [dav.sanin@gmail.com] y Jorge L. Peña-Núñez, Programa de Biología, Facultad de Ciencias Básicas, Universidad de la Amazonía, Florencia, Caquetá, Colombia.  
 [fieldguides.fieldmuseum.org] [777] versión 1 06/2016



1 *S. adnatum*



2 *S. adnatum*



3 *S. adnatum*



4 *S. adnatum*



5 *S. articulatum*



6 *S. articulatum*



7 *S. articulatum*



8 *S. articulatum*



9 *S. caceresii*



10 *S. caceresii*



11 *S. caceresii*



12 *S. caceresii*



13 *S. dasyleuron*



14 *S. dasyleuron*



15 *S. dasyleuron*



16 *S. dasyleuron*



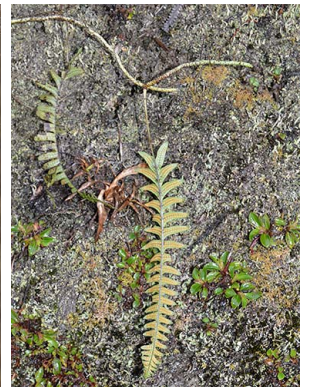
17 *S. fraxinifolium*



18 *S. fraxinifolium*



19 *S. fraxinifolium*



20 *S. funkii*

# Corregimiento El Caraño, Florencia, Caquetá – Colombia SERPOCAULON (Polypodiaceae) de EL CARAÑO

David Sanín<sup>1,2</sup> & Jorge Luis Peña-Núñez<sup>2</sup>

<sup>1</sup>Grupo de Investigación en Agroecosistemas y Conservación de Bosques Amazónicos (GAIA), Universidad de la Amazonía  
<sup>2</sup>Semillero de Investigación en Biogeografía Evolutiva (SIBE), Programa de Biología, Facultad de Ciencias Básicas, Universidad de la Amazonía

Fotos de: Jorge L. Peña y David Sanín. Producido por David Sanín y Jorge L. Peña-Núñez con el apoyo de Keller Science Center del Field Museum.  
© David Sanín [dav.sanin@gmail.com] y Jorge L. Peña-Núñez, Programa de Biología, Facultad de Ciencias Básicas, Universidad de la Amazonía, Florencia, Caquetá, Colombia.

[fieldguides.fieldmuseum.org] [777] versión 1 06/2016



21 *S. funckii*



22 *S. funckii*



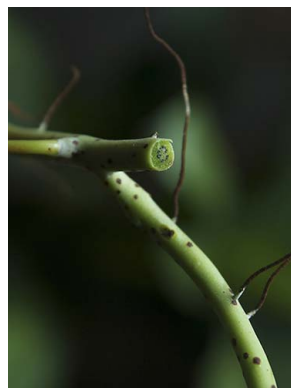
23 *S. levigatum*



24 *S. levigatum*



25 *S. levigatum*



26 *S. levigatum*



27 *S. nanegalense*



28 *S. nanegalense*



29 *S. nanegalense*



30 *S. obscurinervium*



31 *S. obscurinervium*



32 *S. obscurinervium*



33 *S. obscurinervium*



34 *S. obscurinervium*



35 *S. obscurinervium*



36 *S. obscurinervium*



37 *S. obscurinervium*



38 *S. sessilifolium*



39 *S. sessilifolium*



40 *S. sessilifolium*

# Corregimiento El Caraño, Florencia, Caquetá – Colombia

## SERPOCAULON (Polypodiaceae) de EL CARAÑO

3

David Sanín<sup>1,2</sup> & Jorge Luis Peña-Núñez<sup>2</sup>

<sup>1</sup>Grupo de Investigación en Agroecosistemas y Conservación de Bosques Amazónicos (GAIA), Universidad de la Amazonía  
<sup>2</sup>Semillero de Investigación en Biogeografía Evolutiva (SIBE), Programa de Biología, Facultad de Ciencias Básicas, Universidad de la Amazonía.

Fotos de: Jorge L. Peña y David Sanín. Producido por David Sanín y Jorge L. Peña-Núñez con el apoyo de Keller Science Action Center del Field Museum.  
 © David Sanín [dav.sanin@gmail.com] y Jorge L. Peña-Núñez, Programa de Biología, Facultad de Ciencias Básicas, Universidad de la Amazonía, Florencia, Caquetá, Colombia.

[fieldguides.fieldmuseum.org] [777] versión 1 06/2016



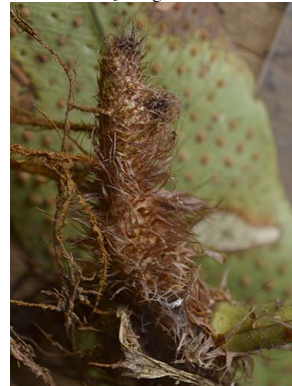
41 *S. triseriale*



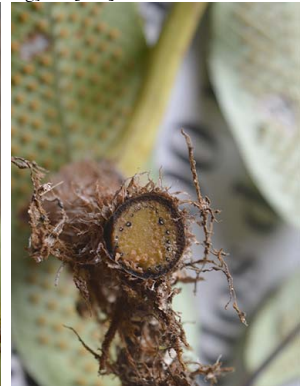
42 *S. triseriale*



43 *S. triseriale*



44 *S. triseriale*



45 *S. triseriale*



46 *S. sp.*



47 *S. sp.*



48 *S. sp.*



49 Bosques de Niebla a 1000 msnm / Cloud forest at 1000 a.s.l.m.



50 Salto de El Caraño / Caraño waterfall

El corregimiento El Caraño se ubica sobre el flanco oriental de la Cordillera Oriental de Colombia. Incluye zonas de vida como Bosque muy húmedo tropical (bmh-T), Bosque muy húmedo premontano (bmh-PM) y Bosque muy húmedo montado bajo (bmh-MB). Estas dos últimas zonas hacen parte de la Reserva Forestal Ley 2 de 1989, sin embargo las presiones antrópicas derivadas de actividades como la tala ilegal, ganadería, cultivos ilícitos y la extracción de carbón, representan una amenaza para la conservación de estos ecosistemas. Históricamente esta región ha permanecido casi inexplorada en términos botánicos debido al orden público. Al ser parte de la zona de transición Andino-Amazónica, estos ecosistemas representan un importante centro de diversificación, donde en los últimos años se han descrito varias especies nuevas para la ciencia y novedades corológicas para el Caquetá (Abalo & Morales 1982, Croat 2013, Galeano & Bernal 2002, Trujillo *et al.* 2015, Trujillo & Callejas 2016). Esta situación justifica incluir estos bosques en programas de conservación que redireccionen el ordenamiento del territorio del municipio de Florencia, Caquetá.

The El Caraño corregimiento is located on the eastern slopes of the Cordillera Oriental and includes the following life zones: Tropical wet forest (wf-T), Premontane wet forest (wf-PM), and Lower montane wet forest (wf-LM). The latter two are part of the 1989 Forest Reserve Law 2, but anthropic pressures from illegal activities like logging, cattle ranching, illicit crop cultivation, and charcoal extraction represent a threat to the conservation of these ecosystems. Historically, this region has been almost unexplored by botanists due to a lack of public order. As part of the Andes-Amazon transition zone, these ecosystems represent an important center of diversification that in recent years have seen the description of several new species and chorological novelties for Caquetá (Abalo & Morales 1982, Croat 2013, Galeano & Bernal 2002, Trujillo *et al.* 2015, Trujillo & Callejas 2016). The importance of this region argues for its inclusion in conservations programs that reinvigorates land-use planning in the municipality of Florencia, Caquetá.

### Literatura citada / Literature cited

Abalo J.E. & Morales L.G. 1982. Veinticinco heliconias nuevas de Colombia. *Phitologia* 51 (1): 1-64  
 Croat T.B. 2013. New Species of *Philodendron* (Araceae) from South America. *Aroidiana*. 36E (1): 16-70  
 Galeano G. & Bernal R. 2002. New species and new records of Colombian palms. *Caldasia* 24 (2): 277-292  
 Trujillo C.W. & Callejas Posada R. 2015. *Piper andakiensis* (Piperaceae) a new species from Amazonian slope of the Eastern cordillera of Colombia. *Caldasia* 37(2): 261-269  
 Trujillo C.W. Trujillo-Trujillo F. & Perdomo O. 2015. New distribution record for *Chelocarpus ulrei*