RESEARCH ARTICLE

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Phylogeny of Selaginellaceae: There is value in morphology after all![1]

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PREMISE OF THE STUDY: The cosmopolitan lycophyte family Selaginellaceae, dating back to the Late Devonian–Early Carboniferous, is notorious for its many species with a seemingly undifferentiated gross morphology. This morphological stasis has for a long time hampered our understanding of the evolutionary history of the single genus *Selaginella*. Here we present a large-scale phylogenetic analysis of *Selaginella*, and based on the resulting phylogeny, we discuss morphological evolution in the group.

METHODS: We sampled about one-third of the approximately 750 recognized *Selaginella* species. Evolutionary relationships were inferred from both chloroplast (*rbcL*) and single-copy nuclear gene data (*pgiC* and *SQD1*) using a Bayesian inference approach. The morphology of the group was studied and important features mapped onto the phylogeny.

KEY RESULTS: We present an overall well-supported phylogeny of *Selaginella*, and the phylogenetic positions of some previously problematic taxa (i.e., *S. sinensis* and allies) are now resolved with strong support. We show that even though the evolution of most morphological characters involves reversals and/or parallelisms, several characters are phylogenetically informative. Seven major clades are identified, which each can be uniquely diagnosed by a suite of morphological features. There is value in morphology after all!

CONCLUSIONS: Our hypothesis of the evolutionary relationships of *Selaginella* is well founded based on DNA sequence data, as well as morphology, and is in line with previous findings. It will serve as a firm basis for further studies on *Selaginella* with respect to, e.g., the poorly known alpha taxonomy, as well as evolutionary questions such as historical biogeographic reconstructions.

KEY WORDS lycophytes; morphology; *pgiC*; phylogeny; *rbcL*; *Selaginella*; Selaginellaceae; *SQD1*

When Korall and coauthors published their phylogenetic analyses of the lycophyte family Selaginellaceae Willk. (Korall et al., 1999; Korall and Kenrick, 2002, 2004), we got the first insights into the evolutionary history of a group dating back to the Late Devonian–Early Carboniferous (370–345 Ma; Kenrick and Crane, 1997; Korall et al., 1999; and references therein), but with an extant species diversity of only some 750 species (Jermy, 1990). These early phylogenetic studies produced a backbone phylogeny, but the limited taxon sampling (ca. 10% of the species) meant that many questions remained unanswered.

Selaginellaceae is a herbaceous, cosmopolitan plant group with greatest diversity in the tropics and subtropics (Jermy, 1990). Most species in the single genus *Selaginella* P.Beauv. are delicate and adapted to warm and humid conditions. However, there are also arctic-alpine species as well as drought-tolerant xerophytes (Jermy, 1990). The family is the sister group to Isoëtaceae Dumort. (see, e.g., Wikström and Kenrick, 1997; Korall et al., 1999), with which it shares the heterosporous condition, i.e., they produce two kinds of spores, mega- and microspores, in separate sporangia. Two morphological synapomorphies for Selaginellaceae are that the stele is found in an air-filled cavity connected to the surrounding tissue by so-called trabeculae and that the megasporangia contain only four megaspores (Jermy, 1990; Kenrick and Crane, 1997).

Species of *Selaginella* range from creeping to ascending and erect, sometimes with long and scandent shoots. Some 50 species have shoots with monomorphic vegetative leaves. Most of these species have the leaves helically arranged, but in three species the leaves are decussately arranged. The remaining 80–90% of the species have anisophyllous, flattened shoots with vegetative leaves in four rows, where the two dorsal rows have smaller leaves than the

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ventral rows (Jermy, 1990; see fig. 3 of Korall and Kenrick, 2002). The sporangia with subtending sporophylls are arranged in strobili at branch tips, either helically (in *Selaginella selaginoides* and *S. deflexa*) or in tetrastichous strobili. Most species have sporophylls that are uniform in size, but some 60 species with dimorphic vegetative leaves also exhibit dimorphic sporophylls. For a few species, these bilateral strobili are nonresupinate, with smaller sporophylls in the same plane as the smaller vegetative leaves. However, most of the taxa with dimorphic sporophylls have resupinate strobili with the smaller sporophylls on the ventral side, i.e., in the same plane as the larger vegetative leaves (Quansah and Thomas, 1985). All but two species (*S. selaginoides* and *S. deflexa*) have root-like organs (rhizophores) arising along the stems, commonly in a ventral or dorsal position in branch dichotomies. Interspecific morphological differences in *Selaginella* are often indistinct or unclear, and the group is notorious for problems associated with species identification. These problems are also partly due to lack of knowledge of the alpha taxonomy (but see, e.g., Valdespino, 1993, 2015; Gardner, 1997; Stefanović et al., 1997; Jermy and Holmes, 1998; Mickel et al., 2004; Zhang et al., 2013; Valdespino et al., 2015).

The classification of Selaginellaceae has been debated for the last 200 years, and several morphology-based classifications have been proposed (e.g., Palisot de Beauvois, 1804; Reichenbach, 1828; Spring, 1840, 1849; Baker, 1883; Hieronymus and Sadebeck, 1901; Walton and Alston, 1938; Rothmaler, 1944; Tryon and Tryon, 1982; Jermy, 1986; Soják, 1993; see Zhou and Zhang, 2015 for a historical overview). The most widely used classification during the recent decades is the one by Jermy (1986). Jermy recognized five subgenera in the single genus *Selaginella* and based these subgenera on phyllotaxy and leaf heteromorphism (isophylly vs. anisophylly): *Selaginella* (2 species), *Tetragonostachys* Jermy (ca. 50 species), *Ericetorum* Jermy (3 species), *Heterostachys* Baker (ca. 60 species), and *Stachygynandrum* (P.Beauv. ex Mirb.) Baker (ca. 600 species).

The first phylogenetic analyses based on DNA sequence data confirmed that *Selaginella* is monophyletic (Wikström and Kenrick, 1997; Korall et al., 1999), something that had been assumed on morphological grounds for a long time. Since then, our knowledge of the phylogenetic relationships of the group has significantly increased. Korall and coauthors (Korall et al., 1999; Korall and Kenrick, 2002, 2004) analyzed a maximum of 62 species based on plastid (*rbcL*) and nuclear (26S rDNA) data. Recently, two phylogenetic analyses using *rbcL* and ITS data have been published. Arrigo et al. (2013) focused on subg. *Tetragonostachys* Jermy, whereas Zhou et al. (2015c) addressed the phylogeny of the genus as a whole, including a total of some 200 species, with a strong focus on taxa in China. The studies generally agree and show a basal dichotomy that resolves subg. *Selaginella* as sister to all species having rhizophores—the so-called rhizophoric clade. Studies by Korall and coauthors (Korall et al., 1999; Korall and Kenrick, 2002, 2004) and Arrigo et al. (2013) showed that the rhizophoric clade is divided into two lineages (called clades A and B by Korall and Kenrick, 2002). With an expanded taxon sampling, Zhou et al. (2015c) also retrieved a clade of two species (*S. sanguinolenta* and *S. nummularifolia*) as sister to clades A and B together. A number of subclades within clades A and B are identified with strong support. The relationships among these subclades are, however, still partly unresolved, especially within clade A. In addition, many of the more recent lineages are unresolved or weakly supported. For some species, such as *S. sinensis* and close allies, the phylogenetic position is unclear and varies depending on the analysis (Korall and Kenrick, 2002, 2004; Zhou et al., 2015c).

The phylogenetic analyses based on DNA sequence data also show that morphological features, including those traditionally used for classification (e.g., leaf heteromorphism, phyllotaxy, bilateral strobili, stelar arrangement, sporangial arrangement, and growth form), show complex evolutionary patterns with reversals and/or parallelisms (Korall and Kenrick, 2002; Zhou et al., 2015c). A consequence of the homoplastic characters is that none of the earlier proposed morphology-based classifications accurately reflect the phylogeny of the family. The first classification based on a phylogenetic analysis was presented by Zhou and Zhang (2015), who relied on the phylogenetic study by Zhou et al. (2015c). Zhou and Zhang (2015) divided *Selaginella* into six subgenera and 18 sections, and, despite the problems of finding unequivocal morphological synapomorphies for most of the groups, they used both morphological and chromosome data in their classification.

During the last 15 years, we have seen significant progress in our understanding of phylogenetic relationships within *Selaginella*. Nevertheless, there are issues still needing consideration. To facilitate further studies on the evolutionary history of the group, such as historical biogeographical analyses, as well as to serve as a broad basis for alpha-taxonomical work and classification, we needed to address in particular: (1) taxon sampling in a geographical perspective (especially with focus on African diversity) and with strong attention paid to the identification of specimens, (2) phylogenetic uncertainty (e.g., resolution of the phylogenetic positions of enigmatic groups such as *S. sinensis* and close allies), and (3) our lack of knowledge on (gross) morphology.

The aim of our study was to present a well-supported, large-scale phylogenetic analysis of *Selaginella* based on a broad taxon sampling, including previously undersampled African species diversity. We expanded previous phylogenetic studies by sampling about one-third of the recognized *Selaginella* species, many of which are represented by multiple accessions; our sampling was worldwide, of most recognized clades, and of morphological diversity in the genus. For the first time, we have also included data from two single-copy nuclear genes, together with chloroplast data. In light of our new phylogeny, which reveals more complex morphological patterns than previously reported (Korall and Kenrick, 2002; Korall and Taylor, 2006; Zhou et al., 2015c), we discuss morphological character evolution within *Selaginella*.

MATERIALS AND METHODS

Species identification and nomenclature—The alpha taxonomy of *Selaginella* is poorly investigated, and taxonomic treatments and floras are for many regions of the world in need of revision or wanting. Species identification is thus problematic, and many herbarium accessions are misidentified. For herbarium specimens, we have, as far as possible, chosen accessions verified by experts in the field; when verification was not possible, we compared our samples to accessions identified by experts. To address species identification and the taxonomy and nomenclature at the species level, we have used species descriptions, floras, Reed (1965–1966), online checklists (e.g., Hassler and Schmitt, 2001), and other recent publications affecting nomenclature of the species (e.g., Smith et al., 2016).

Taxon sampling—The ingroup included a total of 340 accessions, representing 223 species. We have tried to cover morphological, taxonomic, and geographical diversity found in the group. To allow

for an evaluation of within-species variation, we included several accessions for 68 species, based on preliminary results indicating problematic species delimitations and availability of plant material. Accessions in GenBank were used with caution since preliminary analyses, including all GenBank *rbcL*-accessions of *Selaginella*, retrieved many species as nonmonophyletic, indicating possible problems with identification. The outgroup comprised six species of *Isoëtes* L., a genus for which the sister relationship with *Selaginella* is well established in several studies (e.g., Wikström and Kenrick, 1997; Korall et al., 1999). Taxa included in the study, information on vouchers, geographic origin, and GenBank accession numbers are listed in Appendix 1. A total of 478 sequences are newly generated our study.

DNA extraction, PCR, and sequencing—Total DNA was extracted from silica-dried tissue or herbarium material (the oldest collected in 1921) using a modified Carlson–Yoon protocol (Yoon et al., 1991). Dried plant material (20–30 mg) was added to a 2 mL tube with silica beads and ground for 30 s using a Mini-Beadbeater (BioSpec Products, Bartlesville, Oklahoma, USA), 750 µL Carlson buffer and 7.5 µL mercaptoethanol were then added, whereupon the sample was ground for another 30 s, then incubated at 65°C for 60 min. After incubation, 750 µL chloroform–isoamyl alcohol (24:1) was added, and the samples slowly shaken for 30 min. Following 15 min of centrifugation, 2/3 volumes of isopropyl alcohol was added to the water phase and the samples were left for 1–3 weeks at –20°C for DNA to precipitate. The DNA pellets were collected after centrifugation, washed in buffer (76% ethanol, 10 mM ammonium acetate), and dissolved in 100 µL 10 mM Tris-HCl (pH 8.0). In most cases, the DNA worked for direct PCR, but if not, it was purified using the Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Little Chalfont, UK) according to the manufacturer's instructions.

Data from three different genes were included in the study: the chloroplast gene *rbcL*, and two nuclear genes *pgiC* and *SQD1*. *rbcL* has previously been shown to be useful for phylogenetic analyses in *Selaginella* (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Arrigo et al., 2013; Zhou et al., 2015c). *pgiC* and *SQD1* have hitherto not been used in phylogenetic studies involving lycophytes, but have recently been reported to be single-copy in ferns (Rothfels et al., 2013).

Primers used in PCR amplification and sequencing are listed in Table 1. One primer for *rbcL* was designed for this study, whereas the other *rbcL* primers used were taken from Wikström and Kenrick (1997) and Korall et al. (1999). *Selaginella* specific *pgiC* and *SQD1* primers were designed using transcriptome data obtained from the 1000 Plants Initiative (1KP, onekp.com). A slightly adapted version of the script lasseblaste (Larsson, 2013) in combination with MAFFT version 7.127b (Katoh and Standley, 2013) were used to extract and align relevant sequences from transcriptome data of eight *Selaginella* species. Primers were designed using the program AliView version 1.18-beta7 (Larsson, 2014). Our analyses of the transcriptome data, subsequent laboratory work, and phylogenetic analyses indicated that both genes are single-copy in *Selaginella*, as they are in ferns. Nuclear sequences from the published genome of *S. moellendorffii* were excluded from the study since the specimen shows two haplotypes with substantial DNA polymorphism (Banks et al., 2011) and a hybrid origin cannot be ruled out.

PCR reactions were performed in 15 µL volumes using standard *Taq* polymerase. The long *rbcL* region was mostly amplified in two separate PCR reactions to be able to get PCR products, despite the poor quality of the total DNA obtained from many of the herbarium accessions used. The following PCR protocol was used: an initial denaturation step of 95°C for 5 min; then 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 2 min; followed by a final elongation step of 72°C for 10 min. The *pgiC* and *SQD1* regions were both amplified following the PCR protocol: initial denaturation of 95°C for 5 min; then 40 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 2 min; followed by a final elongation of 72°C for 10 min.

PCR products were purified using Illustra ExoProStar 1-Step (GE Healthcare) and later sequenced by Macrogen (Amsterdam, Netherlands) using the same primers as were used for PCR amplification.

Sequences for outgroup species were obtained from GenBank and the 1000 Plants Initiative (1KP, onekp.com), from the latter by using a slightly adapted version of the script lasseblaste (Larsson, 2013).

Multiple sequence alignments and phylogenetic analyses—All sequences were assembled and edited using Pregap4 and Gap4, both modules of the Staden package version 2.0 (Staden, 1996). For each of the three regions, a multiple sequence alignment was performed with MAFFT version 7.127b (Katoh and Standley, 2013), followed by visual inspection in AliView version 1.18-beta7 (Larsson, 2014). Introns were present in the two nuclear data sets (*pgiC* and *SQD1*), but not in *rbcL*. Sections with ambiguous alignment, including all intron regions, were manually excluded. If two or more *rbcL* sequences obtained from different accessions of the same species

TABLE 1. Primers used in amplification and sequencing.

Region	Primer	Usage	Sequence (5′–3′)	Reference
rbcL	rbcL1F	Forward	ATGTCACCACAAACGGA	Wikström and Kenrick (1997)
rbcL	rbcL406F	Forward	GAAGATCTGCGAATTCCCCCCGCTTATTC	Korall et al. (1999)
rbcL	SWRBCL-648F	Forward	AYCGTTTCGTATTYGTAGCRGAAGC	This study
rbcL	rbcL770R	Reverse	GCGAATTCTGCCCTTTTCATCATTTCCTCGCA	Korall et al. (1999)
rbcL	rbcL1192R	Reverse	AATCATCTCCAAATATTTCAGTCAAAGCGGGCA	Korall et al. (1999)
rbcL	rbcL1402R	Reverse	CAAACTTGATTTCTTTCCATACC	Korall et al. (1999)
rbcL	rbcL1409R	Reverse	TCAAATTCAAACTTGATTTCTTTCCA	Wikström and Kenrick (1997)
pgiC	SWPGIC-1666F	Forward	VTTYGCTTTYTGGGAYTGGG	This study
pgiC	SWPGIC-2523R	Reverse	GTCGTGGTTRCTSACAATCTC	This study
SQD1	SWSQD1-817F	Forward	GCBTTYACTTGCAAAGCTTG	This study
SQD1	SWSQD1-1432R	Reverse	ATCTTCTTCCAVGARACGTC	This study

Notes: The whole *rbcL* region was amplified with primer pair rbcL1F + rbcL1409R. When the *rbcL* region had to be amplified in two separate PCR reactions, rbcL1F + rbcL770R and rbcL406F + rbcL1402R were the most successful primer pairs. Occasionally, primer pairs rbcL406F + rbcL1192R or SWRBCL-648F + rbcL1402R worked better for amplification of the second half of the region. Primers used for sequencing were always the same as the ones used for amplification.

were identical, the accession with the most regions sequenced was kept for further analyses. However, the identical multiple accessions are noted in the phylogenetic trees and in Appendix 1. Sequences obtained have been deposited in GenBank. Alignments and the phylogenetic tree (Fig. 2) are available in the Dryad Digital Repository (http://doi.org/10.5061/dryad.88fh0).

Prior to the phylogenetic analyses, the best-fitting nucleotide substitution model for each of the three single-region data sets was determined based on the corrected Akaike information criterion (AICc) as implemented in the program MrAIC version 1.4.6 (Nylander, 2004; Table 2). To evaluate congruence among the regions, we then analyzed each single data set using Bayesian inference as implemented in the parallel version of MrBayes 3.2.4 (Ronquist and Huelsenbeck, 2003). For the *rbcL* data set, four independent runs with eight chains each were run for 40 million generations, employing a temperature parameter of 0.05. For the two nuclear data sets, *pgiC* and *SQD1*, four independent runs with four chains each were run for 20 million generations. The temperature parameter was set to 0.1. In all three analyses, parameters were sampled every 2000 generations. The increased number of chains and the lowered temperature parameter in the analysis of the *rbcL* data set was used to enhance chain-mixing. Sampled values were visually inspected for convergence using the programs Tracer version 1.6 (Rambaut et al., 2014) and AWTY (Wilgenbusch et al., 2004; Nylander et al., 2008), as well as by evaluating the standard deviation of the split frequencies among the independent runs and the PSRF values. For each run, the first 20% of the samples was discarded as burn-in before summarizing the posterior as a 50% majority-rule consensus tree.

To evaluate potential conflicts among the single-region data sets, we manually compared the consensus topologies and considered incongruences supported by a Bayesian posterior probability (PP) of 0.95 or higher as a conflict. Only three minor conflicts were identified, and the three single-region data sets were concatenated using abioscripts (Larsson, 2010). All accessions with data present for at least the *rbcL* region were included. The combined data set was then subject to Bayesian inference analyses in the parallel version of MrBayes 3.2.4 (Ronquist and Huelsenbeck, 2003), where each single region was assigned its own partition with substitution model parameters unlinked between partitions. The combined analyses were run for 20 million generations with the same settings as for the single-region *pgiC* and *SQD1* analyses. For each run, 4 million generations (20%) were discarded as burn-in before summarizing the posterior as a 50% majority-rule consensus tree. We considered a PP > 0.95 as well (or strongly) supported, a PP between 0.90–0.95 as moderately supported, and a PP < 0.90 as weakly supported.

Preliminary phylogenetic analyses based on data from all three DNA regions, but with different taxon samplings, showed instability with respect to the position of the clade containing *S. sanguinolenta* and *S. nummularifolia* (henceforth called the "*sanguinolenta* group"). This clade (eight accessions) changes position depending on taxon sampling. To evaluate this instability, we performed Bayesian inference analyses of the combined three-region data set with different taxon sampling as follows: (1) all taxa included and (2) exclusion of the *sanguinolenta* group.

Computation-intensive analyses were run on the Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX). Trees were inspected in FigTree version 1.4.2 (Rambaut, 2006). All trees were rooted with *Isoëtes* as the outgroup.

Morphology—Vouchers of the included accessions were studied morphologically with respect to traits that in earlier phylogenetic studies and classifications (e.g., Spring, 1840, 1849; Baker, 1883; Hieronymus and Sadebeck, 1901; Jermy, 1986; Korall and Kenrick, 2002; Korall and Taylor, 2006; Zhou et al., 2015c) have been proposed to be of interest for recognizing major groups within the family: vegetative leaf and sporophyll heteromorphism (unrelated to the size differences sometimes observed between mega- and microsporophylls), phyllotaxy, rhizophore position, presence/absence of articulations (i.e., swellings below stem dichotomies that in dried specimens often are seen as dark, constricted segments), and, for some species, also stelar arrangement and megaspore features. In addition, presence of xerophytism was noted. Our focus has been on features observable with the naked eye or stereomicroscope, i.e., features that are more easily and more often studied during identification of taxa, rather than microscopic features, such as microspore ornamentation. Whenever possible, we verified our observations of morphological features using literature data (e.g., Baker, 1885; Harvey-Gibson, 1894; Hieronymus and Sadebeck, 1901; Bower, 1908; van Alderwerelt van Rosenburgh, 1915; Brause, 1921; Steel, 1923; Wardlaw, 1925; Alston, 1934; Tryon, 1949; Horner and Arnott, 1963; Hellwig, 1969; Mickel and Hellwig, 1969; Crabbe and Jermy, 1973; Alston et al., 1981; Mukhopadhyay and Sen, 1981; Tryon and Tryon, 1982; Minaki, 1984; Quansah and Thomas, 1985; Dahlen, 1988; Quansah, 1988; Taylor, 1989; Rauh and Hagemann, 1991; Tryon and Lugardon, 1991; Valdespino, 1993; Gardner, 1997; Stefanović et al., 1997; Jermy and Holmes, 1998; Morbelli and Rowley, 1999; Moran and Smith, 2001; Morbelli et al., 2001; Korall and Kenrick, 2002; Liu et al., 2002; Mickel et al., 2004; Korall and Taylor, 2006; Roux, 2008; Al-Shehri and Lashin, 2009; Roy and Borthakur, 2011; Maideen et al., 2013; Schulz et al., 2013; Zhang et al., 2013; Singh et al., 2014a, b; Zhou and Zhang, 2015; Zhou et al., 2015a–c).

TABLE 2. Number of accessions and characters, proportion of variable characters, and nucleotide substitution models used in the Bayesian inference analyses for the different data sets.

Data set	No. of accessions		No. of characters	Variable characters (%)	Substitution model
	Ingroup	Total			
rbcL [a]	340	346	1362	58	GTR+I+G
pgiC [a]	111	113	427	54	HKY+I+G
SQD1 [a]	127	129	534	56	HKY+I+G
Combined three region-data set [b]	332 (340)	338 (346)	2323 (2323)	57 (57)	— [c]

[a] The eight accessions from the *sanguinolenta* group included.
[b] The eight accessions from the *sanguinolenta* group excluded and included, outside and inside the parentheses, respectively.
[c] Partitioned data set, each partition with substitution model for respective region.

RESULTS

Tree statistics—Summary statistics for the four data sets analyzed in this study (the three single-region data sets and the combined data set) can be seen in Table 2. The number of characters analyzed for the *rbcL*, *pgiC*, and *SQD1* data sets were 1362, 427, and 534 bp, respectively. Taxon-wise, the *rbcL* data set was the most complete, with sequence data for all taxa, totaling 346 accessions (identical sequences included), whereas the two nuclear data sets *pgiC* and *SQD1* included data for 113 and 129 accessions, respectively (identical sequences included). The proportion of variable characters was very high for all of the three single-region data sets: *rbcL* 58%, *pgiC* 54%, and *SQD1* 56%. The combined three-region data set comprised 338 accessions (the *sanguinolenta* group accessions excluded), and 2323 characters, of which 57% were variable. Of 588 sequences analyzed, 478 were newly generated in this study (Appendix 1).

Phylogenetic analyses of single-region data sets—The resulting phylogenies from the three single-region Bayesian inference analyses (*rbcL*, *pgiC*, and *SQD1*, respectively) showed very similar topologies. The single-region phylogenies involved only three conflicts concerning the positions of *S. effusa*, *S. mairei*, and *S. pulvinata* (Appendices S1–S3, see Supplemental Data with the online version of this article). The first two conflicts relate to topological differences between the *rbcL* and *pgiC* phylogenies, whereas *S. pulvinata* shows different positions in the *rbcL* and the *SQD1* phylogenies. These conflicts were considered minor, and the data sets were therefore combined for further analysis.

As noted above, the proportion of variable characters is high in all three single-region data sets (Table 2). In the nuclear single-region analyses, the substitutions are more or less evenly distributed over the tree, whereas in the chloroplast phylogeny branch length differences indicate rate heterogeneity between the major clades (Appendices S1–S3).

Phylogenetic analyses of combined three-region data set—*Position of the sanguinolenta group*—The two species in the *sanguinolenta* group are, based on eight accessions, shown to be a monophyletic group with strong support, with *S. nummularifolia* nested within *S. sanguinolenta* (online Appendix S4). However, the phylogenetic position of the group differs between analyses (Appendices S1–S4). Although the incongruent positions are not strongly supported and therefore were not seen as conflicts, the differences in positions were substantial. Bayesian inference analysis of the combined three-region data set indicates, with strong support (PP 1.0/PP 1.0, referring to the older and younger nodes involved, respectively), that the group is sister to all *Selaginella* species except *S. selaginoides* and *S. deflexa* (position α in Fig. 1; Appendix S4). This position is also found in the single-region analyses of *rbcL* and *SQD1*, with weak (PP 1.0/PP 0.82; Appendix S1) and strong support (PP 1.0/PP 0.96; Appendix S3), respectively. However, in the single-region analysis of *pgiC*, *S. sanguinolenta* is sister to clade B with moderate support (PP 0.91/PP 0.93; position β in Fig. 1; Appendix S2). Due to the disparate positions of the *sanguinolenta* group, two analyses with the combined three-region data set were performed: one with and one without the eight accessions belonging to the group. The well-supported lineages are identical between the two analyses, but with slightly lower support in the analysis including the *sanguinolenta* group.

Hereafter, only the results from the combined analysis with *S. sanguinolenta* and *S. nummularifolia* excluded will be presented (see section *Enigmatic phylogenetic position of the sanguinolenta group* for further discussion).

Phylogenetic relationships—We identified seven major, well-supported clades (schematic tree in Fig. 1; detailed phylogeny in Fig. 2A–G, summarized in one figure in online Appendix S5) here referred to as the selaginella, rupestrae, lepidophyllae, gymnogynum, exaltatae, ericetorum, and stachygynandrum clades. If an unnamed clade is discussed, it is referred to by its outermost (top and bottom) species as depicted in Fig. 2.

Our results showed a phylogenetic tree with an overall well-supported topology (Fig. 2). All relationships discussed below are supported by a PP of 1.0 unless otherwise stated. The selaginella clade, including *S. selaginoides* and *S. deflexa*, is monophyletic (Fig. 2A) and sister to the rhizophoric clade (sensu Korall et al., 1999) comprising all other *Selaginella* species. The rhizophoric clade is further subdivided into two groups: clades A and B (sensu Korall and Kenrick, 2002). Clade A includes 63 species (102 accessions) in our analysis, divided into the five clades: rupestrae (33 species), lepidophyllae (2 species), gymnogynum (19 species), exaltatae (3 species), and ericetorum (6 species). The rupestrae clade is sister to the lepidophyllae clade, but the relationship between this clade and the three other clades in clade A is unresolved (Fig. 2A, B).

The majority of the *Selaginella* taxa, 156 species (224 accessions) in our analysis, are found in clade B, which corresponds to the stachygynandrum clade (PP 0.97; Fig. 2C–G). The topology of the stachygynandrum clade is well resolved, with several well-supported subclades. Within stachygynandrum, the *sinensis* group (*S. yemensis*–*S. sechellarum*, including 10 species and 13 accessions; Fig. 2C) is sister to the other species. The remaining species of stachygynandrum are divided into five larger clades: a dry-tolerant clade of 10 species (*S. nubigena*–*S. digitata*; 13 accessions; Fig. 2C), three clades containing species mainly from Asia and Australasia: *S. douglasii*–*S. arbuscula* (68 species, 98 accessions; Fig. 2D, E), *S. versicolor*–*S. roxburghii* (20 species, 30 accessions; Fig. 2F), and *S. moellendorffii*–*S. biformis* (6 species, 8 accessions; Fig. 2F), with the last clade sister to the predominantly Central and South American clade *S. hirsuta*–*S. contigua* (42 species, 62 accessions; Fig. 2G).

Morphology—By relating morphology to our phylogenetic hypothesis based on DNA sequence data, we show that the presence of rhizophores and tetrastichous strobili are synapomorphies of the rhizophoric clade. All other morphological characters studied involve reversals and/or parallelisms, including vegetative leaf and sporophyll isophylly/anisophylly, phyllotaxy of vegetative leaves, articulations, stelar arrangement, megaspore morphology, and possibly rhizophore position (Table 3; Fig. 2). Many of these characters are, nevertheless, at some level, phylogenetically informative with character states that define clades. Examples include the solenostelic rhizome that is unique to species in the ericetorum clade (Fig. 2B) and the tristelic condition in species in a subclade of the stachygynandrum clade (Fig. 2D). The interpretation of other characters is more complex with, e.g., the presence of bilateral resupinate strobili being strongly homoplastic (Fig. 2D, E, G).

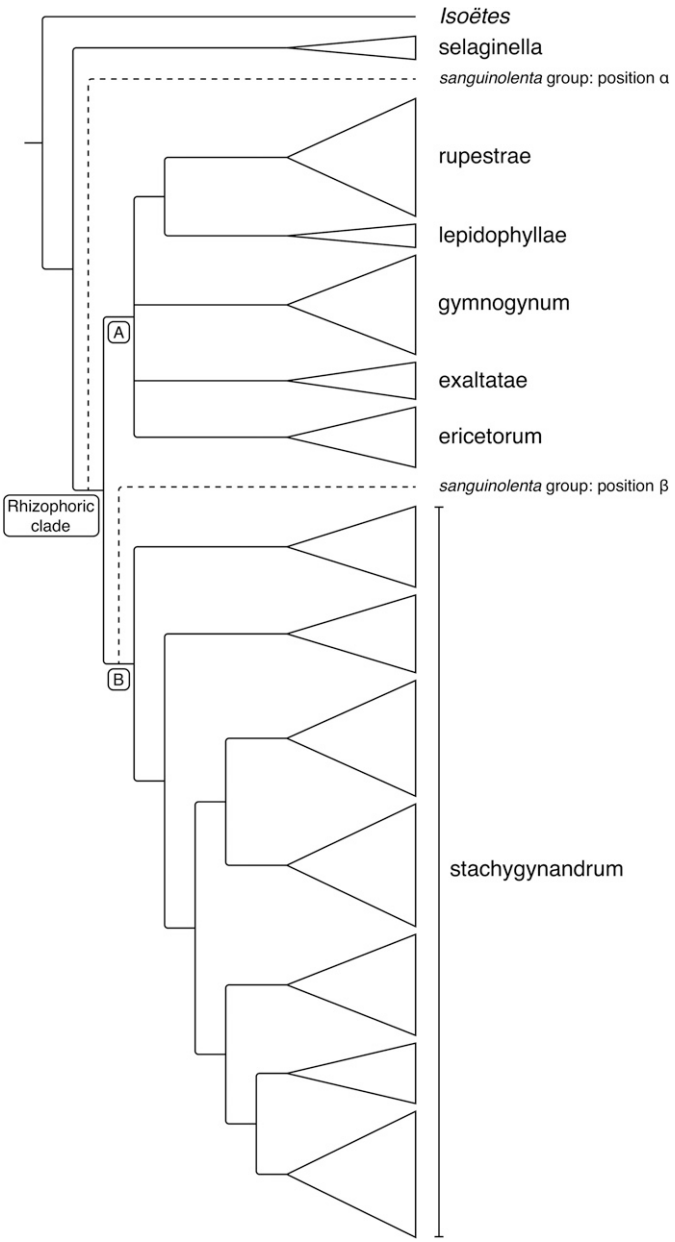


FIGURE 1 A schematic overview of the phylogenetic relationships of *Selaginella* retrieved by a Bayesian inference analysis of the combined three-region data set (*rbcL*, *pgiC*, and *SQD1*), depicting the seven major clades discussed. The two alternative positions for the *sanguinolenta* group are marked as "position α" and "position β". All nodes are supported by a Bayesian posterior probability (PP) of 1.0, except for clade B (PP 0.97). Clade size is based on number of species, scaled logarithmically.

DISCUSSION

This study presents a well-resolved phylogeny of the lycophyte family Selaginellaceae. The group has, for the first time, been analyzed using two single-copy nuclear markers along with the commonly used plastid data. Furthermore, this study includes several newly sampled African taxa, a geographical region that has hitherto been undersampled. Three topological conflicts were found among the analyses of the single-region data sets, involving the positions of *S. effusa*, *S. mairei*, and *S. pulvinata* (Appendices S1–S3). The positions in the plastid data set agree with the positions found in the analysis of the combined three-region data set, as well as the results found by Zhou et al. (2015c). We therefore suggest that the divergent results retrieved by the analyses of the respective nuclear data sets are explained by the smaller and somewhat skewed taxon sampling in these data sets. The result of the combined analysis, including both the nuclear regions and the plastid data, shows an overall well-supported phylogeny, with strong support for both deep nodes in the tree as well as relationships closer to the tips (Fig. 2).

Both the plastid and the nuclear data sets show a large number of substitutions, with the percentage of variable characters ranging from 54–58%. These high numbers are also reported for other regions (26S rDNA: Korall and Kenrick, 2004; ITS: Arrigo et al., 2013 and Zhou et al., 2015c). We also confirm the finding by Korall and Kenrick (2002) that the plastid data set differs remarkably in branch lengths between clades A and B, a pattern not mirrored in the nuclear data sets. The large number of substitutions and the heterogeneous distribution of these have in previous studies contributed to analytical problems, with ambiguous phylogenetic positions of clades with long branches, such as the *sinensis* group (see, e.g., Korall and Kenrick, 2002, 2004). In this study, however, we found a well-supported position of this group (see section *The sinensis group*).

The larger groups retrieved in our study are in concordance with previous studies (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Arrigo et al., 2013; Zhou et al., 2015c). The high support for many of the clades is, however, new to this study; it has not been retrieved in earlier studies based on plastid data alone, or plastid data in combination with the nuclear 26S rRNA or ITS region (see, e.g., Korall and Kenrick, 2004; Arrigo et al., 2013; Zhou et al., 2015c). As in previous studies (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Zhou et al., 2015c), we found that Jermy's classification (1986) includes only two monophyletic subgenera, i.e., *Selaginella* and *Tetragonostachys*. Subgenera *Ericetorum*, *Heterostachys*, and *Stachygynandrum* are nonmonophyletic. The classification presented by Zhou and Zhang (2015) is based on monophyletic groups found in their phylogenetic analysis of *Selaginella* (Zhou et al., 2015c).

Our phylogenetic analysis, combined with the morphological data presented here, provides a well-supported hypothesis for future discussions on the evolution of the single genus *Selaginella*, as well as a robust framework for a new subgeneric classification (Weststrand and Korall, 2016, in this issue). Names of the seven major clades presented below (selaginella, rupestrae, lepidophyllae, gymnogynum, exaltatae, ericetorum, and stachygynandrum) correspond to the subgeneric names in this classification (Weststrand and Korall, 2016). Subgenera and other well-established groups that are circumscribed differently by previous authors will for clarity be referenced to with an accompanying "sensu".

***Phylogenetic relationships within* Selaginella**—In the following section, only the result of the phylogenetic analysis where the accessions of *S. sanguinolenta* and *S. nummularifolia* (the *sanguinolenta* group) were excluded will be discussed (Fig. 2). For a discussion on the *sanguinolenta* group, see the section *Enigmatic phylogenetic position of the sanguinolenta group* below.

Relationships among larger groups—We confirm the sister relationship between the selaginella clade (which corresponds to the subgenus

Selaginella in our proposed classification; Weststrand and Korall, 2016), and the rest of the genus, the so-called rhizophoric clade (sensu Korall et al., 1999), which is characterized by having rhizophores (Fig. 1). This large clade is, in turn, divided into two groups: clades A and B (sensu Korall and Kenrick, 2002). These relationships were recovered in previous studies (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Arrigo et al., 2013; Zhou et al., 2015c). However, Zhou et al. (2015c) also retrieved a clade of *S. sanguinolenta* and *S. nummularifolia* (i.e., the *sanguinolenta* group) as sister to clades A plus B. Our clade A includes five of the seven major clades (rupestrae, lepidophyllae, gymnogynum, exaltatae, and ericetorum). Clade B is equivalent to the clade stachygynandrum (Fig. 1).

The five major clades in clade A are all well supported, with the rupestrae clade and the lepidophyllae clade in a strongly supported sister relationship (Figs. 1 and 2). The relationships among these two and the other three clades (gymnogynum, exaltatae, and ericetorum) are, however, only weakly supported (Figs. 1 and 2). Nevertheless, this topology is in line with all previous studies that use a model-based approach (Korall and Kenrick, 2002; Arrigo et al., 2013; Zhou et al., 2015c). The proposed presence of a "dorsal rhizophoric clade" within clade A (Korall and Kenrick, 2002) lacks support in this study, as in other studies (Korall and Kenrick, 2004; Arrigo et al., 2013; Zhou et al., 2015c). All species in clades rupestrae, lepidophyllae, gymnogynum, and exaltatae, as well as the *sanguinolenta* group, possess dorsal rhizophores, whereas the ericetorum clade, which is nested among the other clades, has rhizophores restricted to the base of the stem and rhizome in the perennials. Thus, the terminology of ventral or dorsal rhizophores cannot easily be applied to the ericetorum clade.

The selaginella clade—Two species are found in the selaginella clade: the type of the genus, *S. selaginoides,* which is circumboreal, and the Hawaiian endemic *S. deflexa* (Fig. 2A). The monophyly of the clade and its sister relationship to the rhizophoric clade have not been questioned in any previous phylogenetic analyses (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Arrigo et al., 2013; Zhou et al., 2015c). The group corresponds to the subgenus *Selaginella* as circumscribed by both Jermy (1986) and Zhou and Zhang (2015). With sequence data from five accessions of *S. selaginoides*, our data indicate that the species is monophyletic, as found by Zhou et al. (2015c). The two species are recognized by having both monomorphic vegetative leaves and monomorphic sporophylls, all helically arranged. In addition, the species lack rhizophores. The group does not include any other species than the two included in this study. See Table 3 for a comparison of morphological characters among the seven major clades.

The rupestrae clade—Based on 33 species (35 accessions), the monophyly of the rupestrae clade is unequivocal, as in all previous phylogenetic studies (Fig. 2A; Korall et al., 1999; Korall and Kenrick, 2002, 2004; Arrigo et al., 2013; Zhou et al., 2015c). The group was treated as subg. *Tetragonostachys* by Jermy (1986), and as subg. *Ericetorum* sect. *Homoeophyllae* Spring by Zhou and Zhang (2015), and VII. *Homoeophyllae* clade in superclade *Ericetorum*/superclade A by Zhou et al. (2015c). This group is easily recognized by having monomorphic (to slightly dimorphic) and helically arranged vegetative leaves, isophyllous (sometimes slightly anisophyllous) and tetrastichous strobili, and dorsal rhizophores. The rupestrae clade includes ca. 50 xerophytic species mainly in North America (Tryon, 1955; Jermy, 1990; Arrigo et al., 2013). In line with previous phylogenetic studies, which included more taxa, the relationships within the group are mostly unresolved (Fig. 2A; Arrigo et al., 2013; Zhou et al., 2015c).

The lepidophyllae clade—Selaginella lepidophylla and *S. novoleonensis*, distributed in the southwestern Unites States and Mexico, and Mexico, respectively, represent the lepidophyllae clade (3 accessions in our analysis; Fig. 2A). The group is characterized by having dorsal rhizophores, being xerophytic, and a rosetted habit with branches that curl inwards during drought. Vegetative leaves are dimorphic and arranged in four rows, and sporophylls are monomorphic in tetrastichous strobili. The sister group relationship between the lepidophyllae and rupestrae clades is here strongly supported, and seen in earlier studies as well, where only *S. lepidophylla* was included (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Arrigo et al., 2013; Zhou et al., 2015c). A possible morphological synapomorphy uniting these two clades is the presence of granules on the inner surface of the exospore of the megaspores (Korall and Taylor, 2006), a character not reported for species in any other group. Rosette-forming *Selaginella* species are also found in two clades in the stachygynandrum clade: *S. nubigena–S. digitata* and *S. hirsuta–S. contigua* (Fig. 2C, G). However, these species possess ventral rhizophores. The group was treated as subg. *Ericetorum* sect. *Lepidophyllae* Li Bing Zhang & X.M.Zhou by Zhou and Zhang (2015), and VIII. *S. lepidophylla* clade in superclade *Ericetorum*/superclade A by Zhou et al. (2015c).

The gymnogynum clade—This clade is well supported with 19 species (43 accessions) in our analysis (Fig. 2B). Species of the gymnogynum clade have dimorphic vegetative leaves in four rows (at least on the distal parts of the plant) and monomorphic sporophylls in tetrastichous strobili. The species have previously been included in series *Articulatae* (Spring) Hieron. & Sadeb. However, *Articulatae* as circumscribed by Spring is here shown to be nonmonophyletic, as in other studies (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Arrigo et al., 2013; Zhou et al., 2015c), and correspond to two clades: gymnogynum and exaltatae. Species included in the gymnogynum clade all possess the morphological characters used to describe the series *Articulatae* and have the following key characters: articulated stems, dorsal rhizophores, a single (rarely two) basal megasporangium surrounded by enlarged sterile sporophylls in an otherwise microsporangiate strobilus, and large reticulate megaspores with a grid-like pattern in cross sections of the exospore (Somers, 1982; Korall and Taylor, 2006). *Selaginella* species have a protostele in the stem, and for most species in the gymnogynum clade, the protostele is a simple, circular to elliptic monostele or a bistele, but three or more steles are found in the stems of a few gymnogynum species (Fig. 2B). The group was treated as subg. *Ericetorum* sect. *Articulatae* (Spring) Li Bing Zhang & X.M.Zhou by Zhou and Zhang (2015), and VI. *Articulatae* clade in superclade *Ericetorum*/superclade A by Zhou et al. (2015c).

Previous studies have shown a basal split in the gymnogynum clade, with the African *S. kraussiana* and the Asian *S. remotifolia* as sister to the Central and South American species. Here, we show that the Central and South American *S. silvestris* is sister to *S. remotifolia* with strong support. The report of *S. apoda* in this clade by Arrigo et al. (2013) is based on a GenBank sequence from a misidentified specimen of *S. kraussiana* (Korall et al., 1999).

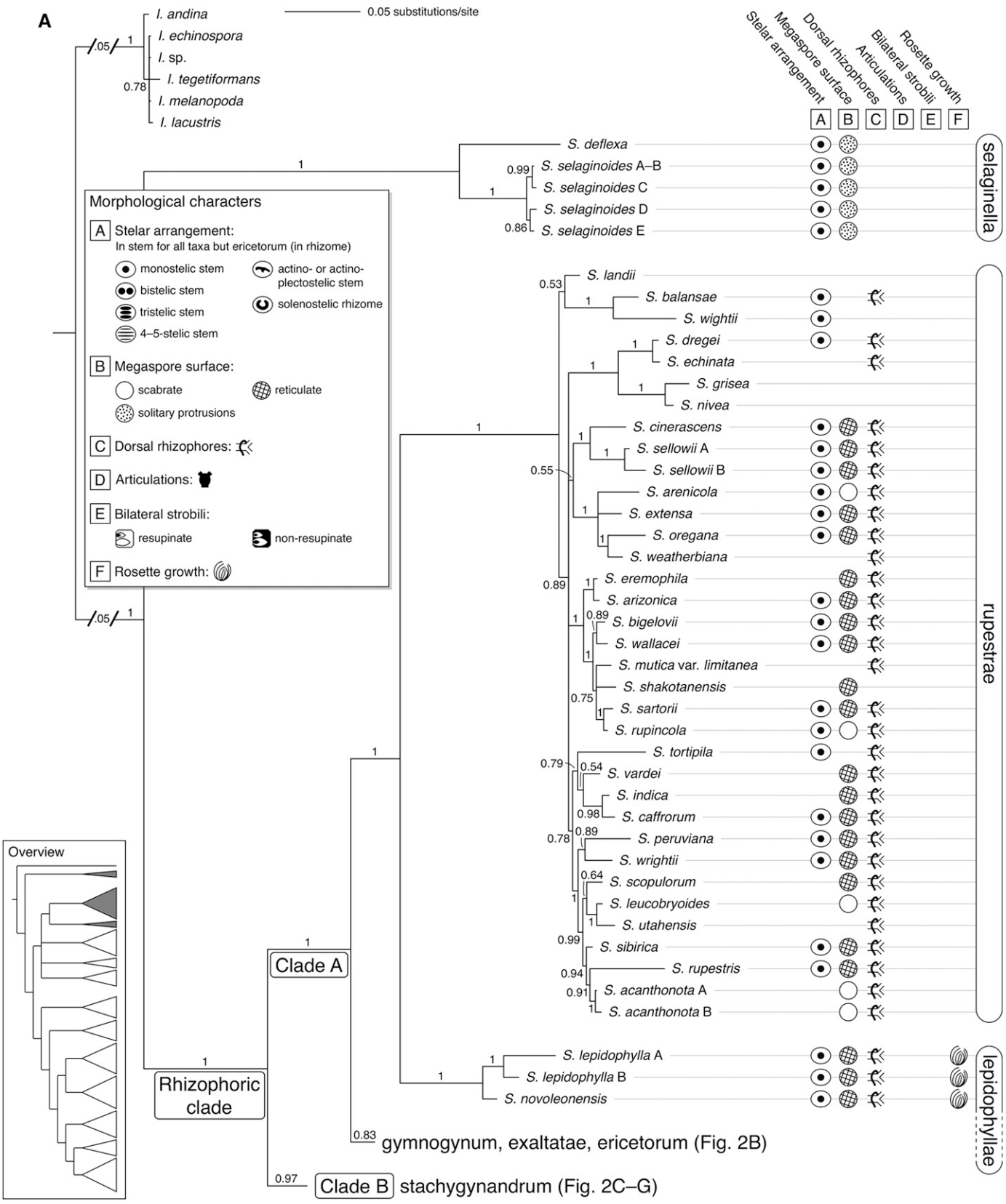
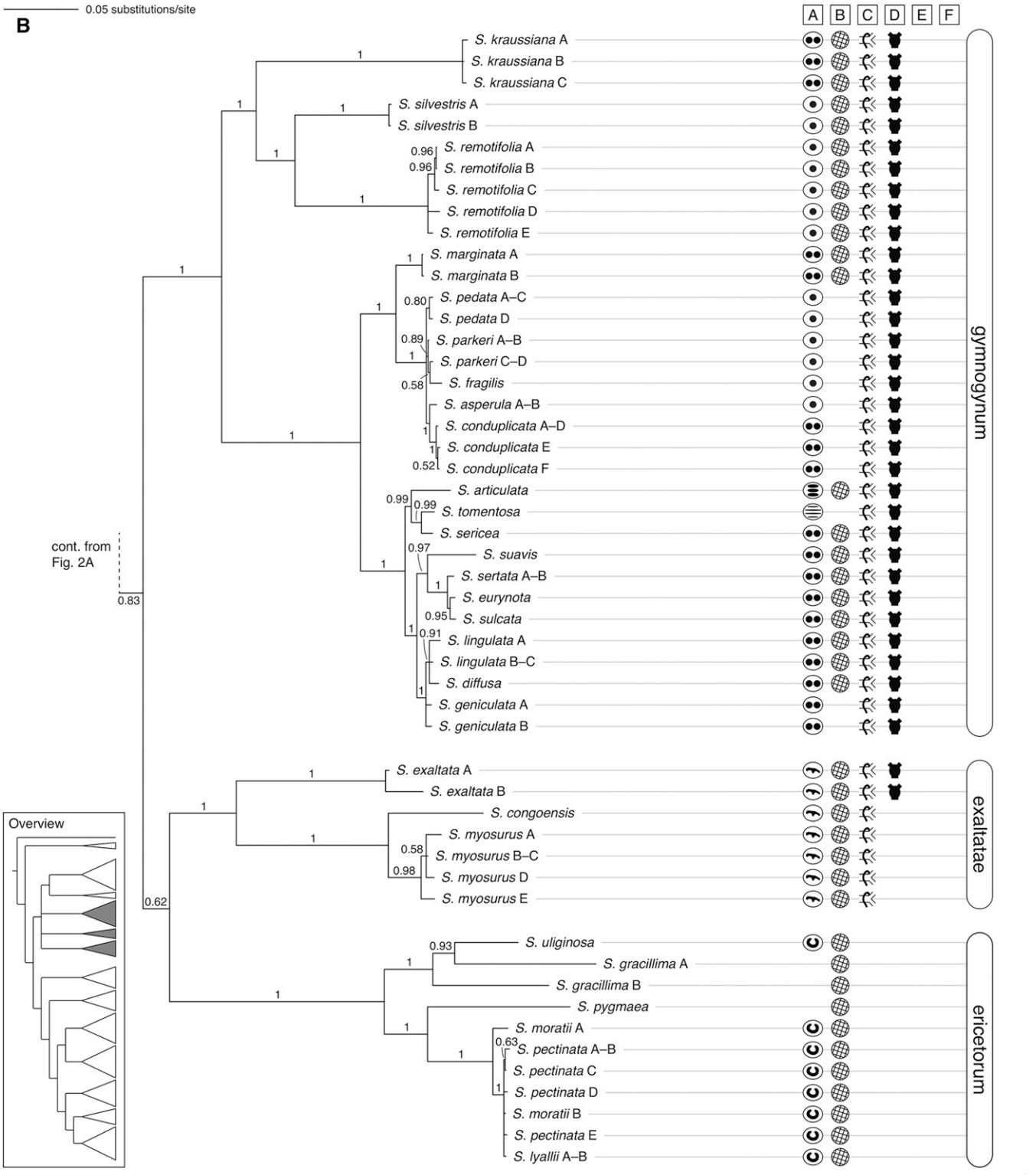


FIGURE 2 The 50% majority-rule consensus tree of *Selaginella* resulting from a Bayesian inference analysis of the combined three-region data set (*rbcL*, *pgiC*, and *SQD1*), divided into panels A–G. Data for the *sanguinolenta* group excluded. Numbers associated with internal branches denote Bayesian posterior probabilities. The numbers /0.05/ and /0.1/ refer to cut branches where the missing length corresponds to 0.05 substitutions/site and



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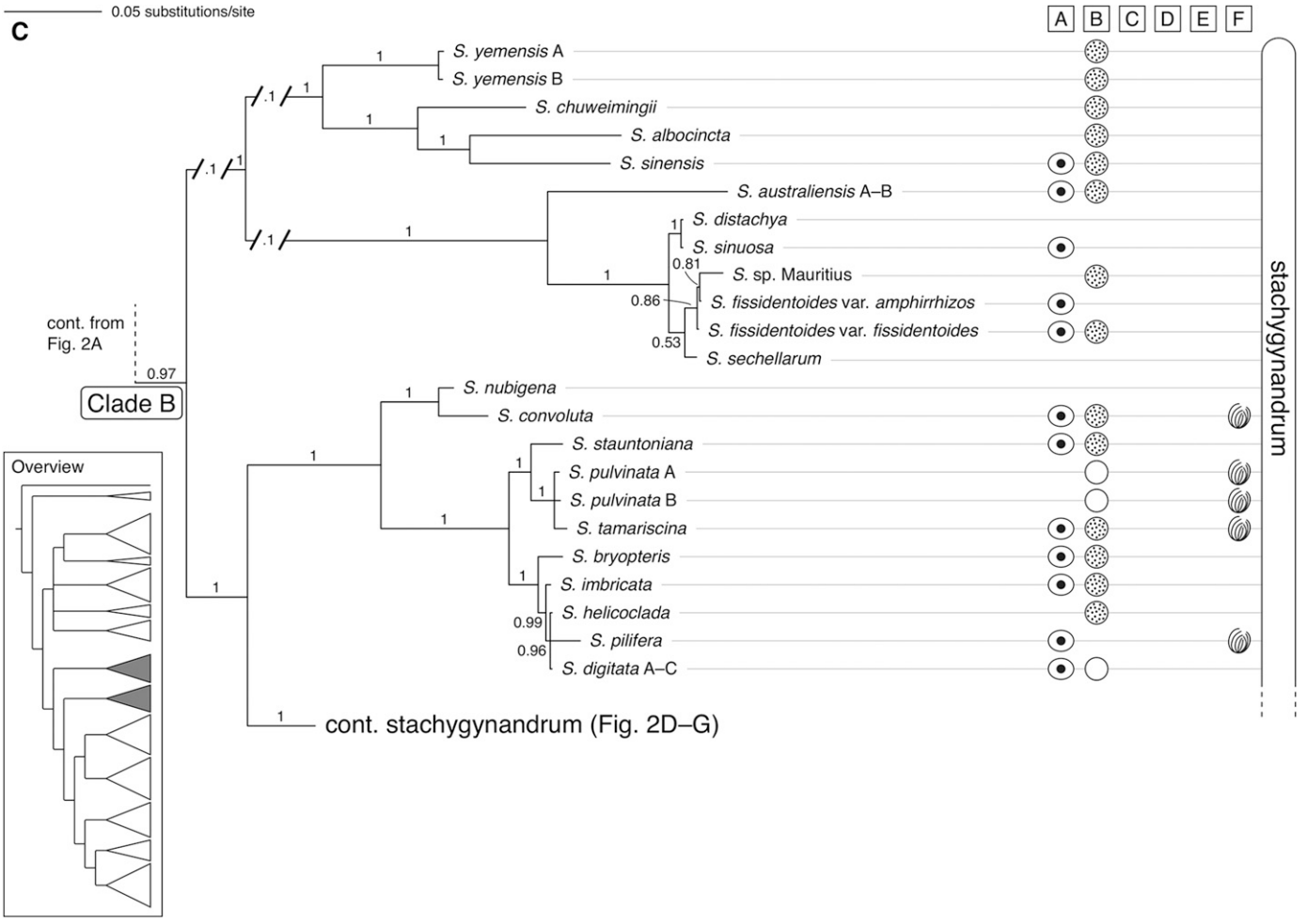
0.1 substitutions/site, respectively. The presence of a number of morphological characters is mapped. Character states are defined in panel A. *I.*, *Isoëtes*; *S.*, *Selaginella*. Panel (A) shows the outgroup (the *Isoëtes* clade), and the selaginella, rupestrae, and lepidophyllae clades, (B) shows the gymnogynum, exaltatae, and ericetorum clades, (C) shows the *S. yemensis*–*S. sechellarum* and *S. nubigena*–*S. digitata* subclades within the stachygynandrum clade, (D) shows the *S. douglasii*–*S. mayeri* subclade within the stachygynandrum clade, (E) shows the *S. monospora*–*S. arbuscula* subclade within the stachygynandrum clade, (F) shows the *S. versicolor*–*S. roxburghii* and *S. moellendorffii*–*S. biformis* subclades within the stachygynandrum clade, and (G) shows the *S. hirsuta*–*S. contigua* subclade within the stachygynandrum clade.



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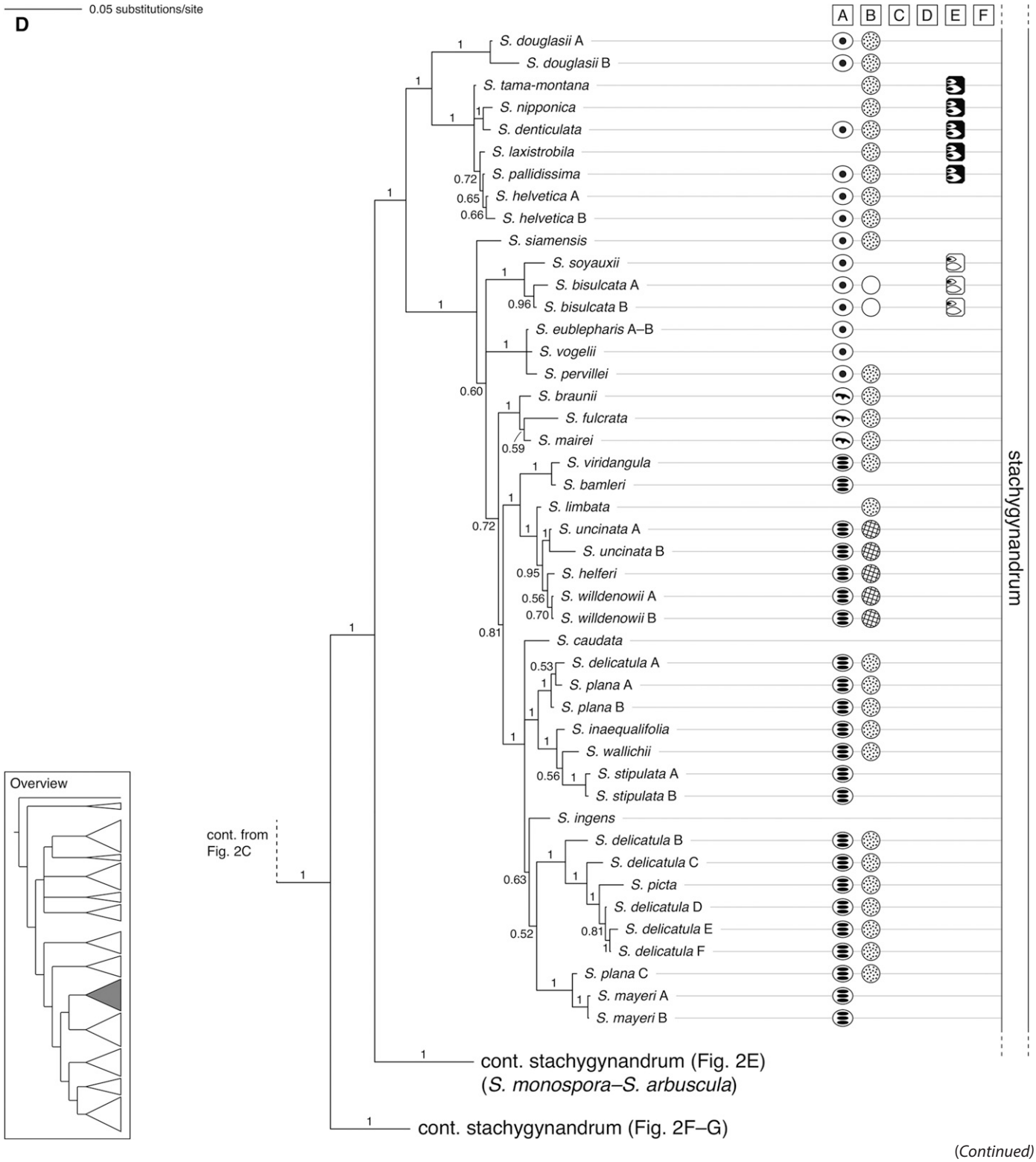
We estimate that the group comprises ca. 40 species (Spring, 1840, 1849; Hieronymus and Sadebeck, 1901; Walton and Alston, 1938; Somers, 1982). They are nearly all neotropical, with the exception of the African *S. kraussiana* and a few Asian species (represented by *S. remotifolia* in our analysis, the others possibly conspecific with it) (Somers, 1982; Moran and Smith, 2001).

The exaltatae clade—This small clade comprises three species (8 accessions) in our analysis: *S. exaltata* from Central and South America, and *S. congoensis* and *S. myosurus* from Africa (Fig. 2B). *Selaginella exaltata* and *S. myosurus* have been included in previous phylogenetic studies but with unclear positions, both with respect to each other and to species here referred to the gymnogynum and ericetorum clades (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Arrigo et al., 2013; Zhou et al., 2015c). We show that the group is strongly supported as monophyletic, but its sister relationship to the ericetorum clade is only weakly supported. Zhou et al. (2015c) analyzed two of these species, *S. exaltata* and *S. myosurus*, but did not find them as sisters. The two species were referred to as subg. *Ericetorum* sect. *Megalosporarum* Li Bing Zhang & X.M.Zhou and subg. *Ericetorum* sect. *Myosurus* Li Bing Zhang & X.M.Zhou, respectively, by Zhou and Zhang (2015).

The exaltatae clade shares most morphological features with the gymnogynum clade, but only *S. exaltata* has traditionally been included in series *Articulatae* (Spring) Hieron. & Sadeb. There are two main morphological differences between the gymnogynum and exaltatae clades. First, not all species in the exaltatae clade have articulations; these are lacking in both *S. congoensis* and *S. myosurus* (Fig. 2B). Second, a unique type of stele, where the protostele is at least three-lobed, seems to support the monophyly of the exaltatae clade. In comparison, species in the gymnogynum clade have simple circular or elliptic protosteles. Mickel and Hellwig (1969) did a thorough study of the stelar arrangement of the stems of *S. exaltata* and found what they called an "actino-plectostele", a type of stele not previously described in *Selaginella*. They described it as a "three-lobed plectostele", i.e., a lobed protostele with xylem in several strands and these strands surrounded by phloem. We confirm their observation in our accessions of *S. exaltata*. The two other species, *S. congoensis* and *S. myosurus*, have a three-lobed actinostele in their main stems, i.e., a lobed protostele with xylem in a solid strand (S. Weststrand and P. Korall, personal observation). Besides these features, all members of the exaltatae clade also seem to share a more or less scandent growth form, a habit not common among other species in *Selaginella* (but see, e.g., *S. helferi* and *S. willdenowii* from Asia).

The ericetorum clade—Our results, based on six species (13 accessions), show that the three species previously included in



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subg. *Ericetorum* sensu Jermy (*S. gracillima*, *S. pygmaea*, and *S. uliginosa*) do not form a monophyletic group (Fig. 2B). Instead, they are strongly supported in a clade with three Madagascan species (*S. lyallii*, *S. moratii*, and *S. pectinata*), and the ericetorum clade as defined here includes all six species. The monophyly of this expanded group has been shown in previous studies (Korall and Kenrick, 2002; Arrigo et al., 2013; Zhou et al., 2015c; all three studies were based on the same six *rbcL* sequences). The group is treated as subg. *Ericetorum* sect. *Lyallia* (Rothm.) Li Bing Zhang & X.M.Zhou by Zhou and Zhang (2015), and III. *Lyallia* clade in superclade *Ericetorum*/superclade A by Zhou et al. (2015c).

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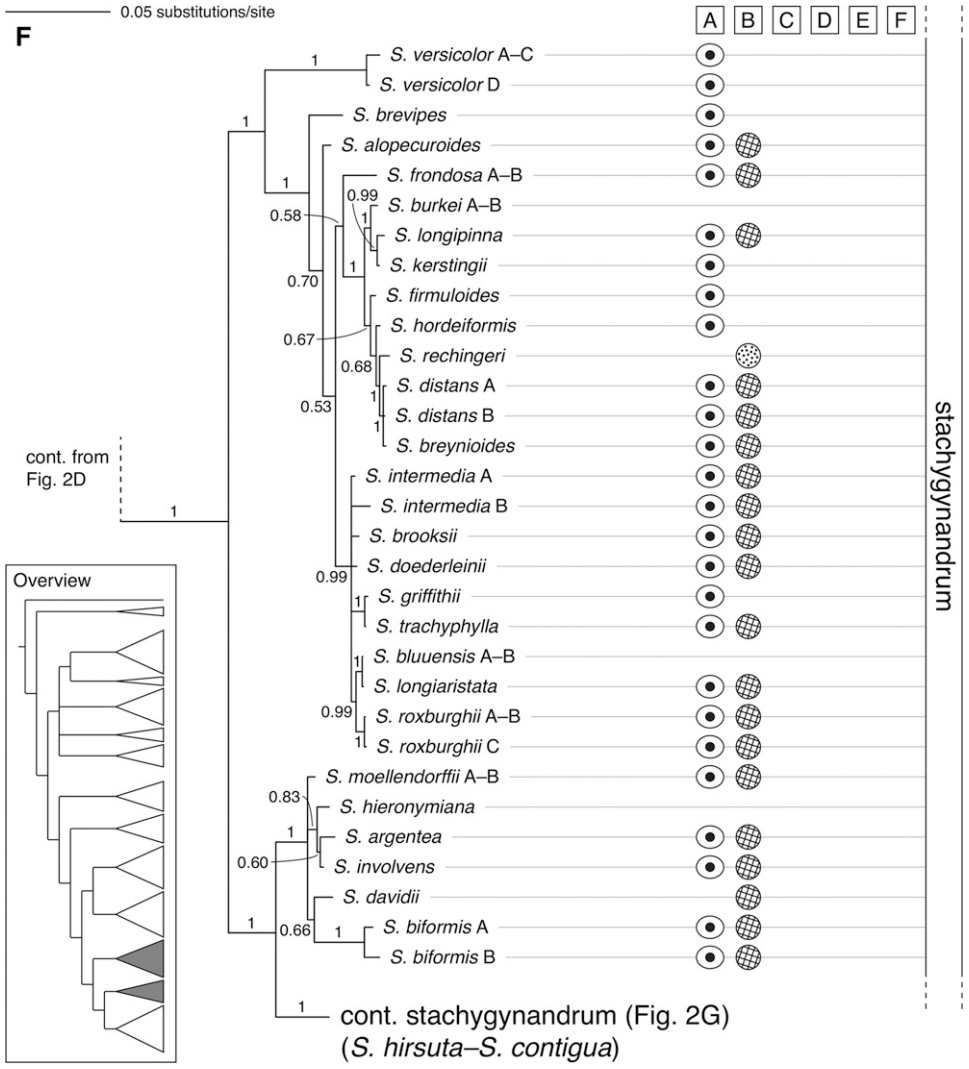
Relationships within the ericetorum clade have hitherto lacked support. Here we show that the South African specimen of *S. pygmaea* is sister to the three Madagascan species, and that these are, in turn, sister to the Australian *S. gracillima* and *S. uliginosa*. However, species boundaries are unclear in the group. Our results indicate that *S. gracillima* is not monophyletic, with *S. uliginosa* nested within, and that accessions of *S. lyallii*, *S. moratii*, and *S. pectinata* form an unresolved complex.

Selaginella uliginosa, *S. gracillima*, and *S. pygmaea* are small plants, the last two annuals, whereas the three Madagascan species (*S. lyallii*, *S. moratii*, and *S. pectinata*) are larger and perennial. At least some of the vegetative leaves are monomorphic and decussately



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arranged in all species, but leaves become dimorphic (arranged in four rows) in the distal parts of the shoots of the Madagascan species. Sporophylls are monomorphic and the strobili tetrastichous for all members of the ericetorum clade. Two morphological characters are unique for the group. First, the megaspores have wing-like laesurae, which are seen also in other species; in the ericetorum clade, this feature co-occurs with a porous surface between the laesurae. In some species, the laesurae are more or less convoluted at the pole, forming a "complex mass" (see, e.g., *S. gracillima*) (Minaki, 1984; Stefanović et al., 1997; Korall and Taylor, 2006; Schulz et al., 2013). Second, the perennials have a creeping solenostelic rhizome, commonly with polystelic stems (Harvey-Gibson, 1894; Hieronymus and Sadebeck, 1901; Bower, 1908; Steel, 1923; Jermy, 1986; Rauh and Hagemann, 1991). These two characters are not known in any other species of *Selaginella* to our knowledge.

Schulz et al. (2013) provided a morphology-based taxonomic revision of subg. *Ericetorum* sensu Jermy and suggested that *S. pygmaea* should be divided into two species: *S. pygmaea* in South Africa and *S. aboriginalis* C.Schulz & Homberg restricted to Australia. They also identified *S. royenii* Alston from New Guinea as belonging to the subgenus. All species are shown to have the wing-like laesurae with the complex mass at the proximal pole on the megaspore (Schulz et al., 2013). Thus, depending on species delimitation, the ericetorum clade might comprise at least eight species. The results of Schulz et al. (2013) and our phylogenetic analysis highlight the need to evaluate the species delimitations in the group, including analysis of DNA sequence data and additional sampling.

The stachygynandrum clade—Clade B, with 156 species (224 accessions) in our analysis, corresponds to the stachygynandrum clade (Fig. 2C–G). The group has mostly been retrieved with strong support in earlier studies, but the varying positions of the *sinensis* group have affected the interpretations (see section The *sinensis* group, below) (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Arrigo et al., 2013; Zhou et al., 2015c). Species in this clade were referred to as subgenera *Stachygynandrum*, *Heterostachys*, and *Pulviniella* Li Bing Zhang & X.M.Zhou by Zhou and Zhang (2015), and to superclade *Stachygynandrum*/superclade C, superclade *Heterostachys*/superclade B, and IX. *Rosulatae* clade by Zhou et al. (2015c).

All species in this clade have dimorphic vegetative leaves in four rows (at least on the distal parts of the plants), and most species have

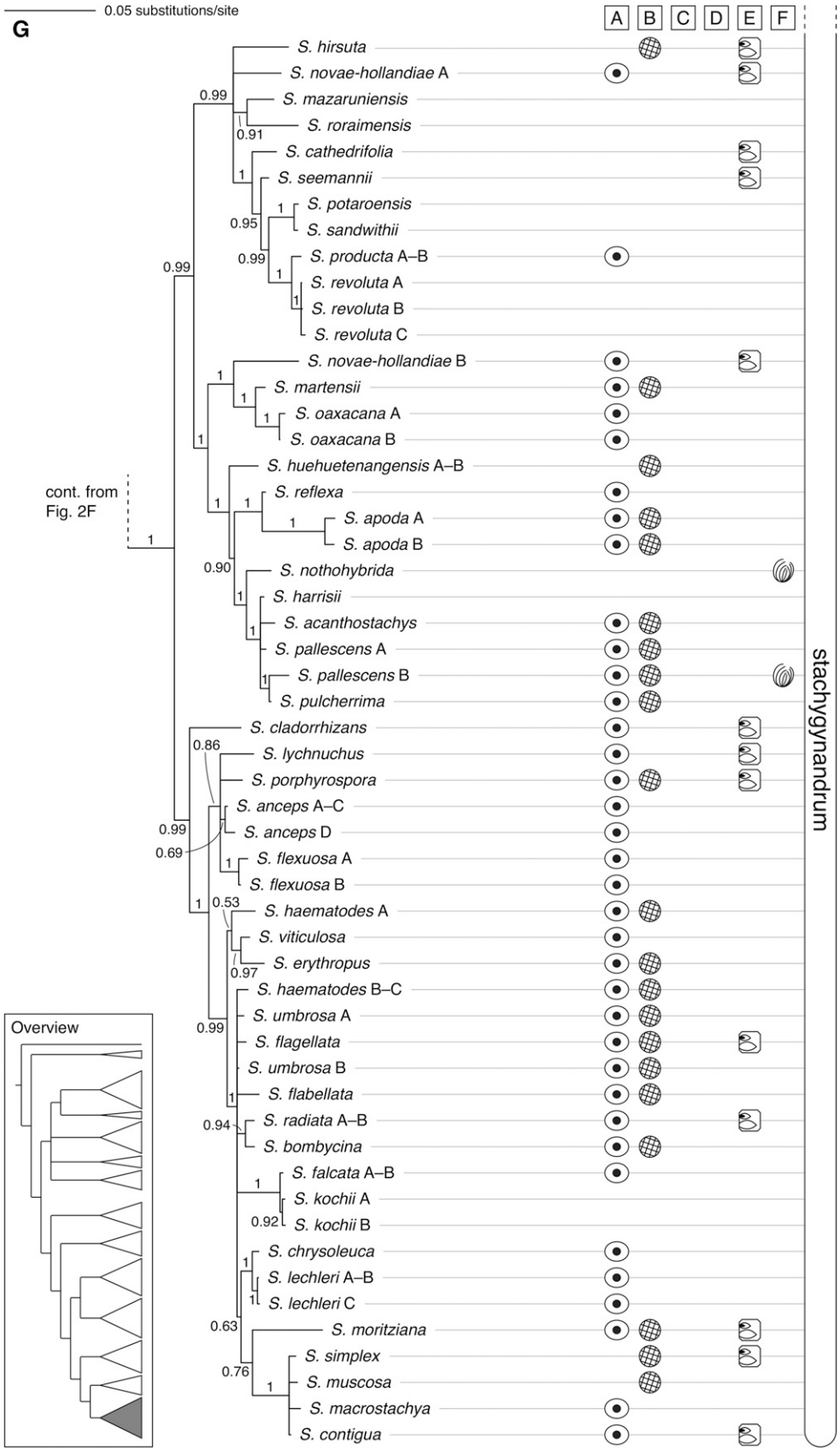
0.05 substitutions/site
G
A
B
C
D
E
F
S. hirsuta
S. novae-hollandiae A
S. mazaruniensis
S. roraimensis
S. cathedrifolia
S. seemannii
S. potaroensis
S. sandwithii
S. producta A–B
S. revoluta A
S. revoluta B
S. revoluta C
S. novae-hollandiae B
S. martensii
S. oaxacana A
S. oaxacana B
S. huehuetenangensis A–B
S. reflexa
S. apoda A
S. apoda B
S. nothohybrida
S. harrisii
S. acanthostachys
S. pallescens A
S. pallescens B
S. pulcherrima
S. cladorrhizans
S. lychnuchus
S. porphyrospora
S. anceps A–C
S. anceps D
S. flexuosa A
S. flexuosa B
S. haematodes A
S. viticulosa
S. erythropus
S. haematodes B–C
S. umbrosa A
S. flagellata
S. umbrosa B
S. flabellata
S. radiata A–B
S. bombycina
S. falcata A–B
S. kochii A
S. kochii B
S. chrysoleuca
S. lechleri A–B
S. lechleri C
S. moritziana
S. simplex
S. muscosa
S. macrostachya
S. contigua
cont. from Fig. 2F
Overview
stachygynandrum

TABLE 3. Morphological characteristics for the seven major clades presented in Fig. 1.

	Phyllotaxy		Anisophylly?			
Clade	**Vegetative leaves**	**Sporophylls**	**Vegetative leaves**	**Sporophylls**	**Rhizophores**	**Other features**
selaginella	Helical	Helical	No	No	No	—
rupestrae	Helical	Tetrastichous	No [a]	No [a]	Dorsal	—
lepidophyllae	Four rows	Tetrastichous	Yes	No	Dorsal	Rosette-forming
gymnogynum	Four rows	Tetrastichous	Yes [b]	No	Dorsal	Articulations
exaltatae	Four rows	Tetrastichous	Yes [b]	No	Dorsal	Some articulate species; actino- or actino-plectostelic stems
ericetorum	Decussate [c] or four rows [d]	Tetrastichous	No/Yes	No	Yes (position unclear)	Megaspore pole; solenostelic rhizomes
stachygynandrum	Four rows	Tetrastichous	Yes [b]	No/Yes	Ventral [e]	—

[a] A few species in the rupestrae clade may have a tendency toward slightly dimorphic vegetative leaves and/or sporophylls.
[b] At least on distal parts of plant.
[c] Monomorphic vegetative leaves.
[d] Dimorphic vegetative leaves.
[e] Members of the *sanguinolenta* group have dorsal rhizophores.

rhizophores that clearly originate on the ventral side in branch dichotomies. In some species, however, the rhizophores are restricted to the base of the stems and are difficult to refer to either a ventral or dorsal position. Sporophylls can be either monomorphic or dimorphic, the latter resulting in bilateral strobili. Subgenus *Stachygynandrum* sensu Jermy (1986) is, both in previous studies (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Zhou et al., 2015c) and in our analysis, shown to be nonmonophyletic. Jermy (1986) included most but not all species found in our clade B in his subg. *Stachygynandrum*, as well as all species in the gymnogynum, exaltatae, and lepidophyllae clades, and the Madagascan species in the ericetorum clade.

The stachygynandrum clade is by far the most diverse of the seven clades here recognized, and we identified a number of subclades, which are discussed below.

The *sinensis* group—The *sinensis* group, the sister clade to the rest of the stachygynandrum clade comprises 10 species (*S. yemensis*–*S. sechellarum*; 13 accessions; Fig. 2C). The phylogenetic position of the group has been inconclusive in previous studies, and the very long branches involved have been suggested as being responsible for the problems seen (Korall and Kenrick, 2002, 2004). In our present study, we expanded the taxon sampling and analyzed the data using Bayesian inference. Both approaches are known to decrease long-branch attraction effects (Felsenstein, 1978; Anderson and Swofford, 2004), and we here show an unequivocal position of the group.

Morphology supports the inclusion of the *sinensis* group in the stachygynandrum clade. All species in the *sinensis* group have dimorphic vegetative leaves in four rows, monomorphic sporophylls in tetrastichous strobili, and ventral rhizophores—all features that define the stachygynandrum clade. Morphological synapomorphies supporting the group itself have, however, hitherto been difficult to identify. The *sinensis* group is always well supported as monophyletic based on DNA sequence data (Korall and Kenrick, 2002; Zhou et al., 2015c; this study), but the gross morphology is variable; species are adapted to drought (e.g., *S. sinensis*) and to rain-forested regions (e.g., *S. australiensis*). Confusion about the group's phylogenetic position has further been fuelled by the disjunct distribution of the group, with species in China, Australia, and Africa, as well as on islands in the Indian Ocean; Madagascar, La Réunion, and the Seychelles. In a preliminary analysis, Zhou et al. (2015c) found the *sinensis* group to be well supported as monophyletic, but nevertheless excluded the clade from further analyses. They argued that more work was needed and referred to the lack of clear morphological synapomorphies; erroneously they cited Korall and Kenrick (2002) to have reported a possible pseudogene of *rbcL* in this clade.

We find that the species within the *sinensis* group share some morphological features. They all possess one (to few) basal megasporangia on the lower side of the strobili (Hieronymus and Sadebeck, 1901; Stefanović et al., 1997; Zhang et al., 2013; S. Weststrand and P. Korall, personal observation), a feature also reported for the gymnogynum and exaltatae clades. However, in the *sinensis* group the megasporangia usually contain fewer than four megaspores. Most of the specimens we have studied have three megaspores per sporangium, and Hieronymus and Sadebeck (1901) reported 1–2 megaspores for "Gruppe der *fissidentoides*" (including, e.g., *S. australiensis* and *S. fissidentoides*). Further, the specimens we have examined have a megaspore surface ornamentation with verrucae, blunt spines, and sometimes more elongated sculptural elements that often are sparsely distributed on the surface (Stefanović et al., 1997; Korall and Taylor, 2006; Zhou et al., 2015b; S. Weststrand and P. Korall, personal observation). We note that *S. australiensis* also has megaspores with sparsely distributed verrucae, and the reticulate pattern reported by Korall and Taylor (2006), based on a single megaspore, is most probably due to a misidentified specimen. Other species that may belong in this group are *S. fruticulosa* (Bory ex Willd.) Spring, *S. obtusa* Spring, and *S. viridula* Spring (Zhou et al., 2015c), all from islands in the Indian Ocean.

The *S. nubigena*–*S. digitata* clade—The clade (10 species, 13 accessions) is drought-adapted and sister to all species in the stachygynandrum clade except the *sinensis* group (Fig. 2C). The morphology varies within the clade, from species that have slightly curled branch tips when dry (e.g., *S. digitata*) to species with a rosetted habit, forming tight balls (e.g., *S. tamariscina*). The clade and its position were retrieved also by Korall and Kenrick (2002) and by Zhou et al. (2015c; IX. *Rosulatae* clade), and it corresponds to subg. *Pulviniella* of Zhou and Zhang (2015). We have increased the taxon sampling, showing that the clade has an even more cosmopolitan distribution than previously thought.

The megaspore surface ornamentation ranges from scabrate (e.g., *S. digitata*) to mostly low and irregular verrucae that often

cover the surface (e.g., *S. imbricata*), thus differing from the sparser and coarser ornamentation seen in the *sinensis* group (Minaki, 1984; Korall and Taylor, 2006; Zhou et al., 2015b, c).

The three mainly Asian–Australasian clades—Three clades with a mainly Asian–Australasian distribution, *S. douglasii–S. arbuscula* (Fig. 2D, E), *S. versicolor–S. roxburghii* (Fig. 2F), and *S. moellendorffii–S. biformis* (Fig. 2F), are all well supported based on DNA sequence data, as are the relationships among them and closely related groups.

The large *S. douglasii–S. arbuscula* clade (68 species, 98 accessions; Fig. 2D, E) comprises all species outside Central and South America with bilateral strobili. Also included, and intermixed with these species, are species with monomorphic sporophylls in tetrastichous strobili (Fig. 2D, E). The *S. douglasii–S. arbuscula* clade was also retrieved with strong support in earlier studies (Korall and Kenrick, 2002; subg. *Heterostachys* of Zhou and Zhang, 2015; clades X–XIII in superclade *Heterostachys*/superclade B of Zhou et al., 2015c).

Species with nonresupinate, bilateral strobili are members of the subclade *S. tama-montana–S. helvetica*, and many of these species have "loose" strobili with sporophylls somewhat distant from each other (Fig. 2D; corresponding to subg. *Heterostachys* sect. *Homostachys* (Baker) Li Bing Zhang & X.M.Zhou of Zhou and Zhang, 2015; and XII. *Homostachys* clade of Zhou et al., 2015c). Zhou et al. (2015c) suggested that the clade can be divided into two groups, one with species having loose, nonresupinate strobili, and the other with almost monomorphic sporophylls, i.e., *S. denticulata* and *S. helvetica*. However, neither their result nor ours support this division. Moreover, the result of Zhou et al. (2015c), which included several accessions of *S. helvetica*, indicates with low support that the species is nonmonophyletic. The *S. monospora–S. arbuscula* subclade predominantly includes species with resupinate strobili (Fig. 2E; corresponding to subg. *Heterostachys* sect. *Heterostachys* Li Bing Zhang & X.M.Zhou and subg. *Heterostachys* sect. *Tetragonostachyae* (Hook. & Grev.) Hieron. & Sadeb. of Zhou and Zhang, 2015; and XIII. *Heterostachys* clade of Zhou et al., 2015c). Species found on Pacific Islands (*S. behrmanniana–S. arbuscula*) form a well-supported clade nested within this mainly Asian clade, a pattern also retrieved by Zhou et al. (2015c). African species with bilateral strobili (e.g., *S. goudotiana*, *S. sambiranensis*, and *S. soyauxii*) are for the first time included in a phylogenetic analysis, and they are seen scattered among the other species in the *S. douglasii–S. arbuscula* clade (Fig. 2D, E).

The stele in species in the *S. douglasii–S. arbuscula* clade (Fig. 2D, E) ranges from simply monostelic to actinostelic and tristelic, with a simple monostele being the plesiomorphic condition. In the weakly supported subclade of *S. viridangula–S. mayeri* (Fig. 2D), all studied species for which we have information are tristelic. The group was not retrieved as monophyletic by Zhou et al. (2015c), but their result also showed low support. Zhou et al. (2015c) placed the neotropical *S. hoffmannii* Hieron. as a member of this clade, but we have examined one of the two vouchers (*C. J. Rothfels 08-088*, DUKE) and conclude that the specimen is actually *S. plana*, a species from Asia–Australasia. Both specimens included by Zhou et al. (2015c) were collected in a botanical garden in Costa Rica, which may explain the misidentification. A three-lobed actinostele occurs in stems of the three species in the *S. braunii–S. mairei* subclade (Fig. 2D; Harvey-Gibson, 1894; Wardlaw, 1925; S. Weststrand and P. Korall, personal observation). Detailed studies of *S. braunii* by Harvey-Gibson (1894) indicate that this actinostele differs from the actino- and actino-plectosteles seen in species in the exaltatae clade. In *S. braunii*, the actinostele of the erect stems arises from fusion of two vascular bundles in a bistelic rhizome (Harvey-Gibson, 1894). This character has not been reported in members of the exaltatae clade.

Megaspore ornamentation in the *S. douglasii–S. arbuscula* clade varies from verrucate and rugulate with protrusions of different size and density, to reticulate (Fig. 2D, E). Zhou et al. (2015c) highlighted some differences in the group, suggesting, e.g., a "disconnected laesurae" to be unique to the *S. tama-montana–S. helvetica* species (Fig. 2D).

The second of the mainly Asian–Australasian clades, the well-supported *S. versicolor–S. roxburghii* clade (20 species, 30 accessions; Fig. 2F), includes species with simple monosteles and isophyllous strobili. The group was also retrieved by Korall and Kenrick (2002) and Zhou et al. (2015c; XX. *S. doederleinii* clade in superclade *Stachygynandrum*/superclade C), but with an unclear phylogenetic position. It corresponds to subg. *Stachygynandrum* sect. *Ascendentes* (Baker) Li Bing Zhang & X.M.Zhou in the classification by Zhou and Zhang (2015). Many species in the group have megaspores with a "zona", a thin projecting structure at the equator (Minaki, 1984; Korall and Taylor, 2006; Zhou et al., 2015b, c). However, this feature is not unique to the clade; for example, *S. involvens* in the *S. moellendorffii–S. biformis* clade (Fig. 2F) has a prominent zona, and a few Central and South American species have similar structures. The distinction between zona and curvaturae perfectae (Punt et al., 1994) is sometimes unclear and may confuse the interpretation of this feature.

For the small *S. moellendorffii–S. biformis* clade (6 species, 8 accessions; Fig. 2F), which includes the genome-sequenced model species *S. moellendorffii* (Banks et al., 2011), we have not found a unique synapomorphy. As in the *S. versicolor–S. roxburghii* clade (and in many other species), the species have a simple monostele and isophyllous strobili. The *S. moellendorffii–S. biformis* clade is here strongly supported. The Zhou et al. (2015c) analysis showed species in this group to be in two separate clades, albeit with weak support (XIV. *S. biformis* clade and XV. *S. involvens* clade in superclade *Stachygynandrum*/superclade C). Despite this result, Zhou et al. (2015c) mentioned morphological similarities, including megaspore ornamentation and vegetative leaves. The *S. moellendorffii–S. biformis* clade corresponds to subg. *Stachygynandrum* sect. *Plagiophyllae* (Warb.) Li Bing Zhang & X.M.Zhou and subg. *Stachygynandrum* sect. *Circinatae* (Hook. & Grev.) Li Bing Zhang & X.M.Zhou in the classification by Zhou and Zhang (2015).

The mainly Central and South American clade—We found a strongly supported clade of almost exclusively Central and South American species, *S. hirsuta–S. contigua* (42 species, 62 accessions; Fig. 2G), as sister to the *S. moellendorffii–S. biformis* clade. The group was retrieved also in earlier studies, with strong (Korall and Kenrick, 2004) or weak support (Korall and Kenrick, 2002; Zhou et al., 2015c). It corresponds to subg. *Stachygynandrum* sect. *Austroamericanae* Li Bing Zhang & X.M.Zhou, subg. *Stachygynandrum* sect. *Heterophyllae* Spring, subg. *Stachygynandrum* sect. *Pallescentes* Li Bing Zhang & X.M.Zhou, and subg. *Stachygynandrum* sect. *Proceres* (Spring) Li Bing Zhang & X.M.Zhou in the classification by Zhou and Zhang (2015), and clade XVI–XIX in superclade *Stachygynandrum*/superclade C of Zhou et al. (2015c). About 20% of the species sampled possess bilateral resupinate strobili, but these do not form a monophyletic group (Fig. 2G). Even

though the species of the *S. hirsuta*–*S. contigua* clade occur mainly in the neotropics, the temperate North American species *S. apoda* and the African *S. cathedrifolia* were found to be members of this clade.

Enigmatic phylogenetic position of the* sanguinolenta *group—In our study, we identified a well-supported group, the *sanguinolenta* group, as having an unclear phylogenetic position that changes depending on the DNA region analyzed. The two species, *S. sanguinolenta* and *S. nummularifolia*, form a well-supported clade with the *S. nummularifolia* accession nested within *S. sanguinolenta*. In our analyses, the group was found in two different positions. The combined three-region data set, the *rbcL* data set, and the *SQD1* data set indicate, with weak to strong support, a position as sister to the rhizophoric clade (position α; Fig. 1). On the other hand, an analysis of the single-region *pgiC* data set shows, with moderate support, a position as sister to clade B (position β; Fig. 1). Zhou et al. (2015c) also retrieved these two topologies, the former using maximum likelihood and Bayesian inference analyses, the latter with parsimony analysis. They interpreted the position found in the parsimony analysis as a possible result of GC-biased chloroplast data and ignored it without further discussion. However, we got the same topology using nuclear data (*pgiC* data set), which were reported to not show a GC bias (Smith, 2009). The *sanguinolenta* group corresponds to subg. *Boreoselaginella* Warb. by Zhou and Zhang (2015) and II. *S. sanguinolenta* clade of Zhou et al. (2015c).

The *sanguinolenta* group has a morphology that suggests that a position as part of the stachygynandrum clade may reflect the true phylogeny (i.e., in line with position β; Fig. 1). Vegetative leaves are dimorphic, grading into monomorphic, and arranged in four rows. Sporophylls are monomorphic in tetrastichous strobili (Zhang et al., 2013). Furthermore, the two species are xerophytic, and the sporangial arrangement shows megasporangia and microsporangia intermixed, or with megasporangia on the lower side of the strobili (Zhang et al., 2013). This indicates a phylogenetic position close to drought-adapted species, except for the *sinensis* group with their unique sporangial arrangement. Studies of megaspores further support this conclusion. *Selaginella sanguinolenta* and *S. nummularifolia* both have megaspores with an outer surface covered with densely packed verrucae (Minaki, 1984; Liu and Yan, 2005; Zhou et al., 2015c; S. Weststrand and P. Korall, personal observation). A cross section of the exospore of *S. sanguinolenta* shows sheet-like structures (Minaki, 1984; S. Weststrand and P. Korall, personal observation; applying the terminology used by Korall and Taylor, 2006), and the innermost layer has free rod-ends/protrusions (S. Weststrand and P. Korall, personal observation). This combination of megaspore features is seen only in the stachygynandrum clade and more specifically in the *S. nubigena*–*S. digitata* subclade (Fig. 2C; Minaki, 1984; Korall and Taylor, 2006). Species belonging to *S. nubigena*–*S. digitata* also share the xerophytic habit, and some species have nearly monomorphic vegetative leaves. In contrast to the ventral rhizophores seen in species in the stachygynandrum clade, as presented here (Fig. 2), members of the *sanguinolenta* group possess dorsal rhizophores. However, there are many examples of reversals and/or parallelisms in the evolution of morphological characters in *Selaginella* (e.g., Korall and Kenrick, 2002; this study), and the occurrence of both ventral and dorsal rhizophores in the same plant is known, for example, in *S. martensii* (see, e.g., Harvey-Gibson, 1902). A possible position of the *sanguinolenta* group as part of the stachygynandrum clade should therefore not be rejected. Further studies with denser taxon sampling are needed to resolve unequivocally the affinities of the group.

Based on megaspore morphology, Minaki (1984) suggested *S. rossii* (Baker) Warb. to be closely related to *S. sanguinolenta*, although they differ in gross morphology (Zhang et al., 2013).

Morphological character evolution—Morphology in *Selaginella* has often been considered hard to interpret in an evolutionary context, and characters commonly used for defining subgroups have been shown to have complex evolutionary histories, with reversals and/or parallelisms (e.g., Korall and Kenrick, 2002; this study). Nevertheless, in our study we showed that some of these characters (or at least character states) are phylogenetically informative.

Gross morphology—Rhizophores have probably originated once in *Selaginella*, in the ancestor of the rhizophoric clade (the sister group Isoëtaceae lacks rhizophores). However, it is unclear how the position of the rhizophore has evolved in the rhizophoric clade. All species in the stachygynandrum clade have ventral rhizophores (with the exception of the *sanguinolenta* group, if it belongs here), whereas species in clade A have dorsal rhizophores, with the possible exception of the ericetorum clade where the rhizophores arise from the rhizome (Fig. 2A, B). In our study we distinguished between the major types of rhizophore positions: ventral or dorsal in branch dichotomies, at the base of stems, or along rhizomes. This distinction does, however, not reflect every facet of the variation observed in this feature. Furthermore, as for most of the morphological characters discussed here, a full understanding of rhizophore morphology and evolution needs thorough comparative studies, both within and among species.

Anisophyllous vegetative shoots also probably arose in the ancestor of the rhizophoric clade, since the members of the sister group, the selaginella clade, have isophyllous shoots. Within clade A, there are reversals to isophyllous shoots in the rupestrae clade and in some species in the ericetorum clade. Phyllotaxy of vegetative shoots and strobili follows the same trend. In the selaginella clade, both vegetative leaves and sporophylls are helically arranged, while in the rhizophoric clade the strobili are tetrastichous and the vegetative leaves are mostly arranged in four rows, with a reversal to helically arranged vegetative leaves in the rupestrae clade. Stem articulations are present only in species in the gymnogynum and exaltatae clades in clade A (Fig. 2B), but due to the unresolved relationships among major clades in clade A, the origin of the character is somewhat unclear.

Stelar arrangement—Stem stelar anatomy is complex in *Selaginella*. However, some character states/conditions appear to represent synapomorphies for clades found by analysis of DNA sequence data. A simple monostele seems to be the plesiomorphic condition in the genus. A bistelic stem is found only in the gymnogynum clade, but is scattered among the species in the clade (Fig. 2B). A tristelic stem is found in one species in the gymnogynum clade (*S. articulata*) and is also a distinguishing character for a clade (weakly supported) of Asian–Australasian taxa in the stachygynandrum clade (*S. viridangula*–*S. mayeri*; Fig. 2D). A lobed protostele (at least three-lobed) has arisen twice, in two separate, well-supported clades: once in the exaltatae clade (Fig. 2B) and once in a small clade in the stachygynandrum clade (*S. braunii*–*S. mairei*; Fig. 2D).

We also confirm solenostelic rhizomes in the perennial species of ericetorum, a type of stele not seen for any other *Selaginella* species (Fig. 2B). Thorough studies on more species (such as the study by Mickel and Hellwig, 1969) are needed for a full understanding of stelar anatomy in *Selaginella*.

Megaspore surface ornamentation—It is clear, from this and earlier studies, that megaspore morphology is phylogenetically informative in *Selaginella,* both with respect to surface ornamentation as well as exospore patterns in cross section (Korall and Taylor, 2006; Zhou et al., 2015c). Megaspore morphology varies considerably, with surface ornamentation ranging from scabrate (i.e., almost smooth, with patterning <1 μm), to solitary protrusions (verrucae, spines), to more elongate sculpturing (e.g., rugulae), or more or less closed reticulate patterns. The different types of sculpturing (see Punt et al., 1994, for terminology) grade into each other, and the density of the sculpturing varies (sometimes mentioned but rarely quantified, but see Korall and Taylor, 2006). As a consequence, it is often problematic to define clearly separated states of megaspore ornamentation. These unclear boundaries also affect how the terminology is applied by different authors, leading to difficulties when comparing studies.

Here, we chose to map only three different main types of megaspore ornamentation: scabrate, solitary protrusions, and reticulate. These patterns grade into one another, but assigning discrete character states allows us to study general trends; a caveat is that data on megaspore morphology is missing for ca. 40% of the studied species (Fig. 2). Species in the selaginella clade have megaspores with solitary protrusions. In clade A, we see almost exclusively reticulate megaspore sculpturing. In clade B/the stachygynandrum clade, the plesiomorphic condition seems to be solitary protrusions, with reticulate megaspores found in at least three clades: in a subclade of *S. viridangula*–*S. mayeri* (Fig. 2D), in a subclade of *S. monospora*–*S. arbuscula* (Fig. 2E), and as a possible synapomorphy for a large clade of *S. versicolor*–*S. contigua* (Fig. 2F, G). Scabrate megaspores are found mainly in the rupestrae clade in clade A (Fig. 2A, these species show interspecific variation; Tryon, 1949; Korall and Taylor, 2006) and in the *S. nubigena*–*S. digitata* clade in clade B (Fig. 2C).

Other megaspore features, besides surface ornamentation, are likely synapomorphies of clades within the genus. For example, wing-like laesurae combined with high porosity at the proximal pole occur in the ericetorum clade, and granules on the inner surface of exospores support the clade of lepidophyllae plus rupestrae. Other features are phylogenetically informative but involve parallelisms and/or reversals; examples are very ordered, grid-like pattern in cross sections of exospores in the clades gymnogynum, exaltatae, and parts of ericetorum, or the zona seen mainly in species in the *S. versicolor*–*S. roxburghii* clade (see, e.g., Korall and Taylor, 2006).

This and other studies that have evaluated megaspore morphology in a phylogenetic framework (Korall and Taylor, 2006; Zhou et al., 2015c) indicate that both surface ornamentation and exospore cross section will also be phylogenetically informative for clades closer to the tips of the phylogeny. However, we see problems when trying to unequivocally assign megaspores to specific spore types, where all features are lumped into one description (Minaki, 1984; Zhou et al., 2015b). We therefore suggest that a more analytical approach is needed, where morphological characters and character states (quantitative and qualitative) in megaspores are identified, delimited, and evaluated in a phylogenetic context.

Bilateral strobili—A morphological feature used in earlier *Selaginella* classifications is the presence of bilateral strobili. Baker (1883) included all species with bilateral, resupinate strobili in subg. *Heterostachys* Baker and those with nonresupinate strobili in subg. *Homostachys* Baker. These groups have persisted in later morphology-based classifications (e.g., Walton and Alston, 1938; Jermy, 1986). However, in agreement with earlier phylogenetic studies on *Selaginella* (Korall and Kenrick, 2002; Zhou et al., 2015c), we confirm that species having resupinate strobili are scattered in different well-supported subclades within the stachygynandrum clade (Fig. 2D, E, G). Species with nonresupinate strobili are restricted to the *S. tama-montana*–*S. helvetica* clade, but it is unclear if they represent a monophyletic group. Monomorphic sporophylls seem to be the plesiomorphic state for the family, with several origins of dimorphic sporophylls in the stachygynandrum clade. In their recent classification, Zhou and Zhang (2015) retained subg. *Heterostachys* but with a new circumscription that attempts to define a monophyletic group. They excluded all Central and South American species with resupinate strobili, but included in subg. *Heterostachys* species with monomorphic sporophylls.

Rosette-forming xerophytes—The rosette-forming, xerophytic *Selaginella* species (sometimes referred to as resurrection plants; their branches curl inwards into a ball when dry and uncurl when moisturized) have in some of the earliest *Selaginella* classifications been conjoined in one group (e.g., Spring, 1840, 1849; Baker, 1883). However, we confirm the results of previous phylogenetic studies (Korall and Kenrick, 2002; Zhou et al., 2015c) that show that the rosette-forming species are scattered in *Selaginella*: two species in the lepidophyllae clade and six species in different positions in the stachygynandrum clade (*S. convoluta*, *S. pilifera*, *S. pulvinata*, *S. tamariscina*, *S. nothohybrida*, and one of the two morphological forms of *S. pallescens*, Fig. 2A–G). Thus, the rosetted habit in *Selaginella* appears to have evolved independently at least three times, in clades with other dry-tolerant species. Members of the lepidophyllae clade are distinguished from the other rosette-forming species by having dorsal rhizophores.

Chromosome numbers—Chromosome numbers have been discussed in previous phylogenetic studies of *Selaginella* (Korall and Kenrick, 2002; Zhou et al., 2015c) and have been used in classification (Zhou and Zhang, 2015). Karyological data for *Selaginella* are known for only about 15% of the species, and the base chromosome numbers for the genus are suggested to be $x = 7$, 8, 9, 10, 11, and 12 (e.g., Manton, 1950; Tschermak-Woess and Doležal-Janisch, 1959; Kuriachan, 1963; Jermy et al., 1967; Ghatak, 1977; Takamiya, 1993; Marcon et al., 2005), even though the existence of $x = 11$ has been questioned (Jermy et al., 1967; Takamiya, 1993). The vast majority of the species studied are diploids having $2n = 18$ or $2n = 20$, and both Takamiya (1993) and Marcon et al. (2005) suggested that the karyological pattern for the genus is complex, with dysploidy occurring repeatedly. This conclusion is clearly supported by our data, despite the lack of information for many species. However, given scarce data, it is problematic to reliably reconstruct chromosome number evolution. With the inclusion of new taxa in this study, along with some additional chromosome data from the literature, we have re-evaluated the utility of chromosome numbers as diagnostic features in a classification. With respect to the recent classification of the genus by Zhou and Zhang (2015) the reported chromosome numbers for one of their six subgenera and eight of their 18 sections are problematic. These include their subg.

Pulviniella (*S. nubigena*–*S. digitata* in our phylogeny), which, with our addition of *S. convoluta* ($2n = 24$; Marcon et al., 2005), has new chromosome counts of $2n = 20, 24$, and their subg. *Stachygynandrum* sect. *Ascendentes* (*S. versicolor*–*S. roxburghii* in our phylogeny), which now includes species with chromosome counts of $2n = 16$, 18, and $3n = 27$ (*S. kerstingii* with $2n = 16$ and *S. bluuensis* with $3n = 27$; Jermy et al., 1967). The other seven sections of Zhou and Zhang (2015) for which the given chromosome numbers are doubtful are all in their subg. *Stachygynandrum*: sect. *Circinatae*, *Heterophyllae*, *Heterostachys*, *Oligomacrosporangiatae* Hieron. & Sadeb., *Pallescentes*, *Plagiophyllae*, and *Proceres*. Furthermore, some of the available chromosome counts are reported from cultivated specimens with ploidal levels that likely are a result of human selection (e.g., *S. martensii* with $2n = 50$–60; Jermy et al., 1967). We conclude that with the limited information available on chromosome number, and the demonstrated evolutionary complexity in the genus, chromosome numbers should be used with caution.

Species delimitations and alpha taxonomy—Historically, *Selaginella* is a genus beset with taxonomic confusion, in part because of the large number of species. Contributing to the confusion is the seemingly undifferentiated gross morphology and a paucity of modern monographic treatments that consider the totality of species in a phylogenetic context. These factors have lead to great difficulty in identifying species. A major problem is the lack of basic floristic work in many parts of the world. In this study we have, when possible, included several accessions per species to get a rough idea of the present status of the alpha taxonomy. We have taken a cautious approach when including previously published accessions and have excluded most accessions that would render species nonmonophyletic, unless we could verify the identity of the specimens. Nevertheless, we have shown that nonmonophyletic species definitely are present in our phylogeny (e.g., *S. delicatula*, *S. gracillima*, *S. haematodes*, S. *novae-hollandiae*, *S. pallescens*, and *S. plana*), with the caveat that some specimens still may be misidentified, despite our efforts. With the constraints adopted, we have found fewer nonmonophyletic species than previously reported (Zhou et al., 2015c).

Some of the nonmonophyletic species are variable morphologically, such as *S. novae-hollandiae*, whereas others are represented by accessions that are morphologically very similar, despite their obvious nonmonophyly (e.g., *S. delicatula* and *S. haematodes*). Similar scenarios are seen at the species level, where two species may be almost identical, but are found in different positions in the tree. The two Asian species, *S. chrysocaulos* and *S. labordei* (here represented by two and five accessions, respectively; Fig. 2E), are found in different subclades in the stachygynandrum clade, despite having very similar morphology. The feature that most easily distinguishes herbarium material of the two species is the sporophyll margins that in *S. labordei* are white and slightly more ciliolate than those of *S. chrysocaulos*. Before our reidentifications, herbarium specimens included in our study were variously determined as one or the other, highlighting the difficulty in identification. Contradictions in published phylogenies and the resultant classifications still exist. As an example, contrary to our study, Zhou et al. (2015c) found their *S. chrysocaulos* accession in a position close to *S. labordei*; further study is needed to resolve this issue, which is perhaps due to misidentification. The same pattern is also true for *S. bisulcata* and *S. pennata*, two species with very similar gross morphology, which in our study are found in two separate clades (*S. douglasii*–*S. mayeri* and *S. monospora*–*S. arbuscula*, respectively), in contrast to Zhou et al. (2015c), who depicted the two species as sister taxa.

We also note possible species complexes with unclear species boundaries, such as the three Madagascan species in the ericetorum clade: *S. lyallii*, *S. moratii*, and *S. pectinata*. In the *sanguinolenta* group, *S. nummularifolia* is nested within *S. sanguinolenta* (Appendices S1–S4), with the latter species showing remarkable intraspecific variation at the DNA sequence level, as well as in morphology (Zhang et al., 2013), indicating that the species boundaries are unclear.

Our increased understanding of the evolutionary relationships in *Selaginella*, from this and earlier studies (Korall et al., 1999; Korall and Kenrick, 2002, 2004; Korall and Taylor, 2006; Arrigo et al., 2013; Zhou et al., 2015c), together with supporting morphology, provide a framework for addressing the alpha taxonomy of well-defined smaller groups within the family.

***Concluding remarks on the evolutionary relationships of* Selaginella**—In this study, we have presented a robust hypothesis of the evolutionary relationships within *Selaginella*, building upon earlier phylogenetic studies and with an increased taxon sampling, covering both the morphological and geographical diversity in the genus. We have also shown morphological characters that correlate with this hypothesis, based on analysis of DNA sequence data. We resolved the position of the *sinensis* group, and we addressed the position of another enigmatic group, the *sanguinolenta* group, using morphology.

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APPENDIX 1 **Voucher information and GenBank accession data for material used. Missing data are indicated by —. Herbarium acronyms follow Index Herbariorum (Thiers, 2008). References are indicated by superscript letters, as follows: [a] Korall and Kenrick (2002), [b] Rydin and Wikström (2002), [c] Korall et al. (1999), [d] Arrigo et al. (2013), [e] sequences were obtained from the same DNA extract as used by Korall et al. (1999), [f] Ebihara et al. (2010), [g] Zhou et al. (2015c), [h] sequences were obtained from the same DNA extract as used by Korall and Kenrick (2002), [i] Therrien and Haufler (unpublished), [j] sequences were obtained from the same DNA extract as used by Korall and Kenrick (2004), [k] Manhart (1994), [l] Smith (2009), [m] Yi et al. (unpublished), [n] Wikström and Kenrick (1997), [o] Tsuji et al. (2007), [p] voucher collected by P. Korall in 1998 from the same plant in cultivation as collected by S. Weststrand 2011–2016.**

Taxon; Voucher number and herbarium acronym; GenBank accession numbers: *rbcL*, *pgiC*, *SQD1*; Collection locality.

Isoëtes andina Spruce ex Hook.; —; AF404492[b], —, —; Colombia ♦ ***I. echinospora*** Durieu; P. Korall 2013:61 (UPS); KY022953, —, —; Sweden ♦ ***I. lacustris*** L.; —; AJ010855[c], —, —; Sweden ♦ ***I. melanopoda*** J.Gay & Durieu; —; L11054[k], —, —; USA ♦ ***I. sp.*** NA; The 1000 Plants Initiative (1KP, onekp.com), sample PYHZ; KY023314, KY023185, KY022842; Unknown ♦ ***I. tegetiformans*** Rury; The 1000 Plants Initiative (1KP, onekp.com), sample PKOX; KY022841, KY023186, KY022843; Unknown.

Selaginella acanthonota Underw.; **(A)** The 1000 Plants Initiative (1KP, onekp.com), sample ZYCD; KY023183, KY023187, KY023317; USA (North Carolina); **(B)** R. F. Britt 3041 (S); KY022954, —, —; USA (North Carolina) ♦ ***S. acanthostachys*** Baker; —; AJ295884[a], —, —; Ecuador ♦ ***S. albocincta*** Ching; D. E. Boufford et al. 35039 (A); KY022957, KY022847, —; China (Yunnan) ♦ ***S. alopecuroides*** Baker ; —; AJ295875[a], —, —; East Malaysia (Borneo) ♦ ***S. alutacea*** Spring; P. Korall 2006:9 (S); KY022958, —, KY023191; Peninsular Malaysia ♦ ***S. anceps*** (C.Presl) C.Presl; **(A)** H. Tuomisto 16658 (UPS); KY022962, —, KY023193; Brazil; **(B)** H. Tuomisto 16380 (UPS); KY022961, —, KY023192; Brazil; **(C)** H. Ellenberg 2353 (U); KY022960, —, —; Peru; **(D)** P. J. M. Maas and R. L. Dressler 1682 (U); KY022959, —, —; Panama ♦ ***S. apoda*** (L.) C.Morren; **(A)** The 1000 Plants Initiative (1KP, onekp.com), sample LGDQ; KY023315, KY023188, KY023184; Cultivated; **(B)** —; AJ010854[c], —, —; USA (North Carolina) ♦ ***S. arbuscula*** (Kaulf.) Spring; H. H. Iltis et al. 96 (U); KY022963, —, —; Hawaii ♦ ***S. arenicola*** Underw.; —; AF419084[d], —, —; USA (Louisiana) ♦ ***S. argentea*** (Wall. ex Hook. & Grev.) Spring; P. Korall 2006:55 (S); KY022964, KY022848, KY023194; Peninsular Malaysia ♦ ***S. arizonica*** Maxon; —; AJ010851[c], KY022949[e], KY023195[e]; USA (Arizona) ♦ ***S. articulata*** (Kunze) Spring; —; AJ295894[a], —, —; Ecuador ♦ ***S. asperula*** Spring; **(A)** H. Tuomisto 16903 (UPS); KY022968, KY022850, KY023198; Brazil; **(B)** G. T. Prance et al. 13806 (U); KY022967, —, —; Brazil (Amazonas) ♦ ***S. australiensis*** Baker; **(A)** S. Weststrand 89 (NSW, UPS); KY022969, KY022851, —; Australia (Queensland); **(B)** —; AJ295890[a], —, —; Australia ♦ ***S. balansae*** (A.Braun) Hieron.; J. Gattefossé s.n. (15 January 1935) (S); KY022970, KY022852, KY023199; Morocco ♦ ***S. bamleri*** Hieron. ex Brause; W. J. Baker 856 (L); KY022971, —, KY023200; Indonesia (New Guinea) ♦ ***S. banksii*** Alston; M. L. Grant 3563 (L); KY022972, —, —; French Polynesia ♦ ***S. behrmanniana*** Hieron. ex Brause; R. J. Johns 8937 (L); KY022973, —, KY023201; Indonesia (New Guinea) ♦ ***S. biformis*** A.Braun ex Kuhn; **(A)** F. E. Schmutz SVD 5175 (L); KY022974, —, KY023202; Indonesia (Lesser Sunda Islands); **(B)** —; AB574641[f], —, —; Japan ♦ ***S. bigelovii*** Underw.; —; KT161401[g], —, —; USA (California) ♦ ***S. bisulcata*** Spring; **(A)** Gaoligong Shan Biodiversity Survey 22176 (GH); KY022975, KY022853, —; China (Yunnan); **(B)** C. R. Fraser-Jenkins 5321 (L); KY022976, —, —; Nepal ♦ ***S. bluuensis*** Alderw.; **(A)** B. S. Parris 11530 (L); KY022978, KY022854, KY023204; East Malaysia (Borneo); **(B)** W. M. A. Brooke 9610 (L); KY022977, —, KY023203; East Malaysia (Borneo) ♦ ***S. bombycina*** Spring; —; AJ010848[c], —, —; Cultivated ♦ ***S. boninensis*** Baker; —; AB574642[f], —, —; Japan ♦ ***S. brachystachya*** (Hook. & Grev.) Spring; **(A)** J. Klackenberg 434 (S); KY022980, —, —; Sri Lanka; **(B)** W. A. Sledge 913 (L); KY022979, —, —; Sri Lanka ♦ ***S. braunii*** Baker; —; KT161419[g], —, —; China (Yunnan) ♦ ***S. brevipes*** A.Braun; B. S. Parris 11341 (L); KY022981, —, KY023205; East Malaysia (Borneo) ♦ ***S. breynioides*** Baker; U. Swenson 524 (S); KY022982, KY022855, KY023206; Fiji ♦ ***S. brooksii*** Hieron.; —; AJ295876[a], KY022941[h], KY023207[h]; East Malaysia (Borneo) ♦ ***S. bryopteris*** (L.) Baker; C. R. Fraser-Jenkins 4370 (L); KY022983, —, —; Nepal ♦ ***S. burkei*** Hieron.; **(A)** R. J. Johns 9293 (L); KY022984, KY022856, KY023208; Indonesia (New Guinea); **(B)** R. J. Johns 9259 (L); KY022985, —, KY023209; Indonesia (New Guinea) ♦ ***S. caffrorum*** (Milde) Hieron.; G. J. de Joncheere SAC 237 (L); KY022986, —, —; South Africa ♦ ***S. cathedrifolia*** Spring; A. J. M. Leeuwenberg 6019 (U); KY022987, —, KY023210; Cameroon ♦ ***S. caudata*** (Desv.) Spring; J. R. Croft 1073 (L); KY022988, —, KY023211; Papua New Guinea ♦ ***S. chrysocaulos*** (Hook. & Grev.) Spring; **(A)** Gaoligong Shan Biodiversity Survey 27010 (GH); KY022989, KY022857, KY023212; China (Yunnan); **(B)** H. Li 12202 (GH); KY022990, —, —; China (Yunnan) ♦ ***S. chrysoleuca*** Spring; F. Hekker and W. H. A. Hekking 10415 (U); KY022991, —, —; Ecuador ♦ ***S. chuweimingii*** X.M.Zhou et al.; X. C. Zhang et al. 2737 (L); KY023156, KY022932, KY023298; China (Yunnan) ♦ ***S. ciliaris*** (Retz.) Spring; **(A)** P. Korall 2006:2 (S); KY022992, —, —; Peninsular Malaysia; **(B)** P. Korall 2006:61 (S); KY022993, —, —; Peninsular Malaysia ♦ ***S. cinerascens*** A.A.Eaton; —; KT161429[g], —, —; USA (California) ♦ ***S. cladorrhizans*** A.Braun; Y. Mexia 8725 (S); KY022994, KY022858, —; Mexico (Guerrero) ♦ ***S. conduplicata*** Spring; **(A)** H. Tuomisto 16907 (UPS); KY023000, KY022863, —; Brazil; **(B)** H. Tuomisto 15252 (UPS); KY022997, KY022860, —; Brazil (Amazonas); **(C)** H. Tuomisto 16061 (UPS); KY022998, KY022861, —; Brazil; **(D)** H. Tuomisto 16799 (UPS); KY022999, KY022862, —; Brazil; **(E)** M. J. Jansen-Jacobs et al. 6544 (U); KY022995, KY022859, —; Suriname; **(F)** G. A. Cremers 5331 (U); KY022996, —, —; French Guiana ♦ ***S. congoensis*** Alston ; D. Champluvier 5059 (BR); KY023001, —, —; Republic of the Congo ♦ ***S. contigua*** Baker; P. G. Windisch 08/69 (U); KY023002, —, —; Brazil (Rio de Janeiro) ♦ ***S. convoluta*** (Arn.) Spring; R. M. Harley 16181 (U); KY023003, —, —; Brazil (Bahia) ♦ ***S. crassipes*** Spring; F. Fagerlind and J. Klackenberg 654 (S); KY023004, —, KY023213; Sri Lanka ♦ ***S. davidii*** Franch.; D. E. Boufford et al. 37535 (A); KY023005, KY022864, KY023214; China (Gansu) ♦ ***S. deflexa*** Brack.; —; AF093253[c], —, —; Hawaii ♦ ***S. delicatula*** (Desv.) Alston; **(A)** W. Takeuchi 16765 (L); KY023011, KY022868, KY023219; Papua New Guinea; **(B)** P. Korall 2006:56 (S); KY023007, KY022865, —; Peninsular Malaysia; **(C)** K. U. Kramer et al. 8224 (U); KY023006, —, KY023215; China (Hong Kong); **(D)** E. Schuettpelz 1218A (DUKE); KY023010, KY022867, KY023218; Taiwan; **(E)** K. U. Kramer et al. 7507 (U); KY023009, —, KY023217; Taiwan; **(F)** S. T. Chiu 05474 (NSW); KY023008, KY022866, KY023216; Taiwan ♦ ***S. denticulata*** (L.) Spring; —; AJ010853[c], —, —; Greece ♦ ***S. diffusa*** (C.Presl) Spring; —; AJ010852[c], —, —; Cultivated ♦ ***S. digitata*** Spring; **(A)** N. Wikström et al. 110319-2 (S); KY023013, —, —; Madagascar; **(B)** P. Phillipson 1826 (L); KY023012, —, —; Madagascar; **(C)** —; AJ295895[a], —, —; Madagascar ♦ ***S. distachya*** Cordem.; K. U. Kramer et al. 9248 (U); KY023014, —, —; Réunion ♦ ***S. distans*** Warb.; **(A)** U. Swenson 526 (S); KY023016, KY022869, KY023221; Fiji; **(B)** A. C. Smith 9269 (S); KY023015, —, KY023220; Fiji ♦ ***S. doederleinii*** Hieron.; —; AB574643[f], —, —; Japan ♦ ***S. douglasii*** (Hook. & Grev.) Spring; **(A)** C. J. Rothfels 3863 (DUKE); KY023017, KY022870, —; USA (Oregon); **(B)** —; AF419049[l], —, —; Unkown ♦ ***S. dregei*** (C.Presl) Hieron.; L. Smook 8211 (BR); KY023018, —, KY023222; South Africa ♦ ***S. echinata*** Baker; S. Larsson et al. L048 (S); KY023019, —, KY023223; Madagascar ♦ ***S. effusa*** Alston; Gaoligong Shan Biodiversity Survey 22153 (GH); KY023020, KY022871, KY023224; China (Yunnan) ♦ ***S. eremophila*** Maxon; —; KT161454[g], —, —; USA (California) ♦ ***S. erythropus*** (Mart.) Spring; S. Weststrand 106 (UPS); AJ295877[a, p], KY022872, KY023225; Cultivated ♦ ***S. eublepharis*** A.Braun ex Hieron.; **(A)** E. P. J. Zuidgeest s.n. (6 June 1978) (L); KY023022, —, —; Zanzibar; **(B)** D. K. Harder et al. 1518 (L); KY023021, —, —; Tanzania ♦ ***S. eurynota*** A.Braun; C. J. Rothfels et al. 08-183 (DUKE); KY023023, KY022873, —; Costa Rica ♦ ***S. exaltata*** (Kunze) Spring; **(A)** H. Tuomisto 16359 (UPS); KY023024, KY022874, KY023226; Brazil; **(B)** —; AJ010849[c], —, —; Ecuador ♦ ***S. extensa*** Underw.; —; AF419085[d], —, —; Mexico (Jalisco) ♦ ***S. falcata*** (P.Beauv.) Spring; **(A)** L. Skog and C. Feuillet 7236 (U); KY023025, KY022875, KY023227; French Guiana; **(B)** S. Lehtonen

923 (UPS); KY023026, KY022876, KY023228; French Guiana ♦ ***S. firmuloides*** Warb.; —; AJ295870[a], —, —; New Caledonia ♦ ***S. fissidentoides*** (Hook. & Grev.) Spring **var. *amphirrhizos*** (A.Braun ex Hieron.) Stefanović & Rakotondr.; F. Rakotondrainibe 6664 (BR); KY023027, KY022877, —; Mayotte ♦ ***S. fissidentoides*** (Hook. & Grev.) Spring **var. *fissidentoides***; N. Wikström et al. 110307-1 (S); KY023028, KY022878, —; Madagascar ♦ ***S. flabellata*** (L.) Spring; —; AJ295885[a], —, —; Grenada ♦ ***S. flagellata*** Spring; —; AJ295866[a], —, —; Venezuela ♦ ***S. flexuosa*** Spring; **(A)** J. C. Lindeman and J. H. de Haas 4061a (U); KY023029, —, —; Brazil (Paraná); **(B)** J. A. Steyermark and G. Agostini 91109 (U); KY023030, —, —; Venezuela ♦ ***S. fragilis*** A.Braun; —; AJ295872[a], —, —; Ecuador ♦ ***S. frondosa*** Warb.; **(A)** B. S. Parris 25/85 (L); KY023032, —, KY023230; East Malaysia (Borneo); **(B)** N. Wikström and H. Wanntorp 142 (S); KY023031, —, KY023229; East Malaysia (Borneo) ♦ ***S. fulcrata*** (Ham.) Spring; Unknown s.n. (mounted by Mrs. P. Jaffrey, herbarium no. U0283095) (U); KY023033, —, —; India (West Bengal) ♦ ***S. geniculata*** (C.Presl) Spring; **(A)** C. J. Rothfels et al. 08-113 (DUKE); KY023035, KY022879, KY023231; Cultivated; **(B)** P. J. M. Maas et al. 4562 (U); KY023034, —, —; Peru ♦ ***S. goudotiana*** Spring **var. *abyssinica*** (Spring) Bizzarri; **(A)** J. Kornaś 1172A (BR); KY023037, —, —; Tanzania; **(B)** R. E. G. Pichi Sermolli 6756 (L); KY023038, —, —; Ethiopia; **(C)** J. Kornaś and A. Medwecka-Kornaś 3688 (BR); KY023036, —, —; Zambia ♦ ***S. goudotiana*** Spring **var. *goudotiana***; M. Thulin and H. Razafindraibe 11750 (UPS); KY023039, KY022880, —; Madagascar ♦ ***S. gracillima*** (Kunze) Spring ex Salomon; **(A)** —; AJ010844[c], —, KY023232[e]; Australia (Victoria); **(B)** A. E. Orchard 4319 (L); KY023040, —, —; Australia (Western Australia) ♦ ***S. griffithii*** Spring; C. Charoenphol et al. 3685 (S); KY023041, KY022881, KY023233; Thailand ♦ ***S. grisea*** Alston; —; AF419072[d], —, —; Unknown ♦ ***S. haematodes*** (Kunze) Spring; **(A)** —; AJ010846[c], —, —; Ecuador; **(B)** H. Tuomisto 16353 (UPS); KY023043, KY022883, KY023235; Brazil; **(C)** H. Tuomisto 16198 (UPS); KY023042, KY022882, KY023234; Brazil ♦ ***S. harrisii*** Underw. & Hieron.; J. B. Beck 1124 (DUKE); KY023044, KY022884, KY023236; Mexico (San Luis Potosí) ♦ ***S. helferi*** Warb.; —; KT161470[g], —, —; China (Yunnan) ♦ ***S. helicoclada*** Alston; —; AJ295896[a], —, —; Madagascar ♦ ***S. helvetica*** (L.) Spring; **(A)** D. E. Boufford et al. 35943 (A); KY023045, KY022885, —; China (Sichuan); **(B)** —; AJ295891[a], KY022947[h], —; Georgia ♦ ***S. heterostachys*** Baker; —; AB574645[f], —, —; Japan ♦ ***S. hieronymiana*** Alderw.; P. J. Edwards 4333 (L); KY023046, KY022886, KY023237; Indonesia (New Guinea) ♦ ***S. hirsuta*** Alston ex Crabbe & Jermy; J. Renz 14195 (U); KY023047, —, —; Guyana ♦ ***S. hordeiformis*** Baker; O. H. Selling 1 (S); KY023048, —, KY023238; New Caledonia ♦ ***S. huehuetenangensis*** Hieron.; **(A)** R. D. Worthington 21322 (L); KY023049, —, —; Belize; **(B)** T. G. Yuncker et al. 5707 (U); KY023050, —, —; Honduras ♦ ***S. imbricata*** (Forssk.) Spring; —; AJ295897[a], —, —; Unknown ♦ ***S. inaequalifolia*** (Hook. & Grev.) Spring; K. U. Kramer and G. B. Nair 6154 (L); KY023051, —, —; India (Kerala) ♦ ***S. indica*** Milde (R.M.Tryon); —; KT161487[g], —, —; China (Yunnan) ♦ ***S. ingens*** Alston; B. S. Parris 11420 (L); KY023052, —, KY023239; East Malaysia (Borneo) ♦ ***S. intermedia*** (Blume) Spring; **(A)** P. Korall 2006:14 (S); KY022965, KY022849, KY023196; Peninsular Malaysia; **(B)** P. Korall 2006:48 (S); KY022966, —, KY023197; Peninsular Malaysia ♦ ***S. involvens*** (Sw.) Spring; D. E. Boufford 37638 (A); KY023053, —, KY023190; China (Gansu) ♦ ***S.* cf. *kalbreyeri*** Baker; F. J. Breteler et al. 2239 (BR); KY023054, —, —; Cameroon ♦ ***S. kerstingii*** Hieron.; —; AJ295881[a], KY022945[h], KY023240[h]; Cultivated ♦ ***S. kochii*** Hieron.; **(A)** F. H. F. Oldenburger et al. ON593 (U); KY023055, KY022887, KY023241; Suriname; **(B)** J. J. de Granville 1051 (U); KY023056, —, KY023242; Brazil (Amapá) ♦ ***S. kraussiana*** (Kunze) A.Braun; **(A)** M. Mokoso 3098 (BR); KY023057, KY022888, KY023243; Democratic Republic of the Congo (South Kivu); **(B)** S. Weststrand 105 (UPS); KY023058, —, KY023244; Cultivated; **(C)** —; AJ010845[c], —, —; Cultivated ♦ ***S. labordei*** Hieron. ex Christ; **(A)** E. Schuettpelz 1176A (DUKE); KY023060, KY022889, —; Taiwan; **(B)** D. E. Boufford 37639 (A); KY023062, —, —; China (Gansu); **(C)** H. Smith 2345 (S); KY023059, —, —; China (Sichuan); **(D)** Gaoligong Shan Biodiversity Survey 18378 (GH); KY023061, —, —; China (Yunnan); **(E)** D. E. Boufford et al. 33036 (A); KY022955, KY022845, —; China (Sichuan) ♦ ***S. landii*** Greenm. & N.Pfeiff.; —; KT161506[g], —, —; Mexico (Jalisco) ♦ ***S. laxa*** Spring; T. G. Yuncker 15994 (U); KY023063, —, KY023245; Tonga ♦ ***S. laxistrobila*** K.H.Shing; D. E. Boufford et al. 30527 (A); KY023064, KY022890, —; China (Sichuan) ♦ ***S. lechleri*** Hieron.; **(A)** H. Tuomisto 16754 (UPS); KY023067, KY022893, KY023247; Brazil; **(B)** H. Tuomisto 16564 (UPS); KY023066, KY022892, KY023246; Brazil; **(C)** H. Tuomisto 16090 (UPS); KY023065, KY022891, —; Brazil ♦ ***S. lepidophylla*** (Hook. & Grev.) Spring; **(A)** —; AF093254[c], KY022894[j], KY023313[j]; Unknown; **(B)** —; AF419051[d], —, —; USA (Texas) ♦ ***S. leptophylla*** Baker; —; KT161512[g], —, —; China (Sichuan) ♦ ***S. leucobryoides*** Maxon; —; KT161515[g], —, —; USA (California) ♦ ***S. limbata*** Alston; —; AB574647[f], —, —; Japan ♦ ***S. lingulata*** Spring; **(A)** C. J. Rothfels et al. 08-096 (DUKE); KY023069, KY022895, KY023248; Costa Rica; **(B)** —; AJ295882[a], —, —; Ecuador; **(C)** H. Balslev and E. Madsen 10318 (U); KY023068, —, —; Ecuador ♦ ***S. longiaristata*** Hieron.; —; AJ295873[a], KY022944[h], KY023249[h]; East Malaysia (Borneo) ♦ ***S. longipinna*** Warb.; —; AJ295860[a], —, —; Australia ♦ ***S. lutchuensis*** Koidz.; —; AB574648[f], —, —; Japan ♦ ***S. lyallii*** (Hook. & Grev.) Spring; **(A)** N. Wikström et al. 110311-2 (S); KY023070, KY022896, KY023250; Madagascar; **(B)** —; AJ295898[a], —, —; Madagascar ♦ ***S. lychnuchus*** Spring; Flora Falcón 869 (U); KY023071, KY022897, —; Venezuela ♦ ***S. macrostachya*** (Spring) Spring; A. Lourteig 2358 (U); KY023072, —, —; Brazil (Santa Catarina) ♦ ***S. mairei*** H.Lév.; D. E. Boufford et al. 35219 (A); KY023073, KY022898, —; China (Yunnan) ♦ ***S. marginata*** (Humb. & Bonpl. ex Willd.) Spring; **(A)** E. Schuettpelz 1378 (UPS); KY023075, KY022900, —; Brazil (Minas Gerais); **(B)** R. M. Harley 19601 (U); KY023074, KY022899, —; Brazil (Bahia) ♦ ***S. martensii*** Spring; —; AJ295878[a], —, —; Cultivated ♦ ***S. mayeri*** Hieron.; **(A)** P. Korall 2006:6 (S); KY023076, KY022901, KY023251; Peninsular Malaysia; **(B)** B. Palm s.n. (17 August 1921) (S); KY023077, —, KY023252; Indonesia (Sumatra) ♦ ***S. mazaruniensis*** Jenman; N. Y. Sandwith 1248 (U); KY023078, —, —; Guyana ♦ ***S. menziesii*** (Hook. & Grev.) Spring; D. P. Rogers s.n. (Die XI-10-46) (U); KY023079, —, —; Hawaii ♦ ***S. miniatospora*** (Dalzell) Baker; **(A)** J. Klackenberg and R. Lundin 567 (S); KY023081, KY022902, KY023254; India (Kerala); **(B)** C. van Hardeveld and H. H. van der Werff 120 (U); KY023080, —, KY023253; India (Tamil Nadu) ♦ ***S. minutifolia*** Spring; K. Larsen et al. 1389 (S); KY023082, —, —; Thailand ♦ ***S. mittenii*** Baker; C. G. G. J. van Steenis 24105 (L); KY023083, —, KY023255; South Africa ♦ ***S. moellendorffii*** Hieron.; **(A)** —; FJ755183 (CDS: 51186–52613)[i], —, —; Unknown; **(B)** M. J. M. Christenhusz 52 (U); KY023084, —, —; Philippines ♦ ***S. monospora*** Spring; **(A)** C. R. Fraser-Jenkins 3031 (L); KY023086, —, —; India (Sikkim); **(B)** F. Ludlow et al. 17023 (L); KY023085, —, —; Bhutan ♦ ***S. moratii*** W.Hagemann & Rauh; **(A)** N. Wikström et al. 110319-1 (S); KY023087, KY022903, KY023256; Madagascar; **(B)** —; AJ295899[a], —, —; Madagascar ♦ ***S. morganii*** Zeiller; P. Korall 2006:29 (S); KY023088, —, —; Peninsular Malaysia ♦ ***S. moritziana*** Spring ex Klotzsch; —; AJ010856[c], —, KY023257[e]; Ecuador ♦ ***S. muscosa*** Spring; E. Schuettpelz 1427 (UPS); KY023089, KY022904, KY023258; Brazil (Minas Gerais) ♦ ***S. mutica*** D.C.Eaton **var. *limitanea*** Weath.; —; KT161538[g], —, —; USA (New Mexico) ♦ ***S. myosurus*** (Sw.) Alston; **(A)** M. Cheek 13843 (BR); KY023091, KY022905, —; Guinea; **(B)** M. Cheek 11574 (BR); KY023092, —, —; Unknown; **(C)** W. R. Q. Luke 10377Z (BR); KY023090, —, —; Democratic Republic of the Congo (Orientale); **(D)** M. Mokoso 2842 (BR); KY023093, —, —; Democratic Republic of the Congo (South Kivu); **(E)** —; AJ295863[a], —, —; Nigeria ♦ ***S. nana*** (Desv.) Spring; A. F. Braithwaite 4521 (L); KY023094, —, KY023259; Solomon Islands ♦ ***S. neocaledonica*** Baker; N. Wikström 244 (S); KY023095, —, KY023260; New Caledonia ♦ ***S. nipponica*** Franch. & Sav.; —; AB574649[f], —, —; Japan ♦ ***S. nivea*** Alston; —; AF419073[d], —, —; Madagascar ♦ ***S. nothohybrida*** Valdespino; C. J. Rothfels 3069 (DUKE); KY023096, KY022906, KY023261; Mexico (San Luis Potosí) ♦ ***S. novae-hollandiae*** (Sw.) Spring; **(A)** —; AJ295883[a], KY022946[h], KY023263[h]; Ecuador; **(B)** —; AJ295865[a], KY022942[h], KY023262[h]; Venezuela ♦ ***S. novoleonensis*** Hieron. & Sadeb.; F. Drouet and D. Richards 3942 (S); KY023097, —, —; Mexico (Sonora) ♦ ***S. nubigena*** J.P.Roux; A. Larsson AL810 (UPS); KY023098, —, —; South Africa ♦ ***S. nummularifolia*** Ching; —; KT161546[g], —, —; Tibet ♦ ***S. oaxacana*** Spring; **(A)** C. J. Rothfels 3345 (UPS); KY023100, KY022907, KY023265; Mexico (Oaxaca); **(B)** C. J. Rothfels 3344 (UPS); KY023099, —, KY023264; Mexico (Oaxaca) ♦ ***S. oregana*** D.C.Eaton; —; AF419066[d], —, —; USA (Washington) ♦ ***S. ornata*** (Hook. & Grev.) Spring; **(A)** P. Korall 2006:20 (S); KY023101, KY022908, —; Peninsular Malaysia; **(B)** P. Korall 2006:68 (S); KY023102, KY022909, —; Peninsular Malaysia; **(C)** N. Wikström and H. Wanntorp 138 (S); KY023103, —, —; East Malaysia (Borneo) ♦ ***S. pallescens*** (C.Presl) Spring; **(A)** S. Weststrand 272 (UPS); AJ295858[a, p], KY022910, KY023266; Cultivated; **(B)** —;

AJ295859[a], —, —; Unknown ♦ ***S. pallidissima*** Spring; R. H. Horreüs de Haas 1481 (U); KY023104, —, —; India (Uttarakhand) ♦ ***S. parkeri*** (Hook. & Grev.) Spring; **(A)** H. Tuomisto 16176 (UPS); KY023107, KY022912, —; Brazil; **(B)** O. Poncy et al. 1082 (U); KY023105, —, —; French Guiana; **(C)** H. Tuomisto 16027 (UPS); KY023106, KY022911, —; Brazil; **(D)** H. Tuomisto 16542 (UPS); KY023108, KY022913, —; Brazil ♦ ***S. pectinata*** Spring; **(A)** N. Wikström et al. 110311-3 (S); KY023111, KY022916, KY023269; Madagascar; **(B)** N. Wikström et al. 110307-3 (S); KY023112, —, KY023270; Madagascar; **(C)** N. Wikström et al. 110307-2 (S); KY023109, KY022914, KY023267; Madagascar; **(D)** —; AJ295900[a], —, —; Madagascar; **(E)** N. Wikström et al. 110312-1 (S); KY023110, KY022915, KY023268; Madagascar ♦ ***S. pedata*** Klotzsch; **(A)** H. Tuomisto 15479 (UPS); KY023116, —, —; Brazil (Amazonas); **(B)** P. J. M. Maas and J. A. Tawjoeran 10907 (U); KY023115, —, —; Suriname; **(C)** M. J. Jansen-Jacobs et al. 321 (U); KY023114, —, —; Guyana; **(D)** J. Denslow 2522 (U); KY023113, —, —; Colombia ♦ ***S. pennata*** (D.Don) Spring; P. S. Sabharwal s.n. (14 October 1956) (U); KY023117, —, —; India (Uttarakhand) ♦ ***S. peruviana*** (Milde) Hieron.; —; KT161559[g], —, —; Mexico (Oaxaca) ♦ ***S. pervillei*** Spring; —; AJ295901[a], KY022952[h], —; Madagascar ♦ ***S. picta*** (Griff.) A.Braun ex Baker ; —; KT161561[g], —, —; China (Yunnan) ♦ ***S. pilifera*** A.Braun; —; AJ295862[a], —, —; Mexico (Nuevo León) ♦ ***S. plana*** (Desv.) Hieron.; **(A)** W. Takeuchi 8954 (L); KY023118, KY022917, KY023271; Papua New Guinea; **(B)** A. F. Braithwaite RSNH 2038 (L); KY023119, —, —; Vanuatu; **(C)** —; AJ295880[a], —, —; Cultivated ♦ ***S. porphyrospora*** A.Braun; A. M. Evans and D. B. Lellinger 6 (U); KY023120, —, —; Costa Rica ♦ ***S. potaroensis*** Jenman; M. J. Jansen-Jacobs et al. 6027 (U); KY023121, —, KY023272; Guyana ♦ ***S. producta*** Baker ; **(A)** R. C. Ek et al. 962 (U); KY023123, —, KY023274; Guyana; **(B)** R. C. Ek et al. 1703 (U); KY023122, —, KY023273; French Guiana ♦ ***S. pulcherrima*** Liebm.; —; AJ010847[c], —, —; Cultivated ♦ ***S. pulvinata*** (Hook. & Grev.) Maxim.; **(A)** D. E. Boufford et al. 37879 (A); KY023124, —, KY023275; China (Sichuan); **(B)** D. E. Boufford et al. 35254 (A); KY023125, —, KY023276; China (Yunnan) ♦ ***S. pygmaea*** (Kaulf.) Alston; —; AJ295892[a], —, —; South Africa ♦ ***S. radiata*** (Aubl.) Spring; **(A)** P. Acevedo-Rdgz. 5786 (U); KY023126, KY022918, —; Suriname; **(B)** —; AJ295867[a], —, —; French Guiana ♦ ***S. rechingeri*** Hieron.; L. Craven 299 (L); KY023127, —, KY023277; Bougainville Island ♦ ***S. reflexa*** Underw.; J. B. Beck 1126 (DUKE); KY023128, KY022919, KY023278; Mexico (San Luis Potosí) ♦ ***S. reineckei*** Hieron.; H. S. McKee 2907 P7338 (L); KY023129, —, KY023279; Samoa ♦ ***S. remotifolia*** Spring; **(A)** Gaoligong Shan Biodiversity Survey 21081 (GH); KY023130, KY022920, KY023280; China (Yunnan); **(B)** Gaoligong Shan Biodiversity Survey 19969 (GH); KY023132, —, KY023282; China (Yunnan); **(C)** D. E. Boufford and B. Bartholomew 24306 (A); KY023131, —, KY023281; China (Sichuan); **(D)** —; AB574650[f], —, —; Japan; **(E)** —; AJ295864[a], —, —; Taiwan ♦ ***S. repanda*** (Desv.) Spring; A. J. M. Leeuwenberg and P. P. C. v. Meer 13009 (L); KY023133, —, —; Indonesia (Java) ♦ ***S. cf. reticulata*** (Hook. & Grev.) Spring; C. R. Fraser-Jenkins 1653 (L); KY022956, KY022846, —; Nepal ♦ ***S. revoluta*** Baker; **(A)** H. Tuomisto 16785 (UPS); KY023135, KY022921, KY023284; Brazil; **(B)** H. Tuomisto 16789 (UPS); KY023136, KY022922, KY023285; Brazil; **(C)** R. M. Tryon and A. F. Tryon 5286 (U); KY023134, —, KY023283; Peru ♦ ***S. roraimensis*** Baker; J. Renz 14231 (U); KY023144, —, —; Guyana ♦ ***S. roxburghii*** (Hook. & Grev.) Spring; **(A)** P. Korall 2006:13 (S); KY023138, KY022924, KY023287; Peninsular Malaysia; **(B)** P. Korall 2006:7 (S); KY023137, KY022923, KY023286; Peninsular Malaysia; **(C)** P. Korall 2006:15 (S); KY023139, KY022925, KY023288; Peninsular Malaysia ♦ ***S. rupestris*** (L.) Spring; —; AF093255[c], —, —; USA (Illinois) ♦ ***S. rupincola*** Underw.; —; AJ010850[c], KY022951[e], KY023289[e]; USA (Arizona) ♦ ***S. sambiranensis*** Stefanović & Rakotondr.; F. Rakotondrainibe 1132 (P); KY023140, —, —; Madagascar ♦ ***S. sandwithii*** Alston; J. P. Schulz 10239 (U); KY023141, —, KY023290; Suriname ♦ ***S. sanguinolenta*** (L.) Spring; **(A)** D. E. Boufford et al. 31244 (A); KY023143, —, KY023292; Tibet; **(B)** —; EU197124[m], —, —; Unknown; **(C)** R. H. Horreüs de Haas 1215 (U); KY023142, KY022926, KY023291; Pakistan; **(D)** —; KT161588[g], —, —; China (Sichuan); **(E)** —; KT161590[g], —, —; China (Sichuan); **(F)** —; KT161591[g], —, —; China (Yunnan); **(G)** —; KT161589[g], —, —; China (Sichuan) ♦ ***S. sartorii*** Hieron.; —; KT161592[g], —, —; Mexico (San Luis Potosí) ♦ ***S. scopulorum*** Maxon; —; KT161595[g], —, —; USA (Washington) ♦ ***S. sechellarum*** Baker; H. J. Schlieben 11711 (BR); KY023145, —, —; Seychelles ♦ ***S. seemannii*** Baker; R. M. Tryon and A. F. Tryon 5195 (U); KY023146, —, —; Peru ♦ ***S. selaginoides*** (L.) P. Beauv. ex Schrank & Mart.; **(A)** S. Weststrand 104 (UPS); KY023148, KY022927, —; Sweden; **(B)** A. Larsson AL871:1 (UPS); KY023147, —, —; Italy; **(C)** —; Y07940[n], —, —; Unknown; **(D)** —; AB574651[f], —, —; Japan; **(E)** —; AF419048[d], —, —; Canada (Ontario) ♦ ***S. sellowii*** Hieron.; **(A)** J. C. Lindeman 6272 (U); KY023149, —, KY023293; Brazil (Rio Grande do Sul); **(B)** —; KT161596[g], —, —; Ecuador ♦ ***S. sericea*** A.Braun; —; AJ295871[a], —, —; Ecuador ♦ ***S. sertata*** Spring; **(A)** C. J. Rothfels 3192 (DUKE); KY023151, KY022928, —; Mexico (Jalisco); **(B)** R. D. Worthington 23987 (U); KY023150, —, —; Belize ♦ ***S. shakotanensis*** (Franch. ex Takeda) Miyabe & Kudô; —; AB574652[f], —, —; Japan ♦ ***S. siamensis*** Hieron.; K. Iwatsuki and N. Fukuoka T7491 (L); KY023152, —, KY023294; Thailand ♦ ***S. sibirica*** (Milde) Hieron.; L. A. Viereck and K. Jones 5667 (S); KY023153, KY022929, KY023295; Alaska ♦ ***S. silvestris*** Aspl.; **(A)** J. B. Beck 1218 (DUKE); KY023154, KY022930, KY023296; Mexico (Oaxaca); **(B)** P. Korall 1996:9 (S); KY023155, KY022931, KY023297; Ecuador ♦ ***S. simplex*** Baker; —; AJ295888[a], —, —; Brazil (São Paulo) ♦ ***S. sinensis*** (Desv.) Spring; —; AJ295868[a], KY022943[h], —; China (Beijing) ♦ ***S. sinuosa*** (Desv.) Alston; D. Lorence and T. Cadet 2733 (P); KY023157, —, —; Réunion ♦ ***S. soyauxii*** Hieron.; W. J. Harley 2098 (L); KY023158, —, —; Liberia ♦ ***S. sp. Bismarck Archipelago*** NA; J. R. Croft 1069 (L); KY023160, —, KY023299; Bismarck Archipelago ♦ ***S. sp. Mauritius*** NA; D. H. Lorence M157 (U); KY023159, —, —; Mauritius ♦ ***S. sp. Pohnpei*** NA; B. C. Stone 1746 (U); KY023161, —, —; Federated States of Micronesia (Pohnpei) ♦ ***S. stauntoniana*** Spring; —; AJ295869[a], —, —; China (Beijing) ♦ ***S. stipulata*** (Blume) Spring; **(A)** P. Korall 2006:10 (S); KY023162, KY022933, KY023300; Peninsular Malaysia; **(B)** K. Imin FRI 58604 (L); KY023163, —, KY023301; Peninsular Malaysia ♦ ***S. suavis*** (Spring) Spring; —; AJ295886[a], —, —; Brazil (Rio de Janeiro) ♦ ***S. sulcata*** (Desv. ex Poir.) Spring ex Mart.; —; AJ295887[a], —, —; Brazil (São Paulo) ♦ ***S. tama-montana*** Seriz.; —; AB574654[f], —, —; Japan ♦ ***S. tamariscina*** (P.Beauv.) Spring; —; AJ295861[a], —, —; Russia (Primorsky) ♦ ***S. tomentosa*** Spring; H. P. Fuchs et al. 21992 (U); KY023164, —, —; Colombia ♦ ***S. tortipila*** A.Braun; —; KT161619[g], —, —; USA (North Carolina) ♦ ***S. trachyphylla*** A.Braun ex Hieron.; K. Larsen et al. 1742 (S); KY023165, —, KY023302; Thailand ♦ ***S. uliginosa*** (Labill.) Spring; —; AJ010843[c], KY022950[e], KY023303[e]; Australia (Tasmania) ♦ ***S. umbrosa*** Lem. ex Hieron.; **(A)** P. Wagenaar Hummelinck s.n. (18 January 1955) (U); KY023166, —, —; Tobago; **(B)** —; AJ295879[a], —, —; Cultivated ♦ ***S. uncinata*** (Desv.) Spring; **(A)** —; AB574656[f], —, —; Japan; **(B)** —; AB197035 (CDS: 48412–49839)[o], —, —; Japan ♦ ***S. utahensis*** Flowers; —; AF419067[d], —, —; USA (Utah) ♦ ***S. vaginata*** Spring; **(A)** D. E. Boufford 37640 (A); KY023168, —, KY023304; China (Gansu); **(B)** D. E. Boufford et al. 28593 (A); KY023167, —, —; China (Sichuan) ♦ ***S. vardei*** H.Lév.; D. E. Boufford et al. 32425 (A); KY023169, KY022934, KY023305; Tibet ♦ ***S. versicolor*** Spring; **(A)** G. Benl Ka75/50 (BR); KY023171, —, KY023306; Cameroon; **(B)** J. Cordonnier 254 (BR); KY023172, —, —; Guinea; **(C)** R. Viane 1135 (BR); KY023173, —, —; Côte d'Ivoire; **(D)** A. J. M. Leeuwenberg 6878 (U); KY023170, —, —; Cameroon ♦ ***S. viridangula*** Spring; U. Swenson 528 (S); KY023318, KY022935, KY023307; Fiji ♦ ***S. viticulosa*** Klotzsch; A. Ibáñez et al. 2092AI (U); KY023174, KY022936, KY023308; Panama ♦ ***S. vogelii*** Spring; Carvalho 3727 (S); KY023175, KY022937, —; Equatorial Guinea (Bioko) ♦ ***S. wallacei*** Hieron.; The 1000 Plants Initiative (1KP, onekp.com), sample JKAA; KY023316, KY023189, KY022844; Unknown ♦ ***S. wallichii*** (Hook. & Grev.) Spring; P. Korall 2006:16 (S); KY023176, —, KY023309; Peninsular Malaysia ♦ ***S. weatherbiana*** R.M.Tryon; —; AF419075[d], —, —; USA (Colorado) ♦ ***S. whitmeei*** Baker; M. Karström s.n. (9 August 1998) (S); KY023177, KY022938, KY023310; Samoa ♦ ***S. wightii*** Hieron.; —; AF419062[d], —, —; Sri Lanka ♦ ***S. willdenowii*** (Desv. ex Poir.) Baker; **(A)** P. Korall 2006:3 (S); KY023178, KY022939, KY023312; Peninsular Malaysia; **(B)** S. Weststrand 107 (UPS); AJ295893[a, p], KY022948, KY023311; Cultivated ♦ ***S. wrightii*** Hieron.; —; KT161641[g], —, —; USA (Texas) ♦ ***S. xipholepis*** Baker; K. U. Kramer et al. 8329 (U); KY023179, —, —; China (Hong Kong) ♦ ***S. yemensis*** (Sw.) Spring; **(A)** J. J. F. E. de Wilde 4416 (BR); KY023181, KY022940, —; Ethiopia; **(B)** I. Friis et al. 947 (BR); KY023180, —, —; Ethiopia ♦ ***S. yunckeri*** Alston; T. G. Yuncker 15933 (U); KY023182, —, —; Tonga.