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Splachnum pensylvanicum (Splachnaceae) is Recorded from the Southern Hemisphere

MICHAEL LÜTH
Emmendinger Str. 32, 79106 Freiburg, Germany. e-mail: mail@milueth.de

BERNARD GOFFINET
Department of Ecology and Evolutionary Biology, 75 North Eagleville Road, University of Connecticut, Storrs, CT, 06250 U.S.A. e-mail: goffinet@uconn.edu

Abstract. *The genus* Splachnum *is newly recorded from Brazil based on a collection from Santa Catarina. Inferences from two chloroplast loci, the* trn*L*-trn*F region and the* rps*4 gene, indicate that this sample is closely allied to the primarily North American* S. pensylvanicum*, but differs by several mutations. Examination of populations of* S. pensylvanicum *suggests that this species is fairly variable morphologically, and that the Brazilian specimen, although atypic, cannot be distinguished from the Northern Hemisphere populations. The population from Santa Catarina, Brazil, is only the second record of this species from South America and the first from the Southern Hemisphere.*

Keywords. *Splachnum pensylvanicum*, Splachnaceae, biogeography, South America, phylogeny.

Although the genus *Splachnum* is best known for the spectacular amplification of the capsule into a brightly colored umbrella-shaped hypophysis, some species have only a weakly expanded sterile neck below the sporangium. One of these is *S. pensylvanicum* (Brid.) Grout *ex* H. A. Crum, a species known from eastern North America, from Quebec to Florida and westwards to Texas, as well as from two disjunct populations, one in the Baltic region of Russia, formally known as eastern Prussia (Frisvoll 1978) and one on a tepui (2,300 m) in Venezuela (Gradstein et al. 2001). The species is rather rare throughout its range, although it can be locally abundant. *Splachnum pensylvanicum* is characterized by a short-cylindrical to globose capsule with a weakly expanded hypophysis, and a short seta.

In September 2001, the senior author discovered a large population of Splachnaceae on rotten cow dung in a sloping fen in a pasture in the mountainous area (1,730 m) of the Urubici region in the Serra do Rio do Rastro, Estado Santa Catarina, in southeastern Brazil. The plants were reminiscent of *Tetraplodon angustatus* (Hedw.) Bruch & Schimp., but the chambered peristome clearly distinguishes it from *Tetraplodon*, and the specimen seemed to belong instead to the genus *Splachnum*. The short seta and the rather narrow hypophysis point to an affinity with *S. pensylvanicum*. Initial comparisons with descriptions of North American specimens, and few collections provided by Jan-Peter Frahm (Bonn, Germany), suggested that the individuals from Brazil were morphologically distinct, differing in the size and stature of the seta and the outline of the perigonial leaves. To further test whether the Brazilian population merely represents a phenotypic variant of *S. pensylvanicum* or a genetically distinct lineage worthy of taxonomic recognition, collections of *S. pensylvanicum* spanning its North American distribution were studied and sequence data from two chloroplast loci, the *trn*L-*trn*F region and the *rps*4 gene were compared for material from both North and South America.

METHODS AND MATERIALS

The morphology of 23 specimens (22 from North America and one from Venezuela) of *S. pensylvanicum* held at MO were studied, and compared to the sample from Brazil (Santa Catarina, Serra do Rio do Rastro, Urubici, Morro da Igreja, on a sloping fen in a pasture below the radar station, abundant on rotten, humid cow dung, 1,730 m, 17.09.2001, *M. Lüth 3612,* KA, CONN).

DNA extraction and phylogenetic analysis.—Apices of stems and branches were sampled from a duplicate of the collection of *Splachnum* from Brazil (CONN), from a sample of *S. pensylvanicum* (#2 in Fig. 3) collected in New York by N. Miller (*10523,* Herb. Goffinet), and of *S. adolphi-friederici* Broth. (*D'Arcy 7535,* DUKE). Another specimen of *S. pensylvanicum* collected by Norton Miller in New York was studied by Goffinet et al. (2004) and is referred to as *S. pensylvanicum* #1 in Fig. 3. No other recent material of *S. pensylvanicum* suitable for DNA analysis could be located. DNA was extracted using the DNeasy Plant mini kit from Qiagen (Valencia, California) following the manufacturer's protocol. The amplification and sequencing of the *trn*L-*trn*F region and of the *rps*4 gene (both part of the chloroplast genome) follow the protocol described in Goffinet et al. (2004). Sequencing products were purified using Sephadex G-50 (Amersham) gel filters, and separated

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by capillary electrophoresis using the ABI Prism™ 3100 Genetic Analyzer. Nucleotide sequences were edited using Sequencher 3.1 (Gene Codes Corporation), entered in PAUP*version 4.0b10 for Macintosh-PPC (Swofford 2002), and manually aligned.

Sequences were added to those obtained for other species of *Splachnum* by Goffinet et al. [2004: *S. ampullaceum* (*rps*4: AY039044/*trn*L-*trn*F: AY039069), *S. luteum* (AY499623/ AY501395), *S. melanocaulon* (AY499624/ AY501396), *S. pensylvanicum*1 (AY039046/AY039071), *S. rubrum* (AY039045/AY039070), *S. sphaericum* (AY499621/ AY501393), *S. vasculosum* (AY499625/ AY501397), and *S. weberbaueri* (AY499622/ AY501394)]. We excluded from the analyses the partial 5′exon of the *trn*L gene, the *trn*F sequences (i.e., the 3′ end of the *trn*L-F amplicon), the first 30 bps of the *rps*4 gene and the spacer following the 3′ end of the gene. Determining positional homology in the *trn*L sequences took into account the secondary structure proposed by Quandt and Stech (2004).

Phylogenetic inferences were made under the criterion of maximum parsimony, using the branch and bound algorithm as implemented in PAUP 4.0b10, with the option ''furthers'' selected for the type of sequence addition sequence. Gaps were treated as missing data. Support for the branches was estimated using the bootstrap approach (Felsenstein 1985) using a branch and bound algorithm on 10,000 pseudoreplicates with the furthest addition option invoked.

Results and Discussion

Morphological characters.—A survey of the populations of *S. pensylvanicum* from North America and Venezuela revealed that *S. pensylvanicum* is variable morphologically, except for the hypophysis, which is never much broader than the urn when dry and only slightly swollen when moist. As indicated by Crum and Anderson (1981), the species can be diagnosed by its seta, which never exceeds one cm in length. In some cases, the seta is very short, barely reaching 1.5 mm (e.g., *Reese 1143*—MO) and the capsule, which is typically exserted, is partially hidden by the periachaetial leaves. When the seta is short it appears more robust compared to the slender stature of the longer setae, but no anatomical differences distinguish the two types. The seta is always composed of a central strand of mostly collapsed cells, and the remaining cells, including the peripheral ones, are thin-walled, as illustrated in Frisvoll (1978). Miller (1994) reported that the seta of *S. pensylvanicum* elongates by a factor of three after the dehiscence of the capsule. Hence the variation in seta length observed among populations may not reflect genotypic variation or phenotypic plasticity but merely developmental stages. The material from Brazil (Figs. 1 & 2), which has setae that are shorter (2–4 mm) than those typically reported for *S. pensylvanicum* (4–9 mm fide Crum & Anderson, 1981) may thus simply represent a growth stage prior to seta elongation.

Crum and Anderson (1981) described the leaves of *S. pensylvanicum* as ''usually long-lanceolate and slenderly long-acuminate (but occasionally obovate and rather abruptly short- to long acuminate)'' with margins ''irregularly but strongly and sharply serrate to almost ciliate-dentate in upper half or somewhat more (occasional leaves entire).'' It is, however, not uncommon to find leaves with small teeth, or with entire margins. In fact we would argue that most upper leaves lack serrations. The basalmost leaves differ from the upper ones in their broad obovate shape and margins with long teeth or cilia, which are reminiscent of some Southern Hemisphere species of *Tayloria*. Such leaves occur also in the Brazilian plants, and it appears as though the development of the leaves followed a heteroblastic series, which is not uncommon in mosses. In one population collected in Texas by Eula Whitehouse in 1950 [*22845*—MO (two duplicates)], branches originating from below the perichaetium on the stems have only well-developed obovate leaves with ciliate margins, which differ strikingly from the narrow and long acuminate cauline leaves. This observation alone suggests that leaf shape may be highly plastic in this species.

The Brazilian population was initially also diagnosed by the shape and the entire margin of the perigonial bracts. However, study of material from throughout the range reveals that, like vegetative leaves, these bracts vary from ovate-lanceolate to nearly round in the outline of their clasping bases, and that serrations along the margins vary from absent to well developed.

In *Splachnum pensylvanicum*, as in related taxa, the perigonia are massive and hold numerous antheridia and paraphyses, surrounded by clasping and long subulate bracts. In some populations (e.g., *Miller 10523*—MO), they seem to be sessile (and thus lateral) on stems that are terminated by a perichaetium. Most often, however, they are produced at the end of slender, very loosely foliate axes. In the Brazilian population, the perigonia are not produced below the perichaetium, but occur on axes that are as long as the female plants. We could not find any evidence that the male axes originated from the female stems. Although *S. pensylvanicum* is described as autoicous (Crum 1966; Crum & Anderson 1981; Miller, 1994), Frisvoll (1978, p. 248) considered the plants to be either unisexual or hermaphroditic. Potential dioicy in the Brazilian population is thus not incompatible with current concepts of *S. pensylvanicum*.

Comparison of sequence data.—The following sequences were obtained and deposited in GenBank: DQ020138 (*trn*L-F) and DQ020141 (*rps*4) for the sample from Brazil, DQ020139 (*trn*L-F) and DQ020142 (*rps*4) for the sample of *S. pensylvanicum*2 from New York, and DQ020140 (*trn*L-F) for the sample of *S. adolphi-friederici*. We were unable to obtain *rps*4 sequences for the latter species. Fur-

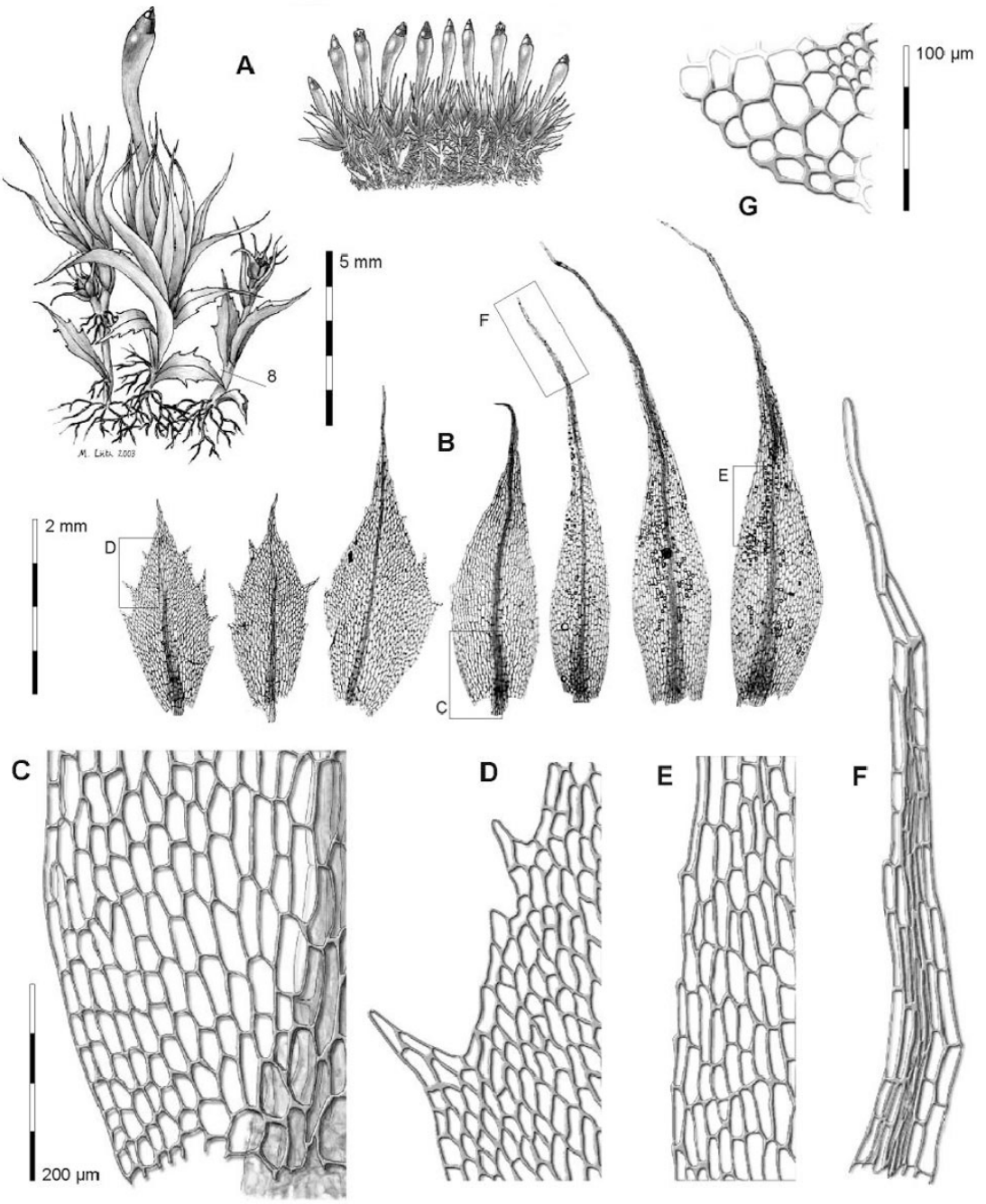

FIGURE 1. *Splachnum pensylvanicum* from Brazil (*Lüth 3612,* KA). A. Habit of two male and one female plant. B. Variation in leaf shape along the stem, from lower (left) to upper (right) leaves. C. Basal laminal cells. D. Upper laminal cells of toothed leaf. E. Upper laminal cells of entire-margined leaf. F. Leaf tip. G. Transverse section of costa. H. Transverse section of stem.

thermore the *rps*4 sequences of *S. pensylvanicum* are incomplete, as sample #1 lacks the last 80 nucleotides and sample #2 (the newly sequenced one) lacks 54 nucleotides. These missing portions represent mostly the intergenic spacer, which was excluded from the analysis.

The length of the *trn*L region (i.e., *trn*L intron, *trn*L 3′exon and *trn*L-*trn*F spacer) varied between 357 (*S. melanocaulon*) and 394 (*S. adolphi-friederici*) nucleotides. Based on Quandt and Stech (2004) the *trn*L intron should be considered two nucleotides longer than the length reported by Gof-

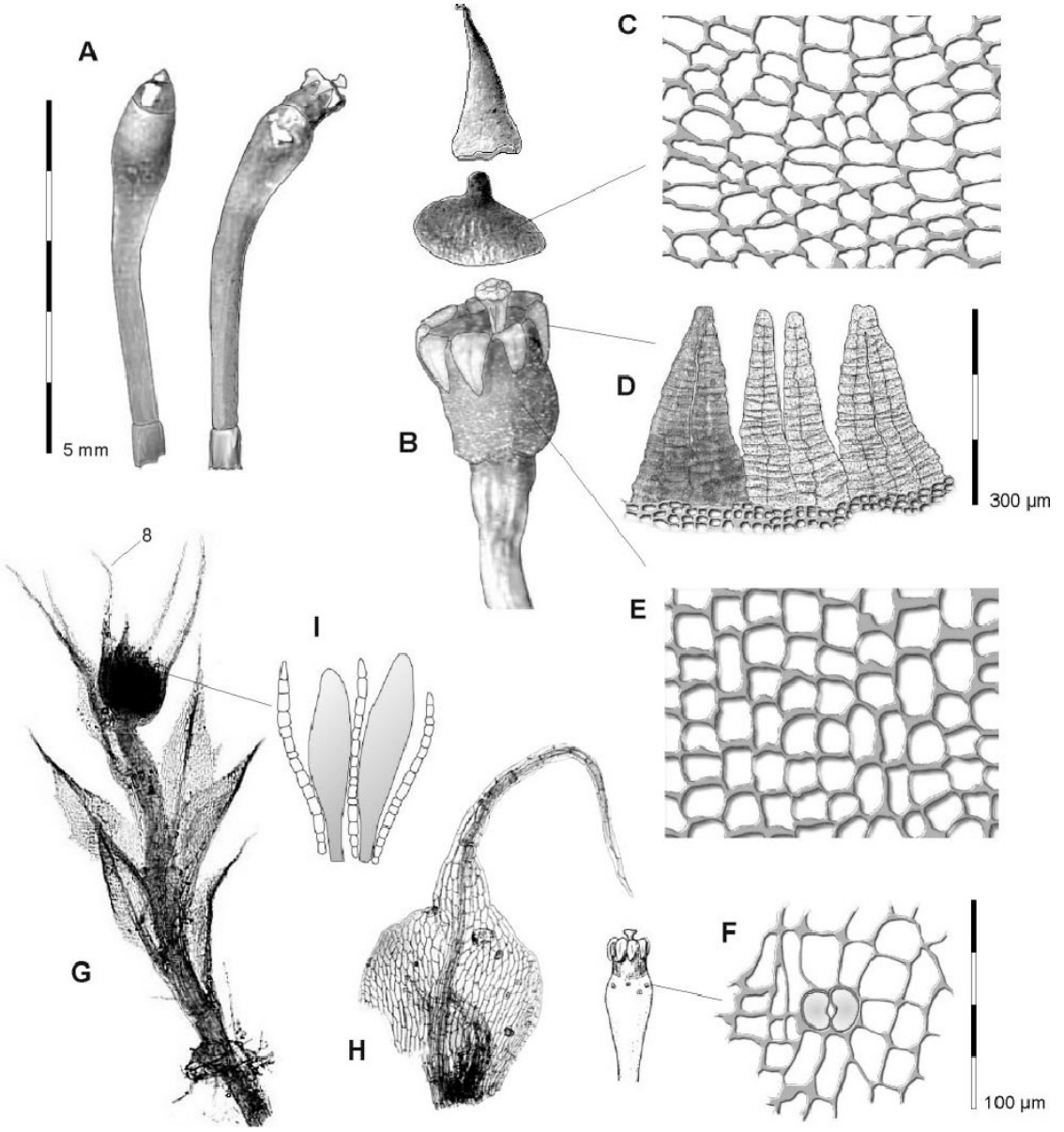

FIGURE 2. *Splachnum pensylvanicum* from Brazil (*Lüth 3612*, KA). A. Young (left) and mature (right) moist sporophyte. B. Dry and old sporophyte, with dehisced operculum and calyptra. C. Exothecial cells of operculum, showing incrassate cell corners. D. Peristome teeth. E. Exothecial cells of urn. F. Stoma. G. Male plant with perigonium. H. Perigonial bract. I. Antheridia and paraphyses.

finet et al. (2004). Insertions of gaps to preserve positional homology of the intron sequence resulted in a matrix of 306 characters. The *rps*4 were all, except as noted for those of *S. pensylvanicum*, 570 nucleotides long. The combined *trn*L-*trn*F/*rps*4-gene matrix included 11 exemplars and 980 characters. A single 12 bp-region of ambiguous homology within the P6a loop was excluded, and hence 968 were retained for the phylogenetic analysis. The sequences obtained for the second specimen of *S. pensylvanicum* (#2 in Fig. 3) from New York were identical to those reported earlier (Goffinet et al. 2004), which is not surprising considering that both specimens were collected in two peatlands close to one another. The sequence derived from the specimen from Brazil differs from those of North American populations of *S. pensylvanicum* in the length (389 nts versus 381 in *S. pensylvanicum*) and the primary nucleotide sequences (at least one transition) of the *trn*L intron, as well as by four point mutations (two transitions and two transversions) in the *rps*4 gene. The increase in length of

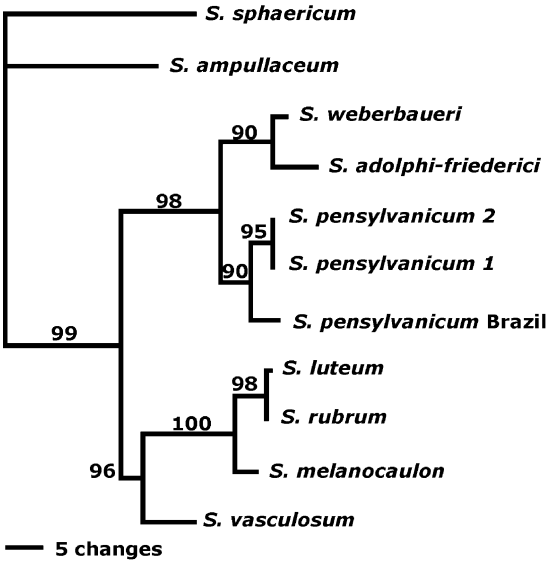

FIGURE 3. Unrooted phylogeny for *Splachnum* based on inferences from *trn*L-*trn*F and *rps*4 data. Single most parsimonious tree obtained using the exhaustive search strategy [Number of phylogenetically informative characters: 52, other variable characters: 59; length = 119 steps; CI (with autapomorphies excluded) = 0.8529; RI = 0.9021]. Numbers above branch indicate bootstrap proportions.

the intron sequence is primarily accounted for by a five bp duplication of the 3′ end of the P6a stem structure (sensu Quandt & Stech 2004).

The search resulted in a single optimal tree (Fig. 3) for which all nodes have high bootstrap values (>90%). Although the tree is unrooted it is fully congruent with the general branching pattern proposed by Goffinet et al. (2004) within the genus *Splachnum*. If rooted with *S. ampullaceum* as suggested by Goffinet et al. (2004), the sample from Brazil appears most closely related to *S. pensylvanicum*. Their degree of divergence is similar to that observed between other closely related species (e.g., *S. luteum* and *S. rubrum*; Fig. 3), and may suggest that the Brazilian population has diverged from the North American populations due to its geographic and hence reproductive isolation. Removal from overall patterns of gene flow likely leads to divergence, and isolation alone may thus support the recognition of a distinct taxon (Avise & Ball 1990). However, given the paucity of recently collected material of *S. pensylvanicum* we are unable to assert that the Northern Hemisphere populations do in fact compose a coherent, monophyletic lineage, from which the Brazilian populations is and has been reproductively isolated long enough for it to have drifted, and become genetically distinct. Furthermore, in the absence of any morphological differentiation of the Brazilian population, we propose to simply assimilate it within *S. pensylvanicum*. In South America, this species was hitherto only known from Venezuela (Gradstein et al. 2001) and is thus an addition to the moss flora of Brazil (Yano 1996).

The current report from Brazil thus adds a third disjunction to the mainly North American distribution of *S. pensylvanicum*. It is possible, and maybe even likely that the species may be more widespread in the Neotropics, but exploration of rain forests rather than pastures has led to it being overlooked.

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LITERATURE CITED

AVISE, J. C. & R. M. BALL, JR. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Survey in Evolutionary Biology 7: 45–67.

CHURCHILL, S. P. & E. L. LINARES. 1995. Prodromus Bryologiae Novo-Granatensis. Introducción a la flora de musgos de Colombia. Parte 2. Grimmiaceae a Trachypodiaceae. Instituto de Ciencias Naturales—Museo de Historia Natural Bibliotheca José Jeronimo Triana 12: 455–924.

CRUM, H. A. 1966. The relationship of *Tetraplodon pennsylvanicus*. THE BRYOLOGIST 69: 205–207.

——— & L. E. ANDERSON. 1981. Mosses of eastern North America, Vol. 1. Columbia University Press, NY.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

FRISVOLL, A. A. 1978. The genus *Tetraplodon* in Norway. A taxonomic revision. Lindbergia 4: 225–246.

GOFFINET, B., A. J. SHAW & C. J. COX. 2004. Phylogenetic inferences in the dung-moss family Splachnaceae from analyses of cpDNA sequence data and implications for the evolution of entomophily. American Journal of Botany 91: 748–759.

GRADSTEIN, S. R., S. P. CHURCHILL & N. SALAZAR-ALLEN. 2001. Guide to the bryophytes of tropical America. Memoirs of the New York Botanical Garden 86: 1–577.

MILLER, N. G. 1994. A study of the moss *Splachnum pensylvanicum* using scanning electron microscopy. Hikobia 11: 471–478.

QUANDT, D. & M. STECH. 2004. Molecular evolution of the *trn*T_{UGU}-*trn*F_{GAA} region in bryophytes. Plant Biology 6: 545–554.

SWOFFORD, D. L. 2002. PAUP*version 4.0b10: Phylogenetic Analysis Using Parsimony (and other methods). Sunderland: Sinauer Associates (unpublished).

YANO, O. 1996. A checklist of the Brazilian bryophytes. Boletim do Instituto de Botânica 10: 47–232.

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