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Genetic analysis of four Île Amsterdam sphagna: high morphological divergence within Sphagnum subgenus Subsecunda

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Corresponding Author:	Eric F. Karlin, Ph.D. Ramapo College Mahwah, NJ UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Ramapo College
Corresponding Author's Secondary Institution:	
First Author:	Eric F. Karlin, Ph.D.
First Author Secondary Information:	
Order of Authors:	Eric F. Karlin, Ph.D.
	Sean C. Robinson, Ph.D.
	Kristian Hassel, Ph.D.
	Kjell Ivar Flatberg, Ph.D.
Order of Authors Secondary Information:	
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Genetic analysis of four Île Amsterdam sphagna: high morphological divergence within *Sphagnum* subgenus *Subsecunda*

Eric F. Karlin[1], Sean C. Robinson[2], Kristian Hassel[3], Kjell Ivar Flatberg[3]

[1]*Environmental Science Program, School of Theoretical & Applied Science, Ramapo College,Mahwah, NJ 07430, USA;* [2] *Biology Department, SUNY College at Oneonta, Oneonta, NY 13820, USA;* [3] *Department of Natural History, NTNU University Museum, Norwegian University of Science and Technology, N-7491 Trondheim, Norway*

Correspondence to: Eric F. Karlin, Environmental Science Program, School of Theoretical & Applied Science, Ramapo College, Mahwah, NJ, 07430, USA.
Email: ekarlin@ramapo.edu Phone: 201-684-7743

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Author details

Eric F. Karlin, Professor of Plant Ecology, Environmental Science Program, School of Theoretical & Applied Science, Ramapo College, Mahwah, NJ, 07430-1680, USA.
Email: ekarlin@ramapo.edu. Telephone: +1-201-684-7743.
ORCID: 0000-0003-4218-8825.

Sean C. Robinson, Assistant Professor of Biology, Curator of the Jewell and Arline Moss Settle Herbarium, Biology Department, 218 Science 1, SUNY-Oneonta, Oneonta, New York 13820, USA. Email: sean.robinson@oneonta.edu. Telephone: +1-607-436-3732.
ORCID: 0000-0003-0961-4968

Kristian Hassel, Associate Professor, Department of Natural History, NTNU University Museum, Norwegian University of Science and Technology, N-7491 Trondheim, Norway, email: kristian.hassel@ntnu.no phone: +47 73592252
ORCID: 0000-0002-1906-8166

Kjell Ivar Fkatberg, Department of Natural History, NTNU University Museum, Norwegian University of Science and Technology, N-7491 Trondheim, Norway, email: kjell.flatberg@ntnu.no phone: +47 73592248

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Abstract

Genetic analyses using both SSRs and nucleotide sequences were carried out on four Île Amsterdam sphagna: *Sphagnum cavernulosum* of unknown subgenus, *S. complanatum* and *S. islei* of subg. *Subsecunda*, and *S. cf. planifolium* representing subg. *Cuspidata*. Genetic analyses show that all four species belong to subgenus *Subsecunda* and none are allopolyploids. This includes *S. cavernulosum*, which is morphologically divergent from all extant subgenera in the genus. *Sphagnum cavernulosum*, *S. complanatum* and *S. islei* are part of the Afro-Australasian clade of subg. *Subsecunda*, with *S. complanatum* and *S. islei* being closely associated with the African *S. capense* complex. *Sphagnum cavernulosum* is an outlier within the Afro-Australasian clade. Preliminary genetic analyses show *S. islei* to be closely related to *S. complanatum* and the two taxa may represent two morphologically divergent genets of one species. Although initial morphological study placed specimens identified as *S. cf. planifolium* in subg. *Cuspidata*, they are shown to belong to the *S. africanum* clade of subg. *Subsecunda*. These plants are closest to the African *S. truncatum* based on morphology. It is concluded that the source population for the Île Amsterdam populations of each of these four species appears to have ultimately been based in Africa. Further study is required to determine the ecological and evolutionary significance, if any, provided by the pronounced morphological variation within species and the high morphological divergence among species in subg. *Subsecunda*. A prior report of *S. recurvum* (subg. *Cuspidata*) possibly occurring on Île Amsterdam is concluded to have been based on laboratory error.

Keywords: Africa, Île Amsterdam, island populations, , morphological divergence, *Sphagnum*, *Sphagnum cavernulosum*, subgenus *Subsecunda*

Introduction

Compared to many isolated oceanic islands, Île Amsterdam has a diverse *Sphagnum* flora, both in terms of species richness and the number of subgenera. Flatberg *et al*. (2011) reported four subgenera and six species to be present there and that four of the six species were apparently either endemic to Île Amsterdam (*S. cavernulosum* Flatberg & Whinam, *S. complanatum* Flatberg & Whinam, *S. islei* Warnst.) or endemic to both Île Amsterdam and the nearby Île Saint-Paul (*S. lacteolum* Besch.). In comparison, just two *Sphagnum* species representing two subgenera are reported for the Hawaiian Islands (Karlin, 2001). From one to three *Sphagnum* species are reported for each of the following isolated oceanic Holantarctic islands, with none being endemic: Australia: Macquarie Island (*S.* ×*falcatulum s.s.* Besch.); New Zealand: Antipodes Islands (*S.* ×*australe s.l.* Mitt., *S.* ×*falcatulum s.l.*), Auckland Islands (*S.* ×*australe s.l.*, *S.* ×*falcatulum s.l.*), Campbell Island (*S.*×*australe s.l.*, *S. novo-zelandicum* Mitt.), Chatham Islands (*S.* ×*australe s.l.*, *S.* ×*cristatum* Hampe, *S.* ×*irritans* Warnst.) (Fife, 1996, Seppelt, 2012; Karlin *et al.,* 2013; Karlin & Robinson, 2017). However, the tallies are a bit ambiguous for the Holantarctic islands because of the occurrence of two cryptic species complexes. *Sphagnum* ×*falcatulum s.l.* has been shown to be a cryptic species complex composed of three genetically distinct taxa: *S.* ×*falcatulum s.s.* (allo-allo-triploid), *S.* ×*irritans* (allo-diploid), and *S. cuspidatum* (haploid) (Karlin *et al.*, 2009, 2011, 2013; Karlin & Robinson, 2017), with *S.* ×*falcatulum s.l.* being used when it is not known which of the three species are present in a given area. *Sphagnum* ×*australe s.l.* has also shown to be a cryptic species complex composed of three genetically distinct taxa: allo-diploid I *S.* ×*australe,* allo-diploid II *S.* ×*australe*, allo-allo-triploid *S.* ×*australe* (Karlin *et al.,* 2009, Karlin, 2014).

The Île Amsterdam flora includes two species in subg. *Subsecunda* (*S. complanatum* and *S. islei*), one species in subg. *Rigid*a (*S. lacteolum*) and one species in subg. *Acutifolia* (*S.*

cf. violascens Műll.Hal.) (Flatberg *et al*., 2011). The morphological characters of another species, *S. cavernulosum* do not fit into any subgenus currently recognized in *Sphagnum*. Finally, Flatberg *et al*. (2011) also reported one subg. *Cuspidata* species for Île Amsterdam. It was based on two identically labelled specimens with morphologically uniform plants that were collected from one locality (AMS 44). We will refer to these two specimens as 'berry' for this study. Flatberg *et al.* (2011) proposed that the plants could belong to the morphologically variable Australasian *S. cf. falcatulum*, but without further comments on taxonomy. These two specimens were finally assigned to and arranged under *S.* cf. *planifolium* Müll. Hal. in the TRH herbarium, a morphologically heterogeneous species traditionally placed in subgenus *Cuspidata* and known from tropical Africa and Madagascar (Eddy, 1985). For either species, the occurrence of a population on Île Amsterdam would represent a notable range extension. Although *S.* ×*falcatulum* and *S.*×*planifolium* have both been traditionally placed in subg. (or section) *Cuspidata* (Warnstorf,1911, Eddy, 1985, Fife,1996, Seppelt, 2012), recent studies have shown both to be cryptic species complexes of allopolyploids having a history of inter-subgeneric hybridization between members of subg. *Cuspidata* and subg. *Subsecunda* (Karlin *et al.*, 2009, 2011, 2014; Karlin, 2014). Karlin (2014) concluded that inter-subgeneric allopolyploids should be unranked at the subgenus level. That 'berry' was associated with both *S.* ×f*alcatulum* and *S.* ×*planifolium* suggests that it may also have a history of inter-subgeneric hybridization involving subg. *Cuspidata* and subg. *Subsecunda*. Three other *Sphagnum* allopolyploids (all gametophytically allo-diploid) have been documented to have a history of hybridization between these two subgenera: *S.* ×*irritans* (Karlin & Robinson, 2017), *S.* ×*mendocinum* (Shaw & Goffinet, 2000; Karlin *et al.*, 2010), and *S.*×*slooveri* (Karlin *et al.*, 2014).

Despite repeated attempts, Flatberg et al. (2011) failed to obtain genetic sequences from the Île Amsterdam specimens they studied. Thus their species delimitations are based

120 solely on morphological characters. Sporophytes were not observed on any of the Île
121 Amsterdam sphagna, suggesting that sexual reproduction was either lacking, or very rare, for
122 the S*phagnum* populations present there.
123 This study uses microsatellites (SSRs) and nucleotide sequences to explore a number
124 of unresolved questions associated with four of the Île Amsterdam taxa: *S. cavernulosum, S.*
125 *complanatum*, *S. islei*, and *S. cf. planifolium*. Specifically, we examined: 1) the relationship of
126 *S. cavernulosum* to the genus *Sphagnum*; 2) the relationship of *S. islei* and *S. complanatum* to
127 each other and to species of the '*S. capense*' complex; 3) the possibility that 'berry' has a
128 history of inter-subgeneric hybridization between subg. *Cuspidata* and subg. *Subsecunda*; 4)
129 the allelic diversity in the Île Amsterdam populations of *S. cavernulosum, S. complanatum,*
130 and *S. islei*.
131 **Material and methods**
132 *Notes on the taxa*
133 We follow the classification of *Sphagnum* of Shaw *et al.* (2010), which divides the genus into
134 six subgenera: *Acutifolia* (which includes sections *Acutifolia, Insulosa*, and *Polyclada*),
135 *Cuspidata, Rigida, Sphagnum, Squarrosa,* and *Subsecunda*.
136 *Sphagnum cavernulosum*
137 Given its morphological peculiarities, it is possible that *S. cavernulosum*: 1) does not belong
138 in *Sphagnum*; 2) belongs in *Sphagnum* and has a history of inter-subgeneric hybridization; 3)
139 is a morphological outlier of an extant subgenus; or 4) represents a previously unrecognized
140 subgenus in *Sphagnum*. Examples of all four possibilities have recently been documented in
141 *Sphagnum*: 1) the transfer of some species placed in *Sphagnum* to different genera and
142 families (Crum & Seppelt,1999; Shaw *et al.*, 2010; Shaw *et al.*, 2016); 2) inter-subgeneric
143 hybridization in *Sphagnum* (Shaw *et al.*, 2000; Karlin *et al.*, 2009, 2014; Karlin, 2014);
144 morphological outliers occurring within a subgenus (Shaw *et al.*, 2004); and 4) genetic

evidence suggesting that one of the three monoploid genomes present in individuals of the double allopolyploid *S. australe s.l.* belongs in *Sphagnum* (based on both nuclear and plastid sequences), but is genetically divergent from the currently recognized subgenera (Karlin, 2014). Subsequent mitochondrial and plastid genomic analyses by Shaw *et al.* (2016) found that this monoploid genome likely represents an early-diverging lineage within subg. *Sphagnum*.

Sphagnum complanatum and *S. islei*

Eddy (1985) placed *S. islei* in synonymy with the African *S. capense*. Noting that both *S. complanatum* and *S. islei* were morphologically close to *S. capense*, Flatberg *et al*. (2011) concluded that they were distinct from that species and also from each other. However, given the absence of sexual reproduction in either species on Île Amsterdam (Flatberg *et al*., 2011), it may be that these two Île Amsterdam taxa are morphologically distinct genets of one species, both of the same sex, which represent independent long distance founding events. Genetic analyses are required to explore the relationships of these three species.

Taxon sampling

The specimens sampled included 24 Île Amsterdam *Sphagnum* specimens collected by J. Whinam in 2007 and three specimens collected by Y. Frenot in 2010. The latter specimens were not included in the study of Flatberg *et al.* (2011). All specimens were housed at herbarium TRH (Norwegian University of Science and Technology) and had been identified by K. I. Flatberg (KIF). The 2007 material included eight specimens of *S. cavernulosum* (collected at seven sites), 11 specimens of *S. complanatum* (collected at 11 sites), three specimens of *S. islei* (collected at three sites) and two identically labelled specimens with morphologically uniform plants (collected from one locality: AMS 44) that were filed under *S. cf. planifolium*. We will refer to the latter two specimens as 'berry'. The three 2010

specimens had been identified KIF as *S. cavernulosum, S. complanatum*, and *S. islei*. Information on specimens yielding usable DNA is provided in Appendix 1.

DNA analysis

DNA was extracted from one gametophyte stem selected from each specimen. The remaining portion of each isolate was placed in a small, labelled packet and returned to the specimen. Two or three separate DNA samples were obtained from each of the two 'berry' specimens and the three 2010 specimens. Extractions were done using Qiagen DNeasy plant mini kits and a modified version of the Qiagen protocol (Qiagen, Valencia, CA). Modifications to the Qiagen protocol included an additional tissue disruption after adding Buffer AP1 (lysis buffer) and RNase A, and an extension of the initial incubation time at 65 °C in Buffer AP1 (lysis buffer) from 10 minutes to four hours. Microsatellites were amplified in 10 µl multiplex reactions, each targeting three loci, using Qiagen multiplex PCR kits and methods described in Shaw *et al.* (2008b). One (1) µl of each PCR product was then mixed with 0.25 µl GS500 LIZ size standard and 10 µl Hi-Di Formamide (Applied Biosystems, Foster City, CA) for electrophoresis on an ABI 3730x1 DNA Analyzer at Cornell University's Biotechnology Resource Center in Ithaca, NY.

Microsatellite (SSR) markers

All samples were assayed for 16 SSRs (numbered as in Shaw *et al*., 2008b): 1, 4, 5, 7, 9, 10, 14, 16, 17, 18, 19, 20, 22, 28, 29, 30. Duplicate runs of all SSRs were undertaken for samples having amplicons at two or more SRRs. Fragments were analyzed using the GeneMarker software, version 2.6.7 (SoftGenetics, State College, PA). Alleles of different sizes (nucleotide pairs) were simply coded as different, regardless of SSR repeat numbers. In addition, SSR data sets of subg. *Subsecunda* species from Africa (9 samples representing five haploid and one allopolyploid species) and Australasia (10 samples representing two haploid

193 species) used in prior studies (Karlin *et al*., 2008, 2014) were also utilized. Information about
194 these specimens is listed in Appendix 1.
195 *Nucleotide sequences*
196 One nuclear and one plastid nucleotide sequence were assayed for the three 2010 samples and
197 the two 2007 samples of 'berry'. The plastid locus was 'trnL (UAA) 59 exon-trnF(GAA)
198 region' (hereafter, trnL). The nuclear locus was '5.8S ribosomal RNA gene, partial sequence;
199 and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial
200 sequence' (hereafter referred to as *ITS2*$^{+}$). Thirty-eight *ITS2*$^{+}$ sequences and thirty *trnL*
201 sequences of additional *Sphagnum* species were obtained from Genbank. All specimens from
202 which nucleotide sequences were obtained (and their GenBank accession numbers) are listed
203 in Appendix 1. Nucleotide BLAST (BLASTn) was used to determine the Genbank sequences
204 having the highest identity to nucleotide sequences obtained from the Île Amsterdam species
205 being studied.
206 *Statistical analyses*
207 Phylogenetic analyses were done separately for the *ITS2*$^{+}$and *trnL* loci. The best-fit models of
208 nucleotide substitution were determined for each locus based on the Akaike information
209 criterion (AIC) as implemented in jModeltest 0.1.1 (Posada, 2008). The program MrBayes
210 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) was used for
211 conducting Bayesian analysis (Yang & Rannala, 1997) of each locus. Except for three indels
212 in the *trnL* data alignment, gaps and missing characters were treated as missing data, and thus
213 did not contribute any phylogenetic information. It was concluded, however, that three indels
214 in the *trnL* alignment had phylogenetic information. Thus the *trnL* data was divided into two
215 partitions for analysis: one partition for the nucleotides and a second partition for the presence
216 or absence of each the three indels.

A genetic distance matrix of haplotypes based on 11 SSRs (1, 4, 7, 12, 14, 17, 18, 19, 22, 28, 29) was created using the program GenAlEx 6.501, with *S. islei* being excluded because of missing data. The other six SSRs were not included in this analysis because of missing data among the specimens in the data set. This distance matrix was used for both Principal Coordinates Analysis (PCoA), using GenAlEx, and neighbor-joining analysis, using the Neighbor program in PHYLIP 3.69 (Felsenstein, 1989). The latter analytical approach was applied by Karlin *et al.*, (2013). A tree based on the neighbor-joining analysis was created by using TreeDyn as implemented on the 'phyogeny.fr' online platform (Chevenet *et al.,* 2006; Deeper *et al.* 2008). SSR data from the two 2007 specimens of *S. islei* having amplicons was pooled to yield a haplotype based on 9 SSRs (out of the 11 SSRs used above, with 8 alleles from one specimen and 1 allele from the second). This was included in a second neighbor-joining analysis based on 9 SSRs (1, 4, 12, 14, 17, 18, 19, 22, 29) that was focused primarily on the Afro-Australasian clade.

Results

DNA extracted from the 2007 specimens of *S. cavernulosum*, *S. complanatum*, and *S. islei* was of low quality and the majority did not yield any SSR amplicons; the few that did yielded amplicons of very low amplitude. The latter included two *S. islei* specimens, which collectively had alleles at 11 SSRs. In contrast, DNA from the two 2007 'berry' specimens and the three 2010 specimens was of higher quality and yielded SSR amplicons with strong peaks as well as *ITS* nucleotide sequences; *trnL* nucleotide sequences were also obtained from the three 2010 specimens. Subsequent to genetic analyses, a closer morphological assessment led KIF to conclude that the 2010 specimen which had initially been identified as *S. islei* was *S. complanatum*. Consequently, we have no nucleotide sequence data for *S. islei*. The number of SSRs producing amplicons varied among the Île Amsterdam species, being lowest for *S. islei* and highest for 'berry' (16 SSRs). The limited results from the 2007 specimens precluded

a study of the genetic diversity in the Île Amsterdam populations of *S. cavernulosum*, *S. complanatum*, and *S. islei*.

Estimated Gametophytic Ploidy

With the exception of SSR-5, the four Île Amsterdam species each had one allele per individual per SSR. This indicates that 1) they are not allopolyploids and 2) that they are likely gametophytically haploid. The exception to this pattern was SSR-5. The one 2010 *Sphagnum cavernulosum* sample had two alleles per individual (alleles '196' & '477') and the two 2010 *S. complanatum* samples had two (alleles '194' & '196') and three (alleles '194', '196', and '480') alleles per individual, respectively. This indicates that more than one copy of SSR-5 is present in each individual of these two species, and this has also been detected in some members of the Afro-Australian clade (Karlin *et al.*, 2008, 2014). However, just one weakly amplified allele per individual was obtained from the 2007 specimens yielding amplicons at SSR-5: *S. cavernulosum* (2 specimens), *S. complanatum* (1 specimen) and *S. islei* (1 specimen). As alleles are apparently not always amplified from each of the copies of SSR-5 present in individuals, particularly with low quality DNA, we conclude that our data set is not sufficient to resolve the total number of copies of SSR-5 present in individuals of *S. cavernulosu*m, *S. complanatum* and *S. islei*. The three separate samples run on both of the two 2007 'berry' specimens each had one strongly amplified allele at SSR-5 and we conclude that these plants had just one copy of SSR-5 per individual.

Nucleotide Sequences

As BLAST searches of GenBank accessions showed the nucleotide sequences of all three species to be associated with subg. *Subsecunda*, phylogenetic analyses focused on that group. Based on the nuclear *ITS2*$^{+}$sequences, three Southern Hemisphere clades within subg. *Subsecunda* are present in the analysis (Fig. 1). *Sphagnum cavernulosum* and *S. complanatum* group with the Afro-Australasian clade, having the highest genetic similarity with the African

species (Fig. 1). However, these two Île Amsterdam species both show some genetic divergence from the African members of the clade. In contrast, *ITS2*$^{+}$ sequences place "berry" in the '*S. africanum*' clade, which includes both African and Neotropical species (Fig. 1). Given the very high genetic similarities among the component species, it is not possible to resolve 'berry' from the other taxa in this clade based on *ITS2*$^{+}$ sequences.

Figure 1 about here

The plastid (*trnL*) sequences yield a phylogenetic tree similar to that based on *ITS* sequences (Figs. 1, 2). However, while divergence between *S. complanatum* and many of the African and Australasian species is slight when based on *trnL* sequences, *S. cavernulosum* is shown to be quite divergent from the other members of this clade (Fig. 2). Although *trnL* sequences for 'berry' are lacking, there is no clear delineation among other members of the *S. africanum* clade based on *trnL* data.

Figure 2 about here

Genetic distance among SSR haplotypes

A neighbor-joining tree showing genetic distance among haplotypes (based on 11 SSRs) of *S. cavernulosum*, *S. complanatum*, 'berry', and members of the *'S. africanum*' clade and the Afro-Australasian clade clearly show *S. cavernulosum* to belong to the Afro-Australasian clade, but that it is notably divergent from the other members of that clade (Fig. 3). 'Berry' is deeply embedded within the '*S. africanum'* clade and *S. complanatum* is associated with African members of the Afro-Australasian clade, having the highest allelic similarity with one of the two specimens of *S. capense* represented in the analysis (Fig. 3). Although the SSR data

shows more variability than that detected with the nucleotide sequences for the *"S. africanum*" clade, with the exception of *S. bordasii* Besch., it does not allow for delineation among the component species included in this analysis. Karlin *et al.* (2014) concluded that *S. bordasii* was likely an allopolyploid, with at least one of its two ancestral species belonging to the *"S. africanum*" clade. Thus its notable divergence from the other members of *"S. africanum*" clade reflects its hybrid history.

Figure 3 about here

Allelic divergence between 'berry' and one specimen of *S. africanum* and one specimen of *S. truncatum* is slight, and falls well within the range of intra-specific divergence detected among individuals of the latter two species (Fig. 3). Likewise, allelic divergence between *S. complanatum* and one specimen of *S. capense* also falls within the range of intra-specific divergence detected within *S. capense* as well as that among individuals of the Australasian *S. comosum* and *S. novo-zelandicum* (Fig. 3).

The first axis of a Principal Coordinates Analysis (PCoA) of the 11 SSR data set (with 28% of the total allelic variation) is associated with the resolution of the '*S. africanum*' clade from the Afro-Australasian clade. The second axis, with 16% of the total allelic variation, focuses on the separation of the Australasian species from the other members of Afro-Australasian clade, with *S. cavernulosum* being divergent from other members of both clades. Finally, the third axis, with 10% of the allelic variation, is associated with the resolution of the two Australasian species (*S. comosum* and *S. novo-zelandicum*).

The neighbor-joining tree based on 9 SSRs shows *S. islei* to be most closely related to *S. complanatum*, with the two species forming a clade sister to a specimen of *S. capense* (Appendix 2). This analysis shows allelic divergence between *S. complanatum* and *S. islei* to

be far less than the respective range of intra-specific divergences detected within each of the three species in the Afro-Australasian clade represented by two or more specimens each in this analysis (i.e. *S capense, S. comosum, S. novo-zelandicum*).

Discussion

Genetic data shows that the diversity of subgenera in genus *Sphagnum* represented at Île Amsterdam is less than previously thought, with the four species investigated in this study all belonging to ubg. *Subsecunda*. Due to low quality of extracted DNA from many of the Île Amsterdam specimens, combined with seemingly high morphological variation, we are in many cases not able to evaluate the morphological species concepts applied.

Sphagnum cavernulosum

Genetic analyses clearly show *S. cavernulosum* to belong in *Sphagnum* and that it is a member of subg. *Subsecunda*. It is part of the Afro-Australasian clade within that subgenus, which closely corresponds to 'lineage B' of Shaw *et al.* (2008a). *ITS2*$^{+}$ data indicate *S. cavernulosum* to be most closely related to African species. However, *trnL* and SSR data both show it to be an outlier within this clade. *Sphagnum cavernulosum* is not unique in having a morphology that is highly divergent from that typically associated with subg. *Subsecunda*. Genetic analyses have shown that three other species that were once placed in different sections (or subgenera) based on their respective highly divergent morphologies belong in subg *Subsecunda*: 1) *S. macrophyllum* (and the closely related *S. cribosum*) were formerly in section *Isocladus*; and 2) *S. pylaseii* was formerly in section *Hemitheca* (Shaw *et al.* 2004). These three latter species (Figs. 1, 2) are all part of a clade that also includes *S. cyclophyllum* and *S. microcarpum* (Shaw *et al.*, 2004). All members of this clade are largely limited to eastern North America. Thus *S. cavernulosum* represents a separate evolutionary innovation which resulted in a large morphological divergence within subg. *Subsecunda*.

342 *Sphagnum complanatum* and *S. islei*

343 Genetic analyses show that both *S. complanatum* and *S. islei* are most closely related to the
344 African members of the Afro-Australasian clade members (i.e. *S. capense, S. davidii, S.*
345 *pycnocladulum*). Based on the data in hand, the very slight allelic divergence between *S.*
346 *complanatum* and *S. islei* suggests that they can be morphologically divergent genets of the
347 same species. However more genetic data is required to make this conclusion. Indeed, this
348 study indicates that further study of the taxonomic relationships among African members of
349 the Afro-Australasian clade is required.

350 *'berry'*

351 Both SSRs and *ITS2*$^{+}$ nucleotide sequences unequivocally show 'berry' to be a member of
352 subg. *Subsecunda*, not *Cuspidata*, and they also show no sign of a history of inter-subgeneric
353 hybridization. The plant belongs to the '*S. africanum'* clade, a group which includes both
354 African members (*S. africanum, S. bordasii, S. truncatum*) as well as Neotropic members (*S.*
355 *acutirameum, S. uleanum*). The clade is a subset of lineage 'D' of Shaw *et al.* (2008a).
356 However, genetic divergence between 'berry' and the other members of the *S. africanum*
357 clade is minimal and it is not possible to delineate 'berry', let alone most of the species in this
358 clade, based on the genetic data in hand. We conclude that not only is it premature to describe
359 'berry' as a new species, but that an in depth study of the '*S. africanum'* complex is also
360 needed to tease out the taxonomic relationships among its component species. A detailed
361 morphological description of 'berry' (which we will subsequently refer to as *S.* cf. *truncatum*
362 Hornsch. solely based on morphological characters) is provided below to facilitate future
363 study.

364 *Morphological description*

365 *Sphagnum* cf. *truncatum* Hornsch.

Shoots medium-sized, slender and rather flaccid, juvenile more or less unbranched shoots common; colour green, yellowish-green to pale brown, sometimes with weak orange stain. *Capitulum* 10–15 mm in diameter, with somewhat laterally and narrowly pointed branches. *Stem* varyingly greenish to pale brownish in parts; in transverse section with predominantly unistratose cortex of enlarged cells, sometimes with 2(–3)–stratose portions, sclerodermis weakly differentiated, 2–4 cells wide, medulla of large, parenchymatous cells; outer cortical wall in superficial view efibrillose and eporose (Fig. 4B). *Stem leaves* scattered to rather densely arranged, varyingly spreading to pendent-spreading along stem, shape varying from broadly lingulate, elongate-lingulate to elliptic-lingulate (Fig. 4A); apex narrowly to broadly rounded to sometimes sub-obtuse, occasionally slightly erose to notched, often somewhat involute; lateral leaf margins in distal part narrowly bordered by elongate cells 2-4 cells wide, border narrower or lacking in proximal part of leaf; length (1.5–)1.7(–2.0) mm, breadth mid-leaf (0.8–)0.9(–1.1) mm; breadth at proximal end (0.8–)0.9(–1.1); hyalocysts in superficial view elongate, fairly straight to narrowly S-shaped, much shorter in distal than proximal part of leaf, many to most cells densely fibrillose from distal to proximal leaf ends, most cells at least one-septate, and often some cells septate in 3–4 parts; cells on adaxial surface with usually 3–10 medium-sized, perfect to partly imperfect (shadow), ± circular, irregularly distributed pores in distal leaf part, in proximal part of leaf mostly eporose: cells on adaxial surface pauciporose except for a few scattered, obscure pores/pseudopores, in proximal leaf part often with one small, perfect pore at one or both cell ends and sometimes with a few additional, minute to medium-sized, free-lying, circular pores; chlorocysts in superficial view short and narrowly S-shaped in distal leaf part to long and nearly straight in proximal part. *Branches* usually in fascicles, rather distantly arranged along stem, consisting of usually (1–)2(–3) divergent and 1–2(–3) somewhat thinner and shorter pendent branches varyingly spreading from stem. *Divergent branches* arched decurved-spreading from stem, broadest in

proximal half, ending acute-obtuse or more gradually tapering into a narrow point above in wetter habitats; length (10–)15–20(–25) mm; branch stems pale except for usually slight pale brown in proximal part, in transverse section with uni-stratose cortex (Fig. 4F), in superficial view with elongate retort cells with one distal end pore with indistinct neck (Fig. 4G). *Leaves of divergent branches* non-ranked, fairly straight to varyingly secund, rather concave and often folded in microscope, particular in proximal part of branch, leaves in middle part of branch broadly lanceolate–ovate to elliptic–ovate, relatively wider towards proximal leaf-end; leaf apex broadly rounded-dentate to truncate-dentate with 5–9 distinct teeth (Fig. 4C, 4D); lateral margins with distinct border of elongate, thickened cells 3–5 cm wide, resorption furrow lacking; length (3.1–)3.7(–4.1) mm, breadth mid-leaf (0.9–)1.3(–1.6) mm; hyalocysts narrowly S-shaped elongate to nearly straight and larger of size in proximal leaf part, fibrillose throughout leaf, non-septate to sometimes some cells septate in 2(–3) parts in proximal leaf part; hyalocysts on abaxial surface eporose to more often pauciporose with small, circular to obscure pores at cell ends and corners (Fig. 4H, 4I); porosity on adaxial surface rather similar to abaxial surface, but usually less pauciporose with often 2–6(–8) mostly circular, commissural to sub-commissural, occasionally free-lying, non-ringed pores in addition to a few cell end pores (Fig. 4J); chlorocyst lumen in transverse section elliptic to elliptic–rectangular/trapezoidal, exposed equally on both surfaces to more broadly exposed on abaxial (convex) surface, without resorption furrow. Leaves of pendent branches smaller and narrower than on divergent branches and often secund to falcate; hyalocysts with pore structure similar to leaves of divergent branches.

Figure 4 about here

Sexuality: not known; plants with perigonial leaves, antheridia, perichaetia and sporophytes unrecorded. The habitat is unspecified, but the growth form of the plants indicates an aquatic habitat.

Morphological comparison: Table 1 compares selected morphological characters of the Île Amsterdam plant with three African species of the *S. africanum* group as circumscribed by Eddy (1985), i.e. *Sphagnum truncatum* Hornsch., *S. rutenbergii* Müll.Hal. and *S. africanum* Welw. & Duby and with one South American species, *S. acutirameum* (Crum, 1992). *Sphagnum truncatum* is known from southern Africa, Madagascar, Mauritius and Réunion, *S. rutenbergii* from Madagascar and Mauritius, *S. africanum* from West Africa, and *S. acutirameum* from Brazil.

Table 1 about here

Our comparison shows that the Île Amsterdam plant, (1) has longer branch leaves and relatively shorter stem leaves than outlined for the other four species. *Sphagnum rutenbergii* is separated by predominantly 2–3-stratose stem cortex, *S. africanum* and *S. acutirameum* have fewer branches in the fascicles and non-truncate/dentate branch leaf apices. *S. rutenbergii* and *S. africanum* also differ by their multi-porose abaxial surfaces of the branch leaves, and *S. acutirameum* by its multi-porose adaxial surfaces. Other branch and stem leaf porosity differences are less obvious, as are cross section shapes and outlines of branch leaf chlorophyllose cells (not included in Table x). The multi-septate stem leaves of the Île Amsterdam plant are shared by the putative allopolyploid *S. bordasii*. Eddy (1985) considered *S.bordasii* to be a variety of *S. truncatum* based solely on morphology (*S. truncatum* var. *bordasii* (Besch.) A.Eddy). However its allopolyploid status clearly shows *S. bordasii* to be evolutionarily quite distinct from *S. truncatum*, which SSR analysis shows to likely be gametophytically haploid (Karlin *et al.*, 2014). This taxon is known from southern Africa, Burundi, Madagascar, Mauritius and Réunion, and with type collections from Mauritius.

The morphological variation of the African members is complex, and Eddy's taxonomical treatment includes several synonymic names at species and intraspecific levels, particularly within *S. truncatum* and *S. rutenbergii*. The influence of phenotypic plasticity is difficult to evaluate only based on herbarium material. The seemingly long branch leaves of the Île Amsterdam plant compared to the other African taxa within the *S. africanum* group compared here, can therefore reflect local microhabitat conditions where plants were collected rather than different genotypes. A more comprehensive genetic and morphological study is therefore necessary for outlying the taxonomic position of the Île Amsterdam plant within the *S. africanum* group in more detail. Morphologically it seems to stand closest to *S. truncatum* and the allopolyploid *S. bardasii* and we thus preliminarily classify 'berry' as *S.* cf. *truncatum*.

Sphagnum tumidulum Besch.

Sphagnum tumidulum represents another example of morphological divergence among members of subg. *Subsecunda.* Although placed in subg. *Subsecunda,* the morphology of *S. tumidulum*'s branch leaf chlorophyll cells is atypical for the subgenus (Warnstorf 1911; Eddy,1985; Liu, 2014). Recent plastid and mitochondrial genomic analyses have confirmed the placement of *S. tumidulum* in this subgenus (Shaw *et al.*, (2016) and SSR analysis by Liu *et al.* (2014) concluded that *S. tumidulum* is likely to be gametophytically haploid (i.e. it is not an allopolyploid). Eddy (1985) found *S. tumidulum* to be morphologically quite distinct from members of section *Acrosphagnum* (i.e. the *S. capense* complex). However, *trnL* sequences hint a close relationship between *S. tumidulum* and the Afro-Australasian clade (Fig 2). Additional genetic data is needed to explore this question.

Sphagnum recurvum P. Beauv.

Based on genetic analysis, Karlin *et al.* (2014) reported the possible occurrence of *S. recurvum* (subg. *Cuspidata*) on Île Amsterdam which would represent a large extension in the

geographic distribution of this species. However, they noted that the provenance of the specimen was equivocal and that laboratory error may have led to the discrepancy. As we find no members of subg. *Cuspidata* to be present on Île Amsterdam, we conclude that their report resulted from laboratory error.

Source populations

Genetic data indicates that the source populations for the Île Amsterdam populations of *S. complanatum, S. islei*, and 'berry' were each ultimately based in Africa. In contrast, although genetic data clearly places *S. cavernulosum* in the Afro-Australasian clade, the location of the source population is ambiguous. However, *ITS2*$^{+}$ data suggests that it may have been African. This fits well with the predominantly westerly winds associated with the island.

Polymorphism in subg. Subsecunda

Morphological variation is pronounced in subg. *Subsecunda* at both intra-specific and inter-specific levels. It is particularly extreme in some allopolyploids having one or more subg. *Subsecunda* monoploid genomes (Karlin *et al.*, 2008, 2009, 2011, 2013; Shaw *et al.* 2012; Karlin and Robinson, 2017), it has also been found to be pronounced among the ramets of a clonal population of the haploid *S. comosum* Müll. Hal. (Karlin *et al.*, 2008). That two of the four Île Amsterdam sphagna examined in this study were not initially placed in subg. *Subsecunda* based on their respective morphological characters highlights the high degree of morphological divergence occurring in this subgenus. This leads to the following question, which is far beyond the scope of this study to address: 'For subg. *Subsecunda*, what ecological and/or evolutionary significance, if any, is provided by having a high morphological variation, both for individual species as well as at the subgenus level?'

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Geolocation Information

Sphagnum cavernulosum — EK1012: 37.8539°S, 77.5400°E; *Sphagnum complanatum* — EK1013 & EK1014: 37.8539°S, 77.5400°E; *Sphagnum islei* — EK945: 37.8402°S, 77.5585°E; EK946: 37.8439°S, 77.5485°E; *Sphagnum* cf. *truncatum* — EK901: 37.8417° S,77.5492°E;

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Taxonomic Additions and Changes: None.

ORCID

Eric F. Karlin http://orcid.org/0000-0003-4218-8825

Sean C. Robinson http://orcid.org/0000-0003-0961-4968

Kristian Hassel http://orcid.org/0000-0002-1906-8166

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Figure 1. Phylogram based on Bayesian analysis of *ITS2*$^{+}$ sequences (nuclear) of *S. cavernulosum, S. complanatum*, 'berry', and other species in subg. *Subsecunda*. Arrows indicate Île Amsterdam samples.

Figure 2. Phylogram based on Bayesian analysis of *trnL* sequences (plastid) of *S. cavernulosum, S. complanatum*, 'berry', and other species in subg. *Subsecunda*. Arrows indicate Île Amsterdam samples.

Fig. 3. Neighbor joining tree of haplotypes (based on 11 SSRs) of *S. cavernulosum, S. complanatum,* and 'berry' plus members of the '*S. africanum*' clade and the Afro-Australasian clade. Arrows indicate Île Amsterdam samples.

Fig. 4. *Sphagnum* cf. *truncatum*. A: Stem leaves. B: Stem in cross section. C: Leaf from middle part of divergent branch. D: Branch leaf apex. E: Chlorophyllose cells of divergent branch leaves in cross section. F: Branch in cross section. G: Retort cells of branch cortex in superficial view. H: Cell structure on mid-leaf abaxial surface of stem leaf. I–J: Cell structure on mid-leaf surface of divergent branch leaves. I: Abaxial surface. J: Adaxial surface. Material: Île Amsterdam, Terres Australes et Antarctiques Françaises, leg. J. Whinam 12.12.2007 (TRH 742253).

Table

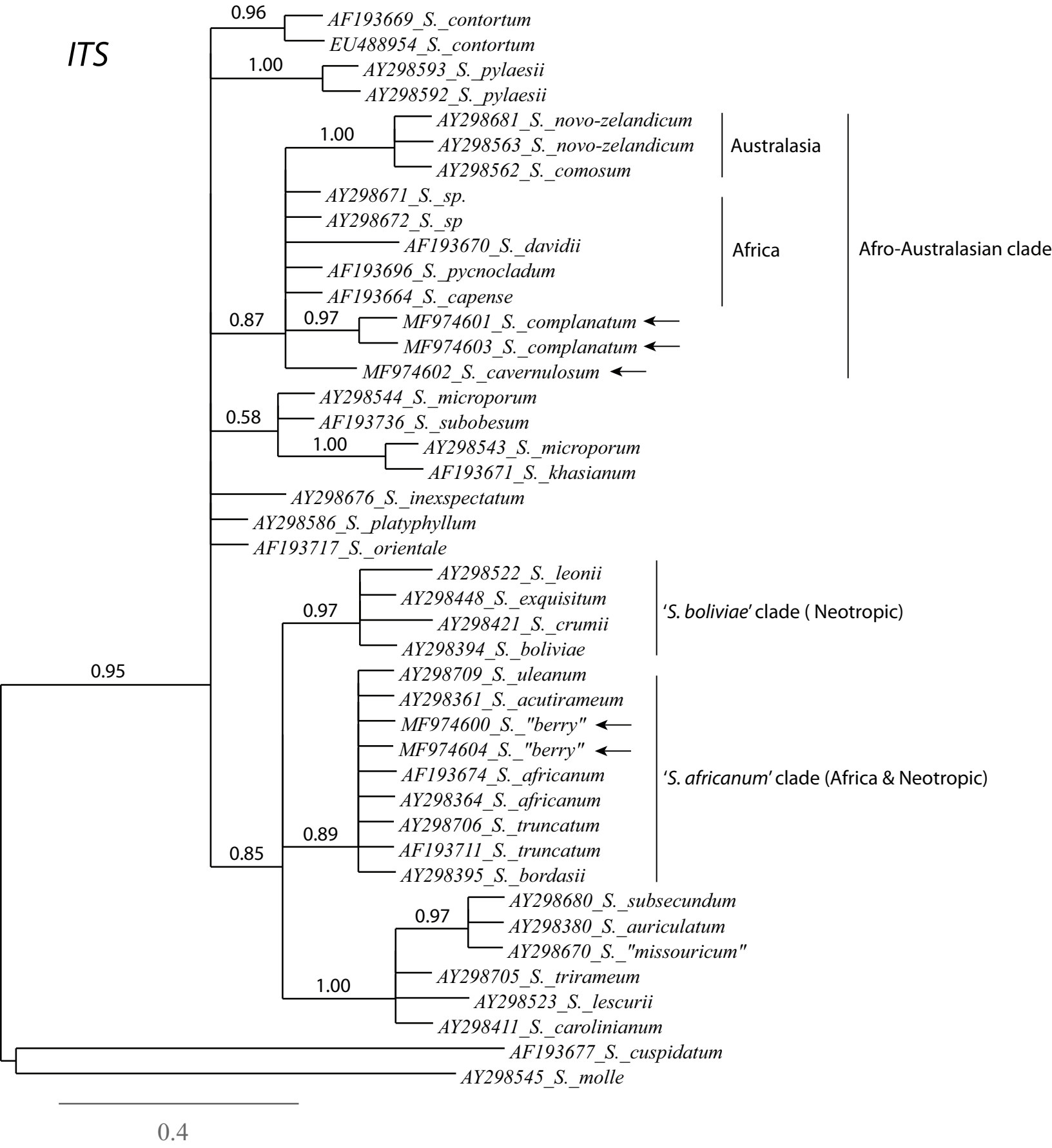
Table. 1. Morphological comparison of the Île Amsterdam plant with four other *Subsecunda* species in the *S. africanum* group.

Morphological characters/ taxon	**Île Amsterdam plant**	***S. truncatum***	***S. rutenbergii***	***S. africanum***	***S. acutirameum***
Stem cortex, cross section	predominantly uni-stratose	predominantly uni-stratose, sometimes with irregular duplication	predominantly 2–3-stratose	predominantly uni-stratose	uni–stratose
Stem leaf size	(1.5-)1.7(–2.0) mm long	1.4–2.8 mm long	1.8–2.8 mm long	1.9–2.8 mm long	2.0–2.2 mm long
Branch leaf size	(3.1–)3.7(-4.1) mm long	1.6–3.0 mm long	(1.4–)1.6–2.0(–2.8) mm long	(1.2–)1.4–1.9(–2.1) mm long	3 mm long
Relative leaf size	stem leaves markedly shorter than branch leaves	stem leaves often as long as branch leaves	stem leaves always longer than branch leaves	stem leaves always longer than branch leaves	stem leaves shorter than branch leaves
Stem leaf hyalocyst septations	abundant throughout most of leaf	non-septate to few cells septate (in var. *truncatum*), to abundant septate throughout most of leaf in var. *bordasii*	not mentioned	not mentioned	not mentioned
Branches in fascicles	3–5(–6)	2–4(–5)	(2–)4–5	never more than 3	1 to 3
Branch leaf apex	truncate, 5–9-dentate	broadly truncate, 8–15-dentate	truncate, 6–9-dentate	broadly rounded and eroded, sub-cucullate, never truncate-dentate	narrowly rounded and slightly erose-dentate

Branch leaf porosity, abaxial surface	pauciporose with cell ends, small, circular to diffuse pores and occasional obscure, cell corner pores	few to very numerous, small, mainly ringed pores, scattered or in series along the commissures	with abundant small, ringed pores in series along the commissures, sometimes with additional pores in the cell midline	with abundant small, ringed commissural pores, with scattered additional midline pores	few or none pores
Branch leaf porosity, adaxial surface	2-6(–8) mostly circular , commissural to sub-commissural, occasionally free-lying, non-ringed pores in addition to cell end pores	equally variable as on abaxial surface, with few to abundant pores, sometimes more abundant than on abaxial surface	without pores or with a few scattered pores mainly in, or near, the apical and upper-lateral cell angles	with usually a few scattered small pores and varying number of pseudopores	many pores in interrupted or nearly continuous commissural rows
Stem leaf porosity, abaxial surface	eporose to more often pauciporose with small, circular to obscure pores at cell ends and corners	more or less as in branch leaves	not mentioned	± identical to branch leaves	pores in interrupted, commissural rows near apex
Stem leaf porosity, adaxial surface	rather similar to abaxial surface, but usually less pauciporose with often 2–6(–8) mostly circular, commissural to sub-commissural, occasionally free-lying, non-ringed pores in addition to a few cell end pores;	more or less as in branch leaves	not mentioned	± identical to branch leaves	more numerous pores over a larger area

Non-colour figure

Click here to download Non-colour figure Figure 1_ITS_FINAL.eps



Non-colour figure

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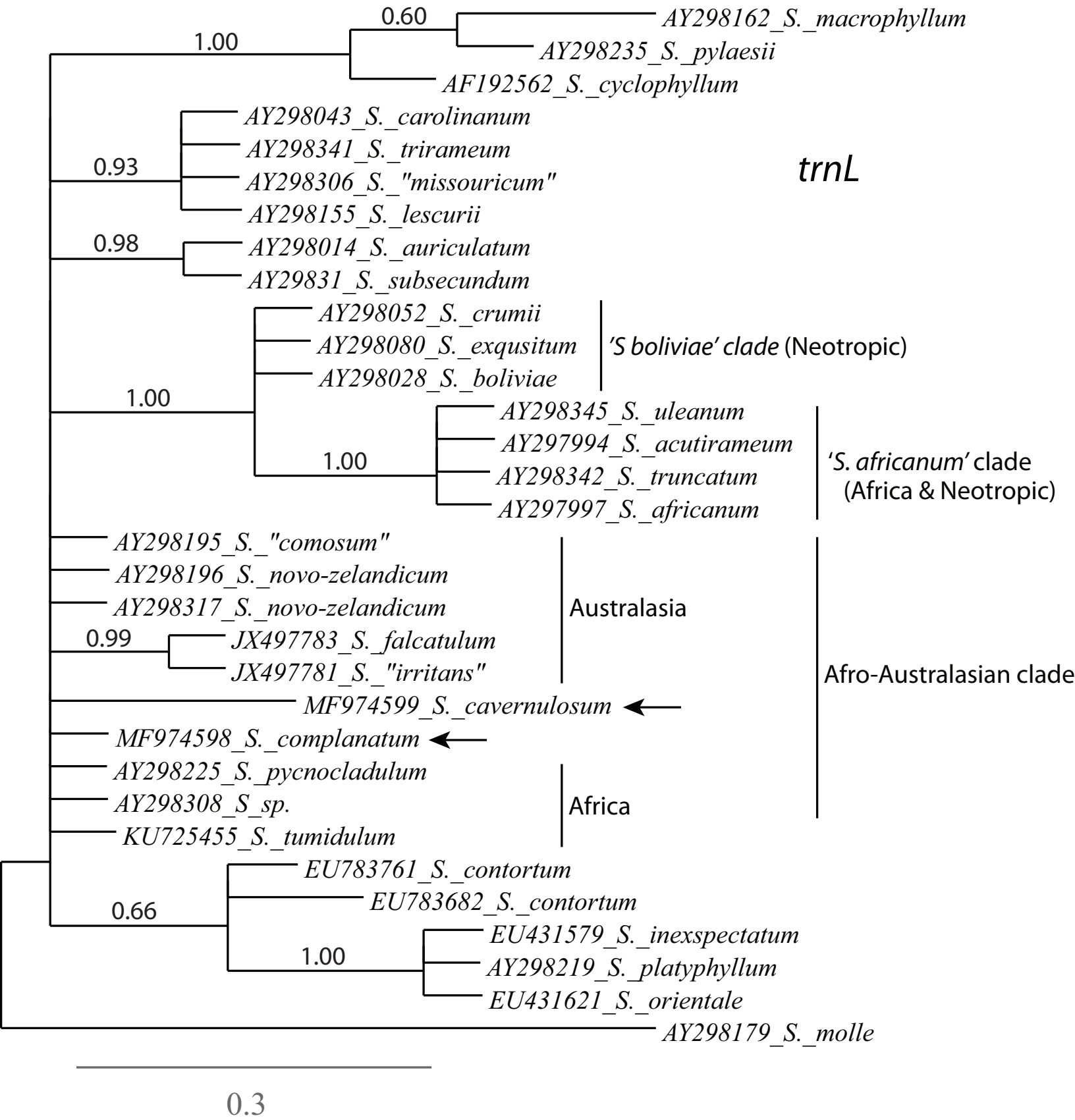

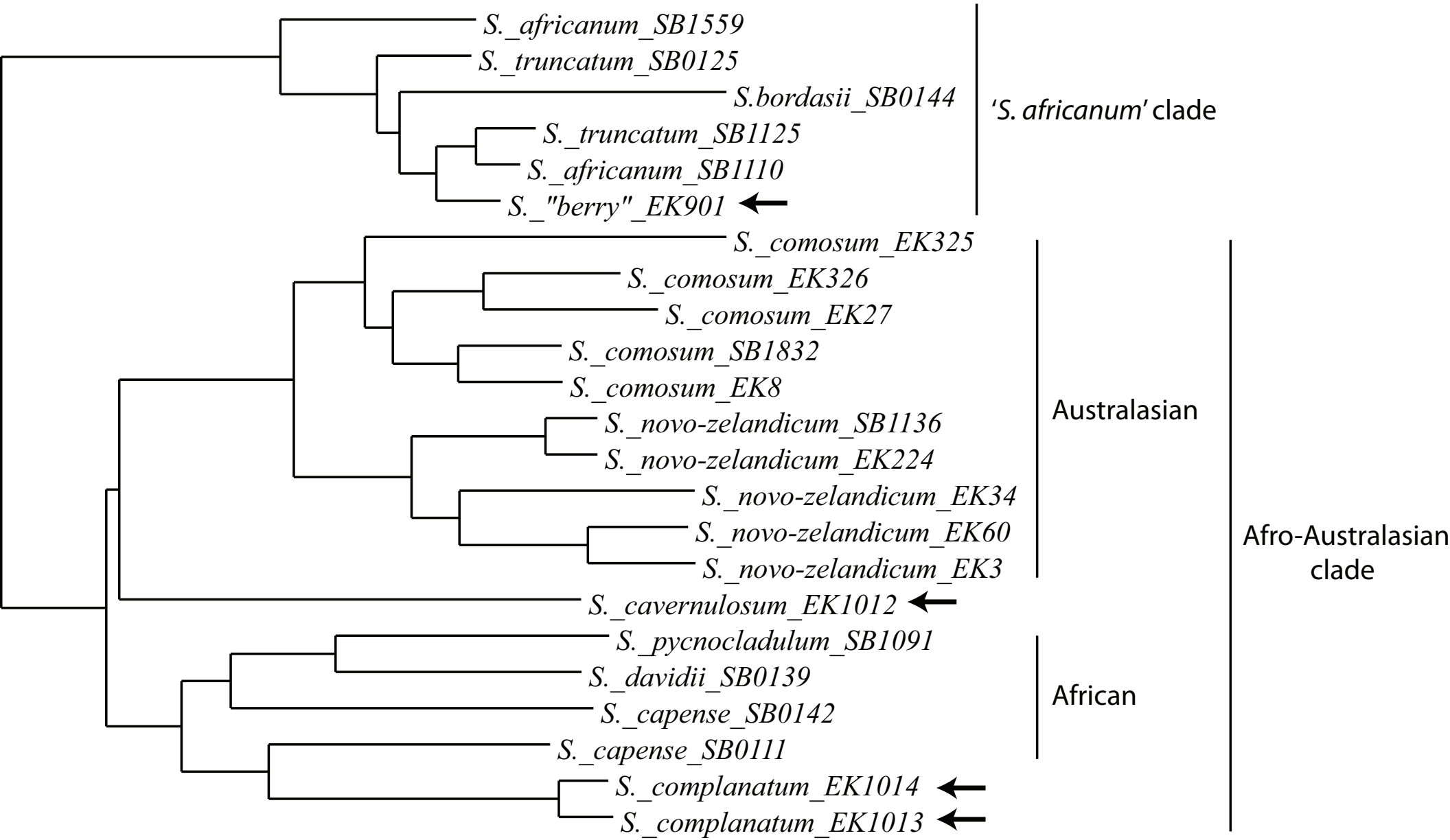

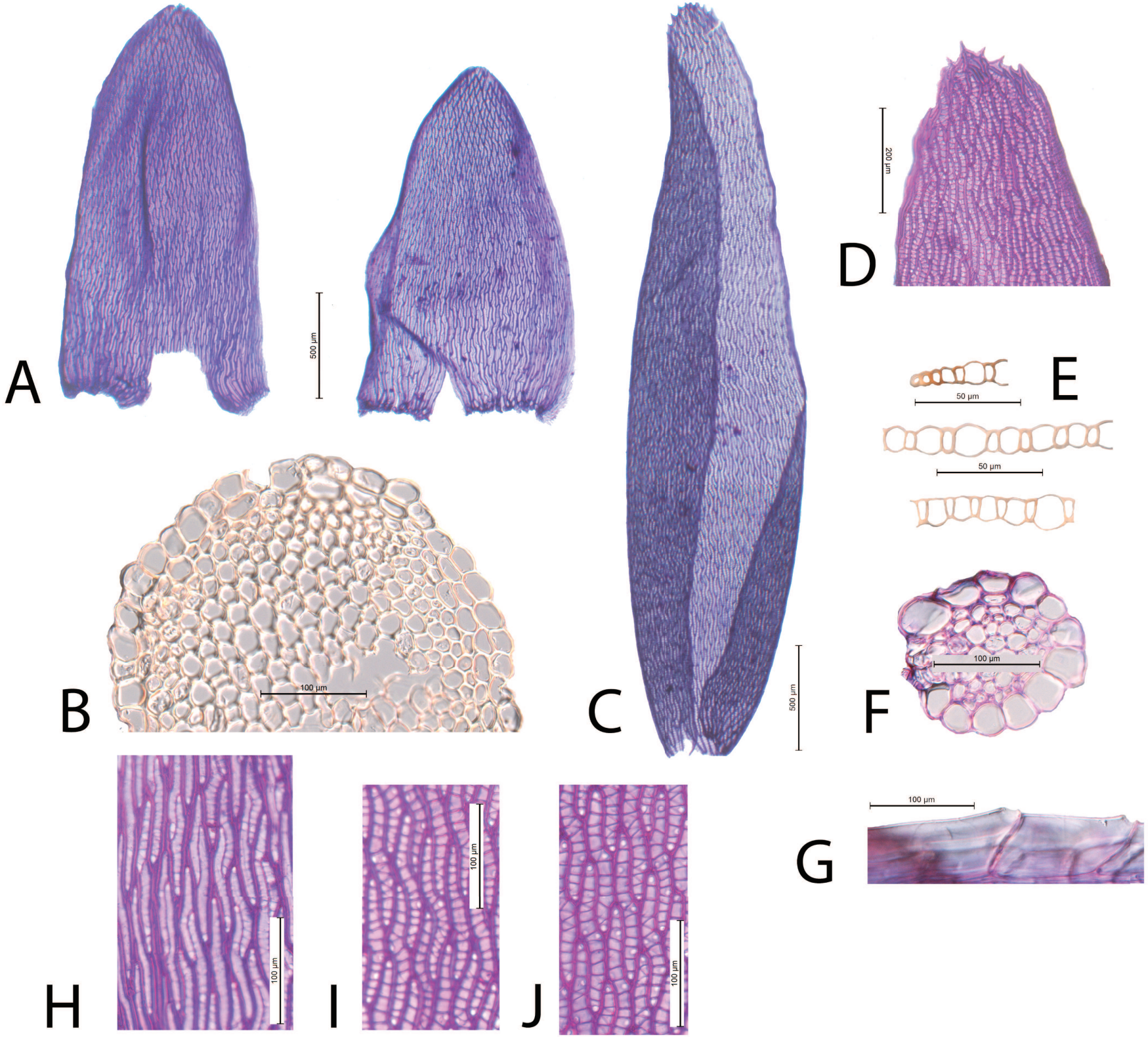
A
500 µm
B
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C
500 µm
D
200 µm
E
50 µm
50 µm
F
100 µm
G
100 µm
H
100 µm
I
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J
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Appendix 1

Voucher information and GenBank accession numbers for nucleotide sequences used in this study was well specimens for which microsatellite data was obtained. Voocher specimens are deposited in the following herbaria: DUKE – Duke University; MICH – University of Michigan, Ann Arbor; MO – Missouri Botanical Garden; NY – New York Botanical Garden; TRH – Norwegian University of Science and Technology.

Taxon — Isolate & voucher specimen: *ITS, trnL*, SSRs (+ = yes);

Note: '---' an updated name is used, with the name the accession is filed under in Genbank listed in brackets

Newly generated DNA data:

Subgenus *Subsecunda*

Sphagnum cavernulosum* Flatberg & Whinam** — EK1150 *Frenot s.n.*, Île Amsterdam (B742385 TRH): MF974602, MF974599, +; ***Sphagnum complanatum* Flatberg & Whinam** — EK1151 *Frenot s.n.*, Île Amsterdam (B742376, TRH): MF974601, MF974598, **−**; ***Sphagnum complanatum — EK1013 *Frenot s.n.*, Île Amsterdam (B742376, TRH): **−**, **−**, +; S***phagnum complanatum*** — EK1152 *Frenot s.n.* (B742379 TRH): MF974603, –, **−**; S***phagnum complanatum*** — EK1152 *Frenot s.n.* (B742379 TRH): **−**, **−**, +; ***Sphagnum islei* Warnst.** — EK945 J. Whinam JW03, Île Amsterdam (B674407 TRH): **−**, **−**, +; ***Sphagnum islei*** — EK946 J. Whinam JW38 (B674409 TRH): **−**, **−**, +; ***Sphagnum cf. truncatum* Hornsch.** — EK1153 *J. Whinam AMS 44*, Île Amsterdam (B742253, TRH): MF974604, –, +; ***Sphagnum cf. truncatum*** — EK1154 *J. Whinam AMS 44* (B742252, TRH): MF974600, –, +;

Extant DNA data:

Unranked at subgenus level (inter-subgeneric allopolyploids)

***Sphagnum falcatulum* Besch.** — EK140 *Karlin 0511-2009*, New Zealand (DUKE): **−**, JX497783, **−**; ***Sphagnum 'irritans'* Warnst. [*falcatulum*]** — EK53 *Karlin 0511-2001*, New Zealand (DUKE): **−**, JX497781, **−**;

Subgenus *Acutifolia*

***Sphagnum molle* Sull.** — SB392 *Andrus 8113*, Ireland (DUKE): AY298545, AY298179, −;

Subgenus *Cuspidata*

***Sphagnum cuspidatum* Ehrh. ex Hoffm.** — SB642 *Shaw 9327*, USA (DUKE): AF193677, −, −;

Subgenus *Subsecunda*

Sphagnum acutirameum* H. A. Crum**— SB873 *Vital & Buck 19692*, Brazil (NYBG): AY298361, AY297994, **−**; ***Sphagnum africanum* Welw. & Duby** — SB1110 *Stoutamire s.n.*, South Africa (MICH): AY298364, AY297997, +; ***Sphagnum africanum — SB97 *Von Rooy 1802*, South Africa (DUKE): AF193674, **−**, **−**; ***Sphagnum africanum*** — SB1559 *Van Rooy 1802*, South Africa (DUKE): −, −, +; ***Sphagnum auriculatum* Schimp.** — SB1093 *Infanti & Heras VIT 16670*, Spain (DUKE): AY298380, AY298014, **−**; ***Sphagnum boliviae* Warnst.**— SB1109 *de Luna 2097*, Boliva (MICH): AY298394, AY298028, **−**; ***Sphagnum bordasii* Besch.** — SB144 *Buck 13594*, South Africa (NYBG): AY298395, −, +; ***Sphagnum capense* Hornsch.**— SB142 *Snook 7352*, South Africa (NYBG): AF193664, −, −; ***Sphagnum carolinianum* R. E. Andrus**— SB1385 *Anderson 27727*, USA (DUKE): AY298411, AY298043, **−**; ***Sphagnum 'comosum'* Müll. Hal. [*S. novo-zelandicum*] —** *SB1132 Wynne s.n.*, Australia (MICH): AY298562, AY298195, +; ***Sphagnum comosum —*** EK8 *Karlin 0511-1703*, New Zealand (DUKE): **−**, **−**, +; ***Sphagnum comosum —*** EK27 *Karlin 0511-0732*, New Zealand (DUKE): −, −, +; EK325 *Streimann 52994*, Australia (MICH): −, −, +; ***Sphagnum comosum —***

EK326 *Streimann 48043*, Australia (DUKE): −, −, +; ***Sphagnum* ‘*comosum*’** [*S. cymbifolioides*] **—** SB1832 *Streimann 43159*, Australia (DUKE): −, −, +; ***Sphagnum contortum* Schultz** — LP16 *Shaw 13143*, Norway (DUKE): EU488954, **−**, **−**; ***Sphagnum contortum*** — LP83 *Shaw 13307*, Norway (DUKE) : **−**, EU783761, **−**; ***Sphagnum contortum*** — LP104 *Shaw 12095*, Sweden (DUKE): **−**, EU783682, **−**; ***Sphagnum contortum*** — SB648 *Anderson 25410*, USA (DUKE): AF193669, **−**, **−**; ***Sphagnum crumii* Schäf.-Verw.**— SB1082 *Schafer-Verwimp & Verwimp 15129*, Brazil (MICH): AY298421, AY298052, **−**; ***Sphagnum cyclophyllum* Sull. & Lesq.** — SB78 *Shaw 8560*, USA (DUKE): −,AF192562, −; ***Sphagnum davidii* Warnst.** — SB139 *Buck 13508*, South Africa (NYBG): AF193670, −, −; ***Sphagnum exquisitum* H. A. Crum** — SB1122 *Schafer-Verwimp & Verwimp 15127*, Brazil (MICH): AY298448, AY298080, **−**; ***Sphagnum inexspectatum* Flatberg** — SB1355 *Uchida 2015*, Japan (DUKE): AY298676, **−**, **−**; ***Sphagnum inexspectatum*** — SB2192 *Shaw 14028*, USA (DUKE): **−**, EU431579, **−**; ***Sphagnum khasianum* Mitt.** — SB134 *Redfern et al. 34401*, China (NYBG): AF193671, −, −; ***Sphagnum leonii* H. A. Crum**— SB1079 *Leoni 2170*, Brazil (MICH): AY298522, −, −; ***Sphagnum lescurii* Sull.** — SB1134 *Vincent 6143* Belize (MICH): AY298523, AY298155, **−**; ***Sphagnum macrophyllum* Bernh. ex Brid.** — SB814 *Risk 6856*, USA (DUKE): **−**, AY298162, **−**; ***Sphagnum microporum* Warnst. ex Cardot** — SB1337 *Yamaguchi 14436*, Japan (DUKE): AY298543, **−**, **−**; ***Sphagnum microporum*** — SB1339 *Higuchi 40841*, Japan (DUKE): AY298544, **−**, **−**; ***Sphagnum ‘missouricum’* Warnst. ex Cardot [*subsecundum*] — *SB1059, Schofield 101087, Canada (DUKE): AY298670, AY298306,* −; −**;** ***Sphagnum novo-zelandicum* Mitt.** — SB1136 *Seppelt 20349*, Australia (MICH): AY298563, AY298196, +; ***Sphagnum novo-zelandicum*** — SB1136 *Seppelt 20349*, Australia (MICH): AY298563, AY298196, +; ***Sphagnum’novo-zelandicum’* [*S. subsecundum*]** — SB881 *Buck 6817*, New Zealand (NYBG):

AY298681, AY298317, **−**; ***Sphagnum novo-zelandicum*** — EK224 *Vitt 2319*, New Zealand (NY): **−**, **−**, +; ***Sphagnum novo-zelandicum*** — EK34 *Karlin 0511-1767*, New Zealand (DUKE): −, −, +; ***Sphagnum novo-zelandicum*** — EK60 *Karlin 0511-1002*, New Zealand (DUKE): −, −, +; ***Sphagnum orientale* L. I. Savicz** — SB630 *Afonina exsicc.*, Russia (MO): AF193717, −, −; ***Sphagnum orientale*** — SB2346 *Shaw 13794*, USA (DUKE): **−**, EU431621, **−**; ***Sphagnum platyphyllum* (Lindb. ex Braithw.) Sull. ex Warnst.** — SB1389 *Schofield 105816*, USA (DUKE): AY298586, AY298219, **−**; ***Sphagnum pycnocladulum* Müll. Hal.** — SB610 *Chapman 6573*, Malawi (MO): AF193696, **−**, **−**; ***Sphagnum pycnocladulum*** — SB1091 *Chapman 6573*, Malawi (MO): **−**, AY298225, **−**; ***Sphagnum pylaesii* Brid.** — SB1165 *Belland & Schofield 16525*, Canada (MICH): AY298592, −, −; ***Sphagnum pylaesii*** — SB1166 *M. Lewis 87449*, Bolivia (MICH): AY298593, −, −; ***Sphagnum pylaesii*** — SB1400 *Durfort s.n.* (DUKE): −,AY298235, −; ***Sphagnum sp.*** — SB1140 *Miehe & Miehe U71-10970*, Uganda (DUKE): AY298672, AY298308, **−**; ***Sphagnum sp.*** — SB1139 *Miehe & Miehe U80-11017*, Uganda (DUKE): AY298671, **−**,**−**; ***Sphagnum subsecundum* Nees** — SB1394 *Heidestein 201032*, Netherlands (DUKE): AY298680, AY298316, **−**; ***Sphagnum trirameum* H. A. Crum**— SB847 *Allen 18212*, Belize (NYBG): AY298705, AY298341, **−**; ***Sphagnum truncatum* Hornsch.** — SB1125 *Stoutamire s.n.*, South Africa (MICH): AY298706, AY298342, +; ***Sphagnum truncatum*** — SB125 *Buck 13599*, South Africa (NYBG): AF193711, **−**, +; ***Sphagnum tumidulum* Besch.** — SB610 *Chapman 6573*, Malawi (MO): **−**, KU725455, **−**; ***Sphagnum uleanum* Müll. Hal.** — SB1103 *Schafer-Verwimp & Verwimp 10616*, Brazil (MICH): AY298709, AY298345, **−**;

Appendix 2

Appendix 2 figure goes here

Neighbor-joining tree of haplotypes (based on 9 SSRs) *S. cavernulosum, S. complanatum, S. islei*, and members of the 'Afro-Australasian clade, with three members of the *S. africanum'* clade as an outgroup. Arrows indicate Île Amsterdam samples.