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Reinterpreting the phylogenetic position, systematics and distribution of the *Raddia-Sucrea* lineage (Poaceae, Olyrinae), with a new monotypic and endangered herbaceous bamboo genus from Brazil

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Herbaceous bamboos (tribe Olyreae) are typical components of Neotropical forests, with a great diversity in Brazil. Olyreae comprise three subtribes, of which Olyrinae are the most heterogeneous, with 19 genera widely distributed in Brazilian biomes but also occurring in Mexico, Central America and northern and western South America. One of the lineages of this group is composed of the genera *Raddia* and *Sucrea*, the relationships of which were recently studied. Although *Raddia* was clearly monophyletic, the three *Sucrea* spp. displayed incongruence between the plastid and nuclear genomes. In the present study, we increased taxon sampling and included additional DNA regions [ITS, *rpl32–trnL*, *trnD–trnT* and *trnS–*(*psbZ*)*–trnG*] and used macro- and micromorphological characters to investigate the relationships in this group. The analyses involved maximum parsimony, Bayesian inference and maximum likelihood estimation methods. The three species traditionally assigned to *Sucrea* are confined to the Brazilian Atlantic Forest and represent two lineages, one sister to *Raddia* and composed of *S. monophylla* (the type species) and *S. maculata*, with open panicles, microechinate-areolate pollen and a distribution from Bahia to Rio de Janeiro states. The other lineage, sister to *Raddia* + *Sucrea*, is composed of an unusual species with contracted panicles, microechinate-rugulate pollen and conspicuous tuberous roots; this is endemic to Rio de Janeiro and Espírito Santo states. This species, traditionally known as *S. sampaiana*, is here transferred to the new genus *Brasilochloa* (as *B. sampaiana*). It is a rare lineage, currently endangered with extinction, as is the case for a number of herbaceous bamboos occurring in the Atlantic Forest.

ADDITIONAL KEYWORDS: Atlantic Forest – *Brasilochloa* – Olyreae – phylogeny – *Sucrea sampaiana*.

INTRODUCTION

Bamboos (Poaceae subfamily Bambusoideae) comprise the major grass lineage that diversified primarily in association with forests, and they are important components of Neotropical floras (Clark, Londoño & Ruiz-Sanchez, 2015; Kellogg, 2015; Clark & Oliveira, 2018). Brazil is considered the most megadiverse country in the world and the humid forests of the eastern coast harbour the main centre of diversity

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*Corresponding author. E-mail: rpatricia@uefs.br of bamboos in the Neotropics, especially in lowlands in Bahia and Espírito Santo states (Soderstrom, Judziewicz & Clark, 1988; Clark, 1990; Judziewicz *et al.*, 1999; Oliveira, Borba & Longhi-Wagner, 2008a). Several genera and species of this group were described based on collections made in Brazil by Thomas Soderstrom and Cleofé Calderón in the 1960–1970s, including woody (tribe Bambuseae) and herbaceous (tribe Olyreae) bamboos.

Olyreae currently include *c.* 22 genera of pluricarpic plants with predominantly unisexual spikelets, which are distinguished mainly by the arrangement of the male and female spikelets in the synflorescences and by the disposition of the synflorescences on the plant (Judziewicz *et al.*, 1999; Vorontsova *et al.*, 2016; Clark & Oliveira, 2018). They are almost exclusive to Neotropical forests (Soderstrom *et al.*, 1988; Judziewicz *et al.*, 1999), but they were poorly known until Soderstrom's studies in Brazil and Central America, which greatly inform our current research. One of the genera belonging to this tribe and described based on collections made during the aforementioned period was *Sucrea* Soderstr., which honoured the Brazilian botanist Dimitri Sucre, from the Botanical Garden of Rio de Janeiro (Soderstrom, 1981).

Soderstrom (1981) described *Sucrea* as 'a group of herbaceous bamboos forming clumps up to 1 m tall, with one-flowered and unisexual spikelets, as typical of this tribe, being endemic to the Atlantic Forest of Brazil, and occurring in the understory, up to 400 m.a.s.l.'. He assigned three species to this genus, one known only from southern Bahia (*S. monophylla* Soderstr., the type species), one occurring in Rio de Janeiro and Espírito Santo states (*S. maculata* Soderstr.) and one formerly recorded only from southern Espírito Santo [*S. sampaiana* (Hitchc.) Soderstr.], but more recently found in northern Rio de Janeiro (Oliveira, Longhi-Wagner & Jardim, 2011).

Since Soderstrom (1981) formally described *Sucrea*, it has been distinguished from allied genera in Olyreae mainly by synflorescence features, including the position on the plant body (always terminal), and by the morphology of the spikelets, which are exclusively one-flowered and unisexual. He first considered *Sucrea* to be related to *Raddia* Bertol. and *Olyra* L., arguing that the synflorescence was probably retained from an *Olyra*-like ancestor but with spikelets of *Raddia*, which led him to refer to it as '*Raddiolyra*' for some time. This author also considered it similar to *Raddiella* Swallen, both sharing with *Raddia* female spikelet characters, such as prominent nerves on the glumes, with the glumes usually twisting at the apex and at the base. However, male and female spikelets in *Sucrea* occur in the same panicle (vs. in different synflorescences in *Raddia* and *Raddiella*), and the female glumes have cartilaginous margins. Based on vegetative morphology only, Clayton & Renvoize (1986) also considered *Sucrea* as positioned between *Raddia* and *Olyra*, with the latter genus similar to *Sucrea*. Some of the morphological diversity of this group is shown in Fig. 1.

Understanding of the relationships of these genera in Olyreae was initially modified with the publication of the genus *Parodiolyra* Soderstr. & Zuloaga, segregated from *Olyra* by Soderstrom & Zuloaga (1989) and considered to be close to *Raddiella* (I.L.C. Oliveira *et al.,* in press). These genera are undoubtedly part of Olyrinae, which are supported by molecular criteria as a distinct subtribe of Olyreae (Zhang & Clark, 2000; Kelchner & BPG, 2013; Oliveira *et al.*, 2014; Saarela *et al.*, 2018). The few molecular phylogenetic analyses exclusively focused on Olyrinae to date recovered at least four supported lineages (Oliveira, 2006; Carvalho, 2013; Oliveira *et al.*, 2014; Oliveira, 2017). These studies corroborated the strong relationship between *Sucrea* and *Raddia*, composing the *Raddia*-*Sucrea* lineage analysed in detail by Oliveira *et al.* (2014). This study recovered the monophyly of *Raddia*, but the data set was uninformative regarding *Sucrea*, which was paraphyletic depending on the genome spacers analysed (the nuclear ITS and the plastid *trnD–trnT*), indicating that internal relationships within this lineage remain obscure.

The main doubt regarding the circumscription of *Sucrea* has been focused on the placement of *S. sampaiana,* first described by Hitchcock (1927) as *Olyra sampaiana* Hitchc., in honour of a former curator of the Brazilian National Museum of Rio de Janeiro, Dr Alberto José de Sampaio, who sent to him some samples collected in southern Espírito Santo by José Vidal (Soderstrom, 1981). *Sucrea sampaiana* was always considered as a distinctive grass species, based on its congested and spiciform synflorescences, with female spikelets distributed on the upper branches and the males on the lower ones and the lower glumes of the female spikelets usually long ciliate on the upper margins. In addition, its male spikelets are acute and < 5 mm long, and it has conspicuous tuberous roots (Soderstrom, 1981; Judziewicz *et al.*, 1999; Oliveira *et al.*, 2011, 2014). These features contrast with those of the other two species of the genus (*S. maculata* and *S. monophylla*), which display open paniculate synflorescences, with female spikelets terminating each branch of the synflorescence, glumes of the female spikelets not ciliate, male spikelets long and acuminate and tuberoids never detected on their roots (Soderstrom, 1981; Judziewicz *et al.*, 1999; Oliveira *et al.*, 2014). Although we have observed these differences for several years in herbarium specimens and in the field, until a more complete analysis synthesizing morphology, geography and molecular phylogenetics could be completed, we have avoided making any new taxonomic proposals within this group.

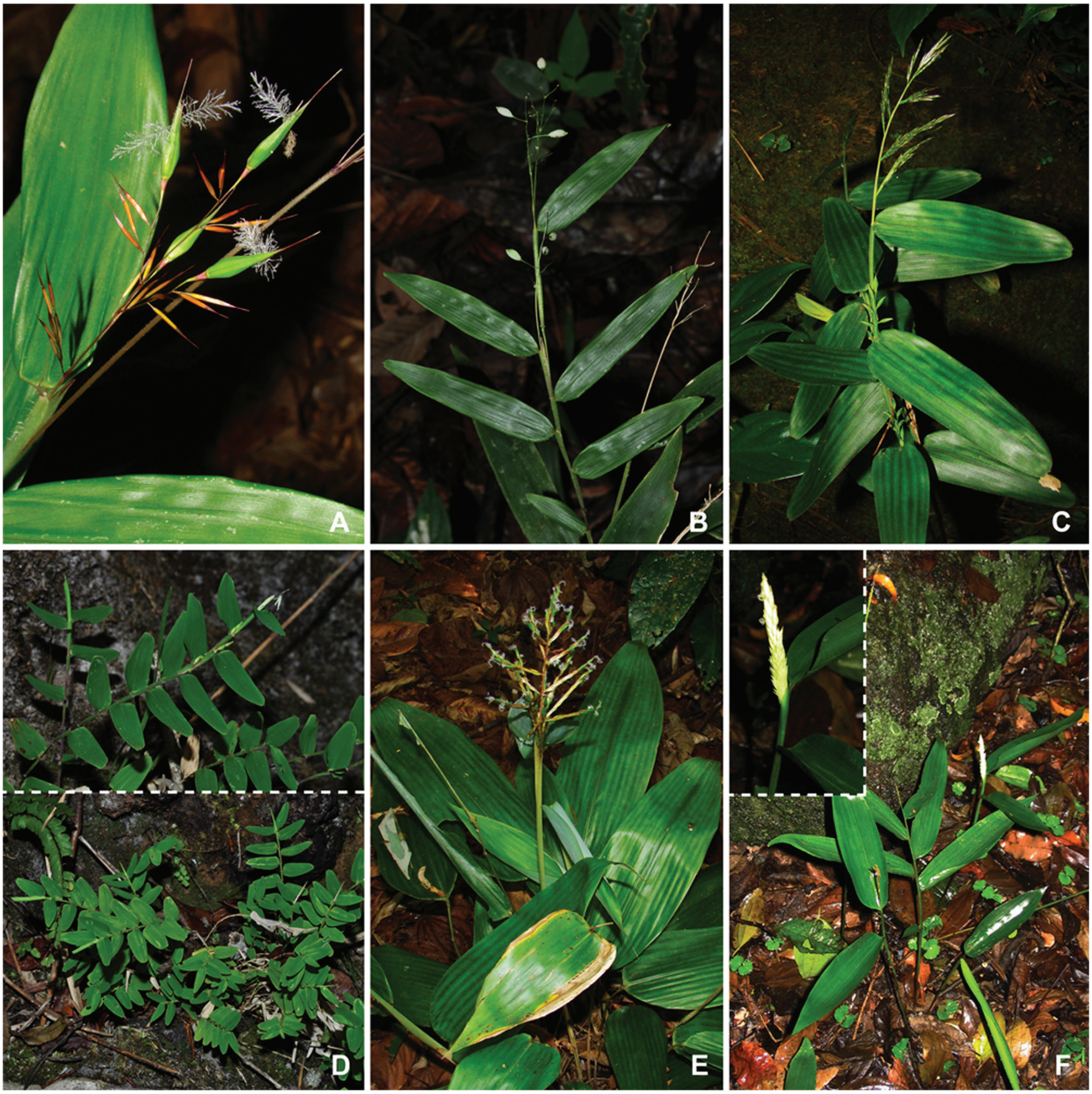

Figure 1. *Sucrea* and related genera in Olyrinae. A, *Olyra glaberrima* Raddi. B, *Parodiolyra ramosissima* (Trin.) Soderstr. & Zuloaga. C, *Raddia brasiliensis* Bertol. D, *Raddiella esenbeckii* (Steud.) C.E.Calderón & Soderstr. E, *Sucrea monophylla* Soderstr. F, *Sucrea sampaiana* (Hitchc.) Soderstr.

On the other hand, based on non-conventional criteria for a morphological phylogenetic analysis, Grande Allende (2016) proposed modifications in the taxonomy of Olyreae, without any complementary molecular analyses. This author expanded the circumscription of *Raddia*, transferring to it the three species previously accepted in *Sucrea*, as *R. maculata* (Soderstr.) J.R.Grande, *R. monophylla* (Soderstr.) J.R.Grande and *R. sampaiana* (Hitchc.) J.R.Grande. This decision was justified on morphological similarities, e.g. between *R. megaphylla* R.P.Oliveira & Longhi-Wagner and *S. maculata*, which both produce a single and large leaf blade per culm in adult individuals. These taxonomic modifications were not adopted as part of the world checklist of bamboos and rattans (Vorontsova *et al.*, 2016) or by Soreng *et al.* (2017), but they are used in the Tropicos database (http://www.tropicos.org).

Other recent studies by Dórea *et al.* (2017) on the ectexine patterns of pollen grains in species of Poaceae from Neotropical forests, including several genera and species of herbaceous bamboos, showed that *Sucrea sampaiana* is distinguishable based on these features. Variation in pollen features in Olyreae recently supported a new genus in Parianinae (*Parianella* Hollowell, F.M.Ferreira & R.P.Oliveira), also distinguishable by molecular phylogenetics, macromophology and biogeography (Ferreira *et al.*, 2013). In a similar way, pollen features have helped us to clarify our concepts regarding the delimitation of *Sucrea* and its relationship with *Raddia* and other genera in Olyrinae.

Besides the aforementioned systematic questions, *Sucrea sampaiana* also deserves to be highlighted from a conservation point of view. It was first collected in the early 20th century, in a region currently modified to pasture, and was not recollected for > 100 years, leading Soderstrom (1981) and Judziewicz *et al.* (1999) to consider it as probably extinct. As part of our studies on Brazilian Olyreae we found a small population in 2003, in the north of Rio de Janeiro state, with apparently no more than 100 individuals, occurring in a remnant of Atlantic Forest in an area surrounded by extensive pastures (Oliveira *et al.*, 2011). For several years it was the single population of this species known in the wild, leading the Brazilian government to include it on the list of Brazilian species threatened by extinction (MMA, 2008; Filgueiras *et al.*, 2013).

Here, our main objective is to improve knowledge of the systematics of herbaceous bamboos, focusing on the relationships of the species currently accepted in *Sucrea*, and their relationships with other genera of Olyrinae, especially *Raddia*. Taxon sampling for this clade and the number of DNA regions analysed were increased and we tested the topologies of the resulting phylogenetic trees, posing the following central questions: (1) Are *Sucrea* and *Raddia* monophyletic and independent genera? (2) May the conflicting placement of *S. sampaiana* be explained by combined DNA sequences, micro- and morphological features? (3) What are the taxonomic implications of our evolutionary analyses? Additionally, as *Sucrea* is endemic to the endangered Brazilian Atlantic Forest, we also aimed to provide updates on the geographical distribution of each species and discuss other important issues regarding habitat and conservation.

MATERIAL AND METHODS

Taxon sampling

All currently accepted *Sucrea* spp. (three) and *Raddia* spp. (nine) were sampled, including their type species (*S. monophylla* and *R. brasiliensis* Bertol., respectively). These were included as ingroup taxa along with 27 other representatives of subtribes Olyrinae [*Arberella* Soderstr. & C.E.Calderón (two species of seven in the genus), *Cryptochloa* Swallen (2/9), *Diandrolyra* Stapf (2/3), *Lithachne* P.Beauv. (2/4), *Olyra* (4/25), *Parodiolyra* (2/5), *Piresia* Swallen (2/5) and *Raddiella* (1/8)] and Parianinae [*Eremitis* Döll (5/5), *Pariana* Aubl. (3/35) and *Parianella* (2/2)]. The New Guinean herbaceous bamboo *Buergersiochloa bambusoides* Pilg. (subtribe Buergersiochloinae) was chosen as the outgroup based on previous studies (Triplett & Clark, 2010; Kelchner & BPG, 2013; Triplett *et al.*, 2014; Wysocki *et al.*, 2015; Saarela *et al.*, 2018). Leaf samples were mostly obtained in the field or from greenhouse-grown specimens and dried in silica gel (Chase & Hills, 1991). A few other samples were obtained from herbarium specimens. Voucher information is given in Table 1.

DNA extraction, amplification and sequencing

DNA extraction was performed using a modified version of the 2× CTAB procedure of Doyle & Doyle (1987) for silica-dried samples and the DNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) for herbarium samples. We selected the internal transcribed spacer (ITS) of the nuclear rDNA and three plastid spacers [*rpl32–trnL*, *trnD–trnT*, and *trnS–*(*psbZ*)*–trnG*] because they have been successfully used in phylogenetic analyses encompassing several lineages across Poaceae, providing satisfactory levels of variation at different taxonomic ranks (e.g. Triplett & Clark, 2010; Kelchner & BPG, 2013; Fisher, Clark & Kelchner, 2014; Oliveira *et al.*, 2014; Silva *et al.*, 2015). These markers were amplified via PCR and sequenced following the procedures described by Oliveira *et al.* (2014) and Silva *et al.* (2015). Primers used for amplification and sequencing, and PCR conditions are shown in Table 2.

PCR reactions were performed using the TopTaq Master Mix Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol, for a final volume of 10 μL, with 0.25 μM each primer and *c.* 30 ng template DNA. For herbarium samples, PCR reactions also included 2 μL TBT-PAR [trealose, bovine serum albumin, polysorbate-20 (Tween-20)] (Samarakoon, Wang & Alford, 2013), and for ITS they also included 0.2 μL dimethyl sulphoxide 99.5% to avoid secondary conformations.

Purification of PCR products was performed using the ExoSAP-IT PCR Clean-up Kit (GE Healthcare, Buckinghamshire, UK) or PEG 11% (Paithankar & Prasad, 1991), and then sequenced in both directions using the Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Austin, TX, USA) according to the following protocol: a hot start followed by

Table 1. Voucher information and GenBank accession numbers for DNA sequences used in this study. Taxon names are in accordance with the classification of the BPG (2012). Specimens are cited only by the name of the first collector and collection number, followed by the country where each collection was made and acronym of the herbarium where they are housed (acronyms according to Thiers, 2019). A single asterisk indicates exceptional cases in which a distinct voucher was used to generate a sequence for a single terminal. Double asterisk indicates new sequences generated in this study

Species	Voucher	ITS	*trnD–trnT*	*rpl32–trnL*	*trnS–(psbZ)–trnG*
Subtribe Buergersiochloinae (outgroup)					
Buergersiochloa bambusoides Pilg.	*S. Dransfield 1365* (New Guinea, K)	KC990734	FJ643988	KY612930	KX027403**
Subtribe Olyrinae					
Arberella bahiensis Soderstr. & Zuloaga	*J.G. Jardim s.n.* (Brazil, HUEFS)	KC990700	KC990735	MK175309	MK175377**
Arberella costaricensis (Hitchc.) Soderstr. & C.E.Calderón	*C.E. Calderón 2109* (Costa Rica, MO)	–	MK175421**	MK175416**	MK175426**
Cryptochloa capillata (Trin.) Soderstr.	*R.P. Oliveira 969* (Brazil, HUEFS)	KC990710	KC990745	–	KX027404**
Cryptochloa decumbens Soderstr. & Zuloaga	*J.G. Sánchez-Ken 664* (Panama, MO)	MK175245	MK175358	MK175311	MK175379**
Diandrolyra bicolor Stapf	*R.P. Oliveira 850* (Brazil, HUEFS)	KC990727	KC990761	MK175313	MK175381**
Diandrolyra tatianae Soderstr. & Zuloaga	*R.P. Oliveira 726* (Brazil, HUEFS)	KC990728	KC990762	MK175314	KX027406**
Lithachne horizontalis Chase	*P.L. Viana 5202* (Brazil, BHCB)	KC990706	KC990741	MK175315	MK175383**
Lithachne pauciflora (Sw.) P.Beauv.	*R.P. Oliveira 970* (Brazil, HUEFS)	KC990707	–	MK175316	KX027407**
	L.G. Clark 1297 (Brazil, ISC)*	–	KC990742	–	–
Olyra bahiensis R.P.Oliveira & Longhi-Wagner	*R.P. Oliveira 977* (Brazil, HUEFS)	KC990705	KC990740	MK175317	MK175384**
Olyra glaberrima Raddi	*R. Verveloet 2206* (Brazil, HUEFS)	KC990702	KC990737	MK175318	KX027408**
Olyra humilis Nees	*H.M. Longhi-Wagner 8001* (Brazil, HUEFS)	KC990701	KC990736	–	KX027409**
Olyra latifolia L.	*R.P. Oliveira 667* (Brazil, HUEFS)	KC990704	KC990739	MK175319	KX027410**
Parodiolyra micrantha (Kunth) Davidse & Zuloaga	*R.P. Oliveira 650* (Brazil, HUEFS)	KC990713	KC990748	MK175330	KX027411**
Parodiolyra ramosissima (Trin.) Soderstr. & Zuloaga	*R.P. Oliveira 688* (Brazil, HUEFS)	KC990714	KC990749	MK175334	MK175396**
Piresia goeldii Swallen	*R.P. Oliveira 1205* (Brazil, HUEFS)	KC990708	KC990743	MK175335	KX027413**
Piresia sympodica (Döll) Swallen	*R.P. Oliveira 1195* (Brazil, HUEFS)	KC990709	KC990744	MK175336	KX027417**
Raddia angustifolia Soderstr. & Zuloaga	*R.P. Oliveira 725* (Brazil, HUEFS)	KC990715	KC990750	MK175337	MK175397**
Raddia brasiliensis Bertol.	*R.P. Oliveira 972* (Brazil, HUEFS)	KC990716	KC990751	–	MK175398**
Raddia distichophylla (Schrad. ex Nees) Chase	*R.P. Oliveira 601* (Brazil, HUEFS)	KC990717	KC99075	MK175338	MK175399**
Raddia guianensis (Brongn.) Hitchc.	*R.P. Oliveira 911* (Brazil, HUEFS)	KC990718	–	MK175339	MK175400**
	R.P. Oliveira 993 (Brazil, HUEFS)*	–	KC990753	–	–

Table 1. Continued

Species	Voucher	ITS	*trnD–trnT*	*rpl32–trnL*	*trnS–(psbZ)–trnG*
Raddia lancifolia R.P.Oliveira & Longhi-Wagner	*R.P. Oliveira 980* (Brazil, HUEFS)	KC990719	KC990754	MK175340	MK175401**
Raddia megaphylla R.P.Oliveira & Longhi-Wagner	*R.P. Oliveira 981* (Brazil, HUEFS)	KC990720	KC990755	MK175341	MK175402**
Raddia portoi Kuhlm.	*R.P. Oliveira 1042* (Brazil, HUEFS)	KC990721	KC990756	MK175342	MK175403**
Raddia soderstromii R.P.Oliveira, L.G.Clark & Judz.	*R.P. Oliveira 722* (Brazil, HUEFS)	KC990722	–	MK175343	KX027427**
	R.P. Oliveira 978 (Brazil, HUEFS)*	–	KC990757	–	–
Raddia stolonifera R.P.Oliveira & Longhi-Wagner	*R.P. Oliveira 1078* (Brazil, HUEFS)	KC990723	KC990758	MK175344	MK175404**
Raddiella esenbeckii (Steud.) C.E.Calderón & Soderstr.	*R.P. Oliveira 664* (Brazil, HUEFS)	KC990712	KC990747	MK175352	KX027428**
Sucrea maculata Soderstr.	*R.P. Oliveira 2276* (Brazil, HUEFS)	–	MK175422**	MK175417**	MK175427**
	R.P. Oliveira 851 (Brazil, HUEFS)	KC990724	MK175423**	MK175355	MK175414**
Sucrea monophylla Soderstr.	*I.L.C. Oliveira 08* (Brazil, HUEFS)	–	MK175424**	MK175418**	MK175428**
	R.P. Oliveira 1072 (Brazil, HUEFS)	KC990725	–	MK175356	KX027430**
Sucrea sampaiana (Hitchc.) Soderstr.	*R.P. Oliveira 2325* (Brazil, HUEFS)	–	MK175425**	MK175420**	MK175429**
	R.P. Oliveira 991 (Brazil, HUEFS)	KC990726	KC990760	MK175357	MK175415**
Subtribe Parianinae					
Eremitis afimbriata F.M.Ferreira & R.P.Oliveira	*F.M. Ferreira 2196* (Brazil, HUEFS)	KX016075	KX016043	KY612894	MK175382
Eremitis linearifolia Hollowell, F.M.Ferreira & R.P.Oliveira	*F.M. Ferreira 2185* (Brazil, HUEFS)	KX016085	KX016050	KY612904	–
Eremitis magnifica F.M.Ferreira & R.P.Oliveira	*F.M. Ferreira 2158* (Brazil, HUEFS)	KX016086	KX016051	KY612905	–
Eremitis parviflora (Trin.) C.E.Calderón & Soderstr.	*F.M. Ferreira 2064* (Brazil, HUEFS)	KX016087	KX016052	KY612906	–
Eremitis robusta Hollowell, F.M.Ferreira & R.P.Oliveira	*F.M. Ferreira 2215* (Brazil, HUEFS)	KX016093	KX016056	KY612912	–
Pariana nervata Swallen	*R.P. Oliveira 1876* (Brazil, HUEFS)	KX016099	KX016060	KY612919	MK175385
Pariana pallida Swallen	*R.P. Oliveira 1194* (Brazil, HUEFS)	KC990731	–	KY612920	MK175386
Pariana vulgaris Tutin	*R.P. Oliveira 1844* (Brazil, HUEFS)	KY674523	KY659797	MK175320	MK175387
Parianella carvalhoi (R.P.Oliveira & Longhi-Wagner) F.M.Ferreira & R.P.Oliveira	*A.C. Mota 298* (Brazil, HUEFS)	KX016105	KX016066	KY612925	MK175388
Parianella lanceolata (Trin.) F.M.Ferreira & R.P.Oliveira	*R.P. Oliveira 681* (Brazil, HUEFS)	KC990729	KC990763	KY612927	KX027433**

Table 2. Primer information and PCR conditions. Primers marked with an asterisk were used for sequencing only. All others were used for amplification and sequencing

DNA region	Primer name	Primer sequence 5′-3′	Reference	PCR Conditions					
				Pre-melting	Denaturation (I)	Primer annealing (II)	Primer extension (III)	Cycles (I + II + III)	Final extension
ITS	17SE (F)	ACG AAT TCA TGG TCC GGT GAA GTG TTC G	Sun *et al.* (1994)	94 °C (1 min)	94 °C (30 s)	50 °C (40 s)	72 °C (40 s)	28	72 °C (5 min)
	26SE (F)	TAG AAT TCC CCG GTT CGC TCG CCG TTA C	Sun *et al.* (1994)						
	92 (F)*	AAG GTT TCC GTA GGT GAA C	Desfeux *et al.* (1996)	Following the sequencing protocol					
	4 (R)*	TCC TCC GCT TAT TGA TAT GC	White *et al.* (1990)						
rpl32–trnL	rpL32-F (F)	CAG TTC CAA AA A AAC GTA CTT C	Shaw *et al.* (2007)	94 °C (1 min)	94 °C (40 s)	50 °C (45 s)	72 °C (1 min, 30 s)	35	72 °C (5 min)
	trnL^{UAG} (R)	CTG CTT CCT AAG AGC AGC GT	Shaw *et al.* (2007)						
trnD–trnT	trnT^{GGU} (F)	CTA CCA CTG AGT TAA AAG GG	Demesure, Sodzi & Petit (1995)	94 °C (1 min)	94 °C (45 s)	55 °C (40 s)	72 °C (1 min, 30 s)	35	72 °C (5 min)
	trnD^{GUC} (R)	ACC AAT TGA ACT ACA ATC CC	Demesure *et al.* (1995)						
trnS–(psbZ)–trnG	trnG^{UUC} (F)	GTA GCG GGA ATC GAA CCC GCA TC	Shaw *et al.* (2005)	94 °C (1 min)	94 °C (45 s)	55 °C (40 s)	72 °C (1 min, 10 s)	35	72 °C (5 min)
	trnS^{GCU} (R)	AGA TAG GGA TTC GAA CCC TCG GT	Shaw *et al.* (2005)						

3 min of initial denaturation at 96 °C, and 30 cycles of 96 °C denaturation for 20 s, 50 °C annealing for 15 s and 60 °C extension for 4 min. Sequencing products were cleaned using 80% isopropanol and 70% ethanol, and analysed on a 3130xl Genetic Analyzer (Applied Biosystems/HITACHI, Tokyo, Japan) at the Laboratório de Sistemática Molecular de Plantas of the Universidade Estadual de Feira de Santana (LAMOL/UEFS). GenBank accession numbers for the newly generated sequences plus others downloaded from GenBank are listed in Table 1.

Phylogenetic analyses

Electropherograms were edited and assembled using Geneious v.6.0.5 (Biomatters, Auckland, New Zealand). The resulting sequences were combined with those from GenBank (Table 1), and the matrices for each marker were aligned using MUSCLE (Edgar, 2004), as implemented in Geneious, and then checked and manually adjusted. The alignments for each data set are available upon request to the authors. Indels were coded by the 'simple indel coding' method using the computer program GapCoder (Young & Healy, 2003). In this conservative approach, all gaps that have different 5′ and/or 3′ ends are coded as separate presence/absence characters (Simmons & Ochoterena, 2000). Regions with ambiguous alignment or suspected sequencing errors (especially 5′ or 3′ ends) were excluded from the analyses, corresponding to the following positions in the total evidence data set (ITS + plastids + all gaps; 5.3% of all sites; Table 3): 1–27, 742–825 (18S 3′ end, and 26S 5′ end; ITS); 1918–1963 (*trnL* 5′ end; *rpl32–trnL*), 1964–1972, 1973–1979, 3414–3435 (*trnM* 3′ end, *trnM–trnT* spacer and *trnY–trnD* spacer 3′ end; *trnD–trnT*); 3436–3465, 4347–4378 [*trnS*^{UGA} 3′ end and *psbZ–trnG*^{UCC} spacer 3′ end; *trnS–*(*psbZ*)*–trnG*]. Individual and combined (plastid only and plastid + ITS) data sets were analysed using maximum parsimony (MP), Bayesian inference (BI) and maximum likelihood (ML) methods, with and without coded indels. Combinability of the individual matrices was assessed by comparing incongruent clades with high bootstrap support (Wiens, 1998).

MP analyses were performed using PAUP v.4.0b10 (Swofford, 2002) with Fitch parsimony as the optimality criterion (Fitch, 1971). Heuristic searches were performed with 1000 random taxon-addition replicates using TBR branch-swapping and a limit of 15 trees saved per replicate to avoid extensive swapping on large islands of trees. The resulting trees were used as starting trees for a subsequent round of TBR swapping with an upper limit of 10 000 trees. Internal support was evaluated using non-parametric bootstrapping (Felsenstein, 1985) with 2000 replicates, as indicated by Hedges (1992) and Müller (2005), simple taxon-addition and TBR algorithm, saving 15 trees per replicate. Bootstrap values (hereafter indicated as MPBS) of 50–70% were considered as weak, 71–85% as moderate and > 85% as strong (Kress, Prince & Williams, 2002). Only MPBS values > 70% were recorded on the trees.

BI analyses were performed using MrBayes v.3.2.3 (Ronquist & Huelsenbeck, 2003) in the CIPRES Science Gateway v.3.3 (Miller, Pfeiffer & Schwartz, 2010). The best-fitting nucleotide substitution models were selected by the Akaike information criterion (AIC) in MrModeltest v.2.3 (Nylander, 2004). To avoid over-parameterization, we estimated single models for: coding genes (*rpl32*, *psbZ*); tRNA genes (*trnT*, *trnE*, *trnY*); rRNA genes (5.8S); nuclear spacers (ITS1, ITS2) and plastid spacers (*rpl32–trnL*, *trnT–trnE*, *trnE–trnY*, *trnY–trnD*, *trnS–psbZ*, *psbZ–trnG*) (Table 3). For the coded indels, we applied the binary model by defining the commands lset to 'coding=variable', and prset to 'symdirihyperpr=fixed(infinity) ratepr=variable' (Ronquist, Huelsenbeck & Teslenko, 2011). Two parallel analyses were run for 10^6 generations using the Metropolis-coupled Markov chain Monte Carlo (MCMC) algorithm with four random-initiated chains (Huelsenbeck & Ronquist, 2001), sampling trees every 1000 generations. The convergence of the runs was assessed by checking if the standard deviation of split frequencies reached a value < 0.01. After discarding the initial 2500 trees of each run as burn-in, and checking if the effective sample sizes of all parameters were > 200, the remaining trees were summarized in a majority-rule consensus including the posterior probabilities (PP) as branch support estimates. Only PP values ≥ 0.95 were considered a strong support (Erixon *et al.*, 2003). The trees were edited using FigTree v.1.3.1 (Rambaut, 2009) and CorelDRAW X3 (Corel Corporation).

A partitioned ML analysis was also performed for the total evidence data set using RAxML-HPC v.8 (Stamatakis, Hoover & Rougemont, 2008; Stamatakis, 2014) on the CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010). Analyses were run under the GTR + G model, using the '-f a' algorithm (rapid bootstrap analysis and search for the best-scoring ML tree in one single program run; Stamatakis, 2014) and 1000 bootstrap replicates (hereafter indicated as MLBS). Only MLBS values > 70% were recorded on the trees. Given the non-monophyly of *Sucrea* detected in the analyses, we also performed the Shimodaira–Hasegawa test (SH test; Shimodaira & Hasegawa, 1999) to test four alternative hypotheses: (1) *Sucrea* monophyletic and sister to *Raddia*; (2) *S. maculata* + *S. monophylla* sister to *S. sampaiana* + *Raddia*; (3) *S. maculata* sister to the clade ((*S. sampaiana* + *S. monophylla*) *Raddia*) and (4) *S. monophylla* sister to the clade ((*S. sampaiana* + *S. maculata*) *Raddia*). We generated constrained maximized likelihood estimate (MLE) trees for these hypotheses under the same

Table 3. Features of the DNA data sets based on one of the most parsimonious trees from the total evidence (plus gaps) parsimony analysis (percentages calculated in relation to aligned length), and nucleotide substitution models selected for the Bayesian analyses based on the Akaike information criterion (AIC). Notes: bp = base pairs; CI = consistency index; RI = retention index

DNA region	Aligned length (bp)	Number of excluded sites (bp)	Number of variable sites (bp; except excluded sites)	Number of potentially parsimony informative sites (bp; except excluded sites)	Number of changes/ variable sites	Fitch tree length	CI	RI	Bayesian model
ITS	825	111 (13.45%)	334 (40.48%)	245 (29.7%)	2.74	916	0.59	0.77	mixed
18S gene	27	27 (100%)	–	–	–	–	–	–	–
ITS1	268	–	153 (57.09%)	109 (40.67%)	2.48	379	0.63	0.79	GTR + G
5.8S gene	164	–	17 (10.37%)	13 (7.93%)	1.94	33	0.64	0.7	K80 + I
ITS2	282	–	164 (58.16%)	123 (43.62%)	3.07	504	0.55	0.75	GTR + G
26S gene	84	84 (100%)	–	–	–	–	–	–	–
rpl32–trnL	1138	46 (4.04%)	246 (21.62%)	155 (13.62%)	1.49	366	0.76	0.89	mixed
rpl32 gene	183	–	29 (15.85%)	20 (10.93%)	1.34	39	0.77	0.87	GTR + G
rpl32–trnL^{UAG} spacer	909	–	217 (23.87%)	135 (14.85%)	1.51	327	0.76	0.9	GTR + G
trnL^{UAG} gene	46	46 (100%)	–	–	–	–	–	–	–
trnD–trnT	1472	38 (2.58%)	289 (19.63%)	178 (12.09%)	1.44	415	0.8	0.89	mixed
trnM^{CAU} gene	9	9 (100%)	–	–	–	–	–	–	–
trnM^{CAU}–*trnT*^{GGU} spacer	7	7 (100%)	–	–	–	–	–	–	–
trnT^{GGU} gene	69	–	20 (28.99%)	9 (13.04%)	1.45	29	0.83	0.9	K80 + I
trnT^{GGU}–*trnE*^{UUC} spacer	734	–	150 (20.44%)	87 (11.85%)	1.39	208	0.81	0.89	GTR + G
trnE^{UUC} gene	73	–	4 (5.48%)	2 (2.74%)	1	4	1	1	K80 + I
trnE^{UUC}–*trnY*^{GUA} spacer	89	–	10 (11.24%)	4 (4.49%)	1.2	12	0.92	0.97	GTR + G
trnY^{GUA} gene	85	–	8 (9.41%)	5 (5.88%)	1.5	12	0.75	0.77	K80 + I
trnY^{GUA}–*trnD*^{GUC} spacer	406	22 (5.42%)	97 (23.89%)	71 (17.49%)	1.55	150	0.78	0.89	GTR + G
trnS–(psbZ)–trnG	943	62 (6.57%)	130 (13.79%)	88 (9.33%)	1.33	173	0.86	0.94	mixed
trnS^{UGA} gene	30	30 (100%)	–	–	–	–	–	–	–
trnS^{UGA}–*psbZ* spacer	399	–	67 (16.79%)	42 (10.53%)	1.25	84	0.89	0.95	GTR + G
psbZ gene	187	–	14 (7.49%)	13 (6.95%)	1.43	20	0.8	0.92	GTR + G
psbZ–trnG^{UCC} spacer	327	32 (9.79%)	49 (14.98%)	33 (10.09%)	1.41	69	0.84	0.92	GTR + G

Table 3. Continued

DNA region	Aligned length (bp)	Number of excluded sites (bp)	Number of variable sites (bp; except excluded sites)	Number of potentially parsimony informative sites (bp; except excluded sites)	Number of changes/ variable sites	Fitch tree length	CI	RI	Bayesian model
Plastid	3553	146 (4.11%)	665 (18.72%)	421 (11.85%)	1.43	954	0.8	0.9	mixed
Plastid + ITS	4378	257 (5.87%)	999 (22.82%)	666 (15.21%)	1.87	1870	0.69	0.84	mixed
Gaps ITS	173	–	172 (99.42%)	78 (45.09%)	1.42	244	0.7	0.78	binary
Gaps *rpl32–trnL*	95	–	95 (100%)	50 (52.63%)	1.37	130	0.73	0.84	binary
Gaps *trnD–trnT*	134	–	132 (98.51%)	56 (41.79%)	1.26	167	0.79	0.84	binary
Gaps *trnS–(psbZ)–trnG*	70	–	70 (100%)	26 (37.14%)	1.2	84	0.83	0.82	binary
Plastid + plastid gaps	3852	146 (3.79%)	962 (24.97%)	553 (14.36%)	1.39	1335	0.79	0.89	mixed
Plastid + ITS + all gaps	4850	257 (5.3%)	1468 (30.27%)	876 (18.06%)	1.7	2495	0.71	0.83	mixed

conditions specified above for the ML analysis, which were thereafter compared to the unconstrained topology. The tests between unconstrained and constrained MLE topologies were performed in PAUP v.4.0b10 (Swofford, 2002) using RELL and 1000 bootstrap replicates. Significant difference was determined by a significance value of $P = 0.05$.

MORPHOLOGICAL DATA

A large morphological data set was used in this study, based on field collections and herbarium specimens, including the study of type material, deposited in the following herbaria: ALCB, ASE, B, BHCB, BM, BR, CEPEC, CVRD, GUA, IAN, INPA, ISC, K, LE, MBM, MBML, MG, MO, NY, P, R, RB, RBR, SP, SPF, UEC, UESC, US and VIC (acronyms according to Thiers, 2019). In order to compare and discuss the topologies, we analysed morphological descriptions available in the literature (Soderstrom, 1981; Clayton & Renvoize, 1986; Judziewicz *et al.*, 1999; Oliveira, 2001, 2006; BPG, 2012; Kellogg, 2015).

SCANNING ELECTRON MICROSCOPY

The surfaces of pollen grains and female anthoecia of the three *Sucrea* spp. were analysed by scanning electron microscopy (SEM). Pollen grains were gathered from fertile anthers taken from mature but closed spikelets from herbarium specimens [*S. maculata*: *Oliveira et al. 2276* (HUEFS); *S. monophylla*: *Kallunki & Pirani 450* (K), *Carvalho et al. 2025* (K); *S. sampaiana*: *Chase 10049* (K)], following the procedures described by Dórea *et al.* (2017). Female anthoecia were also gathered from herbarium specimens [*S. maculata*: *Oliveira et al. 851* (HUEFS); *S. monophylla*: *Oliveira et al.* 1072 (HUEFS); *S. sampaiana*: *Oliveira et al. 991* (HUEFS)]. All materials were mounted on stubs covered with carbon tape without pre-treatment, such that both lemma and palea sides were exposed. The pollen grains of *S. maculata* were coated with gold and analysed at the Laboratório de Microscopia Eletrônica de Varredura of the Universidade Estadual de Feira de Santana (LABMEV/UEFS), whereas the pollen grains of *S. monophylla* and *S. sampaiana* were coated with platinum and analysed at the Jodrell Laboratory of the Royal Botanic Gardens, Kew. Palynological nomenclature followed Punt *et al.* (2007) and Hesse *et al.* (2009), and SEM terminology followed Soderstrom & Zuloaga (1989).

ANATOMICAL AND HISTOCHEMICAL ANALYSES OF TUBEROUS ROOTS

Transverse sections were made in the median region of the tuberous roots of *Sucrea sampaiana*, based on *Oliveira et al. 911* (HUEFS). The material selected was dehydrated in an ethanol series (Johansen, 1940) and placed in glycol-methacrylate, according to the manufacturer's (Leica) instructions. Cross sections of variable thickness (5–10 µm) were made using a Thermo Scientific HM325 rotary microtome, with a disposable steel blade, and afterwards stained with toluidine blue 0.05% in phosphate buffer pH 6.8 (O'Brien, Feder & McCully, 1964). The permanent slides were mounted with Entellan. For the recognition of secondary metabolites, histological sections were also made by hand in the median region of those structures, with the aid of a single-edge razor blade. We used Lugol and Sudan IV to detect the presence of starch (Jensen, 1962) and lipids (Pearse, 1985), respectively, and the histological slides were immediately photomicrographed after each test was performed.

GEOGRAPHICAL DISTRIBUTION AND CONSERVATION STATUS

Data on occurrence points for members of *Sucrea* were gathered during fieldtrips and from herbarium samples and the online database SpeciesLink (available at splink.org.br). The application GeoCAT (available at geocat.kew.org) was also used, assisting the identification of the area of occupancy (AOO, 2 km^2 cell width) and extent of occurrence (EOO) for endangered species, according to the guidelines of the International Union for Conservation of Nature (IUCN, 2017), also indicating their threat categories. To verify whether there are populations of *Sucrea* under protection, we plotted records on a map of designated and proposed reserves, from IUCN data of global protected areas (UNEP-WCMC, 2019).

RESULTS

DATA SETS

For ITS we used 39 sequences and the aligned length was 825 base pairs (bp), of which 334 (40.48%) were variable and 245 (29.7%) were potentially parsimony informative. For *rpl32–trnL* we used 40 sequences and the aligned length was 1138 bp, of which 246 (21.62%) were variable and 155 (13.62%) were potentially parsimony informative. For *trnD–trnT* we used 41 sequences and the aligned length was 1472 bp, of which 289 (19.63%) were variable and 178 (12.09%) were potentially parsimony informative. For *trnS–(psbZ)–trnG* we used 39 sequences and the aligned length was 943 bp, of which 130 (13.79%) were variable and 88 (9.33%) were potentially parsimony informative. In addition, 173, 95, 134 and 70 indel characters were coded for ITS, *rpl32–trnL*, *trnD–trnT* and *trnS–(psbZ)–trnG*, respectively. The combined data matrix including all DNA regions + gaps was 4850 bp, of which 1468 (30.27%) were variable and 876 (18.06%) were potentially parsimony informative (Table 3).

ANALYSIS WITH ALL DATA COMBINED

The phylogenetic trees from the individual nuclear and plastid analyses displayed similar topologies, with no incongruences regarding the *Raddia-Sucrea* clade (trees not shown). In general, the ITS trees had less internal resolution than the plastid trees, but the support values were similar for most nodes. The topology resulting from the BI analysis of all data sets combined is used for discussion since this method recovered more resolved trees than those from the MP and ML analyses.

The ingroup species of Olyreae formed two well-supported clades, which correspond to Parianinae (full support: 1.00 PP, 100% MPBS, 100% MLBS, hereafter in that order) and Olyrinae (1.00/99/100) (Fig. 2). In Parianinae, *Parianella* (full support), *Pariana* (1.00/99/97) and *Eremitis* (full support) were recovered as monophyletic, with *Parianella* as sister to the *Pariana-Eremitis* clade. In Olyrinae, four well-supported lineages were confirmed (Fig. 2). The first one (full support) includes the species of *Diandrolyra*, *Parodiolyra* and *Raddiella* and is sister to the remaining species of Olyrinae (1.00/98/100). The second clade (full support) comprises *Piresia* spp. The third (1.00/89/94) includes the sampled species of *Arberella*, *Cryptochloa*, *Lithachne* and *Olyra*. The fourth clade (full support) comprises the species currently accepted in *Raddia* and *Sucrea*. *Raddia* was fully supported as monophyletic, but the relationships among its species were unclear. The three *Sucrea* spp. did not form a monophyletic group and were recovered in two fully supported clades. One is sister to *Raddia* and includes all specimens analyzed of *S. maculata* and *S. monophylla* (the type of *Sucrea*). The other is composed of the two specimens of *S. sampaiana*, which is sister to the *Raddia* + (*S. maculata* + *S. monophylla*) clade (1.00/99/97) (Fig. 2).

TEST OF ALTERNATIVE TOPOLOGIES

The results obtained from the SH test reject all of the alternative hypotheses: (1) *Sucrea* monophyletic and sister to *Raddia* (P = 0.031); (2) *S. maculata* + *S. monophylla* sister to *S. sampaiana* + *Raddia* (P = 0.012); (3) *S. maculata* sister to the clade ((*S. sampaiana* + *S. monophylla*) *Raddia*) (P = 0) and (4) *S. monophylla* sister to the clade ((*S. sampaiana* + *S. maculata*) *Raddia*) (P = 0).

MICROMORPHOLOGY

The shape of the female anthoecia of all *Sucrea* spp. is similar. They are lanceolate and dorsiventrally compressed

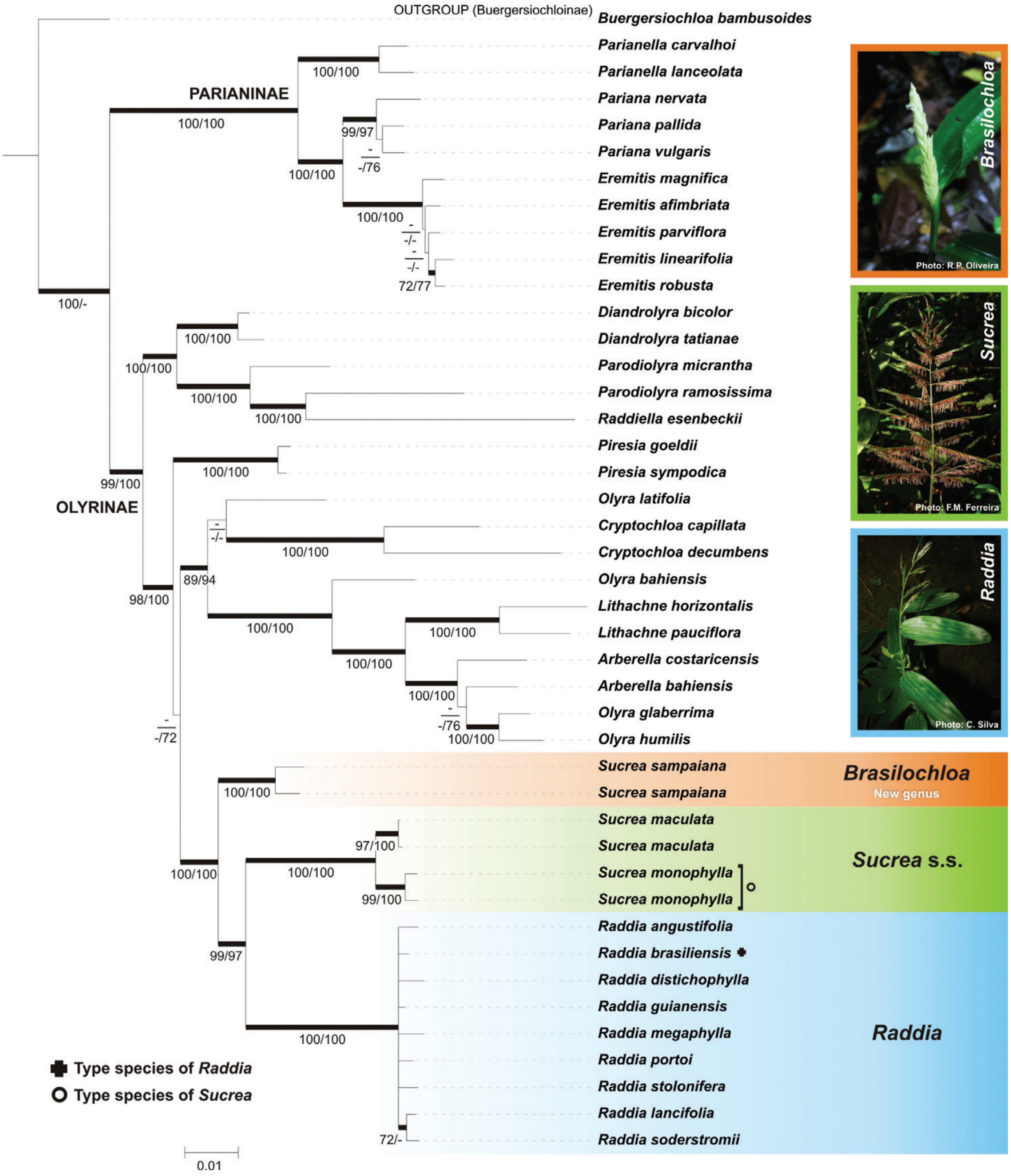

Figure 2. Bayesian consensus phylogram resulting from the combined plastid and nuclear analysis (including gaps). Maximum parsimony (left) and maximum likelihood (right) bootstrap support values are reported below branches. Thick branches are supported by posterior probability = 1.00. Dashes indicate absence of significant bootstrap (> 70%) and posterior probability (≥ 0.95) support values. Representatives of *Sucrea*, *Raddia* and the new genus *Brasilochloa* are indicated.

(Fig. 3A, D, G), and their lemma and palea also have small pits over the surface (Fig. 3A–I). In *S. maculata* and *S. monophylla*, there is a tuft of macrohairs at the apex of the palea and at the upper margins of the lemma (Fig. 3B, E), whereas in *S. sampaiana* the macrohairs cover the entire apex of the lemma and the palea and form a tuft at the base of the palea (Fig. 3H, I).

The pollen grains of *Sucrea* are monads with a single annulate and porate aperture (Fig. 4A–I). They share perforations and microspines, but displayed two different patterns of ectexine ornamentation. *Sucrea maculata* and *S. monophylla* have a microechinate-areolate pattern (Fig. 4A–F) and *S. sampaiana* has a microechinate-rugulate pattern (Fig. 4G–I).

MORPHOLOGY, ANATOMY AND HISTOCHEMISTRY OF THE TUBEROUS ROOTS

The tuberous roots of *S. sampaiana* are *c.* 2.5 cm in length and *c.* 1.8 cm in diameter (Fig. 5A). The outermost layer is brownish (Fig. 5B), composed of suberin (Fig. 5C, white asterisk), with about three layers of thin-walled cells (Fig. 5D, black arrow), about two layers of phellem, produced by divisions of the phellogen (Fig. 5C, black asterisk) and three to six inner layers of phelloderm (Fig. 5C, black arrow head), with thick-walled cells (Fig. 5D, white arrow). The cortical region is differentiated into external, median and inner cortex (Fig. 5D, E). As part of the external cortex, the exodermis (Ex) is formed by three layers of cells with strongly thickened walls (Fig. 5D); the median cortex (Mc) has *c.* 30–40 layers of thin-walled cells (Fig. 5D) and the internal cortex is composed of the endodermis (En), the cells of which exhibit U-shaped inner tangential wall thickening (Fig. 5E, black asterisk). The vascular cylinder is composed of two layers of pericycle (Pe) with sclerified cells and the vascular system has > 20 poles of peripheral metaxylem elements and one central metaxylem element (Fig. 5E). The histochemical tests indicated the presence of dense starch in the region of the median cortex (Fig. 5F), and lipids in the external region to the exodermis (Fig. 5G) and inner cortex and pericycle (Fig. 5H). However, both secondary compounds are absent in the medullary region of the vascular cylinder.

GEOGRAPHICAL DISTRIBUTION AND ECOLOGICAL ASPECTS

Specimens assigned to the genus *Sucrea* are found so far in three Brazilian states (Bahia, Espírito Santo and Rio de Janeiro; Fig. 6). The plants inhabit the understory of the Atlantic Forest, at elevations from 0 to 430 m. *Sucrea monophylla* occurs almost exclusively in southern Bahia, from where we analysed most of the specimens. We also found two newly collected and disjunct populations: Entre Rios, on the northern Bahian coast (*Popovkin 1350*, HUEFS); and one in central Espírito Santo, in Marilândia (*Costa-Lima & Fiaschi 2229*, HUEFS); expanding the knowledge on its distribution. This species has an EOO = 60 623 km^2 and an AOO = 144 km^2.

Most populations of *S. maculata* have been recorded from southern Rio de Janeiro to central Espírito Santo. However, we confirmed a single and disjunct population, represented by one collection (*Santos 2087*, US) from southern Bahia made in 1971, between Teixeira de Freitas and Alcobaça municipalities (approximate location). Just one population was collected recently, in central Espírito Santo (*Oliveira et al. 2276*, HUEFS). The EOO and AOO calculated were 29 861 km^2 and 28 km^2, respectively.

The distribution of *S. sampaiana* was historically based on only two records made in Espírito Santo state, one in the south, near the city of Alegre. Two new populations were found during our recent fieldtrips, one in the north of Rio de Janeiro state (Campos dos Goytacazes, *Oliveira et al. 991*, HUEFS) and a new locality in central Espírito Santo (Santa Leopoldina, *Oliveira et al. 2325*, HUEFS). The species has an EOO = 703 km^2 and an AOO = 12 km^2.

When the distributions of the three species are plotted on a map of protected areas, only *S. monophylla* has records inside protected areas. Some recorded populations fall inside the following protected areas: Environmental Protection Area (Portuguese: Área de Proteção Ambiental: APA) Costa de Itacaré/Serra Grande, APA Lagoa Encantada and Terra Indígena Caramuru-Paraguaçu (indigenous reserve); however, that information is not given in the corresponding exsiccatae.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS AMONG *SUCREA*, *RADDIA* AND OTHER LINEAGES OF OLYRINAE

According to the present analysis, the monophyly of Parianinae and Olyrinae is confirmed, as is their sister relationship (Oliveira, 2006; Sungkaew *et al.*, 2009; Ferreira, 2012; Carvalho, 2013; Kelchner & BPG, 2013; Oliveira *et al.*, 2014; Oliveira, 2017). We increased the sampling of members of Olyrinae, recovering the four lineages identified by Oliveira *et al.* (2014) and I.L.C. Oliveira *et al.* (in press). However, we showed considerable improvement in statistical support for some clades, as in the *Raddia*-*Sucrea* clade, which previously was recovered with low resolution and no significant statistical support (Oliveira *et al.*, 2014).

With regard to other genera in Olyrinae, inclusion of a second species allowed us to test the monophyly

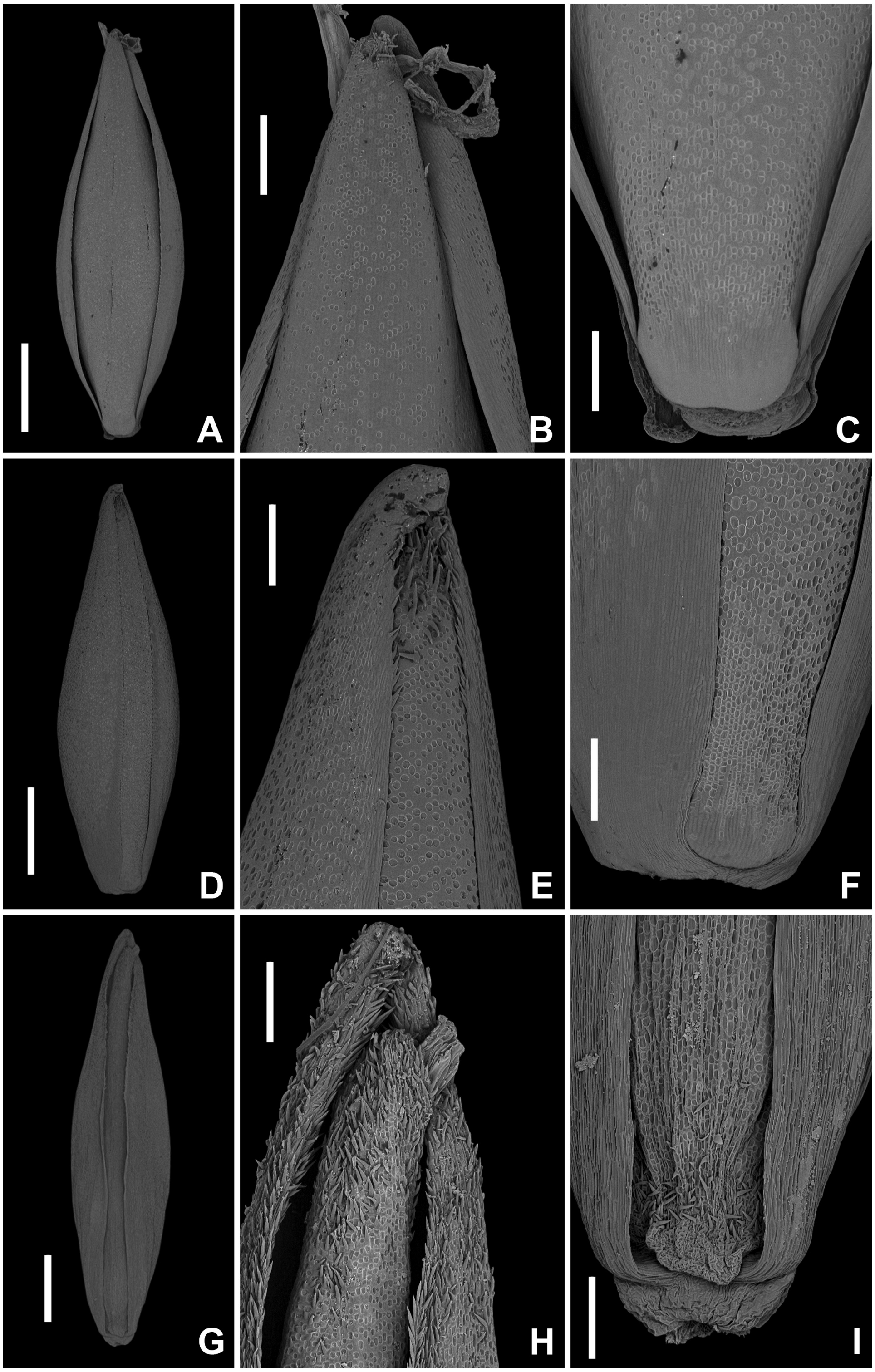

Figure 3. SEM images of the female anthoecium. A–C, *Sucrea maculata* [*Oliveira et al. 851* (HUEFS)]. D–F, *Sucrea monophylla* [*Oliveira et al. 1072* (HUEFS)]. G–I, *Sucrea sampaiana* [*Oliveira et al. 991* (HUEFS)]. Bars A, D, G = 1 mm; B–C, E–F, H–I = 200 µm.

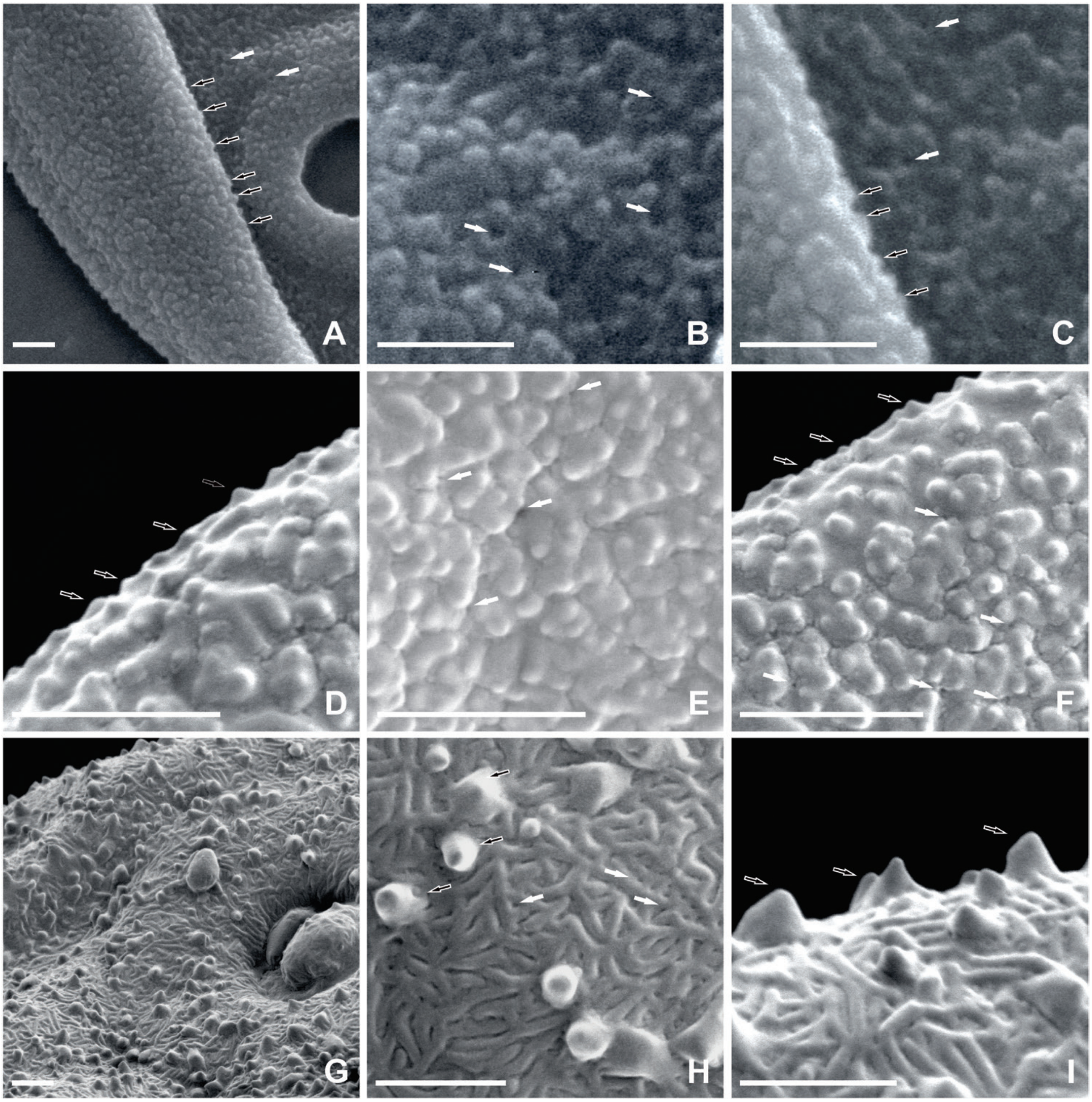

Figure 4. Ectexine of pollen grains in *Sucrea*. A–C, *Sucrea maculata*. D–F, *Sucrea monophylla*. G–I, *Sucrea sampaiana*. Black arrows indicate microspines. White arrows indicate perforations. Scale bar = 1 µm.

of *Arberella*. This genus was represented in previous phylogenetic analyses only by *A. bahiensis* Soderstr. & Zuloaga, which was supported as sister to *Olyra glaberrima* Raddi + *O. humilis* Nees (Oliveira *et al.*, 2014). We also included *A. costaricensis* (Hitchc.) Soderstr. & C.E.Calderón, which was resolved as sister to the previous clade but with low support, indicating for the first time the probable paraphyly of *Arberella*, but confirming its close relationship with these two *Olyra* spp., as indicated by Oliveira *et al.* (2014).

Including *Arberella* in phylogenetic studies has been difficult, due to its rarity in the field and herbaria. As is common in several other genera of herbaceous bamboos, *Arberella* occurs disjunctly between Central America and northern/western South America, where seven species are found (Judziewicz *et al.*, 1999); only one is endemic to the Atlantic Forest of southern Bahia,

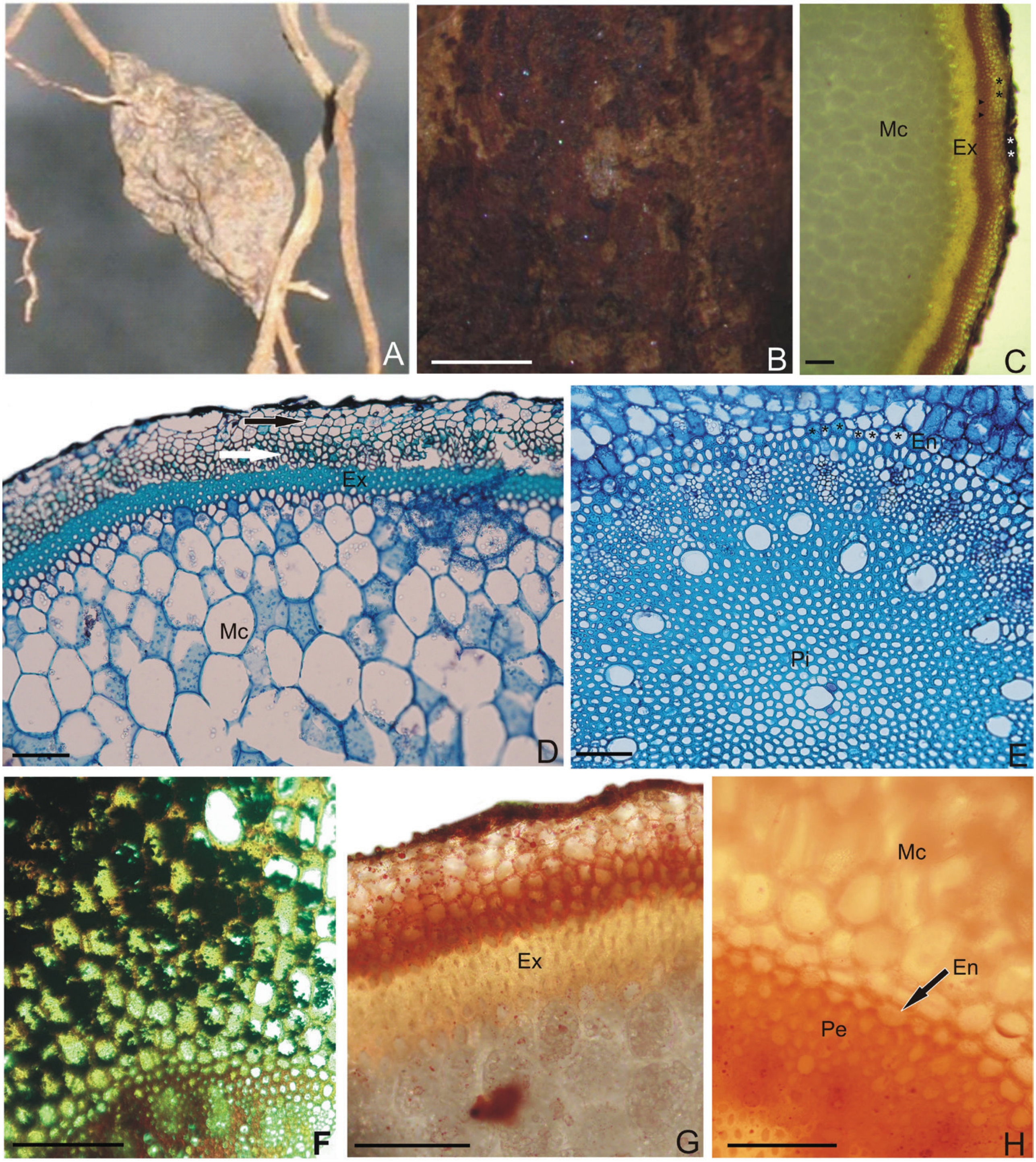

Figure 5. Morphology and anatomy of the tuberous roots of *Sucrea sampaiana*. A, General morphology of the tuberous root. B, General view of the periderm. C–H, Cross section of tuberous root. C, Periderm with exoderm and medium cortex. D, Detail of layers of the periderm and fibrous exoderm. E, Vascular cylinder. F, Mid-cortex with starch grains in the parenchyma. G, Detail of lipids in periderm. H, Detail of endodermis coloured with Sudan IV. (Ex: exodermis; En; Endodermis; Mc: Mid-cortex; Pe: periderm; Pi: Fibrous pith; scale bars: B = 500 µm, C–H = 100 µm).

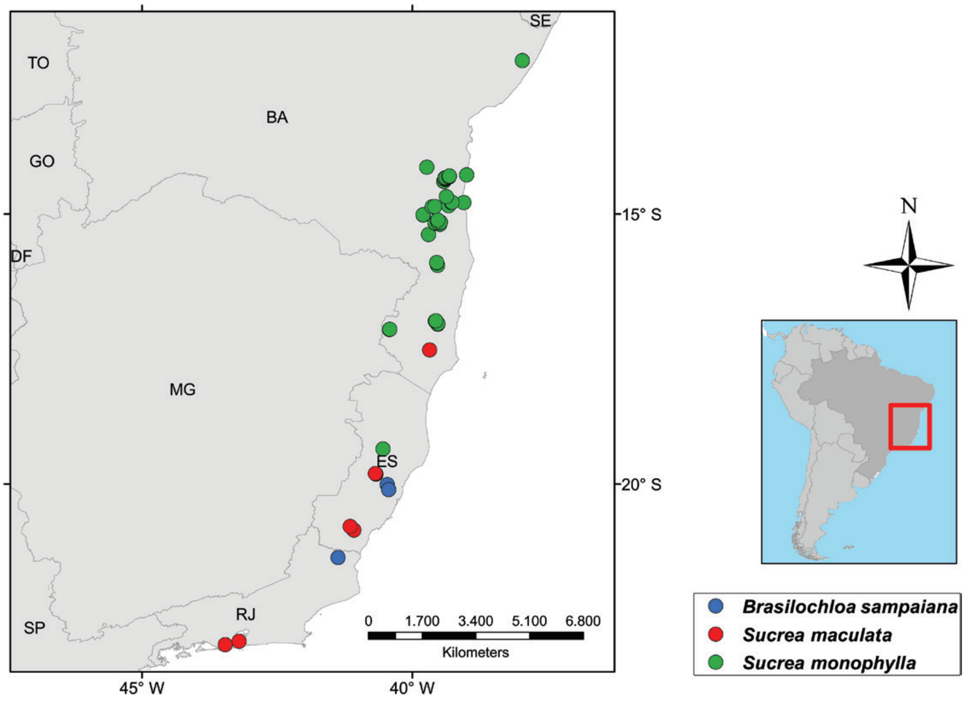

Figure 6. Geographical distribution of *Sucrea* and *Brasilochloa* in the Atlantic Forest, Brazil.

Brazil (*A. bahiensis*; Oliveira *et al.*, 2014). Considering that the results presented here for this genus are preliminary, and that this lineage (including *Olyra* and its allies) is currently the subject of detailed study, based on a broader sampling (I.L.C. Oliveira *et al.*, unpubl. data), it is premature to make any taxonomic changes with respect to *Arberella* or *Olyra* at present.

RESOLVING UNCERTAINTY IN THE *RADDIA-SUCREA* LINEAGE

In accordance with previous phylogenetic analyses including Olyreae (Zhang & Clark, 2000; Oliveira *et al.*, 2014), our results showed that *Sucrea* is closely related to *Raddia*. Oliveira *et al.* (2014) recovered *Raddia* as clearly monophyletic, but they were not able to define more precisely whether *Sucrea* was paraphyletic in some way, or monophyletic, as recovered in their plastid analysis, although with low support. The low number of regions analysed by those authors probably led to weak support for some clades, which may be responsible for that ambiguity. On the other hand, *Raddiella* was resolved by Oliveira *et al.* (2014) as close to *Parodiolyra* and *Diandrolyra* in both of their individual analyses, and only *R. malmeana* (Ekman) Swallen showed up in the *Raddia-Sucrea* clade in the combined analysis, which was probably an artefact since all branches collapsed in that analysis.

As part of the recent phylogenetic study of the *Raddiella-Parodiolyra* clade, I.L.C. Oliveira *et al.* (in press) unambiguously indicated that *Raddiella* and *Raddia*/*Sucrea* belong to independent lineages in Olyrinae, highlighting the presence of a strongly supported *Raddia-Sucrea* clade. They also recovered *Sucrea* as non-monophyletic and suggested the need for more detailed investigation involving this group, as discussed here. We successfully increased the sampling of members of Olyrinae and the number of DNA markers and, using *Buergersiochloa bambusoides* as the outgroup, we obtained high statistical support in all analyses performed (Fig. 2).

Of the morphological features traditionally considered to be shared by *Raddia* and *Sucrea*, the most evident ones are found in the female spikelets, including glumes with prominent nerves and cartilaginous margins, usually twisting at the apex and at the base, clavate pedicels and finely pitted lemmas and paleas (Soderstrom, 1981; Oliveira, 2006; Oliveira *et al.*, 2014). It is important to mention that *Raddiella* shares with these two genera the glumes with prominent nerves, but they lack cartilaginous margins and do not twist at the apex or at the base in the female spikelets (Table 4). Thus, since *Raddiella* is not part of that clade (I.L.C. Oliveira *et al.*, in press), we infer that these features may have evolved independently in Olyrinae.

Raddia and *Sucrea* share the same fruit dispersal strategy, known as ballistochory, which is achieved by the twisting of the female glumes as they dry (Sendulsky, 1993). This mechanism is only known for these two

Table 4. Morphological comparison among genera of Olyrinae related to *Brasilochloa*

Character	*Raddia*	*Sucrea*	*Brasilochloa*
Synflorescence pattern	Male and female spikelets in different synflorescences	Male and female spikelets in the same synflorescence	Male and female spikelets in the same synflorescence
Synflorescence type	male: paniculate, open; female: racemose	paniculate, open	congested to spiciform
Synflorescence position on the plant body	male: terminal or less commonly axillary; female: axillary	terminal	terminal
Distribution of male and female spikelets in each synflorescence	terminal: usually all male, axillary: female	female spikelets terminal on each branch and male below	lower branches: male spikelets only; upper branches: female spikelets only
Disarticulation of female spikelet	above the glumes	above the glumes	above the glumes
Glumes with prominent nerves (in female spikelets)	present	present	present
Colour of the male spikelet	greenish	greenish or purplish	whitish
Ballistochory dispersal	present	present	absent
Tuberous roots	absent	absent	present

genera in Olyreae and is probably a synapomorphy of this clade (Oliveira *et al.*, 2014). Therefore, these two taxa were traditionally circumscribed based on other features, including the synflorescence pattern (male and female spikelets in the same synflorescence in *Sucrea* vs. male and female spikelets in different synflorescences in *Raddia*) and leaves symmetrical at the apex and acuminate in *Sucrea* (vs. asymmetrical and apiculate in *Raddia*) (Soderstrom, 1981; Oliveira, 2006; Oliveira *et al.*, 2014).

According to Oliveira *et al.* (2014), the ITS analysis did not support the inclusion of *Sucrea sampaiana* in this genus, and the *trnD–trnT* data showed *S. maculata* as sister to *Raddia*, making *Sucrea* paraphyletic. However, even though in the combined analysis *S. sampaiana* was recovered as sister (albeit with weak support) to the supported clade formed by *S. monophylla* + *S. maculata*, its phylogenetic position and the circumscription of *Sucrea* were considered questionable. Despite the results found by Oliveira *et al.* (2014), all alternative hypotheses for the placement of *S. sampaiana* using the SH test were rejected in the present work. Instead, our current results strongly support *S. sampaiana* as the sister lineage of the clade including the other two species of *Sucrea* + *Raddia* and are consistent with the morphological heterogeneity evident in *Sucrea*.

Sucrea monophylla (the type species) and *S. maculata* are similar to each other, sharing open paniculate synflorescences, with female spikelets terminal on each branch (Soderstrom, 1981). They differ by the number of leaf blades on each culm (several in *S. maculata* vs. often only one in *S. monophylla*); number of female spikelets at the apex of each synflorescence branch (often one or two in *S. maculata* vs. three or four in *S. monophylla*), colour and indument of male spikelets (reddish and glabrous in *S. maculata* vs. greenish and pubescent or scabrous in *S. monophylla*) (Oliveira, 2001). Besides the tuberous roots, Soderstrom (1981) indicated that *S. sampaiana* is distinct in having a congested, spiciform synflorescence, female spikelets on the upper branches and male spikelets on the lower branches (Fig. 7), with the male spikelets whitish and hirsute (Soderstrom, 1981; Oliveira, 2001).

In contrast to the male anthoecia, the features of the female ones in these species may vary according to the stage of development. When mature, they may be more than twice the width of the young ones. *Sucrea maculata* and *S. monophylla*, when young, often display greenish or straw-coloured anthoecia and *S. sampaiana* displays whitish or straw-coloured young anthoecia. Although all of them become brown at maturity, only *S. sampaiana* possesses maculate female anthoecia, as observed in *Oliveira et al. 991* (HUEFS). Maculate anthoecia have been traditionally documented for *Rehia* Fijten and some *Olyra* spp. (Judziewicz *et al.*, 1999). Therefore, it seems to be widely distributed in this subtribe, being also observed in *Piresia leptophylla* (Döll) Swallen, *Arberella bahiensis* and *Olyra filiformis* Trin. (Soderstrom & Zuloaga, 1985; Oliveira, 2001) and in *Reitzia smithii* Swallen.

The ornamentation (surface and indument) of female anthoecia is frequently used for taxonomic purposes in grasses, including Olyreae, at the species level in

Olyra (Soderstrom & Zuloaga, 1989), *Parodiolyra* (Soderstrom & Zuloaga, 1989; Zuloaga & Davidse, 1999) and *Raddiella* (Zuloaga & Judziewicz, 1991). Microscopic trichomes and pits on the surface of the lemma and the palea were observed, in the present work, in the three species previously assigned to *Sucrea*, but without a clear taxonomic utility. The three species also have small pits along both lemma and palea, which are more evident in *S. sampaiana* (Fig. 3). Oliveira (2006) previously confirmed the presence of a pitted female anthoecium in *Raddia* using both SEM and a stereomicroscope, which indicate that this character occurs throughout the *Raddia*-*Sucrea* clade.

The results of Dórea *et al.* (2017) also help us to better understand the circumscription of this group, since unexpected variation was found in the ectexine of pollen grains in Olyreae. Four ectexine patterns were documented by those authors for forest grasses, three of them found in species of Olyreae. *Raddia* and *Sucrea* (*S. maculata* and *S. monophylla*) also shared the same pollen pattern (microechinate-areolate), whereas *S. sampaiana* was distinct from them in having a rugulate ectexine, in addition to microspines. The presence of perforations in the ectexine of these species is here revealed for the first time (Fig. 4). These perforations seem to be common in grasses, or at least

Figure 7. *Brasilochloa sampaiana*. A, Habitat. B, Habit. C, Tuberous roots. D, Terminal synflorescence. E, Plant with senescent synflorescences at the apex of the culms, in a disturbed locality in the Atlantic Forest.

in Olyreae, but their observation requires detailed analyses, which had not previously been done.

TUBEROUS ROOT ANATOMY AND HISTOCHEMISTRY

Modifications in subterranean vegetative organs, although common in different plant groups, especially herbaceous groups, often differ from the original organ in form and function (Gregory, 1965). This author mentioned that the most common modification is functional, related to the storage of the nutrients, allowing the plant to combine survival mechanisms to function as organs of vegetative propagation under adverse environmental conditions. These adverse conditions were observed in the field, in places where *S. sampaiana* occurs, which was already discussed by Soderstrom (1981).

Grasses often have a shallow root system that withers when there is scarcity of water in the soil. The presence of tuberous roots in a few unrelated genera in Poaceae has been poorly documented in the literature, e.g. *Lophatherum* Brongn. (Shaefer, 1942) and *Puelia* Franch. (Clayton, 1967), members of the subfamilies Panicoideae and Puelioideae, respectively. In the Olyrinae (Bambusoideae), tuberous roots have only been reported in *S. sampaiana* (Soderstrom, 1981); they are unknown in *S. maculata* and *S. monophylla*. However, during our recent fieldtrips in the Amazon, we also observed similar structures in a single member of *Pariana* Aubl. (subtribe Parianinae; R.P.Oliveira, pers. comm.). Based on this, our data indicated that tuberous roots have probably evolved independently in grasses as a whole and in bamboos and that our knowledge about them is still limited.

Modifications in both form and function of the root system of *S. sampaiana*, with the development of tuberous roots associated with the typically adventitious ones, were reported since the first description of this species, as *Olyra sampaiana* (Hitchcock, 1927). Tuberous roots are expected to be composed basically of parenchyma cells, which store water and starch, and protect the individual plants from adverse conditions, promoting their maintenance in the environment (Rizzini & Heringer, 1961; Kellogg, 2015).

We hypothesize that the energetic value given by carbohydrates and water, observed as a high concentration of starch in the median cortex of tuberous roots in *S. sampaiana*, will supply new sprouts of this species in its natural habitat, as we observed in our greenhouse-grown specimen, which sprouted after death of aerial parts due to dehydration. Additionally, probable protection of this species against pathogens, and the maintenance of turgor in those root systems may be due to the lipid layer observed in the periderm, exodermis, internal cortex and pericycle of the tuberous roots analysed.

TAXONOMIC CONSIDERATIONS

Our results strongly refute the recent modifications in the taxonomy of Olyreae made by Grande Allende (2016), concerning the expansion of the circumscription of *Raddia*, to include the three species previously accepted in *Sucrea*. Our phylogenetic results led us to definitively confirm the presence of three supported lineages in this group, with *Sucrea* as traditionally accepted being paraphyletic; for this reason, we propose the following taxonomic modifications. We consider *Sucrea s.s.* to include only *S. maculata* and *S. monophylla*, and we formally transfer *Sucrea sampaiana* to the new genus *Brasilochloa*, which is sister to the *Raddia-Sucrea* clade (Fig. 2). These genera share several similarities, but differ in the following characters.

Brasilochloa R.P.Oliveira & L.G.Clark, **gen. nov.**
Type: *Olyra sampaiana* Hitchc. (≡ *Brasilochloa sampaiana* (Hitchc.) R.P.Oliveira & L.G.Clark).

Brasilochloa is clearly distinguishable from all other genera of Olyrinae by the presence of tuberous roots and synflorescences congested to spiciform, with female spikelets on the upper branches and whitish male spikelets on the lower branches.

Plants monoecious, perennial, caespitose, erect, with tuberous roots. Leaves distributed along the culms; leaf sheaths open, fimbriae at the apex absent, glabrous; leaf blades glabrous, flat, broadly ovate, apex symmetrical and acuminate, base symmetrical, rounded; ligule membranous ciliate. Synflorescences

*KEY TO THE GENERA OF OLYRINAE OF THE **BRASILOCHLOA-SUCREA-RADDIA** CLADE*

1. Male and female spikelets in different synflorescences, the male paniculate and terminal or less commonly axillary, the female racemose and axillary .... *Raddia*
1´. Male and female spikelets in the same synflorescence, with unisexual spikelets mixed in two distinct patterns.
   2. Synflorescences open paniculate, with female spikelets terminal on each branch; male spikelets greenish or purplish; tuberous roots absent .... *Sucrea*
   2´. Synflorescences congested to spiciform, with female spikelets on the upper branches, male spikelets on the lower ones; male spikelets whitish; tuberous roots present.......... *Brasilochloa*

congested to spiciform, terminal, one to five per culm, with female spikelets on the upper branches, male spikelets on the lower ones. Spikelets one-flowered, dimorphic, females larger than the males, dorsiventrally compressed. Female spikelets lanceolate, with pedicels clavate, disarticulating above the two persistent glumes; glumes aristate, chartaceous, with cartilaginous margins, glabrous or scabrous, five-veined, veins prominent; anthoecium coriaceous, lanceolate, microscopically pitted and pilose, brown or greenish, becoming maculate at maturation, the lemma completely enclosing the palea; lodicules three; stigmas two, plumose; caryopsis with a linear hilum. Male spikelets lanceolate, glumes absent, anthoecium membranous, hairy or hirsute, whitish; stamens three.

The genus is monotypic. Its name was given in reference to Brazil, to which it is endemic, occurring only in the Atlantic Forest of Espírito Santo and Rio de Janeiro states.

Brasilochloa sampaiana (Hitchc.) R.P.Oliveira & L.G.Clark, comb. nov. based on *Olyra sampaiana* Hitchc., J. Wash. Acad. Sci. 17: 215. 1927. [≡ *Sucrea sampaiana* (Hitchc.) Soderstr. Brittonia 33: 208, f. 6. 1981; ≡ *Raddia sampaiana* (Hitchc.) J.R.Grande. Bol. Centro Invest. Biol. Univ. Zulia 50(1): 35. 2016]. Type: Brazil, Espírito Santo, Reeve, 6 December 1924, *J. Vidal 44* (holotype US!; isotypes: IAN!, R!).

Comments: Brasilochloa sampaiana is clearly distinguished by the presence of tuberous roots and by synflorescence morphology, but several other features make this species singular and easy to identify, as mentioned in the discussion. The root system with tuberous structures does not occur in any other genus of Olyrinae and is rare in grasses (Soderstrom, 1981).

Distribution, habitat and conservation status: This species is endemic to the Brazilian Atlantic Forest, with a scattered distribution, restricted to Rio de Janeiro and Espírito Santo states, where individuals were found in rocky environments (Fig. 7). Based on IUCN (IUCN, 2017) criteria, we considered this species as Endangered [EN B1 + 2ab(iv)] because it is currently known only from two localities and a reduction of the number of locations was observed, since the Rio de Janeiro population could not be found.

Specimens analysed: BRAZIL: ESPÍRITO SANTO: Alegre, 20 November 1929, *Chase 10049* (B, BM, F, IAN, K, NY, R, RB, US); Santa Leopoldina, Colina Verde (Morro Agudo), 13 September 2006, *Demuner et al. 2865* (MBML); Santa Leopoldina, Colina Verde (Morro Agudo), 22 September 2016, *Oliveira et al. 2325* (HUEFS); Santa Leopoldina, Fazenda Caioaba, Mata de várzea, 6 January 2006, *Magnago et al. 507* (MBML). RIO DE JANEIRO: Campos dos Goytacazes, 13 December 1982, *Carauta & Gurken 4416* (GUA); Campos dos Goytacazes, Morro do Baú, Cruz da Serra, Base do Morro do Baú, 19 December 2003, *Oliveira et al. 991* (CEPEC, HUEFS, ICN).

Sucrea Soderstr., Brittonia 33: 200 (1981). Type: *Sucrea monophylla* Soderstr.

Plants monoecious, perennial, caespitose, erect, tuberous roots absent. Leaves distributed along the culms or less commonly with dimorphic culms, the vegetative ones erect, with leaves well developed, and flowering culms with fewer and smaller leaves; leaf sheaths open, fimbriae at the apex absent, glabrous; leaf blades glabrous, flat, ovate-lanceolate to broadly ovate, apex symmetrical and acuminate, base symmetrical or sometimes asymmetrical, rounded to subcordate; ligule membranous, ciliate. Synflorescences open paniculate, with female spikelets terminal on each branch; terminal, one or two per culm. Spikelets one-flowered, dimorphic, females larger than the males, dorsiventrally compressed. Female spikelets lanceolate, with pedicels clavate, disarticulating above the two persistent glumes; glumes aristate, chartaceous, with cartilaginous margins, glabrous or scabrous, five-veined, veins prominent; anthoecium coriaceous, lanceolate or elliptical, microscopically pitted and glabrous, brown or greenish, uniform in colour (not maculate) at maturity, the lemma completely enclosing the palea; lodicules three; stigmas two, plumose; caryopsis with a linear hilum. Male spikelets filiform, glumes absent, anthoecium membranous, glabrous, green to purplish; stamens three.

This genus is endemic to Brazil. It comprises two species occurring only in the Atlantic Forest of Bahia, Espírito Santo and Rio de Janeiro states.

1. ***Sucrea maculata*** Soderstr., *Brittonia* 33(2): 205. 1981. [≡ *Raddia maculata* (Soderstr.) J.R.Grande, Bol. Centro Invest. Biol. Univ. Zulia 50(1): 35. 2016]. Type: Brazil, Rio de Janeiro, Serra da Bica, Cascadura, 1886, *Schwacke* s.n. (holotype RB!, isotype IAN!).

Comments: Sucrea maculata is similar to *S. monophylla*, from which it is differentiated by having a larger number of leaves per culm, *c*. 12 in the material examined. The ovate-lanceolate to ovate blades, and one or two female spikelets at the apex of each branch of the synflorescence also differentiate them. According to Soderstrom (1981), based on materials from Rio de Janeiro and Espírito Santo, the leaf blades of this species are only 6.0–7.0 × 2.0–2.5 cm.

Distribution, habitat and conservation status: This species is also endemic to the Brazilian Atlantic Forest, distributed from Rio de Janeiro to central Espírito Santo state (Soderstrom, 1981; Judziewicz *et al.*, 1999), with an early record also in Bahia state (Oliveira, 2001). Based on IUCN (2017) criteria, we considered this species as Endangered [EN B2b(iii)], because its distribution is highly fragmented and we observed a steep decline in the quality of its habitat, due to the continuous deforestation of the Atlantic Forest (Fundação SOS Mata Atlântica & INPE, 2017). Only one new population was found in the last 20 years, and most records for this species are > 40 years old, so we cannot be sure if those populations still exist in the face of the high deforestation rates of their habitat.

Specimens analysed: BRAZIL. Habitat in sylvis ad Sebastianopolin, *Martius s.n.* (M); Without locality, *Nees* (B). BAHIA: Teixeira de Freitas, km 6 da rodovia para Alcobaça, 9 October 1971, *Santos 2087* (CEPEC). ESPÍRITO SANTO: Cachoeiro do Itapemirim, estrada de Cachoeiro do Itapemirim para Itabira, 25 April 1972, *Soderstrom 1962* (B, INPA, RB); Cachoeiro do Itapemirim, 10 km de Cachoeiro para Alegre, 26 April 1972, *Soderstrom 1970* (CEPEC, INPA, K, RB); Santa Teresa, São João de Petrópolis, E.A.F.S.T. Valão de S. Braz, 6 October 2001, *Fontana 197* (MBML); Santa Teresa, São João de Petrópolis, Mata da escola de Agronomia, 12 April 2003, *Oliveira et al. 851* (HUEFS); 4 March 2016, *Oliveira et al. 2276* (HUEFS). RIO DE JANEIRO: Búzios, rancho Dez, mata de tabuleiro, 2 October 1996, *Araújo et al. 10478* (GUA); Rio de Janeiro, Grajaú, 25 November 1969, *Emygdio 2683* (R); Rio de Janeiro, Mata da Tijuca, 14 April 1972, *Soderstrom 1941, 1946* (B, INPA, K, RB); 25 November 1969, *Sucre 6359* (RB); Rio de Janeiro, Jacarepaguá, mata da Colônia Juliano Moreira, 27 October 1980, *Penna & Maia s.n.* (GUA); Rio de Janeiro, Parque Estadual da Pedra Branca, Floresta do Camorim, Lage do Ravi, 28 August 1982, *Ribeiro & Costa 298* (GUA, RB); Rio de Janeiro, Serra da Bica, Cascadura, 1886, *Schwacke s.n.* (IAN, R, RB).

2. ***Sucrea monophylla*** Soderstr., *Brittonia* 33: 200. 1981. [≡ *Raddia monophylla* (Soderstr.) J.R.Grande, Bol. Centro Invest. Biol. Univ. Zulia 50(1): 35. 2016]. Type: Brazil, Bahia, Itabuna, Ferradas, Faz. Aberta Grande, 14°47’21”S 39°16’36”W 16/I/1968, *C.E. Calderón 2045* (holotype CEPEC!; isotypes B!, HUEFS!, MBM!, K!, W!).

Comments: As part of the original description of this species, Soderstrom (1981) indicated a single and well-developed leaf blade on each culm. However, in the key to the species included in the same work, he referred to it as ‘usually one leaf per culm’. Under cultivation, we have observed up to four leaf blades per culm in young plants, and also in the field, when the plants have experienced long dry periods. Mattos-Silva, Vinha & Pereira (1988) also reported that only adult individuals display a single leaf per culm, whereas in young plants the number of leaves is actually greater. This may lead to misidentifications of this species as *S. maculata*, in which the number of leaves per culm is more than one. Several specimens from Bahia also have leaves with smaller dimensions compared to what was described by Soderstrom (1981) for this species, which would also approximate the range of leaf size in *S. maculata*. These materials also have two or three female spikelets at the apex of each branch of the synflorescence, and may be considered as intermediate between the two species (Oliveira, 2001).

Distribution, habitat and conservation status: This species is endemic to the Brazilian Atlantic Forest, being considered as endemic to southern Bahia since its description (Soderstrom, 1981; Judziewicz *et al.*, 1999; Oliveira, 2001; Oliveira *et al.*, 2011). However, it also occurs in north-eastern Bahia and the middle of Espírito Santo, in areas of hygrophilous and mesophilous Atlantic Forest, sometimes associated with cacao plantations. Mattos-Silva *et al.* (1988) reported this species as an invader of cacao plantations. Based on IUCN criteria (2017), we considered this species as Vulnerable [VU B2ab(iii)]. Unlike the other species, the number of known population was greater, thus increasing its AOO and EOO, and it is also found in protected areas in Bahia state.

Specimens analysed: BRAZIL. BAHIA: Aurelino Leal, na estrada para Lajes do Banco, 25 March 1992, *Londoño et al. 736*, *739* (CEPEC, IBGE); 21 January 1982, *Hollowell et al. 3006* (CEPEC); 16 February 1994, *Carvalho et al. 4391* (CEPEC); Fazenda Santa Luzia, 23 March 1972, *Calderón & Pinheiro 2187* (CEPEC); Fazenda Santa Maria, mata úmida secundária, 12 March 1972, *Calderón & Pinheiro 2186* (B, CEPEC, HUEFS, K, W); 11,2 km da BR 101, 30 October 2001, *Thomas et al. 12663* (SP); Fazenda Nossa Senhora do Rosário, *Oliveira et al. 979* (HUEFS); Rodovia BR 101, km 5 trecho Ubaitaba/Itabuna, 14 December 1978, *Santos & Mattos-Silva 3161* (CEPEC, HUEFS, MBM); Buerarema, estrada Itabuna-Una, entrada à direita do posto Timbuibão, 12 June 2016, *Oliveira et al. 17* (HUEFS); Esplanada, Algodão, 13 February 2013, *Popovkin et al. 1350* (HUEFS); Ibicaraí, 3 km W da cidade, BA 265, 14 February 1988, *Pirani et al. 2340* (IBGE, SPF); Ibicaraí, BR 101, 5 km W of the gate to the city of Ibicaraí, 7 February 1993, *Kallunki & Pirani 450* (K, SPF); Ibicaraí, 41 km W da BR 101 e 5 km W do portão da cidade de Ibicaraí, 14 February 1994, *Pirani et al. 2981* (SPF); Ibicaraí, entre Ibicaraí e Floresta Azul, 270 m, 3 April 1976, *Soderstrom et al. 2105* (CEPEC, HUEFS, INPA, K); Ipiaú, estrada para

Jequié, 2 November 1970, *Santos 1258* (CEPEC); Itabuna, Fazenda São José, Serra do Correa, 20 June 1972, *Santos 2320* (CEPEC); Ferradas, Fazenda Aberta Grande, 16 January 1968, *Calderón 2045* (B, CEPEC, HUEFS, K, MBM, W); 14 January 1968, *Pinheiro 480* (CEPEC); 14 km SW of Itabuna, mata úmida, 13 March 1972, *Calderón & Pinheiro 2166* (B, CEPEC, HUEFS, K, W); Itabuna, Fazenda Marinêda, 3 March 1986, *Santos 4123* (CEPEC, MBM, RB); Itacaré, *c.* 2 km em direção ao km 13 S de Ubaitaba, 14 February 1986, *Andersson et al. 1657* (CEPEC); Ramal à esquerda da BR 101, 12 June 1979, *Mattos-Silva et al. 431* (CEPEC); Itajuípe, Rodovia Itajuípe/Ubaitaba, 12 km N de Barro Central, 24 April 1965, *Belém & Magalhães 886* (CEPEC, IAN, UB); 50 km N de Itabuna na BR 101, 20 April 1976, *Soderstrom et al. 2151* (CEPEC, HUEFS, INPA, K); Itamaraju, *c.* 4 km N da cidade, mata secundária, 9 April 1972, *Calderón & Pinheiro 2214* (CEPEC, K); Fazenda Pau-brasil, 3 November 1983, *Carvalho et al. 2025* (CEN, CEPEC, K); *c.* 3 km para Eunápolis, Faz. Novo Pau-Brasil, mata higrófila, 11 March 2004, *Oliveira et al. 1051* (HUEFS); *Longhi-Wagner & Oliveira 9228* (HUEFS, ICN); Itapebi, Distrito de Ventania (BR 101 em direção a São João do Paraíso), Fazenda Guarani (cabruca), mata higrófila, 12 March 2004, *Oliveira et al. 1072* (HUEFS); Fazenda Guarany, 12 March 2004, *Longhi-Wagner & Oliveira 9232* (CEPEC, HUEFS); Jussarí, Fazenda Alciato Carvalho, 10 May 1995, *Thomas et al. 10864* (CEPEC); Fazenda Santo Antonio, May 1991, *Carvalho 3348* (CEPEC); estrada para Palmira (mata higrófila), 2 February 1994, *Thomas et al. 10223* (CEPEC); 28 October 1983, *Callejas et al. 1580* (CEPEC, K, MBM, RB); Fazenda Teimoso, 21 February 1998, *Amorim et al. 2691* (CEPEC, SP); RPPN Serra do Teimoso, 20 December 1998, *Carvalho et al. 6693* (CEPEC, HUEFS, SP); 25 September 1999, *Carvalho et al. 6821* (CEPEC); October 2016, *Oliveira et al. 8* (HUEFS); 14 March 2001, *Oliveira & Longhi-Wagner et al. 716*, *717* (CEPEC, HUEFS); 13 March 2004, *Oliveira & Longhi-Wagner 1079* (CEPEC, HUEFS); 14 March 2003, *Oliveira et al. 816* (HUEFS); 3 February 1998, *Jardim et al. 1487* (SP); Ubaitaba, 8 km na estrada para Lages, 25 April 1965, *Belém & Magalhães 951* (CEPEC, IAN, UB); 13 January 1968, *Calderón 2038* (CEPEC, HUEFS, K); Uruçuca, estrada para Ubaitaba, 22 April 1970, *Santos 759* (CEPEC, K). ESPÍRITO SANTO: Marilândia, Alto Liberdade, Pedra do Cruzeiro, 4 February 2016, *Costa-Lima & Fiaschi 2229* (HUEFS, MAC, UFRN).

ON THE CONSERVATION OF *BRASILOCHLOA* AND ALLIES

Many species of herbaceous bamboos often have small populations, both in number of individuals and occupied area, and some of them are known from a single population (Oliveira *et al.*, 2011). They are usually confined to the forest understory, and since herbaceous vegetation is the first to be removed during deforestation, they are becoming increasingly rare due to the fragmentation not only of the Atlantic Forest, but of the other tropical forests where they occur, including the Amazon Forest (Oliveira *et al.*, 2011). Based on our fieldtrips searching for Olyreae in South America for more than two decades, we confirm here that not only *B. sampaiana*, but several other members of Olyreae, including most other members of the *Raddia-Sucrea* lineage, are seriously threatened with extinction.

Herbaceous bamboos are much easier to collect than woody ones, but tend to be overlooked on the forest floor due to their small stature and inconspicuous synflorescences (Soderstrom *et al.*, 1988; Clark, 1990). The previously enigmatic plants of *B. sampaiana* were first collected in the 1920s in the Atlantic Forest of southern Espírito Santo state, in eastern Brazil. This species was not seen in field for > 70 years, and it was considered extinct by Soderstrom (1981). The Atlantic Forest of Bahia and Espírito Santo states has been continuously indicated as the main centre of diversity of American bamboos, especially regarding Olyreae (Soderstrom *et al.*, 1988; Clark, 1990; Oliveira *et al.*, 2008a; Ferreira, 2012) and the *Raddia-Sucrea* lineage, as discussed by Oliveira *et al.* (2014). For this reason we intensified our field trips throughout this region, trying to recollect not only this, but several other species of this tribe.

According to Oliveira *et al.* (2011), the great challenge concerning habitat loss in the Atlantic Forest is related to the fact that deforestation directly affects the persistence and other biological aspects of the species occurring in this biome, including Olyreae. These species tend to have a fragmented distribution of their populations due to the formation of islands of vegetation, generally far apart from each other. The distance between these remnants results in the reduction or even the cessation of gene flow (pollination or seed dispersal) necessary to maintain adequate levels of variability within and between populations (Oliveira *et al.*, 2008b). In turn, this causes a greater differentiation between them, making them increasingly restricted to certain areas, as seems to be the case in *B. sampaiana* and several other taxa.

Based on specimens housed at the herbarium GUA collected in the 1980s, we found a new and small population of *B. sampaiana* in 2003, in a forest remnant in northern Rio de Janeiro (Fig. 7). This species was included in the list of threatened species of Brazil (as *Sucrea sampaiana*, MMA, 2008), in the list of the rare plants of Brazil (Oliveira *et al.*, 2009), and the red list of endangered species of this country (Filgueiras *et al.*, 2013). In 2015, we returned to northern Rio de Janeiro state and found that this population had been destroyed. However, newly collected plants from two localities were found in the MBML herbarium, distributed much more to the north, in central Espírito Santo (Santa Leopoldina municipality). At this moment, we have found *B. sampaiana* in only one of the three recorded localities, and we confirm its scattered distribution, restricted to Espírito Santo and Rio de Janeiro states. Unless new populations are discovered in protected areas, this new genus may rapidly disappear in nature.

Soderstrom (1981) and Judziewicz *et al.* (1999) considered *S. maculata* as endemic to the Atlantic Forest from Espírito Santo and Rio de Janeiro states, but we have confirmed the first record from Bahia state, based on a single collection made in the southern region. The place where the collection was made is now the local airport, so the population must be destroyed. It has a small number of populations registered in databases and only a few individuals in each one. Judziewicz *et al.* (1999) treated it as probably extinct in the wild, but we found two populations in the field in Espírito Santo and Rio de Janeiro, with a small number of individuals in each. The former was almost wiped out due to a serious drought in that region. The species was also recently included in the red list of threatened species of Brazil ([EN B2ab(iii)] category; Filgueiras *et al.*, 2013).

Sucrea monophylla has been considered as endemic to southern Bahia since its description (Soderstrom, 1981; Judziewicz *et al.*, 1999; Oliveira, 2001; Oliveira *et al.*, 2011), but now we know that its distribution is larger, also occurring in north-eastern Bahia and in central Espírito Santo. It is found in areas of both hygrophilous and mesophilous Atlantic Forest, sometimes associated with cacao plantations. Mattos-Silva *et al.* (1988) reported this species as an invader of cacao plantations. It was not included in the red list of threatened species from Brazil (Filgueiras *et al.*, 2013), but we only observed a single population in a protected area, the Reserva Particular do Patrimônio Natural Serra do Teimoso, in Jussarí municipality.

The Atlantic Forest biome historically has been severely affected by deforestation. It is currently one of the most protected by Brazilian laws, but its deforestation is still occurring (Galindo-Leal & Câmara, 2003; Tabarelli *et al.*, 2005). This biome is currently reduced to small patches of vegetation, mainly on private proprieties (Rambaldi & Oliveira, 2003). Most natural reserves are < 100 hectares (Fundação SOS Mata Atlântica, 2017), and thus they are insufficient to guarantee the persistence of species and all biological and environmental processes required for its survival, not only for herbaceous bamboos, but for other plant species endemic to this important biome (Silva & Tabarelli, 2000; Rambaldi & Oliveira, 2003).

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