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ABSTRACT

Campylopus Brid. is a widely distributed genus with some very generalist species. Morphological differences between non and sex-expressing plants and the frequent occurrence of non-sex-expressing shoots have generated difficulties in the identification of the group. Our main objective was to morphologically compare the gametophytes of two species of *Campylopus*: *Campylopus julaceus* A.Jaeger and *Campylopus lamellatus* Mont. We performed morphometric analyses (based on 21 gametophyte characters), quantified their sexual expressions, and tested the viability of the asexual propagula produced by *C. julaceus*. Our results indicate that those putative species do not form two mutually exclusive groups. Sexual expression in *C. julaceus* was significant (84.4%, with 88.2% females and 11.8% males), compared to the low expression of *C. lamellatus* (5.29%, 55.5% females and 44.5% males). Asexual propagula occurred in both species, but were more frequently expressed in *C. julaceus* (100% of the samples; *C. lamellatus* 31%). Those propagula showed 50 to 60% regeneration, forming rhizoids or green protonemata within only two days. *Campylopus lamellatus* is often encountered sterile, while *C. julaceus* is apparently only a sexual form. Additionally, as the sexual success of the species can be restricted by the spatial separation of the sexes, asexual propagula should be useful for population maintenance. Finally, we highlight the importance of studies focusing on DNA analyses at the population level and involving specimens collected globally to better understand the delimitation of sympatric species of *Campylopus*.

KEY WORDS
Campylopus julaceus,
Campylopus lamellatus,
Brazilian ironstone outcrops,
morphometric analysis,
bryophyte reproduction.

RÉSUMÉ

Comparaison morphométrique d'espèces sympatriques de Campylopus *Brid. (Leucobryaceae, Bryophyta).*

Campylopus Brid. est un genre largement distribué avec quelques espèces très généralistes. Les différences morphologiques entre les plantes montrant ou non une expression sexuée et l'occurrence fréquente de pieds n'exprimant pas de sexe ont généré des difficultés dans l'identification de ce groupe. Notre principal objectif est de comparer morphologiquement les gamétophytes de deux espèces de *Campylopus* : *Campylopus julaceus* A.Jaeger et *Campylopus lamellatus* Mont. Nous avons mené des analyses morphométriques (basées sur 22 caractères du gamétophyte), quantifié les expressions sexuelles et testé la viabilité des propagules asexuées produites par *C. julaceus*. Nos résultats indiquent que ces deux espèces possibles ne forment pas deux groupes mutuellement exclusifs. L'expression sexuée de *C. julaceus* était significative (84.4 %, avec 88.2 % de femelles et 11.8 % de mâles), comparée à l'expression basse de *C. lamellatus* (5.29 %, 55.5% de femelles et 44.5 % de mâles). Des propagules asexuées apparaissent chez les deux espèces, mais sont plus fréquentes chez *C. julaceus* (100 % des échantillons) que chez *C. lamellatus* (31 %). Ces propagules montrent 50 à 60 % de régénération, formant, en deux jours seulement, des rhizoïdes ou des protonéma verts. *Campylopus lamellatus* est souvent rencontré stérile, tandis que *C. julaceus* est apparemment seulement sous la forme sexuée. De plus, puisque le succès du sexe de l'espèce peut être restreint par une séparation spatiale des sexes, les propagules asexuées peuvent s'avérer utiles pour la continuité des populations. Enfin, nous soulignons l'importance des études axées sur l'analyse de l'ADN au niveau de la population et impliquant des spécimens récoltés à l'échelle mondiale pour mieux comprendre la délimitation des espèces sympatriques de *Campylopus*.

MOTS CLÉS
Campylopus julaceus,
Campylopus lamellatus,
affleurements ferreux du Brésil,
analyses morphométriques,
reproduction des bryophytes.

INTRODUCTION

Campylopus Brid. is a genus of acrocarpous mosses belonging to the family Leucobryaceae Schimp. (Goffinet & Buck 2004; Goffinet *et al.* 2008), comprising approximately 165 described species (Frahm 1999; Stech 2004). *Campylopus* is considered one of the most successful genera of mosses as it shows wide geographic and ecological distributions, occurring across all continents, including the Antarctic (Gbif.org; Frahm 1987, 1990, 1992; Frahm & Hedderson 2004; Stech & Wagner 2005); 30 species are known to Brazil (Costa & Peralta 2015).

Campylopus species are small, erect dioicous plants that grow up to *c.* 15 cm tall and form dense tufts (Frahm 1991; Sharp *et al.* 1994). Their most visible feature is the wide midrib (costa) on the leaves (which is usually long-excurrent) that promote mechanical rigidity. The costae of *Campylopus* species vary among the different taxa in cross-section, with deuter cells, possibly hyalocytes (large, empty, water-storage cells without chlorophyll), and stereids (thick-walled cells) with or without lamella (filament-like outgrowths in cross-section) (Frahm 1990; Gradstein *et al.* 2001). The leaves are generally lanceolate, with or without notable alar cells at the basal margins of the leaves, often differentiated in terms of their size, shape, or color.

Bryophytes develop sporophytes and can produce high numbers of haploid spores through meiosis. The sexual chromosomes are equally distributed during that process, and the proportions of males and females would be expected to be similar (Glime & Bisang 2014; Maciel-Silva & Pôrto 2014); many dioicous moss species shows sexual biases; however, with studies generally recording a female bias (Bowker *et al.* 2000; Bisang & Hedenäs 2005; Bisang *et al.* 2006; Cronberg *et al.* 2006; Hedenäs *et al.* 2010; Stark *et al.* 2010; Horsley *et al.* 2011; Baughman *et al.* 2017). Due to problems of low fecundity (i.e., the failure to produce sporophytes because of non sex-expression, sex bias among the gametophytes, or spatial separation of sexes), many dioicous mosses produce asexual structures such as gemmae and propagula that influence the sex ratios of their populations (Longton & Greene 1979; Miles & Longton 1992; Bowker *et al.* 2000; Longton 2006; Glime & Bisang 2014), and those asexual structures may promote the maintenance of purely female or male colonies. While gemmae are characterized by recapitulating the ontogeny pattern from the spore and do not contain an apical cell, asexual propagula (e.g. deciduous apices and branches) present an apical cell that originates a new shoot without the protonematal stage (Maciel-Silva & Pôrto 2014).

Some species of *Campylopus* are predominant and abundant in azonal environments, such as rocky outcrops in Brazil (Frahm & Porembski 1994; Moraes & Lisboa 2006; Silva *et al.* 2014; Peñaloza-Bojacá *et al.* 2018a) and show important adaptations (e.g. costal lamellae, hyalocytes, and hyaline leaf points) that allow them to withstand high solar radiation and water deficits in rigorous habitats. Ironstone outcrops (locally known as Cangas) are important geosystems in Brazil, although they are often heavily mined (Jacobi *et al.* 2007; Carmo & Jacobi 2012; Madeira *et al.* 2015; Medina *et al.* 2015). Those ironstone outcrops have varied lithological origins, with the Cangas in the Iron Quadrangle (Minas Gerais State) being unique with respect to their formation and mineral contents – with notable species' diversities, rarity, and endemism (Carmo & Jacobi 2012, 2013; Carmo & Kamino 2015; Fantecelle *et al.* 2017).

Sex-expressing plants are morphologically distinct from non sex-expressing plants in the genus *Campylopus* (Frahm 1991). That trait, combined with a low frequency of sex-expressing plants, can make species delimitations in *Campylopus* difficult. Within that context, we morphologically compared two very similar species of the genus *Campylopus*: *C. julaceus* A.Jaeger and *C. lamellatus* Mont., the latter has been recognized as *C. pilifer* in South America until recently (see Gama *et al.* 2017 for details). Leaves of the apical comal tuft and the slender shapes of plants have been used in taxonomic descriptions to morphologically circumscribe *C. julaceus* and distinguish it from *C. lamellatus* (Frahm 1991; Santos 2011). Those two taxa commonly occur on ironstone outcrops in Minas Gerais State (Brazil), and are sometimes encountered together in the same patch. *Campylopus julaceus* is frequently found with sexual and asexual structures on its apical tuft (Peñaloza-Bojacá *et al.* 2018b). We addressed the following questions: 1) Is *C. julaceus* morphologically distinct from *C. lamellatus*, independent of the region of the stem where leaves are attached (i.e. not just the apical region leaves)?; 2) What are the rates of sexual expressions of both taxa in the Canga formations in the Iron Quadrangle in Minas Gerais?; and 3) Are the asexual propagula of *C. julaceus* viable in terms of regeneration and the propagation of that species?

MATERIAL AND METHODS

Studied species

Campylopus lamellatus (Fig. 1A-E) is widely distributed in Brazil (Frahm 1991; Costa & Peralta 2015; Carmo *et al.* 2016) where it is commonly found on exposed rocks, boulders, dry soils, road and trail banks, and shows a large altitudinal range (up to 4800 m). *Campylopus julaceus* (Fig. 1F-J) has been recorded in the southern, southeastern and northeastern regions of Brazil at elevations above 500 m (Santos 2011), being commonly found on sandy or gravelly soils, rarely directly on rocks. *Campylopus julaceus* differs from *C. lamellatus* mostly in respect to leaf morphology and leaf arrangement along the stem (Santos 2011): its leaves have a julaceous-type orientation, appressed to the stem, but forming a very distinctive tuft at the plant apex; the leaves at the apical tuft are broadly lanceolate to ovate, commonly with their apex obtuse and abruptly acuminate (Frahm 1991).

The two species appear identical in terms of other traits (including the leaves on the lower sections of both plants), and plants with the typical leaves of *C. lamellatus* are commonly found in patches together with other plants showing an apical comal tuft and the morphology of *C. julaceus* (Santos 2011). Broadly, *C. lamellatus* has leaves 5-7 mm long; lanceolate to gradually acuminate; with or without differentiated alar cells (depending on the habitat); costa excurrent, and occupies (in terms of width) ½ to ⅓ of the leaf base; and serrate hairpoints, frequently hyaline. Cells in the upper leaf portion are shortly oval to oblong rhombic; while cells in the basal portion are long-rectangular, hyaline, and thin-walled. In cross section, the leaves have a lamella 3-4 cells tall, groups of 3-5 stereids in the dorsal portion, and hyalocytes in the ventral portion (Frahm 1991). Occasional specialized asexual reproduction by deciduous stem tips has been recorded in *C. lamellatus*, while *C julaceus* is generally characterized by plants producing conspicuous bud-like structures (plants in which their branches end in tufts with gametangia and asexual propagula; Frahm 1991; Sharp *et al.* 1994). In a previous study (Peñaloza-Bojacá *et al.* 2018a; fig. 5A), the presence of deciduous propagula surrounding the sexual branches was observed within the apical tufts of plants having a *C. julaceus* morphology.

Sampling

The study sites were located in two legally protected areas in the Iron Quadrangle, in Minas Gerais State, Brazil: the Serra do Rola-Moça State Park - RM (20°01’28”-20°03’09”S and 43°59’11”-44°00’35”W) and the Gandarela National Park - GA (19°58’37”-20°14’22”S and 43°47’07”-43°31’34”W). Specimens were collected from the soil and from rocks on different ironstone outcrops in the two parks. We collected twenty-four samples (*c.* 25 cm²) from different populations: 14 from GA and 10 from RM, in February and April 2015; deposited in the BHCB herbarium (Tables 1 and 2). We also analyzed ten other samples of *C. julaceus* and *C. lamellatus* from herbarium material from the states of Bahia (SP-135068 and SP-379171), Minas Gerais (holotype Paris-4554, SP-354355, SP-172272, SP-136607, SP147031) Rio de Janeiro (SP-147033), and Santa Catarina (SP-436486 and SP-147022) (Table 1).

Morphometric analyses

Of the 24 samples (*c.* 25 cm² patches), five had only the *C. julaceus* morphotype, while thirteen had only the *C. lamellatus* morphotype (both considered pure samples). Eight samples contained plants having morphological traits that matched *C. julaceus* and outher plants with morphological traits of *C. lamellatus* (mixed samples). We selected three shoots from each pure sample, and for mixed sample three shoots (i.e. a plant unit, or ramet) of *C. julaceus* and three of *C. lamellatus* (totaling six shoots for mixed samples). Samples SP-172272 and SP-136607 (Table 1) were exceptional in that they also showed traits of *C. julaceus*, and we accordingly removed three more shoots. A total of 109 shoots were therefore analyzed, of which 39 were classified as *C. julaceus* and 70 as *C. lamellatus* (Table 1).

The gametophyte length (mm), from shoot base to the apical leaves, of all of the shoots was measured (using a digital caliper); and six leaves were detached from each region of the shoot (base, middle and apex) to measure the leaf length (µm). Microscopic images of the leaves were obtained using a Zeiss Axio Lab.A1 optical microscope and Axio Vs40 V 4.8.2.0. software (Zeiss, Oberkochen, Germany). We also selected 19 other quantitative traits directly or indirectly related to the characters commonly used to distinguish *C. julaceus* and *C. lamellatus* (Frahm 1991, Sharp *et al.* 1994, Santos 2011). ImageJ version 1.51f 17 software (Rasband 1997-2012) was used to measure all microscopic traits. Leaf lengths were calculated as the arithmetic means of six leaves per shoot region (base, middle and apex); the other microscopic traits were calculated as the arithmetic average of three leaves per shoot region. All twenty-one characters are described in Figure 2.

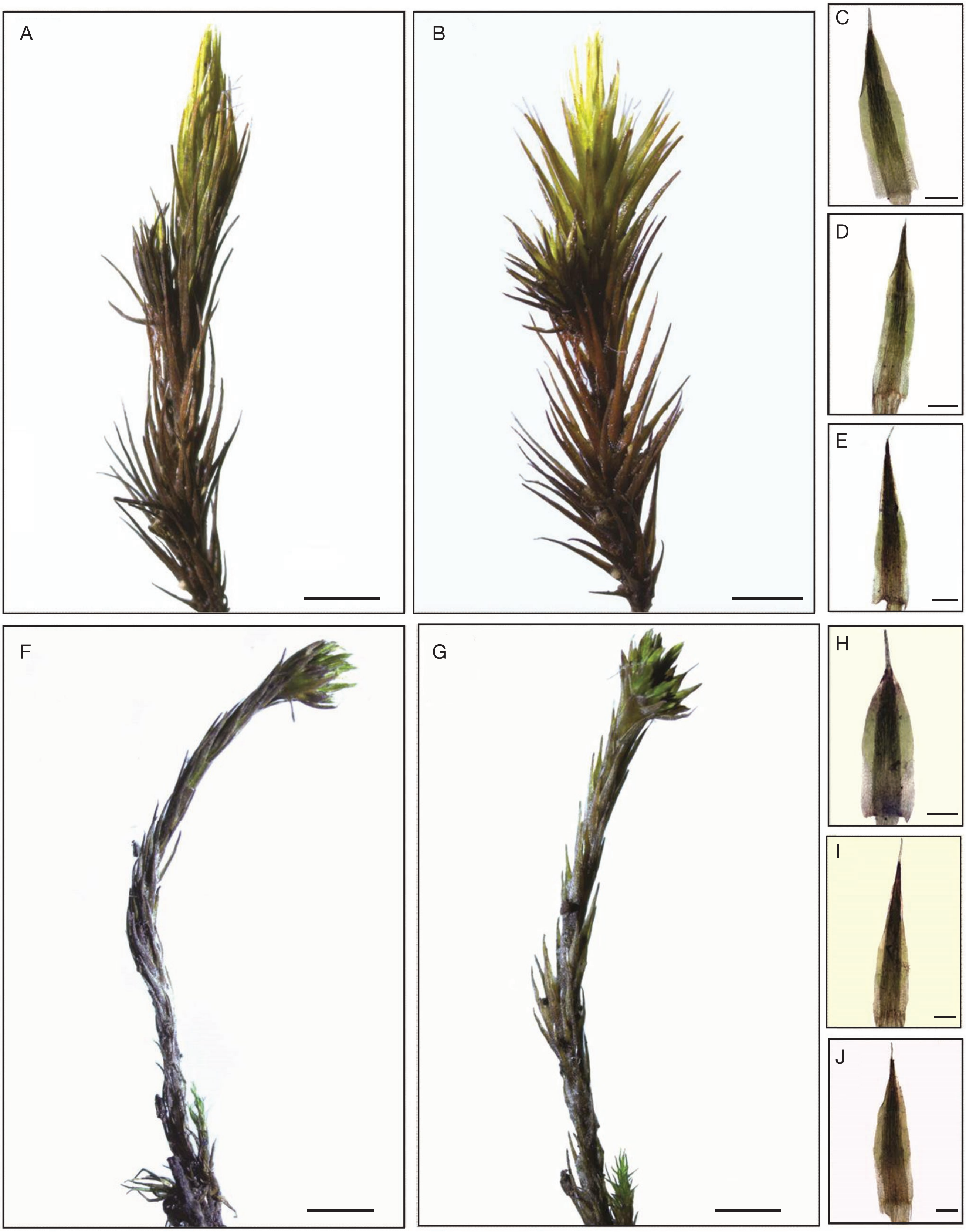

FIG. 1. — Species analyzed: **A**-**E**, *Campylopus lamellatus* Mont.; **F**-**J**, *Campylopus julaceus* A. Jaeger: **A**, **F**, dry plant; **B**, **G**, wet plant; **E**, **J**, basal leaf; **D**, **I**, middle leaf; **C**, **H**, apex leaf. Scale bars: A, B, F, G, 2 mm; C-E, H-J, 500 µm.

Table 1. — Campylopus vouchers used in the morphometric analyses. Shoots: J. *C. julaceus* A. Jaeger; L: *Campylopus lamellatus* Mont.

Species	Shoots	Voucher number	Locality
	J05a – J05b – J05c	Paris-4554 sample type	Brazil, Minas Gerais, Serra dos Orgãos
	J01a – J01b – J01c	SP-135068	Brazil, Bahia, Morro do chapéu
	J02a – J02b – J02c	SP-147022	Brazil, Santa Catarina, Curitiba, An Der Strabe BR116
	J03a – J03b – J03c	SP-354355	Brazil, Minas Gerais, Serra da Grama, Fazenda J. Pequeno
C. julaceus	J04a – J04b – J04c	SP-379171	Brazil, Bahia, County of Lençóis, BR242
	L03a - L03b - L03c - L03d - L03e - L03f	SP-172272	Brazil, Minas Gerais, Santa Barbara, Serra do Caraça
	L04a - L04b - L04c	SP-436486	Brazil, Santa Catarina, Mafra,Paleontological site
	L01a - L01b - L01c - L01d L01e - L01f	SP-136607	Brazil, Minas Gerais, Contry of Santa Barbara
	L02a - L02b - L02c	SP-147031	Brazil, Minas Gerais, Diamantina, ander Strabe MG2
	L02d - L02e - L02f	SP-147033	Brazil, Rio de Janeiro, Parati, Serra do Mar
	L09a - L09b - L09c	BHCB-179839	Brazil, Minas Gerais, Serra do Rola-Moça State Park
	L10a - L10b - L10c	BHCB-179910	
	L11a - L11b - L11c	BHCB-179921	
	L12a - L12b - L12c	BHCB-179935	
	L17a - L17b - L17c	BHCB-187626	
	L18a - L18b - L18c	BHCB-187631	
	L19a - L19b - L19c	BHCB-187632	
C. lamellatus	L20a - L20b - L20c	BHCB-187570	Brazil, Minas Gerais, GandarelaNational Park
	J06a - J06b - J06c - L05a- L05b - L05c	BHCB-179859	
	J07a - J07b - J07c - L06a -L06b - L06c	BHCB-179868	
	J08a - J08b - J08c	BHCB-179912	
	L07a - L07b - L07c	BHCB-179912	
	J09a - J09b - J09c - L08a - L08b - L08c	BHCB-179940	Brazil, Minas Gerais, Serra do Rola-Moça State Park
	J10a - J10b - J10c - L13a - L13b - L13c	BHCB-187650	
	J11a - J11b - J11c - L14a - L14b - L14c	BHCB-187625	
Mixed Samples - *C. julaceus* and *C. lamellatus*	J12a - J12b - J12c - L15a - L15b - L15c - L15d	BHCB-187612	
	J13a - J13b - J13c - L16a - L16b - L16c	BHCB-187582	Brazil, Minas Gerais, Gandarela National Park

Table 2. — Samples used in the viability tests of asexual propagula of *C. julaceus* A. Jaeger.

Name	Sample	Locality
RM1, RM2, RM3, RM4	BHCB-181035, BHCB-181038, BHCB-181042, BHCB-181044, BHCB-181050	Brazil, Minas Gerais, Serra do Rola-Moça State Park.
GA1, GA2, GA3, GA4	BHCB-187650, BHCB-187663, BHCB-187698, BHCB-187703, BHCB-187725.	Brazil, Minas Gerais, Gandarela National Park.

Sexual and asexual expression in *C. julaceus* and *C. lamellatus*

We selected ten shoots of *C. julaceus* and ten shoots of *C. lamellatus* from the same samples used for the morphometric analyses (16 samples; Table 1): 90 shoots of *C. julaceus* and 160 of *C. lamellatus*. Those specimens were dissected and studied under dissecting and compound microscopes. The presence or absence of reproductive structures (asexual – vegetative propagula, and sexual – gametangia and sporophytes) was recorded per shoot.

Viability of asexual propagula

We selected five samples of *C. julaceus* from the Rola-Moça State Park (RM) and five from the Gandarela National Park (GA) to test the viability of their asexual propagula (deciduous branches). We selected eight shoots from each sample, and detached three propagula from each shoot, totaling 120 propagula from each locality (Table 2).

Sixty propagula from each locality were randomized, and then sown in four acrylic boxes (3.5 cm diameter) per locality. Fifteen propagula were placed inside each box under a double layer of filter paper moistened with 0.5 ml of Knop's nutrient solution (Nehira 1983). Additionally, sixty other propagula from each locality were cultivated on *c.* 2 g of sifted soil removed from the ironstone outcrops and moistened with 1 mL of deionized water. The acrylic boxes were placed under red and blue led lights, and were randomized daily. Since the length of propagula was not homogeneous, we performed another assay with 15 propagula from four colonies from each locality to test if propagulum's length had effect on the viability. We examined the propagula for signs of regeneration as protonemata or rhizoids for nine days (average temperature: 26.4°C, min 22.1°C and max 29.3°C; 12h light *c.* 10 µmol . m-2 . s-1).

Table 3. — Statistical values of the morphometric analyses.

Shoot area	**Co-phenetic correlation**	**Simple Euclidean Distance**		**MRPP**			**Discriminant analyses**	
		Minimum	Maximum	T	P	A	Wilks lambda	P
Base	0.8	1.83	15.49	-10.613	<0.001	0.052	0.56416	<0.0001
Middle	0.85	1.65	17.27	-13.926	<0.001	0.064	0.45030	<0.0001
Apex	0.74	2.06	13.46	-12.663	<0.001	0.063	0.52154	<0.0001

Gametophyte histology

Longitudinal and transversal sections were made of the gametophyte stems of the two species (using plant fragments; *c.* 3 mm) in order to better understand the leaf arrangements and anatomies. The material was fixed in 4% Karnovsky in 0.1% phosphate buffer (pH 7.2) for 24 hours, vacuum-dried (Karnovsky 1965, modified), dehydrated in an ethanol series (Johansen 1940), and subsequently infiltrated with a mixture of 50 mL of basic resin and 0.5 g of activator (LeicaHistoResin®) for at least 24 hours. Samples were transferred to plastic molds (mixing with a 1:1 ratio of 0:1 (v/v) ethanol/infiltration solution (1 mL of hardener per 15 mL of activated resin). Polymerization occurred for 10 min at room temperature and 15 min at 60°C. The embedded material was sectioned (5 µm thickness) in a rotating microtome (Zeiss® Hyrax M40), mounted on glass slides, stained with 0.5%-toluidine blue in sodium phosphate buffer (pH 4-6), and photographed using a Zeiss microscope.

Data analysis

We performed Cluster Analysis (UPGMA - unweighted pair-group method with arithmetic means) using the Euclidean distance coefficient to separate groups of specimens. For each species, we analyzed characters from different regions of each shoot (base, middle and apex). We then used a multiple response procedure permutation test (MRPP) to evaluate the cohesion and the distances between the groups (Euclidean Distance Coefficient). In relation to the indices, A describes the homogeneity within the groups, with A = 1 signifying that all members of each group are identical to each other but different from the other groups (McCune & Grace 2002); the standard was adopted that when $A \geq 0.3$ and $P \leq 0.005$ they were considered heterogeneous groups. Principal Components Analysis (PCA), per shoot region (base, middle and apex), using the values of the arithmetic means of each plant trait, was applied to analyze the correlation between them and illustrate the distributions of the groups on the different axes. The analyses were performed using Fitopac software (version 2.1.2.85; Sherpherd 2010). Additionally, we used a Discriminant analysis to test differences between two taxa based on each shoot region (Statistica 10.0, Statsoft Inc.).

A GLIM (Generalized Linear Model) with normal distribution and log link function was used to test if the viability of the asexual propagula of *C. julaceus* differed between the different sampling localities and culture conditions, where localities and culture conditions were predictor categoric factors and viability was the response variable. A logistic regression was applied to ensure that propagula length did not influence the final viability rate (Statistica 10.0, Statsoft Inc.).

RESULTS

Morphometric analyses

UPGMA analyses showed that the morphometric measurements of the basal, middle, and apex leaves of the plants of both species overlapped (Fig. 3), and it was not possible to clearly distinguish the two species (Table 3). Although the MRPP analysis was statistically significant concerning the recognition of two different groups ($P<0.005$), the data from the two species were very heterogeneous, with a lot of overlap between the groups ($A \cong 0.05$-0.06; Table 3). The PCA analysis corroborated that result, revealing a distinction between the two *Campylopus* species, but a consistent overlap of many specimens (Figs 3; 4).

Discriminant analyses for basal, middle and apical regions of shoots showed significant differences between both taxa (Table 3), especially for the characters: shoot base – trait 14 - wilks: 0.606, P: 0.013; in shoot middle – trait 9 - w: 0.494 and P: 0.004, trait 15 and trait 16 - w: 0.479 and P: 0.019; and in the shoot apex – trait 6 - w: 0.551 and P: 0.029, trait 7 - w: 0.554 and P: 0.022, and the trait 11 - w: 0.555 and P: 0.020 (see Fig. 2 for details of traits).

Sexual and asexual expression of *C. julaceus* and *C. lamellatus*

Among the 160 shoots of *C. lamellatus* dissected, only nine (5.6%) were found with sexual reproductive structures; 55.6% of those nine shoots had female gametangia and 44.4% had male gametangia (Table 4). Asexual propagula as deciduous branches were found in 31% of the shoots and deciduous apices in 69%.

Regarding the 90 shoots of *C. julaceus* examined, 84% of the shoots had sexual structures, 74.4% were found to have female gametangia while 10% had male gametangia (Table 4). Curiously, all of the male shoots were found in the same sample. All shoots (100%) had deciduous apices and branches. None of the studied shoots of *C. julaceus* and *C. lamellatus* had sporophytes.

Viability of asexual propagula in *C. julaceus*

The asexual propagula had green protonemata and rhizoids on the second day of cultivation (Fig. 5B, C). In the first assay

Number	Leaf traits
1	Width of the leaf base
2	Width of the costa in the leaf base
3	Length of the hyaline basal lamina
4	Width of the leaf middle
5	Width of the costa in the leaf middle
6	Width of the leaf apex
7	Width of the leaf in the end lamina
8	Length of the basal cell near costa
9	Width of the basal cell near costa
10	Length of the basal cell middle lamina
11	Width of the basal cell middle lamina
12	Length of the basal cell border lamina
13	Width of the basal cell boder lamina
14	Length of the upper cell near costa
15	Width of the upper cell near costa
16	Length of the upper cell middle lamina
17	Width of the upper cell middle lamina
18	Length of the upper cell border lamina
19	Width of the upper cell border lamina
20	Mean length of the leaf
21	Length of the shoot

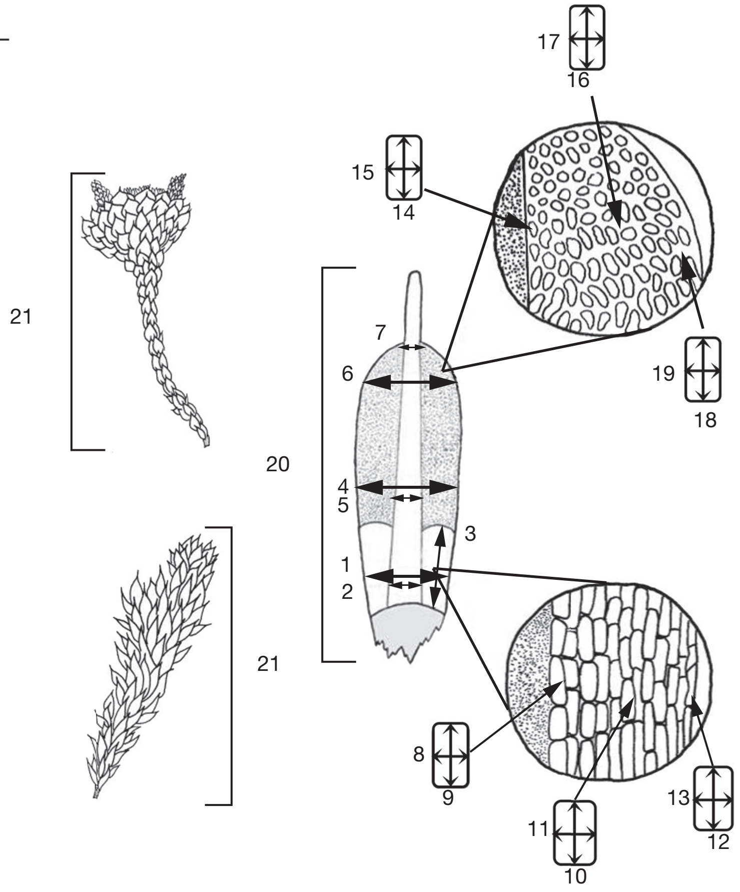

FIG. 2. — Characters measured in the morphometric study and illustrations of each.

(with filter paper and nutrient solution) the regeneration rate was 0.55% for RM and 0.60 for GA after nine days, thus showing similar regeneration responses (Wald-stat = 0.497, n.s) among plants from both sampling localities. Similarly, under soil from the ironstone outcrops and deionized water, the regeneration rate for RM was 0.48% and 0.55 for GA. In the second assay, despite the length variation of the propagula, that trait did not influence the viability rates of RM and GA propagulum (Wald-stat = 2.699, n.s.).

GAMETOPHYTE HISTOLOGY

In relation to the cross-sections of their leaves, *C. julaceus* and *C. lamellatus* were not entirely different (Fig. 6A-B). Both species showed the presence of hyalocysts (thickness mean ± se = 13.3 µm ± 0.4 vs 12.4 ± 0.3, respectively; t-test = 1.516, df = 88, P = 0.06), and dorsal lamellae with similar number of cells. In general, *Campylopus julaceus* presented more 3-cells lamellae and C. *lamellatus* had more 4-cells, resulting in statistically different (not biologically) mean values (3.5 ± 0.1 vs 3.7 ± 0.1; t = -1.985, df = 148, P = 0.02). Additionally, the proportion of hyalocists compared to costa thickness (at cross-section) was not different between species (0.33 ± 0.01 vs 0.32 ± 0.01; t = 0.040, df = 88, P = 0.484).

Transversal and longitudinal sections of *C. julaceus* showed comal tuft arrangements with (from the outside to the inside) differentiated leaves, bud-like asexual propagula, bud-like sexual branches (perichaetia for female gametangia; or perigonia for male gametangia), and the central axis of the gametophyte (Fig. 6C, D). In longitudinal view, the asexual propagula showed a short stem axis with some leaves, and an abscission line containing initial cells that quickly developed into rhizoids and protonemata (Fig. 6E).

DISCUSSION

CAMPYLOPUS JULACEUS AND *C. LAMELLATUS* DO NOT FORM TWO MUTUALLY EXCLUSIVE GROUPS

Among the main characters used to distinguish the two species, statistical differences were recorded when we analyzed variations in “leaf apex widths” (trait 6) and “width of the leaf in the end lamina” (trait 7) measured on the shoot apex (Discriminant analysis). Although other traits related to cell size (wide and length) had contributed to discriminate both groups, when basal and middle sections of shoots were analyzed, all these traits are very unstable among shoots of

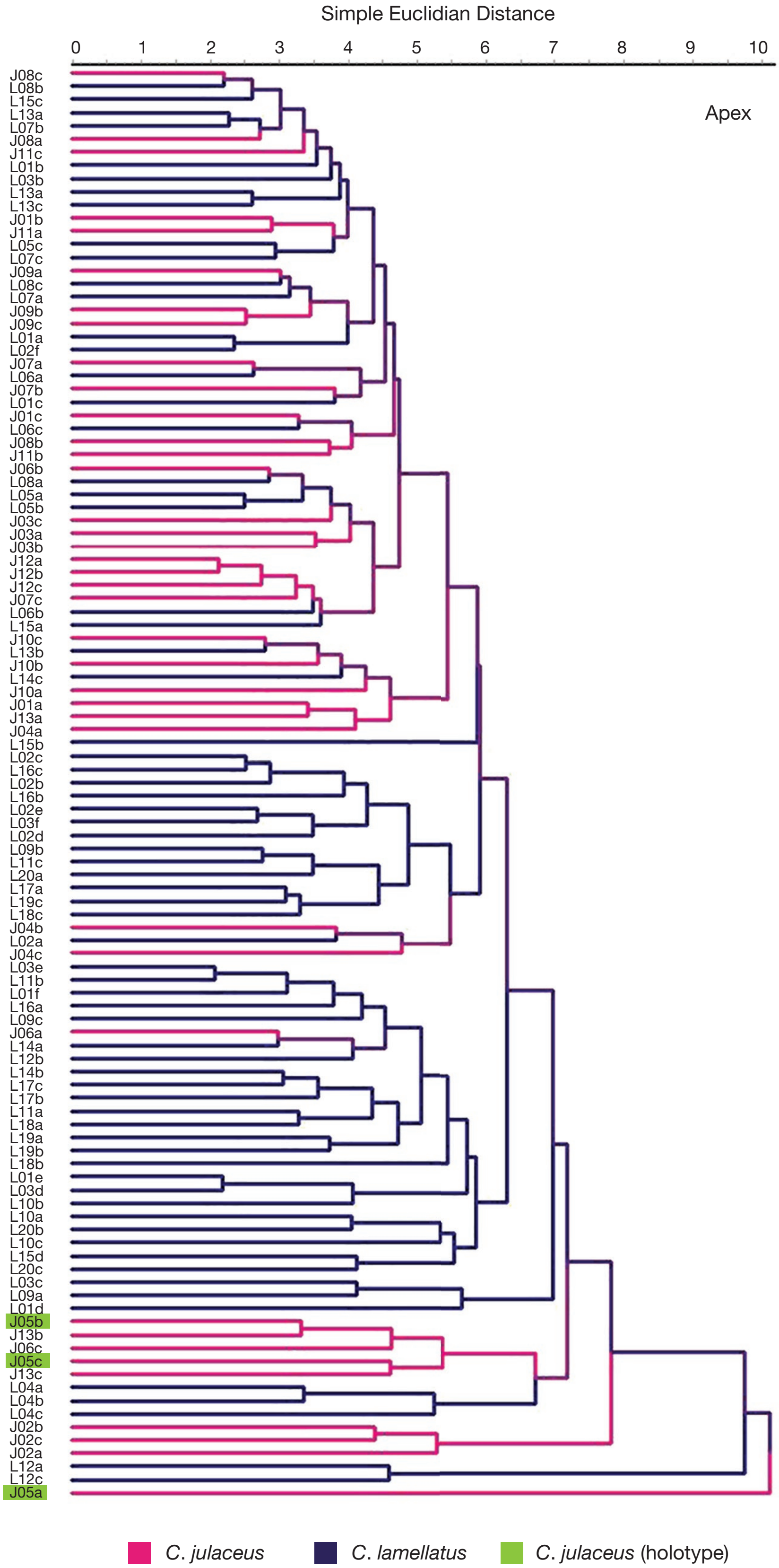

FIG. 3. — Cluster Analysis (UPGMA) for the characters of the basal, middle, and apical parts of leaves of *C. julaceus* A. Jaeger (holotype highlighted in green) and *C. lamellatus* Mont.

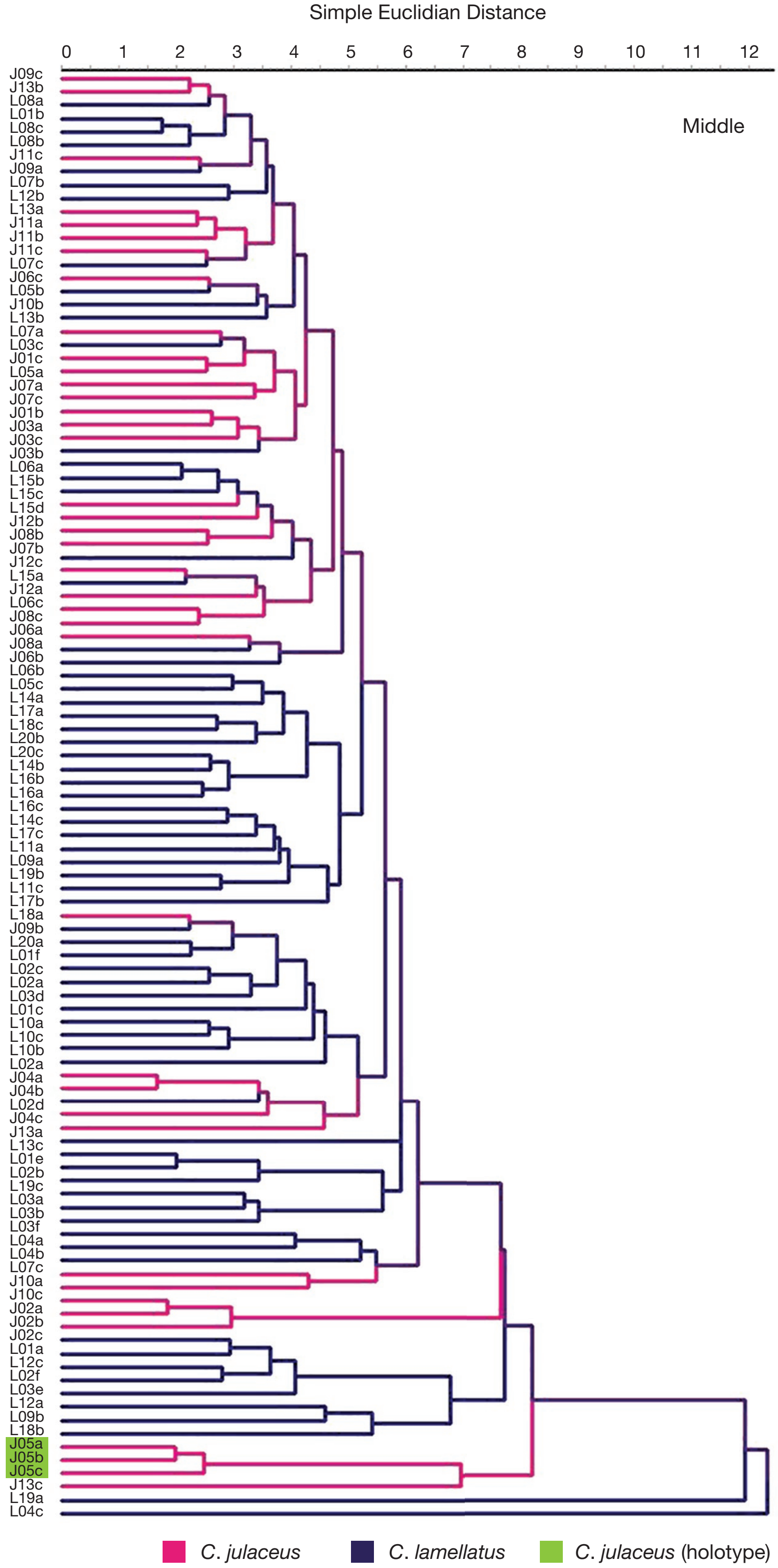

FIG. 3. — Continuation.

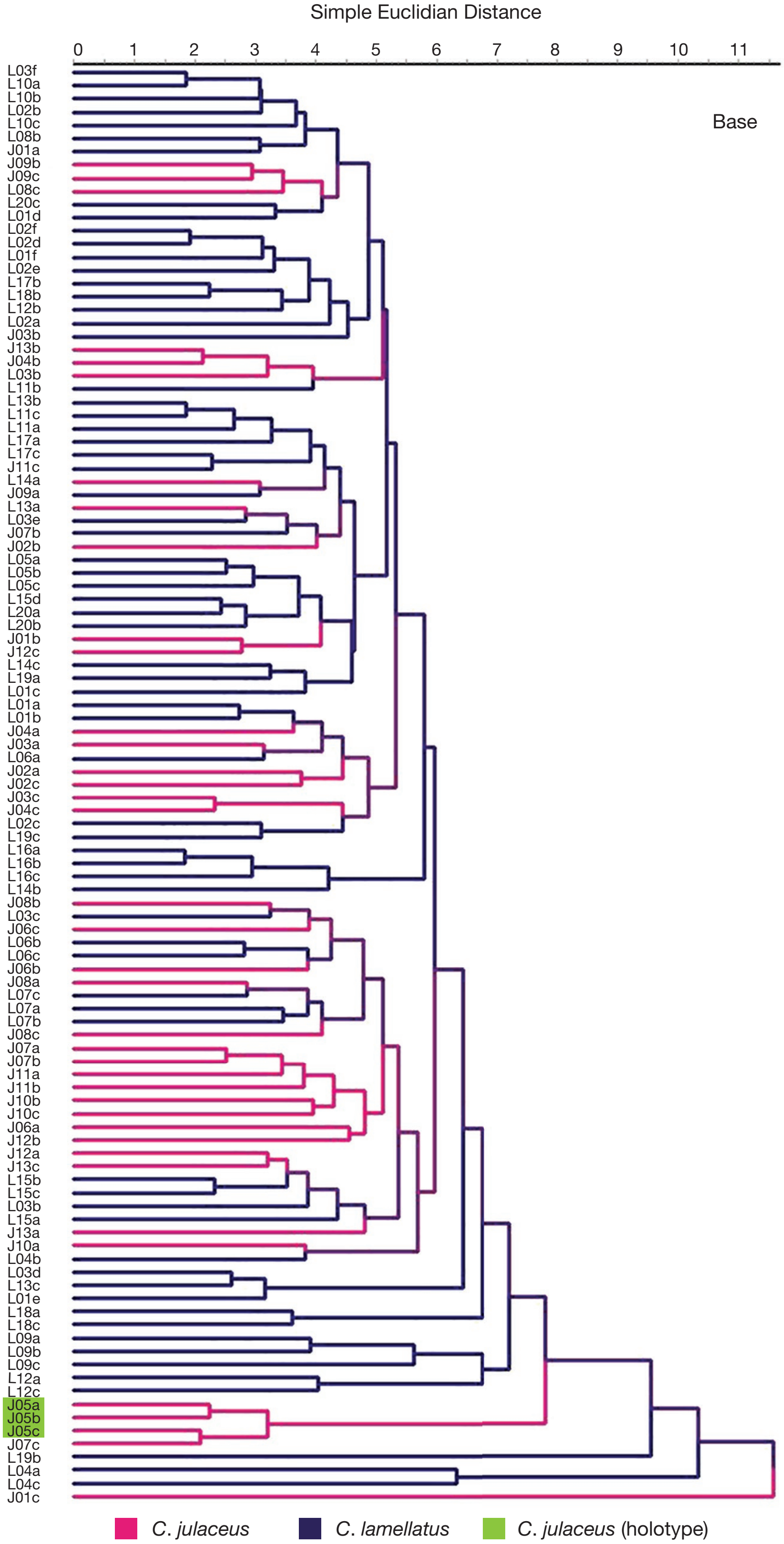

FIG. 3. — Continuation.

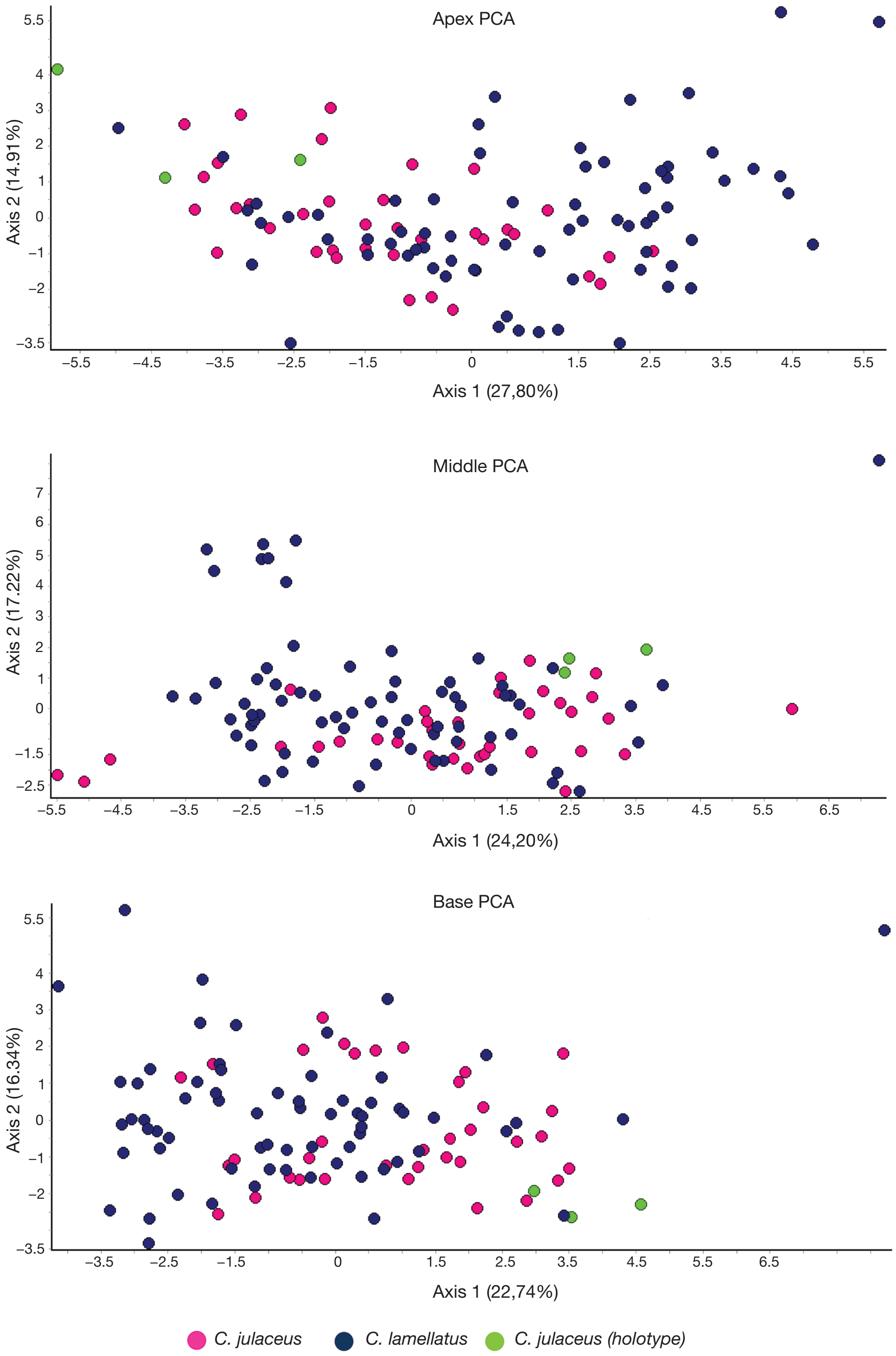

FIG. 4. — Principal Components analysis (PCA) of the leaf characters of the basal, middle, of apical the shoots of *C. julaceus* A. Jaeger (including the holotype) and *C. lamellatus* Mont.

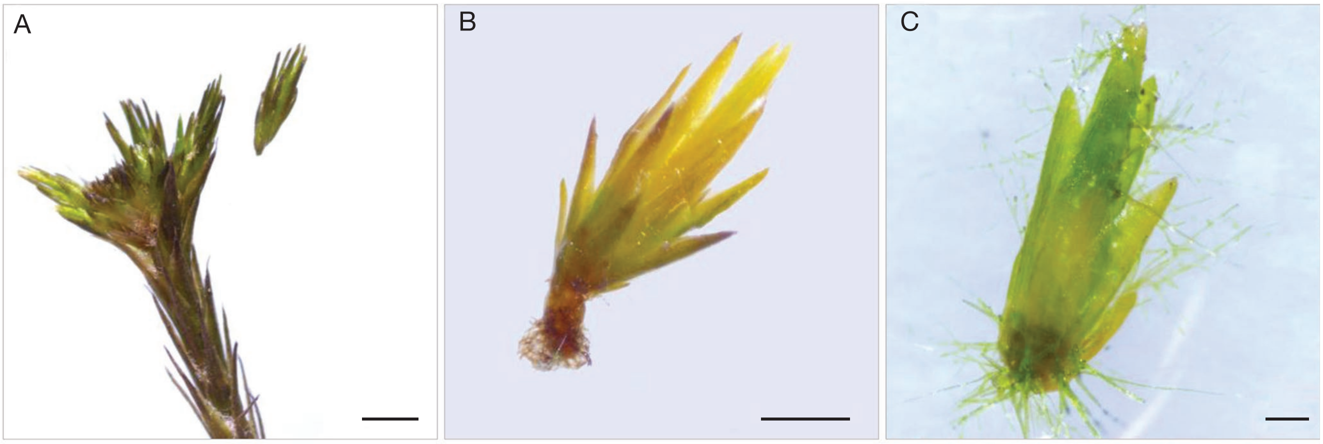

Fig. 5. — Propagula of *C. julaceus* A. Jaeger: **A**, gametophyte comal tuft and a detached propagulum; **B**, detached propagulum, already producing rhizoids; **C**, propagulum cultivated for 2 days, showing several protonematal filaments. Scale bars: A, 1 mm; B, 0.5 mm; C, 0.2 mm..

Table 4. — Analysis of sexual expression by *C. julaceus* A. Jaeger and *C. lamellatus* Mont.. Ten shoots were studied for each voucher.

			Sexual expression	
Sample	**Voucher**	**Species**	♀	♂
Mixed Samples - *C. julaceus* and *C. lamellatus*	BHCB 179859	*C. julaceus*	8	0
	BHCB 179859	*C. lamellatus*	0	0
	BHCB 179940	*C. julaceus*	9	0
	BHCB 179940	*C. lamellatus*	0	0
	BHCB 179868	*C. julaceus*	7	0
	BHCB 179868	*C. lamellatus*	0	0
	BHCB 179912	*C. julaceus*	10	0
	BHCB 179912	*C. lamellatus*	0	0
	BHCB-187650	*C. julaceus*	9	0
	BHCB-187650	*C. lamellatus*	0	0
	BHCB-187625	*C. julaceus*	6	0
	BHCB-187625	*C. lamellatus*	0	0
	BHCB-187612	*C. julaceus*	10	0
	BHCB-187612	*C. lamellatus*	0	0
	BHCB-187663	*C. julaceus*	8	0
	BHCB-187663	*C. lamellatus*	0	0
	BHCB-187641	*C. julaceus*	0	9
C. lamellatus	BHCB 179921	*C. lamellatus*	0	0
	BHCB 179935	*C. lamellatus*	0	3
	BHCB 179910	*C. lamellatus*	0	0
	BHCB 179839	*C. lamellatus*	1	0
	BHCB-187626	*C. lamellatus*	2	0
	BHCB-187632	*C. lamellatus*	1	1
	BHCB-187631	*C. lamellatus*	1	0
	BHCB-187570	*C. lamellatus*	0	0

the same taxon. Thereafter those variations were only slight, because there was a strong overlap (see Fig. 4). In the UPGMA trees, shoots of *C. julaceus* were intermingled with those of *C. lamellatus*. That same pattern was repeated independent of the leaf section considered (base, middle, or apex). Although the MRPP test indicated a significant split between the two groups (*C. julaceus* and *C. lamellatus*), they are weakly cohesive. Additionally, the PCA analysis demonstrated that the two species overlap one another. The morphometric data in our study confirmed the morphological similarities of the two species, highlighting that the observed differences are likely not significant enough to segregate them into two distinct taxa.

In their descriptions of *C. julaceus,* Een (1989) and Santos (2011) mentioned the similarity of that species to *C. lamellatus* (previously as *C. pilifer*). Santos (2011) reported that the morphological traits of *C. lamellatus* leaves are quite similar to those of *C. julaceus*. Additionally, she noted that herbaria voucher specimens containing both species appear to have similar leaves, except for the leaves in the comal tufts (apical section of shoot) of *C. julaceus*. Similarly, Frahm (1991) reported that sex-expressing plants of *C. julaceus* differ in terms of the terminal tuft, with distinct leaves encircling several gametangia. In fact, statistical differences in traits as "leaf apex width" and "width of the leaf in the end lamina" between both taxa emphasize the morphological dissimilarities between *C. julaceus* and *C. lamellatus*. In the present study, plants morphologically recognized as *C. lamellatus* and *C. julaceus* appear to actually represent the same taxonomic unit. As suggested by other authors (Een 1989; Santos 2011), *C. julaceus* could simply represent the reproductive phase of the *C. lamellatus* (or *C. pilifer* in other world places). In addition of the presence of sexual branches containing gametangia in the comal tuft of *C. julaceus*, we also found asexual propagula (e.g., deciduous branches) near them. This system may be beneficial because the plants appear to allocate energy to produce several vegetative propagula simultaneously with sexual branches (female or male gametangia), increasing the chances of offspring output.

Campylopus lamellatus and *C. julaceus* seem to belong to a complex of morphologically similar species, which also include the invasive *C. introflexus* (Hedwig) Bridel and *C. pilifer* (restricted to old world, Gama *et al.* 2017). *Campylopus introflexus* differs from *C. lamellatus*/*C. pilifer* because the strongly recurved hyaline leaf apex and the dorsal costal lamellae composed of only 1-2 cells in the latter (Gradstein & Sipman 1978; Frahm 1991; Gama *et al.* 2016). Several inventories or local floras have

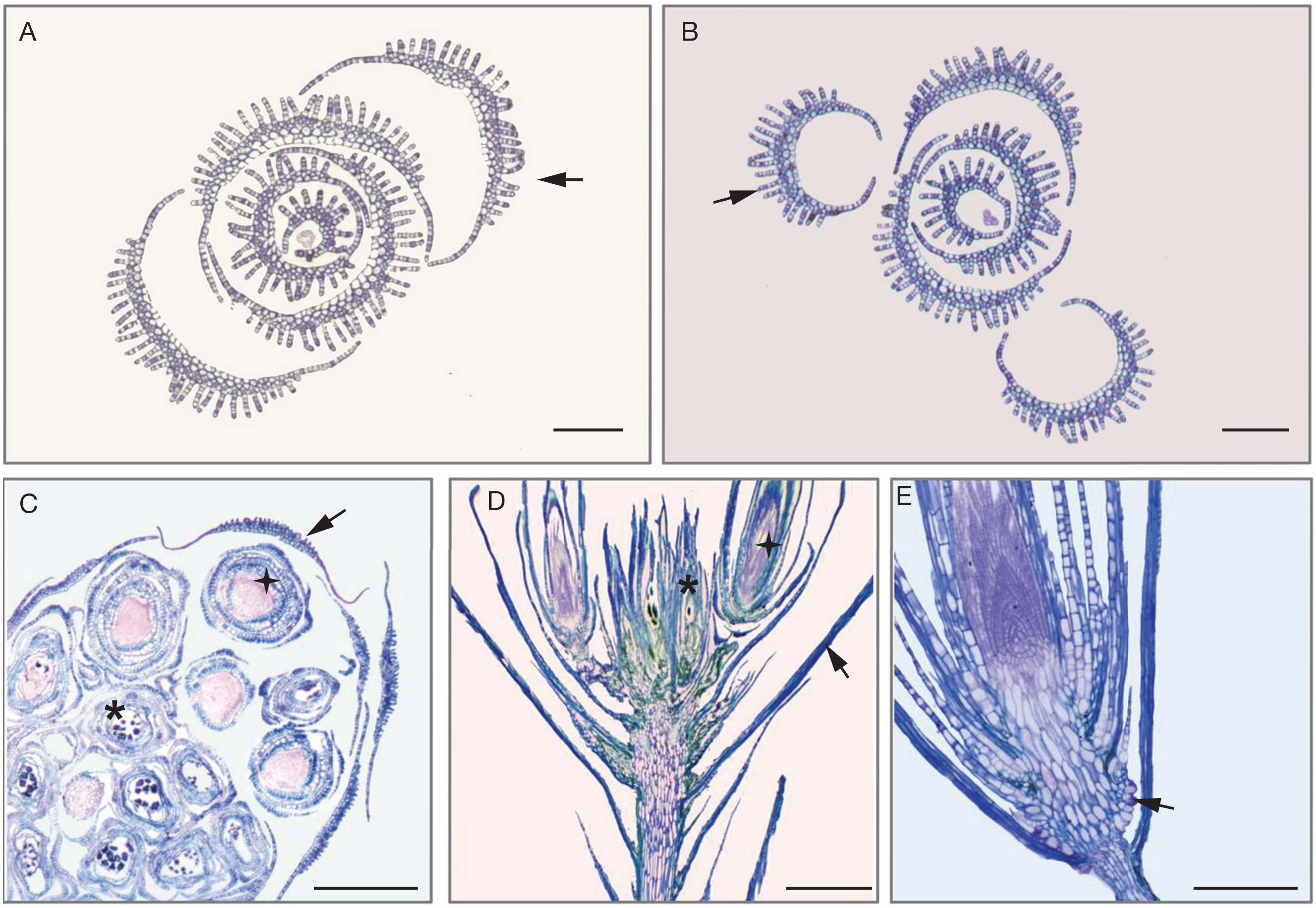

Fig. 6. — Histology of *C. julaceus* A. Jaeger (A, C–E) and *C. lamellatus* (B): **A**, **B**, transversal sections of the leaves; arrows indicate leaf lamella; **C**, transversal section of a comal tuft in the gametophyte tip; **arrow** indicates a modified leaf surrounding a group of asexual propagula (**star symbol**) and female gametangia (**asterisk**); **D**, longitudinal section of a comal tuft, showing modified leaves (**arrow**), asexual propagulum (**star**), and female gametangia (**asterisk**); **E**, longitudinal section of an asexual propagulum still attached to the shoot. The **arrows** indicates inflated basal cells, which may later differentiate into rhizoids and protonemata. Scale bars: A, B, 100 µm; C, E, 200 µm; D, 500 µm.

associated the two species, as intermediate specimens have been described that are often mistakenly identified (Frahm 1991; Frahm & Stech 2006). *C lamellatus* and *C. pilifer* have central lamellae (in cross section) in a conspicuous V-shaped pattern, and the former presents lamellae consisting of 5-6 cells different from *C. pilifer* with 3-4 cells (Frahm 1991; Gama *et al.* 2017). *Campylopus julaceus,* as explained above, is very similar to *C. lamellatus*/*C. pilifer* and it is commonly treated as those species (Sharp *et al.* 1994; Santos 2011). Additionally, Frahm (1991) also recognized *C. julaceus* ssp. *arbogastii* in Africa as a morphologically distinct taxon (i.e. shorter lamellae at the costa) with similar niche to *C. julaceus* in the Southeastern Brazil. Evolutionary studies involving all of those related *Campylopus* species, at a broader geographical scale (phylogeography) will be needed, however, to clarify the phylogenetic relationships of that species complex.

CAMPYLOPUS JULACEUS IS OFTEN FOUND BEARING SEXUAL STRUCTURES, WHILE *C. LAMELLATUS* IS USUALLY FOUND WITHOUT THEM

Santos (2011) highlighted that *C. julaceus* is often found growing alongside *C. lamellatus*, and often so with gametangia, whereas *C. lamellatus* is sterile (non sex-expressing). Variations in the leaves of the comal tuft may therefore be related to morphological differences between individuals expressing or not expressing sex – which is commonly recorded in species of the genus *Campylopus* (Frahm 1991). In fact, among the shoots of *C. lamellatus* analyzed in this study (including the mixed samples), a few plants showed sexual reproduction structures, whereas the shoots of *C. julaceus* commonly had sexual organs. The presence of non sex-expressing gametophytes is quite common, and the absence of sporophytes is frequently associated with a dioicous condition; the spatial segregation of sexes can influence sexual expression, thus *C. julaceus* may simply be the reproductive stage of *C. lamellatus*, with variations in sexual expression being linked to the elevation, year, life cycle stage, substrate, or growing conditions (Longton & Schuster 1983; Korpelainen 1998; Bisang & Hedenäs 2005; Stark *et al.* 2005).

PROPAGULA OF *C. JULACEUS* ARE VIABLE FOR EFFECTIVE PROPAGATION

Approximately 50 to 60% of the *C. julaceus* propagula regenerated in the experiment, readily forming rhizoids and chloronema. Since the comal tuft always present sexual structures associated to several asexual propagula, the above data indicate that the plants invest in the production of sexual structures at the same time as they invest in viable asexual reproduction. As Frey & Kürschner (2011) noted, clonal reproduction confers ecological advantages to the species by balancing the difficulties of mating and the difficulties created by their disproportionate sex ratios. *Campylopus julaceus* (and likely *C. lamellatus*), even failing to produce sporophytes, may take advantage of the asexual reproduction. Regeneration success was similar between the RM and GA sites, revealing that both areas provided suitable conditions for producing viable propagula, and that those propagula are capable of regenerating new plants. All of the samples had propagula, suggesting that they are constantly produced by the gametophytes.

Conclusion

Our study confirms that *C. julaceus* is morphologically very similar to *C. lamellatus*, and that it is unclear whether the morphological characters commonly used to distinguish both species are significant enough to justify considering them as two distinct taxa. However, as *C. julaceus* and *C. lamellatus* are widely distributed throughout Brazil (and the world for *C. pilifer*), a broader global molecular approach will be necessary to securely establish that they belong to the same taxon. Based on our analyses, samples of *C. julaceus* appear to be mostly mixed, having shoots with the *C. lamellatus* morphotype (frequently non sex-expressing plants). *Campylopus julaceus* could not be definitively distinguished here from *C. lamellatus* either anatomically or morphometrically, corroborating the observations of Santos (2011) that *C. julaceus* individuals found among the samples of *C. lamellatus* simply represent the reproductive phase of the latter. We also suggest the asexual propagula have significant roles in the maintenance and rapid growth of *C. julaceus*/*C. lamellatus* communities on ironstone outcrops.

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