- Three new species of *Bertolonia* (Melastomataceae) from Espírito Santo, Brazil 1
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ABSTRACT

- We describe and illustrate three new species of Bertolonia, all endemic to the state of Espírito 19
- Santo, Brazil. Bertolonia duasbocaensis and B. macrocalyx occur close to each other, in the 20
- municipalities of Cariacica and Viana. Bertolonia ruschiana has a wider distribution, occurring 21
- in the municipalities of Santa Leopoldina, Santa Maria de Jetibá and Santa Teresa. The first two 22
- species are respectively classified as endangered (EN) and critically endangered (CR), and the 23
- latter as lower risk (LR). We also present an identification key for the species of Bertolonia that 24
- occur in Espírito Santo. 25

26 **Keywords**. Atlantic Forest; Bertolonieae; endemism; taxonomy.

INTRODUCTION

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- 28 Bertolonia Raddi is a Neotropical genus of Melastomataceae endemic to the Atlantic Forest
- 29 biome (Baumgratz, 1990; Bacci et al., 2016). The genus was previously classified as a member
- 30 of tribe Bertolonieae, together with other genera of Melastomataceae that are also herbs with
- obtriqutrous capsular fruits, like *Monolena* Triana ex Bent. & Hook. f., *Salpinga* Mart. ex DC.
- and Triolena Naudin (Clausing & Renner, 2001). Nevertheless, the tribe is not monophyletic,
- and *Monolena*, *Salpinga* and *Triolena* actually belong to a different evolutionary lineage
- 34 (Goldenberg et al., 2012). Inside the Atlantic Forest Biome, the species of Bertolonia occur
- 35 mostly in the Atlantic Rainforest ("Floresta Ombrófila Densa Submontana/Montana", following
- the official Brazilian system Veloso et al., 1991). It has 22 taxa, including Bertolonia
- 37 margaritacea Naudin [until recently recognized as Salpinga margaritacea (Naudin) DC.; see
- Bacci et al., 2016 and Goldenberg et al., 2016]. Bertolonia species are small, perennial herbs that
- 39 inhabit moist and shaded areas. They grow on forest litter, directly on rocks or on shallow soils,
- on decaying logs or as epiphytes on the base of tree trunks (frequently tree ferns). The plants are
- usually glandulose-punctate or also with other types of trichomes, rarely glabrous (Baumgratz,
- 42 1990). The inflorescences are scorpioid cymes and the fruits are obtriquetrous (Baumgratz,
- 43 1990), bertolonidium-type capsules (following Baumgratz, 1983–1985).
- Four new species of *Bertolonia* have been described in the last five years (Baumgratz et al.,
- 45 2011; Bacci et al., 2016; Silva-Gonçalves et al., 2016), three from the state of Bahia and one
- 46 from Rio de Janeiro. According to "Flora do Brasil" (Baumgratz, 2016), there should be only
- five species of *Bertolonia* occurring in the state of Espírito Santo: *Bertolonia formosa* Brade, *B.*
- 48 foveolata Brade, B. maculata DC., B. sanguinea Saldanha ex Cogn. and B. wurdackiana
- Baumgratz. Since the specimens that have been mentioned as *B. sanguinea* actually belong to
- one of the three new species described here, the total number for *Bertolonia* in Espírito Santo
- should rise to seven species, which makes this state the second richest, after Rio de Janeiro (8
- species), but before Bahia (6 species; Baumgratz, 2016). Espírito Santo was originally and
- almost entirely covered by the Atlantic Forest Biome (Fundação SOS Mata Atlântica & INPE,
- 54 1993). Nowadays only about 10% of the original vegetation remains (Fundação SOS Mata
- 55 Atlântica & INPE, 2014). Despite of the small remaining forested area and the relatively

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Comment [1]: It should be lineages because Triolena and Monolena are in one lineage and Salpinga is in a different one (Merianieae)

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- homogenous vegetation, Espírito Santo has several rare, endemic and threatened species of both fauna and flora, and can be considered a biodiversity hotspot (Dutra et al., 2015).
- We describe here three new species of *Bertolonia* that are endemic to Espírito Santo, Brazil.
- 60 We also provide an identification key for the species that occur in Espírito Santo, comparisons
- with closely related species, information about distribution and conservation status, and
- 62 illustrations of living and dry specimens.

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MATERIAL AND METHODS

- The authors have been collecting *Bertolonia* in Espírito Santo since 2008. Specimens were
- collected and processed following the usual precedures for botanical specimens (Mori et al.,
- 67 1989). Morphological descriptions follow Radford (1974), Baumgratz (1985-1987) and
- 68 Baumgratz (1990). The study was based on literature (Baumgratz, 1990, Baumgratz et al., 2011,
- 69 Bacci et al., 2016) and the analysis of specimens at CEPEC, MBM, MBML, NY, RB, UPCB,
- 70 VIES (acronyms according to Thiers, 2016). Ecological and geographic distribution data were
- 71 also obtained from herbarium labels. Conservation status assessments were based on range size
- 72 (criterion B), according to IUCN (2014). The Extension of Occurrence (EOO) and Area of
 - Occupancy (AOO) were calculated through the GeoCAT tool (Bachman et al., 2011). Collection
 - permits were issued by the "Instituto Estadual do Meio Ambiente" (IEMA/Espírito Santo:
- 75 14489/07) for the plants inside "Reserva Biológica de Duas Bocas" and by the "Instituto Chico
- 76 Mendes de Conservação e Biodiversidade (ICMBio: 49043-2) for the plants inside the "Reserva
- 77 Biológica Augusto Ruschi".

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published work according to the International Code of Nomenclature for algae, fungi, and plants

- (ICN), and hence the new names contained in the electronic version are effectively published
- under that Code from the electronic edition alone. In addition, new names contained in this work
- which have been issued with identifiers by IPNI will eventually be made available to the Global
- Names Index. The IPNI LSIDs can be resolved and the associated information viewed through
- any standard web browser by appending the LSID contained in this publication to the prefix

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89	Key to the species of Bertolonia from the state of Espírito Santo, Brazil.	fabian michelangeli 10/16/2016 9:12 AM
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91	1'. Leaf surfaces, flat 3	Deleted: s with
31	1. Box sarrassy nation	fabian michelangeli 10/16/2016 9:04 AM
92	2. Petioles villose; sepal margins, entire; petals 5.5–7 mm long	Deleted: margins
-		fabian michelangeli 10/16/2016 9:10 AM
93	2'. Petioles hirsute; sepals margins, long-laciniate; petals 7.8–8.3 mm long	Comment [3]: Laciniate means divided into
		deep narrow irregular segments so it is hard to use this term just for the margin.
94		think that it would be better to use "margins fimbriate"
95	3. Leaves abaxial and/or adaxial surfaces permanently covered with sessile and short stalked	fabian michelangeli 10/16/2016 9:06 AM
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96	glands and long unbranched trichomes	fabian michelangeli 10/16/2016 9:05 AM
07	2) I consider the desired conference of with only confident desired about the desired conference	Deleted: with
97	3'. Leaves abaxial and adaxial surfaces covered with only sessile and short stalked glands	fabian michelangeli 10/16/2016 9:05 AM
98	5	Deleted: margins
		fabian michelangeli 10/16/2016 9:12 AM
99	4. Petioles hirsute at the apex; flowers 17–22 mm long; sepals fleshy, with short-lacinate	Deleted: A
100	margins; petals margins entire; anther connective with a dorsal appendage B. formosa	fabian michelangeli 10/16/2016 9:12 AM
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101	4'. Petioles glandulose-pilose or glandulose-villose; flowers 11–13 mm long; sepals	fabian michelangeli 10/16/2016 9:12 AM Deleted: also
402		fabian michelangeli 10/16/2016 9:13 AM
102	membranaceous, with entire margins; petals margins ciliate at the apex; anther connective	Deleted: Both
103	unnapendaged	fabian michelangeli 10/16/2016 9:13 AM
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104	5. Hypanthium covered with only sessile or short stalked glands; sepals with an acute apex;	fabian michelangeli 10/16/2016 9:14 AM
105	petals 6–6.5 mm long, widely obovate	Comment [4]: Fimbriate?
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106	5'. Hypanthium covered with sessile or short stalked glands and with unbranched trichomes;	Deleted: with eciliate
107		fabian michelangeli 10/16/2016 9:14 AM Comment [5]: Only at the apex (as in the
107	sepals truncate; petals 7–12 mm, irregularly elliptic	couplet above) or throughout
108	6. Flowers 13.3–14.7 mm long; calyx external teeth ca. 1 mm long, triangular, covered with only	fabian michelangeli 10/16/2016 9:14 AM Deleted: with the
109	sessile and short stalked glands; petals long-apiculate (ca. 1 mm long), the apiculus without an	fabian michelangeli 10/16/2016 9:15 AM
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127	unbranched trichome at the tip	fabian michelangeli 10/16/2016 9:17 AM
128	duasbocaensis	Deleted: its
129	6'. Flowers 9.5–11.2 mm long; calyx external teeth ca. 2 mm long, ovate, covered with sessile	
130	and short stalked glands and long-stalked glands (0.6–0.8 mm long); petals short-apiculate (ca.	
131	0.5 mm long), the apiculus with an unbranched trichome at the tip	fabian michelangeli 10/16/2016 9:16 AM Comment [6]: In these cases it would be
122		better to place this character first to differentiate the two species
132		fabian michelangeli 10/16/2016 9:15 AM
133	Bertolonia duasbocaensis Bacci & R.Goldenb. sp. nov.	Deleted: also fabian michelangeli 10/16/2016 9:17 AM
134	(Figs 1, 2)	Deleted: its
13 .		
135	Type. Brazil. Espírito Santo: Mun. Cariacica, Reserva Biológica Duas Bocas. Localidade de	
136	Alegre, trilha do Pau-Oco. Floresta Ombrófila Densa Montana 20°16'4"S 40°31'30"W 525 m, 18	
137	January 2009, fl., R. Goldenberg, C.N. Fraga, P.H. Labiak, R.C. Forzza, A.P. Fontana, L.C.	
138	Kollmann and P.B. Schwartsburd 1249 (holotype: UPCB!; isotypes: CEPEC!, MBML!).	
139	Diagnosis. Bertolonia duasbocaensis is similar to B. formosa. The new species has petioles and	
140	leaves covered only with sessile or short-stalked glands (vs. petioles hirsute and leaves also	
141	covered with unbranched trichomes in <i>B. formosa</i>), smaller flowers (13.3–14.7 mm long vs. 17–	
142	22 mm long in <i>B. formosa</i>), inconspicuous and truncate sepals with triangular external teeth (vs.	
143	conspicuous, 3.2-3.7 mm long sepals, these rounded and lacking external teeth) and by the petals	fahian michalangali 10/16/2016 0:17 AM
144	with the apex covered with sessile or short-stalked glands (vs. petals glabrous).	fabian michelangeli 10/16/2016 9:17 AM Deleted:
145	Description . Herbs 10–35 cm tall, terrestrial, reptant; adventitious roots branched, growing from	
146	several points along the stem, but larger next to its base; stem 3-5 mm thick, terete and slightly	
147	costate, the older portions plagyotropic and aphyllous, the young ones erect and bearing leaves.	
148	Branches, leaves, inflorescences and bracts with sparse to dense, sessile or short-stalked (then	
149	less than 0.1 mm long) glands, young branches also with sparse unbranched trichomes. Leaves	
150	opposite, occasionally subopposite; petioles 3–9.5 cm long, quadrangular, slightly costate,	
151	covered with the same trichomes as the branches; blade 7.8–11.7 × 4.5–9 cm, flat, ovate to	
152	elliptic, chartaceous, base rounded to slightly cordate, apex rounded to slightly acute, margins	
153	entire to slightly crenulate and ciliate, adaxial surface dark-green, whitish along the primary	
154	vein, sparsely covered with sessile glands, otherwise glabrescent, abaxial surface light-green or	
155	lilac, sparsely covered with sessile glands, veins three to five, plus a shorter marginal pair that do	

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not reach the leaf apex, basal, main veins at the abaxial surface with pocket domatia at their
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       bases. Thyrsoids 4.5–10.7 cm long, terminal (but pseudo-lateral in older, fruiting specimens),
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       with one pair of paraclades, these cymose, scorpioid, the branches greenish to light-pink;
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       bracteoles 0.5–0.8 mm long, narrow-lanceolate, apex acuminate, both surfaces covered only with
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       sessile glands. Flowers 5-merous, 13.3–14.7 mm long, on pedicels ca. 2 mm long, light green,
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       densely covered with sessile and short-stalked glands. Hypanthium light green, 2.5–2.7 × 2 mm,
       widely campanulate, 10-costate, with the same indument as the pedicel, seldom with scattered
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       unbranched trichomes in its the upper half. Calyx caducous on fruits, tube ca. 0.7 mm long,
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       sepals truncate, external teeth ca. 1 mm long, green to slightly vinaceous, triangular, apex acute,
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       margins entire, eciliate, both surfaces with the same indument as the pedicels. Petals white, with
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       light-pink or light-purple apex, 10.8-12 \times 4.5-5 mm, irregularly elliptic, base slightly atenuate,
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       apex acute and apiculate (ca. 1 mm long), the apiculus bending outwards (to the abaxial surface
       of the petal), lacking trichomes at its tip, margins entire, both surfaces papillose, adaxial surface
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       apex sparsely covered with sessile or short-stalked glands. Stamens 10, 6–7 mm long,
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       isomorphic; filaments 3.5-4 mm long, slightly widened at the base; thecae yellow, 2-3 mm long,
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       oblong-subulate, slightly undulate, pore apical, introrse; connective prolonged ca. 1 mm below
       the thecae, dorsally thickened. Ovary free, apex glabrous, 3-locular, placentation axillary; style
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       ca. 5 mm long, curved at the apex, glabrous; stigma slightly capitate, papillose. Capsules
       bertolonidium-type, 4–5 × 4.5–6 mm, obtriquetrous; seeds 0.4–0.5 mm long, triangular,
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       tuberculate on the dorsal angles and apex.
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       Distribution and conservation status.—Bertolonia duasbocaensis has been collected a few
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       times since 2008, mostly along the same trail ("Pau-Oco") inside Duas Bocas Biological
       Reserve, but with one specimen occurring in a private property near the Reserve. The Biological
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       Reserve is a fully protected area, kept by the state government (IEMA/ES). It is located in the
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       municipality of Cariacica, in central Espírito Santo, between 20°14'40"S - 20°18'30" S and
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       40°28'01"W – 40°32'07"W. Its 2910 ha are covered with well-preserved Atlantic Rainforest that
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       protects threatened species of both fauna and flora (Novelli, 2010). In the last five years, three
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       new plant species were described from Duas Bocas: Eugenia amorimii Fraga & Giaretta
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       (Giaretta & Fraga, 2014), Leandra magnipetala R. Goldenb. & E. Camargo (Camargo &
       Goldenberg 2011), and Ouratea cauliflora Fraga & Saavedra (Fraga & Saavedra, 2014). The
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       plants occur in shaded and moist slopes at 500-600 m elev. Bertolonia duasbocaensis was
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collected with flowers in January and February and with fruits in January, February and July. 191 According to IUCN (2014) criteria B1ab(ii) + B2ab(ii), with EOO (Extent of Ocurrence) = 2.235 192 km² and AOO (Area of Occupancy) = 12.000 km², this species should be classified as 193 "endangered" (EN). It has been collected only in two locaties, one of them in a private property. 194 Paratypes. Brazil. Espírito Santo: Mun. Cariacica, 10 January 2007, fl. and fr., A.P. Fontana et 195 al. 2584 (MBML!, RB!, UPCB!); 10 January 2007, fl., L. Kollmann et al. 9457 (MBML!); 20 196 July 2008, fr., A.M.A. Amorim et al. 7571 (CEPEC!, RB!, UPCB!); 22 July 2008, fr., R. 197 Goldenberg 1210 (CEPEC!, MBML!, RB!, UPCB!); 15 February 2008, fl. and fr., R.C. Forzza. 198 et al. 5035 (CEPEC!, MBM!, MBML!, RB!, UPCB!). Mun. Viana, Arredores da Reserva 199 200 Biológica Duas Bocas, São Paulo de Viana, prop. do Valtinho, 700 m. 19 January 2009, fl. and fr., R. Goldenberg et al. 1257 (MBML!, RB!, UPCB!). 201 **Etymology**— The epithet "duasbocaensis" refers to the Duas Bocas Biological Reserve, the type 202 locality of the new species. 203 Remarks. Bertolonia duasbocaensis is characterized by the ovate to elliptic leaves with rounded 204 to slightly cordate bases, ciliate margins, 3-5 main veins, and covered only with sessile and 205 short-stalked glands, the widely campanulate hypanthium, truncate sepals, triangular external 206 calvx teeth, apiculate petals with the apex of the adaxial surface covered with sessile or short-207 208 stalked glands, and oblong-subulate stamens with a dorsally thickened appendage. The new species closely resembles B. formosa, also endemic of the state of Espírito Santo. For more 209 details on the distinction between the two of them, see the diagnosis above. 210 Bertolonia hoehneana Brade, B. nymphaeifolia Raddi and B. sanguinea share with the new 211 212 species the leaves with cordate bases and also the branches and adaxial surface of the leaves covered only with sessile or short stalked glands. The first is a rare species, endemic to the state 213 of São Paulo (Baumgratz, 1990; Baumgratz, 2016) and differs by the leaves with a cordate-214 lobate base (vs. rounded to slightly cordate in B. duasbocaensis), eciliate margins (vs. ciliate 215 margins) and an acute, attenuate, or sometimes acuminate apex (vs. rounded to slightly acute), 216 by the smaller flowers (10-13 mm long vs. 13.5-15 mm long) with the hypanthium covered only 217 218 with sessile or short-stalked glands (vs. covered also with scattered unbranched trichomes,

mostly on its upper half) and sepals widely ovate, lacking external teeth (vs. sepals truncate, with

external teeth). Both Bertolonia nymphaeifolia and B. sanguinea are endemic to the state of Rio

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de Janeiro and differ from the new species by the bigger leaves, with 7–9 and 5–7 acrodromous 224 veins, respectively (vs. 3–5 in B. duasbocaensis), petioles hirsute at the base or seldom on the 225 whole petiole (vs. petioles covered with sessile or stalked glands, the young ones sometimes with 226 caducous, unbranched trichomes), longer flowers, 18–23 mm long in B. sanguinea (vs. shorter 227 ones, 13.3–14.7 mm long in B. duasbocaensis) and by the long subulate stamens with an acute 228 229 extension at the apex, forming a tube below the apical pore (vs. oblong-subulate stamen, without the extension at the apex). The morphology of the calvx is also different: B. nymphaeifolia has 230 231 widely ovate, membranaceous sepals with fleshy, rounded external teeth, this forming a concave cavity on the sepals and B. sanguinea has fleshy, widely ovate sepals lacking external teeth 232 233 (Baumgratz 1990), while B. duasbocaensis has truncate sepals with a triangular external teeth. Bertolonia duasbocaensis closely resembles B. macrocalyx; both are endemic of Espírito 234 Santo and occur very close to each other. The differences between both species are listed in the 235 diagnosis of B. macrocalyx (see below). 236 237 Bertolonia macrocalyx Bacci & R.Goldenb. sp. nov. 238 (Figs. 3, 4) 239 240 Type. Brazil. Espírito Santo: Mun. Viana, Arredores da Reserva Biológica Duas Bocas. São 241 Paulo de Viana, beira da estrada. Floresta Ombrófila Densa Montana 20°18'3"S 40°32'48"W 575 m, 17 January 2009, fl., R. Goldenberg, C.N. Fraga, P.H. Labiak, R.C. Forzza, A.P. Fontana, 242 and P.B. Schwartsburd 1235 (Holotype: UPCB; Isotypes: CEPEC, MBML). 243 **Diagnosis**. Bertolonia macrocalyx is similar to B. duasbocaensis. Both species have similar 244 245 vegetative morphology, but B. macrocalyx has the calyx with longer (ca. 2 mm long), ovate and ciliate external teeth (vs. shorter, ca. 1 mm long, triangular and eciliate in B. duasbocaensis), 246 short-terete hypanthium (vs. widely campanulate hypanthium), smaller, 7–8.5 × 3–3.5 mm petals 247 248 with the upper half of the adaxial surface densely covered with sessile and short-stalked glands (vs. bigger $10.8-12 \times 4.5-5$ mm petals, with only the apex of the adaxial surface sparsely 249 covered with sessile and short-stalked glands). 250

Description. Herbs ca. 20 cm tall, terrestrial or rupicolous, reptant; adventitious roots branched, growing from several points along the stem, but larger next to its base; stem ca. 3 mm thick, quadrangular and slightly costate, the older portions plagyotropic and aphyllous, the young ones erect and bearing leaves. Branches, leaves, inflorescences and bracts with sparse to dense, sessile or short stalked (then less than 0.1 mm long) glands. Leaves opposite, occasionally subopposite; petioles 1.5–5.3 cm long, quadrangular, slightly costate, covered with the same trichomes as the branches, also with unbranched trichomes, denser at the apex; blade $10-13.5 \times 4.7-6.3$ cm, flat, ovate, narrowly elliptic to lanceolate, chartaceous, base slightly cordate, apex rounded to slightly acute, margins entire to slightly crenulate and ciliate, adaxial surface dark-green, sometimes light green along the primary vein, abaxial surface light-green or lilac, veins three to five, plus a shorter marginal pair that do not reach the leaf apex, basal, main veins at the abaxial surface with pocket domatia at their bases. Thyrsoids 11.3-16.2 cm long, terminal (but pseudo-lateral in older, fruiting specimens), with one pair of paraclades, these cymose, scorpioid, the branches greenish to light-pink; bracteoles ca. 0.5 mm long, linear to narrow-lanceolate, apex aristate and tipped with one glandular trichome ca. 0.5 mm long, both surfaces covered only with sessile glands. Flowers 5-merous, 9.5–11.2 mm long, on pedicels 1.5–2 mm long, light pink, densely covered with sessile and short stalk glands. Hypanthium light green, 2.5–2.7 × 2 mm, shortterete, 10-costate, ridges pink, the ones between the external teeth ending in one trichome, with the same indument as the pedicel, also with unbranched trichomes, on the ridges at the apex of the hypanthium. Calyx caducous on fruits, tube ca. 0.8 mm long, sepals truncate, external teeth ca.2 mm long, with the abaxial surface pinkish, abaxial light green, covered with the same trichomes as the pedicels and also with unbranched trichomes (0.6–0.8 mm long) on the adaxial surface, ovate, apex rounded, margins entire, ciliate at the apex. Petals white, with light-pink apices, $7-8.5 \times 3-3.5$ mm, irregularly elliptic, base slightly atenuate, apex acute and apiculate (ca. 0.5 mm long), the apiculus bending outwards (to the abaxial surface of the petal), ending in a long unbranched trichome, margins entire, both surfaces papillose, upper half of the adaxial surface densely covered with sessile and short stalked glands. Stamens ten, 6–6.5 mm long, isomorphic; filaments .3.5-4 mm long, slightly widened at the base; thecae yellow, 2-2.5 mm long, oblong-subulate, slightly undulate, pore apical, introrse; connective prolonged ca. 1.5 mm below the thecae, with a dorsal calcar, ca. 0.3 mm long. Ovary free, apex glabrous, 3-locular, placentation axillary; style ca. 5 mm long, curved at the apex, glabrous; stigma slightly capitate,

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papillose. Capsules bertolonidium-type (following Baumgratz 1983–1985), 3.5–5 × 4.5–5.3 mm, 282 obtriquetrous; seeds unknown. 283 **Distribution and conservation status.** Bertolonia macrocalyx is endemic to the state of Espírito 284 285 Santo. It had been collected only twice, both in 2008. The species occurs just outside the limits of the Duas Bocas Biological Reserve, in the municipality of Viana, (Fig. 7). The area is covered 286 with well-preserved Atlantic Rainforest. Bertolonia macrocalyx was collected near a local 287 roadside and in a private property. It occurs in shaded and moist slopes, with flowers in January 288 and with old fruits in January, May and June. According to IUCN (2014) criteria B2ab(iii), AOO 289 (Area of Occupancy) = 8.000 km², this species should be classified as "critically endangered" 290 (CR). It has been collected only two times, one of them inside a protected area. 291 292 Paratypes. Brazil. Espírito Santo: Mun. Cariacica, Reserva Biológica Duas Bocas, São Paulo Viana, beira da estrada 575 m, 06 May 2008, fr., C.N. Fraga et al. 2049 (CEPEC!, MBML!, 293 294 UPCB!). Etymology. The epithet "macrocalyx" refers to the calyx with long external teeth, one of the 295 diagnostic features of the new species. 296 297 Remarks. Bertolonia macrocalyx is characterized by the short petioles (1.5–5.3 cm long) covered with sessile and short stalked glands and also with unbranched trichomes at the apex, 298 leaves with 3 main veins, both surfaces covered with sessile and short stalked glands, 299 hypanthium short-terete, light green with pinkish ridges, calyx with truncate sepals and external 300 teeth light green on the abaxial surface, ovate and ciliate (trichomes mostly at the apex), petals 301 with the upper half densely covered with sessile and short-stalked glands, short-apiculate, with 302 303 the apiculus ending in an unbranched trichome, and stamens oblong-subulate, with a dorsally thickened connective appendage. 304 305 Bertolonia duasbocaensis also has petals with the apex covered with sessile and short stalked glands, but they are concentrated at the apex of the adaxial surface and are much more sparsely 306 307 distributed when compared with B. macrocalyx. Another diagnostic feature of B. macrocalyx is the morphology of the calyx. The sepals are truncate (like B. duasbocaensis), but with a long (ca. 308 2 mm), ovate and ciliate external teeth. For more details, see the comments above on Bertolonia 309

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314 Bertolonia ruschiana Bacci & R.Goldenb. sp. nov. (Figs. 5, 6) 315 Type. Brazil. Espírito Santo: Mun. Santa Teresa, Reserva Biológica Augusto Ruschi. Beira da 316 317 estrada para Goiapaba-Açu, após a entrada da Reserva. Floresta Ombrófila Densa Montana 318 19°52'38"S 40°32'20"W 800 m, 14 January 2016, fl. and fr., L.F. Bacci, T. Bochorny and M. Bolson 321 (Holotype: UEC!; Isotypes: CEPEC!, MBML!, NY!, UPCB!). 319 320 Diagnosis. Bertolonia ruschiana is similar to B. nymphaeifolia. Both have branches, adaxial 321 suface of the leaves, pedicels and hypanthium covered only with sessile or short stalked glands, white petals with a pinkish apex, hypanthium widely campanulate and a fleshy calyx with widely 322 323 ovate sepals. The new species differs by the leaves with 5 main acrodromous veins (vs. 7–9 main 324 acrodromous veins in B. nymphaeifolia) and with ciliate margins (vs. eciliate margins), petioles covered with sessile and short-stalked glands, seldom with unbranched trichomes (vs. petioles 325 covered with sessile and short-stalked glands, and also hirsute), shorter flowers (8-9 mm long vs. 326 12-15 mm long), petals apex covered with sessile and short stalked glands on the adaxial surface 327 (vs. petals apex glabrous) and ovary apex glabrous (vs. ovary apex covered with glands). 328 329 **Description**. Herbs 10–40 cm tall, terrestrial, seldom rupicolous, reptant; adventitious roots 330 branched, growing from several points along the stem, but larger next to its base; stem 4-7 mm thick, terete and slightly costate, the older portions plagyotropic, aphyllous, the young ones erect 331 and bearing leaves. Branches, leaves, inflorescences and bracts with sparse to dense, sessile or 332 333 short stalked (then less than 0.1 mm long) glands. Leaves opposite, occasionally subopposite; petioles 1.5–6.4 cm long, quadrangular, costate, covered with the same trichomes as the 334 branches, seldom with scattered unbranched trichomes at the apex; blade $9-18.5 \times 4.5-10.5$ cm, 335 flat, ovate, elliptic to wide elliptic, chartaceous, base slightly cordate, apex rounded to slightly 336 acute, sometimes mucronulate, margins crenulate and ciliate, adaxial surface dark-green or with 337 a whitish stripe along the primary vein, covered with sessile glands, abaxial surface light-green 338 or lilac, covered with sessile or short stalked glands, seldom with small unbranched trichomes on 339

the marginal veins, veins five, plus a shorter marginal pair that do not reach the leaf apex, basal,

main veins at the abaxial surface strongly marked. Thyrsoids 3.2-9.5 cm long, terminal (but

pseudo-lateral in older, fruiting specimens), with one pair of paraclades, these cymose, scorpioid, 342 the branches greenish to light-brownish; bracteoles 0.5–0.6 mm long, narrow-triangular, apex 343 344 acuminate, both surfaces covered only with sessile glands. Flowers 5-merous, 8-9 mm long, on pedicels 1.5–2 mm long, densely covered with sessile and short-stalked glands. Hypanthium 345 light green, $2-2.5 \times 2$ mm, widely campanulate, 10-costate, with the same trichomes as in the 346 347 pedicel. Calvx caducous, tube ca. 1 mm long, sepals ca. 1.5 mm long, widely ovate, margins entire, both surfaces covered with the same trichomes as in the pedicels, seldom with an 348 unbranched trichome on the tip of the ridges between the sepals, external teeth 0.5–0.7 mm long, 349 light pink, fleshy, triangular, slightly concave, sometimes forming a small cavity, with an apex 350 ending in a small trichome, caducous. Petals white, with a light-pink apex, 6-6.5 × 5.7-6 mm, 351 widely obovate, slightly convex, fleshy, base slightly atenuate, apex rounded to obcordate, 352 353 margins entire, both surfaces papillose, adaxial surface apex covered sparsely with sessile or short stalked glands. Stamens ten, 6–7.5 mm long, isomorphic; filaments 3.5–4.5 mm long, 354 papillose, slightly widened at the base; thecae yellow, 2.5–3 mm long, oblong-subulate, slightly 355 356 undulate, pore apical, introrse; connective prolonged 0.7-0.9 mm below the thecae, dorsally thickened (0.3–0.5 mm long). Ovary free, apex glabrous, 3-locular, placentation axillary; style 357 ca. 6 mm long, curved at the apex, glabrous; stigma slightly capitate, papillose. Capsules 358 bertolonidium-type, $5.5-7 \times 6-7.5$ mm; seeds 0.5-0.7 mm long, triangular, tuberculate at the 359 apex. 360 Distribution and conservation status. Bertolonia ruschiana occurs in the central region of the 361 362 state of Espírito Santo, in three neighboring municipalities: Santa Leopoldina, Santa Maria de 363 Jetibá and Santa Teresa (Fig. 7). Most populations were found in Santa Teresa, within three 364

state of Espírito Santo, in three neighboring municipalities: Santa Leopoldina, Santa Maria de Jetibá and Santa Teresa (Fig. 7). Most populations were found in Santa Teresa, within three different conservation units and in several private properties. Two populations with several individuals were found inside the Augusto Ruschi Biological Reserve, a Brazilian Federal Conservation Unit with 3598 ha (IBAMA, 2000). A few specimens of *B. ruschiana* were collected in some trails inside the Santa Lúcia Ecological Station and the São Lourenço Municipal Natural Monument (also called "Caixa D'água") are kept by the Museu de Biologia Professor Mello Leitão (MBML). The first has 400 ha and the second has 363 ha, both covered with Atlantic Rainforest (Mendes & Padovan, 2000). Specimens of *B. ruschiana* usually are terrestrial or rupicolous herbs that inhabit moist and shaded slopes or rocks with shallow soil near water, but sometimes they <u>are epiphytes growing on lower portions of tree trunks</u>. It was

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- 374 collected with flowers from January to March and with fruits all year. According to IUCN
- 375 (2014) this species should be classified as "Lower Risk" (LR), subcategory Near Threatened (nt).
- 376 It has been collected more than 30 times, inside three conservation units and several private
- 377 properties. Nevertheless, its habitat is fragmented and the conservation of the species depends on
- 378 the maintenance of these units.
- 379 Paratypes. Brazil. Espírito Santo: Mun. Santa Leopoldina: Santo Antônio, 24 October 1988, fr.,
- 380 V. Krause s.n. (MBML 5295!); Bragança, Mata do Tyrol, prop.: Assunta Salvador, 31 October
- 381 2006, fr., L.F.S. Magnago 1523 (MBML!); Califórnia, prop. de Albertino Kringer, 16 March
- 382 2007, fr., A.P. Fontana et al. 3050 (MBML!, RB!, UPCB!). Mun. Santa Maria de Jetibá: São
- José do Rio Claro, terreno de L. Butke, 06 May 2001, fr., L. Kollmann et al. 3650 (MBML!).
- 384 Mun. Santa Teresa: Estação Biológica da Caixa D'água, 11 April 1985, fr., W. Boone 333
- 385 (MBML!); Penha, 01 October 1985, fr., H.O.B. Fernandes 1527 (MBML!); Cabeceira do Rio
- Saltinho, 03 July 1986, fr., H.Q.B. Fernandes 2003 (MBML!); Valão de São Lourenço, Estação
- Ecológica da Caixa D'água, 23 December 1993, fr., E. Bausen 53 (MBML!, SPF!, UPCB!);
- Valão de São Lourenço, Estação Ecológica da Caixa D'água, 07 January 1994, fl. and fr., E.
- 389 Bausen 54 (MBML!, UPCB!, SPF!); Estação Biológica de Santa Lúcia, 17 January 1995, fr.,
- 390 C.C. Chamas and R.R. Santos 375 (MBML!); Estação Biológica de Santa Lúcia, 24 January
- 391 1995, fl., C.C. Chamas 380 (MBML!); 05 March 1999, fl. and fr., L. Kollmann et al. 2039
- 392 (MBML!); Estação Biológica de Santa Lúcia, 19 April 2000, fr., L. Kollmann et al. 2869
- 393 (MBML!); Valsugana Velha, Prop. Dr. Pedro, 06 June 2001, fr., L. Kollmann 3816
- 394 (MBML); Valsugana Velha, 23 June 2001, fr., A.P. Fontana et al. 132 (MBML!); Valsugana
- 395 Velha, Estação Biológica de Santa Lúcia, 14 September 2001, fr., L. Kollmann and E. Bausen
- 396 4553 (MBML!); Reserva Biológica de Nova Lombardia, 05 February 2002, fl. and fr., L.
- 397 Kollmann et al. 5499 (MBML!, UPCB!); Reserva Biológica Augusto Ruschi, 22 January 2003,
- 398 fl., R.R. Vervloet et al. 1718 (MBML!); Nova Lombardia, Reserva Biológica Augusto Ruschi,
- estrada Marlene, 05 February 2003, fl., R.R. Vervloet et al. 1777 (MBML!, RB!, UPCB!); APP
- 400 São Lourenço, Trilha Boa, 29 March 2003, fr., A.P. Fontana et al. 545 (MBML!, UPCB!); Nova
- 401 Lombardia, Reserva Biológica Augusto Ruschi, trilha seguindo o córrego, 01 April 2003, fr.,
- 402 R.R. Vervloet and E. Bausen 2095 (MBML!); Reserva Biológica Augusto Ruschi, trilha da
- 403 Cachoeira, 20 January 2005, fl., H.O.H. Fernandes and R. Goldenberg 3373 (MBML!, UPCB!);
- 404 Santo Henrique, 26 January 2005, fl., L. Kollmann 7308 (MBML!); Santo Henrique, beira da

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- 405 estrada, 26 January 2005, fl., L. Kollmann and A.P. Fontana 7328 (MBML!, UPCB!); Penha,
- 406 prop. sr. Tabajara Ribeiro de Oliveira, 22 May 2005, fr., R.C. Britto 58 (MBML!); Santo
- 407 Anselmo, terreno do M. Nandolfo, 830 m, 24 February 2006, fr., L. Kollmann et al. 8675
- 408 (MBML!); Julião, prop. Sr. João Luiz Rodrigues de Souza, 23 February 2007, fr., A.P. Fontana
- 409 2980 (MBML!); Nova Lombardia, Terreno do Furlani, 13 July 2007, fr., R. Goldenberg et al.
- 410 891 (MBML!, NY!, UPCB!); Lombardia, prop. de João Furlane, 19°48'9"S 40°32'14"W, 800 m,
- 4 May 2009, fr., A.P. Fontana et al. 5982 (MBML!, WAG); Nova Lombardia, terreno do
- Furlani, 7 February 2011, fr. and fl., F.A. Michelangeli et al. 1604 (NY!; UPCB!); Nova
- Lombardia, terreno do Furlani, 7 February 2011, fl., F.A. Michelangeli et al. 1606 (NY!;
- 414 UPCB!); Reserva Biológica Augusto Ruschi, 10 December 2012, fr., J.A. Lombardi 9819
- 415 (UPCB!); Reserva Biológica Augusto Ruschi, 27 November 2013, fr., L.F. Bacci and D.F. Lima
- 416 108 (UPCB!); Reserva Biológica Augusto Ruschi, trilha da Roda D'água, 14 January 2016, fl.
- and fr., *L.F. Bacci* et al. 317 (MBML!, NY!, UEC!, UPCB!).
- 418 **Etymology**. The epithet "ruschiana" honors the Brazilian naturalist Augusto Ruschi (1915-
- 419 1986), who fought against the deforestation of the Atlantic Forest, mainly in the state of Espírito
- 420 Santo. In addition, the type locality of the species is in the Augusto Ruschi Biological Reserve.
- 421 **Remarks**. Bertolonia ruschiana is characterized by the adaxial surface of the leaves, pedicels
- and hypanthium covered only with sessile or short stalked glands, the leaves with 5 main
- 423 acrodromous veins and ciliate margins, hypanthium widely campanulate, sepals widely ovate
- 424 and fleshy, with triangular, slightly concave external teeth. The widely depressed obovate,
- slightly convex and fleshy petals with the apex of the adaxial surface covered sparsely with
- 426 sessile or short stalked glands are also important for the recognition of the new species.
- 427 Bertolonia ruschiana closely resembles B. nymphaeifolia. (for more details, see the diagnosis
- 428 above).
- 429 Several specimens of *Bertolonia ruschiana* had been determined as *Bertolonia sanguinea*, due
- 430 to the similar vegetative morphology. Both species share the big leaves with cordate bases, and
- both surfaces of the leaf covered only with sessile or short stalked glands, but B. sanguinea has
- 432 hirsute petioles and eciliate leaf margins (Baumgratz, 1990). The morphology of the flowers is
- 433 also different. Bertolonia sanguinea has sepals lacking external teeth (vs. sepals with a
- triangular, concave external teeth in *B. ruschiana*), longer flowers (18-23 mm vs. 8-9 mm),

435	glabrous petals (vs. adaxial surface apex covered with sessile and short stalked glands), longer
436	stamens (18-19 mm long vs. 6-7.5 mm), these with an extension at the apex, forming a tube (vs.
437	stamens without an extension at the apex).
438	
439	ACKNOWLEDGMENTS
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442	fieldwork of AMA and RG in Espirito Santo State was made possible by support from the
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449	at Santa Lucia. We thank IEMA/ES for the collection permit in Reserva Biológica de Duas
450	Bocas (IEMA #14489/07), and also for the support in the reserve, and ICMBio for the collection
451	permit in Reserva Biológica Augusto Ruschi (ICMBio n°49043-2).
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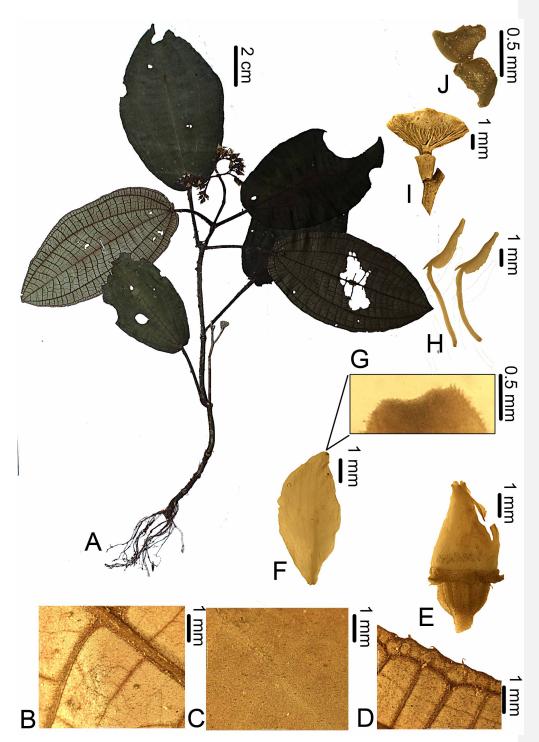


Figure 1. Bertolonia duasbocaensis. A. Habit. B. Detail of the abaxial surface of the leaf. C. Detail of the adaxial surface of the leaf. D. Abaxial surface of the leaf showing the ciliate margin. E. Flower bud. F. Adaxial view of the petal. G. Detail of the petal apex covered with sessile and short stalked glands. H. Lateral view of the stamens. I. Fruit. J. Seeds. (A-D from R.C. Forzza et al. 5035; E-H from R. Goldenberg et al. 1249; I and J from R. Goldenberg et al. 1210).

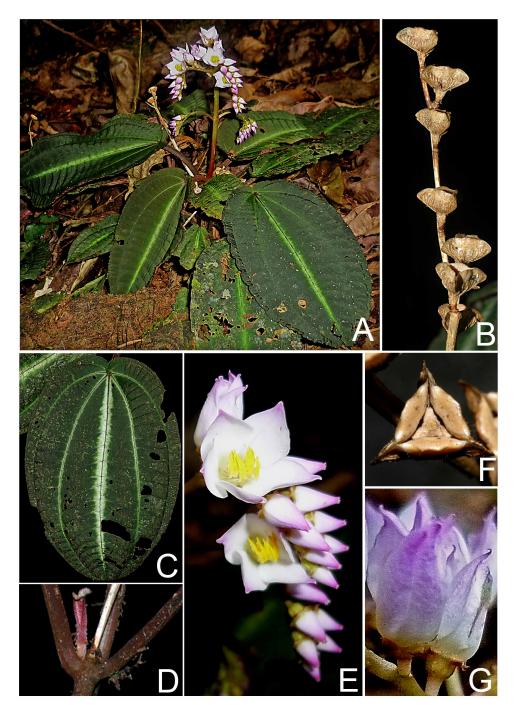
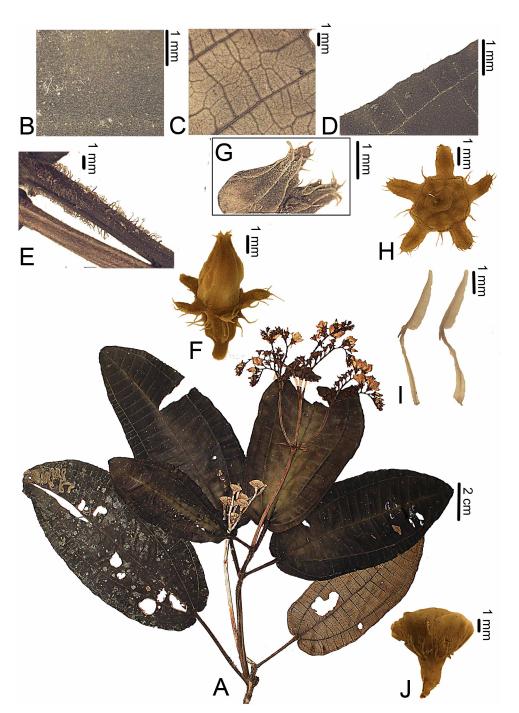


Figure 2. Bertolonia duasbocaensis. A. Habit. B. Infructescence. C. Adaxial surface of the leaf. D. Stem and petioles. E) Inflorescence. F. Fruit. G. Flowers on lateral view. (Photos by R. Goldenberg).



of the abaxial surface of the leaf. D. Adaxial surface of the leaf showing the ciliate margin. E. Petioles. F. Flower. G. Petals of a flower bud showing the sesille and short stalked glands on the upper half. H. Frontal view of the calyx. I. Lateral view of the stamens. J. Fruit. (A-I from R. Goldenberg et al. 1235; J from C.N. Fraga et al. 2049).

Figure 3. Bertolonia macrocalyx. A. Habit. B. Detail of the adaxial surface of the leaf. C. Detail

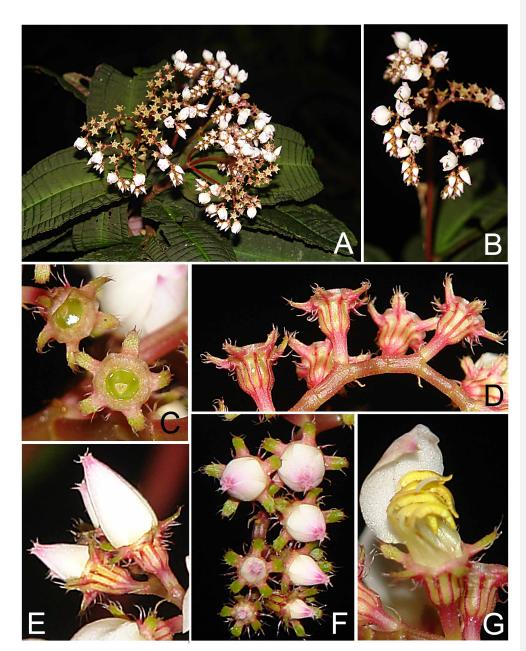


Figure 4. Bertolonia macrocalyx. A. Habit. B. Inflorescence. C. Frontal view of the calyx and ovary apex. D. Lateral view of old flowers. E. Lateral view of the flowers. F. Frontal view of the flowers. G. Stamens (Photos by C.N. Fraga).

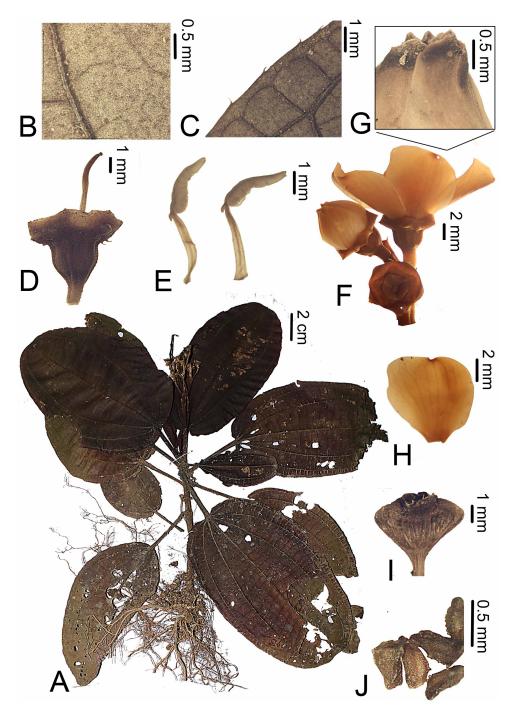


Figure 5. Bertolonia ruschiana. A. Habit. B. Detail of the abaxial surface of the leaf. C. Detail of the abaxial surface of the leaf showing the ciliate margin. D. Hypanthium, calyx and style. E. Lateral view of the stamens. F. Flowers and flower bud. G. Detail of the petals apex covered with sessile and short stalked glands. H. Adaxial view of the petal. I. Fruit. J. Seeds. (A, I and J from L. Kollmann and E. Bausen 4553; B-H from L.F. Bacci et al. 321).

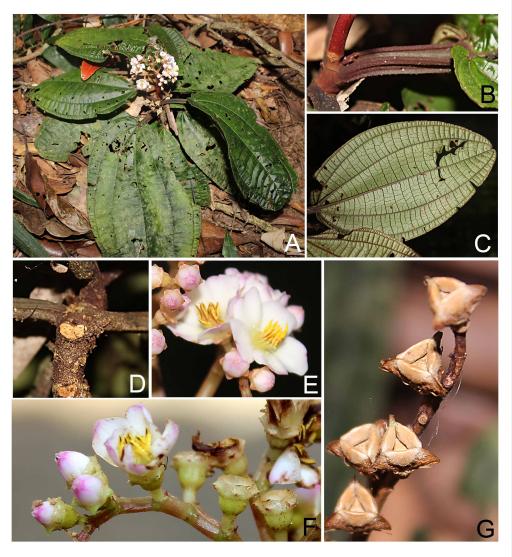


Figure 6. *Bertolonia ruschiana*. A. Habit. B. Petiole. C. Abaxial surface of the leaf blade. D. Stem. E. Flowers and flower buds. F. Inflorescence. G. Infructescense. (Photos by L.F. Bacci).

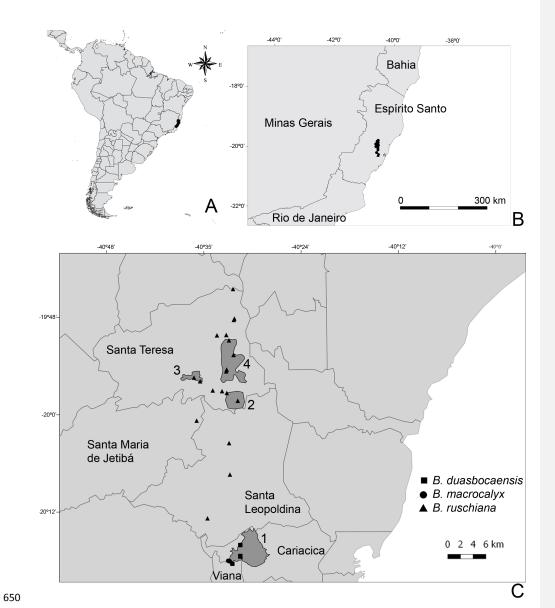


Figure 7. Map with the collection localities of the new species: A) South America, the state of
Espírito Santo in black; B) Detail of the state of Espírito Santo and the neighboring states, the
collection points in black; C) Collection points. Conservation Units: 1- Duas Bocas Biological
Reserve, 2- Santa Lúcia Ecological Reserve, 3- São Lourenço Natural Municipal Reserve, 4Augusto Ruschi Biological Reserve.

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