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

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

**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
- 31 <u>obtriquetrous obtriqutrous</u> capsular fruits, like *Monolena* Triana ex Bent. & Hook. f., *Salpinga*
- 32 Mart. ex DC. and *Triolena* Naudin (Clausing & Renner, 2001). Nevertheless, the tribe is not
- 33 monophyletic, and Monolena, Salpinga and Triolena actually belong to a different evolutionary
- 34 lineage (Goldenberg et al., 2012). Inside the Atlantic Forest Biome, the species of *Bertolonia*
- 35 occur mostly in the Atlantic Rainforest ("Floresta Ombrófila Densa Submontana",
- 36 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
- 38 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,
- 40 on decaying logs or as epiphytes on the base of tree trunks (frequently tree ferns). The plants are
- 41 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
  - from Rio de Janeiro. According to "Flora do Brasil" (Baumgratz, 2016), there should be only
- 47 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
- 49 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
- 51 should rise to seven species, which makes this state the second richest, after Rio de Janeiro (8
- 52 species), but before Bahia (6 species; Baumgratz, 2016). Espírito Santo was originally and
- 53 almost entirely covered by the Atlantic Forest Biome (Fundação SOS Mata Atlântica & INPE,
- 54 1993). Nowadays only about 10% of the original vegetation remains preserved (Fundação SOS
- 55 Mata Atlântica & INPE, 2014). Despite of the small remaining forested area and the relatively

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. 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 illustrations of living and dry specimens.

## 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., 1989). Morphological descriptions follow Radford (1974), Baumgratz (1985-1987) and Baumgratz (1990). The study was based on literature (Baumgratz, 1990, Baumgratz et al., 2011, Bacci et al., 2016) and the analysis of specimens at CEPEC, MBM, MBML, NY, RB, UPCB, VIES (acronyms according to Thiers, 2016). Ecological and geographic distribution data were also obtained from herbarium labels. Conservation status assessments were based on range size (criterion B), according to IUCN (2014). The Extension of Occurrence (EOO) and Area of Occupancy (AOO) were calculated through GeoCAT (Bachman et al., 2011). Collection permits were issued by the "Instituto Estadual do Meio Ambiente" (IEMA/Espírito Santo: 14489/07) for the plants inside "Reserva Biológica de Duas Bocas" and by the "Instituto Chico Mendes de Conservação e Biodiversidade (ICMBio: 49043-2) for the plants inside the "Reserva Biológica Augusto Ruschi".

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84	"http://ipni.org/". The online version of this work is archived and available from the following
85	digital repositories: PeerJ, PubMed Central, and CLOCKSS.
86	
87	RESULTS
88	Key to the species of Bertolonia from the state of Espírito Santo, Brazil.
89	1. Leaves with bullate/foveolate surfaces
90	1'. Leaves with flat surfaces
91	2. Petioles villose; sepals with entire margins; petals 5.5–7 mm long <i>B. foveolata</i>
92	2'. Petioles hirsute; sepals with long-laciniate margins; petals 7.8–8.3 mm long
93	B. wurdackiana
94	3. Abaxial and/or adaxial surfaces of the leaf blade permanently covered with sessile and short
95	stalked glands and also long unbranched trichomes
96	3'. Both surfaces of the leaf blade covered with only sessile and short stalked glands
97	4. Petioles hirsute at the apex; flowers 17–22 mm long; sepals fleshy, with short-lacinate
98	margins; petals with eciliate margins; anther connective with a dorsal appendage B. formosa
99	4'. Petioles glandulose-pilose or glandulose-villose; flowers 11–13 mm long; sepals
100	membranaceous, with entire margins; petals with the margins ciliate at the apex; anther
101	connective unnapendaged
102	5. Hypanthium covered with only sessile or short stalked glands; sepals with an acute apex;
103	petals 6–6.5 mm long, widely obovate
104	5'. Hypanthium covered with sessile or short stalked glands and also with unbranched trichomes;
105	sepals truncate; petals 7–12 mm, irregularly elliptic
106	6. Flowers 13.3-14.7 mm long; calyx external teeth ca. 1 mm long, triangular, covered with only
107	sessile and short stalked glands; petals long-apiculate (ca. 1 mm long), the apiculus without an
108	unbranched trichome its tip

6'. Flowers 9.5-11.2 mm long; calyx external teeth ca. 2 mm long, ovate, covered with sessile 109 and short stalked glands and also long-stalked glands (0.6-0.8 mm long); petals short-apiculate 110 111 112 113 Bertolonia duasbocaensis Bacci & R.Goldenb. sp. nov. 114 (Figs 1, 2) 115 Type. Brazil. Espírito Santo: Mun. Cariacica, Reserva Biológica Duas Bocas. Localidade de Alegre, trilha do Pau-Oco. Floresta Ombrófila Densa Montana 20°16'4"S 40°31'30"W 525 m, 18 116 January 2009, fl., R. Goldenberg, C.N. Fraga, P.H. Labiak, R.C. Forzza, A.P. Fontana, L.C. 117 Kollmann and P.B. Schwartsburd 1249 (holotype: UPCB!; isotypes: CEPEC!, MBML!). 118 119 Diagnosis. Bertolonia duasbocaensis is similar to B. formosa. The new species has petioles and 120 leaves covered only with sessile or short-stalked glands (vs. petioles hirsute and leaves also 121 covered with unbranched trichomes in B. formosa), smaller flowers (13.3-14.7 mm long vs. 17-122 22 mm long in B. formosa), inconspicuous and truncate sepals with triangular external teeth (vs. 123 conspicuous, 3.2–3.7 mm long sepals, sepals, these rounded and lacking external teeth) and by the 124 petals with the apex covered with sessile or short-stalked glands (vs. petals glabrous). **Description**. Herbs 10–35 cm tall, terrestrial, reptant; adventitious roots branched, growing from 125 several points along the stem, but larger next to its base; stem 3-5 mm thick, terete and slightly 126 costate, the older portions plagyotropic and aphyllous, the young ones erect and bearing leaves. 127 Branches, leaves, inflorescences and bracts with sparse to dense, sessile or short-stalked (then 128 129 less than 0.1 mm long) glands, young branches also with sparse unbranched trichomes. Leaves 130 opposite, occasionally subopposite; petioles 3–9.5 cm long, quadrangular, slightly costate, covered with the same trichomes as the branches; blade  $7.8-11.7 \times 4.5-9$  cm, flat, ovate to 131 elliptic, chartaceous, base rounded to slightly cordate, apex rounded to slightly acute, margins 132 133 entire to slightly crenulate and ciliate, adaxial surface dark-green, whitish along the primary 134 vein, sparsely covered with sessile glands, otherwise glabrescent, abaxial surface light-green or 135 lilac, sparsely covered with sessile glands, 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 136 137 bases. Thyrsoids 4.5–10.7 cm long, terminal (but pseudo-lateral in older, fruiting specimens),

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bracteoles 0.5-0.8 mm long, narrow-lanceolate, apex acuminate, both surfaces covered only with 139 140 sessile glands. Flowers 5-merous, 13.3-14.7 mm long, on pedicels ca. 2 mm long, light green, 141 densely covered with sessile and short-stalked glands. Hypanthium light green,  $2.5-2.7 \times 2$  mm, widely campanulate, 10-costate, with the same indument as the pedicel, seldom with scattered 142 unbranched trichomes in its the upper half. Calyx caducous on fruits, tube ca. 0.7 mm long, 143 sepals truncate, external teeth ca. 1 mm long, green to slightly vinaceous, triangular, apex acute, 144 margins entire, eciliate, both surfaces with the same indument as the pedicels. Petals white, with 145 light-pink or light-purple apex,  $10.8-12 \times 4.5-5$  mm, irregularly elliptic, base slightly atenuate, 146 147 apex acute and apiculate (ca. 1 mm long), the apiculus bending outwards (to the abaxial surface 148 of the petal), lacking trichomes at its tip, margins entire, both surfaces papillose, adaxial surface 149 apex sparsely covered with sessile or short-stalked glands. Stamens 10, 6–7 mm long, isomorphic; filaments 3.5–4 mm long, slightly widened at the base; thecae yellow, 2–3 mm long, 150 oblong-subulate, slightly undulate, pore apical, introrse; connective prolonged ca. 1 mm below 151 152 the thecae, dorsally thickened appendage. Ovary free, apex glabrous, 3-locular, placentation axillary; style ca. 5 mm long, curved at the apex, glabrous; stigma slightly capitate, papillose. 153 Capsules bertolonidium-type, 4–5 × 4.5–6 mm, obtriquetrous; seeds 0.4–0.5 mm long, triangular, 154 155 tuberculate on the dorsal angles and apex. 156 **Distribution and conservation status.**—Bertolonia duasbocaensis has been collected a few times since 2008, mostly along the same trail ("Pau-Oco") inside Duas Bocas Biological 157 158 Reserve, but with one specimen occurring in a private property near the Reserve. The Biological 159 Reserve is a fully protected area, kept by the state government (IEMA/ES). It is located in the municipality of Cariacica, in central Espírito Santo, between 20°14'40"S - 20°18'30" S and 160  $40^{\circ}28'01"W - 40^{\circ}32'07"W$ . Its 2910 ha are covered with well-preserved Atlantic Rainforest that 161 protects threatened species of both fauna and flora (Novelli, 2010). In the last five years, three 162 new plant species were described from Duas Bocas: Eugenia amorimii Fraga & Giaretta 163 (Giaretta & Fraga, 2014), Leandra magnipetala R. Goldenb. & E. Camargo (Camargo & 164 Goldenberg 2011), and Ouratea cauliflora Fraga & Saavedra (Fraga & Saavedra, 2014). The 165 plants occur in shaded and moist slopes at 500-600 m elev. Bertolonia duasbocaensis was 166 collected with flowers in January and February and with fruits in January, February and July. 167 According to IUCN (2014) criteria B1ab(ii) + B2ab(ii), with EOO (Extent of Ocurrence) = 2.235 168

with one pair of paraclades, these cymose, scorpioid, the branches greenish to light-pink;

"endangered" (EN). It was collected only in two locations, one of them in a private property. 170 Paratypes. Brazil. Espírito Santo: Mun. Cariacica, 10 January 2007, fl. and fr., A.P. Fontana et 171 al. 2584 (MBML!, RB!, UPCB!); 10 January 2007, fl., L. Kollmann et al. 9457 (MBML!); 20 172 July 2008, fr., A.M.A. Amorim et al. 7571 (CEPEC!, RB!, UPCB!); 22 July 2008, fr., R. 173 Goldenberg 1210 (CEPEC!, MBML!, RB!, UPCB!); 15 February 2008, fl. and fr., R.C. Forzza. 174 et al. 5035 (CEPEC!, MBM!, MBML!, RB!, UPCB!). Mun. Viana, Arredores da Reserva 175 Biológica Duas Bocas, São Paulo de Viana, prop. do Valtinho, 700 m, 19 January 2009, fl. and 176 fr., R. Goldenberg et al. 1257 (MBML!, RB!, UPCB!). 177 Etymology— The epithet "duasbocaensis" refers to the Duas Bocas Biological Reserve, the type 178 locality of the new species. 179 180 Remarks. Bertolonia duasbocaensis is characterized by the ovate to elliptic leaves with rounded 181 to slightly cordate bases, ciliate margins, 3–5 main veins, and covered only with sessile and short-stalked glands, the widely campanulate hypanthium, truncate sepals, triangular external 182 183 calyx teeth, apiculate petals with the apex of the adaxial surface covered with sessile or shortstalked glands, and oblong-subulate stamens with a dorsally thickened appendage. The new 184 species closely resembles B. formosa, also endemic of the state of Espírito Santo. For more For 185 186 more details on the distinction between the two of them, see the diagnosis above. 187 Bertolonia hoehneana Brade, B. nymphaeifolia Raddi and B. sanguinea share with the new 188 species the leaves with cordate bases and also the branches and adaxial surface of the leaves 189 covered only with sessile or short stalked glands. The first is a rare species, endemic to the state 190 of São Paulo (Baumgratz, 1990; Baumgratz, 2016) and differs by the leaves with a cordatelobate base (vs. rounded to slightly cordate in B. duasbocaensis), eciliate margins (vs. ciliate 191

margins) and an acute, attenuate, or sometimes acuminate apex (vs. rounded to slightly acute),

with sessile or short-stalked glands (vs. covered also with scattered unbranched trichomes,

by the smaller flowers (10-13 mm long vs. 13.5-15 mm long) with the hypanthium covered only

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

de Janeiro and differ from the new species by the bigger leaves, with 7-9 and 5-7 acrodromous

veins, respectively (vs. 3–5 in B. duasbocaensis), petioles hirsute at the base or seldom on the

km² and AOO (Area of Occupancy) = 12.000 km², this species should be classified as

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Comentado [PJFG1]: The variation is greater in the description: "margins entire to slightly crenulate and ciliate"

**Comentado [PJFG2]:** "short-stalked glands" Not present in the description of the leaf blade.

whole petiole (vs. petioles covered with sessile or stalked glands, the young ones sometimes with caducous, unbranched trichomes), longer flowers, 18–23 mm long in *B. sanguinea* (vs. shorter ones, 13.3–14.7 mm long in *B. duasbocaensis*) and by the long subulate stamens with an acute 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 calyx is also different: *B. nymphaeifolia* has 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 (Baumgratz 1990), while *B. duasbocaensis* has truncate sepals with a triangular external teeth.

*Bertolonia duasbocaensis* closely resembles *B. macrocalyx*; both are endemic of Espírito Santo and occur very close to each other. The differences between both species are listed in the diagnosis of *B. macrocalyx* (see below).

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- Bertolonia macrocalyx Bacci & R.Goldenb. sp. nov.
- 212 (Figs. 3, 4)
- 213 Type. Brazil. Espírito Santo: Mun. Viana, Arredores da Reserva Biológica Duas Bocas. São
- 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,
- and P.B. Schwartsburd 1235 (Holotype: UPCB; Isotypes: CEPEC, MBML).
- 217 **Diagnosis**. Bertolonia macrocalyx is similar to B. duasbocaensis. Both species have similar
- 218 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),
- short-terete hypanthium (vs. widely campanulate hypanthium), smaller,  $7-8.5 \times 3-3.5$  mm petals
- 221 with the upper half of the adaxial surface densely covered with sessile and short-stalked glands
- 222 (vs. bigger  $10.8-12 \times 4.5-5$  mm petals, with only the apex of the adaxial surface sparsely
- 223 covered with sessile and short-stalked glands).
- 224 **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,
- 226 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 × 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, papillose. Capsules bertolonidium-type (following Baumgratz 1983–1985), 3.5–5 × 4.5–5.3 mm, obtriquetrous; seeds unknown.

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Santo. It had been collected only twice, both in 2008. The species occurs just outside the limits 258 259 of the Duas Bocas Biological Reserve, in the municipality of Viana, (Fig. 7). The area is covered 260 with well-preserved Atlantic Rainforest. Bertolonia macrocalyx was collected near a local roadside and in a private property. It occurs in shaded and moisty slopes, with flowers in January 261 and with old fruits in January, May and June. According to IUCN (2014) criteria B2ab(iii), AOO 262 (Area of Occupancy) = 8.000 km<sup>2</sup>, this species should be classified as "critically endangered" 263 264 (CR). It has been collected only two times, one of them inside a protected area. Paratypes. Brazil. Espírito Santo: Mun. Cariacica, Reserva Biológica Duas Bocas, São Paulo 265 Viana, beira da estrada 575 m, 06 May 2008, fr., C.N. Fraga et al. 2049 (CEPEC!, MBML!, 266 267 UPCB!). Etymology. The epithet "macrocalyx" refers to the calyx with long external teeth, one of the 268 269 diagnostic features of the new species. 270 **Remarks.** Bertolonia macrocalyx is characterized by the short petioles (1.5–5.3 cm long) 271 covered with sessile and short stalked glands and also with unbranched trichomes at the apex, leaves with 3 main veins, both surfaces covered with sessile and short stalked glands, 272 hypanthium short-terete, light green with pinkish ridges, calyx with truncate sepals and external 273 274 teeth light green on the abaxial surface, ovate and ciliate (trichomes mostly at the apex), petals 275 with the upper half densely covered with sessile and short-stalked glands, short-apiculate, with 276 the apiculus ending in an unbranched trichome, and stamens oblong-subulate, with a dorsally 277 thickened connective appendage. 278 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 279 distributed when compared with B. macrocalyx. Another diagnostic feature of B. macrocalyx is 280 the morphology of the calyx. The sepals are truncate (like B. duasbocaensis), but with a long (ca. 281

2 mm), ovate and ciliate external teeth. For more details, see the comments above on Bertolonia

Distribution and conservation status. Bertolonia macrocalyx is endemic to the state of Espírito

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<del>duasbocaensis</del>

**Comentado [PJFG3]:** The variation is greater in the description "veins three to five"

**Comentado [PJFG4]:** It makes no sense because these differences are described here only. "The differences between both species are listed in the diagnosis of *B. macrocalyx* (see below)."

Bertolonia ruschiana Bacci & R.Goldenb. sp. nov.

Type. Brazil. Espírito Santo: Mun. Santa Teresa, Reserva Biológica Augusto Ruschi. Beira da 287 estrada para Goiapaba-Açu, após a entrada da Reserva. Floresta Ombrófila Densa Montana 288 19°52'38"S 40°32'20"W 800 m, 14 January 2016, fl. and fr., L.F. Bacci, T. Bochorny and M. 289 Bolson 321 (Holotype: UEC!; Isotypes: CEPEC!, MBML!, NY!, UPCB!). 290 291 **Diagnosis**. Bertolonia ruschiana is similar to B. nymphaeifolia. Both have branches, adaxial suface of the leaves, pedicels and hypanthium covered only with sessile or short stalked glands, 292 293 white petals with a pinkish apex, hypanthium widely campanulate and a fleshy calyx with widely ovate sepals. The new species differs by the leaves with 5 main acrodromous veins (vs. 7–9 main 294 acrodromous veins in B. nymphaeifolia) and with ciliate margins (vs. eciliate margins), petioles 295 covered with sessile and short-stalked glands, seldom with unbranched trichomes (vs. petioles 296 covered with sessile and short-stalked glands, and also hirsute), shorter flowers (8-9 mm long vs. 297 298 12-15 mm long), petals apex covered with sessile and short stalked glands on the adaxial surface (vs. petals apex glabrous) and ovary apex glabrous (vs. ovary apex covered with glands). 299 300 **Description**. Herbs 10–40 cm tall, terrestrial, seldom rupicolous, reptant; adventitious roots branched, growing from several points along the stem, but larger next to its base; stem 4-7 mm 301 thick, terete and slightly costate, the older portions plagyotropic, aphyllous, the young ones erect 302 and bearing leaves. Branches, leaves, inflorescences and bracts with sparse to dense, sessile or 303 short stalked (then less than 0.1 mm long) glands. Leaves opposite, occasionally subopposite; 304 305 petioles 1.5-6.4 cm long, quadrangular, costate, covered with the same trichomes as the branches, seldom with scattered unbranched trichomes at the apex; blade  $9-18.5 \times 4.5-10.5$  cm, 306 flat, ovate, elliptic to wide elliptic, chartaceous, base slightly cordate, apex rounded to slightly 307 acute, sometimes mucronulate, margins crenulate and ciliate, adaxial surface dark-green or with 308 a whitish stripe along the primary vein, covered with sessile glands, abaxial surface light-green 309 or lilac, covered with sessile or short stalked glands, seldom with small unbranched trichomes on 310 311 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 312 pseudo-lateral in older, fruiting specimens), with one pair of paraclades, these cymose, scorpioid, 313 the branches greenish to light-brownish; bracteoles 0.5–0.6 mm long, narrow-triangular, apex 314 acuminate, both surfaces covered only with sessile glands. Flowers 5-merous, 8-9 mm long, on 315

(Figs. 5, 6)

pedicels 1.5–2 mm long, densely covered with sessile and short-stalked glands. Hypanthium 316 light green,  $2-2.5 \times 2$  mm, widely campanulate, 10-costate, with the same trichomes as in the 317 318 pedicel. Calyx caducous, tube ca. 1 mm long, sepals ca. 1.5 mm long, widely ovate, margins 319 entire, both surfaces covered with the same trichomes as in the pedicels, seldom with an unbranched trichome on the tip of the ridges between the sepals, external teeth 0.5–0.7 mm long, 320 321 light pink, fleshy, triangular, slightly concave, sometimes forming a small cavity, with an apex ending in a small trichome, caducous. Petals white, with a light-pink apex,  $6-6.5 \times 5.7-6$  mm, 322 widely obovate, slightly convex, fleshy, base slightly atenuate, apex rounded to obcordate, 323 margins entire, both surfaces papillose, adaxial surface apex covered sparsely with sessile or 324 325 short stalked glands. Stamens ten, 6–7.5 mm long, isomorphic; filaments 3.5–4.5 mm long, 326 papillose, slightly widened at the base; thecae yellow, 2.5-3 mm long, oblong-subulate, slightly 327 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 328 ca. 6 mm long, curved at the apex, glabrous; stigma slightly capitate, papillose. Capsules 329 330 bertolonidium-type, 5.5–7 × 6–7.5 mm; seeds 0.5–0.7 mm long, triangular, tuberculate at the 331 apex. 332 Distribution and conservation status. Bertolonia ruschiana occurs in the central region of the 333 state of Espírito Santo, in three neighboring municipalities: Santa Leopoldina, Santa Maria de 334 Jetibá and Santa Teresa (Fig. 7). Most populations were found in Santa Teresa, within three 335 different conservation units and in several private properties. Two populations with several 336 individuals were found inside the Augusto Ruschi Biological Reserve, a Brazilian Federal 337 Conservation Unit with 3598 ha (IBAMA, 2000). A few specimens of B. ruschiana were collected in some trails inside the inside the Santa Lúcia Ecological Station and the São 338 Lourenço Municipal Natural Monument (also called "Caixa D'água") are kept by the Museu de 339 Biologia Professor Mello Leitão (MBML). The first has 400 ha and the second has 363 ha, both 340 341 covered wthwith Atlantic Rainforest (Mendes & Padovan, 2000). Specimens of B. ruschiana 342 usually are terrestrial or rupicolous herbs that inhabit moist and shaded slopes or rocks with 343 shallow soil near water, but sometimes they epiphesepithets growing on lower portions of tree trunks. It was collected with flowers from January to March and with fruits all year. According 344 to IUCN (2014) this species should be classified as "Lower Risk" (LR), subcategory Near 345 Threatened (nt). It has been collected more than 30 times, inside three conservation units and 346

- 347 several private properties. Nevertheless, its habitat is fragmented and the conservation of the
- 348 species depends on the maintenance of these units.
- 349 Paratypes. Brazil. Espírito Santo: Mun. Santa Leopoldina: Santo Antônio, 24 October 1988, fr.,
- 350 V. Krause s.n. (MBML 5295!); Bragança, Mata do Tyrol, prop.: Assunta Salvador, 31 October
- 351 2006, fr., L.F.S. Magnago 1523 (MBML!); Califórnia, prop. de Albertino Kringer, 16 March
- 352 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!).
- 354 Mun. Santa Teresa: Estação Biológica da Caixa D'água, 11 April 1985, fr., W. Boone 333
- 355 (MBML!); Penha, 01 October 1985, fr., H.Q.B. Fernandes 1527 (MBML!); Cabeceira do Rio
- 356 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!);
- 358 Valão de São Lourenço, Estação Ecológica da Caixa D'água, 07 January 1994, fl. and fr., E.
- 359 Bausen 54 (MBML!, UPCB!, SPF!); Estação Biológica de Santa Lúcia, 17 January 1995, fr.,
- 360 C.C. Chamas and R.R. Santos 375 (MBML!); Estação Biológica de Santa Lúcia, 24 January
- 361 1995, fl., C.C. Chamas 380 (MBML!); 05 March 1999, fl. and fr., L. Kollmann et al. 2039
- 362 (MBML!); Estação Biológica de Santa Lúcia, 19 April 2000, fr., L. Kollmann et al. 2869
- 363 (MBML!); Valsugana Velha, Prop. Dr. Pedro, 06 June 2001, fr., L. Kollmann 3816
- 364 (MBML); Valsugana Velha, 23 June 2001, fr., A.P. Fontana et al. 132 (MBML!); Valsugana
- 365 Velha, Estação Biológica de Santa Lúcia, 14 September 2001, fr., L. Kollmann and E. Bausen
- 366 4553 (MBML!); Reserva Biológica de Nova Lombardia, 05 February 2002, fl. and fr., L.
- 367 Kollmann et al. 5499 (MBML!, UPCB!); Reserva Biológica Augusto Ruschi, 22 January 2003,
- 368 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
- 370 São Lourenço, Trilha Boa, 29 March 2003, fr., A.P. Fontana et al. 545 (MBML!, UPCB!); Nova
- 371 Lombardia, Reserva Biológica Augusto Ruschi, trilha seguindo o córrego, 01 April 2003, fr.,
- 372 R.R. Vervloet and E. Bausen 2095 (MBML!); Reserva Biológica Augusto Ruschi, trilha da
- Cachoeira, 20 January 2005, fl., H.Q.H. Fernandes and R. Goldenberg 3373 (MBML!, UPCB!);
- 374 Santo Henrique, 26 January 2005, fl., L. Kollmann 7308 (MBML!); Santo Henrique, beira da
- estrada, 26 January 2005, fl., L. Kollmann and A.P. Fontana 7328 (MBML!, UPCB!); Penha,
- 376 prop. sr. Tabajara Ribeiro de Oliveira, 22 May 2005, fr., R.C. Britto 58 (MBML!); Santo
- 377 Anselmo, terreno do M. Nandolfo, 830 m, 24 February 2006, fr., L. Kollmann et al. 8675

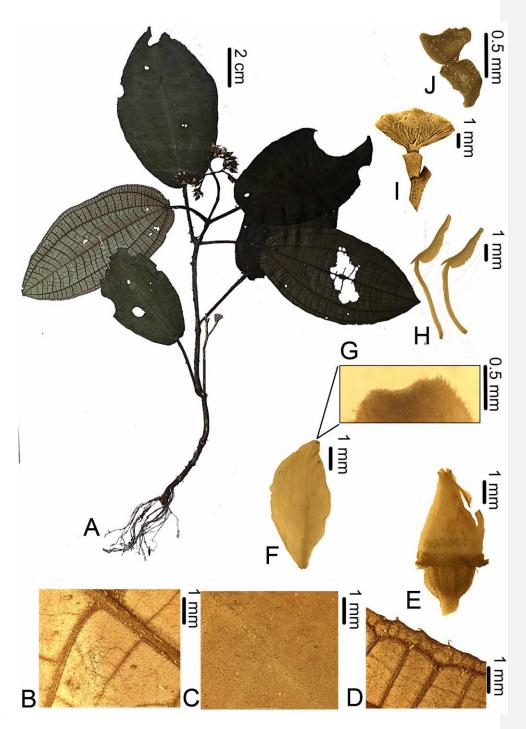
- 378 (MBML!); Julião, prop. Sr. João Luiz Rodrigues de Souza, 23 February 2007, fr., A.P. Fontana
- 379 2980 (MBML!); Nova Lombardia, Terreno do Furlani, 13 July 2007, fr., R. Goldenberg et al.
- 380 891 (MBML!, NY!, UPCB!); Lombardia, prop. de João Furlane, 19°48'9"S 40°32'14"W, 800 m,
- 381 4 May 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
- 383 Lombardia, terreno do Furlani, 7 February 2011, fl., F.A. Michelangeli et al. 1606 (NY!;
- 384 UPCB!); Reserva Biológica Augusto Ruschi, 10 December 2012, fr., J.A. Lombardi 9819
- 385 (UPCB!); Reserva Biológica Augusto Ruschi, 27 November 2013, fr., L.F. Bacci and D.F. Lima
- 386 108 (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!).
- 388 **Etymology**. The epithet "ruschiana" honors the Brazilian naturalist Augusto Ruschi (1915-
- 389 1986), who fought against the deforestation of the Atlantic Forest, mainly in the state of Espírito
- 390 Santo. In addition, the type locality of the species is in the Augusto Ruschi Biological Reserve.
- 391 **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
- 393 acrodromous veins and ciliate margins, hypanthium widely campanulate, sepals widely ovate
- and fleshy, with triangular, slightly concave external teeth. The widely depressed obovate,
- 395 slightly convex and fleshy petals with the apex of the adaxial surface covered sparsely with
- sessile or short stalked glands are also important for the recognition of the new species.
- 397 Bertolonia ruschiana closely resembles B. nymphaeifolia. (for more details, see the diagnosis
- 398 above).
- 399 Several specimens of *Bertolonia ruschiana* had been determined as *Bertolonia sanguinea*, due
- 400 to the similar vegetative morphology. Both species share the big leaves with cordate bases, and
- 401 both surfaces of the leaf covered only with sessile or short stalked glands, but B. sanguinea has
- 402 hirsute petioles and eciliate leaf margins (Baumgratz, 1990). The morphology of the flowers is
- 403 also different. Bertolonia sanguinea has sepals lacking external teeth (vs. sepals with a
- 404 triangular, concave external teeth in *B. ruschiana*), longer flowers (18-23 mm vs. 8-9 mm),
- 405 glabrous petals (vs. adaxial surface apex covered with sessile and short stalked glands), longer
- 406 stamens (18-19 mm long vs. 6-7.5 mm), these with an extension at the apex, forming a tube (vs.
- stamens without an extension at the apex).

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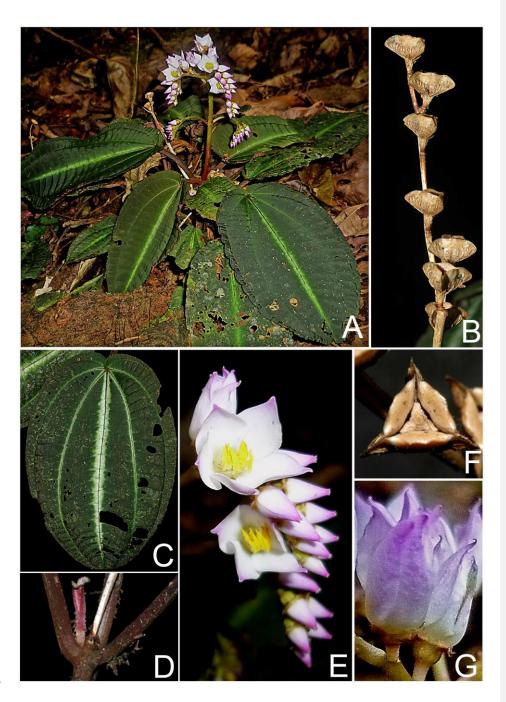
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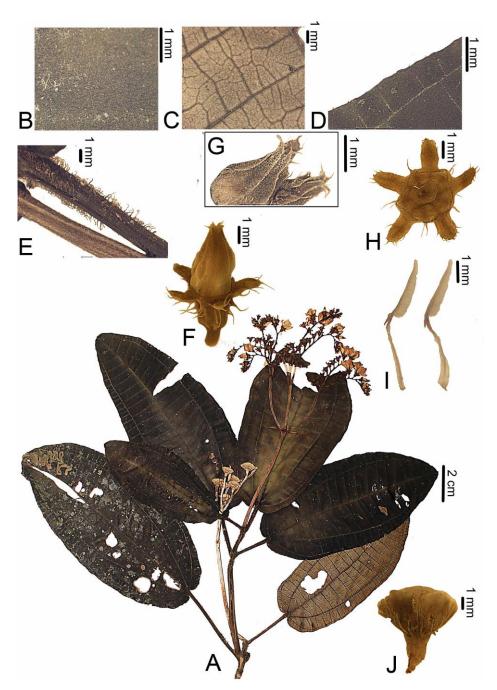
Detail of the adaxial surface of the leaf. D. Abaxial surface of the leaf showing the ciliate margin. E. Flower bud. F. Adaxial view of the petal. G. Detail of the petal apex covered with sessile and short stalked glands. H. Lateral view of the stamens. I. Fruit. J. Seeds. (A-D from R.C. Forzza et al.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

Figure 1. Bertolonia duasbocaensis. A. Habit. B. Detail of the abaxial surface of the leaf. C.



D. Stem and petioles. E) Inflorescence. F. Fruit. G. Flowers on lateral view. (Photos by R. Goldenberg). 

Figure 2. Bertolonia duasbocaensis. A. Habit. B. Infructescence. C. Adaxial surface of the leaf.



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

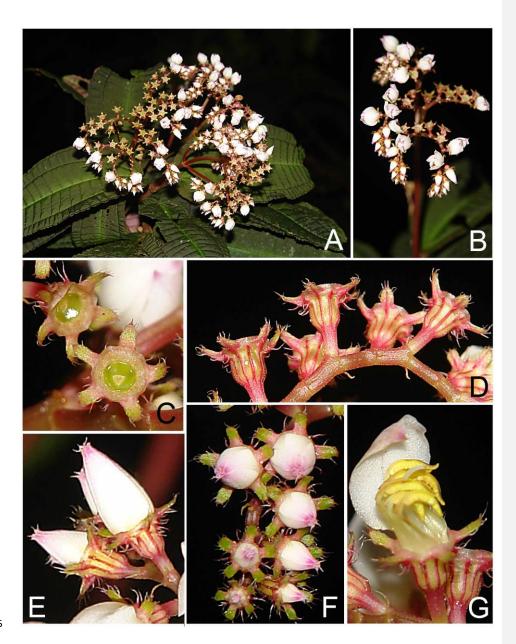


Figure 4. Bertolonia macrocalyx. A. Habit. B. Inflorescence. C. Frontal view of the calyx and ovary apex. D. Lateral view of old flowers. E. Lateral view of the flowers. F. Frontal view of the flowers. G. Stamens (Photos by C.N. Fraga). 

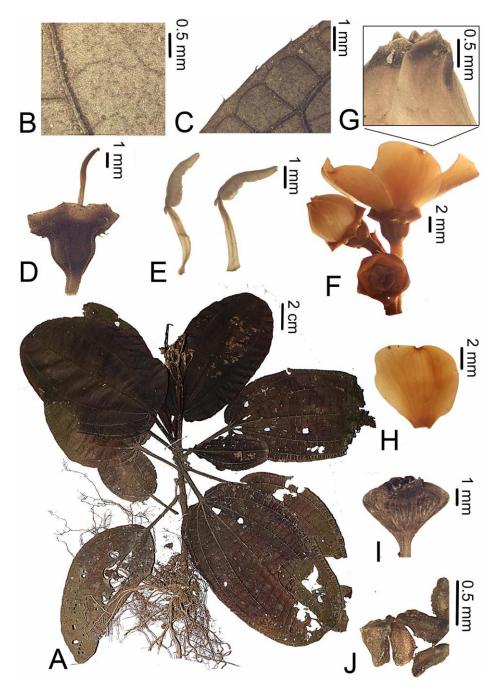


Figure 5. Bertolonia ruschiana. A. Habit. B. Detail of the abaxial surface of the leaf. C. Detail of the abaxial surface of the leaf showing the ciliate margin. D. Hypanthium, calyx and style. E. Lateral view of the stamens. F. Flowers and flower bud. G. Detail of the petals apex covered with sessile 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).

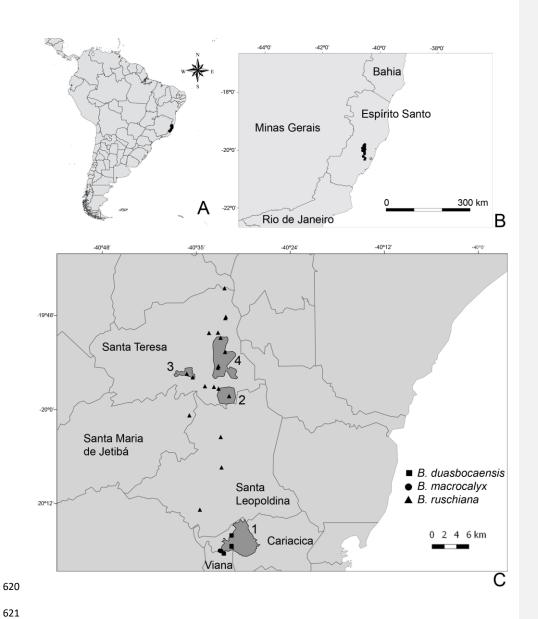


Figure 7. Map with the collection localities of the new species: A) South America, the state of
Espírito Santo in black; B) Detail of the state of Espírito Santo and the neighboring states, the
collection points in black; C) Collection points. Conservation Units: 1- Duas Bocas Biological
Reserve, 2-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